Stable Carbon and Nitrogen Isotope Analysis of Captive Northern Galagos (*Otolemur garnettii*) Fed Experimental "Frugivorous" and "Invertebrate" Diets

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Otolemur garnettii are small (~ 770g), semi-solitary, omnivorous primates and although there have been studies examining the behavioral ecology of the species very little is known about its diet. This is due in part to the species cryptic nature, fast and sporadic movements, and nocturnal activity patterns. To address this gap in the literature this study employs stable isotope analysis (SIA) on a captive population (n=11) of *O. garnettii* that resided at the University of Southern Mississippi to examine dietary patterns and establish feces-diet isotopic fractionation. Over a six-week period, the population was fed experimental diets that mimic the seasonal availability of food resources, these diets have been traditionally referred to as 'frugivory' and 'insectivory.' As part of a larger galago digestibility project examining the nutritional properties of these foods and feces, this study used isotope ratio mass spectrometry (IRMS) to additionally record the stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta^{15}N$) values of these samples. I hypothesize that the $\delta^{13}C$ and $\delta^{15}N$ values of the feces will track the dietary changes presented to the galagos and that each diet will be isotopically distinct from one another. This study revealed that inter-individual variation in the digestibility of the diets can be identified but may not lead to an overall significant degree of difference among a large captive colony, isotopically speaking. This

study demonstrated that even short-term dietary changes can be tracked accurately using SIA which is an important result for field primatologists studying free-ranging galagos and other small, nocturnal strepsirrhine primates. The implementation of these methods in field settings may prove useful to researchers attempting to understand nonhuman primate dietary patterns across time which has become increasingly more difficult to observe as primate populations decline globally.

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A Thesis Presented to the Faculty of the Department of Anthropology

East Carolina University

In Partial Fulfillment of the Requirements of the Degree of

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By

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Table of Contents

List of Tables

List of Figures

Chapter One - Introduction

This study employs the method and theory of stable isotope analysis (SIA) to advance our understanding of the behavior and biology of the northern Garnett's galago (*Otolemur garnettii*). To date, little is known regarding the behavioral ecology and evolution of *O. garnettii,* and SIA can be used to address this gap. SIA is frequently employed by primatologists interested in non-human primate (NHP) ecology given that stable isotope values are faithfully recorded in the tissue and excreta of animals, and these values can provide dietary data on the order of days to years (Sandberg et al. 2012). However, thus far, most of the SIA research has been conducted on flagship NHP species including baboons (*Papio ursinus*), bonobos (*Pan paniscus*), chimpanzees (*Pan troglodytes*), and orangutans (*Pongo pygmaeus*) (Leader-Williams & Dublin 2000; Codron et al. 2006; Loudon et al. 2016; Loudon et al. 2019; Tsutaya et al. 2017; Tsutaya et al. 2021). Although many of these species are experiencing population decline and may face extinction, continued focus on these large-bodied primates limits our understanding of overall primate isotope ecology. This is because large-bodied primates, specifically those historically receiving the brunt of scientific focus, have very different dietary regimes and behavioral ecology than other primate species. Furthermore, bonobos, chimpanzees, and orangutans rely heavily on fruits which can often have different isotopic values compared to leaves and invertebrates and are not representative of the dietary breadth of the estimated 504 NHP species alive today (Estrada et al. 2017). Thus, there is a need to conduct dietary studies of these smaller-bodied primates as well, to better understand the breadth of SIA's applicability for primatological studies and ensure we aren't narrowing our understanding by focusing on a specific minority of NHP species.

To date, little is known about the feeding ecology of *O. garnettii* due to its cryptic nature, fast and sporadic movements, and nocturnal activity patterns. At present, only two projects using

conventional methods to study feeding ecology have been conducted on *O. garnettii* both occurring in the 1980s (Harcourt & Nash 1986; Masters et al. 1988). As such, SIA is an ideal methodological approach for expanding our understanding of the biology, behavior, and evolution of this species given that stable isotope values record dietary behavior that is frequently difficult or impossible to observe among nocturnal NHPs. Harcourt and Nash (1986) performed visual examinations of *O. garnettii* fecal samples that were collected from traps. Though informative, this approach only identified the undigested components of the fruits and invertebrates that the population consumed. In addition, Masters et al. (1988) examined the gut contents of *O. garnettii* across six study sites and found that invertebrates consisted of approximately half of their diet, and the contributions of seeds and fruits represented the other half. Most dietary studies on *O. garnettii* end in the 1980s until Schoeninger et al. (1998) performed SIA on galago hair samples from two separate populations residing at the Gedi Ruins Monument reserve, located in Eastern Kenya. The Schoeninger et al. (1998) study was among the first applications of SIA in primatology. Although this study provided critical data regarding broad dietary behaviors and habitat utilization of the two study species of galagos, it did not include the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values of the local flora and invertebrates that were consumed by the galagos. Without dietary stable isotope values, the galago hair values examined in the study were difficult to attribute to any particular food resource(s).

This study is the first to quantify and compare the stable carbon and nitrogen isotope values of foods from experimental diets with the fecal matter of *O. garnettii*. Comparing known $\delta^{13}C$ and $\delta^{15}N$ of foods and fecal matter provides accurate calculations of stable isotopic fractionation values. Here, fractionation refers to the relative partitioning of light and heavy

stable carbon and nitrogen isotopes in an animal's excreta or tissue. Once the fractionation values were calculated, we examined how the $\delta^{13}C$ and $\delta^{15}N$ galago fecal values track diet and dietary shifts. The data that were generated during this study contributes to a better understanding of both the dietary patterns and stable isotope ecology of *O. garnettii*, and more broadly NHPs. SIA has become an important tool for primatologists, improving the primatological community's understanding of how primates interact with their environments. These data may be especially critical to many species in the Order Primates as some species continue to decline, as a result of the impacts of unsustainable anthropogenic activity (Estrada et al. 2017).

Chapter Two - Background and Theory

Background

The name 'Garnett's galago' was given to the species by William Ogilby (1805-1873), a British naturalist, in 1838. Ogilby named the species after George Howson Garnett (Ogilby 1838, Olson 1979). The species is the most geographically restricted of the greater galagos (*Otolemur*), only residing in Kenya, Somalia, and Tanzania (Figure 1). The term 'greater' simply refers to their size, as they are one of the two largest galagid species, along with their sister species, *Otolemur crassicaudatus. O. garnettii* is a small, semi-solitary, nocturnal primate that spends most of its time within the dense canopy of highland, riverine, and coastal forests at or above 5m from the forest floor. They rarely descend to forage terrestrially or move within the canopy quadrupedally as their hindlimbs are long and adapted to vertical clinging and leaping (Gebo 2011). Thus, *O. garnettii*, are not well adapted for moving about the ground as it increases their susceptibility to predation (Olson 1979). Along with hindlimb adaptations for leaping, *O. garnettii* possesses a suite of traits that make them successful nocturnal hunters and foragers including an acute sense of smell due to an increased reliance on olfaction, large ears for communication and surveillance, and large eyes with *Tapetum luciduma* that allow for enhanced vision in low light conditions (Nash et al. 1981).

Nash and Harcourt (1986) found that within a wild population of adult *O. garnettii* from Diana, Kenya males weighed 820g ± 98g, while females weighed 720g ± 60g. The species is omnivorous, primarily consuming invertebrates and fruits with the contribution of each dietary resource fluctuating throughout the year based on seasonal availability (Harcourt & Nash 1986).

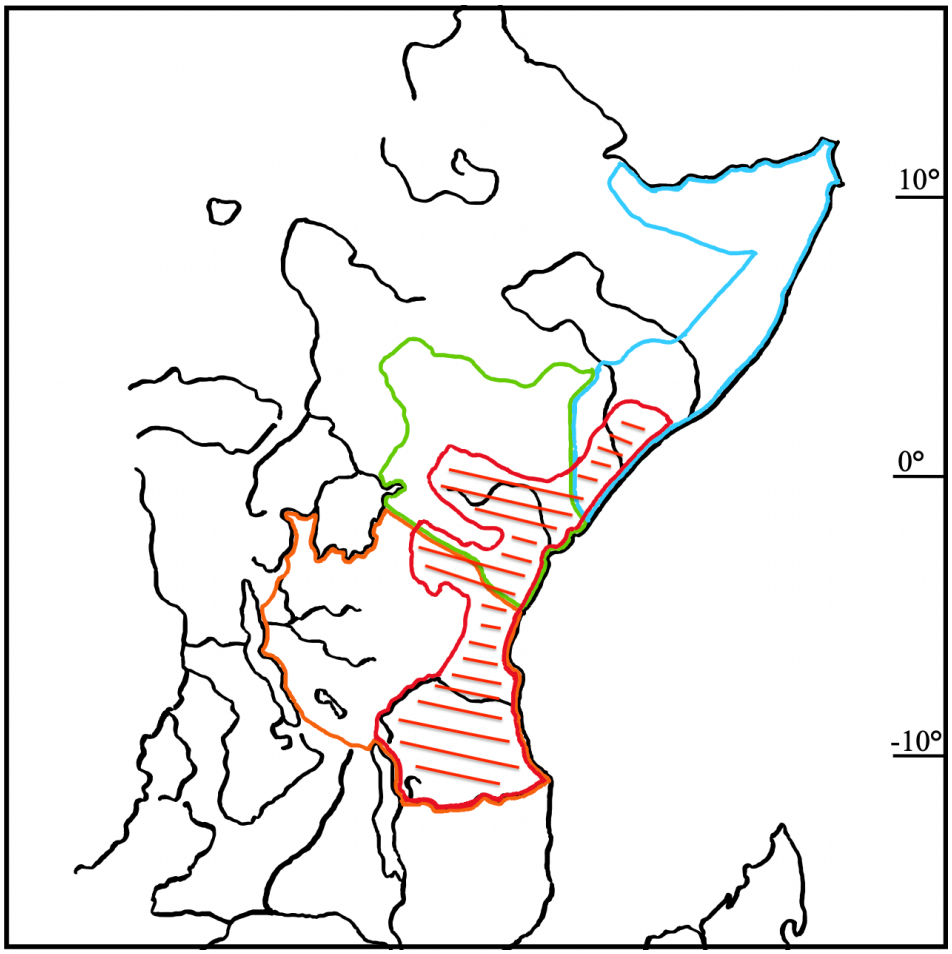

Figure 1. Map estimating the geographic range of *Otolemur garnettii*. The orange represents the border of Tanzania, green is the border of Kenya, blue is the border of Somalia, and red outlines the natural range of *O. garnettii*. Data for geographic distributions from Nash et al. (1989).

Hladik (1979) suggested that strepsirrhine primates weighing 500g to 2kg are most commonly "Insectivorous" (i.e., concentrating on invertebrates) and "Frugivorous" (i.e., concentrating on fruits). In such instances, where species follow mixed/omnivorous feeding patterns, fruits generally provide the bulk of the species' caloric needs yet may lack sufficient proteinaceous tissue, and therefore the consumption of invertebrates is necessary to maintain a balanced diet of carbohydrates, proteins, amino acids, and lipids (Hladik 1979). Hladik's assertions are supported in part by radio tracking studies conducted on two populations of *O.*

garnettii and a sympatric species of galago, *O. zanzibaricus*, which took place at the Gedi Ruins National Monument and the coastal forests of Diana, located within 100km of Mombasa, Kenya (Harcourt & Nash 1986).

Nash and Harcourt's (1986) results documented that the larger-bodied *O. garnettii*'s movement throughout the night was intermittent, and the authors attributed their movement patterns to locomoting between patches of fruit, while the smaller species, *O. zanzibaricus*, ranged continuously throughout the night hunting invertebrates. Although some conclusions can be made regarding the feeding ecology of *O. garnettii* based on their dental morphology, body mass, visual examinations of fecal matter, and radio tracking, these methods do not provide researchers with a complete understanding of their dietary patterns (Kay 1975; Gingerich 1980; Hladik 1979; Harcourt & Nash 1986; Nash & Harcourt 1986). Since SIA is a useful method for documenting dietary patterns and habitat utilization, the approach complements existing behavioral observations and fecal examinations of the dietary ecology of *O. garnettii* (Newsome et al. 2007; West et al. 2006).

Theory

Isotopes are atoms of elements with the same atomic number (protons) and a different number of neutrons. For example, carbon 12 (^{12}C) consists of 6 protons and 6 neutrons, while carbon 13 (^{13}C), a stable isotope of carbon, has 6 protons and 7 neutrons. Since protons and neutrons account for most of an atom's mass, a difference in isotopic mass affects an element's dynamics during natural biogeochemical processes as well as organism-level metabolism. For example, $H_2{}^{18}O$ is heavier than $H_2{}^{16}O$. Both water molecules occur naturally but the ratio of the two water molecules will vary with evaporation and condensation, due to the mass difference

between the heavier and lighter isotopes. This mass difference results in “fractionation” which can be traced throughout biogeochemical systems in nature. Thus, the relative abundance of ^{12}C to ^{13}C (and ^{14}N to ^{15}N) in foods can be used to make reliable interpretations of mass-dependent metabolic processes (Ben-David & Flaherty 2012).

The application of this isotopic fractionation to understand food web dynamics is defined as stable isotope analysis (SIA). SIA can provide insights into a species’ biology, behavioral ecology, and evolution. SIA has also been implemented to clarify the ecosystem and food-web dynamics of individuals or populations in both free-ranging and captive settings (Loudon et al. 2007; Vogel et al., 2011; Bădescu et al. 2022). SIA is used to discriminate the ratio of heavy to light isotopes of elements, offering insight into metabolic pathways, chemical transport, and acting as biological/ecological tracers (Fry & Arnold 1982; Harvey et al. 2002; West et al. 2006).

This study examines stable carbon ($\delta^{13}C$) and nitrogen isotopes ($\delta^{15}N$), although there are other common elements used in ecological studies including hydrogen, oxygen, strontium, and sulfur (Ben-David & Flaherty 2012). Carbon and nitrogen can be used to make inferences about the local flora and fauna that contribute to a species' diet through biological assimilation (DeNiro & Epstein 1978; 81). During digestion, dietary carbon and nitrogen are incorporated into new cells during cell production and maintenance. The isotopic signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) associated with that food in the diet can be used to make inferences about the local flora and fauna that contribute to a species' diet through biological assimilation (DeNiro & Epstein 1978; 81), which leaves the excreted product with an isotopic ratio that is different than that of the food consumed. The food an animal consumes is referred to as the substrate (starting component or source of a chemical reaction) and the tissue (fur, bone, muscle, etc.) or excreta (feces or urine) is known as the product (the result of a chemical reaction on a substrate) (Ben-

David & Flaherty 2012). The difference in the isotopic ratios between the substrate and the product is known as fractionation. These differences are due to the rate of chemical and physical reactions that occur within the body of an animal. Furthermore, various products (i.e., animal tissues or excreta) have differing turnover times in which the extent of fractionation varies. For example, feces-diet fractionation is representative of short-term dietary data on the order of days or weeks, while keratin-diet (hair) or collagen-diet (bone) would be representative of long-term dietary trends spanning days to years (Ben-David and Flaherty 2012).

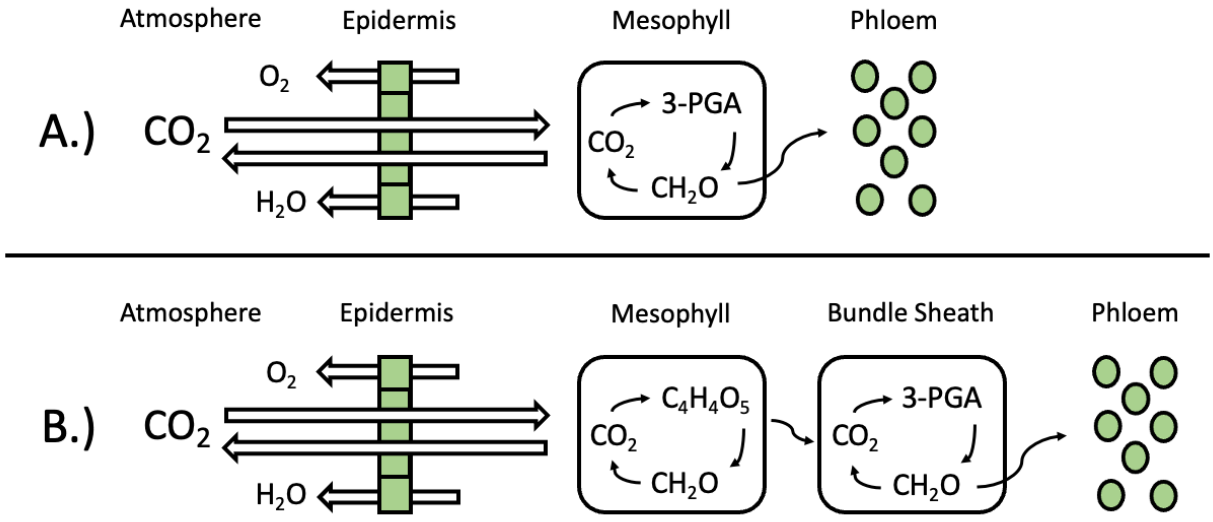

Figure 2. Adapted from O'Leary (1988). This figure displays the photosynthetic pathways of both C_3 (A) and C_4 (B) plants, with arrows representing the relative flux of CO_2 throughout their individual pathways. The increased complexity of the C_4 photosynthetic pathway (B)(Hatch-Slack cycle), coupled with a lower rate of CO_2 diffusion back into the atmosphere is why C_4 plants are ^{13}C enriched relative to C_3 plants (O'Leary 1988).

Plants have signature ratios of ^{13}C and ^{12}C in their tissues, which are linked to their photosynthetic pathways that are referred to as C_3, C_4, and crassulacean acid metabolism (CAM) plants. C_3 plants proceed through the Calvin cycle producing a three-carbon acid (3-

Phosphoglyceric acid (3-PGA)), while C_4 plants utilize the Hatch-Slack cycle producing a four-carbon acid (Oxaloacetic acid ($C_4H_4O_5$)) (Benson et al. 1950; Slack and Hatch 1967). These differences are due to the heavier isotopes diffusing slower through metabolic pathways due to their greater mass.

Throughout each step of these reactions, the heavy isotope becomes relatively less abundant, as the lighter isotope moves quicker throughout these processes and is therefore more readily incorporated into the next step in a reaction pathway. The rate C_3 plants process CO_2 during photosynthesis is limited by the rate at which CO_2 diffuses back into the atmosphere limiting their retention of ^{13}C; while the increased complexity of the C_4 photosynthetic pathway (Hatch-Slack cycle), coupled with a lower rate of CO_2 diffusion allows C_4 plants to process and incorporate more ^{13}C into the newly created organic matter (O'Leary 1988)(Figure 2). As such, C_4 plants are referred to as being enriched in ^{13}C (Ben-David and Flaherty 2012). These ratios are measured against international standards to ensure precision and comparability. The international standard for carbon is Vienna PeeDee Belemnite (VPDB) (Craig 1953). The average $\delta^{13}C$ values of C_4 plants are about -14‰ (parts per mil, or parts per thousand) while the $\delta^{13}C$ values of C_3 plants average around -27‰ (O'Leary 1988; Sandberg et al. 2012; Marshall et al. 2007; Smith and Epstein 1971).

In animals, nitrogen can be used as an indicator of the trophic transfer of energy. The assimilation of nitrogen in animals, both ^{14}N and ^{15}N, is associated with the consumption of various proteinaceous tissues that differ in their amino acid structure (Ben-David et al. 2012). Animal tissues are typically ^{15}N-enriched relative to diet, consequently, there is generally a stepwise increase in $\delta^{15}N$ values (~3‰) per trophic level (Figure 3) (DeNiro & Epstein 1981; Schoeninger & DeNiro 1984). This stepwise increase is due to the fact that trophic levels are

indicative of food web positioning, the higher trophic levels consist of animals that have consumed animals on the preceding trophic levels thus leading to enriched $\delta^{15}N$ values when compared to lower trophic levels (Ben-David and Flaherty 2012). The international standard for nitrogen is atmospheric nitrogen (AIR) and most biological samples are enriched compared to the standard resulting in mostly positive values (Crowley et al. 2010).

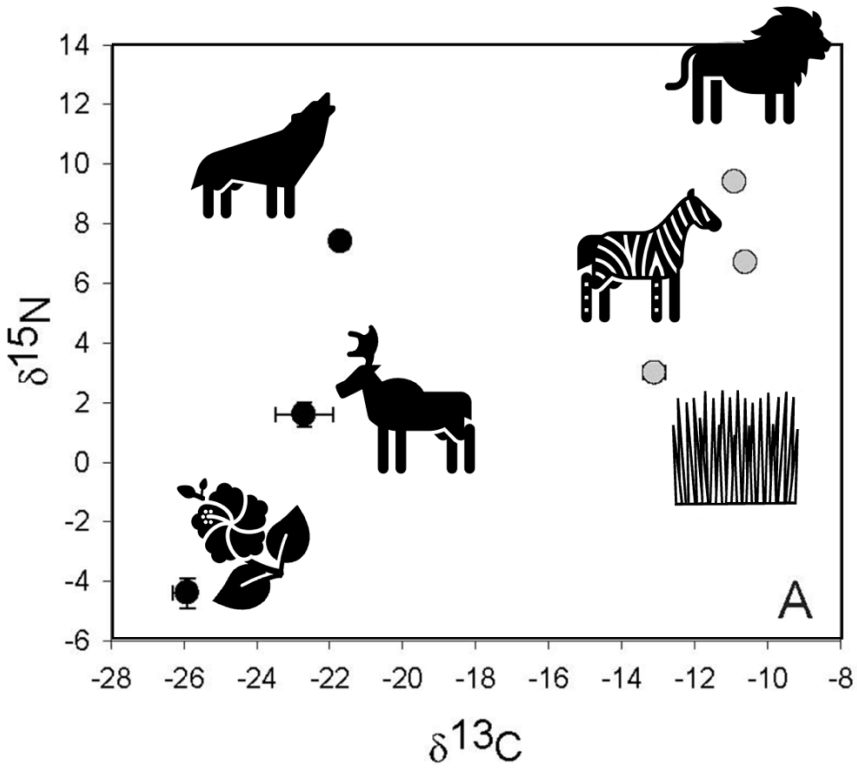

Figure 3. $\delta^{13}C$ and $\delta^{15}N$ biplot adapted from Ben-David and Flaherty (2012). This figure displays a primary producer, herbivore, and carnivore from two separate systems (C_3: willow bud, moose, and C_4: wolves; grasses, zebra, and lions) and how their relative trophic positions affect their individual isotopic signatures due to biological assimilation.

SIA can be used to study ecology, niche differentiation, and even allows for comparison across time and space using archived museum specimens and fossils (Sandberg et al. 2012). SIA may also be used to identify cryptic or secretive behaviors that are difficult for primatologists to record using behavioral observations. For example, Loudon et al. (2014) studied the stable

carbon and nitrogen isotope hair values of groups of free-ranging vervet monkeys (*Chlorocebus pygerythrus*) across South Africa. Comparisons of the $\delta^{13}C$ values of the groups demonstrated that some groups secretly consumed C_4 crops (i.e., corn or maize).

Although these vervet monkeys had adopted secretive behaviors to avoid detection, the application of stable isotope analysis confirmed cryptic feeding behavior that was difficult to observe using conventional observations. Given that it is also difficult to observe the nocturnal behavioral patterns of *O. garnettii* in its natural habitat, SIA can expand our understanding of their behavioral ecology. Furthermore, galagids retain many 'ancestral' morphological and behavioral traits and have been used as an extant ecological analog for understanding the ecology, evolution, and origins of the earliest extinct primate species (Fleagle 2013).

A handful of captive NHP studies have examined the fractionation of stable carbon and stable nitrogen between diet and feces and hair to understand both short-term and long-term metabolic trends and dietary patterns (Tsutaya et al. 2017; Tsutaya et al. 2021; Macharia et al. 2014). Tsutaya et al. (2021) suggested that more controlled stable isotope studies of primate fecal matter should be conducted, to better understand the compositional heterogeneity of feces, as it is a mixture of the digested and undigested portions of an animal's diet. Controlled studies, such as this one, allow researchers to better understand the relationships in question as they are less impacted by the external variables and possible unknowns of wild studies. However, there are many challenges associated with SIA, including an understanding of hair-diet fractionations among wild primate populations. For example, hair is easier to acquire from captive NHPs but can be difficult to obtain in the wild. This may include acquiring hair from arboreal nests or using sticky darts for collection, but these methods may be stressful for NHPs. Additionally, behavioral observations of cryptic species in the wild could lead to their stress, creating artifacts

in metabolism and affecting fractionation. Thus, collecting feces may be a more favorable approach to understanding their dietary patterns. Although hair provides $\delta^{13}C$ and $\delta^{15}N$ data over a longer temporal scale, similar information could be derived with long-term fecal studies collected throughout the year to capture seasonal variation.

Research Questions and Hypotheses

The research design implemented in this study provides a greater understanding of the diet and ecology of captive and free-ranging *O. garnettii*. This study will address the following research questions and hypotheses.

Q_1: What are the feces-diet fractionation values for a captive colony of northern Garnett's galago (*Otolemur garnettii*) that were provided a controlled "Frugivorous" and "Invertebrate" diet that mimicked seasonal dietary shifts?

H_1: The "Invertebrate" diet will result in higher $\delta^{15}N$ fecal values and $\delta^{13}C$ fecal values will reflect the $\delta^{13}C$ of the invertebrates consumed.

H_2: The "Frugivorous" diet will result in lower $\delta^{15}N$ fecal values and $\delta^{13}C$ fecal values that are less variable than those of the invertebrates.

Q_2: How do the $\delta^{13}C$ and $\delta^{15}N$ fecal values of this captive population of *O. garnettii* vary as a function of dietary shifts?

H_3: The $\delta^{13}C$ and $\delta^{15}N$ values of the feces will change to be more representative of the diets they were fed during the time of collection and be isotopically distinct from one another.

Chapter Three - Methods

Study Subjects

This study examines the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope fractionation between diet and feces among a captive colony of eleven adult northern greater galagos (*Otolemur garnettii*). The colony was located at the Primate Behavior Research Center at the University of Southern Mississippi (USM). The galagos were housed in individual cages organized in a linear row to facilitate social interactions between adjacent individuals. Since the galagos were caged individually, accurate measures of their food intake and fecal collections were recorded. Collections began on 31 May 2019 and concluded on 14 July 2019. The colony included five females and six males, with individual galagos being between the ages of 54 to 191 months at the time the study began (Table 1). Mean weights were recorded weekly, given the galagos cooperation, to monitor each individual's physical health throughout the duration of study.

Table 1. Sex, age, and weight (mean and standard deviations) of individual galagos (*Otolemur garnettii*) housed at the University of Southern Mississippi.

Individual	Sex	Date of Birth	Age (months)	Mean Weight ± SD (g)
Baker	F	11/13/2014	54	954 ± 23
Brandine	F	2/6/2006	159	845 ± 15
Emily	F	4/27/2013	73	837 ± 16
Houdini	F	6/3/2014	59	959 ± 32
Piper	F	6/10/2003	191	951 ± 35
Chris	M	1/14/2013	76	1067 ± 57
Curious	M	5/19/2011	96	1016 ± 48
Hercules	M	3/26/2010	110	1033 ± 40
Joey	M	2/6/2006	159	916 ± 29
Kyle	M	3/16/2009	122	957 ± 26
Tim	M	10/29/2008	127	874 ± 25

Research Design

The colony was presented with four broad diets including a Baseline Diet, Transition Diet, Frugivorous Diet, and Invertebrate Diet. The Baseline Diets represented the standard dietary regimes that the galagos normally consumed and reflected the nutritional needs of the species and individuals. The galagos were placed on two experimental diets; the Frugivorous Diet which included no invertebrates and the Invertebrate Diet which included no fruits. These diets were chosen to mimic seasonal dietary shifts experienced by galagos in the wild. Before and after the galagos were placed on the experimental diets, they were put on Transitional Diets that included both invertebrates and fruits, to minimize the risk of any adverse gastrointestinal complications associated with abrupt dietary changes (Figure 4 and Table 2). All diets included primate biscuits (Lab Diet® 5045) to ensure that each individual's minimum macro and micro nutritional needs were met.

Table 2. Diets including food items provided to the galago colony. Redworms were excluded from the final Baseline Diet because all suppliers within a 30-mile radius of the research facility were sold out or carried dead stock.

Diet	Foods Provided
Initial Baseline	Primate biscuits, Blackberries, Raspberries, Tamarind, Redworms, Mealworms
Transition 1	Primate biscuits, Blackberries, Raspberries, Tamarind, Mealworms
'Frugivorous'	Primate biscuits, Blackberries, Raspberries, Tamarind
Transition 2	Primate biscuits, Blackberries, Raspberries, Tamarind, Redworms, Mealworms, Nightcrawlers, and Crickets
'Invertebrate'	Primate biscuits, Redworms, Mealworms, Nightcrawlers, and Crickets
Transition 3	Primate biscuits, Blackberries, Raspberries, Tamarind, Redworms, Mealworms, Nightcrawlers, and Crickets
Final Baseline	Primate biscuits, Blackberries, Raspberries, Tamarind, Mealworms

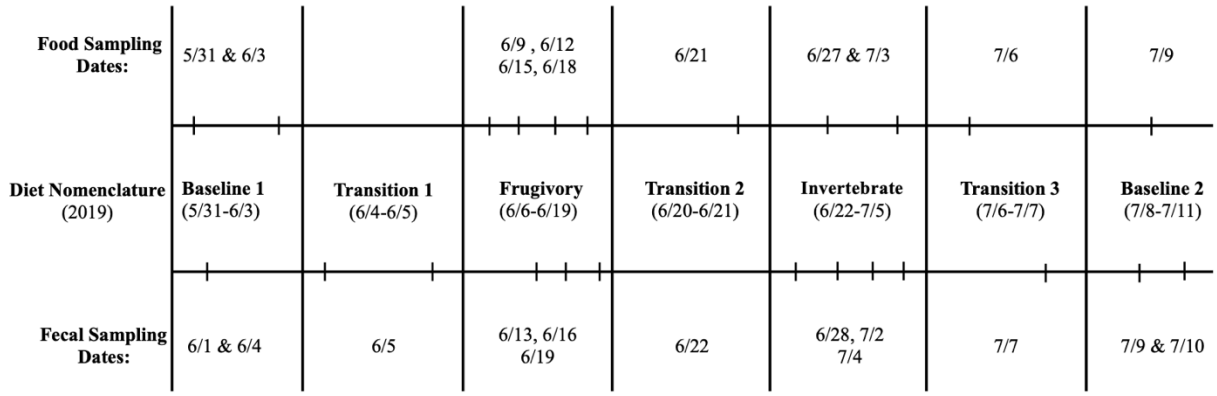

Figure 4. Food and fecal sampling timeline with each diet and its corresponding dates in the center row, food sampling dates above, and fecal sampling dates below. Hair was extracted from some fecal samples from galagos during the Baseline 1 and Baseline 2.

The foods presented to the galagos consisted of the following fruits: blackberries (*Rubus sp. 1*), raspberries (*Rubus sp. 2*), and tamarind (*Tamarindus indica*), while the invertebrates consisted of crickets (*Acheta domesticus*), earthworms (*Lumbricus terrestris*), redworms (*Eiscenia fetida*), and mealworms (*Tenebrio molitor*). The quantity of each food provided to each galago was recorded, along with how much remained after feeding to establish the quantities consumed by each galago every day. These quantities aid in the establishment of dietary mass fraction (%)(DMF), defined here as the amount of an individual substrate consumed (g)/the amount of total substrate consumed (g) (Table 3). This measurement allowed for more accurate interpretations of both the $\delta^{13}C$ and $\delta^{15}N$ values for the establishment of feces-diet fractionations. The study population lived in individual enclosures that prevented them from incorporating extraneous food sources. All methods used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Southern Mississippi (USM), and the galagos were regularly visited and accessed by USM veterinarians (See appendices: Protocol #: 15111301.1).

Table 3. The dietary mass fraction (%) of each food (substrate) provided to the galagos during the experimental diets, including Baseline 1 (B1), Frugivory (F), Invertebrate (I), and Baseline 2 (B2). The asterisks (*) in B1 for *T. molitor* and *E. fetida* indicate a substrate that was rotating during the feeding of the diet. On days they were provided *T. molitor*, they were not provided *E. fetida*, each making up 18.4 % on their respective days.

Foods (substrate)	B1 (%)	F (%)	I (%)	B2 (%)
Primate Biscuits	61.5	24.5	29.8	68.4
Blackberries (*Rubus sp. 1*)	9.0	33.8	0.0	9.9
Crickets (*Acheta domesticus*)	0.0	0.0	4.2	0.0
Mealworms (*Tenebrio molitor*)	18.4*	0.0	14.9	9.2
Nightcrawlers (*Lumbricus terrestris*)	0.0	0.0	25.9	0.0
Raspberries (*Rubus sp. 2*)	8.9	33.4	0.0	10.0
Redworms (*Eiscenia fetida*)	18.4*	0.0	25.2	0.0
Tamarind (*Tamarindus indica*)	2.2	8.3	0.0	2.5

Methods

Food and Fecal Sample Preparation and Stable Isotope Analysis

The galagos were presented foods each morning, and on the following morning a fecal sample from each galago was collected. Fecal and food samples were refrigerated directly after collection. The samples were then desiccated for 24 hours at 40°C in a Fischer Scientific Isotemp drying oven. Each sample was placed in a labeled individual bag and sealed with desiccant and stored in a freezer (-20°C). Galago feces represented the digested and undigested portions of the prior day's diet. Feeding experiments using non-toxic glitter and food coloring with the colony revealed a gut transit rates of approximately 12 hours (Smith unpublished data).

All fecal and food samples were ground into a fine powder using a mortar and pestle until relative homogeneity was achieved. Each sample was placed into small sample bags prior to encapsulation. Between each sample, the mortar and pestle were wiped with KIMWIPES and acetone (greater than or equal to 99.5% ACS) and allowed dry. Approximately 2mg (current SD ± 0.19) of sample was encapsulated into 3.5mm x 5mm tin capsules which were then crimped

and closed, all weighing was conducted using a Sartorius CP2P microbalance. After encapsulation, each sample was placed into a 96-well sample tray, where it was assigned an identifier (A1, A2, B1, etc.) and then cataloged into a master inventory sheet used by the University of North Carolina-Wilmington (UNCW) Isotope Ratio Mass Spectrometry (UNCW-WIRMS) facility.

Hair Sample Preparation

During fecal sample preparation, some samples consisted of relatively large quantities of galago hair that was removed via auto-grooming using their toothcombs and subsequently passed through their gastrointestinal tracts. The hair acted as a binder maintaining the structure of the feces. Hair samples from each galago were collected from both the Baseline 1 and Baseline 2 Diets for encapsulation and analysis. Fecal samples were rehydrated using approximately 25ml of type I deionized water, from an Elga Ultralab UHQ water purification system, and sonicated for 30 minutes to agitate the samples. After sonification, samples were individually placed on 20cm x 20cm ashed squares of aluminum foil where an average of 1.11mg ± 0.20mg of hair was manually separated using forceps and placed into 5mm x 9mm tin capsules and crimped.

Stable Isotope Analysis

The stable carbon and nitrogen values for food, fecal, and hair samples were analyzed at the University of North Carolina – Wilmington Isotope Ratio Mass Spectrometry (UNC-WIRMS) facility located at their Marine Science Center. The lab utilizes a Costech® 4010 Elemental Analyzer coupled with a zero-blank autosampler to measure the solid organic materials' carbon and nitrogen isotope ratios (tissue, feces, etc.). The mass spectrometer provided

the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios which are expressed using the lowercase Greek delta (δ) notation in parts per thousand or per mil (‰) relative to Acetate and two separate standards, USGS 40 and USGS 41a, both L-glutamic acid (see Equation 1). These standards were prepped on-site at UNC-WIRMS and placed into each individual sample tray. Originally the amount of standard was between 0.2-0.9mg, however, the samples were relatively nitrogen-poor and high carbon peaks led to this being increased to 0.5-2.0mg, this range was intentional to be able to accurately calibrate the mass spectrometer using a wider range of C:N quantities. For carbon, the standard was the Vienna PeeDee Belemnite (VPDB) and for nitrogen, the standard was atmospheric N_2 (AIR), respectively.

Equation 1.) This equation is used to quantify the $\delta^{13}C$ and $\delta^{15}N$ values. X = the element's heavier form, and R = the ratio of the heavier form of the element/lighter form of the element (Craig 1953, O'Leary 1988).

$$\delta X(‰) = \left[\frac{R\ sample - R\ standard}{R\ standard}\right] \times 1000$$

Data Analysis

All data pertaining to the $\delta^{13}C$ and $\delta^{15}N$ of the samples (n=395) (which included food, feces, standards, and blanks) were provided to by the UNC-WIRMS lab vial Microsoft Excel files which contained measured stable carbon and nitrogen isotope values, linear corrections, two-point normalizations, and important data summarization. These files were reviewed for any outliers using the 1.5 x IQR (Interquartile Range) rule, with values bordering this range of tolerance or exceeding it theorized to be associated with the heterogeneity of both the foods and feces. Foods such as blackberries (*Rubus sp. 1*) and raspberries (*Rubus sp. 2*) contain many small

seeds which could potentially affect measured values. The statistical program JMP (John's Macintosh Project) Pro 16 was used to create figures and conduct statistical analyses. Some data pertaining to the study population's digestibility was supplemented by Loudon et al. (2023). Transitional diets are excluded from the statistical analyses as the primary aims of this project were to establish an isotopic baseline and better understand the feasibility of tracking diets that mimic seasonal variation in the wild using feces. Transitional diets were included to aid the galagos in transitioning between diets based on substrates with major structural differences, but such transitions are unlikely to occur in nature.

Chapter Four - Results

The mean $\delta^{13}C$ and $\delta^{15}N$ values for the foods provided to the galagos during all diets are displayed in Table 4. The invertebrates' $\delta^{15}N$ values are enriched compared to those of the fruits, which is likely linked to their higher degrees of proteinaceous tissues. The invertebrates also displayed the greatest degree of variability in $\delta^{13}C$ values with crickets (*Acheta domesticus*) measuring -20.8 ± 0.2‰ and mealworms (*Tenebrio molitor*) measuring -29.3 ± 0.5‰. The $\delta^{13}C$ values of the invertebrates could not be reliably predicted because we did not have any dietary data on what the invertebrates were fed by our providers. Based on their $\delta^{13}C$ values, it is likely that the crickets which were consumed by the galagos were fed predominantly a corn-based diet, as the $\delta^{13}C$ value of corn (a C_4 plant) is approximately -11‰. In contrast, the mealworms' diet consisted primarily of C_3 plants, possibly rice, wheat, or potatoes, which would have a $\delta^{13}C$ value of approximately -27‰ (O'Leary 1988; Suzuki et al. 2010). Since corn, wheat, rice, and potatoes are each grown domestically, and are heavily subsidized, they are likely cost-effective foods for the invertebrates we sampled (Edwards 2018).

Table 4. The foods (substrates) provided to the galagos during the experimental diets and dietary composition of each diet including the Baseline 1 (B1), Frugivory (F), Invertebrate (I), Baseline 2 (B2), and the mean $\delta^{13}C$ and $\delta^{15}N$ (‰) and standard deviation (SD) of each food.

Foods (substrate)	Diets	Mean $\delta^{13}C$ ± SD (‰)	Mean $\delta^{15}N$ ± SD (‰)
Primate Biscuits	B1, F, I, B2	-22.4 ± 0.7	1.8 ± 0.8
Blackberries (*Rubus sp. 1*)	B1, F, B2	-26.2 ± 0.9	5.1 ± 0.6
Crickets (*Acheta domesticus*)	I	-20.8 ± 0.2	4.3 ± 0.4
Mealworms (*Tenebrio molitor*)	B1, I, B2	-29.3 ± 0.5	2.8 ± 0.3
Nightcrawlers (*Lumbricus terrestris*)	I	-25.1 ± 1.0	7.1 ± 0.5
Raspberries (*Rubus sp. 2*)	B1, F, B2	-26.3 ± 1.3	1.0 ± 0.5
Redworms (*Eiscenia fetida*)	B1, I	-22.6 ± 3.4	5.4 ± 1.4
Tamarind (*Tamarindus indica*)	B1, F, B2	-28.2 ± 1.5	2.9 ± 0.7

H_1: The "Invertebrate" diet will result in higher $\delta^{15}N$ fecal values and $\delta^{13}C$ fecal values will reflect the $\delta^{13}C$ of the invertebrates consumed.

The mean $\delta^{13}C$ and $\delta^{15}N$ values of each individual's feces during the four diets, Baseline 1 (B1), Frugivory (F), Invertebrate (I), and Baseline 2 (B2) are displayed in Table 5. When averaged, across all foods within the diet, the foods in the "Invertebrate" diet were significantly lower in $\delta^{13}C$ values ($P<0.05$) and higher in $\delta^{15}N$ values ($P<0.001$). Towards this end, the $\delta^{15}N$ fecal values of the galagos were significantly higher ($P<0.01$) when they consumed the Invertebrate diet (Table 4, Table 5). The significantly lower $\delta^{13}C$ values of the Invertebrate diet are likely due to diets characterized by higher amounts of C_4 resources which would be enriched compared to the Frugivorous diet presented to the galagos, which consisted of exclusively C_3 plants.

Although there were significantly higher $\delta^{15}N$ fecal values while the galagos consumed the Invertebrate diet, much of the variation was driven by individuals. For example, Piper's (♀) average $\delta^{15}N$ fecal values on the Invertebrate diet were 4.9 ± 1.0‰, while Houdini's (♀) average $\delta^{15}N$ fecal values consuming the same diet were 3.7 ± 0.5‰. During the study, the galagos were presented with the same quantities of each food, yet they consumed different amounts due to their individual preferences. During the Invertebrate diet, Piper's dietary mass fractionation (i.e., the weight of invertebrates she consumed/the weight of all substrates consumed) during this regime consisted of 65.2% of her diet, while Houdini's invertebrate consumption was 71.4% of her diet. As a result, Houdini consumed a greater percentage of invertebrates on the Invertebrate diet, yet she also had lower $\delta^{15}N$ fecal values in comparison to Piper (by approximately ~1.2‰).

Table 5. The mean $\delta^{13}C$ and $\delta^{15}N$ (‰) fecal values with standard deviations (SD) for each galago on each diet. Baseline (B1) (n=2), Frugivory (F) (n=3), Invertebrate (I) (n=3), and Baseline 2 (B2) (n=2).

Individual & Sex	B1 (Mean ± SD (‰))		F (Mean ± SD (‰))		I (Mean ± SD (‰))		B2 (Mean ± SD (‰))	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
BAKER ♀	-23.6±1.0	3.4±0.6	-24.7±0.3	3.9±0.4	-24.2±0.8	4.0±1.1	-24.0±1.1	4.6±0.4
BRANDINE ♀	-23.3±2.0	3.7±0.1	-24.9±0.7	3.7±0.1	-24.8±0.2	3.9±0.6	-24.2±1.5	4.3±1.5
CHRIS ♂	-23.3±3.4	3.4±0.5	-25.3±0.4	3.5±0.4	-23.9±0.1	4.4±0.7	-23.7±1.1	4.1±1.5
CURIOUS ♂	-23.9±2.2	3.3±0.1	-25.2±0.5	3.7±0.5	-25.4±0.2	3.7±0.8	-23.8±1.0	4.1±1.6
EMILY ♀	-23.5±1.2	3.4±0.5	-23.8±0.9	3.6±0.2	-25.1±0.8	4.1±0.5	-25.2±0.8	3.8±0.6
HERCULES ♂	-22.7±0.3	3.2±0.3	-24.9±0.3	3.9±0.6	-24.6±0.4	4.6±0.6	-23.5±1.5	4.6±1.1
HOUDINI ♀	-24.7±1.6	3.0±0.3	-24.2±1.5	3.8±0.3	-25.6±0.6	3.7±0.5	-24.6±1.0	3.9±1.2
JOEY ♂	-23.9±2.1	3.1±0.2	-25.1±0.4	3.8±0.2	-25.1±0.3	4.1±0.5	-23.8±0.8	3.8±0.8
KYLE ♂	-21.1±1.3	3.14±0.1	-24.3±0.2	3.4±0.7	-24.5±0.2	4.3±0.7	-22.1±0.1	3.0±0.4
PIPER ♀	-24.2±2.1	2.8±0.3	-24.9±0.4	4.1±0.4	-23.8±0.8	4.9±1.0	-24.0±1.3	4.2±1.2
TIM ♂	-23.9±1.0	3.6±0.5	-24.1±0.5	3.5±0.7	-24.9±1.4	3.9±0.6	-24.9±0.7	4.0±1.2
Average:	-23.4±1.6	3.3±0.4	-24.7±0.7	3.7±0.4	-24.7±0.8	4.1±0.7	-24.0±1.1	4.0±0.9

The median $\delta^{13}C$ and $\delta^{15}N$ fecal values for each individual during Baseline 1 are shown in Figure 5. Both Baseline diets represent the regular dietary regimes of the captive population and are included to establish an isotopic baseline, as well as show how the galagos fecal baseline $\delta^{13}C$ and $\delta^{15}N$ shifted when compared to the experimental diets (Frugivory and Invertebrate). Throughout the Baseline 1 diet, the galagos' average dietary mass fraction consisted of 61.5% primate biscuits, 20.1% fruits, and 18.4% invertebrates.

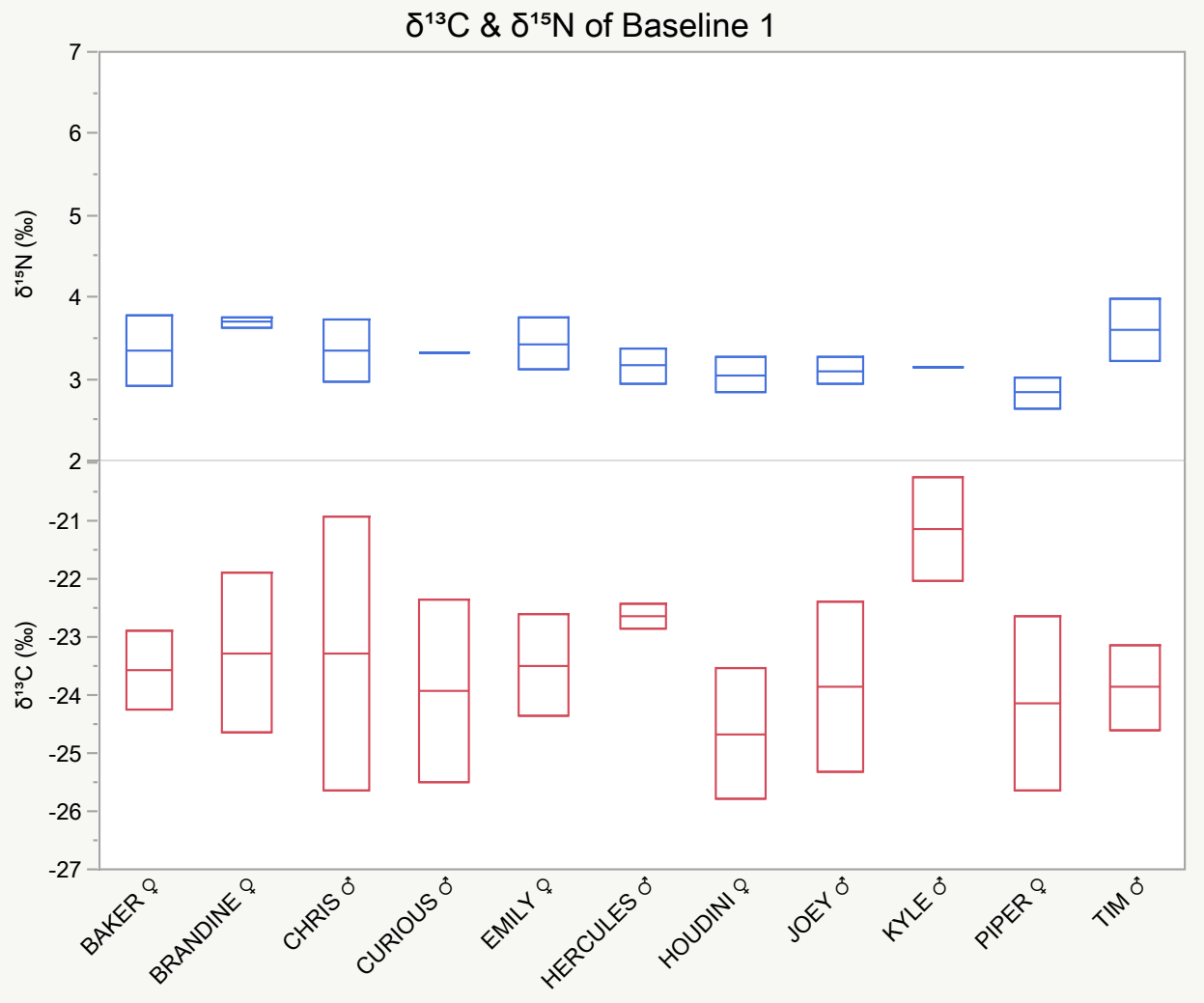

Figure 5. Box-and-whisker plots showing the $\delta^{13}C$ and $\delta^{15}N$ fecal values (‰), for each galago on the Baseline 1 diet. The line represents the median, and the boxes represent the lower (25%) and upper quartiles (75%).

While consuming the Baseline 1 diet there were slightly lower $\delta^{15}N$ fecal values compared to the Frugivory diet. This appears to be linked to the lower $\delta^{15}N$ values of the Primate Biscuits which averaged 1.8 ± 0.8‰. The dietary mass fraction of Primate Biscuits in each diet with Baseline 1 averaging 61.5% and Frugivory averaging 24.5%. Baseline 1 also had more variability in $\delta^{13}C$ fecal values (-23.4 ± 1.6‰), than the Frugivory diet (-24.7 ± 0.7‰). This is due to the inclusion of redworms (*E. fetida*) and mealworms (*T. molitor*), which were the most

enriched and depleted foods (when including standard deviation) with mean values of -22.6 ± 3.4‰ and -29.3 ± 0.5‰, respectively.

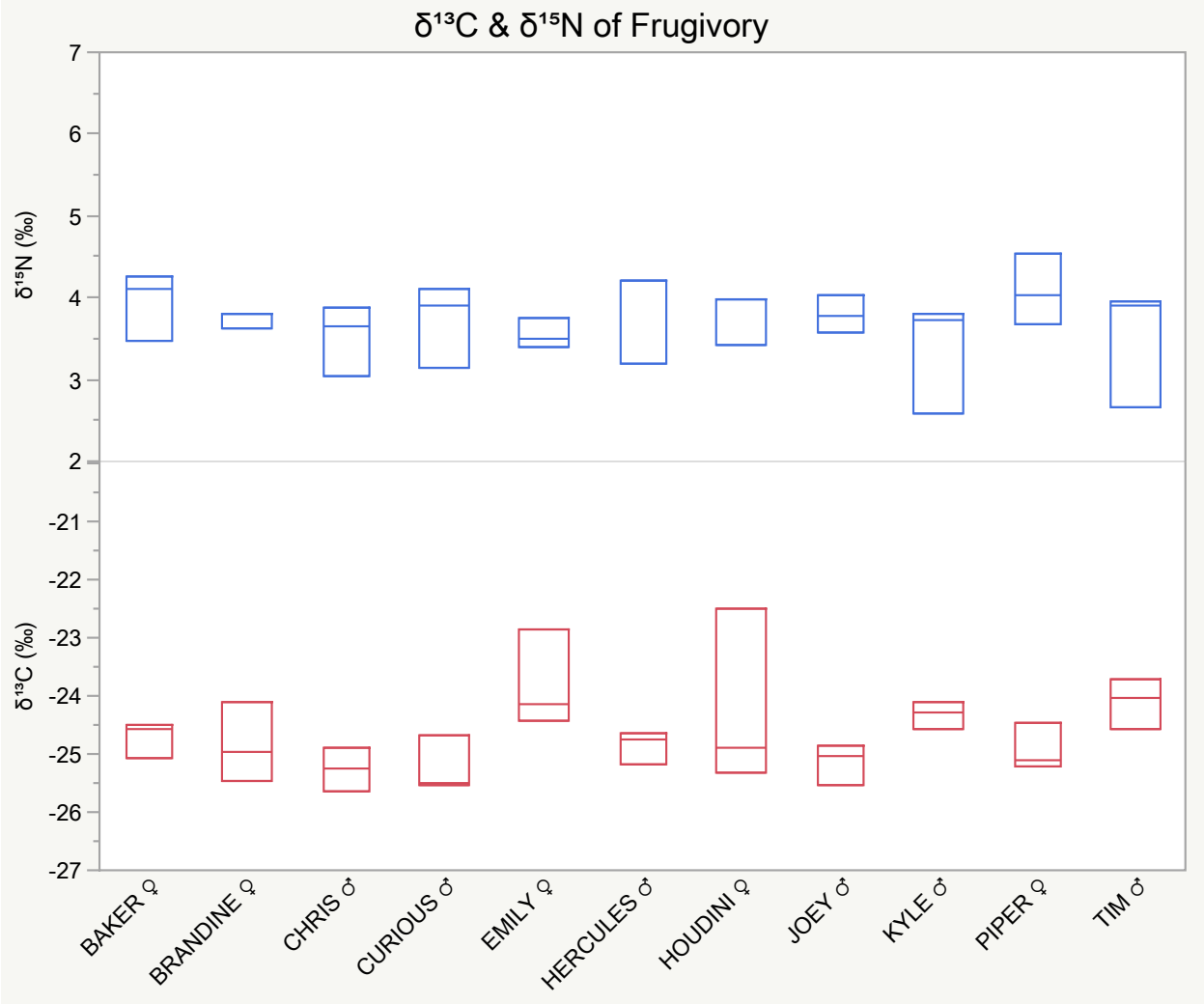

Figure 6. Box-and-whisker plots showing the $\delta^{13}C$ and $\delta^{15}N$ fecal values (‰), for each galago on the Frugivory diet. The line represents the median, and the boxes represent the lower (25%) and upper quartiles (75%).

H_2: The "Frugivorous" diet will result in lower $\delta^{15}N$ fecal values and $\delta^{13}C$ fecal values that are less variable than those of the invertebrates.

The median $\delta^{13}C$ and $\delta^{15}N$ fecal values for each individual during the Frugivory diet are displayed in Figure 6. The Frugivorous diet had significantly lower $\delta^{15}N$ fecal values ($P<0.01$),

when compared to the $\delta^{15}N$ fecal values of the Invertebrate diet. The digestibility data shows the percent of crude protein (CP) averaged across all invertebrates in the study is 58.8 ± 1.1%, while the average percent CP of all fruits is 7.3 ± 1.1%. Percent CP acts as an indicator of a food's protein concentrations (Loudon et al. 2023). The lower $\delta^{15}N$ fecal values of the Frugivory diet are as predicted, due to the assorted fruits' low concentration of proteinaceous tissue. Additionally, the lesser degree of variability recorded in the $\delta^{13}C$ values of the fruits was exhibited in the galago fecal values. This decrease in variability is most likely related to the fact that all fruits in the Frugivory diet were C_3 plants, and the most ^{13}C enriched fruits were raspberries (*Rubus sp.* 2) at -26.3 ± 1.3‰, and the most ^{13}C depleted fruits were tamarinds (*T. indica*) at -28.2 ± 1.5‰. In contrast, the most ^{13}C enriched invertebrate species included in the Invertebrate diet were crickets (*A. domesticus*) which were -20.8 ± 0.2‰, which suggests that they were fed comparatively high amounts of C_4 resources and account for the ^{13}C enriched values that were observed.

One explanation for why these enriched ^{13}C values were not present in the galago feces while the colony was consuming the Invertebrate diet is that the crickets (*A. domesticus*) exhibited comparatively high $\delta^{13}C$ values, but they only consisted of 4.2% of their dietary mass fraction during the invertebrate diet, while the other invertebrates (mealworms, nightcrawlers, and red worms) accounted for a combined 66% of the diet, with the remaining 29.8% consisting of primate biscuits. Therefore, the average $\delta^{13}C$ fecal values during the Invertebrate diet of -24.7 ± 0.8‰ predominately reflect the average $\delta^{13}C$ value of the three invertebrates that consisted of the majority of the Invertebrate diet which was -25.7 ± 1.6‰ and is slightly more depleted compared to the primate biscuits (biscuits accounted for a dietary mass fraction of 29.8%) with an average $\delta^{13}C$ value of -22.4 ± 0.7‰.

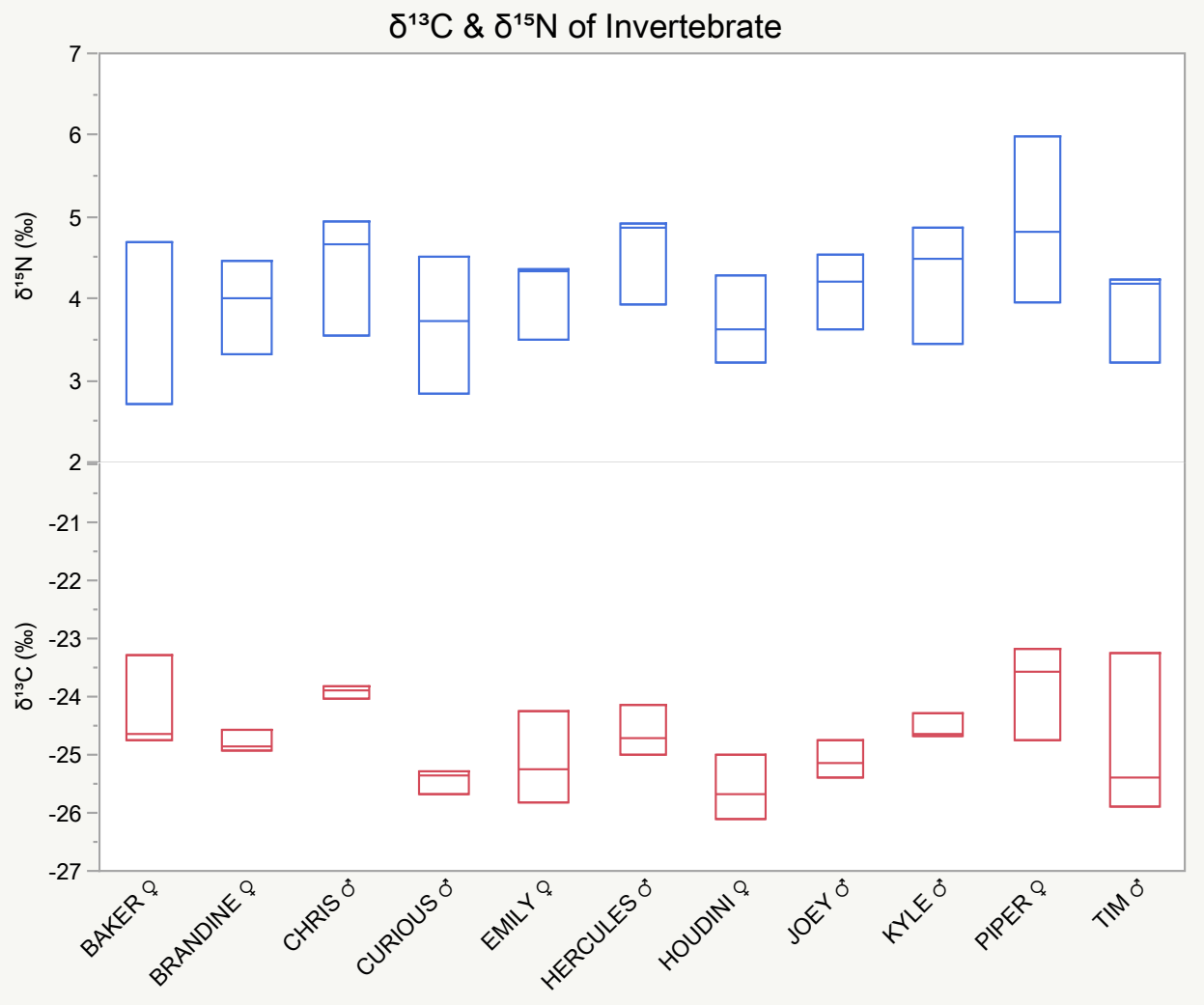

Figure 7. Box-and-whisker plots showing the $\delta^{13}C$ and $\delta^{15}N$ fecal values (‰), for each galago on the Invertebrate diet. The line represents the median, and the boxes represent the lower (25%) and upper quartiles (75%).

The median $\delta^{13}C$ and $\delta^{15}N$ fecal values for each galago during Baseline 2 are presented in Figure 8. Redworms (*E. fetida*) were not included in Baseline 2, but they were included in the Baseline 1, because all suppliers within a 30-mile radius of the research facility were sold out or carried dead stock. The absence of *E. fetida*, with an average $\delta^{13}C$ value of -22.6 ± 3.4‰ and $\delta^{15}N$ value of 5.4 ± 1.4‰, decreases the overall isotopic variability of Baseline 2's $\delta^{13}C$ fecal

values (-24.0 ± 1.1‰) when compared to Baseline 1 (-23.4 ± 1.6‰). Additionally, Baseline 2's increased average $\delta^{15}N$ fecal value of 4.0 ± 0.9‰ (Baseline 1 average $\delta^{15}N$: 3.3 ± 0.4‰) is most likely due to the fact Baseline 2 was preceded by the Invertebrate diet.

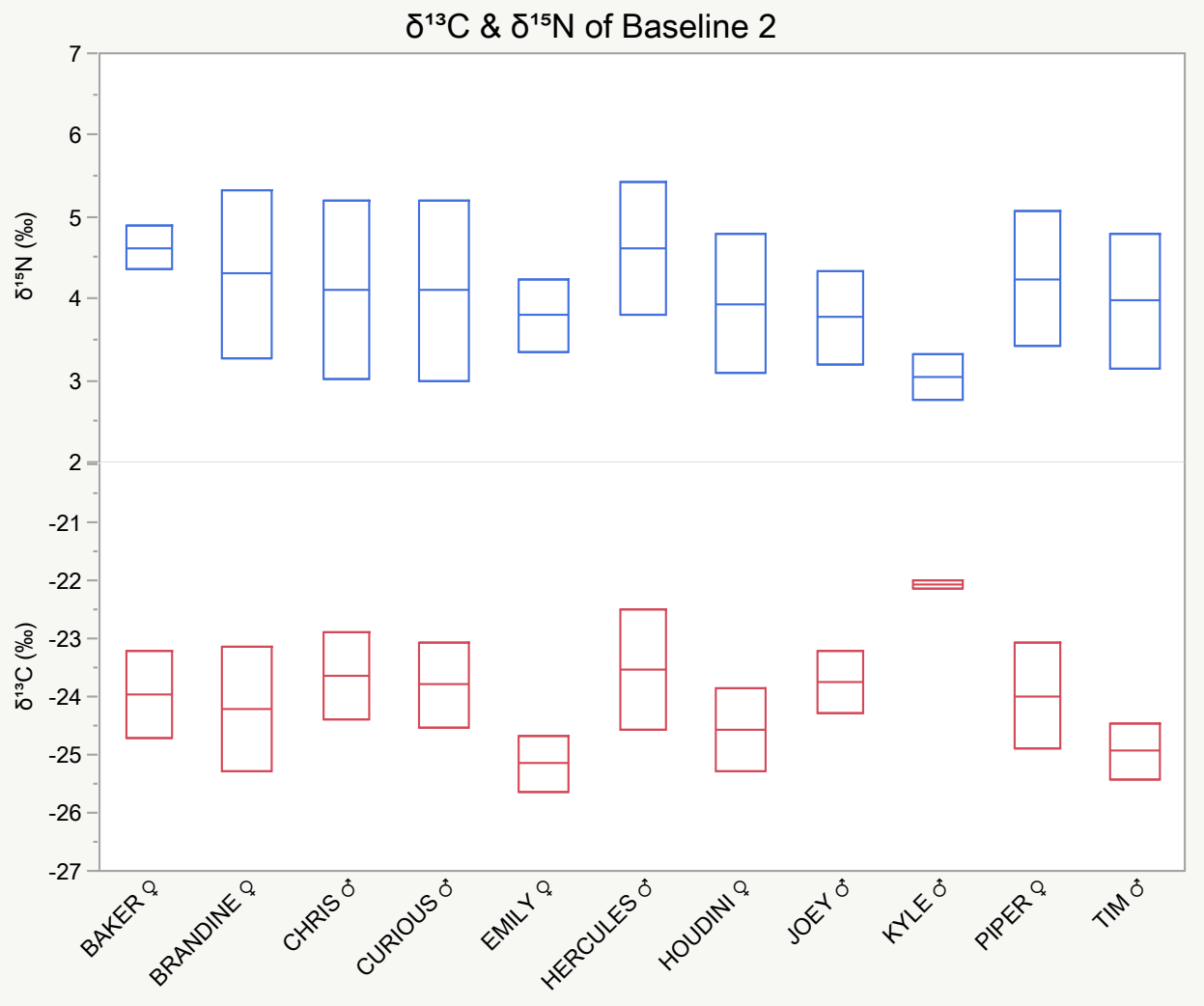

Figure 8. Box-and-whisker plots showing the $\delta^{13}C$ and $\delta^{15}N$ fecal values (‰), for each galago on the Baseline 2 diet. The line represents the median, and the boxes represent the lower (25%) and upper quartiles (75%).

H_3: The $\delta^{13}C$ and $\delta^{15}N$ values of the feces will change to be more representative of the diets they were fed during the time of collection and be isotopically distinct from one another.

The $\delta^{13}C$ and $\delta^{15}N$ values of the foods that the galagos consumed are presented in Figure 9A. This included a total of 55 food samples. Figure 9B presents the 143 $\delta^{13}C$ and $\delta^{15}N$ values of the galago fecal samples analyzed in this study from each diet. The axes for Figure 9A ranged from -32.0‰ to -20.0‰ for the X axis, and 2.0‰ to 8.5‰ for the Y axis. Figure 9A shows that there is little isotopic overlap among the foods from the experimental diets, other than the partial overlap between blackberries and nightcrawlers, blackberries and redworms, raspberries and tamarind, and complete overlap of mealworms by tamarind.

Figure 9Bs' axes are different from 9A. The X-axis range is -27.0‰ to -20.0‰ and the Y-axis is 2.0‰ to 6.0‰. The $\delta^{13}C$ and $\delta^{15}N$ values of the feces show how the fecal values shift to be more representative of the different foods that each diet included. While there was an overlap in these values, each of the diets were distinguishable from one another and the eclipses reflect what was expected. Baseline 1 has the most variable $\delta^{13}C$ values (-23.4 ± 1.6‰) and the lowest $\delta^{15}N$ values (3.3 ± 0.4‰). The Frugivorous diet shows the least variability of $\delta^{13}C$ values (-24.7 ± 0.7‰) and relatively low $\delta^{15}N$ values (3.7 ± 0.4‰). The Invertebrate diet has a similar degree of variability in $\delta^{13}C$ values (-24.7 ± 0.8‰) as the Frugivorous diet but significantly higher $\delta^{15}N$ values (4.1 ± 0.7‰; $P<0.001$). During Baseline 2 the $\delta^{13}C$ values (-24.0 ± 1.1‰) shift to be more similar to Baseline 1 while having the highest degree of variability in $\delta^{15}N$ values (4.0 ± 0.9‰).

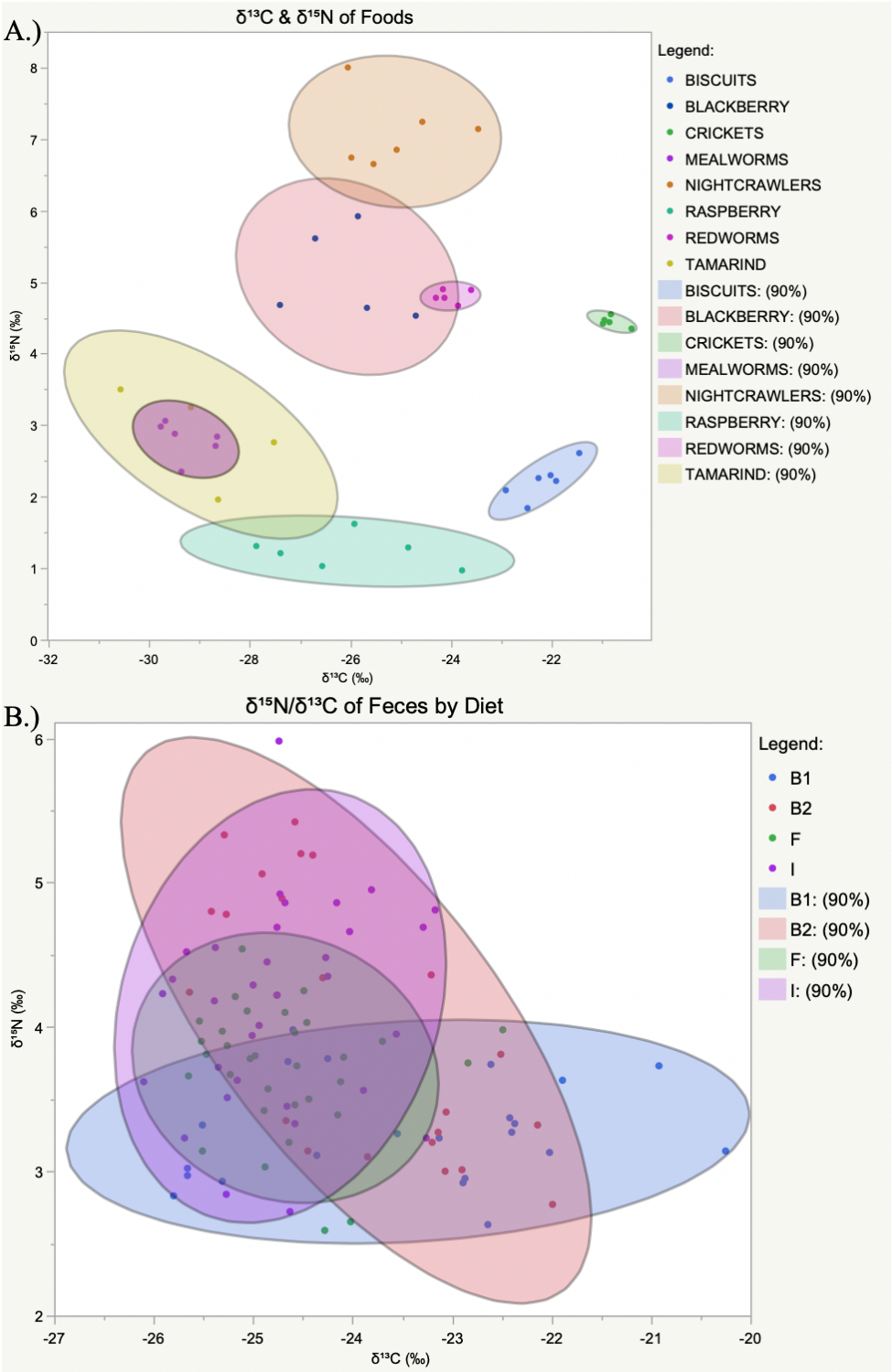

Figure 9. The scatterplot (A) shows the $\delta^{13}C$ & $\delta^{15}N$ values of the foods the galagos were presented, which included primate biscuits, blackberries, crickets, mealworms, nightcrawlers, raspberries, redworms, and tamarind (n=55). Ellipses represent a 90% CI of variation. The scatterplot (B) shows the $\delta^{13}C$ & $\delta^{15}N$ values of all fecal samples analyzed from each diet; Baseline 1 (B1), Frugivory (F), Invertebrate (I), and Baseline 2 (B2) (n=143). Ellipses represent a 90% CI of variation.

Fractionation and Dietary Mass Fraction

Table 6 displays the average $\delta^{13}C$ and $\delta^{15}N$ (‰) food and fecal values of each experimental diet, which were then used to establish feces-diet fractionation rates during each diet. During the Frugivorous diet, the approximate fractionation represented by the delta symbol (Δ) for both $\delta^{13}C$ and $\delta^{15}N$ was + 1‰ (+1.1‰ and +1.0‰ respectively). While consuming the Invertebrate diet, the approximate Δ for both $\delta^{13}C$ and $\delta^{15}N$ was -0.5‰ (-0.7 and -0.2 respectively). Table 6 also displays the dietary mass fraction (DMF) of each diet, broken down into classes. These classes, which consist of Biscuits, Fruits, and Invertebrates, are groups of foods differentiated by their structural differences (i.e., composition of proteins, lipids, carbohydrates) that affect their predicted isotopic values. The table shows what percentage of each class (Biscuits, Fruits, and Invertebrates) made up each diet (B1, F, I, B2) and the mean $\delta^{13}C$ and $\delta^{15}N$ values of each class. This allows for a better understanding of which classifications may have more impact on the $\delta^{13}C$ and $\delta^{15}N$ values of the feces during each diet.

Table 6. The upper half of this table presents the mean $\delta^{13}C$ and $\delta^{15}N$ (‰) values for food and feces with standard deviations (SD) for each experimental diet (Baseline (B1) (n=2), Frugivory (F) (n=3), Invertebrate (I) (n=3), and Baseline 2 (B2) (n=2)), as well as the Δ value or rate of fractionation between the substrate and the product. The lower half of the table presents the dietary mass fraction (DMF) of each classification of substrate during each diet as well as the mean $\delta^{13}C$ & $\delta^{15}N$ of each classification.

Population Level δ	B1 (Mean ± SD(‰))		F (Mean ± SD(‰))		I (Mean ± SD(‰))		B2 (Mean ± SD(‰))	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
δ:Food	-25.8±1.4	3.0±1.0	-25.8±1.1	2.7±0.7	-24.0±1.2	4.3±0.7	-26.5±1.0	2.5±0.9
δ:Feces	-23.4±1.6	3.3±0.4	-24.7±0.7	3.7±0.4	-24.7±0.8	4.1±0.7	-24.0±1.1	4.0±0.9
+/- Δ	+ 2.4	+ 0.3	+ 1.1	+ 1.0	- 0.7	- 0.2	+ 2.5	+ 1.5

DMF/ Population Level δ		B1 (% & ‰)		F (% & ‰)		I (% & ‰)		B2 (% & ‰)	
		DMF (%)	δ (‰)	DMF (%)	δ (‰)	DMF (%)	δ (‰)	DMF (%)	δ (‰)
$\delta^{13}C$:Food	**Biscuits**	61.5	-22.4	24.5	-22.4	29.8	-22.4	68.4	-22.4
	Fruits	18.4	-25.6	75.7	-26.9	0.0	N/A	22.4	-26.9
	Inverts	20.1	-26.9	0.0	N/A	70.2	-24.2	9.2	-29.3
$\delta^{15}N$:Food	**Biscuits**	61.5	1.8	24.5	1.8	29.8	1.8	24.5	1.8
	Fruits	20.1	3.0	75.7	3.0	0.0	N/A	75.7	3.0
	Inverts	18.4	4.1	0.0	N/A	70.2	4.9	9.2	2.8

Chapter Five - Discussion

Feces-diet fractionation

Q_1: What are the feces-diet fractionation values for a captive colony of northern Garnett's galago (*Otolemur garnettii*) that were provided a controlled "Frugivorous" and "Invertebrate" diet that mimicked seasonal dietary shifts?

H_1: The "Invertebrate" diet will result in higher $\delta^{15}N$ fecal values and $\delta^{13}C$ fecal values will reflect the $\delta^{13}C$ of the invertebrates consumed.

H_2: The "Frugivorous" diet will result in lower $\delta^{15}N$ fecal values and $\delta^{13}C$ fecal values that are less variable than those of the invertebrates.

Invertebrate Diet

H_1 was supported by the results of this study. These data showed, when averaged across all foods within the diet, the galagos' "Invertebrate" diet was significantly lower in ^{13}C ($P<0.05$) and higher in ^{15}N ($P<0.001$), and their $\delta^{15}N$ fecal values were significantly higher ($P<0.01$) when the galagos consumed the Invertebrate diet. H_1 states we expect higher $\delta^{15}N$ values and $\delta^{13}C$ that reflect those of the invertebrates consumed during the diet. Anticipated differences in $\delta^{15}N$ were because fruit is relatively low in proteinaceous tissue while invertebrates are high in protein and comparatively low in carbohydrates (Crowley et al. 2010). The exact differences in the $\delta^{13}C$ values of the diets were difficult to predict without knowing the diets of the farm-raised invertebrates.

There is a difference in magnitude between the significance of the $\delta^{15}N$ values, which decreased between the substrates ($P<0.001$) and the products ($P<0.01$). This decrease could be due to the structural differences in the substrates themselves and how readily they are digested by the galagos. *O. garnettii* possesses three paralogs for Acidic Mammalian Chitinase Genes (CHIA), meaning the species has either evolved or retained genes that allow them to efficiently

breakdown and digest chitin, the major structural component of invertebrate exoskeletons (Janiak et al. 2017). Digestibility trials revealed the galagos could more efficiently digest the "Invertebrate" diet (DMD = 76.6 ± 7.0%) compared to the "Frugivorous" diet (DMD = 72.0 ± 5.1%), which could mean "Invertebrate" foods are more readily incorporated into their tissues than "Frugivorous" foods (Loudon et al. 2023). Therefore, although the foods that compromised the Invertebrate diet had a higher degree of variation than that observed in the galago feces, this is most likely due to their efficient digestion as the product we sampled was feces or the undigested portion of their diet.

Frugivorous Diet

H_2 was supported by the result of this study, as the Frugivorous diet showed significantly lower $\delta^{15}N$ values ($P<0.001$), with the average $\delta^{15}N$ value of the fruit species measuring 3.0 ± 0.6‰ compared to an average $\delta^{15}N$ of 4.9 ± 0.7‰ for invertebrates; as well as significantly lower $\delta^{15}N$ fecal values for this diet ($P<0.01$). H_2 states that the galagos average $\delta^{15}N$ fecal value would be lower during the Frugivorous diet due to a lack of proteinaceous tissue and their $\delta^{13}C$ fecal values would be less variable as a result of fewer fruit species being consumed in comparison to the invertebrate species. A review of the current literature showed an absence of isotopic data on the fruit species included in the Frugivorous diet, leading to our generalized expectations.

Although, this is not always the case, as Oelze (2014) found chimpanzees (*P. troglodytes*) displayed significant seasonal variation in their $\delta^{15}N$ values and suggested that their elevated stable isotope nitrogen values were due to the consumption of nitrogen-rich fruits. Similarly, we found the average $\delta^{15}N$ value of blackberries (5.1 ± 0.6‰) was higher than two of the species of

invertebrates, the crickets and the mealworms (4.3 ± 0.4‰ and 2.8 ± 0.3‰ respectively). Interestingly, raspberries' average $\delta^{15}N$ values were 1.0 ± 0.5‰ and were the most nitrogen-depleted fruit, which may be attributed to the $\delta^{15}N$ values of the soils where they were grown, or the application of synthetic fertilizers (Bateman & Simon 2007).

Rate of Fractionation or Δ

Additionally, Q_1 set out to establish feces-diet fractionation rates for the captive colony of northern Garnett's galagos during the experimental diets. As noted in the results, during the Frugivorous diet, the approximate rate of fractionation or Δ for both $\delta^{13}C$ and $\delta^{15}N$ was + 1‰ (+1.1‰ and +1.0‰ respectively). While during the Invertebrate diet, the approximate Δ for both $\delta^{13}C$ and $\delta^{15}N$ was -0.5‰ (-0.7‰ and -0.2‰ respectively). These results are interesting because, unlike keratin-diet (hair) fractionation or collagen-diet (bone) fractionation where there is generally a positive stepwise increase between the substrate and the product because of processes including cell reconstruction increasing the body's retention on the heavier isotope, the product or feces is a mixture of digested and undigested substrates (Ben-David & Flaherty 2012). This means that the internal biological processes within the galagos' creates a product that is more positive in both $\delta^{13}C$ and $\delta^{15}N$ during the frugivorous diet and more negative during the Invertebrate diet.

Individual Variation

Furthermore, there seem to be factors affecting fractionation rates between individuals, which needs to be further investigated. One outlier that can be observed was the fecal values of

Piper, a 191-month-old female, and Houdini, a 59-month-old female, during the Invertebrate diet.

Table 7. Sub-sample of sex, age, weight (mean and standard deviations), and mean $\delta^{15}N$ fecal values (during Invertebrate diet) of individual galagos (*O. garnettii*) within the study population.

Individual	Sex	Age (months)	Mean Weight ± SD (g)	Mean $\delta^{15}N$ ± SD (‰)
Houdini	F	59	959 ± 32	3.7 ± 0.5
Baker	F	54	945 ± 23	4.0 ± 1.1
Chris	M	76	1067 ± 57	4.4 ± 0.7
Piper	F	191	951 ± 35	4.9 ± 1.0
Brandine	F	159	845 ± 15	3.9 ± 0.6
Joey	M	159	916 ± 29	4.1 ± 0.5

There are multiple variables that could possibly affect how an individual fractionates different isotopes (^{13}C, ^{15}N, ^{18}O) because the various biological processes that route each isotope may change overtime. This could be attributed to changes in physical health, age, and substrate digestibility (Hobson et al 1993; Oelze et al. 2023; Loudon et al. 2023). It should be noted that although health was not directly measured, all individuals among the study population were regularly monitored by staff before, during, and after the study with no health discrepancies occurring during the study. All individuals were recorded as being in overall good health during the study, and therefore health should not be a variable. Health is a variable because poor health, due to factors such as insufficient diets and old age, can lead to an animal catabolizing its own tissues to make up for the caloric deficit (Hobson et al. 1993). Loudon et al. (2007) found that ring-tailed lemurs (*Lemur catta*), particularly those in poor health, indicated by an increased frequency of individuals suffering from tooth loss, hair loss, and low body weight had elevated $\delta^{15}N$ values.

Table 7 presents three of the youngest individuals as well as three of the oldest individuals, whose average $\delta^{15}N$ fecal value during the Invertebrate diet was 4.2 ± 0.7‰ which closely mirrors the total populations' average $\delta^{15}N$ fecal value of 4.1 ± 0.7‰. Based on this subsample it would appear that age alone cannot account for the differences in fecal fractionation observed between Piper and Houdini. However, age has been found to affect the fractionation rates of bonobos (*P. paniscus*) (Oelze et al. 2023). They found the age of the bonobo, classified as either subadult (age < 9 years) or adult (age > 9 years), significantly affected their $\Delta^{15}N$ fecal values. Identifying species that do have age-based differences in rates of fractionation is important, especially for studies that examine behaviors during periods of breastfeeding and weaning. Often such studies use $\delta^{15}N$ values as the subadults' $\delta^{15}N$ values are typically ^{15}N-enriched relative to their mother, generally a stepwise increase in $\delta^{15}N$ values of ~2‰ (Reitsema 2012).

Furthermore, even though there is little published data to support sex or mass-based differences in primate isotopic fractionation, the impacts of these variables cannot be completely excluded from consideration. Additional analyses were conducted on a subsample containing both male and female galagos, as well as a variety of masses, which included the second lightest individual, Brandine at 845 ± 15g, and the heaviest individual, Joey at 1067 ± 57g. Our findings did not indicate that sex or mass were the cause of observed variation. The findings of the subsample were later confirmed by random effects models which were run on the entire study population to see if any of the independent variables (age, sex, mass) had a significant effect on individual feces-diet fractionation with no significance found. In general, very little is known about how age, sex, or even mass (across single species) affects fractionation (Oelze et al. 2023; Kim et al. 2021; Kurle et al.2014). Even though there are known sex-based differences in the

impacts of energy homeostasis, and it would be reasonable to suggests such variables would influence isotopic fractionation, there is not any published sources to support such hypotheses to date (Kim et al. 2021). Additionally, how age and mass affect fractionation (among members of the same species) have not been examined. This could be associated with the difficulty of acquiring such demographic information from subjects in the wild, especially among wild primate studies, but this further demonstrates the usefulness of captive studies in understanding such relationships.

It may be that structural differences in the substrates themselves are the source of this inter-individual variation. Digestibility trials conducted on this colony of galagos revealed they digested significantly higher quantities of crude fat, crude protein, and non-detergent fiber during the Invertebrate diet when compared to the Frugivorous diet (Loudon et al. 2023). Additionally, they noted a wide range of variability in rates of digestion among galagos across all diets. As feces is a mixture of both digested and undigested portions of an animals' diet, their apparent differences in relative ability to digest different substrates would alter the rate at which each is represented in their feces. However, this study cannot reliably conclude what the source of individual variation is, but simply note its existence and the need for further research.

Seasonal dietary shifts effects on $\delta^{13}C$ & $\delta^{15}N$ fecal values

Q_2: How do the $\delta^{13}C$ and $\delta^{15}N$ fecal values of this captive population of *O. garnettii* vary as a function of dietary shifts?

> **H_3**: The $\delta^{13}C$ and $\delta^{15}N$ values of the feces will change to be more representative of the diets they were fed during the time of collection and be isotopically distinct from one another.

H_3 predicted the $\delta^{13}C$ & $\delta^{15}N$ fecal values would change to be more representative of the diets they were fed during the time of collection. This prediction was based on similar studies examining the feces-diet fractionation rates of NHP's, we expected to find significant differences in the $\delta^{15}N$ fecal values of the "Frugivorous" and "Invertebrate" diets (Codron et al. 2006; Loudon et al. 2016; Loudon et al. 2019; Tsutaya et al. 2017; Tsutaya et al. 2021). Significance being defined as: isotopic data supporting a degree of distinction between the stable carbon and stable nitrogen ratios of the two main food types, fruits and invertebrates, and the ability to verify large-scale dietary shifts.

This can be broken down into two major themes, 1.) the isotopic signature of the feces would change to be more representative of the diet they were fed during the time of collection and 2.) be isotopic signature of the feces would be distinct from one another. First, we predicted the isotopic signature would be more representative of the diets they were fed during the time of collection based on their gut transit time of approximately 12 hours which is based on feeding experiments using non-toxic glitter and food coloring (Smith unpublished data). Tissues such as keratin or collagen require more time for such a shift in their isotopic ratios as the process is contingent upon their rates of cell maintenance and turnover (Ben-David & Flaherty 2012). Part 1 of H_3 is supported by our findings which show the $\delta^{13}C$ and $\delta^{15}N$ fecal values shifting to be more representative of the $\delta^{13}C$ and $\delta^{15}N$ values of the foods from each diet (B1, F, I, and B2; Table 6). This is important because it shows quantifiable changes in their dietary regimes on the scale of hours to days. Second, we also predicted that the isotopic signature of feces sampled during each diet would be isotopically distinct from feces sampled during other diets. This portion of H_3 is supported by significant differences in the $\delta^{15}N$ fecal values of both the Frugivorous and Invertebrate diets ($P<0.01$). The observed changes in their $\delta^{13}C$ fecal values

proved to be less informative, as the invertebrates' $\delta^{13}C$ values were highly varied with crickets and mealworms measuring in at -20.8 ± 0.2‰ and -29.3± 0.5‰, respectively.

Chapter Six - Conclusion

Very few primate species, if any, live in static habitats. Their habitats are constantly changing due to natural seasonal variations, climate change, and unsustainable anthropogenic activities (ex. logging, agriculture, settlement expansion, and other means of habitat fragmentation and deforestation; Estrada et al. 2017). Understanding temporal dietary variation is key to understanding a species' ecology, especially in regions characterized by extreme seasonal shifts that many NHP species face (i.e., a distinct wet and dry season). During such periods, NHPs may rely on completely different food resources linked to seasonal availability (Lambert & Rothman 2015). This means NHP stable isotopic studies, which use hair that provides rather homogenous isotopic values due to the structural nature of keratin, may not be as representative for identifying a species' overall dietary breadth or utilization of their habitat.

Although very little is known about the feeding ecology of *O. garnettii* due to its cryptic nature, fast and sporadic movements, and nocturnal activity patterns, this research demonstrates that applying stable isotope analyses and establishing feces-diet fractionation rates may prove useful for future researchers who are examining similar questions in free-ranging settings. Studies examining the feeding ecology of wild populations using stable isotope analyses will require proper planning and implementation. They will additionally have to account for more variables than were necessary during this controlled study. However, this study provides some of the groundwork necessary for future researchers to make informed decisions when asking similar questions and deciding which methodologies to implement.

This study also revealed the impact of digestibility on fecal stable isotope analyses. This study also highlighted individual variations in the $\delta^{13}C$ and $\delta^{15}N$ values of the galagos which would be difficult to determine among wild NHPs. The ultimate sources of the stable isotopic

variation revealed in this study remain uncertain and will require further research to be better understood. However, our primary findings indicate that not only were there significant stable isotopic differences between the foods of each experimental diet but that these differences carried over into the feces from each diet as well. This indicated that not only do they digest substrates differently, based on their structural composition, but they also had differing rates of fractionation during diets composed of substrates with differing structural compositions.

This research addresses gaps in the literature of both this species and the genus as well, even though this study doesn't address the gaps in the literature specifically related to the behavioral ecology or evolution of *O. garnettii*, it does establish feces-diet fractionation rates for a captive colony in the hopes that future research may be able to utilize our findings in the design of wild studies looking to address similar questions. Our current understanding of NHP isotope ecology is skewed towards apes and monkeys. Using geographic radiation as a marker of relative species success or adaptability, the family Galagidae is extremely successful yet among the most understudied group of NHPs. The data generated during this study improves our understanding of their dietary ecology and may prove useful for developing durable conservation initiatives. Just like how Loudon et al. (2007) found previously unrecorded behaviors such as sugar cane crop raiding from a population of vervets living on a nature reserve, a better understanding of how primate populations utilize their habitat can help policymakers and researchers make informed decisions when designating protective areas/ nature reserves.

At present, hair collection is commonly used as a noninvasive sampling approach among primatologists using stable isotope ecology. However, fecal samples are easier to collect and fecal matter can be more heterogenous in nature, and reflective of short-term dietary trends. The results of this research demonstrate the utility of fecal sampling to expand over longer periods of

time for captive and free-ranging study populations. Additionally, this study found hair in large enough quantities within the feces of each individual to test for hair-diet fractionation, this should be possible in the wild as well because they are semi-solitary and adapted to self-grooming via the use of a dental adaptation called a dental scrapper (tooth comb) (Gingerich 1980).

References

Bădescu I., Watts D.P., Katzenberg M.A., and Sellen D.W. 2022. "Maternal lactational investment is higher for sons in chimpanzees. *Behav Ecol Sociobiol*, Vol. 76, No. 44, pp. 1-19.

Bateman A.S., and Kelly S.D. 2007. "Fertilizer nitrogen isotope signatures." *Isotopes in environmental and health studies*, Vol. 43, No. 3, pp. 237-247.

Ben-David M. and Flaherty E.A. 2012. "Stable isotopes in mammalian research: a beginner's guide." *Journal of Mammalogy*, Vol. 93, No. 2, pp. 312-328.

Ben-David M., Newsome S.D., and Whiteman J.P. 2012. "Lipid and amino acid composition influence incorporation and discrimination of ^{13}C and ^{15}N in mink." *Journal of Mammalogy*, Vol. 93, No. 2, pp. 399-412.

Benson A.A., Bassham J.A., Calvin M., Goodale T.C., Haas V.A. and Stepka W. 1950. "The path of carbon in photosynthesis. v. paper chromatography and radioautography of the products[1]." *Journal of the American Chemical Society*, Vol. 72, No. 4, pp.1710-1718.

Craig H. 1953. "The Geochemistry of the Stable Carbon Isotopes." *Geochimica et Cosmochimica Acta*, Vol.3, pp. 53-92.

Craig H. 1957. "Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide." *Geochimica et Cosmochimica Acta*, Vol. 12 No. 1-2, pp.133-149.

Codron D., Lee-Thorp J.A., Sponheimer M., de Ruiter D., and Codron J. 2006. "Inter- and Intrahabitat Dietary Variability of Chacma Baboons (*Papio ursinus*) in South African Savannas Based on Fecal $\delta^{13}C$, $\delta^{15}N$, and %N." *American Journal of Primatology*, Vol. 129, pp. 204-214.

Crowley B.E., Carter M.L., Karpanty S.M., Zihlman A.L., Koch P.L., and Dominy N.J. 2010. "Stable carbon and nitrogen isotope enrichment in primate tissues." *Oecologia*, Vol. 164, pp. 611-626.

DeNiro M.J. & Epstein S. 1978. "Influence of diet on the distribution of carbon isotopes in animal." *Geochimica et Cosmochimica Acta*, Vol. 42, pp. 495-506.

DeNiro M.J. & Epstein S. 1981. "Influence of diet on the distribution of nitrogen isotopes in animal." *Geochimica et Cosmochimica Acta*, Vol. 45, pp. 341-351.

Edwards C. 2018. "Agricultural subsidies." Available at http:// www.downsizinggovernment.org/agriculture/subsidies (accessed date Apr 11, 2023).

Ellam, R. 2016. "Isotopes: A Very Short Introduction." *Oxford University Press*, Ch. 3

Estrada A., Garber P.A., Rylands A.B., Roos C., Fernandez-Duque E., Di Fiore A., Nekaris K.A., Nijman V., Heymann E.W., Lambert J.E., Rovero F., Barelli C., Setchell J.M., Gillespie T.R., Mittermeier R.A., Arregoitia L.V., de Guinea M., Gouveia S., Dobrovolski R., Shanee S., Shanee N., Boyle S.A., Fuentes A., MacKinnon K.C., Amato K.R., Meyer A.L.S., Wich S., Sussman R.W., Pan R., Kone I., and Li B. 2017. "Impending extinction crisis of the world's primates: Why primates matter." *The American Association for the Advancement of Science,* Vol. 3, No. 1, pp. 1-16.

Flaherty E.A. and Ben-David M., 2010. Overlap and partitioning of the ecological and isotopic niches. *Oikos*, Vol. 119, pp.1409–1416.

Fleagle J.G. 2013. "Primate Adaptation and Evolution." *Elsevier*, 3rd Edition

Fry B. and Arnold C.1982. "Rapid 13C/12C turnover during growth of brown shrimp (*Penaeus aztecus*)." *Oecologia*, Vol. 54, No. 2, pp. 200-204.

Gebo D. L. 2011. "Vertical clinging and leaping revisited: vertical support use as the ancestral condition of strepsirrhine primates." *American Journal of Physical Anthropology*, Vol. 146, No. 3, pp. 323-335.

Gingerich P.D. 1980. "Dental and cranial adaptations in Eocene Adapidae." *Zeitschrift für Morphologie und Anthropologie*, Vol. 71, No. 2, pp. 135-142.

Harcourt C.S. & Nash L.T. 1986. "Species Differences in Substrate Use and Diet Between Sympatric Galagos in Two Kenyan Coastal Forests." *Primate*, Vol. 27, No. 1, pp. 41-52.

Harvey Chris J., Paul C. Hanson, Timothy E. Essington, Paul B. Brown, and James F. Kitchell. 2002. "Using bioenergetics models to predict stable isotope ratios in fishes." *Canadian Journal of Fisheries and Aquatic Sciences* 59, No. 1, pp. 115-124.

Hladik C.M. 1979. "Diet and ecology of prosimians." G.A. Doyle. R.D. Martin. "The study of prosimian behavior." *Academic Press*, NY, pp. 307-357.

Hobson K.A., Alisauskas R.T., and Clark R.G. 1993. "Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses for diet." *Condor*, Vol. 95, pp. 388-394.

Janiak M.C., Chaney M.E., and Tosi A.J. 2017. "Evolution of Acidic Mammalian Chitinase Genes (CHIA) Is Related to Body Mass and Insectivory in Primates." *Mol. Biol. Evol.,* Vol. 35, No. 3, pp. 607-622

Jesus F.M., Pereira M.R., Rosa C.S., Moreira M.Z. and Sperber C.F. 2015. "Preservation methods alter carbon and nitrogen stable isotope values in crickets (*Orthoptera: Grylloidea*)." *PloS One*, Vol. 10, No. 9, p.e0137650.

Kay R.F. 1975. “The Functional Adaptations of Primate Molar Teeth.” *American Journal of Physical Anthropology*, Vol. 43, pp. 195-216.

Kim SL, Jones C, Santollo J. 2021. The influence of sex, hormone status, and growth rate on dietary isotope fractionation: a case study with laboratory rats. *bioRxiv*. Mar 25:2021-03.

Kurle C.M., Koch P.L., Tershy B.R. and Croll D.A., 2014. The effects of sex, tissue type, and dietary components on stable isotope discrimination factors (Δ13C and Δ15N) in mammalian omnivores. *Isotopes in environmental and health studies*, *50*(3), pp.307-321.

Lambert J.E. and Rothman J.M. 2015. “Fallback foods, optimal diets, and nutritional targets: primate responses to varying food availability and quality.” *Annual Review of Anthropology,* Vol. 44, pp. 493-512.

Leader-Williams N.A. and Dublin H.T. 2000. “Charismatic megafauna as flagship species.” *Conservation Biology Series-Cambridge*, pp.53-84.

Loudon J.E., Sponheimer M., Sauther M.L., and Cuozzo, F. P. 2007. “Intraspecific variation in hair δ13C and δ15N values of Ring-Tailed Lemurs (*Lemur catta*) with known individual histories, behavior, and feeding ecology.” *American Journal of Physical Anthropology*, Vol. 133, pp. 978-985.

Loudon J.E., Grobler J.P., Sponheimer M., Moyer K., Lorenz J.G., & Turner, T. R. 2014. “Using the stable carbon and nitrogen isotope compositions of vervet monkeys (*Chlorocebus pygerythrus*) to examine questions in ethnoprimatology.” *PloS One*, Vol. 9, No. 7, e100758.

Loudon J.E., Sandberg P.A., Wrangham R.W., Fahey B., & Sponheimer M. 2016. “The Stable Isotope Ecology of *Pan* in Uganda and Beyond.” *American Journal of Primatology*, Vol. 78, pp. 1070-1085.

Loudon J.E., Wakefield M.L., Kimel H.M., Waller M.T., Hickmott A.J., White F.J., and Sponheimer M. 2019. “Stable isotope data from bonobo (*Pan paniscus*) faecal samples from the Lomako Forest Reserve, Democratic Republic of the Congo.” *African Journal of Ecology*, pp. 1-6.

Loudon J.E., Smith B.K., Bianchi S., Howells M.E., Krowka M.A., Gomez A.M., Davison S., Paine O.C.C., and Sponheimer M. 2023. “Variable digestibility of captive northern greater galagos (*Otolemur garnettii*) fed experimental “frugivorous” and “invertebrate” diets.” *Zoo Biology*.

Nash L.T. and Harcourt S.C. 1986. “Social Organization of Galagos in Kenyan Coastal Forests: II. *Galago garnettii*.” *American Journal of Primatology*, Vol. 10, pp. 357-369.

Nash L.T., Bearder S.K., and Olson T.R. 1989. “Synopsis of *Galago* Species Characteristics.” *International Journal of Primatology*, Vol. 10, No. 1, pp. 57-80.

Newsome S.D., Martinez del Rio C., Bearhop S., and Phillips D.L. 2007. “A niche for isotopic ecology.” *Front Ecol Environ*, Vol. 5, No. 8, pp. 429-436.

Macharia A.N., Cerling T.E., Jorgensen M.J., and Kaplan J.K. 2014. “The hair-diet ^{13}C and ^{15}N fractionation in *Chlorocebus aethiops sabaeus* based on a control diet study.” *Ann. Zool. Fennini*, Vol. 51, pp. 66-72.

Marshall J.D., Brooks J.R., Lajtha K. 2007. “Sources of variation in the stable isotopic composition of plants.” Pp.22-60 in Stable isotopes in ecology and environmental science (R. Michener and K. Lajtha, eds.). 2nd ed. *Blackwell Publishers,* Boston, Massachusetts.

Master J.C, Lumsden W.H.R., and Young D.A. 1988. “Reproductive and Dietary Parameters in Wild Greater Galago Populations.” *International Journal of Primatology*, Vol. 9, No. 6, pp. 573-592.

Ogilby W. 1838. On a new species of galago. *Proc. Zool. Soc. London*. pp. 846-860.

Oelze V.M., Head J.S., Robbins M.M., Richards M., and Boesch C. 2014. “Niche differentiation and dietary seasonality among sympatric gorillas and chimpanzees in Loango National Park (Gabon) revealed by stable isotope analysis”. *Journal of Human Evolution*, Vol. 66, pp. 95-106.

Oelze V.M., O'Neal I., Wittig R. M., Kupczik K., Schulz-Kornas E., and Hohmann, G. 2023. “A skew in poo: Biases in primate fecal isotope analysis and recommendations for standardized sample preparation.” *American Journal of Primatology*, Vol. 85, No. 1.

O’Leary M. H. 1988. “Carbon Isotopes in Photosynthesis.” *Bioscience*, Vol. 38, No. 5, pp. 328-336.

Olson T.R. 1979. “Studies on Aspects of the Morphology and Systematics of the Genus Otolemur, 1859.” (Primates: Galagidae), Ph.D. thesis, *University of London*, London (University Microfilms No. 79-70, 038, Ann Arbor, Mich.).

Reitsema L.J. (2012). “Introducing fecal stable isotope analysis in primate weaning studies.” *American Journal of Primatology*, Vol. 74, pp.926–939.

Sampedro L. and Domínguez J. 2008. “Stable isotope natural abundances (δ13C and δ15N) of the earthworm *Eisenia fetida* and other soil fauna living in two different vermicomposting environments”. *applied soil ecology*, Vol. 38, No. 2, pp.91-99.

Sandberg P.A., Loudon J.E., and Sponheimer M. 2012. “Stable Isotope Analysis in Primatology: A Critical Review.” *American Journal of Primatology*, Vol. 74, pp. 969-989.

Schmidt O., Scrimgeour C.M. and Curry J.P., 1999. “Carbon and nitrogen stable isotope ratios in body tissue and mucus of feeding and fasting earthworms (*Lumbricus festivus*)”. *Oecologia*, Vol. 118, No. 1, pp.9-15.

Schoeninger M. J., and DeNiro M. J. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica Et Cosmochimica Acta, 48*(4), 625-639.

Schoeninger M.J., Iwaniec U.T., and Nash L.T. 1998. "Ecological attributes recorded in stable isotope ratios of arboreal prosimian hair." *Oecologia*, Vol. 113, pp. 222-230.

Slack C.R. and Hatch M.D. 1967. "Comparative studies on the activity of carboxylases and other enzymes in relation to the new pathway of photosynthetic carbon dioxide fixation in tropical grasses." *Biochemical Journal*, Vol. 103, No. 3, pp. 660-665.

Smith B.N. and Epstein S. 1971. "Two categories of 13C/12C ratios for higher plants". *Plant Physiol*, Vol. 47, pp. 380-384.

Suzuki Y., Akamatsu F., Nakashita R. and Korenaga T. 2010. A "Novel Method to Discriminate between Plant and Petroleum-derived Plastics by Stable Carbon Isotope Analysis." *Chemistry Letters*, Vol. 39. pp. 998-999.

Tsutaya T., Fujimori Y., Hayashi M. Yoneda M., and Miyabe-Nishiwaki T. 2017. "Carbon and nitrogen isotopic offsets between diet and hair/feces in captive chimpanzees." *Rapid Communications in Mass Spectrometry*, Vol. 31, pp. 59-67.

Tsutaya T., Ogawa N.O., Nomura T. Shimizu M., Ohkouchi N., and Kuze N. 2021. "Carbon and nitrogen stable isotopic offsets between diet and hair/feces in captive orangutans." *Primates*, Vol. 62, pp. 945-954.

Vogel E.R., Crowley B.E., Knott C.D., Blakely M.D., Larsen M.D., and Dominy N.J. 2012. "A Noninvasive Method for Estimating Nitrogen Balance in Free-Ranging Primates." *Int J Primatol*, Vol. 33, pp.567–587.

West J.B., Bowen G.J., Cerling T.E., and Ehleringer J.R. 2006. "Stable isotopes as one of nature's ecological recorders." *TRENDS in Ecology and Evolution*, Vol. 21, No. 7, pp. 408-414

Yang Y., Hu L., Li X., Wang J. and Jin G. 2021. "Nitrogen fixation and diazotrophic community in plastic-eating mealworms Tenebrio molitor L". *Microbial Ecology*, pp.1-13.

Appendices

Table 8. Displays the age (months), $\delta^{13}C$ and $\delta^{15}N$ values (‰), C/N Ratio, date (2019), and diet of all measured fecal samples during the study grouped by galago. Highlighted values in either the $\delta^{13}C$ or $\delta^{15}N$ have been identified as outliers using the 1.5 IQR rule.

Age (months)	Specimen Name	Corrected δ13C (‰)	Corrected δ15N (‰)	C/N Ratio	Date (2019)	Diet
54	BAKER ♀	-22.89	2.92	19.83	June 1, 2019	B1
Youngest Female	BAKER ♀	-24.25	3.78	12.53	June 4, 2019	B1
Youngest Overall	BAKER ♀	-25.58	3.65	12.59	June 5, 2019	T1
	BAKER ♀	-24.58	3.46	15.22	June 13, 2019	F
	BAKER ♀	-24.49	4.25	14.59	June 16, 2019	F
	BAKER ♀	-25.06	4.11	11.56	June 19, 2019	F
	BAKER ♀	-23.63	4.83	7.90	June 22, 2019	T2
	BAKER ♀	-23.29	4.69	8.63	June 28, 2019	I
	BAKER ♀	-24.76	4.69	11.72	July 2, 2019	I
	BAKER ♀	-24.63	2.72	13.62	July 4, 2019	I
	BAKER ♀	-24.72	4.89	12.60	July 7, 2019	T3
	BAKER ♀	-24.71	4.89	14.06	July 9, 2019	B2
	BAKER ♀	-23.21	4.36	10.11	July 10, 2019	B2
159	BRANDINE ♀	-21.89	3.63	17.06	June 1, 2019	B1
	BRANDINE ♀	-24.65	3.76	13.28	June 4, 2019	B1
	BRANDINE ♀	-25.33	3.11	11.11	June 5, 2019	T1
	BRANDINE ♀	-24.12	3.62	13.42	June 13, 2019	F
	BRANDINE ♀	-24.98	3.80	10.08	June 16, 2019	F
	BRANDINE ♀	-25.47	3.81	13.52	June 19, 2019	F
	BRANDINE ♀	-23.26	4.65	7.32	June 22, 2019	T2
	BRANDINE ♀	-24.86	4.45	11.42	June 28, 2019	I
	BRANDINE ♀	-24.94	4.01	10.33	July 2, 2019	I
	BRANDINE ♀	-24.58	3.33	12.84	July 4, 2019	I
	BRANDINE ♀	-24.91	3.95	11.50	July 7, 2019	T3
	BRANDINE ♀	-25.29	5.33	14.92	July 9, 2019	B2
	BRANDINE ♀	-23.14	3.27	7.40	July 10, 2019	B2
76	CHRIS ♂	-20.92	3.73	8.15	June 1, 2019	B1
Youngest Male	CHRIS ♂	-25.66	2.97	13.37	June 4, 2019	B1
	CHRIS ♂	-24.55	3.95	10.95	June 5, 2019	T1
	CHRIS ♂	-25.26	3.87	9.64	June 13, 2019	F
	CHRIS ♂	-24.88	3.03	14.27	June 16, 2019	F

	CHRIS ♂	-25.65	3.66	13.45	June 19, 2019	F
	CHRIS ♂	-24.77	4.02	8.47	June 22, 2019	T2
	CHRIS ♂	-24.03	4.66	7.35	June 28, 2019	I
	CHRIS ♂	-23.81	4.95	9.10	July 2, 2019	I
	CHRIS ♂	-23.89	3.56	13.56	July 4, 2019	I
	CHRIS ♂	-24.49	5.66	13.47	July 7, 2019	T3
	CHRIS ♂	-24.40	5.19	14.59	July 9, 2019	B2
	CHRIS ♂	-22.90	3.01	13.30	July 10, 2019	B2
96	CURIOUS ♂	-22.37	3.33	14.18	June 1, 2019	B1
	CURIOUS ♂	-25.51	3.32	15.23	June 4, 2019	B1
	CURIOUS ♂	-25.15	3.22	18.66	June 5, 2019	T1
	CURIOUS ♂	-25.51	3.14	15.41	June 13, 2019	F
	CURIOUS ♂	-24.68	4.10	14.35	June 16, 2019	F
	CURIOUS ♂	-25.52	3.90	16.86	June 19, 2019	F
	CURIOUS ♂	-24.37	5.08	8.04	June 22, 2019	T2
	CURIOUS ♂	-25.67	4.52	9.14	June 28, 2019	I
	CURIOUS ♂	-25.35	3.72	12.57	July 2, 2019	I
	CURIOUS ♂	-25.27	2.84	9.31	July 4, 2019	I
	CURIOUS ♂	-25.20	4.99	12.21	July 7, 2019	T3
	CURIOUS ♂	-24.52	5.20	14.71	July 9, 2019	B2
	CURIOUS ♂	-23.07	3.00	12.33	July 10, 2019	B2
73	EMILY ♀	-22.61	3.74	12.83	June 1, 2019	B1
	EMILY ♀	-24.36	3.11	13.20	June 4, 2019	B1
	EMILY ♀	-24.62	3.22	12.46	June 5, 2019	T1
	EMILY ♀	-24.44	3.50	14.26	June 13, 2019	F
	EMILY ♀	-24.15	3.39	15.02	June 16, 2019	F
	EMILY ♀	-22.84	3.75	8.35	June 19, 2019	F
	EMILY ♀	-23.71	4.21	6.02	June 22, 2019	T2
	EMILY ♀	-24.25	4.35	9.06	June 28, 2019	I
	EMILY ♀	-25.81	4.33	8.94	July 2, 2019	I
	EMILY ♀	-25.26	3.51	13.68	July 4, 2019	I
	EMILY ♀	-25.54	4.33	8.76	July 7, 2019	T3
	EMILY ♀	-25.64	4.24	10.76	July 9, 2019	B2
	EMILY ♀	-24.67	3.35	8.42	July 10, 2019	B2
110	HERCULES ♂	-22.42	3.37	21.36	June 1, 2019	B1
	HERCULES ♂	-22.87	2.95	14.72	June 4, 2019	B1
	HERCULES ♂	-24.76	3.60	15.10	June 5, 2019	T1
	HERCULES ♂	-25.18	4.21	15.39	June 13, 2019	F
	HERCULES ♂	-24.76	4.22	13.58	June 16, 2019	F

	HERCULES ♂	-24.64	3.20	9.73	June 19, 2019	F
	HERCULES ♂	-23.78	4.52	7.43	June 22, 2019	T2
	HERCULES ♂	-24.16	4.86	7.84	June 28, 2019	I
	HERCULES ♂	-24.73	4.92	8.14	July 2, 2019	I
	HERCULES ♂	-25.01	3.94	10.11	July 4, 2019	I
	HERCULES ♂	-24.78	5.16	11.37	July 7, 2019	T3
	HERCULES ♂	-24.58	5.42	13.31	July 9, 2019	B2
	HERCULES ♂	-22.51	3.81	9.00	July 10, 2019	B2
59	HOUDINI ♀	-23.55	3.26	16.10	June 1, 2019	B1
	HOUDINI ♀	-25.80	2.83	15.71	June 4, 2019	B1
	HOUDINI ♀	-25.76	3.19	9.37	June 5, 2019	T1
	HOUDINI ♀	-24.89	3.42	14.18	June 13, 2019	F
	HOUDINI ♀	-22.49	3.98	11.65	June 16, 2019	F
	HOUDINI ♀	-25.31	3.97	13.04	June 19, 2019	F
	HOUDINI ♀	-23.44	4.67	7.95	June 22, 2019	T2
	HOUDINI ♀	-25.00	4.29	9.99	June 28, 2019	I
	HOUDINI ♀	-26.10	3.62	7.80	July 2, 2019	I
	HOUDINI ♀	-25.69	3.23	11.03	July 4, 2019	I
	HOUDINI ♀	-25.23	4.94	13.17	July 7, 2019	T3
	HOUDINI ♀	-25.27	4.78	12.41	July 9, 2019	B2
	HOUDINI ♀	-23.85	3.10	12.96	July 10, 2019	B2
159	JOEY ♂	-22.40	3.27	13.59	June 1, 2019	B1
Oldest Male	JOEY ♂	-25.31	2.93	14.77	June 4, 2019	B1
	JOEY ♂	-21.54	3.33	7.18	June 5, 2019	T1
	JOEY ♂	-24.85	3.57	12.69	June 13, 2019	F
	JOEY ♂	-25.03	3.78	13.18	June 16, 2019	F
	JOEY ♂	-25.54	4.04	4.22	June 19, 2019	F
	JOEY ♂	-24.00	4.52	8.22	June 22, 2019	T2
	JOEY ♂	-25.16	3.63	8.49	June 28, 2019	I
	JOEY ♂	-24.76	4.22	7.95	July 2, 2019	I
	JOEY ♂	-25.38	4.55	7.52	July 4, 2019	I
	JOEY ♂	-24.91	4.63	13.11	July 7, 2019	T3
	JOEY ♂	-24.30	4.34	10.97	July 9, 2019	B2
	JOEY ♂	-23.20	3.20	11.52	July 10, 2019	B2
122	KYLE ♂	-22.02	3.13	10.93	June 1, 2019	B1
	KYLE ♂	-20.25	3.14	10.67	June 4, 2019	B1
	KYLE ♂	-24.61	3.57	11.88	June 5, 2019	T1
	KYLE ♂	-24.09	3.79	11.79	June 13, 2019	F

	KYLE ♂	-24.28	2.59	10.86	June 16, 2019	F
	KYLE ♂	-24.56	3.73	13.13	June 19, 2019	F
	KYLE ♂	-24.12	4.79	8.45	June 22, 2019	T2
	KYLE ♂	-24.68	4.86	8.81	June 28, 2019	I
	KYLE ♂	-24.27	4.48	9.65	July 2, 2019	I
	KYLE ♂	-24.66	3.45	11.72	July 4, 2019	I
	KYLE ♂	-25.29	4.85	10.56	July 7, 2019	T3
	KYLE ♂	-22.14	3.32	13.44	July 9, 2019	B2
	KYLE ♂	-21.99	2.77	11.10	July 10, 2019	B2
191	PIPER ♀	-22.64	2.63	14.64	June 1, 2019	B1
Oldest Female	PIPER ♀	-25.66	3.02	16.79	June 4, 2019	B1
Oldest Overall	PIPER ♀	-22.69	2.46	9.35	June 5, 2019	T1
	PIPER ♀	-25.11	4.54	9.67	June 13, 2019	F
	PIPER ♀	-25.23	3.67	14.55	June 16, 2019	F
	PIPER ♀	-24.46	4.03	15.61	June 19, 2019	F
	PIPER ♀	-23.85	4.29	11.67	June 22, 2019	T2
	PIPER ♀	-23.17	4.81	9.93	June 28, 2019	I
	PIPER ♀	-24.74	5.98	16.64	July 2, 2019	I
	PIPER ♀	-23.56	3.95	11.99	July 4, 2019	I
	PIPER ♀	-24.71	5.14	14.46	July 7, 2019	T3
	PIPER ♀	-24.91	5.06	16.17	July 9, 2019	B2
	PIPER ♀	-23.06	3.41	12.77	July 10, 2019	B2
127	TIM ♂	-23.13	3.23	15.08	June 1, 2019	B1
	TIM ♂	-24.60	3.98	9.05	June 4, 2019	B1
	TIM ♂	-23.23	3.19	11.70	June 5, 2019	T1
	TIM ♂	-24.02	2.65	12.26	June 13, 2019	F
	TIM ♂	-24.58	3.96	12.50	June 16, 2019	F
	TIM ♂	-23.70	3.90	11.72	June 19, 2019	F
	TIM ♂	-24.03	4.48	8.08	June 22, 2019	T2
	TIM ♂	-25.39	4.18	8.58	June 28, 2019	I
	TIM ♂	-25.91	4.23	9.91	July 2, 2019	I
	TIM ♂	-23.26	3.23	8.62	July 4, 2019	I
	TIM ♂	-24.34	4.17	12.31	July 7, 2019	T3
	TIM ♂	-25.42	4.80	12.20	July 9, 2019	B2
	TIM ♂	-24.45	3.14	5.29	July 10, 2019	B2

Table 9. Displays the age (months), $\delta^{13}C$ and $\delta^{15}N$ values (‰), C/N Ratio, date (2019), and diet of all measured food samples during the study grouped by species. Highlighted values in either the $\delta^{13}C$ or $\delta^{15}N$ columns have been identified as outliers using the 1.5 IQR rule. Additionally, some of the highlighted values in the $\delta^{15}N$ column are nonquantifiable.

Specimen Name	Corrected δ13C (‰)	Corrected δ15N (‰)	C/N Ratio	Date (2019)	Diet
BLACKBERRY	-27.00	0.45	21.72	31-May	B1
BLACKBERRY	-25.85	-2.33	30.89	3-Jun	B1
BLACKBERRY	-26.70	5.61	30.62	9-Jun	F
BLACKBERRY	-27.40	4.68	29.08	12-Jun	F
BLACKBERRY	-25.67	4.64	33.32	18-Jun	F
BLACKBERRY	-24.70	4.53	25.65	21-Jun	T2
BLACKBERRY	-25.85	5.92	16.47	6-Jul	T3
BLACKBERRY	-26.43	1.86	25.62	9-Jul	B2
BISCUITS	-23.30	0.92	9.13	31-May	B1
BISCUITS	-21.45	2.61	10.28	3-Jun	B1
BISCUITS	-22.91	2.09	9.60	9-Jun	F
BISCUITS	-22.26	2.26	9.41	12-Jun	F
BISCUITS	-23.19	0.35	9.94	15-Jun	F
BISCUITS	-21.91	2.22	9.63	21-Jun	T2
BISCUITS	-22.02	2.30	10.17	3-Jul	I
BISCUITS	-22.48	1.84	9.77	9-Jul	B2
CRICKETS	-20.95	4.47	5.05	21-Jun	T2
CRICKETS	-20.82	4.55	4.90	24-Jun	I
CRICKETS	-20.98	4.42	4.87	27-Jun	I
CRICKETS	-20.85	4.44	4.67	30-Jun	I
CRICKETS	-20.41	4.35	4.94	3-Jul	I
CRICKETS	-20.80	3.38	2.27	6-Jul	T3
MEALWORMS	-28.68	2.71	6.44	3-Jun	B1
MEALWORMS	-29.68	3.06	6.87	21-Jun	T2
MEALWORMS	-29.77	2.98	2.78	27-Jun	I
MEALWORMS	-28.65	2.84	5.28	3-Jul	I
MEALWORMS	-29.36	2.35	1.92	6-Jul	T3
MEALWORMS	-29.49	2.88	7.02	9-Jul	B2
NIGHTCRAWLERS	-25.98	6.74	4.89	21-Jun	T2
NIGHTCRAWLERS	-25.54	6.65	4.77	24-Jun	I
NIGHTCRAWLERS	-26.05	8.00	2.22	27-Jun	I
NIGHTCRAWLERS	-24.57	7.24	4.69	30-Jun	I
NIGHTCRAWLERS	-25.08	6.85	4.48	3-Jul	I
NIGHTCRAWLERS	-23.46	7.14	2.16	6-Jul	T3

RASPBERRY	-25.92	1.62	23.26	31-May	B1
RASPBERRY	-26.54	0.46	26.49	3-Jun	B1
RASPBERRY	-23.78	0.97	32.12	9-Jun	F
RASPBERRY	-24.85	1.29	29.86	12-Jun	F
RASPBERRY	-26.13	0.35	32.49	15-Jun	F
RASPBERRY	-26.56	1.03	44.24	18-Jun	F
RASPBERRY	-27.87	1.31	34.34	21-Jun	T2
RASPBERRY	-27.39	1.21	10.52	6-Jul	T3
RASPBERRY	-27.21	0.30	34.96	9-Jul	B2
REDWORMS	-15.74	8.10	5.05	31-May	B1
REDWORMS	-0.15	0.68	11.83	21-Jun	T2
REDWORMS	-23.60	4.89	6.23	24-Jun	I
REDWORMS	-23.86	4.67	2.82	27-Jun	I
REDWORMS	-24.16	4.90	6.32	30-Jun	I
REDWORMS	-24.30	4.78	6.27	3-Jul	I
REDWORMS	-24.13	4.78	3.03	6-Jul	T3
TAMARIND	-25.82	1.12	47.46	31-May	B1
TAMARIND	-27.52	2.76	55.83	3-Jun	B1
TAMARIND	-27.71	-2.70	55.47	9-Jun	F
TAMARIND	-28.63	1.96	58.96	15-Jun	F
TAMARIND	-28.16	-5.62	83.94	18-Jun	F
TAMARIND	-29.17	3.25	79.41	21-Jun	T2
TAMARIND	-30.57	3.50	42.40	9-Jul	B2

Table 10. Displays the $\delta^{13}C$ and $\delta^{15}N$ values, C/N Ratio, and the Diet and date of hair that was sampled from the galagos feces during processing. Hair was specifically sampled from Baseline 1 at the beginning of the study and Baseline 2 at the end of the study.

Specimen Name	Corrected δ13C (‰)	Corrected δ15N (‰)	C/N Ratio	Date	Diet
BAKER	-21.12	5.64	3.82	4-Jun	B1
BAKER	-19.75	5.92	3.35	10-Jul	B2
BRANDINE	-19.93	5.89	3.47	4-Jun	B1
BRANDINE	-19.84	5.93	3.38	10-Jul	B2
CHRIS	-20.98	5.64	3.88	4-Jun	B1
CHRIS	-20.46	5.73	3.65	10-Jul	B2
CURIOUS	-21.11	4.81	4.43	4-Jun	B1
CURIOUS	-19.74	6.05	3.34	10-Jul	B2
EMILY	-22.21	5.68	4.51	4-Jun	B1
EMILY	-19.93	5.85	3.32	10-Jul	B2
HERCULES	-19.16	6.76	3.43	4-Jun	B1
HERCULES	-18.97	7.09	3.21	10-Jul	B2
HOUDINI	-20.48	5.52	3.52	4-Jun	B1

HOUDINI	-20.14	5.41	3.36	10-Jul	B2
JOEY	-19.95	5.75	3.33	4-Jun	B1
JOEY	-19.85	5.79	3.31	10-Jul	B2
KYLE	-23.89	5.16	6.28	4-Jun	B1
KYLE	-19.34	6.52	3.48	10-Jul	B2
PIPER	-20.60	5.72	3.52	4-Jun	B1
PIPER	-19.67	5.86	3.25	10-Jul	B2
TIM	-21.44	5.29	3.98	4-Jun	B1
TIM	-21.06	5.42	3.87	10-Jul	B2

Figure 10. Copy of The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) approval for the proposed research on their captive colony of *O. garnettii*, date October 14, 2018.

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

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NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER:	**15111301.1 (Replaces 15111301)**
PROJECT TITLE:	**Examining the Health and Wellbeing of Captive Housed Otolemur garnetti**
PROPOSED PROJECT DATES:	**10/2018 – 09/2020**
PROJECT TYPE:	**Renewal**
PRINCIPAL INVESTIGATOR(S):	**B. Katherine Smith**
DEPARTMENT:	**School of Social Science and Global Studies**
FUNDING AGENCY/SPONSOR:	**NSF and Startup Funds**
IACUC COMMITTEE ACTION:	**Designated Review Approval**
PROTOCOL EXPIRATON DATE:	**September 30, 2020**

Jake Schaefer, PhD IACUC Chair	Date: October 24, 2018