PRIMATE NUTRITIONAL ECOLOGY: THE ROLE OF FOOD SELECTION, ENERGY INTAKE, AND NUTRIENT BALANCING IN MEXICAN BLACK HOWLER MONKEY (*ALOUATTA PIGRA*) FORAGING STRATEGIES

BY

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DISSERTATION

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ABSTRACT

Studying primate nutritional ecology is critical for addressing questions related to individual and group-based decision making, feeding ecology, life history, and reproductive success. However, understanding food selection is a complex task, and it requires integrating information on physiology, behavior, and the ecological and social environments in which the animals live. In this dissertation, I examined the nutritional ecology of Mexican black howler monkeys (*Alouatta pigra*), an endangered nonhuman primate species characterized by a high intraspecific variability in time spent feeding on leaves, fruits, flowers, and other items (such as bark and stems) across seasons and study sites. Howler monkeys are considered the most folivorous New World primates, with leaves accounting for up to 100% of feeding time during certain months. Given assumptions regarding the challenges faced by foragers exploiting difficult to digest or high fiber foods that also may contain plant secondary compounds, howlers are considered energy-limited. While howler monkeys do consume a leaf-heavy diet during certain seasons of the year, and possess certain anatomical and physiological traits such as a capacious colon where fermentation occurs, a relatively long food transit time for their body mass, and molars with high shearing crests that contribute to the efficient processing of leafy material, describing them as folivores is an oversimplification of their dietary ecology. In this 15-month field study, I combined ecological, behavioral, and phytochemical data to analyze patterns of patch and food choice, nutrient and energy intake, and nutrient balancing in two groups (n = 14) of black howler monkeys inhabiting a 1400-ha semi-deciduous forest (“El Tormento”) in Campeche, Mexico. By following a single individual and recording its complete diet over the course of a single day, the amount in grams of each resource consumed, and the phytochemical characteristic of the food ingested, I constructed complete daily dietary profiles for each focal animal, and analyzed

individual food choices using the Geometric Framework for nutrition. The GF is a multidimensional approach in which variables such as different food components and the amount of ingested nutrients are viewed in geometric space. The first chapter examines the role of resource mixing (i.e., switching between patches characterized by different types of resources and proportions of macronutrients) in individual feeding patch choice and patch leaving decisions. The second chapter analyzes the effects of plant phytochemical characteristics, including macronutrients and minerals, on individual food selection. The third chapter utilizes nutritional geometry to explore the synergistic effects of multiple nutrients and energy requirements on howler food choice across three different seasons (rainy, dry, and *nortes*). Finally, in the fourth chapter I outline the major conclusions and contributions of this research.

I found that resource mixing offered the strongest explanation for feeding patch choices of black howler monkeys. This is based on data indicating that individuals frequently switched among complementary food items (e.g., from mature fruits to young leaves, from young leaves to immature fruits), moving from a lower protein patch to a higher protein food patch and *vice versa*. Moreover, neither patch depletion, satiation, nor social factors (e.g., intra-group aggression) were found to play an important role in individuals' decisions to leave a patch. During the dry and rainy seasons, indices of howler food selectivity did not correlate with the nutrient and energetic content of foods consumed. This is not expected in a nutrient maximization model, but is consistent with the expectations of nutrient balancing. Based on the amount of food ingested (grams dry weight), howler monkeys were characterized by a fruit dominated diet (58% fruits, 37% leaves, 5% flowers), but this pattern differed among seasons. Leaves (mainly mature) were the most consumed food items during the *nortes* (49.5%). However, despite temporal changes in food consumption and food availability, and despite the

fact that the food items consumed by howlers at El Tormento contained on average ~11% available protein, high levels of condensed tannins, and a low protein-to-fiber ratio (0.4 for young and mature leaves), across seasons the howlers consumed on average 102 kJ of available protein per metabolic body mass per day and 628 kJ/mbm of total energy. These values surpassed their daily requirements for protein and metabolizable energy, and were higher than those reported for primates considered ripe fruit specialists such as spider monkeys (*Ateles* spp.). Maintaining a balance in daily protein and non-protein energy intake was the most consistent strategy adopted by howler monkeys across all seasons of the year. These findings support the idea that howler monkey feeding strategies enable them to translate energy into rapid growth rates and high reproductive output compared to other atelines. Finally, this research supports the increasing recognition of nutrient balancing as a dietary strategy used by nonhuman primates to exploit nutritionally imbalanced and complementary foods in order to meet their dietary needs.

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CHAPTER 1

GENERAL INTRODUCTION

A major question in biological anthropology is how do male, female, and juvenile primates obtain sufficient nutrients required for growth, maintenance, and reproduction (Milton, 1987; Lambert, 2007; Leonard et al., 2007; Lambert, 2011; Kuzawa and Bragg, 2012). During the course of human evolution, changes in energy requirements associated with increased brain size and social complexity, an extended juvenile period, and the production of altricial young resulted in altered patterns of habitat exploitation and the ability to colonize new habitats (Leonard and Robertson, 1994; Leonard et al., 2003). For example, it has been argued that early in their evolutionary history, human ancestors were successfully able to exploit more open savanna habitats by consuming resources such as underground tubers, rhizomes, terrestrial fibrous foods, and large hard seeds and nuts (Teaford and Ungar, 2000). Processing these types of resources was facilitated by cognitive changes in foraging strategies, changes in social cooperation, and technological innovations in food processing, such as the use of wooden tools as digging sticks, stone tools to cut and pound tough plant material, and the use of fire as an aid in the breakdown of difficult to process nutrients and in the denaturation of toxins (Ambrose, 2001; Wrangham, 2007; Sponheimer and Dufour, 2009). According to Milton (2000: 480), “diet influenced, indeed drove, human evolution”. Thus, the study of food choice and nutritional ecology of wild prosimians, monkeys and apes offers critical insight into the factors that have shaped human and nonhuman primate diet, nutrition, social behavior, and cognition.

Nutritional ecology also is relevant for the study of primate ontogeny and life history (Leigh, 1994), since the intake of particular macro and micronutrients, and especially protein and energy balance, is known to affect infant and juvenile growth patterns and female reproductive success and fitness (Lee, 1987; Altmann, 1991; Robbins, 1993a; Altmann, 1998; Altmann and Alberts, 2005; Altmann, 2006; Emery Thompson et al., 2007; Robbins et al., 2007; Terasawa et al., 2012; Vogel et al., 2012; Emery Thompson, 2013). For example, Altmann (2006) found that energy shortfalls (as a proportion of a calculated “optimum”) in female baboons (*Papio cynocephalus*) were directly correlated with a decrease in female reproductive success, as measured by the number of surviving juveniles. These findings were based on the assumptions that energy was the main factor affecting the baboons' fitness and that optimal diets were those that maximized energy intake (and at the same time complied with minimum nutrient requirements) (Altmann, 1991). This approach builds on models of Optimal Foraging Theory, focusing on the maximization of a single “currency”, such as energy or protein, as the main determinant of individual fitness (Pyke et al., 1977; Harrison, 1984; Stephens and Krebs, 1986a; Altmann, 2006; Ydenberg et al., 2007).

In contrast to these single-currency models, more recent frameworks are based on a multi-dimensional and nutritionally explicit approach to test the hypothesis that foragers attempt to balance the intake of multiple nutrients by adjusting the amount and proportion of protein, lipids, and carbohydrates consumed (Behmer, 2009; Raubenheimer et al., 2009; Felton et al., 2009b; Simpson and Raubenheimer, 2011; Simpson and Raubenheimer, 2012). In the past several years, nutritional geometry and the “Geometric Framework for nutrition” (Simpson and Raubenheimer, 1993; Raubenheimer and Simpson, 1993) have been incorporated into primatology to explore the synergistic effects of multiple nutrients, secondary metabolites, and energy requirements on the food choice of spider monkeys, baboons, and gorillas (Felton et al., 2009a; Felton et al., 2009c; Rothman et al., 2011; Johnson et al., 2013). Using this framework, wild spider monkeys (*Ateles chamek*) were found to prioritize protein intake, which means that they maintained a constant daily protein intake across seasons independently of the percentage of leaves in the diet and fluctuations in food availability (Felton et al., 2009a; Felton et al., 2009c). Johnson et al. (2013) found that a single wild female chacma baboon (*Papio hamadryas ursinus*), followed during 30 consecutive days, maintained a balanced intake of protein and non-protein energy (i.e., lipids and carbohydrates) despite high variation in the nutritional content and amount of foods consumed. A different pattern was found in wild mountain gorillas (*Gorilla beringei*), which maintained a relatively constant non-protein energy intake throughout the year, and they did so by over-ingesting protein during periods of high leaf consumption (Rothman et al., 2011).

Given the fact that individual primate species appear to solve problems of nutrient balancing in alternative ways, in this dissertation I used nutritional geometry to analyze patterns of food selection and nutrient balancing in two groups of Mexican black howler monkeys (*Alouatta pigra*) living in a 1400-ha semi-deciduous forest in Campeche, Mexico, during three seasons characterized by fluctuations in food availability. From August 2010 to October 2011, I collected ecological, behavioral, and phytochemical data to address a set of questions concerning black howler monkey nutritional ecology. These questions include: 1) Is resource mixing a strong explanation for feeding patch choice in black howler monkeys? 2) Do individuals leave an undepleted feeding patch before satiation in order to balance nutrients or to limit and dilute the effects of ingesting high levels of particular toxins? 3) Is howler food choice correlated with particular phytochemical characteristics of the selected food items during different seasons? 4) Do black howler monkeys show patterns of protein prioritization such as those presented by *Ateles chamek*, due to their phylogenetic relationship?

In this dissertation I build on previous research on howler monkey (*Alouatta* spp.) feeding ecology to provide a detailed analysis of the foraging tradeoffs and choices that howlers make in exploiting nutritionally heterogeneous food resources, including leaves which are high in fiber, lignin, and secondary compounds; fruits that are characterized by high amounts of non-structural carbohydrates and lipids, but also secondary compounds; and flowers, which are high in available protein, but also potentially high in fiber. This research moves beyond traditional models of energy maximization and, by focusing on the importance of nutrient balancing in understanding primate responses to resource availability and distribution, contributes to advance the field of primate nutritional ecology.

Howler monkeys have been described as behavioral folivores (Milton, 1978; Milton, 1979) to differentiate the ecological challenges they face in consuming difficult to digest resources (such as fibrous leaves and bark) from those faced by primates such as colobines and indriids, which are referred to as anatomical folivores due to the specializations of their stomachs and ceca, respectively, where efficient fiber fermentation occurs. Howler monkeys are hindgut fermenters, and they appear to overcome difficulties in digesting the structural carbohydrates present in leaves using a strategy that includes a relatively long food transit time for a platyrrhine (20.4 hours for *A. palliata*, weighing 7 kg (Milton, 1984)), high shearing crests that contribute to the efficient processing of leafy material (Kay, 1975; Kay, 1990), and an activity pattern characterized by behaviors that conserve energy, such as long periods of rest, limited within-group social interactions, and a small day range (Milton, 1980). Howlers have a capacious colon compared to other atelines (Milton 1981), and in fact colon and cecum volume in *A. palliata* is larger than what is predicted for a primate given their body size (Table 1.1). However, when comparing the ratios of intestine length to body length, howlers differ considerably from other hindgut fermenters such as indriids (*Propithecus* has a ratio of intestine length to body length of 15.5:1), and instead show greater similarity with spider monkeys, a highly frugivorous ateline relative (2.8:1 for *A. palliata* and 2.7:1 for *Ateles geoffroyi*) (Milton, 1981).

Nevertheless, describing howler monkeys as folivores is an oversimplification of their dietary ecology. In fact, there is considerable evidence that howler monkeys are characterized by high intraspecific variability in time spent feeding on leaves, fruits, flowers, and other items such as bark and stems across seasons and study sites (Di Fiore et al., 2011; Chaves and Bicca-Marques, 2013). For example, in the semi-deciduous tropical forest of the Calakmul Biosphere Reserve, Mexico, ripe and unripe fruits accounted for more than 85% of black howler monkey (*A. pigra*) feeding time in the wet season (Rizzo, 2004). In contrast, in the riverine gallery forest of Northern Belize, black howlers spent on average 86% of feeding time on young and mature leaves in the dry season (Pavelka and Knopff, 2004). However, several studies have repeatedly demonstrated that time spent feeding on different items is not a precise measure of the contribution of those items to the foragers' diet (see Kurlan and Gaulin (1987) for a thorough assessment of this methodological problem). A recent review analyzing dietary patterns (or syndromes) in several howler monkey species (Garber et al., in press) showed that, based on grams consumed, Mesoamerican howler monkeys (*A. palliata* and *A. pigra*) and *A. seniculus* are characterized by a relatively balanced leaf and fruit diet, and were found to eat similar amounts of leaves and fruit across the year. In contrast, Amazonian howler monkeys (*A. belzebul* and *A. macconnelli*) are characterized by a fruit-enriched diet (55-57% of grams ingested were from fruit), whereas Atlantic Forest and southern howler monkeys (*A. caraya* and *A. guariba*) appear to have a leaf-enriched diet (>60% of grams ingested were from leaves) (Garber et al., in press). Yet it remains unclear the degree to which differences in dietary patterns reflect the type of forest inhabited, seasonal differences in forest productivity, the relative availability of different food items, or the phytochemistry of the plant species present at each site. Thus, a more detailed knowledge of the spatial and temporal availability of foods and nutrients present in tropical forests, and of individual patterns of nutrient and energy intake is needed to understand dietary selectivity and the factors that affect food choice in howler monkeys.

Alouatta pigra has a limited geographical distribution, and is found only in southeastern Mexico, northern Guatemala, and Belize (Marsh et al., 2008). It is listed as “endangered” in the IUCN Red List of Threatened Species, mainly due to the rapid fragmentation and transformation of natural habitats across its range (Marsh et al., 2008). Two species of howlers are present in Mexico, *A. pigra* and *A. palliata*. Based on genetic and biogeographic information, current evolutionary hypotheses on the colonization of Central America by *Alouatta* present a scenario in which the ancestors of *A. pigra* first crossed the Panamanian isthmus approximately 3 million years ago (Cortes-Ortiz et al., 2003; Ford, 2006). One million year later, the ancestors of *A. palliata* independently migrated into Central America (Ford, 2006). However, whereas black howler populations have become restricted to small forest refugia across a circumscribed range in northern Mesoamerica, mantled howlers (*A. palliata*) have expanded their range and now are found from Ecuador through the Mexican state of Veracruz (Cortes-Ortiz et al., 2003). At present these two howler species are sympatric, and sometimes interbreed, along a narrow region of southern Mexico (Cortes-Ortiz et al., 2007; Kelaita and Cortes-Ortiz, 2013). Ecological distinctions between *A. palliata* and *A. pigra* have been hypothesized to reflect greater ecological tolerance and superior dispersal and competitive abilities of mantled howlers (Ford, 2006, but see Baumgarten and Williamson, 2007). However, in the absence of detailed comparative data on diet, foraging strategies, and nutritional ecology of *A. pigra* across a range of habitat types and geographical areas, our understanding of species differences in resource exploitation and dietary adaptability in disturbed areas, forest fragments, and continuous forests is limited.

Each chapter of this dissertation, excluding this General Introduction and the Conclusions, is structured as an independent research article. In Chapter 2, I analyzed the set of factors affecting individual decisions regarding howler feeding patch choice. In particular, my goal was to explore the role of resource mixing in black howler decisions of when to leave a patch and which patch to visit next. The results showed that patch depletion, satiation, and within-group contest competition were not frequent motivations for leaving a patch. Resource mixing, and in particular switching from young leaf to immature fruit feeding patches, was found to offer the strongest explanation of patch choice in this species. I also found that protein intake rates differed across fruit, leaf, and flower patches, resulting in alternating the amount of protein ingested in different patch types.

Chapter 3 describes the phytochemical composition of the foods consumed by black howler monkeys, and examines how plant nutritional chemistry (i.e., content of macronutrient and minerals) affected individual food selection. Based on indices of selectivity that reflected seasonal changes in the amount of different phenophases of the most consumed plant species and their availability in the environment, I found that howlers did not significantly select food items containing high levels of protein, sugar, or with a high protein-to-fiber ratio. In addition, howlers did not avoid foods characterized by a high fiber content. Only in one season of the year, the *nortes*, leaf ingestion was higher than fruit and flower consumption. During that season, howler selectivity indices correlated positively with the lipid, potassium, and phosphorus content of the ingested foods. The importance of lipids in howler monkey diets has largely been overlooked, and in this chapter I argue that, since energy intake was not higher during this season, a strategy of selecting fruits high in lipids (17-41% dry matter) served to balance higher protein intake resulting from increased leaf consumption. Overall, the data did not support the prediction that food choice in howlers was based on a protein or protein-to-fiber maximization strategy.

In Chapter 4, I applied a nutritional geometry framework to determine how howler monkeys adjusted nutrient and energy intake during different periods of the year characterized by fluctuations in food availability. Overall, I found evidence that, despite fluctuations in the daily intake of protein, carbohydrates, and lipids, black howler monkeys attempted to balance the intake of protein and non-protein (i.e., carbohydrates plus lipids) energy within the same day or over the course of four-five days. Regulating the ratio of nutrients ingested can be considered as a strategy for buffering the deficits and surpluses related to an imbalanced diet. Finally, both daily protein and non-protein intake were found to be relatively high when compared to primates characterized by a different dietary emphasis, such as highly frugivorous spider monkeys (*Ateles chamek*), or genera described as omnivorous such as baboons (*Papio hamadryas ursinus*). This finding has been highlighted in a recent study of black howler monkey nutritional ecology at a different site in Mexico (Amato and Garber, in press), and calls into question the characterization of howler monkey diets as energy-limited. Thus, we need to reevaluate the nutritional strategies of howler monkeys and other atelines in order to better understand the effects of phylogeny, habitat, diet, and digestive adaptation on primate life history and nutrient requirements of growth and reproduction.

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Table 1.1. Residuals (listed in decreasing order) from the regressions of colon volume (cm^3) (**A**) and cecum+colon volume (cm^3) (**B**) on body mass (g) for 17 primate species. Positive values represent higher colon and cecum volumes than what would be predicted for a primate species given its body mass. Data obtained from Chivers and Hladik (1980)

A)

Species	Residual log colon volume
Alouatta palliata	**0.318211**
Symphalangus syndactilus	0.251731
Pan troglodytes	0.104217
Alouatta seniculus	**0.097366**
Pongo pygmaeus	0.077707
Papio anubis	0.075855
Lagothrix lagotricha	0.064387
Gorilla gorilla	0.055484
Semnopithecus entellus	0.038891
Hylobates lar	0.020097
Saimiri oerstedii	-0.078791
Avahi laniger	-0.110392
Colobus polykomos	-0.168217
Nasalis larvatus	-0.197421
Leontopithecus rosalia	-0.573990
Ateles paniscus	**-0.621109**
Cercopithecus aethiops	-0.655427

B)

Species	Residual log(cecum+colon) volume
Alouatta palliata	**0.348465**
Symphalangus syndactilus	0.194082
Alouatta seniculus	**0.143122**
Avahi laniger	0.121453
Lagothrix lagotricha	0.108833
Pan troglodytes	0.106576
Gorilla gorilla	0.088465
Pongo pygmaeus	0.059578
Papio anubis	0.041269
Semnopithecus entellus	0.017087
Hylobates lar	-0.074492
Saimiri oerstedii	-0.105527
Nasalis larvatus	-0.220788
Colobus polykomos	-0.241300
Ateles paniscus	**-0.513441**
Leontopithecus rosalia	-0.621664
Cercopithecus aethiops	-0.654831

CHAPTER 2

FEEDING PATCH CHOICE AND RESOURCE MIXING IN BLACK HOWLER MONKEYS (*ALOUATTA PIGRA*)

Introduction

Food items ingested by primates usually occur in discrete food patches that can be monopolized by one or several group members (Isbell, 2012). However, the definition of a "food patch" can be challenging. Most field researchers have agreed to consider a patch as an aggregation of food items arranged in such a way that the forager can feed in it without interruption, and it can be represented by an isolated tree or a group of food trees of the same species with adjoining canopies (Leighton and Leighton, 1982; White and Wrangham, 1988; Chapman, 1988). Here I also defined a patch as a single feeding tree. The availability, distribution, and "quality" (e.g., size, density of food items) of feeding patches have been considered to have an important effect on primate social organization, group cohesion, and feeding competition (Wrangham, 1980; Isbell, 1991; van Hooff and van Schaik, 1992; Peres, 1996; Koenig, 2000). Due to this, studies of patch choice have traditionally analyzed individual foraging decisions in relation to patterns of social foraging, dominance, partner preferences, and feeding competition (Di Bitetti and Janson, 2001; Kazahari and Agetsuma, 2008; King et al., 2009; Garber et al., 2009; Marshall et al., 2012; Kazahari et al., 2013). However, information on detailed ecological and nutritional characteristics of the patches also is needed to understand the basis of feeding patch preferences (Marshall et al., 2012; Leighton, 1993).

Traditional ecological models based on Optimal Foraging Theory offer a set of predictions to explain patch choice based on a “currency” (usually energy) that is expected to contribute significantly to fitness (Harrison, 1984; Stephens and Krebs, 1986b; Altmann, 2006; Ydenberg et al., 2007). Optimal Foraging Theory was modified by Charnov (1976) to include the Marginal Value Theorem (MVT), which predicts that foragers will remain in a food patch until the energetic intake from that patch drops below the average value of other food patches in the environment. Once the current patch drops below this level, the forager is expected to search for another patch and feed there until resources in the new patch fall below the average patch value or the forager is satiated. In this model, a forager is expected to consider a patch to be functionally depleted prior to the time that all the food items are removed. In patches that minimally exceed the productivity of the average patch, changes in food density, resulting from the forager’s feeding activity or the feeding behavior of other foragers, are expected to lower the patch value relative to non-exploited patches (Chapman and Chapman, 2000a).

However, recent models of primate nutritional ecology have stressed the importance of nutrient balancing as a primary factor in individual foraging decisions (Felton et al. 2009b). Nutrient balancing is defined as a process in which decisions concerning where to feed and when to leave a patch are based on balancing the intake of protein, lipids, carbohydrates, minerals, and secondary compounds rather than maximizing the intake of energy or protein (Felton et al., 2009b). For example, a forager could leave a feeding patch before satiation or patch depletion and move to a different patch that contains complementary resources in order to ingest a set of nutrients that will contribute to a more nutritionally balanced diet. Such a pattern has been reported in a variety of organisms ranging from invertebrates to primates (Houston et al., 2011; Simpson and Raubenheimer, 2012); and feeding trials with captive animals showed that foragers tend to choose “mixed diets” even when foods that are considered preferred are offered *ad libitum* (e.g., tortoises (*Kinixys spekii*): (Hailey et al., 1998); brown bears (*Ursus arctos*): (Erlenbach et al., 2014)). However, studies that have applied this framework to the analysis of feeding patch preferences of foragers in the wild are scarce (Felton et al., 2009a; Felton et al., 2009b; Felton et al., 2009c).

In this research, I investigated a set of factors affecting feeding patch choice in black howler monkeys (*Alouatta pigra*) during a 15-month field study in Campeche, Mexico. First, I identified the most commonly visited feeding patches and determined whether time spent feeding in each patch correlated with the amount of food consumed. Then, I examined how factors such as patch depletion, satiation, resource mixing (i.e., switch from one food type another), and social factors affected howler monkeys decisions concerning when to leave a patch. Finally, I examined whether protein intake in a patch could explain patch choice and patch leaving patterns. I used these data to test the following hypotheses: **1)** Considering the relatively small group sizes of *A. pigra* (5-15 individuals), and the fact that howler monkeys are reported to frequently feed on large trees bearing fruits and leaves (Chapman et al., 1988; Rivera and Calme, 2006), I expect an individual to leave a feeding patch prior to depletion (here I define patch depletion based on observations of any group member returning to feed in the same patch during the same day or over the course of the next two days); **2)** Considering that leaves may contain higher amounts of potentially harmful secondary metabolites than fruits, and that fruits and flowers may be patchily distributed in space and time (Milton, 1980), I expect that a howler exits leaf patches before satiation, but exploits fruit and flower patches more intensively until satiated (i.e., not engaging in another feeding bout for at least 1 hr); **3)** An unsatiated individual who has not been threatened or displaced by a conspecific will leave a patch before it is depleted in order to locate a new food type that offers a complementary set of nutrients (e.g., switch from young leaves to mature fruits, or from flowers to mature leaves); **4)** If frequent switching between feeding patches is a strategy to mix resources and balance nutrients (Milton, 1980), a howler monkey will move from a feeding patch characterized by high (above average patch value) protein intake (kJ/min) to a patch characterized by lower protein intake (below average patch value) and *vice versa*.

Methods

Study Site and Subjects

This study was carried out in the state of Campeche, Mexico, at El Tormento (18º36′44″N; 90º48′31″W), a 1400-ha forested area owned by Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The dominant vegetation types in this area of the Yucatan peninsula are semi-deciduous and evergreen seasonal forests (*sensu* Miranda & Hernández 1963). The climate is hot and humid, and three seasons can be recognized: a dry and hot season from February to May (~100 mm rain during four months, 28°C mean daily temperature), a rainy season from June to September (~900 mm rain during four months), and a colder season ("*nortes*") from October to January (~160 mm rain during four months, 22.8°C mean daily temperature). According to data recorded by the Comisión Nacional del Agua (CONAGUA, 2013), between 1971 and 2000 total annual precipitation at El Tormento averaged 1291 mm, and mean annual temperature was 25.6°C. During the study period (September 2010-August 2011) total annual rainfall was 1246 mm, and average mean and maximum annual temperatures were 26.3°C and 33.4°C, respectively.

Two neighboring groups of black howler monkeys were followed during 15 months. Group M contained 10-12 individuals (including 4-5 adult males and 2 adult females) and group J contained 6-7 individuals (no more than 2-3 adult males at the same time and 2 adult females). The monkeys were individually recognized through their facial features, scars, and broken digits, however ten individuals were darted and marked with color anklets in August 2010, prior to the start of the behavioral data collection. Between September 2010 and November 2011, groups M and J ranged in areas of 14.5 and 4.5 ha respectively, with an overlap of 1.3 ha.

Field Data Collection

Behavioral data

From September 2010 to November 2011, I collected data on the howler monkey activity budget, diet, feeding patch occupancy, and within and between-group social interactions, conducting all-day follows of one adult individual per day. I obtained 1300 hours of behavioral data on 14 focal animals (ten males: 674 hours; four females: 626 hours) using two-minute instantaneous samples on focal animals (Martin and Bateson, 2007). The activities recorded instantaneously included: feeding (i.e., ingestion of food items), resting, traveling, and social interactions (vocalizations, howling bouts, aggression, play, sexual interactions, affiliative behavior such as grooming). When the focal animal started feeding, I temporarily switched to a method of continuous data collection, recording the duration (to the nearest second) of each feeding event and the quantity (number of items, parts of the item, or bites per minute), phenophase (i.e. young/mature leaf, immature/mature fruit, flower/inflorescence, other), and species of the items ingested. Then, I resumed the two-minute instantaneous data collection. All feeding trees (n = 689) were tagged, measured (DBH) and identified. Data on displacements and agonistic interactions were recorded both by instantaneous focal animal sampling (% of activity budget) and *ad libitum* (rates/hour), together with the identity of participants.

Feeding patches

I defined a feeding patch as a single feeding tree visited by the focal animal. A feeding bout was recorded when the focal animal was observed to handle and ingest a food item of a particular phenophase. A feeding bout ended when the focal animal stopped feeding for at least 30 minutes or left the food patch. In more than 50% of the cases, a feeding bout corresponded to the time the focal animal spent exploiting one food type in a feeding patch, but there were a few exceptions: 1) cases in which the focal animal fed on two or more phenophases in a single patch (e.g., switched from young leaves to mature leaves on the same tree); 2) cases in which the focal animal stopped feeding on a phenophase, began resting or performing other activities, and then resumed feeding on the same phenophase in the original patch after >30 minutes. In both circumstances, I counted these as two separate feeding bouts in one feeding patch.

I assumed that the focal animal might leave a feeding patch for the following reasons: 1) patch depletion, 2) satiation, 3) resource mixing, 4) social factors (e.g., aggression). Patch depletion was excluded if the focal animal or any group member was observed returning to feed in the same patch during the same day or over the course of the next two days. Moreover, I collected phenological scores (estimating the percentage of the crown containing food items on a scale of 0 − 4 [0; 1: 1−25%; 2: 26−50%; 3: 51−75%; 4: 76−100%]), and assigned a score to each patch immediately after the focal animal ended the feeding bout. I considered an individual to be satiated if it did not engage in a feeding bout for a period of at least 1 hour after terminating its previous feeding bout. Resource mixing was scored when the focal animal switched from one phenophase to another (e.g. fruits to leaves, or leaves to flowers) during successive feeding bouts within a period of < 1 hour. Other factors that I could not account for, might be involved in the decisions concerning when to leave a patch, for example the accumulation of particular plant secondary metabolites. Social factors included intra-group aggression over food, intergroup encounters (and howling bouts), mating behavior such as copulations and mate guarding, and other affiliative behavior such as play. Overall, I analyzed a total of 1678 focal animal feeding bouts.

Plant sample collection

Food items were collected from feeding trees either on the same day or within two days of the observed feeding bout. I attempted to gather at least 50 g of flowers/inflorescences and 100 g of leaves and fruits (wet weight) per tree. The plant items were transported to the field laboratory, where they were measured and weighed to the nearest 0.01 g. Leaves, flowers, and inflorescences were air dried at a constant weight in a dark area. After discarding the parts that were not eaten (e.g. seeds), fruits were cut into small slices and kept in a drying oven (<50ºC) until reaching a stable dry weight. All samples (n = 146) were then stored in paper bags labeled with date, tree number, and phenophase, and maintained in a dry place with a silica desiccant until phytochemical analyses were performed.

Laboratory Analyses

Plant samples were analyzed in the Nutritional Ecology Laboratory at Hunter College, City University of New York, from March to June 2012. All samples were analyzed in duplicate. The samples were ground using a Wiley® mill fitted with a 1-mm screen. Dry matter was calculated by drying the field-dried samples in an oven (105ºC) immediately before each analysis to remove atmospheric moisture (Rothman et al., 2012).

Total nitrogen (N) was determined via combustion according to AOAC (1995) using a Leco TruSpec Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA). Crude protein (CP) was calculated by multiplying N by 6.25 (Rothman et al., 2012). To take into account nitrogen bound to fiber and secondary metabolites, I estimated available protein (AP) in two ways: *1)* to determine the amount of fiber-bound nitrogen, I subtracted acid detergent insoluble nitrogen (ADIN) from N, multiplied this value by 6.25, and then subtracted it from CP (Rothman et al., 2008); *2)* to estimate the effect of tannins on N digestibility, we measured the polyethylene glycol (PEG, a tannin-blocking agent) binding capacity in a subset of plant samples (n= 42, those that were positive for the qualitative acid-butanol assay for condensed tannins [Waterman and Mole 1994]), followed by in vitro digestion with pepsin and cellulase (DeGabriel et al., 2008). In all the subsequent analyses regarding patch choice I used values of available protein instead of crude protein. Available protein is reported in energy units, calculated using the standard conversion factors (or physiological fuel values) of 17 kJ/g (Conklin-Brittain et al., 2006).

Data Analysis

<u>*Nutrient intake*</u>

I constructed a complete dietary profile for each adult group member by quantifying feeding rates and estimating daily nutrient and energy intake during at least two full day focal follows per individual per month. In all analyses related to nutrient intake, I included 91 focal sample days of 9 males (n = 44) and 4 females (n = 47), which correspond to 91 observation days in which the focal animal could be successfully followed for a period of 9 − 12 consecutive hours without being out of sight for more than 10 minutes, and all feeding bouts engaged in by the focal animal were recorded in detail. To analyze the effect of protein intake on patch choice, I divided each of the 91 days of observation in two blocks of 5-6 hours each (morning and afternoon). In most cases, the end of the morning block coincided with the start of a long resting bout that lasted on average 3.7 (± 1.6) hours. This was done to determine whether protein intake earlier in the day influenced protein intake later in the day.

To calculate the daily amount of food ingested (g dry weight) by each focal animal, I multiplied the feeding bout length on food item *i* (minutes) by the corresponding feeding rate (g dry weight/min), and then summed all daily events. To estimate daily nutrient intake, I multiplied the nutrient content of each food item *i* by the estimated amount of item *i* ingested (g dry weight) in each feeding bout. I obtained daily values (converted to kilojoule equivalents) of protein intake for each focal animal. To take into account the possible effects of different body weights on nutrient intake in male and female howler monkeys, I divided the amount of food consumed (grams dry weight) and nutrient intake by the metabolic body mass (mbm = $M^{0.762}$) of the corresponding focal animal. Body mass for nine of the 14 focal animals was obtained in August 2010 during the darting and marking phase (adult males: 7.6 ± 0.9 kg, adult females: 6 ± 0.3 kg). For those individuals whose weights were not available (n = 4), I used data on mean body weight of male and female *A. pigra* from Kelaita et al. (2011), since they were based on a larger sample size (37 males, 32 females).

Statistical analyses

To test differences among fruit, leaf, and flower patches in the amount of food consumed, time spent feeding, and rate of protein intake in each patch, I used Kruskal-Wallis tests, since these variables did not conform to normality and homoscedasticity assumptions. Spearman's rank order correlations were used to analyze the relationship between time spent feeding in a patch and amount of food ingested, and between morning and afternoon protein intake. I used chi-square tests to determine if the frequencies of switching between different patch types differed from expected values based on a random distribution.

I used Linear Mixed-Effect Models (LMM) (Crawley, 2007; Zuur et al., 2009) to analyze 1) if the number of feeding patches visited in the afternoon (response variable) could be predicted by the number of feeding patches visited in the morning (fixed factor), and 2) if the afternoon protein intake (response variable) could be predicted by the morning protein intake. Other predictor variables included in the latter model were sex, season, and the number of patches visited in the morning. All models included the identity of individuals as a random factor. Data were square root transformed to conform to assumptions of normality. All the analyses were run in *STATISTICA 12* (StatSoft, Inc. 2011) and R (version 3.0.1), fitting the linear models by REML (Restricted Maximum Likelihood criterion), and using the nlme package (Pinheiro et al., 2013).

Results

Visited feeding patches

A total of 690 feeding patches were visited throughout the study period (1300 hours of observation from September 2010 to November 2011). Sixteen percent of the feeding patches were visited on two of 145 observation days, not necessarily consecutive; 7.2% were visited on three observation days; 3% on four observation days; and 4.7% on 5-13 observation days. The most visited patch (on 13 days) was a single *Brosimum alicastrum* (Moraceae) tree, in which the focal animals fed on mature and young leaves, immature fruits, and inflorescences across the three seasons. Overall, young leaf patches were the most visited (31.6% of the total number of visited feeding trees), followed by immature fruit (21.6%), mature leaf (19.3%), mature fruit (18.7%) and flower/inflorescence (8.2%) patches.

The time spent feeding per patch visit differed significantly according to the type of food consumed (Kruskal-Wallis, $H = 143.1$, $df = 4$, $n = 1667$, $p<0.0001$). Individuals spent on average more time feeding in flower (11.6 ± 12.8 min), mature (10 ± 11.7 min) and immature (8.9 ± 12.2 min) fruit patches than in young (5.2 ± 6.3 min) and mature (3.9 ± 4.9 min) leaf patches (Figure 2.1, Table 2.1). In addition, the amount of food (in grams) consumed per feeding bout by the focal animals differed significantly according to the food type (Kruskal-Wallis, $H = 106.6$, $df = 4$, $n = 1667$, $p<0.0001$). On average, individuals consumed more food in mature (20.3 ± 33.1 g dry weight) and immature (20.6 ± 25.6 g) fruit patches than in mature leaf (11.9 ± 17.6 g), young leaf (10.9 ± 15.6 g), and flower/inflorescence (9.3 ± 10.5 g) patches (Figure 2.2, Table 2.2). Time spent in a feeding patch and the amount of food ingested correlated positively (Spearman correlation, $r = 0.83$, $n = 1665$, $p<0.001$). However, while howlers tended to feed on flowers/inflorescences for as much time as fruits (11.6 min vs. 9.5 min on average), the amount in grams of flowers ingested per feeding bout was significantly lower due to their low dry weight (9.3 g vs. 20.5 g).

Leaving a feeding patch

Despite the fact that the majority of the feeding trees were not re-visited by the focal animal within two days of the initial feeding bouts, the same feeding patch was visited more than once during the same day on 53% of the 145 observation days, and I only recorded four instances of patch depletion. Each of these occurred in flower patches (i.e., there were no flowers left in the patch after the feeding bout). In more than 80% of the cases, the phenological score (0-4) recorded at the beginning and at the end of the feeding bout did not show a decrease (i.e., reduced by at least a factor of two). Moreover, time spent feeding in a patch (mean: 7.28 min) was not influenced by patch size (mean DBH: 41.6 cm) (Figure 2.3). Thus, overall, patch depletion was unlikely to explain the majority of cases in which the howlers left a feeding patch.

I recorded evidence of satiation in 19.6% of the cases: of these, 20.4% of the time the forager appeared satiated after feeding in a fruit patch, 17.3% after feeding in a leaf patch, and 10.9% after a feeding bout in a flower patch. These values are standardized according to the total number of fruit, leaf, and flower patches visited. Despite the fact that, as hypothesized, satiation was more frequently associated with fruit patches, these results did not differ from what expected based on an equal distribution of satiation among patch types (Chi-square test: $\chi^2 = 2.85$, df = 2, $p = 0.23$).

In the majority of the cases (80.3%), the focal animal left the feeding patch prior to satiation. On those occasions, resource mixing, or moving from one food type to another food type, accounted for 49.4% of the patch leaving events. The most frequent observations included switching from a young leaf to an immature fruit patch (12.5%), switching from feeding on mature fruits to young leaves (12.2%) and from immature fruits to young leaves (12.2%) (Figure 2.4). These values differed significantly from the frequencies expected if switching between patch types was based on no preference ($\chi^2 = 6.40$, df = 1, $p < 0.02$; $\chi^2 = 5.75$, df = 1, $p < 0.05$, respectively). In contrast, switching from mature fruits to flowers was observed significantly less than expected ($\chi^2 = 4.10$, df = 1, $p < 0.05$). These data support the hypothesis that howler monkeys frequently alternate between food patches to consume different plant parts, in particular frequently switching from fruits to young leaves.

Finally, social factors (n = 100) accounted for only 8.1% of patch leaving events. In these cases, intergroup encounters (in the form of howling bouts or chases) were the most common social factor (53%, n = 53) that resulted in leaving a patch, followed by feeding related intra-group aggression (24%), mating behavior (e.g., copulation, mate guarding) (19%), and other reasons (e.g., play) (4%). Overall, contest feeding competition occurred at a rate of 0.018 events/hr (24 aggressive interactions across 1300 hours of observation), or 0.0019 events/min (considering total feeding time, i.e., 200 hr), and therefore appeared to play a minimal role in howler patch choice. Moreover, these intra-group direct aggression or overt displacement events were not associated with a specific type of food patch; nine occurred in young leaf patches, six during immature fruit consumption, four during mature leaf eating bouts, three in mature fruit patches, and two while feeding on inflorescences.

Patch choice from a nutritional perspective

To analyze the nutritional basis of individual patch choice, I examined data based on 91 observation days during which the focal animals were observed continuously for 9–12 hours per day. The average number (±SD) of feeding bouts per day was 14.0 (± 5.01); 7.3 (± 3.5) in the morning and 6.7 (± 3.5) in the afternoon (Table 2.3). On average, the howlers fed on a similar number of trees in each time block (One-way ANOVA, F(1,180) = 1.3, p = 0.25), but the number of patches visited in the afternoon was not predicted by the number of patches visited in the morning (LMM, t = -0.05, df = 77, p = 0.95). Moreover, the number of patches visited in the morning did not have a significant effect on the amount of protein ingested in the morning (t =

0.78, df = 77, p = 0.49) or in the afternoon (t = -0.75, df = 76, p = 0.45). However, the number of feeding patches visited in the afternoon did affect positively afternoon protein intake (t = 3.45, df = 77, p <0.0001), indicating a change in howler behavior such that higher intakes were achieved by visiting a greater number of feeding patches.

The rate of protein intake (kJoule/min) was similar in young and mature leaf patches (Kruskal-Wallis, H = 368.2, n = 1352, p = 0.9), and higher (p <0.0001) than protein intake in mature fruit, immature fruit, and flower patches (which, instead, had similar rates, p = 0.9) (Table 2.4). This pattern was consistent in the morning and in the afternoon, and it confirms the fact that switching from leaf to fruit patches was associated with different nutritional gains for howler monkeys. These data lend support to the hypothesis that individuals alternated amounts of protein ingested across patches to achieve a balanced nutrient target.

The data also indicate that total protein intake during the afternoon feeding bouts was significantly predicted by the protein intake in the morning (LMM, t = 2.55, df = 77, p = 0.012). Figure 2.5 shows the positive correlation between protein ingested in the morning and protein ingested in the afternoon. Thus, a higher cumulative intake of protein in the morning was followed by a higher intake in the afternoon. Likewise, mornings characterized by a lower than average nutrient intake, were followed by a similar cumulative nutrient intake in the afternoon. This is also is shown by the fact that patterns of protein intake were extremely similar during the two temporal blocks (morning and afternoon) (Table 2.3). The sex of the focal animal (p >0.13) and season (p>0.26) did not have significant effects on these patterns.

Discussion

Studies of patch choice offer critical insights into the mechanisms used by individuals to decide which patch to visit, when to leave a patch, and which patch to visit next. These decisions are affected by patterns of food distribution and availability, within-group and inter-group feeding competition, and nutrient needs (Pyke et al., 1977; Stephens et al., 2007). In this analysis of the feeding patch choices of black howler monkeys, I found that howlers rarely depleted a food patch prior to leaving. Similarly, howlers left a patch in response to satiation only 19.6% of the time. However, instances of satiation were more frequent during fruit feeding than in leaf patches, but the difference was not significant. Thus, it appears that, according to my definitions, neither satiation nor patch depletion explained the majority of patch choice decisions made by howlers. In addition, aggression over food occurred at very low rates (I recorded only 24 aggressive interactions across 1300 hours of observation of 14 focal animals), indicating that within group contest competition was not a significant factor primarily affecting individual foraging decisions, as has been reported in other howler monkey populations (Wang and Milton, 2003; Kowalewski, 2007). Instead, the data presented here support a resource mixing strategy, with howlers frequently leaving an undepleted patch of a particular food type to move to another patch containing a complementary food type. In particular, howlers frequently switched between young leaves and immature fruits, mature fruits and young leaves, and immature fruits and young leaves during successive feeding bouts. Data presented in Chapter 3 indicate that these resources differ in nutritional content, with young and mature leaves being higher in available protein (~13.4% dry weight) and neutral detergent fiber (48.8%) than mature fruits (7.5% and 33.6%, respectively), and mature fruits having a higher lipid (11.1%) and sugar (23.6%) content than leaves (2.19% and 5.5%, respectively). Protein intake rate was significantly higher in leaf patches than in fruit patches, and the most common pattern observed in howlers across successive feeding patches was to move from a lower protein patch to a higher protein patch, and from a higher protein patch to a lower protein patch. However, despite these fluctuations in protein intake from one patch to the other, the cumulative amount of protein obtained in the afternoon was positively affected by the cumulative amount ingested in the morning, indicating that howler monkeys maintained a specific daily intake strategy that allowed them to achieve a targeted proportion of nutrients.

Do black howler monkeys leave a feeding patch due to patch depletion?

Models of optimal foraging generally assume that foragers deplete (or functionally deplete) a patch prior to leaving it. In primates, this has been examined in the context of within-group feeding competition (Snaith and Chapman, 2005, 2008). In the case of scramble or indirect feeding competition, access to food resources is generally unaffected by rank or aggression. Rather, the first individual to arrive in a patch may obtain a finder's advantage and consume all the foods in the patch, especially in food patches characterized by small, concentrated food items (Janson and van Schaik, 1988; Bicca-Marques and Garber, 2005; Garber et al., 2009). It also has been argued that, in small food patches, foraging efficiency of lower ranking individuals declines with increasing group size, as a limited number of high ranking individuals can deplete the patch. Under these conditions, aggression at feeding sites is expected to be high and foragers are expected to switch frequently between patches and travel greater distances to encounter a larger number of food patches (Janson and van Schaik, 1988). These assumptions, which are part of the ecological constraints model (Chapman and Chapman, 2000a; Chapman and Chapman, 2000b), also imply that most food patches encountered by wild primates are depletable. Despite the fact that this model was initially presented to explain patterns of subgrouping and patch choice in frugivorous primates, it has been extended to explain the socioecology of leaf-eating primates (Snaith and Chapman, 2005; Snaith and Chapman, 2007; Snaith and Chapman, 2008). For example, whereas red colobus monkeys (*Procolobus rufomitratus*), living in groups of up to 18 individuals, depleted patches of young leaves (Snaith and Chapman, 2005), groups of sympatric guerezas (*Colobus guereza*), ranging in size from 4 to 11 individuals, did not appear to deplete feeding patches, based on the fact that time spent feeding in a patch was not a function of either patch size or feeding party size (Tombak et al., 2012).

In the present study of black howler monkeys, within group contest competition at feeding sites was very low. Moreover, time spent feeding in a patch was not a function of patch size (i.e., tree DBH), and in over 80% of the cases, howlers left a feeding patch before depletion. Similar results were found for *Alouatta caraya* in Argentina, where several neighboring groups were observed feeding in the same trees during consecutive days; the same feeding tree was revisited on the same day during 36-65% of days; and within-group aggression at feeding sites was very low (0.002-0.004 agonistic interactions per individual per hour) (Kowalewski, 2007). Additionally, neither patch size, nor the number of individuals in the feeding party had an effect on time spent feeding in a patch (Kowalewski, 2007). These patterns differed from those reported in a study of *A. palliata* in Costa Rica (Chapman, 1988). When mantled howlers fed in fruit patches, patch size and the number of individuals in the subgroup were positive predictors of time spent feeding. These interspecific differences in foraging strategies and patch use might be analogous to those found in colobines, especially considering that group size in *A. palliata* is much larger than in *A. pigra* and *A. caraya* (for example, the group studied by Chapman [1988] consisted of 40 individuals).

Do black howler monkeys leave a feeding patch due satiation?

According to Optimal Foraging Theory and the Marginal Value Theorem, food intake rates decline as time spent feeding in a patch increases (Charnov, 1976; Stephens and Krebs, 1986b). However, two different factors affect feeding rates. Declining feeding rates could indicate both patch depletion (or declining abundance of food resources) and/or forager satiation (Grether et al., 1992). Tombak et al. (2012) reported that, in the case of guerezas, individuals left feeding patches most likely due to satiation rather than to patch depletion because they maintained a constant feeding rate (bites/min) without an increase in patch movement, which was assumed to be a measure of feeding effort. A recent study modeling foraging preferences and analyzing the decision rules of three groups of *A. pigra* in Calakmul, Mexico (Plante et al., 2014) reported that decisions on when to leave a tree were strongly affected by the satiation state of the individuals, which was defined as the amount of time spent eating earlier in the day, weighted by total foraging time. In contrast, in my study of black howler monkeys, I considered that individuals were satiated if they spent at least one hour without feeding after their last feeding bout. Using this definition, more than 80% of the feeding bouts did not result in satiation.

Do black howler monkeys leave a feeding patch due to resource mixing?

I found that resource mixing was the most common reason for leaving a patch for unsatiated howlers. In general, howlers left productive food patches before they were depleted in order to locate a new food type that offered a different set of nutrients or secondary compounds. Other studies of patch choice and patch depletion in howler monkeys (*A. caraya*), colobines (*Colobus guereza*), and tamarins (*Saguinus* spp.) have suggested that resource mixing can be a major factor in primate foraging decisions (Kowalewski, 2007; Garber and Kowalewski, 2010; Tombak et al., 2012). Moreover, feedback mechanisms and physiological factors, such as specific gut capacity and toxin thresholds, or the interacting effects of nutrients and secondary compounds (e.g., inhibition of glucose absorption by flavonoids [Karasov, 2011] or increases in glucose absorption associated with higher carbohydrate intake [Karasov and Diamond, 1988]) provide internal signals that drive resource mixing decisions. The fact that black howler monkeys alternated feeding bouts between fruit and leaf patches, as well as alternating bouts of higher and lower protein intake, suggest that this pattern could be dictated by the need to balance nutrients.

Few studies of other vertebrates have analyzed patch choice in the wild from a resource mixing perspective. In a field experiment set out to test the "complementarity hypothesis" (i.e., daily foraging patterns characterized by a switch among different currencies, such as total energy and protein), highly frugivorous habituated curassows (*Mitu salvini* and *Crax alector*) were found to combine and alternate feeding bouts on energy-rich fruits with feeding bouts on protein-rich leaves and invertebrates, at the expenses of maximizing energy intake (Jimenez, 2004). This suggests that the observed foraging patterns were best explained by a need to meet daily macro and micronutrient requirements. In addition to achieving their protein requirements, curassows might have foraged for specific essential amino-acids present in leaves but not found in fruits, or to increase calcium intake, which is considered limiting for birds that feed on fruits and invertebrates (Levey and Martinez del Rio, 2001). A recent study of nutrient balancing in free-ranging spider monkeys (*Ateles chamek*) in Bolivia analyzed individual cumulative trajectories of daily nutrient intakes (Felton et al., 2009c)(Felton et al., 2009c)(Felton et al., 2009c). Spider monkeys could reach their average balance of protein and non-protein energy using two strategies: consuming food items characterized by a constant balance of protein and non-protein energy, or alternating between foods characterized by high or low ratios of protein and non-protein energy ("zigzagging" nutritional trajectory). The latter was the strategy most frequently used by spider monkeys (83% of days). In the present study, black howler monkeys followed an analogous pattern by alternating feeding bouts in patches offering complementary nutrients.

From a decision-making perspective, the fact that primates may be able to retain information on foraging success in particular food patches (Garber, 2000), can facilitate decisions of leaving a patch to travel to a distant patch that will offer different nutritional rewards. Thus, models of patch use should incorporate data on spatial memory on previous experiences and expectations of future rewards, with data on the sequential intake of nutrients in different types of food patches in testing alternative explanations of feeding patch choice in howler monkeys.

In conclusion, the analysis of feeding patch choice revealed that moving between feeding patches characterized by resources differing in their nutritional composition was the most common strategies used by black howler monkeys during their feeding bouts. Howlers rarely depleted feeding patches, and it was evident that social factors and aggressive interactions were not significant factors affecting howler patch choice decisions. In addition to suggesting the importance of nutrient balancing, these results also offer insights into the role of within-group feeding competition and ecological constraints on primate food choice and decision-making.

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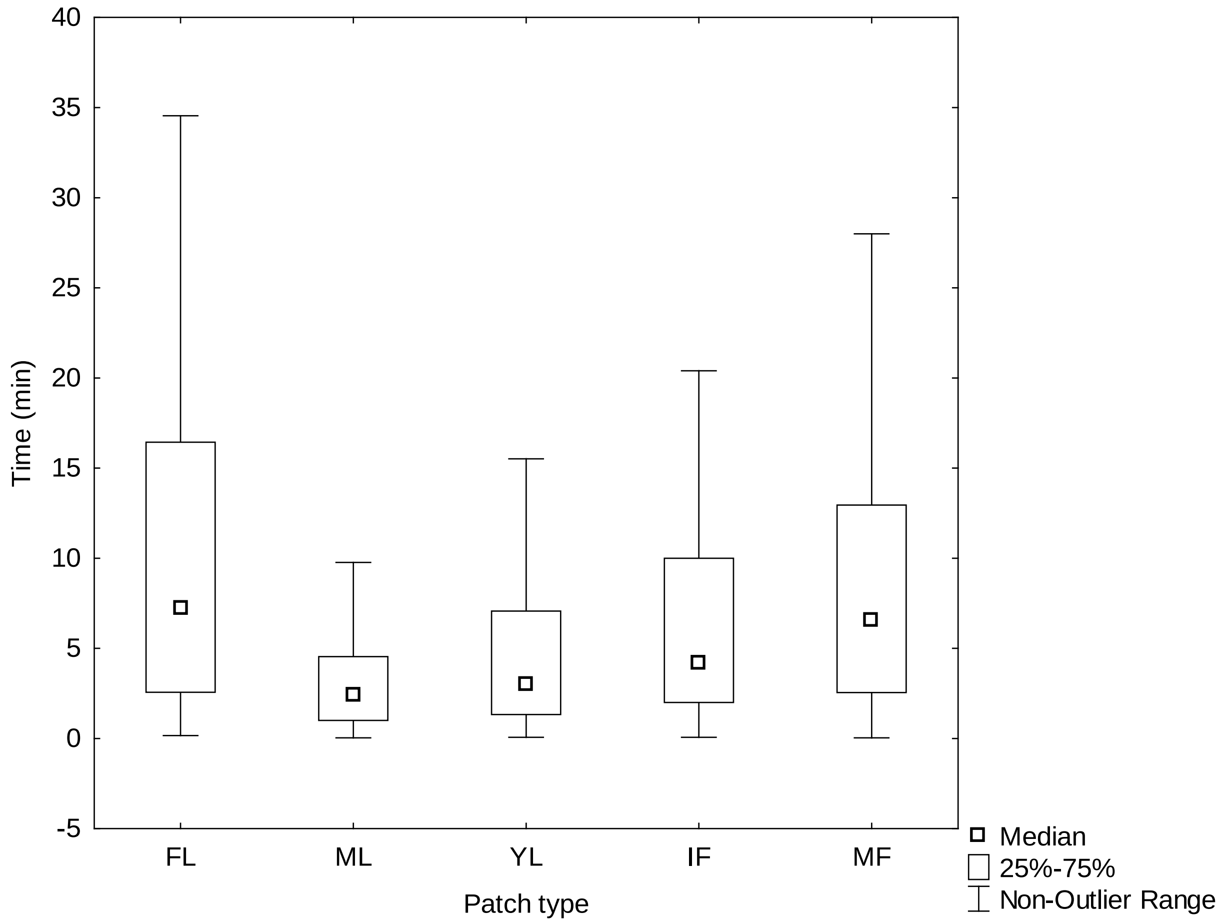

Figure 2.1. Time spent feeding (minutes) in different types of feeding patches

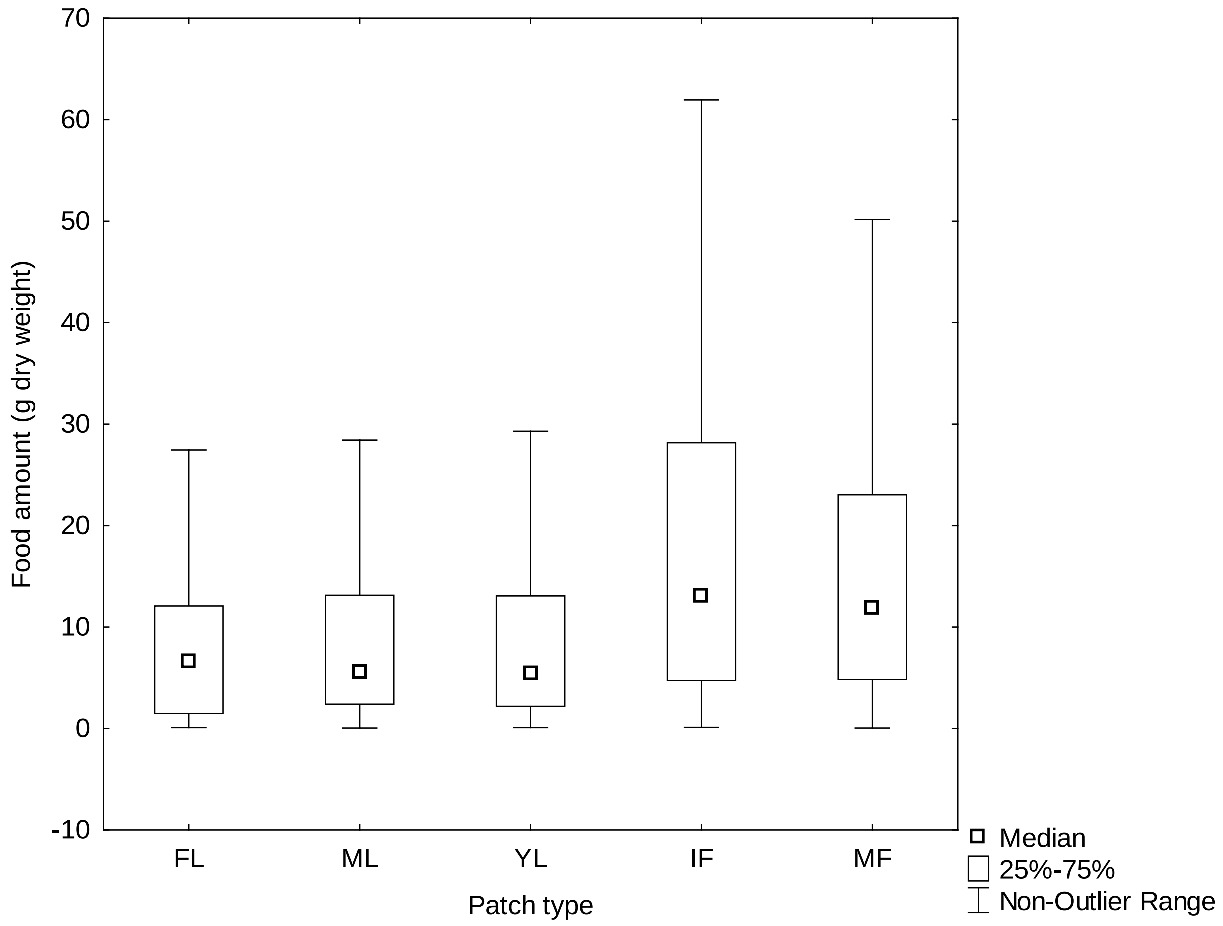

Figure 2.2. Amount of food (grams dry weight) consumed in different types of feeding patches

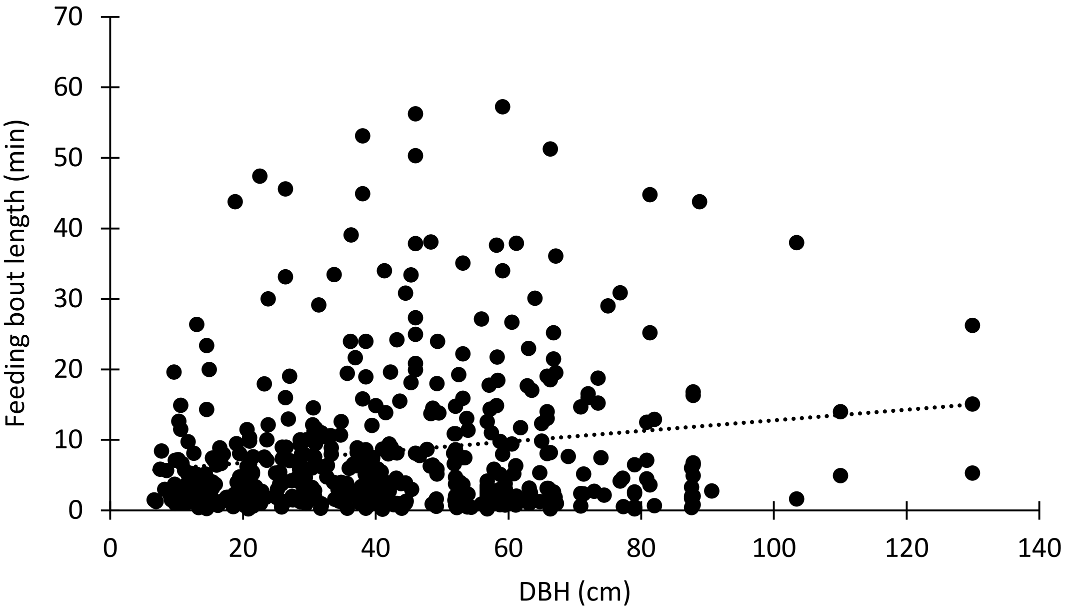

Figure 2.3. Relationship between patch size (i.e. DBH of feeding trees) and feeding bout length

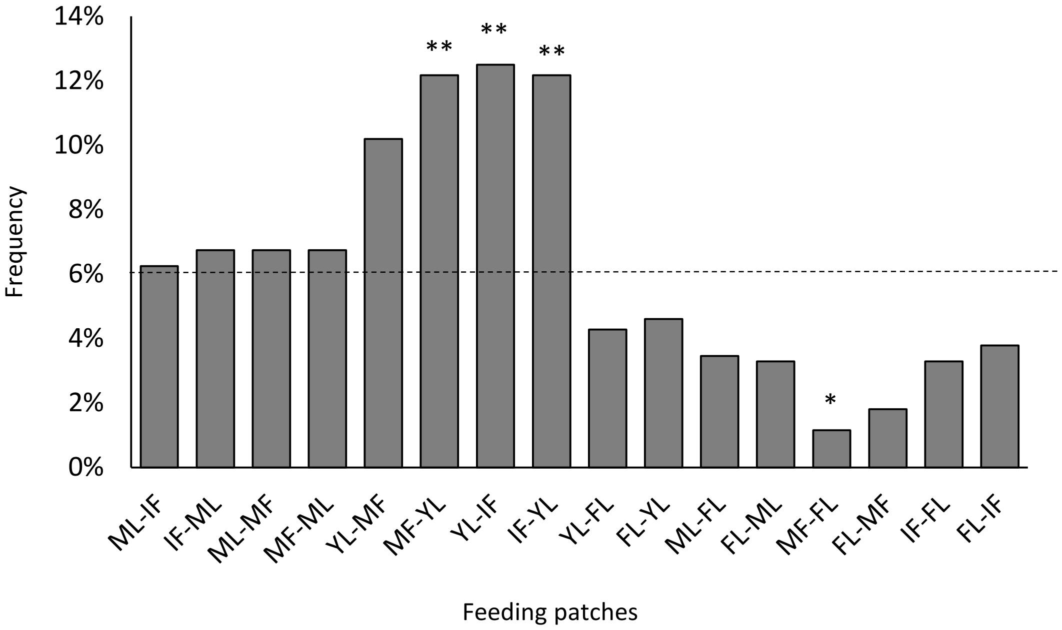

Figure 2.4. Resource mixing by black howler monkeys. Frequency of switching from one feeding patch type to a different patch type. Frequencies are calculated taking into account the total number of patch-switching events. The dashed line shows the expected frequency of switching between patch types based on the total number of events and no preference. Asterisks represent significant differences from the expected values (** p<0.02; * p<0.05). Feeding patches: ML, mature leaves; YL, young leaves; MF, mature fruits; IF, immature fruits; FL, flowers and inflorescences.

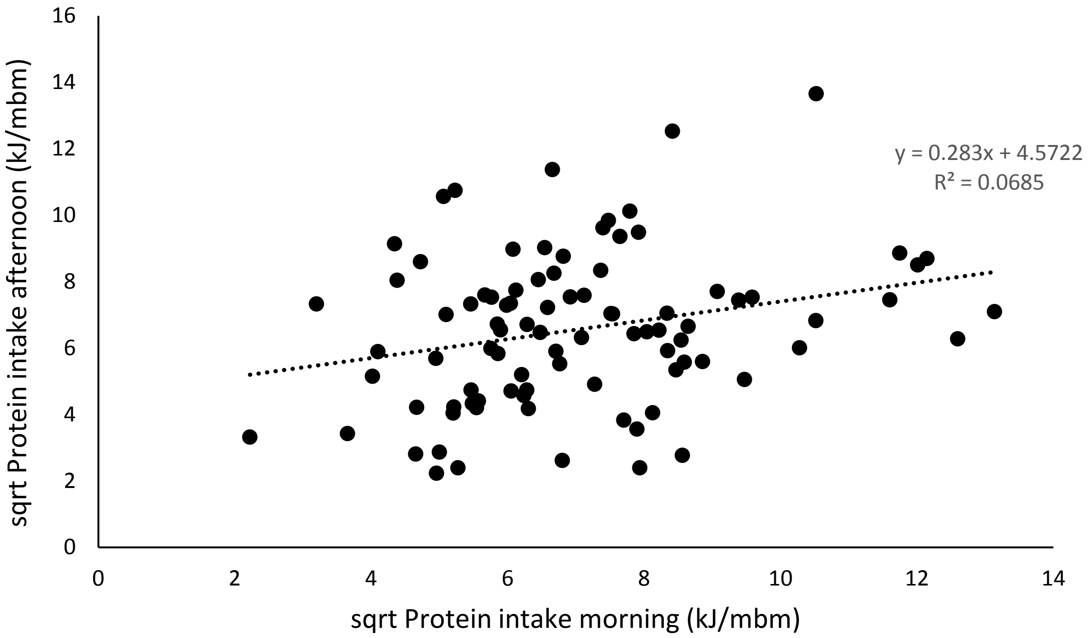

Figure 2.5. Positive correlation between protein intake during the morning bouts and protein intake during the afternoon bouts (protein values in kjoule per metabolic body mass are square root transformed)

Table 2.1. Amount of food consumed (g dry weight) by focal animals in different types of feeding patches

	FL	**ML**	**YL**	**IF**	**MF**
n	137	324	530	361	314
Min	0.10	0.06	0.09	0.11	0.05
Max	63.56	140.58	100.02	219.15	380.07
Sum	1274.83	3870.94	5798.58	7462.28	6364.21
Mean	9.31	11.95	10.94	20.67	20.27
Std. error	0.90	0.98	0.64	1.35	1.87
Variance	110.02	312.56	219.92	655.36	1096.84
Stand. dev	10.49	17.68	14.83	25.60	33.12
Median	6.58	5.58	5.45	13.07	11.94

Table 2.2. Time spent feeding (minutes) by focal animals in different types of feeding patches

	FL	**ML**	**YL**	**IF**	**MF**
n	137	324	529	363	314
Min	0.17	0.03	0.07	0.07	0.03
Max	83.97	41.73	50.33	93.42	92.00
Sum	1586.33	1271.82	2731.23	3241.38	3156.38
Mean	11.58	3.93	5.16	8.93	10.05
Std. error	1.09	0.27	0.26	0.64	0.66
Variance	163.38	23.94	34.59	148.05	137.16
Stand. dev	12.78	4.89	5.88	12.17	11.71
Median	7.20	2.42	3.00	4.18	6.53

Table 2.3. Daily protein intake by focal individuals and characteristics of feeding bouts during two temporal blocks (morning and afternoon) (n = 91 observation days)

	Number of feeding patches visited	Food amount consumed (g dry weight/mbm)	Protein (kJ/mbm)
Morning			
mean	7.32	27.57	53.95
SD	3.52	15.43	33.91
Afternoon			
mean	6.75	25.16	48.33
SD	3.52	14.79	33.08

Table 2.4. Individual rates of protein intake (kJ/min) in different types of feeding patches during the morning and afternoon (n = 91 observation days)

	Morning					**Afternoon**				
	n	**Mean**	**Min**	**Max**	**SD**	**n**	**Mean**	**Min**	**Max**	**SD**
FL	76	2.17	0.19	10.56	1.88	46	3.44	0.14	10.83	3.11
MF	118	2.77	0.30	11.72	2.15	121	2.71	0.59	11.72	1.78
IF	172	2.25	0.13	10.09	1.80	143	2.70	0.50	11.72	2.24
ML	96	6.71	1.42	23.02	4.90	124	5.89	0.61	18.62	4.53
YL	249	4.99	0.86	17.25	2.84	207	5.06	0.44	23.03	3.01

CHAPTER 3

THE EFFECTS OF NUTRITIONAL CHEMISTRY ON FOOD SELECTION IN MEXICAN BLACK HOWLER MONKEYS (*ALOUATTA PIGRA*)

Introduction

Compared to many groups of mammals, primates include an extremely broad range of food types in their diet such as leaves, fruits, flowers, seeds, gums, nectar, fungi, lichens, and animal prey (Garber, 1987; NRC, 2003; Robbins and Hohmann, 2006). These resources differ in terms of nutritional content, chemical and mechanical properties, renewal rate, and spatial and temporal availability, and therefore have a significant influence on primate foraging strategies (Freeland and Janzen, 1974; Glander, 1982; Kinzey and Norconk, 1993; Chapman et al., 2012). Food choice also is affected by factors such as the forager's body mass and basal metabolic rate; its anatomical and digestive adaptations (including gut morphology, food transit time, and activity of gut microbes); its nutritional and energetic needs; and social factor such as rank, feeding competition, and partner alliances (Janson, 1988; McNab, 2002; Mackie, 2002; Robbins and Hohmann, 2006; Lambert, 2011; Chapman et al., 2012). In this research I examine one aspect of diet: the nutritional basis of food choice in black howler monkeys, *Alouatta pigra*.

Despite the importance of nutritional and energetic requirements in determining food choice, studies of feeding ecology in non-human primates have focused primarily on time spent feeding as a measure or index of dietary preference, food intake, and the importance of particular food items in the diet (Clutton-Brock, 1975; Rudran, 1978; Milton, 1980; Glander, 1981). While some authors suggest that a time-based approach is useful in facilitating intraspecific comparisons among primate populations exploiting habitats that differ in resource availability and distribution (Felton et al., 2008), the advantages of this measure are principally related to the repeatability and simplicity of data collection compared to studies designed to obtain data on food intake rates and weights of food items ingested, especially in forest habitats where conditions of visibility are limited (Chivers, 1998). In fact, time spent feeding is not a strong proxy of nutrient intake, since it does not reflect the amount of food consumed or differences in the nutrient content of specific animal and plant tissue ingested (Kurland and Gaulin, 1987; Felton et al., 2009). In several studies directly comparing time budgets with amount ingested, researchers have found that whereas time spent eating fruits underestimates grams of fruit consumed, time spent feeding on leaves, flowers, and animal matter markedly (up to 600% for animal matter) overestimates their intake (Gaulin and Gaulin, 1982; Chivers, 1998; Amato, 2013; Garber et al., in press). Thus, a more complete understanding of primate diets requires quantification of feeding time, ingestion rates, weight of the items ingested, and the nutritional composition of plant parts and animal tissues consumed in order to calculate daily nutrient intake (Schuelke et al., 2006; Felton et al., 2009; Rothman et al., 2012).

Moreover, general assumptions of the nutrient content of plant tissue (for example that mature leaves contain more fiber and less protein than young leaves, or that ripe fruits are low in protein compared to young leaves and insects (Coley, 1983; Milton, 2008)) must be viewed cautiously, since there is evidence of marked variation in the phytochemical content of a given food item depending on the plant species exploited, individual plant, location of food item in the tree crown, time of day, season, site, and habitat (Chapman et al., 2003; Worman and Chapman, 2005; Houle et al., 2007; Carlson et al., 2013). In this regard, Gaulin and Gaulin (1982) found that young leaves consumed by *Alouatta seniculus* were characterized by lower protein/minute intake than mature leaves and fruits. Schuelke et al. (2006) reported that mature fruits consumed by *Semnopithecus entellus* in Nepal contained significantly more crude protein (grams/dry mass) than young and mature leaves. Similarly, the sugar content of certain fruit species in the diet of red colobus (*Piliocolobus tephrosceles*) and redtail monkeys (*Cercopithecus ascanius*) in Uganda was lower than the average value of young leaves (Danish et al., 2006). Finally, there can be significant intrageneric differences in the nutrient content of fruits; for example the pulp of nine species of figs (*Ficus* spp.) in Uganda was highly variable in crude protein (4.3-20.7%), fiber (NDF) (23-65%), and water soluble carbohydrates (6.6-23.2%) (Conklin and Wrangham, 1994). Thus, in the absence of nutritional analyses of plant parts collected from the particular feeding tree exploited by primate consumers, generalizations regarding the nutritional content of individual food items are likely to misrepresent the basis of food choice.

Studies of the role of particular nutrients, secondary compounds, and minerals in primate food choice also have suggested that factors such as the protein-to-fiber ratio of leaves may significantly affect consumption (e.g., in mantled howlers, *A. palliata* (Milton, 1979; Milton, 1998); colobine monkeys such as *Presbytis rubicunda* and *P. melalophos* (Davies et al., 1988), *Procolobus badius* (Chapman and Chapman, 2002), and *Colobus guereza* (Fashing et al., 2007); and several lemur species (Ganzhorn, 1992)). The consumption of leaves with higher protein-to-fiber ratios over leaves with lower ratios (e.g., 0.73 *vs*. 0.45 of eaten and uneaten leaves by *C. guereza* in Kenya (Fashing et al., 2007)) appears to be related to the fact that fiber, and especially some of its components such as cellulose and hemicellulose, are difficult to degrade and digest (Barboza et al., 2009) and thus there is a limit to the amount of fibrous plant material that can be processed by an individual. A large bulk of digesta in the gut may reduce the efficiency of microbial activity, and this also can affect protein uptake (Milton 1979, 1998), favoring a ratio of protein-to-fiber consumption that is generally above 0.5 (Mowry et al., 1996). However, there are several cases in which this ratio does not explain primate leaf choice (Wallis et al., 2012; Simmen et al., 2014), and leaves with a lower protein to fiber ratio (e.g., 0.48 in mature leaves eaten by *A. pigra* in Belize (Behie and Pavelka, 2012b)) also are consumed (Oates et al., 1990; Dasilva, 1994; Mowry et al., 1996; Behie and Pavelka, 2012b). Therefore, several factors (including better estimations of available protein in leaves) must be taken into account when analyzing the effect of this variable on primate food choice.

Dietary selectivity also may be influenced by the mineral content of food items (Behie and Pavelka, 2012a). Macrominerals (such as Na, K, Ca, P) are required by vertebrates given their role as structural components of hard tissues (i.e., bone and teeth) and their involvement in osmotic balance; and trace minerals, such as Mn, Fe, Cu, Zn, participate in biochemical pathways and have catalytic functions in activating enzymes and hormones (Barboza et al., 2009). Different wild plants are considered to be relatively high in certain minerals (Milton, 2003), and it has been suggested that mixing food items from several different plant species or plant tissues should provide primates with a sufficient amount of minerals to satisfy their daily requirements (Nagy and Milton, 1979; Milton, 2003). However, there also is evidence that some tropical forests (e.g., in the Amazon and Southeast Asia) are characterized by nutrient- and mineral-poor acidic soils, low primary productivity and, as a consequence, low mineral availability in the environment, especially calcium, magnesium, and potassium (Janzen, 1974; Waterman and Mole, 1989). The high soil acidity, in fact, causes rapid weathering and high leaching of cations such as Ca and K resulting from the decomposition of organic matter (Janzen, 1974), which may cause primate foragers to consume aquatic plants, plant exudates, invertebrates, or decaying wood to achieve their mineral requirements (Garber, 1980; Rothman et al., 2006a; Chaves et al., 2011).

Despite the limited information on the precise mineral requirements of wild primates (Felton et al., 2009; Chapman et al., 2012), it has been shown that mineral content can have a direct influence on primate dietary choices (Power et al., 1999; Laska et al., 2000; Rode et al., 2003; Rothman et al., 2006a; Fashing et al., 2007; Behie and Pavelka, 2012a). For example, leaves consumed by *Nasalis larvatus* in Indonesia contained significantly higher concentrations of P (0.15%) and K (1.28%) than leaves not included in the diet, which instead were higher in Ca (0.62%) and Mn (175 mg/Kg) (Yeager et al., 1997). The selection of leaves high in phosphorus by *N. larvatus* appeared to be related to the fact that P was a limiting element in their environment. Inadequate supplies of this mineral can produce net loss of mineral from bone, loss of appetite, and decreased body growth (Robbins, 1993; Barboza et al., 2009). Analogously, in a population of *A. pigra* living in an environment affected by a hurricane that caused drastic long-term changes in food supply, Behie and Pavelka (2012a) demonstrated that Zn and Ca concentration in leaves significantly predicted howler food selection independent of food availability and macronutrient content. In addition, sodium is often deficient in primate diets, since the majority of terrestrial plants contain low Na levels. Thus, several primate taxa such as mountain gorillas, chimpanzees, colobines, and spider monkeys are reported to engage in geophagy, consume decaying wood, visit salt licks, or consume plants with high Na concentration such as *Eucalyptus* sp. (Huffman and Wrangham, 1994; Rode et al., 2003; Rothman et al., 2006a; Chaves et al., 2011; Link et al., 2011).

Thus, given both the different and complementary nutritional profiles of particular plant tissues available to primate foragers, I examine the concept of nutrient balancing and its effect on seasonal food selection in black howler monkeys (*A. pigra*). Nutrient balancing represents a foraging strategy in which decisions concerning where to feed and what to eat are based on balancing the intake of macronutrients, micronutrients, and secondary compounds rather than on energy or protein maximization (Westoby, 1974; Felton et al., 2009; Simpson and Raubenheimer, 2012). Studies on invertebrates (*Schistocerca gregaria* (Waldbauer and Friedman, 1991; Raubenheimer and Simpson, 1993)) and vertebrates (tortoises, *Kinixys spekii* (Hailey et al., 1998); bee-eaters, *Merops apiaster* (Krebs and Avery, 1984); nectar-feeding sunbirds, *Cinnyris talatala* (Köhler et al., 2012); laboratory rats (Markison et al., 2000) and mice (Sørensen et al., 2008); and mink, *Mustela vison* (Mayntz et al., 2009)) have suggested that balanced diets are selected via macro and micronutrient regulation (Raubenheimer et al., 2009). Nutrient balancing can occur through a “continuous regulation of food intake that involves frequent shifts between foods” of different nutritional composition (Waldbauer and Friedman, 1991: 43), through adjustments in the amounts consumed of one type of food, or through selective extraction of specific nutrients from a given food type (Mayntz et al., 2005).

Here I analyze the diet and phytochemical composition (macronutrients, minerals, and condensed tannins) of foods consumed during a year-long study of two groups of Mexican black howler monkeys (*A. pigra*) inhabiting a semideciduous tropical forest in Southern Mexico. Previous studies of *A. pigra* indicate strong seasonal monthly shifts in dietary emphasis. For example, in the semideciduous tropical forest of the Calakmul Biosphere Reserve, Mexico, ripe and unripe fruits accounted for more than 85% of black howler monkey feeding time in the wet season (Rizzo, 2004). In contrast, in the riverine gallery forest of Northern Belize black howlers spent on average 86% of feeding time on young and mature leaves in the dry season (Pavelka and Knopff, 2004). Thus, a more detailed understanding of dietary preferences, nutrient balancing, and seasonal changes in nutrient and energy intake is needed to examine the basis of dietary selectivity in black howler monkeys.

The goals of this research are to: 1) compare and contrast howler feeding patterns determined through time-based versus amount-based assessments; 2) describe the phytochemical composition of the foods consumed by black howler monkeys; and 3) relate patterns of food choice (based on seasonal amounts of food ingested) with the phytochemical characteristics of plant parts consumed. Specifically, I test the hypothesis that black howler monkey food choice during three different seasons is strongly correlated with particular phytochemical characteristics of the selected food items, according to the following predictions:

Prediction 1. As suggested by previous studies of the feeding ecology of mantled howler monkeys (Milton 1979) and other leaf-eating primates (Rogers et al., 1990; Mowry et al., 1996; Chapman and Chapman, 2002), black howler monkeys are expected to exhibit a preference for food items characterized by a high content of available protein and a high protein-to-fiber ratio. Therefore, if this is correct, selectivity indices (a measure of food preference based on the amount of different phenophases in the diet and their seasonal availability in the environment) for the most consumed items (>80% of the diet) by howlers in each of the three seasons studied, will show significant positive correlation with these two nutritional variables.

Prediction 2. Studies analyzing how food nutrient content affected food selectivity (using selectivity indices based on time spent feeding on different plant phenophases and plant species abundance) in mantled howler monkeys (Williams-Guillén, 2003) and black and white colobus monkeys (Fashing et al., 2007) found that fiber correlated negatively with selectivity. Moreover, taking into account a food availability index, simple sugars appeared to be positively influencing food selection in black howler monkeys (Behie and Pavelka, 2012b). Based on these findings, I expect that selectivity indices of the most consumed food items (>80% of the diet) by black howlers correlate negatively with fiber content (NDF, ADF, ADL), positively with carbohydrates (either WSC and/or TNC), and show no correlation with the amount of other nutrients such as protein and lipids in consumed in particular foods.

Prediction 3. Sodium is not an essential element for terrestrial plants (Maathuis, 2014), but it is required by animals, mainly for its role in impulse transmission in nerves and muscles, being fundamental for growth and reproduction (Robbins, 1993; Chapin III et al., 2011). Thus, due to the general low Na levels found in plants (Rode et al., 2003; Rothman et al., 2006a; Chapin III et al., 2011), plant eating animals like black howler monkeys are expected to show a significant correlation between food item selectivity and Na content.

Methods

Study Site and Subjects

The research was carried out in the state of Campeche, Mexico, at El Tormento (18º36′44″N; 90º48′31″W), a 1400-ha forested area owned by Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The dominant vegetation types in this area of the Yucatan peninsula are semi-deciduous and evergreen seasonal forests (*sensu* Miranda and Hernández 1963). The Yucatecan soils are characterized by a limestone bedrock and a shallow soil surface layer, and by the absence of surface water (Aguila-Alcantara, 2007). The climate is hot and humid, and three seasons can be recognized: a dry and hot season from February to May (~100 mm rain during four months, 28°C mean daily temperature), a rainy season from June to September (~900 mm rain during four months), and a colder season ("*nortes*") from October to January (~160 mm rain during four months, 22.8°C mean daily temperature). According to data recorded by the Comisión Nacional del Agua (CONAGUA, 2013), between 1971 and 2000 total annual precipitation at El Tormento averaged 1291 mm, and mean annual temperature was 25.6°C. During the study period (September 2010-August 2011) total annual rainfall was 1246 mm, and average mean and maximum annual temperatures were 26.3°C and 33.4°C, respectively.

Two neighboring groups of individually-recognized black howler monkeys were followed during 15 months. Group M contained 10-12 individuals (including 4-5 adult males and 2 adult females) and group J contained 6-7 individuals (no more than 2-3 adult males at the same time and 2 adult females). Ten out of 16 adult individuals in the study groups were darted and marked with color anklets in August 2010, prior to the start of the behavioral data collection. The remaining adults were individually recognized based on facial features, scars, and broken digits. Between September 2010 and November 2011, the two groups ranged in areas of 14.5 (M) and 4.5 ha (J), with an overlap of 1.3 ha.

Field Data Collection

Behavioral Data

From September 2010 to November 2011, I collected data on the howler monkey activity budget and diet, conducting all-day follows of one adult individual per day. I obtained 1300 hours of behavioral data of 14 focal animals (ten males: 674 hours; four females: 626 hours) using two-minute instantaneous samples on focal animals (Martin and Bateson, 2007). When the focal activity was feeding, I temporally switched to a method of continuous data collection, in which I recorded the duration (to the nearest second) of each feeding event and the quantity (number of items, parts of the item, or bites per minute), phenophase (i.e. young/mature leaf, immature/mature fruit, flower/inflorescence, other), and species of all items ingested. Then I resumed collecting two-minute instantaneous samples of the focal animal. All feeding trees (n = 689) were tagged, measured (DBH, tree height, crown height, two perpendicular axes of the crown) and identified. Unless otherwise indicated, percentages of food items in the howler diet (e.g., % leaves, % fruit) are based on amounts of food ingested in grams, and not on the percentage of feeding observations.

Plant sample collection

Food items were collected from feeding trees either on the same day or within two days of the observed feeding bout. I attempted to gather at least 50 g of flowers/inflorescences and 100 g of leaves and fruits (wet weight) per tree. The plant items were transported to the field laboratory, where they were measured and weighed to the nearest 0.01 g. Leaves, flowers, and inflorescences were air dried at a constant weight in a dark area. After discarding the parts that were not eaten (e.g., exocarp, seeds), fruits were cut into small slices and kept in a drying oven (<50ºC) until reaching a stable dry weight. All samples were then stored in paper bags labeled with date, tree number, and phenophase, and maintained in a dry place with a silica desiccant until phytochemical analyses were performed.

Food availability

I carried out phenological censuses to monitor the presence and abundance of different plant food items during different seasons (i.e., young and mature leaves; ripe and unripe fruits; flowers, inflorescences, and flower buds). Two types of censuses were carried out: 1) 10 Gentry's belt transects (50×2 m each) (Gentry, 1988) in each group's home range were walked bimonthly (n= 196 trees); 2) five trees/sp of each of the 10 most important tree species in the howler monthly diet were monitored. In both cases I estimated the percentage of the crown containing food items for each monitored tree on a scale of 0 − 4 (0; 1: 1−25%; 2: 26−50%; 3: 51−75%; 4: 76−100%). To measure tree abundance and calculate basal area of the monitored species, I established six randomly placed 50×50 m quadrats and 33 additional Gentry's transects, tagging and identifying all trees with DBH ≥ 10 cm. The ecological sampling covered 12 − 25% of the home ranges of the two study groups. For each food item of a given species, I calculated a food availability index (FAI) by multiplying the average bimonthly phenology score by its total estimated basal area. I then summed FAI across items and averaged those values across the two bimonthly surveys to obtain total monthly FAI for young and mature leaves; mature and immature fruits; and flowers (see Foerster et al., 2012 for a similar analysis).

Laboratory Analyses

Plant samples were analyzed in the Nutritional Ecology Laboratory at Hunter College, City University of New York, from March to June 2012. All samples were analyzed in duplicate. The samples were ground using a Wiley® mill fitted with a 1-mm screen. Dry matter was calculated by drying the field-dried samples in an oven (105ºC) immediately before each analysis to remove atmospheric moisture (Rothman et al., 2012).

Total nitrogen (N) was determined via combustion according to AOAC (1995) using a Leco TruSpec Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA). Crude protein (CP) was calculated by multiplying N by 6.25 (Rothman et al., 2012). Although this conversion factor likely overestimates the amount of protein present in tropical plant samples (Milton and Dintzis, 1981), other proposed values (e.g., 4.3) might result in an underestimation (Conklin-Brittain et al., 1999). Moreover, since the majority of primate nutritional studies quantify crude protein in this way, we used the 6.25 factor for comparative purposes. However, to take into account nitrogen bound to fiber and secondary metabolites, I estimated available protein (AP) in two ways: *1)* to determine the amount of fiber-bound nitrogen, I subtracted acid detergent insoluble nitrogen (ADIN) from N, multiplied this value by 6.25, and then subtracted it from CP (Rothman et al., 2008); *2)* to estimate the effect of tannins on N digestibility, I measured the polyethylene glycol (PEG, a tannin-blocking agent) binding capacity in a subset of plant samples (n= 42, those that were positive for the qualitative acid-butanol assay for condensed tannins (Waterman and Mole, 1994)), followed by in vitro digestion with pepsin and cellulase (DeGabriel et al., 2008).

Lipids were determined by extraction with petroleum ether using an XT15 Fat Analyzer (ANKOM, Macedon, NY, USA) (AOCS 2009). I subtracted 1 from the percentage of ether extract to account for all the non-lipid non-nutritive components extracted by the procedure (e.g., waxy substances, cutin, essential oils) (Rothman et al., 2012).

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially using filter bags in an ANKOM 200/220 fiber analyzer (Van Soest et al., 1991). Fruit samples containing >5% fat were pre-extracted in acetone before the NDF procedure, since lipids might interfere with the detergent (Rothman et al., 2012). Water soluble carbohydrates (WSC) were measured with the phenol-sulfuric acid assay (Dubois et al., 1956), using sucrose as a standard. Total nonstructural carbohydrates (TNC) were calculated by subtraction: %TNC = 100 – %NDF – %lipids – %AP – %ash (Rothman et al., 2012).

Plant samples were extracted with 70% acetone to measure condensed tannins using the acid-butanol assay (Waterman and Mole, 1994). I did not use external standards (e.g., quebracho), since it has been demonstrated that they may overestimate condensed tannin content by up to 35% (Rothman et al., 2009). Absorbance values, measured in a spectrophotometer at 550 nm, were reported according to a qualitative system: <0.10 (0, i.e. tannins are not present); 0.10 – 0.50 (+); 0.50 – 1.00 (++), and >1.00 (+++) (Rothman et al., 2006b). Twenty-one samples were sent to Dairy One Inc. for mineral analyses. Minerals (Na, Ca, P, Mg, K, Fe, Zn, Cu, Mn, Mo) were determined using a Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Radial Spectrometer (Thermo Optek Corp, Franklin, MA).

Data analysis

To calculate the daily amount of food ingested (g dry weight) by each focal animal, I multiplied the feeding bout length on food item *i* (minutes) by the corresponding feeding rate (g dry weight/min), and then summed all daily events. To estimate daily nutrient intake, I multiplied the nutrient content of each food item *i* by the estimated amount of item *i* ingested (g dry weight) in each feeding bout. The metabolizable energy content of plant samples was calculated using standard conversion factors (or physiological fuel values): 16 kJ/g for TNC, 17 kJ/g for AP, and 37 kJ/g for lipids (Conklin-Brittain et al., 2006). Since howler monkeys obtain energy from fiber fermentation, I calculated a conversion factor based on mean fiber digestibility (47.7%) reported by Edwards and Ullrey (1999) for captive *A. palliata*, *A. caraya*, and *A. seniculus* consuming a diet of 42% NDF. Considering that gut microbes might retain at least 4 kJ/g during hemicellulose and cellulose fermentation for their own growth (Conklin-Brittain et al., 2006), I multiplied the digestion coefficient (0.477) by 12 kJ/g. Thus I used a conversion factor of 5.7 kJ/g for NDF. I defined the energy derived from TNC, NDF, and lipids as non-protein energy.

I computed Jacobs selectivity index (D) (Jacobs, 1974) to identify preferences in the food choices of the study animals, using dietary items that accounted for more than 80% of the total diet in individual seasons. The index was calculated as $D = (r_{is} - p_s) / (r_{is} + p_s - 2r_{is}p_s)$, where r_{is} is the percentage of item *i* belonging to species *s* in the diet (based on grams consumed), and p_s is calculated as the availability (FAI) of item *i* belonging to species *s*. This index varies from -1 to +1, with positive values indicating preference, negative values avoidance, and 0 indicating no selection.

Differences in nutrient content among different plant parts were analyzed using one-way ANOVAs and unequal n HSD *post-hoc* tests, and Kruskal-Wallis tests with subsequent multiple comparisons when variables were not normally distributed even after transformation. Spearman rank order correlations were used to analyze the relationship between the phytochemical characteristics (i.e., nutrients, minerals, and metabolizable energy) of the food consumed and 1) the contribution (percent amount) of different items to the diet, and 2) the selectivity indices of different items. I used an alpha level of 0.05 for all statistical analyses. These tests were run in *STATISTICA 12* (StatSoft, 2011).

Results

Diet and feeding patterns

The annual feeding patterns of the two study groups differed consistently according to the methodology used to describe them. Based on feeding time, howlers appeared to consume more leaves (50.6%) than mature and immature fruits (40.2%). However, data on grams consumed indicated that leaves accounted for 37% of the diet, and fruits for 58.2%. According to amount consumed, during the dry and rainy seasons the howler diet was predominantly fruit based (54.1% and 69.2%, respectively), whereas during the *nortes*, howler monkeys increased their consumption of leaves (49.5%) and flowers/inflorescences (9.7%). Table 3.1 highlights the differences in the howler diet when values are based on percentage of time spent feeding compared to the weight of food items ingested. Black howler monkeys devoted 10% more time to consuming leaves than fruits, however, the amount of fruit pulp consumed was 21% greater than the amount of leaf tissue consumed. Flower ingestion was slightly overestimated (3%) by using a time-based approach. These differences reflect the fact that average feeding rates for mature leaves (3.45 ± 1.9 g/min), mature fruits (2.56 ± 1.6 g/min) and immature fruits (2.52 ± 2.2 g/min) were greater than rates for young leaves (2.10 ± 1.4 g/min) and flowers (1.00 ± 0.6 g/min). However, feeding rates within each food type varied greatly among different plant species (e.g., from 0.6 g/min for *Blepharidium mexicanum* mature leaves to 7.8 g/min for mature leaves of an undetermined vine species ("Vine sp. 2")). Thus, I estimated the amount of food ingested using actual feeding rates for each individual plant species, and that resulted in the observed dietary patterns of 58.2% fruits, 37% leaves, and 4.7% flowers/inflorescences.

Overall, black howler monkeys fed on 44 plant species (20 families). Fourteen of these accounted for 81% of the yearly diet (based on food amount ingested). In addition, the three highest ranking species, *Brosimum alicastrum* (Moraceae), *Manilkara zapota* (Sapotaceae), and *Acacia usumacintensis* (Fabaceae), comprised 52.5% of the annual diet. The most consumed items throughout the year were *chicozapote* (*M. zapota*) immature fruits (14%), followed by *ramón* (*B. alicastrum*) mature (12%) and immature (7%) fruits (Table 3.2). The consumption of these fruits differed temporally, but during each season mature and/or immature fruits of either or both species were found among the three most consumed foods (Table 3.2).

During the dry season, the howlers consumed food items from 36 plant species, 11 of which (14 different plant parts) accounted for 81% of the diet. The most consumed items were *Manilkara zapota* (29%) and *Pseudolmedia oxyphyllaria* (14%) immature fruits. During the *nortes*, black howlers fed on a total of 28 plant species; seven species (11 plant parts) accounted for 80% of the diet, and the most consumed items were the immature fruits of *Acacia usumacintensis* mature leaves (16%), *Brosimum alicastrum* (14%), and *M. zapota* immature fruit (12%). Finally, during the rainy season, individuals consumed items belonging to 32 species, six of which (nine plant parts) constituted 83% of the seasonal diet. The most consumed items were *B. alicastrum* (26%) and *Pimenta dioica* (13%) mature fruits. Overall these data indicate that black howlers exhibited a consistent dietary pattern across seasons, with 6-11 species accounting for 80% of food consumed. However, whereas during the dry and rainy seasons, fruits (mature and immature) of two or three plant species accounted for 47% of grams consumed, and leaves (young and mature) from two or three species accounted for ~16%, during the *nortes* fruits and leaves of three plant species accounted for 33% and 28%, respectively, of the total amount of food consumed.

Given that food selection could be affected by plant species abundance and temporal availability of food resources, I calculated the selectivity index D taking into account the availability of different food phenophases throughout the study period. D ranges from -1 (negative selection or avoidance) to +1 (positive selection), with 0 indicating no selection or neutrality. In the dry season, *Pseudolmedia oxyphyllaria* immature fruits (D = 0.98), *Protium copal* young leaves (D = 0.96), and *Brosimum alicastrum* young leaves (D = 0.95) were highly selected. *Pseudolmedia oxyphyllaria* accounted for 14.6% of the dry season diet. Trees of this species were characterized by a relatively low abundance (basal area/ha: 0.54) in the study area, and their mature and immature fruits were consumed intensively during this season. During the *nortes*, the howlers showed a high preference for consuming *Dendropanax arboreus* mature fruits (D = 0.97) and *Trophis racemosa* inflorescences (D = 0.96). During the rainy season, *B. alicastrum* mature fruit (D = 0.86) and *Acacia usumacintensis* mature leaves (D = 0.82) had the highest selection indices (Table 3.3). These data indicate a black howler behavioral pattern characterized by feeding on foods that were relatively abundant and available in the environment (such as *Manilkara zapota* fruits, which were intensively consumed, and highly abundant), as well as actively seeking particular food items that were present in the environment at relatively low availability (e.g., *P. oxyphyllaria* immature fruits).

Phytochemical data

Phytochemical analyses of 127 plant samples collected from feeding trees belonging to 29 species (Figure 3.1, Table 3.4) indicated that plant tissues consumed by *A. pigra* differed significantly in their nutrient, mineral and secondary compound content.

Protein. Crude protein (CP) ranged from 3.3% in *Manilkara zapota* mature fruits to ~33% in mature and young leaves of one vine species and some tree species of the *Fabaceae* family (e.g., *Platymiscium yucatanum*). CP content differed significantly among plant parts (Kruskal-Wallis: H = 40.46, df = 4, $p < 0.0001$), being higher in young ($p < 0.005$) and mature ($p < 0.008$) leaves (average CP = 20.5% and 20%, respectively), and flowers/inflorescences (19.7%, $p < 0.03$) than in fruit, both mature (9%) and immature (11.7%). Available protein (AP), which is a more accurate measure than CP of protein accessible to the consumer since it does not include non-digestible nitrogen (bound to secondary compounds and fiber), also differed with respect to plant part (H = 19.44, df = 4, p = 0.0006), with flowers (AP = 15.8%) exhibiting higher levels than mature (7.5%, p = 0.001) and immature (9.1%, p = 0.01) fruits. In the case of young and mature leaves (13.7% vs. 13.2%, respectively), AP did not differ significantly from each other and from flowers. AP ranged from 0.7% in *M. zapota* immature fruits, to 27.5% in *P. yucatanum* young leaves. Overall, these results indicate that flowers and inflorescences consumed by howler monkeys were as high in available protein as were mature and young leaves, which contained more available protein than fruits. However, the variability in protein content among the same food types across plant species was high (for example the average amount of available protein in mature fruits was 9.1%, and its standard deviation 9.3), thus suggesting that particular species may be targeted or substituted for their nutrient content. The difference between CP and AP was greater in mature and young leaves, in which almost 7% of nitrogen was bound to fiber and tannins, and thus resistant to digestion, than in mature fruits (1.6%), immature fruits (2.6%) and flowers (3.8%). Thus, protein present in fruits and flowers was more digestible than in young and mature leaves.

Lipids. Lipids ranged from 0% in certain young and mature leaves to 30-35% in mature and immature fruits of *Dendropanax arboreus*. Lipid content differed among phenophases (H = 15.35, df = 4, p =0.004). Lipids were significantly higher in mature fruit (average = 11.1%) than young leaves (1.9%, p = 0.003) and mature leaves (2.4%, p = 0.03). Average lipid content in inflorescences (2.2%) was lower than in mature fruits, but the difference was marginally significant (p = 0.05). Lipid content in immature fruits (5.4%) did not differ significantly from that of other plant parts.

Fiber. Fiber, which included neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), differed according to plant part (NDF, Kruskal-Wallis: H = 20.40, df = 4, p = 0.0004; ADF, ANOVA: F(4,106) = 5.1, p = 0.0008; ADL, ANOVA: F(4,96) = 2.55, p = 0.04). Specifically, NDF was higher in leaves (young leaves = 50%, p = 0.0007; mature leaves = 47%, p = 0.02) than in mature fruit (33.6%). NDF in young leaves (but not mature leaves) also was significantly higher than in flowers/inflorescences (39.5%, p = 0.02). Similarly, ADF was significantly higher in young leaves (ADF = 39.5%) than mature fruit (25.2%, p = 0.001). ADL content also tended to be higher in young leaves (ADL = 24.7%) than in all other items (e.g., ADL mature leaves = 19.2%; immature fruit = 16.4%), but the differences between specific plant parts were not significant. Finally, the protein to fiber ratio (AP:ADF) did not differ between young and mature leaves (Mann-Whitney U-test: U = 260, n_1= 22, n_2 = 26, p = 0.59) (young leaves = 0.41 ± 0.3; mature leaves = 0.45 ± 0.3). Overall, mature and young leaves consumed by howlers were similar in their nutrient composition, including the percentage of fiber, available protein, and protein to fiber ratio.

Non-structural Carbohydrates. Water soluble carbohydrates (WSC), which include simple sugars and fructans, ranged from 1-2% in mature and young leaves of different plant species, to 41.7% in *Simarouba glauca* and *Vitex gaumeri* mature fruit. WSC were significantly higher in mature fruits (average = 23.6%) than mature leaves (6.2%, p = 0.0003), flowers (5.6%, p = 0.01), and young leaves (4.9%, p< 0.0001), and higher in immature fruit (13%) than young leaves (p = 0.01). In contrast, there were no significant differences in total non-structural carbohydrates (TNC) present in different plant parts, despite the fact that on average they appeared to be lower in young and mature leaves (~28 ± 10%) than in mature fruit (42 ± 22%), immature fruit (36 ± 18%), and inflorescences (36 ± 8%).

Condensed Tannins. Of the 78 samples analyzed for condensed tannins, 69% resulted in a positive reaction (Table 3.5). According to the qualitative system used to report absorbance of the plant samples (acid-butanol assay), the highest amounts of condensed tannins (+++) were found in nine samples of mature leaves belonging to six species, five samples of young leaves (five species), two inflorescence samples (two plant species), two samples of one species of immature fruit, and one sample of one species of mature fruit. The presence of high amounts of condensed tannins in young and mature leaves, inflorescences, mature fruits and immature fruits included in the howler diet is likely to reduce the availability of proteins and thus potentially act as an antifeedant. However, sixty-three percent of the samples with high amounts of tannins (+++) were included in the 10 species most consumed by howler monkeys during the study period (*Bursera simaruba*, *Manilkara zapota*, *Metopium brownei*, *Pseudolmedia oxyphyllaria*, and a vine of unknown species), indicating that howlers appear to be able to tolerate high tannin levels.

Ash. Ash constitutes the inorganic portion of the plant sample that remains as residue after combustion, and represents the total mineral content of the sample. Ash content differed among items (ANOVA on transformed data: F(4,115) = 6.12, p= 0.0001), being higher in flowers (ash = 7%, p = 0.03) and mature leaves (7.6%, p = 0.0006) than mature fruit (4.2%), suggesting that these phenophases are richer in mineral content.

Minerals. Content of macrominerals in howler foods are shown in Table 3.6. Mature leaves contained significantly higher levels of Ca than mature fruit (H = 11, df = 3, p =0.01), but were lower in K than young leaves (H = 9.1, df = 3, p = 0.02). Mature leaves also were higher in Mn than mature fruits (H = 9.3, df = 3, p = 0.02)**.** The concentration of P, Mg, Na, Fe, Zn, and Cu did not differ significantly among plant parts. However, P content tended to be higher in inflorescences (0.29%), and young leaves (0.23%), and Fe content higher in young (50.6 ppm) and mature leaves (49.5 ppm) and inflorescences (77 ppm in *Trophis racemosa*). Only mature leaves and inflorescences contained higher levels of Ca (>1.4%) than the estimated minimal dietary concentration recommended by the National Research Council for non human primates (0.55%) (NRC, 2003). Ca content was particularly high in *Brosimum alicastrum* (2.17%) and *Metopium brownei* (2.16%) mature leaves. Average concentrations of Mg (>0.18%) and K (>1.3%) in all plant parts surpassed the estimated Mg adequate dietary levels (0.08%) and minimum K requirements (>0.4%) for non human primates. In contrast, P, Na, Fe, Cu (except for young leaves), and Mn in the analyzed foods did not meet NRC recommendation. In general, leaves and flowers were higher in mineral content than fruits, and all of the plant samples analyzed tended to be low in sodium, phosphorus, iron, and calcium, with the exception of some mature leaves such as those of *M. brownei* and *B. alicastrum*, which were high in calcium.

Feeding preferences: relationship between selectivity index and food phytochemistry

The amount of particular food items (e.g., *Manilkara zapota* immature fruits) in the diet of black howler monkeys (expressed as %) during each of the three seasons did not correlate significantly with mineral, nutrient, and energy content. In contrast, the selectivity index based on diet consumption and food availability (D), which is a measure of howler food preferences, showed a positive correlation during the *nortes* with lipid ($r_s = 0.648$, n = 10, p = 0.04), P ($r_s = 0.719$, n = 8, p = 0.04), and K content ($r_s = 0.747$, n = 8, p = 0.03) (Table 3.7). During this season, the consumption of leaves (40.3% mature leaves) and flowers (8.4%) increased compared to other periods, and mature fruits of *Dendropanax arboreus* (7.35% of the diet), which were rich in lipids, were highly selected. No significant correlations were found during the rainy and the dry season, suggesting that during these periods, individual plant parts and species were not preferentially selected based solely on their individual phytochemical characteristics.

Figure 3.2 shows the percentage of nutrients (calculated by multiplying the nutrient content of different plant parts by their percentage contribution, based on grams ingested, to the seasonal diet) ingested by howler monkeys from each of the food items that constituted ~80% of the seasonal diet (listed in Table 3.3). General patterns of nutrient ingestion did not change significantly during the three seasons; for example the average protein-to-fiber ratio of the most consumed food items did not vary significantly across seasons (dry: 0.57 ± 0.4; rainy: 0.65 ± 0.3; *nortes*: 0.58 ± 0.3; Kruskal-Wallis, $H = 0.87$, $n = 31$, $p = 0.6$). Analogously, dietary mineral content (Figure 3.3) was similar across seasons, regardless of the fact that mineral content differed among certain plant parts (see results). These data suggest that howlers were successful in regulating their food intake and obtained a relatively consistent balance of nutrients throughout the year.

There was, however, a trend of increasing the intake of sugars in the rainy season (1.8-fold increase relative to the *nortes*); of Mn, available protein, and lipids in the *nortes* (2-fold, 1.4-fold, and 1.8-fold increase, respectively, relative to the dry season); and lignin in the dry season (1.9-fold increase relative to the rainy season) (Figure 3.2). Despite these seasonal fluctuations in the intake of certain nutrients, the selectivity indices only showed significant correlations between the amount of foods consumed and their phytochemical characteristics during one season, the *nortes*, in which one plant species appeared to be targeted for its lipid content. This indicates that during most of the year, howlers did not obtain higher intake of nutrients by targeting specific food items rich in that particular nutrient, but instead exhibited a foraging pattern in which the consumption of the fruits, leaves, and flowers of several common plant species resulted in a consistent intake of macronutrients and minerals.

Discussion

In this research I analyzed the nutritional basis of food selection in two groups of Mexican black howler monkeys. Integrating data on diet, plant phytochemical characteristics, and selectivity towards species-specific plant phenophases, I tested if black howler monkeys selected for or against specific nutrients and minerals, and if food choice could be explained in the context of a nutrient balancing framework. Nutrient balancing posits that a forager's goal is not to maximize the intake of any single nutrient over others, but to achieve a targeted proportion of macro and micronutrients (*intake target*, *sensu* Simpson and Raubenheimer, 2012). Here I tested three predictions regarding howler monkey nutrient intake.

The data indicate that black howler monkeys' selection of food items (based on indices of selectivity calculated considering seasonal changes in the amount of phenophases consumed and their availability in the howler home ranges) did not show a significant positive correlation with the protein content or protein-to-fiber ratio of the resources consumed, thus not supporting Prediction 1 (protein and protein-to-fiber maximization). As examined in Prediction 2, food choice was not negatively correlated with fiber content, or positively correlated with sugar concentration. Thus, Prediction 2 also was not supported. Given that I did not examine the fiber content of food items in the environment, it is possible that foods not eaten had a different nutritional profile from foods consumed. Finally, Prediction 3, which examined evidence for dietary selectivity based on Na content of food items, was not supported, despite the fact that this mineral was found in low amounts in foods consumed (lower than the estimated NRC requirements).

These results indicate that, based on abundance and availability, during one of the three season considered (*nortes*), lipid, K, and P content appeared to play an important role in howler monkeys' decisions to consume particular food items. In general, lipids have not been argued to be involved in howler monkey food selection (Norconk et al., 2009). There currently exist one report of the fatty acid profile of the howler diet (Chamberlain et al., 1993) and only three nutritional studies (Williams-Guillén, 2003; Behie and Pavelka, 2012b; Aristizabal, 2013) that reported lipid content of howler foods. In all cases, the items ingested contained low amounts of lipids (<9% dry matter).

In contrast, howler patterns of food choice during the rainy and dry seasons failed to indicate that the maximization of any particular nutrient drove food choice. This indicates that, despite the changing food preferences (i.e., selectivity indices) shown by howlers across seasons for different plant species and plant parts, food items appeared to be selected in an attempt to balance the relative proportions of protein, lipids, and carbohydrates ingested, rather than to prioritize a particular nutrient. This is consistent with a behavioral pattern of nutrient balancing (Milton, 1998, Felton et al., 2009).

Phytochemical composition of the diet

Previous studies of the nutrient composition of foods consumed by howler monkeys suggest that their diet is mostly composed of leaves and flowers with high protein-to-fiber ratios and fruits rich in carbohydrates and sugars (Milton, 1979; Glander, 1981; Estrada, 1984); however, they also seem to tolerate high fiber content (up to 60% ADF and 40% ADL) in the foliage ingested (Silver et al., 2000).

Phytochemical analyses of plant parts collected from feeding trees visited by howler monkeys during this 15-month study showed that young leaves, mature leaves, and flowers/inflorescences were richer than mature and immature fruits in available protein, ash, and minerals (especially calcium), but they were not a good source of sugars. Leaves did not differ in fiber content from immature fruit, but were higher in NDF and ADF than mature fruits. In contrast, mature fruits were rich in lipids and water soluble carbohydrates and relatively lower in sodium than other plant parts (Figure 3.1, Table 3.4). Overall, these results were consistent with standard descriptions of neotropical primate food characteristics (Norconk et al., 2009). However these data also revealed that assumptions regarding the nutrient content of individual foods consumed by primates are sometimes incorrect. For example, although it is often assumed that immature *vs*. mature leaves ingested by primates differ in fiber, available protein, and secondary metabolites (Milton, 1979; Mowry et al., 1996; Chapman and Chapman, 2002; Tombak et al., 2012), mature and young leaves consumed by black howler monkeys in the present study did not differ in their nutritional components. Based on a sample size of 26 (young leaves) and 32 (mature leaves), I found similar amounts of crude and available protein, fiber, total non-structural carbohydrates, and ash (Figure 3.1, Table 3.4). Given that I only analyzed plant samples that were included in the howler diet, these data indicate that mature leaves chosen were similar to young leaves in nutrient content, possibly to avoid higher amounts of antifeedants, such as indigestible cell wall material and secondary compounds, which may have been present in uneaten mature leaves (Glander, 1981; Norconk et al., 2009). Nevertheless, during all seasons howlers were able to locate and consume mature and young leaves of similar nutrient content without significant changes in day range and time spent traveling.

Similarly, average protein-to-fiber (AP/ADF) ratio did not differ between mature and immature leaves, and was low (~0.4) compared to the values provided in the literature for leaves eaten by howler monkeys (Silver et al., 2000; Williams-Guillén, 2003; Behie and Pavelka, 2012b) (Table 3.8) and colobines such as *Procolobus rufomitratus* (ratio = 0.8) (Gogarten et al., 2012) and *C. guereza* (ratio = 0.7) (Fashing et al., 2007). In general, young leaves are reported to have higher protein-to-fiber ratios than mature leaves (e.g., 0.81 vs. 0.48 for leaves in the diet of black howler monkeys in Belize [Behie and Pavelka, 2012b]), and to be preferentially selected for this reason (Milton, 1998). However, the majority of the studies calculated the ratio using crude protein rather than available protein, which leads to higher values, since CP also includes the amount of nitrogen bound to fiber and tannins, and therefore unavailable to the consumer (Wallis et al., 2010). Silver et al. (2000) calculated the ratio as AP/ADF in leaves consumed by black howler monkeys in Belize, and also did not find significant differences between the protein-to-fiber ratio of young and mature leaves. This highlights the importance of measuring the amount of nutrients that can be digested and metabolized by the forager rather than the absolute amount of nutrients in foods (DeGabriel et al., 2008; Wallis et al., 2010; Wallis et al., 2012; DeGabriel et al., 2013). Measuring AP/ADF in foods consumed by black howler monkeys, we found that individuals included leaves characterized by a low protein-to-fiber ratio among their most consumed or preferred items (e.g., ratio = 0.2 in *Protium copal* young leaves, which were highly selected in the dry season [D = 0.96]). These low values may be similar to those found in other primate species, when available protein rather than crude protein is included in the calculation of the protein-to-fiber ratio. For example, in the present study, AP and CP differed by

44% (± 30%) and 47% (± 26%) in young and mature leaves, respectively. Assuming a similar relationship, other studies of howler monkeys are likely to find an AP/ADF ratio similar to that reported here.

The nutritional composition of leaves in the howler diet at El Tormento showed a trend of a lower percentage of available protein and minerals and higher fiber compared to values reported for other food items consumed by howler and spider monkeys at other sites (Gaulin and Gaulin, 1982; Silver et al., 2000; Williams-Guillén, 2003; Felton, 2008; Righini et al., 2013; Aristizabal, 2013) (Table 3.8). For example, young leaves collected at El Tormento contained generally higher amounts of ADF and ADL than those found in other studies (e.g., ADF: 39.5% *vs* 25.3% [Behie and Pavelka, 2012b]; ADL: 24.7% *vs* 20.3% [Williams-Guillén, 2003] and 14.6% [Fernández, in prep.]). Increased fiber might be linked to the geochemical characteristics of the soils, since nutrient-poor soils are usually characterized by vegetation with low quality foliage (e.g. presence of secondary compounds, low protein to fiber ratio) (McKey, 1978; Waterman et al., 1988). Protein and phenolics share the same precursor, phenylalanine (Jones and Hartley, 1999). Nitrogen limitation in the soil reduces the production of proteins, therefore phenylalanine can be incorporated into phenolic synthesis, resulting in an increased amount of lignin and condensed tannins in the leaves (Wright et al., 2010). The Yucatan peninsula in Mexico (location of this study) was formed from limestone on a karstic landscape and is characterized by a shallow soil surface layer and a low content of organic matter, favoring the rapid leaching of nutrients (Perry et al., 2003; Aguila-Alcantara, 2007). Limestone soils are identified by the presence of the mineral calcium carbonate, high concentrations of bicarbonate ions, and high pH (Kishchuk et al., 1999; Misra and Tyler, 1999), which may result in reduced phosphate and micronutrient (e.g., zinc, iron) availability (Leytem and Mikkelsen, 2005). These characteristic of the soils are likely responsible for the presence of condensed tannins (found in ~80% of the leaves sampled), the low protein-to-fiber ratio (< 0.45), low iron content (< 51 ppm), and moderate P concentration (< 0.2%) of the foliage collected at El Tormento.

Feeding preferences and selectivity indices

Based on the amount of food ingested throughout the year, three plant species accounted for over 50% of the study groups' diet: *Brosimum alicastrum* (Moraceae), which is considered an important food source for atelines such as *A. palliata* (Milton, 1980; Chapman, 1988; Estrada et al., 1999), *A. pigra* (Silver et al., 1998; Rizzo, 2004; Rivera and Calmé 2006) and *Ateles* spp. (Ramos-Fernández and Ayala-Orozco, 2003; Russo et al., 2005; Di Fiore et al., 2008); *Manilkara zapota* (Sapotaceae), also commonly included in the diet of howler and spider monkeys (Silver et al., 1998; Di Fiore et al., 2008; Dias et al., 2011; Scherbaum and Estrada, 2013; Plante et al., 2014); and *Acacia usumacintensis* (Fabaceae). The latter species is only found in Southern Mexico, Belize and Guatemala (Seigler et al., 2006), and a related species, *A. dolichostachya* has been reported as a food source for *A. pigra* (Dias et al., 2011) and *Ateles geoffroyi* (Scherbaum and Estrada, 2013). Howlers fed on young leaves, mature leaves, mature fruits and immature fruit of *B. alicastrum* and *M. zapota*, and on young and mature leaves of *A. usumacintensis*. *Brosimum alicastrum* and *M. zapota* were common trees in the study groups home ranges (basal area/ha = 8.88 m^2 and 14.02 m^2, respectively), whereas *A. usumacintensis* basal area was lower (1.39 m^2).

Despite fluctuations in fruit and young leaf availability across different seasons, different phenophases belonging to at least one of these species were always found among the three most commonly consumed items in each season. Similar patterns of substantial reliance on few plant species (mostly in the Moraceae family), and low use of the majority of the other species, are reported in several other howler monkey species (Milton, 1980; Kowalewski, 2007; Chaves and Bicca-Marques, 2013) and other atelines such as *Ateles* spp. (Chapman, 1988; Wallace, 2005; Felton et al., 2008; González-Zamora et al., 2009) and *Lagothrix lagotricha poeppigii* (Di Fiore, 2004). For example, Milton (1980) reported that four species, including *Ficus yoponensis*, *B. alicastrum*, *Poulsenia armata*, and *F. insipida* accounted for ~40% of the overall feeding time of one group of *A. palliata* on Barro Colorado, Panama, followed during 9 months. However, while in my study only 25 species (out of 44) accounted for <1% of the total amount of foods consumed, in Milton's study, 54 species (out of 73) accounted for <1% of feeding time, indicating that most species were only consumed on one day during the total study period (Milton, 1980). This suggests that the dietary breadth of *A. pigra* at El Tormento was lower than what is reported for some populations of mantled (*A. palliata*) and brown (*A. guariba clamitans*) howler monkeys living in continuous forests or large fragments (>500 ha) (Chaves and Bicca-Marques, 2013). Feeding on a wide variety of plant species to limit food intake from any single plant species has been suggested as a strategy that howlers use to dilute the effects of potentially harmful secondary compounds when feeding on leaves (Milton, 1980; Glander, 1981). However, food species diversification strategies are best identified on a daily scale, rather than considering the number of species included in the total diet (Glander, 1981). This is due to the fact that strategies of resource mixing to avoid ingesting high daily amounts of any single toxin include increasing the number of leaf or fruit species eaten per day or across several consecutive days, but this pattern may be repeated across weeks resulting in the exploitation of a limited number of plant species (Glander, 1981). Milton (1980) reported that mantled howlers on Barro Colorado

Island fed on average on 7.6 plant species daily. In *A. pigra*, I found that individuals fed on 6.8 ± 2.3 plant species per day, showing that there were not marked differences between *A. pigra* and *A. palliata* in the number of plant species visited per day, despite the fact that overall the number of plant species included in the yearly diet was smaller in *A. pigra.* I also found that howlers visited on average 14.0 ± 5.0 different feeding trees daily, a higher number than what was reported for five groups of *A. seniculus* in the Colombian Andes, which visited 5.7 ± 1.6 feeding trees/day in a secondary forest and 9.1 ± 4.4 in a mature forest (Gomez-Posada et al., 2007). The increased number of individual trees visited by *A. pigra* may be a local response to the fact that ~70% of the howler foods that I analyzed contained condensed tannins, and intraspecific variability in tannin content was high (in 43% of the cases, tannin content differed among samples of the same phenophase collected from different trees of the same species during the same season) (Table 3.5), indicating that feeding on several different trees of the same species on the same day is one solution to the problem of ingesting high levels of secondary compounds. In fact, on 95% of the days, howlers fed on more than one tree of the same species each day, the mode being two, 3.1 ± 1.3 the average, and seven the maximum (for *Pseudolmedia oxyphyllaria*).

Despite consuming up to 42% of fruits and leaves (combined) of *Brosimum alicastrum, Manilkara zapota* and *Acacia usumacintensis* in a given season, the selectivity index D, which takes into account not only the relative abundance of a species, but also the availability of its phenophases, showed that these species were not always the most preferred by the howlers (Table 3.3). I defined preference according to the selectivity index D, with D = 1 indicating high selectivity or preference (see Methods). This index also showed that there were some food items (e.g., *Vitex gaumeri* young leaves) that were consumed according to their availability, others that were exploited at a lower rate than their availability (e.g., *M. zapota* young leaves during the dry season, D = -0.3), others that were moderately preferred (e.g., *V. gaumeri* mature fruits during the rainy season, D = 0.4), and several plant parts that were strongly preferred (e.g., *Trophis racemosa* inflorescences and *Dendropanax arboreus* mature fruits during the *nortes*, and *Pseudolmedia oxyphyllaria* immature fruits during the dry season, D >0.9). The same phenophase of a given species, due to its changing availability across seasons, could go from being highly preferred during one season (e.g., *B. alicastrum* young leaves, D = 0.95 in the dry season), to moderately preferred in others (e.g., *B. alicastrum* young leaves, D = 0.44 in the *nortes* and 0.36 in the rainy season), reiterating the importance of considering current availability of resources when analyzing primate food choice (Leighton, 1993; Takemoto, 2003; Boesch et al., 2006), rather than tree species abundance, species density/ha, or total basal area/ha, which are consistent across the year despite marked fluctuations in resource availability (Rogers et al., 1990; Williams-Guillén, 2003; Fashing et al., 2007; Felton et al., 2008; Bowler and Bodmer, 2011; Suarez, 2013). These results indicate that, although during all seasons *A. pigra* individuals showed significantly stronger (or weaker) preferences for certain plant species and parts (consumed at a greater frequency than expected based on availability in the environment), in general these preferences were not based on the targeting of a particular nutrient. This means that, with the exception of lipids, K, and P, which drove howler food selection during the *nortes*, preferences toward specific food items in the howler diet were not driven by the macro or micro nutrient content of these resources.

Plant phytochemistry and food choice

Previous studies of howler monkey (*Alouatta* spp.) feeding ecology and phytochemistry have shown that several different factors affect food choice (Milton, 1979; Behie and Pavelka, 2012a). In the case of *A. palliata,* it has been argued that protein and the protein to fiber ratio of leaves are strong predictors of dietary preferences (Milton, 1979). This was based on observations indicating a feeding preference for young leaves, which were higher in crude protein and lower in fiber than mature leaves (Milton, 1980) (but see Table 3.8). Positive selectivity towards leaves with a high (relative to average values of leaves in the environment) protein-to-fiber ratio is consistent with patterns found in other folivorous primates such as *Trachypithecus johni* (Oates et al., 1980), *Colobus satanas* (McKey et al., 1981), and *Presbytis rubicunda* (Davies et al., 1988), and these findings might have led researchers to suggest that in general, leaf eating primates should select food items that maximize protein while minimizing fiber intake (McKey et al., 1981; Waterman et al., 1988). Sugars also have been considered important in primate diets (Rogers et al., 1990; Espinosa-Gomez et al., 2013), and it has been noted that prosimians such as *Microcebus murinus*, spider monkeys, gorillas, and chimpanzees commonly tolerate higher tannin concentrations if they are found in high sugar solutions (Reynolds et al., 1998; Simmen et al., 1999; Laska et al., 2000; Remis, 2006). In this regard, a study of *A. pigra* by Behie and Pavelka (2012b) reported that simple sugars and protein were positively associated with food choice. When analyzing only the leaves consumed, however, food choice was positively associated with simple sugars, negatively associated with fiber, and unrelated to protein content (Behie and Pavelka, 2012b). In this study, as in the present research, the protein-to-fiber ratio of leaves was not a significant factor in howler food choice.

Nutritional factors driving food choice in howler monkeys may be affected by the characteristics of the howlers' habitat, including forest primary productivity and soil geochemistry, whose characteristics have a major influence on leaf chemistry (Janzen, 1974). The need to target a specific nutrient and maximize its intake might arise under conditions in which that nutrient is limiting in the environment, as has been reported for several colobine populations (Davies et al., 1988; Yeager et al., 1997). For example, some authors argue that if in a given environment all plant phenophases are generally high in protein, primate food choice will not likely be based on protein prioritization, but rather on the targeted intake of nutrients such as sugars, fiber, or minerals (Kool, 1992, Yeager et al., 1997; Behie and Pavelka, 2012a), since the physiological requirements for protein should be easily met. The fact that overall young and mature leaves consumed by *A. pigra* at El Tormento were not particularly rich in available protein (averaging ~13% dry matter) and did not have a high protein-to-fiber ratio (~0.4) compared to other sites inhabited by howler monkeys (Table 3.8), might have resulted in protein prioritization by howler monkeys. This was not the case, since I did not find that protein content significantly affected food choice. Nevertheless, the howlers were able to meet and surpass their protein requirements (4.9-5.2 g/mbm according to Nagy and Milton, 1979), consuming on average 6 g/mbm of available protein per day (see Chapter 4). Thus, I argue that howlers met their protein requirement not by targeting only protein-rich plant species/phenophases, but by ingesting plant parts from a core set of common tree species, and mixing these with a limited number of seasonally preferred items. For example, young leaves of *Protium copal* were characterized by a high selectivity index (D = 0.96) during the dry season, despite the fact that they had a low protein-to-fiber ratio (0.26) and below average content of available protein (10.8% dry matter). Given the howler small day range (<500 m), a highly selected species like *P.*

copal may have been consumed because of its proximity to a group of staple species, rather that based on nutrient content only.

The significant correlation between selectivity and food lipid content found during the *nortes* was driven principally by the consumption (7.35% of the seasonal diet) of *Dendropanax arboreus* mature fruits, which contained >35% lipids and were highly selected (D = 0.97). The average lipid content of neotropical fruits is reported to be around 18.5% (Jordano, 2000), but the majority of the fruits do not usually contain more than 10% (Worman and Chapman, 2005), thus indicating that the fruits of *D. arboreus* were particularly rich in lipids. This food resource alone accounted for 58% of total lipid intake during the *nortes*. Other lipid sources during the *nortes* were *Manilkara zapota* immature fruits, which accounted for 14% of lipid intake, and *Brosimum alicastrum* immature fruit, which contributed 7.5%. The importance of lipids in the diet of howler monkeys has largely been overlooked because it was assumed that the lipid content of the diet of leaf-eating primates was very low (Rogers et al. 1990, Norconk et al. 2009). This could have been related to the fact that it has generally been assumed that fatty fruits are mostly consumed by fruit-eating vertebrates, while leaf-eating species prefer sugar-rich fruits (Janzen, 1975; Rogers et al., 1990). In general, increased consumption of lipid-rich foods is associated with increased energy requirements, since fats provide twice the energy of sugars and protein (NRC, 2003). Some primate species (*Pan troglodytes*, *Lophocebus albigena*, *Cercopithecus ascanius*) consume a higher amount of lipids during peaks of ripe fruit availability (Conklin-Brittain et al., 1998), which may coincide with reproduction (e.g., in Sanje mangabeys, *Cercocebus sanjei* (McCabe et al., 2013)) (Lee, 1987; Schneider, 2004). However, in *A. pigra*, average daily individual intake of energy derived from lipids plus carbohydrates was lower during the *nortes* (414 kJ/mbm) than during the other two seasons of the year (541 and 579 kJ/mbm), and daily individual lipid intake was lower in the *nortes* (75 ± 45 kJ/mbm) than in the rainy season (84 ± 64 kJ/mbm) (see Chapter 4). Moreover, the *nortes* was not the season with higher mature fruit availability (average FAI = 8.8 during the *nortes vs*. 53.9 during the rainy season). On the contrary, mature leaves were highly abundant (average FAI = 461) and they were the most consumed items (37.8%) during this season. Thus, in absence of higher energetic needs during the *nortes*, selecting fruits high in lipids could have served to balance the higher protein consumption characterizing the *nortes* (105 ± 49 kJ/mbm), in order to maintain a relatively stable ratio of protein and non-protein energy. The yearly average protein to lipid intake ratio was 1.8:1, and this value was consistent during the three seasons (1.8:1 during the rainy and *nortes*, and 1.9:1 during the dry season).

In conclusion, during this 15-month field study of the nutritional ecology of black howler monkeys in Mexico, I found that:

- based on grams consumed, the diet of *A. pigra* at El Tormento was mainly fruit-based, and only during one season (*nortes*) the consumption of leaves (49.5%) and flowers (9.7%) increased with respect to fruits (40.8%).
- young and mature leaves did not differ in their content of crude and available protein, fiber, lipids, non-structural carbohydrates, ash, and protein-to-fiber ratio.
- among the seasonally preferred plant parts in the howler diet, there were two fruit species that were particularly high in lipids (*Dendropanax arboreus*: 35 ± 6% dry weight; and *Metopium brownei*: 18 ± 5% dry weight).
- sixty-nine percent of the plant samples analyzed (n =78) contained condensed tannins.
- based on phytochemical analysis of plant parts included in the howler diet, it appeared that leaves at El Tormento had lower protein-to-fiber ratios compared to leaves consumed

by howler and spider monkeys at other sites. This is possibly linked to the geochemical characteristics of limestone soils in the Yucatan peninsula.

- only during one season, food selectivity of black howler monkeys correlated positively with certain food phytochemical characteristics (i.e., lipids, K, P), whereas during the rest of the year, the selectivity indices for the food items accounting for >80% of the seasonal diet did not correlate with content of available protein, fiber, sugars, lipids, energy, minerals, and protein-to-fiber ratio.

According to a nutrient balancing strategy, over the course of one or several consecutive days, individuals are expected to consistently ingest foods that enable them to achieve (or approach to) a targeted proportion of nutrients (e.g., protein/non protein energy), rather than maximizing the ingestion of any single nutrient or energy. This proportion or balance is defined as the *intake target* (Behmer, 2009; Simpson and Raubenheimer, 2012). In order to achieve this target, foragers can: **1)** consume individual food items that are characterized by a balance of nutrients similar to the target. This pattern has been reported for *Ateles chamek* in Bolivia, where it was found that during parts of the year, six types of foods, including mature figs, were nutritionally balanced and showed a protein/non protein energy content that coincided with the spider monkeys' target, or average yearly intake of protein/non protein energy (Felton et al. 2009); **2)** show preference for individual nutrients, whose intake can be targeted by feeding on one or few phenophases or species containing high amounts of that nutrient(s), or by preferentially feeding on several different phenophases or species whose cumulative consumption provides the targeted amount of that nutrient. In this case it is expected that individuals will prioritize the consumption of the targeted nutrient(s) until their daily nutritional requirements are met [Raubenheimer and Simpson, 1993]); **3)** not exhibit a preference for any single nutrient, and instead mix resources, feeding on a variety of different phenophases and plant species in order to achieve a balanced diet from individually nutritionally imbalanced foods. This is analogous to the strategy employed by several herbivorous insects such as *Locusta migratoria,* in which the *intake target* was achieved by frequently switching between foods containing different combinations of protein and carbohydrates (Chambers et al., 1995; Raubenheimer and Simpson, 1997).

I did not find strong evidence for the first possibility, since the most consumed items in the three season were not characterized by ratios of protein and non-protein energy coinciding with the seasonal average. For example during the *nortes*, the most consumed items, *Acacia usumacintensis* mature leaves, *Brosimum alicastrum* immature fruits, and *Manilkara zapota* immature fruits had protein/non protein ratios of 0.45:1, 0.44:1, and 0.009:1, respectively, which differed from the seasonal intake ratio of 0.25:1. The data indicate, however, that black howler monkeys followed the second pattern during the *nortes* (showing selectivity towards food lipid content as a way of maintaining a balanced protein/non protein energy seasonal intake), and the third pattern during the rest of the year, when no single nutrients was prioritized to approach the *intake target*. Overall, these data lend support to the hypothesis that a nutrient balancing foraging strategy offers the strongest explanation for the decisions taken by black howler monkeys in selecting or avoiding particular feeding trees and food items.

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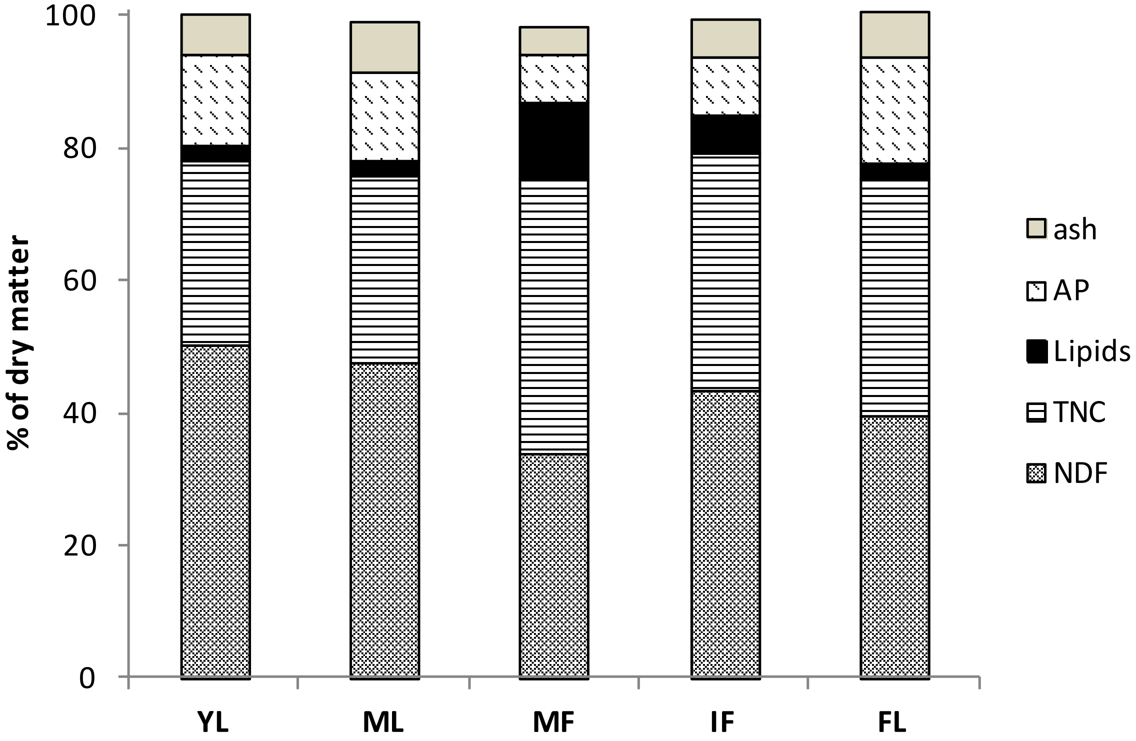


Figure 3.1. Nutritional composition of different categories (young and mature leaves, mature and immature fruits, flowers and inflorescences) of food items collected from black howler monkey feeding trees. AP: available protein, TNC: total nonstructural carbohydrates, NDF: neutral detergent fiber

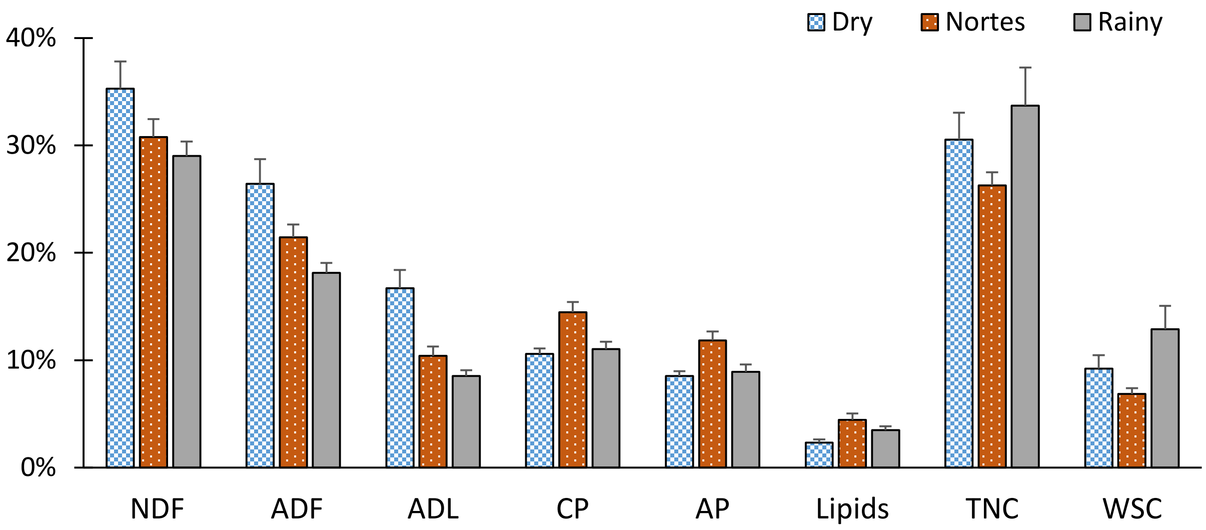
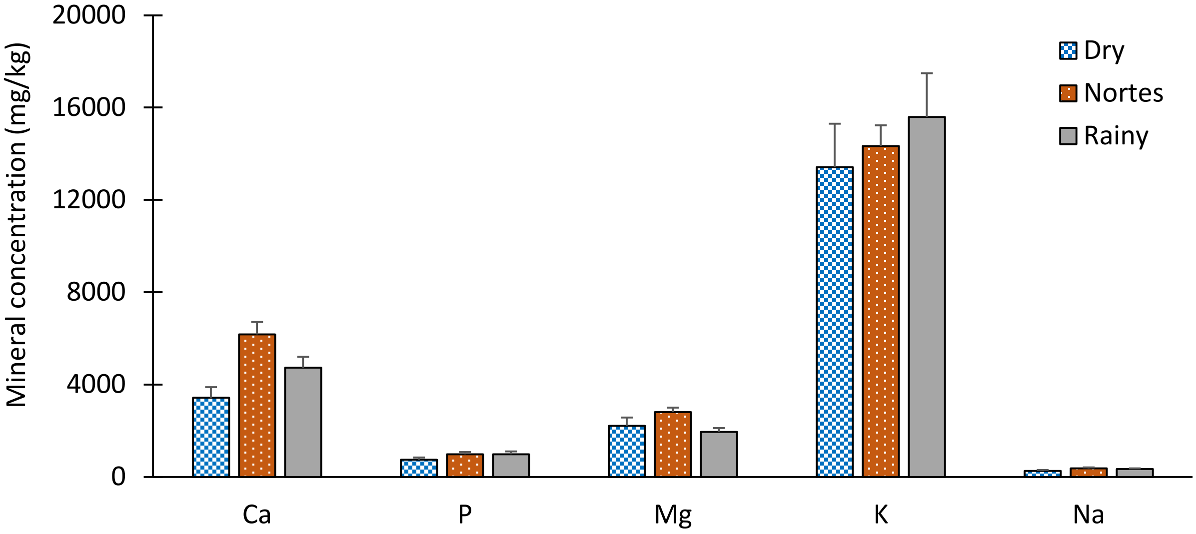


Figure 3.2. Percentage of nutrients (±SD) ingested by black howler monkeys across different seasons from all the food items that constituted ~80% of the diet (see Table 3.3). These values were obtained multiplying the nutrient content of different plant parts by the percentage contribution (based on grams ingested) of each plant part to the seasonal diet. NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, CP: crude protein, AP: available protein, TNC: total nonstructural carbohydrates, WSC: water soluble carbohydrates



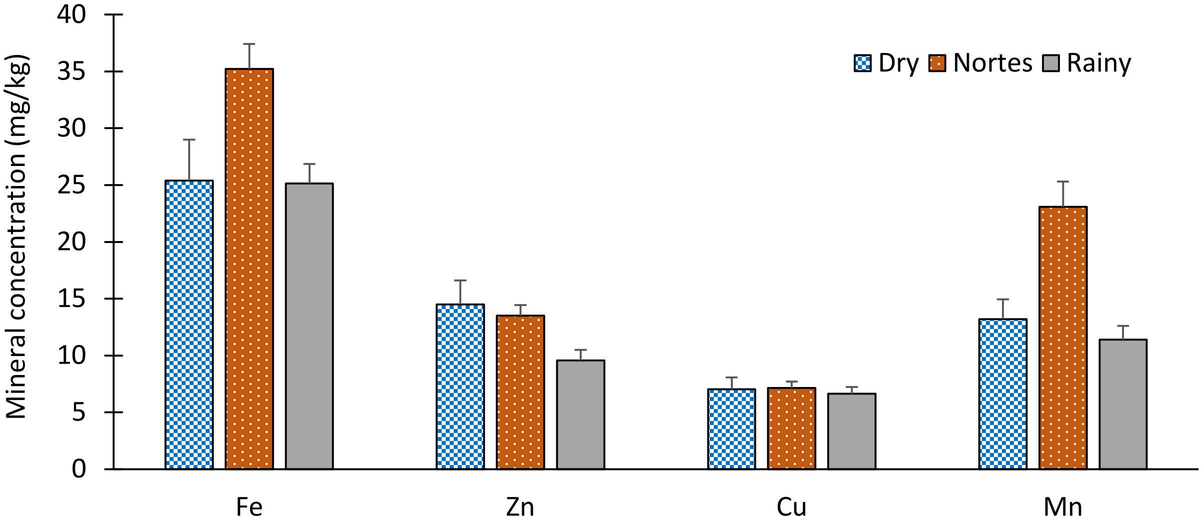


Figure 3.3. Concentrations of macrominerals and trace elements (±SD) ingested seasonally by black howler monkeys from all the food items that constituted ~80% of the diet (see Table 3.3)

Table 3.1. Composition of the diet of the two howler monkey study groups. ML: mature leaves, YL: young leaves, MF: mature fruits, IF: immature fruits, Flowers and inflorescences

	% based on feeding time				% based on amount consumed (grams)			
	Dry	***Nortes***	**Rainy**	**Total**	**Dry**	***Nortes***	**Rainy**	**Total**
YL	45.6%	10.6%	31.3%	31.6%	32.9%	11.7%	18.0%	22.2%
ML	9.9%	43.0%	15.5%	19.0%	5.6%	37.8%	12.2%	14.8%
Tot Leaves	**55.5%**	**53.6%**	**46.8%**	**50.6%**	**38.4%**	**49.5%**	**30.2%**	**37.0%**
MF	6.6%	12.3%	36.9%	19.5%	7.7%	12.2%	54.0%	29.0%
IF	25.4%	28.1%	13.7%	20.6%	46.4%	28.6%	15.2%	29.2%
Tot Fruit	**32.0%**	**40.4%**	**50.6%**	**40.2%**	**54.1%**	**40.8%**	**69.2%**	**58.2%**
Flowers/Inflo	**11.7%**	**6.0%**	**2.4%**	**7.6%**	**7.1%**	**9.7%**	**0.6%**	**4.7%**

Table 3.2. Composition of the annual diet (% based on grams ingested) of black howler monkeys (ML: mature leaves, YL: young leaves, MF: mature fruits, IF: immature fruits, INFLO: inflorescences)

Family	**Species**	**Plant part**	**Diet**
Sapotaceae	*Manilkara zapota*	IF	14.03%
Moraceae	*Brosimum alicastrum*	MF	12.01%
Moraceae	*Brosimum alicastrum*	IF	7.14%
Mimosaceae	*Acacia usumacintensis*	ML	6.46%
Moraceae	*Brosimum alicastrum*	YL	6.23%
Myrtaceae	*Pimenta dioica*	MF	6.07%
Moraceae	*Pseudolmedia oxyphyllaria*	IF	5.33%
Anacardiaceae	*Metopium brownei*	MF	3.38%
Lamiaceae	*Vitex gaumeri*	MF	2.40%
Mimosaceae	*Acacia usumacintensis*	YL	2.34%
Lamiaceae	*Vitex gaumeri*	YL	2.24%
-	Vine sp. 1	YL	1.84%
Moraceae	*Ficus* sp.	IF	1.73%
Burseraceae	*Bursera simaruba*	ML	1.50%
Araliaceae	*Dendropanax arboreus*	MF	1.43%
Moraceae	*Trophis racemosa*	INFLO	1.43%
Burseraceae	*Protium copal*	YL	1.37%
Simaroubaceae	*Simarouba glauca*	MF	1.28%
Mimosaceae	*Lysiloma latisiliquum*	YL	1.22%
Sapotaceae	*Manilkara zapota*	MF	1.21%

Table 3.3. Seasonal composition of the diet (% based on grams ingested) of black howler monkeys and Jacobs selectivity index based on species-specific phenophase availability (D) for food items belonging to different species

Season	**Species**	**Plant part**	**Diet**	**D**
Dry	*Manilkara zapota*	IF	29.29%	0.907
	Pseudolmedia oxyphyllaria	IF	14.64%	0.987
	Vitex gaumeri	YL	6.14%	0.041
	Brosimum alicastrum	YL	5.27%	0.957
	Vine sp. 1	YL	3.62%	-[1]
	Simarouba glauca	MF	3.51%	0.926
	Lysiloma bahamensis	YL	3.30%	-0.270
	Protium copal	YL	3.09%	0.961
	Manilkara zapota	YL	2.70%	-0.360
	Lysiloma bahamensis	FL/inflo	2.56%	-0.114
	Swartzia cubensis	INFLO	1.96%	0.865
	Brosimum alicastrum	IF	1.81%	0.676
	Platymiscium yucatanum	YL	1.44%	-[2]
	Vine sp. 2	L	1.42%	-[1]
Nortes	*Acacia usumacintensis*	ML	16.13%	0.876
	Brosimum alicastrum	IF	14.45%	0.887
	Manilkara zapota	IF	11.69%	0.527
	Dendropanax arboreus	MF	7.35%	0.975
	Trophis racemosa	INFLO	7.31%	0.968
	Brosimum alicastrum	YL	6.08%	0.447
	Bursera simaruba	ML	5.78%	0.413
	Acacia usumacintensis	YL	3.37%	0.834
	Brosimum alicastrum	MF	3.33%	0.846
	Blepharidium mexicanum	ML	2.53%	0.921
	Brosimum alicastrum	FL	2.14%	0.766
Rainy	*Brosimum alicastrum*	MF	25.76%	0.867
	Pimenta dioica	MF	13.42%	-[2]
	Brosimum alicastrum	IF	8.39%	0.608
	Metopium brownei	MF	7.65%	0.789
	Acacia usumacintensis	ML	7.41%	0.826
	Brosimum alicastrum	YL	7.09%	0.362
	Vitex gaumeri	MF	5.45%	0.420
	Ficus sp.	IF	3.81%	0.200
	Acacia usumacintensis	YL	3.64%	0.597

[1] Basal area could not be determined for vines and climbers

[2] Food availability could not be determined for these species since the few visited trees were at the margins/outside the forested area and did not fall in any of the transects and quadrats.

Table 3.4. Nutritional composition of food items included in the diet of black howler monkeys at El Tormento, Mexico

Sp.	**Family**	**Item**	**% DM**	**% Ash**	**% NDF**	**% ADF**	**% ADL**	**% CP**	**% AP**	**% Lipids**	**% TNC**	**Sucrose**	**AP:ADF**	**n**
Acacia sp.	Fabaceae	ML	93.51	5.94	47.73	31.46	19.31	26.21	17.41	5.37	23.55	3.32	0.57	2
Acacia gaumeri	Fabaceae	ML	93.35	7.22	36.04	24.16	9.96	21.73	15.70	1.83	39.21	3.73	0.65	1
Acacia usumacintensis	Mimosaceae	ML	91.50	10.33	39.07	25.16	15.51	24.99	20.08	1.80	28.73	1.24	0.87	4
		YL/LB	-	0.43	43.50	29.65	20.79	28.50	25.05	2.67	28.35	-	0.84	1
Adelia barbinervis	Euphorbiaceae	IF	92.28	2.98	56.72	41.44	20.95	9.70	7.75	3.04	29.52	3.70	0.14	1
Blepharidium mexicanum	Rubiaceae	ML	91.80	6.52	44.21	28.15	14.83	19.44	10.65	2.90	35.09	6.08	0.34	6
Brosimum alicastrum	Moraceae	ML	90.83	14.42	39.04	23.47	5.53	17.07	14.78	1.38	30.38	4.61	0.65	2
		MF	91.63	5.14	24.32	15.70	3.76	11.92	10.84	2.08	57.62	30.59	0.73	2
		INFL	90.27	13.19	37.16	19.90	8.31	22.81	19.98	2.67	27.00	1.37	1.09	3
		IF	90.42	7.88	42.54	26.38	8.49	22.24	20.23	2.34	22.30	5.74	0.89	5
Bursera simaruba	Burseraceae	ML	90.36	6.61	42.37	33.12	17.30	13.82	7.69	0.96	42.36	16.71	0.19	3
		YL	90.07	5.66	49.68	42.50	27.26	13.11	9.98	2.86	31.81	4.00	0.06	1
Cydista aequinoctialis	Bignoniaceae	FL	92.71	2.00	26.62	14.46	-	15.11	13.24	2.47	55.68	-	0.92	1
Dendropanax arboreus	Araliaceae	MF	94.86	7.10	27.38	20.12	7.88	12.63	10.89	35.19	19.44	8.31	0.55	3
		IF	94.64	7.38	32.33	23.17	9.84	13.35	11.17	30.41	18.71	7.10	0.48	2
Ficus sp.	Moraceae	ML	90.12	3.56	47.49	38.57	21.14	10.29	-	1.42	37.24	-	-	1
		YL/LB	88.53	8.14	38.83	31.89	21.91	19.22	13.26	1.39	38.38	1.88	0.28	1
Guettarda combsii	Rubiaceae	IF	92.01	4.58	74.38	59.04	26.58	6.36	4.04	0.04	16.96	19.40	0.07	1
Lysiloma latisiliquum	Mimosaceae	INFL	92.62	5.56	38.24	27.22	15.12	20.33	15.93	0.83	39.44	6.50	0.62	3
Mangifera indica	Anacardiaceae	MF	89.80	2.57	12.57	10.28	1.71	4.50	3.97	1.98	79.62	34.58	0.38	2
Manilkara zapota	Sapotaceae	ML	91.77	10.91	48.27	35.52	14.65	10.25	5.54	3.22	32.06	3.13	0.16	2
		YL/LB	91.31	5.45	49.00	41.29	21.64	12.40	9.75	3.21	32.33	1.86	0.21	3
		MF	91.32	2.20	34.55	30.53	23.92	3.31	1.36	4.27	57.62	35.24	0.05	3
		IF	92.20	2.39	46.35	39.67	29.10	3.73	0.69	5.23	47.26	19.64	0.02	7
Metopium brownei	Anacardiaceae	ML	90.93	7.36	44.99	39.80	23.67	12.00	9.41	4.35	33.89	12.13	0.16	2
		MF	94.19	3.25	45.03	32.42	28.50	11.89	9.63	18.20	23.94	6.56	0.28	6
Petrea volubilis	Verbenaceae	FL	91.87	8.23	-	-	-	21.34	19.75	-	-	9.76	-	1

Table 3.4 (cont.)

Pimenta dioica	Myrtaceae	MF	91.04	5.85	40.28	29.01	14.23	6.46	-	6.35	41.06	10.86	-	1
Pithecellobium platylobum	Fabaceae	ML	91.83	7.49	56.64	40.38	25.98	25.44	11.18	0.00	24.69	2.13	0.42	1
Platymiscium yucatanum	Fabaceae	YL	91.77	7.42	24.61	17.09	8.92	30.55	27.53	0.96	39.48	4.73	1.61	3
Pouteria campechiana	Sapotaceae	ML	90.38	6.05	71.70	62.20	44.60	22.19	9.62	3.80	8.82	1.18	0.22	1
		YL/LB	88.92	6.61	74.55	66.61	48.23	20.85	7.24	6.04	5.56	1.42	0.17	1
Protium copal	Burseraceae	ML	91.84	4.22	68.63	53.30	28.37	17.80	9.76	0.55	16.84	-	0.18	6
		YL/LB	91.64	5.40	61.22	48.69	29.36	20.17	10.86	1.12	21.40	4.20	0.26	3
Pseudolmedia oxyphyllaria	Moraceae	YL	91.66	8.90	46.80	31.67	18.31	20.76	15.59	1.55	27.17	-	0.50	1
		MF	90.07	6.14	50.57	35.45	19.98	15.49	10.04	1.37	31.87	8.82	0.28	2
		IF	91.28	6.83	44.16	32.81	18.33	19.55	18.33	1.35	29.78	6.80	0.51	3
Simarouba glauca	Simaroubaceae	MF	91.01	4.99	20.20	-	-	7.23	6.13	2.13	64.82	41.71	-	1
Swartzia cubensis	Caesalpiniaceae	YL/LB	90.43	3.97	46.54	34.45	23.03	26.74	21.47	0.66	27.36	2.87	0.64	1
		IF	91.64	4.90	40.61	21.36	12.18	26.37	24.81	0.55	29.13	8.34	1.16	2
		FL	90.92	3.92	43.50	30.86	18.45	26.08	21.02	0.42	31.14	-	0.77	3
Trophis racemosa	Moraceae	INFL	92.09	11.80	25.74	20.00	-	22.95	21.29	2.72	38.59	6.64	1.02	5
Vitex gaumeri	Lamiaceae	YL/LB	91.68	7.66	47.26	38.95	26.71	21.19	11.18	2.24	31.76	3.80	0.31	2
		MF	93.83	2.68	21.49	16.35	8.78	4.34	2.23	1.18	72.30	39.25	0.14	3
		IF	93.24	4.25	23.32	19.43	10.38	5.02	2.54	1.57	68.51	41.70	0.13	2
Vine sp. 1		ML	91.49	5.70	55.05	37.08	17.36	32.49	26.80	0.27	12.18	2.31	0.72	1
		YL/LB	91.85	5.23	45.97	34.29	21.77	32.37	26.31	1.24	21.25	-	0.77	1
Vine sp. 2		ML	90.36	7.79	33.88	34.17	17.55	12.36	8.66	0.94	48.74	-	0.25	1

ML: mature leaves; YL: young leaves; LB: leaf buds; MF: mature fruits; IF: immature fruits; INFL: inflorescences

Table 3.5. Plant species and plant parts (young and mature leaves, mature and immature fruits, flowers and inflorescences) in the howler monkey diet containing condensed tannins.
A qualitative system was used to report the absorbance values following the acid-butanol assay: <0.10 (0, i.e. tannins are not present); 0.10 – 0.50 (+); 0.50 – 1.00 (++), and >1.00 (+++) (Rothman et al. 2006).

Species	**Plant part**	**Absorbance level**	**n**
Acacia gaumeri	ML	++	1
Acacia usumacintensis	ML	0	3
	ML	+	2
	YL	0	1
Adelia barbinervis	IF	++	1
Blepharidium mexicanum	ML	++	2
	ML	+++	1
	YL	++	1
Brosimum alicastrum	IF	+	1
	MF	0	2
	ML	0	1
	ML	+	1
Bursera simaruba	ML	+++	2
	YL	+++	1
Coccoloba barbadensis	IF	+	1
Dendropanax arboreus	MF	0	2
Ficus sp.	YL	+++	1
Guettarda combsii	IF	++	1
Lysiloma bahamensis	INFLO	++	2
Mangifera indica	MF	0	1
Manilkara zapota	FL	++	1
	IF	++	2
	IF	+	2
	MF	+	2
	ML	+++	2
	YL	+++	1
	YL	++	1
Metopium brownei	ML	++	1
	ML	+++	1
	MF	0	2
Mimosa sp.	ML	++	1
	ML	+	1
Pimenta dioica	MF	++	1
Platymiscium yucatanum	YL	0	1
Pouteria campechiana	ML	+	2
	YL	0	1
Protium copal	INFLO	+++	1
	YL	++	4
	YL	+	1

Table 3.5 (cont.)

Pseudolmedia oxyphyllaria	IF	+++	2
	INFLO	+++	1
	MF	+++	1
	ML	0	1
Simarouba glauca	MF	0	2
Sp. 1	YL	+++	1
Swartzia cubensis	IF	0	1
	INFLO	0	2
	YL	+	1
Trophis racemosa	INFLO	0	2
Vine (unkn. sp. 1)	ML	++	1
	ML	+++	1
Vine (unkn. sp. 2)	ML	+++	2
Vitex gaumeri	IF	0	1
	MF	0	1
	YL	0	1
	YL	+	1
	YL	+++	1

Table 3.6. Content of macrominerals (% dry weight) and trace elements (parts per million, or mg/kg) in plant parts (ML: mature leaves, YL: young leaves, MF: mature fruits, IF: immature fruits, INFLO: inflorescences) consumed by black howler monkeys at El Tormento, Mexico

Item	**Species**	**Ca %**	**P %**	**Mg %**	**K %**	**Na %**	**Fe (ppm)**	**Zn (ppm)**	**Cu (ppm)**	**Mn (ppm)**	**Mo (ppm)**
ML	*Acacia* sp.	1.92	0.09	0.26	0.80	0.06	84	26	2	54	<0.1
ML	*Acacia usumacintensis*	0.91	0.09	0.35	1.55	0.07	45	15	7	23	<0.1
ML	*Brosimum alicastrum*	2.17	0.06	0.50	1.21	0.04	33	11	5	56	0.30
ML	*Bursera simaruba*	1.29	0.08	0.35	0.74	0.06	45	8	3	23	0.30
ML	*Bursera simaruba*	0.93	0.12	0.37	1.67	0.01	55	22	9	36	<0.1
ML	*Metopium brownei*	2.16	0.05	0.32	0.67	0.13	40	5	1	13	0.10
ML	*Pouteria campechiana*	0.43	0.22	0.16	2.27	0.02	45	23	13	18	<0.1
	Avg ML	**1.40**	**0.10**	**0.33**	**1.27**	**0.05**	**49.57**	**15.71**	**5.71**	**31.86**	-
	St. Dev.	0.69	0.06	0.10	0.59	0.04	16.55	8.12	4.27	17.30	-
YL	*Manilkara zapota*	0.45	0.15	0.18	2.03	0.06	45	10	6	24	<0.1
YL	*Protium copal*	0.29	0.26	0.23	2.51	0.07	64	21	18	11	<0.1
YL	*Vitex gaumeri*	0.49	0.29	0.41	3.41	0.01	43	44	21	16	<0.1
	Avg YL	**0.41**	**0.23**	**0.27**	**2.65**	**0.05**	**50.67**	**25.00**	**15.00**	**17.00**	-
	St. Dev.	0.11	0.07	0.12	0.70	0.03	11.59	17.35	7.94	6.56	-
MF	*Brosimum alicastrum*	0.26	0.16	0.21	2.47	0.04	25	13	8	8	<0.1
MF	*Dendropanax arboreum*	0.16	0.18	0.26	2.49	0.04	63	20	20	15	0.30
MF	*Metopium brownei*	0.23	0.14	0.15	1.37	0.03	49	11	14	8	<0.1
MF	*Pimenta dioica*	0.62	0.09	0.14	2.04	0.04	27	6	8	5	<0.1
MF	*Simarouba glauca*	0.10	0.07	0.16	1.67	0.02	32	4	5	6	<0.1
	Avg MF	**0.27**	**0.13**	**0.18**	**2.01**	**0.03**	**39.20**	**10.80**	**11.00**	**8.40**	-
	St. Dev.	0.20	0.05	0.05	0.49	0.01	16.32	6.30	6.00	3.91	-
IF	*Brosimum alicastrum*	0.58	0.20	0.39	2.02	0.05	40	19	11	17	2.10
IF	*Dendropanax arboreum*	0.42	0.21	0.23	2.55	0.09	94	26	23	24	<0.1
IF	*Manilkara zapota*	0.21	0.02	0.39	2.02	0.05	40	19	11	17	2.10
IF	*Pseudolmedia oxyphyllaria*	0.70	0.20	0.21	1.70	0.02	29	29	8	19	0.50
IF	*Vitex gaumeri*	0.05	0.05	0.07	2.16	0.01	12	10	7	2	<0.1
	Avg IF	**0.39**	**0.14**	**0.26**	**2.09**	**0.04**	**43.00**	**20.60**	**12.00**	**15.80**	-
	St. Dev.	0.27	0.09	0.14	0.31	0.03	30.72	7.37	6.40	8.23	-
INFLO	*Trophis racemosa*	1.58	0.29	0.46	2.65	0.03	77	30	7	105	0.90
	Estimated requirements (NRC 2003)	**0.55**	**0.3 - 0.4**	**0.04 - 0.08**	**0.24**	**0.20**	**100**	**13 - 20**	**15**	**44**	-

Table 3.7. Spearman correlation coefficients (r_s) showing the relationship between the phytochemical characteristics of the food consumed during the *nortes* by black howler monkeys and the selectivity indices (D) of different items based on species-specific phenophase availability in the habitat. Sample sizes (n) and p-values (p) also are shown. NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, CP: crude protein, AP: available protein, TNC: total nonstructural carbohydrates, WSC: water soluble carbohydrates, ME: metabolizable energy

	Macronutrients and energy										
	Ash	NDF	ADF	ADL	CP	AP	Lipids	TNC	WSC	AP/ADF	ME
r_s	0.212	-0.273	-0.515	-0.317	0.212	0.333	**0.648**	-0.442	-0.150	0.430	0.527
n	10	10	10	9	10	10	10	10	9	10	10
p	0.556	0.446	0.128	0.406	0.556	0.347	**0.043**	0.200	0.700	0.214	0.117

	Minerals								
	Ca	P	Mg	K	Na	Fe	Zn	Cu	Mn
r_s	-0.168	**0.719**	-0.024	**0.747**	-0.217	0.386	0.614	0.491	-0.169
n	8	8	8	8	8	8	8	8	8
p	0.691	**0.045**	0.955	**0.033**	0.606	0.346	0.106	0.217	0.690

Table 3.8. Nutrient content and protein to fiber ratio of food items consumed by howlers

Plant part	**% NDF**	**% ADF**	**% ADL**	**% Lipids**	**% CP**	**% AP**	**% TNC**	**Prot:Fiber**	**Reference**
YL	52.70	40.80	21.70	3.30	19.70	17.00	32.10	0.5*	Aristizábal 2013
	41.04	25.35		2.62	20.49			0.81	Behie & Pavelka 2012
					16.70				Estrada 1984
	41.04	31.01	14.59	1.74	24.59	20.20	29.22	0.81*	Fernández in prep
					21.7				Gaulin & Gaulin 1982
		33.71			12.03				Glander 1981
	54.40	36.40	21.10		21.20			0.58	Oftedal 1991
	50.22	**39.50**	**24.70**	**1.99**	**20.50**	**13.74**	**27.90**	**0.41***	**Present study**
	50.69	35.85			22.34	16.88		0.56*	Silver et al. 2000
	44.20	37.20	20.30		22.30			0.72	Williams-Guillén 2003
ML	56.20	43.10	21.80	2.90	17.60	14.90	30.90	0.4*	Aristizábal 2013
	53.12	33.91		2.60	16.43			0.48	Behie & Pavelka 2012
					12.60				Estrada 1984
	40.13	24.54	10.79	2.58	16.92	14.23	32.50	0.66	Fernández in prep
		37.54			12.39				Glander 1981
	57.20	40.50	20.40		16.60			0.41	Oftedal 1991
	47.35	**34.70**	**19.30**	**2.39**	**20.05**	**13.18**	**28.20**	**0.45***	**Present study**
	56.63	37.09			17.07	12.51		0.37*	Silver et al. 2000
	48.40	36.60	17.60		19.50			0.60	Williams-Guillén 2003
MF	43.50	34.40	17.70	8.60	11.90	10.50	41.00	0.4*	Aristizábal 2013
	45.01	32.50		2.44	9.74				Behie & Pavelka 2012
	26.09	19.30	9.12	1.97	11.33	9.16	56.53		Fernández in prep
	33.60	**26.38**	**17.06**	**11.13**	**8.95**	**7.50**	**41.80**	**0.34***	**Present study**
					7.77	5.04			Silver et al. 2000
	36.38	37.24		4.41	17.26				Urquiza-Haas et al. 2008
	49.90	40.20	20.00		8.80			0.29	Williams-Guillén 2003
IF	42.60	29.70	10.60	2.90	6.40	5.80	48.40	0.2*	Aristizábal 2013
	22.31	16.20	5.06	8.34	17.54	16.51	42.56		Fernández in prep
	43.19	**31.42**	**16.43**	**5.48**	**11.73**	**9.10**	**36.30**	**0.43***	**Present study**
FL	45.80	28.60	19.13	5.48	16.13				Behie & Pavelka 2012
	29.99	19.84	8.33	1.63	21.36	18.42	46.23		Fernández in prep
	50.60	35.80	17.10		14.40				Oftedal 1991
	39.50	**28.80**	**18.79**	**2.21**	**19.67**	**15.86**	**36.20**	**0.61***	**Present study**
		35.56			17.10	11.64		0.35*	Silver et al. 2000
	41.20	33.30	18.50		21.10			0.71	Williams-Guillén 2003

*Calculated as AP/ADF

CHAPTER 4

RETHINKING HOWLER MONKEY FEEDING ECOLOGY: NUTRIENT BALANCING IN FREE-RANGING *ALOUATTA PIGRA*

Introduction

Understanding how animals select foods and balance nutrients is critical for addressing questions related to feeding ecology and social behavior and their effects on reproductive success and life history strategies (Milton, 2006; Karasov and Martinez del Rio, 2007; Lambert, 2011; Simpson and Raubenheimer, 2012). Nutrient imbalances, deficiencies, and/or overconsumption can seriously compromise activity, health, growth, and reproduction (Oftedal, 1991; Barboza et al., 2009). However "quantifying the underpinnings of diet selection is challenging, especially in studies of wild animals, because it requires precise feeding observations of individuals over continuous periods, relevant analysis of all foods consumed, and a framework to analyze the complex, multivariate nature of the data" (Felton et al., 2009c: p.676).

Foods represent complex mixtures of different organic and inorganic compounds that are consumed by animals to satisfy their requirements for growth, reproduction, and health (Lambert, 2011; Simpson and Raubenheimer, 2012). Certain foods can be difficult to masticate or digest, and foods can contain potentially harmful substances that consumers must avoid or denature (Norconk et al., 2009; Lambert, 2011). The precise behavioral, physiological, and hormonal mechanisms used by foragers to obtain a nutritionally balanced diet remain unclear; however, laboratory studies with invertebrates and vertebrates have demonstrated that individuals can alter food choice based on their nutrient needs and the phytochemical composition of available foods (Robbins et al., 2007; Marsh et al., 2007; Simpson and

Raubenheimer, 2012). Previous nutritional experience, current nutritional imbalances, or changes in physiological state may change the dietary response of animals, resulting in a preference to consume particular nutrients over others (Simpson and Raubenheimer, 2000). For example, locusts (*Locusta migratoria*) and caterpillars (*Spodoptera littoralis*) tend to select protein-rich foods after having consumed diets lacking in protein (Simmonds et al., 1992; Chambers and Simpson, 1995). Similarly, vertebrates such as salmonid fish (*Coregonus lavaretus*) overconsume lipids and carbohydrates to obtain protein, when offered a low protein diet (Ruohonen et al., 2007).

Nutritional Geometry

Several theories and nutritional models, including energy or protein:fiber maximization, nutrient mixing, and toxin avoidance, have been proposed to explain the food choice and nutritional ecology of primates (Glander, 1978; Milton, 1980; Felton et al., 2009b). Among these, nutrient balancing models (Felton et al., 2009a; Felton et al., 2009b; Felton et al., 2009c; Rothman et al., 2011; Behie and Pavelka, 2012) argue that individual food choices are based on the regulation of multiple nutrients in response to the consumers' changing nutrient needs (Simpson et al., 2004). When no single food provides the optimal "nutrient target" that maximizes fitness, animals may be required to regulate the intake of multiple nutrients. Nutrient balancing requires the presence of internal feedback mechanisms and neural pathways often referred to as appetites that serve to regulate daily nutrient intake across seasons and periods of growth and reproduction (Raubenheimer, 2011; Simpson and Raubenheimer, 2012). Recent research on a variety of taxonomically distinct organisms (from locusts to gorillas) has shown that the Geometric Framework (GF), which examines patterns of macro and micronutrient intake in multidimensional space, offers an instructive model to understand the interactive effects of nutrients on animal food choices (Simpson and Raubenheimer, 1993; Raubenheimer et al., 2009; Simpson and Raubenheimer, 2012; Raubenheimer et al., 2012).

For example, a recent 8-month study of wild spider monkeys (*Ateles chamek*) in Bolivia using the GF (Felton et al., 2009a; Felton et al., 2009c) revealed that individuals regulated their daily intake of available protein, while total energy intake fluctuated according to the nutritional content of available food items. This pattern has been described as the “protein leverage effect”, since it causes excess energy intake when the percentage of protein in the diet is low (Simpson et al., 2003; Simpson and Raubenheimer, 2005). Spider monkeys maintained a relatively stable protein intake across seasons, independent of fluctuations in food availability and abundance, by mixing nutritionally complementary foods such as leaves, fruits, and flowers, or by eating nutritionally balanced items such as *Ficus boliviana* fruits. In contrast, Rothman et al. (2011) found that, in Ugandan mountain gorillas (*Gorilla beringei*), the intake of non-protein energy (i.e., energy contribution from lipids, total non structural carbohydrates, and neutral detergent fiber) was relatively constant throughout the year, while protein intake varied according to the availability of leaves and fruits in the environment. During periods of fruit scarcity, gorillas consumed high amounts of proteinaceous leaves, thus over-ingesting protein, to reach their target intake of carbohydrates and lipids (which are present in leaves, but in lower proportions). For this gorilla population, prioritizing non-protein energy was a higher priority than prioritizing protein intake. Despite exhibiting two different patterns of nutrient regulation, both studies provide strong evidence that primates regulate nutrient intake in order to balance the consumption of protein, lipids, and carbohydrates, and appear to do so over the course of a single day.

Howler Monkey Diet

Howler monkeys (*Alouatta* spp.) represent the most geographically widespread genus of New World monkeys and range from Mexico to Argentina (IUCN, 2013). Their diet and feeding ecology have been extensively studied for more than 30 years (Milton, 1979; Milton et al., 1979; Nagy and Milton, 1979a; Nagy and Milton, 1979b; Milton, 1980; Glander, 1981; Milton, 1981; Glander, 1982; Milton and McBee, 1983; Estrada, 1984; Julliot, 1996; Stoner, 1996; Milton, 1998; Silver et al., 1998; Estrada et al., 1999; Silver et al., 2000; Dunn et al., 2010; Palma et al., 2011; Behie and Pavelka, 2012; Amato et al., 2013; Chaves and Bicca-Marques, 2013; Garber et al., in press). Despite their relatively enlarged hindgut, relatively long food transit times (for platyrrhines), robust mandibles, and molars with high shearing crests, howler monkeys have been described as behavioral folivores (Milton, 1978; Milton, 1979). Milton offered this term to differentiate the ecological challenges howler monkeys face in consuming difficult to digest resources such as fibrous leaves from those faced by colobines and indriids, which she referred to as anatomical folivores due to their specialized stomachs and ceca that enable them to more efficiently ferment foliage and seeds with the aid of symbiotic bacterial and protozoan microflora (Kay and Davies, 1994). Clearly, both behavior and anatomy/physiology play an important role in howler monkey nutritional ecology.

In order to effectively consume large amounts of leaves, potentially high in fiber and secondary compounds, howlers are reported to reduce or limit energy costs, for example by resting during 60-80% of their daily activity budget, traveling less than 500 m/day (based on 20 studies; (Di Fiore et al., 2011)), and selectively avoiding foods high in fiber and toxins (Glander, 1981). Glander (1981) hypothesized that nutritional, phytochemical, and structural differences in the available foods play an important role in howler dietary selectivity. In contrast, Milton (1979;

1998) proposed that the most important factor influencing leaf choice in howler monkeys was the protein:fiber ratio of plant material. However, the precise nutritional factors affecting food choices in howlers remain poorly understood. This is due mainly to the fact that previous studies have tended to lack: 1) precision in assessing the weight and nutrient characteristics of the foods ingested, 2) data on total daily nutrient intake of an individual, and 3) an integrative and nutritionally explicit framework to identify how sequential patterns of food choice contribute to nutrient balancing (Schuelke et al., 2006; Felton et al., 2009c).

In the present study, I apply a nutritional geometry model to the study of black howler monkey (*A. pigra*) foraging strategies and food choice. My goal is to identify short and long term dietary strategies used by adult howler monkeys to regulate energy and macronutrient intake. I test the following hypotheses: **1)** due to the close phylogenetic relationship with spider monkeys and relatively similar diets including seasonally varying amounts of fruits, leaves, and flowers, black howler monkeys will show patterns of protein prioritization similar to *A. chamek* in Bolivia; **2)** due to the fact that both howler monkeys and gorillas can subsist during periods of the year on a diet mainly consisting of leaves and fibrous vegetation, black howler monkeys will show patterns of carbohydrate and lipid prioritization similar to *G. beringei* in Uganda; **3)** since individual patterns of nutrient regulation respond to changes in food and nutrient availability in the environment, howler monkeys will regulate and prioritize protein intake when the daily diet is dominated by items rich in carbohydrates and lipids (for example during periods when fruits account for >50% of the diet), but will prioritize nonstructural carbohydrate and lipid intake when the daily diet is mostly leaf-based (e.g. during seasons when fruits are less abundant).

Methods

Study Site and Subjects

The research was carried out in the state of Campeche, Mexico, at El Tormento (18º36′44″N; 90º48′31″W), a 1400-ha forested area owned by Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The dominant vegetation types in this area of the Yucatan peninsula are semi-deciduous and evergreen seasonal forests (*sensu* Miranda & Hernández 1963). The climate is hot and humid, and three seasons can be recognized: a dry and hot season from February to May (~100 mm rain during four months, 28°C mean daily temperature), a rainy season from June to September (~900 mm rain during four months), and a colder season ("*nortes*") from October to January (~160 mm rain during four months, 22.8°C mean daily temperature) (Figure 4.1). According to data recorded by the Comisión Nacional del Agua (CONAGUA, 2013), between 1971 and 2000 total annual precipitation at El Tormento averaged 1291 mm, and mean annual temperature was 25.6°C. During the study period (September 2010-August 2011) total annual rainfall was 1246 mm, and average mean and maximum annual temperatures were 26.3°C and 33.4°C, respectively.

Two neighboring groups of black howler monkeys were followed during 15 months. Three complementary data sets were recorded: ecological, behavioral, and phytochemical. Group M contained 10-12 individuals (including 4-5 adult males and 2 adult females) and group J contained 6-7 individuals (no more than 2-3 adult males at the same time and 2 adult females). All of the monkeys were individually recognized, with ten individuals darted and marked with color anklets in August 2010, prior to the start of the behavioral data collection (Table 4.1). Between September 2010 and November 2011, groups M and J ranged in areas of 14.5 and 4.5 ha respectively, with an overlap of 1.3 ha. Although focusing on two groups limited the number of individuals sampled, the behavioral and ecological hypotheses tested required detailed sampling of the same individuals throughout an entire day and across consecutive days, and during all months of the year. This necessitated a focus on all adult individuals (n = 14) of the two study groups to determine accurate dietary and nutritional profiles and patterns of sequential food choice.

Field Data Collection

Phenological data

I conducted phenological censuses to monitor the presence and abundance of different phenophases (i.e. young and mature leaves; ripe and unripe fruits; flowers, inflorescences, and flower buds). Two types of censuses were carried out: 1) 10 Gentry's belt transects (50×2 m each) (Gentry, 1988) in each group's home range were walked bimonthly (n= 196 trees); 2) five trees/sp for each of the 10 most important tree species in the howler monthly diet were monitored. In both cases I estimated the percentage of the crown containing food items for each monitored tree on a scale of 0 − 4 (0; 1: 1−25%; 2: 26−50%; 3: 51−75%; 4: 76−100%). To measure tree abundance and calculate basal area of the monitored species, I established six randomly placed 50×50 m quadrats and a 33 additional Gentry's transects, tagging and identifying all trees with DBH ≥ 10 cm. My ecological sampling covered 12 − 25% of the home ranges of the two study groups. For each phenophase of a given species I obtained a food availability index (FAI) by multiplying the average bimonthly phenology score by its total estimated basal area. I then summed FAI across items and averaged those values across the two bimonthly surveys to obtain total monthly FAI for young and mature leaves; ripe and unripe fruits; and flowers (see Forester et al. (2012) for a similar analysis).

Behavioral Data

From September 2010 to November 2011, I collected data on the howler monkey activity budget, diet, feeding patch occupancy, and within and between-group social interactions, conducting all-day follows of one adult individual per day. I obtained 1300 hours of behavioral data on 14 focal animals (ten males: 674 hours; four females: 626 hours). I used two-minute instantaneous sampling (individual activity records) of focal animals. When the focal animal started feeding, I temporarily switched to a method of continuous data collection, recording the duration (to the nearest second) of each feeding event. I also calculated feeding rates using a stopwatch and recorded the quantity (number of items, parts of the item, or bites per minute), phenophase (i.e. young/mature leaf, immature/mature fruit, flower/inflorescence, other), and species of the items ingested. Once the feeding ended, I resumed instantaneous focal animal sampling at two minute intervals. All feeding trees (n = 689) were tagged, measured (DBH, tree height, crown height, two perpendicular axes of the crown) and identified. Unless otherwise indicated, percentages of food items in the howler diet (i.e., % leaves, % fruit) are based on amounts of food ingested in grams, and not on the percentage of feeding observations.

Plant sample collection

Food items were collected from feeding trees either on the same day or within two days of the observed feeding bout. I attempted to gather at least 50 g of flowers/inflorescences and 100 g of leaves and fruits (wet weight) per tree. The plant items were transported to the field laboratory, where they were measured and weighed to the nearest 0.01 g. Leaves, flowers, and inflorescences were air dried at a constant weight in a dark area. After discarding the parts that were not eaten (e.g. seeds), fruits were cut into small slices and kept in a drying oven (<50ºC) until reaching a stable dry weight. All samples (n = 146) were then stored in paper bags labeled with date, tree number, and phenophase, and maintained in a dry place with a silica desiccant until phytochemical analyses were performed.

Laboratory Analyses

Plant samples were analyzed in the Nutritional Ecology Laboratory at Hunter College, City University of New York, from March to June 2012. All samples were analyzed in duplicate. The samples were ground using a Wiley® mill fitted with a 1-mm screen. Dry matter was calculated by drying the field-dried samples in an oven (105ºC) immediately before each analysis to remove atmospheric moisture (Rothman et al., 2012).

Total nitrogen (N) was determined via combustion according to AOAC (1995) using a Leco TruSpec Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA). Crude protein (CP) was calculated by multiplying N by 6.25 (Rothman et al., 2012). Although this conversion factor likely overestimates the amount of protein present in tropical plant samples (Milton and Dintzis, 1981), other proposed values (e.g., 4.3) might result in an underestimation (Conklin-Brittain et al., 1999). Moreover, since the majority of primate nutritional studies quantify crude protein in this way, I used the 6.25 factor for comparative purposes. However, to take into account nitrogen bound to fiber and secondary metabolites, I estimated available protein (AP) in two ways: *1)* to determine the amount of fiber-bound nitrogen, I subtracted acid detergent insoluble nitrogen (ADIN) from N, multiplied this value by 6.25, and then subtracted it from CP (Rothman et al., 2008); *2)* to estimate the effect of tannins on N digestibility, I measured the polyethylene glycol (PEG, a tannin-blocking agent) binding capacity in a subset of plant samples (n= 42, those that were positive for the qualitative acid-butanol assay for condensed tannins (Waterman and Mole, 1994)), followed by in vitro digestion with pepsin and cellulase (DeGabriel et al., 2008).

Lipids were determined by extraction with petroleum ether using an XT15 Fat Analyzer (ANKOM, Macedon, NY, USA) (AOCS, 2009). I subtracted 1 from the percentage of ether extract to account for all the non-lipid non-nutritive components extracted by the procedure (e.g. waxy substances, cutin, essential oils) (Rothman et al., 2012).

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially using filter bags in an ANKOM 200/220 fiber analyzer (Van Soest et al., 1991). Fruit samples containing >5% fat were pre-extracted in acetone before the NDF procedure, since lipids might interfere with the detergent (Rothman et al., 2012). Water soluble carbohydrates (WSC) were measured with the phenol-sulfuric acid assay (Dubois et al., 1956), using sucrose as a standard. Total nonstructural carbohydrates (TNC) were calculated by subtraction: %TNC = 100 – %NDF – %lipids – %AP – %ash (Rothman et al., 2012). To calculate individual daily nutrient end energy intake I used TNC instead of WCS, since TNC account for additional non-structural carbohydrate fractions such as starch and soluble fiber, which I did not measure separately.

The metabolizable energy content of plant samples was calculated using standard conversion factors (or physiological fuel values): 16 kJ/g for TNC, 17 kJ/g for AP, and 37 kJ/g for lipids (Conklin-Brittain et al., 2006). Since howler monkeys obtain energy from fiber fermentation, I calculated a conversion factor based on mean fiber digestibility (47.7%) reported by Edwards and Ullrey (1999) for captive *A. palliata*, *A. caraya*, and *A. seniculus* consuming a diet of 42% NDF. Considering that gut microbes might retain at least 4 kJ/g during hemicellulose and cellulose fermentation for their own growth (Conklin-Brittain et al., 2006), we multiplied the digestion coefficient (0.477) by 12 kJ/g. Thus we used a conversion factor of 5.7 kJ/g for NDF.

Data analysis

I constructed a complete dietary profile for each group member by quantifying feeding rates and estimating daily nutrient and energy intake of individual adult males and females (at least two full day focal follows per individual per month). In the following analysis I include only 91 focal samples of 9 males ($n = 44$) and 4 females ($n = 47$), i.e., those corresponding to 91 days in which the focal animal could be successfully followed for $9 - 12$ consecutive hours without being out of sight for more than 10 minutes, and all feeding bouts were recorded in detail.

To calculate the daily amount of food ingested (g dry weight) by each focal animal, I multiplied the feeding bout length on food item *i* (minutes) by the corresponding feeding rate (g dry weight/min), and then summed all daily events. To estimate daily nutrient intake, I multiplied the nutrient content of each food item *i* by the estimated amount of item *i* ingested (g dry weight) in each feeding bout. I then obtained daily values of AP energy (total AP intake, converted to kilojoule equivalents), non-protein energy (sum of the intake of TNC, lipids, and NDF, converted to kilojoule equivalents), and total energy (sum of AP and non-protein energy) for each focal animal. To take into account the possible effects of different body weights on nutrient and energy intake in male and female howler monkeys, I divided each value of daily energy intake by the estimated metabolic body mass ($\text{mbm} = M^{0.762}$) of the corresponding focal animal. Body mass for nine of the 14 focal animals was obtained in August 2010 during the darting and marking phase of this project (adult males: 7.6 ± 0.9 kg, adult females: 6 ± 0.3 kg). For those individuals whose weights were not available ($n = 4$), I used data on mean body weight of male and female *A. pigra* from Kelaita et al. (2011), which were based on a larger sample size (37 males, 32 females).

I analyzed daily nutrient intake data using a Geometric Framework (GF) for nutrition. I constructed two- and three-dimensional models, showing in geometric space the individual daily intake of available protein, TNC, and lipids. In the two-dimensional models I represented available protein energy on the x-axis, and non-protein energy (TNC+NDF+lipids) on the y-axis. The metabolism of protein differs from that of lipids and carbohydrates in that amino acids cannot be synthesized from other macronutrients and cannot be stored, which contrasts with the capacity of lipids and carbohydrates to be stored as triacylglycerol and glycogen, respectively (Brosnan et al., 2011). Moreover, carbohydrates and lipids are generally used for energy in vertebrates (Raubenheimer and Simpson, 1999), thus, considering protein and non-protein energy separately reflects their different metabolic characteristics.

In a geometric framework, each point in nutritional space represents the nutrient composition of the daily diet of one focal animal, and the nutritional "rails" (vectors passing through the origin) represent the balance of those nutrients for the food ingested. To assess the effect of sex on several response variables such as daily nutrient intake, I ran repeated-measures ANOVAs using *STATISTICA 10* (StatSoft, 2011), with season as a within-subjects factor, and sex as a between-subjects factor. To analyze temporal patterns of nutrient prioritization, I plotted the cumulative daily intake of AP and NPE intake during periods (ranging from four to 40 days) for which I had intake data for consecutive days.

I also used right-angled mixture triangles (RMT) to compare the relative (% of total energy) daily intake of protein, lipids, and carbohydrates of focal individuals with the nutrient composition of foods that comprised at least 1% of the annual diet. Slope differences were tested using one-sample tests based on correlations between residual and fitted values (“smatr” package in R) (Warton et al., 2006).

I used Linear Mixed-Effect Models (LMM) (to avoid problems of non-independence of errors due to temporal pseudoreplication, i.e. repeated measurements from the same individual (Crawley, 2007)) to analyze the effects of different variables on individual protein and non-protein energy intake and their ratio. All models included an individual's identity as a random factor, and predictor variables as fixed factors. Included among the hypothesized predictor variables were: season; percentage of fruit in the daily diet (based on amounts ingested); percentage of leaves in the daily diet (based on amounts ingested); daily total feeding time (minutes spent feeding in a day per unit of metabolic body mass); and the food availability indices. Data were log or arcsine square root transformed to conform to assumptions of normality. I initially included all the predictor variables in the model, and then dropped one by one those that were not significant based on hypothesis testing procedures (backward selection). I refitted the model after each removal, and used the Akaike (AIC) selection criterion to identify the best model (Zuur et al., 2009). I conducted the analyses in R (version 2.15.1), fitting the linear models by REML (Restricted Maximum Likelihood criterion), and using nlme (Pinheiro et al., 2013) and MuMIn (Barton, 2013) packages (R Core Team, 2012).

Results

Behavioral data

Based on the complete behavioral data set (1300 hours of observation of 10 adult males and 4 adult females), the yearly diet of *A. pigra* at El Tormento consisted of 37% leaves, 58.2% fruit, and 4.7% flowers and inflorescences (based on grams ingested). The same dietary profile was present in males (37.5% leaves, 57.8% fruits, and 4.4% flowers) and females (36.3% leaves, 58.5% fruits, and 5% flowers). Howler monkeys' diets broadly coincided with seasonal changes in food availability (Figure 4.2). For example, at the onset of the dry season (February) several species of deciduous trees that were important in the howler diet (e.g., *Bursera simaruba* (L.) Sarg., *Manilkara zapota* (L.) P. Royen, *Metopium brownei* Roxb.) began to shed some or all of the leaves. Consequently, during the next three months the availability of mature leaves in the forest diminished considerably (>50%). During the mid-dry season there was an increase in leaf bud and young leaf availability that peaked in mid-April (FAI = 243.4). This corresponded to a relative decrease in the consumption of mature leaves, and a relative increase in the intake of young leaves during the dry season. Immature and mature fruits were more abundant during the rainy season, from June to September (average monthly FAI for mature fruit: 66.9 ± 16.4, and immature fruit: 34.9 ± 8.2), however September and October 2010 were characterized by a scarcity of mature (FAI = 1.6 ± 0.6) and immature (FAI = 12.1 ± 2.7) fruit compared to the following year (September - October 2011) (FAI mature fruit: 57.4 ± 33.9; FAI immature fruit: 43 ± 21.7) (Figure 4.2). For example, the production of *Brosimum alicastrum* Sw. fruits, which accounted for 33% of the howler fruit portion of the total diet, was eight times more abundant (based on FAI scores) in 2011 than in 2010. Thus, as a general pattern, during the dry and rainy seasons howler monkey diet was fruit-based (54-69%), whereas during the *nortes*, leaf consumption was greatest (49.5%) (Table 4.2).

Individuals fed on leaves, fruits, and flowers belonging to 44 species (36 species during the dry season, 32 during the rainy season, and 28 during the *nortes*) representing 20 families. Fourteen plant species comprised at least 1% (each) of the annual diet, with three species (*B. alicastrum* [Moraceae], *M. zapota* [Sapotaceae], and *Acacia usumacintensis* Lundell [Fabaceae]) accounting for 52% of the total diet, based on the amount of food ingested.

Nutritional data

Based on 91 complete days of dietary information, individuals consumed an average (±SD) of 218.5 ± 95.3 g of dry matter per day, spending 103.3 ± 40.9 minutes feeding (range: 30 - 241 min). Daily total feeding time varied across seasons, being lowest (94.3 ± 46.2 min) during the dry season (repeated measures ANOVA: $F(2,10) = 4.99$, $p = 0.03$), which also was the time of the year characterized by the longest periods of resting (408.9 ± 67.5 min). This may be in response to the daily maximum temperatures, which averaged 36.1°C. Patterns of daily food consumption (grams), and energy and macronutrient ingestion per unit metabolic body mass did not differ by sex (repeated-measures ANOVAs: $p>0.05$) (Figure 4.3), and therefore I combined male and female data in all subsequent analyses.

Available protein constituted on average 16.6 ± 5% of total daily energy intake, carbohydrates (TNC) 52 ± 9%, and lipids 12 ± 7%. The daily intake of available protein energy (AP), non-protein energy (NPE), and total energy did not vary significantly across seasons (LMM: $p>0.05$); however, based on their coefficients of variation (CV) (50%, 47%, and 45% respectively) howler monkeys were found to vary nutrient intake across days. This may reflect the fact that I sampled 13 different individuals per month. In contrast, the ratio of protein and non-protein energy (AP:NPE) was found to differ significantly across seasons, being higher during the *nortes* than the dry season (LMM: $p = 0.0006$). Across days, however, the ratio fluctuated less (CV = 36%) than AP, NPE, and total energy (Figure 4.4). Fluctuation in the daily intake of AP and NPE revealed a trend, with four-to-five days of lower-than-average energy intake generally followed by four-five days of correspondingly higher-than-average energy intake. This was particularly evident for NPE (Figure 4.5). The cumulative intake plots show that the daily AP:NPE intake was constant over consecutive days ($R^2 = 0.99$) and over periods of consecutive four-five days ($R^2 = 0.98$) (Figure 4.6). These data suggest that howler monkeys regulate energy intake over the course of a single day as well as over a period of several days, so that several consecutive days of low energy intake were balanced by several days of high energy intake.

Nutrient balancing strategies (Hypotheses 1-3):

Available Protein Intake - Overall, the howler monkey daily intake of available protein averaged 102 kJ/mbm (or 6 ± 3 g/mbm) and ranged from 16 to 271 kJ/mbm (Table 4.3). The best model indicated that daily total feeding time was a strong predictor of available protein intake (Table 4.4), which tended to be higher on days when individuals spent more time feeding (i.e. during the rainy and *nortes* seasons). Given that the daily intake of available protein was not correlated with food availability, or with the percentage of leaves ($r_s = 0.77$, $n = 91$, $p = 0.4$), leaves and flowers ($r_s = 0.91$, $n = 91$, $p = 0.3$), or fruit ($r_s = -0.09$, $n = 91$, $p = 0.3$) in the diet, I tested whether AP intake was regulated more tightly than NPE consumption, as predicted by the protein leverage hypothesis. My data did not support a pattern of protein regulation when analyzing the entire data set and when each of the three seasons was analyzed separately. In fact, NPE intake was not significantly higher on days in which AP constituted a small proportion of the daily diet (<10% of total energy) (Figure 4.7). However, I did find evidence supporting a protein regulation effect when I analyzed daily patterns of food intake. On days (n =12) in which the diet was relatively balanced and contained between 40-60% fruit and 60-40% leaves, protein intake was more stable than on days characterized by a predominantly (>70%) leaf-based or fruit-based diet (Figure 4.8). This means that when the daily fruit-leaf consumption was relatively equal in terms of amount consumed, AP intake fluctuated less (CV = 30%) than NPE intake (CV= 43%). This indicates that the focal animals maintained a relatively constant daily AP intake (106 ± 32 kJ/mbm, 18 ± 3% of total energy) by consuming a diet characterized by relatively equal amounts of fruits and leaves. Thus, while Hypothesis 1 (constant protein prioritization) and Hypothesis 3 (seasonal protein prioritization, during periods of high fruit intake) were not supported, these findings suggest that howler monkeys are able to regulate protein intake, possibly by mixing specific plant items and species, and by adjusting their feeding rates.

Non-Protein Energy Intake - Daily intake of non-protein energy averaged 526 kJ/mbm and ranged from 140 to 1282 kJ/mbm. Patterns of NPE intake were best explained by daily total feeding time (higher intake on days when individuals spent more time feeding), and % fruit consumed (higher intake when fruit ingestion was higher) (Table 4.5). These data show that black howler monkeys neither prioritized nor maintained a constant intake of carbohydrates and lipids during the whole year or during different seasons. Thus, these results fail to support Hypothesis 2 (non-protein energy prioritization) and Hypothesis 3 (non-protein energy prioritization during periods of a leaf-based diet).

AP:NPE Ratio - Within the Geometric Framework, the AP:NPE ratio (which ranged from 0.04 to 0.44 in this study) is represented by the slope of nutritional rails (Figure 4.9). Since I found evidence of a consistent balance of AP and NPE over consecutive days (**Figure 4.6**), I tested the hypothesis that howler monkeys adopted a strategy of AP:NPE regulation. The best model identified by AIC to explain patterns of AP:NPE revealed three significant effects: 1) season (higher ratios during the *nortes* than the dry season) (Figure 4.10); 2) daily total feeding time (higher ratios with increased feeding time) (Figure 4.11); and 3) % of fruit ingested (lower ratios when fruit consumption was higher) (Table 4.6). Based on the 91 sample days, howler monkeys consumed higher amounts of protein-rich items, such as leaves (47.4%) and flowers (11.2%), than fruit (41.4%) during the *nortes* (Figure 4.12), with mean energy intake for all individuals represented by a nutritional rail with a slope of 0.25. In contrast, during the rainy and dry seasons, when fruit consumption was greater, the nutritional rails exhibited slopes of 0.19 and 0.17, respectively (Figure 4.9), indicating a lower intake of available protein relative to non-protein energy. Finally, the AP:NPE ratio was not significantly correlated with the amount of food ingested daily (grams) (Spearman correlation, r = -0.13, n = 91, p = 0.2).

As was the case for protein intake, when I analyzed the data according to season or daily diet, I found that during the rainy season, and on days in which individuals ingested >70% leaves (n = 11) , howlers followed a strategy of AP:NPE prioritization, attempting to maintain a relatively constant balance of protein and non-protein energy intake (Figure 4.13). Moreover, during the rainy season, energy derived from AP constituted 16.2 ± 3.7% of total energy intake, and fluctuated less (CV = 22.8%) than the AP energy content in the foods ingested (AP energy/total energy = 8.1 ± 4.3%, CV = 53%). This suggests that howlers ingest different plant items to reach their nutritional goals rather than consume nutrients according to their availability in foods present in the environment. This also is evident when plotting a right-angle mixture triangle (Figure 4.14), which shows that the nutritional composition of the consumed foods differed from the composition of the daily diet (slope test, F = 6.6, r = 0.26, p = 0.01). These results indicate that black howler monkeys exhibit a foraging and dietary strategy that prioritizes proportions of AP and NPE rather than prioritize their intake (Figure 4.15).

Discussion

The main goal of this study was to examine the nutritional ecology of adult black howler monkeys using a Geometric Framework in order to test hypotheses of nutrient regulation and food choice based on alternative nutrient mixing strategies. Traditional foraging models such as Optimal Foraging Theory have had limited success in explaining what nutrient or combination of nutrients guide primate feeding decisions (Post, 1984; Barton et al., 1992; Grether et al., 1992; Felton et al., 2009b). This is due to the fact that optimization models are typically based on the maximization of a "currency" (usually energy or protein) that is expected to contribute disproportionately to fitness (Stephens and Krebs, 1986; Ydenberg et al., 2007), whereas a nutrient-explicit and multidimensional approach is predicated on the benefits of balancing nutrient intake in response to fluctuating resources, nutrient heterogeneity, and changes in the nutritional requirements of the consumers.

I tested several hypotheses concerning the nutritional ecology of black howler monkeys living in a semi-deciduous forest characterized by seasonal changes in food availability. I hypothesized that black howler monkeys show nutrient regulation strategies that prioritize 1) daily protein intake, 2) daily non-protein energy intake, or 3) limiting nutrients according to changes in food availability (e.g. leaf scarcity), switching prioritization patterns seasonally. I found instead a fourth pattern of nutrient regulation, characterized by a balance in the daily intake of available protein and non-protein energy, which was achieved despite fluctuations in the intake of protein, carbohydrates, and lipids.

Available Protein Intake - Based on the fact that daily protein intake was not consistent during the study period, my hypothesis that howler monkeys show a daily regulation of available protein intake was not supported. Moreover, I did not find evidence of protein prioritization during periods of leaf scarcity (dry season). One possible reason for this lack of protein prioritization is that, during all seasons of the year, individuals in the howler monkey study groups surpassed protein requirements for adult non-human primates (4−7.5% of metabolizable energy (Oftedal, 1991; NRC, 2003), consuming a diet in which AP constituted on average 16.6 ± 5% of total energy. This was achieved despite the fact that foliage at El Tormento is not particularly rich in available protein (young leaves: 13.7% ± 6.3; mature leaves: 13.2% ± 6.7 [percentage of dry matter]) (see chapter 2) compared to leaves consumed by primates at other field sites (Conklin-Brittain et al., 1999; Silver et al., 2000; Aristizabal, 2013). This suggests that howler nutrient intake did not match the nutrient composition of available foods, but that individuals selected and mixed different food items to obtain a relatively high daily intake of available protein. However, this also indicates that overall, protein availability in the environment was sufficient to allow black howler monkeys to consume a diet that was not deficient in protein during any season of the year. In general, the fact that selected foods are high in available protein is not sufficient to guarantee that individual daily protein intake also is high, as illustrated by the following example. Available protein in young and mature leaves consumed by two groups of black howler monkeys at a nearby site in Tabasco, Mexico, was reported to be 17 ± 4% and 14.9 ± 4%, respectively (higher than at my field site); however, daily available protein intake was only 3.9 g/mbm, or 65.6 ± 30 kJ/mbm (Aristizabal, 2013). This may have resulted from limited amount of food ingested daily (116 ± 78 g dry weight) or possibly to the ecological characteristics of the small (< 3.9 ha) and anthropogenically disturbed fragments inhabited by these howler monkeys (Aristizabal, 2013).

The need for prioritization of protein intake, as reported in humans and a number of vertebrate and invertebrate species (e.g., locusts, mice, mink, spider monkeys) (Simpson and Raubenheimer, 2000; Simpson and Raubenheimer, 2005; Sørensen et al., 2008; Mayntz et al., 2009; Felton et al., 2009a; Felton et al., 2009c; Martinez-Cordero et al., 2012) appears to be related to the negative effects of protein deficiency (e.g., slower growth, muscle atrophy, higher risk of infections), since amino-acids are necessary for maintenance and repair of body tissues and for growth. In addition, there may be deleterious health consequences in the overconsumption of protein. Studies of several fruit fly species (Lee et al., 2008; Fanson et al., 2009; Fanson and Taylor, 2012), ants (Dussutour and Simpson, 2009), and crickets (Maklakov et al., 2008) have shown that there are fitness costs (e.g. reduced lifespan) associated with excessive protein intake. For example, in *Drosophila melanogaster*, the maximum individual lifespan was obtained on a diet characterized by a 1:16 protein to carbohydrate ratio, and it progressively decreased as the ratio rose, up to 1.9:1 (Lee et al., 2008). The negative effects of high protein intake might be due to an increase in mitochondrial production of reactive oxygen species (ROS), which cause high incidence of oxidative damage to cells (Ayala et al., 2007). In humans, excessive sustained daily protein intake (>30% of total energy intake) can result in increased uric acid levels (leading to gout), urinary calcium loss (leading to osteoporosis) (St. Jeor et al., 2001), hyperaminoacidemia, hyperammonemia, hyperinsulinemia, and even death (Bilsborough and Mann, 2006).

During periods in which protein intake of black howler monkeys might have exceeded their requirements, protein-precipitation capacity of tannins found in the foods ingested might have aided the excretion of excess nitrogen (Rothman et al., 2011). The diet of the focal animals included items containing tannins; 60% of the samples containing high amounts of condensed tannins belonged to five species (*Bursera simaruba*, *Manilkara zapota*, *Metopium brownei*, *Pseudolmedia oxyphyllaria* Donn. Sm., and an undetermined vine) and included mature and immature leaves, mature and immature fruits, and inflorescences. These were among the 10 most commonly consumed plant species which accounted for 75% of the howler diet during the study period (see Chapter 3).

Non-Protein Energy Intake

The daily intake of non-protein energy by black howler monkeys fluctuated throughout the year, and was not prioritized during periods of fruit scarcity (*nortes* and dry season) as has been reported for mountain gorillas (Rothman et al., 2011). In the case of howler monkeys, NPE intake was positively correlated with the daily amount of fruit ingested, and thus the lowest NPE intake was recorded during the *nortes*, when fruits accounted for 40% and leaves for 49.5% of the howler diet. As was the case for protein intake, the daily amounts of carbohydrates and lipids ingested by adult black howlers were high (526 ± 248 kJ/mbm) compared to values reported for other species (Table 4.3). For example, daily intake of carbohydrates plus lipids was 512 kJ/mbm in *Gorilla beringei*. In contrast, NPE in *Ateles chamek* varied between 143 and 1271 kJ/mbm (Felton et al. 2009a), which coincides with the values of non-protein energy intake that I found in *A. pigra* (140-1282 kJ/mbm). These data show that whether they consume a fruit-based diet or a balanced fruit-and-leaf based diet, Atelines show similar intakes of non-protein energy, highlighting the importance of fruit eating in primates of this subfamily (Di Fiore et al., 2011).

AP:NPE regulation

Balancing the intake of AP:NPE was the most consistent strategy adopted by black howler monkeys in the study population. Over the course of 15 months, the daily ratio of protein and non-protein energy ingested fluctuated less (0.04 - 0.44; CV = 36%) than AP (50%) and NPE (47%) intake. In contrast, the daily ratio of AP to NPE fluctuated to a much greater extent in Ugandan mountain gorillas than in black howler monkeys, ranging from 0.06 to 2.9 (Rothman et al. 2011). This was due to the fact that, over the course of a year, the gorillas went from a fruit-based diet (>40% on a wet weight basis) to a primarily leaf-based diet (<5% fruit), resulting in a change in daily available protein intake from 16% of total energy during fruit periods to 31% of total energy when leaf-eating predominated.

Although the diet of black howler monkeys at El Tormento also varied seasonally from mostly fruit-based during the dry and rainy seasons (54-69% fruit in the diet) to leaf-based during the *nortes* (49.5% leaves), daily AP intake varied on average only from 15.2% of total energy in the dry season to 20% during the *nortes*. Moreover, in black howlers, the contribution of non-protein energy to total energy varied little across the year (CV = 6%), being 80-85% during the dry, rainy, and *nortes* seasons. Thus, even during periods of lower fruit availability, howler monkeys maintained high intakes of carbohydrates and lipids by being highly selective in the fruit species consumed. Lipids in particular fluctuated more than carbohydrates, contributing on average 12% to the total energy intake (CV = 59%). Ripe and unripe fruits of *Metopium brownei* and *Dendropanax arboreus* (L.) contained relatively high concentrations of lipids (17-41% dry matter) compared to other fruits or plant parts. These fruits were mainly consumed during the *nortes* and the rainy season, thus their ingestion contributed significantly to seasonal variation in lipid intake, even during periods when leaves dominated the howler diet.

A similar patterns of AP:NPE regulation has recently been reported by Johnson et al. (2013) in a study of daily diet composition and nutrient intake of a single female chacma baboon (*Papio hamadryas ursinus*). During a period of 30 consecutive days, this female baboon was able to maintain an AP:NPE balance (CV of the ratio = 31%) ingesting a relatively stable intake of available protein each day, whereas the contribution of lipids and carbohydrates to total energy fluctuated to a greater extent. Although my data on howler monkeys indicate that the AP:NPE balance was maintained across the study period, unlike the baboon study I found that both daily protein and non-protein energy intake fluctuated.

The benefits of balancing nutrients are well documented in several organisms. In insects it has been demonstrated that, when restricted to imbalanced diets, individuals are able to mix nutritionally complementary foods to reach a specific balance of nutrients required to facilitate weigh gain, enhanced survival and higher fecundity (Waldbauer and Friedman, 1991; Simpson et al., 2004; Behmer, 2009; Simpson and Raubenheimer, 2012). For example, the consistent choice of a specific ratio of casein and sucrose (4:1) in caterpillars (*Heliothis zea*) was associated to significant increases in growth rate and survival (Waldbauer et al., 1984). In contrast, nutrient imbalance has been shown to cause fitness costs in insects, since it leads to increasing the consumption of the deficient nutrient by ingesting an excess of other nutrients (Raubenheimer, 1992; Raubenheimer and Simpson, 2003). Thus, regulating the ratio of nutrients ingested can be considered as a strategy for buffering the deficits and surpluses related to an imbalanced diet (Raubenheimer and Simpson, 1993).

My data provide evidence that black howler monkeys attempted to balance nutrients on a daily basis. My experimental design did not allow us to collect data from the same individual over consecutive days (see Johnson et al. 2013), however, given black howler small group size, highly cohesive feeding patterns, and low levels of agonistic behavior at feeding sites (e.g., feeding competition), I assume similar patterns of nutrient intake across individuals. I found indications that howler monkeys also attempted to balance energy and nutrient intake over periods of several days, as documented in humans (de Castro, 2000). This could be one of the reasons why I did not find a clear pattern of daily protein or non-protein energy regulation. Another explanation is that overall, my data show that the average daily energy intake of black howler monkeys was high compared to previous reports (628 ± 286 kJ/mbm), and was in fact higher than what had been previously reported for *A. palliata* based on rates of CO_2 production of temporarily caged wild monkeys (~ 355 kJ/kg/day, equivalent to ~ 553 kJ/mbm) (Nagy and Milton, 1979a) and in one study based on food nutritional analysis and behavioral observations of wild individuals (441 kcal/day, equivalent to ~ 548 kJ/mbm) (Williams-Guillén, 2003) (Table 4.3). Average daily energy intake did not differ significantly between males and females (see chapter 2), and was stable across seasons, showing that it was not affected by the availability of food items in the environment or by the amount of different plant parts in the diet. This is consistent with a dietary pattern of selective feeding characterized by an attempt to balance nutrient intake throughout the year.

Similarly, both daily protein and non-protein intake appear to be relatively high when compared to primates characterized by a different dietary emphasis (Table 4.3), such as highly frugivorous spider monkeys (55-92% feeding time on fruit) (Di Fiore et al., 2011), or genera described as omnivorous such as baboons (3-90% of feeding time on fruit; 1-65% underground plant parts; 13-40% nuts, seeds, and pods; 1-33% leaves; 3-29.5% flowers; 1-15% sap and gum; 1-2% bark and pith; 0.6-4% animal food) (Swedell, 2011). For example, spider monkeys, despite being commonly described as having an energy maximizing dietary and ranging patterns, may be more energy-limited than howler monkeys. The population of *A. chamek* studied by Felton et al. (2009a,c) was characterized by a daily total energy intake of approximately 412 kJ/mbm, 34% lower than the average for my howler monkey population. These results demonstrate that the howler diet was not energetically constrained, and suggest that this howler population was not experiencing nutritional stress even during periods of relatively low fruit availability. The latter also is confirmed by preliminary results from a study on physiological indicators of stress in the howler population of El Tormento; daily AP intake and fecal glucocorticoids (fGC) in adult individuals were negatively correlated, but neither total energy, nor protein and non-protein intake significantly predicted fGC concentration, possibly resulting from the fact that energy intake was high throughout the year (Righini et al., 2013). Thus, I argue that characterizations of howler monkeys as energy minimizers need to be reassessed.

Conclusions

This population of black howler monkeys was able to maintain a high energy diet by balancing the intake of non-protein energy to available protein despite seasonal changes in food availability and dietary emphasis. Individuals did not show significant evidence of protein prioritization patterns as reported in spider monkeys (Felton et al., 2009a; Felton et al., 2009c), or NPE prioritization as reported in mountain gorillas (Rothman et al., 2011). Instead, howlers exhibited a different pattern, regulating and maintaining the balance of protein and non-protein energy (AP:NPE) rather than prioritizing the ingestion of one nutrient over others. These findings highlight the fact that the grouping of primates using broad dietary classifications (e.g., folivory, frugivory, omnivory) fails to adequately represent their nutritional strategies, and that different species of fruit-leaf eating primates, independent of their phylogenetic relationship, might differ in their nutrient regulation priorities. Thus, for example, classifying gorillas and howler monkeys as “folivores” or spider monkeys and howler monkeys as "frugivores" fails to account for different nutrient prioritizing strategies.

The finding that my howler population was characterized by a highly energetic diet offers some indications of how howler monkeys can translate energy into the high reproductive rates and relatively fast pace of development that characterize them compared to other atelines (Ross, 1991; Di Fiore et al., 2011). Female howler monkeys reach reproductive maturity earlier, have shorter interbirth intervals, shorter gestation length, and shorter periods of infant dependence than *Ateles*, *Brachyteles*, and *Lagothrix* (Kappeler and Pereira, 2003). Energy expenditure and daily energy balance must clearly be taken into account too, since here I am only examining patterns of energy intake. However, howler activity budgets, characterized by long daily resting periods, short traveling bouts, and low levels of social interactions (Di Fiore et al., 2011), may allow howlers to invest energy in growth and reproduction rather than in other activities such as wide ranging behavior, territorial defense and patrolling (Wallace, 2008), and male-to-female aggression in the form of prolonged chases (Slater et al., 2008; Link et al., 2009), which are commonly reported in spider monkeys. Low energy expenditure and low basal metabolic rates are in general associated with diets that are limited in energy and nutrient content, have a low digestibility or high amounts of plant secondary metabolites (McNab, 1986). Howler monkeys, though, apparently avoid the problems associated with these types of foods using a series of behavioral (e.g., being selective in food choice, modifying food intake, mixing food resources daily, and regulating nutrient ingestion) and digestive (e.g. adjustments of food retention times and digestibility) mechanisms (Batzli et al., 1994; Cruz-Neto and Bozinovic, 2004). Thus, the capacity of howler monkeys to maintain high energy intake even during periods of leaf consumption might offer them an advantage over other atelines. For example, muriquis

(*Brachyteles* spp.), which also spend 41-93% of their monthly feeding time on leaves (Di Fiore et al., 2011), are instead characterized by a relatively short food transit time (~ 8 hrs), habits of defecating 10-14 times during the day, and less selective foraging patterns than howler monkeys (Milton (1984b) reported that *B. arachnoides* generally feeds from several trees and vines while traveling, instead of targeting selected feeding trees). This suggests that muriquis cannot obtain much energy from the more difficult to degrade plant material in their foods, which is expelled and not completely fermented (Milton, 1984a; Milton, 1984b). Howler monkeys are considered colonizing species, with a high ecological adaptability, and able to survive and reproduce in seasonal environments and fragmented landscapes (Rosenberger et al., 2009; Rosenberger et al., 2011); these characteristics might have arisen as a consequence of their ability to meet and surpass their energetic requirements, which in turn have allowed them to differentiate from an ancestral ateline life history strategy.

Finally, the use of geometric analysis in studies of free-living animals contributes importantly to understanding different patterns of nutrient regulation and provides additional evidence that primates actively regulate macronutrient intake. The behavioral and physiological mechanisms that control ingestive behaviors and specific appetites in human and non-human primates, however, require additional study.

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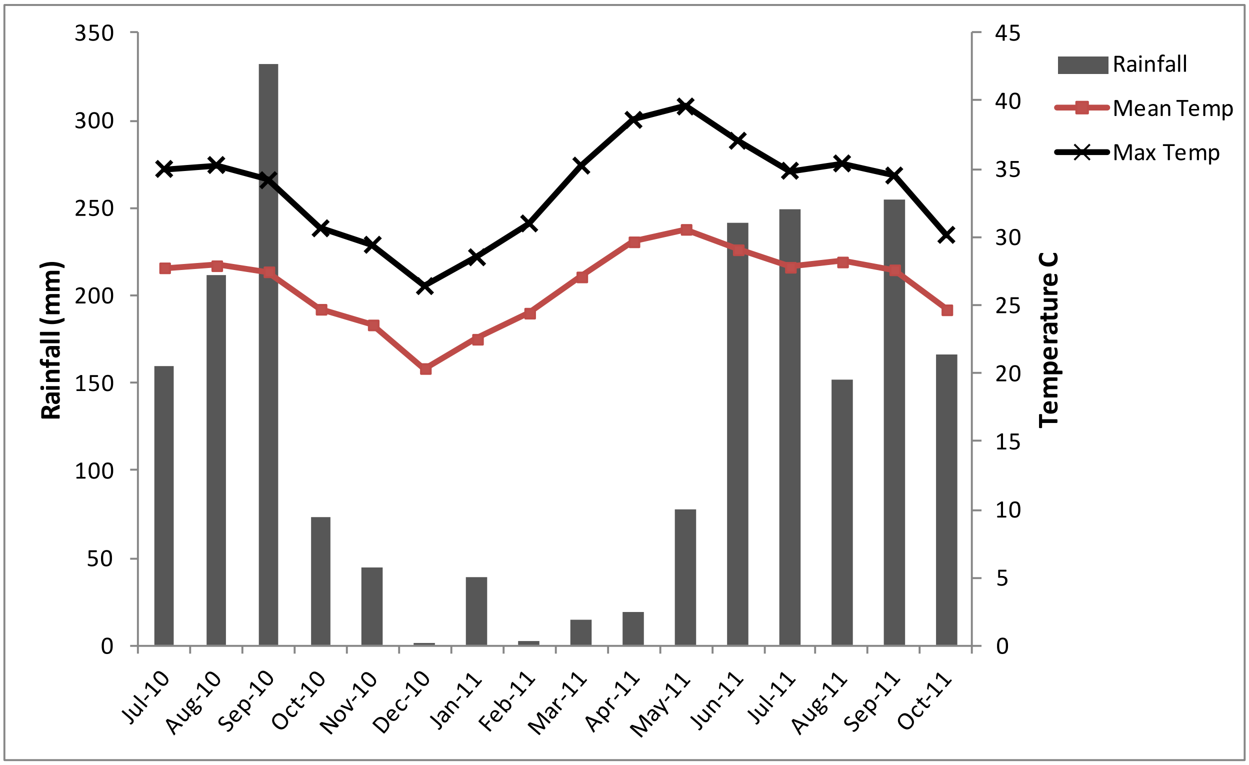

Figure 4.1. Average rainfall and temperatures at El Tormento during the study period. The rainy season spans from June to September, the *nortes* from October to January, and the dry season from February to May.

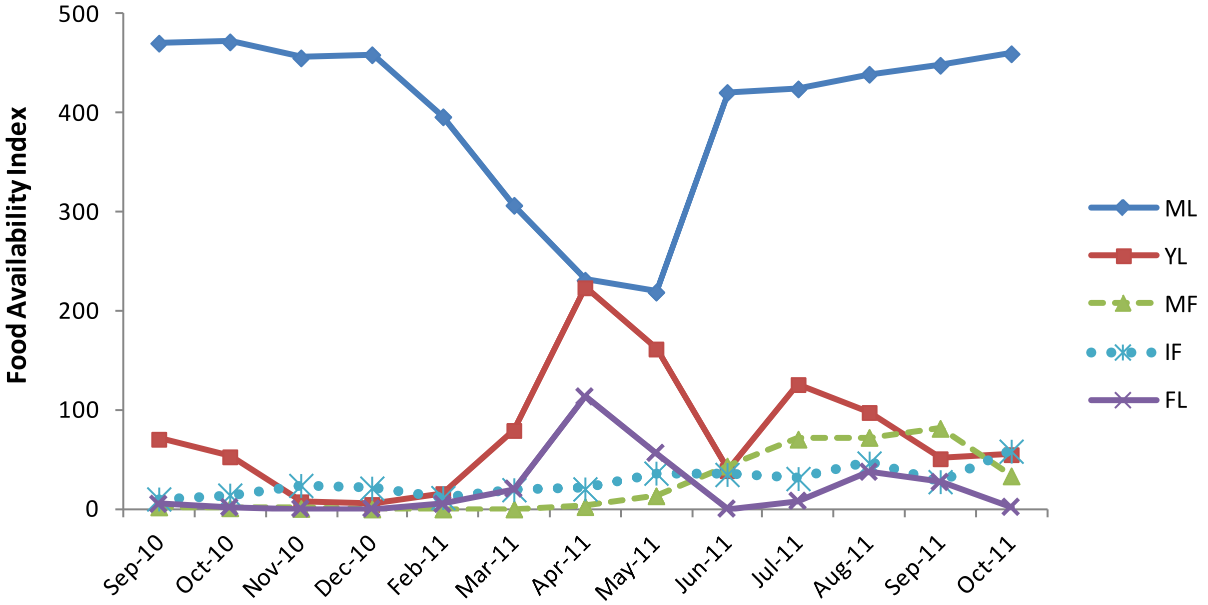

Figure 4.2. Food availability index for leaves (ML, YL), fruits (MF, IF), and flowers/inflorescences (FL) during the study period.

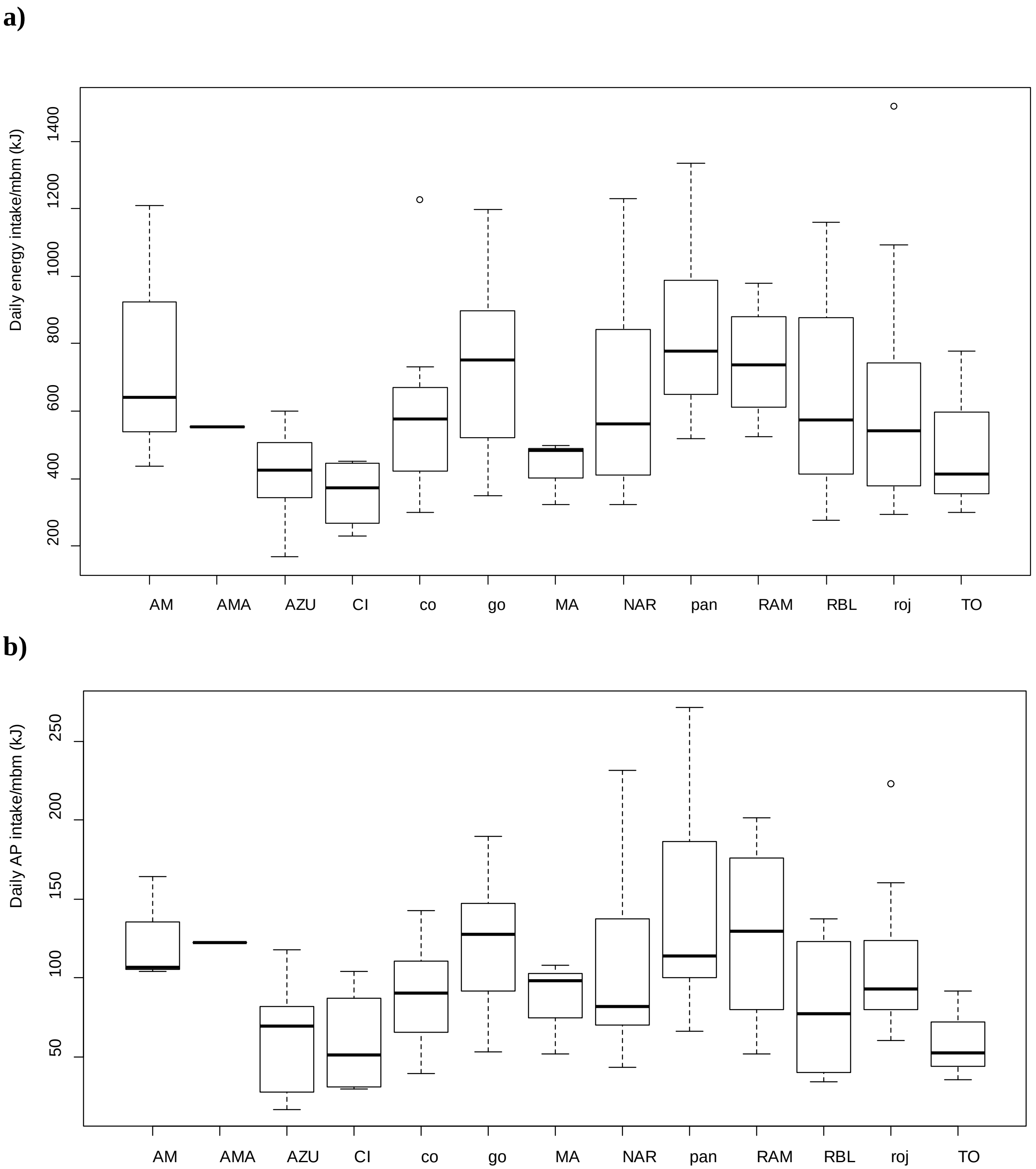

Figure 4.3. Daily total energy intake (**a**) and available protein intake (**b**) for each individual (kJ/mbm) (n= 91 focal follows). Females (n = 4) have lowercase names, males have uppercase names (n = 9). Box and whisker plots show median, percentiles (25% and 75%) and minimum and maximum values. Circles represent outliers.

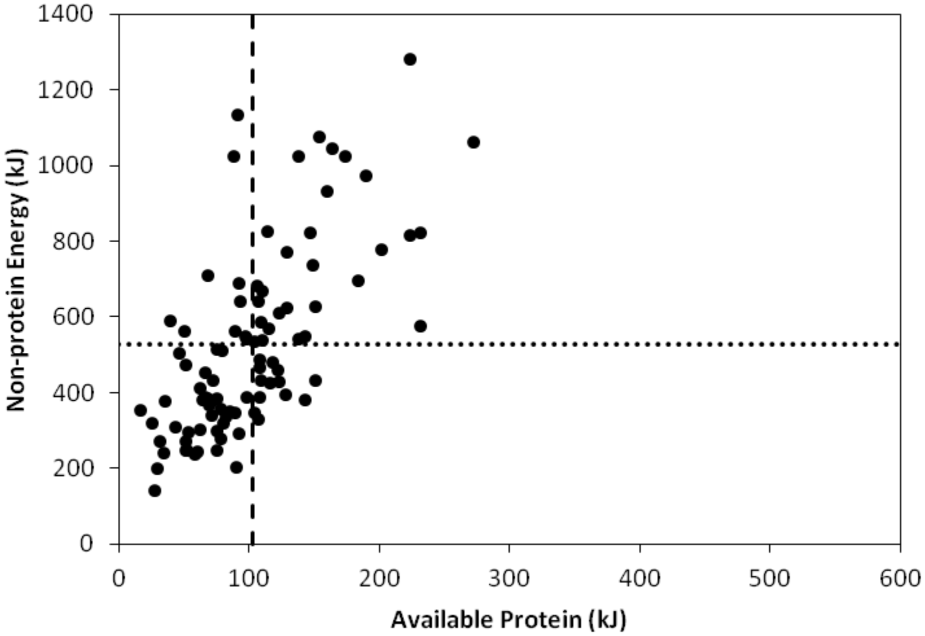

Figure 4.4. Daily intake of available protein (AP) and non-protein energy (NPE) (kJ) of adult male and female black howler monkeys. Each point represents the daily intake of one focal individual (n= 91). The vertical dashed line shows mean protein intake. The horizontal dotted line shows mean non-protein energy intake. Energy values are divided by the individual estimated metabolic body mass ($M^{0.762}$).

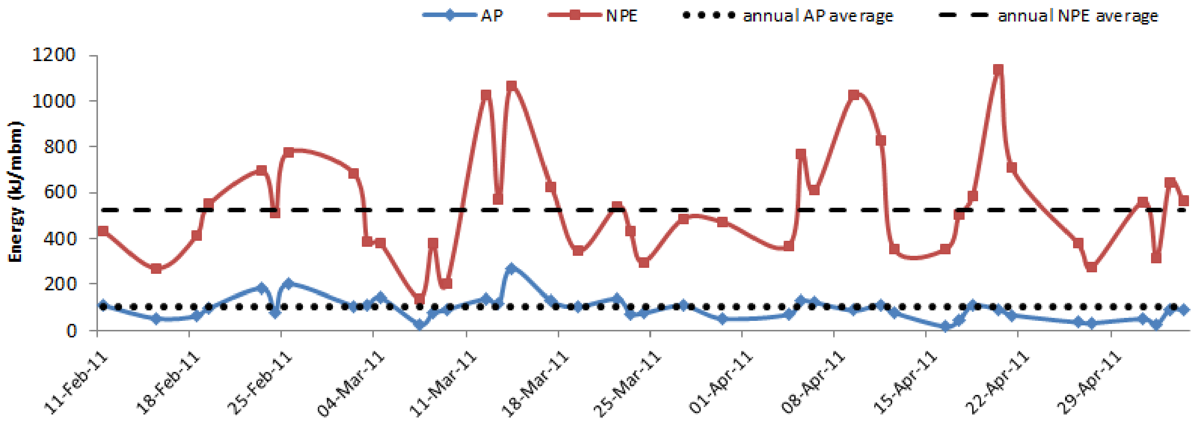

Figure 4.5. Daily fluctuations of protein (AP) and non-protein energy (NPE) intake during the dry season. Each point represents daily energy intake of a different individual on consecutive days.

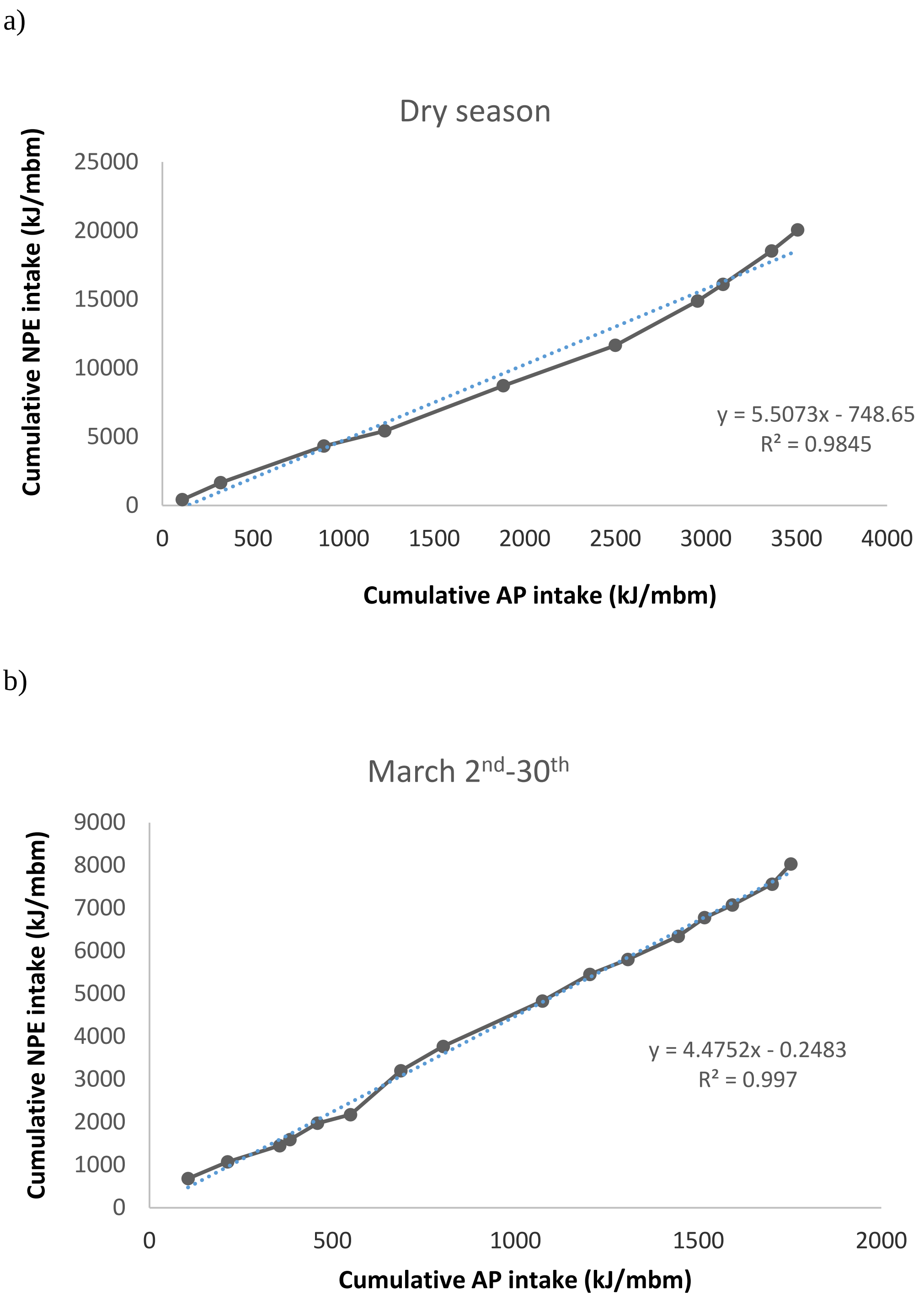

Figure 4.6. **a)** Cumulative protein and non-protein energy during 4-5 days-periods in the dry season. **b)** Cumulative daily intake of protein and non-protein energy during a month in the dry season.

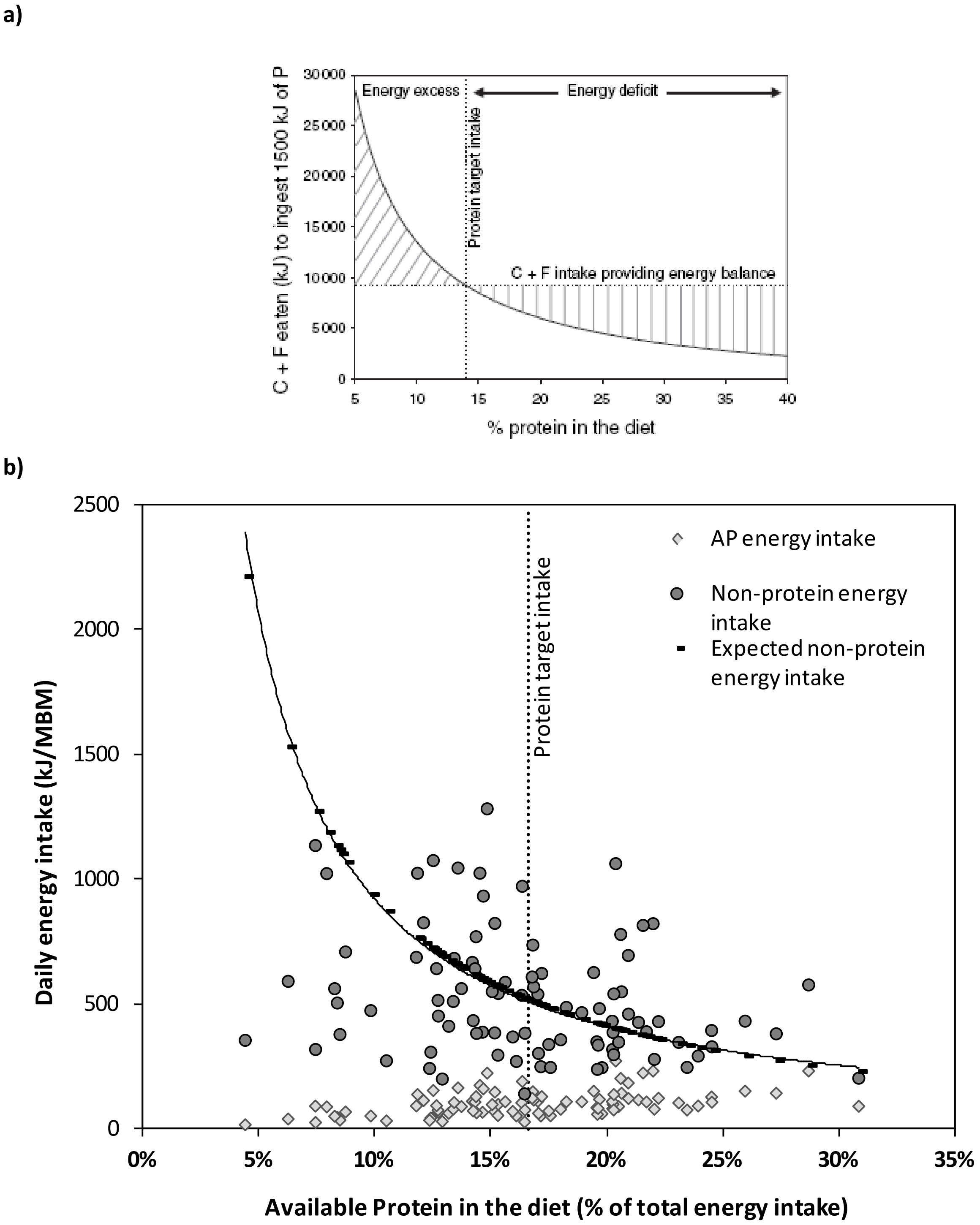

Figure 4.7. **(a)** The protein leverage effect, taken from Simpson and Raubenheimer 2005. When protein (P) intake is strictly regulated, energy balance is affected through the leverage over

carbohydrate and fat (C+F) intake. For example, a small decrease in %P in the diet results in an excess of C+F intake (diagonal hatched area), while a small increase in %P causes under-consumption of C+F (vertical hatched area). **(b)** Plot of the daily nutrient intake data obtained from 91 howler monkey focal follows to test if protein intake is regulated according to the protein leverage hypothesis. Expected non-protein energy intake (hyperbolic trend line) was calculated as: (Pt/AP) − Pt; where Pt is the target intake of protein (assumed to approximate the observed mean protein intake) and AP is the proportion of available protein in the diet. According to the protein leverage hypothesis, as the % of protein in the diet increases, non-protein energy intake decreases, but available protein intake should remain constant. Despite that available protein intake did not fluctuate greatly, our data do not show the typical protein leverage pattern (non-protein energy intake is not higher on daily diets containing low % of protein, i.e. observed non-protein energy intake does not follow the expected hyperbolic trend).

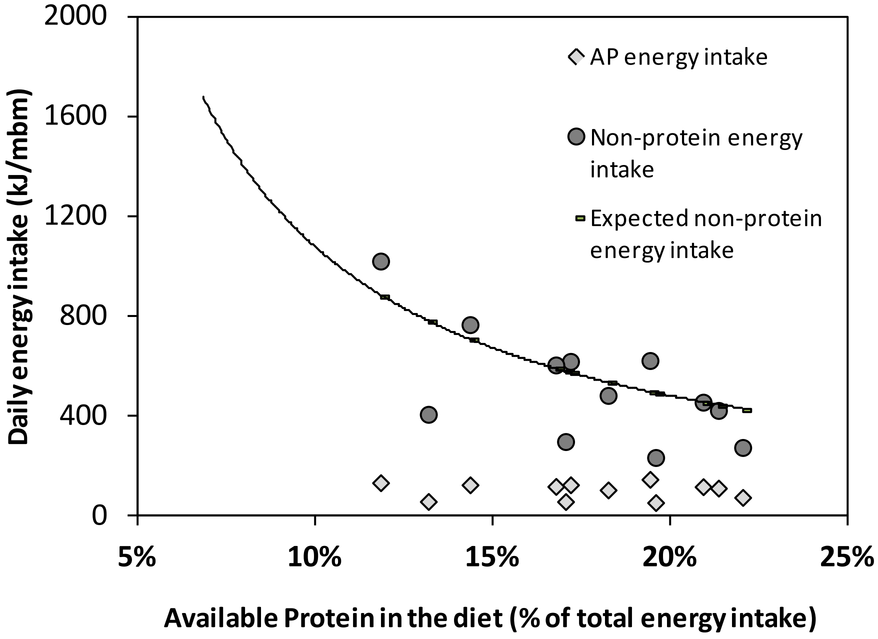

Figure 4.8. Plot of the daily nutrient intake data obtained from 12 focal samples on days in which the diet was balanced (40-60% fruit and 40-60% leaves, based on amounts consumed). Daily AP intake remains relatively constant and NPE decreases as the % of protein in the diet increases.

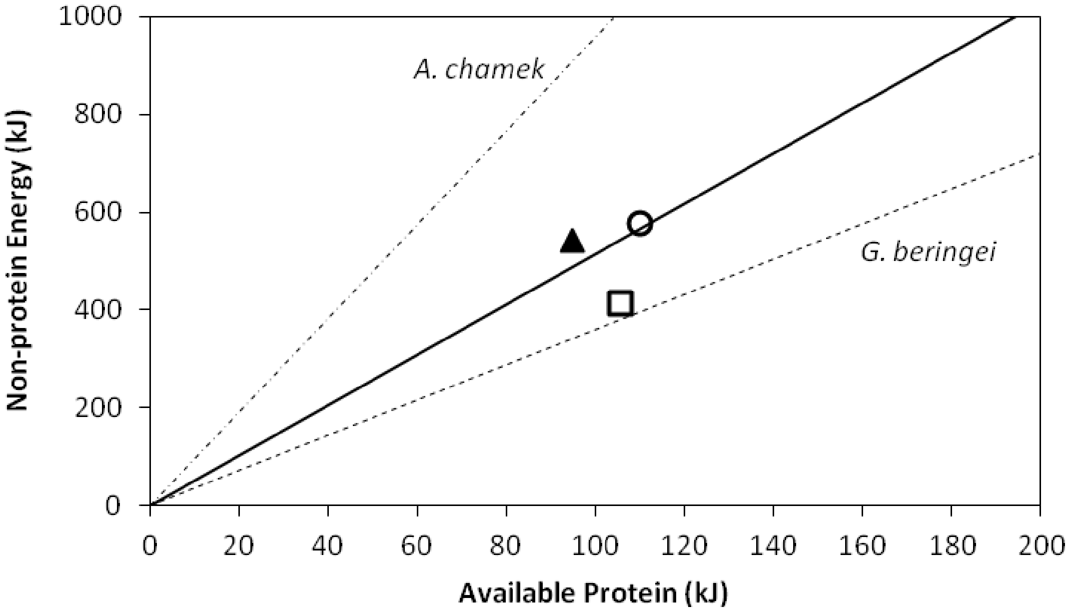

Figure 4.9. Geometrical plot of mean intake of available protein (AP) and non-protein energy (NPE) (kJ) of black howler monkeys (n= 91 daily focal follows). The continuous nutritional rail indicates the average balance of AP:NPE during the entire study period. The triangle represents mean AP:NPE during the dry season, the square represents mean AP:NPE during the *nortes* season, and the circle represents mean AP:NPE during the rainy season. Dotted nutritional rails indicating average AP:NPE for *Ateles chamek* (Felton et al. 2009) and *Gorilla beringei* (Rothman et al. 2011) are shown for comparative purposes. All energy values are divided by the individual estimated metabolic body mass ($M^{0.762}$).

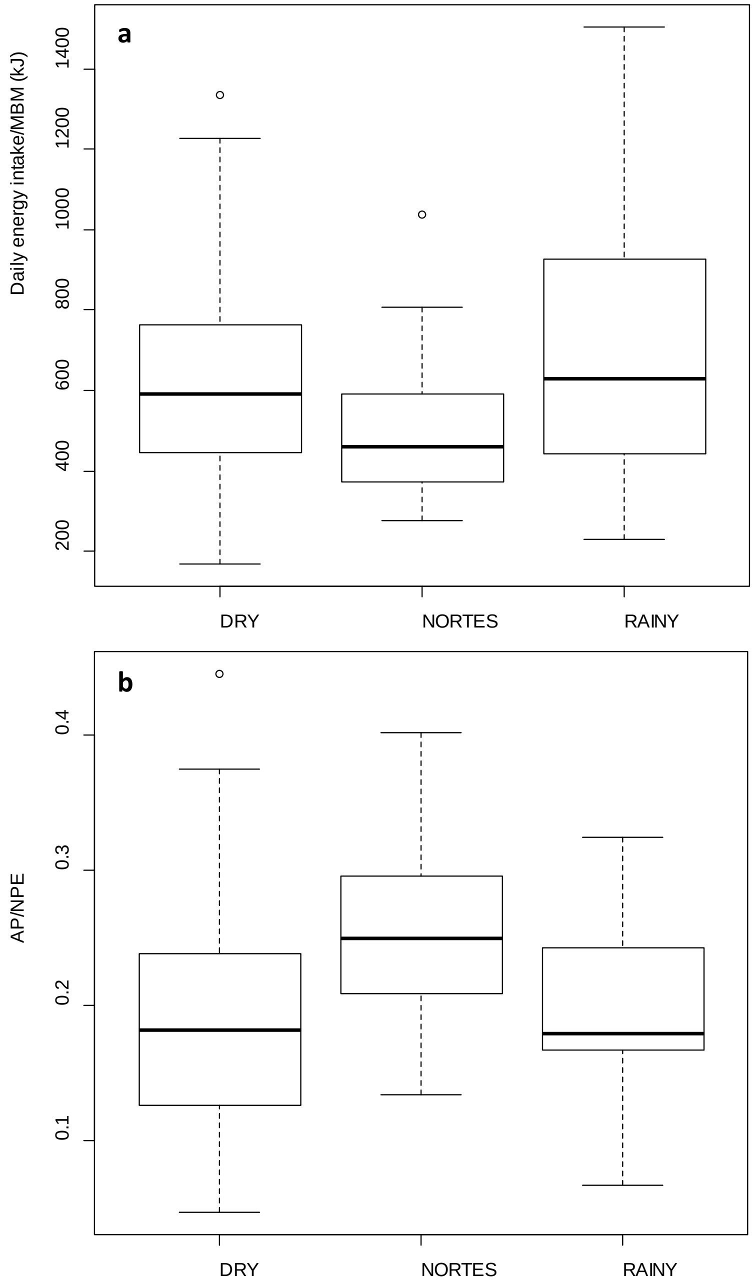

Figure 4.10. Variation of daily energy intake (kJ/mbm) (**a**), and available protein:non-protein energy ratio (**b**) during three different seasons. Box and whisker plots show median, percentiles (25% and 75%) and minimum and maximum values. Circles represent outliers.

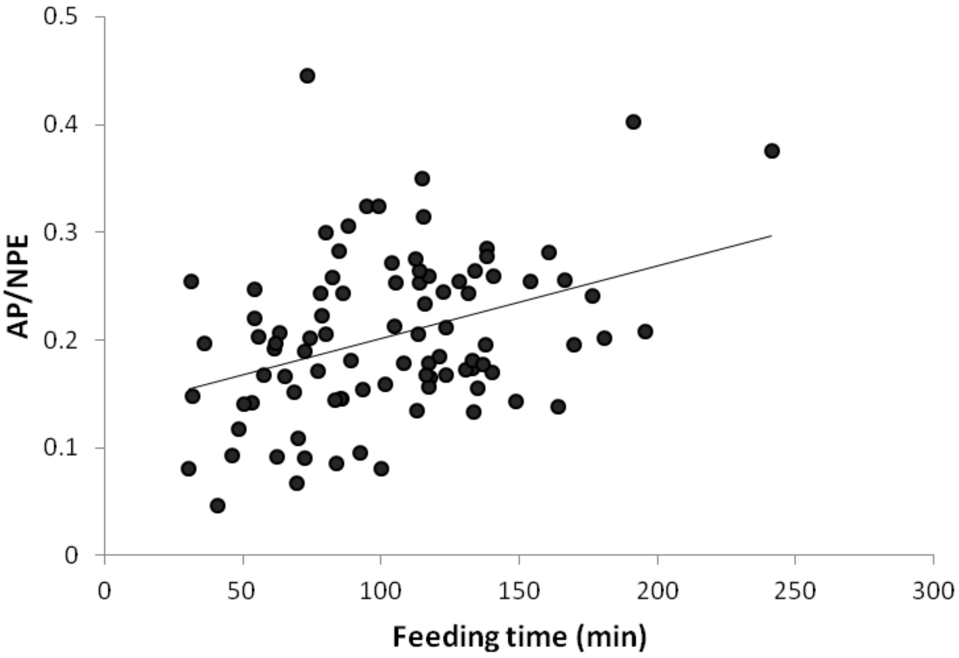

Figure 4.11. Bivariate plot of feeding time vs. ratio of available protein and non-protein energy. The trend line is shown for descriptive purposes only.

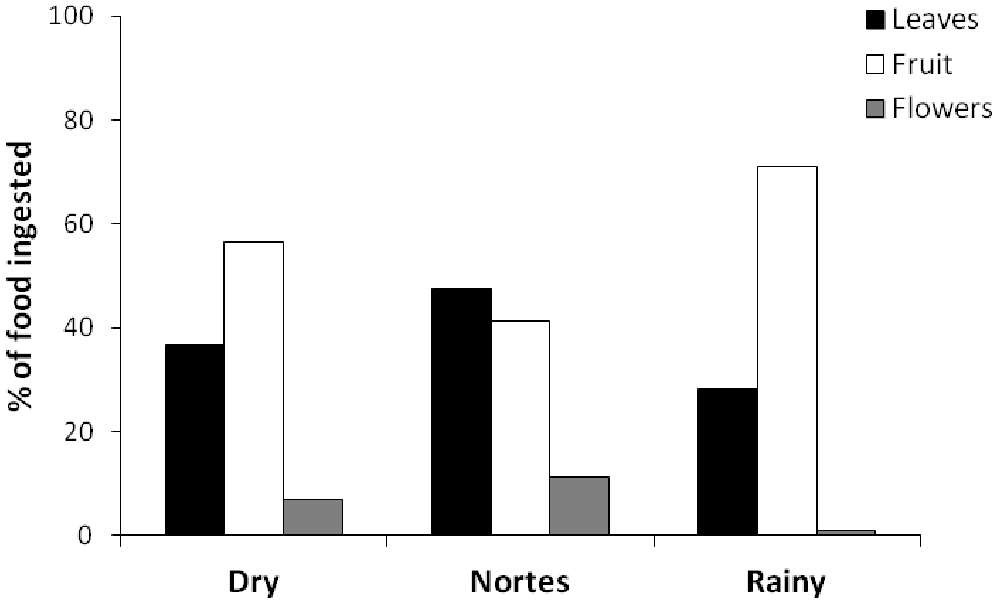

Figure 4.12. Leaf, fruit, and flower consumption during three different seasons. Percentages were calculated as grams of different items out of the total amount ingested.

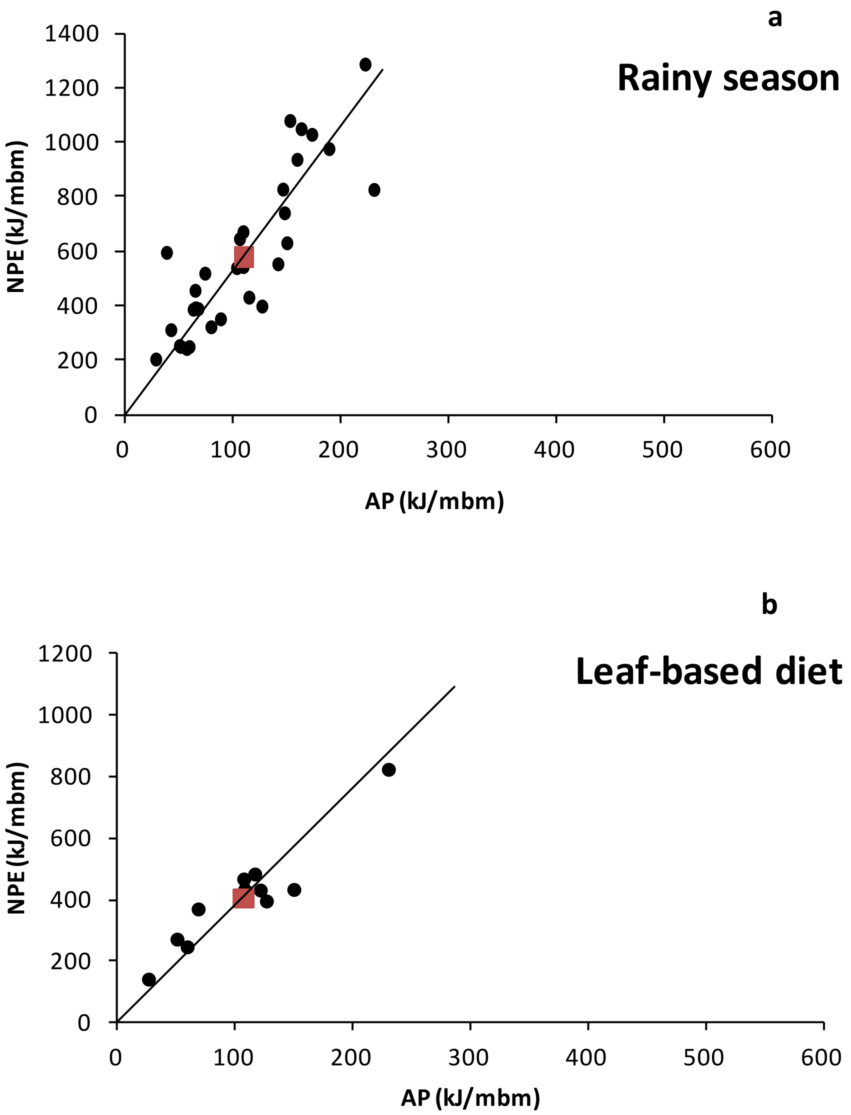

Figure 4.13. Daily intake of available protein (AP) and non-protein energy (NPE) (kJ/mbm) of adult male and female black howler monkeys in the rainy season (a), and on days in which leaves constituted >70% of the food ingested (b). Both patterns show a strategy of AP:NPE prioritization. The red square represents the average AP:NPE (assumed to approximate the intake target).

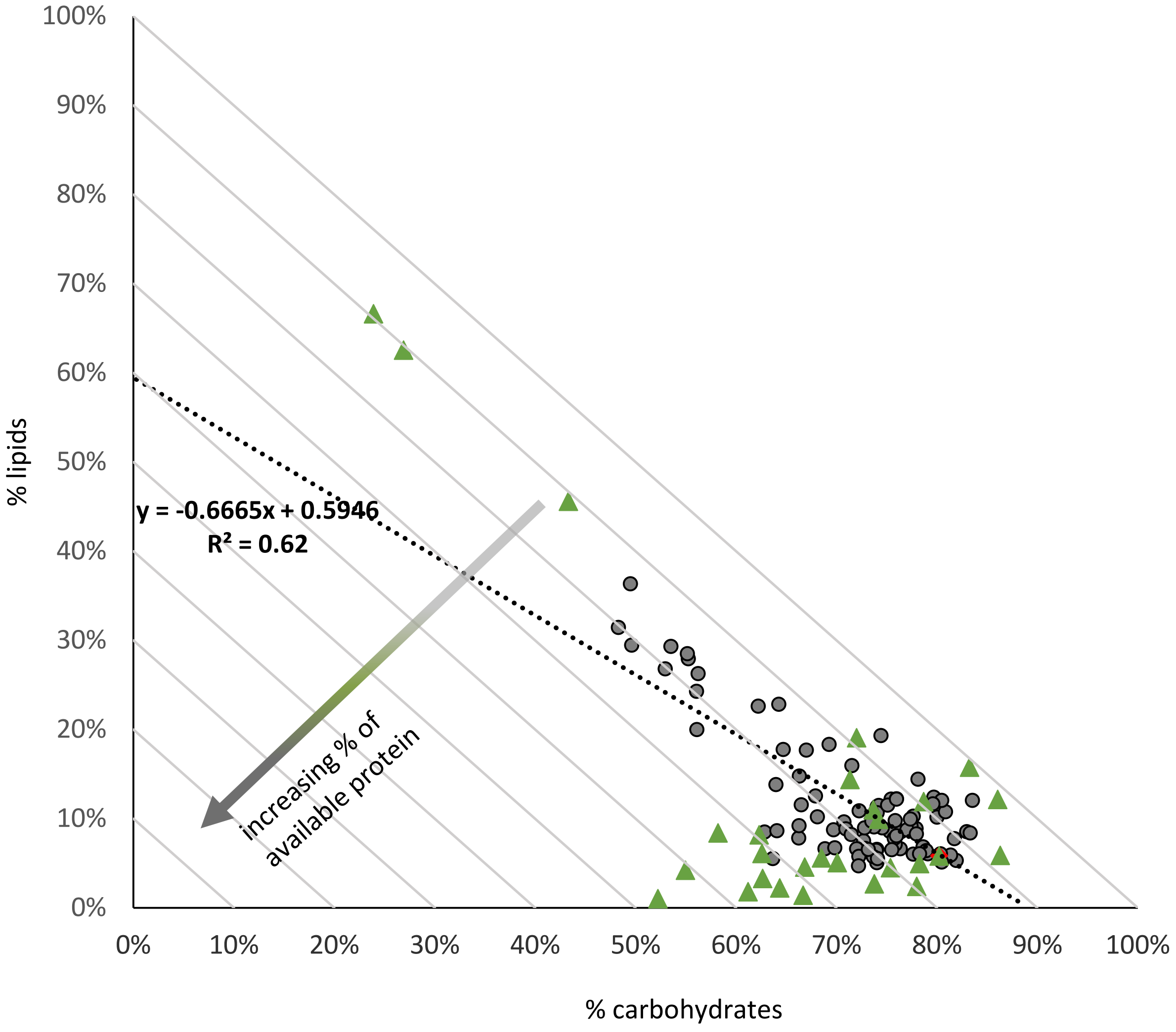

Figure 4.14. Right-angle mixture triangle showing the relative contribution of lipids, carbohydrates, and available protein (implicit axis) to daily metabolizable energy intake of black howler monkeys. Circles represent the nutritional composition of the daily diet and triangles the nutritional composition of foods representing >1% of the howler diet. The dotted line represents the linear regression of diet composition.

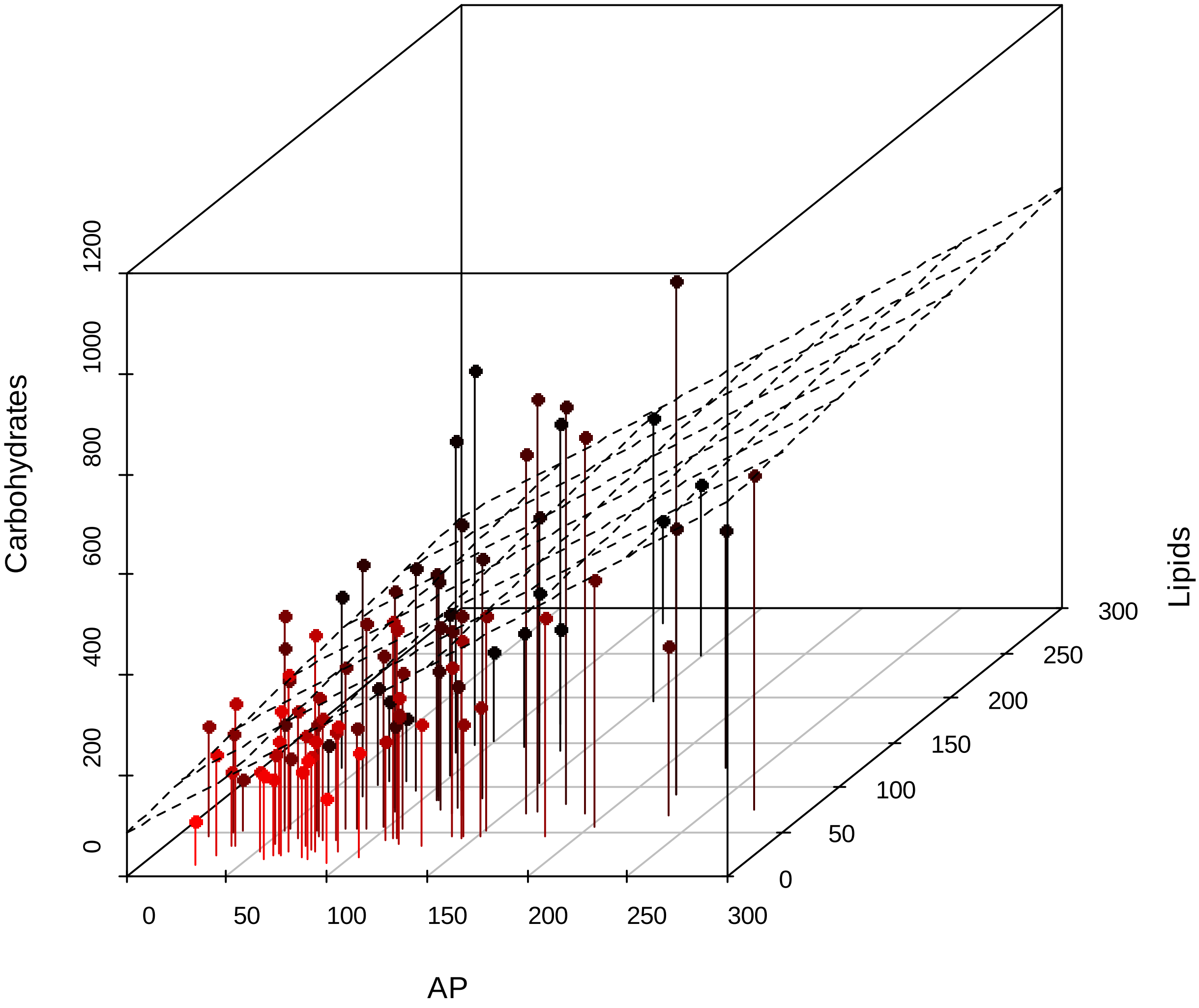

Figure 4.15. Three-dimensional scatterplot showing daily intake of energy (kJ/mbm) from AP, carbohydrates, and lipids. A regression plane also is shown.

Table 4.1. Composition of the two study groups (J and M), including data on body weight and body mass index of nine darted and marked adult individuals.

Group	ID	Age	Sex	Body weight (kg)	BMI[a] (kg/m^2)	Born (Mother)	Entered group	Left group
J	MA	Adult	Male	6.9	28.16			Mar 2011
J	MR[*]	Adult	Male	8.7	26.78			Mar 2011
J	CI	Adult	Male				Mar 2011	May 2011
J	TO	Adult	Male	7.5	23.08		Apr 2011	
J	AM	Adult	Male				Apr 2011	
J	CO	Adult	Female					
J	GO	Adult	Female					
J	MI	Juvenile	Male					Sept 2011
J	JJ	Infant	Male			Aug 2010 (GO)		
M	AZU	Adult	Male	8.4	36.46			
M	AMA	Adult	Male	7.9	30.37			Mar 2011
M	RBL	Adult	Male	7.7	25.45			
M	NAR	Adult	Male	5.7	25.80			
M	RAM	Adult	Male	7.9	27.60			
M	PAN	Adult	Female	5.8	29.96			
M	ROJ	Adult	Female	6.3	24.70			
M	AUG	Juvenile	Male					
M	JOR	Infant	Female			Nov 2009?		
M	IMC	Infant	Male			Feb 2010? (PAN)		
M	NAT	Infant	Female			Dic 2010 (ROJ)		
M	VAL	Infant	Male			Feb 2011 (PAN)		

[a] Body mass index (BMI) was calculated as body weight (kg) divided by the square of crown-rump length (m^2)

[*] Peripheral male even before leaving the group definitively. Focal samples of this male were not included in the analysis.

Table 4.2. Composition of the diet (% based on amount of foods consumed in grams) of the two study groups based on the entire data set (1300 focal hours).

	Dry	***Nortes***	**Rainy**	**Total**
YL	32.9%	11.7%	18.0%	22.2%
ML	5.6%	37.8%	12.2%	14.8%
Tot Leaves	**38.4%**	**49.5%**	**30.2%**	**37.0%**
MF	7.7%	12.2%	54.0%	29.0%
IF	46.4%	28.6%	15.2%	29.2%
Tot Fruit	**54.1%**	**40.8%**	**69.2%**	**58.2%**
Flowers	**7.1%**	**9.7%**	**0.6%**	**4.7%**

Table 4.3. Estimated mean (±SD) daily nutrient and energy intake (kJ per unit of metabolic body mass) for different non-human primate species

Species	Site	Food ingested (g dry weight/day)	Protein intake (g/mbm)	Protein (kJ/mbm)	NPE (kJ/mbm)	Total energy (kJ/mbm)	Reference
Alouatta caraya	Argentina	322 ± 60		168 ± 35[e]	842 ± 117	1011 ± 149	Fernández, unpub. data
Alouatta palliata	Panama	348	4.9 – 5.2			553	Nagy & Milton 1979, Milton 1979[a]
	Nicaragua	173 ± 58	7.0			548[c]	Williams-Guillén 2003
Alouatta pigra	Campeche, Mexico	218 ± 95	6.0[e]	102 ± 51[e]	526 ± 248 (140 – 1282)[*]	628 ± 286	This study
	Chiapas, Mexico	278 ± 69	8.6	130	430 (25 – 1500)[*]	580	Amato 2013[**]
	Tabasco, Mexico	116 ± 78	3.9[e]	66 ± 31[e]	296 ± 163	361	Aristizábal 2013
Ateles chamek	Bolivia	262		39[e]	373 (143 – 1271)[*]	412	Felton et al. 2009a,b
Gorilla beringei	Uganda			182[e]	512	852	Rothman et al. 2008, 2011
Pan troglodytes	Uganda					610[b]	Conklin-Brittain et al. 2006
Papio hamadryas ursinus	South Africa			(26 – 196)[*de]	(186 – 893)[*d]	499 ± 226[c]	Johnson et al. 2013
Pongo pygmaeus	Indonesia					647[b]	Conklin-Brittain et al. 2006

[a] All values are estimations. Energy intake is based on rates of CO_2 production in temporarily caged mantled howler monkeys

[b] Values calculated assuming a high level of fiber fermentation

[c] Original values in kcal converted to kJoules and divided by metabolic body mass

[d] Extrapolated from Figure 2 in Johnson et al. 2013

[e] Available Protein

[*] Minimum and maximum values are reported

[**] Calculations are based on literature estimates of nutritional values of the foods ingested

Table 4.4. LMM analysis of factors affecting daily available protein intake.

Parameter	**Estimate**	**SE**	**df**	**95% Confidence Intervals***	
				Lower	**Upper**
Intercept	1.43	0.25	76	0.92	1.93
Daily total feeding time	**0.93**	**0.07**	**76**	**0.78**	**1.08**
% of leaves consumed (based on grams)	0.21	0.11	76	-0.01	0.44

*If zero is not included in the CI, the effect of the parameter is significant (in bold)
SE = standard error, df = degrees of freedom

Table 4.5. LMM analysis of factors affecting daily non- protein energy intake.

Parameter*	**Estimate**	**SE**	**df**	**95% Confidence Intervals**	
				Lower	**Upper**
Intercept	5.22	0.13	76	4.96	5.47
Daily total feeding time	**0.02**	**0.003**	**76**	**0.01**	**0.02**
% of fruit consumed (based on grams)	**0.62**	**0.14**	**76**	**0.33**	**0.91**

*If zero is not included in the CI, the effect of the parameter is significant (in bold)
SE = standard error, df = degrees of freedom

Table 4.6. LMM analysis of factors affecting daily AP:NPE

Parameter	**Estimate**	**SE**	**df**	**95% Confidence Intervals***	
				Lower	**Upper**
Intercept	0.13	0.04	74	0.04	0.2
Season (Nortes)	**0.04**	**0.01**	**74**	**0.01**	**0.07**
Season (Rainy)	0.02	0.01	74	-0.003	0.05
Daily total feeding time	**0.04**	**0.01**	**74**	**0.02**	**0.07**
% of fruit consumed (based on grams)	**-0.11**	**0.02**	**74**	**-0.16**	**-0.07**

*If zero is not included in the CI, the effect of the parameter is significant (in bold)
SE = standard error, df = degrees of freedom

CHAPTER 5

CONCLUSIONS

This dissertation examined the nutritional ecology of black howler monkeys (*Alouatta pigra*) during a 15-month study incorporating ecological, behavioral, and phytochemical data, and provided new insights into ateline feeding ecology, nutrition, and energetics. Three different approaches, addressed in the three main chapters of the dissertation, support the overall result that resource mixing and nutrient balancing are integral components of the foraging and feeding decisions of howler monkeys. The main findings of this research and their implications are outlined in this chapter.

On an annual basis, the black howler monkey diet was found to be more fruit-based than expected, considering that howler monkeys have been described as behavioral folivores (Milton, 1978) and semifolivores (Rosenberger et al., 2011). I found that individuals consumed an equal amount (~29% of total dry weight consumed) of mature and immature fruits, which accounted for 58.2% of their diet. Leaves, in contrast, accounted for 37% of the diet. This fruit-biased pattern found in black howler monkeys is consistent with data reported for other howler species such as *Alouatta macconnelli* and *A. belzebul* (>50% fruits, when considering amount of food consumed) (Pinto and Setz, 2004; Garber et al., in press).

In *A. pigra*, leaves accounted for approximately 50% of the diet only during one season of the year (the *nortes*, a relatively colder period with lower precipitation than the rainy season, and low young leaf and mature fruit availability). Mature leaves were highly consumed during the *nortes* (38% *vs* 12% young leaves) and daily protein intake accounted for 20 ± 4% of total energy during this season. Phytochemical data indicated, however, that there were no significant differences in the nutrient content of mature and young leaves selected by howler monkeys, including available protein and protein-to-fiber ratio. Both young and mature leaves of some of the most commonly consumed species (e.g., *Bursera simaruba*) were found to contain moderate to high amounts of condensed tannins. How howlers are able to detoxify large amounts of condensed tannins remains unclear, however proline-rich proteins present in the howlers' saliva could counteract the negative effects of tannins due to their high binding affinity for these molecules (Milton, 1998; Espinosa-Gomez et al., 2012).

The analysis of how food selection was affected by the phytochemical characteristics of foods (Chapter 3) showed that howler monkey food selection was not driven by the protein content of foods or by the protein-to-fiber ratio of leaves; instead, individuals showed temporal preferences for foods high in lipids (specifically, *Dendropanax arboreus* and *Metopium brownei* mature fruits), whose importance in the diets of howler monkeys has been traditionally overlooked. A similar pattern of selecting foods high in lipids is reported for sakis (*Pithecia* spp.), which feed on seeds and arils that can contain >60% lipids (dry matter) (Norconk and Conklin-Brittain, 2004). These results indicate that howlers generally did not target specific food items or plant species based on their nutrient content, but when they did, as in the case of preference towards lipids during the *nortes*, it was to balance the intake of protein and non-protein ingested (i.e., to maintain a relatively constant ratio of protein and non-protein energy), rather than maximizing the intake of energy or a particular nutrient.

The analysis of feeding patch choice (Chapter 2) revealed that black howler monkeys moved between food patches characterized by resources of differing nutritional composition. In only four occasions the howlers did deplete a feeding patch. In addition, behavioral observations indicated that aggression at feeding sites was rare (0.018 aggressions/hour), and therefore feeding competition was not a primary factor affecting howler patch choice decisions. In the majority of the cases (49.4%), howler monkeys left a patch to move from one food type to a different food type. Switching between young leaves to immature fruits, and from fruits to young leaves, occurred more frequently than expected by chance. Protein intake was higher in leaf than in fruit patches, thus moving between fruit and leaf patches was associated with different nutritional intakes. Based on this evidence, nutrient balancing appeared to be the most critical factor in howler monkey patch choice.

The analysis of howler daily nutrient intake using a nutritional geometry approach (Chapter 4) provided direct evidence that howler monkeys maintained a constant ratio of protein and non-protein energy despite seasonal changes in the specific food items consumed and the proportion of food types exploited (i.e., when they changed from a primarily fruit-based diet, to a diet characterized by a higher intake of leaves and flowers). In this regard, black howler monkeys differed from Ugandan mountain gorillas (*Gorilla beringei*) and Bolivian spider monkeys (*Ateles chamek*) in their nutrient regulation strategy. Whereas gorillas were found to prioritize, and maintain a constant seasonal intake of non-protein energy, spider monkeys were found to prioritize and maintain a constant daily intake of protein (Felton et al., 2009a; Rothman et al., 2011). Protein prioritization, or a strategy associated with the regulation of protein intake rather than other nutrients, and a feeding pattern of maintaining a constant daily protein intake despite variation in the intake of carbohydrates and lipids, also has been suggested for a population of black howler monkeys in Palenque, Mexico (Amato and Garber, in press). However, in that study the authors did not conduct focal follows of the same individual during the entire day, calculated nutrient and energy intake based on published nutritional values for neotropical plant species, and used values of crude protein estimates rather than available protein. Thus, their finding of protein prioritization is either best considered as a general estimate of howler monkey nutritional strategies, or, based on differences in forest productivity and habitat types, it can be considered as evidence that the nutritional strategies of a given species might vary according to site-specific ecological characteristic. Protein prioritization also has been described in humans (Simpson et al., 2003; Simpson and Raubenheimer, 2005) and linked to problems of overingestion of fat- and carbohydrate-rich foods, leading to obesity. Overall, these studies suggest that 1) nutrient prioritization strategies are common among a wide range of primate taxa, and 2) nutrient regulation may be less tied to a specific dietary emphasis (e.g., leaf-enriched or fruit-based diet), but a response to ecological factors. For example, site-specific characteristics such as the nutritional composition of available foods, the presence/absence of staple food species, and the intensity of fluctuations in resource availability appear to play an important role in determining the type of nutritional strategy adopted by different primate populations. Thus, the use of nutritional geometry in identifying the nutritional prioritization and nutrient balancing strategies of free-ranging animals is an important tool that should be implemented to analyze food choice, feeding ecology, and decision-making associated with why leave a patch and which patch to visit next.

Another significant finding of this dissertation was that the average daily metabolizable energy intake of individuals (628 kJ/mbm) was higher than what was reported for a population of “ripe-fruit specialists” such as spider monkeys (*Ateles chamek*) (~400 kJ/mbm) (Felton et al., 2009a; Felton et al., 2009b; Di Fiore et al., 2011). Also daily energy obtained from lipids and carbohydrates was on average higher in *A. pigra* (526 kJ/mbm) than in *A. chamek* (373 kJ/mbm). These results highlight the capacity of howler monkeys to maintain high energy intake even during periods of leaf consumption, and offer an explanation as to how howlers can afford the high rates of reproduction and rapid growth that characterize them compared to other atelines (Ross, 1991; Leigh, 1994). Given that life history strategies are characterized by a series of dissociable components that respond to different selective pressures (Blomquist et al., 2009), the importance of diet and energetics in shaping life history characteristics should not be underestimated. Future studies should focus on estimating energy balance in howler monkeys, taking into account not only energy intake, but also daily energy expenditure to more precisely assess the amount of energy available to invest in processes such as rapid growth and reproduction. Additional research also is needed to determine if patterns of high energy intake and balancing protein and non-protein energy intake are shown by black howler populations at other sites, by other howler monkey species, and by other atelines. Similar studies are currently being conducted on *A. pigra* in Tabasco, Mexico (Aristizabal, 2013), and on *A. caraya* in Argentina (Fernandez, in prep.), thus offering opportunities for comparisons that will be valuable for advancing the field of nutritional ecology.

Howler monkeys are one of the most studied neotropical primates, and studies that focus on basic aspects of their diet are numerous (Di Fiore et al., 2011). A recent review shows a much higher sampling effort towards atelines, and *Alouatta* spp. in particular (37.4% of all studies analyzed), compared to other neotropical primate taxa, even when differences in geographic distribution were taken into account (Hawes et al., 2013). However, this dissertation revealed that the results obtained using traditional methods of feeding time and assumptions on the nutritional content of fruits, leaves, and flowers, are at odds with new analytical frameworks that focus on nutritional geometry. Thus, we need to re-examine what is known of howler feeding ecology and nutrition. In particular, the quantification of feeding rates; the estimation of daily amounts of food ingested; the calculation of daily nutrient and energy intake; the assessment of available protein rather than crude protein in the plant parts consumed by howler monkeys; the calculation of food selectivity indices based on measures of food availability rather than on plant species abundance; and the identification of nutrient prioritization patterns, all contributed to offer a more comprehensive picture of howler monkey nutritional ecology. This research, in fact, showed that hypotheses regarding primate dietary strategies, energy conservation, and adaptations to overcome energetic stresses are based on assumptions (e.g., that the howler monkey diet is energy-limited) that are not supported by empirical data. One of the goals of this dissertation was to demonstrate that using direct measures of nutrient and energy intake, it is possible to reliably assess the nutritional status and nutritional goals of wild primates. This approach is applicable to many primate species, and understanding the nutritional and energetic strategies of primate taxa characterized by different dietary patterns will offer critical insight into the behavioral, digestive, and physiological adaptations that human and non-human primates have acquired to exploit resources varying in their phytochemical characteristics under different environmental pressures.

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