Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Amy K. Cobden Date

Party Animals: Food, Sociality and Stress in Wild Bonobos (*Pan paniscus*) of Iyema, Lomako Forest,
Democratic Republic of Congo

By

Amy K. Cobden
Doctor of Philosophy
Graduate Division of Anthropology

_____

Sarah Gouzoules
Advisor

_____

Craig Hadley
Committee Co-Chair

_____

Kim Wallen
Committee Member

_____

Frans de Waal
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Party Animals: Food, Sociality and Stress in Wild Bonobos (*Pan paniscus*) of Iyema, Lomako Forest, Democratic Republic of Congo

By
Amy K. Cobden
B.A. Oberlin College 2003
M.A. Emory University 2010

Advisor: Sarah Gouzoules, Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Graduate Division of Anthropology

Abstract

Party Animals: Food, Sociality and Stress in Wild Bonobos (*Pan paniscus*) of Iyema, Lomako Forest, Democratic Republic of Congo

By Amy K. Cobden

Stress is a part of everyday life for everyone. But how has it shaped our ability to adapt to unpredictable environments without compromising our capacity to keep our friends close (or our enemies closer)? My research explores this question through the lens of one of our closest cousins, the bonobo (*Pan paniscus*), to see how a species known for its gregarious and relaxed social structure responds to pressures from the outside world. Bonobos and Chimpanzees (*Pan troglodytes*) are the two closest primate relatives to humans. We share over 98% of our genetic blueprint with them, and they share more than 99% with one another. However, behaviorally they are remarkably different. Chimpanzees are hierarchical, male-dominant and use aggression to resolve conflicts. Bonobos, on the other hand are egalitarian, female-biased and use a wide array of sexual behaviors to resolve conflict, regardless of age or sex. Their diverse and elaborate sexual repertoires are pronounced when food is present, suggesting that food-related pressures fostered this notable behavioral adaptation. This raises the question: Do bonobos experience food stress, and if so, how do they respond? Popular theories that attempt to explain differences in social behaviors between both species of *Pan* often include the hypothesis that terrestrial herbaceous vegetation (THV) is a key factor in maintaining large party sizes seen in bonobos. Although this theory has been negated repeatedly over the years and across sites, there are still gaps in our understanding about its role in wild bonobo diets. Using a year's worth of behavioral and ecological data from a community of wild bonobos and their environment, I examined seasonal patterns in resource availability and diet in the Iyema community, corresponding seasonal patterns in bonobo nest party sizes (a measure of sociality), and energetic hormonal profiles that accompanied these changes to see if shifts in resource availability and sociality were reflected in three key hormonal metabolites associated with different kinds of stress. I found that the Iyema forest displayed seasonal patterns in both fruit abundance and diversity, and that both these factors proved significant in predicting nest party sizes, using a generalized linear mixed model. THV was the third most frequently consumed food item in the Iyema bonobo diet, but its consumption was significantly negatively correlated with an increase in party size. Moreover, THV consumption showed distinctly seasonal patterns, suggesting that its nutritional properties should be reexamined on a seasonal scale. Urinary C-peptide (which is a direct measure of insulin) and urinary cortisol (a glucocorticoid involved in multiple stress responses) were predictably inversely correlated in the context of diet, environmental diversity and shifts in party sizes, showing that a) bonobos experience periods of environmentally-related energetic stress that affect their sociality and b) long-term use of non-invasively collected hormonal metabolites show merit in demonstrating complex social and energetic relationships in wild primates.

Party Animals: Food, Sociality and Stress in Wild Bonobos (*Pan panisciscus*) of Iyema, Lomako Forest, Democratic Republic of Congo

By
Amy K. Cobden
B.A. Oberlin College 2003
M.A. Emory University 2010

Advisor: Sarah Gouzoules, Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Graduate Division of Anthropology

Acknowledgements

I was 16 when my older brother told me what a bonobo was, and I immediately had the sense that studying them in the wild was directly on par with other wild childhood fantasies of adult jobs, like being an astronaut, a professional athlete or the president of the United States; highly unlikely and completely awesome. So I suppose I should thank my brother first here, for planting such a ridiculous idea as to go to the congo to study the elusive "hippie-ape" in my head.

This project could not have happened without a major cooperative effort on part of many, many people, over multiple continents and over almost an entire decade. I suspect that anyone reading this will appreciate that conducting a project where you have to spend over a year and a half in the DRC collecting data, followed by over a year in the lab is a challenging endeavor by itself. Add onto that managing a camp, a staff and a hefty budget, losing field assistants mid-way through data collection, other people then losing your data, coming home and losing your lab and your mentor in quick succession and finally running out of money before you can finish- that is challenging. Keeping track of who to thank has been a task in and of itself.

First and foremost, my humble thanks to Pat Whitten. 10 years ago, when I decided I wanted to study non-invasive endocrinology in bonobos, I wrote to four labs, asking if I could stand in the corner and learn their methods, and with some brilliant luck Pat happened to need a lab technician. She has been nothing but supportive, helpful, encouraging and entertaining over the years. Were it not for Pat, I have no doubt my life would be very different today.

My committee has undergone many transformations since I first proposed this project, and I am deeply grateful that in the end, I had a committee that was flexible, supportive and widely balanced in their fields and criticisms. Thanks to Sally Gouzoules for challenging me to raise my own bar early on in this process and for standing by me through it, with all the changes that have taken place over the last 5 years. Thank you, Kim Wallen, for tolerating my unfortunate use of the word, "handful" so frequently when referring to fecal samples, for your advice, your critique and above all your patience. I am not sure if I can tell you, or anyone how satisfying it is to gain your slow-coming acceptance of my methods. I bought Frans de Waal's book, Bonobo The Forgotten Ape from a used bookstore in college and it was largely thanks to Frans and his popular science writing that I pursued this particular field of study during and after college. It is a strange and satisfying kind of gratification to gain the attention and approval of someone whose work inspired you to go forth with your own. Thank you, Frans, for helping to hook my interest early on, and for helping me close this chapter over a decade later. Thank you as well, to Craig Hadley for stepping up, and into my patchwork committee. And a collective thank you to all of you for some of the most meaningful compliments I have, or might ever receive.

Sarah Pruett opened the door for me at the Biomarker Core Lab, at Yerkes, and in doing so, also opened a door in a dark cloud to reveal a very important silver lining. This project took a decidedly positive turn thanks to her and her expertise, particularly with the LC/MS. Thank you, Sarah, for putting up with my stinking samples, my tardy ways, my incapacity to do conversions, and for patiently walking me through the astoundingly intimidating and complex process of learning liquid-chromatography/mass-spectrometry in under a year.

This study was designed and executed over a span of years that saw major advances in the ways that researchers in my field statistically analyze extremely complex systems ecology data. I have never been good at statistics, and was presented with a tremendous dilemma when it came to choosing how to present the outcomes of my data to my peers, who span a wide range of thinking when it comes to statistics. By some unbelievable stroke of luck, I found a friend who has a passion for complex statistical modeling and primate ecology. I can't put a second author on this dissertation, but if I could, it would be Brendan Barrett. Thank you for spinning all this straw into gold as well as your shared enthusiasm of R. Kelly. I wonder if you had any idea what you were getting yourself into when you agreed to help me with all of this.

Thanks to Teague O'Mara for continuously setting a bar that I will probably never reach (you are much taller than I am), but which I keep jumping for. You have been an invaluable friend and peer over the years and I think that I am profoundly lucky to say as much.

It's a small community of people who study primates in the DRC, and I'm fortunate to have many of them as friends and peers. Thanks in particular go to Zanna Clay, for her unflinching support and good humor even now as she is all the way over in my old stomping grounds at Lui Kotal. I wish you were here, but I'm happy for you that you're there. Thanks to Laura Darby for her timely help with power points, maps, graphics and for being a willing recipient of my incipient rants and for holding the mirror up when I needed it most. Thanks to Frances White, without whom I would never have been introduced to Lomako. Thanks to Michel Waller, for his friendship and support in my early stages of pilot data collection; I also hope we get to play Weo again some day. Thanks to Jef Dupain, for hiring a stranger to do the impossible and for providing me with so many unforgettable and priceless lessons in self-reliance. Thanks to Ethan Gicker, for your good humor during so many days with a compass and a machete and nothing else, and for making the best of getting stranded in uncomfortable places. Thanks to Kate Roach and Doris Wu for their help with data collection and entry, for their insights, their stories, their uplifting company at the end of a long year and a half in the jungle, and for continuing to be marvelous friends.

Big thanks to Angie Kilduff, Chelsea Martinez, Katie Hall, Sarah Barks, Sarah Freeman, Vicki Alla, and Winnie Eckhardt for their helpful comments, criticisms and questions while I was writing this; your remarks and encouragement kept me awake in spite of myself and kept me motivated when I really needed it most. I can't believe how many of you actually read my drafts and kept going.

Many friends of mine have provided support of all kinds over the years in ways that have directly and indirectly helped me move forward with this work, and I'm sure I'm forgetting many of them in this paragraph. Thanks go to Ollie Green and Dave Hughes, whose friendship, cooking and impeccable humor have kept my spirits up through so many frustrating times. Everyone should be so lucky as to have friends like them. Betsy McCann, thank you for being absurd, and hilarious, and for sitting up with me in the middle of the night proof-reading my reports, and preventing me from killing other people or myself in the process- you've saved more lives than you can imagine. Randi Cappelletti regularly awes me with her energy, her wit and her heart. Thanks, Sarah Crossley and Aminda Warburton for keeping me fed for over a year when I was clearly not going to cook for myself; you probably saved me from some kind of gastronomic-

related cancer.Beth Turner and Matt Livermore, thank you as well, for the food, the drinks, the advice and for being so weird. Thank you, Joanna Newsom, for shooting the moon (albeit a different moon) with me; growing up with you has been one of the truest privileges of my life.

One of the greatest things about getting older is the depth to which you begin to understand and appreciate your parents. I don't have children yet, so I have a hard time imagining my reaction if my 22 year old daughter told me that she wanted to go to a remote snake-filled jungle, days away from the nearest hospital in a country mostly known for its very recent, very violent civil war to study an *exotic animal*. I would probably tell her to pay for it herself. I am fortunate not to have myself for a parent. Thank you, Mom and Dad, and Eric, Laura and Carone, for cheering me on for so many years even though the odds of this working out to financially support you in your retirement are slim-to-none. I hope some day I can be so optimistically selfless to my own offspring. It's good for all of us that my two siblings are attorneys. Thank you Michael and Cameron, for choosing more lucrative careers, and for having grandchildren earlier so that I could go off and chase monkeys in peace.

Thanks to the Leakey Foundation, for their financial support and for extending their humanitarian attitudes as far as to humor my tardy attitude regarding reporting periods. Thanks to the Oberlin College Alumni Fund, for helping to fund pilot work in 2007. Thanks to African Wildlife Foundation for both supporting important long-term research that I could not have afforded otherwise, and simultaneously deterring me from a path in life I would probably deeply regret.

Lastly, I offer my deepest thanks to the men and women of the Lomako Conservation Science Center: Bofaso-Longi Pauline, Estelle Loleko, Mangue Bofaso Jonas, Bomposo Ikwa Dipon, Lokuli Impempe Rena, Boyou Banana, Bompoma Longondo Sayo, Bompoma Beken, Nkombe National, Ikwa Bofaso Depot, Iyambe Ifulu Charment, Koral Baarda-Boraso, Lokuli Lomboto Isaac, Lofoko Gedeon, Bofoloa Ilombe Cyrri, Christian Djambo, Iyambe Belvu, and of course, Ikala Lokuli Mila and Mawa. Merci de m'avoir permis de gagner votre respect, et pour le mien gagner. Nous vous remercions de permanence me mettant au défi de devenir une meilleure personne, et me laisser vous mets au défi de retour. Merci de m'avoir aidé à recueillir un données que c'est incroyablement vaste, pour prendre les choses comme les selles à laver, balayer les sentiers forestiers avec des balais faits maison, et apprivoiser poulets sérieux (peut-être pas autant de la part de la poule apprivoiser). Merci pour tant de jours et de nuits d'histoires hilarantes, tristes, terribles, bizarre et sérieux autour de la table du dîner. Je ne regrette pas toujours le cas, mais je m'ennuie chacun de vous tous les jours.

Table of Contents

Table of Figures and Tables:

Chapter 1

Introduction

Introduction:

Bonobos are the least studied of the great apes, and the ecological factors that influence their social dynamics in the wild are unclear on many levels, particularly with respect to feeding competition. The social behavior of bonobos (*Pan paniscus*) is different than that of chimpanzees (*Pan troglodytes*), and in many ways, most other primates. The female-based, egalitarian social structure of bonobos differs strikingly from the male-dominated hierarchies found in most wild chimpanzee communities. The hallmarks of these differences in bonobos are best described in terms of bonding between non-related females, in addition to a suite of elaborate non-reproductive social sexual behaviors.

Scientific field studies on bonobos (*Pan paniscus*) did not begin until the 1970's, and in spite of decades of dedicated efforts by a small but persistent community of researchers, there are still large and cumbersome gaps in our understanding of bonobo socioecology. In contrast, the depth and breadth of what we do know about the bonobo's closest relative, the chimpanzee (*Pan troglodytes*) has repeatedly and increasingly demonstrated that variability of behavior and social structures in spite of ecological overlap is possible within the same species. Generally, differences between the two species have been highlighted in terms of social relationships between the sexes and conflict resolution/avoidance. Both species live in fission-fusion social structures where females leave their natal group when they reach maturity, and emigrate into communities where they are unlikely to be related to other individuals. Female chimpanzees are generally understood to experience hostility and aggression from other females during this time, presumably because they present competition for limited resources. Emigrating

bonobos, however, have been observed to directly approach dominant females and solicit sexual behaviors that are suspected to ease their transition by establishing a kind of rapport within the community. While male chimpanzees, more likely to be related to one another will form alliances and spend more time proximally to one another, and generally form the core of feeding party compositions, bonobos demonstrate the opposite. Females form the core of feeding parties, and when related individuals are bonded, it has long appeared to be the case that mother-son bonds play a much more important role than brother-brother bonds. Conflict, often seen in the form of feeding and mate competition is typically resolved with aggression in chimpanzees, whereas in bonobos an elaborate repertoire of sexual behaviors that transcend sex and age are used. These differences are general, and have been observed with a great degree of variation in chimpanzees, and some degree of variation in bonobos. We have yet to fully comprehend the extent to which bonobos differ between communities and across their range, but it seems likely given what we know about chimpanzees that they will also present variations on what has so far been a common theme of egalitarian, female-based social structure.

Humans and both species of *Pan* diverged from a common ancestor approximately 8 million years ago. Sometime between 800,000 and 2.7 million years ago, the ancestral population of both species of *Pan* was separated (Morin et al. 1994; Gagneux et al. 1999; Yu et al. 2003; Fischer et al. 2004), probably due to a global warming event during the Pleistocene that caused the Congo Basin to flood, creating a geographic barrier (the Congo River) that continues to draw a physical line between chimpanzees and bonobos to this day (Eriksson et al. 2004; Hvilsom et al. 2014).

In light of the significant overlap they share in both genetic heritage and geography (historical and current), as well as the clear differences in their social behavior and structure, the evolutionary processes behind the behavioral divergence seen in *Pan* have been the subject of debate for decades. In the early 1980's, it was proposed that differences in their feeding ecology played a critical role in their behavioral divergence (Wrangham 1986). In particular, the hypothesis that chimpanzees and allopatric gorillas experienced harsher seasonal shortages in food and increased competition for resources at critical periods during their evolution took a major foothold in the debate about differences in *Pan*. Presumably, these pressures are what led chimpanzees, particularly males to select for more aggressive behaviors. Bonobos, the theory assumes, evolved in an environment supposedly free of feeding competition with other similar megafauna. If, or when, food shortages occurred, the argument continues that they had a readily available supply of terrestrial herbaceous vegetation (THV) to graze on while moving through the forest looking for fruit patches. This steady supply of THV was key in supporting large feeding parties in the absence of large fruit patches, and it consequently enabled the gregarious social structure we see in bonobos today. The hypothesis that THV supports large party sizes in bonobos has driven decades of research investigating their socioecology: the degree to which their environment is seasonal (White 1998a; Furuichi et al. 2008; Mulavwa et al. 2008) similarities and differences in their feeding ecology to chimpanzees (Chapman, White, and Wrangham 1994; White 1996b; Kano and Mulavwa 1984), and the extent to which THV plays a role in their diet (Malenky, Kuroda, and Ono 1994; Malenky and Stiles 1991). Although much has been revealed about the socioecology of bonobos, particularly the degree to which they overlap with

chimpanzees (e.g. Stanford 1998), no single factor by itself has been shown to account for shifts in party sizes (feeding or nesting) in bonobos (Furuichi 2009). The question remains whether or not bonobos experience periods where availability of resources, be it low or high affects their social dynamics. Additionally, understanding how physiological stress relates to these environmental and social shifts is a meaningful link that is currently missing in this picture.

Changes in feeding party size and composition have been reported to shift in sync with seasonal variation of fruit abundance; feeding parties became smaller (presumably due to increased feeding competition), females continued to maintain all-female social groups, while males became more solitary (White, 1998). Bonobo females are also widely considered to dominate males in feeding contexts, sometimes through the formation of coalitions, and sometimes possibly due to male deference as a mating strategy (Kano 1980; Parish 1996; Hashimoto et al. 2008; Hohmann and Fruth 2007). Priority of access to resources is accordingly linked with dominance, a relationship that has served as a proxy for reproductive success in other species (Abbott et al. 2003). Bonobos are known to exhibit dominance hierarchies (though, not strict ones) in the wild (White 1996; Furuichi et al. 1998; Hohmann and Fruth 2002). Yet the link between their ecology and social organization as it relates to fitness remains unclear. The ecological models that have been used to explain fundamental differences in the social organization of *Pan* assumes that the stress of feeding competition (which is expressed and experienced via aggression, or malnutrition) directs the social and reproductive strategies of both males and females.

Have female bonobos been selected to be social in the absence of stress associated with severe feeding competition? Or has the kind of feeding competition that they have been (and continue to be) subject to led them to adapt in ways not yet accounted for by existing theories of kin selection and feeding ecology? The relationship between party dynamics and stress in bonobos is only recently coming into focus (e.g. Surbeck et al. 2012). This dissertation is one of the first in a new wave of long-term (more than 2 months) studies that are closely re-examining aspects of bonobo socio-ecology with new tools, such as non-invasive endocrine assessment. Each of the chapters here build upon one another as a means of revisiting old, unfinished attempts at explaining bonobo social structure through environmental mechanisms.

In addition to this new layer of analysis, I am also contributing a full year data set on a study community (Iyema), discussed in Chapter 2, which had previously been studied, but for which very little long-term socio-ecological literature has been published. In Chapter 3, Seasonality, I address the question of stability in the Iyema forest using a year long data set that measured environmental resource availability. In Chapter 4, I examine the consumption rates of THV by the Iyema community, and its connection to sociality, showing (in Chapter 5) that if anything, increased consumption of THV prevents large-scale social cohesiveness. Chapter 5, Nest Parties addresses the relationship between environmental resource availability (measured through abundance of resources and diversity of available preferred fruit species) and show that changes in environmental diversity most strongly predict nest party size. In Chapter 6, I reconsider all of the socio-ecological questions addressed in previous chapters with the added lens of energetic hormones, collected non-invasively through feces and urine and analyzed using state of

the art technology, paving the way for further studies to build upon. Ultimately I find that relationships, which are not totally clear from a single perspective (e.g. nest party sizes vs. seasonal shifts in fruit availability) become evident when contextualized with metabolic hormones.

This study contributes a small, but timely footnote in the story of human evolution through the new perspective it gives on forces acting on bonobo socioecology. Primatology is a relatively new field compared to other fields that inform how we understand what it means to be human. Within that framework, our understanding of chimpanzees in particular has been extremely influential in how we perceive ourselves, creating a false, and lopsided understanding of how and why certain human traits "make sense" in light of our deeper evolutionary past. Bonobos present a counter-reference to a biological narrative that highlights aggression and male dominance, but are too often delegated to the post-scripts and footnotes of theories that propose to account for *Homo* and its evolutionary baggage because of the contradiction they present to popular socioecological theories (Wrangham and Peterson 1997; Parish et al. 2000). Taken parsimoniously, this study tells us that simple ecological correlates, such as preferred species of fruits, general abundance and the potential for unlimited fall back foods is not likely to be the sole pressure that led to differences between these two species. In turn, this should tell us that it is not just the constant search for food in an uncertain environment that led us to be distinctly human. It was probably a much more complicated mix of things. Why our ancestors "left" the trees and then the forest should be the major question in understanding our deepest roots that distinguish us from our closest cousins.

It could have been in search of food, or it could have been because they had an innate curiosity to move and explore in search of greener pastures.

What is clear with this study is that the Iyema forest, at least for the duration of one year, was as close to as it could be to what many have hypothesized the ancestral bonobo forest to be: Eden. The hormonal data suggests that within the confines of "Eden," however, that subtle changes in the environment are likely to impose costs that limit bonobo sociality, and that bonobos maintain their levels of sociality in spite of these costs.

Chapter 2:

Lomako Forest and the Iyema Study Site

Chapter 2 Abstract

The study site Iyema (00°55) North, 21°06) East) is located north of the Lomako river, inside of the greater Lomako Forest area, within the Equateur Province in north-western Democratic Republic of Congo. It is approximately 15km North of the well-known Ndele study site (also more generally referred to as Lomako). The Iyema study site was started by Jef Dupain and colleagues in 1995, who established a research program that continued through 1999, when civil conflict took over the entire country. At the time they were able to identify 11 individuals and estimated the community to be as large as 50 individuals. In February of 2010 I returned to Iyema with a team composed of local staff and assistants from the U.S. and DRC to recommence long-term research on the Iyema bonobo community. We rebuilt a small camp, established over 35km of trails, including 28.8 main transects and 14.4km of phenological trails. 1805 trees and lianas known to be consumed by bonobos were identified and labeled for monthly monitoring. A general survey of the study site's forest types show that it is composed mainly of old growth primary forest on terra firma soil, with very little swamp compared to other well studied bonobo sites; the terrestrial herbaceous vegetation species known as *Haumania librechtstiana* is present across the majority of the study area. The diversity and density of preferred fruiting trees across the study site was relatively evenly spread. The community, in spite of some hunting pressure over the years was found regularly (approximately 3 times/week) within a relatively small core area (16km2) during the course of the study period. The community was not habituated enough for detailed behavioral follows, but tolerant enough to permit observers for hours at a time. It is too early to estimate the full home range and size of the Iyema community, but by the end of the study period 21 independent individuals were identified.

The Iyema Study Site

2.1 Lomako Forest

The study site Iyema (00°55) North, 21°06 East) is located north of the Lomako river, inside of the greater Lomako Forest area, within the Equateur Province in north-western Democratic Republic of Congo (Van Krunkelsven et al. 1999; fig.'s 2.1.a &b). It is approximately 15km as the bird flies from the more well known study site, Ndele (fig. 2.1.c). Ndele was established as a research site in the early 1970's by Randall Susman, and Noel and Alison Badrian (Badrian and Badrian 1984) Ndele eventually became one of two primary locations for seminal research on wild bonobo behavior and ecology (Badrian and Badrian 1984; Susman 1984; Malenky and Stiles 1991; Doran 1993; Malenky, Kuroda, and Ono 1994; White 1996b). In the early 1990's, Gottfried Hohmann and Barbara Fruth began their work at the Eyengo study site, approximately 3 km from the Ndele site, with a neighboring community of bonobos (Fruth and Hohmann 1993). Research at the Iyema study site was begun in 1995 by Jef Dupain and colleagues, who established a camp, a series of phenological trails and a research program that focused on habituating the Iyema community and collecting basic information about their environment (Van Krunkelsven et al. 1999; Dupain et al. 2000). Iyema ran semi-continuously from 1995 to 1999, when Dupain and his colleagues were forced out of the study site at gunpoint by members of the national army. Between 1999 and 2010 the site was visited semi-regularly by local members of Dupain's team, and was occasionally walked through by census teams conducting surveys on bonobo density in the area, but

was not reopened as a study site until my arrival in February 2010. As a result, members of the local community frequently hunted within it between 1999 and 2010.

The new Iyema study site was set up with the assumption that Dupain's study community was still occupying the same home range, based off of promising encounters and observations with the Iyema community in August 2009 and February 2010. My team and I established our trail system based on trail systems used by Dupain et al. in the 1990's (Fig. 2.2.b).

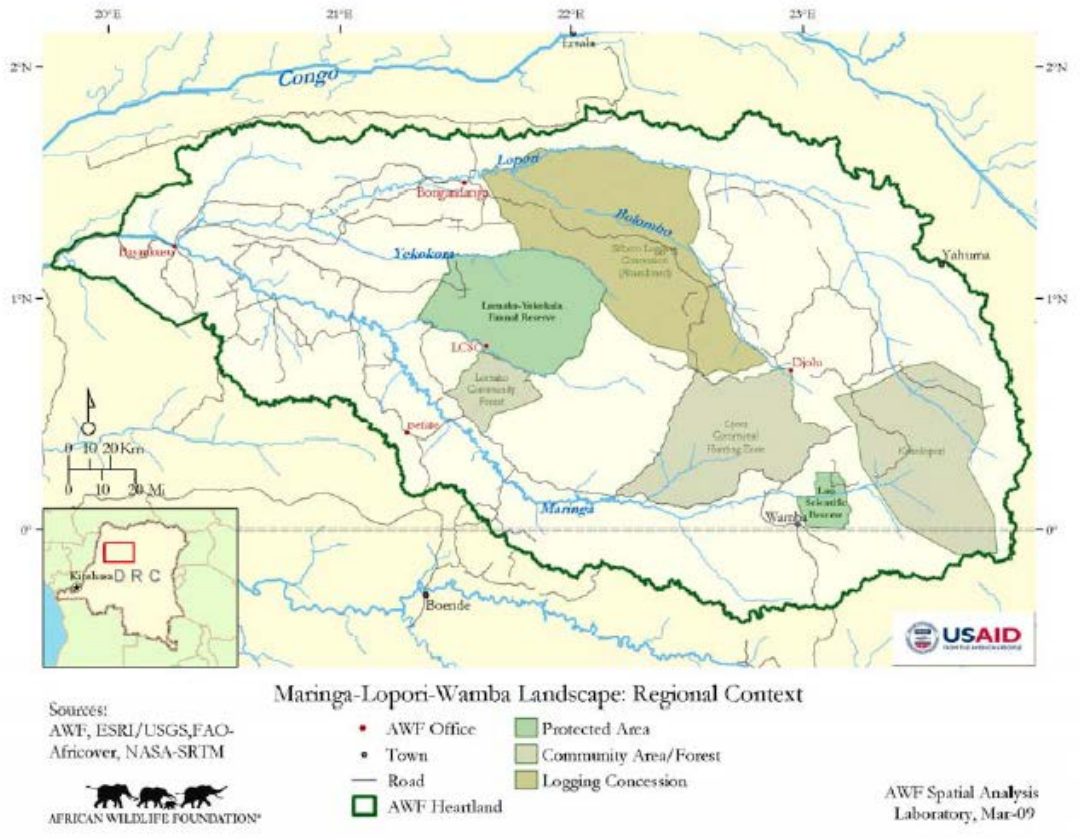

Figure 2.1.a The Maringa-Lopori-Wamba Landscape (magnified within DRC) is an area in the Equateur Province that is the focus of conservation efforts by African Wildlife Foundation. The Lomako Yokokola Faunal Reserve was established in 2005 as a protected space, free of logging, hunting, farming and other destructive forms of exploitation. As of 2011, various forms of exploitation (fishing, hunting, farming) were still ongoing in small patches through the area.

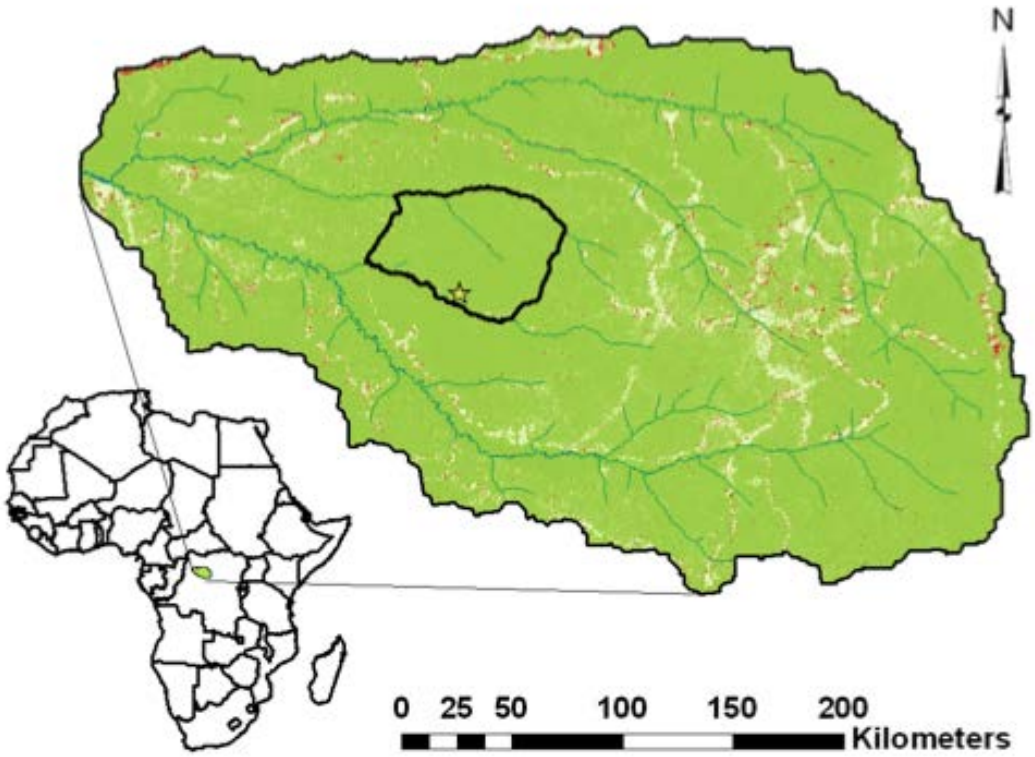

Figure 2.1.b Location of the Iyema study site (yellow star) within the to the Lomako-Yokokola Faunal Reserve, within the Maringa-Wamba-Lopori Landscape.

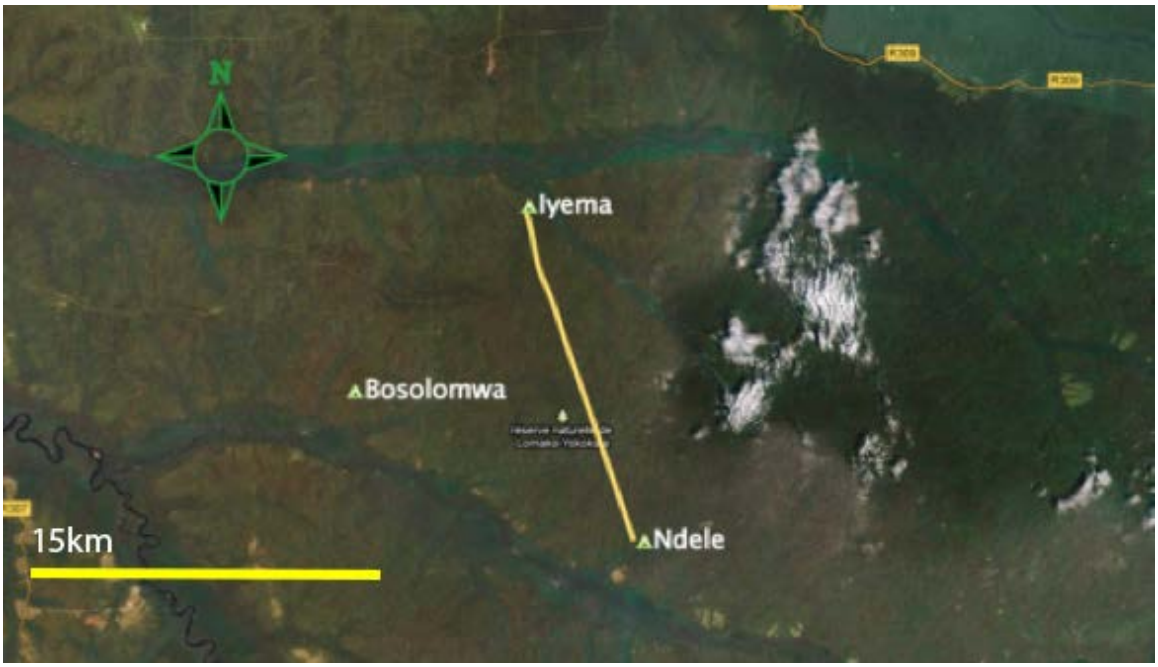

Fig. 2.1.c Satellite image of Lomako Forest, showing Ndele, Iyema, and Bosolomwa. The yellow line is a direct trail between the Ndele and Iyema sites. Bosolomwa is a semi-permanent human settlement 9km from Iyema.

2.2 Forest Composition at Iyema

In May and June of 2010, my team and I established a series of trails in grid formations across the study site formed by 6 trails, each 4.8km long (total major trails = 28.8km)(see fig. 2.2.a, map). Smaller trails were added during the study period as we became more familiar with the bonobos' ranging habits. Trails were marked at 50m intervals and regularly maintained (cleared and pruned) throughout the study period.

To identify forest types, I walked all main trails with an assistant using methods described by Reinartz et al. (2008), which involved estimating canopy cover, estimating the dominant forest understory, identifying the ground type every 100 meters, and recording GPS information. Information on forest structure is summarized in Table 2.2.b The Iyema study area is mainly composed of undisturbed primary forest with sub-canopy dominated by the presence of *Marantacea* species (e.g. *Haumania liebrechtsiana*; discussed further in Chapter 4), more commonly referred to as terrestrial herbaceous vegetation (THV). Small streams run through the site, but swamp and seasonally inundated forest are uncommon, in contrast to other study sites with more heterogeneity and forest mosaic compositions, such as Wamba and Lui Kotal (Kano and Mulavwa 1984; Idani et al. 1994; Hohmann and Fruth 2003) . Homogenous forest types, dominated by the species (rarely frequented by bonobos), *Gilbertiodendron* were only counted once (from 432 sample points).

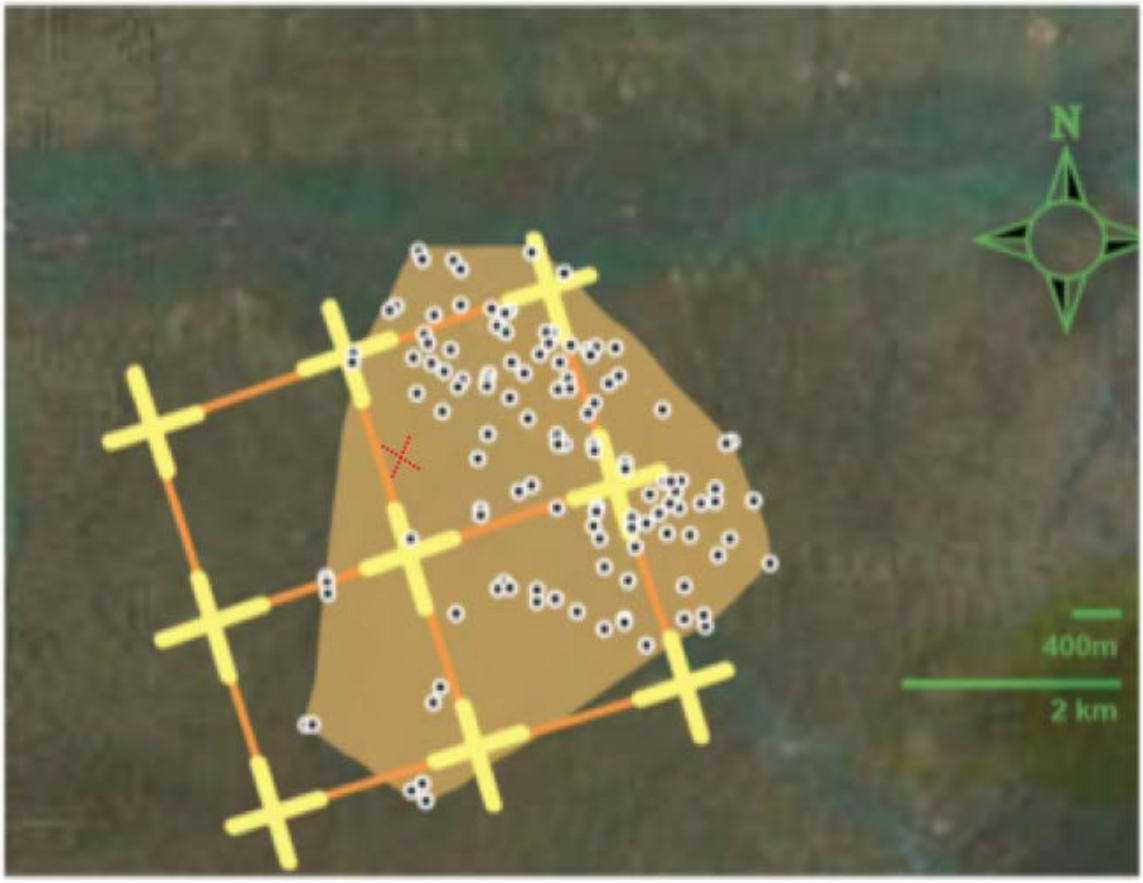

Figure 2.2.a Iyema trail system and locations of nest parties (satellite view). Orange lines indicate major trails, yellow lines indicate phenological trails. The red cross is placed at the location of the campsite. Opaque area highlights known community range, indicated by cumulative observed nest sites (black dots) for the 2010-2011 study period.

	% Canopy Cover		**% Understory**		**% Ground Type**
25%>	3.61	**Marantacea**	72.29	**Seasonally Inundated**	1.61
25%-50%	13.25	**Woody**	20.88	**Terra Firma**	98.39
51%-75%	18.07	**Liana**	6.83		
75%<	65.06				

Table 2.2.b Breakdown of forest types at Iyema.

2.2.1 Resource Density and Distribution

At the intersection of the 9 main trails, we established phenological transects that were 800m x 800m x 20m (totaling 1.6km, or 3.2Ha each). With the help of local guides, we identified and labeled trees within 10m of either side of the trail, which were over 10cm diameter at breast height (DBH), using a list of species of trees and lianas known to be preferred by bonobos, which was based on both published literature (Bermejo, Illera, and Pí 1995) and collective knowledge of experienced guides and myself. I had intended to sample a minimum of 10 of each species from this list on each transect, but quickly realized that this was not always possible: not surprisingly, some species are hyper-abundant, while others can be sparsely distributed. 45 species were monitored monthly for production of new leaves, flowers and fruit.

Trees and lianas were identified and labeled using flagging tape, and a spreadsheet was created with the names and ID's of all trees and lianas for each transect for uniform data collection. The number of species represented in each transect did not vary drastically, ranging between 26 and 37 different species per transect (see fig. 2.2.1.a). However, more variation is seen in figure 2.2.1.b (below), where the *total number* of each of these preferred species were counted per transect to determine the density at which they are represented throughout the study site.

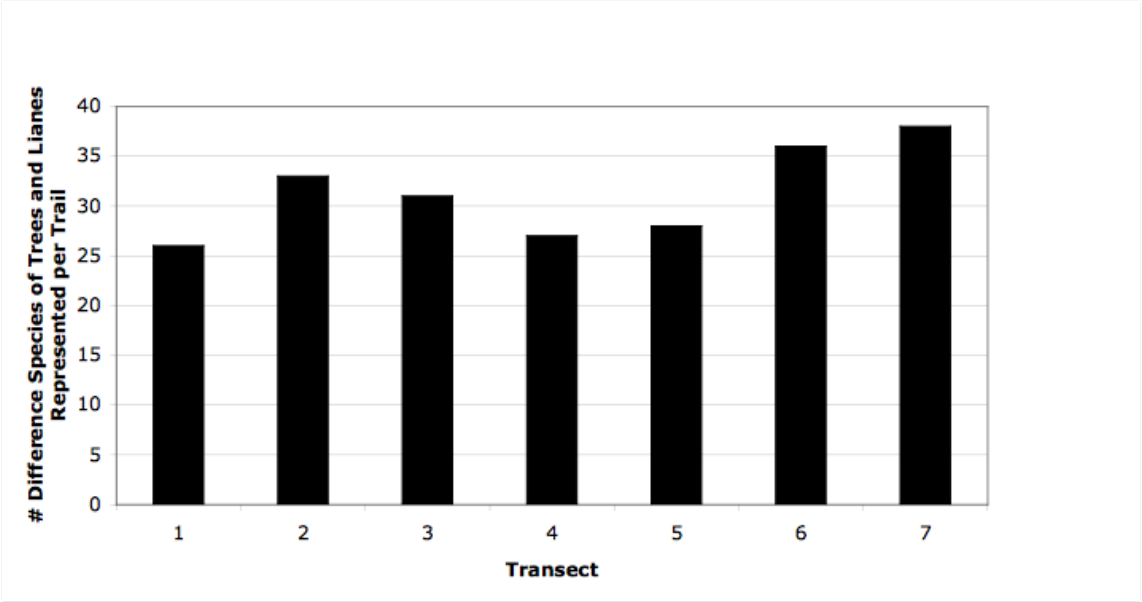

Figure 2.2.1.a Number of *different species* locally known to be consumed by bonobos represented by 7 phenological transects. Data includes plants thought to be consumed for medicinal purposes, in addition to "preferred" species that are eaten more regularly. Data for transects 8 and 9 unavailable.

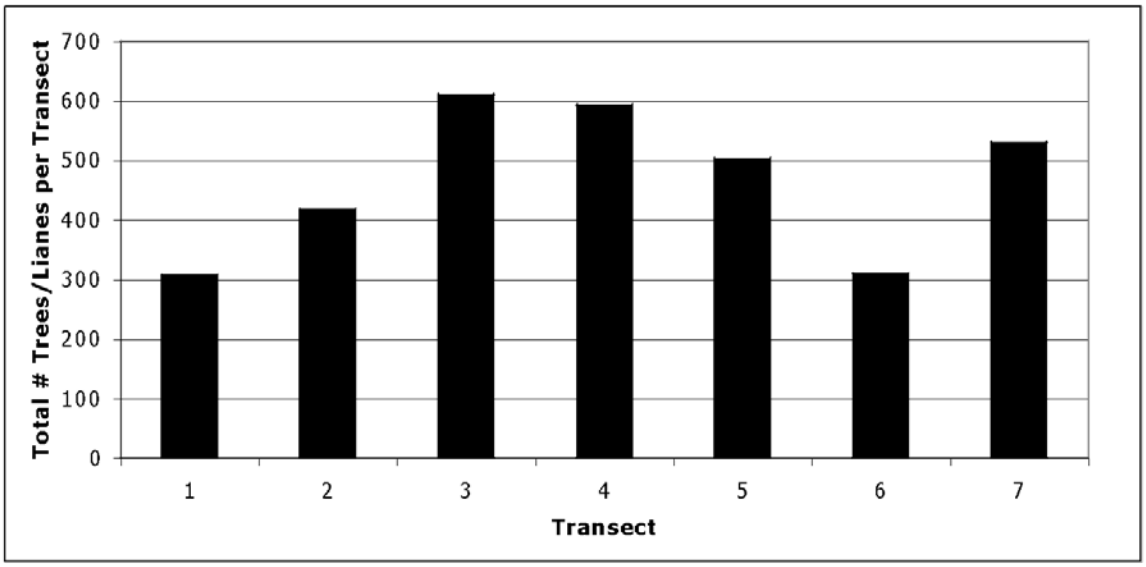

Figure 2.2.1.b *Total number* of preferred species of tree and liana within 10m to either side of phenological transects, per transect. Demonstrates that density of preferred species varied across the forest.

2.3 Community Structure

The exact number of residents in the Iyema community is uncertain. By the time I left Iyema (after a full year of observations approximately 3 times a week), I estimated the community was between 25-35 individuals. I had been able to confidently ID the following 21 bonobos with the help of a 400mm zoom lens and DSLR camera I carried with me during follows.

- 7 adult males
- 8 adult females (2 with infants/dependents)
- 2 juvenile males
- 3 juvenile females
- 1 sub-adult male

All the individuals above are photographed. There were some "cliques" (parties whose composition seemed consistent over the year, suggesting that they preferred each other to other members of the community; (Waller 2011) that were quickly identifiable, but often there were other individuals on the periphery of these parties that I could not reliably identify, and who were less comfortable with my presence than their identified peers.

The Iyema community had been semi-habituated from the mid-to-late 1990's and a single male from the same community was still clearly identifiable (Dupain, personal communication). However, intervening years characterized by intermittent hunting and a shift in local taboos that had previously prevented the hunting of bonobos appeared to have affected at least the one previously habituated male, and very possibly other members of the community who were more cryptic in the presence of researchers. van Krunkelsven et al. estimated that there were 50 individuals in the Iyema community, but

were only able to clearly identify 11 during their time there; methods for estimation of that community size were not described (1999). A number of younger individuals appeared genuinely curious, but cautious about our presence, suggesting that hunting pressures had not been severe in recent years.

Bonobos are notoriously difficult to habituate, due to both the dense forest that they live in, which makes visibility on part of both humans and *Pan* difficult, but also because they will often go for hours through the day not making any noise, especially if they are concerned about being followed. Nonetheless, we averaged 3 follows per week throughout the year, and had our first nest-to-nest follow in October of 2010 (five months into the study period). This benchmark is significant for two separate reasons: 1- this steady level of contact rate was unusual in my personal experience, having worked at both Lui Kotal during its 2nd year and in Ndele for two short seasons, which had been significantly more habituated in recent history. The Ndele community is notorious for disappearing for months at a time, in spite of being considered to have one of the smallest home ranges of known *Pan* communities. In contrast, the bonobos at Iyema were considerably easier to find than the other two study sites. 2- We consistently found the Iyema community within 4km of our campsite, and on multiple occasions we would hear them within 500m from the site in late afternoons or evenings. The consistency and proximity with which we found them during this study period leads me to believe that they live within a relatively small home range that contains everything they need (to be discussed further in Chapter 3,4 and 6).

Chapter 3:

Seasonality and Diet of the Iyema Forest and Bonobo Community

Chapter 3 Abstract
The relationship between environment and social structure and behavior in primates has been a major focus of primatological research since the discipline's foundations. Within this focus, seasonal changes in primate environments have been shown repeatedly to have profound effects on a variety of primate species. Attempts to explain differences in the social dynamics of *Pan* have also often focused on differences in their environments, largely examining how changes in resource abundance and diversity affect fission-fusion patterns within and across communities. A long-held assumption about differences in bonobos and chimpanzees asserts that bonobos, confined to the Congo Basin, live in an environment that is less seasonal than that of most chimpanzees. However, multiple comparisons of habitats occupied by both bonobos and chimpanzees have revealed a wide variety of both seasonal expression and community dynamics. Studies thus far on the relationship between bonobos and seasonal changes in their environment are largely limited to two sites: Ndele and Wamba, leaving us to wonder if bonobo responses to environmental changes vary to the same degree seen in chimpanzees. In order to address the question of whether or not bonobos are affected by changes in their environment, we must first examine the environment itself: is it seasonal in ways that might be meaningful to the bonobos living within it. Two rainy seasons and two dry seasons were identified (one long and one short for both). Temperatures remained relatively stable throughout the year. My team and I monitored 1805 tagged trees and lianas each month over the course of a full year for the production of new leaves, flowers and fruit on 14.4km of phenological transects. Abundance and environmental diversity both showed clear seasonal patterns. Following this, I identified 14 species of fruit consumed most frequently by the Iyema bonobo community to examine how availability of preferred species in their environment affected their dietary choices. Based off of fecal analysis, the Iyema bonobos appeared to always choose their diets selectively, in spite of seasonal variation, suggesting that they can afford to be choosey in a rich environment.

Seasonality and Diet of the Iyema Forest and Bonobo Community

3.1 Background:

Seasonal changes in tropical environments are not as easily discernable as in temperate environments, but shifts in the food availability of tropical environments have been repeatedly demonstrated to fluctuate in both space and time (Peres 1994; Conklin-Brittain et al. 1998; Knott 1998; van Schaik et al. 2005). These fluctuations affect multiple aspects of primate sociality, including group size (Mulavwa et al. 2008; Pride 2005), levels of competition (Erhart and Overdorff 1998; Curtis and Zaramody 1998), daily travel costs (Chapman and Chapman 2000) and reproductive costs (Kitaysky et al. 2010; Brockman et al. 2007) (Also, see Isbell 1991 for review).

Primate societies were initially characterized based on their social organization alone (Crook and Gartlan 1966; Eisenberg, Muckenhirn, and Rudran 1972; Clutton-Brock and Harvey 1977). The vast majority of primates live in bisexual communities with more than 3 adults, setting them apart from other mammals (Van Schaik and Kappeler 1997). The relationship between female reproductive strategies and food distribution has been hypothesized and demonstrated to play a critical role in the organization of primate social groups (Trivers 1972; Wrangham 1980; Isbell and Young 2002; Kappeler and van Schaik 2002). Summarized: females, who bear the main energetic load of producing and rearing offspring will organize themselves according to the distribution of resources in their environment. When food is patchy and defendable, females are likely to form alliances in order to defend those resources. When food is broadly and evenly spread, females are more likely to disperse and compete with one another for food by “scrambling” for these resources. In the former scenario, females are expected to form strict hierarchies in the

process of defending patches, whereas in the latter it is assumed that the lack of defensible patches results in a lack of female bondedness. Males are expected to structure their activities (feeding and social bonding) around the physical distribution of females. Consequently, the control of resources is tantamount with priority of access to, or control of female sexuality and reproduction.

3.1.1 Abundance and Diversity

Abundance and diversity are two important and distinct factors that are frequently discussed in terms of primate socioecology. Abundance, as discussed here, refers to the relative volume or biomass of available consumable resources. It is one means of measuring forest productivity patterns, and helpful in conceptualizing the environment broadly. Diversity refers to the number of different species that are ripe and edible; it does not necessarily equate to, but *contributes* to overall abundance. "Availability" is used here as a general descriptor for the combination of abundance and diversity, generally.

3.1.2 Differences in *Pan* Social Structure

Since the early 1980's, attempts to explain the differences between bonobo and chimpanzee social organizations have highlighted ecological variation between the two species as critical in their respective social and physiological adaptations (Wrangham 1980; Wrangham 1986; Wrangham 1993). Underlying these arguments is the assumption that the crucial difference between the female-based *Pan paniscus* and the male-bonded *Pan troglodytes* was that bonobos were not subject to the same seasonally lean periods that limited feeding party size and, by extension, female sociality in chimpanzees

(Wrangham 1980, 1986). A critical component to this hypothesis was the assertion that terrestrial herbaceous vegetation (THV) was a widely available resource in both environments, consumed primarily by gorillas and not chimpanzees north of the Congo River, and by bonobos, unhindered by competition with comparable megafauna as they traveled on the ground in search of feeding trees. Multiple examinations of THV's role in the diet of chimpanzees and bonobos have shown that it is not critical to supporting large feeding parties, but does play an important role in bonobo diets (discussed further in Chapter 4).

The known range of the bonobos' habitat is centered on the equator, with some variation in habitat type, ranging from thick, evergreen forest to savannah mosaics (Kano 1983; Hohmann and Fruth 2003a; Thompson 2002). The degree of variation of chimpanzee habitats is more extreme, as they range much farther to the north, east and west, resulting in a wider expression of seasonality. Gombe (Tanzania) goes through notable dry periods that result in periodic dearths of fruit, while Tai Forest (Ivory Coast) and Kibale (Uganda) tend to be more steady in terms of fruit availability (Yamakoshi 2004; Chapman et al. 1999). The effects of these environments on the social behavior and structure of the chimpanzee communities living within them have been the subject of focus for decades of continuous research (e.g. Sugiyama and Koman 1979; Boesch and Boesch-Achermann 2000; Newton-Fisher, Reynolds, and Plumptre 2000; Pienkowski et al. 1998; Mitani, Watts, and Lwanga 2002)

Bonobos have been observed to have feeding parties that are on average larger than those of most chimpanzee communities, which were partially explained by correspondingly large feeding patches (White and Wrangham 1988). Wider examinations

of ecological correlates in chimpanzees and bonobos across study sites have produced variable results, where the range of chimpanzee party sizes varies almost as much as that of unprovisioned bonobos (reviewed in Stumpf 2011). In the Budongo community (Uganda) abundance has little to do with feeding party sizes (Newton-Fisher 2000), whereas in the Gashaka community (Nigeria) seasonal changes in abundance was found to be a strong predictive factor of party size (Hohmann et al. 2006). In the Ngogo chimpanzee community (Uganda) the combination of fruit availability *and* estrous females together best predict party sizes (Mitani et al. 2002), while in the Tai forest (Ivory Coast), estrous females are the *only* factor that predicts party size, unless females are entirely absent at which point resource availability corresponds to all-male party sizes (Anderson et al. 2002). When chimpanzee feeding parties decrease in size, the party composition virtually always biases males (Wrangham 2000; Janson and Goldsmith 1995; Williams et al. 2002; Anderson et al. 2002), whereas the opposite is true for bonobos (White 1998, Kano 1982, Furuichi 1987). Estrous females play a large role in attracting larger parties in chimpanzee communities, but when estrous females are not present, general food decreasing fruit abundance has been shown to limit parties composed entirely of males, suggesting that the role of abundance is more nuanced than a simple direct correlation. The presence of estrous females in bonobo feeding parties has not been shown to singularly direct party size (Furuichi 2009), and as party sizes go down in size it is males that are peripheralized, supporting the interpretation that abundance levels are never so low as to limit female sociality (White 1998; Furuichi et al. 2008; Stumpf 2011). To date, no single predictive model has been successful in predicting bonobo party size (Furuichi 2009), highlighting the need for more integrative forms of

analysis of different aspects of bonobo socioecology, which I will address in Chapter 6. However, in order to establish that the community I studied experiences seasonal changes in their environment that are meaningful in ways that affect their social dynamics, I address the following questions (3.2).

3.2 Organizing Questions:

3.2.1 Does the Iyema bonobo community live in a seasonal environment?

3.2.1.1 Are there months where abundance is significantly higher than others?

3.2.1.2 Are there months when environmental diversity is significantly higher than others?

3.2.1.3 Are diversity and abundance related?

3.2.2 Are seasonal fluctuations in the environment reflected in the Iyema bonobos' diet?

3.2.2.1 Does dietary diversity (measured through the number of species found in bonobo feces) reflect environmental diversity of available ripe fruit?

3.3 Methods

3.3.1 Rainfall and Temperature

Maximum and minimum temperatures were recorded daily in the morning (fig. 3.3.1a.) Daily rainfall was measured using a standard rain gauge placed in the center of camp (fig. 3.3.1.b&c).

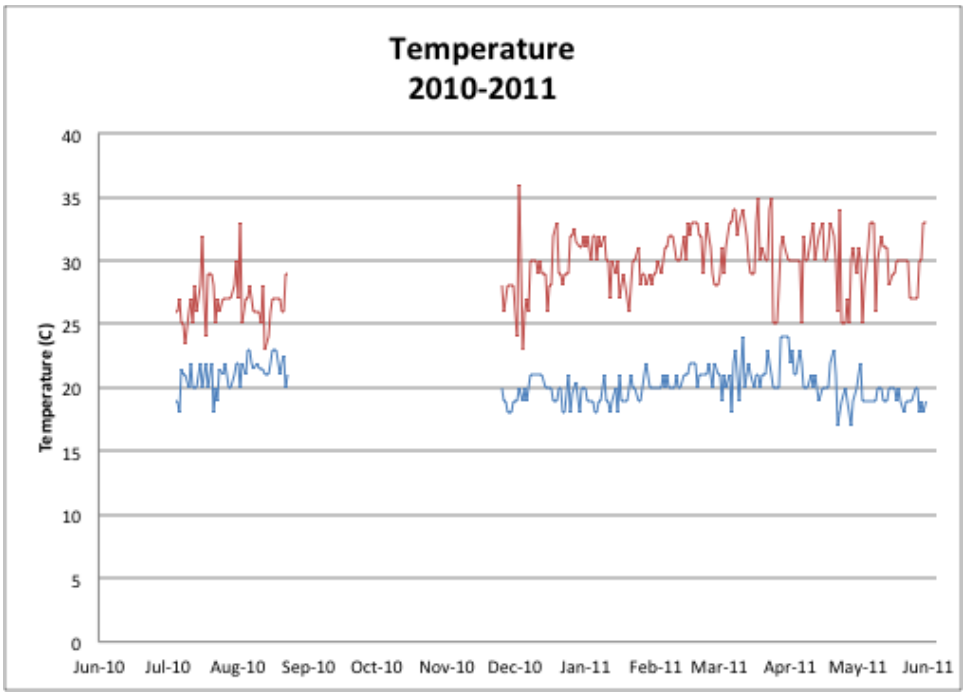

Figure 3.3.1.a Minimum and maximum temperatures between late June 2010 and June 2011. Gaps between August and October were the result of broken thermometers.

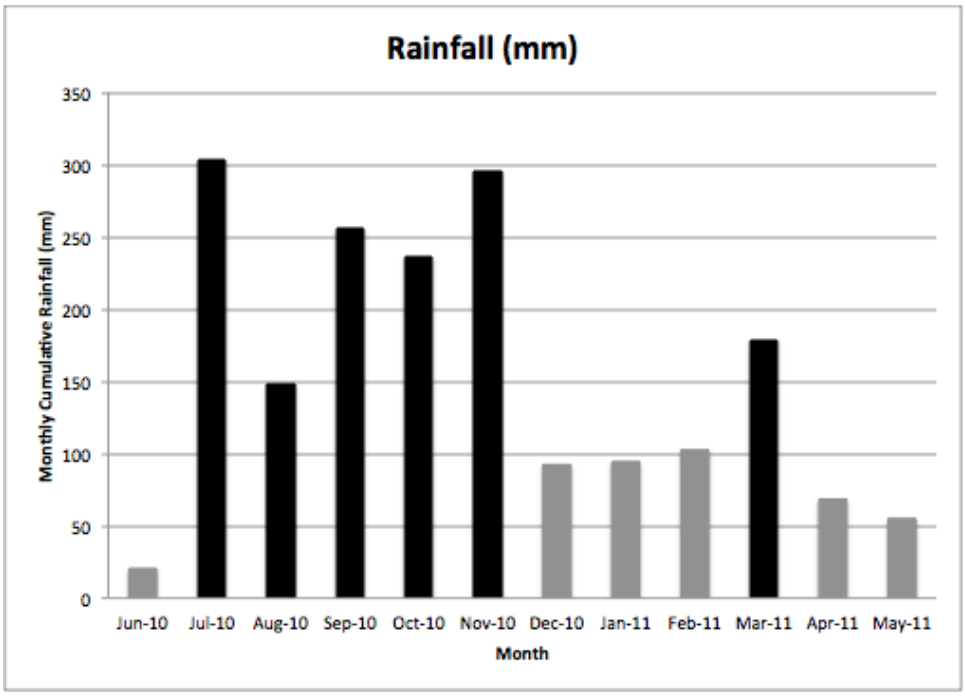

Figure 3.3.1.b Monthly cumulative rainfall between June 2010 and May 2011. Grey indicates dry months (rainfall ≤ 100mm), while black indicates rainy months (rainfall ≥ 100mm).

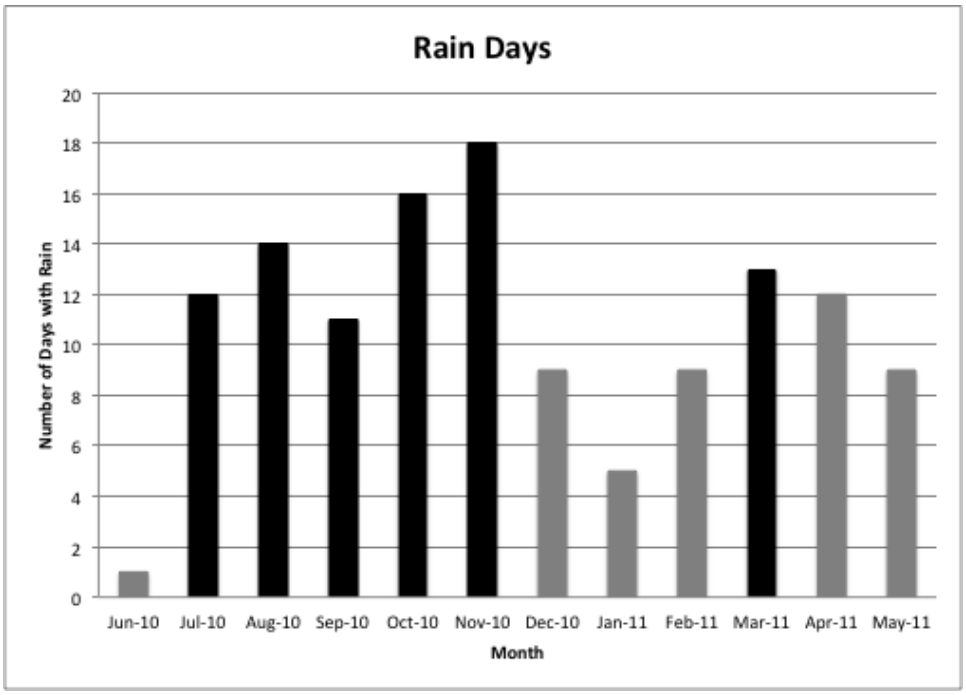

Figure 3.3.1.c Total number of rainy days per month. Grey indicates dry months, while black indicates rainy months.

3.3.2 Phenological Transects

3.3.2.1 Identification of trees and lianas

To assess the distribution and abundance of potential fruiting trees across the study area, I compiled a list of 51 species known to be consumed by bonobos, based on lists obtained from other researchers (Jef Dupain and Frances White) and published literature. In May of 2010 over 2000 trees and lianas were identified, marked, and observed each month. Due to errors in the data and occasional deaths of trees, a total of 1805 were statistically analyzed.

3.3.2.2 Density and distribution of species

In May 2011 we recorded the number of all species of trees (DBH>10cm) and lianas known to be eaten by bonobos within 10m of either side of all 9 phenology transects. I totaled these numbers to create a density index of individual species in order to control for overrepresentation in the data (some trees were disproportionately monitored compared to others because of oversights made when identifying individual trees and lianas in June, 2010). For each species, the total number per species counted on all transects was divided by the total number of all trees and lianas.

3.3.2.3 Monitoring of trees and lianas

Transects were systematically monitored for the production of new leaves, flowers and fruit each month between June 2010 and October 2011. There are several ways to estimate growth and fruit production on trees and lianas, which can vary from estimating absolute number of fruits to estimating a logarithmic-based number (e.g. 1, 10, 100,

1000, etc.) (e.g. Chapman et al. 1992; Chapman et al.1994). After testing different methods during the months preceding data collection, I decided to assess production of fruit by visually estimating the percentage of canopy occupied by leaves, flowers and fruit. This proved to be the most repeatable method for data collection with field assistants.

Standing at the same spot each month, the observer would look at the entire canopy of the tree in question and estimate the percentage of canopy occupied by new leaves, flowers, or ripe fruit. Depending on the size of the canopy, up to three estimates were recorded and averaged, though it was typically only one measure for the whole canopy. Although an estimate of 90% saturation does not actually mean the cubic meters of the tree in question are 90% full of fruit, it helps more to describe a month-to-month *pattern*, which stays consistent.

Estimation of fruit ripeness was evaluated on a scale of 0-10 (table 3.3.2.3.a). For statistical analysis, I included all instances where a tree had fruit ranging from 5-8, based on my observations that within the course of a month (e.g. the intervals between observations) most species would ripen, or would have *been* ripe in the weeks preceding. During the month of June and July observations were done by myself and Malou Nsambo, a Congolese university student. We were joined in July by Boyou Banana (a trained local guide who had done similar work in the 1990's), who took over the phenology monitoring completely by the end of August 2010. Once per month I would join Banana to ensure that the methods were consistent and that we were in agreement about our observations.

Ripeness	Description
0-1	Small, barely visible emerging green bud from flower, no petals left
2-3	Small, hard, green fruits; visible but not immediately apparent.
4-5	Medium-sized hard, greenish fruits; moderately visible and some color where species changes color.
6	Medium-large hard fruits; more color than green where species change color.
7	Large fruits, visibly ripe, or close to; edible, but not ripe (usually indicated by remnants of half-eaten remains on ground).
8	Large fruits, visibly ripe, fully colored where species change color; ripe.
9	On ground, past ripe with black splotches; unlikely to be eaten by primates on ground.
10	On ground, blackened, rotten, past edible.

Table 3.3.2.3a Criteria for scoring fruit ripeness. Based on personal observations, scores between 5 and 8 were included in calculating estimates for forest-wide abundance and diversity of ripe fruit because of the rate at which fruit was seen to ripen.

3.3.3 Collection and assessment of feces

Fresh fecal samples were collected from beneath night nests on the mornings of behavioral follows by myself and my assistants. Depending on the height from which

they dropped and the consistency of the feces, as much of the partially intact samples as we could recover was packaged in debris-free *Marantacea* leaves (large, banana-like leaves), which were clearly labeled with the nest ID they were associated with. When nests and fecal samples were close (within a meter of one another), only the largest portions of intact samples were collected, and samples were differentiated from one another based on color and consistency.

Following collection, transport of feces to camp, and hormonal sampling (see Chapter 6 for methods, the) remaining feces were washed in a special area outside of camp, in order to clearly discern grains and fibers. This was done by placing individual samples in a sieve made of mosquito netting (with no-see-um mesh) and pouring water over them to wash away fecal matter until seeds and fruit pits were clearly visible. The mesh was fine enough that very small seeds (e.g. figs) and fibers were not washed away. For each fecal sample (n=712), we recorded the local name and number of all seeds and grains in the sample (see table 3.4.1.1.c). Fibers and seeds that were too small to count individually (e.g. fig seeds) were visually estimated on a quadrant scale in relation to the entire sample including the grains. For example, if the amount of fig seeds when separated into their own clump appeared to be equal or less than half the entire sample, it was scored “2”; if it clearly composed the vast majority of the sample, it was scored “4”.

3.4 Results

3.4.1 Does the Iyema bonobo community live in a seasonal environment?

There is a very clear picture of seasonal fruit production, prior to adjusting for density of species or ripeness of fruit (fig. 3.2.1.a). Although the seasonal fruit production

appears clear at this level, the lack of discernable patterns in nesting behaviors (see Chapter 5, figure 5.3.a) suggests that any impact of seasonality on bonobo social behavior occurs on a much more nuanced level, or simply not at all. This raised the question of which fruits amongst those monitored were being consumed the most by the bonobos. We monitored 45 species of trees and lianas, but 31 of those species were rarely seen in the feces of the bonobos (see figure 3.4.1.b below). I selected 16 species of the most frequently consumed and seasonally important foods (including THV, as a general category; see table 3.4.1.c for species names and values).

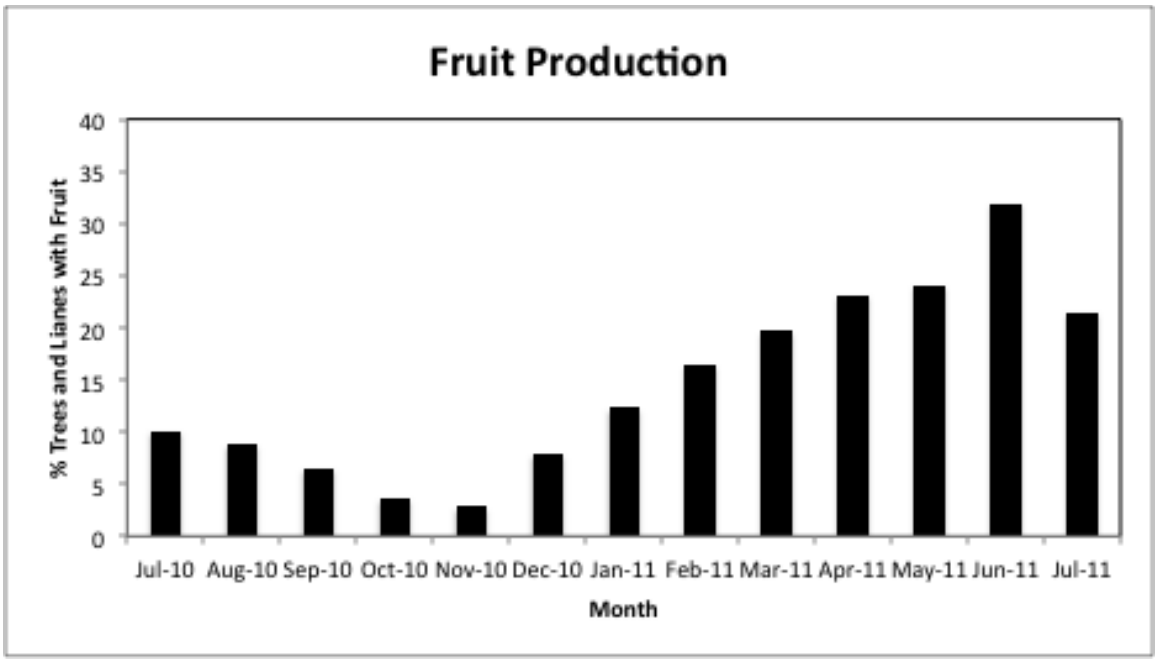

Figure 3.4.1.a The percentage of trees and lianas fruiting per month illustrates basic seasonal fluctuation in fruit production in the Iyema forest. Percentages were obtained by assigning a binomial value to trees that were fruiting or not fruiting, and dividing the sum of all species of fruiting tree by the total observed.

.

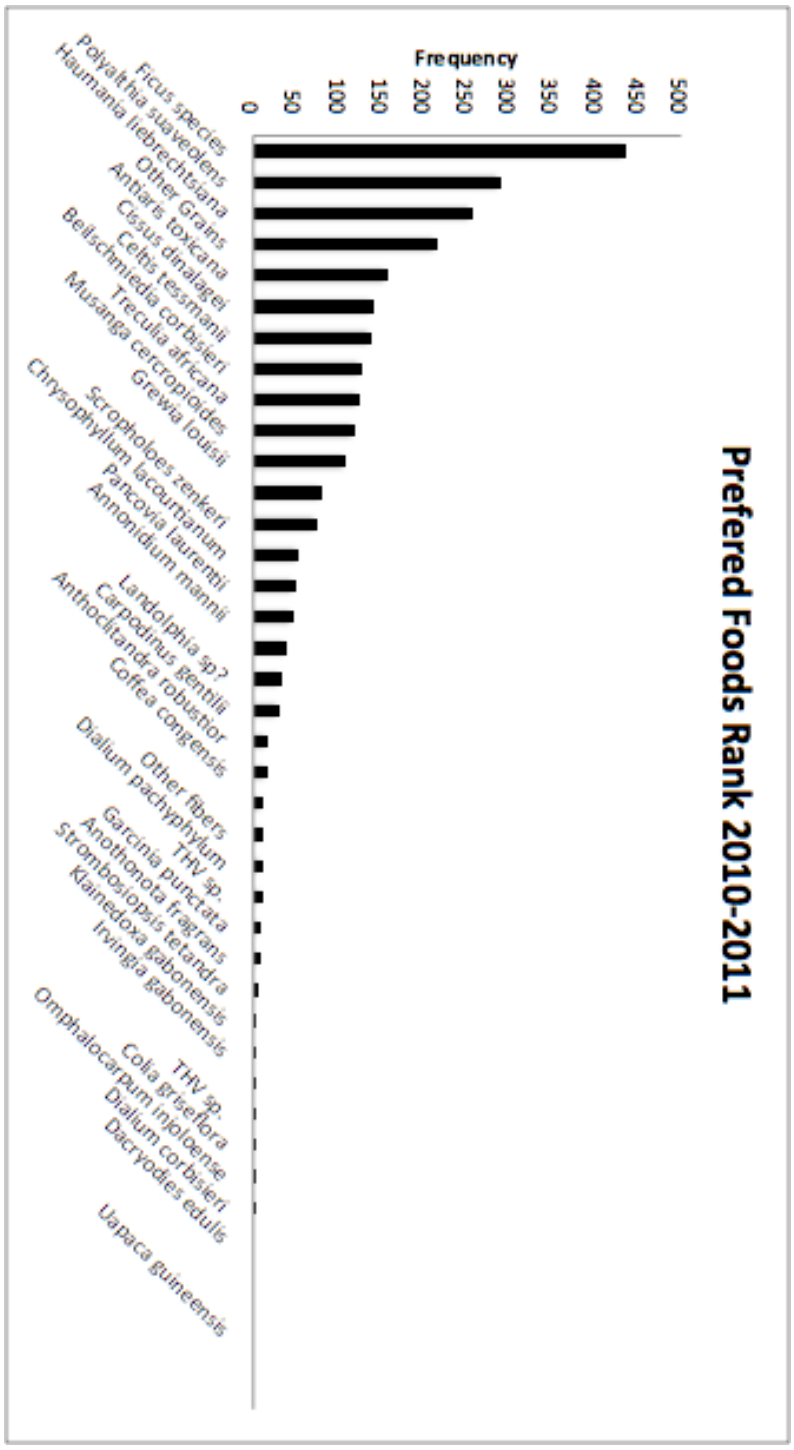

Figure 3.4.1.b Frequency of fruits and fibers (leaves and THV species) found in bonobo feces between June 2010 and June 2011. N = 712. Note: In the basic fecal content analysis, leaves were frequently present, but rarely the bulk of the feces.

Species	***Family***	**Local Name**	**Rank**	**Frequency (x/712)**	**% of all feces**
Ficus spp.	*Moraceae*	Lokumo	1	435	61.10
Polyalthia suaveolens	*Annonaceae*	Bolinda	2	289	40.59
Haumania liebrechtsiana		Bekombe	3	256	35.96
Other Grains		Other grains	4	215	30.20
Antiaris toxicana	*Moraceae*	Linkoko	5	157	22.05
Cissus dinalagei	*Vitaceae*	Bolalenga	6	140	19.66
Celtis tessmanii	*Ulmaceae*	Mbeko	7	138	19.38
Beilschmiedia corbisieri	*Lauraceae*	Bongolu	8	127	17.84
Treculia africana	*Moraceae*	Boimbo	9	124	17.42
Musanga cercropioides	*Moraceae*	Bomambo	10	119	16.71
Grewia louisii	*Tiliaceae*	Bofumbo	11	109	15.31
		Bolole	12	81	11.38
Scropholoes zenkeri	*Cesalpiniaceae*	Bofili	13	75	10.53
Chrysophyllum lacourtianum	*Sapotaceae*	Bofambu	14	54	7.58
Pancovia laurentii	*Sapindaceae*	Botende	15	49	6.88
Annonidium mannii	*Annonaceae*	Bonenge	16	47	6.60

Table 3.4.1.c Top species' names, rank and frequencies for 2010- 2011. Calculations based on a presence/absence dichotomy. Note: *Haumania* (#3) is a species of THV, not a fruit. "Other grains" were a conglomerate of species that were not identifiable to the team and is composed of multiple unknown species. Neither *Haumania* nor "Other" were included in the abundance analysis.

3.4.1.1 Are there months where abundance is significantly higher than others?

Abundance, measured by total metric volume of estimated resources (including leaves and the top 14 preferred fruits species) and by fruit alone (also the to 14 preferred fruit species) showed seasonal patterns (fig. 3.4.1.1.a &b). We standardized abundance measures against a mean of total fruit abundance in order to compare deviations from the standard mean. Results show that 6 months out of the year abundance was lower than the mean, while during the months of January, February and March all showed abundance measures more than ½ a standard deviation above the mean (fig. 3.4.1.1.c)

The top 16 most frequently consumed species included a species of preferred THV (see Chapter 4) and a general category for grains unrecognizable by myself, or my local staff; these were not included in analysis of abundance and diversity. I used the 14 most frequently consumed species to calculate general abundance of ripe fruit for each month. For smaller species, and for leaves, respectively, I calculated the sums of each individual tree's estimated metric volume per month, and divided the sum of all trees by the total number of hectares sampled:

$$\frac{\text{Sum(individual tree canopy volume x \% with fruit)}}{\text{28.8 Ha}} = \text{cubic meters available fruit/Ha}$$

For larger species, which could not be estimated in terms of occupied canopy, I assigned an estimate of cubic meters per individual fruit and multiplied it by the number of fruits on individual trees, and divided by the total number of hectares sampled. Sums for both types of fruits were added together with estimated metric volume of leaves and produced the graphs below. Overall, the total abundance of the preferred species appears to be highly seasonal. However, it is primarily young leaves that produce this effect (fig.

3.4.1.1.d, below), which should be kept in mind when considering the negative relationship between overall abundance and nest party sizes.

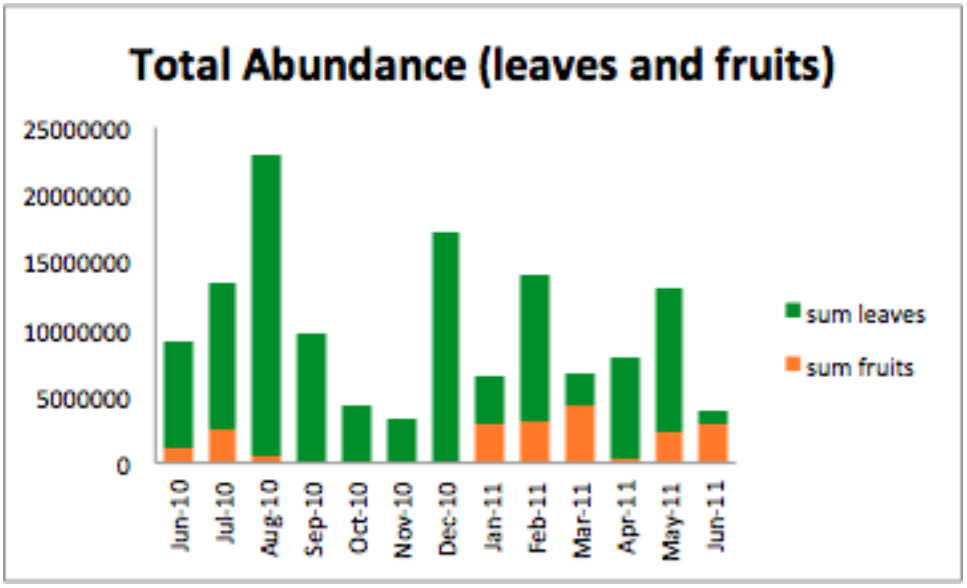

Figure 3.4.1.1.a Abundance (measured on the Y axis, in m3/Ha) broken down between young leaves and ripe fruits. Young leaves by these measures vastly exceed fruits in terms of abundance and year round availability.

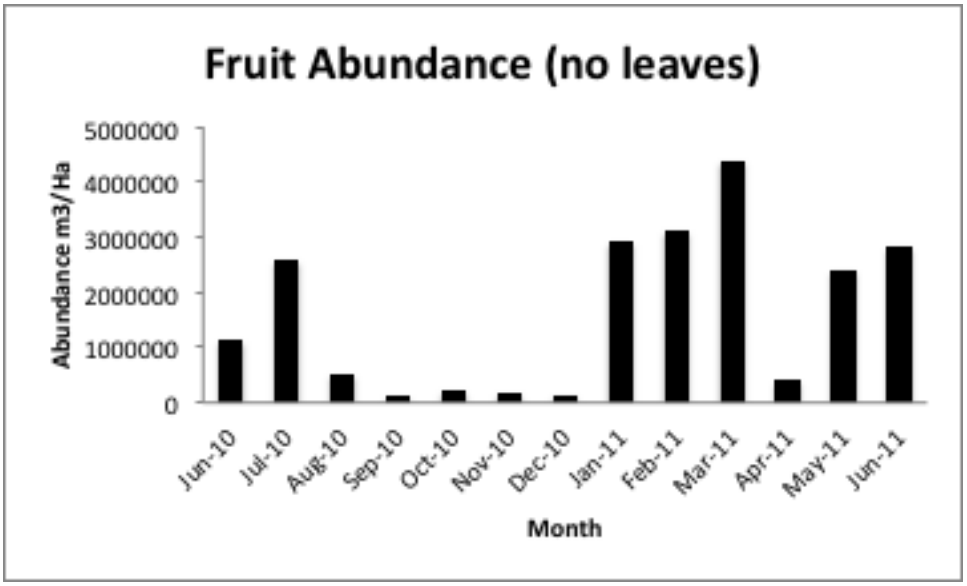

Figure 3.4.1.1.b Fruit abundance without leaves shows clear seasonal fluctuation.

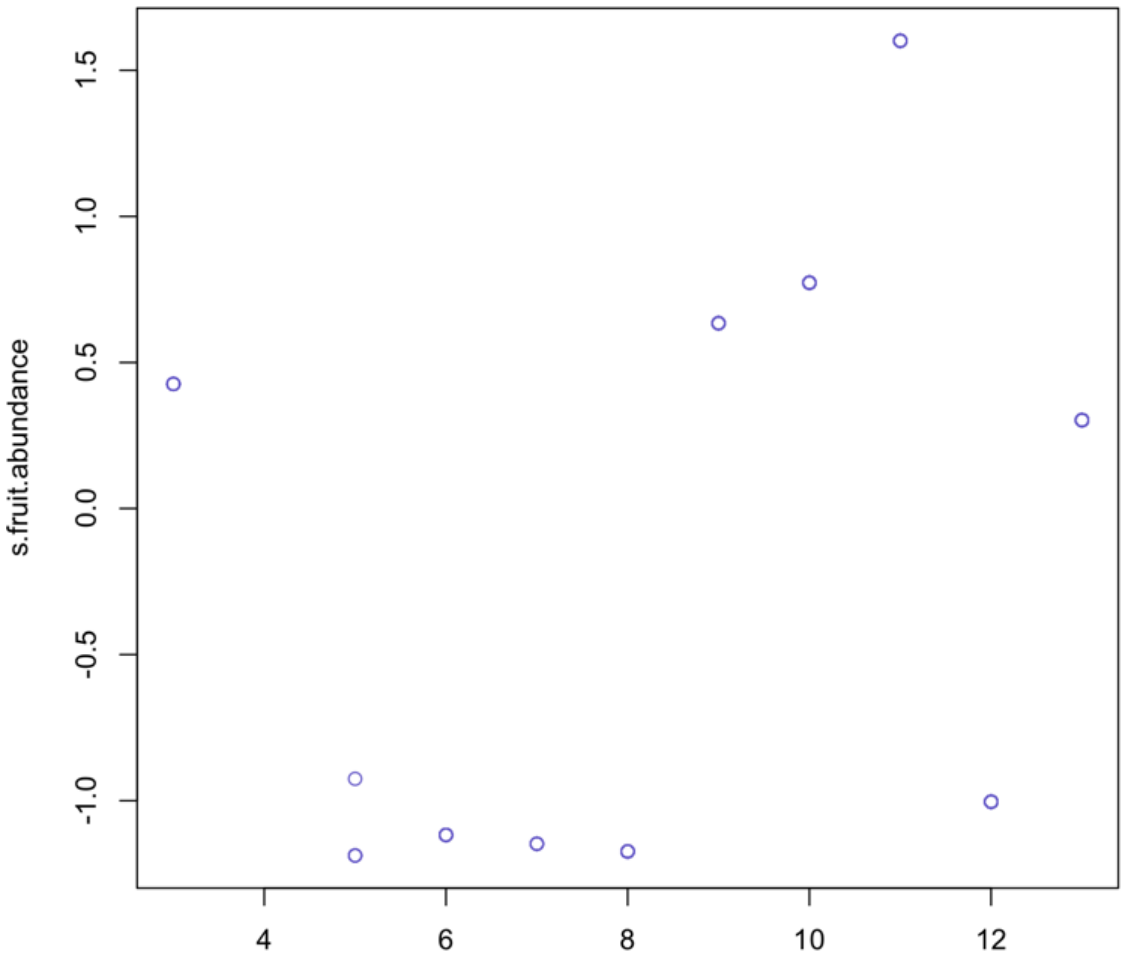

Figure 3.4.1.1.c General abundance of the 14 most preferred species as it deviates from average by month (x axis). Due to the degree of variation in monthly abundance (largely from young leaves production), we standardized measures by subtracting mean yearly abundance from the monthly measurement, and dividing by the standard deviation of all months. (Monthly Abundance – Mean Monthly Abundance)/SD(All Months)

3.4.1.2 Are there months when environmental diversity is significantly higher than others?

Environmental diversity showed a seasonal pattern, ranging from 5 to 10 preferred ripe species per month (fig. 3.4.1.2.a).

3.4.1.3 Are diversity and abundance related?

Diversity and total abundance showed a weak, positive relationship (fig. 3.4.1.3.a), while the relationship between diversity and fruit abundance was also positive, but not as strong (fig. 3.4.1.3.b).

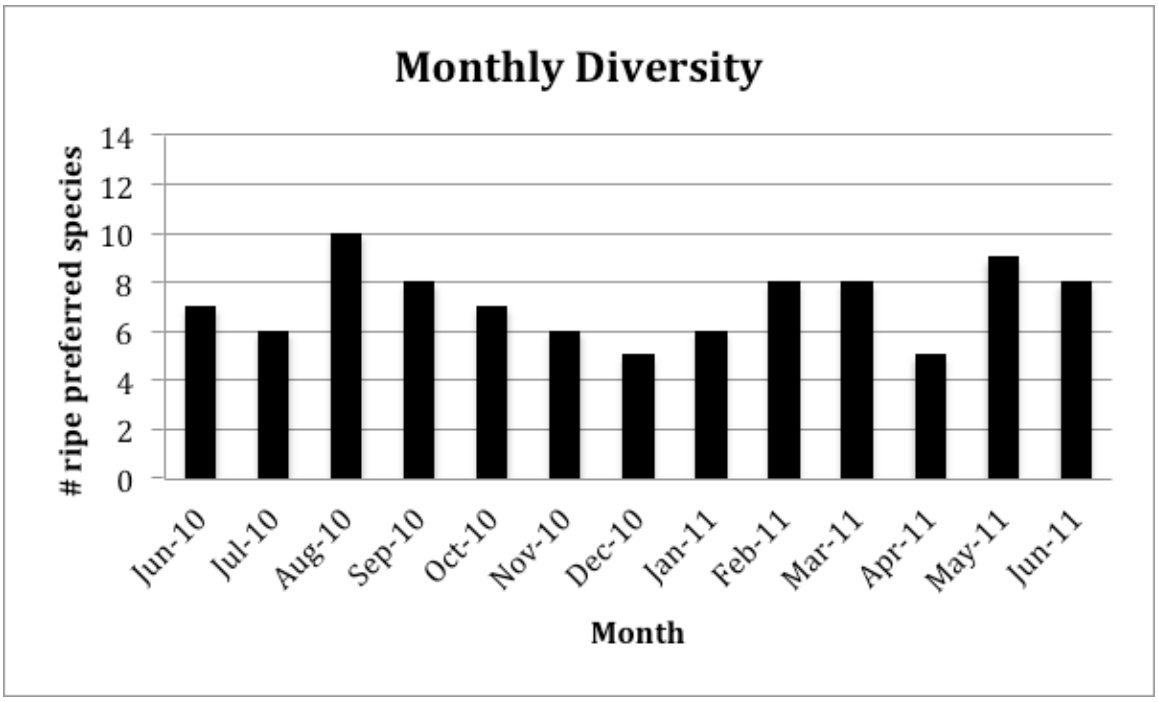

Figure 3.4.1.2.a Monthly diversity of top preferred species eaten by the Iyema community

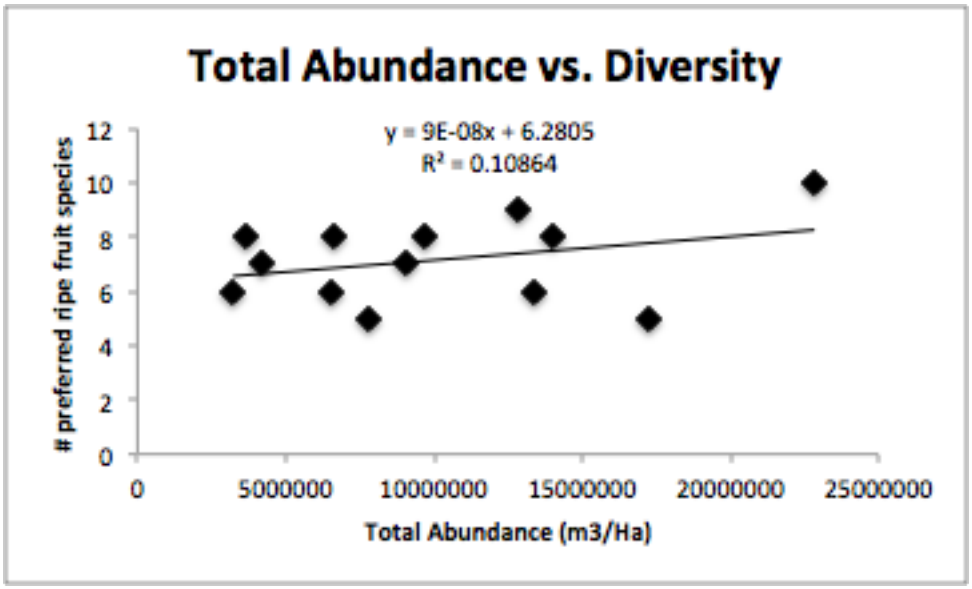

Figure 3.4.1.3.a Demonstrates small, but positive relationship between the number of ripe preferred fruit species and estimated total abundance (includes young leaves) (m3/Ha).

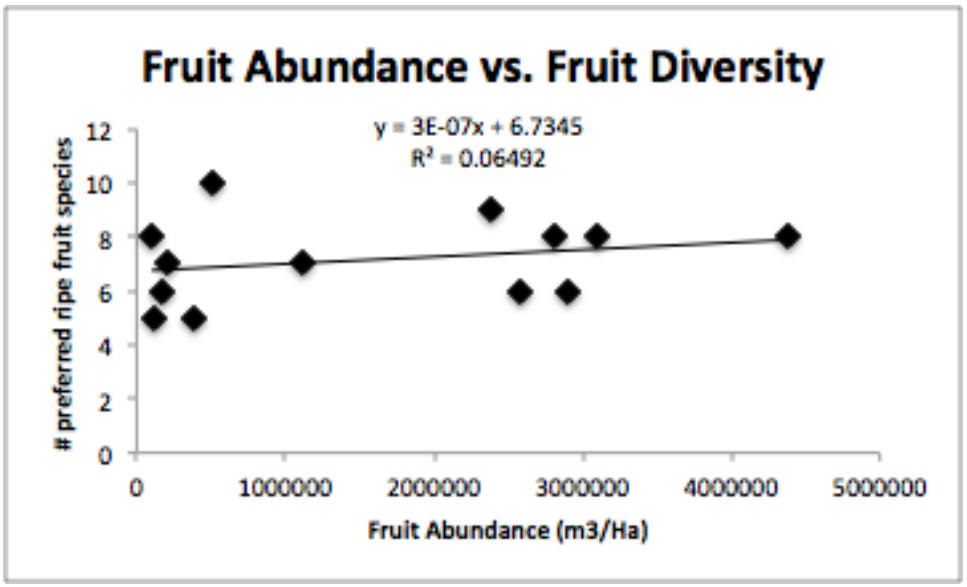

Figure 3.4.1.3.b Demonstrates small, but positive relationship between the number of ripe preferred fruit species and estimated fruit abundance (m3/Ha).

3.4.2 Are seasonal fluctuations in the environment reflected in the Iyema bonobos' diet?

3.4.2.1 Does dietary diversity (measured through the number of species found in bonobo feces) reflect environmental diversity of available ripe fruit?

Dietary diversity, represented by the number of species total (including fibers) found in the bonobos' feces does not correspond with changes in their environment. Using a GLMM model, which used environmental diversity and fruit abundance to predict dietary diversity, we found that there appears to be no relationship between the number of ripe fruit species preferred by bonobos, the abundance of those species and the number of species consumed on average per month by the bonobos (fig. 3.4.2.a)

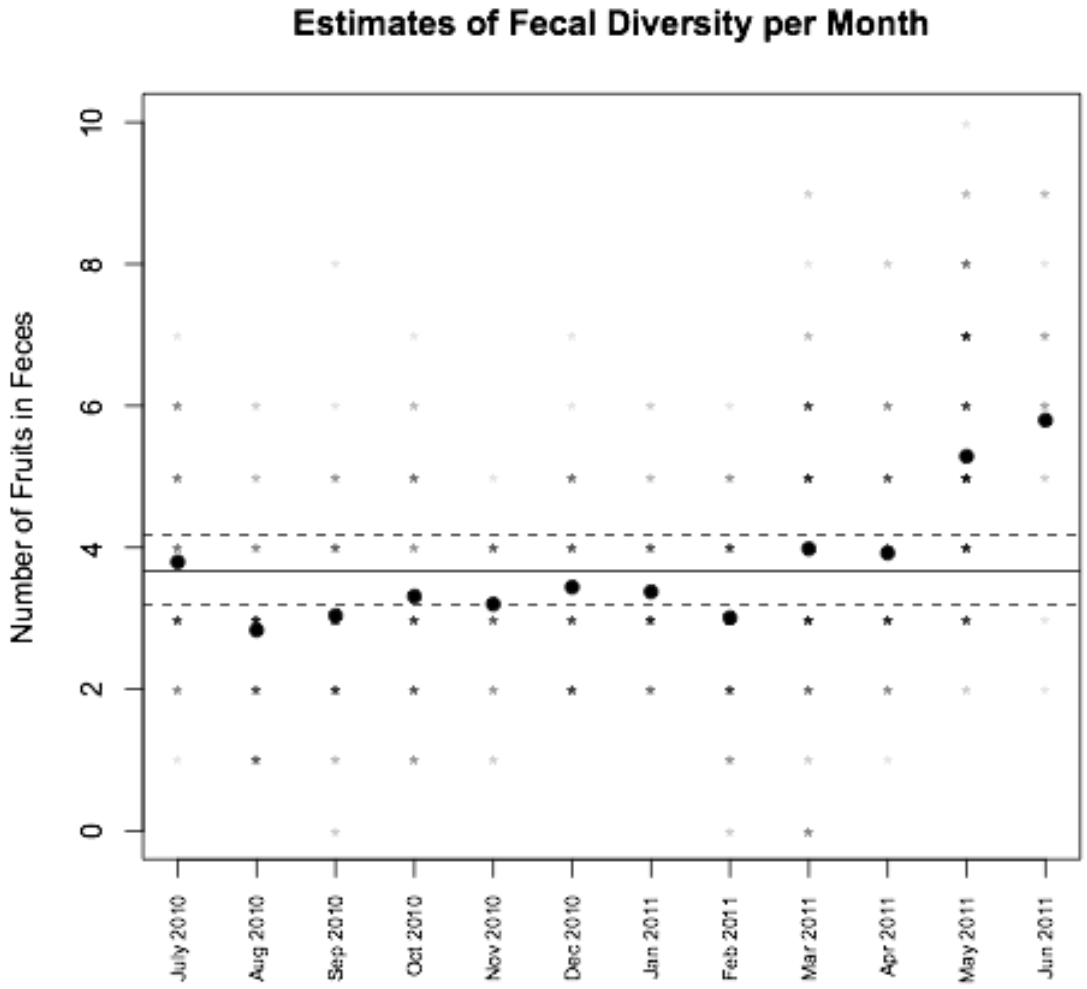

Figure 3.4.2.1.a Outcome of a GLMER model that predicts monthly average of dietary diversity (black dots), contrasted against raw data of dietary diversity (black stars).

3.5 Discussion

The Iyema forest is in many ways an "idyllic" bonobo habitat: primary forest with some swamp, abundant terrestrial herbs, and a relatively even distribution of tree and liana species that are preferred by bonobos. Rainy seasons, while they occurred, were not severe.

My data contributes to previous records from nearby Ndele that there are seasonal shifts in fruit production within and across years in the Lomako forest (White 1998; White 1996; Malenky 1990; Badrian, Badrian, and Susman 1981). Furthermore, the productivity of the majority of species that are preferred by bonobos appears to follow the rainy seasons. This sets the stage for examining whether or not a) seasonal fluctuations in resource abundance and diversity affect basic bonobo social behavior (to be measured via nest party sizes; Chapter 5) and b) metabolic stress levels in the community shift with shifts in seasonal resource availability (Chapter 6).

Chapter 4:

Terrestrial Herbaceous Vegetation and the Iyema Bonobo Diet

Chapter 4 Abstract

Terrestrial herbaceous vegetation (THV) is a key component in the diets of chimpanzees and bonobos, but varies in availability and quality across study sites and time. One of the most influential theories that has attempted to explain key differences in divergent behavioral evolution of *Pan* asserts that year-round access to THV was critical in maintaining large party sizes in bonobos during periods of food scarcity. I tested this hypothesis using data collected over the course of one year, where seasonal patterns in fruit availability were established. Analysis from 712 fecal samples showed that the species *Haumania librechtstiana* was one of the most commonly eaten food items by the Iyema bonobo community. However, it clearly and significantly was eaten less as party sizes increased, negating the role it plays in supporting bonobo social cohesion. Its consumption increased along with increases in both fruit abundance in the environment and increases in dietary diversity of the bonobos. Conversely, its consumption decreased with decreases in environmental diversity. Results strongly suggest its utility as a fallback food in this regard. However, consumption rates showed a clear and distinct seasonal pattern, suggesting that its consumption is also strongly linked to the times of year when it provides the most nutritional pay-off in terms of protein.

Terrestrial Herbaceous Vegetation: Consumption patterns by the Iyema bonobos and implications for diet and sociality

4.1 Background/lineage

Terrestrial herbaceous vegetation (THV) refers to a variety of pithy plants that can be found on the forest floor in equatorial environments, which is consumed by the African apes in virtually all observed study sites. In 1986, Richard Wrangham published a seminal chapter in a co-edited volume, Ecological Aspects of Social Evolution, theorizing that year-round access to THV was critical in supporting large party sizes during lean periods throughout bonobo evolution. By extension, THV's role in bonobo diet was hypothesized to have enabled increased gregarious behaviors mainly through a lack of intra-group feeding competition (Wrangham 1986).

4.1.2 THV consumption patterns across *Pan*

Tests of the THV theory have largely been limited to two bonobo study sites (Lomako and Wamba), but have also been applied to both eastern and western chimpanzees (Malenky, Kuroda, and Ono 1994; Furuichi 2009; Yamakoshi 2004). THV does form a large component of bonobo diet year-round in both Wamba (Kano and Mulavwa 1984) and Lomako (Malenky and Stiles 1991). However, the species of THV preferred by bonobos are not consumed as fallback food during periods of low fruit abundance, nor has it been associated with larger than average feeding parties in either bonobos or chimpanzees (Malenky, Kuroda, and Ono 1994; White 1998a; Yamakoshi 2004). Numerous studies have found low rates of time spent feeding on THV by both bonobos and chimpanzees (e.g. White and Wrangham 1988; Malenky and Stiles 1991; Stanford and Nkurunungi 2003). THV is clearly an important and consistent part of the diet of wild bonobos, but no evidence thus far supports that it is singularly responsible for

enabling sociality when resources are in decline (Malenky, Kuroda, and Ono 1994; White 1998a; Furuichi et al. 2008; Furuichi 2009).

It is possible that low rates of observed consumption reported for sites like Lomako may have had more to do with the habituation of the Lomako communities. Observations of less-habituated bonobos bias time in the trees, out of reach of THV. Alternatively, on the ground, in dense patches of THV, direct observation of feeding behaviors is difficult (pers. obs.). Examination of feces provides a clearer picture, where all feeding behaviors are represented in one convenient package. Yamakoshi (2004) compared consumption rates of THV across *Pan* study sites, including sites where chimpanzees had to compete with gorillas and found that at three sites (Lope, Ndoki and Kahuzi-Biega) the fibrous content of chimpanzee feces was greater during the non-fruiting season (Tutin and Fernandez 1993; Kuroda et al. 1996; Basabose 2002), but was unable to connect these patterns to party sizes in these communities.

4.1.2 Preferences for THV

Detailed studies of THV feeding ecology at the Ndele study site determined that THV (broadly defined) was ubiquitously distributed, but that bonobos had selective preferences for one species in particular (*Haumania liebrechtsiana*), and within that species, preferences for specific plant parts and patches. The manner in which bonobos at Ndele fed in these patches revealed that it should be considered more like fruit species, with discrete patches than a ubiquitous resource (Malenky and Stiles 1991). THV, here onwards refers to *Haumania liebrechtsiana*.

4.1.3 Nutritional Content of THV

Nutritional information of THV across study sites is lacking, though we do know that THV consumed by bonobos has a higher protein value than that of the Kanywara and Kibale chimpanzees (Wrangham et al. 1991; Malenky and Stiles 1991) (fig. 4.1.3.a). The digestible carbohydrate content for THV in Lomako is still unknown.

The consumption patterns of THV by Ndele bonobos were not statistically linked to fruit availability (measured through weekly diversity of available preferred fruits) or to rainfall patterns, whereas Kibale chimpanzees have been found to consume more THV during times of fruit scarcity (Malenky and Wrangham 1994). This suggests that while chimpanzees are probably consuming THV as a means of substituting carbohydrate loads, missing from fruit scarcity, bonobos appear to be supplementing an already carbohydrate-rich diet with added protein.

Nutritional analyses of ripe fruit consumed by bonobos showed that on average, preferred fruits contained 4.5% dry weight protein and an average carbohydrate content of 31.96% (dry weight), while the carbohydrate content of *H. liebrechtsiana* is 2.22%. The average lipid content in *H. liebrechtsiana* (1.61% dry weight) is also lower than that of ripe fruit (5.48% dry weight) (Malenky & Stiles 1991). This (in addition to relatively stable *abundance* of fruit) may explain why bonobos feed on THV year round, and not only when preferred fruits are less available: THV provides a source of consistent protein, not carbohydrates. Comparing fruits to THV is literally like comparing apples to bamboo shoots.

TABLE IV. Nutrient content of *Haumania liebrechtsiana* (% dry wt.)

Plant part	Protein	Carbohydrates	Lipids
New leaves	40.35	2.35	1.59
New shoots	44.60	2.65	1.98
New pith	30.62	1.65	1.26

Figure 4.1.3.a Nutrient content analysis of *Haumania* from the Ndele study site (Lomako Forest) by Malenky & Stiles (1991). Preferred parts of *Haumania* contain between 30 and 40% protein per gram of dry weight.

4.2 Organizing questions

Given that variation in THV consumption occurs between chimpanzee sites, it was possible that the Iyema community would express different THV consumption patterns 30 years later, and 15km away from the Ndele community. Having already shown that the Iyema community experienced seasonal changes in fruit abundance and diversity, the questions now become,

4.2.1 Do Bonobos eat more THV when dietary resources are low?

4.2.2 Is the consumption of THV correlated with larger party sizes?

4.3 Methods

4.3.1 Distribution across the study site

Marantacae species were widely distributed across the study site, occurring over approximately 72% of the transects in the study site (see Chapter 2).

4.3.2 Fecal Analysis

712 fecal samples were analyzed for dietary composition following collection from night nests (see Chapter 3). The presence of *Haumania* in the bonobos' feces was assessed on a quadrant scale: my team and I estimated the volume of the feces composed by fibers (including leaves and other species of THV), giving an impression of how much they ate in relationship to the rest of their diet. Not every fecal sample was collected in its entirety, so in order to avoid sampling bias, *Haumania* was analyzed statistically on a present/absent basis.

4.4 Results

Results from this point onwards often involve a form of statistical analysis referred to as General Linear Mixed Models (GLMMs), which were run with the help of a friend and colleague, Brendan Barrett, a graduate student at UC Davis (thus, my use of "we" when referring to choices of analysis and results). Data sets such as this one, which incorporate information from complex ecological systems and equally complex social systems that respond in part to them have presented an analytical challenge where statistics are concerned as long as ecology has been a discipline. In recent years, there has been a general push within ecological fields to use GLMMs to assess these systems with use of predictive models in lieu of null hypotheses. The advantages to this approach are myriad, especially insofar as these methods can control much better for random variation, repeat sampling from unknown individuals and "nest" relationships in a way that ANOVA's and t-tests cannot (e.g. Johnson and Omland 2004, Bolker et al. 2009).

Figures I present here are visual representations of raw data and expected outcomes based off of a series of model selections Brendan and I did, where all "predictive" factors that we hypothesized might influence resource availability in meaningful ways to a bonobo (e.g. total abundance, fruit abundance only, environmental diversity, THV consumption, etc.) were used to model the outcome of something like nest parties. Multiple models indicate different ways of statistically analyzing relationships and interactions between these factors. For the sake of space and clarity, I did not include all models here, and often present only the best model, which was chosen based on AIC.w or DIC scores, which indicate a model's predictive power in comparison to the other models. From that point, while p values are not always present, the best way of

interpreting the data is to consider the slope, or intercept, and the confidence interval around it in order to understand its power in explaining the outcome in question.

4.4.1 Do Bonobos eat more THV when dietary resources are low?

Bonobos do not eat more *H. liebrechtsiana* when either total abundance or fruit abundance are low, nor does fruit diversity have an effect on THV consumption rates. To test this, we first calculated the percentage of feces from each nest party that contained *H. liebrechtsiana* and regressed it against the respective abundance measures for that month (fig.'s 4.4.1.a, b, c). Interestingly, the percentage of individuals' feces containing *H. librechtstiana* per party vs. those without clearly followed a distinct seasonal shift (fig. 4.4.1.d).

Taking this analysis a step further, we next evaluated the interacting effects of fruit abundance, available fruit diversity, and dietary diversity by generating 4 Binomial GLMMs where the outcome was the presence of absence of THV in the feces. Month and nest party were used as varying intercepts and fecal diversity was used as a varying slope. Model comparison, using DIC and AIC favored the model that included all parameters (fruit abundance, environmental diversity, and dietary diversity) (M2, table 4.4.1.e). Predictors were centered or standardized before model fit. M2, which included all parameters was the best supported model by both DIC and AICc. Coefficients shown are direct model output that have not yet been transformed using a logistic link function (fig.'s 4.4.1.e & f). 24.6% (logistic(-1.12)) of all bonobos consumed *Haumania* across all months. Fecal diversity was positively related to *Haumania* consumption. Environmental diversity was negatively related, and as we moved one standard deviation in diversity above the mean, only 11.3% of bonobos consumed *Haumania*. As we increase the fecal

diversity by one standard deviation, we see an increase to 41.6% of bonobos who were consuming *Haumania*. Fruit abundance had a slightly positive effect, although with wide confidence intervals on both sides of zero, suggesting that there was great variation within this pattern. More variation in *Haumania* consumption was observed across months than within nest parties, suggesting two things: *Haumania* consumption is seasonal, and not randomly occurring across social composition.

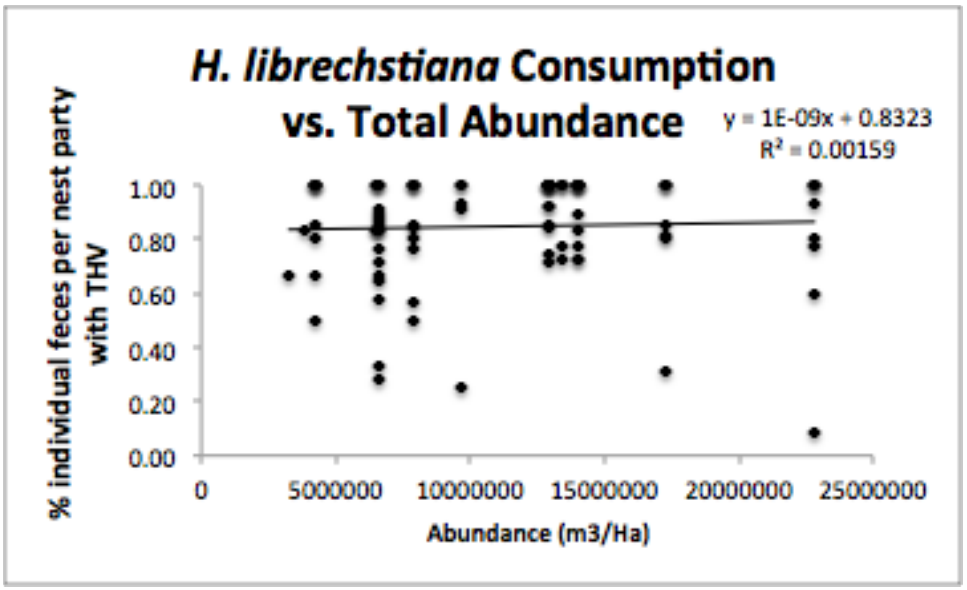

Figure 4.4.1.a THV consumption vs. total abundance. Consumption was calculated by taking the percentage of feces containing *H. liebrechtsiana* in each nest party and plotting it against the abundance measure for that month. *n*= 92

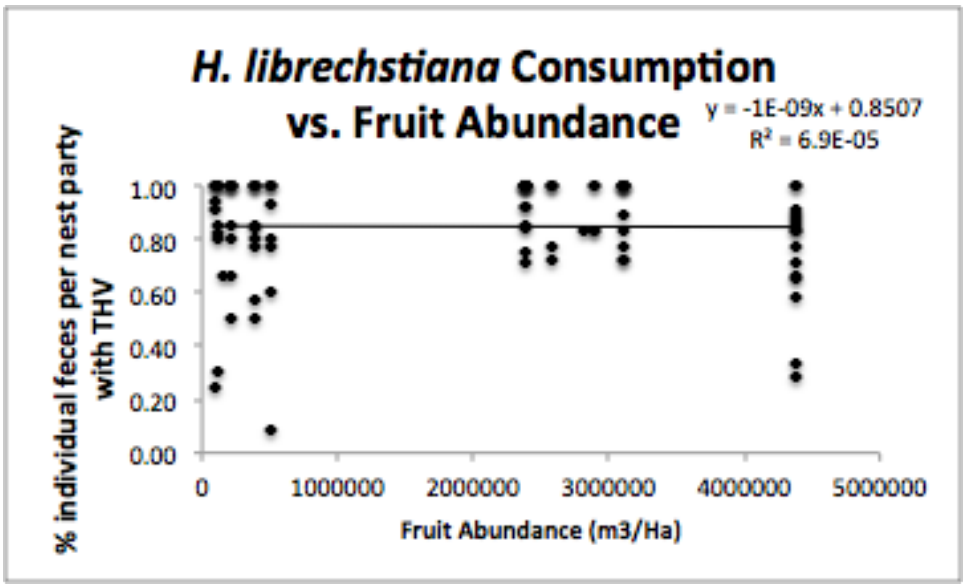

Figure 4.4.1.b THV consumption vs. fruit abundance. Consumption was calculated by taking the percentage of feces containing *H. liebrechtsiana* in each nest party and plotting it against the abundance measure for that month. *n*= 92

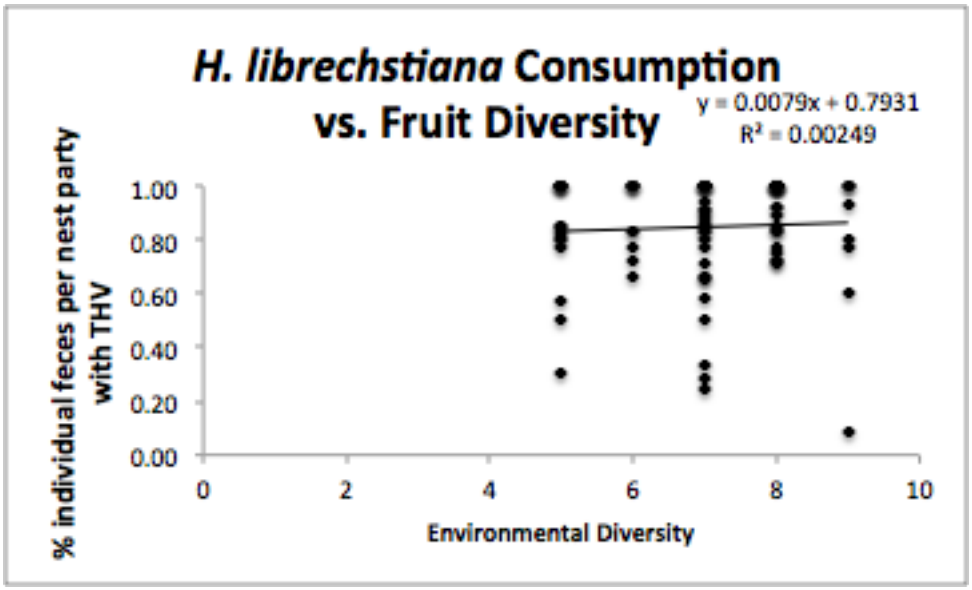

Figure 4.4.1.c THV consumption vs. fruit diversity (environmental diversity). Consumption was calculated by taking the percentage of feces containing *H. liebrechtsiana* in each nest party and plotting it against the number of species of ripe fruit preferred by bonobos for that month. *n*= 92

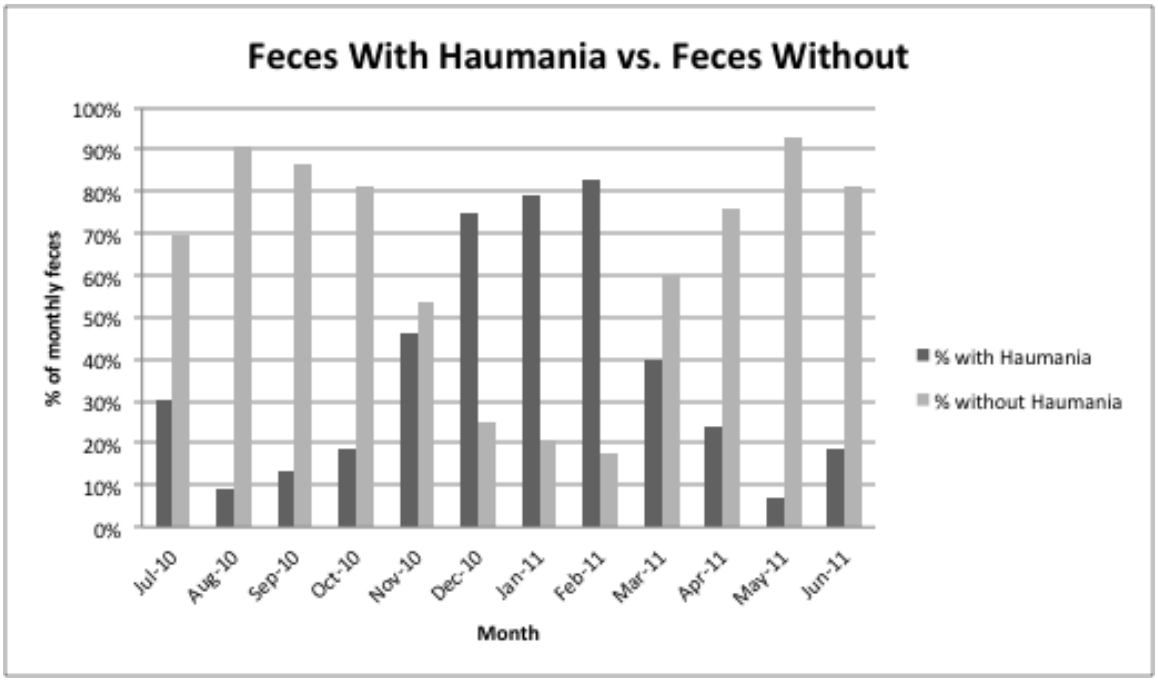

Figure 4.4.1.d Seasonal consumption rates of THV by the Iyema bonobos. Shows the percentage of feces each month where *H. liebrechtsiana* is either present or absent (does not control for total composition). The probability that THV is consumed shows a clear seasonal arc.

Model predictors of *Haumania librechtstiana* consumption	M1	M2	M3	M4
Intercept (alpha)	-0.63	-1.14	-0.93	-0.97
Effect of fruit abundance (beta1)	0.35	0.38	0.15	0.77
Effect of environmental diversity (beta2)		-0.94	NA	-0.66
Effect of dietary diversity (beta3)		0.78	0.79	
DIC	723.2487	594.0735	598.9285	670.4086
AICc	729.28	614.39	617.19	680.49
n =	715	711	711	715

Table 4.4.1.e 4 models used to predict the consumption of *Haumania librechtstiana*, using fruit abundance, number of available resources in the environment (environmental diversity) and the number of items found in the bonobos' feces as predictive factors of THV consumption. Models 2 and 3 show lower DIC and AIC scores, indicating that all factors together are important when bonobos choose to eat or not eat THV.

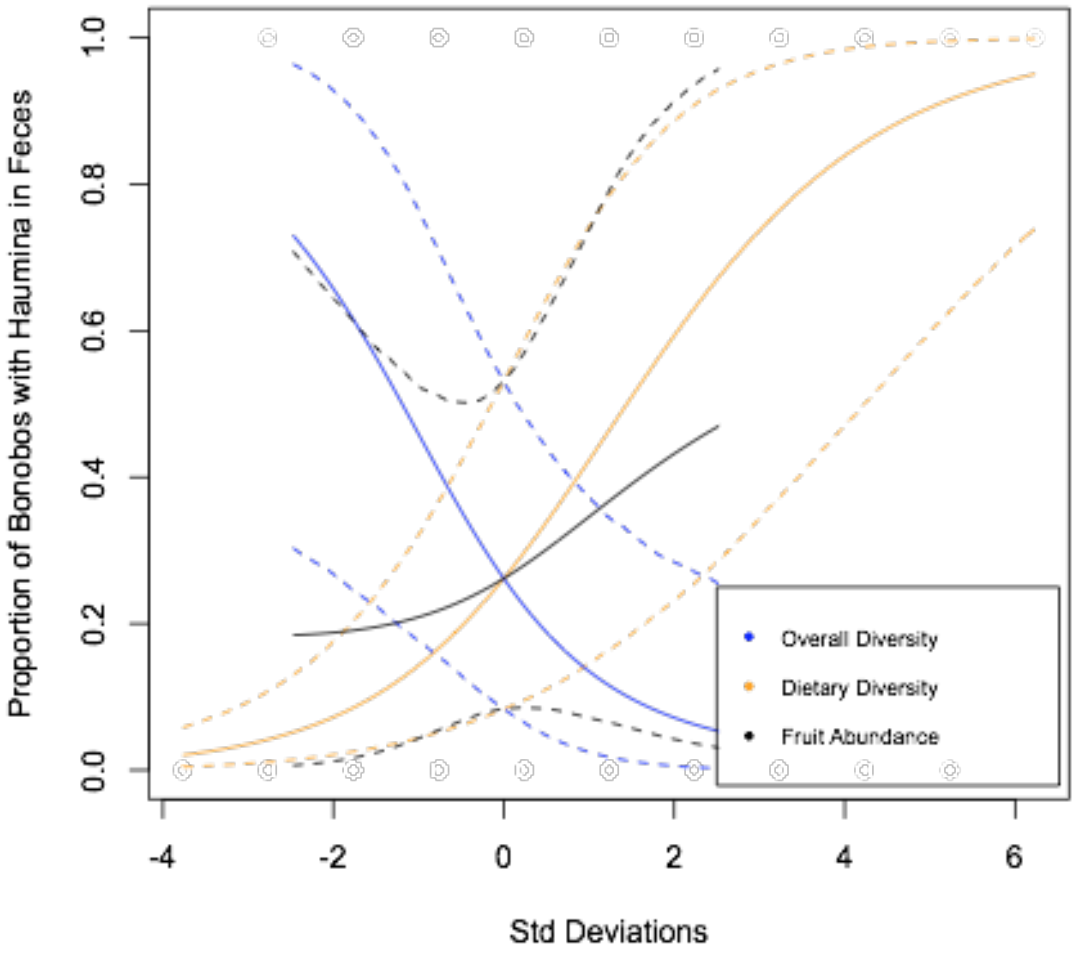

Figure 4.4.1.f "M2" relationships between *Haumania* consumption environmental diversity (blue line) dietary diversity (yellow line) and fruit abundance (green line). Dotted lines signify confidence intervals for respective factors. Fruit abundance and dietary diversity both have positive correlations with presence of *Haumania* in the feces, while the number of fruits in the environment (environmental diversity, referred to above as overall diversity) has a negative effect.

Predictors of THV consumption	Estimate(95% CI)	SE	z value	Pr(>|z|)	N
Intercept (alpha)	-1.12 (-2.42 - 0.14)	0.65	-1.744	0.0812	711
Effect of fruit abundance (beta1)	0.38 (-0.77 - 1.52)	0.58	0.648	0.5171	
Effect of environmental diversity (beta2)	-0.94 (-1.69 - - 0.19)	0.38	-2.465	0.0137	
Effect of dietary diversity (beta3)	0.78 (0.47 - 1.09)	0.16	4.929	8.27E-007	

Table 4.4.1.g “M2” results summary: effects of fruit abundance, fruit diversity and dietary diversity as predictors for the consumption of *Haumania librechtstiana*. Fruit abundance and dietary diversity both have positive correlations with presence of *Haumania* in the feces, while the number of fruits in the environment (environmental diversity) has a negative effect.

4.4.2 Is the consumption of THV correlated with larger party sizes?

The consumption of THV does not support larger party sizes. We used both a general regression of the percentage of feces containing *H. liebrechtsiana* in a nest party and nest party sizes, and a poisson GLMM with random effects for month and controlled for overall abundance. The presence of THV in the feces decreased significantly ($p=0.027$) as mean monthly party size increased (fig. 4.4.2.a) suggesting that at least, on a month-to-month basis, big parties are not associated with THV consumption. The mean party size here was estimated as 7.02 (but see Ch. 5), and when considered on a party-by-party basis the presence of *Haumania* in feces had a near zero effect on party size (Table 4.4.2.b). These two results are two different ways of viewing a complicated picture: on one hand, monthly means offer a way of looking at broad, temporal relationships. On the other, viewing a nest-to-nest variation shows that the link between THV consumption and nest parties is not singular.

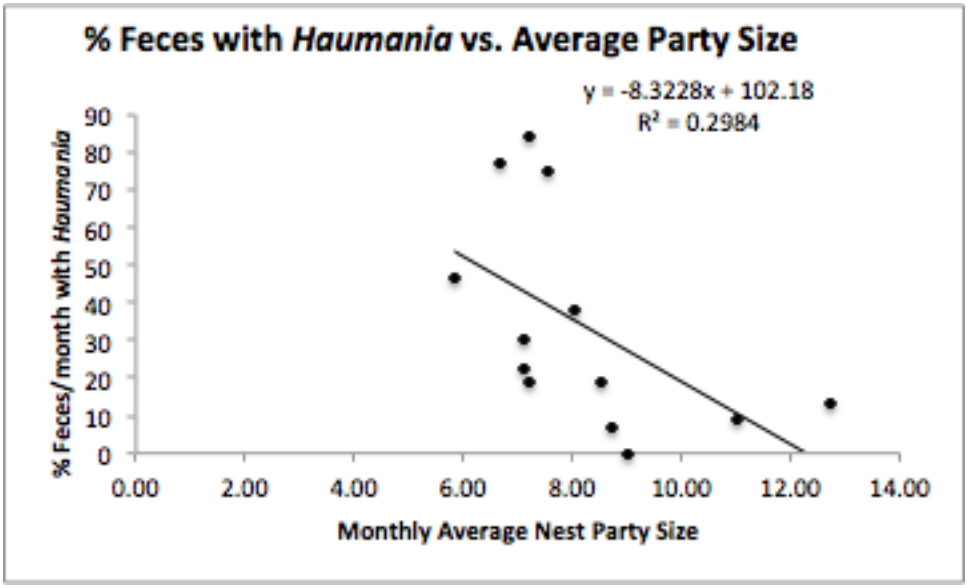

Figure 4.4.2.a Monthly percentage of individual feces with *H. liebrechtsiana* vs. average monthly nest party size between June 2010 and June 2011.

GLMM for the effects of *H. liebrechtsiana* on nest party size:
(*Note that "Bekombe" refers to *H. liebrechtsiana*)

Model: m1<- glmer(num.nests ~ Bekombe.f + (1+ Bekombe.f|project.month) + (1+ Bekombe.f|nest.party) , data=d , family="poisson")

***Haumania* and Nest Party Size**	**Mean Parameter Estimates (95%CI)**	**SE**	**pr(>abs(z))**	**N**
Intercept (alpha)	1.95 (1.83 - 2.06)	0.05907	<2e-16 ***	712
***Haumania* Effect on NPS**	0.002 (-0.07 - 0.07)	0.035375	0.939	

Table 4.4.2.b Effect of *H. liebrechtsiana* on nest party size (abr. NPS in table). *H. liebrechtsiana* has no effect on nest party size.

4.5 Discussion

My observations fall in line with previous tests of the THV hypothesis: THV is not associated with larger parties. In fact, it is significantly and negatively correlated with nest party size. THV, specifically *Haumania liebrechtsiana* does comprise a significant portion of the Iyema bonobo diet, being present in over a third of all feces and ranking the 3rd most frequently consumed food source out of 712 samples, over the course of a year of observations.

Haumania consumption increased both when abundance increased, and to greater effect when dietary diversity increased; conversely, increases in environmental diversity saw a decline in *Haumania* consumption. Increased environmental diversity offers more dietary options for wild animals, though does not necessarily directly correlate with an increase in dietary diversity: having more options increases the likelihood of satisfying energetic and nutritional needs with a few key items, which might explain the negative relationship with *Haumania*. Conversely, a wide dietary breadth may indicate the need to satisfy energetic and nutritional needs through multiple avenues, which would explain the positive relationship between dietary diversity and *Haumania* consumption. In other words, this paints *Haumania* as a fallback food.

However, the periods when *Haumania* is consumed at the highest rates directly coincide with the longer dry season during the study period, which probably reflects seasonal patterns of *Haumania* growth: *Haumania* produces higher rates of fresh young shoots and stems following the rain season (Frances White, pers. com.). It is possible that *Haumania* acts as both a fallback food, and as a seasonally preferred food: it can be consumed year round, but may provide higher energetic and nutritional pay-off at key

times during the year, which would explain both its statistical relationships to environmental and dietary diversity, as well as the highly seasonal pattern of its consumption in this community.

In spite of repeated efforts on part of field workers who study bonobos and their diet, the notion that THV has supported bonobos' large party sizes remains enigmatically present in the kinds of basic ecological comparisons of *Pan* that are taught in intro anthropology courses. My data, like so much data before it reaffirms that while THV is clearly important in wild bonobo diets where it is present, it does not in any way shape or form support large party sizes. In fact, it may be more clearly associated with restricting them (see conclusion chapter). The question of what a large party size is, is of course, relative and the focus of the next chapter.

Chapter 5:

Nest Parties and Their Ecological Correlates at Iyema

Chapter 5 Abstract
A fundamental theme in socioecology is the relationship between an organisms environment and its social structure. The fission-fusion system found in *Pan* has been thought to minimize feeding competition, by forcing groups into small parties when intra-community feeding competition rises. A commonly observed difference in the social dynamics between bonobos and chimpanzees is the tendency for bonobos to have a) larger parties on average than chimpanzees and b) a higher ratio of females to males than chimpanzees. Explanations for this have often assumed that bonobos have been able to maintain larger party sizes on average than chimpanzees because they live in an environment that is hyper-abundant. Using ecological measures on seasonal shifts in fruit abundance and diversity in the Iyema study site, along with information from 105 separate nest parties over a year-long period, we tested predictors that best explained variations in bonobo party sizes (represented through nest parties). The average nest party size across the year was 7.7, which was similar to other bonobo sites, as well as chimpanzee sites, such as Tai and Bossou. We used GLMMs to model ecological correlates that influence sociality, and found that abundance had little to no effect on predicting nest party sizes, while the number of species preferred by bonobos that were ripe per month were the best predictor of bonobo grouping patterns. We also found that bonobo nesting patterns showed seasonal variation, indicating that they experience environmental pressures that limit their sociality.

Nest Parties at Iyema: Basic patterns of seasonal sociality and their ecological correlates

5.1 Background

5.1.1 Factors that affect primate social dynamics

The relationship between environment and the social structure and behavior in primates has been a major focus of primatological research since the discipline's foundations (Crook and Gartlan 1966; Altmann 1974; Wrangham 1980; Van Schaik and Van Hooff 1983; Terborgh and Janson 1986; Isbell and Young 2002; Newton-Fisher, Reynolds, and Plumptre 2000). For decades, fundamental themes of relationships between reproduction and access to resources have been the basis of what and how we understand primate ecology and evolution. In summary, the logic usually goes that females, burdened with the weight of long-term reproductive investment will organize themselves according to resource distribution, which will either be patchy or "evenly" distributed. Males will follow suit, organizing themselves around access to females; in some cases, threat of predation will increase or decrease group sizes (Wrangham 1980; van Schaik 1989; Sterck, Watts, and Van Schaik 1997). As discussed in chapter 3, ecological correlates of social dynamics in *Pan* have yielded mixed results, showing some support for the effects of fruit diversity on party sizes, no effects from abundance, and the most strong effects from estrous females (see fig. 5.1.1.a)

Travel costs have been hypothesized to restrict party sizes in primate communities (Chapman and Chapman 2000), and received some attention and support in chimpanzee studies. However, the importance of direct costs associated with travel as they influence group dynamics in *Pan* seem to be largely outweighed by the significant pull that estrous females have in attracting members to a party (Thompson et al. 2007; Wrangham 2000;

Anderson et al. 2002). I was unable to include time spent traveling in my behavioral observations, and my observations of party composition were not consistent enough to be useful for analysis, so travel distance and the number of estrous females are two factors that were not considered directly in my statistical analysis (though, they are discussed in the conclusion).

Humans are the only verified predatory threat to bonobos; leopards and venomous snakes pose obvious threats, but the direct effects that leopard predation may have on bonobo socioecology remain unexamined. Only one observation that suggests predation on bonobos by leopards exists, but the observation was indirect: a bonobo toe was found in fresh leopard scat in 2004 (D'Amour et al. 2006).

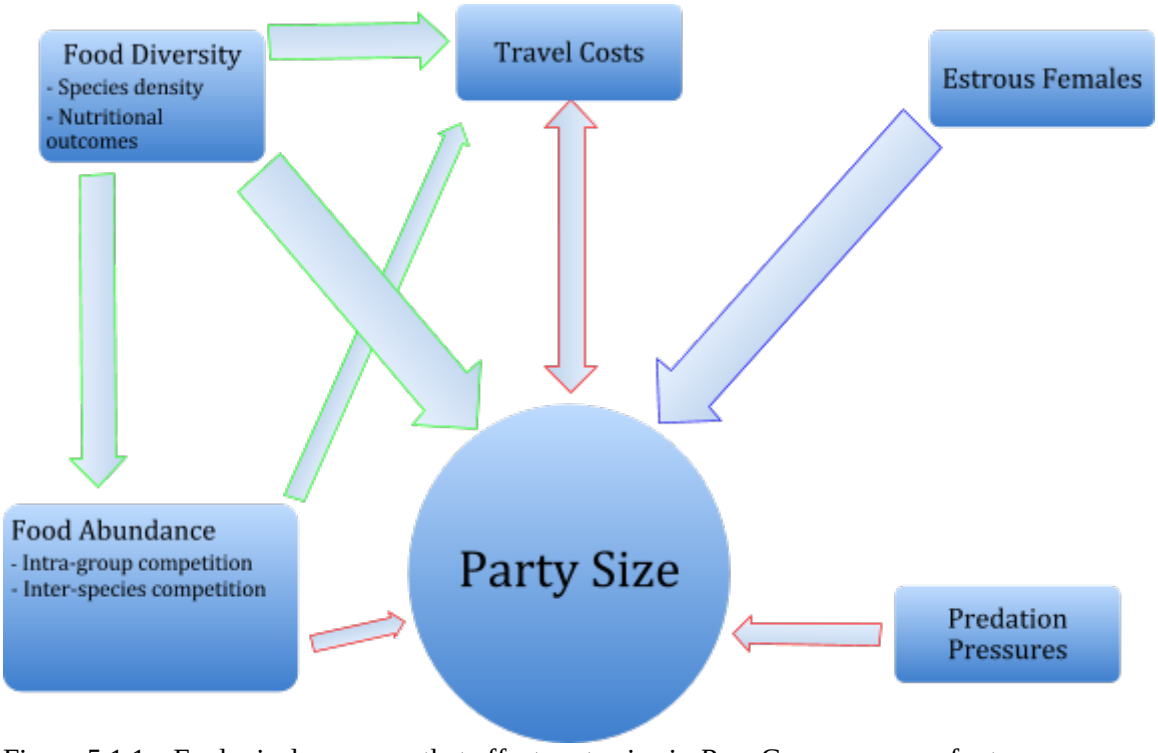

Figure 5.1.1.a Ecological pressures that affect party size in *Pan.* Green arrows refer to resource-based pressures. Blue arrows refer to social pressures. Red arrows refer to factors that are hypothesized to affect social behaviors, but which either lack support or data. Larger arrows suggest larger influence on outcomes.

5.1.2 Feeding parties in bonobos and chimps:

5.1.2.1 Fission Fusion Communities in Pan

Chimpanzees and bonobos live in multi-male/multi-female fission-fusion communities, composed of anywhere between a handful of individuals to over 100 residents who will divide into smaller sub-groups that move, feed and sleep within their respective home-ranges (Mitani, Watts, and Muller 2002; Furuichi 1989). These communities, due to their daily and hourly flexibility, provide an excellent means by which to examine socioecological dynamics between sociality and short-term environmental changes. The fision-fusion structure may limit intragroup feeding competition and improve foraging efficiency. Smaller parties form at times when resources dip in both species (though, the methods for measuring declines in resource availability vary) (White 1996b; Doran 1997; Hashimoto et al. 2003; Mitani et al. 2002; Chapman et al. 1995)

The ecological and social correlates between party size (usually expressed as a percentage of total community size) has been a repeatedly examined in chimpanzees for decades, producing a wide range of results that demonstrate more than anything the flexibility that chimpanzees are capable of (e.g. Boesch, Hohmann, and Marchant 2002). Far fewer studies on bonobo communities have contributed to this line of research, both within and across three study sites: Ndele (Lomako), Wamba and most recently, Lui Kotal (Hohmann and Fruth 2003b).

Changes in bonobo feeding party size and composition have been observed to occur in sync with seasonal shifts in kind and availability of fruits in Lomako Forest, suggesting that bonobo sociality is constrained by their physical environment (White 1998). It should not be surprising that, if there are indeed seasonal periods in the

bonobos' environment where resources decline in quantity and quality, that they would respond in kind by constraining their party sizes as a means of cutting back on immediate feeding competition. The implications of a clear cut response to seasonal shifts in resources by reducing feeding parties is important in the debate on *Pan* evolution. If both bonobos and chimpanzees reduce their party size according to regular periods of low resource availability, then why do we see such different outcomes in terms of group composition? In other words, if the qualities that bonobos are best known for result from having led an evolutionary path where environment played no role in restricting sociality, we would not expect to see reductions in party size. Nor would we expect to see such specific responses to small occurrences of food stress (e.g. brief sexual encounters with members of the same and opposite sex when entering a food patch). It seems clear that food has played an important role in the development of bonobo social behavior and dynamics, but when and how those pressures have occurred remain unclear, prompting the question, how do changes in food availability affect bonobo social dynamics?

5.1.2.1 Feeding parties vs. Nesting Parties

Feeding parties are typically used as a measure of sociality in fission-fusion species such as bonobos and chimpanzees, which presents a number of challenges for field studies. In dense forests visibility is limited, and depending on the level of the community's habituation, observers run the risk of chronically underestimating party size when not all members are both visible and/or vocal (pers. observation). Nests, however, provide a static and consistent form of party measurement that have been shown to reflect average feeding party sizes throughout the days surrounding their construction (Mulavwa et al. 2010; but see Hohmann and Fruth 2003b).

All of the great apes build fresh nests every evening, and sometimes during the day for the primary purpose of resting (Goodall 1962; Kano 1983; Fruth and Hohmann 1993; Schaller 1963; MacKinnon 1974). The density of nests observed on transects is commonly used as a means of estimating density for bonobo populations (Mohneke and Fruth 2008; Reinartz et al. 2008). Nests themselves have been argued to demonstrate nuanced aspects of social behavior in bonobos, acting as "taboo zones" for any individuals other than the occupants during rest and play time (Fruth and Hohmann 1993). They are not, however, commonly used as an index of bonobo sociality. Reasons for why nest party sizes are used less than feeding party sizes are not entirely clear, except that typically in this field behavioral observations are prioritized over indirect observations when possible, and often the amount of time and effort that field workers can expend in collecting this kind of data is limited. It can also be argued that feeding party sizes are a better reflection of sociality because they directly affect animals' ability to gain access to energetic and social resources, while nest parties represent a period of time during the day when their proximity to one another is largely passive (because they are sleeping). However, a major handicap in this line of reasoning is that our perception of a meaningful feeding party of bonobos may not be the same as the bonobos' understanding. Measures of proximity used in these behavioral observations are well-thought out and standardized, but risk exclusion of peripheralized animals out of sight to us, who are still well perceived by their conspecifics- and this is important when considering something like stress. Nest parties, in this case are a valuable measure because they provide a static, consistent summary of the previous day's activities in terms

of both sociality (through the number of nests in the party) and through the contents of the feces and urine beneath each individual nest each morning.

5.2 Organizing questions

This study aimed to re-address the issues summarized above with a new study community and study site in order to contribute to the larger picture of diversity in *Pan* with the following questions:

5.2.1 Do party sizes shift seasonally?

5.2.2 Do seasonal shifts in resource availability, measured through abundance and diversity affect party size?

5.3 Methods

Collection of phenological and fecal/dietary data are described in chapter 3 (seasonality).

5.3.1 Nest Parties

My team and I collected information on the size of 129 nest parties for every morning follow between June 2010 and June 2011, and opportunistically when we came across nest sites that were within 1-2 weeks old (identified by freshness of leaves, occasional presence of older fecal material, and color of broken branches). A nest party was defined according to methods used by Mulavwa et al. (2010), where a congregation of nests was defined as all nests within 30 m of the nearest neighbor. If nests that appeared to be part

of the nest party occurred outside of the 30 m cut-off, they were counted as a separate party.

5.4 Results

5.4.1 Do party sizes shift seasonally?

Using month as a measure of season, Iyema bonobos showed some seasonally-related shifts in nest party sizes, but variations of party sizes (between 2 and 24 nests in the same week) make any clear patterns difficult to discern (fig. 5.4.1.a). However, using a GLMM that controlled for variation across months, there was a clear effect of the month on the number of nests in a party (fig. 5.4.1.c and table 5.4.1.d). Figure 5.4.1.c demonstrates variation across months in weighted means of measured nest party sizes; it does not predict nest party sizes based on other factors. The average party size over all months was 7.7, with a standard deviation of 4.5.

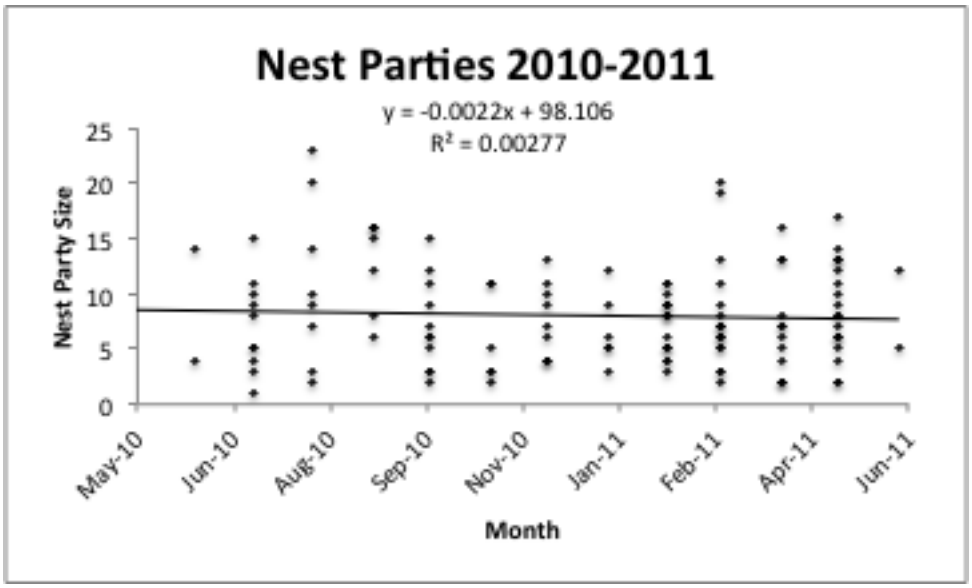

Fig. 5.4.1.a Nest party sizes between May 2010 and June 2011. Each dot represents an individual nest party from that month; n= 129

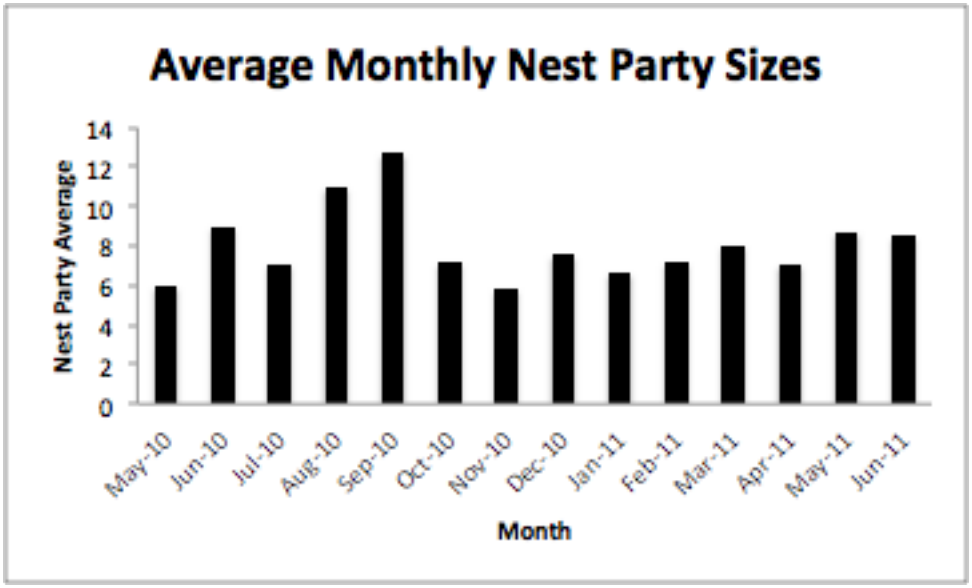

Fig 5.4.1.b Average nest party sizes across months; suggests some degree of seasonality.

GLMM code: **m1 <- glmer(num.nests~ 1+ (1|project.month) , data=dd , family="poisson")**

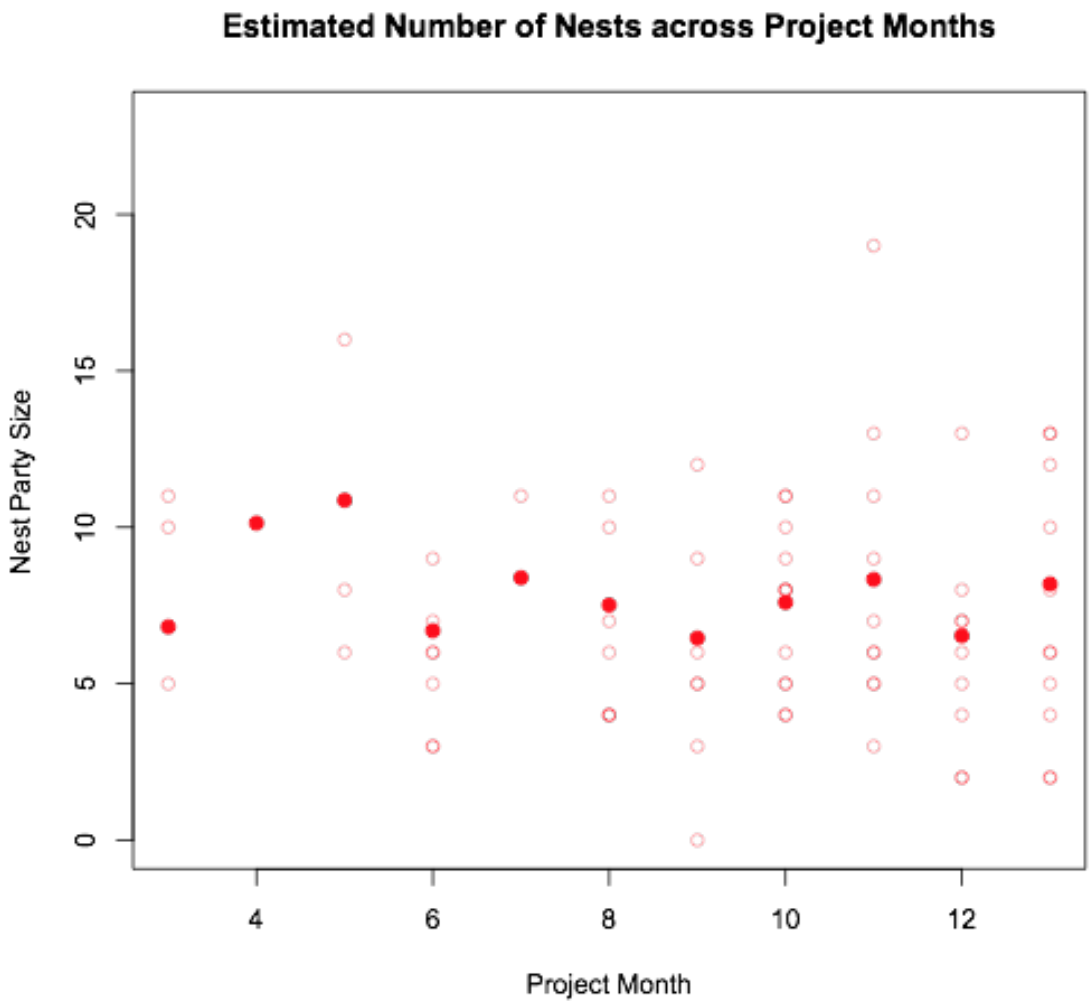

Figure 5.4.1.c Modeled estimates of nest party sizes per month. Shows clear shifts in average nest party estimates (solid red dots) against raw data (pale red dots). Project month 1=May 2010.

Effect of Month on Nest Party Size	Estimate	S.E.	2.5%-97.5%	*p*
Intercept	2.06	.07	1.93-2.19	<2e-16***

Table 5.4.1.e Effect of Month as a predictor of nest party size. *n* = 105

5.4.2 Do seasonal shifts in resource availability, measured through abundance and diversity affect party size?

It appears that diversity and abundance in conjunction explain party size. Both DIC and AIC favored the model(s) with environmental diversity as a predictor of the number of nests. Below are summaries from several poisson GLMMs using month as a varying intercept. M3 is the best-supported model in this case, although all models make similar predictions. While it is typically advisable to average models, when predictions between them are similar, we chose to use M3 because it's AIC.w score was highest, accounting for more variation in outcomes in spite of similar AIC and DIC scores. Basic regressions, using scatterplots showed no relationship (fig. 5.4.2.c &d)

Predictors for Number of Nests	M0	M1	M2	M3	M4
Intercept (alpha)	2.24	2.23	2.26	2.25	2.24
Effect fruit abundance (beta1)	NA	-0.06	NA	-0.09	-0.09
Effect of environmental diversity (beta2)	NA	NA	0.06	0.08	0.1
Interaction between fruit diversity and abundance (beta3)	NA	NA	NA	NA	0.03
DIC	4167.924	4166.826	4165.141	4161.43	4169.49
AICc	4171.94	4172.86	4171.17	4169.49	4170.91
n =	723	723	723	723	723

Table 5.4.2.a Different models and their criteria for predicting nest party size. Best model was chosen based on the lowest DIC (deviance information criterion) value

Predictors of Nest Party Size	Estimate(95% CI)	SE	z value	Pr(>|z|)
Intercept (alpha)	2.25(2.17 - 2.33)	0.04	53.05	< 2e-16
Fruit abundance (beta1)	-0.09(0.03 - 0.13)	0.03	2.98	0.00284
Environmental diversity (beta2)	0.08 (-0.18 -0.01)	0.04	-2.1	0.0361

Table 5.4.2.b Result summary for M3, predicting nest party size using combination of fruit abundance and diversity measures.

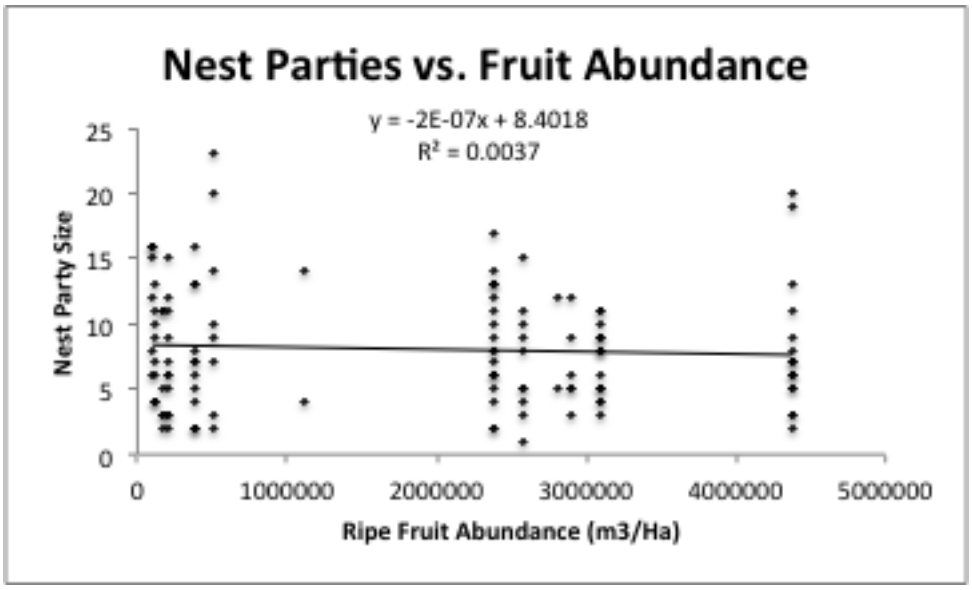

Fig. 5.4.2.c.a Basic correlation of individual nest party sizes and monthly estimated abundance (m3) of preferred ripe fruit.

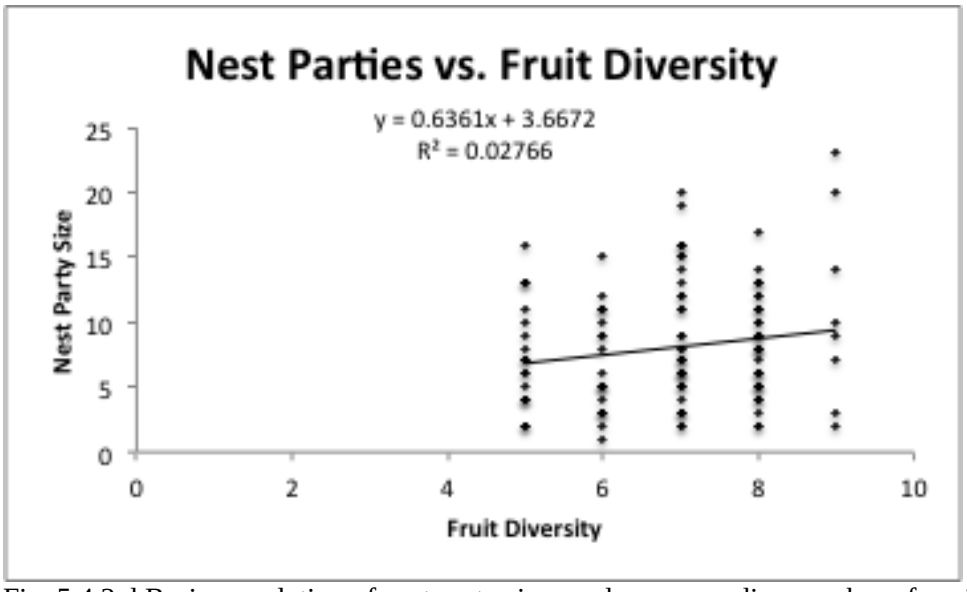

Fig. 5.4.2.d Basic correlation of nest party sizes and corresponding number of available preferred species in the environment.

5.5 Discussion

Nest party sizes showed a small seasonal pattern, in spite of wide fluctuations in the short term, sometimes manifesting as a party of two and then a party of twenty or more in the same day or week. Likewise, seasonal patterns detected in Chapter 3 appear to have a predictive effect on nest party sizes in spite of outward appearances represented in basic scatterplots. This highlights the value of using integrative statistical modeling when addressing complex socioecological relationships such as these; basic regressions would have shown no relationships. The models I use here incorporate ecological factors that influence bonobo living at the same time, neither of which necessarily have an effect on party size on their own, but which interact meaningfully. This is better exemplified in the next chapter, where I demonstrate how shifts in abundance can augment the relationship between energetic hormones and environmental diversity; the latter two appear to interact on their own in a way that might be detectable with a scatterplot and regression, but the interaction is best contextualized in terms of other factors that a single regression by itself would not show.

The average nest party size was 7.7, with a standard deviation of 4.5. This is comparable to results seen at Wamba in the last decade, post-provisioning (Mulavwa 2010) but smaller than earlier reports for Wamba (fig. 5.4.a; Kano 1992), and comparable to those reported for Ndele and Tai Forest (Lehmann and Boesch 2003; Badrian and Badrian 1984; White 1996). Consideration of nest party sizes is preferably done as a percentage of total community size, but this was not possible due to unfinished habituation of the community. In spite of this, the implications for the nest parties seen

here present an opportunity for considering at least, some continuity between two close study sites (Iyema and Ndele), similar to Kanywara and Ngogo, where nest party sizes differ significantly, presumably due to differences in resource availability and competition (e.g. Emery Thompson 2009).

The question here now becomes, if these shifts follow what appears to be a seasonal pattern, do we see energetic trade-off's on a micro scale through expression of energetic hormones?

Chapter 6:

Hormonal correlates of seasonality, sociality and diet in the Iyema bonobo community

Chapter 6 Abstract

The relationship between changes in social party sizes and environmental correlates remains variable across chimpanzee communities and unclear in bonobos. Similar to multiple chimpanzee communities, resource abundance appears to have no clear effect on party size in bonobos. Rather, the number of available preferred species (environmental diversity) is the best predictor of bonobo party size in bonobo communities. I reexamined the relationship between sociality in bonobos (measured through night nest party sizes) and their environment through the filter of metabolic hormones, such as fecal glucocorticoids, urinary cortisol and urinary C-peptide of insulin. We found that both urinary hormones were better correlated to changes in party size, and changes in resource availability than fecal glucocorticoids. Urinary cortisol was most likely to be highest when nest party sizes were above average size in the context of low environmental diversity, and lowest when nest party sizes were below average size in the context of high dietary diversity. Conversely, C-peptide levels were likely to be highest when party sizes were small and environmental diversity was high, and declined as nest party size increased and environmental diversity decreased. We also found a strong relationship between nest party size and cortisol levels, which were lowest in average party sizes, after controlling for resource availability, suggesting that above average party sizes create more opportunity for stressful environments, thus limiting hyper-sociality. C-peptide levels were highest in the context of medium-to-low dietary diversity, suggesting that in the bonobo diet, quantity does not necessarily equate with quality in terms of energetic pay-off. No significant relationships were found with any of the three energetic hormones and the THV species *Haumania librechtstiana.* Ketones were not found to be accurate predictors of energetic stress (measured via C-peptide levels), due to several field-specific problems. These relationships bring to focus the complexities of bonobo social relationships in the context of diet and resource availability: although they appear to live in a plentiful environment, bonobos clearly experience limitations in sociality, which are reflected through various manifestations of physiological stress.

Hormonal Correlates of Seasonality, Sociality and Diet in the Iyema Bonobo Community

6.1 Introduction:

Theoretical frameworks that account for variation of group sizes in social animals assume that there are fitness consequences associated with social living, and that membership in groups of favorable size is a means of optimizing fitness (Wrangham 1980). Costs and benefits of social grouping in primates have been examined on many levels (e.g. Sterck et al. 1997), largely through comparisons of behavior (Chapman and Chapman 2000) and fitness parameters (Takahata et al. 1998; van Noordwijk and van Schaik 1999). Cost and benefit analyses of different aspects of primate living have also been successfully examined using glucocorticoids as a means of assessing fitness in the short term. The acquisition and maintenance of rank (Virgin and Sapolsky 1997), reproduction costs (Brockman et al. 2007; Muller et al. 2007), development and maintenance of close social bonds (Crockford et al. 2008) have all demonstrated the nuanced role that glucocorticoids play in the social behavior of wild primates. Costs and benefits of variation of group size using GCs as a measure of fitness has been examined successfully in lemurs, but no other primates to date (Pride 2005).

Non-invasive collection of metabolic hormones through urine and feces (e.g. urinary cortisol, fecal corticosterone, urinary C-peptide of insulin) has been used in a variety of species to address complex intersections between social and physical environments (Whitten, Brockman, and Stavisky 1998; Emery Thompson and Knott 2008; Kitaysky, Piatt, and Wingfield 2007; Weingrill et al. 2004; Pride 2005; Tecot 2010; Surbeck et al. 2012). Shifts in energetic hormones that accompany natural shifts in resource availability

have also been well-documented in other primate, mammalian, bird and reptile species (Kitaysky & Wingfield 2007). Steroid metabolites are detectable in the feces of a wide range of mammals as well as birds (Palme et al. 2005), and have been shown to accurately represent preceding hormonal events in plasma though the lag time between those events and defecation is largely species-specific (e.g. Bahr et al. 2000).

With these factors in mind, I aimed to re-examine unresolved questions about bonobo socioecology with the added lens of hormones involved in both metabolic and psychosocial stress (figure 6.1.a).

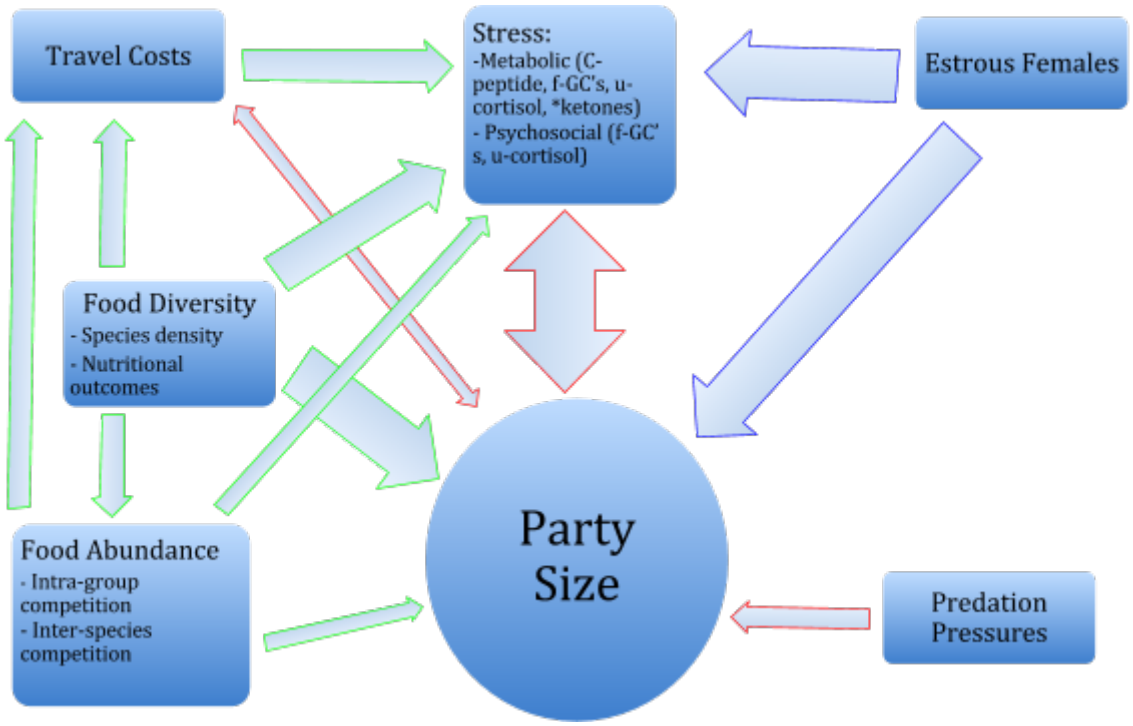

Figure 6.1.a Socioecological model of predictors of party size for *Pan*, including stress as a predictive factor. Green arrows refer to resource-based pressures. Blue arrows refer to social pressures. Red arrows refer to factors that are hypothesized to affect social behaviors, but which either lack support or have yet to be studied in depth. Larger arrows suggest larger influence on outcomes.

6.1.1 Bonobo (and chimpanzee) behavior, ecology, social structure and their hormonal correlates

Examination of the energetic states of bonobos is long overdue, considering that the main hypotheses explaining differences in *Pan* center on differences in food availability (Wrangham 1986, White 1998, Furuichi 2009). Urinary cortisol, fecal glucocorticoids, C-peptide of insulin, and ketones have all been shown to reflect various states of energetics in the great apes in captivity (Deschner et al. 2008) and in the wild (Emery Thompson and Knott 2008; Vogel et al. 2012). A pronounced effect of seasonality on energetic hormones has also been found in species that experience a range of environmental seasonal expression, such as chimpanzees (Emery Thompson et al. 2009), orangutans (*Pongo pygmaeus)*(Knott 1998), ring-tailed lemurs (*Lemur catta*)(Pride 2005). Changes in levels of urinary cortisol have been examined on an interpersonal, but not seasonal scale in bonobos at Lui Kotal by (Surbeck 2012). No other published studies exist to date on energetic hormone levels in wild bonobos.

6.1.1.1 Glucocorticoids: fecal corticosterone metabolites and urinary cortisol

Glucocorticoids (GCs) are metabolic hormones secreted in the adrenal glands that act on both the brain and body to affect both behavior and fitness. They, along with catecholamines, are essential in responding to an acute stressor but become pathogenic when secreted in excess over long periods (reviewed by Sapolsky et al. 2000). In the absence of detailed life-history data, the physiological measure of stress can be useful as a means of identifying key relationships between an organism and its environment, especially insofar as identifying which factors most strongly influence adaptive behaviors. As an adaptive physiological mechanism, stress should theoretically signal the

need for a change in behavior, or predict a chronic condition in the absence of change. Thus, stress is a useful analytical tool for modeling how adaptive behaviors emerge on a micro-temporal level in an evolutionary framework.

Long-term exposure to stress hormones has been well demonstrated to have suppressive effects on both immune and reproductive functions in other species of primate, such as baboons (Sapolsky et al. 2000; Sapolsky 1993), while in some shorter-lived species, such as ring-tailed lemurs (*Lemur catta*) they have been linked to decreased life expectancy (Pride 2005b). The physiological effects of exposure to different levels of stress can be used to infer the meaning of life-strategy choices. For example, in some cases, the physiological costs associated with obtaining and maintaining high rank may be outweighed by the benefits that come with high rank (Wingfield et al. 1990; Muller and Wrangham 2004). In other cases, obtaining a higher rank may be possible, but due to the stress involved an individual may choose another strategy that may not reap the benefits of high rank, but avoid the costs as well (Virgin and Sapolsky 1997).

GCs are commonly employed as a means to assess general "stress" levels in highly social, wild-living primates. Stress is a broad term, but typically refers to energetic stress incurred from lack of food or physical exertion, or psychological stress from perceptions of threats from the outside world. Outside of controlled conditions, it is impossible to discern the extent to which observed levels of GCs reflect psychological states, physiological states, or both, due to the multiple functions GCs play in highly social animals, which highlights the need to look more broadly at other hormones specifically involved in metabolism and unaffected by psychosocial conditions.

6.1.1.2 Urinary C-peptide of Insulin

Urinary C-peptide of Insulin (C-peptide) is a by-product of pro-insulin that specifically reflects levels of insulin in the blood prior to expulsion and is unaffected by psychosocial stressors. C-peptide has been used as a reliable signal of energetic status in orangutans (Emery Thompson and Knott 2008), chimpanzees (Sherry and Ellison 2007; Emery Thompson et al. 2009), and bonobos (Deschner et al. 2008). In principle, C-peptide can be used to a) contextualize measurements of GCs (measured here, through urinary cortisol or fecal corticosterone) and b) test whether or not seasonal shifts in fruit availability do impose physiological costs on bonobo foraging patterns.

6.1.1.3 Expected Relationships between glucocorticoids and C-peptide

C-peptide is released in the conversion of pro-insulin to insulin and is produced on an equimolar basis with insulin, a hormone critical in the transfer and storage of energy. C-peptide is an effective way of measuring energetic load (e.g. levels of insulin) in the context of socioecological relationships, such as social rank and fruit availability: high levels of C-peptide represent high energetic intake, while low levels are linked to diminished energy intake (Sherry and Ellison 2007; Emery Thompson and Knott 2008). Simply put, if rising monthly levels of GCs inversely correspond to decreasing levels of C-peptide (fig. 6.1.1.a), the change in GCs is probably related to energetic costs incurred during that period, whereas if C-peptide levels remain high in an individual with high levels of GCs, then psychosocial factors are more likely to be the cause of stress indicated by GCs (fig. 6.1.1.b). In this case, fluctuations in GCs should correspond between urinary cortisol and fecal GCs. Likewise, if the changes in levels of these hormonal markers do not correspond with changes in fruit abundance and diversity, it can be interpreted that

declining fruit abundance and diversity are not major motivating factors in driving bonobo sociality.

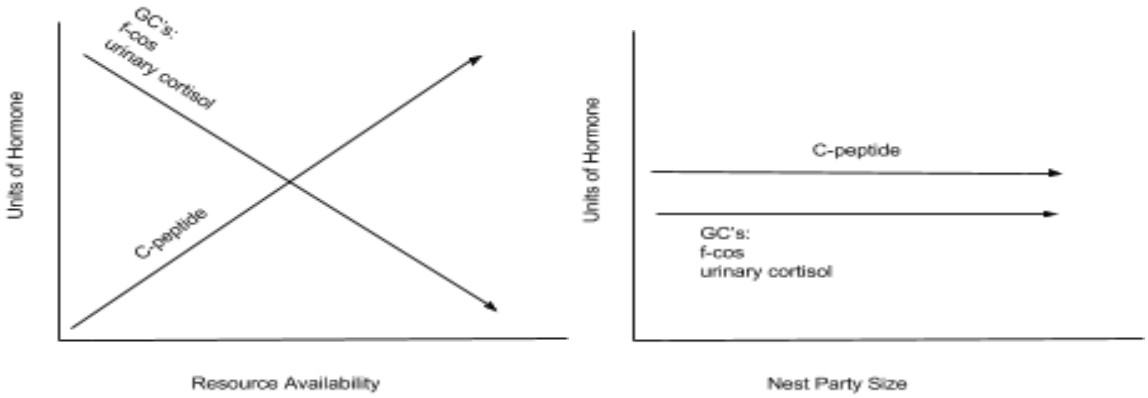

Figure 6.1.1.a Demonstrates the predicted relationships between glucocorticoids (GCs) and urinary C-peptide as resource availability (abundance and/or diversity) and nest party sizes, respectively increase (assuming that resource competition does not increase as well). Models assume that GCs reflect energetics and that nest parties increase as resource availability permits.

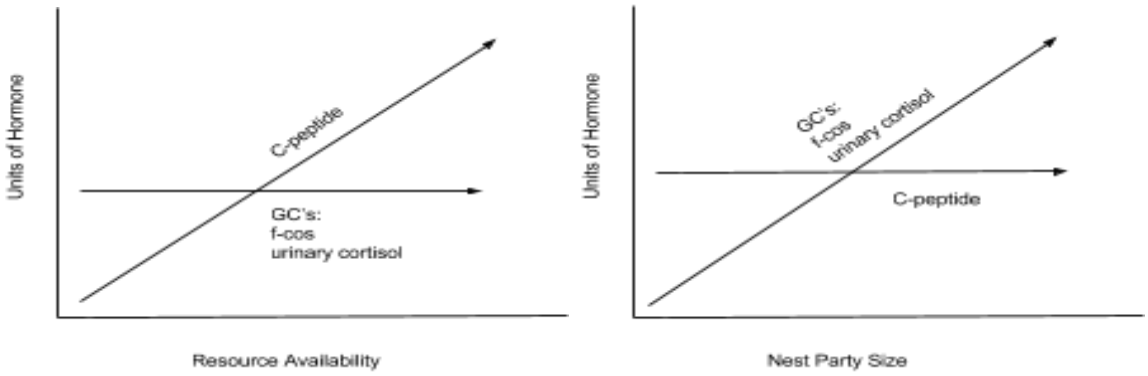

Figure 6.1.1.b Demonstrates the predicted relationships between glucocorticoids (GCs) and urinary C-peptide as resource availability (abundance and/or diversity) and nest party sizes, respectively increase (assuming that resource competition does not increase as well). Models assume that increased numbers of individuals result in stressful interactions, either psychologically, physiologically or both. Predicts that larger social groups trump energetic pay-off.

6.1.1.4 Ketones

Ketones have also been proposed as a promising means of a quick and effective way of measuring energetic load in wild primates, as they are produced in circumstances where little to no carbohydrates are available in the body either due to a hyper-consumption of protein (in the case of an "Atkin's" diet), or from the breakdown of the body's own fat and protein reserves (in the case of starvation). Ketones are expelled through urine, and have been used to demonstrate low energetic states to greatest effect in the case of wild orangutans (Knott 1998). There is a notable dearth of published literature on ketone collection in other primate species, other than orangutans. This is probably because they have not been successfully employed with chimpanzees (Tobias Deschner, pers. comm.), and urine is difficult to collect in the wild as primate body size decreases. Because they are a relatively cheap and quick way to examine health status in wild primates, I included them in my initial analysis, with the expectation that if they were present, their levels would directly negatively correspond to C-peptide levels.

6.2 Research Goals and Organizing Questions

The questions I address in this chapter aim to re-examine the long-held view that bonobos live in an environment that is more "forgiving" than that of most chimpanzees, using the lens of metabolic hormones to examine energetic stress and its relationship to bonobo socioecology. The assumptions here are that in spite of living in a stable equatorial environment, environmental pressures in the form of resource availability can still be observed to impact bonobo social behaviors (reflected in party sizes, see Chapter

5), and the trade-offs between sociality and energetics can be seen in accompanying shifts in energetic hormones.

Below I've outlined the original organizing questions posed in previous chapters, which I have reframed to consider the relationships between bonobo environment, diet and social dynamics in terms of energetic hormones (figure 6.2.a):

Chapter	**Original Question**	**Modified Questions (this chapter)**
3. Seasonality	*3.2.1 Does the Iyema bonobo community live in a seasonal environment?* *3.2.2 Are seasonal fluctuations in the environment reflected in the Iyema bonobos' diet?*	*6.2.1.1 Do energetic hormones, such as C-peptide and f-GCs reflect seasonal shifts in resource abundance?* *6.2.1.2 Do energetic hormone levels reflect seasonal shifts in fruit diversity?* *6.2.1.3 Do energetic hormone levels reflect shifts in dietary diversity?*
4. Terrestrial Herbaceous Vegetation	*4.2.1 Do Bonobos eat more THV when dietary resources are low?*	*6.2.2.1 Is the consumption of THV associated with lower metabolic stress levels (lower f-GCs, lower cortisol) and higher measures of energetic surplus (high C-peptide)?*
5. Nest Parties	*5.2.1 Do party sizes shift seasonally?* *5.2.2 Do seasonal shifts in resource availability, measured through abundance and diversity affect party size?*	*6.2.3.1 Are seasonal shifts in metabolic hormones associated with shifts in party size?* *6.2.3.2 Following observations that nest parties averaged at 7.7, is this number the "optimal" party size where a balance is seen between energetic hormones?*

Figure 6.2.a Organizing questions from Chapters 3-5 reframed to address relationship between bonobo sociality and resource availability in terms of energetic hormones.

6.3 Methods

6.3.1 Collection

Samples were collected between 6am and 10am, and carried back to camp. Urine samples accompanying fecal samples were collected from beneath night nests using 1ml transfer pipets, and temporarily stored in VWR 5 ml centrifuge tubes until they were transferred to filter paper at camp. Care was taken to ensure that a) samples were not contaminated (e.g. feces mixed with urine or debris) and b) that only one sample was collected per nest. Time between collection and preservation varied between 5 and 12 hours. Collection of fecal samples is described in more detail in chapter 2 (3.3.6).

6.3.2 Preservation and Field Processing

Collection and preservation of fecal and urine samples officially began in June 2010 and ended in June 2011. Extraction of fecal samples began in October 2011, at the Laboratory of Reproductive Ecology in the Anthropology department at Emory University, and was interrupted in December 2011. Extraction of both fecal and urine samples resumed in November 2012 and January 2013, respectively in the Biomarker Core Lab at the Yerkes Main Station.

6.3.2.2 Urine Preservation

Following collection and transport from the nest site to camp (between 6 and 18 hours following ejection), 400ul of urine was aliquotted onto labeled Whatman 903 Protein

Saver Cards, using a 200ul precision pipetter (VWR), following methods described by Knott (2005): Cards were closed following application of urine to filter paper, and placed in a sealed plastic container with color-indicating silica gel overnight to dry. My methods differed from Knott's in 2 primary ways. First, Knott's methods for hormonal preservation in orangutan urine, using filter paper calls for 200ul, and I used 400ul . Second, cards were transferred into whirlpack bags with approximately 1 teaspoon of silica gel the day following collection, instead of being stored in plastic sleeves. This should not be an issue in terms of recovery because samples were not touching one another. Bags were sealed and stored in the same large SeaLine waterproof duffle as the feces. Filter paper urine samples were transferred to a -80F freezer in August 2012. The delay between preservation and storage in the freezer ranged between 1 year and 2.5 years. Filter paper has been shown to be a reliable means of storing hormones for a period of 1-5 years in terms of *qualitative* recovery, although hormones and their metabolites do degrade over time (on a scale of hours to months depending on the hormone and conditions) rendering absolute quantitative recovery problematic for analysis in a study such as this (Shideler et al. 1995; Knott 2005). Both Shideler et al. and Knott found that recovery of hormones stored on filter paper, which had degraded with time were still highly correlated with matched samples that had been frozen immediately after collection.

6.3.2.3 Urinary Chemstrips

Following preservation of the urine on filter paper, remaining urine (if there was any) was pipetted onto Urinary Chemstrips (Roche Diagnostics) to check for specific gravity, pH, leukocytes, nitrites, protein, glucose, ketones, urobilinogen, bilirubin, and blood/hemoglobin. For the purposes of this dissertation, I only discuss the results of ketones.

Wild bonobo urine ranges in color from clear to the shade of espresso. This presented a dilemma when interpreting the change in colors on the chemstrips, particularly for non-red-based shades, which were easier to distinguish in spite of urinary color variation. Starting in August 2010 (project month 4), I established a color code for urine that ranged from 0 to 10 (where 0 was clear and 10 was as dark as the urine became) in order to control for any bias the color variation may have created in the results. Results, indicated by color changes on chem strips were written down, along with all information about each urine sample, including its estimated color on a scale of 1-10, based off of visual estimation by myself and my assistants.

6.3.2.4 Controlling for pregnancy

Glucocorticoid levels are known to rise significantly in pregnant females (Cavigelli 1999), so I tested all urine samples with home pregnancy tests prior to preservation. I did not find any positive results for pregnancy throughout the study period, which struck me as implausible. This method for determining early reproductive state in the wild is known to work for chimpanzees (Tobias Deschner, pers. com.), but has not been successful with

wild bonobos (Martin Surbeck, pers. com.). The inclusion of samples from unknown pregnant females with high levels of glucocorticoids may present a pitfall in the interpretation of my data where significantly high levels of urinary cortisol and fecal-GCs are concerned.

6.3.2.5 Fecal Preservation

For individual fecal samples, I removed approximately 10g of grain-free feces from the center (to best avoid any chance of contamination and for consistency), and squished it between folded sides of a clean piece of labeled tinfoil to evenly distribute fecal matter for consistent drying. These samples were then placed in the Coleman oven at 55-60^{o} C**,** following Brockman & Whitten (1996) for 30 minutes to an hour and a half, depending on the amount of moisture in the sample. Samples were considered to be dry when they crumbled between the foil like a cracker. They were then moved to individually labeled whirlpack bags with approximately 1 teaspoon of color-indicating silica gel, sealed, and stored in a large SeaLine waterproof duffle, also containing a larger bag of silica.

Preservation of fecal samples in the absence of lab grade alcohol and/or liquid nitrogen presented a major challenge in this study. Brockman and Whitten described methods for drying feces in a Coleman Camp Oven, using NuWick candles to maintain a steady temperature of 55-60^{o} C (1996). I initially tried this, and alternatives during brief field seasons in 2007 and 2009 and quickly realized that I would not be able to fit enough of these candles in my luggage to last an entire year. I also experimented with jerry-rigging a small tomato paste can with a tinfoil cover and some local moonshine to

produce a reliable, sustainable flame, but the process was too high maintenance for the conditions I would have at Iyema. I ultimately decided that the most consistent form of heat for the oven would be wood-fire, with a clay chimney I made myself. We kept a regular supply of small, dry kindling for the cleanest burn possible, and would heat up the oven until a temperature of 55-60^{o} C was stable for 15 minutes or more. It is possible that smoke from the wood fire may have contributed to some kind of interference when samples were extracted later- however, this seems unlikely given the number of studies that use similar methods and results that have been produced (including my own from 2007). Nonetheless, it is worth noting that these methods were not controlled in an ideal manner. Samples were stored in a -80F freezer after August 2012, resulting in a collection-to-freezer delay of 1-2 years.

6.3.3 Extraction

Fecal extractions began in October, 2011 in the Reproductive Ecology and Environmental Toxicology Lab, in the Anthropology Department, at Emory University. Work ceased in December, 2011 and recommenced at the Biomarkers Core lab, run by Sarah Pruett, at the Yerkes National Primate Research Center Main Station in December of 2012. All urine extractions took place in the Biomarkers Core lab, during February and March 2013.

6.3.3.1 Urine

Samples included in analysis were analyzed for creatinine following C-peptide assays using microplate assay kits made by Oxford Biomedical Research. All samples included

in analysis had a creatinine level of 0.1ng/ml or higher, and levels presented here are corrected for creatinine unless stated otherwise.

6.3.3.1.a RIA Extraction:

Urine was extracted from protein saver cards following methods described by Knott (1997). The entire filter paper component was cut away from the attached paper cover, and cut into smaller strips that fit inside a 13mm x 100mm borosilicate glass test tube. Strips were then covered and soaked overnight in 5ml of HPLC grade MeOH at 4C. The following day, strips were removed with forceps, and remaining MeOH was dried down in a 40C warm bath with nitrogen. All tubes received a second wash with approximately 1ml MeOH to concentrate dried urine to the bottom of the tubes, and dried again. Samples were then reconstituted with 400ul of assay buffer from a Human C-peptide RIA Kit (Millipore), vortexed, and immersed in a sonicator for 5 minutes each, prior to assay. Samples were stored at -80C between assays.

6.3.3.1.b LC/MS Extraction

Following C-peptide RIA's, remaining samples were extracted further for analysis using liquid chromatography mass spectrometry (LC/MS). Hormones were removed from the buffer using a standard ether extraction. 100ul of sample suspended in assay buffer were pipetted from the sample tube and transferred to fresh 12x75mm borosilicate tubes. 1ml of liquid ether was added to each tube, capped with cork plugs, and vortexed for 4 minutes, allowed to rest for 3 minutes to facilitate separation of ether from buffer. Tubes

were submerged in a bath of dry ice and MeOH in order to freeze the aqueous buffer layer (approx. 2 minutes) and the ether layer with extracted hormones was decanted into fresh tubes. Ether was dried down using a warm bath and nitrogen, and samples were finally re-suspended in 500ul of 70/30 LC/MS grade H2O/Acetonitrile.

6.3.3.1.2 Recovery

A) *Basic Recovery Test*

To test for basic hormonal recovery from filter paper (not taking into consideration degradation over time), I added known levels of hormones to 11 samples from August 2010 and June 2011 preserved on filter paper, using LC/MS grade hormone standards. I let these filter papers dry over night in silica, and extracted them in two phases identical to the methods used in the C-peptide assay. Samples were then prepared with the methods used for LC/MS analysis (extraction with MEOH and reconstitution in C-peptide RIA kit buffer, followed by ether extraction and reconstitution in 70/30 H2O/Acetonitrile).

In order to determine basic recovery, and to see if the addition of hormone standards was interfering with endogenous hormones, I set up 4 conditions (table 6.3.3.1.2.a):
1- The entire card received a total of 50 ul of Androstenedione (A4), Testosterone (T), Progesterone (Prog) and Cortisol (Cort), in addition to 50ul of each of 3 heavy radiolabeled standards that were used in the LC/MS assays distributed evenly across the card.

2- The card was cut in half and one half received 25ul of each of the four hormones in addition to 25ul of the heavy labeled hormones while the second half received nothing at all.

3- The entire card received 50ul of only the 3 heavy labeled standards.

4- The entire card received 50ul of only the four hormones, and no heavy labels. Due to some malfunction of the automatic pipetters, some cards were botched, and as a result, my N values vary per condition (table 6.3.3.1.2.a)

After extracting hormones from the filter papers, I assayed them using the same methods I had used for all other assays and calculated recovery rates varying between 56%-77% (Table 6.2.3.1.2.b).

B) *Relative Recovery*

I do not have a measure of absolute hormone recovery for this study. This is because I did not preserve pieces of filter paper with known levels of hormones as controls at the beginning of my study. However, levels of hormone recovered from my samples are still comparable in relative terms, and questions asked here have more to do with relationships in terms of high-vs-low than absolute levels.

Emery Thompson and Knott (2008) found that C-peptide and creatinine recovery from filter paper resulted in a lower recovery than samples immediately frozen at −20°C, but that the rate of degradation was uniform across specimens (also see Knott 2005; Sherry and Ellison 2007). Similar results have been detected with urea concentration in frozen and non-frozen samples (Sykes 2007). In addition, C-peptide and creatinine recovery from filter paper stored under varying temperatures or lengths of time tends to be robust, showing no major differences that should raise concern about the time lag

between collection and extraction for samples across the year (Emery Thompson and Knott 2008; Knott 2005).

The absolute values of the urinary hormonal levels presented here are most certainly underestimates of the concentrations at the time of collection. However, given that all the samples were stored together, the values should be comparable across the samples. Below are graphs of the results of all samples used, by month with basic regression lines to demonstrate that the time lag between collection and assay did not have an effect on measured levels. If degradation over time had an effect, we would expect to see a positive correlation between time of collection and the assayed hormone levels in all samples (fig.'s 6.3.3.1.2.c, d and e).

1: Full Card Spike	2: Half-Card Comparison	3: Only Heavy Labels*	4: Urine with Basic Spike
50ul A4, T, Prog, Cort /400ul urine + heavy labels	a) No hormones added b) 25ul A4, T, Prog, Cort/200ul urine + heavy labels	50ul D3, C13 100ug/dL D4 per 100ul urine; no T, Prog or Cort (accidentally added A4)	50ul A4, T, Prog, Cort; No heavy labels
N= 4* 2 samples have unreliable progesterone levels due to pipette malfunction	N= 4/condition	N= 2	N= 1 *Because I ran out of D4

Table 6.2.3.1.2.a Conditions for testing recovery from filter paper with bonobo urine. I applied known levels of LCMS grade hormone within the confines of the standard curve I used for my assays to each of 11 cards left over from my field work to test for basic recovery.

Hormone	Recovery
A4	77%
Testosterone	56%
Progesterone	64%
Cortisol	61%

Table 6.2.3.1.2.b Calculated recovery from filter paper for four steroid hormones assayed in LC/MS

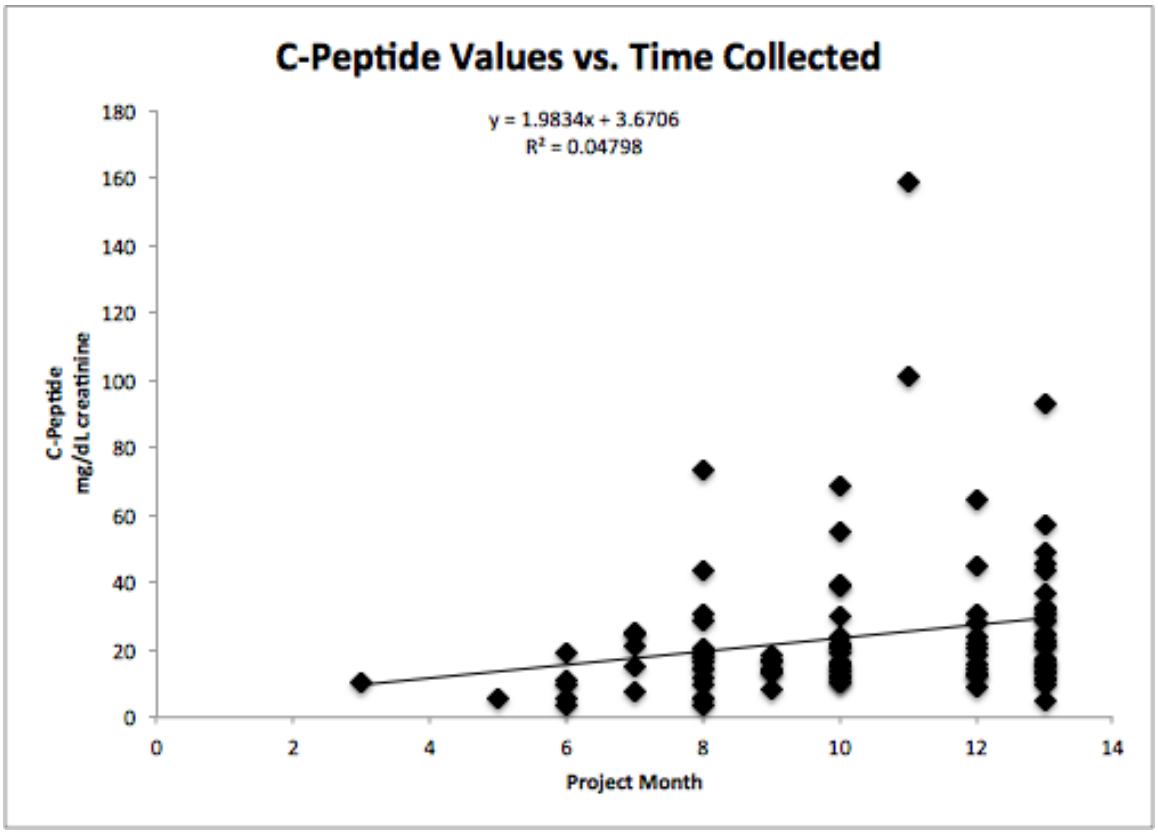

Figure 6.3.3.1.2.c The values of C-peptide sampled across months shows no significant difference across months. If time affected recovery significantly, we would expect to see a stronger relationship between the month collected and the values reported.

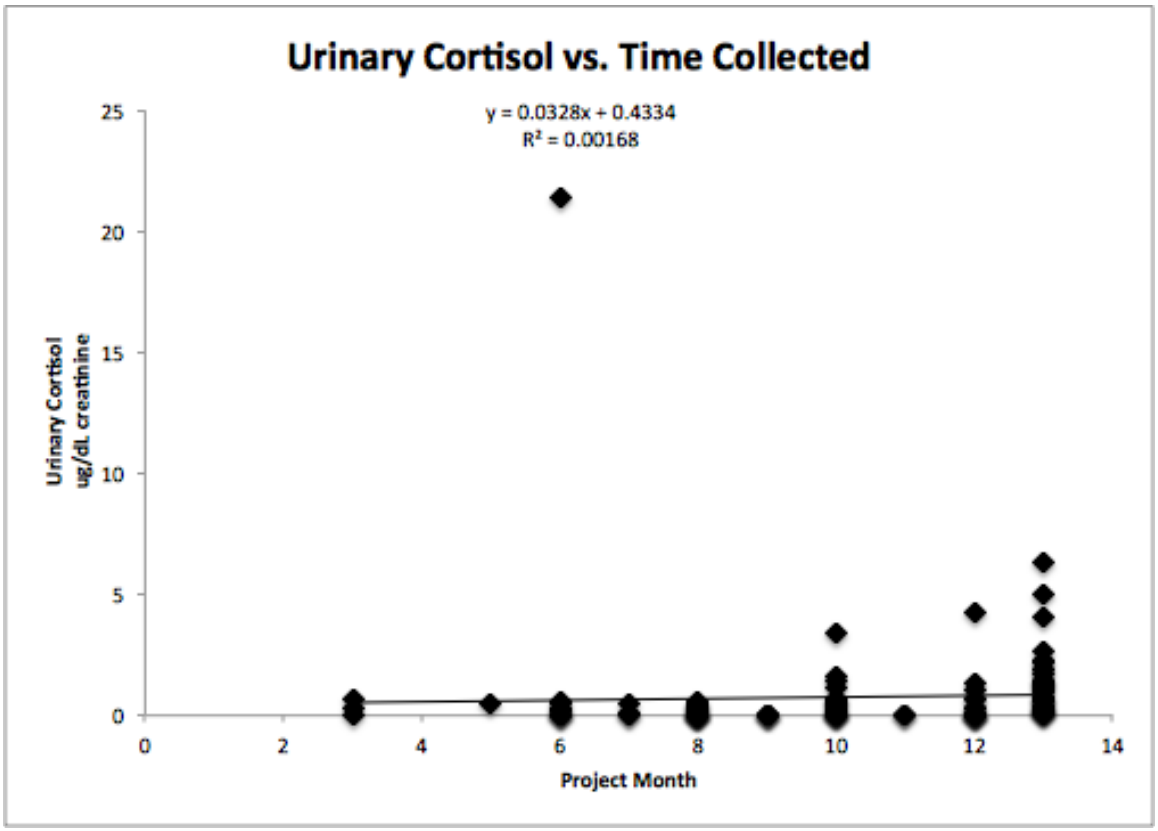

Figure 6.3.3.1.2.d The values of urinary cortisol sampled across months shows no significant difference across months. If time affected recovery significantly, we would expect to see a stronger relationship between the month collected and the values reported.

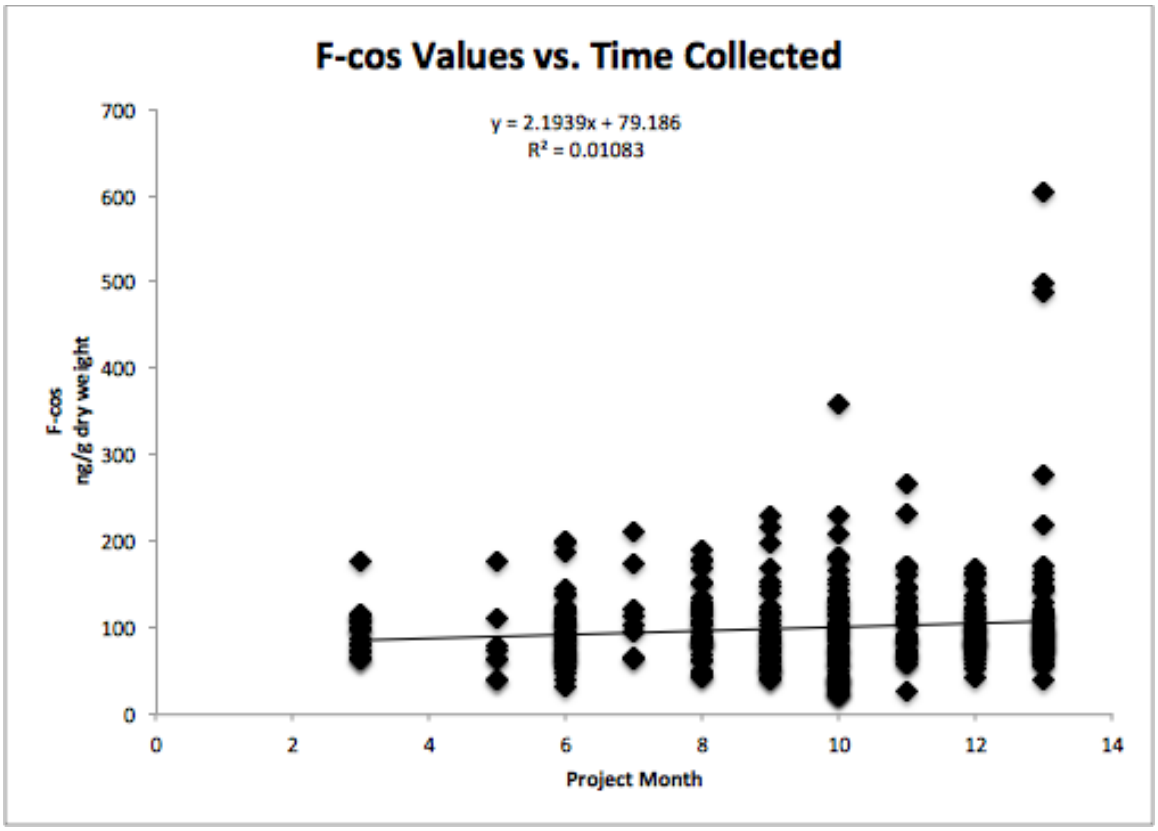

Figure 6.3.3.1.2.e The values of fecal GCs sampled across months shows no significant difference across months. If time affected recovery significantly, we would expect to see a stronger relationship between the month collected and the values reported.

6.3.3.2 Feces extraction

Dried fecal samples were ground using either a glass or stainless steel mortar and pestle until they resembled a fine powder. 0.5g of pulverized fecal matter was weighed into a 12x75mm polypropylene test tube, and vortexed for 10 minutes in 2ml of a 4:1 HPLC grade MeOH:Acetone solution. Samples were then transferred to 0.2um pore 5ml centrifuge tubes (Centrex), and spun for 15 minutes at 3000RPM at 4C. 2ml of deionized water was added to the filtered solution and left for 10 minutes, before eluting with SepPak cartridges (Waters) primed with 5ml of MeOH, followed by 10ml of deionized water. 5ml of DH2O was pipetted into columns after the sample, and a vacuum pump was used to remove remaining water from the columns before adding a final 3ml of HPLC grade MeOH for elution into individually labeled 12x75mm polypropylene test tubes. All tubes were stored in a -80F freezer.

6.3.4 Analysis and Validation

All samples were assayed in duplicate.

6.3.4.2 Radioimmunoassays

6.3.4.2.1 Validation of fecal glucocorticoids

6.3.4.2.1.a Serial Dilution

I used four samples to test for serial dilutions in the fecal glucocorticoid RIA assay: 2 known males (Ma and Mb) and 2 known females (Fa and Fb) from the month of October (fig. 6.3.4.2.1.a). Based on previous work in 2007, I started the dilution at 1:4 anticipating that higher concentrations would be less reliable. Results in the graph below demonstrate

relatively consistent values for all dilutions, except for 1:4 with both females. For subsequent assays, I used a 1:16 dilution of sample reconstituted in buffer.

6.3.4.2.1.b Replicability

For each assay, two kit controls containing high and low levels of known corticosterone were assayed, in addition to a pool comprised of aliquots from multiple samples in order to monitor the consistency of the assay (Table 6.4.2.1.b).

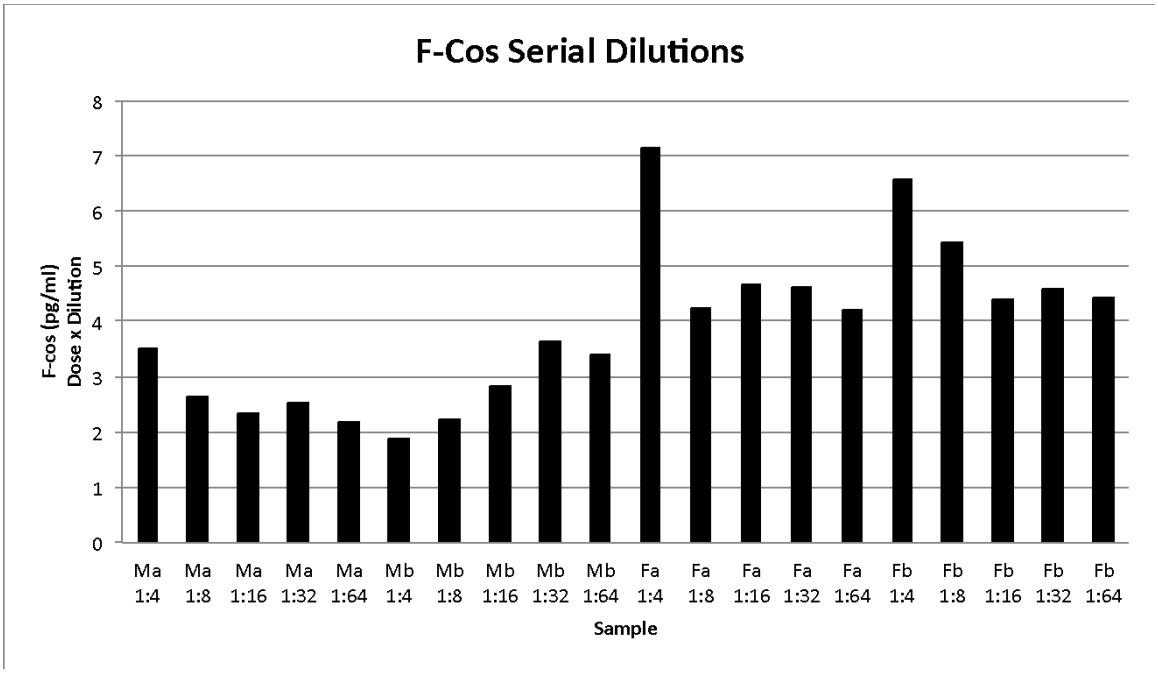

Figure 6.3.4.2.1.a Serial dilutions for extracted and assayed fecal samples from 2 males and 2 females. This step was taken to determine the ideal dilution at which to assay fecal samples with the corticosterone RIA. Concentrations such as 1:4 appeared to be too concentrated, while concentrations at 1:16 and beyond appeared to produce reliably similar results after correcting for dilution.

Assay Control	Average ng/ml	%CV	*n* =
Kit 1 (low)	0.14	16.38	8
Kit 2 (high)	1.18	16.51	8
Pool	0.23	9.47	8

Table 6.3.4.2.1.b Results of repeat assays on the same three controls. The assay manufacturer provided kit controls (Kit 1 and 2) showed higher inter-assay variability than my own internal pool. Internal pool values are not corrected for dose per dry weight.

6.3.4.2.2 Validation of C-peptide

6.3.4.2.2.a Serial Dilution

In order to test the efficacy of the kit, I began by assaying two pools of samples, which I expected to have relatively high or low levels of C-peptide, based on recorded presence or absence of ketones. The “high” pool consisted of five urine samples that had tested negative for ketones, while the “low” pool consisted of five urine samples that had tested positive for any ketones. I reconstituted all samples in 400ul of buffer provided by the kit, vortexed, and withdrew 300ul of reconstituted urine from each tube for a total pool of 1.5 ml, at a “1:1” dilution. I then created a serial dilution from the 1:1 concentration to a 1:8 concentration to be sure that the kit was binding predictably to the target metabolites (fig. 6.3.4.2.2.a).

Results from my first assay showed that my highest concentration was at the low end of the detection limit for the kit standards, when prepared according to kit instructions. To adjust for this, I extended the standard curve 4 additional steps, from a range of 2.5ng/nmol - 0.331ng/nmol to 2.5ng/nmol- 0.008ng/nmol, by making a serial dilution of the standard provided by the kit. I reconstituted all dried down samples at a 1:1 concentration of buffer to original sample. All other steps in the process followed kit instructions.

6.3.4.2.2.b Replicability

For each assay, two kit controls containing high and low levels of known C-peptide were assayed, in addition to a pool comprised of aliquots from multiple samples in order to monitor the consistency of the assay (Table 6.4.2.1.b).

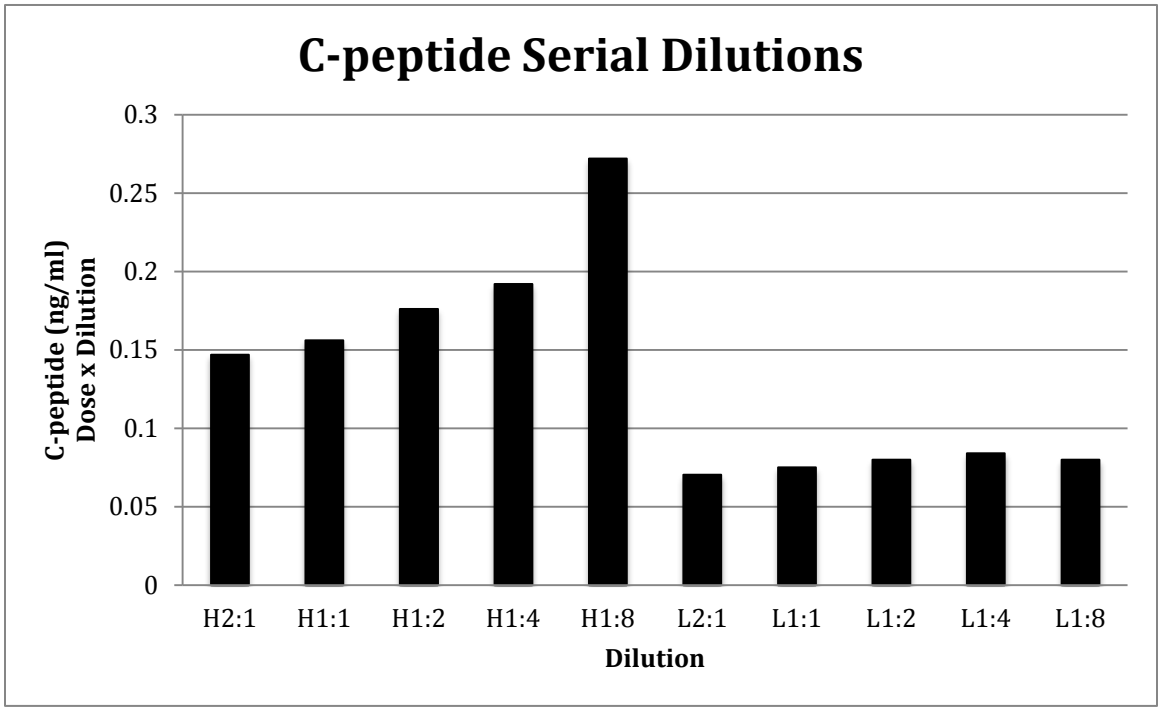

Figure 6.3.4.2.2.a Serial dilutions for urinary C-Peptide. H indicates high levels of ketones, while L indicates low levels of ketones; ketones proved to be an unreliable measure (see 6.3.5). Assays were run at a 1:1 dilution. Values above are not corrected for creatinine.

Repeated Measures for C-peptide of Insulin Assay:

Assay Control	Average ng/ml	%CV	*n* =
Kit 1 (low)	0.26	13.33	4
Kit 2 (high)	1.34	9.36	4
Internal Pool 1	0.13	17.61	3
Internal Pool 2	0.11	16.63	4

Table 6.4.2.1.b Results of repeat assays on the same four controls. The assay manufacturer provided kit controls (Kit 1 and 2) showed lower inter-assay variability than my own internal pools. Internal pool values are not corrected for creatinine.

6.3.4.3 Validation of LC/MS

6.3.4.3.1 Matrix effects

To ensure that the C-peptide kit buffer was not creating any interference, we reconstituted known levels of hormone in buffer and extracted using ether. Figure 6.3.4.3.1.a demonstrates the clarity of the standard spikes for each of the four hormones at a low dose, 1ng/ml (next page).

6.3.4.2.2 Linearity

Below are 4 examples of standard curves from assays, demonstrating the assay's linearity for all four hormones assayed.

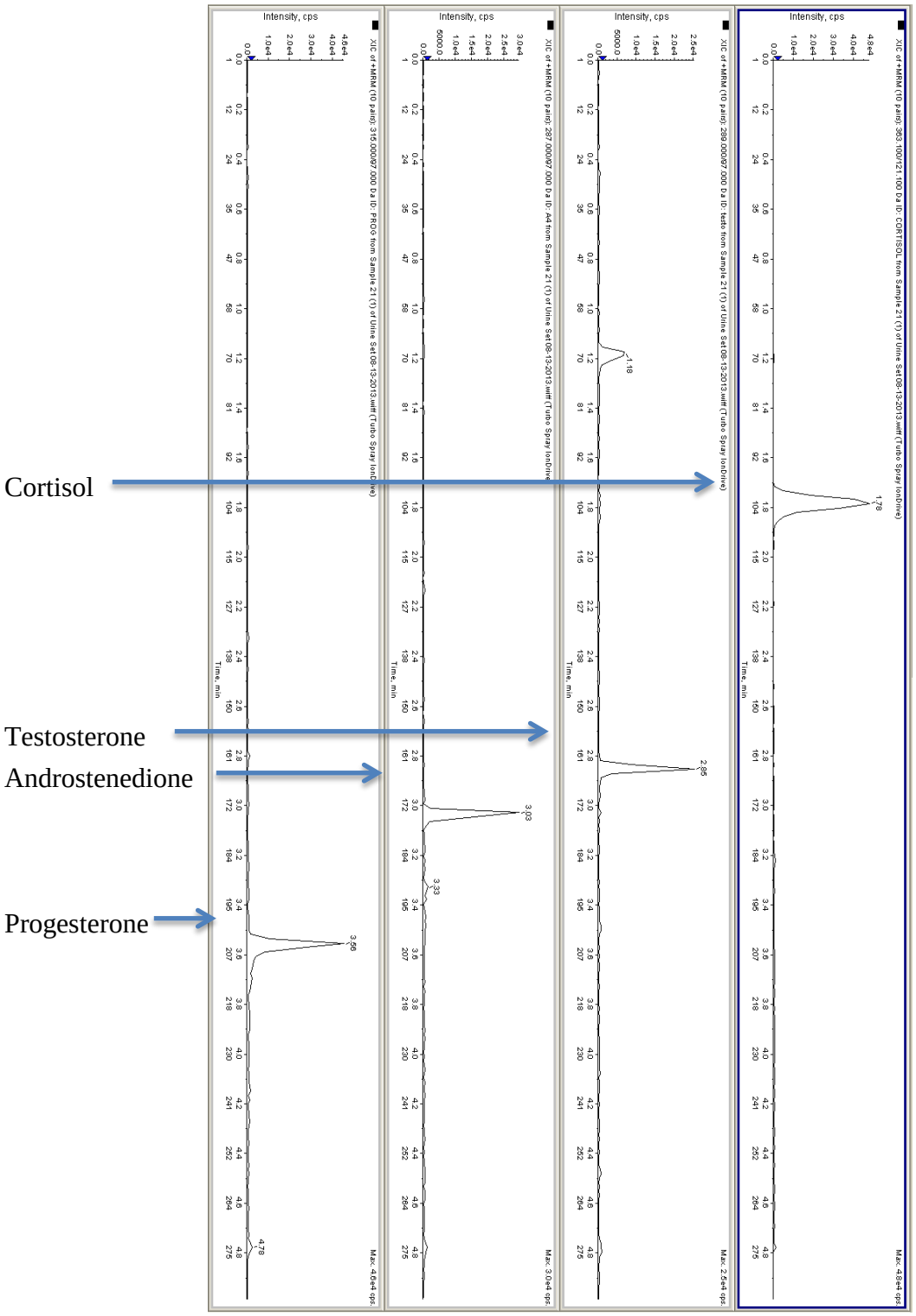

Figure 6.3.4.3.1.a Steroid peaks for cortisol, testosterone androstenedione and progesterone at 1ng/ml in extraction buffer on LC/MS. Demonstrates clear signals and lack of interference from buffer.

Androstenedione (A4):

Expected Concentration	Number of Values	Mean Calculated Concentration	% Accuracy	Std. Deviation	%CV
0.1	*3*	*0.10*	*96.7*	*0.01*	*14.9*
0.5	*4*	*0.54*	*108.7*	*0.08*	*15.6*
1	*4*	*1.08*	*107.6*	*0.18*	*16.4*
5	*4*	*5.01*	*100.3*	*0.21*	*4.3*
10	*4*	*10.10*	*101.0*	*0.33*	*3.3*
50	*4*	*46.63*	*93.3*	*1.13*	*2.4*
100	*4*	*91.66*	*91.7*	*1.51*	*1.6*

Regression Equation: *y = 0.026 x + 2.75e-005 (r = 0.9922)*

Testosterone:

Expected Concentration	Number of Values	Mean Calculated Concentration	% Accuracy	Std. Deviation	%CV
0.1	*2*	*0.10*	*102.8*	*0.01*	*6.0*
0.5	*4*	*0.43*	*86.9*	*0.03*	*7.6*
1	*4*	*1.11*	*110.8*	*0.27*	*24.0*
5	*4*	*5.22*	*104.3*	*0.09*	*1.7*
10	*4*	*10.49*	*104.9*	*0.22*	*2.1*
50	*4*	*48.38*	*96.8*	*1.02*	*2.1*
100	*4*	*94.90*	*94.9*	*2.05*	*2.2*

Regression Equation: y = 0.0213 x + 0.00155 (r = 0.9910)

Progesterone:

Expected Concentration	Number of Values	Mean Calculated Concentration	% Accuracy	Std. Deviation	%CV
0.1	*2*	*0.10*	*97.9*	*0.01*	*6.0*
0.5	*4*	*0.49*	*99.0*	*0.07*	*14.8*
1	*4*	*1.14*	*114.4*	*0.23*	*20.5*
5	*4*	*4.70*	*94.1*	*0.10*	*2.2*
10	*4*	*9.57*	*95.7*	*0.63*	*6.6*
50	*4*	*50.06*	*100.1*	*1.91*	*3.8*
100	*4*	*97.79*	*97.8*	*1.67*	*1.7*

Regression Equation: $y = 0.0187 x + 0.00694$ ($r = 0.9916$)

Cortisol:

Expected Concentration	Number of Values	Mean Calculated Concentration	% Accuracy	Std. Deviation	%CV
0.1	*2*	*0.10*	*99.0*	*0.03*	*33.9*
0.5	*4*	*0.49*	*98.1*	*0.03*	*6.1*
1	*4*	*1.08*	*107.9*	*0.03*	*2.7*
5	*4*	*5.16*	*103.3*	*0.17*	*3.3*
10	*4*	*10.20*	*102.0*	*0.28*	*2.8*
50	*4*	*48.98*	*98.0*	*1.64*	*3.3*
100	*4*	*91.23*	*91.2*	*8.13*	*8.9*

Regression Equation: $y = 0.726 x + 0.0254$ ($r = 0.9948$)

6.3.4.2.3 Replicability

I used both pools (taken from multiple samples of my own) and extra urine from an adult chimpanzee (“Foxy”, in table 6.3.4.2.3.a) from the Biomarkers Core Lab to monitor the consistency of the assays. I ran the chimpanzee urine at both 100ul and 50ul to double check for consistency, and because some samples had to be run at 50ul, having been depleted from previous assays.

Generally speaking the 50ul and 100ul values produced similar values, although the CV’s (coefficient of variance) for both varied widely in the case of testosterone and cortisol. The wide variation in testosterone for Foxy seems clear: her levels never exceeded 1ng/ml in assays, and were typically below detection limits (0.1ng/ml). Consistency for the bonobo urine pools was strong between assays, which supports the accuracy of the LC/MS assays.

Hormone	Average 50ul (ng/ml)	%CV	Average 100ul (ng/ml)	%CV
A4 Pool	-	-	2.88	3.78
T Pool	-	-	1.58	8.71
Prog Pool	-	-	0.09	6.67
Cort Pool	-	-	0.97	8.71
A4 Foxy	0.15	49.73	0.42	14.36
T Foxy	0.04	76.02	0.10	101.31
Prog Foxy	0.29	5.29	0.62	7.26
Cort Foxy	1.35	110.46	1.01	79.35

Table 6.3.4.2.3.a Replicability for pools used in LC/MS assays, with %CV. n = 6 for Pool values; n = 10 for Foxy values. Values were not corrected for creatinine.

6.3.5 Ketones: problems with color and reliability

Bonobo urine manifests in a range of color, from clear to the dark brown of espresso (fig. 6.3.5.a). This presented a dilemma when assessing the results of the color-indicating chemstrips. To test whether the color of the urine was affecting the interpretation of ketones and other biomarkers, I ran a basic correlation with color vs. results (fig. 6.3.5.b) and also ran an order probit model with the presence or absence of ketones as the dependent variable and color code as the independent variable. Darker colored samples significantly predicted the presence of ketones ($p > .01$), although clear samples with ketones were sometimes the case, as were dark samples without ketones. Based on this outcome, I would not recommend chemstrips as the sole means of identifying health and energetic status in wild apes. However, I would recommend re-attempting this method if a) there were a means of taking light-controlled photos of the results, and b) if the urine could be analyzed within minutes of urination.

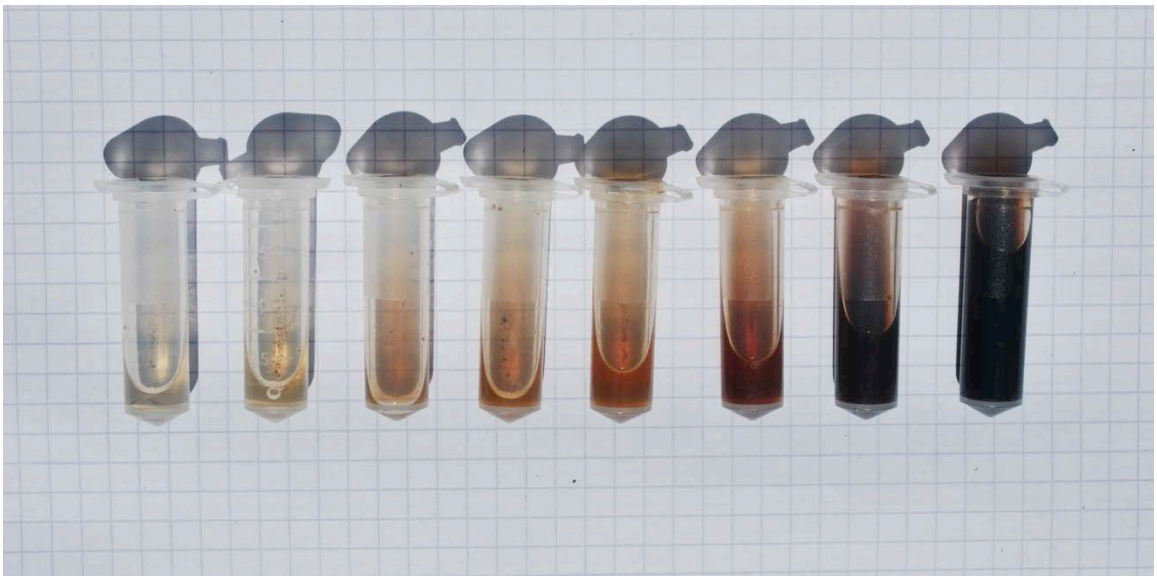

Figure 6.3.5.a Variation in the color of wild bonobo urine. Clear (far left) was scored as 0, while an espresso-like tone (far right) was scored 10. Not all shades are depicted here.

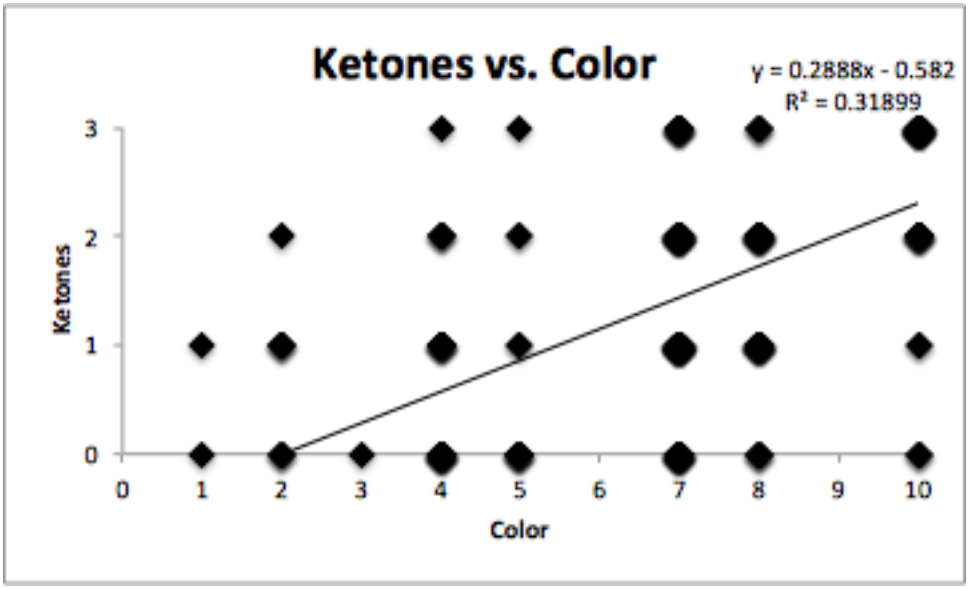

Figure 6.3.5.b Color of Urine vs. ketone results from urinary chemstrips. Demonstrates the significant relationship between color of urine and outcome of tests for ketones on urinary chemstrips ($p > 0.01$). Color scale ran from 0 (clear) to 10 (dark like espresso). Note: shadows indicate large numbers of samples with the same outcome.

6.4 Results

Fecal: 392 fecal samples assayed for immunoreactive corticosterone metabolites had %CV's of less than 20% (the %CV cut-off for the Biomarker Core Lab), and were included in statistical analysis.

Urine: 152 samples had creatinine values higher than 0.1mg/dL and were included in analysis. All samples were assayed in duplicate and for each hormone assayed, an estimated concentration and %CV was produced. Within the same sample, %CV's for different hormones (e.g. urinary cortisol and c-peptide) varied, explaining the difference in n values between the two urinary hormones. Below is a table summarizing the number of samples analyzed for the three hormones (table 6.4).

Assay Summary	n=	units	low	high	median	average	%CV range
F-cos	392	ng/g dry wt	21.60	603.36	89.76	100.11	0.1-19.7%
U-Cort	123	ug/dL cr	0.00	21.30	0.24	0.77	0-23.9%
C-peptide	104	mg/dL cr	3.44	158.65	17.91	24.11	0.1-24.2%

Table 6.4 Summary of number, units, and range of hormones assayed and their results.

6.4.1 Hormones and Seasonality

6.4.1.1 Do energetic hormones, such as C-peptide, urinary cortisol and f-GCs reflect seasonal shifts in resource abundance?

To analyze this we used multivariate generalized linear mixed effects models (GLMM) using the lme4 package in R. For each specific question below the best predictive model, predicting respective hormone levels with consideration to total abundance, fruit diversity and nest party sizes are included, followed by a descriptive graph and data summary table. Confidence intervals are visualized with dashed lines.

6.4.1.1.1 C-peptide vs. abundance

Total abundance (leaves and fruit) does not accurately predict C-peptide levels, nor does fruit abundance by itself (fig's 6.4.1.1.1.a and b). For total abundance (including the metric volume of young leaves in the environment), we used month and nest party to account as varying intercepts, and allowed varying slopes for our predictors (s.sum.abundance and c.diversity). We also allowed for an interaction between diversity and abundance, assuming the two were related. The mean fixed effect estimate for C-peptide levels was found to be 18.81 mg/dL creatinine (2.53-35.10, 95% CI). Total abundance had a negative effect, but with a wide confidence interval -3.85 (-28.45 - 20.75 95% CI) for each unit in abundance, suggesting the relationship is not a strong or predictive one (figures 6.4.1.1.1.a and b).

Using another multivariate GLMM for fruit abundance only, we found that fruit abundance was positively related to C-peptide levels. C-peptide levels went up by 4.28

ug/dL for each unit of fruit abundance, and the 95 % CI was largely on the positive side of zero (figures 6.4.1.1.1.c and d).

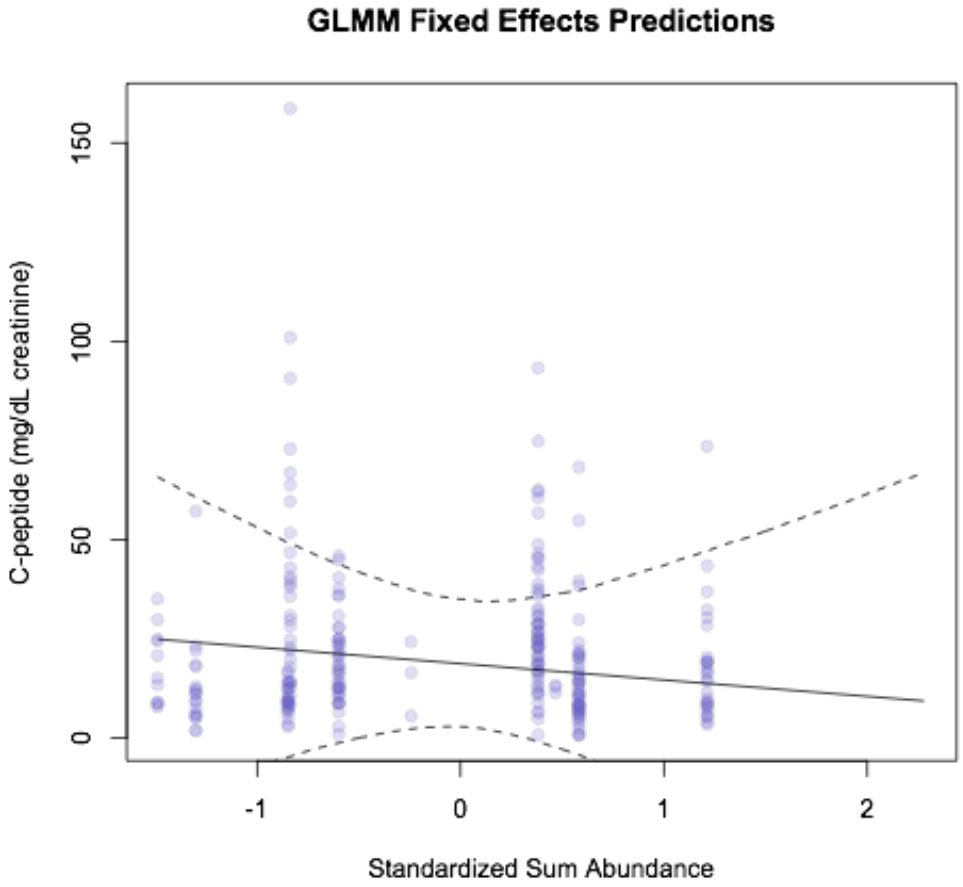

Figure 6.4.1.1.1.a Small, negative effect of abundance (standardized for diversity and nest party size) on C-peptide levels.

Model code:

m4 <- lmer(C_pep ~ s.sum.abundance*c.diversity + (1 + s.sum.abundance + c.diversity|project.month) + (1 + s.sum.abundance + c.diversity|nest.party) , data=d)

Effects of Total Abundance and Diversity on C-peptide	Mean (95%CI)	StdDev	T-value	N
Intercept (alpha)	18.81 (3.53 - 35.10)	8.31	2.264	268
Effect of total abundance (beta1)	-3.85 (-28.45-20.75)	12.55	-0.307	
Effect controlling for diversity	3.12 (-8.16 – 14.39)	5.75	0.542	
Interaction between total abundance and diversity	-2.52 (-20.73 - 15.69)	9.29	-0.271	

Figure 6.4.1.1.1.1.b Result summary of GLMM for effect of total abundance on C-peptide levels.

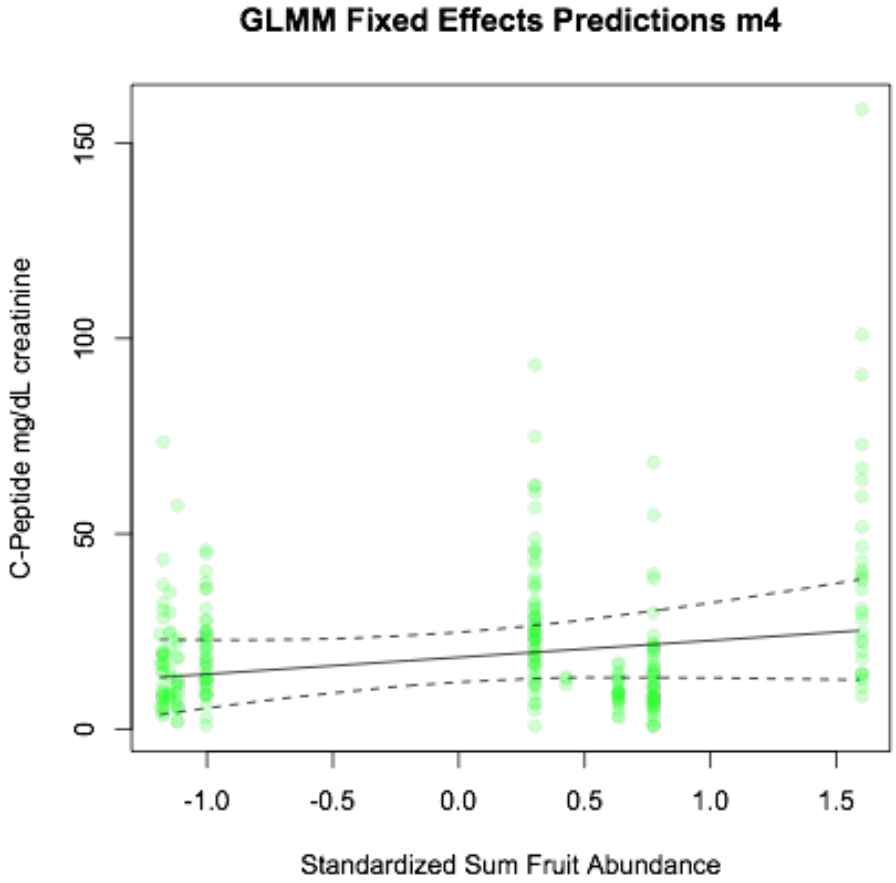

Figure 6.4.1.1.1.c positive effect of fruit abundance (standardized for diversity and nest party size) on C-peptide levels.

Effect of Fruit Abundance on C-peptide levels	Mean (95%CI)	SE	T-value	N
Intercept (alpha)	18.35 (12.04 – 24.66)	3.22	5.698	268
Effect of fruit abundance	4.28 (-2.47 – 11.02)	3.44	1.242	
Effect controlling for diversity	3.8 (-0.81 – 8.40)	2.35	1.615	
Interaction between fruit abundance and diversity	5.17 (.22 – 10.12)	2.53	2.048	

Figure 6.4.1.1.1.d Result summary of GLMM for effect of fruit abundance on C-peptide levels.

6.4.1.1.2 Urinary cortisol vs. abundance

Overall summed abundance shows a negative relationship with urinary cortisol levels (figures 6.4.1.1.2.a and b). We used month and nest party to account as varying intercepts, and allowed varying slopes for our predictors (s.sum.abundance and c.diversity). We also allowed for an interaction between diversity and abundance, assuming the two were related. The mean fixed effect estimate for cortisol levels was found to be 1.89ng/g creatinine (0.93 – 2.85, 95% CI). Total abundance had the strongest negative effect (figures 6.4.1.1.2.a and b). Cortisol also has a negative relationship with fruit abundance, while diversity does not seem to be related to urinary cortisol in this model (small effect wide CI).

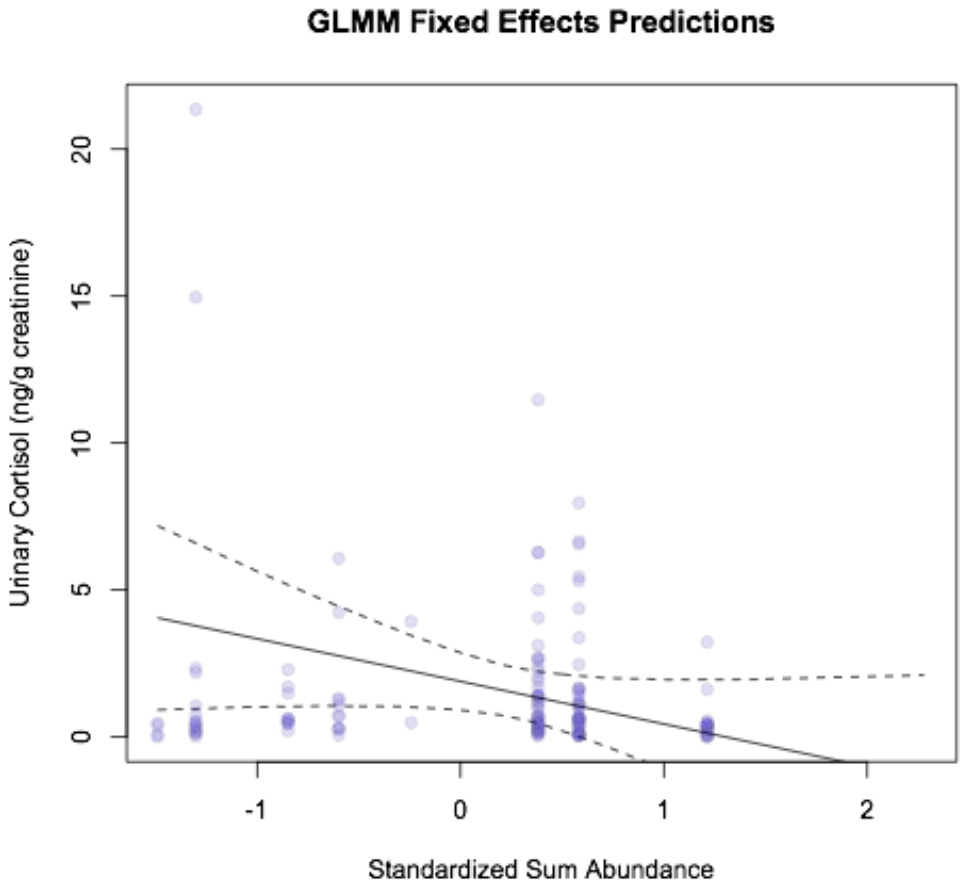

Figure 6.4.1.1.2.a Effect of abundance (standardized for diversity and nest party size) on urinary cortisol levels.

Model code:

m4 <- lmer(Cort ~ s.sum.abundance*c.diversity + (1 + s.sum.abundance|project.month) + (1|nest.party) , data=d)

Effects of Abundance and Diversity on Cortisol	Mean (95%CI)	StdDev	T-value	**N**
Intercept (alpha)	1.89 (0.93 – 2.85)	0.49	3.84	**151**
Effect of total abundance	-1.46 (-3.16 - 0.25)	0.87	-1.671	
Effect of diversity	0.34 (-0.35-1.03)	0.35	0.974	
Interaction between diversity and abundance	-0.46 (-1.51 - 0.59)	0.53	-0.863	

Figure 6.4.1.1.2.b Result summary of GLMM for effect of abundance and diversity on cortisol levels.

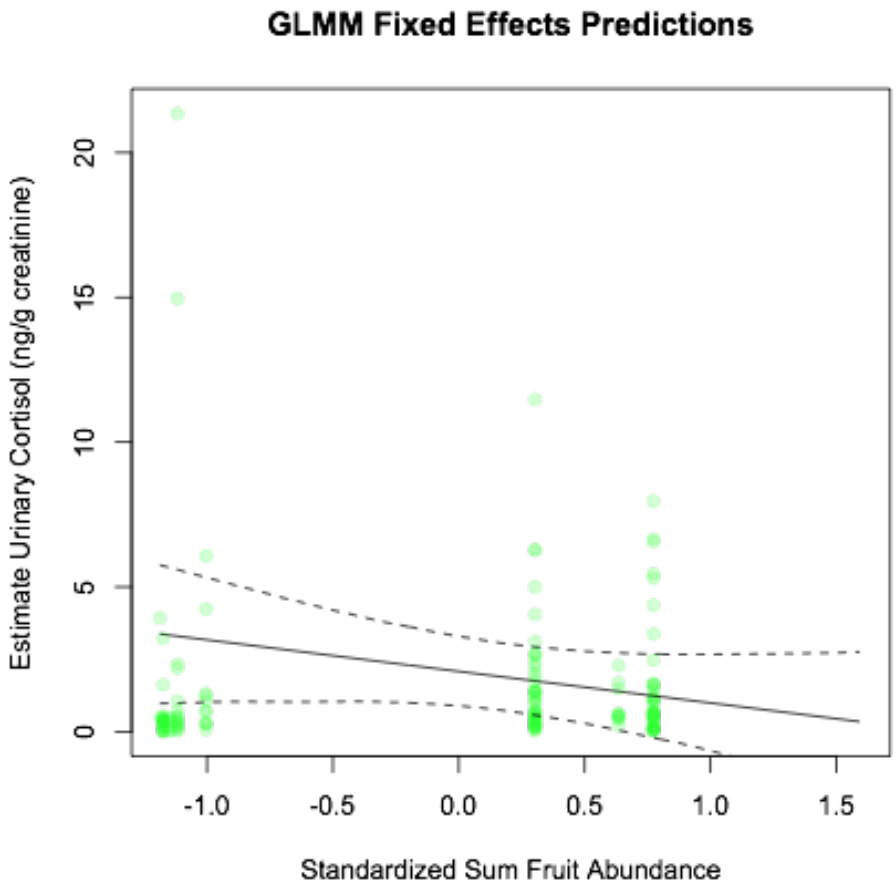

Figure 6.4.1.1.2.c Effect of fruit abundance (standardized for diversity and nest party size) on urinary cortisol levels.
Model: **m4 <- lmer(Cort ~ s.fruit.abundance*c.diversity + (1 +s.fruit.abundance|project.month) + (1|nest.party) , data=d)**

Effect of Fruit Abundance and Diversity on Urinary Cortisol	Mean (95%CI)	SE	T-value	N
Intercept (alpha)	2.09(0.88-3.30)	0.62	3.382	151
Effect of fruit abundance	-1.10 (-2.59 - 0.39)	0.76	-1.443	
Effect of diversity	0.15(-.69-0.99)	0.43	0.345	
Interaction between fruit abundance and diversity	-0.69 (-1.79 - 0.41)	0.56	-1.224	

Figure 6.4.1.1.2.d Result summary of GLMM for effect of fruit abundance and diversity on cortisol levels.

6.4.1.1.3 Fecal corticosterone vs. abundance

Abundance appears to have little or no effect on f-GCs (fig's 6.4.1.1.3a and b). Model selection using DIC suggested that diversity was a more important predictor of f-GC levels than abundance, and that an interaction term or multivariate model did not help explain the data. So an interaction term was not included in this model and only total abundance was modeled. F-GCs showed no meaningful relationship with fruit abundance, with a small estimate and wide confidence interval -1.62 (-12.8-9.58) (figure 6.4.1.1.3.c).

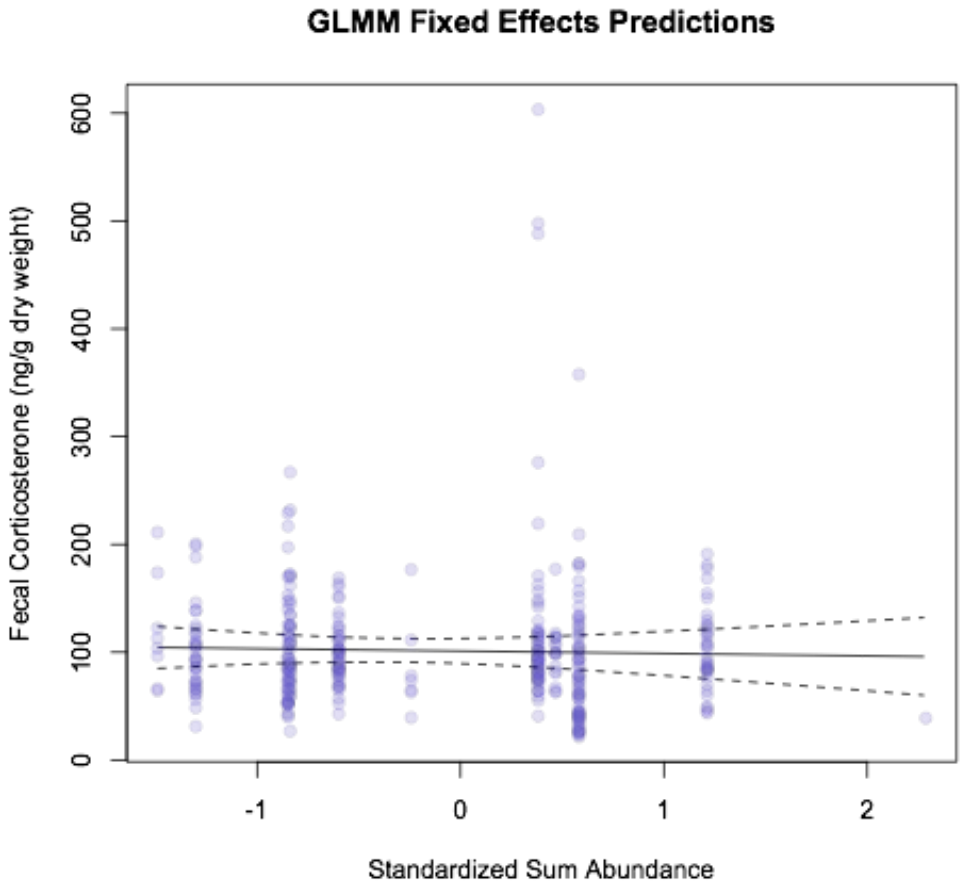

Figure 6.4.1.1.3.a Effect of total abundance on urinary cortisol levels.

Model: **m2 <- lmer(F_cos ~ s.sum.abundance + (1 + s.sum.abundance|project.month) + (1|nest.party) , data=d)#**

Effect of Abundance on f-GCs	Mean (95%CI)	StdDev	T-value	N
Intercept (alpha)	101.5 (89.68 – 112.63)	5.86	17.28	372
Effect of total abundance (beta)	-2.21 (-15.35 - 10.92)	6.7	-0.33	

Figure 6.4.1.1.3.b Result summary of GLM for effect of abundance on f-GC levels.

6.4.1.2 Do energetic hormone levels reflect seasonal shifts in fruit diversity?

6.4.1.2.1 Fruit Diversity and C-peptide

Environmental diversity is positively related to C-peptide levels (beta/slope =2.35) with similarly sized estimate and narrower confidence interval as can be seen by the estimate in the below graph with the black line and 95% confidence interval (figure 6.4.1.2.1.a).

There was also an important interaction effect between fruit abundance and diversity as it related to C-peptide levels. In low abundance environments (measured as 1 standard deviation below the mean fruit abundance) a near zero and slightly negative relationship between diversity and abundance was observed (green line). In high fruit abundance environments (measured as 1 standard deviation above the mean fruit abundance), diversity had a stronger effect on increased C-peptide levels (red line) (figure 6.4.1.2.1.a).

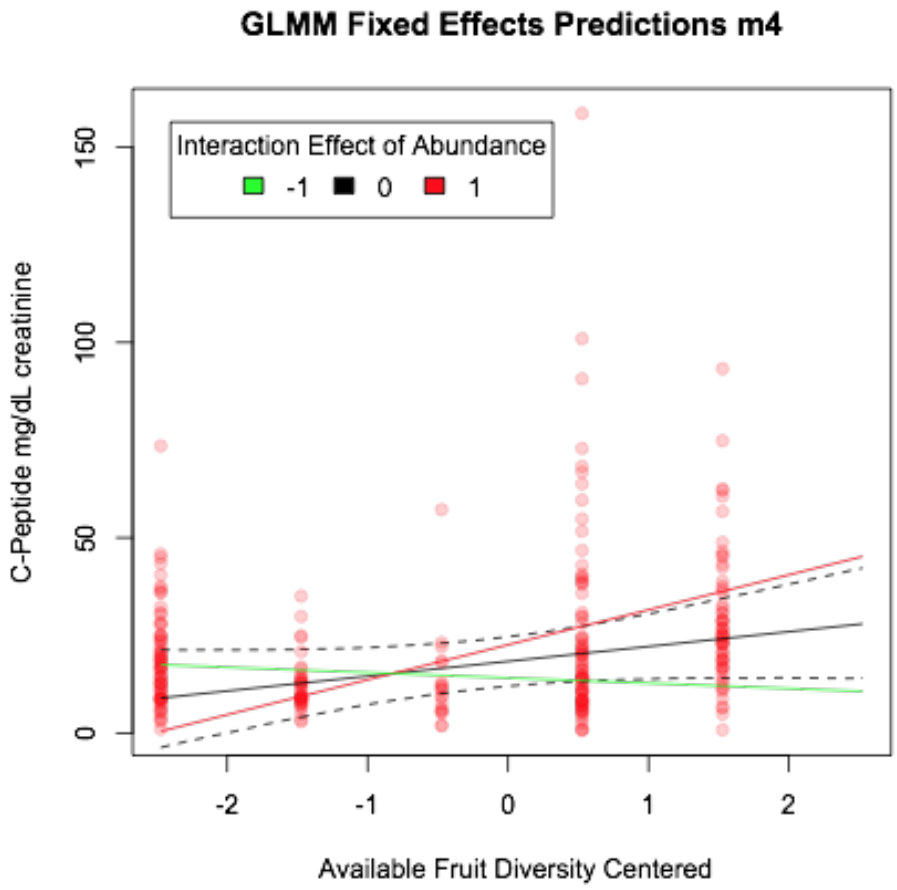

Figure 6.4.1.2.1.a Effect of fruit diversity on C-peptide levels (black line), also showing interaction with periods of increased (red) and decreased (green) abundance.

6.4.1.2.2 Fruit Diversity and Urinary Cortisol

Fruit diversity on its own had little to no effect on urinary cortisol levels: 0.15 ng/g creatinine (-.69 -0.99) (black line). However, fruit diversity interacted with fruit abundance in meaningful ways that were related to urinary cortisol outcomes. In low abundance, high diversity environments, cortisol was at its highest. However, in high diversity, high abundance environments (red line) urinary cortisol levels were at their lowest (figure 6.4.1.2.2.a &b).

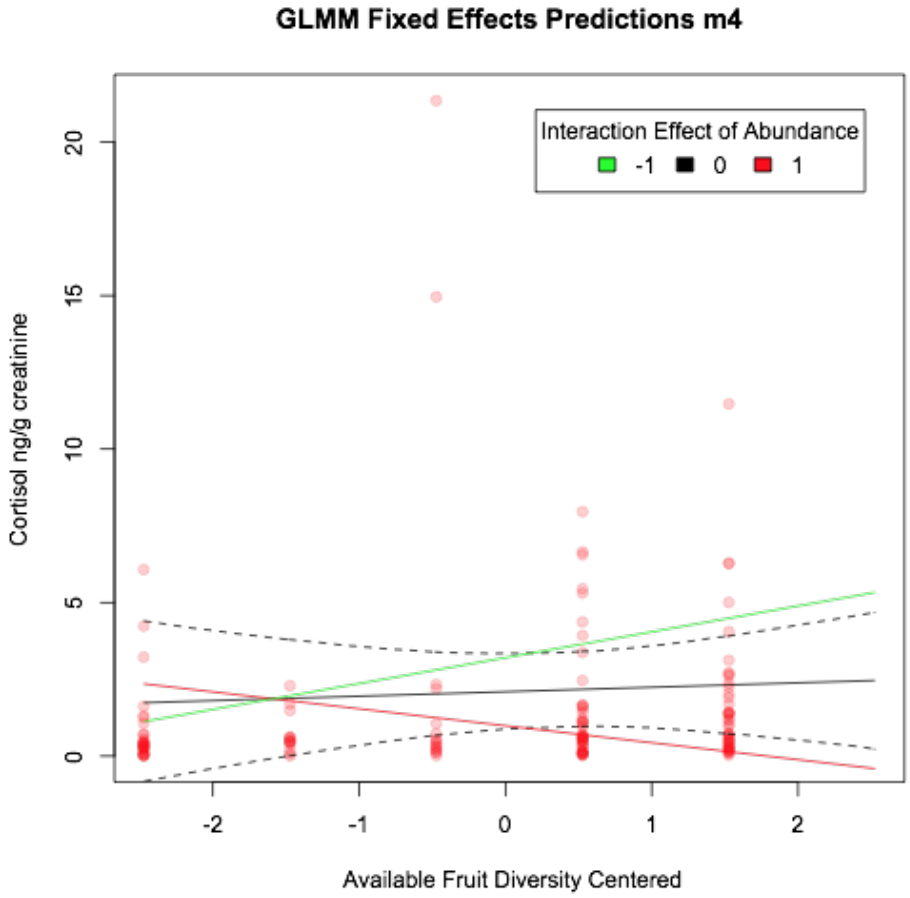

Figure 6.4.1.2.2.a Effect of fruit diversity on cortisol levels (black line), also showing interaction with periods of increased (red) and decreased (green) abundance.

Effects of Fruit Diversity on Cortisol	Mean (95%CI)	SE	T-value	N
Intercept (alpha)	2.09(0.88-3.30)	0.62	3.382	151
Effect of fruit abundance (beta1)	-1.10 (-2.59 - 0.39)	0.76	-1.443	
Effect of fruit diversity	0.15(-.69-0.99)	0.43	0.345	
Interaction between fruit diversity and abundance	-0.69 (-1.79 - 0.41)	0.56	-1.224	

Table 6.4.1.2.2.b Results summary of effects on urinary cortisol levels from fruit diversity and abundance.

6.4.1.2.3 Fruit Diversity and f-GCs

DIC model comparison favored diversity over abundance as a means of estimating f-GC levels. Diversity, estimated in a separate GLMM showed no relationship with f-GC values (wide confidence interval estimate near 0 1.62 (-12.8-9.58)) (figures 6.4.1.2.3.a and b).

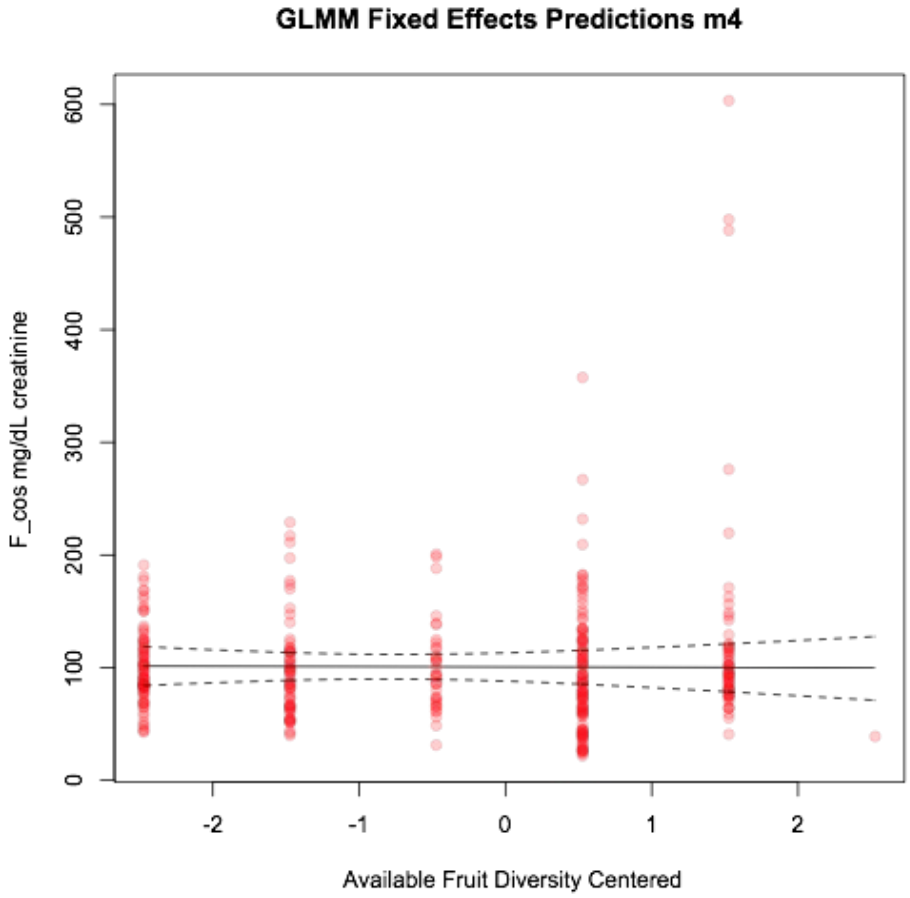

Figure 6.4.1.2.3.a Effect of fruit diversity on f-GC levels. No relationship.

Effect of Fruit Diversity on f-GCs	Mean (95%CI)	SE	T-value	N
Intercept (alpha)	100.63(87.98-113.28)	6.45	15.5	372
Effect of fruit diversity	-0.3 (-8.13 - 7.54)	4	-0.074	

Figure 6.4.1.2.3.b Result summary of GLM for effect of fruit diversity on f-GC levels.

6.4.1.3 Do energetic hormone levels reflect shifts in dietary diversity?

Dietary diversity had little to no effect on any of the measured hormones. C-peptide shows a small increase in levels as dietary diversity increases, but a closer look at the graph shows that the highest levels of C-peptide are when dietary diversity is moderate (between 3 and 7 species) (fig. 6.4.1.3.1.a &b). Urinary cortisol showed a slightly negative relationship with dietary diversity, where levels started to peak at both low and high ends of diversity, in contrast to the peak seen in C-peptide levels at moderate levels of dietary diversity (fig. 6.4.1.3.2.a). f-GC levels showed little to no relationship with measures of dietary diversity (fig. 6.4.1.3.3.a & b). There is a near zero effect size with confidence intervals on both side of zero. This was done using a GLMM with varying intercepts for nest party, month, and varying slopes for fecal diversity. Multivariate models were also run using fecal and forest diversity measures and this did not change model predictions.

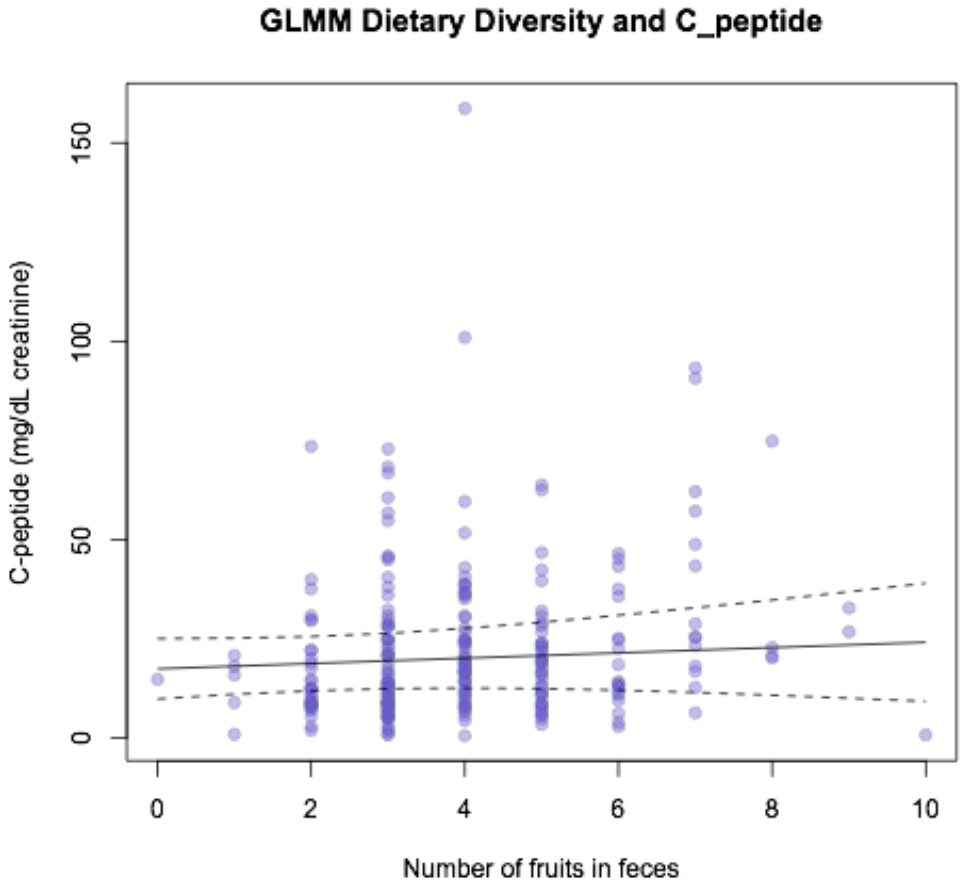

Figure 6.4.1.3.1.a Effect of dietary diversity on urinary C-peptide. The number of fruit species found in bonobo feces shows a small positive relationship to levels of C-peptide. Levels are highest at moderate dietary levels (between 3 and 7).

C-peptide and Dietary Diversity	Mean (95%CI)	SE	T-value	N
Intercept (alpha)	17.52 (9.76-25.28)	10.63	4.427	265
Effect of dietary diversity (Beta)	.67 (-0.98 – 2.31)	2.18	0.793	

Table 6.4.1.3.1.b Result summary of GLM for effect of dietary diversity on urinary C-peptide levels. Highest values occur between 2 and 8 species, but do not necessarily rise as diversity increases, suggesting a balance struck between foraging and energetic returns.

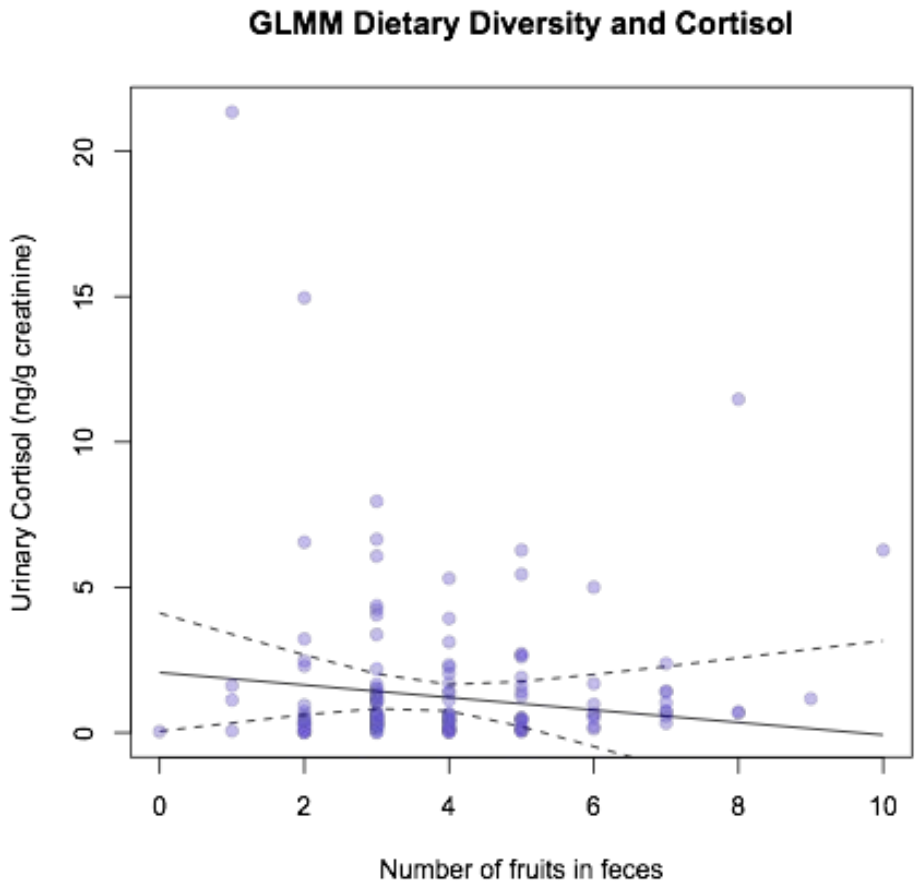

Figure 6.4.1.3.2.a Effects of dietary diversity on urinary cortisol. Shows small negative relationship where cortisol goes down as the number of fruits in diet go up. Patterns suggest that there may be an ideal middleground between too few and too many species in the diet.

Cortisol and Dietary Diversity	Estimate(95% CI)	SE	t value	N
Intercept (alpha)	2.06 (0.05 - 4.07)	1.03	2.006	148
Effect of dietary diversity (Beta)	-0.21 (-0.73 0.31)	0.27	-0.792	

Table 6.4.1.3.2.b Result summary of GLM for effect of dietary diversity on urinary cortisol levels. Lowest values occur between 2 and 8 species, but do not necessarily rise as diversity increases, suggesting a balance struck between foraging and energetic returns.

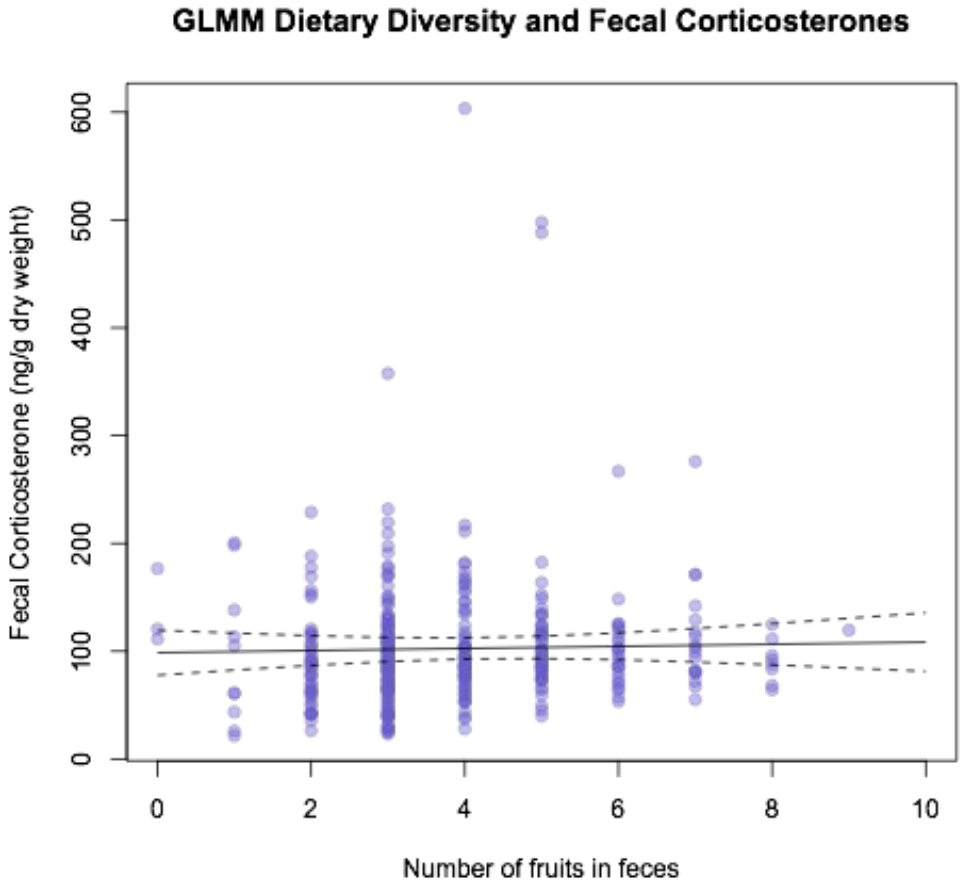

Figure 6.4.1.3.3.a Effect of dietary diversity on f-GCs. The number of fruit species found in bonobo feces does not show a relationship to levels of fecal glucocorticoids. Interestingly, f-GCs are also highest at moderate dietary levels (between 3 and 7).

Model: m2 <- lmer(F_cos ~ fecal.diversity +(1+fecal.diversity|project.month) + (1+fecal.diversity|nest.party) , data=d)

f-GCs and Fecal Diversity	Mean (95%CI)	SE	T-value
Intercept (alpha)	98.73 (77.9-119.56)	10.63	9.29
Effect of Fecal Diversity (Beta)	.96 (-3.32 – 5.24)	2.18	0.439

Figure 6.4.1.3.3.b Result summary of GLM for effect of dietary diversity on f-GC levels.

6.4.2 Hormones and terrestrial herbaceous vegetation (THV)

If the role of THV in bonobo diet is truly significant in terms of fostering increased sociality and egalitarian behaviors during periods of lowest resource availability, we would expect to see its presence in the diet associated with lower GCs and increased C-peptide levels. We ran a GLMM testing the relationships between fecal GCs, urinary cortisol, C-peptide and *Haumania librechtstiana.*

6.4.2.1 Is the consumption of THV associated with lower metabolic stress levels (lower f-GCs, lower cortisol) and higher measures of energetic surplus (high C-peptide)?

6.4.2.1.1 THV and C-peptide

Using a GLMM with varying intercepts for month, nest party size, consumption of *H. liebrechtsiana* and overall fruit abundance as predictors, we found that on average there is virtually no difference between C-peptide levels between bonobos who consumed *H. liebrechtsiana* (20.01 ng/dL creatinine) compared to bonobos who did not consume *H. liebrechtsiana* (20.95 ng/dL creatinine). Using this model, fruit abundance was a stronger predictive factor of C-peptide levels than the consumption of *H. liebrechtsiana*. Little variation was observed across months after accounting for overall resource abundance. C-peptide levels varied more between nest parties than across months. Below are model predictions plotted across months with the grand means shown in dotted lines. *Note that in the model, “bekombe” refers to *H. liebrechtsiana*.

6.4.2.1.2 THV and Urinary Cortisol

Using a similar model as above, with C-peptide, but with cortisol as a predictor variable, we found that *H. liebrechtsiana* consumption was marginally related to lower cortisol levels. On average cortisol levels were higher in bonobos who consumed *H.*

liebrechtsiana (1.75 mg/dL) compared to those who did not (1.32 mg/dL) (Figure 6.4.2.1.2.a and b)

6.4.2.1.2 THV and f-GCs

No notable difference was observed between f-GC levels and *H. liebrechtsiana* consumption. There was little variation across months, however f-GC levels in nest parties differed greatly.

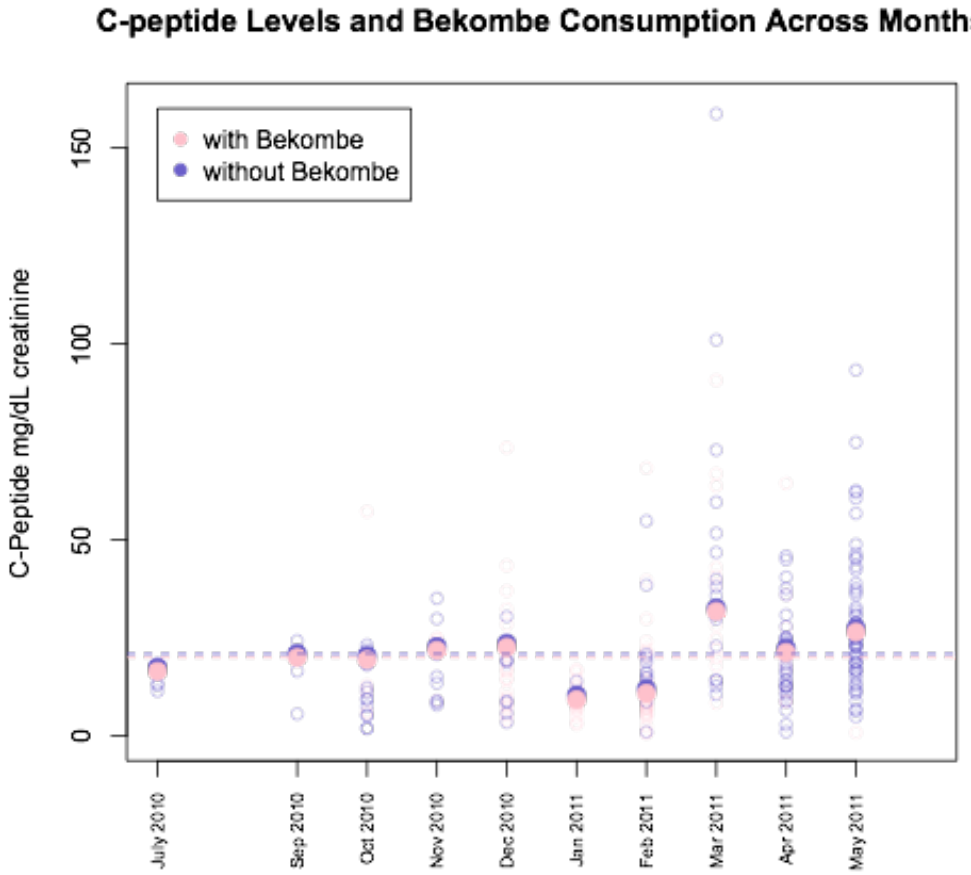

Figure 6.4.2.1.1.a Model predictions (dashed line) vs. raw data (pink and purple circles) for the effect of THV on C-peptide levels, across months. Bekombe is a local name for *Haumania librechtstiana*.

Model:**m4 <- lmer(C_pep ~Bekombe.f + s.fruit.abundance + (1 + Bekombe.f |project.month) + (1 + Bekombe.f|nest.party), data=d)**

C-peptide, Abundance and *H. liebrechtsiana*	Mean (95%CI)	SE	T-value	N
Intercept (alpha)	20.95 (13.42-28.47)	3.84	5.457	267
Effect of *H. liebrechtsiana* (beta1)	-0.94 (-6.15-4.27)	2.66	-0.354	
Effect of total abundance (beta2)	4.35 (-2.18 – 10.86)	3.33	1.3404	

Figure 6.4.2.1.1.b Result summary of GLMM using THV as a predictive factor for C-peptide levels, controlling for abundance.

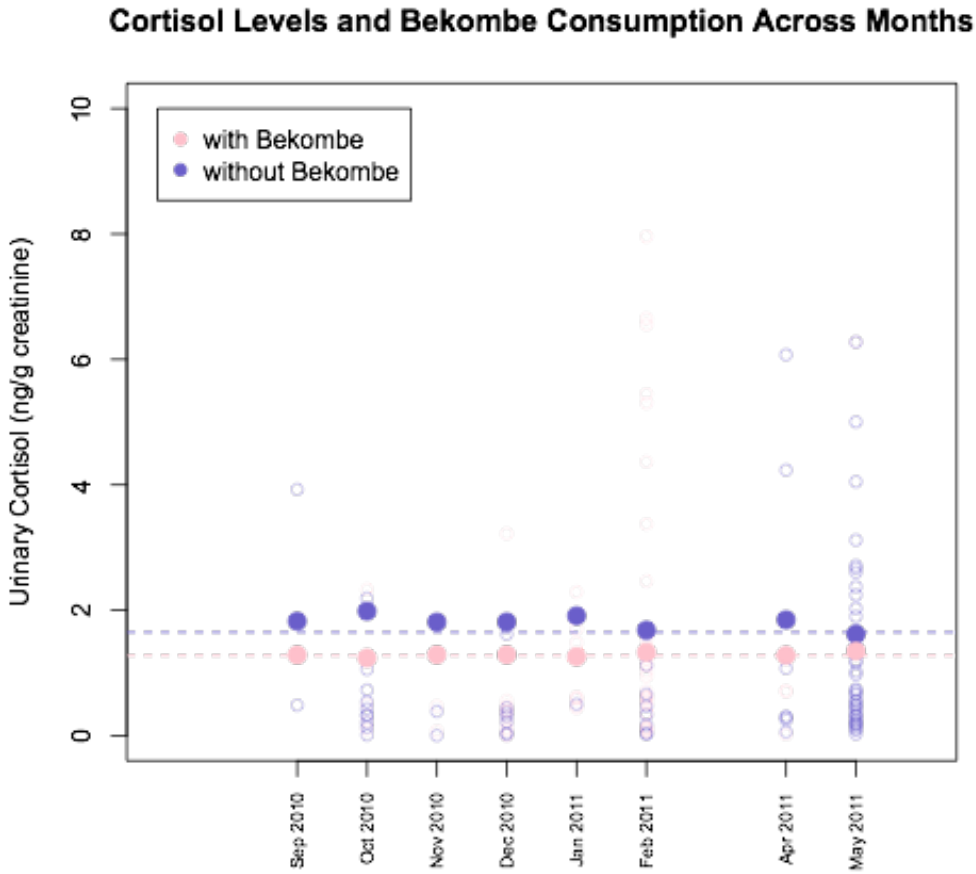

Figure 6.4.2.1.2.a Model predictions (dashed lines) vs. raw data (pink and purple circles) for the effect of THV on urinary cortisol levels, across months. Bekombe is a local name for *Haumania librechtstiana*. Levels of cortisol in the urine were marginally lower in individuals who had eaten THV than those who had not, but the biological significance is very small.

Model: **m4 <- lmer(Cort~Bekombe.f + s.fruit.abundance + (1 + Bekombe.f |project.month) + (1 + Bekombe.f|nest.party), data=d)**

Cortisol, Abundance and *H. liebrechtsiana*	Mean (95%CI)	SE	T-value	N
Intercept (alpha)	1.75(0.51-3.00)	0.64	2.756	149
Effect of *H. liebrechtsiana* (beta1)	-0/43 (-1.71-0.85)	0.65	-0.662	
Effect of fruit abundance (beta2)	.41(-.12-.95)	0.27	1.516	

Figure 6.4.2.1.2.b Result summary of GLMM using THV as a predictive factor for urinary cortisol levels, controlling for fruit abundance.

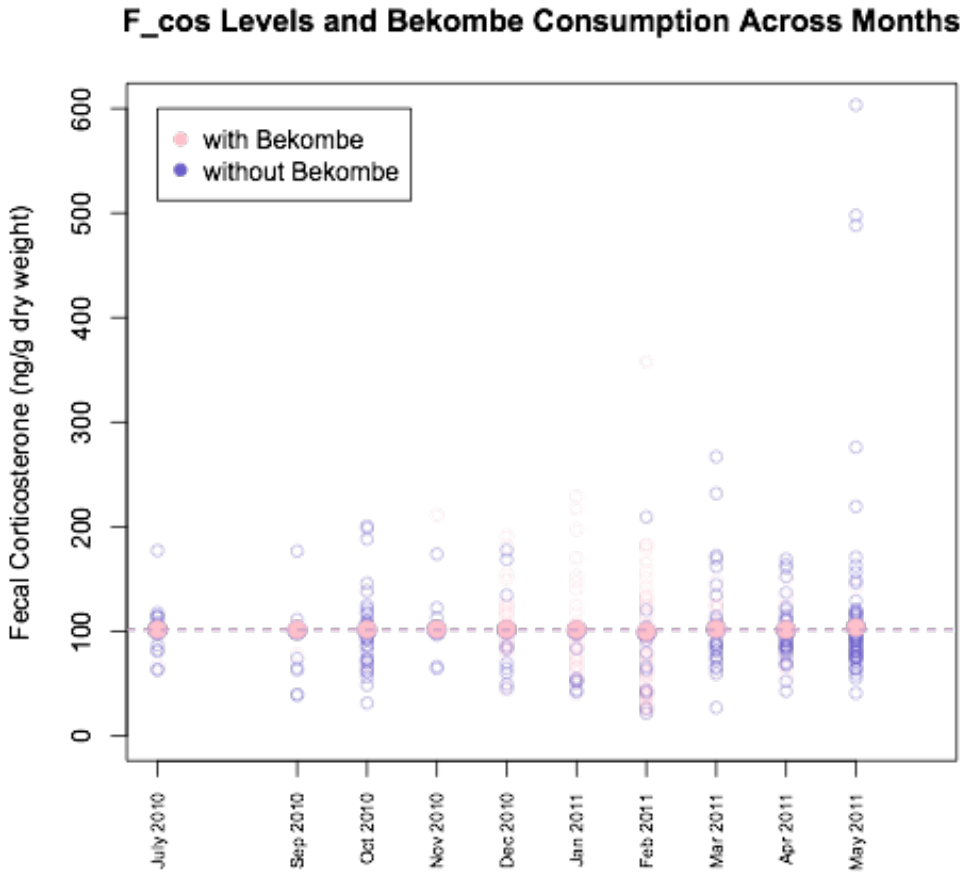

Figure 6.4.2.1.3.a Model predictions (dashed lines) vs. raw data (pink and purple circles) for the effect of THV on f-GC levels, across months. Bekombe is a local name for *Haumania librechtstiana*.

f-GCs, *H. librechtstiana,* Abundance	Mean (95%CI)	SE	T-value	N
Intercept (alpha)	101.46 (89.34-113.58)	6.18	16.413	369
Effect of *H. librechtstiana*(beta1)	-.07 (-12.88-12.74)	6.53	-0.011	
Effect of fruit abundance (beta2)	-2.1 (-12.4-8.19)	5.25	-0.4	

Figure 6.4.2.1.3.b Result summary of GLMM using THV as a predictive factor for urinary cortisol levels, controlling for fruit abundance.

6.4.3 Hormones and nest parties

6.4.3.1 Are seasonal shifts in metabolic hormones associated with shifts in party size?

Cortisol seems to be most closely linked to seasonal shifts in party size, although the effect size has little meaning biologically speaking. Using a series of linear poisson GLMMs we compared 3 models with different and the model with Cortisol had the lowest DIC values and largest effect sizes. (Tables 6.4.3.1.a&b)

6.4.3.2 Following observations that nest parties averaged at 7.7, is this number the "optimal" party size?

Parties that exceeded 7-8 individuals showed a much higher likelihood of experiencing increasing levels of cortisol (fig. 6.4.3.2.1.a). In order to see if an optimal party size could be viewed in terms of C-peptide, we ran another GLMM, this time controlling for variation across nest party so as to remove the effects of the linear decrease in C-peptide as nest party size increased. The relationship was less clear with C-peptide (fig. 6.4.3.2.2.a &b).

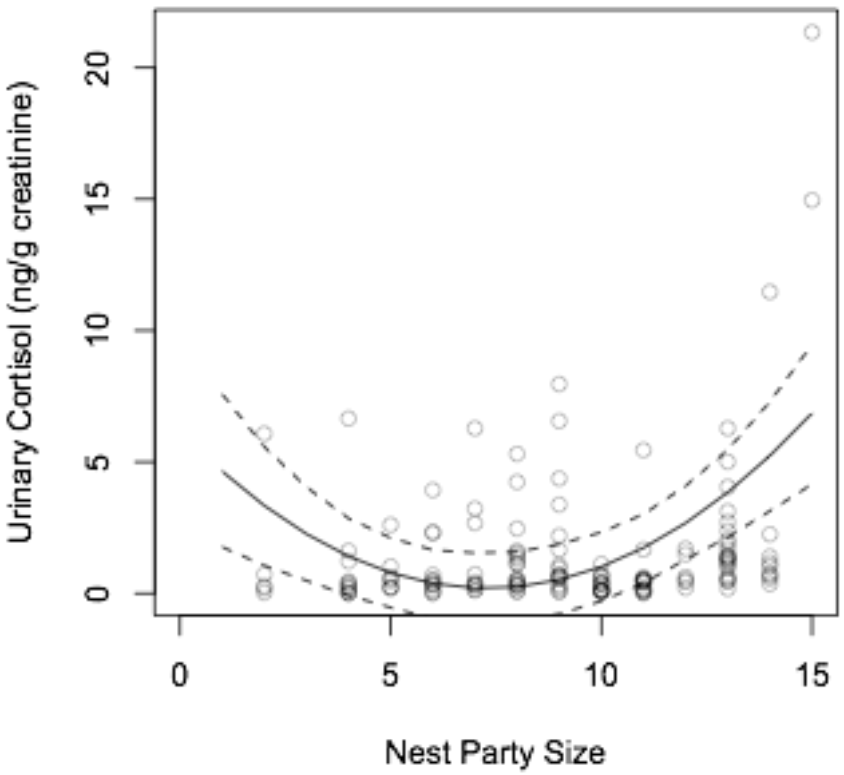

Figure 6.4.3.2.1.a Optimal balance between nest party size and urinary cortisol levels, controlling for dietary and environmental diversity.

Model: **m5Cort <- lmer(Cort ~ num.nests + I(num.nests^2) + s.fruit.abundance*s.fecal.diversity + (1 |project.month) + (1|nest.party) , data=d)**

Effect of Nest Party Size on Cortisol Levels	Mean (95%CI)	SE	T-value
Intercept (alpha)	6.18 (2.48 9.87)	1.89	3.27
Effect of number of nests (beta1)	-1.64 (-2.59 -0.70)	0.48	-3.40
Effect of number of nests squared	0.11 (0.06 0.17)	0.03	3.88
Effect of fruit abundance (beta2)	-0.06 (-1.37 1.25)	0.67	-0.09
Effect of dietary diversity	0.24 (-0.18 0.66)	0.21	1.14
Interaction bt fruit abundance and dietary diversity	0.71 (0.11 1.31)	0.31	2.30

Table 6.4.3.2.1.b Effect of nest party size on urinary cortisol levels, controlling for dietary and environmental diversity.

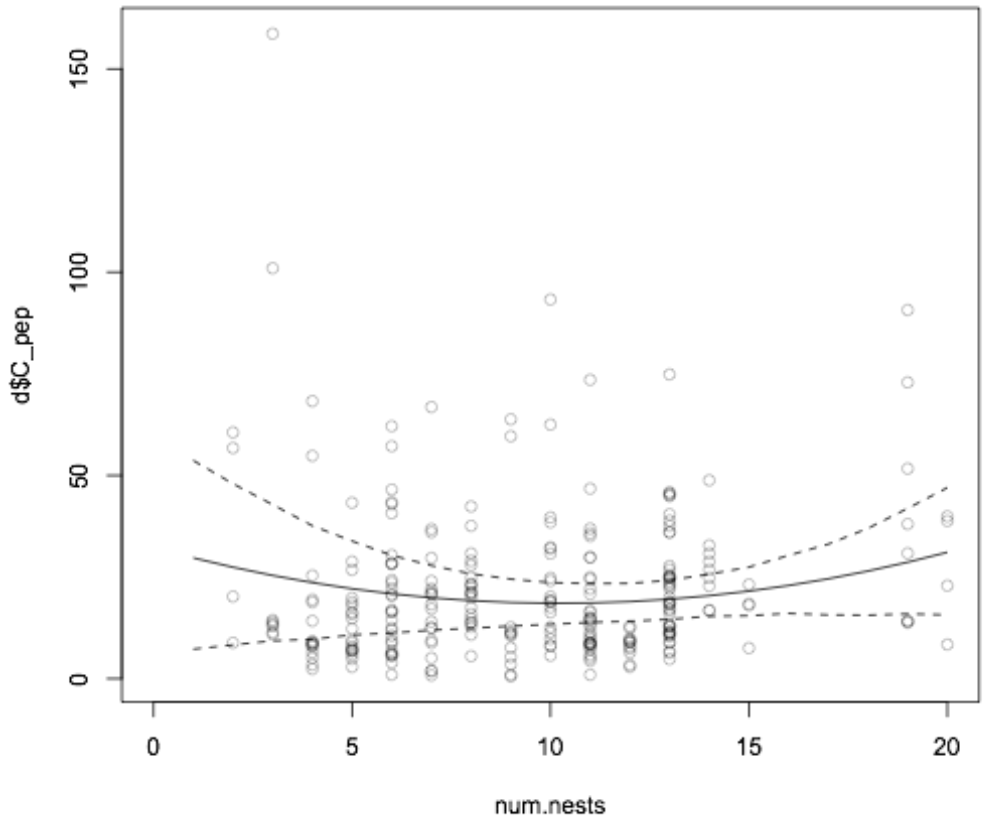

Figure 6.4.3.2.2.a Curvilinear balance between C-peptide levels and party size, controlling for dietary and environmental diversity. Highest values occur in smallest party sizes.

Model: **m7C_pep <- lmer(C_pep ~ num.nests*s.fecal.diversity + I(num.nests^2)*s.fecal.diversity + (1 + num.nests + I(num.nests^2)|project.month) + (1|nest.party) , data=d)**

C-peptide and Optimal NPS	Mean (95%CI)	SE	T-value
Intercept (alpha)	32.20 (4.90 59.51)	13.93	2.31
Effect of number of nests (beta1)	-2.68 (-6.94 1.58)	2.17	-1.23
Effect of dietary diversity	-1.04 (-12.78 10.70)	5.99	-0.17
Effect of number of nests squared	0.13 (-0.05 0.31)	0.09	1.45
Interaction bt number of nests and dietary diversity	0.44 (-1.94 2.81)	1.21	0.36
Interaction bt dietary diversity and # of nests ^2	-0.02 (-0.13 0.10)	0.06	-0.27

Table 6.4.3.2.2.b Effect of nest party size on urinary C-peptide levels, controlling for dietary and environmental diversity.

6.5 Discussion:

6.5.1 Discussion of Methods:

6.5.1.1 Non-invasive sampling: pro's and con's

There are many advantages and disadvantages associated with analysis of non-invasively collected hormones (Wasser et al. 2000; Whitten et al. 1998; Ziegler and Wittwer 2005; Goymann 2000). In terms of advantages, non-invasive collection of hormones when working with wild animals is particularly useful because it avoids creating stress responses produced from coming into contact with unhabituated animals. Fecal and urine samples are also readily available on a daily basis. Accordingly, the use of fecal and urine is employed in a wide variety of investigations that examine relationships between hormones and behaviors, as well as in various questions in the realms of stress and animal welfare, reproductive physiology, behavioral ecology, conservation biology, and biomedical research. However, there are important drawbacks that merit attention when designing and interpreting results that employ non-invasive hormonal collection. Degradation of metabolites at ambient temperature and contamination are both potentially confounding factors of collecting and storing fecal and urine samples in non-sterile environments. Unfortunately, environments where non-invasive fecal and urine sampling is most useful, such as my study site, do not typically have electricity and are not sterile. In spite of this, a wide range of studies that have used non-invasive methods to investigate endocrinology in the wild have shown compelling results that support the efficacy of their methods (Strier and Ziegler 2005).

One of the major dilemmas of using feces and urine to assess hormones, is the uncertainty of whether of not steroid hormones that have been excreted rapidly through the kidneys and/or gut undergo major changes to their composition. Though opinions and

results vary across authors and studies, the resulting metabolites of both steroid and peptide hormones have been found to be stable across long periods of time in some notable cases (Lasley and Kirkpatrick 1991; Lasley and Shideler 1993).

6.5.1.2 Diurnal patterns and time of day of collection

Some metabolic hormones (e.g. testosterone and cortisol) in chimpanzee and bonobo urine show a clear diurnal pattern with elevated levels in the early morning followed by a steady decline through the day (Muller and Lipson 2003). I argue that this actually worked to my advantage when it came to urinary cortisol: fresh samples were collected at the same time every day. Bonobos usually urinate and defecate directly outside of their nests immediately after waking up. Fresh urine and feces are not hard to differentiate from urine and feces that have been out for more than an hour once you are familiar with the characteristics of both. I collected the first urinations of the day from individuals (we took special care to be sure that only fresh samples were collected). In doing so, I avoided confounding effects of diurnal changes in cortisol levels by standardizing all urinary collections time (although, see Anestis and Bribiescas 2004). Conveniently, early morning collection also allowed me to collect the majority of my urine samples with paired fecal samples, allowing for a comparison of levels of GCs in both urine and feces from the same day. Deschner et al. (2008) found no significant circadian effects on C-peptide titers in bonobo urine, so time of day of collection should not be of concern for C-Peptide.

6.5.1.3 Radioimmunoassays vs. Liquid Chromatography/Mass-Spectrometry

The efficacy and accuracy of identifying and measuring hormone metabolites using commonly employed assays, such as radioimmunoassays (RIA's) has been also been increasingly called into question, as other more accurate methods such as enzyme immunoassays and liquid chromatography mass spectrometry (LC/MS) have become more widely available (Penning et al. 2010; Goymann 2012). However, RIA's are relatively cost-effective, and have been shown repeatedly to effectively show levels of GCs that reflect serum levels from the previous day (e.g. Bahr et al. 2000, Whitten et al. 1998).

This project incorporated the use of three different measures of energetic hormonal activity: fecal corticosterone, urinary cortisol, and urinary C-peptide of insulin (referred to simply as C-peptide). Corticosterone is not directly produced by the adrenal glands in primates, nor is it excreted in feces (Palme 2005). However, the metabolites of fecal GCs following the digestive process have a higher affinity to corticosterone-specific antibodies than to antibodies specific to cortisol in multiple primate species (Wasser et al 2000; Whitten et al. 1998). Thus, radioimmunoassays for corticosterone have been a popular and affordable means of f-GC analysis for almost two decades. It is important to note that the following analysis uses the term fecal corticosterone interchangeably with f-GCs, all of which refer to *immunoreactive corticosterone metabolites*, not corticosterone itself. Results here neither support nor negate the use of f-GCs entirely. On the one hand, they appeared to respond to the corticosterone RIA predictably, suggesting that levels of hormone are being reliably measured. On the other hand, their lack of correlation with

any of the ecological or social measures in contrast to the strong correlations seen in the urinary hormones call to question the efficacy of using them.

C-peptide from great ape urine has been successfully assayed with both enzyme immunoassays and RIA's (Emery Thompson et al. 2009; Emery Thompson and Knott 2008; Deschner, Kratzsch, and Hohmann 2008). To date, no successful validations of C-peptide from wild great ape urine for LC/MS have occurred. This is probably because the metabolite is different enough from the C-peptide standard that it does not produce a peak in LC/MS, but still similar enough to C-peptide antibodies that it binds in competitive assays. Results here strongly support the efficacy of using urinary C-peptide RIA's to assess changes in energetic shifts in the context of broad ecological measures, and in light of its predictably inverse relationship with shifts in urinary cortisol.

Urinary cortisol can be assessed using radioimmunoassays (Bahr et al. 1998; Robbins and Czekala 1997). Controlled studies on marmosets, macaques, and chimpanzees have shown that cortisol is virtually absent in feces, but largely present in urine 5.5h following injection with radiolabeled hormone (Bahr et al. 2000). Their findings also showed that approximately 61-87% of the cortisol was expressed as conjugates, highlighting the limitations of interpreting results from non-invasive studies: reported levels do not necessarily reflect total physiological levels. However, assuming that metabolization of hormones remains consistent over time, changes in levels of non-invasively collected hormones over days, weeks and months can still be used to reflect endogenous responses to the environment. The sterane skeletal structure of steroid hormones, including cortisol remain intact enough following digestion and defecation such that excreted metabolites still bind with reasonable specificity to assay antibodies,

and can also be measured using LC/MS, which is far more accurate and specific than RIA's (Taylor 1971, Lindner 1972, Macdonald 1983). In recent years methods for the analysis of steroid hormones found in urine from wild primates using LC/MS have proven to be both more accurate and potentially more cost-effective, offering the possibility to assess a full profile of hormones from a single sample instead of a single metabolite (Penning et al. 2010; Hauser et al. 2008). Results here strongly support the use of LC/MS in assessing urinary cortisol.

6.5.2 Summary of Results:

Urinary C-peptide and urinary cortisol both appear to have inverse relationships, while f-GCs do not appear to have any strong relationship across categories (table 6.5.2.a).

Summary: Ecological Predictors of Energetic Hormones	**Total Abundance**	**Pref. Fruit Abundance**	**Environ-mental Diversity**	**Abundance + Diversity**	**THV**
C-Peptide	sm. negative (could be due to leaves)	positive	positive	strong positive effect when *both* are high	none
Urinary Cortisol	negative	strong negative	no relationship	strong negative effect when abundance is low and diversity is high	small negative
F-Cos	no relationship	no relationship	no relationship	NA	none

Table 6.5.2.a Summary of ecological predictors of energetic hormones: total abundance, abundance of preferred fruit species (no leaves), environmental diversity (number of ripe fruit species in home range preferred by bonobos), and interaction between abundance and diversity.

Results Summary (Basic):

Chapter	Original Question	Modified Question	Results
3. Seasonality	*3.2.1 Does the Iyema bonobo community live in a seasonal environment?* *3.2.2 Are seasonal fluctuations in the environment reflected in the Iyema bonobos' diet?*	*6.2.1.1 Do energetic hormones, such as C-peptide and f-GCs reflect seasonal shifts in resource abundance?* *6.2.1.2 Do energetic hormone levels reflect seasonal shifts in fruit diversity?* *6.2.1.3 Do energetic hormone levels reflect shifts in dietary diversity?*	*6.4.1.1* *Yes.* *6.4.1.2* *Yes.* *6.4.1.3* *Yes*
4. Terrestrial Herbaceous Vegetation	*4.2.1 Do bonobos eat more THV when abundance is low?*	*6.2.2.1 Is the consumption of THV associated with lower metabolic stress levels (lower f-GCs, lower cortisol) and higher measures of energetic surplus (high C-peptide)?*	*6.4.2.1* *Yes/No: associated with lower levels of u-cort, lower levels of f-GCs, lower levels of C-peptide.*
5. Nest Parties	*5.2.1 Do party sizes shift seasonally?* *5.2.2 Do seasonal shifts in resource availability, measured through abundance and diversity affect party size?*	*6.2.3.1 Are seasonal shifts in metabolic hormones associated with shifts in party size?* *6.2.3.2 Following observations that nest parties averaged at 8, is this number the "optimal" party size?*	*6.4.3.1* *Yes, primarily cortisol, but not supported in C-peptide or f-GCs* *6.4.3.2* *It appears to be, in terms of cortisol, but is not as strongly linked to C-peptide or f-GCs*

Chapter 7 Conclusion

Iyema bonobos live in a seasonal environment, defined by rainfall and monthly estimates of fruit production. Fruit abundance and diversity were found to be the best predictors of party size in this community, in surprising contrast to findings in other communities of *Pan*. This suggests that resource abundance is more important to bonobos than has previously been considered, and also highlights the importance of viewing a complex socioecological scenario such as this with nuanced methods, such as GLMMs.

Analysis of 712 fecal samples over a complete year showed that bonobos in the Iyema community showed a clear preference for the THV species *Haumania liebrechtsiana*, which was the third most frequently consumed food item in the bonobos' diet, after figs and a commonly occurring drupe species (*Polyalthia suaveolens).* The consumption of *Haumania* in the bonobo community does appear to be seasonal (fig. 4.4.1.a). Fruit abundance had a small positive relationship with *Haumania* consumption (fig. 4.4.e), demonstrating that contrary to the predictions of the THV model, *Haumania* consumption was more likely to decrease as abundance also decreased. The inverse relationships of environmental diversity and dietary diversity with *Haumania* consumption were initially perplexing: why would *Haumania* consumption decrease as variety in the environment increases, yet increase as the number of species in the bonobo diet increases? The short answer is that a) bonobo dietary choices seem to have little to do with environmental diversity directly (Ch. 3) and b) a wider dietary breadth is often an indication that energetic and/or nutritional needs can not be met with just a few items.

Thus, in the latter case, we would predict that the likelihood of consuming *Haumania* would rise if more species are being consumed in response to low quality options, because it is always available, even if it is not at peak production of young shoots and leaves; *Haumania* can be (but is not always) consumed as a fallback food. Since I have no nutritional information on the bonobo diet for this study, I use C-peptide levels instead, as an indication of energetic returns. If consumption of more species is indeed a means of balancing energetic requirements when food quality is low, you would expect to see a steady (flat) or even negative relationship between C-peptide levels and the number of species consumed. This was the case (Ch. 6, fig. *6.4.1.3.1*.b)

THV growth patterns appear to correlate with rainfall (White, unpublished data/personal communication), and the rate of THV consumption in this community steadily increased as the long rain season of 2010 began to transition into the long dry period, suggesting that much like fruit species, there are distinct periods when its nutritional pay-off are highest (Ch. 4, fig. 4.4.1.a). Nutritional content of THV in mountain gorilla (*Gorilla beringei*) habitats in Uganda have been found to change seasonally (Rothman et al. 2011). The lack of a relationship between *Haumania* and energetic hormones was somewhat confusing, but not very surprising. Although it is high in protein, *Haumania* is not high in carbohydrates (Yamakoshi, 2004), which likely accounts for the lack of correlation with elevated C-peptide levels.

Not much is known about the ways in which bonobos seek out THV, nor have there been any close examinations of whether or not bonobos will choose it over another preferred resource. The “typical” interpretation of Wrangham’s 1986 hypothesis depicts bonobos as feeding on hyper-abundant THV while they move between fruit patches in

the forest. However, accounts from field observers draw a distinction between feeding-directed styles of travel, where the bonobos will either be on the move in a manner that is more akin to a "mission," moving very fast through the forest as though there is a specific destination in mind (A. Fowler, pers. comm.), or moving through *Haumania* patches in particular, at a slow and deliberate pace. Likewise, Isaac Schamberg's studies of vocalizations by bonobos at Lui Kotal have shown that they will differentiate vocally between THV patches are particularly exciting and those that are of moderate quality or preference (Isaac Schamberg, pers. comm.).

What might be more interesting about my results is the alternative perspective they pose to Wrangham's original hypothesis: Is it possible that THV is a preferred resource, and due to the way in which it is distributed in their environment, does it actually *restrict* sociality as opposed to enabling it?

Bonobos experience seasonal fluctuations in fruit diversity and availability (e.g. White 1998, Mulavwa 2008, Chapter 3). Based on what we understand from other studies that have closely examined nutritional variation within and between species in chimpanzee environments (over time and across different study sites), we know many species of fruit are inconsistent in terms of nutritional content and calories across geography and time (Chapman et al. 2005). Protein consumption by other wild primates is often considered to be relatively stable compared to total ingested energy across periods with high and low fruit availability (Conklin-Brittain et al. 1998; Felton et al. 2009; N'guessan, Ortmann, and Boesch 2009), but recent studies have shown that protein gained from young leaves and shoots also varies seasonally (Irwin et al. 2014; Rothman, Raubenheimer, and Chapman 2011).

The need for protein during times when THV is “ripe” would likely direct the movements and activities of bonobos to bias THV patches. However, in doing so they are limited by the physical reality of a THV patch, which is much like a maze of bamboo: dense and constricting. THV patches of all kinds are not environments that easily facilitate social behavior between more than three or four individuals at a time (Pers. Obs.).

Malenky & Stiles (1991) noted that observations of bonobos on the ground were rare in part because visibility was hindered by dense vegetation, but also because bonobos tend to become shy on the ground (see also, Kuroda, 1979). From the bonobos’ point of view, this makes sense: you can’t see predators as well from a dense thicket as you can from a lofty tree branch, and while there is safety in numbers, you can’t keep your numbers high when you can’t see more than a few individuals away from you. On the other hand, in the absence of predatory pressures, it may just be that maintaining socially rewarding feeding parties during times that bias feeding in THV patches is cumbersome. The relationship in my data show that a) nest party sizes with the highest consumption rates of THV are small, relative to the mean, and b) consumption of THV is marginally associated with lower levels of cortisol. It may simply be that THV is a seasonally prioritized food and smaller parties are less stressful on all counts.

Party sizes showed a seasonal pattern generally across months, but were highly variable from day to day. My statistical models showed that nest party size was best predicted by the interaction of both fruit abundance and diversity of preferred ripe fruit that was available in the environment. These results contrasted with numerous other studies that also found that as fruit abundance in the environment increased, its relative

importance in predicting foraging party sizes in chimpanzees declined (Newton-Fisher, Reynolds, and Plumptre 2000; Hashimoto et al. 2003; Wrangham, Conklin-Brittain, and Hunt 1998). Aforementioned studies argue that these patterns suggest that for in these forests abundance rarely if ever falls below a low-end threshold of fruit abundance, thus satisfying basic energetic needs of the *Pan* communities within them. This interpretation was also supported by the most recent analysis of the ranging patterns of bonobos, living in nearby Ndele (15km away) which has the smallest annual ranges of any of the *Pan* study sites at an astonishing 2.5km2 (Waller 2011). Waller's findings, contextualized by archival data of fruit availability in the Ndele forest lend support for the notion that bonobos in the Lomako area do not need to range far to get what they need. My estimated core range of the Iyema community (~16km2) was not as small as the estimate for the Ndele community, but falls well within the range of other *Pan* communities (e.g. 22km2 for Wamba, 13-26km2 for Tai, 15-20km2 for Bossou, 16 for Kanyawara, summarized in Waller 2011). The conclusions here, that abundance of fruit and fruit diversity play an important role in determining bonobo party sizes suggests that a more nuanced analytical approach to the interaction between these factors may reveal different conclusions.

Nest party sizes never exceeded 26, and averaged 7.7 individuals, which was slightly higher than feeding parties reported for Lomako by White and Wrangham (1988), but similar to the most recent reports from the more habituated Wamba (Mulawva 2010), as well as Tai forest (8.3), Mahale (6.1) and smaller than average party sizes reported for Ngogo (10.3) (Lehmann and Boesch 2003; Nishida et al. 2003; Mitani, Watts, and Lwanga 2002, respectively). The consistency of parties at 7.7 individuals was striking across the year, and suggests that in spite of regular variation there was clear preference

for that party size. It is entirely possible that there was a core group of 7-8 individuals that preferred each other's company, but it is impossible to say without knowing the inter-individual dynamics of this community. What is clear is that in addition to the frequency of seeing this particular number of nests throughout the year, parties that exceed this size showed an increased likelihood of rising cortisol levels after controlling for variation in environmental diversity and fruit abundance (Chapter 6, Figure 6.4.3.2.1.a). This is a strong indication that larger parties equate with increased competition for food, sex, or both. Sporadic predation by large cats or humans may also have been the causes for both the large parties and urinary cortisol levels, but data was not available for this. Upward deviation from the optimum party size equates with stress, and stress is always best avoided. The question remains, why have larger parties, when smaller parties are associated with lower stress levels and higher energetic pay-off?

The answer is not in these data, but may already be in Martin Surbeck's socioendocrine work with the Lui Kotal bonobo community, where he has observed urinary cortisol levels rise in males in the presence of estrous females in feeding parties (Surbeck et al. 2012). Likewise, my own data from a small pilot study using only fecal glucocorticoids showed that in adult females, levels of f-GCs were positively and significantly correlated to the presence of males in the previous night's nesting parties (ANOVA $F=13.52$, $p<0.02$). In juvenile females, a suggestive negative correlation was found between f-GC levels and the number of adult females present in the previous night's nesting party (Correlation coefficient $r= -0.773$, $p= .07$).

These results make sense in light of observations across study sites that when party size decreases, it is the females who stay together, while males tend to become

peripheralized (White 1998). In addition, it appears as though young females incur the benefits of female-female associations in this social system. Bonobo females appear to experience physiological costs associated with the presence of males, though it is unclear exactly what that cost is. It is reasonable to assume that as party sizes increase, the likelihood of sexual competition increases as well, as seen in Surbeck's study. However, my results here show a clear relationship between energetic pressures and large party sizes.

In other words, for bonobos, the *presence* of members of the opposite sex is associated with increased levels of stress when everything else is accounted for, but whether that is because their presence incites inter- or intra-sex competition is still unclear. In the Iyema community, there are at least 8 identified adult females. Is it possible that these 8-nest parties are largely composed of only females? Given the fission-fusion dynamics of *Pan*, it seems unlikely that the same 8 individuals would consistently form exclusive nest parties, but it is an avenue worth exploring.

My data also showed clear ecological correlates between nest party size and energetic hormones: as party size increased and the *variety* of items to eat in the environment decreased, cortisol increased and C-peptide decreased, as predicted (Chapter 6, figure 6.1.1.a). This relationship seems to be fairly straightforward: increased food competition equals increased stress and decreased carbohydrate intake. However, in the absence of detailed behavioral observations, it is impossible to tease apart social causes of stress from energetic causes of stress: it is possible that increases in nest party sizes increased the likelihood of other stressful encounters that were not necessarily food-related, such as sexual competition. Chimpanzee parties that include estrous females tend to be larger,

regardless of food availability (Nishida 1979; Goodall 1986; Hashimoto, Furuichi, and Tashiro 2001). It is also important to consider that while seasonal shifts in fruit diversity are affecting all members of this community, other factors, such as differences in rank will affect who gets what and when (Janson and Vogel 2006; Furuichi 1997; Murray et al. 2006). This was exemplified during the month of March, when diversity and nest party sizes were both higher than normal, and C-peptide level variation was also at its highest within nest parties, suggesting that even when resources are at their richest, not all individuals experience the same energetic benefits.

References

Abbott, D. H., E. B. Keverne, F. B. Bercovitch, et al.
2003 Are Subordinates Always Stressed? A Comparative Analysis of Rank Differences in Cortisol Levels among Primates. Hormones and Behavior 43(1): 67–82.

Altmann, J.
1974 Observational Study of Behavior: Sampling Methods. Behaviour 49: 227–265.

Anderson, DEAN P., ERIK V. Nordheim, C. Boesch, and T. C. Moermond
2002 Factors Influencing Fission-Fusion Grouping in Chimpanzees in the Taï National Park, Côte d'Ivoire. Behavioural Diversity in Chimpanzees and Bonobos. Cambridge University Press, Cambridge: 90–101.

Anestis, S. F., and R. G. Bribiescas
2004 Rapid Changes in Chimpanzee (Pan Troglodytes) Urinary Cortisol Excretion. Hormones and Behavior 45(3): 209–213.

Badrian, AJ, and NL Badrian
1984 Group Composition and Social Structure of Pan Paniscus in the Lomako Forest. *In* The Pygmy Chimpanzee. R.L. Susman, ed. Pp. 325–346. New York: Plenum Press.

Badrian, N., A. Badrian, and R. L. Susman
1981 Preliminary Observations on the Feeding Behavior ofPan Paniscus in the Lomako Forest of Central Zaïre. Primates 22(2): 173–181.

Bahr, N. I., R. Palme, U. Möhle, J. K. Hodges, and M. Heistermann
2000 Comparative Aspects of the Metabolism and Excretion of Cortisol in Three Individual Nonhuman Primates. General and Comparative Endocrinology 117(3): 427–438.

Bahr, N. I., C. R. Pryce, M. Dobeli, and R. D. Martin
1998 Evidence from Urinary Cortisol That Maternal Behavior Is Related to Stress in Gorillas. Physiology and Behavior 64(4): 429–438.

Basabose, A. Kanyunyi
2002 Diet Composition of Chimpanzees Inhabiting the Montane Forest of Kahuzi, Democratic Republic of Congo. American Journal of Primatology 58(1): 1–21.

Bermejo, M., G. Illera, and J. S. Pí
1995 Animals and Mushrooms Consumed by Bonobos (Pan Paniscus): New Records from Lilungu (Ikela), Zaire. International Journal of Primatology 16(1): 879–898.

Boesch, C., and H. Boesch-Achermann

2000 The Chimpanzees of the Tai Forest. Behavioural Ecology and Evolution. Oxford: Oxford University Press.

Boesch, Christophe, Gottfried Hohmann, and Linda Marchant
2002 Behavioural Diversity in Chimpanzees and Bonobos. Cambridge University Press. http://books.google.com/books?hl=en&lr=&id=-E7QdC6Q8cIC&oi=fnd&pg=PR8&dq=hohmann+bonobo&ots=ZXp9eJGMrt&sig=SYhD4b46qyR2X8VfvtifxXfz2j8, accessed March 17, 2014.

Benjamin M., Mollie E. Brooks, Connie J. Clark, Shane W. Geange, John R. Poulsen, M. Henry H. Stevens, and Jada-Simone S. White. "Generalized Linear Mixed Models: A Practical Guide for Ecology and Evolution." *Trends in Ecology & Evolution* 24, no. 3 (2009): 127–35.

Brockman, D. K., A. Cobden, and P. L. Whitten
2007 Birth Season Glucocorticoids Are Related to the Presence of Infants in Male Sifaka (Propithecus Verreauxi). Am J Phys Anthropol 44: 81.

Brockman, DK, and PL Whitten
1996 Reproduction in Free-Ranging Propithecus Verreauxi: Estrus and the Relationship between Multiple Mating Partners and Fertilization. AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 100: 57–69.

Cavigelli, S. A.
1999 Behavioural Patterns Associated with Faecal Cortisol Levels in Freeranging Female Ring-Tailed Lemurs, Lemur Catta. Animal Behaviour 57: 935–944.

Chapman, C. A., L. J. Chapman, T. T. Struhsaker, et al.
2005 A Long-Term Evaluation of Fruiting Phenology: Importance of Climate Change. Journal of Tropical Ecology 21(01): 31–45.

Chapman, C. A., F. J. White, and R. W. Wrangham
1994 Party Size in Chimpanzees and Bonobos: A Reevaluation of Theory Based on Two Similarly Forested Sites. Chimpanzee Cultures Edited by Wrangham RW, McGrew WC, de Waal FBM, Heltne PG.

Chapman, C. A., R. Wrangham, and L. J. Chapman
1994 Indices of Habitat-Wide Fruit Abundance in Tropical Forest. Biotropica 26(2): 160–171.

Chapman, C. A., R. W. Wrangham, L. J. Chapman, D. K. Kennard, and A. E. Zanne
1999 Fruit and Flower Phenology at Two Sites in Kibale National Park, Uganda. Journal of Tropical Ecology 15(02): 189–211.

Chapman, Colin A., and Lauren J. Chapman
2000 Determinants of Group Size in Primates: The Importance of Travel Costs. On the

Move: How and Why Animals Travel in Groups. University of Chicago Press, Chicago 24: 41.

Chapman, Colin A., Lauren J. Chapman, Richard Wangham, et al.
1992 Estimators of Fruit Abundance of Tropical Trees. Biotropica 24(4): 527–531.

Chapman, Colin A., Lauren J. Chapman, and R. W. Wrangham
1995 Ecological Constraints on Group Size: An Analysis of Spider Monkey and Chimpanzee Subgroups. Behavioral Ecology and Sociobiology 36(1): 59–70.

Clutton-Brock, T. H., and P.H. Harvey
1977 Primate Ecology and Social Organization. J Zool Lond 183: 1–39.

Conklin-Brittain, Nancy Lou, Richard W. Wrangham, and Kevin D. Hunt
1998 Dietary Response of Chimpanzees and Cercopithecines to Seasonal Variation in Fruit Abundance. II. Macronutrients. International Journal of Primatology 19(6): 971–998.

Conklin-Brittain NL, Wrangham RW, Hunt KD
1998 Dietary Response of Chimpanzees and Cercopithecines to Seasonal Variation in Fruit Abundance. II. Macronutrients. Int J Primatol 19: 971–998.

Crockford, C., R. M. Wittig, P. L. Whitten, R. M. Seyfarth, and D. L. Cheney
2008 Social Stressors and Coping Mechanisms in Wild Female Baboons (Papio Hamadryas Ursinus). Hormones and Behavior 53(1): 254–265.

Crook, J.H., and J.C. Gartlan
1966 Evolution of Primate Societies. Nature 210: 1200–1203.

Curtis, Deborah J., and Alphonse Zaramody
1998 Group Size, Home Range Use, and Seasonal Variation in the Ecology of Eulemur Mongoz. International Journal of Primatology 19(5): 811–835.

D'Amour, Danielle E., Gottfried Hohmann, and Barbara Fruth
2006 Evidence of Leopard Predation on Bonobos (Pan Paniscus). Folia Primatologica 77(3): 212–217.

Deschner, T., J. Kratzsch, and G. Hohmann
2008 Urinary C-Peptide as a Method for Monitoring Body Mass Changes in Captive Bonobos (Pan Paniscus). Hormones and Behavior.

Doran, D. M.
1993 Comparative Locomotor Behavior of Chimpanzees and Bonobos: The Influence of Morphology on Locomotion. AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 91: 83–83.

Doran, Diane
1997 Influence of Seasonality on Activity Patterns, Feeding Behavior, Ranging, and Grouping Patterns in Tai Chimpanzees. International Journal of Primatology 18(2): 183–206.

Dupain, J., E. Van Krunkelsven, L. Van Elsacker, and R. F. Verheyen
2000 Current Status of the Bonobo (Pan Paniscus) in the Proposed Lomako Reserve (Democratic Republic of Congo). Biological Conservation 94(3): 265–272.

Eisenberg, J.F., N.A. Muckenhirn, and R. Rudran
1972 The Relation between Ecology and Social Structure in Primates. Science 176: 863–874.

Emery Thompson, M., and C. D. Knott
2008 Urinary C-Peptide of Insulin as a Non-Invasive Marker of Energy Balance in Wild Orangutans. Hormones and Behavior 53(4): 526–535.

Emery Thompson, Melissa, Martin N. Muller, Richard W. Wrangham, Jeremiah S. Lwanga, and Kevin B. Potts
2009 Urinary C-Peptide Tracks Seasonal and Individual Variation in Energy Balance in Wild Chimpanzees. Hormones and Behavior 55(2): 299–305.

Erhart, Elizabeth M., and Deborah J. Overdorff
1998 Infanticide in Propithecus Diadema Edwardsi: An Evaluation of the Sexual Selection Hypothesis. International Journal of Primatology 19(1): 73–81.

Felton, Annika M., Adam Felton, David Raubenheimer, et al.
2009 Protein Content of Diets Dictates the Daily Energy Intake of a Free-Ranging Primate. Behavioral Ecology 20(4): 685–690.

Fischer, Anne, Victor Wiebe, Svante Pääbo, and Molly Przeworski
2004 Evidence for a Complex Demographic History of Chimpanzees. Molecular Biology and Evolution 21(5): 799–808.

Fruth, B., and G. Hohmann
1993 Ecological and Behavioral Aspects of Nest Building in Wild bonobos(Pan Paniscus). Ethology. Berlin, Hamburg 94(2): 113–126.

Furuichi, T.
1989 Social Interactions and the Life History of Female Pan Paniscus in Wamba, Zaire. International Journal of Primatology 10: 173–197.
1997 Agonistic Interactions and Matrifocal Dominance Rank of Wild Bonobos (Pan Paniscus) at Wamba. International Journal of Primatology 18(6): 855–875.
2009 Factors Underlying Party Size Differences between Chimpanzees and Bonobos: A Review and Hypotheses for Future Study. Primates 50(3): 197–209.

Furuichi, T., G. Idani, H. Ihobe, et al.
1998 Population Dynamics of Wild Bonobos (Pan Paniscus) at Wamba. International Journal of Primatology 19(6): 1029–1043.

Furuichi, T., M. Mulavwa, K. Yangozene, et al.
2008 Relationships among Fruit Abundance, Ranging Rate, and Party Size and Composition of Bonobos at Wamba. The Bonobos: Behavior, Ecology, and Conservation. Springer, New York: 135ñ149.

Gagneux, Pascal, Christopher Wills, Ulrike Gerloff, et al.
1999 Mitochondrial Sequences Show Diverse Evolutionary Histories of African Hominoids. Proceedings of the National Academy of Sciences 96(9): 5077–5082.

Goodall, J.
1986 The Chimpanzees of Gombe: Patterns of Behavior. Belknap Press of Harvard University Press Cambridge, Mass.(USA).

Goodall, Jane M.
1962 Nest Building Behavior in the Free Ranging Chimpanzee. Annals of the New York Academy of Sciences 102(2): 455–467.

Goymann, Wolfgang
2012 On the Use of Non-Invasive Hormone Research in Uncontrolled, Natural Environments: The Problem with Sex, Diet, Metabolic Rate and the Individual. Methods in Ecology and Evolution 3(4): 757–765.

Hashimoto, C., T. Yasuko, E. Hibino, et al.
2008 Longitudinal Structure of a Unit-Group of Bonobos: Male Philopatry and Possible Fusion of Unit-Groups. The Bonobos: Behavior, Ecology, and Conservation. Springer, New York: 107ñ119.

Hashimoto, Chie, Takeshi Furuichi, and Yasuko Tashiro
2001 What Factors Affect the Size of Chimpanzee Parties in the Kalinzu Forest, Uganda? Examination of Fruit Abundance and Number of Estrous Females. International Journal of Primatology 22(6): 947–959.

Hashimoto, Chie, Shigeru Suzuki, Yuji Takenoshita, et al.
2003 How Fruit Abundance Affects the Chimpanzee Party Size: A Comparison between Four Study Sites. Primates 44(2): 77–81.

Hauser, B., D. Schulz, C. Boesch, and T. Deschner
2008 Measuring Urinary Testosterone Levels of the Great apes—Problems with Enzymatic Hydrolysis Using Helix Pomatia Juice. General and Comparative Endocrinology.

Hohmann, G., A. Fowler, V. Sommer, and S. Ortmann
2006 Frugivory and Gregariousness of Salonga Bonobos and Gashaka Chimpanzees: The Abundance and Nutritional Quality of Fruit. *In* Feeding Ecology in Apes and Other Primates. G. Hohmann, M. Robbins, and C. Boesch, eds. Cambridge: Cambridge University Press.

Hohmann, G., and B. Fruth
2002 10• Dynamics in Social Organization of Bonobos (Pan Paniscus). Behavioural Diversity in Chimpanzees and Bonobos.

Hohmann, Gottfried, and Barbara Fruth
2003a Lui Kotal—a New Site for Field Research on Bonobos in the Salonga National Park. Pan African News 10: 25–27.
2003b Culture in Bonobos? Between-Species and Within-Species Variation in Behavior1, vol.44. , 4. JSTOR. http://www.jstor.org/stable/10.1086/377649, accessed March 17, 2014.
2007 New Records on Prey Capture and Meat Eating by Bonobos at Lui Kotale, Salonga National Park, Democratic Republic of Congo. Folia Primatologica 79(2): 103–110.

Idani, G., S. Kuroda, T. Kano, and R. Asato
1994 Flora and Vegetation of Wamba Forest, Central Zaire with Reference to Bonobo (Pan Paniscus) Foods. Tropics 3(3-4): 309–332.

Irwin, Mitchell T., Jean-Luc Raharison, David Raubenheimer, Colin A. Chapman, and Jessica M. Rothman
2014 Nutritional Correlates of the “lean Season”: Effects of Seasonality and Frugivory on the Nutritional Ecology of Diademed Sifakas. American Journal of Physical Anthropology 153(1): 78–91.

Isbell, L. A.
1991 Contest and Scramble Competition: Patterns of Female Aggression and Ranging Behavior among Primates. Behavioral Ecology 2(2): 143–155.

Isbell, L. A., and T. P. Young
2002 Ecological Models of Female Social Relationships in Primates: Similarities, Disparities, and Some Directions for Future Clarity. Behaviour 139(2-3): 177–202.

Janson, C. H., and M. L. Goldsmith
1995 Predicting Group Size in Primates: Foraging Costs and Predation Risks. Behavioral Ecology 6(3): 326–336.

JANSON, CHARLES, and ERIN VOGEL
2006 11 Hunger and Aggression in Capuchin Monkeys. Feeding Ecology in Apes and Other Primates 48: 285.

Johnson, Jerald B., and Kristian S. Omland. “Model Selection in Ecology and Evolution.” *Trends in Ecology & Evolution* 19, no. 2 (February 2004): 101–8. doi:10.1016/j.tree.2003.10.013.

Kano, Takayoshi
1980 Social Behavior of Wild Pygmy Chimpanzees (< I> Pan Paniscus</i>) of Wamba: A Preliminary Report. Journal of Human Evolution 9(4): 243–260.
1983 An Ecological Study of the Pygmy Chimpanzees (Pan Paniscus) of Yalosidi, Republic of Zaire. International Journal of Primatology 4(1): 1–31.

Kano, Takayoshi, and Mbangi Mulavwa
1984 Feeding Ecology of the Pygmy Chimpanzees (Pan Paniscus) of Wamba. *In* The Pygmy Chimpanzee Pp. 233–274. Springer. http://link.springer.com/chapter/10.1007/978-1-4757-0082-4_10, accessed March 17, 2014.

Kappeler, P. M., and C. P. van Schaik
2002 Evolution of Primate Social Systems. International Journal of Primatology 23(4): 707–740.

Kitaysky, A. S., J. F. Piatt, and J. C. Wingfield
2007 Stress Hormones Link Food Availability and Population Processes in Seabirds. MARINE ECOLOGY-PROGRESS SERIES- 352: 245.

Kitaysky, Alexander S., John F. Piatt, Scott A. Hatch, et al.
2010 Food Availability and Population Processes: Severity of Nutritional Stress during Reproduction Predicts Survival of Long-Lived Seabirds. Functional Ecology 24(3): 625–637.

Knott, C. D.
1997 Field Collection and Preservation of Urine in Orangutans and Chimpanzees. Tropical Biodiversity 4: 95–102.
1998 Changes in Orangutan Caloric Intake, Energy Balance, and Ketones in Response to Fluctuating Fruit Availability. International Journal of Primatology 19(6): 1061–1079.

Knott, Cheryl D
2005 Radioimmunoassay of Estrone Conjugates from Urine Dried on Filter Paper. American Journal of Primatology 67(1): 121–135.

Van Krunkelsven, E., J. Dupain, L. Van Elsacker, and R. Verheyen
1999 Habituation of Bonobos (Pan Paniscus): First Reactions to the Presence of Observers and the Evolution of Response over Time. Folia Primatologica 70(6): 365–368.

Kuroda, Suehisa, Tomoaki Nishihara, Sigeru Suzuki, and Rufin A. Oko

1996 Sympatric Chimpanzees and Gorillas in the Ndoki Forest, Congo. Great Ape Societies: 71–81.

Lasley, B. L., and S. E. Shideler
1993 Methods for Assessing Reproduction in Nondomestic Species. Zoo and Wild Animal Medicine. Current Therapy 3: 79–86.

Lasley, Bill L., and Jay F. Kirkpatrick
1991 Monitoring Ovarian Function in Captive and Free-Ranging Wildlife by Means of Urinary and Fecal Steroids. Journal of Zoo and Wildlife Medicine: 23–31.

Lehmann, Julia, and Christophe Boesch
2003 Social Influences on Ranging Patterns among Chimpanzees (Pan Troglodytes Verus) in the Taï National Park, Côte d'Ivoire. Behavioral Ecology 14(5): 642–649.

MacKinnon, John
1974 The Behaviour and Ecology of Wild Orang-Utans (< I> Pongo Pygmaeus</i>). Animal Behaviour 22(1): 3–74.

Malenky, R. K., S. Kuroda, and E. Ono
1994 The Significance ofTerrestrial Herbaceous Foods For Bonobos, Chimpanzees, and Gorillas. Chimpanzee Cultures.

Malenky, R.W.
1990 Ecological Factors Affecting Food Choice and Social Organization in Pan Paniscus. State University of New York at Stony Brook.

Malenky, R.W., and E.W. Stiles
1991 Distribution of Terrestrial Herbaceous Vegetation and Its Consumption by Pan Paniscus in the Lomako Forest, Zaire. American Journal of Primatology 23: 153–169.

Malenky, R.W., and R. W. Wrangham
1994 A Quantitative Comparison of Terrestrial Herbaceous Food Consumption by Pan Paniscus in the Lomako Forest, Zaire, and Pan Troglodytes in the Kibale Forest, Uganda. American Journal of Primatology 32: 1–12.

Mitani, J. C., D. P. Watts, and J. S. Lwanga
2002 Ecological and Social Correlates of Chimpanzee Party Size and Composition. Behavioural Diversity in Chimpanzees and Bonobos. Cambridge University Press, Cambridge: 102–111.

Mitani, J. C., D. P. Watts, and M. N. Muller
2002 Recent Developments in the Study of Wild Chimpanzee Behavior. EVOLUTIONARY ANTHROPOLOGY 11(1): 9–25.

Mohneke, M., and B. Fruth

2008 Bonobo (Pan Paniscus) Density Estimation in the SW-Salonga National Park, Democratic Republic of Congo: Common Methodology Revisited. The Bonobos. Behavior, Ecology, and Conservation: 151ñ166.

Morin, Phillip A., James J. Moore, Ranajit Chakraborty, et al.
1994 Kin Selection, Social Structure, Gene Flow, and the Evolution of Chimpanzees. Science 265(5176): 1193–1201.

Mulavwa, M., T. Furuichi, K. Yangozene, et al.
2008 Seasonal Changes in Fruit Production and Party Size of Bonobos at Wamba. *In* The Bonobos: Behavior, Ecology, and Conservation. Pp. 121–134. New York, NY: Springer.

Mulavwa, M. N., K. Yangozene, M. Yamba-Yamba, et al.
2010 Nest Groups of Wild Bonobos at Wamba: Selection of Vegetation and Tree Species and Relationships between Nest Group Size and Party Size. American Journal of Primatology 71: 1–12.

Muller, M. N., S. M. Kahlenberg, M. Emery Thompson, and R. W. Wrangham
2007 Male Coercion and the Costs of Promiscuous Mating for Female Chimpanzees. Proceedings of the Royal Society B: Biological Sciences 274(1612): 1009–1014.

Muller, M. N., and R. W. Wrangham
2004 Dominance, Cortisol and Stress in Wild Chimpanzees (Pan Troglodytes Schweinfurthii). Behavioral Ecology and Sociobiology 55(4): 332–340.

Muller, Martin N., and Susan F. Lipson
2003 Diurnal Patterns of Urinary Steroid Excretion in Wild Chimpanzees. American Journal of Primatology 60(4): 161–166.

Murray, C. M., L. E. Eberly, and A. E. Pusey
2006 Foraging Strategies as a Function of Season and Rank among Wild Female Chimpanzees (Pan Troglodytes). Behavioral Ecology 17(6): 1020.

N'guessan, Antoine K., Sylvia Ortmann, and Christophe Boesch
2009 Daily Energy Balance and Protein Gain among Pan Troglodytes Verus in the Taï National Park, Côte d'Ivoire. International Journal of Primatology 30(3): 481–496.

Newton-Fisher, Nicholas E., Vernon Reynolds, and Andrew J. Plumptre
2000 Food Supply and Chimpanzee (Pan Troglodytes Schweinfurthii) Party Size in the Budongo Forest Reserve, Uganda. International Journal of Primatology 21(4): 613–628.

Nishida, Toshisada
1979 The Social Structure of Chimpanzees of the Mahale Mountains. The Great Apes 5(7).

Nishida, Toshisada, Nadia Corp, Miya Hamai, et al.
2003 Demography, Female Life History, and Reproductive Profiles among the Chimpanzees of Mahale. American Journal of Primatology 59(3): 99–121.

Van Noordwijk, Maria A., and Carcl P. van Schaik
1999 The Effects of Dominance Rank and Group Size on Female Lifetime Reproductive Success in Wild Long-Tailed Macaques, Macaca Fascicularis. Primates 40(1): 105–130.

Palme, R., S. Rettenbacher, C. Touma, S. M. El-Bahr, and E. Möstl
2005 Stress Hormones in Mammals and Birds: Comparative Aspects Regarding Metabolism, Excretion, and Noninvasive Measurement in Fecal Samples. Annals of the New York Academy of Sciences 1040(1): 162–171.

Parish, A. R.
1996 Female Relationships in Bonobos (Pan Paniscus). Human Nature 7(1): 61–96.

Parish, Amyr, F. B. M. De Waal, and D. Haig
2000 The Other“ Closest Living Relative”: How Bonobos (Pan Paniscus) Challenge Traditional Assumptions about Females, Dominance, Intra-and Intersexual Interactions, and Hominid Evolution. Annals of the New York Academy of Sciences 907(1): 97–113.

Penning, Trevor M., Seon-Hwa Lee, Yi Jin, Alejandro Gutierrez, and Ian A. Blair
2010 Liquid-Chromatography Mass Spectrometry (LC-MS) of Steroid Hormone Metabolites and Its Applications. J Steroid Biochem Mol Biol. 121((3-5)): 546–555.

Peres, Carlos A.
1994 Composition, Density, and Fruiting Phenology of Arborescent Palms in an Amazonian Terra Firme Forest. Biotropica 26(3): 285–294.

Pienkowski, M. W., A. R. Watkinson, Gillian Kerby, et al.
1998 Temporal Patterns of Crop-Raiding by Primates: Linking Food Availability in Croplands and Adjacent Forest. Journal of Applied Ecology 35(4): 596–606.

Pride, Ethan R.
2005b High Faecal Glucocorticoid Levels Predict Mortality in Ring-Tailed Lemurs (Lemur Catta). Biology Letters 1(1): 60–63.

Pride, R.E.
2005 Optimal Group Size and Seasonal Stress in Ring-Tailed Lemurs (Lemur Catta). Behavioral Ecology 16: 550–560.

Reinartz, Gay Edwards, Patrick Guislain, TD Mboyo Bolinga, et al.
2008 Ecological Factors Influencing Bonobo Density and Distribution in the Salonga National Park: Applications for Population Assessment. *In* The Bonobos Pp. 167–188.

Springer. http://link.springer.com/chapter/10.1007/978-0-387-74787-3_10, accessed March 17, 2014.

Robbins, Martha M., and Nancy M. Czekala
1997 A Preliminary Investigation of Urinary Testosterone and Cortisol Levels in Wild Male Mountain Gorillas. American Journal of Primatology 43(1): 51–64.

Rothman, Jessica M., David Raubenheimer, and Colin A. Chapman
2011 Nutritional Geometry: Gorillas Prioritize Non-Protein Energy While Consuming Surplus Protein. Biology Letters 7(6): 847–849.

Sapolsky, R.
1993 Endocrinology Alfresco: Psychoendocrine Studies of Wild Baboons. Recent Progress in Hormone Research 48: 437–465.

Sapolsky, R. M., L. M. Romero, and A. U. Munck
2000 How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions 1. Endocrine Reviews 21(1): 55–89.

Van Schaik, C. P., and J. Van Hooff
1983 On the Ultimate Causes of Primate Social Systems. Behaviour 85(1-2): 91–117.

Van Schaik, C. P., and P. M. Kappeler
1997 Infanticide Risk and the Evolution of Male-Female Association in Pimates. Proceedings of the Royal Society B: Biological Sciences 264: 1687–1694.

Van Schaik, C. P., R. Madden, and J. U. Ganzhorn
2005 Seasonality in Primates. *In* Seasonality in Primates: Studies of Living and Extinct Human and Non-Human Primates. DK Brockman and CP van Schaik, eds. Cambridge: Cambridge University Press.

Van Schaik, C.
1989 The Ecology of Social Relationships amongst Female Primates. *In* Comparative Socioecology. V. Standen and R.A. Foler, eds. Pp. 195–218. Oxford: Blackwell.

Schaller, George E.
1963 The Mountain Gorilla: Ecology and Behavior. http://psycnet.apa.org/psycinfo/1964-00458-000, accessed March 18, 2014.

Sherry, D.S., and P.T. Ellison
2007 Potential Applications of Urinary C-Peptide of Insulin for Comparative Energetics Research. AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 133(771-778).

Shideler, S. E., C. J. Munro, H. K. Johl, H. W. Taylor, and B. L. Lasley

1995 Urine and Fecal Sample Collection on Filter Paper for Ovarian Hormone Evaluations. American Journal of Primatology 37(4): 305–315.

Stanford, C. B.
1998 The Social Behavior of Chimpanzees and Bonobos: Empirical Evidence and Shifting Assumptions 1. Current Anthropology 39(4): 399–420.

Stanford, Craig B., and J. Bosco Nkurunungi
2003 Behavioral Ecology of Sympatric Chimpanzees and Gorillas in Bwindi Impenetrable National Park, Uganda: Diet. International Journal of Primatology 24(4): 901–918.

Sterck, E. H. M., D. P. Watts, and C. P. Van Schaik
1997 The Evolution of Female Social Relationships in Nonhuman Primates. Behavioral Ecology and Sociobiology 41(5): 291–309.

Strier, Karen B., and Toni E. Ziegler
2005 Advances in Field-Based Studies of Primate Behavioral Endocrinology. American Journal of Primatology 67(1): 1–4.

Stumpf, R. M.
2011 Chimpanzees and Bonobos: Inter-and Intraspecies Diversity. Primates in Perspective: 340–356.

Sugiyama, Yukimaru, and Jeremy Koman
1979 Social Structure and Dynamics of Wild Chimpanzees at Bossou, Guinea. Primates 20(3): 323–339.

Surbeck, Martin, Tobias Deschner, Anja Weltring, and Gottfried Hohmann
2012 Social Correlates of Variation in Urinary Cortisol in Wild Male Bonobos (Pan Paniscus). Hormones and Behavior 62(1): 27–35.

Susman, R. L.
1984 The Pygmy Chimpanzee. New York: Plenum Press.

Sykes, Brian D.
2007 Urine Stability for Metabolomic Studies: Effects of Preparation and Storage. Metabolomics 3(1): 19–27.

TAKAHATA, Yukio, Yoshi KAWAMOTO, Hirohisa HIRAI, et al.
1998 Ticks Found among the Wild Ringtailed Lemurs at the Berenty Reserve, Madagascar. African Study Monographs 19(4): 217–222.

Tecot, Stacey R.
2010 It’s All in the Timing: Birth Seasonality and Infant Survival in Eulemur Rubriventer. International Journal of Primatology 31(5): 715–735.

Terborgh, J., and C. H. Janson
1986 The Socioecology of Primate Groups. Annual Review of Ecology and Systematics 17(1): 111–136.

Thompson, Joam
2002 Bonobos of the Lukuru Wildlife Research Project JO A. MYERS THOMPSON. Behavioural Diversity in Chimpanzees and Bonobos.

Thompson, M. E., S. M. Kahlenberg, I. C. Gilby, and R. W. Wrangham
2007 Core Area Quality Is Associated with Variance in Reproductive Success among Female Chimpanzees at Kibale National Park. Animal Behaviour 73(3): 501–512.

Trivers, R. L.
1972 Parental Investment and Sexual Selection. Sexual Selection and the Descent of Man 1971: 136–179.

Tutin, Caroline EG, and Michel Fernandez
1993 Composition of the Diet of Chimpanzees and Comparisons with that of Sympatric Lowland Gorillas in the Lopé Reserve, Gabon. American Journal of Primatology 30(3): 195–211.

Virgin, C. E., and R. M. Sapolsky
1997 Styles of Male Social Behavior and Their Endocrine Correlates among Low-Ranking Baboons. American Journal of primatology(Print) 42(1): 25–39.

Vogel, Erin R., Brooke E. Crowley, Cheryl D. Knott, et al.
2012 A Noninvasive Method for Estimating Nitrogen Balance in Free-Ranging Primates. International Journal of Primatology 33(3): 567–587.

Waller, Michel Tyler
2011 The Ranging Behavior of Bonobos in the Lomako Forest. University of Oregon. https://scholarsbank.uoregon.edu/xmlui/handle/1794/11648, accessed March 17, 2014.

Wasser, S. K., K. E. Hunt, J. L. Brown, et al.
2000 A Generalized Fecal Glucocorticoid Assay for Use in a Diverse Array of Nondomestic Mammalian and Avian Species. General and Comparative Endocrinology 120(3): 260–275.

Weingrill, Tony, David A. Gray, Louise Barrett, and S. Peter Henzi
2004 Fecal Cortisol Levels in Free-Ranging Female Chacma Baboons: Relationship to Dominance, Reproductive State and Environmental Factors. Hormones and Behavior 45(4): 259–269.

White, F. J.

1998a Seasonality and Socioecology: The Importance of Variation in Fruit Abundance to Bonobo Sociality. International Journal of Primatology 19(6): 1013–1027.
1996b Comparative Socioecology of Pan Paniscus. *In* Great Ape Societies. W.C. McGrew, T. Nishida, and L. Marchant, eds. Pp. 29–41. Cambridge: Cambridge University Press.
1996 Pan Paniscus 1973 to 1996: Twenty-Three Years of Field Research. Evolutionary Anthropology Issues News and Reviews 5(1): 11–17.

White, F. J., and R. W. Wrangham
1988 Feeding Competition and Patch Size in the Chimpanzee Species Pan Paniscus and Pan Troglodytes. Behaviour 105(1-2): 148–164.

White, Frances J.
1998 The Importance of Seasonality in Primatology. International Journal of Primatology 19(6): 925–927.

Whitten, P. L., D. K. Brockman, and R. C. Stavisky
1998 Recent Advances in Noninvasive Techniques to Monitor Hormone-Behavior Interactions. YEARBOOK OF PHYSICAL ANTHROPOLOGY 41: 1–23.

Williams, J.M., A. E. Pusey, J.V. Carlis, B.P. Farms, and J. Goodall
2002 Female Competition and Male Territorial Behaviour Influence Female Chimpanzees' Ranging Patterns. Animal Behaviour 63: 523–532.

Wingfield, J. C., R. E. Hegner, Jr A. M. Dufty, and G. F. Ball
1990 The" Challenge Hypothesis": Theoretical Implications for Patterns of Testosterone Secretion, Mating Systems, and Breeding Strategies. American Naturalist 136(6): 829.

Wrangham, R. W.
1980 An Ecological Model of Female-Bonded Primate Groups. Behaviour 75(3-4): 262–300.
1993 The Evolution of Sexuality in Chimpanzees and Bonobos. Human Nature 4(1): 47–79.

Wrangham, R. W., N. L. Conklin, C. A. Chapman, et al.
1991 The Significance of Fibrous Foods for Kibale Forest Chimpanzees [and Discussion]. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 334(1270): 171–178.

Wrangham, R. W., and D. Peterson
1997 Demonic Males. London, England: Bloomsbury.

Wrangham, Richard W.
2000 21• Why Are Male Chimpanzees More Gregarious than Mothers? A Scramble

Competition Hypothesis. Primate Males: Causes and Consequences of Variation in Group Composition: 248.

Wrangham, Richard W., Nancy Lou Conklin-Brittain, and Kevin D. Hunt
1998 Dietary Response of Chimpanzees and Cercopithecines to Seasonal Variation in Fruit Abundance. I. Antifeedants. International Journal of Primatology 19(6): 949–970.

Wrangham, RW
1986 Ecology and Social Evolution in Two Species of Chimpanzees. *In* Ecology and Social Evolution: Birds and Mammals. D.I. Rubenstein and R. W. Wrangham, eds. Pp. 352–378. Princeton, NJ: Princeton University Press.

Yamakoshi, G.
2004 Evolution of Complex Feeding Techniques in Primates: Is This the Origin of Great Ape Intelligence? *In* The Evolution of Thought. A.E. Russon and D.R. Begun, eds. Pp. 140–171. Cambridge: Cambridge University Press.

Yu, Ning, Michael I. Jensen-Seaman, Leona Chemnick, et al.
2003 Low Nucleotide Diversity in Chimpanzees and Bonobos. Genetics 164(4): 1511–1518.

Ziegler, Toni E., and Daniel J. Wittwer
2005 Fecal Steroid Research in the Field and Laboratory: Improved Methods for Storage, Transport, Processing, and Analysis. American Journal of Primatology 67(1): 159–174.