EXAMINING FORAGING MODELS USING DIETARY DIVERSITY AND GUT MICROBIOTA IN BONOBOS (*PAN PANISCUS*)

by

ALEXANA J. HICKMOTT

A DISSERTATION

Presented to the Department of Anthropology
and the Division of Graduate Studies of the
University of Oregon in
partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

September 2021

DISSERTATION APPROVAL PAGE

Student: Alexana J. Hickmott

Title: Examining Foraging Models Using Dietary Diversity and Gut Microbiota in Bonobos (*Pan paniscus*)

This dissertation has been accepted and approved in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Anthropology by:

Frances J. White	Chairperson
Larry Ulibarri	Core Member
Nelson Ting	Core Member
Brendan Bohannan	Institutional Representative

and

Andrew Karduna	Interim Vice Provost of the Division of Graduate Studies

Original approval signatures are on file with the University of Oregon Division of Graduate Studies.

Degree awarded September 2021

© 2021 Alexana J. Hickmott

DISSERTATION ABSTRACT

Alexana J. Hickmott

Doctor of Philosophy

Department of Anthropology

September 2021

Title: Examining Foraging models Using Dietary Diversity and Gut Microbiota in Bonobos (*Pan paniscus*)

Optimal diet and functional response models are used to understand the evolution of primate foraging strategies. The predictions of these models can be tested by examining the changes in dietary diversity. Primate gut microbiome communities are of increasing interest due to their important role in nutrition, development, health, and disease. Recent evidence from gorillas suggests fecal glucocorticoid metabolite concentration (FGMC) has no significant role in structuring gorilla gut microbiomes. We investigated dietary diversity and the gut microbiota in bonobos (*Pan paniscus*) at two research camps within the same protected area (N'dele and Iyema) in Lomako Forest, Democratic Republic of Congo (DRC). We compared dietary diversity results from behavioral observation (1984/1985, 1991, 1995, 2014, & 2017) and fecal washing analysis (2007 & 2009) between seasons and study period using three diversity indices (Shannon's, Simpson's, and SW evenness). We describe gut microbiome, $\delta^{13}C$, $\delta^{15}N$ data, and FGMC for eighteen bonobo fecal samples from separate individuals, collected in June 2014 at Iyema, Lomako Forest, DRC. The average yearly dietary diversity indices at N'dele were Shannon H' = 2.04, Simpson's D = 0.18, and SW evenness = 0.88 while at Iyema, the indices were Shannon H' = 2.02, Simpson's D = 0.18, and SW evenness = 0.88. Shannon's index was lower during when fewer bonobo dietary items were available for consumption. The results of the gut microbiome analyses found that $\delta^{13}C$ were significant [PERMANOVA $F_{1,17}$ =0.17261, p = 0.023] in explaining beta diversity in gut microbiota but only when sex was a predictor in the model. Females had slightly higher $\delta^{13}C$ values than males perhaps due to lower consumption of C4 plants by females. We found FGMC did not significantly explain the variation in bonobo gut microbiota beta diversity. We ran linear regressions on the abundance of the microbial genera and found eighty genera were significantly explained by FGMC. Overall, this research suggests that

optimal diet models best explained bonobo foraging strategies and patterns in bonobo gut microbiota, diet, and stress may need to center around the differential consumption of C4 plants like *Ficus* spp. and terrestrial herbaceous vegetation (THV) by males and females.

This dissertation includes previously published and unpublished co-authored material.

CURRICULUM VITAE

NAME OF AUTHOR: Alexana J. Hickmott

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene, Oregon
University of Wisconsin: Madison, Madison, Wisconsin
University of California: San Diego, La Jolla, California

DEGREES AWARDED:

Doctor of Philosophy, Anthropology, 2021, University of Oregon
Master of Arts, Physical Anthropology, 2015, University of Wisconsin: Madison
Bachelor of Arts, 2012, Anthropology, University of California: San Diego

AREAS OF SPECIAL INTEREST:

Primatology, primate foraging strategies, feeding ecology, gut microbiota, stress

PROFESSIONAL EXPERIENCE:

Graduate Teaching Employee, Department of Anthropology, University of Oregon, 2016-2021

Adjunct Professor, Department of Sociology, Anthropology, and Philosophy, Northern Kentucky University, 2016

Graduate Teaching Assistant, Department of Anthropology, University of Wisconsin: Madison, 2013-2015

GRANTS, AWARDS, AND HONORS:

Gary Smith Summer Professional Development Award, University of Oregon, 2020

Pauline W. Juda Memorial Endowment Fund Award, University of Oregon, 2020

Anthropology Department Graduate Research Award, University of Oregon, 2020

Pauline W. Juda Memorial Endowment Fund Award, University of Oregon, 2019

Special “OPPS” Travel Award, Graduate School, University of Oregon, 2018

Leakey Research Grant, Leakey Foundation, Northern Kentucky University, 2016

Sherwood L. Washburn Award, Eleanor Roosevelt College, University of California: San Diego, 2012

PUBLICATIONS:

Hickmott, A. J., Waller, M. T., Wakefield, M. L., Malone, N., Brand, C. M., & White, F. J. (*Submitted*). Foraging Models and the Dietary Diversity of the Lomako Forest Bonobos. *Folia Primatologia.*

White, F. J., Brand, C. M., **Hickmott, A. J.,** Minton, I. R. (2020). Sex differences in bonobo (*Pan paniscus*) terrestriality: implications for human evolution. *Journal of Anthropological Sciences. 98*, 5-14.

Loudon, J. E., Wakefield, M. L., Kimel, H. M., Waller, M. T., **Hickmott, A. J**., White, F. J., & Sponheimer, M. (2019). Stable isotope data from bonobo (*Pan paniscus*) faecal

samples from the Lomako Forest Reserve, Democratic Republic of the Congo. *African Journal of Ecology*, *57*(3), 437-442.

Wakefield, M. L., **Hickmott, A. J.,** Brand, C. M., Takaoka, I. Y., Meador, L. M., Waller, M. T., & White, F. J. (2019). New Observations of Meat Eating and Sharing in Wild Bonobos (*Pan paniscus*) at Iyema, Lomako Forest Reserve, Democratic Republic of the Congo. *Folia Primatologica*, *90*(3), 179-189.

Mrinalini Watsa, Gideon Erkenswick, Deirdre Halloran, Erin E. Kane, Alice Poirier, Karina Klonoski, Santiago Cassalett, Elizabeth Maciag, Mihnea R. Mangalea, Mary P. Dinsmore, Holly McCready, Breanne K. Boughan, Chelsea Parker, **Alexana J. Hickmott**, Inés Esperanza Nole Bazan, Alfonso Zuñiga. (2015). A field protocol for the capture and release of callitrichids. *Neotropical Primates* 22 (2): 59-68.

Nowak-Lovato, K. L., **Hickmott, A. J.,** Maity, T. S., Bulyk, M. L., Dunbar, J., & Hong-Geller, E. (2012). DNA binding site analysis of *Burkholderia thailandensis* response regulators. *Journal of microbiological methods*, *90*(1), 46-52.

ACKNOWLEDGEMENTS

I am sincerely grateful to my advisor, Dr. Frances J. White, for her support and encouragement. Frances has generously shared her knowledge and wisdom, always been my biggest advocate. She pulled me back into academia when I was ready to leave. She helped me navigate this journey and always gave the utmost support as a fellow woman in STEM. She allowed me to be my own independent researcher but was always there when I needed a sounding board or advice. I appreciate all the opportunities she has given me and credit her with much of my academic and personal growth over this journey.

Dr. Nelson Ting has similarly generously given me his time, advice, knowledge and laboratory training. Nelson kindly opened his laboratory and lab group to me and I am eternally grateful to him and Dr. Kirsten Sterner for including me. Nelson and his lab group helped me to be a better scientist and helped inspire my love of outreach.

I am similarly indebted to Dr. Larry Ulibarri who was always eager to discuss my research with me and who has given me encouragement and hope throughout my entire dissertation. I am deeply grateful to Dr. Brendan Bohannan for welcoming me into his lab group and for his helpful advice throughout all steps in this marathon.

I am deeply grateful to Dr. Monica Wakefield at the Northern Kentucky University for giving me the opportunity, funding, and training to collect my field data. Anyone who has done primate field work knows it is difficult at the best of times and Monica's guidance and friendship made it all the more fun. I am lucky to count Monica as a friend and colleague, and I am also grateful to Dr. Josh Snodgrass, Dr. Klaree Boose, Dr. Colin Brand, Dr. Michel Waller, Dr. Nick Malone, and Dr. Geeta Eick,

Most importantly, I would like to thank the following people without whom this work would not be possible: Jef Dupain, Hugues Akpona, and the African Wildlife Foundation, Dipon Bomposo, Beken Bompoma, Teddy Bofaso, Christian Djambo, Mathieu Esaola, Bellevie Iyambe, Augustin Lofili, Isaac Lokoli, Gedeon Lokofo, Thomas Lokuli, Joel Bontambe, Abdulay Bokela, Papa Siri, and Paco Bertolani, who generously provided logistical support.

This dissertation was financially supported by the UO Gary Smith Summer Professional Development Award, the UO Department of Anthropology Pauline W. Juda Memorial Endowment Fund Award, UO Anthropology Department Graduate Research Award, the UO Graduate School Special "OPPS" Travel Award, a Leakey Research Grant, and a UO Faculty Research Grant.

I would like to thank my friends, Elisabeth, Colin, Diana, Hannah, Kylen, Jenneca, Tian, and Klaree for their unwavering support. Elisabeth, for all the bird watching. Colin, for being the best academic brother ever. Diana, for being my primate gut microbiome sister. Hannah and Jenneca, for being my fellow chronic illness warriors. Tian, for all the SPFT. Kylen, for our hot tub hangs. Klaree, for being my barn buddy. I'm so thankful for your friendship and love. I wouldn't be here is it wasn't for my amazing social network.

Lastly, special thanks to my cat Zorro who kept me sane and made sure I took breaks to feed him. Love you Z!

To my friends and family, especially:

To my mother and father,
Cathy Rocke and Donald Hickmott,
the bedrock on which I’m built.

To my sister,
Emily Hickmott,
the best sister in the world and the smartest person I know.

To my grandmother,
Barbara Jane Rocke,
the person who taught me to read and inspired me to ask questions.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

1.1 Background 1

1.2 Primate foraging models 1

1.3 Primate gut microbiomes and diet 6

1.4 Primate gut microbiomes and stress 8

1.5 Bonobos as models 9

1.6 Research sites 12

II. A TEST OF FORAGING MODELS USING DIETARY DIVERSITY INDICIES FOR THE LOMAKO FOREST BONOBO 13

2.1 Introduction 13

2.2 Materials and Methods 21

2.2.1 Study Camps 21

2.2.2 Data Collection 23

2.2.3 Data Analysis 24

2.3 Results 26

2.3.1 Percentage of foraging by species and food type 26

2.3.2 Dietary Diversity Variation by Method, Camp, and Study Period 26

2.3.3 Monthly Dietary Diversity 28

2.4 Discussion/ Conclusion 32

2.4.1 Dietary Diversity Comparisons Across Apes 35

III. GUT MICROBIOTA AND DIET FROM FECAL STABLE ISOTOPIC VALUES FOR BONOBOS (*PAN PANISCUS*) OF THE LOMAKO FOREST, DEMOCRATIC REPUBLIC OF CONGO 37

Chapter Page

3.1 Introduction ... 37

3.2 Methods ... 39

3.2.1 Data collection ... 39

3.2.2 Social group determination ... 40

3.2.3 16S rRNA sequencing data ... 42

3.2.4 Stable isotopic values ... 42

3.2.5 Model predictors ... 43

3.2.6 Data analysis ... 44

3.3 Results ... 44

3.3.1 $\delta^{13}C$ and $\delta^{15}N$ results ... 46

3.3.2 Alpha diversity ... 46

3.3.3 Beta diversity ... 49

3.3.4 Abundance models ... 51

3.4 Discussion ... 52

IV. A COMPARISON OF FECAL GLUCOCORTICOID METABOLITE CONCENTRATION AND GUT MICROBIOTA DIVERSITY IN BONOBOS (*PAN PANISCUS*) ... 58

4.1 Introduction ... 58

4.2 Methods ... 59

4.2.1 Study Camps ... 60

4.2.2 Data Collection and Analysis ... 60

4.2.3 Model predictors ... 63

4.2.4 Data analysis ... 64

Chapter Page

4.3 Results 65

4.3.1 Alpha diversity 65

4.3.2 Beta diversity 65

4.3.3 Abundance models 65

4.4 Discussion 72

V. CONCLUSION 74

REFERENCES CITED 78

LIST OF FIGURES

Figure Page

1.1 Schematic of the different dietary models examined in this dissertation. 3

1.2 Map of Iyema and N'dele field sites, Democratic Republic of Congo (DRC)........ 12

2.1 Schematic of different dietary models. 14

2.2 Map of the Lomako Forest Reserve (dark green). 23

2.3 The method comparison (behavioral observation vs. fecal washing) for the three indices (Shannon's, Simpson's, and SW evenness). 28

2.4 Site comparison (N'dele vs. Iyema) for the three indices (Shannon's, Simpson's, and SW evenness)........ 29

2.5 Monthly comparison of FAI, Shannon's (H'), Simpson's D, and SW evenness for bonobos at Iyema, Lomako, DRC, June – Oct 2017. 31

3.1 Association map from Brand. et al., (2016)........ 41

3.2 A map of the sampling locations based on a figure from Brand et al. (2016). 43

3.3 Boxplot of read counts 46

3.4 $\delta^{13}$and δ^{15}N values plotted against sex. 47

3.5 Shannon's diversity index against A. community membership, B. sex, C. food item, D. δ^{13}C and E. δ^{15}N values. 48

3.6 PCOA of samples clustered by predictor variable. 49

4.1 Map of Iyema field site circled in blue Lomako, DRC........ 61

4.2 There are no significant differences in alpha diversity for A. nesting group, B. logFCMC (referred here after as FCMC), and C. rawFGCM........ 66

4.3 PCOA for nesting group and FGMC. 68

LIST OF TABLES

Table Page

2.1 Percentage of foraging time by species
and food type. 17
2.2 Conditions under which the functional response and optimal diet
models will give different results for Shannon's (H′), Simpson's (D)
index, and Shannon-Weiner (SW) evenness. 19
2.3 Study periods with sample size and methods were used to study
bonobo feeding ecology at N'dele and Iyema, Lomako Forest, (DRC). 22
2.4 Yearly diversity indices for bonobos at two sites in Lomako Forest, DRC. 27
2.5 Monthly dietary diversity indices for Shannon's
(H′), Simpson's D, and Shannon-Weiner (SW) evenness. 30
2.6 Percentage of trees with ripe fruit, new leaves, and flowers from
phenology transects at Iyema, Lomako Forest, DRC for five months in 2017. 32
2.7 Comparisons of dietary diversity indices across hominoids. 36
3.1 Predictor variables included in analysis and how they were collected. 45
3.2 Results for the significant models from the PERMANOVAs 50
3.3 Taxa that co-varied with $\delta^{13}C$ and $\delta^{15}N$ values. 53
4.1 Predictor variables included in analysis and how they were collected. 64
4.2 Results for the non-significant models from the PERMANOVAs. 67
4.3 Taxa that co-varied with logFGMC and rawFGMC. 69

CHAPTER I

INTRODUCTION

1.1 Background

The evolution of diet in humans and non-human primates (NHP) has been an important line of inquiry since the first primates were studied almost a decade ago. Why primates eat certain food items has long been of interest to primatologists, yet there remain many unanswered questions about diet throughout human evolutionary history. Evolutionary models are necessary to understand the relative importance of ecological variables in the evolution of primate dietary diversity (Lambert and Rothman, 2015). These models incorporate dietary breadth, energy return, nutrition quality, mechanical properties, digestibility, food species distribution and abundance, and seasonality (Richardson, 1985; Strier, 2016). What many of these models fail to consider is the primate gut microbiome. A gut microbiome or the gut microbiota is the community of bacteria and microbes inhabiting the distal gastrointestinal tract or gut. It is essential to recognize the ongoing debate with many scientists arguing "microbiome" should only be used when referring to the combined genetic material of a particular community of microbes, and "microbiota" should be used to refer to the community of microbes living in a particular environment (Stulberg et al., 2016). However, many scientists use them interchangeably. Thus, for this dissertation, the two terms will be used interchangeably. Additionally, it is important to recognize that other body sites within and outside of the gut house communities of microbes. Locations like the stomach and small intestine play host to their unique community of microbes, but the most accessible location to study these communities reside in the distal portion of the mammalian gut. Nutrient consumption and the foraging decisions an individual makes may significantly impact the gut microbiome and are essential in understanding dietary adaptations.

1.2 Primate foraging models

The four major models that have been used to understand primate diets are functional response models, optimal foraging or optimal diet models, fallback food feeding models, and geometric framework models (Figure 1.1; MacArthur and Pianka, 1966; Holling, 1959; Leighton, 1993; Simpson and Raubenheimer, 1995). Each of these models places different emphasis and significance on the different ecological factors. Functional response models emphasize food species distribution and abundance (Figure 11.; Holling 1965). Optimal diet models focus on dietary breadth, energy return, and abundance (Figure 1.1; MacArthur and Pianka, 1966, Charnov, 1976; Pyke et al., 1977). Fallback food models predict how a diet will respond under conditions when preferred foods are unavailable (Figure 1.1; Lambert, 2007; Marshall and Wrangham, 2007). At the same time, geometric framework models focus on nutrient quality, digestibility, and mechanical properties of food items (Rothman et al., 2011; Raubenheimer et al., 2009). Geometric framework models, also, focus on how the nutritional components incorporated into a primate's diet are required for a primate to grow and reproduce (Rothman et al., 2011). While helpful, geometric framework models will not be examined in this dissertation but represent another avenue of investigation and are essential to recognize as one of the types of foraging models used in primatology.

Among these models, functional response and optimal diet models incorporate aspects of dietary breadth that can be applied to questions of dietary diversity and how dietary diversity changes with resource availability in a highly productive forest where periods of scarcity are rare (Marshall and Wrangham, 2007; Lambert ,2007; Raubenheimer et al., 2009). In contrast, fallback food models, while necessary for understanding primate diets, focus more on adaptations to periods of food scarcity or nutrient deficiencies and focus less on how dietary diversity would be expected to change during periods of non-scarcity and are thus more important for examining primate gut microbiota (Marshall and Wrangham, 2007; Lambert, 2007). Functional response models are focused on how the number of food items eaten by an individual changes as a function of food density or availability (Holling, 1959; Holling, 1965). One of the components of functional response models is the prediction that when food abundance increases then food consumption will also increase (Holling, 1959; Lambert and Rothman, 2015). The functional response model is often an underlying assumption in

those studies of primate foraging strategies that predict that consumption is linked to the abundance of a particular food or food type in the environment. However, direct tests of the predictions associated with functional response models using dietary diversity are rare in these studies. The main prediction of functional response models is that consumption of a particular food will increase as the density of that food increases in the environment (Holling, 1965, Krebs & McCleery, 1984).

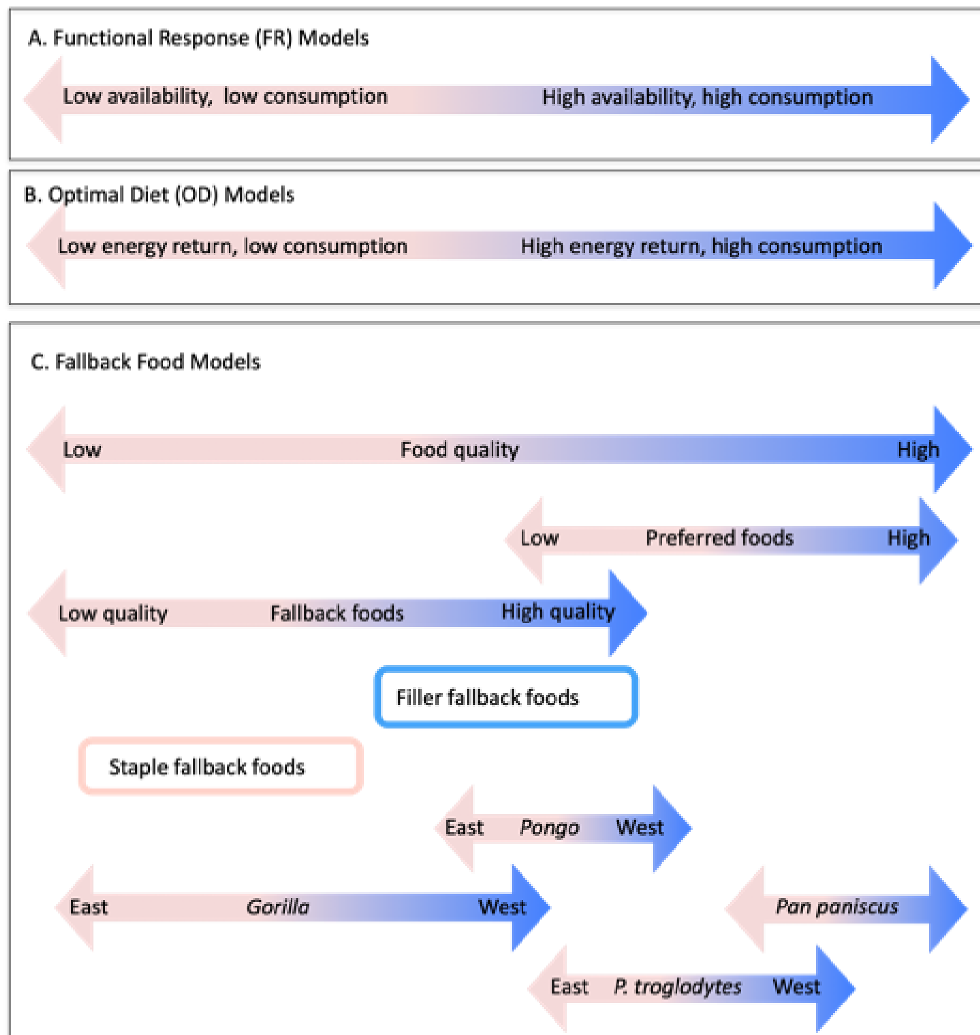

Figure 1.1. Schematic of the different dietary models examined in this dissertation. Figure based on A. Holling, 1959, B. MacArthur and Pianka, (1966), and C. Marshall et al., (2009) and Lambert, (2007).

In contrast, optimal diet models examine how animals obtain food resources and predict which patches a species feed. Optimal diet models suggest that the items that

compose a diet are based on decisions that maximize energy return and economic foraging effort (MacArthur and Pianka, 1966, Charnov, 1976; Pyke et al., 1977). The major components to consider when looking at optimal diet models in primates are the currency or food resource value, the constraints of time and energy, and the decision rules which assume a primate consumer will act in a way that maximizes their energy gain per unit of time (MacArthur and Pianka, 1966; Verlinden and Wiley, 1989). There are three major components to optimal diet models. Firstly, these models predict that every food item has a value equal to the energy content of a particular food item minus the energy expenditure when foraging for that food item. Secondly, these models rank all food items based on the energy return. Finally, the optimal diet is determined by starting with the highest-ranked item and consuming items in decreasing order of rank; thus, when high-value resources are available dietary breadth decreases (Richardson, 1985). Both functional response and optimal diet models, when applied to primates, have previously examined species richness (e.g., Altman & Wagner, 1978; Harrison, 1984; Montalvo et al., 2019). We add an examination of dietary diversity to assess not only species richness but also species evenness when comparing functional response and optimal diet models. Therefore, in order to understand how dietary diversity, and especially the number and evenness of species consumed, would be expected to change in a non-seasonal rainforest frugivore, like bonobos (*Pan paniscus*), we examine the predictions of functional response and optimal diet models using percent of foraging time and dietary diversity in chapter one.

Much of the primatological literature has focused on fallback food models compared to functional response models and optimal diet models. A fallback food is a resource that a primate taxon will turn to in periods of food scarcity and is a food item eaten in times where preferred food items are unavailable (Constantino & Wright, 2009; Lambert and Rothman, 2015.; Lambert, 2007; Marshall et al., 2009; Marshall & Wrangham, 2007). Fallback food models incorporate some of the aspects of optimal diet models in that these models are concerned with preferred vs. non-preferred food. Marshall & Wrangham, (2007) defined fallback food as those foods used when there is a decrease in the availability of preferred foods. This decrease in preferred foods and increased consumption of fallback foods is associated with increased dietary stress (*ibid*).

These foods primates fall back upon are typically low quality in terms of nutrient density and energy return (Lambert, 2009; Rothman et al., 2012). Fallback foods can be "staple" fallback foods that are annually available and can more frequently be found in the environment; therefore, they will be reliable during seasonal shortages of other more preferred foods. Alternatively, "filler" fallback foods can be seasonally or annually available but fill in the diet during periods when more preferred foods are unavailable (Marshall et al., 2009; Marshall and Wrangham, 2007). Other ways of classifying fallback foods include Lambert's (2007)'s classification of those with lower nutritional density and energy return. However, they require more handling time or anatomical adaptation, and the with higher nutritional density and energy return are rare and difficult to process and require tool use or processing. These fallback foods models incorporate preference, energy return, seasonal food availability, and periods of increased dietary stress.

Fallback food models in bonobos are related to the terrestrial herbaceous vegetation (THV) hypothesis. The THV hypothesis was proposed to explain the significant differences in bonobo and chimpanzee (*Pan troglodytes*) social behavior. This hypothesis suggests that chimpanzees almost always occur with sympatric gorilla (*Gorilla spp*.) populations (Wrngham, 1986; Sistiaga et al., 2015; Tutin et al., 1991). Gorillas tend to be much more folivorous than chimpanzees and consume much of the herbaceous vegetation that grows on the ground where they occupy the same forests as chimpanzees (Doran et al., 2002; Ganas et al., 2004). This THV represents a potential fallback food that is no longer available for these chimpanzee populations (Tutin and Fernandez, 1993). Bonobos, comparatively, do not face the same feeding competition from gorillas, as bonobos are only found south of the Congo river while most gorillas populations are found north of this boundary (Gruber and Clay, 2016; Rogers et al., 2004). Therefore, the THV hypothesis proposes that because bonobos have access to the low quality, ubiquitous resource of this ground growing herbaceous vegetation it allows female bonobos to form closer social bonds and have lower levels of stress (Malenky and Wrangham, 1994; White and Wrangham, 1988). We will use bonobo gut microbiomes to examine parts of the THV hypothesis in chapters two and three.

1.3 Primate gut microbiomes and diet

Nutrient processing is an essential part of the gut microbiome and is important in understanding dietary adaptations. In their review, Candela et al., (2012) suggest that the human intestinal microbiome represents a physiological phenotype and guarantees rapid adaptation of the metabolic preference of the super-organism (host and microbiota) in response to diet. However, there is an issue with their word, guarantee, the microbiota may not guarantee they will be able to break a dietary item down, but what it may do is provide a potential means of non-host breakdown, which could be particularly important in shaping host plasticity. The gut microbiota's role in nutrient breakdown may represent a physiological phenotype (Candela et al., 2012) that allows for expanding an individual host's nutrient breakdown capabilities. There has been an ongoing area of research investigating the effects of diet and phylogeny on the gut microbiome of NHP primates. Several studies have investigated this, including a study in nine captive colobine species, which found diet was a strong predictor of colobine gut microbiota composition than phylogeny (Hale et al., 2018). Fundamental to the understanding of the evolution of primate diets and their gut microbes were early investigations like Bruorton et al., (1991), who investigated the differences between the omnivorous vervet (*Cercopithecus aethiops*) and samango monkey (*Cercopithecus mitis*), a folivorous hindgut fermenter. They found evidence for bacterial fermentation in both monkey's cecum and colon but that the folivorous samango monkeys had higher fermentation capabilities than the less specialized vervets. Howler monkeys in more intact habitats exhibited higher diversity in their gut microbiota. Other more recent investigations into three NHP species frugivorous (fruit-eating) *Varecia variegata*, generalist *Lemur catta*, and folivorous (leaf-eating) *Propithecus coquereli* tested the relationship between host lineage, captive diet, life stage, and the composition of the gut microbiota found that diets and phylogeny are confounded. However, diet does appear to be an essential factor in gut microbial composition (McKenny et al., 2015). This comparison between frugivores, generalists, and folivores primates is fundamental to understanding variation in primate diets and gut microbiomes.

Other recent investigations into the relationship between nutrient processing and the primate gut microbiome found that howler monkeys in suboptimal or fragmented habitats had lower diversity in their gut microbiomes, potentially due to the less diverse diet in fragmented forests (Amato et al., 2013). Shifts in composition and activity of gut microbiota provide additional energy and nutrients to compensate for changes in diet. The gut microbiota was found to provide additional energy and essential nutrients to compensate for changes in diet. Thus, studies in howler monkeys (*Alouatta pigra*) supports the idea that gut microbiota provides an effective buffer against seasonal fluctuations in energy and nutrient intake while shifting in response to howler monkey diets (Amato et al., 2014a). Investigations into human gut microbiota found a large degree of temporal stability in human gut microbiomes. However, subtle shifts in the microbiome occur between seasons for human populations like the Hutterites which consume higher fresh produce seasonally (Coyte et al., 2015). Other hypotheses around the stability of the gut microbiome in primates need to be addressed.

In folivorous primates, variation in microbiota richness and diversity reduces due to dietary changes resulting from habitat disturbances in red colobus monkeys. Functional analysis suggests that these shifts may be due to reducing food element diversity in fragments in human-modified landscapes (Barelli et al., 2015). Amato et al., (2016) reviewed much of the evidence surrounding primate gut microbiota. They concluded that current approaches are insufficient to directly link the gut microbiota and the variation found in the gut microbial community's composition to NHP health and behavior on both proximate and ultimate time scales. The analysis of the composition of specific taxa within black howler monkeys found that there are environmental and dietary changes that influence shifts in gut microbiota for captive housed monkeys (Nakamura et al., 2011). Other folivorous primates, like Verreaux's sifakas (*Propithecus verreauxi*) gut microbiota, changed based on seasonal conditions, conditional on fruit and fiber consumptions, and were influenced by group membership. Investigations into the gut microbiome of Yuan snub-nosed monkeys (*Rhinopithecus bieti*) found broad diversity of bacteria, and numerous glycosides hydrolases responsible for lignocellulose biomass degradation suggest that the gut microbiome is key to folivorous primates and the breakdown of essential nutrients necessary for survival and reproduction (Xu et al.,

2015). In addition, gorilla (*Gorilla gorilla gorilla*) gut microbiomes support the idea that geographical range and dietary composition may be an essential modulator of gut microbiome composition and found that gut microbiome composition and function potentially reflect the external host environment (Gomez et al., 2015). Comparisons between two gorilla species (*G. g. gorilla and G. b. beringi*) demonstrate that gut microbiome and metabolome exhibit significantly different patterns and may be related to fiber breakdown in the mountain gorilla population. These samples exhibited enrichment of markers associated with simple sugar, lipid, and sterol digestion (Gomez et al., 2016).

For frugivorous primates, investigations into chimpanzee (*Pan troglodytes*) gut microbiomes began with Degnan et al., (2012). They found that in chimpanzees from Gombe, geography, time, sex, and age were associated with the stability, diversity, and composition of the microbiome (Degnan et al., 2012). Additionally, research into macaque (*Macaque fuscat*a) gut microbiomes examined several factors including maternal diet, post-natal diet, obesity, and post-weaning diet. They found that only high-fat maternal diet and post-natal diet structure offspring distal gut microbial composition (Ma et al., 2014). However, microbial communities are diverse, dynamic and have been found to vary by location and show within and between host variations. In addition, they are influenced by host species and phylogeny (Stumpf et al., 2016). Understanding the gut microbiota in terms of its overall patterns and factors affecting microbial diversity is extremely important for primate health, broader biodiversity, and conservation strategies worldwide (Stumpf et al., 2016).

1.4 Primate gut microbiomes and stress

One of the significant relationships that lie at the interface of host-microbe communication is stressor-induced infection susceptibility and systemic immunomodulation (Bailey, 2012). While stress has long been associated with reduced immune system function, there are other compounding effects through a potential decrease in gut microbial community diversity. The field of microbial endocrinology is a relatively novel and emerging field of research that has only been investigated in model or laboratory systems (reviewed in Sandrini et al., 2015). Specifically, this field

investigates the human body as home to microbes, but that affects and are affected by the hormonal signaling in their host's body. Stress seems to be an essential modulator of diversity found within the gut microbiome, but stress appears to have mixed effects at other body sites like the vaginal tract. However, compared to the skin microbial community, the gut microbial community appears to be more sensitive to stress-related changes in the host (Sandrini et al., 2015). Outside of model and laboratory-based systems, the effects of stress in NHP have established some critical patterns.

Among NHP, it was clear from the very early studies using sequencing technologies to examine primate gut microbiota that there was some level of communication going on with the gut's environment (Wireman et al., 2006). We can use what we know about stress in wild-living primate populations to understand the primate gut microbiome and its stress response. For example, Stanton et al., (2015) found that at Gombe increased fecal glucocorticoid metabolite concentration (FGMC) were related to maternal motivation, increased female chimpanzees' response to infant cues, and increased infant handling. All of these results could be incorporated into hypotheses surrounding primate gut microbiome responses. Increased FGMC in the mother could potentially reduce diversity in the maternal gut microbiome, while increased infant handling could increase the diversity in the gut microbiome of the infant for other primate taxa. However, evidence from bonobos suggests the communication between a host's gut microbiome and their overall health may be system dependent as blood parasites like malaria do not seem to be influenced but the composition of the gut microbiome (Liu et al., 2017).

1.5 Bonobos as models

Bonobos are an excellent model species for examining the effects of diet and stress on the gut microbiome because of their similarities with humans in their dietary and social behavior. Bonobos live in communities of mixed male-female groups that fission-fusion daily (Gruber and Clay, 2016). These fission-fusion events mean that a party of bonobos will change composition throughout the day (Aureli et al., 2008). Bonobos are male philopatric, which means females leave their natal group at sexual

maturity (Furuichi 1989; White 1996b). Bonobos exhibit female dominance, have low levels of aggressive behaviors, exhibit high levels of affiliative social bonds, and high levels of socio-sexual behaviors compared to chimpanzees (Gruber and Clay, 2016). Due to the fact that bonobos share 98% of their DNA with humans and chimpanzees, they are an excellent model for examining questions related to the gut microbiome, diet, and stress (Gruber and Clay, 2016; King and Wilson, 1975). Due to this genetic and phylogenic similarity, if bonobos and chimpanzees share similarities and humans differ in their patterns of gut microbiome, then we can assume that humans have a derived gut microbiome trait. In comparison, when all three have similar patterns, we can conclude that this gut microbial pattern is a shared derived trait. When all three species differ in their gut microbial patterns, then we can assume there is no *Pan*/human pattern, and we need to look to the other great ape species to understand the evolutionary trends in how the gut microbiome co-evolved with its host.

Previous bonobo gut microbiota studies only included samples from a single atypical bonobo site (Moeller et al., 2016; Nishida and Ochman, 2019). The only cross-site comparison of bonobo gut microbiomes concluded that malaria parasite infection did not affect bonobo gut microbiota composition (Liu et al., 2017). In contrast, gorillas (*Gorilla* spp.) gut microbiome characteristics shift with shifts in seasonal variation in fruit availability and vary with season and metabolite composition during times of the year classified as "high" fruit and "low" fruit (Gomez et al., 2015; Gomez, Rothman, et al., 2016). These broad classifications do not adequately quantify fruit availability within a habitat nor measure what fruits are available for gorillas and other primates to utilize (Gomez et al., 2015; Gomez, Rothman, et al., 2016). Thus, there is a gap in the great ape gut microbiome literature around how subtle shifts in specific resources change gut microbiota.

Factors such as female dominance, high levels of socio-sexual behaviors, and fluidity in community members potential influence why bonobo gut microbiota patterns may differ from other great apes. Bonobos represent a complement to the chimpanzee evolutionary model for human evolution and related gut microbiome dynamics. Dietary signatures in great ape gut microbiomes have been investigated in chimpanzees (Degnan et al., 2012) and gorillas (Gomez et al., 2015; Gomez, Rothman, et al., 2016) but have yet

to be examined in bonobos. Thus, there is a gap in knowledge on the relationship between bonobo's diet and the gut microbiota, specifically, whether diet and stress change bonobo microbial communities. We examine the relationship between diet and the bonobo gut microbiome in chapter two.

While diet may play an important role in the composition of primate gut microbiomes, stress also needs to be evaluated as a critical factor. Stress may mediate between a host and its commensal gut microbes (reviewed in Keay et al., 2006). Stress is linked to a decrease in the diversity of species found in the gut microbiome (Konturek et al., 2011). Maternal stress increased *Lactobacillus* microbes found in captive macaque (*Macaca mulatta*) gut microbiomes (Bailey, 2009; 2012; Bailey and Coe, 1999). Recent evidence from gorillas found no relationship between fecal glucocorticoids and gut microbiome composition but found a positive correlation between family *Anaerolineaceae,* genus *Clostridium,* and genus *Oscillibacter* suggesting that stress may select for certain types of bacteria within a gut microbiome (Vlčková et al., 2018). The ability of a host and its gut microbiome to potentially communicate is vital to the survival of both, and an increasing interest in this communication has emerged (Sandrini et al., 2015). There may be analogous communication between a host and its microbiome in humans (Konturek et al., 2011). This relationship remains unclear among primates and understanding how an individual primate communicates with its gut microbes remains to be determined.

The communication between the gut microbiome and hormonal systems has far-reaching implications for host physiology that help elucidate co-evolutionary forces (Davenport et al., 2017). Bonobos undergo stressors that can be systematically measured, and they express many of the same life-history traits as humans (Gruber and Clay, 2016). Thus, bonobos represent good models to understand aspects of human development and physiology (de Waal, 2005; Jaeggi, Burkart, and Van Schaik, 2010; Parish, De Waal, and Haig, 2000; White, 1996b). The genetic similarity between humans and bonobos allows for a test of whether the pattern of decreasing microbiome diversity and increased stress found in humans applies to a genetically similar species (Bailey, 2009; Gruber and Clay, 2016; King and Wilson, 1975). We examine the relationship between stress and the bonobo gut microbiome in chapter three.

1.6 Research sites

Data was collected from two field sites, Iyema and N'dele, in the Lomako Forest Reserve, Tshuapa Province, Democratic Republic of Congo (DRC) (Figure 1.2). The Iyema field site (00°55 N, 21°06 E) consists of a trail system encompassing ~30 km^2. Behavioral observations and preliminary genetic analyses suggest 26 - 66 individual bonobos in the trail system surrounding the Iyema camp, likely in two or more communities (Bertolani, personal communication; Sakamaki, personal communication; Brand et al., 2016). The study area consists mostly of undisturbed primary forest with an understory plant community dominated by *Marantacea* species. Several small streams run through the study area, but swamp forest, seasonally inundated forest, and homogenous *Gilbertiodendron* stands are relatively rare (Cobden, 2014). The N'dele field site is located about 15 km southeast of Iyema (Figure 1.2) and consists of a 40 km^2 trail system. The latter site includes the overlapping ranges of two bonobo communities: Bakumba and Eyengo. Between 1983 and 1985, a group formed around immigrating females and inhabited the region before transitioning into the Bakumba community (White & Wood, 2007). The study area at N'dele includes a mosaic of forest types, including secondary forest and homogenous *Gilbertiodendron* forest, but is mostly undisturbed primary forest. Several other habitat types at N'dele include streams, swamp forest, swamp grassland, and river habitats (White, 1992).

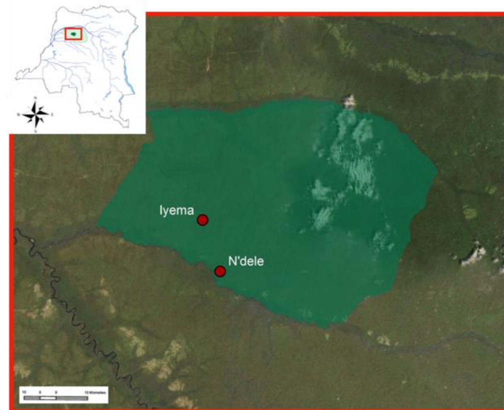

Figure 1.2. Map of Iyema and N'dele field sites, Lomako Forest, Democratic Republic of Congo (DRC).

CHAPTER II

A TEST OF FORAGING MODELS USING DIETARY DIVERSITY INDICIES FOR THE LOMAKO FOREST BONOBOS

From: Hickmott, A. J., Waller, M. T., Wakefield, M. L., Malone, N., Brand, C. M., & White, F. J. (*In review*). Foraging Models and the Dietary Diversity of the Lomako Forest Bonobos. *Folia Primatologia.*

2.1 Introduction

The acquisition of food resources is necessary for survival, gestation, and lactation and has influenced primate behavior (Boubli and Dew, 2005; Bray et al., 2018; Clutton-Brock, 1974). Evolutionary models provide a framework for understanding the relative importance of ecological variables in the evolution of primate dietary diversity (Lambert, 1998; Lambert, 2004; Lambert and Rothman, 2015). These models help us understand how different ecological variables influence foraging strategies and incorporate dietary breadth, energy return, nutritional quality, mechanical properties, digestibility, food species abundance and distribution, and seasonality (Richard, 1985; Strier, 2015). Three major model types have been used to understand primate diets: 1) functional response models (Holling, 1959); 2) optimal foraging or optimal diet models (MacArthur and Pianka, 1966); and 3) fallback food models (Lambert, 2007; Marshall & Wrangham, 2007). Each of these model types places emphasis and significance on different ecological factors (Figure 2.1). For example, functional response models emphasize food-species distribution and abundance (Holling, 1965), whereas optimal diet models focus on aspects of dietary breadth, energy return, and abundance (Charnov, 1976; MacArthur and Pianka, 1966; Pyke, Pulliam, and Charnov, 1977) and fallback food models predict how animals will change their diets under conditions when preferred foods are unavailable (Marshall et al., 2009). These models differ in their usefulness for addressing different hypotheses. For example, functional response and optimal diet models incorporate aspects of dietary breadth that are useful in testing model predictions in highly-productive forests where periods of scarcity are rare (Lambert, 2007; Marshall

& Wrangham, 2007; Raubenheimer et al., 2009), while fallback food models test predictions under conditions of food scarcity.

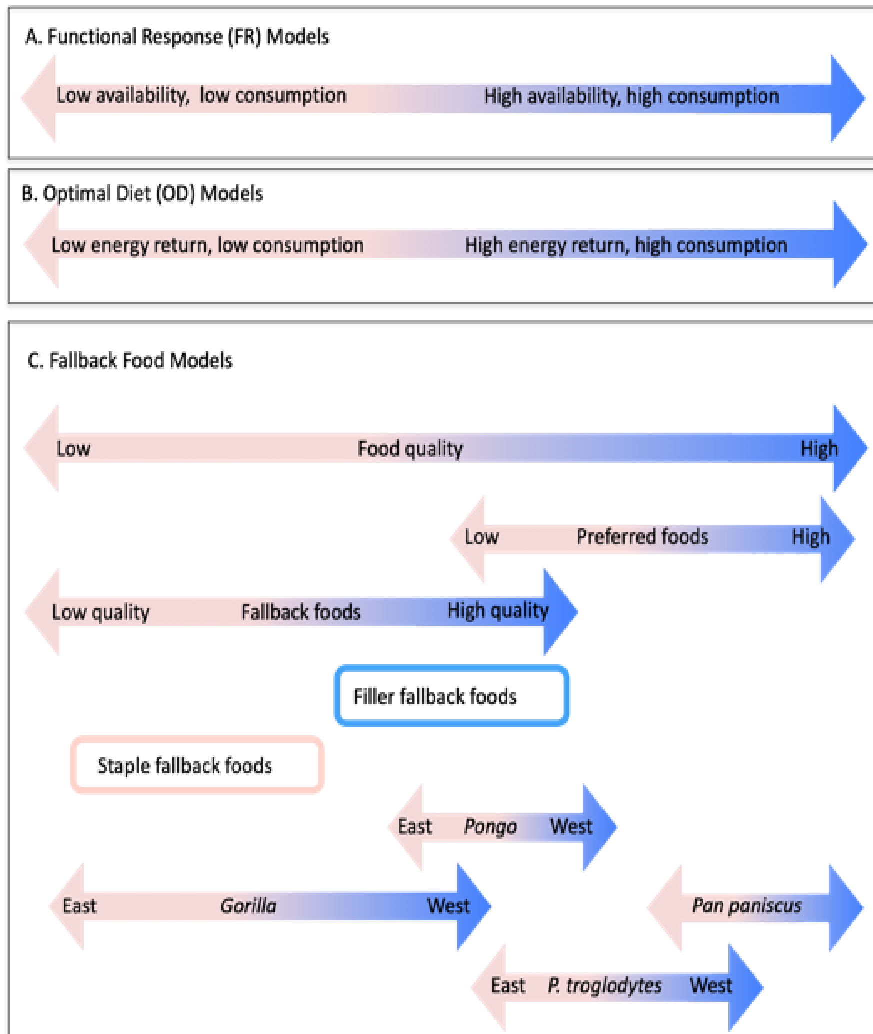

Figure 2.1 Schematic of different dietary models. Figure based on A. Holling, 1959, B. MacArthur and Pianka, (1966) C. Marshall et al., (2009), Lambert, (2007).

Functional response models are often the underlying assumption in most primate feeding ecology studies (Krebs, 1984; Lambert and Rothman, 2015). The concept that food abundance predicts consumption of those same food items has been documented in Taihangshan macaques (*M. mulatta tcheliensis*), black and white colobus (*Colobus guereza*), masked titi-monkeys (*Callicebus personatus melanochir*), orangutans (*Pongo*

pygmaeus), and western lowland gorillas (*Gorilla gorilla gorilla*) (Cui et al., 2019; Doran et al., 2002; Heiduck, 1997.; Leighton, 1993). Optimal diet models focus on aspects of dietary breadth, energy return, and abundance but have only sporadically been directly tested in primatology (Altmann & Wagner, 1978; Sayers et al., 2009). Among yearly data collected on baboons (*Papio cynocephalus*), tests of optimal diet models found mean energy shortfall was a predictor of female baboon reproductive lifespan (Altmann, 1991). In Himalayan langurs (*Semnopithecus entellus*), the energetic currency of food resources generally predicted their consumption (Sayers, Norconk, and Conklin-Brittain, 2009). Fallback food models predict how a diet will respond under conditions when preferred foods are unavailable (Lambert, 2007; Marshall & Wrangham, 2007). Fallback foods are essential in primate diets, including Japanese macaques (*Macaca fuscata*), sportive lemurs (*Lepilemur ruficaudatus*), chimpanzees (*Pan troglodytes*), and others (Constantino and Wright, 2009; Furuichi, Hashimoto, and Tashiro, 2001; Hanya and Chapman, 2013). However, to our knowledge, no studies in primates use dietary diversity indices as a tool to test the model predictions of functional response and optimal diet models.

Dietary diversity indices help examine variation in primate diets because they facilitate comparisons across methods, including behavioral observations and fecal washing, and geography (Basabose, 2002; Erhart et al., 2018; McGrew et al., 1988; William C. McGrew et al., 2009; Phillips & McGrew, 2014; Potts et al., 2011; Tutin et al., 1991). The three most commonly used indices are the 1) Shannon-Weaver, also known as Shannon's (*H'*) index, 2) Simpson's index (D), and 3) Shannon-Wiener evenness index (SW evenness), all of which incorporate two main factors: 1) species richness (N); and 2) species evenness (Magurran, 1988). Dietary species richness describes the number of species eaten, whereas dietary species evenness is concerned with the relative predominance of different species in the diet (Mittelbach and McGill, 2019). Shannon's index aims to combine evenness and richness into a single metric of diversity and assumes that sampling is from an infinitely large population (Magurran, 1988; Shannon and Weaver, 1949). In contrast, Simpson's index measures the probability that two randomly sampled items in the diet are the same and is a measure of

concentration (Magurran, 1988; Simpson, 1949). Finally, SW evenness takes the same basic approach as the other indices but detects patterns due to shifts in the overall species availability. When abundant species dominate, the value of the index will be higher (Magurran, 1988; Pielou, 1974). For example, in examining faunal loss from bushmeat hunting at Bioko Island, Equatorial Guinea, the SW evenness index was higher than Shannon's index in examining temporal variation in the diversity of species taken as bushmeat (Albrechtsen et al., 2007). Thus, these diversity indices can be used to examine model predictions that deal with changes in richness and evenness.

In community ecology, where many of these indices were developed, the use of these diversity indices to compare across data sets collected at different times and from different locations is the main strength of the diversity indices (Mittelbach and McGill, 2019; Pielou, 1974). The strength of a diversity index is that it compresses data into a single comparable index (Lehman and Tilman, 2000; Magurran, 1988). The weakness of these indices is that they do lose resolution when examining the specifics of what species are consumed at which frequencies, which is why for this paper, we have also provided the frequency of consumption for the different food species across the different datasets (Table 2.1). In studying the dietary ecology for Steller sea lions (*Eumetopias jubatus*) and Holarctic martens (*Martes* spp.*),* dietary diversity indices were used to investigate changes in diet over space and time using spatially separated field sites over multiple years (Lozano, Moleón, and Virgós, 2006; Zhou et al., 2011). Thus, borrowing from community ecology, we seek to use dietary diversity indices and how they shift across a year and between datasets to inform our understanding of primate foraging strategies. The Shannon's index, Simpson's index, and SW evenness index have been used in primatology primarily to compress a year's worth of feeding ecology data into a single index (Cui et al., 2019; Erhart, Tecot, and Grassi, 2018; Potts, Watts, and Wrangham, 2011). Typically, only the Shannon's or Simpson's index is reported in primatology and is only reported as a single metric in a feeding ecology paper (*ibid*).

Table 2.1 Percentage of foraging time by species and food type. Light grey columns represent the fecal washing datasets while the white cells represent the behavioral observation datasets. The dark gray cells represent the top three food items consumed for each dataset.

Year (Site)	1984 (N’dele)	1991 (N’dele)	1995 (N’dele)	2007 (N’dele)	2009 (N’dele)	2009 (Iyema)	2014 (Iyema)	2017 (Iyema)
Species								
Annonidium mannii	0.74%					6.45%		
Anothonota fragrans								2.88%
Anthoclitandra robustior							33.33 %	
Antiaris toxicana	16.3%							4.32%
Autranella congolensis	0.74%	1.47%						
Beilschmiedia corbisieri	0.74%							
Blighia welwitschii		1.47%						
Carpodinus gentilii	4.44%	7.35%						0.72%
Celtis mildbraedii	10.37%	0.98%						13.67%
Celtis tessmanii								0.72%
Cephalophus weynsii[1]								2.16%
Chrysophyllum lacourtianum	1.48%							
Cissus dinalagei		0.98%	1.52%		2.99%	16.13%		0.72%
Crudia laurentii			2.27%					2.16%
Dialium corbisieri								2.88%
Dialium sp.	3.70%			4.76%				
Entandrophragma sp.								0.72%
Ficus spp.	20.00%	3.43%		28.57%	22.39 %	25.8%	22.22 %	1.44%
Funtumia elastica		0.98%						
Garcinia cola			2.27%					
Garcinia species	0.74%							
Gilbertiodendron dewevrei		1.96%					5.56%	
Haumania liebrechstsiana	11.85%	7.35%	12.88 %					2.16%
Irvingia gabonensis			13.64 %					
Irvingia wombulu	2.22%							
Klainedoxa gabonensis								1.44%
Macarange sp.								0.72%
Musanga cercropioides	0.74%	0.49%					5.56%	
Nauclea diderichii	3.70%	8.82%						
Omphalocarpum mortehanii								1.44%
Palisita sp.								0.72%

Table 2.1 (continued).

Pancovia laurentii	1.48%							
Paramacrolobium coerulum	0.74%							
Parinaria excelsa	0.74%							
Polyalthia suaveolens	4.44%	18.14 %		4.76%	47.76 %	32.25%		15.83%
Pterygota beguaertii	2.22%							
Scropholoes zenkeri	7.41%	13.24 %	32.58 %				11.11 %	35.97%
Staudtia stipitata		0.49%						0.72%
Strombosia glaucescens							16.67 %	3.6%
Strombosia grandifolia								0.72%
Strombosiopsis tetandra		6.37%						2.88%
Strombosiopsis zenkeri		4.41%		4.76%				
Trachyphylum braunianum			7.58%					
Treculia africana	1.48%	9.80%	18.18 %					0.72%
Uapaca guineensis	0.74%	11.76 %						
Unknown sp.	1.48%	0.49%	9.09%	23.81%	26.86 %	12.90%	5.56%	0.72%
Unknown sp.	0.74%			19.04%		3.22%		
Unknown sp.	0.74%			14.28%		3.22%		

These dietary diversity indices generate different predictions about the different primate foraging models. Functional response models predict that Shannon’s index will correlate positively with food density (Table 2.2). In contrast, Simpson's index is predicted to be lower when more food items are available for consumption. For SW evenness, functional response models predict that H' will parallel changes in N, but H'/ln(N) will depend weakly, if at all, on N since individuals are not selective in their choice of dietary items. Optimal diet models predict that Shannon's index will be lower during periods of the year when preferred or highly valued food items are available. In contrast, Simpson's index will be higher during periods of the year when a few highly dominant species are being consumed (Table 2.2). Meanwhile, H/ln(N) should be inversely related to overall food availability, specifically for high-quality items, such as fruit. When high-quality items are abundant, SW evenness will be low. Under optimal diet models, H may increase with N, but the relationship is expected to be weaker than under functional response models and could be inversely related (Table 2.2).

Table 2.2 Conditions under which the functional response and optimal diet models will give different results for Shannon's (H'), Simpson's (D) index, and Shannon-Weiner (SW) evenness.

Model	**Shannon's index (H')**	**Simpson's index (D)**	**Shannon-Weiner (SW) evenness index (H' /ln(N))**
Index Description	Combines evenness and richness into a single metric; Assumes that sampling is from an infinitely large population.	Measures the probability that two randomly sampled items in the diet are the same and is a measure of concentration	Detects patterns due to shifts in the overall species availability such that when abundant species dominate, the value of the index will be higher
Functional Response	High when fruit is available	Low when fruit is available	H' will parallel changes in N, but will depend weakly, if at all, on N
Optimal Diet	Low when preferred fruits are available	Higher when a few food items dominate diets	Inversely related to overall food availability for high-quality items (fruit).

Optimal diet models suggest that dietary items incorporated in a diet are based on decisions that maximize energy return and economic foraging effort (Altmann & Wagner,

1978; Harrison, 1984; Sayers et al., 2009). Broadly, there are three significant components to optimal diet models. First, these models predict that every food item has a value equal to the energy content of the food minus the energy it takes to obtain that item (net energy return) (Harrison, 1984; Lambert and Rothman, 2015). Second, these models rank all food items based on the net energy return (Koenig et al., 1998; MacArthur and Pianka, 1966). Finally, the optimal diet is determined by starting with the highest-ranked item and consuming items in decreasing order of rank; thus, when high-value resources are available, dietary breadth decreases (Altmann & Wagner, 1978; Charnov, 1976; Richard, 1985). Optimal diet models are essential when considering what will happen when high-quality foods are abundant, whereas functional response models better explain primate foraging decisions (e.g., Altmann & Wagner, 1978; Harrison, 1984). Functional response and optimal diet models have been tested in studies of primate diets (Altmann, 1991; Chapman et al., 2004a; Cui et al., 2019; Doran et al., 2002; Heiduck, 1997.; Sayers et al., 2009).

Bonobos (*Pan paniscus*) are considered primarily frugivorous and consume fruits, new leaves, insects, vertebrates, terrestrial herbaceous vegetation, and flowers (Furuichi, 1989; Hohmann & Fruth, 2003; Kano & Mulavwa, 1984; Loudon et al., 2019; Serckx et al., 2015; Wakefield et al., 2019; White, 1986; White, 1992, 1998). Some populations may use fallback foods (e.g., bonobos living in forest-savannah mosaic habitats at Malebo (Serckx et al., 2015)). However, no direct test of optimal diet models has been undertaken using dietary diversity indices at Lomako, in the Democratic Republic of Congo (DRC) (White, 1998).

We aim to test the predictions of functional response and optimal diet models in bonobos, using dietary diversity to capture species richness and species evenness when comparing models. We characterize variation in bonobo dietary diversity between seasons, study periods, and two research camps within the same protected contiguous forest. We also consider the efficacy of using fecal washing to capture dietary diversity. We predict that if bonobo foraging behavior fits functional response models, then Shannon's index will follow seasonal shifts in measures of food density and abundance. In contrast, Simpson's index will be lower when there is an overall higher abundance of food items in the environment than when there is a lower abundance of food items. Under

functional response models, SW evenness will be correlated with changes in species richness (N) such that when species richness is high, SW evenness will be high and low when species richness is low. If functional response models do not explain bonobo foraging patterns, this index will be weakly linked to species richness. Suppose optimal diet models better explain bonobo foraging strategies, then Shannon's index will be lower during periods of the year when fewer items are available for consumption and high-value items are abundant. Comparatively, we predict SW evenness to be inversely related to food availability. Simpson's index will be higher during periods of the year when a few dominant species and less high-quality food items were consumed. High-value food items, in this case, fruit, are considered high-value food items under the model predictions for optimal diet models.

2.2 Materials and Methods

2.2.1 Study Camps

Non-invasive behavioral observations and fecal washings were collected over seven field seasons (Table 2.3) between 1984 and 2017 at Iyema and N'dele field camp in Lomako Forest Reserve, Tshuapa Province (formerly Equateur Province), DRC (Figure 2.2). Iyema field camp (00°55 N, 21°06 E) consists of a trail system encompassing ~30 km^2. Behavioral observations and preliminary genetic analyses suggest 26 - 66 individual bonobos in the trail system surrounding the Iyema camp, likely in two or more communities (Bertolani, personal communication; Sakamaki, personal communication; Brand et al., 2016). The study area consists mostly of undisturbed primary forest with an understory plant community dominated by *Marantacea* species. Several small streams run through the study area, but swamp forest, seasonally inundated forest, and homogenous *Gilbertiodendron* stands are relatively rare (Cobden, 2014). N'dele is located about 15 km southeast of Iyema (Figure 2.2) and consists of a 40 km^2 trail system. N'dele contains the overlapping ranges of two bonobo communities: Bakumba and Eyengo. Between 1983 and 1985, a group formed around immigrating females and inhabited the region before transitioning into the Bakumba community

(White & Wood, 2007).

Table 2.3 Study periods with sample size and methods that were used to study bonobo feeding ecology at N'dele and Iyema, Lomako Forest, Democratic Republic of Congo (DRC).

Months of data collection	Site	Method	Hours of observation	Community size	Number of fecal samples collected
Oct. 1984 – July 1985	N'dele	Behavioral observation	248.0	~85	-
June – Sept. 1991	N'dele	Behavioral observation	43.3	~85	-
July – Aug. 1995	N'dele	Behavioral observation	26.8	~85	-
July 2007	N'dele	Fecal washing	-	-	7
July 2009	N'dele	Fecal washing	-	-	52
July 2009	Iyema	Fecal washing	-	-	22
June – July 2014	Iyema	Behavioral observation	9.75	~26 - 66	
June – Oct. 2017	Iyema	Behavioral observation	176.5	~26 - 66	

The study area at N'dele includes a mosaic of forest types, including secondary forest and homogenous *Gilbertiodendron* forest, but is mostly undisturbed primary forest. Several other habitat types at N'dele include streams, swamp forest, swamp grassland, and river habitats (White, 1992).

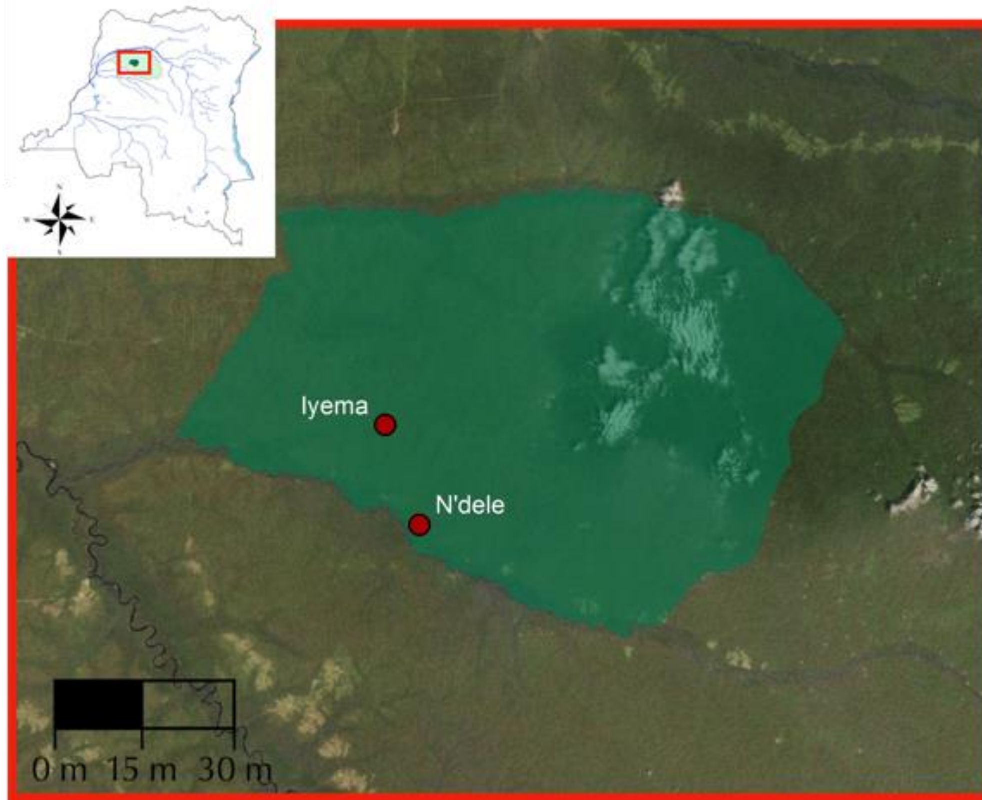

Figure 2.2 Map of the Lomako Forest Reserve (dark green). Circles represent the two field sites where data were collected. Iyema and N'dele is ~15 km away from each other. The inset map shows the location in the Democratic Republic of Congo (DRC).

2.2.2 Data Collection

We collected dietary diversity information using two years of fecal washing data and seven years of behavioral observation using two methods (Table 2.3). We calculated yearly and monthly Shannon's, Simpson's, and SW evenness diversity indices for each study period (1984/1985, 1991, 1995, 2007, 2009, 2014, & 2017 for a total of 27 months) using the frequency of a particular plant species consumed using the 'vegan' package in R version 3.4.3 (R Core Team, 2017; Oksanen et al., 2013). We recorded feeding behavior during focal follows and group scans, identifying which individuals were eating, what they were eating, and the plant part they were eating at each time point (Altmann, 1974). We also recorded the plant food species and plant parts consumed during both the focal observations and group scans. We followed nesting parties from their night nests or as we

contacted them while walking trails. We recorded party composition, social behavior, activity, and GPS location during 15-minute scans.

The fecal washing data sets used 80 non-invasively collected fecal samples from underneath bonobo night nests (White, 1992). We transported fecal samples to the camp at N'dele. We identified seeds to species level and counted them to obtain approximate amounts of each fruit eaten. We estimated the percentage fiber by weighting the feces before washing of each sample to approximate the amount of fiber recently consumed.

We used phenology transects to measure seasonal food abundance using the transect methods in Chapman et al., (1992). We marked known bonobo food species trees located within 3 m of each transect and scored them monthly for young leaves, fruit, and flowers on a 0-4 scale, where 0 is 0% of a particular resource (fruit, new leaves, or flowers), 1 is 1%-24%, 2 is 25-49%, 3 is 50-74%, and 4 is 75-100% of a particular food resource. When fruit was present on the tree, we recorded the percentage of ripe fruit by examining the total area of the tree crown and estimating the percentage (0-4) of that area covered by ripe fruit (Chapman et al., 1992; Chapman, Wrangham, and Chapman, 1994). We calculated food availability indices (FAI) following Mitani et al., (2002). While our measure of fruit abundance is crude, it is the standard established by Chapman et al., (1992) and was used to make our fruit abundance data comparable to chimpanzee sites, like Ngogo (Mitani et al., 2002). To quantify seasonal shifts in fruit abundance in 2017, we monitored four 1 km phenology transects with 513 marked trees of 27 different species once a month during the entire study period, and in 2007 we monitored two 1 km phenology transects with 53 marked trees of 29 species once a month during the study period. To evaluate prevalent food items for each season, we determined the three most dominant species in the diet for that year based on the behavioral observation datasets (Table 2.1).

2.2.3 Data Analysis

To test if each dietary diversity index depended on the month or year it was collected, we created a dissimilarity matrix for each diversity index to see if diversity indices were comparable given the long-time spans between data sets. We ran Mantel

tests on each diversity index's dissimilarity matrix and the time dissimilarity matrix to determine whether they correlated. To test the conditions under which the functional response and optimal diet models will give different results, we ran six Kruskal-Wallis tests separately on each index (Shannon's, Simpson's, and SW evenness), comparing between methods and then within methods but across two research camps within the same protected area. During the behavioral observation data collection, food items were identified when bonobos entered a feeding patch. The tree or food type was identified, and then the plant part was identified as the feeding bout began. We calculated the Shannon index as $H' = \sum[p_i \log p_i]$, where p_i is the proportion of species i in the sample area (Pielou, 1974). We calculated Simpson's index as $D = \sum(n_i^2)$, where n_i represents the probability that two randomly selected individuals in the community belong to the same category (Simpson, 1949). We report indices based on fecal washing and behavioral observations separately. We used Kruskal-Wallis tests in R to test differences in dietary diversity indices using behavioral observations and fecal washing. Correlating the seeds to plant species was done by trained local guides for the fecal washing datasets. Percent fiber was estimated by taking the weight before washing and post washing to estimate the approximate weight of the fiber in the fecal sample. We used a Kruskal- Wallis to test for differences in dietary diversity across two research camps within the same protected area (Iyema vs. N'dele) and between study periods for each method. Sampling was unequal between study periods, but dietary indices weight the values according to richness and evenness, accounting for differences in sample size and allow comparisons across different sample sizes and across time and space (Lehman and Tilman, 2000; Mittelbach and McGill, 2019). We compared the FAI calculated from our available monthly phenology data to two diversity indices calculated per month for 2017. We used linear regression to test whether food availability was related to dietary diversity as measured using the three diversity indices under the predictions of functional response models, using FAI to measure food quantity. We used 'ggplot2' to visualize our data (Wickham, 2009).

2.3 Results

2.3.1 Percentage of foraging by species and food type

Highly consumed items varied by study period and included *Anthoclitandra robustior* (2014), *Antiaris toxicana* (1984), *Celtis mildbraedii* (1984, 2017), *Ficus* spp. (1984, 2014), *Irvingia gabonensis* (1995), *Polyalthia suaveolens* (1991, 2017), *Scropholoes zenkeri* (1991,1995, 2017), *Strombosia glaucescens* (2014), *Treculia africana* (1995), and *Uapaca guineensis* (1991) (Table 2.1). Species richness (N) of food items varied between study periods: bonobos at N'dele consumed 25 (1984 – 1985), 19 (1991), 9 (1995), 7 (2007), and 4 (2009) species, while those at Iyema consumed 7 (2009, 2014) and 24 (2017) species (Table 2.1). Most of the top three food items across years are fruit, but *Scropholoes zenkeri*, a top food item consumed for 1991,1995, and 2017, is notable because only the leaves of this tree are consumed (Table 2.1).

2.3.2 Dietary Diversity Variation by Method, Camp, and Study Period

The results of the Mantel tests for the time matrix compared to Shannon's diversity index (H') (R: -0.048; p = 0.238), Simpson's diversity index (D) (R: 0.041; p = 0.483), and SW evenness (R: 0.047; p = 0.416) were all not significantly different across time indicating that the variation in diversity index was not a consequence of the time between sampling periods. Overall, Shannon's diversity index (H') ranged from 1.25 – 2.67, Simpson's diversity index (D) ranged from 0.10 – 0.33, and SW evenness ranged from 0.73 – 1.01. The mean dietary diversity indices for all study periods for N'dele were $H' = 2.04 \pm 0.58$, $D = 0.18 \pm 0.09$, and SW evenness = 0.88 ± 0.03 while for Iyema they were $H' = 2.02 \pm 0.28$, $D = 0.18 \pm 0.02$, and SW evenness = 0.88 ± 0.14 (Table 2.4).

Behavioral observation had a significantly higher Shannon's (H') index than fecal washing data with a mean difference of 0.51 (Kruskal-Wallis: H = 5, df = 1, p = 0.03). Simpson's (D) index for behavioral observation was significantly lower than for fecal washing data with a mean difference of 0.10 (Kruskal-Wallis: H = 5, df = 1, p = 0.03). SW evenness index did not differ significantly different between methods (Figure 2.3).

Table 2.4 Yearly diversity indices for bonobos at two sites in Lomako Forest, DRC. Shaded cells indicate data from fecal washing. Other cells indicate data from behavioral observation.

Year	**Months of data collection**			Simpson's D		SW evenness	
		N'dele	**Iyema**	**N'dele**	**Iyema**	**N'dele**	**Iyema**
1984/ 1985	10	2.67	.	0.10	.	0.83	.
1991	4	2.55	.	0.10	.	0.87	.
1995	2	1.93	.	0.18	.	0.88	.
2007	1	1.79	.	0.20	.	0.92	.
2009	1	1.25	.	0.67	.	0.90	.
2009	1	.	1.77	.	0.80	.	0.91
2014	2	.	1.96	.	0.17	.	1.01
2017	6	.	2.32	.	0.18	.	0.73
Mean		2.04	2.02	0.18	0.18	0.88	0.88

We found no significant differences between two research camps within the same protected area (Iyema and N'dele) for Shannon's (*H'*), Simpson's D, and SW evenness index (Figure 2.4) for behavioral observation datasets. There were no significant differences in all three diversity indices between study periods (Kruskal-Wallis: H = 7, df = 7, p = 0.43).

2.3.3 Monthly Dietary Diversity

There is a large range of variation in monthly dietary diversity indices (Table 2.5). Comparing the fruit, new leaf, and flower availability data for Iyema in 2017, we found that August had the highest fruit availability (Figure 2.5). We regressed Shannon's (*H'*) index against ripe fruit availability (F = 0.013, df = 1, 3, P = 0.91, R^2adj = -0.32), flower availability (F =5.4, df = 1, 3, P = 0.65, R^2 adj = -0.23), and new leaf availability (F = 0.24, df = 1, 3, P = 0.10, R^2adj = 0.52).

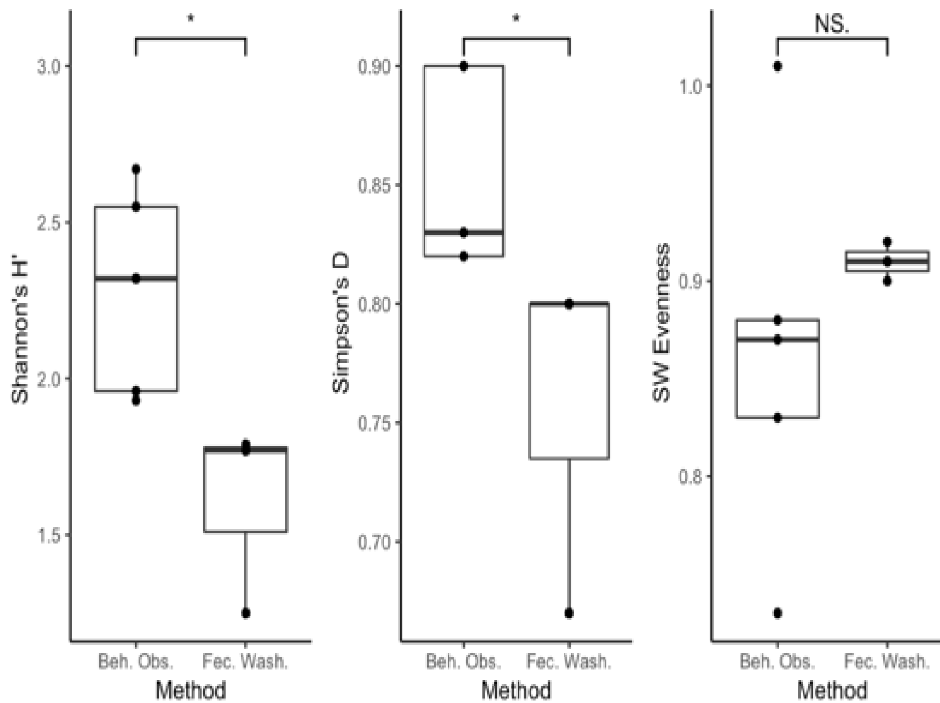

Figure 2.3 The method comparison (behavioral observation vs. fecal washing) for the three indices (Shannon's, Simpson's, and SW evenness). Asterisks indicate significant differences (<0.05). Shannon's and Simpson's index showed significant differences.

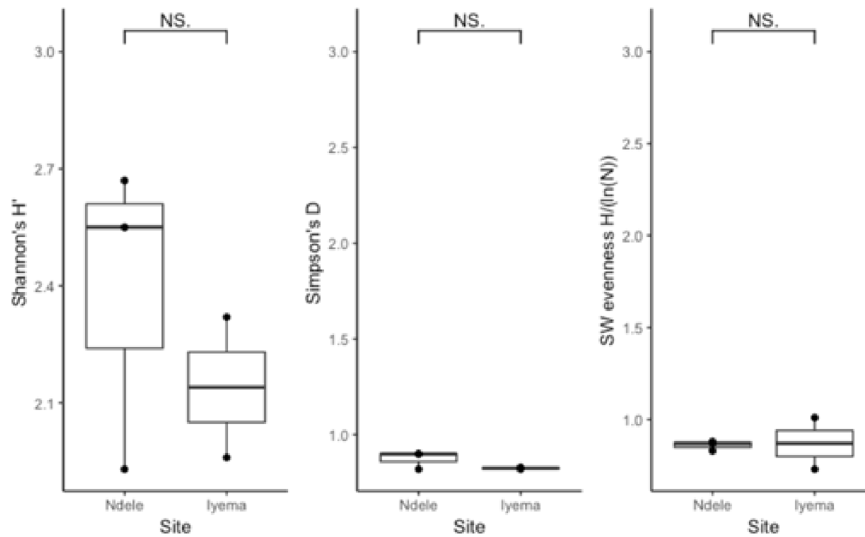

Figure 2.4 Site comparison (N'dele vs. Iyema) for the three indices (Shannon's, Simpson's, and SW evenness). Asterisks indicate significant differences (<0.05). No significant differences were found between sites.

Shannon's diversity ranged from 0.86 – 1.77 for this period, whereas Simpson's diversity index ranged from 0.34 – 0.5. In 2017, the decrease in dietary diversity during October occurred when food availability was highest. Comparatively, September had relatively low fruit availability (Fig. 2.5). Three species, *Scropholoes zenkeri* (35.97%), *Polyalthia suaveolens* (15.89%), and *Celtis mildbraedii* (13.67%), were highly dominant in the diet (Table 2.1). All three species had relatively high numbers of available fruit, new leaves, and flowers during September, the month with the highest Simpson's index. The percent of trees with fruit, new leaves, and flowers during September were *Scropholoes zenkeri* (Fruit: 0%, New leaves: 54.55%, Flowers: 0%), *Polyalthia suaveolens* (Fruit: 10.20%, New leaves: 93.88%, Flowers: 24.49%), and *Celtis mildbraedii* (Fruit: 0%, New leaves: 0%, Flowers: 50.00%) (Table 2.6).

Table 2.5 Monthly dietary diversity indices for Shannon's (H'), Simpson's D, and Shannon-Weiner evenness. Shaded rows represent data from Iyema, Lomako Forest, DRC. Unshaded rows are data from N'dele, Lomako Forest, DRC. Indices are presented in the following order: Shannon's (H'); Simpson's D; SW evenness.

Month/Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1984/1985	1.58; 0.26; 0.88	2.10; 0.17; 0.85	0.89; 0.46; 0.81	0.84; 0.58; 0.60	1.31; 0.29; 0.94	2.17; 0.14; 0.90	0.95; 0.44; 0.86			0.87; 0.50; 0.79	1.59; 0.24; 0.89	1.50; 0.24; 0.93
1991						1.64; 0.22; 0.92	2.10; 0.16; 0.82	1.36; 0.26; 0.98	1.45; 0.33; 0.75			
1995							1.34; -0.67; 0.83	1.67; 0.20; 0.93				
2007							1.79; 0.80; 0.92					
2009							1.25; 0.67; 0.90					
2009							1.77; 0.80; 0.91					
2014							1.63; 0.08; 0.91					
2017						1.60; 0.29; 0.77	1.43; 0.39; 0.69	1.61; 0.28; 0.73	1.77; 0.23; 0.81	0.87; 0.50; 0.79		

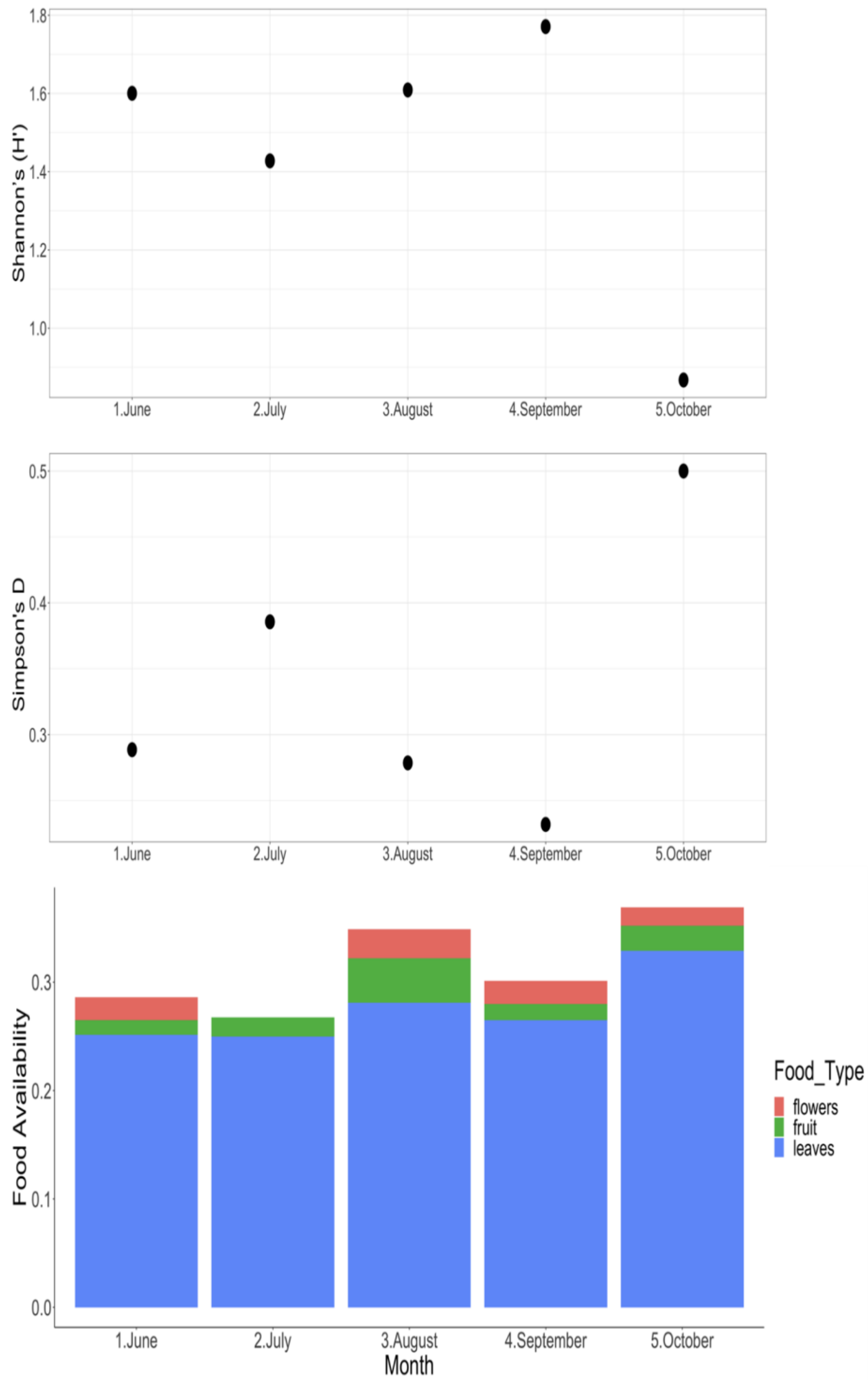

Figure 2.5 Monthly comparison of FAI, Shannon's (H'), Simpson's D, and SW evenness for bonobos at Iyema, Lomako, DRC, June – Oct 2017.

Table 2.6 Percentage of trees with ripe fruit, new leaves, and flowers from phenology transects at Iyema, Lomako Forest, DRC for five months in 2017. Gray highlights indicate the three highest consumed species and may represent high-value food items in the bonobo diet.

	Jun. 2017			Jul. 2017			Aug. 2017			Sept. 2017			Oct. 2017		
Species	Fruit	New leaf	Flower	Fruit	New leaf	Flower	Fruit	New leaf	Flower	Fruit	New leaf	Flower	Fruit	New leaf	Flower
Annonidium mannii	0.00	0.24	0.03	0.03	0.45	0.00	0.13	0.34	0.00	0.00	0.26	0.00	0.00	0.21	0.00
Anothonota fragrans	0.00	0.71	0.43	0.29	0.43	0.00	0.14	0.00	0.00	0.00	0.14	0.00	0.00	0.29	0.00
Antiaris toxicana	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
Beilschmiedia corbisieri	1.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Blighia welwitschii	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Celtis mildbraedii	0.00	0.50	0.00	0.00	0.50	0.00	0.00	1.00	0.00	0.00	0.00	0.50	0.00	0.00	0.50
Celtis tessmanii	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.50	0.50	0.00	1.00	0.00	0.00	1.00	0.00
Chrysophyllum lacourtianum	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.50	0.00	0.50	0.00
Colia griseflora	0.00	0.27	0.00	0.00	0.51	0.00	0.04	0.25	0.00	0.02	0.27	0.02	0.02	0.57	0.00
Dacryodies edulis	0.00	1.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dialium pachyphylum	0.21	0.14	0.00	0.07	0.50	0.00	0.00	0.43	0.00	0.00	0.36	0.07	0.00	0.07	0.00
Diospyros crassiflora	0.00	0.43	0.00	0.00	0.43	0.00	0.00	1.00	0.00	0.00	0.71	0.00	0.00	1.00	0.00
Diospyrus alboflavescens	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Ficus species	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
Garcinia punctata	0.00	0.65	0.00	0.05	0.68	0.00	0.08	0.65	0.03	0.08	0.83	0.00	0.15	0.95	0.00
Garcinia species	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.60	0.00	0.00	0.40	0.00	0.00	0.00	0.00
Grewia louisii	0.00	0.75	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.75	0.00	0.00	0.75	0.00
Irvingia gabonensis	0.50	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.50	0.50	0.00
Klainedoxa gabonensis	0.00	1.00	0.00	0.00	0.50	0.00	0.00	1.00	0.00	0.00	0.50	0.00	0.50	0.50	0.00
Macrolobium pynaertii	0.00	0.33	0.00	0.00	0.50	0.00	0.00	0.17	0.00	0.00	0.33	0.00	0.00	0.50	0.00
Musanga cercropioides	0.25	1.00	0.00	0.50	1.00	0.00	0.75	1.00	0.00	0.75	1.00	0.00	0.50	1.00	0.00
Pentaclethra macrophylla	0.14	0.71	0.57	0.00	0.14	0.00	0.00	0.43	0.14	0.14	0.29	0.00	0.14	0.86	0.00
Polyalthia suaveolens	0.00	0.69	0.00	0.08	0.94	0.00	0.16	0.92	0.00	0.10	0.94	0.24	0.08	0.98	0.08
Scropholoes zenkeri	0.02	0.66	0.00	0.01	0.39	0.00	0.01	0.63	0.01	0.00	0.55	0.00	0.01	0.81	0.00
Strombosia glaucescens	0.00	0.92	0.00	0.08	1.00	0.00	0.42	1.00	0.04	0.04	0.92	0.00	0.13	0.83	0.00
Strombosiopsis tetandra	0.13	0.75	0.00	0.13	0.75	0.00	0.38	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
Treculia africana	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.50	0.00

2.4 Discussion/ Conclusion

Anthoclitandra robustior (2014), *Antiaris toxicana* (1984), *Celtis mildbraedii* (1984, 2017), *Ficus* spp. (1984, 2014), *Irvingia gabonensis* (1995), *Polyalthia suaveolens* (1991, 2017), *Scropholoes zenkeri* (1991,1995, 2017), *Strombosia glaucescens* (2014), *Treculia africana* (1995), and *Uapaca guineensis* (1991) were our top consumed food items. We found that Shannon's indices, which assess dietary evenness and richness, were lower when fewer items were available for consumption. Simpson's index was higher during periods of the year, where a few highly dominant species and less high-value food items were consumed. SW evenness indices had a weak inverse relationship with food availability, supporting the predictions of optimal diet models (Figure 2.5). Based on the results of the linear regressions, abundance was not significantly related to dietary diversity indices, possibly indicating that bonobos do not select food under the

functional response model for the periods where we collected data. Shannon's indices were lower when fewer items were available for consumption and higher when high-value items were abundant.

These results demonstrate that measures of bonobo dietary diversity are dependent on the method. Fecal washing data yielded significantly lower Shannon's diversity index and Simpson's diversity index than behavioral observation. This result is not surprising due to the loss of information that occurs with fecal washing, however fecal washing datasets are still useful for non-habituated primate groups (Rothman, Chapman, and Van Soest, 2012). Behavioral observation data collection confirms what is consumed by the individual and can consider the amount of time spent feeding on a particular dietary item. Thus, it is logical to assume that this method would be more accurate in measuring the diversity of food items consumed in the bonobo diet.

Bonobos diets are understood to be primarily frugivorous with new leaves, insects, vertebrates, terrestrial herbaceous vegetation, and flowers consumed at different rates at different field sites (Furuichi, 1989; Hohmann & Fruth, 2003; Kano & Mulavwa, 1984; Loudon et al., 2019; Serckx et al., 2015; Wakefield et al., 2019; White, 1986; White, 1992, 1998). The extent to which forest ecology has shaped bonobo feeding ecology is still debated today (Cobden, 2014; Fruth & Hohmann, 2018; Kano, 1989; Kano & Mulavwa, 1984; Loudon et al., 2019; White & Wood, 2007; White & Wrangham, 1988). What is needed is long-term data on bonobo field sites, and our study provides that, along with much-needed measures of food availability (Gruber & Clay, 2016; White, 1996). Bonobo foraging behavior exhibits variation depending on the environment (Fruth & Hohmann, 2018; Takayoshi Kano & Mulavwa, 1984; Oelze et al., 2011; Surbeck & Hohmann, 2008). In a savannah-mosaic environment, fallback foods are important in the diet of bonobos in more secondary mosaic environments (Serckx et al., 2015). The tropical forests bonobos inhabit are characterized by a high abundance of dense food patches and ubiquitous terrestrial herbaceous vegetation (THV), yet long term measures of consumption paired with food availability are important for understanding what foraging models structure feeding behavior (Gruber & Clay, 2016; White, 1996; White & Wrangham, 1988). Our study suggests that in productive, intact, primary forests, bonobo foraging strategies may fit with the predictions of optimal diet models rather than

fallback food models as found in more mosaic habitats (Loudon et al., 2017; Oelze et al., 2016; Serckx et al., 2015). Our approach using dietary diversity indices to examine these models is just a piece of the puzzle in understanding the variation in bonobo foraging strategies across bonobo field sites.

One of the strengths of our approach is that diversity indices make data from different field data collection periods easily comparable and allow for a direct comparison across different field sites. Additionally, they shift in predictable ways that make them a good tool for testing foraging models, especially when using multiple diversity indices, as we did in this study. However, the weakness of using a diversity index is that it does compress data losing resolution. Thus, we recommend including the frequencies of food item consumption along with the different dietary diversity indices (e.g., Table 2.1, Table 2.4, Table 2.5). However, it might be expected that the length of the study period used to calculate the diversity index matters. We tested for similarity in adjacent months to the same month in different years, and time did not drive the pattern in the three diversity indices.

We recommend using all of Shannon's, Simpson's, and SW evenness index on behavioral observations in the future, as each index has its strengths and weakness. Most primatology papers only report one of the indices and using all three give a more complete picture. Fecal washing datasets are helpful in certain circumstances and may be used to gain a rough picture of the diet when other data are challenging to obtain. Our results need to be taken as a first attempt to understand the foraging behavior of the Lomako forest bonobos and need to be evaluated in light of the limitations of using previously collected datasets. These sample sizes are small, and ideally, there would be even sampling across study periods, but as the logistics of studying primates are complicated, especially in light of the COVID-19 pandemic, utilizing diversity indices to answer questions about primate foraging behavior is necessary. Additionally, the strength of these dietary indices lies in that they weigh the values according to richness and evenness, accounting for differences in sample size from previously collected datasets (Lehman and Tilman, 2000; Mittelbach and McGill, 2019).

Our results appear to support the predictions of the optimal diet model and not the functional response model. Abundance did not relate to consumption using dietary

diversity indices, indicating that bonobos are not selecting food under the functional response model. This interpretation is, however, preliminary given the limited sample size. Thus, when understanding dietary diversity and its relationship to bonobo diets, optimal diet models and optimizing energy return may be the main factor in structuring bonobo foraging strategies. Bonobos as optimal foragers seem to suggest that we need to incorporate aspects of optimality into future models of great ape foraging research.

2.4.1 Dietary Diversity Comparisons Across Apes

Among apes, the dietary diversity in the Lomako bonobos is relatively high, particularly compared to chimpanzees from multiple sites (Table 2.7). However, our results need to be considered with the caveat of our small sample size. Our data are limited in the hours of observation and months of observation when comparing to other species. Interestingly, gibbons and bonobos exhibit similarly high levels of dietary diversity, which may be due to behavioral or ecological similarities (Kim et al., 2012; McConkey et al., 2003; Newton-Fisher, 1999). Investigations into the plastic and flexible nature of dietary diversity of several species have documented intraspecific variation including, e.g., gorillas, *Gorilla gorilla beringei*: (Watts, 1984); red colobus, *Procolobus tephrosceles*: (Chapman and Chapman, 1999), black and white colobus *Colobus guereza*: (Harris and Chapman, 2007), *Cercopithecus* spp. (Chapman et al., 2004b); chimpanzees, *Pan troglodytes*: (Potts, Watts, and Wrangham, 2011).

We see the potential for future investigations to elucidate some of the remaining challenges to understanding bonobo diets. Examining the extent of diversity captured by fecal washing by focusing on seed dispersal and fiber breakdown through comparisons between behavioral observation, and genetic barcoding methods would be interesting as an avenue of future research. Future research projects will determine if a correction factor could be applied to fecal washing data to estimate dietary diversity indices. Additionally, bonobo foraging behavior appears to be explained by optimal diet models, but future research could focus on a more direct test of optimal diet models.

Table 2.7 Comparisons of dietary diversity indices across hominoids.

Species	**Bonobo, *Pan paniscus***		**Eastern chimpanzee, *Pan troglodytes schweinfurthii***			**Mountain gorilla, *Gorilla gorilla beringei***	**Müeller & agile gibbon, *Hylobates muelleri x agilis***
Site	**N'dele**†	**Iyema**†	**Ngogo**‡	**Kanyawara**‡	**Budongo**§	**Karisoke**‖	**Barito Ulu**¶
Months of data collection	18	9	19	19	16	17	12
Mean Shannon's index	2.04	2.02	1.55	1.78	1.78	1.55	2.67
Range of Shannon's index	1.25-2.67	1. 77 - 2.32	-	-	1.37 – 2.29	0.12 – 2.17	1.80 – 3.60

Table 2.7 (continued).

†This study, ‡Potts et al. 2011, §Newton-Fisher 1999, ‖Watts 1985, ¶McConkey et al. 2003

CHAPTER III

GUT MICROBIOTA AND FECAL STABLE ISOTOPIC VALUES FOR BONOBOS (*PAN PANISCUS*) OF THE LOMAKO FOREST, DEMOCRATIC REPUBLIC OF CONGO

3.1 Introduction

Primate gut microbiome communities are of increasing interest due to their important role in nutrition, development, health, and disease (Allaband et al. ,2019; Dantas et al., 2013; Dillon et al., 2005; Koch and Schmid-Hempel, 2011). In humans, one of the great debates among gut microbiome researchers is what factors influence the composition and diversity of the gut microbiome most: diet, sociality, or lifestyle (Falony et al., 2016). More recently, hormones such as glucocorticoids have been hypothesized to influence primate gut microbiomes (Mallott et al., 2020; Vlčková et al., 2018). Diet is important to the gut microbiome for many types of mammals (Ley et al., 2008; Ley et al., 2008; McKenzie et al., 2017; Moeller & Sanders, 2020). In non-human primates (NHP), diet helps structure the gut microbiome in multiple taxa including geladas (*Theropithecus gelada*, Baniel et al., 2020; Trosvik et al., 2018), black howler monkeys (*Alouatta pigra,* Amato et al., 2014, 2015), white-faced capuchins (*Cebus capucinus*, Mallott et al., 2017; Mallott et al., 2018), saddleback tamarins (*Leontocebus weddelli*; Garber et al., 2019), lowland gorillas (*Gorilla beringei beringei;* Gomez et al., 2016; Hicks et al., 2018*)*, mountain gorillas (*Gorilla gorilla gorilla*; Gomez et al., 2016), and chimpanzees (*Pan troglodytes*; Hicks et al., 2018).

Research on humans shows that even accounting for multiple factors including medication, blood and bowel parameters, diet, health status, anthropometrics, and lifestyle, diet is an important factor in explaining the gut microbiome's variation (Falony et al., 2016). Among NHP, specifically captive colobines, diet has a more substantial effect than host phylogeny on gut microbial communities (Hale et al., 2018). Additionally, in folivores, a loss of dietary fiber is associated with a loss of microbial diversity, paralleling the loss seen in modern human microbiomes (Clayton et al., 2016).

Diet and gut microbiome studies of great apes have focused on gorillas; these found that gut microbiome composition is linked to shifts in seasonal variation in fruit availability (Gomez et al., 2015; Gomez, Rothman et al., 2016). Such availability varies with season and metabolite composition during times of the year classified as "high" fruit and "low" fruit (*ibid*). The chimpanzee gut microbiome was investigated in relation to seasonal shifts in diet in captivity. (Hicks et al., 2018; Kišidayová et al., 2009; Yildirim et al., 2010).

Early studies of the evolution of NHP diets and their gut microbiomes investigated the differences between the omnivorous vervet (*Cercopithecus aethiops*) and samango monkey (*Cercopithecus mitis*), a folivorous hindgut fermenter (Bruorton, Davis, and Perrin, 1991). Black howler monkeys in more intact habitats exhibit higher diversity in their gut microbiome, likely due to dietary differences (Amato et al., 2013). Other investigations into three NHP species, frugivorous *Varecia variegata*, generalist *Lemur catta*, and folivorous *Propithecus coquereli*, tested the relationship between host lineage, captive diet, life stage, and the composition of the gut microbiome; these studies found that diets and phylogeny are confounded, yet, diet appears to be an important factor in gut microbial composition (McKenney, Rodrigo, and Yoder, 2015; McKenney et al., 2018). The gut microbiome provides additional energy and essential nutrients to compensate for dietary changes. In black howler monkeys, gut microbiomes provide a sufficient buffer against seasonal fluctuations in energy and nutrient intake and shift in response to diet changes (Amato et al., 2014). White-faced capuchin gut microbiomes were found to be a structured by the high and low fruit periods, and the high and low invertebrate periods of capuchin diets (Mallott et al., 2017; Mallott et al., 2018).

While diet is significant, sociality, as an avenue for dispersal and maintenance of gut microbiomes, has also been found to be important in NHP (Amato et al., 2017; Moeller et al., 2016; Raulo et al., 2018; Sarkar et al., 2020; Tung et al., 2015; Wikberg et al., 2012). Investigations into NHP gut microbiomes emphasized sociality's impacts on the gut microbiome (Archie and Theis, 2011; Archie and Tung, 2015). Moeller et al., (2016) examined sociality related to the *Pan* microbiome and concluded that sociality was an important factor in structuring bonobo and chimpanzee microbiomes. However, the degree to which sociality influences gut microbiome composition at sites other than

LuiKotale where Moeller et al., (2016) collected samples has not yet been studied, limiting our understanding of inter-site and inter-population variation relations between sociality and the bonobo gut microbiome. The impact and interaction between diet and social group on the Lomako forest bonobo gut microbiomes have not been investigated.

Bonobos are an excellent model species for examining the effects of diet and sociality on the gut microbiome because of the similarities they share with humans in their omnivorous diet and fission fusion social dynamics. Bonobos are primarily frugivorous and have been reported to share and consume meat peacefully, even going as far as sharing meat with neighboring groups (Fruth & Hohmann, 2018). Bonobos live in communities of mixed male-female groups that fission-fusion daily (Gruber and Clay, 2016). These fission-fusion events mean that a party of bonobos will change composition throughout the day (Aureli et al., 2008). Bonobos are male philopatric, which means females leave their natal group at the age of sexual maturity (Furuichi, 1989; White, 1996b). Bonobos tend to exhibit female dominance, have low levels of aggressive behaviors, exhibit high levels of affiliative social bonds, and high levels of socio-sexual behaviors compared to chimpanzees (*Pan troglodytes*) (Gruber and Clay, 2016). Due to the fact that bonobos, share 98% of their DNA with humans and chimpanzees they are an excellent model for examining questions related to the gut microbiome, diet, and sociality (Gruber and Clay, 2016; King and Wilson, 1975). Due to this genetic similarity when patterns in how the diet and the gut microbiome are similar to humans and chimpanzees, we can conclude there is a *Pan*/human pattern. When they are different, then more research is needed to understand why a species that humans are so genetically similar to displays different patterns in how diet and the gut microbiome interact.

3.2 Methods

3.2.1 Data collection

The research site is the Iyema field camp, located just north of the Lomako river at (00°55) North, 21°06 East) in the Democratic Republic of Congo (DRC). The site is

mostly covered by primary forest in *terra firma* soil with some swamps (Cobden, 2014; Dupain et al., 2000). For data collection, we followed bonobos to their night nests as part of the Antwerp Zoo's ongoing habituation efforts from June 2014 – July 2014. Night nest locations were marked, and each nesting site revisited the next morning. We identified each bonobo as it exited the nest and collected 10 ml of fecal sample into 50 mL tubes with 10 ml of RNALater for each individual in the nesting party. While there is debate about whether RNALater is the best sample preservation method for examining NHP gut microbiomes, several research groups have demonstrated that RNALater does not significantly alter the results for gut microbial community composition and diversity when used as a sample preservative (Blekhman et al., 2016; Hayakawa et al., 2018). The samples were stored in a cool, dry place until they were shipped to the Molecular Anthropology Laboratory at the University of Oregon. The remainder of each fecal sample after collection in RNALater was brought back to camp, dried using a camp stove, and placed into bags with desiccant for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis and fecal glucocorticoid analysis. Thus, for each fecal sample, we can obtain data on gut microbiota composition and diversity, stable carbon ($\delta^{13}C$), and nitrogen ($\delta^{15}N$) isotope values.

3.2.2 Social group determination

Social group was determined by genetic capture-recapture of individuals in each nesting party (Brand et al., 2016). We used the nesting maps to obtain measures of nesting proximity and association patterns. In collecting the nesting map data, we watched as each bonobo exited its night nest, and we recorded its sex. After the bonobos had exited their nests, we would take a GPS waypoint at the site of each nest/ fecal sample. For each nesting group or conglomerate of individual nests, we categorized the nesting party's spread using three categories 1 =<10 meters, 2 = 10-40 meters, and 3 = >40 meters. For example, if all the identifiable nests in the nesting group were within less than ten meters, we categorized the nesting party as a one. We also measured the distances between all nest in a nesting party. We then created a nesting map that we could

use to link a fecal sample to an individual nest. We completed genetic analyses to confirm the identification of individual bonobos using a method similar to Brand et al., (2016). We combined these nesting maps with individual's genetic identifications to create a social group determination (Figure 3.1).

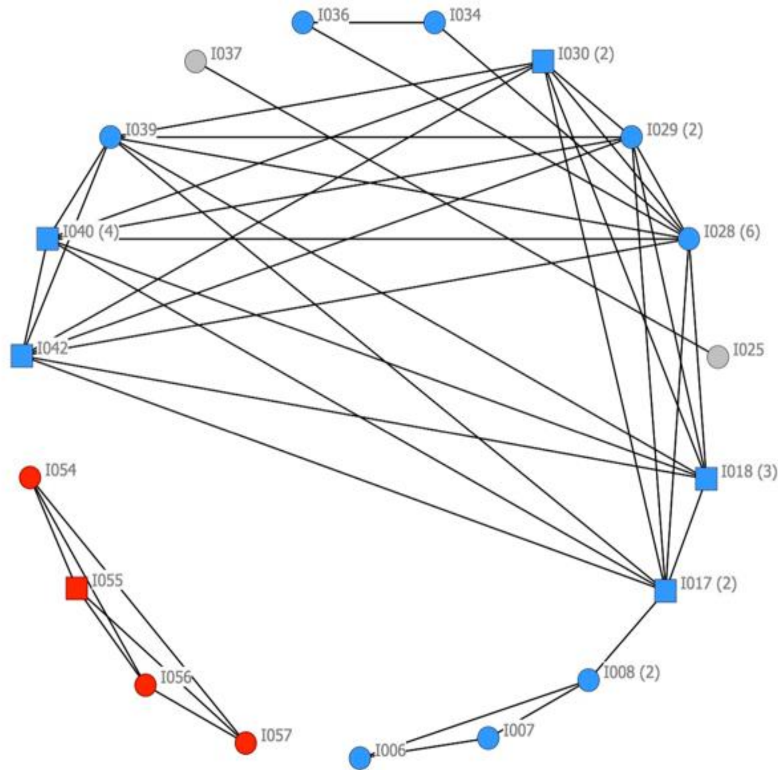

Figure 3.1. Association map from Brand. et al., (2016). For our analyses we used the community membership determinations of the genetic capture/recapture method. Most individuals sampled belonged to the blue group, which corresponds to the Tolende community, while the red individuals belong to the Nyombenyombe community and the gray individuals which likely belong to the Ota community. There was one individual in the sample for which that we were unable to determine community membership.

With this genetic identification, we also used a sexing assay to determine if individuals were male or female. Each nest is linked to the results of the sequencing data and stable isotopic values from the corresponding fecal sample and analyzed to determine if there is a link between social group and the composition and diversity of the gut

microbiome.

3.2.3 16S rRNA sequencing data

We analyzed the gut microbiomes, $\delta^{13}C$, and $\delta^{15}N$ data for 18 bonobo fecal samples, from separate individuals, collected into tubes of RNA Later. DNA were extracted from each fecal sample using QIAamp DNA Mini Stool kit (QIAGEN) in the Molecular Anthropology lab at the University of Oregon. DNA was then quantified using a Qubit dsDNA HS Assay Kit protocol using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Samples containing at least 1.0 ng/µl were sent for sequencing of the V-4 hypervariable region of the bacterial 16S ribosomal RNA at the Genomics and Cell Characterization Core Facility at the University of Oregon. Samples were barcoded using Illumina 515F and 806R primers and these barcodes were targeted during amplification (Illumina, San Diego, CA). Barcoded amplicons were sequenced up to 150 base pair reads on an Illumina NextSeq platform (Illumina, San Diego, CA). Resulting sequences were then demultiplexed and denoised using DADA2 (Caporaso et al., 2010). Operational taxonomic units were assigned using the QIIME2 pipeline (*ibid*).

3.2.4 Stable isotope values

For the isotopic data, samples were desiccated in the field, ground, weighed, and combusted in an elemental analyzer to measure the carbon and nitrogen abundances. The relationship between stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values have been used to supplement behavioral observations and are an additional tool in quantifying diets, given that these values reflect dietary behavior. The stable isotope values were obtained following the methods in Loudon et al., (2019). Plant organs were collected at various heights understory, mid-canopy, and high canopy. Samples were ground into a powder and weighed to ~1.5mg, placed in tin capsules, and combusted in an elemental analyzer. Carbon and nitrogen isotope abundances were quantified using a flow-through inlet system on a continuous flow isotope ratio mass spectrometer. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are expressed using the delta (δ) notation in parts per thousand or permil

(‰) relative to the Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 (AIR) standards.

3.2.5 Model predictors

We tested social group, sex, food item, $\delta^{13}C$ and $\delta^{15}N$ as our predictor variables. Social group was determined from the results of the genetic-capture protocol. We had thirteen individuals in from the Tolende community, three individuals from the Nyombenyombe community, one individual from the Ota community, and one individual whose social group was unable to determined (Figure 3.2). Sex was determined through observation of individuals at the time of collection and then later verified with the results of the sexing assay. We had six males and twelve females in our sample. Food item refers to the primarily undigested food item found in the fecal sample. This was taken as a note on the day of collection. $\delta^{13}C$ and $\delta^{15}N$ values were determined by Loudon et al., (2019).

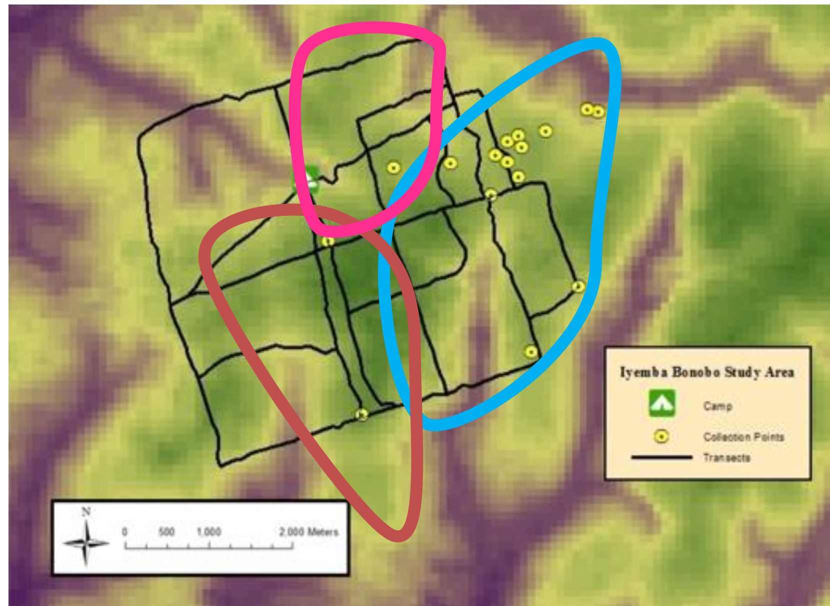

Figure 3.2. A map of the sampling locations based on a figure from Brand et al. (2016). Lines are drawn where each community's range extends. Tolende is in blue, Nyombenyombe is in orange, and Ota is pink.

3.2.6 Data analysis

Statistics were run in R version 4.0.2. For alpha diversity, Shannon's index was calculated in R using the 'vegan' package. Shannon's index is a common measure of alpha diversity. We calculated the Shannon index as $H' = \sum[p_i \log p_i]$, where p_i is the proportion of species *i* in the sample area (Pielou, 1974). We ran linear models against the variables social group, sex, food item, $\delta^{13}C$ and $\delta^{15}N$, and Shannon's index to study alpha diversity or within individual diversity. To examine the relationship between stable isotope values on bonobo gut microbiome, we ran permutational multivariate analysis of variance (PERMANOVA) with 999 permutations using the 'adonis' function in the 'vegan' R package. PERMANOVAs were run on beta diversity using a Bray-Curtis, Jaccard's, and Chao dissimilarity matrix with social group, sex, food item, $\delta^{13}C$ and $\delta^{15}N$ values as predictor variables (Table 3.1). It is of note that PERMANOVAs factor in the order in which variable are entered into the model, so we ran all the variations that we could. We also ran abundance models to test whether a member of the gut microbiota varies with one of our predictor variables. We used a general linear model with a Poisson error distribution.

3.3 Results

During our initial examination of the data, we identified two samples I039 that fell 2.5 standard deviations away from the mean. Samples had on average 52,371.05 ± 24,888.70 reads per sample and sample I039 only had 475 reads. Additionally, it is of note that sample I022 feel barely within two standard deviations from the mean with 2787 reads, but because this sample fell within two standard deviations of the mean we included it for all analyses (Figure 3.3). For the rest of the analyses, we ran all analyses without sample I039.

Table 3.1. Predictor variables included in analysis and how they were collected.

Predictor variable	Categorical/Continuous	Method of collection	Lab analysis
Social group	Tolende. Nyombenyombe, Ota, unknown	Genetic Capture/Recapture results	-
Sex	Male, female	Behavioral observations corroborated with genetic sexing assay	Sexing assay
Food item	Bonenge (fruit), Fiber, Meat, Seed (fruit)	Observation of fecal sample	-
$\delta^{13}C$ values	Continuous value	Non-invasive fecal sample collection	Combusted using an elemental analyzer
$\delta^{15}N$ values	Continuous value	Non-invasive fecal sample collection	Combusted using an elemental analyzer

When sample I039 was collected nothing was noted as out of the ordinary about the individual bonobo. Sample I039 was the fourth sample collected out of a nesting group of eleven. Nothing out of the ordinary or stood out in the behavioral observations for this nesting group in the behavioral observations that would suggest an individual sick or in distress. We conclude that the issue with sample I039 was in the downstream laboratory analyses, perhaps an extraction or sequencing mistake.

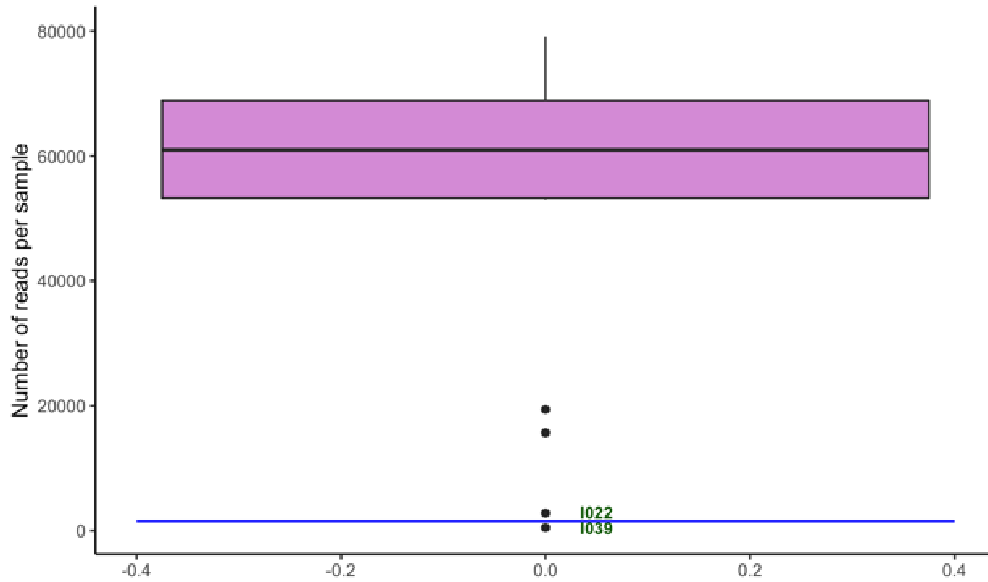

Figure 3.3 Boxplot of read counts. Sample 1039 represents a sample that is more than two standard deviations from the mean, while sample I022 falls just within two standard deviations from the mean. The large black line represents the mean reads per sample and the blue line represents two standard deviations from the mean.

Alpha diversity

3.3.1 $\delta^{13}C$ and $\delta^{15}N$ results

Females had slightly higher δ^{13}C values, than males. This difference was not significant using an ANOVA [F (1,16) =0.132, p = 0.721] (Figure 3.4A). Females had slightly higher δ^{15}N values, than males. This difference was not significant using an ANOVA [F (1,16) = 0.076, p = 0.786] (Figure 3.4B).

3.3.2 Alpha diversity

By plotting against Shannon's diversity, a measure of within individual variation, we are determining the factors that explain within host community diversity in bonobos.

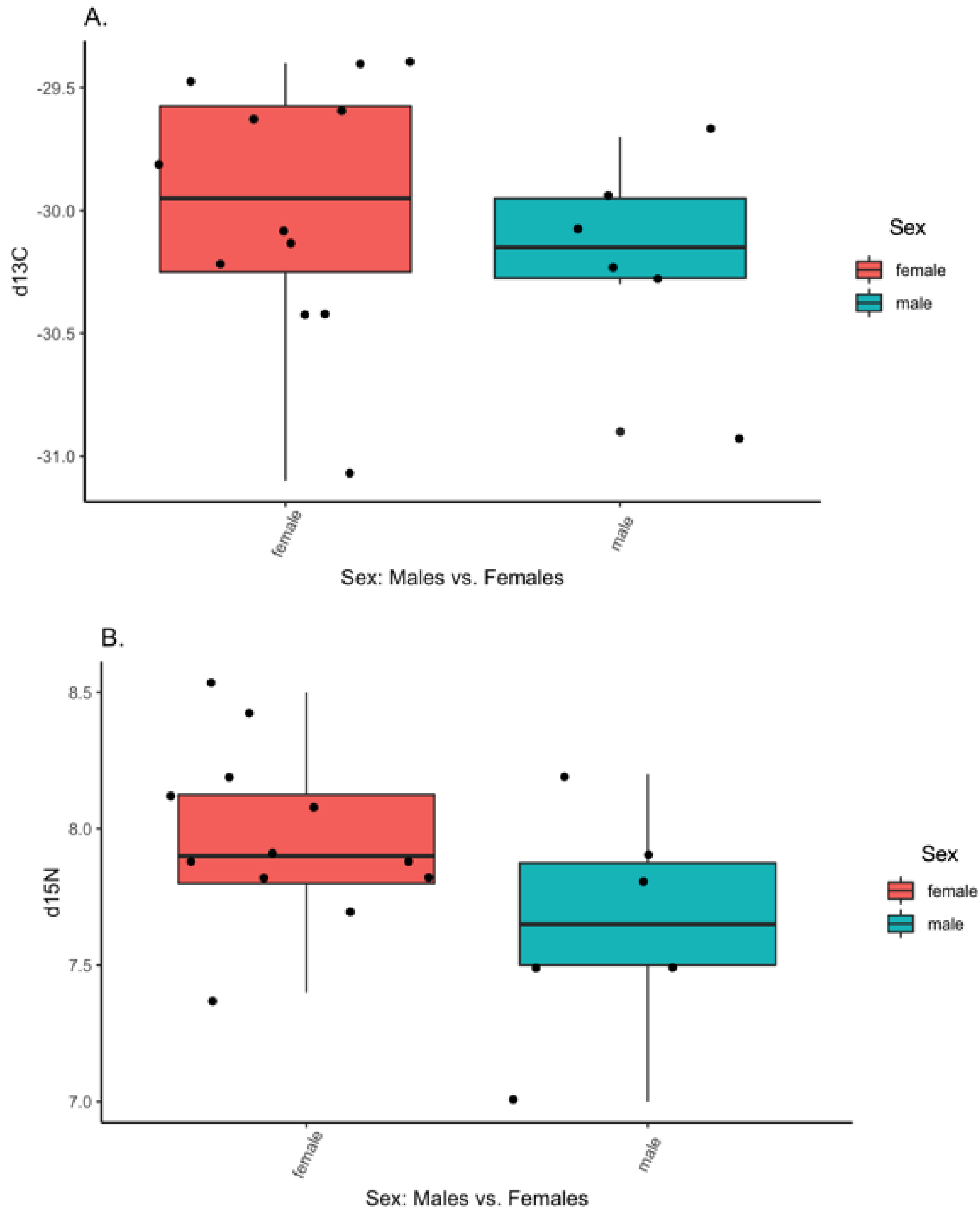

Figure 3.4. $\delta^{13}$and δ^{15}N values plotted against sex.4. A. δ^{13}C values plotted against sex. Females have a slightly higher δ^{13}C value than males, however this difference is not significant. B. δ^{15}N values plotted against sex. Females have a slightly higher δ^{15}N value than males, however this difference is not significant.

The predictors of community membership, sex, food item, δ^{13}C and δ^{15}N values were not significant in explaining alpha diversity as measured by Shannon's index (Figure 3.5). Males have slightly higher alpha diversity than females although this result is not

significant. (Figure 3.6). None of our explanatory variables significantly explain alpha diversity or within host diversity of the microbial community among bonobos.

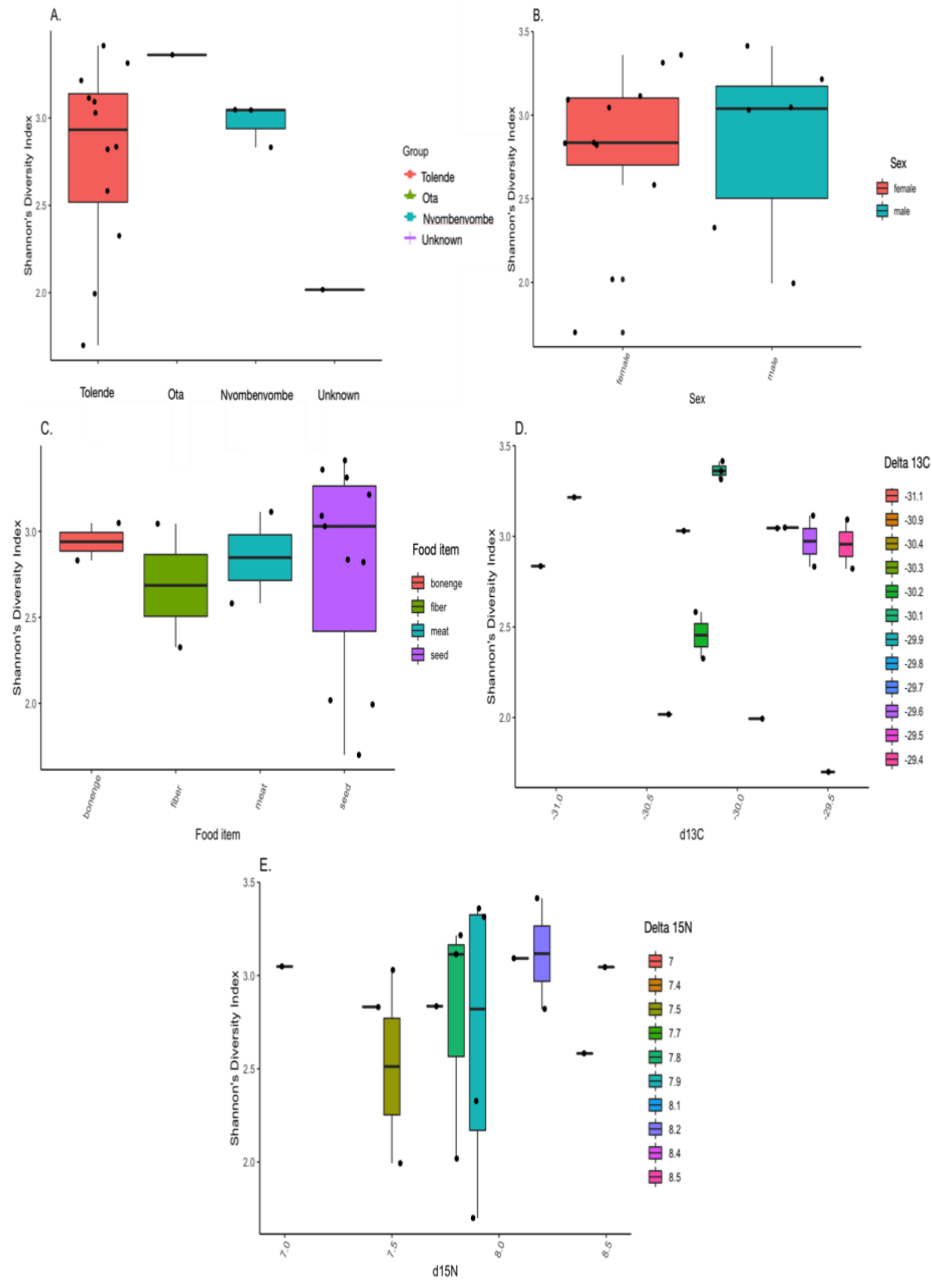

Figure 3.5. Shannon's diversity index against A. community membership, B. sex, C. food item, D. δ¹³C and E. δ¹⁵N values. They are all non-significant.

3.3.3 Beta diversity

We calculated Bray-Curtis, Jaccard's, and Chao dissimilarity matrices to use in the PERMANOVAs. The results were all not significant for models containing only predictors social group, sex, food item, $\delta^{13}C$ and $\delta^{15}N$ values. When the predictor variable, sex, was entered into the model before $\delta^{13}C$ and $\delta^{15}N$ values, $\delta^{13}C$ was a significant explanatory variable explaining 14.5% - 19.0% of the variation in the bonobo gut microbiota. For the model order, sex, social group, $\delta^{13}C$ and $\delta^{15}N$ values social group explained 16.3% and $\delta^{13}C$ values explained 14.5%. When $\delta^{13}C$ and $\delta^{15}N$ values were entered into the model before the other predictor variables, sex was a significant predictor explaining 12.9% – 14.7% of the variation (Figure 3.7; Table 3.3).

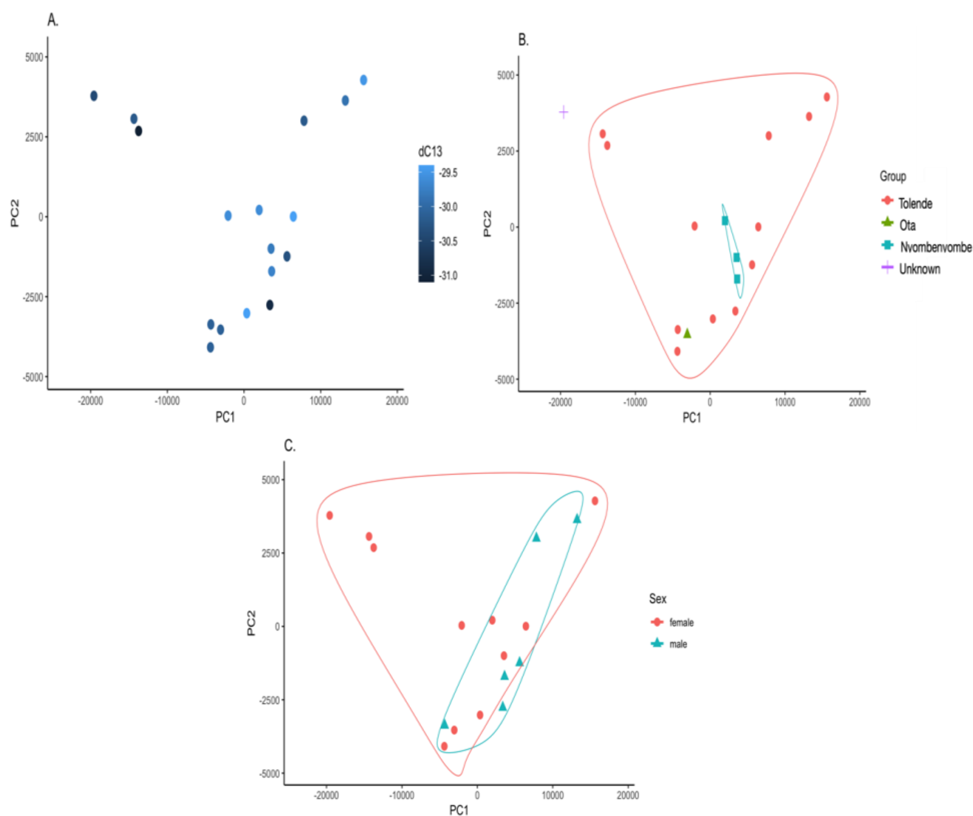

Figure 3.6. PCOA of samples clustered by predictor variable A. $\delta^{13}C$ significantly explains 14.5% - 19.0% of beta diversity for bonobo gut microbiota. B. Social group significantly explains 16.3% of beta diversity for bonobo gut microbiota. C. Sex significantly explains 12.9% – 14.7% of beta diversity for bonobo gut microbiota.

Table 3.2 Results for the significant models from the PERMANOVAs. Social group, Sex, and $\delta^{13}C$ were significant predictors of bonobo gut microbiota composition.

Beta diversity index	**Factor**	***df***	**Sums of squares**	**Mean squares**	***F***	***R*²**	***P***
Bray Curtis dissimilarity	Sex	1	0.14564	0.145642	1.6473	0.07980	0.110
	Social group	**3**	**0.29869**	**0.298695**	**3.3784**	**0.16365**	**0.035** *
	d13C	**1**	**0.26628**	**0.266282**	**3.0118**	**0.14589**	**0.042** *
	d15N	1	0.05359	0.053594	0.6062	0.02936	0.647
	Sex	1	0.14564	0.14564	1.4574	0.07980	0.181
	d13C	**1**	**0.34766**	**0.34766**	**3.4791**	**0.19048**	**0.041** *
	d15N	1	0.03278	0.03278	0.3281	0.01796	0.882
	d13C	1	0.23580	0.235803	2.56361	0.12919	0.055
	d15N	1	0.05344	0.053440	0.58099	0.02928	0.639
	Social group	3	0.20604	0.206037	2.24000	0.11289	0.106
	Sex	**1**	**0.26893**	**0.268932**	**2.92378**	**0.14735**	**0.035** *
	Food item	3	0.23314	0.077712	0.84487	0.12773	0.525
Jaccard's	Sex	1	0.20291	0.20291	1.57210	0.07192	0.109
	Social group	**3**	**0.91697**	**0.30566**	**2.36815**	**0.32501**	**0.037** *
	d13C	**1**	**0.33384**	**0.33384**	**2.58647**	**0.11833**	**0.039** *
	d15N	1	0.07692	0.07692	0.59597	0.02726	0.737
	Sex	1	0.20291	0.20291	1.26317	0.07192	0.234
	d13C	**1**	**0.45638**	**0.45638**	**2.84104**	**0.16176**	**0.021** *
	d15N	1	0.07377	0.07377	0.45926	0.02615	0.885
	Social group	3	0.95272	0.31757	2.51458	0.33768	0.057
	Food item	3	0.37730	0.12577	0.99583	0.13373	0.409

Table 3.2 (continued).

	Sex	1	0.11652	0.11652	0.92262	0.04130	0.470
	d13C	**1**	**0.36179**	**0.36179**	**2.86465**	**0.12823**	**0.031 ***
	d15N	1	0.12896	0.12896	1.02111	0.04571	0.415
	d13C	**1**	**0.32392**	**0.32392**	**2.50964**	**0.11481**	**0.030 ***
	d15N	1	0.10543	0.10543	0.81683	0.03737	0.532
	Social group	3	0.85047	0.28349	2.19641	0.30144	0.069
	Sex	1	0.25082	0.25082	1.94330	0.08890	0.072
Chao	Sex	1	0.006358	0.0063585	1.9159	0.04549	0.281
	Social group	**3**	**0.082875**	**0.0276251**	**8.3239**	**0.59287**	**0.029 ***
	d13C	1	0.010859	0.0108592	3.2721	0.07768	0.098
	d15N	1	0.006507	0.0065071	1.9607	0.04655	0.202
	Sex	1	0.006358	0.0063585	0.79403	0.04549	0.516
	d13C	1	0.015965	0.0159652	1.99369	0.11421	0.202
	d15N	1	0.013361	0.0133615	1.66854	0.09558	0.249
	Social group	3	0.086355	0.0287851	8.1906	0.61776	0.053
	Food item	3	0.009183	0.0030612	0.8710	0.06570	0.415
	Sex	1	0.002832	0.0028321	0.8058	0.02026	0.542
	d13C	1	0.011825	0.0118250	3.3647	0.08459	0.081
	d15N	1	0.004991	0.0049907	1.4201	0.03570	0.327
	d13C	1	0.012175	0.0121748	3.6685	0.08709	0.090
	d15N	1	0.004499	0.0044994	1.3558	0.03219	0.307
	Social group	**3**	**0.082135**	**0.0273784**	**8.2496**	**0.58757**	**0.041 ***
	Sex	1	0.007791	0.0077906	2.3474	0.05573	0.171

3.3.4 Abundance models

To test whether a genus of the gut microbiota varies with dietary stable isotopes, we ran abundance models on $\delta^{13}C$ and $\delta^{15}N$ values. 73 taxa co-varied with $\delta^{13}C$ after Bonferroni correction for multiple models. The results of the abundance models for $\delta^{15}N$

values after Bonferroni correction for multiple models 87 taxa co-varied with $\delta^{15}N$ (Table 3.4).

3.4 Discussion

Our results indicate there are sex-specific and social group patterns in the gut microbiota and diet of the Lomako forest bonobos, especially as it related to the $\delta^{13}C$ stable isotope values. $\delta^{13}C$ values are primarily differentiated by the consumption of C4, C3, and CAM plants in the diet (Crowley et al., 2010; Schoeninger, 2014). The most frequently consumed plants for this period and from later field season were 1. *Anthoclitandra robustior 2. Ficus* spp. 3. *Scropholoes zenkeri 4. Polyalthia suaveolens 5. Celtis mildbraedii*. Of these plants most *Ficus* spp. and many of the species of terrestrial herbaceous vegetation (THV) are considered to be plants utilizing a C3 photosynthetic pathway (*Isotopes: Advances in Research and Application: 2011 Edition,* 2012; Ting et al., 1987). Plants that use a C4 strategy tend to have ~12-13% higher $\delta^{13}C$ values while C3 plants tend to have lower $\delta^{13}C$ values (O'Brien, 2015). In our sample females had slightly higher $\delta^{13}C$ values, indicating a potentially more C4 plants in their diet. These results may indicate that females may use resources like *Ficus* spp. or THV to a lesser extent than males (Figure 4A). We suggest that differential resource consumption of *Ficus* spp. and THV between males and females may be driving the patterns in sex and $\delta^{13}C$ values we observed in the Lomako forest bonobo gut microbiota. However, in order to fully address this question, we need more data is needed to fully draw this conclusion.

Sex differences in gut microbiota are a well-established pattern (Amato et al., 2013; 2014; 2015). In captivity, these sex differences in gut microbiota have been found for macaques (*Macca mulatta*) (Joers et al., 2020). Additionally, in the wild this pattern of sex-differences in gut microbiota have been found for ring-tailed lemurs (*Lemur catta),* black howler monkeys (*Alouatta pigra*), yellow baboons (*Papio cynocephalus*), and chimpanzees (*Pan troglodytes*) (Amato et al., 2014; Bennett et al., 2016; Degnan et al., 2012; Ren et al., 2016; Tung et al., 2015).

Table 3.3. Taxa that co-varied with $\delta^{13}C$ and $\delta^{15}N$ values. The darker the blue the higher the significance value. The lighter blues indicate taxa that were significant, but not after the Bonferroni correction 0.05/106 = 0.00047.

Genus	dC13	dN15
Methanobrevibacter	0.655	7.62E-07
Methanosphaera	0.482	0.616
unk Methanomassiliicoccaceae	2.00E-16	2.00E-16
vadinCA11	2.00E-16	2.00E-16
unk Bacteria	2.00E-16	2.11E-08
unk Koribacteraceae	1	1
unk Actinobacteria	2.00E-16	2.00E-16
unk Bifidobacteriaceae	2.00E-16	2.00E-16
Bifidobacterium	0.127	2.00E-16
unk Coriobacteriaceae	0.223	2.00E-16
unk Coriobacteriaceae	2.00E-16	2.00E-16
Adlercreutzia	0.000528	2.00E-16
Collinsella	3.04E-06	2.00E-16
Slackia	2.74E-10	5.53E-14
unk OPB41	0.2567	0.205
unk Bacteroidales	2.72E-13	2.93E-08
unk Bacteroidales	0.701	0.928
unk Bacteroidales	0.544	2.00E-16
Bacteroides	2.00E-16	0.037
Paludibacter	2.00E-16	2.00E-16
Parabacteroides	2.00E-16	2.00E-16
unk Prevotellaceae	0.617	2.00E-16
Prevotella (Prevotellaceae)	2.00E-16	2.00E-16
unk RF16	1.17E-06	2.00E-16
unk S24-7	2.00E-16	2.00E-16
unk Paraprevotellaceae	2.00E-16	2.00E-16
YRC22	2.00E-16	2.00E-16
Prevotella (Paraprevotellaceae)	2.00E-16	2.00E-16
SHD-231	2.00E-16	2.00E-16
unk S2	2.00E-16	7.00E-14
unk Streptophyta	2.00E-16	2.00E-16
Fibrobacter	2.00E-16	2.00E-16
unk Firmicutes	4.38E-06	1.66E-07

Table 3.3 (continued).

Lactococcus	0.0608	0.999
Streptococcus	0.475	0.61
unk Clostridia	4.38E-05	0.729
unk Clostridiales	2.48E-14	2.00E-16
unk Clostridiales	2.00E-16	0.000544
unk Christensenellaceae	0.0203	3.14E-05
unk Christensenellaceae	1.50E-05	1.04E-06
unk Clostridiaceae	0.431	2.00E-16
02d06	0.00429	0.999
Clostridium	2.00E-16	2.00E-16
unk Lachnospiraceae	2.00E-16	2.00E-16
unk Lachnospiraceae	2.00E-16	2.00E-16
Anaerostipes	0.00416	0.35399
Blautia	3.38E-06	2.00E-16
Butyrivibrio	2.00E-16	2.00E-16
Clostridium	5.49E-15	2.00E-16
Coprococcus	2.00E-16	2.00E-16
Dorea	1.68E-15	1.70E-09
Lachnobacterium	1.03E-05	2.55E-15
Lachnospira	1.04E-12	2.00E-16
Oribacterium	0.0123	2.00E-16
Roseburia	2.00E-16	2.00E-16
Ruminococcus	0.00156	2.00E-16
Peptococcus	6.31E-16	2.00E-16
unk Ruminococcaceae	2.00E-16	6.08E-05
unk Ruminococcaceae	2.00E-16	2.00E-16
Anaerofilum	0.254	0.868
Anaerotruncus	1.62E-12	0.999
Faecalibacterium	2.00E-16	7.83E-07
Oscillospira	2.00E-16	2.00E-16
Ruminococcus	0.88	2.00E-16
unk Veillonellaceae	0.201	0.727
unk Veillonellaceae	2.00E-16	2.45E-06
Anaerovibrio	2.00E-16	0.03
Dialister	2.00E-16	2.00E-16
Phascolarctobacterium	2.00E-16	0.000464
unk Mogibacteriaceae	0.775	2.00E-16
Mogibacterium	0.63	5.44E-13

Table 3.3 (continued).

Erysipelotrichaceae	3.38E-16	2.00E-16
Bulleidia	2.00E-16	2.00E-16
RFN20	2.00E-16	2.00E-16
Eubacterium	0.192	6.23E-12
p-75-a5	2.00E-16	2.81E-08
unk Victivallaceae	0.992	8.09E-09
unk R4-45B	0.0505	2.00E-16
unk Proteobacteria	2.84E-12	0.006922
unk Alphaproteobacteria	0.0493	2.00E-16
unk RF32	2.00E-16	1.12E-05
unk Rickettsiales	0.532	1.23E-05
unk Rickettsiales	6.08E-15	2.00E-16
Phytophthora	3.17E-05	1.06E-07
unk Betaproteobacteria	2.00E-16	1.71E-07
unk Burkholderiales	2.00E-16	3.73E-16
unk Burkholderiales	2.00E-16	2.00E-16
Sutterella	2.00E-16	9.81E-13
unk Oxalobacteraceae	0.374	1.33E-09
unk Desulfovibrionaceae	4.34E-07	1.01E-06
Bilophila	1.04E-05	4.65E-10
Desulfovibrio	2.00E-16	2.00E-16
Campylobacter	2.00E-16	2.00E-16
Flexispira	6.11E-07	1.24E-08
Succinivibrio	2.00E-16	3.55E-09
unk Enterobacteriaceae	0.000148	8.22E-08
Escherichia	1.11E-12	0.0532
Aggregatibacter	0.73	0.0158
Acinetobacter	0.0486	0.000403
Treponema	0.359	2.37E-13
unk Anaeroplasmataceae	0.272	2.00E-16
unk RF39	2.00E-16	2.00E-16
unk ML615J-28	0.000372	7.65E-05
unk HA64	3.01E-10	0.438
unk Cerasicoccaceae	2.00E-16	2.00E-16
unk RFP12	1.06E-12	2.00E-16

These patterns may be driven by social behavior which suggests that the more affiliative sex experiences horizontal microbial transfer at a greater rate than the less affiliative sex (Amato et al., 2017). Among bonobos, females tend to be the more affiliative sex (Furuichi & Hashimoto, 2002; Hashimoto & Furuichi, 2015; Parish et al., 2000; White, 1998; White & Wood, 2007). Female bonobos also exhibit high levels of socio-sexual behaviors compared to chimpanzees (*Pan troglodytes*) including gentio-genito (GG) rubbing that has been thought to be important in maintaining female social bonds (Brand et al., 2018; Gruber and Clay, 2016; Hohmann and Fruth, 2003b). Our results suggest that there may be sex-specific differences in gut microbiota, perhaps due to differences in female affiliation or diet, but more data is needed to full address this claim.

While these patterns of sex differences in primate gut microbiota exist, there have been many studies that look at differences between social groups. Distinct gut microbial communities were found in ring-tailed lemur belonging to different social groups (Bennett et al., 2016). In black and white colobus (*Colobus vellerosus*) social group was the second best explanatory variable explaining 18-28% of gut microbial composition in gut microbiota composition (Wikberg et al., 2020). Additionally, in black and white colobus distinct gut microbial profiles can emerge less than a year after social groups fission (Goodfellow et al., 2019). Across different primates, social group explains 18.6% of the total variation in yellow baboon's gut microbiota, 11.4-15.4% in Verreaux's sifakas (*Propithecus verreauxi*), and 5.8% among geladas (*Theropithecus gelada*) (Springer et al., 2017; Trosvik et al., 2018; Tung et al., 2015). Our results for bonobos suggest that social group explains 16.3% which only a slightly higher amount of variation than Verreaux's sifakas another primate that exhibits female dominance. These results suggest that there may be social group specific differences in gut microbiota, perhaps due to female priority of access to food resources and female dominance (Boesch, Hohmann, and Marchant, 2002; Parish, De Waal, and Haig, 2000; White, 1996b; 1996a; White and Wood, 2007).

The relationship between diet and the gut microbiota has been well documented across different taxa (Amato et al., 2020; Bruorton, Davis, and Perrin, 1991; Burns et al., 2017; Hicks et al., 2018; Li et al., 2016; Stephens et al., 2015). Many of these studies, in

primates, examining the diet and the gut microbiota have occurred in captive primates which have highly disrupted gut microbial communities (Amato et al., 2013; Hale et al., 2018), or they occur on wild populations using behavioral observations to quantify diets (Amato et al., 2015; Gomez, Rothman et al., 2016; Hicks et al., 2018; Trosvik et al., 2018). While behavioral observations are the gold standard in primatology for quantifying diet, there are populations where detailed behavioral observations are difficult. Additionally, the process of habituation is a long process therefore using non-invasive measures of diet allow primatologist to quantify diets and gut microbiota as fecal samples are easily obtained. We took advantage of these easily obtainable fecal samples to examine non-invasive measures of diet and gut microbiota on paired fecal samples, where the $\delta^{13}C$ and $\delta^{15}N$ values and the gut microbial community composition data came from the same fecal sample.

This approach allowed us to gain a snapshot of what factors are structuring the Iyema bonobos' gut microbiota in a non-invasive way. Intermittent follows on un-provisioned bonobos are difficult and different populations have different historical contexts including anthropogenic threats like hunting or war (Waller and White, 2016). There are cases where full habituation of primate populations is not possible especially when populations face hunting pressure (Allan, Bailey, and Hill, 2020). The ethical dilemma for these populations is if habituated the fear response to humans diminishes making populations more vulnerable to human hunting or in areas that are undergoing civil conflict (Green and Gabriel, 2020). The advantage of the methods we present in this paper is the non-invasive nature of our approach. We were able to get a relative snapshot of the Lomako forest diet and gut microbiota on bonobos that were not fully habituated.

CHAPTER IV

A COMPARISON OF FECAL GLUCOCORTICOID METABOLITE CONCENTRATION AND GUT MICROBIOTA DIVERSITY IN BONOBOS (*PAN PANISCUS*)

4.1 Introduction

The gastrointestinal tract microbiome plays essential roles in host nutrition and health (Allaband et al., 2019; Dantas et al., 2013; Dillon et al., 2005; Koch and Schmid-Hempel, 2011). Physiological stress, hereafter referred to as stress, has been found to have a negative effect on the gastrointestinal tract and associated microbiome. Stress may mediate host processes and commensal gut microbiota (reviewed in Keay et al., 2006). Stress has been linked to a decrease in the diversity of species found in the gut microbiome (Konturek et al., 2011). The ability of a host and its gut microbiome to potentially communicate is vital to the survival of both. There is an increasing interest in how host produced hormones, like fecal glucocorticoids that are a measure of stress, interact with the gut microbiota (Sandrini et al., 2015). This relationship remains unclear among primates and understanding how an individual primate communicates with its gut microbes remains to be determined.

The relationship between the gut microbiome and hormonal systems has far-reaching implications for host physiology which helps elucidate co-evolutionary forces (Davenport et al., 2017). Maternal stress increased *Lactobacillus* microbes found in captive macaque (*Macaca mulatta*) gut microbiomes (Bailey, 2009; 2012; Bailey and Coe, 1999). More recently, hormones such as glucocorticoids have been hypothesized to influence primate gut microbiomes (Mallott et al., 2020). Recent evidence from gorillas found no relationship between fecal glucocorticoids and gut microbiome composition but found a positive correlation between family *Anaerolineaceae,* genus *Clostridium,* and genus *Oscillibacter* suggesting that stress may select for certain types of bacteria within a gut microbiome (Vlčková et al., 2018). Additional investigations have attempted to understand the role of host reproductive hormones as a factor influencing the composition

and diversity of primate gut microbiota in leaf monkeys. In trying to understand primate evolution, primate gut microbiomes represent a major piece of the evolutionary puzzle (Amato, 2016). Yet how a host's gut microbiome responds to various stress-based fluctuations during short-term variation in stress remains to be examined in many primate taxa.

While there has been an increase in the number of investigations into primate gut microbiomes for some species, research questions regarding bonobo gut microbiota is relatively new (Liu et al., 2017). Comparisons across great ape microbiota found a correlation between phylogeny and gut microbiome composition (Ochman et al., 2010). Bonobos (*Pan paniscus*) undergo stressors that can be systematically measured, and they express many of the same life-history traits as humans (Gruber and Clay, 2016). Thus, bonobos represent good models to understand aspects of human development and physiology (de Waal, 2005; Jaeggi, Burkart, and Van Schaik, 2010; Parish, De Waal, and Haig, 2000; White, 1996b). The genetic similarity between humans and bonobos allows for a test of whether the pattern of decreasing microbiome diversity and increased stress found in humans holds up for a genetically similar species (Bailey, 2009; Gruber and Clay, 2016; King and Wilson, 1975). Due to this genetic similarity when patterns in how the stress and the gut microbiome are similar to humans and chimpanzees, we can conclude there is a *Pan*/human pattern. When they are different, then more research is needed to understand why a species that humans are so genetically similar to displays different patterns in how stress and the gut microbiome interact. Bonobos and other great apes exhibit a broader diversity in their gut microbes than populations of humans living across different societies in Africa (Moeller et al., 2016). Therefore, we need an understanding of how different bonobo populations vary from each other and from other primates. The only cross-site comparison of bonobo gut microbiomes concluded that malaria parasite infection did not affect gut microbiome composition (Liu et al., 2017). None of these studies investigated how stress affects the composition of bonobo gut microbiomes. Thus, how stress and the bonobo gut microbiota interact is not understood.

4.2 Methods

4.2.1 Study Camps

The research site is the Iyema field camp, located just north of the Lomako river at (00°55) North, 21°06) East) in the Democratic Republic of Congo (DRC) (Figure 4.1). The site is mostly covered by primary forest in *terra firma* soil with some swamps (Cobden 2014; Dupain et al. 2000). For data collection, we followed bonobos to their night nests as part of the Antwerp Zoo's ongoing habituation efforts from June 2014 – July 2014. Night nest locations were marked, and each nesting site revisited the next morning. We identified each bonobo as it exited the nest and collected 10 ml of fecal sample into 50 mL tubes with 10 ml of RNALater for each individual in the nesting party. While there is debate about whether RNALater is the best sample preservation method compared to freezing fecal samples for examining non-human primate (NHP) gut microbiomes. Many scientists argue that freezing fecal samples is the best way to examine gut microbial communities, however due to the remote nature of many primate sites freezing is not always possible or practical. Several research groups have demonstrated that RNALater does not significantly alter the results for gut microbial community composition and diversity when used as a sample preservative, and give consistent results with freezing a fecal sample (Blekhman et al., 2016; Hayakawa et al., 2018). The samples were stored in a cool, dry place until they were shipped to the Molecular Anthropology Laboratory at the University of Oregon. They were stored in a minus 20°C freezer until extraction. The remainder of each fecal sample after collection in RNALater was brought back to camp, dried using a camp stove, and placed into bags with desiccant for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis and fecal glucocorticoid analysis. Thus, for each fecal sample, we can obtain data on gut microbiota composition and diversity and fecal glucocorticoid metabolite concentration (FGMC).

4.2.2 Data Collection and Analysis

We collected eighteen paired fecal samples that were first stored in tubes with RNALater with the remaining portion of the fecal sample then dried for hormonal

analyses, as a part of the initiation of genetic demographic monitoring (Brand et al., 2016). To evaluate FGMC, we used eighteen dried fecal samples in ELISA assays to quantify cortisone as a measure of FGMC. We used the Arbor Assay's DetectX® Corticosterone Enzyme Immunoassay Kit as it is designed to be used on dried fecal samples.

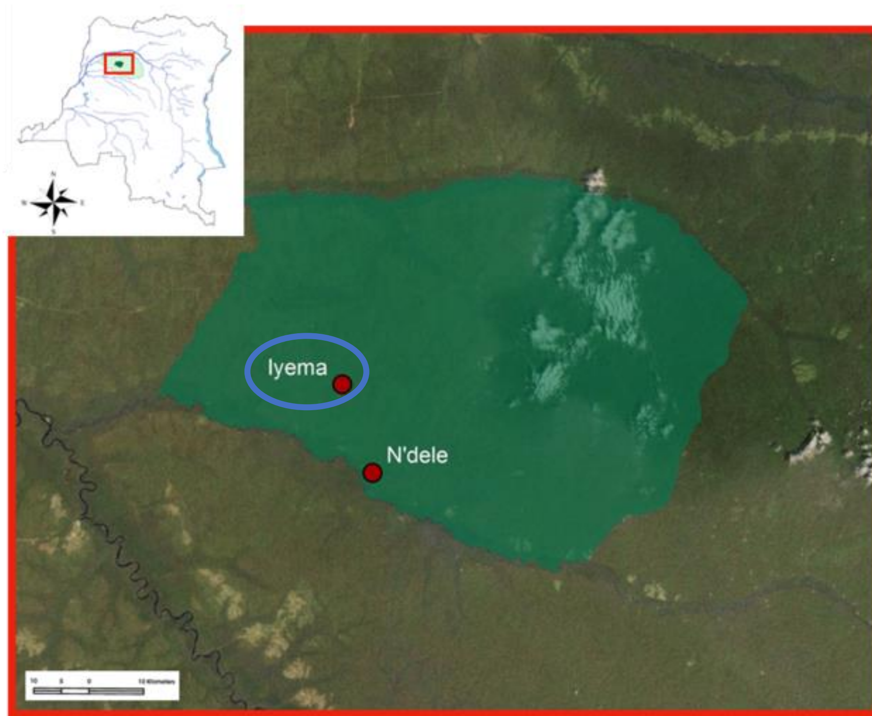

Figure 4.1. Map of Iyema field site circled in blue Lomako, DRC.

The Corticosterone Enzyme Immunoassay Kit measures the glucocorticoid cortisone, and standard curves have been generated for this assay kit (Arbor Assay's DetectX®). We included known controls provided for Cincinnati Zoo bonobos for each plate run. Corticosterone is known to be related to chronic stress and is more closely tied to dietary stress rather than acute stress (Mason, Myers, and Kendall, 1936; Mason, Hoehn, and Kendall, 1938). Since we are measuring corticosterone, we are capturing the stress an individual experienced in the preceding forty-eight hours from when the fecal sample was collected (Millspaugh & Washburn, 2004). It must be noted that due to the fact we were

not watching the bonobos from waking up to going to sleep, we were not able to determine the cause of this stress. Corticosterone has been associated with dietary stress rather than social stress but we cannot rule out social stress as a factor (Mason, Myers, and Kendall, 1936; Mason, Hoehn, and Kendall, 1938). Fecal samples were ground to a powder using a mortar and pestle, weighed out to the protocol's recommended ≥ 0.2 g. of fecal material, avoiding any plant or partially digested food material. Samples were then diluted (1:4) in assay buffer supplied in the 96-well DetectX® Corticosterone Enzyme Immunoassay Kit from Arbor Assays (catalog no. K017-H5) and assayed according to the kit manufacturer's instructions. The manufacturer of this kit reported the detection limit for this assay as 100 pg/mL. To control for shifts in circadian rhythm for FGMC, we used those samples collected under night nests to make sure all bonobo samples are from the same time point. All plates were read using a BioTek microplate reader and analyzed with Gen5 software version 2.0. For the FGMC controls, 100 µl aliquots of re-constituted fecal sample from 10 zoo control samples were pooled, diluted (1:4) in assay buffer, and divided into seven aliquots. We then spiked six of the aliquots with 100 µl of standards such that each aliquot of sample received one of the 6 concentrations of standard (1000, 500, 250, 125, 62.5, 31.2 pg/ml). One aliquot was left neat. Both the spiked and neat aliquots were assayed according to kit instructions (Arbor Assay's DetectX®). We log transformed our raw FGCM to better fit the assumptions of normality (Figure 4.2).

To evaluate gut microbiome composition samples were selected for 16S rRNA library preparation and sequenced on an Illumina NextSeq platform. We used the RNALater fecal samples to extract and sequence microbial DNA. Microbial DNA was extracted from each fecal sample using the QIAamp PowerFecal DNA kit (QIAGEN) in the Molecular Anthropology lab at the University of Oregon. Negative controls were included in extraction batches to test for contamination. Microbial DNA was quantified using a Qubit dsDNA HS Assay Kit protocol using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Samples containing at least 1.0 ng/µl were prepared and sent for sequencing of the V4 hypervariable region of the bacterial 16S ribosomal RNA at the Genomics and Cell Characterization Core Facility at the University of Oregon. We diluted 200 ng of DNA in ten µl of H_2O and PCR amplified. Samples were then be

barcoded using Illumina 515F and 806R primers, and these barcodes were targeted during amplification (Illumina, San Diego, CA). For the amplification reactions one µl DNA, 1.25 µl of 10 µM primer mix, 10.25µl H_2O, and 12.5µl NEB Q5 hot start 2× Master Mix was added to each sample. The thermal cycling consists of initial denaturing at 98°C for 0:30, 20-30 cycles of 98°C for 0:10, 61°C for 0:20, 72°C for 0:20, and a final extension of 72°C for 2:00. PCR products were cleaned using Ampure XP beads (Beckman Coulter, Brea, CA), quantified, and normalized. Barcoded amplicons were sequenced up to 150 base pair reads on an Illumina NextSeq platform (Illumina, San Diego, CA). The resulting sequences were demultiplexed and denoised using DADA2 (Caporaso et al., 2010). Operational taxonomic units (OTUs) were assigned using the QIIME2 pipeline (Caporaso et al., 2010). Quality filtering and assembly was done using the QIIME2 pipeline for microbial analyses (Caporaso et al., 2010). Samples had a mean frequency of 47,756 reads/sample and identified 123 operational taxonomic units (OTUs).

4.2.3 Model predictors

We tested nesting group and log transformed FGMC (here after referred to as FCMC) as our predictor variables with bonobo gut microbiota composition and diversity as the response variable. Nesting group was determined when the fecal sample was collected. The first thing bonobos do on waking is typically defecate over the edge of the nest, such that the distribution of morning fecal samples corresponds to the nest locations. After the bonobos had exited their nests, we would take a GPS waypoint at the site of each nest and fecal sample. We recorded the height and tree species for each nest and if the nest was under an open or closed canopy. For each nesting group or conglomerate of individual nests, we categorized the nesting party's spread using three categories 1 =<10 meters, 2 = 10-40 meters, and 3 = >40 meters. For example, if all the identifiable nests in the nesting group were within less than ten meters, we categorized the nesting party as a one. We also measured the distance between each nest in a nesting party. We then created a nesting map that we could use to link a fecal sample to an individual nest.

4.2.4 Data analysis

Statistics were run in R version 4.0.2. For alpha diversity, Shannon's index was calculated in R using the 'vegan' package. We calculated the Shannon index as $H' = \sum[p_i \log p_i]$, where p_i is the proportion of species i in the sample area (Pielou, 1974). We ran linear models against the individual variables nesting group, FGMC, and Shannon's index to study alpha diversity or within individual diversity. To examine the relationship between FGMC and bonobo gut microbiome we ran permutational multivariate analysis of variance (PERMANOVA) with 999 permutations using the 'adonis' function in the R package 'vegan.' PERMANOVAs use the calculated beta diversity from a Bray-Curtis dissimilarity matrix taking the model predictors, nesting group, and FGMC sequentially (Table 4.1). It is of note that PERMANOVAs factor in the order in which variable are entered into the model. We also ran abundance models to test whether a member of the gut microbiota varies with one of our predictor variables. We used a general linear model with a Poisson error distribution for the abundance models.

Table 4.1. Predictor variables included in analysis and how they were collected.

Predictor variable	**Categorical/Continuous**	**Method of collection**	**Lab analysis**
Nesting group	nest62814, nest7414, nest7514, nest7614, nest7114, forage7114	Observation of at fecal sample defecation	-
FGMC	Continuous log transformed value	Non-invasive fecal sample collection	ELISA assays to quantify corticosterone

4.3 Results

During our initial examination of the data, we identified one sample I039 that fell 2.5 standard deviations away from the mean. Samples had on average 52,371.05 ± 24,888.70 reads per sample and sample I039 only had 475 reads. Additionally, it is of note that sample I022 fell just barely within two standard deviations from the mean with 2787 reads, but because this sample fell within two standard deviations of the mean we included it for all analyses. For the rest of the analyses, we ran all analyses without sample I039 (see Chapter III; Figure 3.3).

4.3.1 Alpha diversity

The predictors nesting group and FGMC were not significant in explaining alpha diversity as measured by Shannon's index (Figure 4.2).

4.3.2 Beta diversity

We calculated Bray-Curtis dissimilarity matrices to use in the PERMANOVAs. The results were all not significant for models containing predictors nesting group and FGMC (Figure 4.3; Table 4.3). Nesting group there is clustering by nesting group for some of the smaller nesting groups but the largest nesting group on 7/5/14 with eight individuals encompasses all of the variation in the smaller nesting groups from other dates (Figure 4.3A). There is very little variation in FGCM and gut microbiota composition (Figure 4.3B).

4.3.3 Abundance models

To test whether a genus of the gut microbiota varies with dietary stable isotopes, we ran abundance models on logFGMC, and rawFGMC. The results of the abundance models for logFGMC after Bonferroni correction for multiple models 80 taxa co-varied with logFGMC (Table 4.3). The results of the abundance models for rawFGMC values

after Bonferroni correction for multiple models 76 taxa co-varied with rawFGMC (Table 4.3).

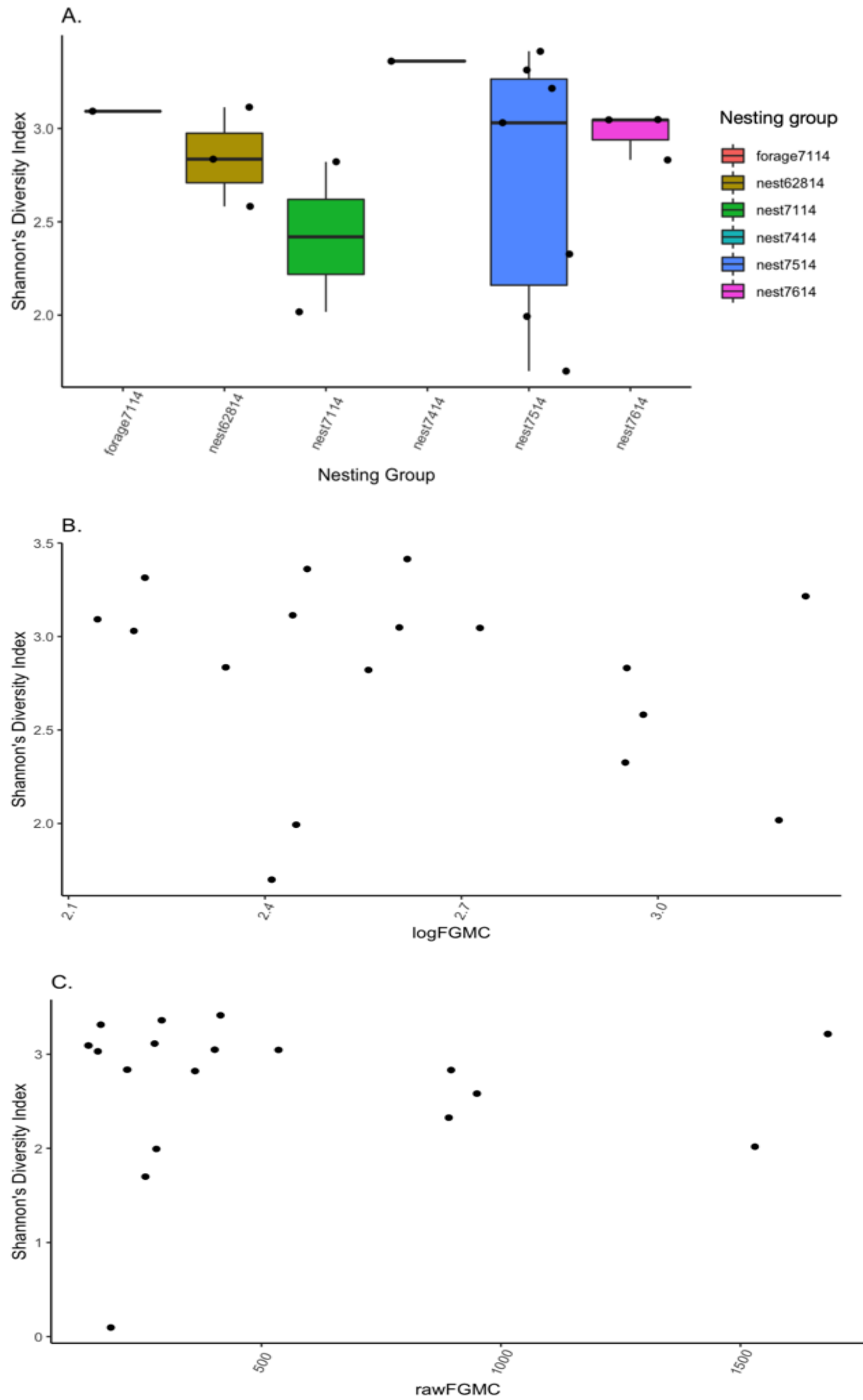

Figure 4.2. There are no significant differences in alpha diversity for A. nesting group, B. logFCMC (referred here after as FCMC), and C. rawFGCM.

Table 4.2. Results for the non-significant models from the PERMANOVAs. None of the predictor variables were significant in explaining beta diversity.

Beta diversity index	**Factor**	***df***	**Sums of squares**	**Mean squares**	***F***	***R*²**	***P***
Bray Curtis dissimilarity	Nesting group	5	0.83720	0.167441	1.8923	0.45870	0.155
	FGMC	1	0.10313	0.103132	1.1655	0.05651	0.280
	FGMC	1	0.18581	0.18580	1.7001	0.1018	0.147
	Nesting group	5	0.83720	0.167441	1.8643	0.4587	0.165
Jaccard dissimilarity	Nesting group	5	1.2078	0.24155	1.6467	0.42809	0.105
	FGMC	1	0.22358	0.22358	1.291	0.07925	0.229
	FGMC	1	0.22358	0.22358	1.5182	0.07925	0.173
	Nesting group	5	1.12512	0.22503	1.5280	0.39879	0.150
Chao dissimilarity	Nesting group	5	0.080229	0.0160458	2.9636	0.57394	0.116
	FGMC	1	0.023145	0.0231450	2.9764	0.16557	0.112
	FGMC	1	0.023145	0.0231450	4.2122	0.16557	0.069
	Nesting group	5	0.061695	0.0123391	2.2456	0.44135	0.170

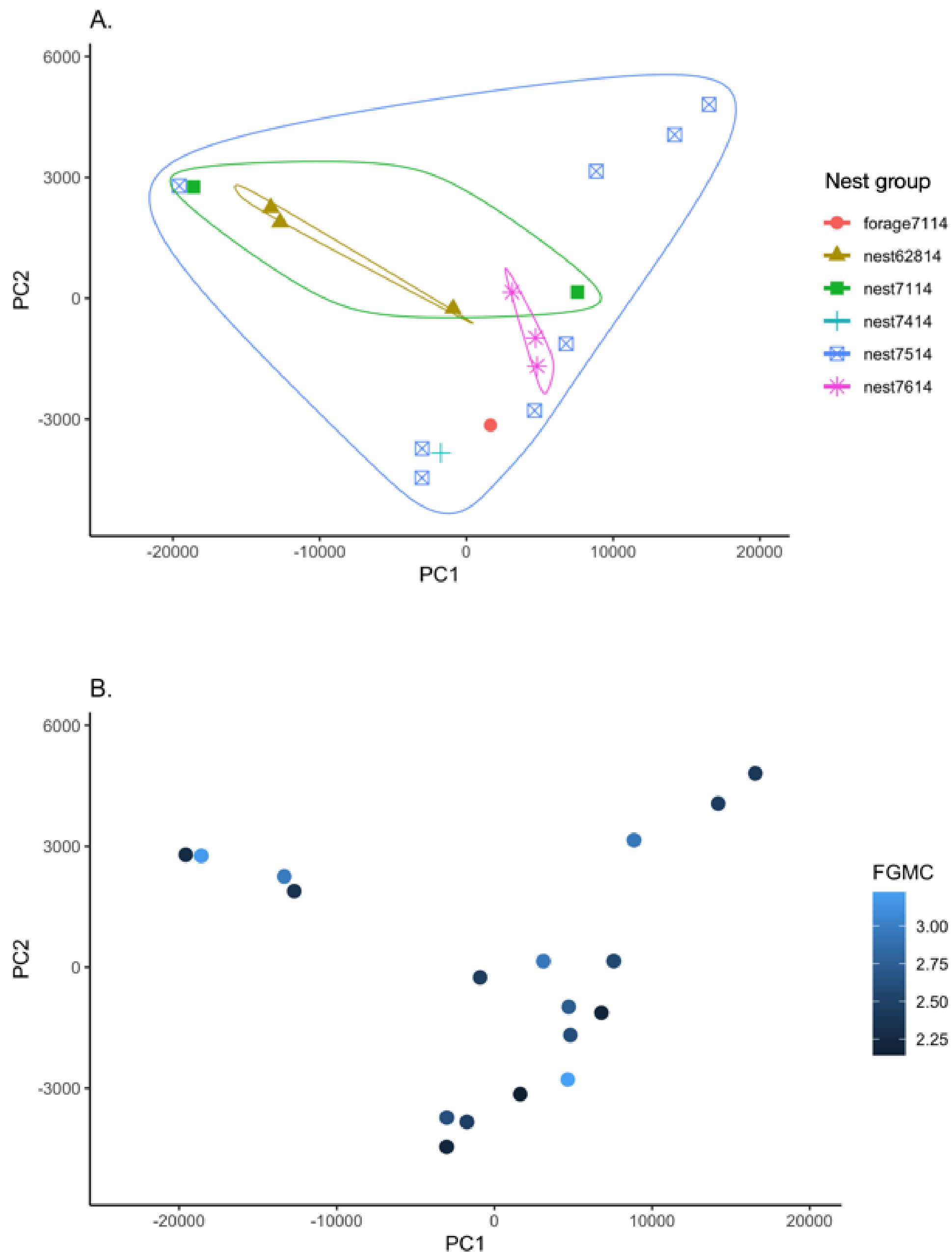

Figure 4.3. PCOA for nesting group and FGMC.3. A. Nesting group does not significantly explain beta diversity for bonobo gut microbiota B. FGMC does not significantly explain beta diversity for bonobo gut microbiota.

Table 4.3 Taxa that co-varied with logFGMC and rawFGMC.

Genus	logFGMC	rawFGMC
Methanobrevibacter	0.0692	0.0372
Methanosphaera	0.0336	0.00987
unk Methanomassiliicoccaceae	3.90E-08	7.04E-14
vadinCA11	2.00E-16	2.00E-16
unk Bacteria	2.00E-16	2.00E-16
unk Koribacteraceae	1	1
unk Actinobacteria	0.0766	5.77E-15
unk Bifidobacteriaceae	1.99E-05	0.383
Bifidobacterium	2.00E-16	2.00E-16
unk Coriobacteriaceae	2.00E-16	2.00E-16
unk Coriobacteriaceae	2.00E-16	2.00E-16
Adlercreutzia	2.00E-16	2.00E-16
Collinsella	2.00E-16	2.00E-16
Slackia	2.00E-16	2.24E-09
unk OPB41	0.568	0.622
unk Bacteroidales	2.00E-16	2.00E-16
unk Bacteroidales	0.312	5.70E-06
unk Bacteroidales	3.25E-11	6.24E-06
Bacteroides	4.67E-09	4.47E-15
Paludibacter	2.00E-16	2.00E-16
Parabacteroides	2.00E-16	2.00E-16
unk Prevotellaceae	2.21E-11	3.15E-11
Prevotella (Prevotellaceae)	2.00E-16	2.00E-16
unk RF16	5.28E-13	2.00E-16
unk S24-7	2.00E-16	2.00E-16
unk Paraprevotellaceae	2.00E-16	2.00E-16
YRC22	1.26E-06	2.00E-16
Prevotella (Paraprevotellaceae)	2.00E-16	2.00E-16
SHD-231	2.00E-16	2.00E-16
unk S2	2.00E-16	2.00E-16
unk Streptophyta	2.00E-16	2.00E-16
Fibrobacter	2.00E-16	0.175
unk Firmicutes	0.00303	0.0115

Lactococcus	0.138	0.999
Table 4.3 (continued).	1.81E-07	4.00E-06
unk Clostridia	2.19E-09	1.50E-13
unk Clostridiales	2.00E-16	2.00E-16
unk Clostridiales	2.00E-16	2.00E-16
unk Christensenellaceae	5.01E-05	0.000419
unk Christensenellaceae	0.018	2.00E-16
unk Clostridiaceae	7.48E-12	1.80E-07
02d06	0.913	0.239
Clostridium	1.22E-06	5.23E-16
unk Lachnospiraceae	2.00E-16	2.82E-11
unk Lachnospiraceae	2.00E-16	2.00E-16
Anaerostipes	0.152	0.396
Blautia	2.00E-16	2.00E-16
Butyrivibrio	0.452	0.0197
Clostridium	0.000471	0.0144
Coprococcus	2.00E-16	2.00E-16
Dorea	2.44E-06	5.55E-08
Lachnobacterium	1.30E-14	2.03E-11
Lachnospira	2.05E-11	0.274
Oribacterium	6.19E-11	2.00E-16
Roseburia	2.00E-16	2.00E-16
Ruminococcus	5.85E-05	1.46E-08
Peptococcus	9.22E-07	0.412
unk Ruminococcaceae	0.00193	0.778
unk Ruminococcaceae	2.00E-16	2.00E-16
Anaerofilum	0.00121	0.000138
Anaerotruncus	0.7872	0.00361
Faecalibacterium	2.00E-16	2.00E-16
Oscillospira	2.00E-16	2.00E-16
Ruminococcus	2.00E-16	2.00E-16
unk Veillonellaceae	0.000389	0.00612
unk Veillonellaceae	3.57E-07	0.501
Anaerovibrio	1.18E-11	2.00E-16
Dialister	3.04E-09	0.00714
Phascolarctobacterium	2.00E-16	1.63E-15
unk Mogibacteriaceae	7.27E-05	0.000152
Mogibacterium	5.42E-12	0.0193

Table 4.3 (continued).

Erysipelotrichaceae	1.61E-06	4.02E-16
	1.36E-12	2.00E-16
RFN20	0.798	0.376
Eubacterium	2.00E-16	2.00E-16
p-75-a5	0.00454	0.174
unk Victivallaceae	0.462	0.0206
unk R4-45B	1.41E-05	0.00571
unk Proteobacteria	1.25E-07	6.00E-07
unk Alphaproteobacteria	2.00E-16	1.08E-15
unk RF32	0.74	3.91E-09
unk Rickettsiales	0.762	0.2088
unk Rickettsiales	2.00E-16	5.09E-09
Phytophthora	0.804	0.0584
unk Betaproteobacteria	6.84E-09	0.000108
unk Burkholderiales	4.38E-13	1.80E-06
unk Burkholderiales	2.00E-16	7.77E-15
Sutterella	0.00186	8.74E-06
unk Oxalobacteraceae	1.33E-13	1.66E-09
unk Desulfovibrionaceae	0.293	0.000341
Bilophila	7.50E-07	0.000118
Desulfovibrio	2.00E-16	2.00E-16
Campylobacter	9.57E-10	6.27E-07
Flexispira	3.65E-08	4.73E-05
Succinivibrio	2.00E-16	3.52E-06
unk Enterobacteriaceae	2.06E-05	0.000921
Escherichia	6.82E-14	2.30E-06
Aggregatibacter	0.867	0.488
Acinetobacter	0.000569	0.00272
Treponema	2.00E-16	2.00E-16
unk Anaeroplasmataceae	2.00E-16	2.00E-16
unk RF39	2.00E-16	2.00E-16
unk ML615J-28	1.00E-15	9.93E-05
unk HA64	9.00E-04	0.000123
unk Cerasicoccaceae	2.00E-16	1.96E-07
unk RFP12	0.023	0.0758

4.4 Discussion

These results suggest that stress has little impact on the composition of the bonobo gut microbiota. Our results are similar to what Vlčková et al., (2018) found in western lowland gorillas in that FGMC did not significantly explain bonobo gut microbiota composition. However, our results differed from Vlčková et al., (2018) in the number and taxa that have a linear relationship with FGMC. Vlčková et al., (2018) only found three taxa that significantly correlated while we found seventy- six taxa that significantly correlated with FGMC, of those, two were similar to those found in western lowland gorillas.

Of the three taxa that correlated FGMC in western lowland gorillas were family *Anaerolineaceae*, genus *Clostridium* cluster XIVb and genus *Oscillibacter*. We found similar correlations in family *Anaerolineaceae* and genus *Clostridium* for bonobos. Although we found similar patterns in the genus *Clostridium*, we did not find genus *Clostridium* cluster XIVb in the eighteen bonobos that we sampled, but we may find genus *Clostridium* cluster XIVb with a larger sample size. We also had no genus *Oscillibacter* detected in our bonobo samples nor did we detect any of the higher family level Oscillospiraceae. However, as the order level Clostridiales is similar to that of the genus *Oscillibacter* found in western lowland gorillas, we found correlations for 23 of the order Clostridiales in our abundance models.

Other notable genus level associations we found with our abundance model results were with the genus *Bifidobacterium*. Various *Bifidobacterium* genera have been found to be associated with wild and captive chimpanzees, wild baboons, and a novel species was detected in ring tailed lemurs (Modesto et al., 2015; Ren et al., 2016; Uenishi et al., 2007). *Bifidobacterium* have also been found to be important in host health, pathogen inhabitation, the production of essential vitamins, and immune system modulation ("Mayo: Bifidobacteria: Genomics and Molecular Aspects - Google Scholar," n.d.). Other notable taxa we found that were significantly correlated with FGMC were a genus belonging to the family Methanomassiliicoccaceae which are associated with the methanogenic activity and the production of volatile fatty acids (Iltchenco et al., 2020). Many species that have been isolated from the human gut microbiota including

Coprococcus, *Dorea, Oribacterium*, *Ruminococcus*, *Faecalibacterium, Ruminococcus, Anaerovibrio, Sutterella, Bilophila,* and *Escherichiawe* found linear correlations within the gut microbiota of bonobos with FGCM (Amato, 2017; Kurilshikov et al., 2020; Moeller, 2017; Yatsunenko et al., 2012).

We also found a significant linear relationship between FGMC concentration and two genera of *Prevotella* that have been associated with non-Western human gut microbiota in populations eating a high fiber or plant based diet and that are known opportunistic pathogens in humans (De Filippo et al., 2010; Yolken et al., 2020). Additionally, we found significant linear relationships between several potentially pathogenic bacteria in humans including *Streptococcus*, *Clostridium, Campylobacter,* and *Treponema*. Other associations we found were in the genus *Blautia* and *Oscillospira* which have been found to be important to the immune system (Lin et al., 2020; Yang et al., 2017). We also found *Roseburia* which has been found to be significant in weight loss in mice (Ryan et al., 2014). We found several species that are known in the mammalian gut to be important in the degradation of plant-based cellulose including members of the genus *Fibrobacter*, *Lachnobacterium,* and *Succinivibrio* (Ley et al., 2008; McKenzie et al., 2017; Moeller and Sanders, 2020).

Our results demonstrate that stress, as measured by FGMC has little to no significant effect on the overall composition of the bonobo gut microbiota. However, we did find a significant linear relationship with seventy-six different individual taxa. These different taxa and their overall effect on the bonobo gut microbiota are difficult to determine beyond broad associations without metagenomic sequencing. Compared to western lowland gorillas our specific taxa results had more taxa that were significant in our linear relationships, therefore there may be species specific patterns in FGMC and gut microbiota across different great ape species. Incorporating FGMC and gut microbiome data can provide a more robust understanding of how stress impacts the gut microbiomes of primates. Future directions for this work include increasing our sample size and incorporating more functional results using shotgun metagenomic sequencing.

CHAPTER V

CONCLUSION

Conclusion

Our results supported predictions of the optimal diet model and not the functional response model in chapter two. Interestingly, the result that abundance did not relate to consumption using dietary diversity indices would suggest that bonobos are not selecting food under the functional response model. However, our results supported the claim that bonobo foraging fit with the predictions of optimal diet models. We found that Shannon's index was lower during periods of the year when fewer items were available for consumption and high-value items are abundant and that Simpson's index was higher during periods of the year where a few highly dominant species, less high-value food items are consumed. Thus, when understanding dietary diversity and its relationship to bonobo diets, optimal diet models and optimizing energy return seems to be structuring bonobo foraging strategies. Diet is an important selective pressure, has shaped primate behavior because the acquisition of food resources is necessary for survival and reproduction (Clutton-Brock, 1977; Dew and Boubli, 2005; Bray et al., 2018). After finding support for the optimal diet model and not the functional response model, we turned to the gut microbiota to examine fallback food models in bonobos.

In chapter three, our results indicate there are sex-specific and social group patterns in the gut microbiota and diet of the Lomako forest bonobos, especially as it related to the δ13C stable isotope values. δ13C values are primarily differentiated by the consumption of C4, C3, and CAM plants in the diet (Crowley et al., 2010; Schoeninger, 2014). The most frequently consumed plants for this period and from later field season were 1. *Anthoclitandra robustior 2. Ficus* spp. 3. *Scropholoes zenkeri 4. Polyalthia suaveolens 5. Celtis mildbraedii*. Of these plants, most *Ficus* spp. and many of the species of terrestrial herbaceous vegetation (THV) are considered to be plants utilizing a C3 photosynthetic pathway (*Isotopes: Advances in Research and Application: 2011 Edition,* 2012; Ting et al., 1987). Plants that use a C4 strategy tend to have ~12-13%

higher δ13C values, while C3 plants tend to have lower δ13C values (O'Brien, 2015). Our sample females had slightly higher δ13C values, indicating potentially more C4 plants in their diet. These results may indicate that females may use *Ficus* spp. or THV to a lesser extent than males. The results suggest the fallback food hypothesis put forth by Wrangham, (1986) may not be supported.

In chapter four, our results demonstrate that stress, as measured by FGMC has little to no significant effect on the overall composition of the bonobo gut microbiota. However, we did find a significant linear relationship with seventy-six different individual taxa. These different taxa and their overall effect on the bonobo gut microbiota are challenging to determine beyond broad associations without metagenomic sequencing. Compared to western lowland gorillas, our specific taxa results had more taxa that were significant in our linear relationships. Therefore, there may be species-specific patterns in FGMC and gut microbiota across different great ape species. Incorporating FGMC and gut microbiome data can provide a more robust understanding of how stress impacts the gut microbiomes of primates. However, this approach may not be the best way to examine stress as it relates to diet as it relates to the use of fallback foods. More specific questions about certain taxa found in the gut microbiome need to be incorporated into the predictions of fallback food models in order to make meaningful conclusions.

Other evidence against the THV hypothesis comes from White and Wrangham, (1988), who tested the THV hypothesis by comparing bonobos at Lomako with chimpanzees at Gombe and concluded feeding competition in smaller food tress was higher in bonobos and the availability of more large fruit trees at Lomako better-explained differences in group size (White and Wrangham, 1988). Additionally, ground use was similar for both sites indicating that bonobos did not utilize ground resources at higher rates than chimpanzees (White and Wrangham, 1988). This conclusion fits with our results from chapter two as bonobos usually eat the fruit of seven of the ten top consumed food items. *Anthoclitandra robustior* (2014), *Antiaris toxicana* (1984), *Celtis mildbraedii* (1984, 2017), *Ficus* spp. (1984, 2014), *Polyalthia suaveolens* (1991, 2017), *Treculia africana* (1995), and *Uapaca guineensis* (1991) are all primarily consumed for their fruit, while the seeds of *Irvingia gabonensis* (1995 and *Strombosia*

glaucescens (2014) are eaten. *Scropholoes zenkeri* (1991,1995, 2017) is the only food item of the top ten that bonobos choose to eat the leaves. Anecdotally, THV is a dispersed food, and bonobos feeding on THV are spread out; therefore, THV as a food resource is not responsible for increased sociality (Hickmott and White, personal observations).

Our results from chapter three indicate that perhaps differential resources use between the sexes may contribute to the structure of the gut microbiome, but a more detailed investigation is needed to determine what resources drive this pattern. Female feeding priority might be a reason why there are differences in male vs. female dietary stable isotopes that are driving patterns in the bonobo gut microbiome. Female feeding priority and female dominance have long been established in bonobos (Parish, De Waal, and Haig, 2000). In the wild, female feeding priority is linked to patch size (White and Wood, 2007). There is no significant difference in male and female bonobo feeding behavior in large patches, but in smaller, more monopolize patches, females enter the food trees first and feed more than males at the start of the bout (*ibid*). Males use alternative strategies like higher levels of terrestriality to compensate for the priority of access females have to smaller food patches.

Males and female terrestriality which would result in different diets and access to THV. Typically, females will travel arboreally at a slower rate to food trees, while males will travel terrestrially (White et al., 2020). Terrestrial travel is typically much more rapid than arboreal travel, allowing males to dispute control of the primary access points with males resulting in the eviction of other males from the tree (White et al., 2020; White and Wood, 2007). This winning and high-ranking male is then able to mate with females as they enter the food tree (White et al., 2020; White and Wood, 2007). The losing male typically goes to the adjacent trees or waits until the dominant male allows them access to the tree (White et al., 2020). This pattern may also result in different male and female diets. We know from nutritional analyses that not all fruits are equal (Rothman, Chapman, and Van Soest, 2012), and the combined effects of female priority of access and male terrestriality may work together to create enough differences to drive patterns of bonobo gut microbiota. However, this is simply a hypothesis and needs to be more thoroughly tested to develop a more robust conclusion.

In chapter four, an aspect of fallback food models, namely changes in stress, does not seem to have an effect on the structure of the gut microbiome. Previous research on stress in the Lomako forest bonobos found a significant effect of increased FGMC when males were in a nesting party (Cobden, Waller, and White, 2010). The follow-up to this pattern suggests that increased stress was due to large party sizes (Cobden, 2014). This result indicates that social stress may be a factor in the Lomako forest bonobos' stress levels. Small groups may be less stressed, and all-female groups may be less stressed because these feeding parties are able to use and monopolize smaller food patches. Additionally, bonobos may not be food stressed because food abundance is much higher than other chimpanzees (White and Wrangham, 1988; Malenky et al., 1993). If bonobos experience less food stress, perhaps stress related to social, or dominance related stressors are more critical, or perhaps the stress that immigrating females experience before social integration plays a role. However, from our data, these are not patterns we can tease apart.

The Lomako forest bonobos represent a unique study population to examine how social, dietary, and sex-based differences contribute to the composition and diversity of the bonobo gut microbiota. The Lomako forest bonobos have some of the lowest δ13C values and highest δ15N values of any other *Pan* community. The highest dietary diversity of any great ape species makes them an excellent dietary model to investigate the effects of diet on bonobo gut microbiota. The high levels of female affiliation, GG rubbing, female dominance, and fission-fusion social dynamics make bonobos a good model for examining various factors and the gut microbiota. Combining these factors associated with bonobo dietary diversity, dietary stable isotopes, and fecal glucocorticoids with our gut microbiome results may yield further results important in understanding how diet and stress shape primate gut microbiota.

REFERENCES CITED

Albrechtsen, Lise, David W. Macdonald, Paul J. Johnson, Ramon Castelo, and John E. Fa. 2007. "Faunal Loss from Bushmeat Hunting: Empirical Evidence and Policy Implications in Bioko Island." *Environmental Science & Policy* 10 (7–8): 654–67. https://doi.org/10.1016/j.envsci.2007.04.007.

Allaband, Celeste, Daniel McDonald, Yoshiki Vázquez-Baeza, Jeremiah J. Minich, Anupriya Tripathi, David A. Brenner, Rohit Loomba, et al. 2019. "Microbiome 101: Studying, Analyzing, and Interpreting Gut Microbiome Data for Clinicians." *Clinical Gastroenterology and Hepatology* 17 (2): 218–30. https://doi.org/10.1016/j.cgh.2018.09.017.

Allan, Andrew T. L., Annie L. Bailey, and Russell A. Hill. 2020. "Habituation Is Not Neutral or Equal: Individual Differences in Tolerance Suggest an Overlooked Personality Trait." *Science Advances* 6 (28): eaaz0870. https://doi.org/10.1126/sciadv.aaz0870.

Altmann, Jeanne. 1974. "Observational Study of Behavior: Sampling Methods." *Behaviour* 49 (3–4): 227–66.

Altmann, Stuart A. 1991. "Diets of Yearling Female Primates (*Papio cynocephalus*) Predict Lifetime Fitness." *Proceedings of the National Academy of Sciences* 88 (2): 420–23. https://doi.org/10.1073/pnas.88.2.420.

Altmann, Stuart A., and Stephen S. Wagner. 1978. "A General Model of Optimal Diet." *Recent Advances in Primatology* 1: 407–14.

Amato, Katherine R. 2016. "Incorporating the Gut Microbiota into Models of Human and Non-Human Primate Ecology and Evolution." *American Journal of Physical Anthropology* 159 (S61): 196–215. https://doi.org/10.1002/ajpa.22908.

———. 2017. "An Introduction to Microbiome Analysis for Human Biology Applications." *American Journal of Human Biology* 29 (1): e22931.

Amato, Katherine R., Sahana Kuthyar, Marcy Ekanayake-Weber, Roberta Salmi, Noah Snyder-Mackler, Lasanthi Wijayathunga, Rajnish Vandercone, and Amy Lu. 2020. "Gut Microbiome, Diet, and Conservation of Endangered Langurs in Sri Lanka." *Biotropica 52*(5), 981-990. https://doi.org/10.1111/btp.12805.

Amato, Katherine R., Steven R. Leigh, Angela Kent, Roderick I. Mackie, Carl J. Yeoman, Rebecca M. Stumpf, Brenda A. Wilson, Karen E. Nelson, Bryan A. White, and Paul A. Garber. 2014. "The Role of Gut Microbes in Satisfying the Nutritional Demands of Adult and Juvenile Wild, Black Howler Monkeys (*Alouatta pigra*)." *American Journal of Physical Anthropology* 155 (4): 652–64. https://doi.org/10.1002/ajpa.22621.

———. 2015. "The Gut Microbiota Appears to Compensate for Seasonal Diet Variation in the Wild Black Howler Monkey (*Alouatta pigra*)." *Microbial Ecology* 69 (2): 434–43. https://doi.org/10.1007/s00248-014-0554-7.

Amato, Katherine R., Sarie Van Belle, Anthony Di Fiore, Alejandro Estrada, Rebecca Stumpf, Bryan White, Karen E. Nelson, Rob Knight, and Steven R. Leigh. 2017. "Patterns in Gut Microbiota Similarity Associated with Degree of Sociality among Sex Classes of a Neotropical Primate." *Microbial Ecology* 74 (1): 250–58. https://doi.org/10.1007/s00248-017-0938-6.

Amato, Katherine R, Carl J Yeoman, Angela Kent, Nicoletta Righini, Franck Carbonero, Alejandro Estrada, H Rex Gaskins, et al. 2013. "Habitat Degradation Impacts Black Howler Monkey (*Alouatta pigra*) Gastrointestinal Microbiomes." *The ISME Journal* 7 (7): 1344–53. https://doi.org/10.1038/ismej.2013.16.

Archie, Elizabeth A., and Kevin R. Theis. 2011. "Animal Behaviour Meets Microbial Ecology." *Animal Behaviour* 82 (3): 425–36. https://doi.org/10.1016/j.anbehav.2011.05.029.

Archie, Elizabeth A, and Jenny Tung. 2015. "Social Behavior and the Microbiome." *Current Opinion in Behavioral Sciences* 6 (December): 28–34. https://doi.org/10.1016/j.cobeha.2015.07.008.

Aureli, Filippo, Colleen M. Schaffner, Christophe Boesch, Simon K. Bearder, Josep Call, Colin A. Chapman, Richard Connor, et al. 2008. "Fission-Fusion Dynamics: New Research Frameworks." *Current Anthropology* 49 (4): 627–54. https://doi.org/10.1086/586708.

Bailey, Michael T. 2009. "The Effects of Psychological Stressors on the Intestinal Microbiota." *Bioscience and Microflora* 28 (4): 125–34. https://doi.org/10.12938/bifidus.28.125.

———. 2012. "The Contributing Role of the Intestinal Microbiota in Stressor-Induced Increases in Susceptibility to Enteric Infection and Systemic Immunomodulation." *Hormones and Behavior* 62 (3): 286–94.

Bailey, Michael T., and Christopher L. Coe. 1999. “Maternal Separation Disrupts the Integrity of the Intestinal Microflora in Infant Rhesus Monkeys.” *Developmental Psychobiology* 35 (2): 146–55. https://doi.org/10.1002/(SICI)1098-2302(199909)35:2<146::AID-DEV7>3.0.CO;2-G.

Baniel, Alice, Katherine R Amato, Jacinta C Beehner, Thore J Bergman, Arianne Mercer, Rachel F Perlman, Lauren Petrullo, et al. 2021. "Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas." *Microbiome*, *9*(1), 1-20. https://doi.org/10.1101/2020.07.07.192336.

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. https://doi.org/10.1002/ajp.10049.

Bennett, Genevieve, Matthew Malone, Michelle L. Sauther, Frank P. Cuozzo, Bryan White, Karen E. Nelson, Rebecca M. Stumpf, Rob Knight, Steven R. Leigh, and Katherine R. Amato. 2016. “Host Age, Social Group, and Habitat Type Influence the Gut Microbiota of Wild Ring-Tailed Lemurs (*Lemur catta*).” *American Journal of Primatology* 78 (8): 883–92. https://doi.org/10.1002/ajp.22555.

Blekhman, Ran, Karen Tang, Elizabeth A. Archie, Luis B. Barreiro, Zachary P. Johnson, Mark E. Wilson, Jordan Kohn, et al. 2016. “Common Methods for Fecal Sample Storage in Field Studies Yield Consistent Signatures of Individual Identity in Microbiome Sequencing Data.” *Scientific Reports* 6 (1): 31519. https://doi.org/10.1038/srep31519.

Boesch, Christophe, Gottfried Hohmann, and Linda Marchant. 2002. *Behavioural Diversity in Chimpanzees and Bonobos*. Cambridge University Press.

Boubli, Jean Philippe, and J. Lawrence Dew. 2005. *Tropical Fruits and Frugivores: The Search for Strong Interactors*. Springer.

Brand, Colin M., Frances J. White, Nancy Thompson Handler, Alexana J. Hickmott, and Klaree J. Boose. 2018. “Adaptive Functions of GG Rubbing among Female Bonobos (*Pan paniscus*) at Lomako, DRC.” *American Journal of Physical Anthropology*, 165:35–35.

Brand, Colin M., Frances J. White, Monica L. Wakefield, Michel T. Waller, Maria Jose Ruiz-López, and Nelson Ting. 2016. “Initiation of Genetic Demographic Monitoring of Bonobos (*Pan paniscus*) at Iyema, Lomako Forest, DRC.” *Primate Conservation* 30: 103–11.

Bray, Joel, Melissa Emery Thompson, Martin N. Muller, Richard W. Wrangham, and Zarin P. Machanda. 2018. “The Development of Feeding Behavior in Wild Chimpanzees (*Pan troglodytes schweinfurthii*).” *American Journal of Physical Anthropology* 165 (1): 34–46.

Bruorton, M. R., C. L. Davis, and M. R. Perrin. 1991. "Gut Microflora of Vervet and Samango Monkeys in Relation to Diet." *Applied and Environmental Microbiology* 57 (2): 573–78.

Burns, Adam R., Elizabeth Miller, Meghna Agarwal, Annah S. Rolig, Kathryn Milligan-Myhre, Steve Seredick, Karen Guillemin, and Brendan J. M. Bohannan. 2017. "Interhost Dispersal Alters Microbiome Assembly and Can Overwhelm Host Innate Immunity in an Experimental Zebrafish Model." *Proceedings of the National Academy of Sciences* 114 (42): 11181–86. https://doi.org/10.1073/pnas.1702511114.

Caporaso, J. Gregory, Justin Kuczynski, Jesse Stombaugh, Kyle Bittinger, Frederic D Bushman, Elizabeth K Costello, Noah Fierer, et al. 2010. "QIIME Allows Analysis of High-Throughput Community Sequencing Data." *Nature Methods* 7 (5): 335–36. https://doi.org/10.1038/nmeth.f.303.

Chapman, Colin A., and Lauren J. Chapman. 1999. "Implications of Small Scale Variation in Ecological Conditions for the Diet and Density of Red Colobus Monkeys." *Primates* 40 (1): 215.

Chapman, Colin A., Lauren J. Chapman, Richard Wangham, Kevin Hunt, Daniel Gebo, and Leah Gardner. 1992. "Estimators of Fruit Abundance of Tropical Trees." *Biotropica*, 527–31.

Chapman, Colin A., Richard Wrangham, and Lauren J. Chapman. 1994. "Indices of Habitat-Wide Fruit Abundance in Tropical Forest." *Biotropica*, 160–71.

Charnov, Eric L. 1976. "Optimal Foraging, the Marginal Value Theorem."

Clayton, Jonathan B., Pajau Vangay, Hu Huang, Tonya Ward, Benjamin M. Hillmann, Gabriel A. Al-Ghalith, Dominic A. Travis, et al. 2016. "Captivity Humanizes the Primate Microbiome." *Proceedings of the National Academy of Sciences* 113 (37): 10376–81. https://doi.org/10.1073/pnas.1521835113.

Clutton-Brock, Timothy H. 1974. "Primate Social Organisation and Ecology." *Nature* 250 (5467): 539–42.

Cobden, Amy K. 2014. "Party Animals: Food, Sociality and Stress in Wild Bonobos (*Pan paniscus*) of Iyema, Lomako Forest, Democratic Republic of Congo." PhD Thesis, Emory University.

Cobden, Amy K., Michel Waller, and Frances J. White. 2010. "Pilot Study for the Analysis of Stress and Kinship in Wild Bonobos (*Pan paniscus*), Lomako Forest, Democratic Republic of Congo." *American Journal of Physical Anthropology*, 82–83.

Constantino, Paul J., and Barth W. Wright. 2009. “The Importance of Fallback Foods in Primate Ecology and Evolution.” *American Journal of Physical Anthropology* 140 (4): 599–602. https://doi.org/10.1002/ajpa.20978.

Crowley, Brooke E., Melinda L. Carter, Sarah M. Karpanty, Adrienne L. Zihlman, Paul L. Koch, and Nathaniel J. Dominy. 2010. “Stable Carbon and Nitrogen Isotope Enrichment in Primate Tissues.” *Oecologia* 164 (3): 611–26. https://doi.org/10.1007/s00442-010-1701-6.

Cui, Zhenwei, Qi Shao, Cyril C. Grueter, Zhenlong Wang, Jiqi Lu, and David Raubenheimer. 2019. “Dietary Diversity of an Ecological and Macronutritional Generalist Primate in a Harsh High-latitude Habitat, the Taihangshan Macaque (*Macaca mulatta tcheliensis*).” *American Journal of Primatology* 81 (4): e22965. https://doi.org/10.1002/ajp.22965.

Dantas, Gautam, Morten O.A. Sommer, Patrick H. Degnan, and Andrew L. Goodman. 2013. “Experimental Approaches for Defining Functional Roles of Microbes in the Human Gut.” *Annual Review of Microbiology* 67 (1): 459–75. https://doi.org/10.1146/annurev-micro-092412-155642.

Davenport, Emily R., Jon G. Sanders, Se Jin Song, Katherine R. Amato, Andrew G. Clark, and Rob Knight. 2017. “The Human Microbiome in Evolution.” *BMC Biology* 15 (1): 1–12.

De Filippo, Carlotta, Duccio Cavalieri, Monica Di Paola, Matteo Ramazzotti, Jean Baptiste Poullet, Sebastien Massart, Silvia Collini, Giuseppe Pieraccini, and Paolo Lionetti. 2010. “Impact of Diet in Shaping Gut Microbiota Revealed by a Comparative Study in Children from Europe and Rural Africa.” *Proceedings of the National Academy of Sciences* 107 (33): 14691–96. https://doi.org/10.1073/pnas.1005963107.

Degnan, Patrick H., Anne E. Pusey, Elizabeth V. Lonsdorf, Jane Goodall, Emily E. Wroblewski, Michael L. Wilson, Rebecca S. Rudicell, Beatrice H. Hahn, and Howard Ochman. 2012. “Factors Associated with the Diversification of the Gut Microbial Communities within Chimpanzees from Gombe National Park.” *Proceedings of the National Academy of Sciences* 109 (32): 13034–39. https://doi.org/10.1073/pnas.1110994109.

Dillon, R. J., C. T. Vennard, A. Buckling, and A. K. Charnley. 2005. “Diversity of Locust Gut Bacteria Protects against Pathogen Invasion.” *Ecology Letters* 8 (12): 1291–98. https://doi.org/10.1111/j.1461-0248.2005.00828.x.

Doran, Diane M., Alastair McNeilage, David Greer, Carolyn Bocian, Patrick Mehlman, and Natasha Shah. 2002. "Western Lowland Gorilla Diet and Resource Availability: New Evidence, Cross-Site Comparisons, and Reflections on Indirect Sampling Methods." *American Journal of Primatology* 58 (3): 91–116. https://doi.org/10.1002/ajp.10053.

Dupain, Jef, 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): 265–72. https://doi.org/10.1016/S0006-3207(00)00004-5.

Erhart, E. M., Stacey R. Tecot, and C. Grassi. 2018. "Interannual Variation in Diet, Dietary Diversity, and Dietary Overlap in Three Sympatric Strepsirrhine Species in Southeastern Madagascar." *International Journal of Primatology* 39 (2): 289–311.

Falony, Gwen, Marie Joossens, Sara Vieira-Silva, Jun Wang, Youssef Darzi, Karoline Faust, Alexander Kurilshikov, et al. 2016. "Population-Level Analysis of Gut Microbiome Variation." *Science* 352 (6285): 560–64. https://doi.org/10.1126/science.aad3503.

Fruth, Barbara, and Gottfried Hohmann. 2018. "Food Sharing across Borders: First Observation of Intercommunity Meat Sharing by Bonobos at LuiKotale, DRC." *Human Nature* 29 (2): 91–103. https://doi.org/10.1007/s12110-018-9311-9.

Fruth, Barbara, Nikki Tagg, and Fiona Stewart. 2018. "Sleep and Nesting Behavior in Primates: A Review." *American Journal of Physical Anthropology* 166 (3): 499–509. https://doi.org/10.1002/ajpa.23373.

Furuichi, Takeshi. 1989. "Social Interactions and the Life History of Female *Pan paniscus* in Wamba, Zaire." *International Journal of Primatology* 10 (3): 173–97.

Furuichi, Takeshi, and Chie Hashimoto. 2002. "Why Female Bonobos Have a Lower Copulation Rate during Estrus than Chimpanzees." *Behavioural Diversity in Chimpanzees and Bonobos*, 156.

Furuichi, Takeshi, Chie Hashimoto, and Yasuko Tashiro. 2001. "Fruit Availability and Habitat Use by Chimpanzees in the Kalinzu Forest, Uganda: Examination of Fallback Foods." *International Journal of Primatology*, 17.

Ganas, Jessica, Martha M. Robbins, John Boscoe Nkurunungi, Beth A. Kaplin, and Alastair McNeilage. 2004. "Dietary Variability of Mountain Gorillas in Bwindi Impenetrable National Park, Uganda." *International Journal of Primatology,* 25 (5): 1043–72. https://doi.org/10.1023/B:IJOP.0000043351.20129.44.

Garber, Paul A., Elizabeth K. Mallott, Leila M. Porter, and Andres Gomez. 2019. "The Gut Microbiome and Metabolome of Saddleback Tamarins (*Leontocebus weddelli*): Insights into the Foraging Ecology of a Small-Bodied Primate." *American Journal of Primatology* 81 (10–11): e23003. https://doi.org/10.1002/ajp.23003.

Gomez, Andres, Klara Petrzelkova, Carl J. Yeoman, Klara Vlckova, Jakub Mrázek, Ingrid Koppova, Franck Carbonero, et al. 2015. "Gut Microbiome Composition and Metabolomic Profiles of Wild Western Lowland Gorillas (*Gorilla gorilla gorilla*) Reflect Host Ecology." *Molecular Ecology* 24 (10): 2551–65. https://doi.org/10.1111/mec.13181.

———. 2016. "Gut Microbiome Composition and Metabolomic Profiles of Wild Western Lowland Gorillas (*Gorilla gorilla gorilla*) Reflect Host Ecology." *Molecular Ecology* 24 (10): 2551–65. https://doi.org/10.1111/mec.13181.

Gomez, Andres, Jessica M Rothman, Klara Petrzelkova, Carl J Yeoman, Klara Vlckova, Juan D Umaña, Monica Carr, et al. 2016. "Temporal Variation Selects for Diet–Microbe Co-Metabolic Traits in the Gut of *Gorilla* spp." *The ISME Journal* 10 (2): 514–26. https://doi.org/10.1038/ismej.2015.146.

Goodfellow, Claire K., Tabor Whitney, Diana M. Christie, Pascale Sicotte, Eva C. Wikberg, and Nelson Ting. 2019. "Divergence in Gut Microbial Communities Mirrors a Social Group Fission Event in a Black-and-white Colobus Monkey (*Colobus vellerosus*)." *American Journal of Primatology* 81 (10–11). https://doi.org/10.1002/ajp.22966.

Green, Victoria M., and Kara I. Gabriel. 2020. "Researchers' Ethical Concerns Regarding Habituating Wild-Nonhuman Primates and Perceived Ethical Duties to Their Subjects: Results of an Online Survey." *American Journal of Primatology* 82 (9): e23178. https://doi.org/10.1002/ajp.23178.

Gruber, Thibaud, and Zanna Clay. 2016. "A Comparison Between Bonobos and Chimpanzees: A Review and Update." *Evolutionary Anthropology: Issues, News, and Reviews* 25 (5): 239–52. https://doi.org/10.1002/evan.21501.

Hale, Vanessa L., Chia L. Tan, Kefeng Niu, Yeqin Yang, Rob Knight, Qikun Zhang, Duoying Cui, and Katherine R. Amato. 2018. "Diet Versus Phylogeny: A Comparison of Gut Microbiota in Captive Colobine Monkey Species." *Microbial Ecology* 75 (2): 515–27. https://doi.org/10.1007/s00248-017-1041-8.

Hanya, Goro, and Colin A. Chapman. 2013. "Linking Feeding Ecology and Population Abundance: A Review of Food Resource Limitation on Primates." *Ecological Research* 28 (2): 183–90. https://doi.org/10.1007/s11284-012-1012-y.

Harris, Tara R., and Colin A. Chapman. 2007. “Variation in Diet and Ranging of Black and White Colobus Monkeys in Kibale National Park, Uganda.” *Primates* 48 (3): 208–21. https://doi.org/10.1007/s10329-006-0036-8.

Harrison, Michael JS. 1984. “Optimal Foraging Strategies in the Diet of the Green Monkey, *Cercopithecus sabaeus*, at Mt. Assirik, Senegal.” *International Journal of Primatology* 5 (5): 435.

Hashimoto, Chie, and Takeshi Furuichi. 2015. “Sex Differences in Ranging and Association Patterns in Chimpanzees in Comparison with Bonobos.” In *Dispersing Primate Females*, edited by Takeshi Furuichi, Juichi Yamagiwa, and Filippo Aureli, 105–26. Primatology Monographs. Tokyo: Springer Japan. https://doi.org/10.1007/978-4-431-55480-6_5.

Hayakawa, Takashi, Akiko Sawada, Akifumi S. Tanabe, Shinji Fukuda, Takushi Kishida, Yosuke Kurihara, Kei Matsushima, et al. 2018. “Improving the Standards for Gut Microbiome Analysis of Fecal Samples: Insights from the Field Biology of Japanese Macaques on Yakushima Island.” *Primates* 59 (5): 423–36. https://doi.org/10.1007/s10329-018-0671-x.

Heiduck, Stefanie. 1997. “Food Choice in Masked Titi Monkeys (*Callicebus personatus melanochir*): Selectivity or Opportunism?,” *International Journal of Primatology*, *18*(4), 487-502.

Hicks, Allison L., Kerry Jo Lee, Mara Couto-Rodriguez, Juber Patel, Rohini Sinha, Cheng Guo, Sarah H. Olson, et al. 2018. “Gut Microbiomes of Wild Great Apes Fluctuate Seasonally in Response to Diet.” *Nature Communications* 9 (1): 1786. https://doi.org/10.1038/s41467-018-04204-w.

Hillier, Stephen G. 2007. “Diamonds Are Forever: The Cortisone Legacy.” *Journal of Endocrinology* 195 (1): 1–6. https://doi.org/10.1677/JOE-07-0309.

Hohmann, Gottfried, and Barbara Fruth. 2003a. “Lui Kotal - A New Site for Field Research on Bonobos in the Salonga National Park,” 4.

———. 2003b. “Lui Kotal - A New Site for Field Research on Bonobos in the Salonga National Park,” 4.

Holling, Crawford S. 1959. “Some Characteristics of Simple Types of Predation and Parasitism.” *Canadian Entomologist* 91 (7): 385–98.

Holling, Crawford Stanley. 1965. “The Functional Response of Predators to Prey Density and Its Role in Mimicry and Population Regulation.” *The Memoirs of the Entomological Society of Canada* 97 (S45): 5–60.

Iltchenco, Janaina, Leandro G. Almeida, Lademir L. Beal, Leticia Marconatto, Luiz Gustavo dos Anjos Borges, Adriana Giongo, and Suelen Paesi. 2020. "Microbial Consortia Composition on the Production of Methane from Sugarcane Vinasse." *Biomass Conversion and Biorefinery* 10 (2): 299–309. https://doi.org/10.1007/s13399-019-00426-0.

Isotopes: Advances in Research and Application: 2011 Edition. 2012. Scholarly Editions.

Jaeggi, Adrian V., Judith M. Burkart, and Carel P. Van Schaik. 2010. "On the Psychology of Cooperation in Humans and Other Primates: Combining the Natural History and Experimental Evidence of Prosociality." *Philosophical Transactions of the Royal Society B: Biological Sciences* 365 (1553): 2723–35.

Joers, Valerie, Gunasingh Masilamoni, Doty Kempf, Alison R. Weiss, Travis M. Rotterman, Benjamin Murray, Gul Yalcin-Cakmakli, et al. 2020. "Microglia, Inflammation and Gut Microbiota Responses in a Progressive Monkey Model of Parkinson's Disease: A Case Series." *Neurobiology of Disease* 144 (October): 105027. https://doi.org/10.1016/j.nbd.2020.105027.

Johnson, Dominic D.P., Roland Kays, Paul G. Blackwell, and David W. Macdonald. 2002. "Does the Resource Dispersion Hypothesis Explain Group Living?" *Trends in Ecology & Evolution* 17 (12): 563–70. https://doi.org/10.1016/S0169-5347(02)02619-8.

Kano, Takayoshi. 1989. "The Sexual Behavior of Pygmy Chimpanzees." *Understanding Chimpanzees*.

Kano, Takayoshi, and Mbangi Mulavwa. 1984. "Feeding Ecology of the Pygmy Chimpanzees (*Pan paniscus*) of Wamba." In *The Pygmy Chimpanzee*, edited by Randall L. Susman, 233–74. Boston, MA: Springer US. https://doi.org/10.1007/978-1-4757-0082-4_10.

Keay, Jessica M., Jatinder Singh, Matthew C. Gaunt, and Taranjit Kaur. 2006. "Fecal Glucocorticoids and Their Metabolites as Indicators of Stress in Various Mammalian Species: A Literature Review." *Journal of Zoo and Wildlife Medicine* 37 (3): 234–44. https://doi.org/10.1638/05-050.1.

King, Mary-Claire, and A. C. Wilson. 1975. "Evolution at Two Levels in Humans and Chimpanzees." *Science* 188 (4184): 107–16.

Kišidayová, Svetlana, Zora Váradyová, Peter Pristaš, Mária Piknová, Katarína Nigutová, Klára J. Petrželková, Ilona Profousová, Kateřina Schovancová, Jiří Kamler, and David Modrý. 2009. "Effects of High- and Low-Fiber Diets on Fecal Fermentation and Fecal Microbial Populations of Captive Chimpanzees." *American Journal of Primatology* 71 (7): 548–57. https://doi.org/10.1002/ajp.20687.

Koch, Hauke, and Paul Schmid-Hempel.2011. “Socially Transmitted Gut Microbiota Protect Bumble Bees against an Intestinal Parasite.” *Proceedings of the National Academy of Sciences* 108 (48): 19288–92. https://doi.org/10.1073/pnas.1110474108.

Koenig, Andreas, Jan Beise, Mukesh K. Chalise, and J.U. Ganzhorn. 1998. “When Females Should Contest for Food - Testing Hypotheses about Resource Density, Distribution, Size, and Quality with Hanuman Langurs (*Presbytis entellus*).” *Behavioral Ecology and Sociobiology* 42 (4): 225–37. https://doi.org/10.1007/s002650050434.

Konturek, Peter C., T. Brzozowski, and S. J. Konturek.(2011). "Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options." *Journal of Physiological Pharmacology*, *62*(6), 591-599.

Krebs, John R. 1984. “Optimization in Behavioural Ecology.” *Behavioural Ecology : An Evolutionary Approach*, 91–121.

Kurilshikov, Alexander, Carolina Medina-Gomez, Rodrigo Bacigalupe, Djawad Radjabzadeh, Jun Wang, Ayse Demirkan, Caroline I. Le Roy, et al. 2020. “Genetics of Human Gut Microbiome Composition.” *BioRxiv*, June, 2020.06.26.173724. https://doi.org/10.1101/2020.06.26.173724.

Lambert, Joanna E. 2007. “Seasonality, Fallback Strategies, and Natural Selection : A Chimpanzee and Cercopithecoid Model for Interpreting the Evolution of Hominin Diet.” *Evolution of the Human Diet : The Known, the Unknown, and the Unknowable*, 324–43.

Lambert, Joanna E. 1998. “Primate Digestion: Interactions among Anatomy, Physiology, and Feeding Ecology.” *Evolutionary Anthropology: Issues, News, and Reviews: Issues, News, and Reviews* 7 (1): 8–20.

———. 2009. “Summary to the Symposium Issue: Primate Fallback Strategies as Adaptive Phenotypic Plasticity—Scale, Pattern, and Process.” *American Journal of Physical Anthropology* 140 (4): 759–66. https://doi.org/10.1002/ajpa.21203.

Lambert, Joanna E., and Jessica M. Rothman. 2015. “Fallback Foods, Optimal Diets, and Nutritional Targets: Primate Responses to Varying Food Availability and Quality.” *Annual Review of Anthropology* 44 (1): 493–512. https://doi.org/10.1146/annurev-anthro-102313-025928.

Lehman, Clarence L., and David Tilman. 2000. “Biodiversity, Stability, and Productivity in Competitive Communities.” *The American Naturalist* 156 (5): 534–52. https://doi.org/10.1086/303402.

Leighton, Mark. 1993. "Modeling Dietary Selectivity by Bornean Orangutans: Evidence for Integration of Multiple Criteria in Fruit Selection." *International Journal of Primatology* 14 (2): 257–313. https://doi.org/10.1007/BF02192635.

Ley, Ruth E., Micah Hamady, Catherine Lozupone, Peter J. Turnbaugh, Rob Roy Ramey, J. Stephen Bircher, Michael L. Schlegel et al. 2008. "Evolution of Mammals and Their Gut Microbes." *Science* 320 (5883): 1647–51. https://doi.org/10.1126/science.1155725.

Ley, Ruth E., Catherine A. Lozupone, Micah Hamady, Rob Knight, and Jeffrey I. Gordon. 2008. "Worlds within Worlds: Evolution of the Vertebrate Gut Microbiota." *Nature Reviews Microbiology* 6 (10): 776–88. https://doi.org/10.1038/nrmicro1978.

Li, Jun, Cecilia Ying Ju Sung, Nikki Lee, Yueqiong Ni, Jussi Pihlajamäki, Gianni Panagiotou, and Hani El-Nezami. 2016. "Probiotics Modulated Gut Microbiota Suppresses Hepatocellular Carcinoma Growth in Mice." *Proceedings of the National Academy of Sciences* 113 (9): E1306–15.

Lin, Jiajia, Xiaoli Nie, Ying Xiong, Zhangbin Gong, Jiulin Chen, Chuan Chen, Yongyi Huang, and Te Liu. 2020. "Fisetin Regulates Gut Microbiota to Decrease CCR9+/CXCR3+/CD4+ T-Lymphocyte Count and IL-12 Secretion to Alleviate Premature Ovarian Failure in Mice." *American Journal of Translational Research* 12 (1): 203.

Liu, Weimin, Scott Sherrill-Mix, Gerald H. Learn, Erik J. Scully, Yingying Li, Alexa N. Avitto, Dorothy E. Loy, et al. 2017. "Wild Bonobos Host Geographically Restricted Malaria Parasites Including a Putative New *Laverania* Species." *Nature Communications* 8 (1): 1635. https://doi.org/10.1038/s41467-017-01798-5.

Loudon, James E., Monica L. Wakefield, Heather M. Kimel, Michel T. Waller, Alexana J. Hickmott, Frances J. White, and Matt Sponheimer. 2019. "Stable Isotope Data from Bonobo (*Pan paniscus*) Faecal Samples from the Lomako Forest Reserve, Democratic Republic of the Congo." *African Journal of Ecology* 57 (3): 437–42.

Loudon, James E., Heather M. Kimel, Michel T. Waller, Monica L. Wakefield, Alexana Hickmott, Francis J. White, and Matt Sponheimer. 2017. "Pairing Feeding Observations with Stable Isotope Data from Bonobo (*Pan paniscus*) Fecal Samples from the Lomako Nature Reserve, Democratic Republic of the Congo." *American Journal of Physcial Anthropology*, 162:267–267.

Lozano, Jorge, Marcos Moleón, and Emilio Virgós. 2006. "Biogeographical Patterns in the Diet of the Wildcat, *Felis silvestris schreber*, in Eurasia: Factors Affecting the Trophic Diversity." *Journal of Biogeography* 33 (6): 1076–85. https://doi.org/10.1111/j.1365-2699.2006.01474.x.

MacArthur, Robert H., and Eric R. Pianka. 1966. “On Optimal Use of a Patchy Environment.” *The American Naturalist* 100 (916): 603–9. https://doi.org/10.1086/282454.

Magurran, Anne E. 1988. *Ecological Diversity and Its Measurement*. Princeton University Press.

Malenky, Richard K., Richard Wrangham, Colin A. Chapman, and Evelyn Ono Vineberg. 1993. “Measuring Chimpanzee Food Abundance.” *Tropics* 2 (4): 231–44. https://doi.org/10.3759/tropics.2.231.

Malenky, Richard K., and Richard 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): 1–12.

Mallott, Elizabeth K., Carola Borries, Andreas Koenig, Katherine R. Amato, and Amy Lu. 2020. “Reproductive Hormones Mediate Changes in the Gut Microbiome during Pregnancy and Lactation in Phayre’s Leaf Monkeys.” *Scientific Reports* 10 (1): 1–9.

Mallott, Elizabeth K., Katherine R. Amato, Paul A. Garber, and Ripan S. Malhi. 2018. “Influence of Fruit and Invertebrate Consumption on the Gut Microbiota of Wild White-Faced Capuchins (*Cebus capucinus*).” *American Journal of Physical Anthropology* 165 (3): 576–88. https://doi.org/10.1002/ajpa.23395.

Mallott, Elizabeth K., Paul A. Garber, and Ripan S. Malhi. (2017). "Integrating feeding behavior, ecological data, and DNA barcoding to identify developmental differences in invertebrate foraging strategies in wild white-faced capuchins (*Cebus capucinus*)." *American Journal of Physical Anthropology*, *162*(2), 241-254. https://onlinelibrary.wiley.com/doi/full/10.1002/ajpa.23113.

Marshall, Andrew J., Corin M. Boyko, Katie L. Feilen, Ryan H. Boyko, and Mark Leighton. 2009. “Defining Fallback Foods and Assessing Their Importance in Primate Ecology and Evolution.” *American Journal of Physical Anthropology* 140 (4): 603–14. https://doi.org/10.1002/ajpa.21082.

Marshall, Andrew J., and Richard W. Wrangham. 2007. “Evolutionary Consequences of Fallback Foods.” *International Journal of Primatology* 28 (6): 1219–35. https://doi.org/10.1007/s10764-007-9218-5.

Mason, Harold L., Willard M. Hoehn, and Edward C. Kendall. 1938. “Chemical Studies of the Suprarenal Cortex IV: Structures of Compounds C, D, E, F, AND G.” *Journal of Biological Chemistry* 124 (2): 459–74.

Mason, Harold L., Charles S. Myers, and Edward C. Kendall. 1936. “Chemical Studies of the Suprarenal Cortex II. The Identification of a Substance Which Possesses the Qualitative Action of Cortin; Its Conversion into a Diketone Closely Related to Androstenedione.” *Journal of Biological Chemistry* 116 (1): 267–76.

“Mayo: Bifidobacteria: Genomics and Molecular Aspects - Google Scholar.” n.d. Accessed April 29, 2021. https://scholar.google.com/scholar_lookup?hl=en&publication_year=2010&author=B.+Mayo&author=D.+van+Sinderen&title=Bifidobacteria%3A+Genomics+and+molecular+aspects.

McConkey, Kim R., Anton Ario, Firmann Aldy, and David J. Chivers. 2003. “Influence of Forest Seasonality on Gibbon Food Choice in the Rain Forests of Barito Ulu, Central Kalimantan.” *International Journal of Primatology* 24 (1): 19–32. https://doi.org/10.1023/A:1021490327385.

McGrew, William C., P. J. Baldwin, and C. E. G. Tutin. 1988. “Diet of Wild Chimpanzees (*Pan troglodytes verus*) at Mt. Assirik, Senegal: I. Composition.” *American Journal of Primatology* 16 (3): 213–26. https://doi.org/10.1002/ajp.1350160304.

McGrew, William C., Linda F. Marchant, and Caroline A. Phillips. 2009. “Standardised Protocol for Primate Faecal Analysis.” *Primates* 50 (4): 363–66. https://doi.org/10.1007/s10329-009-0148-z.

McKenney, Erin A., Thomas M. O’Connell, Allen Rodrigo, and Anne D. Yoder. 2018. “Feeding Strategy Shapes Gut Metagenomic Enrichment and Functional Specialization in Captive Lemurs.” *Gut Microbes* 9 (3): 202–17.

McKenney, Erin A., Allen Rodrigo, and Anne D. Yoder. 2015. “Patterns of Gut Bacterial Colonization in Three Primate Species.” Edited by Markus M. Heimesaat. *PLOS ONE* 10 (5): e0124618. https://doi.org/10.1371/journal.pone.0124618.

McKenzie, Valerie J., Se Jin Song, Frédéric Delsuc, Tiffany L. Prest, Angela M. Oliverio, Timothy M. Korpita, Alexandra Alexiev, et al. 2017. “The Effects of Captivity on the Mammalian Gut Microbiome.” *Integrative and Comparative Biology* 57 (4): 690–704. https://doi.org/10.1093/icb/icx090.

Millspaugh, Joshua J., and Brian E. Washburn. 2004. “Use of Fecal Glucocorticoid Metabolite Measures in Conservation Biology Research: Considerations for Application and Interpretation.” *General and Comparative Endocrinology* 138 (3): 189–99. https://doi.org/10.1016/j.ygcen.2004.07.002.

Mittelbach, Gary G., and Brian J. McGill. 2019. *Community Ecology*. Oxford University Press.

Modesto, Monica, Samanta Michelini, Ilaria Stefanini, Camillo Sandri, Caterina Spiezio, Annamaria Pisi, Gianfranco Filippini, Bruno Biavati, and Paola Mattarelli. 2015. "*Bifidobacterium lemurum* sp. Nov., from Faeces of the Ring-Tailed Lemur (*Lemur catta*)." *International Journal of Systematic and Evolutionary Microbiology* 65 (Pt 6): 1726–34. https://doi.org/10.1099/ijs.0.000162.

Moeller, Andrew H. 2017. "The Shrinking Human Gut Microbiome." *Current Opinion in Microbiology* 38 (August): 30–35. https://doi.org/10.1016/j.mib.2017.04.002.

Moeller, Andrew H., Steffen Foerster, Michael L. Wilson, Anne E. Pusey, Beatrice H. Hahn, and Howard Ochman. 2016. "Social Behavior Shapes the Chimpanzee *Pan*-Microbiome." *Science Advances* 2 (1): e1500997. https://doi.org/10.1126/sciadv.1500997.

Moeller, Andrew H., and Jon G. Sanders. 2020. "Roles of the Gut Microbiota in the Adaptive Evolution of Mammalian Species." *Philosophical Transactions of the Royal Society B: Biological Sciences* 375 (1808): 20190597. https://doi.org/10.1098/rstb.2019.0597.

Newton-Fisher, Nicholas E. (1999). The diet of chimpanzees in the Budongo Forest Reserve, Uganda. *African Journal of Ecology*, *37*(3), 344-354.

Nishida, Alex H., and Howard Ochman. 2019. "A Great-Ape View of the Gut Microbiome." *Nature Reviews Genetics* 20 (4): 195–206. https://doi.org/10.1038/s41576-018-0085-z.

O'Brien, Diane M. 2015. "Stable Isotope Ratios as Biomarkers of Diet for Health Research." *Annual Review of Nutrition* 35: 565–94. https://doi.org/10.1146/annurev-nutr-071714-034511.

Ochman, Howard, Michael Worobey, Chih-Horng Kuo, Jean-Bosco N. Ndjango, Martine Peeters, Beatrice H. Hahn, and Philip Hugenholtz. 2010. "Evolutionary Relationships of Wild Hominids Recapitulated by Gut Microbial Communities." Edited by Mark Achtman. *PLoS Biology* 8 (11): e1000546. https://doi.org/10.1371/journal.pbio.1000546.

Oelze, Vicky M., Benjamin T. Fuller, Michael P. Richards, Barbara Fruth, Martin Surbeck, Jean-Jacques Hublin, and Gottfried Hohmann.2011. "Exploring the Contribution and Significance of Animal Protein in the Diet of Bonobos by Stable Isotope Ratio Analysis of Hair." *Proceedings of the National Academy of Sciences* 108 (24): 9792–97. https://doi.org/10.1073/pnas.1018502108.

Oelze, Vicky M., Geraldine Fahy, Gottfried Hohmann, Martha M. Robbins, Vera Leinert, Kevin Lee, Henk Eshuis, et al. 2016. "Comparative Isotope Ecology of African Great Apes." *Journal of Human Evolution* 101 (December): 1–16. https://doi.org/10.1016/j.jhevol.2016.08.007.

Oksanen, Jari, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, and Helene Wagner. 2013. "Package 'Vegan.'" *Community Ecology Package, Version* 2 (9): 1–295.

Parish, Amy R., Frans BM De Waal, and David 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.

Phillips, Caroline A., and William C. McGrew. 2014. "Macroscopic Inspection of Ape Feces: What's in a Quantification Method?" *American Journal of Primatology* 76 (6): 539–50. https://doi.org/10.1002/ajp.22246.

Pielou, Evelyn Chris 1974. *Population and Community Ecology: Principles and Methods*. CRC Press.

Potts, Kevin B., David P. Watts, and Richard W. Wrangham. 2011. "Comparative Feeding Ecology of Two Communities of Chimpanzees (*Pan troglodytes*) in Kibale National Park, Uganda." *International Journal of Primatology* 32 (3): 669–90. https://doi.org/10.1007/s10764-011-9494-y.

Pyke, Graham H., H. Ronald Pulliam, and Eric L. Charnov. 1977. "Optimal Foraging: A Selective Review of Theory and Tests." *The Quarterly Review of Biology* 52 (2): 137–54. https://doi.org/10.1086/409852.

Raulo, Aura, Lasse Ruokolainen, Avery Lane, Katherine Amato, Rob Knight, Steven Leigh, Rebecca Stumpf, Bryan White, Karen E. Nelson, and Andrea L. Baden. 2018. "Social Behaviour and Gut Microbiota in Red-Bellied Lemurs (*Eulemur rubriventer*): In Search of the Role of Immunity in the Evolution of Sociality." *Journal of Animal Ecology* 87 (2): 388–99.

Ren, Tiantian, Laura E. Grieneisen, Susan C. Alberts, Elizabeth A. Archie, and Martin Wu. 2016. "Development, Diet and Dynamism: Longitudinal and Cross-Sectional Predictors of Gut Microbial Communities in Wild Baboons." *Environmental Microbiology* 18 (5): 1312–25. https://doi.org/10.1111/1462-2920.12852.

Richard, Allison. 1985. *Primates in Nature*.

Rogers, M. Elizabeth, Kate Abernethy, Magdalena Bermejo, Chloe Cipolletta, Diane Doran, Kelley McFarland, Tomoaki Nishihara, Melissa Remis, and Caroline EG Tutin. 2004. "Western Gorilla Diet: A Synthesis from Six Sites." *American Journal of Primatology: Official Journal of the American Society of Primatologists* 64 (2): 173–92.

Rothman, Jessica M., Colin A. Chapman, and Peter J. Van Soest. 2012. "Methods in Primate Nutritional Ecology: A User's Guide." *International Journal of Primatology* 33 (3): 542–66. https://doi.org/10.1007/s10764-011-9568-x.

Ryan, Karen K., Valentina Tremaroli, Christoffer Clemmensen, Petia Kovatcheva-Datchary, Andriy Myronovych, Rebekah Karns, Hilary E. Wilson-Pérez, et al. 2014. "FXR Is a Molecular Target for the Effects of Vertical Sleeve Gastrectomy." *Nature* 509 (7499): 183–88. https://doi.org/10.1038/nature13135.

Sandrini, Sara, Marwh Aldriwesh, Mashael Alruways, and Primrose Freestone. 2015. "Microbial Endocrinology: Host–Bacteria Communication within the Gut Microbiome." *Journal of Endocrinology* 225 (2): R21–34. https://doi.org/10.1530/JOE-14-0615.

Sarkar, Amar, Siobhán Harty, Katerina V.-A. Johnson, Andrew H. Moeller, Elizabeth A. Archie, Laura D. Schell, Rachel N. Carmody, Timothy H. Clutton-Brock, Robin I. M. Dunbar, and Philip W. J. Burnet. 2020. "Microbial Transmission in Animal Social Networks and the Social Microbiome." *Nature Ecology & Evolution* 4 (8): 1020–35. https://doi.org/10.1038/s41559-020-1220-8.

Sayers, Ken, Marilyn A. Norconk, and Nancy L. Conklin-Brittain. 2009. "Optimal Foraging on the Roof of the World: Himalayan Langurs and the Classical Prey Model." *American Journal of Physical Anthropology*, NA-NA. https://doi.org/10.1002/ajpa.21149.

Schoeninger, Margaret J. 2014. "Stable Isotope Analyses and the Evolution of Human Diets." *Annual Review of Anthropology* 43 (1): 413–30. https://doi.org/10.1146/annurev-anthro-102313-025935.

Serckx, Adeline, Hjalmar S. Kühl, Roseline C. Beudels-Jamar, Pascal Poncin, Jean-François Bastin, and Marie-Claude Huynen. 2015. "Feeding Ecology of Bonobos Living in Forest-Savannah Mosaics: Diet Seasonal Variation and Importance of Fallback Foods: Bonobo Diet in Forest-Savannah Mosaics." *American Journal of Primatology* 77 (9): 948–62. https://doi.org/10.1002/ajp.22425.

Shannon, Claude E., and Warren Weaver. 1949. "A Mathematical Model of Communication." *Urbana, IL: University of Illinois Press* 11.

Sistiaga, Ainara, Richard Wrangham, Jessica M. Rothman, and Roger E. Summons. 2015. "New Insights into the Evolution of the Human Diet from Faecal Biomarker Analysis in Wild Chimpanzee and Gorilla Faeces." Edited by Roscoe Stanyon. *PLOS ONE* 10 (6): e0128931. https://doi.org/10.1371/journal.pone.0128931.

Springer, Andrea, Claudia Fichtel, Gabriel A. Al-Ghalith, Flávia Koch, Katherine R. Amato, Jonathan B. Clayton, Dan Knights, and Peter M. Kappeler. 2017. "Patterns of Seasonality and Group Membership Characterize the Gut Microbiota in a Longitudinal Study of Wild Verreaux's Sifakas (*Propithecus verreauxi*)." *Ecology and Evolution* 7 (15): 5732–45. https://doi.org/10.1002/ece3.3148.

Stephens, W. Zac, Travis J. Wiles, Emily S. Martinez, Matthew Jemielita, Adam R. Burns, Raghuveer Parthasarathy, Brendan J. M. Bohannan, and Karen Guillemin. 2015. "Identification of Population Bottlenecks and Colonization Factors during Assembly of Bacterial Communities within the Zebrafish Intestine." Edited by Andrew Goodman and Richard Losick. *MBio* 6 (6): e01163-15. https://doi.org/10.1128/mBio.01163-15.

Strier, Karen B. 2015. *Primate Behavioral Ecology*. Routledge.

Stulberg, Elizabeth, Deborah Fravel, Lita M. Proctor, David M. Murray, Jonathan LoTempio, Linda Chrisey, Jay Garland, et al. 2016. "An Assessment of US Microbiome Research." *Nature Microbiology* 1 (January): 15015. https://doi.org/10.1038/nmicrobiol.2015.15.

Surbeck, Martin, and Gottfried Hohmann. 2008. "Primate Hunting by Bonobos at LuiKotale, Salonga National Park." *Current Biology* 18 (19): R906–7. https://doi.org/10.1016/j.cub.2008.08.040.

Ting, I. P., J. Hann, N. M. Holbrook, F. E. Putz, L. da S. L. Sternberg, D. Price, and G. Goldstein. 1987. "Phosotynthesis in Hemiepiphytic Species of Clusia and Ficus." *Oecologia* 74 (3): 339–46. https://doi.org/10.1007/BF00378927.

Trosvik, Pål, Eric J. de Muinck, Eli K. Rueness, Peter J. Fashing, Evan C. Beierschmitt, Kadie R. Callingham, Jacob B. Kraus, et al. 2018. "Multilevel Social Structure and Diet Shape the Gut Microbiota of the Gelada Monkey, the Only Grazing Primate." *Microbiome* 6 (1): 84. https://doi.org/10.1186/s40168-018-0468-6.

Tung, Jenny, Luis B Barreiro, Michael B Burns, Jean-Christophe Grenier, Josh Lynch, Laura E Grieneisen, Jeanne Altmann, Susan C Alberts, Ran Blekhman, and Elizabeth A Archie. 2015. "Social Networks Predict Gut Microbiome Composition in Wild Baboons." *ELife* 4 (March): e05224. https://doi.org/10.7554/eLife.05224.

Tutin, Caroline E. G., 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. https://doi.org/10.1002/ajp.1350300305.

Tutin, Caroline E. G., Michel Fernandez, M. Elizabeth Rogers, Elizabeth A. Williamson, and William C. McGrew. 1991. "Foraging Profiles of Sympatric Lowland Gorillas and Chimpanzees in the Lopé Reserve, Gabon." *Phil. Trans. R. Soc. Lond. B* 334 (1270): 179–86. https://doi.org/10.1098/rstb.1991.0107.

Uenishi, Gentaro, Shiho Fujita, Gaku Ohashi, Akino Kato, Shino Yamauchi, Tetsuro Matsuzawa, and Kazunari Ushida. 2007. "Molecular Analyses of the Intestinal Microbiota of Chimpanzees in the Wild and in Captivity." *American Journal of Primatology* 69 (4): 367–76. https://doi.org/10.1002/ajp.20351.

Vlčková, Klára, Kathryn Shutt-Phillips, Michael Heistermann, Barbora Pafčo, Klára J. Petrželková, Angelique Todd, David Modrý, et al. 2018. "Impact of Stress on the Gut Microbiome of Free-Ranging Western Lowland Gorillas." *Microbiology,* 164 (1): 40–44. https://doi.org/10.1099/mic.0.000587.

Waal, Frans B. M. de. 2005. "A Century of Getting to Know the Chimpanzee." *Nature* 437 (7055): 56–59. https://doi.org/10.1038/nature03999.

Wakefield, Monica L., Alexana J. Hickmott, Colin M. Brand, Ian Y. Takaoka, Lindsey M. Meador, Michel T. Waller, and Frances J. White. 2019. "New Observations of Meat Eating and Sharing in Wild Bonobos (*Pan paniscus*) at Iyema, Lomako Forest Reserve, Democratic Republic of the Congo." *Folia Primatologica* 90 (3): 179–89.

Waller, Michel T., and Frances J. White. 2016. "The Effects of War on Bonobos and Other Nonhuman Primates in the Democratic Republic of the Congo." In *Ethnoprimatology*, edited by Michel T. Waller, 179–92. Developments in Primatology: Progress and Prospects. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-30469-4_10.

Watts, David. 1984. "Composition and Variability of Mountain Gorilla Diets in the Central Virungas." *American Journal of Primatology*. *7*(4), 323-356. https://onlinelibrary.wiley.com/doi/abs/10.1002/ajp.1350070403.

White, Frances. 1986. "Census and Preliminary Observations on the Ecology of the Black-Faced Black Spider Monkey (*Ateles paniscus chamek*) in Manu National Park, Peru." *American Journal of Primatology* 11 (2): 125–32.

White, Frances J. 1992. "Pygmy Chimpanzee Social Organization: Variation with Party Size and between Study Sites." *American Journal of Primatology* 26 (3): 203–14.

———. 1996a. "Comparative Socio-Ecology of *Pan paniscus*." *Great Ape Societies*, 29–41.

———. 1996b. “Pan Paniscus 1973 to 1996: Twenty-Three Years of Field Research.” *Evolutionary Anthropology: Issues, News, and Reviews: Issues, News, and Reviews* 5 (1): 11–17.

———. 1998. “Seasonality and Socioecology: The Importance of Variation in Fruit Abundance to Bonobo Sociality.” *International Journal of Primatology* 19 (6): 1013–27.

White, Frances J., Colin M. Brand, Alexana J. Hickmott, and India R. Minton. 2020. “Sex Differences in Bonobo (*Pan paniscus*) Terrestriality: Implications for Human Evolution.” *Journal of Anthropological Sciences* 98: 5–14.

White, Frances J., and Kimberley D. Wood. 2007. “Female Feeding Priority in Bonobos, *Pan paniscus*, and the Question of Female Dominance.” *American Journal of Primatology: Official Journal of the American Society of Primatologists* 69 (8): 837–50.

White, Frances J., and Richard W. Wrangham. 1988. “Feeding Competition and Patch Size in the Chimpanzee Species *Pan paniscus* and *Pan troglodytes*.” *Behaviour* 105 (1/2,): 148–64.

Wikberg, Eva C., Diana Christie, Pascale Sicotte, and Nelson Ting. 2020. “Interactions between Social Groups of Colobus Monkeys (*Colobus vellerosus*) Explain Similarities in Their Gut Microbiomes.” *Animal Behaviour* 163 (May): 17–31. https://doi.org/10.1016/j.anbehav.2020.02.011.

Wikberg, Eva C., Pascale Sicotte, Fernando A. Campos, and Nelson Ting. 2012. “Between-Group Variation in Female Dispersal, Kin Composition of Groups, and Proximity Patterns in a Black-and-White Colobus Monkey (*Colobus vellerosus*).” *PLOS One* 7 (11).

Wrangham, Richard W. 1980. “An Ecological Model of Female-Bonded Primate Groups.” *Behaviour* 75 (3/4): 262–300.

Yamagiwa, Juichi., Maruhashi, T., & Yumoto, T. (1996). "Dietary and Ranging Overlap in Sympatric chimpanzees in Kahuzi-Biega National Park, Zaïre." *Great ape societies*, 82.

Yang, Lingyu, Shuyun Liu, Jinmei Ding, Ronghua Dai, Chuan He, Ke Xu, Christa F. Honaker, Yan Zhang, Paul Siegel, and He Meng. 2017. “Gut Microbiota Co-Microevolution with Selection for Host Humoral Immunity.” *Frontiers in Microbiology* 8 (July). https://doi.org/10.3389/fmicb.2017.01243.

Yatsunenko, Tanya, Federico E. Rey, Mark J. Manary, Indi Trehan, Maria Gloria Dominguez-Bello, Monica Contreras, Magda Magris, et al. 2012. "Human Gut Microbiome Viewed across Age and Geography." *Nature* 486 (7402): 222–27. https://doi.org/10.1038/nature11053.

Yildirim, Suleyman, Carl J. Yeoman, Maksim Sipos, Manolito Torralba, Brenda A. Wilson, Tony L. Goldberg, Rebecca M. Stumpf, Steven R. Leigh, Bryan A. White, and Karen E. Nelson. 2010. "Characterization of the Fecal Microbiome from Non-Human Wild Primates Reveals Species Specific Microbial Communities." Edited by Roy Martin Roop Ii. *PLoS ONE* 5 (11): e13963. https://doi.org/10.1371/journal.pone.0013963.

Yolken, Robert, Emese Prandovszky, Emily G. Severance, Glen Hatfield, and Faith Dickerson. 2020. "The Oropharyngeal Microbiome Is Altered in Individuals with Schizophrenia and Mania." *Schizophrenia Research*.

Zhou, You-Bing, Chris Newman, Wen-Ting Xu, Christina D. Buesching, Andrzej Zalewski, Yayoi Kaneko, David W. Macdonald, and Zong-Qiang Xie. 2011. "Biogeographical Variation in the Diet of Holarctic Martens (Genus *Marte*s, Mammalia: Carnivora: Mustelidae): Adaptive Foraging in Generalists." *Journal of Biogeography* 38 (1): 137–47. https://doi.org/10.1111/j.1365-2699.2010.02396.x.