The Dietary Ecology of Ring-Tailed Lemurs As Reflected By

δ^{13}C and δ^{15}N Stable Isotope Values

by

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Madagascar is one of the top global hotspots for biodiversity (Yoder & Nowak, 2006). Yet it is experiencing high rates of habitat loss, especially gallery forest, an important resource to many endemic primate species (Sauther et al., 1999; Sussman & Rakotozafy, 1994). Natural disasters such as Madagascar's cyclical droughts and cyclones may compound the effects of habitat loss (Gould et al., 1999; Whitelaw, 2010; Wright, 1999). Changes in dietary ecology as a result of these events may be reflected in altered hair δ^{13}C and δ^{15}N values from the study population. By studying how lemurs respond to cyclone and drought habitat disturbance, researchers can better understand how forest destruction and non-native species introduction by humans might impact the ability of lemurs to effectively react for continued survival.

This study looks at the effects of habitat disturbance on four endemic Malagasy ring-tailed lemur troops via stable carbon and nitrogen isotope values of hair samples collected between 2003-2006. Severe droughts affected the region in 2004 and 2006. In January 2005, cyclone Ernest hit southwestern Madagascar causing widespread defoliation, tree falls, and a decrease in food availability (Whitelaw, 2010).

δ^{13}C and δ^{15}N values shifted in a non-uniform pattern based on both troop and year. This suggests ring-tailed lemur dietary strategies may vary between troops and depending on what environmental effects are present. During normal years, troops may compete over highly desirable resources. During a drought, they may focus on a few less preferred but abundant resources, reducing their dietary diversity. After a cyclone, the drought resistant resources may no longer be widely available, increasing intra-troop competition and requiring ring-tailed lemurs to broaden their dietary

diversity. Because they are synchronous group feeders (Sauther et al., 1999), a low availability of any single resource may require them to also increase dietary diversity within the troop, forcing individuals to focus on different types of resources. This explanation is one possible interpretation supported by the $\delta^{13}C$ and $\delta^{15}N$ values presented in this thesis.

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CHAPTER 1

INTRODUCTION

1.1 Study Overview

The purpose of this thesis is to support and expand upon our understanding of primate responses to environmental variability. In this thesis, I examine the effects of extreme habitat disturbance on physical and behavioral responses of ring-tailed lemur (*Lemur catta*) troops through stable carbon and nitrogen isotope analysis of hair samples. I focus on four ring-tailed lemur troops in Beza Mahafaly Special Reserve, Madagascar between the years 2003 through 2006, and how their dietary ecology adapts to the extreme conditions caused by Madagascar's periodic droughts and cyclones. Stable carbon and nitrogen isotope analysis provides a means through which to explore ecological responses that might not be obvious by only using behavioral observation.

Cyclone Ernest affected the study site in January 2005 and the region experienced severe drought in 2004 and 2006. Because this study consists of samples taken from 2003-2006, comparisons can be drawn between dietary strategies during drought, cyclone and regular rainfall and ecological conditions (see Chapter 6 for ecological category criteria). Using stable isotope analysis allows a measurable comparison of relative dietary states of troops between years with typical rainfall and food resources, and periods of habitat disturbance. Pairing isotope analysis with lemur behavioral analysis during similar events, this study expands upon current literature and strengthens our understanding of how lemurs respond and adapt to habitat disturbance.

1.2 Study Importance

Faunal records indicate species extinction rates are on the rise (Bellard et al., 2012; Pimm et al., 1995). Biodiversity conservation is a growing concern, and one of the major threats to species persistence is habitat destruction and disturbance (Cuarón, 2000; Gould et al., 1999; LaFleur & Gould, 2009). Natural disasters such as cyclones and droughts can damage existing food resources and limit lemur reproductive potential (Gould et al., 1999; Whitelaw, 2010; Wright, 1999).

Habitat destruction and disturbance is a broad phrase which includes a variety of causes. Two major forms of habitat destruction are anthropogenic disturbance and natural disasters. Anthropogenic disturbance (environments changing due to human activity) can affect lemur populations through activities such as hunting and the logging of trees lemurs use for protection, food and sleeping (Chapman et al., 2000; Cuarón, 2000; Fa et al., 1995; Whitelaw, 2010). Natural disasters and climatic events can cause significant destruction to food resources through water stress, loss of crown cover, limb loss, the uprooting of trees and blossom destruction, all of which limit fruit, blossom and leaf availability to consumers (Behie & Pavelka, 2005; Erhart & Overdorff, 2008; Pavelka et al., 2007; Ratsimbazafy et al., 2002; Wright, 1999). This thesis explores how species that rely on these resources respond to its loss.

1.3 Chapter Outline

The next four chapters provide the background necessary to understand and interpret the remainder of the material and data presented. Chapter 2 introduces the Malagasy study site and explores its unique geologic and faunal history which is necessary to understand the evolutionary context of ring-tailed lemurs and the ecological challenges they face. Understanding the context in which lemurs evolved facilitates later discussion of the suite of adaptive traits available to ring-tailed

lemurs when responding to habitat disturbance. Chapters 3 and 4 cover primate and ring-tailed lemur physical and behavioral responses to habitat disturbance. Chapter 3 includes all primate literature while Chapter 4 focuses exclusively on ring-tailed lemurs. The discussions of both chapters revolve around environmental effects on dietary ecology, group size and organization, birth rate, and infant mortality. Chapter 5 begins with a brief description of what isotopes are and how the data they provide can be applied to ecological studies. This provides readers with the knowledge necessary to understand the chapter's following literature section, which reviews isotopic responses to environmental variables. The literature focuses on how extreme environmental changes can produce a variety of isotopic patterns based on the behavioral responses utilized. I include relevant data from non-primate species.

Chapters 6 through 8 present my study including its methodology, the data analysis, and interpretation of my results. Chapter 6 provides a more detailed description of my sampling breadth and the methods used for animal capture, sample collection and preparation, determination of isotopic abundances, and data analysis. Chapter 7 presents my results. It includes an explanation of the variables used in my analysis, a summary statistics table, and a variety of graphs and plots to support my presentation. The presentation of data primarily focuses on the variability in means, the magnitude of ranges, and how the data shift by year. Chapter 8 discusses the extreme variability of the data, explores environmental effects which may be reflected in the data, and provides one possible interpretation to account for the data variability and observed data shift.

The conclusion briefly reviews the intent and scope of this study, major findings, and suggestions for future research to support a more complete understanding of the data.

CHAPTER 2

BACKGROUND TO MADAGASCAR AND BEZA MAHAFALY SPECIAL RESERVE

<u>2.1 Geological History of Madagascar</u>

Because I am looking at lemurs, it is important to understand the environmental context of Madagascar to better isolate potential effects to lemur ecology.

Madagascar is an island country located in the Indian Ocean off the southeast coast of Africa. However, the land currently known as Madagascar was landlocked within the supercontinent Gondwana approximately 175 million years ago. At 175 Ma, the land to the north of Madagascar would eventually break apart to become South America and Africa. The land to the south would eventually break apart to become the separate landmasses of India, Antarctica and Australia (Yoder & Nowak, 2006). Gondwana began to break apart between 165 and 155 Ma, separating into northern and southern landmasses, with Madagascar being the last major land connection between the landmasses. From 130-118 Ma, the landmass containing modern Madagascar and India broke away from both the supercontinent Gondwana and the other southern landmasses Antarctia and Australia, and IndoMadagascar shifted to Madagascar's current location relative to Africa. Madagascar broke away from the western edge of India between 100-87 Ma (estimates vary). India eventually shifted north, colliding with Asia in the early Eocene, and Madagascar has since remained marooned in the Indian Ocean off the southeastern African coast (Yoder & Nowak, 2006).

2.2 Faunal History of Madagascar: Origin of Lemurs

There are two main types of events that may have contributed to Madagascar's current faunal diversity: vicariance and dispersal. Vicariance occurs when a previously single species inhabiting an area becomes multiple isolated groups, often leading to independent evolution of these isolated groups into separate species. This could have occurred both when Gondwana split in half and later when Madagascar separated from India, which would have isolated the ancestral lemur species and led to largely independent evolution on Madagascar for the last 87 million years (Yoder & Nowak, 2006). Madagascar's history of geographic isolation has convinced many researchers that vicariant events are largely responsible for Madagascar's high endemism.

However, while these events likely do apply to some Malagasy species, current studies tracing the phylogenetic origins of multiple taxa indicate dispersal events may have been more influential to modern Malagasy diversity, including the existence of the endemic lemurs (Yoder & Nowak, 2006). Dispersal occurs when a species travel across an existing border. In this case, it would mean ancestral lemurs arrived on Madagascar after it was already geographically isolated. From what we currently understand of Madagascar's biogeographical history, it is believed ancestral lemurs were able to travel to Madagascar sometime between 80-50 Ma (estimates vary) via a rafting event (live animals traveling on a floating piece of land) using ocean currents (Stankiewicz et al., 2006). Yoder and Nowak (2006) suggest this ocean voyage was made possible through two small areas of the Mozambique Channel (which separates Madagascar from mainland Africa) that extended above the water, breaking the otherwise extensive oceanic trip into three short trips following normal oceanic currents from Africa to Madagascar.

2.3 Contemporary Endemism and Habitat Disturbance in Madagascar

Although ancestral lemurs existed on mainland Africa, the separation between Africa and Madagascar would likely have facilitated independent evolution in the populations. Only the Malagasy population evolved into modern lemurs, which is why lemurs are considered endemic to Madagascar. Lemurs are not the only organisms endemic to Madagascar. Goodman and Benstead (2005) estimate approximately 85% of vascular plant species, 86% of macroinvertebrates, and 84% of all land vertebrate species are endemic to Madagascar. Other studies have estimated that 95% of reptile species, 99% of amphibians, 100% of land mammals (excluding bats) and 10,000 of the estimated 12,000 plant species are endemic to Madagascar (Yoder & Nowak, 2006). This high rate of species endemism is related to Madagascar's unique biogeographic history. As another by-product of Madagascar's geographic isolation, it has also been relatively isolated from significant human influences until approximately 2,000 years ago, when it is estimated humans began colonizing the region (Goodman & Benstead, 2005). The combined factors of high rates of biodiversity, high endemism, limited historical anthropogenic effects and geographic isolation make Madagascar one of the top areas of primate research and conservation efforts (Dunham et al., 2011; Goodman & Benstead, 2005).

There are two significant types of habitat disruption occurring in Madagascar: anthropogenic disturbance and dramatic climatic events, for which Madagascar is well known (Dunham et al., 2011). Although I intend to focus on studies of natural events leading to changes in lemur ecology (with a focus on ring-tailed lemurs), it is essential for researchers to first understand and, ideally, isolate the effects of anthropogenic disturbance. Biological anthropologists and ecologists know the potentially disastrous effects anthropogenic disturbance can have on environments and biodiversity, so it is useful for researchers to study Madagascar, which has a much shorter history of anthropogenic disturbance to account for when studying the effects of natural events on faunal ecology. Ideally, our more complete understanding of human colonization in Madagascar combined with its recent occurrence allows

researchers to better understand how humans have altered the environment to its current state. Once we can account for these effects, we can better isolate factors which affect how natural events are altering lemur ecology.

The significant natural events in Madagascar that I am going to focus on are droughts and cyclones. These events periodically disturb floral and faunal wildlife populations (Dunham et al., 2011; Gould et al., 1999; LaFleur & Gould, 2009). Because these extreme events have occurred so frequently throughout known Malagasy history, many researchers have suggested Malagasy organisms have evolved to better adapt to these events (LaFleur & Gould, 2009; Wright, 1999). Madagascar frequently experiences several cyclones per year (Whitelaw, 2010). The potential effect of these events should not be underestimated. Cyclone Gretelle in 1997 was reported to have destroyed 75% of the trees in Manombo Forest in southeastern Madagascar, including 90% of native trees, and lemur populations were reduced by 50% (Ralainasolo et al., 2008). The surviving native trees lost 85% of their crown volume and lost an estimated 70% of their annual fruit production (Whitelaw, 2010). This level of destruction can have huge impacts on local faunal populations, especially in species such as ring-tailed lemurs that frequently rely on trees for protection and food.

2.4 Beza Mahafaly and Berenty Reserves

Many lemur studies occur within two separate reserves in southern Madagascar: Berenty Reserve and Beza-Mahafaly Special Reserve. Berenty is a semiarid reserve which includes an area of spiny forest as well as two large areas of gallery forest, one of which is the main Berenty Reserve. The main Berenty Reserve consists of gallery and scrub forest with spiny forest on the western side (Jolly et al., 2006). Beza-Mahafaly consists of two separate sections, Parcel 1 which grades from eastern gallery forest to western spiny forest, and Parcel 2 consisting of primarily spiny forest (Gould et al., 1999). Ring-tailed lemurs must adapt to different circumstances, and populations vary in their tolerance and

responses to disturbed habitats (Cuarón, 2000; Whitelaw, 2010). The studies I explore constitute an overview of research previously reported in this field and establish a baseline for comparison with my study's isotope results.

CHAPTER 3

HOW PRIMATES RESPOND TO HABITAT DISTURBANCE

3.1 Effects on Infant Mortality, Birth Rate, Group Size and Organization

Many studies have reported a correlation between major habitat disturbances and shifts in population and social organization. Erhart and Overdorff (2008) found that, with the increase in the number of cyclones between 1994 and 2003, fruit availability decreased and average group size of the Eulemur *fulvus rufus* declined by 46%. Along with cyclone Gretelle's 1997 destruction of 75% of trees and 80% of the canopy in Manombo Forest, researchers noted a significant decrease in fruit production correlated with a population reduction of over half of all active day lemurs (Erhart & Overdorff, 2008). Cyclone Gretelle also affected black and white ruffed lemur populations, in which study groups produced no surviving infants for at least 3 years after the devastating cyclone (Ratsimbazafy et al., 2002).

After the 2001 hurricane Iris on Belize, the black howler monkey (*Alouatta pigra*) population 4 months after the hurricane was reduced by 40%, and the population continued to drop as a 3.5 year follow-up study estimated a total 88% reduction from pre-hurricane population estimates (Behie & Pavelka, 2005; Pavelka et al., 2007). There were also social shifts post-hurricane. The black howlers immediately increased the amount of time they spent resting (possibly due to shade loss from crown damage), although resting time still stayed within the expected range for their species. There was also a 12-week period of social disorganization in which there were high numbers of solitary or transient individuals and existing groups were smaller (Behie & Pavelka, 2005).

Similarly, spider monkeys were found to spend more time resting after hurricanes Emily and Wilma moved through the Yucatan peninsula in 2005. While their social organization is normally characterized by a high degree of fission-fusion behavior, the study group was less likely to form larger subgroups during the hurricane recovery period. The authors note previous research suggesting increased rest periods may result either as a necessity of the shift to exploiting lower quality food resources or because foliage is a fairly continuous resource allowing for reduced home range (Schaffner et al., 2012).

Contrary to the previous studies, verreaux's sifaka population density after 2009 cyclone Fanele were not significantly altered. Cyclone Fanele hit the sifaka food resources hard, damaging ~86% of their food trees with 25-50% of the largest food trees experiencing severe damage in the form of snapped trunks or major delimbing. In addition to population estimates, researchers took skinfold and body mass readings as an indicator of health and found no significant changes to the population within the 2 year study period. The study did not include feeding strategy, so it was not reported how the verreaux's sifaka maintained health despite food resource damage, but the authors note the sifaka's high folivory may afford it some foraging protection from cyclone effects. Cyclones cause significant reduction in immediate crown volume and loss of flowers and fruits, but the following period is characterized by flora concentrating their energy into new growth which results in uncharacteristically high levels of new leaves and thus provides a useful food source for folivores until post cyclone forest structure returns (Lewis & Rakotondranaivo, 2011).

The abundance of new leaves may buffer folivores during the cyclone recovery period, which may have allowed for the verraeux's sifaka continued health in Lewis and Rakotondranaivo's study. Following a 1978 cyclone off Sri Lanka, Dittus reported the destruction of more than 50% of the woody vegetation responsible for producing the majority of the food for two langur species (langurs eat approximately equal proportions of flowers/fruits and leaves with a significant amount of leaf

consumption consisting of young leaves) (Dittus, 1985; Hladik, 1977). However, there was an incongruence in immediate post-cyclone damage and long-term tree deaths. Dittus suggested this was caused by langur populations becoming more folivorous post-cyclone and, because a greater proportion of the foliage available was new leaves, langurs ate a greater proportion of new leaves, which hindered the ability of trees to recover from the cyclone's effects. Thus, Dittus believes the post cyclone near disappearance of 3 tree species was due to langur overbrowsing of new leaves.

3.2 Effects on Dietary Ecology

Researchers frequently predict changes in diet associated with natural disaster related habitat disturbance, but how primate feeding ecology responds to such disturbance is variable. Known responses include shifting their dietary focus to previously minimally consumed resources, continuing to eat the same type of resources but shifting to non-preferred species, and exploiting food resources not previously consumed (Ambrose & DeNiro, 1986; Behie & Pavelka, 2005; Berenstain, 1986; Gould et al., 1999; Ratsimbazaft et al., 2002; Schaffner et al., 2012).

Black and white ruffed lemurs (*Varecia v. variegata*) responded to cyclone Gretelle by shifting their focus from ripe fruits of preferred native species to the unripe fruits of two non-endemic plant species, *Clidemia hirta* (Melastomataceae) and *Cecropia peltata* (Euphorbiaceae). Although Gretelle resulted in 85% canopy loss and the cessation of 95% of trees taller than 15 meters flowering and fruiting, these non-endemic species have greater reproductive potential manifested in two ways exploited by *Varecia*: continuing to fruit post-cyclone and producing fruit over a much greater period of time annually compared to native species (Ratsimbazafy et al., 2002).

Black howler monkeys, which previously spent 43% of time feeding from reproductive plant parts (primarily fruits) and 57% of time eating leaves, shifted to 99.75% folivory following the 2001 hurricane Iris. Because black howlers have adaptations that allow for some folivory and experience

high dietary seasonality, they are able to survive short-term on a almost pure folivorous diet. Whether this shift to almost exclusive folivory is sustainable long-term or how it impacts future reproductive cycles is less understood. Increased folivory in the black howler study population was accompanied by a variety of behavioral changes in feeding including the incorporation of previously unexploited species, consuming different parts of a preferred species, and altered feeding times for consumed species (Behie & Pavelka, 2005).

Spider monkeys adapted similarly to the black howler monkeys. After hurricanes Emily and Wilma in 2005, spider monkeys of the Yucatan peninsula shifted from primarily frugivorous to primarily folivorous (although this trend was initially obscured by a seasonal shift to feeding on larvae). Even though spider monkeys are normally highly frugivorous, leaves occasionally make up a moderate portion of their diet depending on fruit availability. However, it is debatable whether spider monkeys can survive long term on highly folivorous diets without experiencing negative health and reproductive effects (Schaffner et al., 2012).

CHAPTER 4

HOW RING-TAILED LEMURS RESPOND TO HABITAT DISTURBANCE

4.1 Effects on Infant Mortality, Birth Rate, Group Size and Organization

Jolly et al. (2002) studied ring-tailed lemur demographics from the birth years 1983 through 2000. Although they studied droughts instead of the cyclone effects mentioned by Erhart, Overdorff and Ratsimbazafy, Jolly et al. (2002) also found a correlation between habitat disturbance and lemur populations. Significant droughts occurred in 1984, 1991-1992 and 1997. The post-drought years of 1985 and 1998 experienced good rain, and there was also good rain in 1993 through 1994 following a two-year drought. Although there was a correlation between drought years and low birth rates, it was not exactly the type of correlation expected. In 1985, 1994 and 1998, all years of good rain after a recent drought, less than 10% of females in natural (not human occupied) forest successfully bore and raised a surviving juvenile. These results make sense once you consider the life cycle of the ring-tailed lemur. Ring-tailed lemur females feed and gain weight during the summer rainy season and conserve energy while gestating over the winter dry season. However, the females must survive birth and early lactation while still being largely dependent on fat reserves from the previous wet season (with supplementation from lower quality foods). Once you consider this, it makes sense that the lowest juvenile survivability, one of the effects of droughts, would not become obvious until the year after a drought (Jolly et al., 2002).

A long-term study of nine ring-tailed lemur groups from 1987 to 1996 by Gould et al. (1992) followed mortality rates through a severe 1991-1992 drought and recovery years. In 1992, infant

mortality reached 80%, juvenile mortality was 57% and adult female mortality was 21%. Comparatively, adult female mortality was only 3% in a non-drought year. After these two years of drought, there was not significant population recovery until 1996. Interestingly, of the 21% of adult females who died, all of them had lactating infants at the time of death. Also, compared to the 80% infant mortality rate in 1992, in 1987 (which was 5 years after the last drought) there was a 48% infant mortality rate. Now, compare this to the 1994 birth season, 2 years after the drought, which only saw an 18% infant mortality rate (Gould et al., 1999). These mortality rates might reflect natural selection, in which females or infants less adapted to nutritional stress died off, leaving individuals more able to survive nutritional stress. Or, these rates may reflect some biophysical response triggered in adult females during periods of prolonged stress.

While the actual reasoning behind these differing mortality rates are not fully understood, it is likely these low infant mortality rates in recovery seasons facilitate ring-tailed lemur population survival despite Madagascar's extreme climatic changes.

4.2 Effects on Dietary Ecology

In 2005-2006, Whitelaw (2010) studied ring-tailed lemur populations at Beza Reserve. She compared the feeding ecology of four groups, two in the protected reserve and two in anthropogenically disturbed and unprotected habitats. A cyclone had also disrupted the environment seven months prior to her study, which consequently disrupted the natural forest ecology. Whitelaw determined groups within the protected gallery forest ate less diverse diets compared to groups in unprotected, marginal habitats. The groups in marginal habitats also inhabited a smaller home range, but they traveled further each day to find food. These troops also rested less, groomed less, and showed lower group cohesion compared to protected gallery forest groups. Unfortunately, cyclone Ernest hit Madagascar in January 2005, immediately previous to the study. It is known to have stripped a majority

of trees of their flowers, but how the cyclone differentially affected the habitats was not able to be observed, which makes it difficult to separate which ring-tailed lemur behaviors might be due to anthropogenic or climatic effects (Whitelaw, 2010).

Rasamimanana and Rafidinarivo (1993) reached a similar conclusion in their 1987-1989 study at Berenty reserve. During the dry season (when food stress should be most extreme), the researchers studied two troops in separate areas of the reserve, one that inhabited primarily gallery forest and one that inhabited an anthropogenically disturbed area. They concluded the gallery troop, overall, ate less, had reduced dietary diversity, and reduced activity during the dry season as compared to the disturbed habitat troop. Further, they found the gallery forest troop, despite lower energy intake, increased its troop size by 50% compared to the disturbed habitat troop increase of only 17% (Rasamimanana & Rafidinarivo, 1993).

What I think is most interesting about these studies is their conclusions on dietary diversity. The studies did not overly speculate on the causes of increased dietary diversity in marginal habitats, but I think this is an interesting area for further study. Because cyclones are a brief but intense event, they should most strongly affect the reproductive output of whatever plants are flowering at the time of the cyclone. This idea is supported by a report that a 2005 Malagasy cyclone stripped a majority a tamarind trees of their flowers, dramatically reducing this trademark fallback fruit's availability to ring-tailed lemurs (Whitelaw, 2010). Other researchers including O'Brien (2003) and Pavelka and Behie (2005) have also noted there was a reduction of food species available post-cyclone (Whitelaw, 2010). Although many primate studies have concluded dramatic climatic events disturb habitats, leading to a less diverse diet, Whitelaw (2010) and Rasamimanana and Rafidinarivo's (1993) results conclude marginal habitat groups (which permanently occupy disturbed habitats) have a more diverse diet. What is causing this difference, if there even is a difference? Because Whitelaw's study occurred post-cyclone, is this opposite trend in dietary diversity a false effect? That is to say, could marginal habitats

be less affected by cyclones or other climatic events, which then could account for low post-cyclone gallery diversity while simultaneously allowing relatively high levels of marginal diversity?

Of course, there is also the possibility lemurs (compared to other primates), specific species of lemurs, or even different populations of a species could experience the same environmental effects, in the same ecological contexts, and yet react in different ways. Studies on other primate species have documented post-cyclone responses that sometimes contradict Whitelaw (2010) and Rasamimanana and Rafidinarivo's (1993) studies, including coping mechanisms by spending less time active, foraging greater distances or decreasing travel time and distances (Whitelaw, 2010). These sometimes opposing conclusions should not negatively reflect on the marginal diversity studies. However, it does show the need for further research using multiple different comparison methods to better isolate the causes for these opposing conclusions.

CHAPTER 5

STABLE ISOTOPE ECOLOGY

5.1 Chapter Overview

      Isotopic analysis is a common tool used by modern studies in anthropology, paleontology, geology and earth sciences. This chapter begins with a brief overview on what stable isotopes are, how carbon and nitrogen stable isotopes are incorporated into terrestrial ecosystems, and how anthropologists have come to utilize them in studies. I then discuss isotopic literature relevant to my study, focusing on factors which contribute to isotopic variability.

5.2 Stable Isotope Ecology: Overview

      Chemical elements are defined by their number of protons, neutrons and electrons. There are multiple forms each element can take based on the number of neutrons in its nucleus. These forms are called isotopes, and each isotopic form of an element varies only minutely from the others. Yet these differences result in isotopes cycling through the environment in unique ways, which I discuss in more detail later in this chapter (Crowley, 2012; Fry, 2006; Hoeff, 2004; Van der Merwe, 1982).

      Isotopes are referred to by their mass number, which is the total number of protons and neutrons in the nucleus. Because isotopes of an element each vary by their neutron number, each isotope is referred to by a different number (Fry, 2006). I focus on stable isotopes of carbon and nitrogen. The stable isotopes of carbon are ^{12}C and ^{13}C which, for reference, contain 6 and 7 neutrons. The stable isotopes of nitrogen are ^{14}N and ^{15}N. Research of stable isotopes generally revolves around the relative

abundance of two different stable isotopes of the same element, expressed as a ratio. Referring to an isotope as "stable" means it does not undergo radioactive decay, so ratios of stable isotopes in an environment can remain the same over time. This allows researchers analyzing stable isotopes to study materials from a wide age range (Hoeff, 2004).

As I mentioned previously, each isotope cycles through the environment differently, with each step through the environment resulting in isotopes with different masses being preferentially retained or discarded. This process is called fractionation, and it is responsible for the differential isotopic ratios found throughout the environment. When the fractionation cycle for a specific environment is known, researchers can use the isotope ratios to interpret changes in the environment. For example, anthropologists and primatologists can study environments via animal tissues because the isotopes in ingested food and water vary based on habitat, type of food, and environmental conditions, and these isotopes are used to form tissues, providing a record of environmental variables related to diet at the time of tissue deposition (Crowley, 2012; Hoefs, 2008; Van der Merwe, 1982).

Hair is particularly useful for isotopic studies. Hair grows continuously from the base of a follicle and, after it is formed, it is no longer metabolically active. This means the isotopic signature for that specific hair segment will no longer change (Crowley, 2012; Loudon et al., 2007; Sandberg et al., 2012). Because the hair is mostly keratin, the isotopic signature remains preserved over time. Thus, researchers can sample hair from either living or dead animals incrementally to obtain seasonal dietary information (Sponheimer et al., 2009).

Differences in plant carbon isotopic abundances are largely dependent on which photosynthetic pathway the plant takes. The two major categories of plants are C_3 and C_4 plants. Plants that follow the C_3 pathway tend to have slower rates of CO_2 uptake during photosynthesis which results in their $\delta^{13}C$ averaging around -26.5‰ with a typical range of -20‰ to -35‰ based on environmental variables. Comparatively, C_4 plants exhibit more rapid CO_2 uptake and their $\delta^{13}C$ averages around -12.5‰ with a

range typically near -9‰ to -16‰ (estimates by Van der Merwe, 1982 based on data provided by Vogel et al., 1978). If the isotopic value is known for plants consumed by a study group, the $\delta^{13}C$ value of an individual can thus be used by researchers to reconstruct the relative abundances of different plants consumed by the individual (Van der Merwe, 1982).

Differences in nitrogen isotopic abundances are largely dependent on environmental variables and trophic level. Researchers should focus on comparing relative $\delta^{15}N$ values within and between similar types of environments. Plant $\delta^{15}N$ is dependent on the nitrogen composition of the soil it grows in, which in turn is a product of the interaction between many environmental variables. This can include the interactions between fertilizer use in agricultural areas, industrial emissions, volatilization of soil nitrogen, type and quantity of nitrogen-fixing organisms, rate of litter decomposition, and the level of surface soil disturbance (which can also compound the effects of leeching of ^{15}N due to excess precipitation) (Amundson et al., 2003; Evans, 2007; Lee-Thorp & Sponheimer, 2006). Within a given environment, differences in nitrogen isotopic abundances are largely dependent on the individual's trophic level. As trophic level increases, so does $\delta^{15}N$. Once plant $\delta^{15}N$ values are known, each subsequent trophic level exhibits a $\delta^{15}N$ enrichment of around 3‰ to 5‰, although this is variable (Ben-David & Flaherty, 2012; Lee-Thorp & Sponheimer, 2006).

My study focuses on the carbon and nitrogen isotopic ratios of animal hair, which can be of particular use to researchers studying feeding ecology. I discuss stable isotope ratios using the δ notation to express ratio values as a comparison to the international standard in parts per thousand (‰). For example, $\delta^{13}C$ (‰) = ($[^{13}C/^{12}C]_{sample}$ / $[^{13}C/^{12}C]_{standard}$ -1)*1000. For nitrogen stable isotopes, $\delta^{15}N$ (‰) refers to the ratio of ^{15}N to ^{14}N. Observed carbon stable isotope ratios are expressed in values compared to the PeeDee Belemnite (PDB) international standard, which is a Cretaceous marine fossil that has higher ^{13}C values than most the terrestrial biosphere, so most $\delta^{13}C$ values are expressed as negative values. The international standard for nitrogen is atmospheric nitrogen (AIR). $\delta^{15}N$ values tend to be

higher in the terrestrial biosphere relative to atmospheric values, and so are expressed as positive

numbers (Ben-David & Flaherty, 2012).

5.3 Carbon and Nitrogen Stable Isotope Studies: Overview

There is extensive literature on the applications of stable isotope analysis in archaeology,

paleontology and ecology which cannot be covered within the scope of this paper. My focus in this

section is not to explain the developmental history and application of isotopic studies or provide an

exhaustive list of tangential studies. Instead, I focus on material surrounding the utilization of carbon

and nitrogen stable isotopes to the understanding of intra-species variability in dietary ecology,

especially as it applies to local environmental changes. This provides the context for which I describe

ring-tailed lemur dietary variability and response to highly disrupted environments.

5.4 The "Canopy Effect"

In terrestrial ecosystems, the atmosphere provides the carbon dioxide incorporated by plants. If

the $\delta^{13}C$ atmospheric value changes, so does the carbon values in the plants that utilize it. This

phenomenon is relevant to many primate studies due to the "canopy effect." In dense, closed canopy

forest like that utilized by many primate species, rotting leaf litter releases isotopically light carbon

dioxide. The dense canopy minimizes the mixing of this air with the rest of the atmosphere, creating a

pocket of foliage with distinctly low $\delta^{13}C$ values (Van der Merwe, 1982). $\delta^{13}C$ can also be the affected

by light intensities, with denser canopies having increasingly lower light intensities associated with

lower $\delta^{13}C$ (Ehleringer et al., 1986).

This "canopy effect" is also reflected in the isotopic composition of consumer tissues. In a study

of New World Monkeys, the canopy effect contributed more than diet to the carbon isotopic signature

of hair samples. Groups utilizing primarily closed canopy dietary sources had lower $\delta^{13}C$ values, but

$\delta^{15}N$ values remained as expected based on dietary trophic incorporation with omnivores showing the highest $\delta^{15}N$ (Schoeninger et al., 1997). This trend has also been found in a study of 43 East African mammals in the authors were able to clearly distinguish herbivores consuming primarily forest resources from herbivores consuming primarily grassland resources based on the former's lower $\delta^{13}C$ values (Ambrose & DeNiro, 1986).

5.5 Impact of Drought and Water Stress

Drought can have a significant impact on animals both through more direct water stress and indirectly by impacting their available food resources. How animals respond to these stressors has the potential to affect their isotopic signatures.

Ambrose and DeNiro (1986) found in a study of 43 East African herbivore and carnivore species that, within herbivores, more drought-tolerant species showed $\delta^{15}N$ values more similar to carnivore values with higher mean $\delta^{15}N$ compared to herbivores that required regular water consumption. In some cases, drought-tolerant species showed mean $\delta^{15}N$ values as much as 9 permil higher. Ambrose and DeNiro have hypothesized enriched $\delta^{15}N$ could be a side effect of urea excretion mechanisms utilized by drought-adapted species when they are exposed to water stress (1986).

In North American white-tailed deer samples from 46 locations, the authors found bone collagen $\delta^{15}N$ increased as precipitation decreased in animals that consumed more than 10% C_4 plants (Cormie & Schwarcz, 1996). While the authors noted white-tailed deer do not normally consume significant amounts of C_4 grasses, they suggest consumption of them may be indicative of water or nutrient stress. This could also explain the enriched $\delta^{15}N$ values when eating these plants, assuming the $\delta^{15}N$ and water stress concept is valid. The authors suggested nitrogen recycling and urea excretion as the mechanisms that facilitate $\delta^{15}N$ enrichment during water or food stress (Cormie & Schwarcz, 1996).

In a comparative study of two galago and one lepilemur species, Schoeninger et al attributed the

mean 1.8‰ δ^{13}C enrichment of the lepilemurs to the lesser rainfall in Malagasy forests, which are otherwise structurally similar to the forests inhabited by the galago species (1998). Within the lepilemur sample, they also noted a potential correlation of enriched δ^{13}C with increasingly xeric individual home ranges (Schoeninger et al., 1998).

5.6 Isotopic Variability and Body Condition

More recently, studies have attempted to explain individual health in terms of isotopic variability. While animals are anesthetized for health data collection, hair samples can also be easily taken for isotopic studies. By comparing the data, researchers hope to find isotopic indicators of animal health. The previously discussed study of East African animals by Ambrose and DeNiro is also relevant to this health discussion (1986). They found drought-tolerant species showed higher than expected δ^{15}N, which they believed could be the result bodily mechanisms triggered by water stress (Ambrose & DeNiro, 1986). Further studies have suggested this effect may occur in the presence of different stressors.

Hobson et al. (1993) compared isotopic data of birds in a controlled feeding study with values in wild birds. They concluded both wild and captive birds showed enriched δ^{15}N during periods of nutritional stress, regardless of whether this stress resulted in any body mass loss. The authors suggested higher δ^{15}N values were due to bodily mechanisms triggered by nutritional stress in which tissues are catabolized and new tissues become increasingly enriched in δ^{15}N (Hobson et al., 1993). Two studies of whales also used this interpretation to explain cyclical δ^{15}N spikes which seemed to correlate with their seasonal migration patterns during which feeding was reduced (Best & Schell, 1996; Hobson & Schell, 1998).

In a study of ring-tailed lemurs at Beza Mahafaly Special Reserve in Madagascar, Loudon et al. (2007) combined health examinations with hair sample collection and compared body condition to δ^{13}C

and $\delta^{15}N$ values. They found individuals categorized as being in suboptimal health also showed enriched $\delta^{15}N$ values as well as slightly elevated $\delta^{13}C$. This was intensified for individuals in the worst body condition, and individuals across all sexes and troops responded isotopically in the same way. The authors suggested the enriched values could be associated with nutritionally stressed individuals catabolizing their own tissues, as described by previous researchers (Best & Schell, 1996; Hobson & Schell, 1998; Hobson et al., 1993; Loudon et al., 2007).

If higher than expected $\delta^{15}N$ values could be positively associated with nutritional stress, this would be a great advancement in our interpretation of stable isotope variability. However, research is not yet conclusive. In a study of martens, fluctuations in prey availability led to the utilization of non-preferred resources, which was thought to be an indicator of nutritional stress. However, the authors were unable to show body weights changed due to alternative food resource use (Ben-David et al., 1997). In arctic ground squirrels with low food availability, individuals of both excellent and poor body condition showed similar $\delta^{15}N$ values (Ben-David et al., 1999).

5.7 Resource Partitioning

Habitat disturbance, species interactions, and feeding patterns can impact how resources are partitioned within a group, which in turn may be reflected in the individual and group isotopic values. Because species able to effectively use a variety of resources may have survival benefits during resource stress, studying this phenomenon has important implications for researchers studying how climate and habitat disturbance impacts species.

Ambrose and DeNiro (1986) found in a study of 43 East African herbivore and carnivore species that, of all studied savannah-dwelling herbivores, baboons had the lowest $\delta^{15}N$ values. This is likely due in part to their unique diet that includes significant quantities of legumes. Legumes are known to have generally lower $\delta^{15}N$ than other plants within a specific area due to their symbiotic

relationship with nitrogen-fixing micro-organisms (Ambrose & DeNiro, 1986, Ehleringer et al., 1986). This study may have further applications to studies of intra-group variability in food choice by comparing relative δ^{15}N with behavioral data on how food resources are partitioned within a group.

Intra-group δ^{15}N ranges may have implications for resource partitioning. Of known δ^{15}N values for primates, Schoeninger et al. (1998) reported 3 species with wider ranges: two galago species (3.4‰ and 3.0‰), and one lepilemur species (3.1‰). Comparatively, other primate δ^{15}N ranges reported are *Cebus* (0.2‰), *Ateles* (0.6‰), *Alouatta* (1.0‰), and *Brachyteles* (1.1‰). The authors noted both lepilemur and *Alouatta* samples were known to feed on legumes, and the lepilemurs showed high intra-group variability in amount of legumes consumed. The authors concluded this variability accounted for the wide range in the lepilemur sample. The *Alouatta,* which travel more closely together, may also be likely to feed similarly, reducing the group's δ^{15}N range. Comparatively, the prosimian galago and lepilemur species tend to feed more solitarily, which may provide them with greater opportunity for variable intra-group resource choices (Schoeninger et al., 1998).

Although there was no relation to δ^{15}N enrichment, a study of arctic ground squirrels in which individuals within each group had access to the same types of foods and with similar body masses showed a δ^{15}N range of up to 1.8‰. The authors concluded that, although overall food availability was low, the range was due to individual variation in food choices rather than nutritional stress (Ben-David et al., 1999). However, it is possible there was little evidence of nutritional stress in δ^{15}N values because variable food selection mitigated potential intra-group resource competition. If this is the case, then δ^{15}N values may be used to describe adaptations to low or variable food environments. A wide δ^{15}N range might suggest the ability of animals to mitigate the negative health effects of low resource availability. Enriched δ^{15}N might suggest resource partitioning was unable to fully mitigate this effect. However, further research is needed before such claims could be adequately associated with δ^{15}N trends.

Another way researchers interpret resource partitioning is by using δ-space, in which values for two isotopic variables are compared simultaneously. When looking at carbon and nitrogen isotope values, $\delta^{13}C$ values are typically plotted on the X axis with $\delta^{15}N$ values on the Y axis (Laymen et al., 2007; Newsome et al., 2007; Sanberg et al., 2012). Although δ-space, often referred to as "niche space," is generally used to compare resource use between species within a community, it may also provide useful insight into intra-species resource partitioning and changes in resource use (Dammhahn & Kappeler, 2010; Laymen et al., 2007; Newsome et al., 2007; Sandberg et al., 2012).

When comparing between species, their position relative to each other is an expression of their ecological niche (habitat use and diet), especially trophic level (Laymen et al., 2007; Newsome et al., 2007). Species with positions near each other may be interpreted as competing for the same resources, while species positioned further away are using different types of resources with little to no competition (Dammhahn & Kappeler, 2010; Schoener, 1974; Post, 2003). Plotting isotope values as an expression of resource partitioning has also been used to study intra-species effects like seasonality, weaning, and ontogenetic shifts that are associated with a change in dietary resources (Dammhahn & Kappeler, 2010; Newsome et al., 2007; Newsome et al., 2006; Post, 2003). Within-species isotopic variation is associated with a wider feeding niche and overall more flexible diet, which may allow individuals to minimize intraspecific competition (Araujo et al., 2011; Bolnick et al., 2003; Dammhahn & Kappeler, 2010).

CHAPTER 6

METHODS

6.1 Study Site

Hair samples were collected from ring-tailed lemurs at Beza Mahafaly Special Reserve (BMSR) (23°30′S latitude, 44°40′E longitude) in southwestern Madagascar (see Figure 1). BMSR has been a government reserve since 1986, and it has been the site of many long-term studies on lemur populations living within the reserve (Loudon et al., 2007; Sauther, 1991). This area is characterized by a highly seasonal wet and dry annual cycle with ~99% of annual rainfall occurring between November and April, and it experiences extreme periodic drought and cyclone events (Gould et al., 1999; Loudon et al., 2007; Wright, 1999).

This study uses data collected from 2003-2006 because this period represents drought, cyclone, and "normal" years, categorized as such based upon rainfall patterns and general ecology. "Normal" years have average rainfall (~750mm) with a wide range of available food resources, drought years are characterized by low rainfall and reduced availability of food resources, and cyclone years are characterized by higher rainfall, reduced availability of food resources, and highly disturbed ecology (Gould et al., 1999; Jolly et al., 2002; LaFleur & Gould, 2009; Ralainasolo et al., 2008; Sauther & Cuozzo, 2009; Whitelaw, 2010; Wright, 1999). 2003 is categorized as a normal year (~500mm annual rainfall), 2004 a drought (~200mm), 2005 a cyclone year (cyclone Ernest plus ~900mm) and 2006 a semi-drought year (~350mm) and cyclone recovery period (M. Sauther, personal communication, May 15, 2015).

The troops included in this study are found in and surrounding Parcel 1 of the Beza Mahafaly Special Reserve, approximately ~80 ha large with its eastern edge bordering the Sakamena River (see Figure 1). This area is closed canopy gallery forest and has a rich understory due to a barbed wire fence protecting the parcel from livestock grazing. Vegetation is dominated by kily trees (*Tamarindus indica*) in the east and becomes dominated by more xerophytic vegetation in the drier western portion of the reserve (Sauther, 1991). The land surrounding the parcel has been variably affected by anthropogenic disturbance including extensive grazing and selective tree removal by nearby villagers (Sauther & Cuozzo, 2009; Whitelaw, 2010). There is also a permanent research camp located south of Parcel 1 (Whitelaw, 2010).

6.2 Study Population

Ring-tailed lemurs are opportunistic omnivores which, although their diets are dominated by fruits and leaves, also consume leaf stems, flowers, flower stems, spiders, spider webs, caterpillars, cicadas, insect cocoons, birds and soils from termite mounds (Sauther et al., 1999). Their dietary composition can quickly alter based on the availability of seasonal foods (Sauther et al., 1999). This dietary flexibility allows them to colonize a wide variety of environments and facilitates their survival in the unpredictable Malagasy environment (Loudon et al., 2007).

The study groups consist of four main troops: Black, Blue, Orange and Yellow. These four troops were chosen as my focus because they were the only troops with hair samples representing each of the four years (2003-2006) covered by this study. Black, Blue, Orange and Yellow troops all have access to and use resources from gallery forest, anthropogenically degraded habitats and the research camp, although the frequency of use varies. Overall, Black troop lives in an area of the forest dominated by kily and mantsake, highly disturbed by humans and with little to no vegetation on the forest floor. Blue troop has the largest range and are able to covertly utilize resources from other troop

ranges with impunity. Orange troop uses primarily forest within the Beza Mahafaly Special Reserve but also commonly uses human resources from the research camp. Yellow troop primarily uses the Beza reserve forest (M. Sauther, personal communication, May 15, 2015). Troops I have incomplete data for include: Green, Hot Pink, Light Blue, Light Purple, Purple, Red, Teal, and Trois Fromage. These troops are not included in the analysis, but their $\delta^{13}C$ and $\delta^{15}N$ values are reported in Table 1 (see appendix).

6.3 Field and Lab Methods

Data for my analysis consist of 94 samples collected from 57 individuals (30 males, 27 females) at Beza Mahafaly Special Reserve from 2003-2006 (2003 = 34 samples, 2004 = 14, 2005 = 24, 2006 = 22). Hair samples from each of the individuals were collected during routine health examinations of animals anesthetized with a mixture of Ketamine and Diazepam. The anesthetics were delivered via a Telinject blow dart system. After individual examinations and sample collection, the animals were released in the area in which they were originally captured. The capture of animals and sample collection, based on protocols developed over 17+ years of study and animal capture at BMSR, followed all IACUC guidelines (Loudon et al., 2007; Sauther et al., 2006).

Each hair sample was processed using a standard protocol, being cut from the proximal end of the tail as close to the skin as possible. The samples were then placed in individual plastic bags with the proximal end down. Hair samples were cleaned with ethyl alcohol, cut with laboratory scissors, weighed (~700-900 micrograms), placed in tin capsules, combusted in an elemental analyzer and analyzed for stable carbon and nitrogen isotope abundances using a continuous flow isotope ratio mass spectrometer (University of California, Davis). $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are expressed as delta (δ) notations in parts per thousand (‰) relative to the PeeDee Belemite and atmospheric N_2 standards (Loudon et al., 2007).

6.4 Data Analysis

     I tested for isotopic differences between troops (Black, Blue, Orange and Yellow), sexes and years using analysis of variance (ANOVA) and Tukey's post hoc test. Leverage plots were created to explore variable interactions. A δ-space plot provides an alternative view of $\delta^{13}C$ and $\delta^{15}N$ responses. Statistical analyses were performed using JMP statistical software with significance set at $P = 0.05$.

CHAPTER 7

RESULTS

7.1 Chapter Overview

   This chapter presents new stable carbon and nitrogen isotope data for ring-tail lemur hair from

Beza Mahafaly Special Reserve in Madagascar between the years 2003-2006. Table 1 in the appendix

provides a complete listing of processed sample data including individual identification numbers, troop

name, sex, year the sample was taken, $\delta^{13}C$ and $\delta^{15}N$ values for each sample. Only the four largest

troops were used in the analysis because no other troop samples contained data for all four years. I

compare the variables sex, troop and year by $\delta^{15}N$ and $\delta^{13}C$ values.

Table 2. Summary Statistics. Data are Mean, ± S.D., Range, (N).

	2003		2004		2005		2006	
Troop	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Black	-23.9 ±0.4 1.2 (9)	7.1 ±0.3 1.0 (9)	-23.9 ±0.1 0.2 (3)	7.3 ±0.2 0.4 (3)	-23.7 ±1.1 3.3 (10)	7.2 ±0.6 1.8 (10)	-24.3 ±0.2 0.6 (5)	7.1 ±0.7 1.6 (5)
Blue	-24.3 ±0.3 0.9 (12)	6.8 ±0.4 1.2 (12)	-24.3 ±0.4 0.7 (3)	7.3 ±0.3 0.6 (3)	-24.4 ±0.5 0.9 (3)	6.8 ±0.2 0.4 (3)	-24.4 ±0.3 0.8 (6)	7.0 ±0.2 0.4 (6)
Orange	-23.7 ±0.6 1.7 (8)	7.9 ±0.4 1.5 (8)	-24.6 ±0.1 0.2 (3)	7.3 ±0.6 1.2 (3)	-24.2 ±0.5 1.5 (7)	7.4 ±0.6 1.5 (7)	-24.5 ±0.2 0.7 (8)	7.7 ±0.2 0.6 (8)
Yellow	-24.8 ±0.2 0.6 (5)	6.7 ±0.5 1.4 (5)	-25.3 ±0.1 0.2 (5)	6.8 ±0.7 1.9 (5)	-25.1 ±0.7 1.5 (4)	6.8 ±0.5 1.0 (4)	-23.4 ±0.9 1.8 (3)	8.8 ±0.8 1.4 (3)

7.2 Male/Female Comparison

Overall, males show enrichment of ^{15}N compared to females (p=0.03), shown in Figure 2. However, much of this difference is driven by the Yellow 2006 values (Figures 4.3; 4.4). If these divergent values are removed, this enrichment is reduced to borderline significance (p=0.05) with only a 0.2‰ difference in mean values. Subsequent analyses combine male and female data to better highlight troop and year differences (see Chapter 8 for explanation of exclusion).
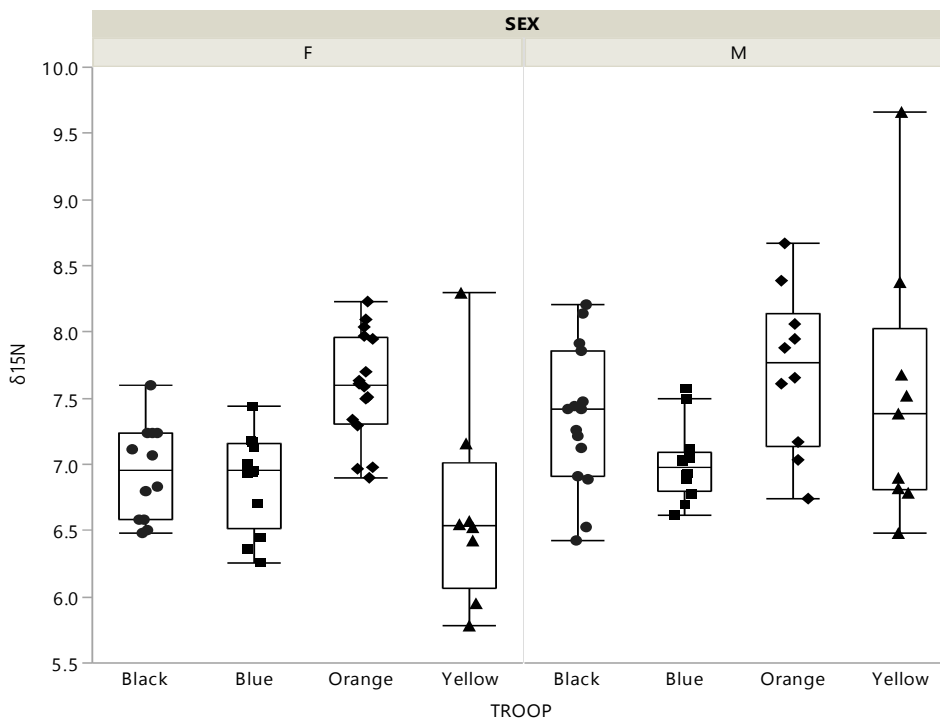
Figure 2: Individual δ¹⁵N by Troop and Sex

7.3 δ^{13}C Values

Figures 3.1 and 3.2 show overall troop comparisons of δ^{13}C values, with Black troop being the most enriched while Yellow is the least enriched (p=0.0019). Blue troop shows the most constrained values over all years while the other troops each show a few particularly enriched values that are mostly contributed by a single year (Black in 2005, Orange in 2003, Yellow in 2006; Figure 3.3). 2004 shows the most distinct carbon isotope ranges, while 2006 shows the most overlap in ranges (Figure

3.4). The overall widest range of $\delta^{13}C$ values occurs in the 2005 cyclone year (Figure 3.4) with Black troop contributing the widest range during that year.

   In terms of range magnitude, Blue consistently showed low $\delta^{13}C$ ranges of less than a 1.0‰ range in any year (Table 2). Black troop's $\delta^{13}C$ values showed a large range magnitude (3.3‰) in 2005. Orange showed the largest range in 2003 (1.7‰), and Yellow had the largest range in 2006 (1.8‰), 1.0‰ larger than any other troop range for that year (Table 2; Figure 3.4). Overall, the four troops showed the most similar ranges in 2004 (0.5‰ difference) and most variable in 2005 (2.4‰ difference) (Table 2; Figure 3.4).
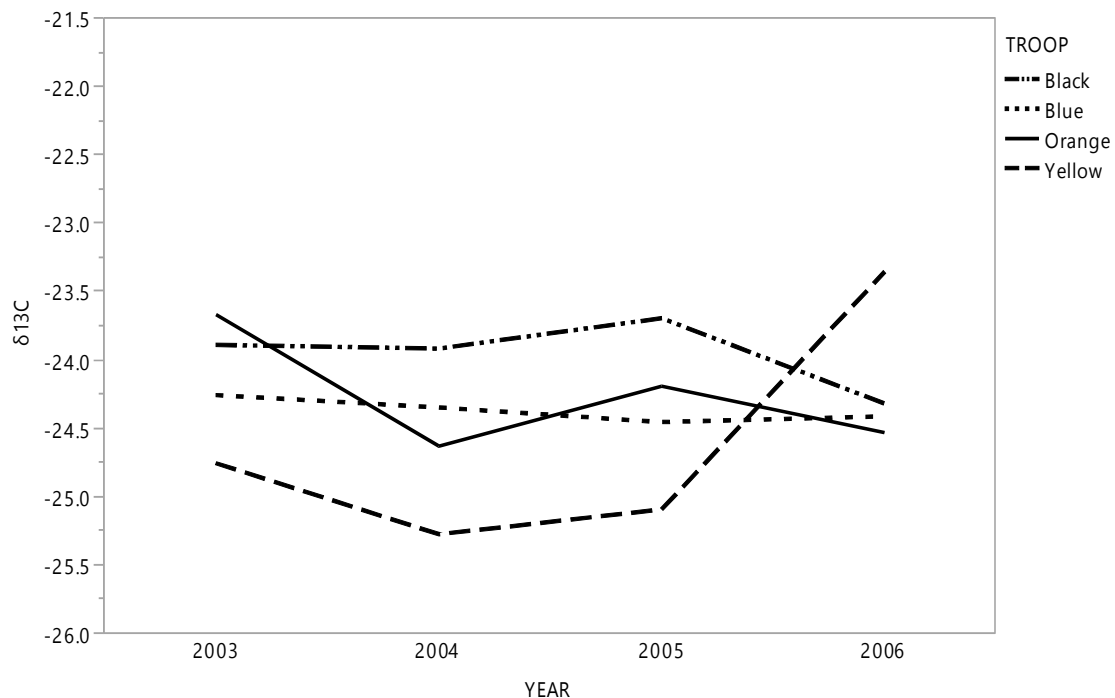
Figure 3.1: $\delta^{13}C$ Means by Troop and Year
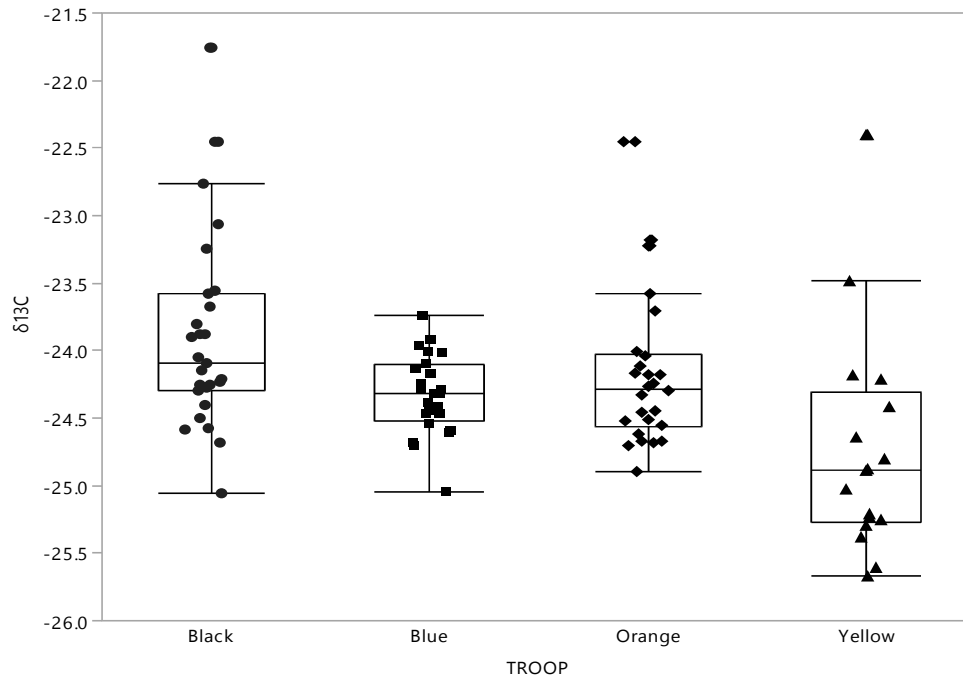
Figure 3.2: Individual δ¹³C by Troop
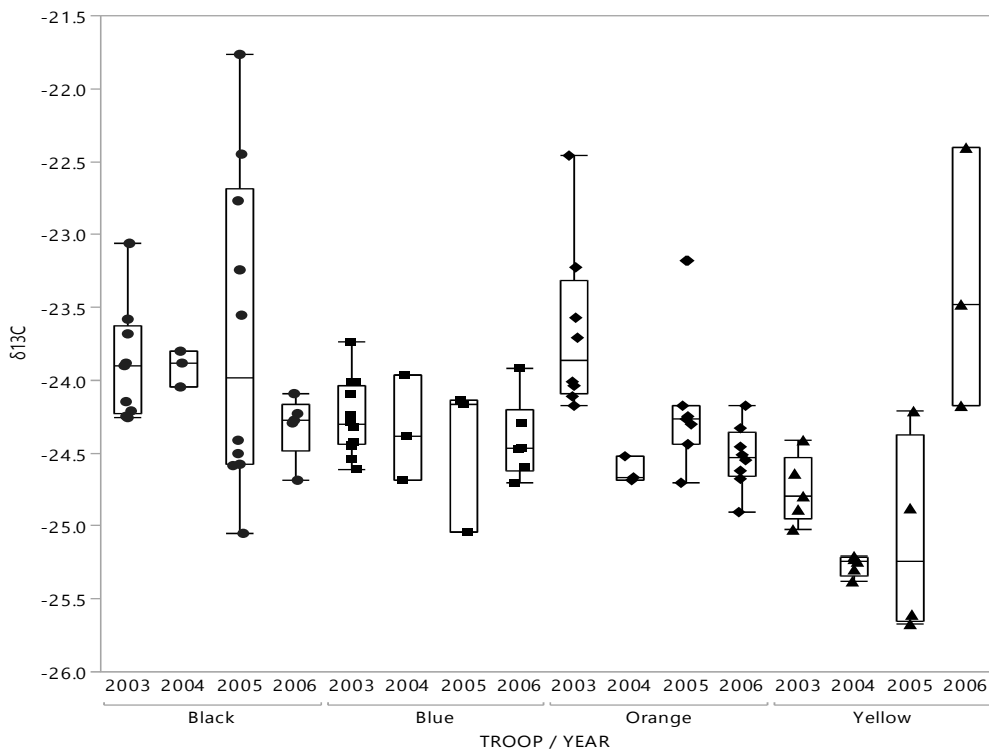
Figure 3.3: Individual δ¹³C by Troop and Year
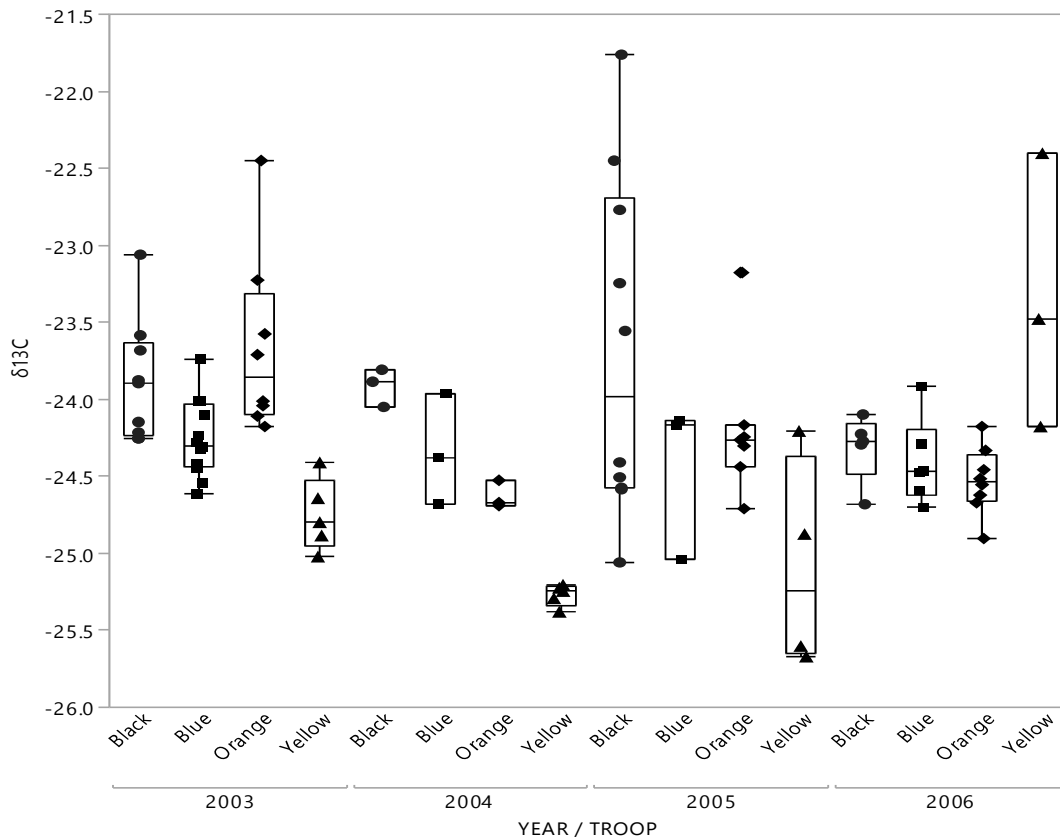
Figure 3.4: Individual $\delta^{13}C$ by Year and Troop

7.4 $\delta^{15}N$ Values

      Orange troop exhibits the highest enrichment of ^{15}N although, due to high Yellow outliers in 2006, Orange is only statistically enriched compared to Blue and Black overall (p=<0.02; Figure 4.2). Blue once again has the most constrained values across the years (Figures 4.2, 4.3). 2006 shows the most distinct nitrogen isotope ranges for each troop, with Yellow showing particularly high values (Figure 4.4). The 2005 wide range and 2006 constrained range seen in $\delta^{13}C$ is not present in $\delta^{15}N$ values (Figure 4.2).

      In terms of range magnitudes, Yellow was the only troop that showed at least a 1‰ $\delta^{15}N$ range

every year, although Orange only dropped below 1.0‰ in 2006 (0.6‰).Black troop showed the largest magnitude of any troop any single year. Overall, the four troops showed the most similar range magnitudes in 2003 (0.5‰ difference) and more varied in 2004, 2005 and 2006 (1.5, 1.4, and 1.2‰ difference, respectively).
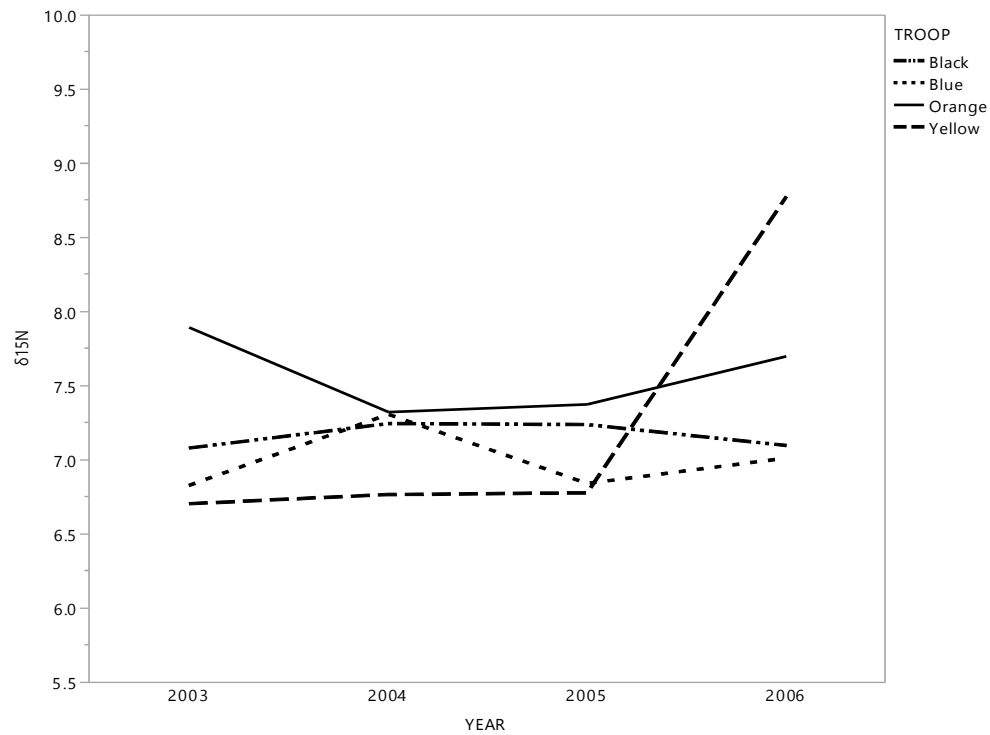
Figure 4.1: $\delta^{15}N$ Means by Troop and Year
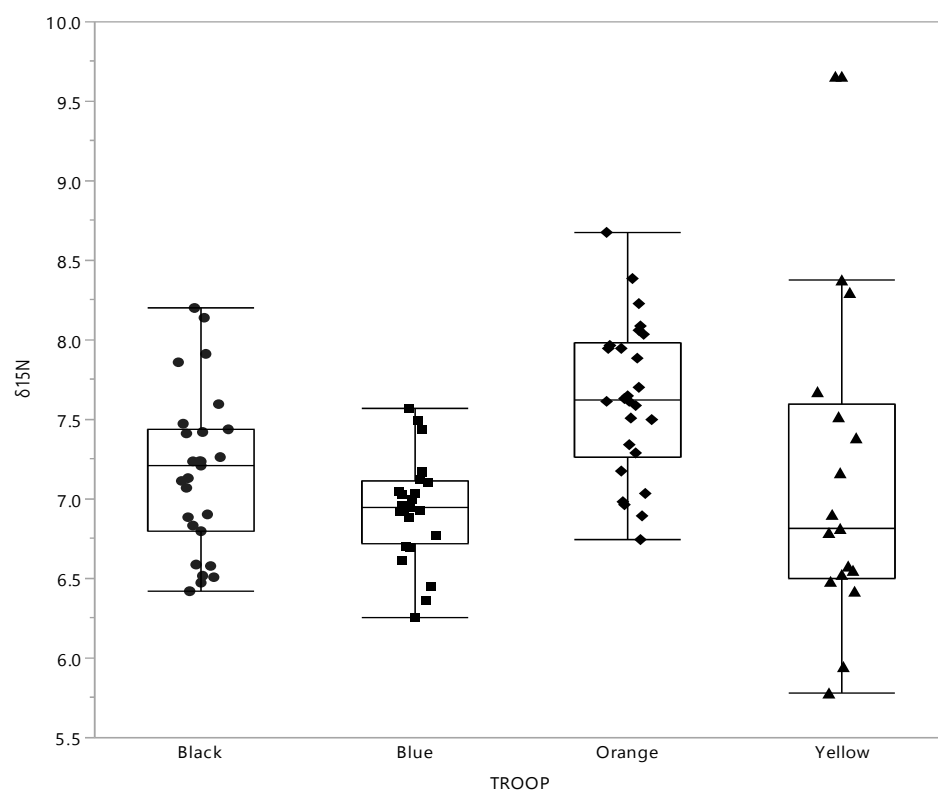
Figure 4.2: Individual δ¹⁵N by Troop
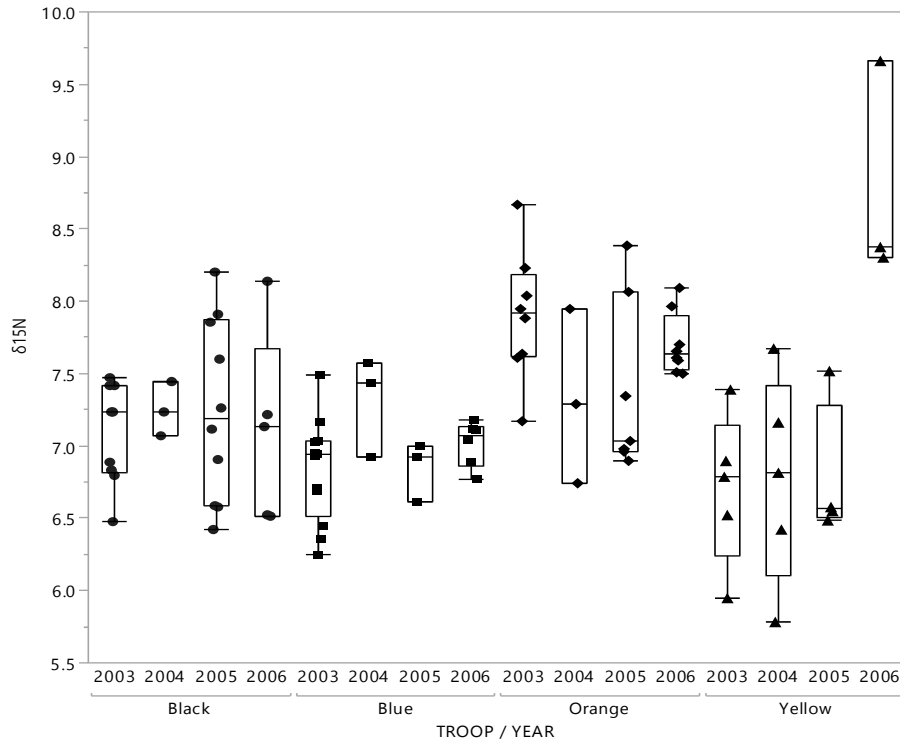
Figure 4.3: Individual δ¹⁵N by Troop and Year
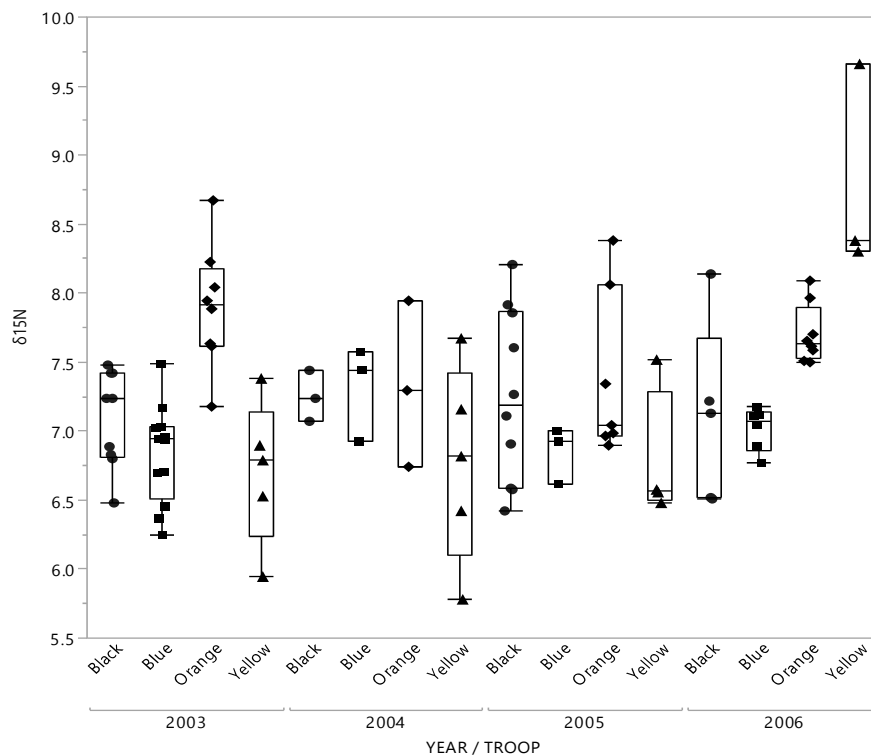
Figure 4.4: Individual δ¹⁵N by Year and Troop

7.5 Troop and Year Interaction

When comparing variable interactions, leverage plots for both δ^{13}C and δ^{15}N showed the interaction between troop and year as being more significant than either troop or year singly (Figures 5.1 and 5.2).
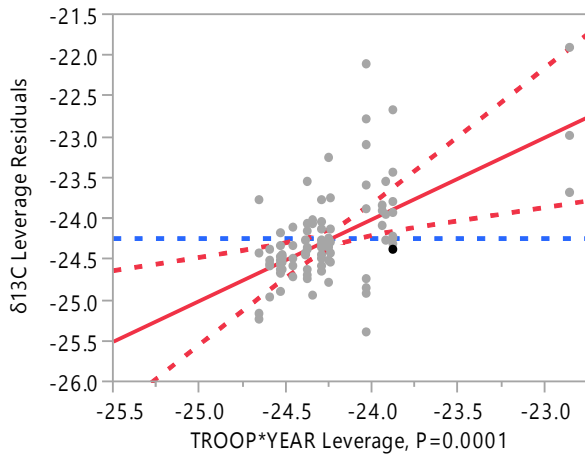
Figure 5.1: δ^{13}C Troop by Year Leverage Plot
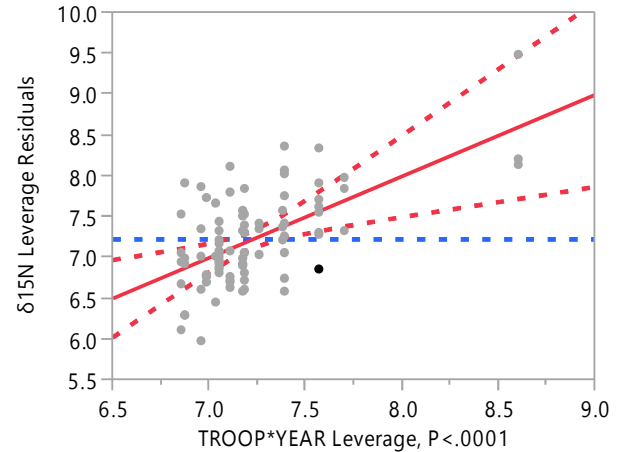
Figure 5.2: δ^{15}N Troop by Year Leverage Plot

7.6 δ-Space

In Figure 7 (see appendix), troops in 2004 and 2006 show the most distinct use of δ-space. In 2004, individuals within each troop showed similar δ^{13}C values but more variable δ^{15}N values, leading to Yellow and Orange troop showing a distinct vertical distribution while Blue and Black values clustered. In 2006, Orange and Blue values were most tightly clustered. 2005 values were the most dispersed of any year.

CHAPTER 8

DISCUSSION

8.1 Overview

At first glance, the isotopic data presented in Chapter 7 may seem chaotic, but that itself can provide useful insight. I think perhaps the most important aspect of my data is that the isotopic values never change in the same way at the same time. This may suggest ring-tailed lemurs have behavioral adaptations that help them utilize resources in different but complimentary ways.

By comparing the results from Chapter 7 to what we know of Malagasy primate behavior, we can create an idea of what could be happening to cause isotopic changes between and within troops. In chapter 5, it was explained how stable isotope values vary based on both flora innate responses and animal behavioral and physiological reactions. Flora have somewhat more limited responses (Amundson et al., 2003; Crowley, 2012; Ehleringer et al., 1986; Evans, 2007; Hoefs, 2008; Lee-Thorp & Sponheimer, 2006; Van der Merwe, 1982), but the wide variability in animal behavior can result in drastically varying isotopic responses (Ambrose & DeNiro, 1986; Araujo et al., 2011; Ben-David et al., 1997; Ben-David et al., 1999; Bolnick et al., 2003; Cormie & Schwarcz, 1996; Dammhahn & Kappeler, 2010; Hobson et al., 1993; Schoener, 1974; Schoeninger et al., 1998; Post, 2003). Although the isotopic responses of ring-tailed lemurs can be the result of a wide variety of potential behaviors, only certain behaviors may provide adaptive advantage (Ambrose & DeNiro, 1986; Behie & Pevelka, 2005; Ben-David et al., 1997; Berenstain, 1986; Gould et al., 1992; Gould et al., 1999; Jolly et al., 2002; Loudon et al., 2007; Rasamimanana & Rafidinarivo, 1993; Ratimbazafy et al., 2002; Schaffner et al., 2012;

Whitelaw, 2010).

Madagascar has been a separate landmass for at least 80 million years, and ancestral lemurs have been evolving in relative isolation there for at least 50 million years. Approximately 84% of all land vertebrate species on Madagascar are endemic, so the species living there should be well-adapted to its extreme climatic fluctuations (Dunham et al., 2011; LaFleur & Gould, 2009; Wright, 1999; Yoder & Nowak, 2006). In addition to high seasonality, Madagascar experiences frequent droughts and up to several cyclones per year with the potential for these events to destroy up to 85% of tree crown volume and 70% of annual fruit production (Whitelaw, 2010). It seems unlikely local fauna could routinely survive such drastic effects without developing behavioral responses to mitigate negative impacts.

One way ring-tailed lemurs may have adapted to Madagascar's dramatically changing conditions is by developing unique foraging strategies. Ring-tailed lemurs are opportunistic omnivores that can quickly change their dietary composition based on food availability (Loudon et al., 2007; Sauther et al., 1999). This is a potentially useful strategy for ring-tailed lemurs as there have been many studies which suggest species with generalist diets are more likely to survive in disturbed environments (Behie & Pavelka, 2005; Ben-David et al., 1997; Gould et al., 1999; Ratimbazafy et al., 2002). The isotopic data presented in Chapter 7 may support this idea when considered in conjunction with other behavioral studies of Malagasy primates and isotopic data from disturbed environments, which I explore later in this chapter.

8.2 Resource Strategies of Generalists

Ambrose and DeNiro (1986) found in a study of 43 East African herbivore and carnivore species that species with wide habitat tolerances, such as omnivores, showed wide ranges in $\delta^{13}C$, reflecting their opportunistic feeding ecology. After a drought followed by a major fire, Berenstain (1986) attributed the survival of a group of long-tailed macaques to their foraging response, a

combination of shifting their diet to less preferred but more readily available food items and increasing their home range. Ratsimbazafy, Ramarosandratana and Zaonarivelo studied black-and-white ruffed lemurs following a severe cyclone which resulted in low fruit production for several years. The lemurs responded by shifting to non-preferred resources and changing their ranging behavior to minimize energy expenditure by choosing resources that require less travel (Ratsimbazafy et al., 2002). Similarly, Gould et al. (1999) found that, during a major drought, ring-tailed lemurs utilized primarily very low quality resources that were within their normal home ranges rather than expanding their ranges to find higher quality resources.

These studies all reinforce the idea that generalists are able to adapt behaviorally to reduce nutritional stress, but that groups may choose to adapt different types of behaviors based on the circumstance. Leverage plots of my ring-tailed lemur $\delta^{13}C$ and $\delta^{15}N$ values may further reinforce this idea. The leverage plots of both $\delta^{13}C$ and $\delta^{15}N$ show that, while troop and year (environmental effects) each affect $\delta^{13}C$ and $\delta^{15}N$ values, the greatest effect is the interaction between troop and year (Figures 5.1 and 5.2). This suggests troops are not all responding to environmental effects in the same way. This concept is the base from which I build my upcoming interpretation of how ring-tailed lemurs respond differently, both between and within troops, to droughts compared to cyclones. There are three significant trends that are highlighted throughout this chapter: Yellow troop's unique enriched 2006 values, Black troop's extreme isotopic range increase (especially in 2005), and Blue troop's stable isotopic ranges over all study years.

8.3 Interpretation of Drought Response

Based on the inconsistent shifting of the data and the flexibility of ring-tailed lemur diets, I would propose resource partitioning is the most significant variable causing isotopic variability. Comparing 2003 (normal year) to 2004 (drought year), all troops decreased in mean $\delta^{13}C$, increased

their $\delta^{13}C$ range, and increased in mean $\delta^{15}N$ values, except for Orange troop (-0.6‰ mean $\delta^{15}N$) (Table 2). However, the amount each troop shifted varied. Blue changed very little between years. Orange and Black both increased their range, but Orange also decreased its mean value by ~1.0‰ while Black had only a negligible decrease (Table 2).

The $\delta^{15}N$ increases could be the result of ring-tailed lemurs experiencing water or nutritional stress during the drought (Ambrose & DeNiro, 1986; Best & Schell, 1996; Cormie & Schwarcz, 1996; Hobson & Schell, 1998; Hobson et al., 1993; Loudon et al., 2007; Schoeninger et al., 1998). However, considering none of these troops increased in $\delta^{15}N$ more than 0.5‰, the increase could also be attributed to natural variation in $\delta^{13}C$ and $\delta^{15}N$ values between plant species (Simmen et al., 2006). It could also be a result of these troops making greater use of animal resources such as insects (Berenstain, 1986; Tutin et al., 1997). Orange troop's ^{15}N decrease could be explained if they chose to highly rely on kily pods (*Tamarindus indica*), which is a leguminous tree potentially associated with lower ^{15}N values in consumers due to the tree's assumed symbiotic relationship with nitrogen-fixing bacteria (Schoeninger et al., 1998). However, whether kily actually fixes nitrogen is highly debatable (El-Siddig et al., 2006). It may be more likely Orange troop used the research camp more often than other troops in 2004. Human foods tend to be higher in ^{13}C and ring-tailed lemurs, including Orange troop, also occasionally eat ^{15}N enriched human feces from Malagasy open-air latrines, which may impact Orange troop isotope values (Fish et al., 2007; Loudon et al., 2007; M. Sauther, personal communication, May 15, 2015; Sponheimer et al., 2003).

Fruits are a significant dietary component of ring-tailed lemurs (Sauther et al., 1999) and there are several fruit trees, including kily and mantsake *(Enterospermum pruinosum),* which continue to fruit during the dry season and may be drought resistant, which would make them useful food resources during this time (Jolly et al., 2002; Sauther & Cuozzo, 2009; Simmen et al., 2006). Kily is also available in both intact and disturbed gallery forest (Sauther & Cuozzo, 2009). In addition to kily and

mantsake, there may also be other trees able to fruit during droughts for which we do not having feeding data (Jolly et al., 2002). If ring-tailed lemurs mainly relied on these trees as a fallback resource during drought, they may have also chosen leaves, their other main dietary component, from nearby trees to minimize excess energy expenditure during this period of stress. This would correlate with studies reporting primates spend more time resting after major climatic event, when resources are less dispersed, and during the dry season, because all of these behaviors suggest a reduced activity budget is preferred when relying on fallback food resources (Schaffner et al., 2012; Whitelaw, 2010).

If ring-tailed lemurs did spend more time in closed canopy forest feeding on a few main fallback resources, this would also explain the falling mean $\delta^{13}C$ values and the reduced isotopic magnitude within and between troops in 2004 (Ambrose & DeNiro, 1986; Schoeninger et al., 1997; Van der Merwe, 1982). It is also worth noting from Figure 3.4 that Black, Orange and Yellow all show very distinct $\delta^{13}C$ ranges in 2004, which could suggest each troop is relying heavily on a few main food resources, but using them in different amounts that result in $\delta^{13}C$ values that are very distinct from each other.

Another way to view 2004 resource use can be seen in Figure 7, which shows individuals from three troops have very similar $\delta^{13}C$ values within each troop, distinct $\delta^{13}C$ values between troops, but differing $\delta^{15}N$ values, leading to a vertical distribution (Figure 7, see appendix). Of the four troops, only Blue $\delta^{13}C$ values overlapped with other troop ranges in 2004 (Figure 3.4, Figure 7). The 2006 drought response showed slightly different $\delta^{13}C$ trends than 2004. Troops did not express the vertical distribution seen in 2004, although Orange and Blue troops did show clustering within the δ-space (Figure 7). This difference may be due to the unique circumstances present in 2006. 2006 was a semi-drought year (~350mm annual rainfall compared to ~500mm in 2003 and ~200mm in 2004) and kily was not present during most of the year (M. Sauther, personal communication, May 15, 2015). As a result, 2006 should be considered as a cyclone-recovery period, distinct from the 2004 drought.

Separating 2004 from 2006 may also support the interpretation that resources are used differently during cyclone and drought periods.

8.4 Interpretation of Cyclone Response

In drought years, it may be possible for ring-tailed lemurs to subsist on a few abundant fallback resources, minimizing the necessity of intra-troop resource partitioning and thus reducing their carbon and nitrogen isotopic magnitude. However, cyclone years require a different strategy. After a cyclone, there are losses of up to 85% of canopy crown volume and 70% of annual fruit production (Whitelaw, 2010). During a drought, ring-tailed lemurs can rely on a few potentially drought-resistant species like kily fruit. However, kily is frequently not an available fallback option after a cyclone, requiring ring-tailed lemurs to adapt a different foraging strategy (LaFleur & Gould, 2009). Kily was not present for 2005 or for most of 2006, which may categorize 2006 as more similar to a cyclone-recovery rather than a drought year (M. Sauther, personal communication, May 15, 2015).

Carbon isotope values between the 2004 drought year and 2005 cyclone year showed none of the troops changed means more than 0.5‰ in either carbon or nitrogen values (Table 2), but their ranges altered dramatically (Figure 3.4). Orange and Yellow broadened their $\delta^{13}C$ isotopic range by 1.3‰, and Black dramatically increased its carbon isotopic range by 3.1‰. Black also shows an increased ^{15}N magnitude of 1.4‰ (Table 2).

None of the troops showed elevated ^{15}N values associated with nutritional or water stress (Best & Schell, 1996; Hobson et al, 1993; Hobson & Schell, 1998), so the troops are likely doing something to mitigate the cyclone effects. One possible explanation is, based on the troops' broader isotopic ranges (especially $\delta^{13}C$), individuals in each troop are feeding either on smaller amounts of more foods or the greater variation between individuals is a result greater intra-troop competition during periods of resource scarcity (Araujo et al., 2011; Bolnick et al., 2003). This should be possible for ring-tailed

lemurs, even after a cyclone, because their generalist feeding ecology allows them to utilize a wide variety of resources (Loudon et al., 2007; Sauther et al., 1999). Even at 70% canopy loss, every individual may be able to feed at a particular foraging site if they are utilizing different types of available food and thus maintain their body condition. A study of spider monkeys (Schaffner et al., 2012) found they were less likely to form large subgroups after a hurricane, which may support the idea that food availability is more dispersed after hurricane and cyclone events, requiring behavior modification in groups of synchronously-feeding ring-tailed lemurs.

This pattern of resource partitioning may also be reflected in δ-space. In Figure 7, carbon and nitrogen isotope values shifted from relatively clustered patterns during the drought years to a very diffuse distribution in the 2005 cyclone year. Black troop shows an especially wide distribution across the δ-space, while Blue troop's distribution pattern changed very little (Figure 7). This makes sense considering Black troop inhabits the most disturbed habitat (M. Sauther, personal communication, May 15, 2015). Disturbed habitats may have fewer high-quality resources, especially after cyclones devastate the kily and mantsake, which may have forced Black troop to respond more extremely than other troops (i.e. low resource availability leads to increased intra-troop competition, requiring a significant broadening of their feeding niche and thus increasing their isotopic ranges). If Black's increased isotopic range is a result of resource stress and a subsequently broader feeding niche, this would correlate with a study showing cortisol levels, commonly associated with increased stress, were highly variable in ring-tailed lemurs during 2005 (Davenport et al., 2006; M. Sauther, personal communication, May 15, 2015). It would also explain the isotopic stability seen in Blue troop. Blue troop would be able to raid other troop resources rather than broadening their feeding niche, thus maintaining a more stable isotopic range. This supports my interpretation because greater variation in isotope values is associated with the utilization of a wider feeding niche (Araujo et al., 2011; Dammhahn & Kappeler, 2010). Also, diffusely distributed intra-species and intra-troop values suggest

differential feeding ecology within groups (Dammhahn & Kappeler, 2010; Schoener, 1974; Post, 2003).

8.5 Yellow Troop

The scenarios I described above are only one possible way of interpreting the data. There are some shifts in the data set which I was not able to account for based on this scenario, such as Yellow troop's ^{15}N magnitude disparity in which they showed an increase in 2004 and decrease in 2005. Yellow troop was also unique in other ways. Except for 2006, they consistently exhibited the lowest means in both carbon and nitrogen values. In 2006, Yellow troop's carbon and nitrogen values increased dramatically (1.7‰ and 2.0‰). This increase corresponds with behavioral data stating they were exploiting local crop resources across the Sakamena river, which separates the crops from their usual home range. When the river flooded, they were stranded in the heavily degraded habitat for five weeks, during which time at least two infants and two adults died (J. Loudon, personal communication, November 17, 2014; M. Sauther, personal communication, October 23, 2013; Whitelaw, 2010). This specific event may support the idea that nutritional stress is associated with elevated ^{15}N. While Yellow troop's 2006 isotope values provide insight into ring-tailed lemur responses to extreme habitat shift, this response was due to circumstances unique to Yellow troop for only this season. As such, Yellow troop's 2006 values should not be directly considered when comparing troop responses between drought and normal years.

8.6 Exclusion of Sex Variable

As I mentioned in Chapter 7, I combined male and female data in my analysis despite a statistically significant δ^{15}N enrichment in males. Ring-tailed lemurs are a female dominated species which affects many aspects of their socioecology (Sauther et al., 1999 and references contained

therein). Sex differences in dietary ecology, as reflected through $\delta^{13}C$ and $\delta^{15}N$, would thus be of great importance to researchers. However, I believe including sex as a major variable in my interpretation would be an error. This decision is largely based on Yellow troop's 2006 influence and whether the difference was able to yield interpretable results.

Overall, Males show higher $\delta^{15}N$ by ~0.3‰. However, Yellow troop in 2006 (N=3) contributed the three highest values, of which one was female ($\delta^{15}N$=8.3) and two were male ($\delta^{15}N$=8.4; 9.7). These values are much higher than the $\delta^{15}N$ (excluding Yellow 2006) average (7.2). I have already addressed why Yellow 2006 values are aberrant and should not be included in overall troop or year comparisons. If Yellow 2006 values are not included, the average $\delta^{15}N$ between males and females drops from 0.28‰ to 0.23‰ and the significance level drops from p=0.03 to p=0.05, resulting in borderline statistical significance.

Even at borderline significance, the sex $\delta^{15}N$ difference may still be useful to some researchers. In this circumstance, however, such a minor difference in $\delta^{15}N$ is nigh uninterpretable. Even at ~0.3‰, this difference is no greater than the $\delta^{15}N$ natural variation seen in mice raised on the same diet (DeNiro & Epstein, 1981). Further, a leverage plot of sex shows minimal effect with r^2=0.05 (Figure 6). From these data, I conclude that any interpretation based on sex differences would have minimal validity. Ultimately, while sex may be biologically and behaviorally significant in ring-tailed lemurs, I believe it should not be included as a significant variable in this analysis.
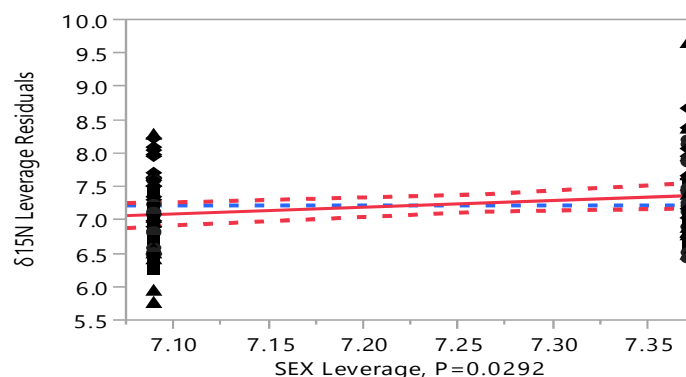
Figure 6: $\delta^{15}N$ Sex Leverage Plot

CONCLUSION

This study has explored physical and behavioral adaptations of ring-tailed lemurs to extreme environmental shifts and proposed some explanations of why this might occur. The data contributed by this study suggest ring-tailed lemurs respond in different ways to droughts and cyclones based on the unique challenges each present. During normal years, there may be greater inter-troop competition for highly preferred resources. Droughts may result in a shift to abundant but less preferred resources, since droughts can limit fruit production in some species but have no negative impact on others, while cyclones have dramatic impacts on overall resource availability, driving greater intra-troop competition (Jolly et al., 2002; LaFleur & Gould, 2009; Ralainasolo et al., 2008; Sauther & Cuozzo, 2009; Whitelaw, 2010). Three significant trends highlight this response: Yellow troop's uniquely enriched 2006 values, Black troop's extreme isotopic range increase in 2005, and Blue troop's overall stable isotopic range.

My interpretation of the data proposes ring-tailed lemurs may respond to droughts by relying primarily on a few abundant species such as kily and mantsake for fruit and preferentially choosing nearby foliage, thus reducing their dietary diversity during droughts, minimizing their travel and maximizing their rest time. The distinct $\delta^{13}C$ ranges and δ-space distributions seen in 2004 may support this interpretation if each troop is utilizing similar resources in slightly different ways, such as consuming different amounts of the same drought-resistant resources (Figure 3.4, Figure 7). This interpretation would also correlate with Orange troop's decreased ^{15}N if Orange troop relied heavily on the potentially $\delta^{15}N$ deficient legume kily, which continues to fruit during drought, or if they engaged in more research camp raiding and coprophagy than other troops (Fish et al., 2007; Jolly et al., 2002; Loudon et al., 2007; Sauther & Cuozzo, 2009; Sponheimer et al., 2003). The other troops' up to 0.5‰ increase in $\delta^{15}N$ may be the result of water and nutritional stress, fractionation differences between

consumed food resources, or increased consumption of animal resources such as insects (Ambrose & DeNiro, 1986; Berenstain, 1986; Best & Schell, 1996; Cormie & Schwarcz, 1996; Hobson & Schell, 1998; Hobson et al., 1993; Loudon et al., 2007; Schoeninger et al., 1998; Simmen et al., 2006; Tutin et al., 1997). The decreased $\delta^{15}N$ range in troops would also support this idea, as lemurs often rely on a few main resources during drought periods rather than expanding their resource base (Gould et al., 1999; Jolly et al., 2002; Sauther & Cuozzo, 2009; Simmen et al., 2006). Orange and Yellow's $\delta^{13}C$ decrease could also potentially be attributed to different $\delta^{13}C$ and $\delta^{15}N$ values between plant species, consuming more resources from dense canopies, or a change in foliage height of the resources being consumed (Ambrose & DeNiro, 1986; Ehleringer et al., 1986; Schoeninger et al., 1997; Van der Merwe, 1982).

After cyclones, ring-tailed lemurs may need to shift their strategy, eating the surviving small amounts of a wider variety of resources and thus increasing dietary diversity under these unique conditions. Kily is frequently not an available fallback resource after cyclones, which can damage canopy volume and annual fruit production in all species (LaFleur & Gould, 2009; Whitelaw, 2010). With fewer resources available per species, ring-tailed lemurs should be able to diversify their resource base (likely seen in Black troop during 2005), possibly with increased intra-troop competition driving individuals to rely on different resource types within each troop, due to their generalist feeding ecology (Araujo et al., 2011; Bolnick et al., 2003; Loudon et al., 2007; Sauther et al., 1999). This response may be reflected in the data via an increase in range magnitude in 2005 (Figure 3.4) and a more diffuse distribution of intra-troop values across δ-space (Figure 7). In the case of Blue troop, they were likely able to maintain a similar diet between normal and cyclone years because of their ability to successfully raid preferred food resources in other troop ranges (M. Sauther, personal communication, May 15, 2015).

Although the data presented in this thesis are useful for interpreting variation in ring-tailed

lemur responses, the scope is temporally constrained. Only four years are represented in the data, which can complicate comparisons between years. For example, without data from several normal years, it is difficult to determine whether the 2003 data accurately represent a normal year. Although I have data from two drought years, the 2006 drought occurs immediately following a cyclone year while the 2004 drought follows a normal year. How ring-tailed lemurs respond to droughts following a devastating cyclone (which may stunt kily fruit production for more than one year) may vary from a typical drought response (LaFleur & Gould, 2009; M. Sauther, personal communication, May 15, 2015; Whitelaw, 2010).

Stable isotope analysis is becoming a common tool for studying dietary ecology, and the literature is continually expanding. Although all new studies contribute to our understanding of this topic, some types of studies may be more effective at furthering our knowledge of primate behavioral adaptations and giving new meaning to previous data. Comprehensive studies will likely be the most effective contributors because they combine behavioral, feeding, health and isotopic data. I believe my study has shown these are essential and intricately linked aspects of studying ring-tailed lemur ecology. Because they are all connected, researchers must combine forces to address all four aspects in their study. Some research projects have already attempted to address this issue but many more are needed to acquire the comprehensive data needed to discover previously obscured trends. Once these data can be routinely combined, stable isotope ecology can recognize its full potential as a powerful research tool.

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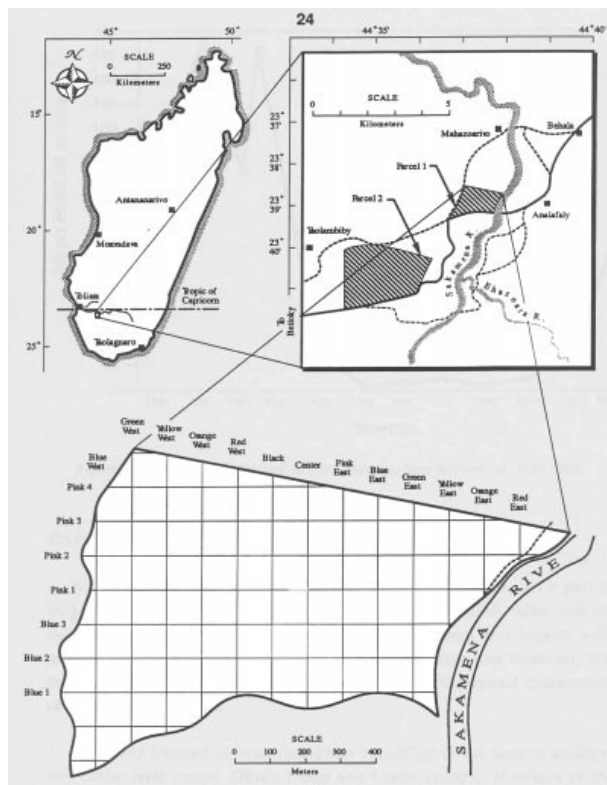
APPENDIX

Figure 1: Map of Madagascar indicating the location of the Beza Mahafaly Special Reserve.
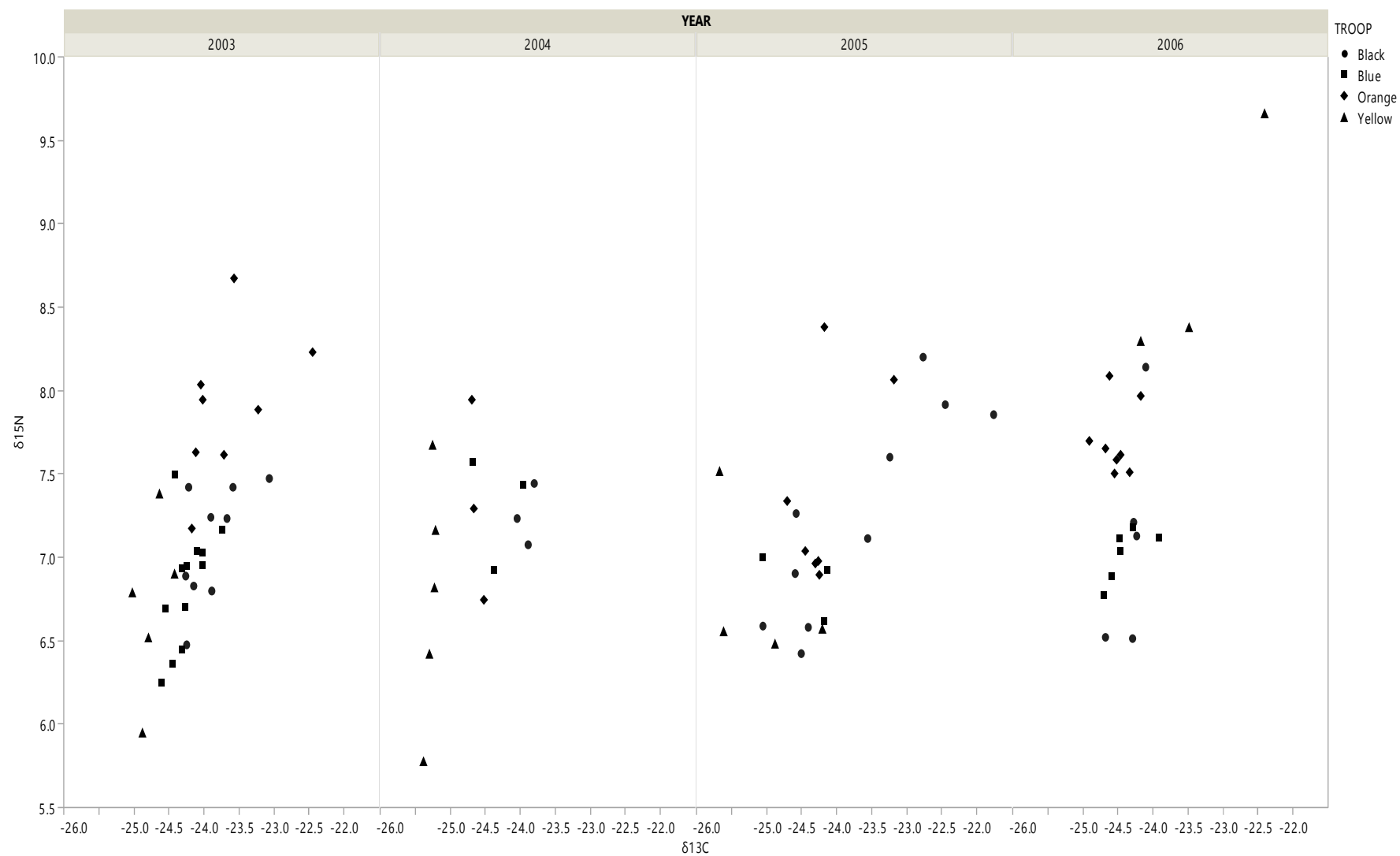
Figure 7: δ-Space

Table 1. Ring-tailed Lemur Hair Raw Carbon and Stable Isotope Data

ID #	Troop	Year	Sex	$\delta^{13}C$	$\delta^{15}N$
110	Black	2003	F	-24.2	6.5
111	Black	2003	F	-24.2	6.8
112	Black	2003	F	-23.9	7.2
116	Black	2003	F	-23.7	7.2
432	Black	2003	F	-23.9	6.8
113	Black	2003	M	-23.6	7.4
114	Black	2003	M	-24.3	6.9
115	Black	2003	M	-23.1	7.5
117	Black	2003	M	-24.2	7.4
111	Black	2004	F	-23.9	7.1
121	Black	2004	F	-24.1	7.2
119	Black	2004	M	-23.8	7.4
110	Black	2005	F	-24.4	6.6
112	Black	2005	F	-25.1	6.6
116	Black	2005	F	-23.6	7.1
121	Black	2005	F	-23.2	7.6
113	Black	2005	M	-24.6	6.9
115	Black	2005	M	-22.8	8.2
119	Black	2005	M	-22.4	7.9
212	Black	2005	M	-21.8	7.9
223	Black	2005	M	-24.5	6.4
226	Black	2005	M	-24.6	7.3
116	Black	2006	F	-24.3	6.5
6	Black	2006	M	-24.2	7.1
223	Black	2006	M	-24.3	7.2
226	Black	2006	M	-24.7	6.5
350	Black	2006	M	-24.1	8.1
126	Blue	2003	F	-24.2	6.9
132	Blue	2003	F	-23.7	7.2
135	Blue	2003	F	-24.0	7.0
137	Blue	2003	F	-24.3	6.7
138	Blue	2003	F	-24.4	6.4
139	Blue	2003	F	-24.3	6.4
141	Blue	2003	F	-24.6	6.3
127	Blue	2003	M	-24.4	7.5
133	Blue	2003	M	-24.3	6.9
134	Blue	2003	M	-24.5	6.7
136	Blue	2003	M	-24.0	7.0
140	Blue	2003	M	-24.1	7.0
138	Blue	2004	F	-24.0	7.4
141	Blue	2004	F	-24.4	6.9
259	Blue	2004	M	-24.7	7.6
227	Blue	2005	F	-25.0	7.0
250	Blue	2005	M	-24.2	6.6
259	Blue	2005	M	-24.1	6.9

138	Blue	2006	F	-23.9	7.1
217	Blue	2006	F	-24.3	7.2
250	Blue	2006	M	-24.7	6.8
259	Blue	2006	M	-24.5	7.1
263	Blue	2006	M	-24.5	7.0
296	Blue	2006	M	-24.6	6.9
9	Green	2005	F	-25.8	6.2
23	Green	2005	F	-26.1	6.0
34	Green	2005	F	-25.1	6.3
235	Green	2005	F	-25.8	5.6
459	Green	2005	F	-26.2	5.3
7	Green	2005	M	-25.5	6.8
16	Green	2005	M	-25.2	7.9
37	Green	2005	M	-25.4	6.9
118	Green	2005	M	-24.9	5.7
118	Green	2006	M	-24.6	7.3
176	Hot Pink	2003	F	-23.7	7.1
177	Hot Pink	2003	F	-23.5	6.5
178	Hot Pink	2003	F	-23.7	5.7
179	Hot Pink	2003	F	-24.3	5.5
188	Hot Pink	2003	F	-24.4	7.0
180	Hot Pink	2003	M	-23.6	6.6
190	Hot Pink	2003	M	-23.9	8.5
197	Hot Pink	2003	M	-24.5	6.6
176	Hot Pink	2004	F	-23.3	8.1
183	Hot Pink	2006	F	-24.4	5.9
184	Hot Pink	2006	F	-24.3	6.5
123	Light Blue	2005	F	-24.3	7.7
128	Light Blue	2005	F	-23.0	8.0
129	Light Blue	2005	F	-23.8	7.7
242	Light Blue	2005	F	-24.5	7.0
124	Light Blue	2005	M	-23.5	8.0
224	Light Blue	2005	M	-24.8	8.5
236	Light Blue	2005	M	-24.2	7.4
247	Light Blue	2005	M	-24.4	7.7
253	Light Blue	2005	M	-24.7	7.1
330	Light Blue	2005	M	-24.7	7.2
271	Light Blue	2006	F	-24.4	7.3
213	Light Blue	2006	M	-24.4	7.1
253	Light Blue	2006	M	-24.6	7.7
330	Light Blue	2006	M	-24.1	7.5
105	Light Purple	2006	F	-25.0	6.0
106	Light Purple	2006	F	-25.2	5.9

154	Orange	2003	F	-24.0	7.9
171	Orange	2003	F	-22.5	8.2
174	Orange	2003	F	-24.1	7.6
368	Orange	2003	F	-24.0	8.0
266	Orange	2003	F	-23.7	7.6
153	Orange	2003	M	-23.6	8.7
166	Orange	2003	M	-24.2	7.2
170	Orange	2003	M	-23.2	7.9
300	Orange	2004	F	-24.7	7.3
249	Orange	2004	M	-24.5	6.7
262	Orange	2004	M	-24.7	7.9
231	Orange	2005	F	-24.3	7.0
300	Orange	2005	F	-24.3	7.0
368	Orange	2005	F	-24.2	6.9
266	Orange	2005	F	-24.7	7.3
166	Orange	2005	M	-24.2	8.4
262	Orange	2005	M	-23.2	8.1
274	Orange	2005	M	-24.4	7.0
231	Orange	2006	F	-24.5	7.6
268	Orange	2006	F	-24.6	7.5
300	Orange	2006	F	-24.3	7.5
364	Orange	2006	F	-24.6	8.1
368	Orange	2006	F	-24.9	7.7
266	Orange	2006	F	-24.2	8.0
153	Orange	2006	M	-24.5	7.6
249	Orange	2006	M	-24.7	7.7
232	Purple	2004	F	-24.5	7.3
215	Purple	2004	M	-24.7	7.0
214	Purple	2006	F	-24.8	6.0
232	Purple	2006	F	-24.5	6.7
44	Red	2004	F	-25.0	6.3
44	Red	2005	F	-25.1	6.3
6	Red	2005	M	-24.4	7.7
53	Red	2005	M	-24.4	7.6
311	Teal	2003	F	-25.0	6.6
151	Teal	2003	M	-24.7	6.2
160	Teal	2003	M	-25.0	6.4
156	Teal	2003	M	-24.0	7.6
230	Teal	2004	M	-24.8	6.1
182	Teal	2005	M	-25.5	6.2
204	Teal	2005	M	-26.4	6.1
230	Teal	2005	M	-25.7	5.7
459	Trois Fromage	2006	F	-24.8	7.2
284	Trois Fromage	2006	M	-25.0	6.9

172	Yellow	2003	F	-24.8	6.5
489	Yellow	2003	F	-24.9	5.9
189	Yellow	2003	M	-24.4	6.9
195	Yellow	2003	M	-24.6	7.4
196	Yellow	2003	M	-25.0	6.8
172	Yellow	2004	F	-25.4	5.8
319	Yellow	2004	F	-25.3	6.4
489	Yellow	2004	F	-25.2	7.2
195	Yellow	2004	M	-25.2	7.7
196	Yellow	2004	M	-25.2	6.8
319	Yellow	2005	F	-24.2	6.6
489	Yellow	2005	F	-25.6	6.6
195	Yellow	2005	M	-25.7	7.5
210	Yellow	2005	M	-24.9	6.5
319	Yellow	2006	F	-24.2	8.3
210	Yellow	2006	M	-23.5	8.4
238	Yellow	2006	M	-22.4	9.7