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Investigating infant feeding development in wild chimpanzees using stable isotopes of hair keratin

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Résumé

Les variations entre les nourrissons quant à la vitesse à laquelle ils atteignent l'indépendance nutritionnelle affectent les trajectoires de développement des nourrissons et entraînent des différences entre les femelles au niveau de la production reproductive. Le moment des transitions alimentaires, notamment l'âge auquel les nourrissons commencent à consommer des aliments solides et l'âge du sevrage (c'est-à-dire le dernier allaitement avec transfert de lait), est difficile à déterminer par l'observation chez les primates sauvages. Il est difficile de distinguer le moment où les nourrissons se livrent à une mise en bouche exploratoire de celui où ils ingèrent réellement des aliments solides, et de déterminer si les contacts observés avec les mamelons reflètent une lactation continue, car les petits sevrés peuvent s'allaiter de confort (sans transfert de lait). Les isotopes stables de carbone et d'azote (δ13C, δ15N, %N) dans les fèces ou les poils peuvent être utilisés pour déterminer les transitions alimentaires. Alors que les isotopes stables fécaux sont sensibles à l'échantillonnage occasionnel d'aliments solides et montrent une forte variation quotidienne, les isotopes stables des poils montrent un amalgame de l'apport alimentaire sur quelques mois. Pour atténuer les limites des isotopes stables fécaux et de l'observation, j'ai appliqué des analyses δ13C, δ15N, %N à la kératine des fils de poils (0-7 ans, N = 169 échantillons de poils) collectés auprès de mères et de nourrissons chimpanzés sauvages (Pan troglodytes) à Ngogo, en Ouganda. En 2013-2014 et 2018, les poils ont été collectés de manière non invasive lorsqu'ils se collaient aux excréments, ou sur le sol après que les mères et les nourrissons se soient toilettés ou reposés. Premièrement, mon objectif était d'utiliser une combinaison de techniques visuelles (longueur et diamètre des fils de poils), associées à des isotopes stables des poils pour distinguer les fils de poils maternels des fils de poils infantiles. Deuxièmement, mon objectif était de déterminer l'âge précis auquel la contribution relative de la nourriture solide l'emporte sur la contribution relative du lait dans le régime alimentaire des bébés chimpanzés sauvages afin de suivre les processus d'alimentation transitoire et de sevrage.

Je n'ai pas pu distinguer les poils du nourrisson de ceux de la mère en utilisant uniquement des techniques visuelles. Les longueurs (cm) et les diamètres (μm) des poils de la mère et du nourrisson ne présentaient pas de différences suffisamment importantes et cohérentes pour pouvoir être utilisées seules pour distinguer les poils. Cependant, les poils des mères et des nourrissons se sont avérés plus fins et plus courts que ceux des mâles adultes (~20 μm de moins pour les

nourrissons). Je me suis donc appuyé sur les valeurs d'isotopes stables elles-mêmes pour distinguer les poils, car les poils des nourrissons étaient enrichis en isotopes lourds de carbone et d'azote par rapport à ceux de leurs mères. Les nourrissons ≤ 2 ans présentaient le plus grand rapport d'isotopes stables de carbone et d'azote (δ13C et δ15N). Les nourrissons âgés de 1,5 à 2 ans présentaient des ratios moyens de δ13C (-22,2 permil, ‰) et de δ15N (9,2‰) supérieurs à ceux des nourrissons ≤ 1,5 ans (valeur moyenne de δ13C de -23,2‰, valeur moyenne de δ15N de 8,6‰). Cela indique que les nourrissons ≤ 2 ans avaient un régime alimentaire global dominé par le lait maternel et consommaient moins d'aliments végétaux solides par rapport aux nourrissons plus âgés. Le changement dans les rapports δ13C une fois que les nourrissons ont dépassé 2 ans a suggéré que les nourrissons ont incorporé une plus grande quantité d'aliments solides dans leur régime alimentaire vers cet âge, ce qui pourrait être le moment où le processus de sevrage a commencé. Nous n'avons pas pu évaluer l'âge de la fin du sevrage, et nous aurons besoin à l'avenir de plus d'échantillons de poils de nourrissons plus âgés pour établir quand le sevrage se termine chez les chimpanzés. Cette étude peut être utilisée dans les évaluations futures des régimes alimentaires des primates, et pour déterminer les âges précis des transitions alimentaires chez les chimpanzés, ce qui fournira des données de base pour reconstruire le chemin évolutif qui a conduit à l'histoire de vie unique (sevrage précoce, fécondité élevée) des humains.

Mots clés: alimentation transitoire, sevrage, développement du nourrisson, isotope stable, développement nutritionnel, chimpanzé, primatologie.

Abstract

Variation between infants in the speed in which they reach nutritional independence affects infant developmental trajectories and leads to differences between females in reproductive output. Timings of feeding transitions, including the age infants begin consuming solid food, and age at the weaned event (i.e. last nursing bout with milk transfer), are hard to determine observationally in wild primates. It is difficult to distinguish when infants engage in exploratory mouthing versus actual ingestion of solid food, and to identify if observed nipple contacts reflect continued lactation, as weanlings can *comfort nurse* (without milk transfer). Stable carbon and nitrogen isotopes ($\delta^{13}C$, $\delta^{15}N$, %N) in feces or hair can be used to determine feeding transitions. While fecal stable isotopes are sensitive to occasional sampling of solid food and show high day-to-day variation, hair stable isotopes show an amalgamation of dietary intake over a few months. To assess fecal stable isotope and observational limitations, I applied $\delta^{13}C$, $\delta^{15}N$, %N analyses to keratin of hair strands (0-7 years, N = 169 hair samples) collected from wild chimpanzee (*Pan troglodytes*) mothers and infants at Ngogo, Uganda. In 2013-2014 and 2018, hairs were collected non-invasively when they stuck to feces, or from the ground after mothers and infants groomed or rested. First, my goal was to use a combination of visual techniques (hair strand length and diameter), combined with hair stable isotopes to distinguish maternal from infant hair strands. Second, my goal was to determine the precise age when the relative contribution of solid food outweighs the relative contribution of milk in the infant diet of wild chimpanzees to track the processes of transitional feeding and weaning.

I could not distinguish infant from maternal hair strands using only visual techniques. The lengths (cm) and diameters (μm) of maternal versus infant hair strands did not show great enough and consistent differences that could alone be used to distinguish the hairs. However, maternal and infant hair strands proved to be thinner and shorter to that of adult male hair (~20 μm lower for infants). I therefore relied on the stable isotope values themselves to distinguish the hairs, as infant hairs were enriched in the heavy carbon and nitrogen isotopes compared to their mothers. Infants ≤ 2 years old had the greatest ratio of stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$). Infants between 1.5 and 2 years old had greater average ratios of $\delta^{13}C$ (-22.2 permil, ‰) and $\delta^{15}N$ (9.2 ‰) than infants ≤ 1.5 years old ($\delta^{13}C$ mean value of -23.2 ‰, $\delta^{15}N$ mean value of 8.6 ‰). This indicated that infants ≤ 2 years old had an overall diet dominated by maternal milk and consumed less solid plant foods compared to older infants. The change in $\delta^{13}C$ ratios once infants surpassed 2 years old

suggested that infants incorporated a greater amount of solid food into their diet near this age. This indicated that the weaning process begins at approximately 2 years old. We could not assess the age of the weaned event, and in the future require more hair samples from older infants to establish when weaning ends in chimpanzees. This study can be used in future assessments of primate diets, and to determine precise ages at feeding transitions in chimpanzees, which will provide basic data to reconstruct the evolutionary path that led to the unique life history (early weaning, high fecundity) of humans**.**

Keywords: transitional feeding, weaning, infant development, stable isotope, nutritional development, chimpanzee, primatology

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Chapter 1 - Literature Review

1.1 Introduction

The length of lactation, or weaning age, serves as a key measure of maternal care and investment, which affects female reproductive rates and offspring survival, and ultimately, the viability of a population (Eckardt et al. 2016). Early weaning marked an important milestone in human evolution and is one of the key characteristics that separate humans from other great apes (Sellen 2007; Sellen 2009). Relatively early weaning in humans shortened inter-birth interval lengths and increased female fecundity (Sellen 2007; Sellen 2009), which led to rapid human population growths in the Pleistocene (Trussel 1979; Tsutaya and Yoneda 2015). Early weaning evolved despite the fact that human life trajectories are characterized by prolonged growth and development, and a more extended period of infant dependency (Kennedy 2005; van Noordwijk et al. 2013; Tsutaya and Yoneda 2015). Among several selection pressures for earlier weaning age, quicker weaning in humans is partly due to the introduction of easily digestible, prepared complementary foods that are used to supplement maternal milk at around six months of age (Hawkes et al. 1998; Hrdy 2009; Kennedy 2005; Sellen 2007; Sellen 2009). It is around this time that babies outgrow the energy available to them through maternal milk, and caregivers need to use highly processed and nutritious weaning foods that are modified versions of adult foods to help meet the nutritional demands of growing babies (Sellen 2009; van Noordwijk et al. 2013; Tsutaya and Yoneda 2015; Bădescu et al. 2017).

Infant nutritional development is the time from complete dependence on maternal milk to nutritional independence and is illustrated by three stages, including exclusive suckling, transitional feeding, and weaning (Sellen 2007; Sellen 2009; Badescu et al. 2017). Nutritional patterns are essential components of life-history models because nutritional development affects early life history parameters, such as weaning age and size at weaning (Sellen 2007; Sellen 2009; Badescu et al. 2017). Maternal milk through lactation provides a steady supply of nutrients to infants that supports proper growth and development prior to the addition of non-milk foods (Sellen 2009; Grote et al. 2016). The dietary needs of newborns during the exclusive suckling stage of development are met exclusively by maternal milk until the nutrient intake needs of infants surpass the available milk energy (Sellen 2007; Sellen 2009; Tsutaya & Yoneda, 2015; Badescu

et al. 2017). At this point, infants must begin to supplement maternal milk by ingesting solid foods due to their growing energy demands. The addition of solid food into the diet marks the second stage of nutritional development, transitional feeding (in non-human mammals) or complementary feeding (in humans) (Sellen 2007; Sellen 2009; Badescu et al. 2017). The final stage of nutritional development, weaning, is a process characterized by increasing nutritional independence from the mother, as infants transition from a diet that includes maternal milk, to one that is comprised entirely of adult foods that they forage themselves, with the exception of humans, where children continue to be provisioned solid foods by caregivers for years after weaning (Lee 1996; Sellen 2001; Sellen 2009; Kennedy 2005, Badescu et al. 2017).

Unlike humans that can be fed through breastmilk or bottle-fed infant formula, non-human primates are entirely dependent on maternal milk that can only be accessed through suckling during nursing (Sellen 2007). Thus, non-human primates consume and rely on maternal milk for a greater number of years relative to the total lifespan than humans, and do not share the trait of relatively early weaning with humans (Kennedy 2005). Great apes exhibit some of the slowest developmental trajectories of all nonhuman primates, although detailed data are lacking on infant feeding development in the great apes (Sellen 2009; Humphrey 2010; Badescu et al. 2017).

Studies on primate nutritional development are often incomplete due to limited information available on feeding transitions, including the introduction of transitional foods and the completion of weaning. To date, most studies of mammal feeding development, including primate studies, focus on two markers of feeding independence: first being the age at first solid food consumption and second being the age at the weaned event (Sellen 2009; van Noordwijk et al. 2013; Bray et al., 2017). However, less is known regarding the nutritional development happening between these two markers, which is a crucial transition period during infancy over a long period of time. **Two potentially important biomarkers are often missing from the literature. The first is when the relative contribution of solid food outweighs that of maternal milk in the infant diet (van Noordwijk et al. 2013). The second is when the weaning process starts within infant nutritional development (Kennedy 2005).** The period between complete and partial dependence on maternal milk, and independent feeding and foraging are potentially important biomarkers of infant growth and nutritional development (Sellen 2007; Sellen 2009; van Noordwijk et al. 2013). It is therefore important to establish reliable and precise primary data on when these feeding transitions occur during infancy for different primate species (Badescu et al. 2017).

Due to observational challenges, it can be difficult to determine infant feeding transitions in wild primates and other mammals. Stable isotope analysis in different tissues, including in non-invasively collected feces and hair keratin, can be used to track infant diets throughout development in living mammals and provide reliable data on the duration and scheduling of nutritional development stages. Determining accurate ages for infant feeding transitions is a crucial factor needed to establish species-specific life-history models. By establishing precise ages at feeding transitions in chimpanzees (*Pan troglodytes*), I hope to provide primary data that can eventually be used to reconstruct the evolutionary path that led to our own species' unique early life history (Sellen, 2006; Sellen, 2009).

In the following pages of the literature review, I will review the three stages of infant nutritional development in wild primates, including exclusive suckling, transitional feeding, and weaning. I will then provide an overview of stable isotope analysis, a dominant field in archaeological science, and provide examples on how it is used to reconstruct diet and offer a window into past human civilizations (Williams and Katzenberg 2012; Makarewicz and Sealy 2015; Reitsema et al. 2017). I will then highlight how stable isotope analysis of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) in hair keratin can be used to track infant diets throughout development in living mammals. I will explain their ability to assist with the methodological constraints of infant nutritional development studies that rely on observational data and fecal stable isotope analysis. I will also detail different hair characteristics that need to be considered when using hair strands for stable isotope analysis in living mammal studies.

1.2 First feeding stage: exclusive suckling

The first stage in infant nutritional development is characterized by the infant having their nutritional needs completely satisfied by milk, from either maternal milk or formula milk, through exclusive suckling (Sellen 2007; Sellen 2009). In this stage, maternal milk is the only nutritional resource that the infant consumes and relies on for growth and development (Sellen 2009; Kennedy 2005: Tsutaya and Yoneda 2015). Human babies can access this resource through nursing on their mother's nipple or can be bottle-fed by their mother and other caregivers. During this first stage, infant growth rates tend to be the highest; thus, nursing and the ingestion of maternal milk is critical for the infant's development (Fairbanks 2000; Tsutaya and Yoneda 2015). Mortality rates are also the highest during early infancy, partly due to the inability of young infants to obtain food

independently (Kennedy 2005). Obtaining adequate amounts of maternal milk is crucial to infant survival during this stage of nutritional development. In primates, the duration of exclusive suckling varies between species and can be influenced by body mass, lifespan, socioecology, and maternal factors including maternal enegetics (Kennedy 2005).

Females must allocate maternal resources to current offspring while also addressing their self-maintenance, remaining development (in the case of immature mothers) and future reproduction (Lee et al. 1991; Fairbanks and McGuire 1995; Kennedy 2005). One of the most energetically costly stages of female mammalian reproductive effort is lactation (Gittleman and Thompson 1988). Primates produce dilute low-energy milk compared to most mammals, which promotes regular and frequent breastfeeding by the mother while minimizing her costs of milk synthesis (Hinde and Milligan 2011).

In some species, the amount of milk that is transferred in one nursing bout and the time spent suckling can vary over time, while in other species, the time spent suckling can remain constant throughout the course of lactation (Hinde and Milligan 2011; Lonsdorf et al. 2014; Badescu 2017). However, the relationship between the volume of milk transferred and suckling rate is currently unknown (Lonsdorf et al. 2014). Non-human primate females can alter their nursing patterns and extend lactation based on environmental stressors, and are able to keep a nutritional base of support for their infant to help ensure their infant's survival (Nowell and Fletcher 2007). For example, gorilla mothers will continue investment in their current offspring and tolerate suckling later in infancy when nutritional resources are scarce, but wean earlier when resources are abundant (Nowell and Fletcher 2007b). The quality and amount of milk ingested during infancy can affect the entire life trajectory of offspring, including how they are able to cope with environmental stress in the future (Hinde and Capitanio 2010).

Besides providing a steady supply of nutrients for infants, maternal milk also contains lymphocytes, macrophages, and secretory immunoglobins that can protect against food-borne and disease-causing pathogens (Kennedy 2005). Due to high mortality rates in infancy (Ross 1998) and a slow maturing immune system in the post-natal period (Kennedy 2005; Tsutaya and Yoneda 2015), having passive immunity through maternal milk provides the infant with an ability to fight off possible infections. In humans, the nutrients and immunological factors that are passed through breast milk have positive effects on infant survival in the first six months of life prior to the ingestion of solid food and the start of complementary feeding (Tsutaya and Yoneda 2015). Infant

growth and development are impacted by both the quality of maternal milk produced and suckling patterns throughout infancy, which affects the length of infant dependency on the mother and the age the infant is weaned (Lee 1997; Ross 1998).

In non-human primates, the age when exclusive suckling ends, and hence, when infants enter transitional feeding, is usually earlier than six months (examples: *Cercopithecus mitis*: 5 weeks (Forster and Cords 2002), *Trachypithecus francoisi* (Reitsema 2012), *Papio hamadryas anubis*: 2 to 3 months (Humphrey et al. 2008), *Papio Cynocephalus*: 5 months (Altmann 1980), *Pan troglodytes*: 4 to 6 months (Lonsdorf et al. 2014; Bray et al. 2017), *Gorilla gorilla*: 6 months (Nowell and Fletcher 2008*); Gorilla beringei*: 6 to 8 months (Watts 1985). The exception is orangutan infants (*Pongo pygmaeus*), who can nurse exclusively for one year before starting to consume solid food items (van Noordwijk and van Schaik 2005).

1.3 Second feeding stage: transitional feeding

The second stage of nutritional development, transitional feeding, takes place over a long period of time between the first instance of solid food ingestion until the complete cessation of suckling (Sellen 2007; Bray et al. 2017; Badescu et al. 2017). It is currently defined as the first time an infant ingests a food item other than maternal milk. Throughout transitional feeding, infants continue to suckle and consume maternal milk to help reach nutritional needs while they learn and develop the skills they need to forage (Lee 1987; Lee 2012; van Noordwijk et al. 2013; Londsdorf et al. 2014; Bray et al. 2017).

The age at first solid food ingestion, and by definition the start of transitional feeding, is defined by the first time an individual eats their first piece of solid or non-milk food (Sellen 2009). However, this definition of transitional feeding does not account for the time when individuals begin to rely on solid food and when solid food constitutes a more substantial proportion of the infant diet. The current definition of transitional feeding does not tell us when infants start to regularly consume solid food. Thus, the age when infants start to regularly consume solid food, which results in a marked increase in the relative contribution of non-milk foods in the diet, may be physiologically more relevant to infant development than the age that infants start to sample food items.

1.3.1 Transitional feeding: the introduction of solid food

Prior to the start of transitional feeding, when infants still rely exclusively on maternal milk, they may engage in exploratory mouthing as well as handling and chewing of food items, without actual ingestion (Watts 1985). In blue monkeys, infants will start to bite and mouth food only 4 days after birth (Forster and Cords 2002). Chimpanzees have been observed to exploratory mouth food at different ages in the first six months of infancy: 1 month at Ngogo (Badescu et al. 2017), 5 months at Kanyawara (Bray et al. 2017), and 6 months at Gombe (Londsdorf et al. 2014). In gorillas, the earliest account of exploratory mouthing was at 4 months old (Watts 1985). The exploration and manipulation of solid food items, also known as "play-feeding," is the first interaction that infants have with food items and is a precursor to actual feeding (Nowell and Fletcher 2007b). Soon after a brief period of play-feeding, infants consume their first non-milk food item.

While infants may first ingest non-milk foods early in infancy, at some other point in the transitional feeding stage, growing infants' nutritional demands and dietary needs surpass the available energy in maternal milk. Infants at this point must begin to supplement maternal milk by regularly ingesting solid foods, to keep up with their growing nutrient intake needs. Infants at this point in transitional feeding need to start incorporating solid food items into their diet or risk starvation (Sellen 2007; Sellen 2009; Nowell and Fletcher 2007; van Noordwijk et al. 2013). The timing of this event, when infants must begin to supplement their diet with non-milk foods, is unknown for most primate species. The age at which great ape infants outgrow the energy available to them in maternal milk and must begin to supplement their diet with plant foods is unknown but is predicted to be around 1 year of age (van Noordwijk 2013). This is similar to the start of complementary feeding in human babies, which begins around six months old, when infants are fed complementary or "weaning foods" that are easily digestible versions of adult food items (Sellen 2007; Sellen 2009).

While direct feeding of infants by a caregiver seems to only occur regularly in humans, food sharing between infants and other group members, usually with the mother, also occurs in non-human primate species (Sarah and Turner 1996; Nowell and Fletcher 2006; Badescu et al. 2020). This is usually in the form of permitted food thefts when an infant is allowed to passively take food away from another individual (Badescu et al. 2020). In captive capuchins, it takes strength and skill to open and manipulate enclosed foods; thus, infants were often permitted to take

food items from adults that they could not access themselves until later in infancy (Fragaszy and Bard 1997). Badescu et al. (2020) found that chimpanzee mothers shared premasticated food with their infants between 6 months and 4 years old. Mouth to mouth premasticated food sharing, or "kiss feeding", by mothers with their infants was proposed as one of the earliest forms of complementary feeding done by humans, and is a behavior that we share with our closest living relatives. In the Mahale Mountains, chimpanzee infants were often successful (65% of solicitations) in obtaining food from their mother until the age of 2 years old (Sarah and Turner 1996). Therefore, food sharing provides an alternative way that infants can gain access to solid food items that decreases the risk of malnutrition and starvation as they start to move away from a diet that relies exclusively on maternal milk (Badescu et al. 2020).

Compared to modern humans, non-human primates endure a relatively longer period of nutritional maturation and dependence on their mother to grow and obtain the skills necessary for independent foraging (Lee 1987; Lee 1997; Lee 2012). Non-human primate infants must have sufficient time to learn the foraging and ecological skills they need to survive independent of their mothers (van Noordwijk et al. 2013; Bray et al. 2017). Multiple studies of non-human primates found that as infants got older, the amount of time dedicated to feeding related activities significantly increased (Nowell and Fletcher 2007; Bray et al. 2017; Matsumoto 2017). As they age, non-human primate infants spend more time feeding on common foods selected by adults, including hard-to-process foods to accommodate an increased need for more calories (Aristizabal et al. 2016; Matsumoto 2017; Bray et al. 2017). Bray et al. (2017) observed that infant chimpanzees over one year old allocated the same amount of time foraging and feeding on solid food items as adult chimpanzees in their community. Infant chimpanzees at Ngogo increased their solid food intake at one year old (Badescu et al. 2017). These studies suggest that 1 year of age is when foraging for food may become necessary in chimpanzees.

There is variation among non-human primate studies in the amount of time infants spend suckling as they progress through transitional feeding. In chimpanzees for example, the time allocated to suckling progressively decreased (Clark 1977; Matsumoto 2017), increased before it started to decrease (Nowell and Fletcher 2007; Bray et al. 2017), or there was no change in the observed amount of time allocated to suckling until infants were weaned (Lonsdorf et al. 2014; Badescu 2017). Because non-human primates are not weaned until later in infancy (see section Weaning in primates below) and forage on solid food items for years before the cessation of

suckling (i.e., the weaned event), it is suggested that this prolonged period of transitional feeding is meant for infants to learn the tools and skills they need to provide for themselves as adults (Boinski et al. 2003; Lonsdorf 2005; van Noordwijk 2013).

1.3.2 Species-specific nutrient needs

In addition to regular nursing that provides a dietary and immunological foundation via maternal milk, growing infants must supplement certain nutrients into their diets via foraging to meet the nutritional demands of growth and development (van Noordwijk and van Shaik 2005; Lonsdorf 2005). These nutrients include macronutrients such as lipids, protein, and carbohydrates, as well as a variety of micronutrients like vitamins, minerals, and fatty acids (Cunnane and Crawford 2014). Fatty acids, the building blocks of lipids within the body, are essential as they play a role in facilitating the growth of the brain and regulating many physiological systems (Cunnane & Crawford 2014). Both human and non-human primates lack the enzymes to synthesize fatty acids and only reach this need through diet (Cunnane and Crawford, 2014). The developing brain is susceptible to brain selective nutrients such as fatty acids, and the lack of proper nutrition during infancy can have deleterious effects on primate brain development (Cunnane and Crawford 2014). The nutritional goal of many omnivores, including primates, is to obtain a balanced intake of necessary macronutrients and to learn the strategies needed to regulate nutrient intake to survive into adulthood (Rothman et al. 2012; Potts et al. 2015). Through the acquisition of foraging skills, infants learn to choose food items eaten by their species. Plant species vary in the energy they can provide and contain different levels of macronutrients and micronutrients; infants therefore must learn how to target what they need (Nowell and Fletcher 2008). Selective feeding by many primates, including chimpanzees (Watts et al. 2012), red colobus (Chapman and Chapman 2002), gorillas (Nowell and Fletcher 2008), and baboons (Hill and Dunbar 2002), reflect this variability, and adults are able to select parts of a plant that are richer in minerals or contain more energy (Nowell and Fletcher 2008). The ability of infants to choose the right foods and stay away from other foods emphasizes the critical need for infants to learn and develop their foraging skills (Galef 2009). During infancy, however, maternal milk provides a buffer against inadequate nutrition as infants learn the foraging and processing skills necessary to establish a balanced diet of adult foods (van Noordwijk et al. 213; Badescu et al. 2017).

1.3.3 Acquisition of skills

Delayed maturation and relatively later weaning in non-human primates are essential for the acquisition of skills to locate and forage for food resources (van Noordwijk and van Schaik 2005; van Noordwijk et al. 2013; Bray et al. 2017). This is critical if infants will become nutritionally independent, and maintain a positive energy balance after weaning. Many non-human primates require time to build the strength to open, process, and eat food that is physically difficult to process, such as fruits, nuts, and seeds that contain a hard shell (van Noordwijk 2013; Matsumoto 2017). Early in infancy, young infants may choose leaves and plant parts that are easiest to obtain and require the least amount of processing (Nowell and Fletcher 2007). For example, leaves of *H. chevalieri* are high in protein and are often favoured by young gorilla infants who require high levels of protein to maintain their growth (Nowell and Fletcher 2007). However, nutrients gained from plants that are easily accessible to infants can still be limiting, and it takes time for infants to learn the skills they need to acquire more nutrients.

Infants must also learn to balance between the time spent processing food items and the time spent ingesting the food items to gain the optimum benefit from their food items without wasting too much time (Nowell and Fletcher 2008). Some non-human primates rely on the use of tools that need to be learned, skilled, and practiced during infancy (Boesch and Boesch-Achermann 2000; van Noordwijk and van Schaik 2005). Chimpanzees exhibit a wide range of tool use and difficult foraging behaviors, including termite fishing (Goodall 1986; Londsdorf 2005) and nut-cracking (Boesch and Boesch-Achermann 2000). Later in adulthood, males can also engage in hunting that takes a skilled level of coordination and social competence to acquire animal protein (Watts and Mitani, 2002). In orangutans, infants spend many years learning from their mothers how to use tools, as infants are not able to engage in tool use independently until the age of four years old (van Noordwijk and van Schaik 2005). For example, it has been documented that the use and handling of old tools in insect holes were not performed by orangutan infants before four years old, and the advanced skills needed to manipulate tools to crack open hard shell Neesia fruit was not performed by infants prior to 5 or 6 years old (van Noordwijk and van Schaik 2005).

Immature non-human primates spend substantial amounts of time observing the food processing techniques of others, practicing for themselves, and overtime perfecting the skills they need (Watts 1985; van Noordwijk and van Schaik 2005). This type of observational learning is usually information transferred from the mother to her infant (Watts 1985; Nowell and Fletcher

2007). During infancy and the juvenile period, young gorillas spent the most time, and were in closest proximity to, their mother compared to any other individual. Most observational learning of foraging and feeding behaviour was, thus, passed down from the mother to the infant (Nowell and Fletcher 2007). Premasticated food sharing and tolerated food thefts between mothers and their offspring also allow infants to gain knowledge on the types of foods eaten by their species and provide information to infants on which foods they should forage (Nishida and Turner, 1996; Badescu et al. 2020). Premasticated food transfers by mothers encourages offspring self-foraging and helps them acquire difficult to process foods (Badescu et al. 2020).

Young primates also need to learn the location and seasonality of food resources (Bray et al. 2017). In many non-human primate species, individuals attain adult feeding patterns and skills long before adulthood (van Noordwijk and van Schaik 2005; Nowell & Fletcher, 2008; Watts, 1985). Independent feeding skills that are learned throughout transitional feeding do not undergo significant changes once infants are weaned (Nowell and Fletcher 2007; Badescu 2017). Thus, transitional feeding is a critical period for the development of skills that infants require post-weaning. While maternal food sharing, observational learning, the practice of tool use, and an increase in foraging effort may encourage independent feeding, these factors do not result in immediate nutritional independence from the mother (i.e., the infant being weaned).

1.4 Weaning in primates

1.4.1 Defining weaning

While weaning can be abrupt, it is often a gradual process that varies in length and is the result of a decreased dependence on maternal milk and increased dependence on solid foods in the diet (Katzenberg et al. 1996; van Noordwijk et al. 2013; Fahy et al. 2014; Bădescu et al.2017). The definition of "weaning" has changed and varied between studies focusing on lactation and nursing patterns of species (Herring et al. 1998). Defining "weaning" as an event that happens in a single moment of time can be limiting and ignores the slow yet important process that unfolds during infant nutritional development (i.e., the transitional feeding stage) (Herring et al. 1998; Tsutaya and Yoneda 2015). Thus, the weaning process should not be used interchangeably with the weaned event, when infants no longer consume maternal milk after the last nursing bout. In species where weaning is gradual, weaning is, therefore, better described as the relative decrease in the consumption of maternal milk relative to the amount of non-milk foods in the infant diet (Horvart

and Kramer 1982; Sellen 2007; Tsutaya and Yoneda 2015; Badescu et al. 2017). Physiological weaning may start with the introduction of non-milk foods and ends with the last suckling bout of maternal milk (Sellen 2007; Tsutaya and Yoneda 2015). Weaning age is the age when direct and/or complete nutritional support by the mother via maternal milk is terminated and occurs at the weaned event (Eckardt et al. 2016).

1.4.2 Weaning age in primates

The age of the weaned event varies across primate species, [e.g., *Microcebus murinus*: 1 month, *Lemur catta*: 3.5 months, (Dettwyler 1995), *Trachypithecus francoisi* 1.3 to 1.8 years (Reitsema 2012); *Papio hamadryas anubis* 1.1 years (Humphrey et al. 2008); *Pan troglodytes*: 4 to 5 years (Clark 1977; Fahy et al. 2014; Bray et al. 2017 ; Badescu et al. 2017); *Gorilla gorilla*: 3.5 to 4.5 years (Watts 199; Nowell and Fletcher 2007); *Pongo pygmaeus*: 7 to 7.7 years (van Noordwijk et al. 2013; Galdikas & Wood 1990)].

Given our relatively long life spans, big body size, large brains, higher energy demands, slow rates of maturation and lengthy dependence on caregivers compared to most other nonhuman primates, one might expect that human babies would be weaned later in infancy than actual data indicate (van Noordwijk et al. 2013; Nowell and Fletcher 2007; Bray et al. 2017). However, human babies are weaned much earlier compared to non-human primates with similar body size and long-life spans (Kennedy 2005; Sellen 2007). Isotopic data from ancient *Homo sapiens* populations confirmed weaning happened between 2 and 3 years (Kennedy 2005; Sellen 2001), while the average weaned age of infants from contemporary hunter-gatherer societies was 2.8 years old (Sellen 2001). The one exception seems to be !Kung children, who were generally weaned between 4 and 4.5 years old (Sellen 2007).

1.4.3 The weaning process

The weaning process is characterized by a reduced dependence on maternal milk and an increased reliance on foraging of solid food (Katzenberg et al. 1996; Sellen 2007; Humphrey et al. 2008). Infants may also experience maternal rejections when they are not allowed access to their mother's nipple to nurse. Near the end of the weaning process, there may be a decrease in the number of suckling attempts by the infant (van Noordwijk et al. 2013; Badescu et al. 2017). In some species where mothers and infants nest together to sleep, like orangutans, infants that were

almost weaned showed a strong reduction in nest sharing with their mother at night (van Noodwijk et al. 2013). After the weaning process is complete, infants are nutritionally independent from their mother, and maternal milk is no longer part of their diet (Lee 1996; Eckardt et al. 2016). Weaned infants should be able to forage, process, and digest adult foods, and should thus exhibit foraging competence similar to that of adults, even in a changing environment (van Noordwijk 2012; Altman 1998). During the weaning process in baboons, the decrease in maternal investment allowed food processing efficiency to increase and was the highest thus far in their development, as infants were forced to independently locate, process, and digest food items (Altmann 1980).

Weaning age is a key measure of maternal investment and can be influenced by many factors, including ecological and social conditions, the mother's ability to cope with nutritional demands of her infant, food availability, risk of predation, and the degree of environmental uncertainty (Brown 2001; Nowell and Fletcher 2007ab; Humphrey et al. 2008; Eckardt et al. 2016). In primates with female dominance hierarchies, one of the greatest determinants of weaned age is female dominance rank (Brown 2011). This is because high-ranking females have greater access to resources and are of higher nutritional status (Brown 2011; Eckardt et al. 2016). Higher-ranking females can thus raise offspring that reach optimal size and weight for weaning earlier, and these mothers can wean their offspring sooner. Experience can also play a role in weaning age; for example, primiparous females (first-time mothers) have been documented to wean their infants later compared to multiparous females. In species that experience infanticide risk (Watts 1989; Saj and Sicotte 2008), such as gorillas, chimpanzees, and colobus monkeys, weaning can be accelerated to ensure the infants survive (Eckardt et al. 2016; Badescu et al. 2016). Mothers may wean their offspring earlier to reduce the chances of external dominant males (for example, external silverbacks) killing their infants (Eckardt et al. 2016). This is because mothers that are still nursing are in postpartum amenorrhea when they are not ovulating, and while they are nursing, they are not available to mate with the new male (Badescu et al. 2016). Therefore, males will commit infanticide to mate and sire offspring with the females sooner. Females living in situations where the risk of infanticide is high may invest more intensely in their infant over a shorter period of time to speed up the process of infant development and to reduce the period that their infant is most vulnerable to infanticide (Badescu et al. 2016).

1.4.4 Methodological constraints of observational data

In the wild, it is difficult to determine when the process of weaning is complete and when the weaned event has occurred. The weaned event can be determined from the last nursing bout, but infants can continue to nurse for comfort, without milk transfer (Martin 1984; Badescu et al. 2017). It is also difficult to observe nursing infants in the wild if the individuals are less habituated to researchers or if mothers are nursing high in the trees that obstruct the view of the infant on the mother. Most behavioural observations take place during the day and therefore nighttime suckling is often missed (Martin 1984; Reitsema 2012; Badescu et al. 2017). Not only is it an obstacle for field primatologists to identify the age of the weaned event, it is also difficult to behaviourally track other feeding transitions throughout infant nutritional development. This includes the stage of transitional feeding when infants first ingest non-milk foods, as well as when infants start to consume solid food items more regularly.

1.4.5 Measuring infant nutritional development in wild chimpanzees

The progression of nutritional development in infancy in chimpanzees has been studied at multiple sites (Taï, Côte d'Ivoire: Fahy et al. 2014; Gombe, Tanzania: Lonsdorf et al. 2014; Kanyawara, Uganda: Bray et al. 2017; Ngogo, Uganda: Badescu et al. 2017; Mahale, Tanzania: Matsumoto 2017). Chimpanzee infants may mouth and possibly ingest solid food as early as 1 month at Ngogo, Kibale National Park, Uganda (Badescu et al. 2017) The earliest observation of solid food consumption in chimpanzee infants at Kanyawara, Kibale National Park, Uganda was at 5 months old (Bray et al. 2017), but in most cases, this did not happen until infants reached 8 months old, comparable to chimpanzee populations of Mahale and Gombe in Tanzania (Fahy et al. 2014; Matsumoto, 2017).

In the Mahale Mountains, Tanzania, it was not until chimpanzee infants were 3 years old that they significantly increased their foraging effort on solid food items, eating leaves and items difficult to process (Matsumoto 2017). Infants over 3 years old were observed to decrease the amount they suckled and significantly reduced their dependence on milk for nutritional support. Infant chimpanzees at Kanywara, Uganda, showed a steady increase in time spent feeding and foraging, and the time spent feeding reached adult levels between 4 and 6 years old (Bray et al. 2017). At the field site in Gombe, Tanzania, infants increased their time feeding on solid food items by 50% at age 5 (Lonsdorf et al. 2014). Stable isotopes revealed that most infants are weaned

between 4.0 and 4.5 years old at Ngogo, Uganda (Badescu et al. 2017) and this age is similar to observations of weaning age at at other field sites (Bray et al. 2017; Londsdorf et al. 2014).

Research that aims to track age-related changes within the extended period of transitional feeding is lacking. Multiple studies have utilized stable isotopes to overcome limits of observational data in infant feeding studies, including in chimpanzees, and to accurately determine infant dietary patterns and nursing signals (Reitsema 2012; Oelze 2015; Fahy et al. 2014; Badescu et al. 2017; Matsumoto 2017).

1.5 Stable isotope analysis: an overview

Stable isotope analysis is widely used to reconstruct long and short-term dietary patterns of both prehistoric and living mammals (Reitsema, 2012; Tsutaya and Yoneda 2015; Williams and Katzenberg, 2012; Fahy et al., 2014; Oelze 2015; Matthews & Ferguson 2015; Badescu et al. 2017; Evacitas 2017; Holá et al. 2015; Herault et al. 2018), and can also offer a window into past human lifeways including mobility, trade routes, and shifts in the way these populations lived (Reitsema et al. 2017; Barrett et al. 2008). An established method used in archaeology, stable isotope analysis, estimates the trophic level of an organism within a food web (Reitsema 2012; Styring et al. 2014; Tsutaya and Yoneda 2015). Stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), and sulfur ($\delta^{34}S$) in animal tissues such as bone, dentine, fingernails, and hair reflect the isotopic signature of the diet and can be used to determine relative contributions of distinct food sources and reconstruct past human diets (Kennedy 2005; Reitsema 2012; Tsutaya and Yoneda 2015; Holá et al., 2015; Casteren et al., 2018; Williams and Katzenberg, 2012). Stable isotopes of carbon and oxygen in tooth enamel can reconstruct herbivore diet and inform past vegetation and hydroclimate (Uno et al. 2018). Stable oxygen isotopes can also be used to dictate how water-dependent a species was (Uno et al. 2018) and is a proxy for the contribution of water sources (Tsutaya and Yoneda 2015). Stable oxygen $\delta^{18}O$ values vary in organisms among different climate zones and seasons since they are controlled by the isotope ratio of precipitation and are influenced by food water, plant water, evaporative processes in soils, plants, and water sources (Luz et al. 1984; Tsutaya and Yoneda 2015; Uno et al. 2018).

Using stable isotopes, dietary information collected over an extensive period of time can reveal the foraging history and foraging behaviour of consumer populations (Hatch et al. 2011;

Matsumoto 2017). For example, fecal stable isotopes from black bears have revealed that black bears shift their behaviour in the spring to consume a more protein-rich diet compared to other seasons in the year (Hatch et al. 2011). Stable isotopes can be a tool to better understand current animal and human diets but is also widely used as a proxy of diet from past civilizations (Ambrose and Katzenberg 2003; Makarewicz and Sealy 2015; Barrett et al. 2008; Barrett et al. 2011; Williams and Katzenberg 2012; Tsutaya and Yoneda 2015; Reitsema et al. 2017). Stable isotopes of carbon and nitrogen have also been used to examine the impact of anthropogenic disturbance on the dietary ecology of multiple primate species (Matthews & Ferguson 2015).

With modernization and changes in agricultural practices, stable isotopes have been a tool used to identify the adoption of new dietary regimes and discover how past populations adjusted their diet based on cultural, environmental, and biological circumstances (Reitsema et al. 2017). Stable isotope analysis used for dietary tracing was a critical tool when understanding the rapid spread of maize in North America (Van de Merwe and Vogel 1978). As well, isotopic ratios in consumer tissues from past civilizations were used to provide insight on how diets shifted during Neolithization in modern Europe (Tauber 1981; Makarewicz and Sealy 2015). By extracting stable nitrogen ($\delta^{15}N$) and stable carbon ($\delta^{13}C$) isotopes from osseous remains, Reitsema et al. (2017) identified that neighboring villages in rural Poland 1000-1400 CE differed in their feeding practices due to local sociodemographic conditions. While some villages relied on agricultural farming, others showed a more comprehensive range and were overall enriched in the heavy carbon and nitrogen isotopes as a result of higher fish consumption. These communities with higher isotope concentrations commonly engaged in more frequent trade between regions (Reitsema et al. 2017). Paleo-diet studies have also utilized stable isotopes to determine what foods individuals consumed leading up to and directly before their death (Williams & Katzenberg 2012).

Along with reconstructing diet, stable isotopes have been used to determine migration and dispersal patterns of species, shifts in food seasonality, and the expansion of traded goods (Makarewicz and Sealy 2015; Barrett et al. 2008; Barrett et al. 2011). Barrett et al. (2011) used $\delta^{13}C$ and $\delta^{15}N$ of cod bone collagen to detect medieval cod trade and the expansion of sea fishing in the Baltic Sea. Stable isotope analysis was used to identify in which region the cod was caught by analyzing bones from medieval settlements that reflected different medieval trade networks (Barrett et al. 2008; Barrett et al. 2001). Using stable isotope analysis of carbon and nitrogen, it was found that since the end of the first millennium, cod fish were being transported and traded

over very long distances, leading to the development of commercial fishing in medieval Europe (Barrett et al. 2008). Thus, stable isotopes can be used to reconstruct the mobility patterns of people, animals, or goods traded and can help us learn about past distribution systems that led to modern expansion (Makarewicz and Sealy 2015; Barrett et al. 2008).

1.6 Stable carbon and stable nitrogen isotopes

Studies that employ stable isotope analysis hold the idea that, isotopically, "you are what you eat" and that the $\delta^{13}C$ and $\delta^{15}N$ values of animals are permanently recorded in body tissues as well as in their waste matter (Louden et al., 2014). The trophic level of humans, animals, and fauna in aquatic, C^3 plant-based, and C^4 plant-based ecosystems can be estimated using stable isotopes (Reitsema 2012; Styring et al. 2014; Tsutaya and Yoneda 2015). Stable carbon and nitrogen isotope analyses are instrumental when tracking the diets of mammals that rely primarily on a vegetarian diet, including primates (Badescu 2017). This is because stable carbon and nitrogen show the relative dietary contribution of plants using photosynthetic pathways C^3 and C^4 and reveal the amount of dietary protein found within these plant sources versus animal food sources (Tsutaya and Yoneda 2015). This is also the case for the animals that feed on these plants. Animals that feed on C^3 plants will show much lower $\delta^{13}C$ values than those that feed on C^4 plants because C^3 plants are more enriched in ^{13}C (Tsutaya and Yoneda 2015). Stable carbon isotopes ($\delta^{13}C$) reflect sources of dietary carbon mostly from vegetation, and stable nitrogen isotopes ($\delta^{15}N$) reflect nitrogen in the amino acids of dietary proteins and, thus, determines an individual's trophic position (Styring et al. 2014; Tsutaya and Yoneda 2015).

1.6.1 Stable isotopes in weaning studies

Several types of stable isotopes can be used in infant weaning studies to track the progression of infant dietary intake through nutritional development. Stable carbon can be used to detect the introduction of solid food into the infant diet, while stable oxygen isotopes ($\delta^{18}O$) or stable nitrogen isotopes ($\delta^{15}N$) can be used to track how the relative contribution of milk in the diet decreases (Kennedy 2005; Fuller et al. 2005; Reitsema 2012; Tsutaya and Yoneda 2015; Badescu et al. 2017). Stable sulfur isotopes ($\delta^{34}S$) can also be used to estimate the contribution of specific weaning foods (Tsutaya and Yoneda 2015). The $\delta^{34}S$ differences between prey and

predator as well as between mothers and nursing infants are very slim (Nechlich et al. 2011; Tsutaya and Yoneda 2015), thus, measuring stable sulfur isotopes would not be as valuable to identify trophic level differences and track weaning. Instead, it can be used to determine differences in the types of protein contributions to the diet within the weaning period, and to determine if, for example, a weanling is relying more heavily on marine or freshwater protein (Tsutaya and Yoneda 2015, Nechlich et al. 2011).

Stable isotope profiles of dentine in marine mammal teeth have shown to be a reliable proxy to assess individual weaning patterns in beluga whales (*Delphinapterus leucas*), killer whales (*Orcinus orca*), northern elephant seals (*Mirounga angustirostris*), and Risso dolphins (*Grampus griseus*) (Evacitas, 2017; Matthews & Ferguson 2015). Teeth of odontocetes accumulate annual dental growth layers and provide a lifetime record of individual feeding patterns. Beluga whales are born with a complete set of retained teeth into and throughout adulthood (Matthews & Ferguson, 2015). Studies found that the $\delta^{15}N$ values over the first several dentin growth layers are consistent with weaning, following a decline in dentine ^{15}N when infants began to transition from milk to solid prey (Evacitas, 2017; Matthews & Ferguson 2015). Using stable isotope analyses, studies have been able to show variation in weaning ages among odontocetes. For example, bottlenose dolphins are nursed for 3-6 years, while beluga whales (*Delphinapterus leucas*) infants showed ^{15}N profiles that indicate they can be weaned between 1 and 3 years old (Matthews & Ferguson 2015).

Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are used routinely in bioarchaeology, and to a much lesser extent, in primatology, to evaluate nursing patterns and identify nursing signals (Badescu et al., 2017; Reitsema, 2012; Tsutaya and Yoneda 2015; Fahy et al., 2014; Oelze 2015; Oelze et al. 2020; Evacitas, 2017; Matthews & Ferguson 2015). Stable carbon and nitrogen isotopes from body tissues and/ or waste can be used to detect trophic level differences between female adult mothers and infants, and can reveal the relative contribution of milk versus non-milk foods in the diets of infants (Reitsema 2012; Tsutaya and Yoneda 2015; Badescu et al. 2017; Oezle 2015). Stable nitrogen isotopes ($\delta^{15}N$) can be used to identify if an infant is still nursing because $\delta^{15}N$ values reflect nitrogen amino acids of dietary proteins and provide an individual's trophic position (Fry 2006; Tsutaya and Yoneda 2015). Fogel and colleagues (1989) first proposed using heavy nitrogen isotopes to identify a nursing signal using fingernails in human babies and observed an increase in $\delta^{15}N$ values as infants nursed, following a decrease at the start of the weaning

process. Once infants are weaned, they show $\delta^{15}N$ values similar to those of their mothers (Herring et al., 1998; Reitsema 2012).

Studies in humans, chimpanzees, and cetaceans indicate that infants show enrichment in the heavy nitrogen isotope with values 2-3 permil (‰) higher than the average $\delta^{15}N$ value found among their adult mothers (Reitsema 2012; Oelze 2015; Badescu et al. 2017; Fahy et al. 2017; Evacitas 2017; Matthews & Ferguson 2015). This trophic level difference gradually decreases through nutritional development and eventually disappears at the weaned event, when the infant and its mother consume the same diet (Reitsema 2012). Therefore, the weaned event occurs when the infant's $\delta^{15}N$ values reach a postweaning baseline value that resembles the average $\delta^{15}N$ value of the mother, and no enrichment is detected due to nursing (Evacitas 2017).

Stable carbon isotopes ($\delta^{13}C$) are more sensitive to the introduction of plant foods in the diet compared to stable nitrogen isotopes (Fuller et al. 2005; Tsutaya and Yoneda 2015). Stable carbon isotopes, therefore, measure the vegetable components and solid food an individual consumes (Reitsema 2012; Tsutaya and Yoneda 2015). This makes analyzing $\delta^{13}C$ helpful in distinguishing between the mouthing of solid food by an infant versus the actual ingestion of solid food and can help track when the infants start to incorporate solid food into their diet.

Reitsema (2012) was first to use fecal stable isotopes from a non-human primate to physiologically track infant nutritional development and determined the timing of weaning. By comparing behavioural data of infant nursing to fecal stable isotope ratios in a captive Francois langur (*Trachypithecus francoisi*) infant, they found that although the infant began to eat solid food at two months old, weaning age was much later than predicted based on previously published data. The results showed that the infant was nighttime nursing, and suggested that the infant was actually weaned later than daytime observations of nursing indicated (Reitsema 2012). Discordances between behaviour and stable isotope data highlighted the importance of using fecal stable isotopes to obtain more accurate data on infant feeding transitions in primates.

The progression of nutritional development in infant chimpanzees has been studied at two sites using stable isotope analysis (Taï, Côte d'Ivoire: Fahy et al. 2014 and Ngogo, Uganda: ; Badescu et al. 2017). First, Fahy et al. (2014) used stable nitrogen ($\delta^{15}N$) data of tooth root dentine to quantify the start of weaning in wild Western Chimpanzees (*Pan troglodytes verus*) in Taï National Park, Taï, Côte d'Ivoire. Stable nitrogen found in serial sections of deciduous lateral incisor root dentine and permanent mandibular molar root dentine showed a clear signal of nursing

infants (Fahy et al. 2014). Both female and male chimpanzee infants showed $\delta^{15}N$ values 2-3‰ higher than the average female, which signified a trophic level difference attributable to nursing. However, staple isotopes from dentine did not reveal any signs of the start of weaning, where the relative contribution of milk to solid food in the diet would typically decrease, until infants were at least 2 years old (Fahy et al. 2014). In this community, females were also found to be weaned earlier than males, as male infants exhibited longer periods of nutritional development and their mothers had longer lactation periods (Fahy et al. 2014).

Second, Badescu et al. (2017) used fecal stable isotopes to investigate the timing of age-related feeding transitions in wild chimpanzee infants at Ngogo, Kibale National Park, Uganda. Fecal stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes and fecal nitrogen concentrations (%N) from wild chimpanzees were successful in identifying physiological dietary indicators from complete dependence on maternal milk to the completion of weaning. In the Ngogo community of chimpanzees, infants less than one year old showed maximum $\delta^{15}N$ values 2.0‰ and $\delta^{13}C$ values 0.8‰ greater than their mothers (Badescu et al. 2017). Infants between one and one and a half years old showed the highest maximum $\delta^{13}C$ value difference of 0.9‰ greater than their mothers (Badescu et al. 2017). The difference between infant and maternal isotopic ratios showed a slow but steady decrease through infant development until infants were weaned at approximately 4.5 years old. Between 4 and 4.5 years old, infants showed $\delta^{15}N$ and $\delta^{13}C$ values nearly identical to their mother. In this community there were, however, infants whose stable isotopes indicated they were nursing until approximately 7 years old, but on average infants were weaned between 4 and 4.5 years old (Badescu et al. 2017). One key finding from this study was that infants were comfort nursing for up to two years post weaning, without milk transfer (Badescu et al. 2017). Compared to stable isotope values, observations of infants nursing would inaccurately indicate that infants were weaned approximately two years later due to comfort nursing.

Although fecal stable isotopes are a beneficial tool to track changes in the infant diet, they present some limitations. First, feces show high daily variations of $\delta^{13}C$, $\delta^{15}N$, and %N, which make it harder to find average isotopic values. Second, fecal stable isotopes account for a short (daily) representation of dietary intake, so one is only able to evaluate the infant diet within 24-48 hours prior to collection of fecal samples. Third, to interpret infant fecal stable isotope values and hone in on the milk component in the infant diet, one needs a reference fecal sample from the mother, collected on the same day, to which infant fecal stable isotope values can be compared.

Collecting fecal samples from the mother and the infant on the same day can be difficult because mothers and infants are hard to keep track of in the wild, as mothers traveling in small female groups can shy away from researchers. It is also difficult to collect samples from young infants who have small or runny feces. To help mitigate observational and fecal stable isotope limitations one can use hair keratin stable isotopes in hair strands that are collected non-invasively (Oelze 2015; Oelze et al. 2020; Schoeninger et al. 1997).

1.7 Hair keratin stable isotope analysis

Stable isotope analysis of hair keratin is a tool that can expand our understanding of diet in both past and present populations. Hair keratin is an ideal sampling material due to its robust structure resilient to contamination or biochemical changes related to UV radiation or abrasion (Oelze 2015). As a result, hair can be recovered and used from archaeological contexts dating back several thousand years or from current study sites to uncover information from modern species. Hair is a protein-rich hard keratin, high in both nitrogen and carbon (Oelze 2015; Oelze 2020). Hair keratin is the element of a hair strand that is used in stable isotope analysis. While hair is generally plucked or cut off from individuals' bodies, it can also be collected non-invasively after hair strands have naturally fallen off of the animals' body, and can be easily preserved and stored (Holá et al., 2015; Williams & Katzenberg 2012). The measurements of nitrogen and carbon isotopes ($\delta^{15}N$, $\delta^{13}C$) can be used in dietary reconstruction to provide time-integrated information of ingested foods and to mitigate limitations from feces and stomach content (Holá et al. 2015; Oelze 2015). A strand of hair, a metabolically inert tissue, can reflect an individual's diet during the hair strand's growth period. Hair is unique as it stores the information of ingested material in chronological order (Holá et al. 2015). Hair strands can be sectioned into sequences that provide a recent feeding history of an animal, while serial strands of hair collected at different time periods can provide long-term dietary intake that spans over a longer period of time (Holá et al. 2015; Oelze 2015). Hair strands that are too short and cannot be appropriately sectioned can also be combined and homogenized to show the average dominant foods an individual ingested during the growth period of the hair strands (Dalerum et al. 2007).

Each hair is a complex tissue, and so, in hair keratin, the biochemical composition, growth cycle, isotope turnover rate, and isotopic fractionation are particularly relevant when undergoing data analysis (Oelze 2015; Schoeninger et al. 1997). Studies that evaluate a nursing signal in infant

hairs use the mother's hair to create isotopic baselines for the infant to aid with fractionation (Oelze, 2015). Fractionation is a characteristic that needs to be considered when using hair strands to reconstruct diet. Previous studies have illustrated that primates and their known solid food items show that the fractionation between diet and hair is approximately 3‰ in $\delta^{15}N$ and $\delta^{13}C$ (Oelze, 2015). Infants that consume a high protein diet due to maternal milk will likely show a higher diet to hair fractionation compared to maternal hair strands.

The rate of hair growth is also an important factor to consider when trying to understand the time frame each strand represents. Estimates of the average growth rate for the study species is needed to create a temporal record using isotopic data. Because hair grows incrementally at a known rate, researchers have used its isotopic composition to explore seasonal variations in diet (Williams & Katzenberg 2012). For example, hair keratin stable isotope analysis can provide information on the types of food leading up to or right before the death of an individual, including the season and altitude of where the individual died (Herring et al. 1998; Williams & Katzenberg 2012).

The metabolic formation rate of hair keratin is also an important factor when using hair to reconstruct diet using stable isotopes. Unlike bones and teeth, hair is constantly formed and replaced throughout an individual's lifetime, which results in a faster turnover rate (Oelze, 2015; Williams & Katzenberg, 2012). Turnover rates vary between species and vary between individuals, making it challenging to estimate hair growth rates. Hair also undergoes three stages within its life cycle: anagen, catagen, and telogen (Oelze 2015). The first phase, anagen, is characterized by hair growth. Catagen, the second phase, is followed by regression, while the final phase, telogen, is the rest period of the hair strand before it is shed and replaced (Schoeninger et al. 1997; Oelze 2015). When a hair strand sheds naturally, it does so in the telogen root phase, however, hair strands can remain inactive on the body in the telogen phase for up ten weeks (Oelze 2015). During this resting period, the hair strand is metabolically inactive and does not absorb any information about what the individual is consuming (Oelze 2015).

1.7.1 Hair keratin stable isotope analysis: nonhuman primates

Stable isotope analysis of carbon and nitrogen in hair is a versatile tool that can be used to reconstruct dietary patterns and feeding behaviours in primate species. The incremental growth of hair makes it possible to record temporal shifts over long periods of time that can be used to

reconstruct the diet of a species (Oelze 2015; Holá et al. 2015). Great Apes (*Gorilla, Pongo, Pan*) are particularly good candidates to use in studies analyzing stable isotopes in hair because their long hair makes it possible to gain dietary data information spanning several feeding seasons throughout the year (Oelze 2015). It is predicted that hair growth rates of approximately 1 cm per month are appropriate for hominoids, including Great Apes (Oelze 2015). Hair can be collected non-invasively from nests, feces, or even left behind on the forest floor due to social activities like grooming (Oelze, 2015). Schoeninger et al. (1997) conducted one of the earliest studies using hair keratin stable isotopes of non-human primates. They emphasized that the use of stable isotopes in primate ecology can be a key advantage in assessing the diet of lesser-known species despite the lack of long-term observational data and to uncover diets of recently extinct primate populations (Oelze 2015; Schoeninger et al. 1997).

Dietary data spanning several weeks can be combined into a comprehensive overview through the analysis of stable isotopes in hair keratin, which can be used to identify when an infant relies on solid foods (Holá et al. 2015; Louden et al. 2014; Oelze 2015). Evaluating when an infant regularly consumes non-milk foods is difficult to detect using fecal stable isotopes alone due to an infant's tendency to sample foods at a young age (Watts 1985; Nowell and Fletcher 2007ab; Reitsema 2012; Bray et al. 2017; Badescu et al. 2017). Because infant hair keratin shows an amalgamation of the infant's diet over several weeks (over the hair growth period), we are less likely to include outliers as data points compared to fecal stable isotope data. Stable carbon isotopes in hair keratin are less sensitive than feces to additions of trace amounts of solid food. Using hair keratin stable isotopes, we are less likely to see rare instances of ingestion of a plant food by very young infants that only occur once over a several weeks. This rare occurrence would be visible using fecal stable analyses if one happened to obtain a fecal sample from the infant on the day that it consumed the solid food. We are thus more capable of quantifying the age at which infants habitually consume solid, non-milks foods when using hair keratin rather than fecal stable isotope analysis.

So far, studies in wild primates have mostly relied on fecal stable isotopes to track infant feeding development. However, there are some benefits over feces to using non-invasively collected hair to assess infant diets in wild primates. Similar to fecal stable isotope analysis, the combination of stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can be applied to hair keratin, but unlike feces, analyses require only trace amounts (0.5 mg) (Oelze 2015). In addition,

as opposed to feces that show high daily variations, hair reflects dietary intake over several weeks or months. Hair strands show lower variations in isotopic values, meaning fewer samples are required from each infant for accurate diet assessments. Unlike feces, hair from the infant and its mother are not needed on the same day to hone in on the milk component in the diet. Therefore, hair strands from infants and mothers collected on different days can be used. Combining hair keratin with fecal stable isotope analysis, and observational feeding data can provide a detailed assessment of infant feeding patterns and provide a more well-rounded model of infant nutritional development in wild primates.

1.8 Thesis goals and predictions

I will apply $\delta^{13}C$ and $\delta^{15}N$ analyses to the keratin of hair collected non-invasively from mothers and infants (0-7 years, N = 169 hair samples) in the Ngogo chimpanzee community at Kibale National Park, Uganda. First, I will establish a protocol to differentiate between chimpanzee infant and adult hairs. Past studies that have used hair keratin stable isotopes will be used as guidelines to help develop my protocol (Oelze, 2015; Louden et al. 2014; Williams and Katzenberg, 2012).

Second, I will evaluate mother-infant trophic-level differences using hair keratin stable isotopes. I predict that infants less than one year old will show the highest maximum difference in their $\delta^{15}N$ values compared to their mothers. I predict that younger infants will show keratin $\delta^{15}N$ values that are elevated by a maximum of 2-3‰ compared to their mothers. This is in line with past studies using stable isotopes to evaluate nursing patterns (Reitsema 2012; Fahy et al 2014; Oelze 2015; Badescu et al. 2017). I predict that after one year of age, and as infants get older, the difference between infant and maternal $\delta^{15}N$ values will progressively decrease and eventually approach zero; this will indicate that infants are weaned and are at this point consuming a diet similar to their mothers.

Third, I aim to determine the precise age when the relative contribution of solid food outweighs the relative contribution of milk in the infant diet of wild chimpanzees. This will allow me to establish the age when infants start to regularly consume solid food, which is an important, developmentally significant milestone in the transitional feeding stage of nutritional development. Past observations and feces showed that infants start sampling solid foods early, before six months

of age and possibly at three months old (Badescu et al. 2017). In contrast, I expect hair stable isotopes will show infants start regularly consuming solid foods at one-year-old (van Noordiwjk et al. 2013). While fecal stable isotopes are sensitive to the occasional sampling of solid food, hair stable isotopes show an amalgamation of dietary intake over a few months and reveal overall diet. Thus, hair stable isotopes may show that foraging is not energetically required for infants until they outgrow the milk energy available, which I predict will be at around one year of age (Van Noordwijk et al., 2013). I expect hair stable isotopes will reveal that infants consume solid food items regularly before two years of age, which contrasts past studies in alternate chimpanzee communities that found no evidence of solid food consumption before two years old(Fahy et al. 2014).

Fourth, I will assess the weaning process in wild chimpanzee infants. I aim to establish when weaning begins and the age of the weaned event in infant chimpanzees. I predict that $\delta^{13}C$ and $\delta^{15}N$ values in hair keratin will drop more dramatically after three years of age, in response to increased foraging and consumption of solid food during this age (Bray et al. 2017; Matsumoto 2017), which would increase the relative contribution of plant foods relative to maternal milk in the infant diet. After three years old, the increase in solid food should cause infant hairs to become more depleted in the heavy carbon and nitrogen isotopes. I predict that the weaned event will occur at 4-5 years old, and infants will show $\delta^{13}C$ and $\delta^{15}N$ values that closely resemble their mothers' at this age (Fahy et al. 2014, Badescu et al. 2017; Bray et al. 2017).

By determining the age when infants regularly consume solid food items, and the ages of infants at the start and end of weaning, I will establish key milestones that occur during the nutritional development of wild chimpanzee infants.

Chapter 2 - Methods

2.1 Study site and species

2.1.1 Ngogo field site

This study was conducted at the Ngogo field site in Kibale National Park, Uganda. Kibale National Park is located in southwestern Uganda and contains 795 km^2 of transitional forest comprised of moist evergreen and semideciduous forests between lowland and montane forests (Watts et a. 2012; Mitani & Watts 2005; Potts and Lwanga 2013; Badescu et al. 2017). Located in the center of Kibale National Park, the Ngogo field site is mostly made up of dry-ground forest. The area is composed of mature forest that is interspersed between areas of regenerating forest, swamp forest, bush, and grassland areas (Sandel et al. 2020; Badescu et al. 2020). The heaviest amount of precipitation occurs in the rainy season that takes place between the months of March to early June and from September to November. The rainy seasons are separated by dry periods with less rainfall, but there is interannual variation in the amount and timing of rain that exists throughout the year. The Ngogo field site receives approximately 1479 mm of rainfall annually, predominantly during the rainy seasons (Watts et al. 2012; Badescu et al. 2017; Badescu et al. 2020).

2.1.2 Study species

Kibale National Park, Uganda, contains eight diurnal primate species including the eastern chimpanzees (*Pan troglodytes schweinfurthii*) (Watts et al. 2012). The Ngogo chimpanzees inhabit the center of the forest and are surrounded by neighbouring chimpanzee communities. The Ngogo chimpanzees make use of all the vegetation found within their home range, and although their home range lies completely within the forest, they predominantly utilize old-growth forest (Watts et al. 2012; Badescu et al. 2020). The Chimpanzee social structure is characterized by fission-fusion dynamics, which means that individuals in the community associate in temporary subgroups that change in size and composition from one day to another as members move through their home range. Individuals are found either in larger or smaller mixed-sex subgroups, or are sometimes alone and, in the case of females, accompanied by their dependent offspring (Badescu et al. 2016; Sandel et al. 2020). The Ngogo chimpanzees currently have a home range of approximately 35

km^2 (Watts et al. 2012; Badescu et al. 2020). Until 2017, individuals of the Ngogo community were found within smaller "neighbourhoods" in the Ngogo field site and were usually consistent in staying in either the western part of their home range, the central part, or the eastern part (Langergraber et al. 2009; Sandel et al. 2020). The chimpanzees at Ngogo are therefore categorized based on the region of the forest they are most likely to be found in. For example, the "western chimpanzee females" are found in the Western part of the home range and spend more time around the "western males" who also often occupy that area. The eastern and central chimpanzees of Ngogo interact and communicate with one another more frequently compared to the western chimpanzees, who are mostly found in the west and interact mostly with the other westerners. While females tend to remain within their own neighbourhood, until 2017, males would often range in neighbourhoods other than their own and interact with the males and females in these other areas of the home range, often returning to their own neighbourhood by the evenings to nest for the night. This was the situation until 2017, when the Ngogo chimpanzees were one unified community comprising different neighbourhoods. However, in 2017-2018, the Ngogo chimpanzee community began undergoing a split between the western chimpanzees and the eastern and central chimpanzee neighbourhoods (Mitani 2020; Sandel and Watts 2021). More recently, the two groups have been acting as two separate communities, and thus, the westerners and the central/easterners rarely, if ever, interact affiliatively anymore (Mitani 2020; Reddy et al. 2021). Since 2018, researchers at Ngogo have observed inter-group aggression between the western and central/easterners, including inter-neighbourhood killings (personal observations), as well as boundary patrols and agonistic inter-group encounters with male dominance displays and with individuals showing signs of fear (Mitani 2020, Sandel and Watts 2021, personal observations). Data for this thesis were collected in 2013-2014, prior to the split, and in 2018, during the intra-community split at Ngogo. Although the chimpanzee community is undergoing a split, in this thesis I will refer to the Ngogo chimpanzee community as a whole, because the split had not been finalized until after my data collection in 2018, and I will refer to the neighbourhoods of chimpanzees at Ngogo as described above: the western, central and eastern Ngogo chimpanzees.

Ngogo is the largest community of chimpanzees ever documented in the wild, and contained approximately 200 individuals (Watts et al. 2012). The Ngogo chimpanzees have been continuously studied at the field site since 1995 by David Watts and John Mitani, and more

recently Kevin Langergraber, who are the co-directors of the Ngogo Chimpanzee Project (NCP). During the data collection period, the Ngogo chimpanzee community included approximately 202 and 207 individuals (Badescu et al. 2017). Our study was made approximately up of approximately 49 female mothers, 31 to 33 adult males, 30 to 32 juvenile females, 33 juvenile males, 33 infant females, and 28 infant males (Badescu et al 2017; Badescu et al. 2020).

In this study an "infant" is defined as the only dependent offspring of its female mother, with no younger siblings (Badescu et al. 2017; Sellen 2009). Infants are generally between 0 and 5 years of age but can be up to 7 years of age when they continue to nurse with milk transfer (Badescu et al. 2017). A "juvenile" refers to an older sibling of an infant before adolescence, and they were individuals who were already weaned, and were generally between 5 and 11 years of age (Badescu et al. 2017; Sandel et al. 2020).

The chimpanzees at the site are well habituated to the presence of researchers. Individuals were recognizable from distinguishing physical characteristics such as size, hair colour, distinctive facial features, and scars. Individuals could also be recognized by other unique and identifiable features. For example, one female had a missing eye while another female in this study had a distinctive and continuous cough that allowed us to identify her even when she was high up in the trees. Individuals were also recognized through group composition and by identifying the members they spent time and traveled with among the smaller subgroups. Although the Ngogo chimpanzee community is large, females were often observed traveling and spending time around the same group of females or members of their family (older juveniles and adult children). Therefore, when identifying individuals in the field, we also relied on information about sub-group compositions and on knowledge about which individuals generally made up the smaller social circles.

2.2 Study subjects

For my study, the subjects used were based on the hair samples that my supervisor, Dr. I Badescu, and I were able to collect in the field between the two field seasons: 2013-2014 and 2018. We collected hair samples from a total of 24 infants, and they were between the ages of 0 and 5 years old. Study infants were then categorized into the 4 following age groups based on known or estimated birth dates: < 1.5 years, 1.5-2 years, 2-3 years, and > 3 years (Table 1). Age categories were chosen based on the availability of hairs collected in different infant age categories. Breaking down the infants into 6 age categories (e.g. < 1 years, 1.5-2 years, 2-2.5 years, 2.5-3 years, 3-3.5

years and > 3 years) resulted in some of the age groups consisting of only one hair sample; therefore, having 4 age categories distributed the samples so that each age group consisted of 2 or more samples. These age groups also represented the ages that chimpanzee infants might be going through different stages of nutritional development. Infants less than 1 to 1.5 years old were completely dependent on maternal milk for growth and development, and may only sample solid foods (Badescu et al. 2020). At approximately 1 to 1.5 years old, infants usually start to consume solid food regularly while still relying on maternal milk to meet their increasing energetic demands (Badescu et al. 2017). Infants between the ages of 2 and 3 years old are fully within the second stage of their nutritional development (transitional feeding) and require increasingly more energy from plant foods than maternal milk can provide on its own (Badecu et al. 2017; Reitsema 2012; Tsutaya & Yoneda, 2015). Weaning can begin for infants over 3 years old and during this process, infants gradually rely less and less on maternal milk in their diet (Badescu et al. 2017; Tsutaya & Yoneda, 2015; Bray et al., 2017; Matsumoto 2017; Fahy et al. 2014). Thus, the relative quantity of milk to other foods should be greatly reduced after 3 years of age, although maternal milk may still provide important immune, hormonal, and micro-nutrient benefits to infants (Badescu et al. 2017; Reitsema 2012).

Table 1. The number of study infants categorized into the different age groups with the number of total hair strands that are within each age category.

Infant age category (years)	Number of study infants	Total number of hair samples
Infants ≤ 1.5 years old	10	17
Infants > 1.5 and 2	4	23
Infants > 2 and 3	9	11
Infants > 3 years old	17	23
Total	**40**	**74**

Each hair sample was labeled with an ID and these ID's made up our sample. Some hair sample ID's matched to a corresponding fecal sample that the hair was found on (see Data Collection below). Although we had 24 study infants, many infants contributed more than one hair sample to our total (Table 1 total, 40 infants due to multiple samples from the same infant). Therefore, rather than categorizing the infants themselves based on age, I categorized the different sample ID's into age groups, thus having all infants' hairs of a particular age group lumped together (Oelze 2015).

Data on new births in the Ngogo chimpanzee community are continuously recorded at Ngogo by researchers and long-term field assistants working full-time at the site. We were thus able to estimate the ages of study infants to the day or within a few weeks of their birth, based on when observers first saw them in the community (Badescu et al. 2020). This yielded a total of 74 hair samples from the 24 study infants. Of the 74 samples, 17 belonged to infants < 1.5 years, 23 from infants 1.5-2 years, 11 from infants 2-3 years, and 23 are from infants > 3 years old (Table 1).

From the sampling, we also obtained 67 hair strands belonging to adult female mothers and 28 hair strands that belonged to adult male chimpanzees. The maternal hair samples were used to interpret our results for the infant samples, so as to identify nursing and foraging signals in infants' diets. The adult male hair samples were used to compare to the isotopic values of the infant and maternal hairs.

2.3 Data collection

Data collection for this project included two distinct periods of time: 2013-2014 and 2018. The first field season took place from September 2013 to June 2014 by my supervisor Dr. Iulia Badescu (University of Montreal). Dr. Badescu collected behavioural data (especially on infant feeding and nursing) and fecal samples from infants, juveniles, and their mothers (See Behavioral data collection below; Badescu 2016; Badescu et al. 2020). The second field season occurred between the months of January and April 2018 from both Dr. Iulia Badescu and myself. Together we collected behavioral feeding data using the same ethogram and behavioral data collection methods used in the first field season. In total, hairs from 24 different infants aged 0-5 (N=74 strands) and their mothers (N=67) were obtained non-invasively (Table 1; see section 2.3.4 Hair Collection for details).

2.3.1 In the field

Data collection would start at 7am and end between 4 and 6 pm. We would leave the camp just after 7 am to find a subgroup of chimpanzees, and data collection could have started right away or several hours later depending on when the chimpanzees were found and on what sub-group of females and infants we wanted to focal that day. The Western chimpanzees' home range was farther from the campsite and therefore took longer to reach. The experienced field assistants that work at the Ngogo field site would sometimes remain with the groups until they went to sleep

and helped us find them in the morning. One method that we heavily relied on for finding the chimpanzees included locating and identifying ripe fig trees (*Ficus mucuso*) since the chimpanzees would spend most of their days feeding and going from one ripe fruit tree to another. Chimpanzees also produced loud vocalizations throughout the day that we were able to track. When a group of individuals were found, we often stayed with them because they were likely to lead us to more females and infants that we needed to find. The amount of data collection varied from day to day depending on when and which females and infants were found each day.

Dr. Iulia Badescu, Kelly Desruelle (another MSc of Dr. Badescu who was in the field at the same time as me) and I collected behavioural data on infants using using 1-hour focal animal sampling of infants with continuous recording, as well as collecting data *ad libitum* on nursing and foraging by infants. While detailed behavioral data were collected on the feeding and social behaviors of the study infants for both the 2013-2014 and 2018 study periods, for my MSc project I only relied on the isotopic analyses of hair samples to evaluate infant diets and feeding transitions. In the future, however, we aim to compare the findings of my research here on the stable isotope profiles of infants at different ages with the behavioural data (presence or absence of nursing and foraging by infants) to aid in determining feeding transitions in infant chimpanzees in future studies.

2.3.2 Hair collection

We collected hair from infants (N=74 hair strands), adult female mothers (N=67 hair strands), and adult males (N=28 hair strands) chimpanzees to use in this study. Although hair collection from mothers and infants at Ngogo occurred in 2013 and 2014 by IB, in 2018, IB and I collected hair from infants, mothers, as well as males. Experienced field assistants aided in the collection of hair samples from males after they groomed and could narrow it down to 2 or 3 individuals based on who was grooming at that time. In previous studies that analyzed bonobo (Oelze 2015) and chimpanzee hair (Schoeninger 1999), the hair strands used were collected almost entirely from nests recently vacated by individuals. However, we obtained most of our hair strands when they were pulled out from the rump areas of the chimpanzees and stuck to feces during defecation, or from the ground or logs after individuals rested or groomed. For the feces, these were also collected for isotopic analysis as part of other projects. Most of the hair strands we obtained that were stuck to feces were single hair strands. We also collected sets of multiple hair

strands at the same time from mothers and infants, which resulted in a hair clump. In this case, the infant and mother were usually grooming and they left behind multiple hair strands that most likely belonged to the mother or the infant.

Every hair strand was stored in a glass vial in the field, if the hairs were found after the chimpanzees rested or groomed. If the hair strands were found after the individual defecated, and therefore on feces, the hairs would be separated from the fecal sample after the fecal sample was dehydrated in a solar dehydrator. The dehydrated fecal sample along with the hair strand were then wrapped in tin foil. Each hair sample that was found on feces had a corresponding label and was named according to the fecal sample. All samples wrapped in tin foil were kept in large plastic collection bags and filled with silica to absorb excess moisture (Oelze 2015; Badescu et al. 2017).

The hair samples were sterilized via an autoclaving process at the Université de Montréal's Department of Biosecurity/ Occupational Health and Safety. The samples were autoclaved for 48 hours to ensure they were in line with CFIA (Canada Food Inspection Agency) protocols, which allowed the samples to be brought to the Université de Montréal and after, to the University of Calgary for processing and data analysis.

2.4 Processing and analyzing hair samples

The processing and analyses of the hair samples were done over two laboratory sessions, from May to July 2018, and from June to August 2019 in the Archaeology Prep Lab and at the Isotope Science Lab, both at the University of Calgary. This study was done in collaboration with Dr. Anne Katzenberg within the Department of Anthropology and Archaeology at the University of Calgary.

Establishing a protocol to differentiate between chimpanzee infant and adult hairs, and to evaluate mother-infant trophic-level differences using hair keratin stable isotopes is critical to my study. In the following sections below I will outline in chronological order the protocol I used to clean, process and prepare the hairs for stable isotope analysis. Past studies of hair keratin stable isotopes were used as guidelines to help develop my own protocol (Oelze, 2015; Louden et al. 2014; Williams & Katzenberg 2012; Schoeninger 199l; L'Herault et al. 2018; Dalerum et al. 2007; Jorkov & Grocke 2016; Rioux et al.2019). The protocol also includes modifications made by me

and Dr. Badescu with the help of Dr. Katzenberg that we found worked best for our study. Dr. Katzenberg offered guidance on creating the protocol and helped us better understand how to carry out stable isotope analysis of hair keratin since she is highly established in the field of archaeology and an expert in stable isotope analysis in a variety of body tissues (Katzenberg 1999; Williams & Katzenberg 2012). Table 2 summarizes the procedure and steps used in our protocol to treat and prepare chimpanzee hairs for isotopic analyses. Small modifications were made between the first lab session in June 2018 and the second lab session in June 2019 that are summarized in the table below. The step added to the protocol in the second lab session were to the second set of hair strands. This step added an extra measurement of the diameter along the shaft of the hair strand for a more accurate total average diameter. We also removed the step of weighing the hairs before any of the cleaning procedures (step 2 and 5 in Table 2) since we concluded this step was not necessary after doing it in June 2018 with the first set of hair samples.

Table 2. Protocol used to process hair samples, broken down into steps and in chronological order. Each step indicates if this procedure happened to the first set of hair strands (N=77), the second set (N=92), or to both sets of hair samples (N=169). The first set of hair strands were processed in June 2018 and the second was processed in June 2019.

Hair Processing Steps	Procedure
1	Hairs separated and put in glass vials
2	First cleaning-ultrasonic bath with double distilled water
3	Weighing the hair strands (first set of samples only)
4	Under a microscope- diameter, length, and root phase recorded and photographed. (diameter measured at 3 points instead of only the center in second set of samples only)
5	Final cleaning-ultrasonic bath with 90% ethanol solution followed by the hairs being rinsed 3 times with double distilled water
6	Weighing the hair strands
7	Hairs folded into tin capsules
8	Isotopic analysis

Measuring the length and shaft diameter of each hair strand was the first step in morphologically separating mother from infant hair strands and allowed us to identify the root phase of each hair strand. All hair strands were evaluated under a microscope and bathed in a solution of 90% ethanol to remove lipids (see section 2.4.5 Final Cleaning and Removal of Lipids). All hair strands from both sets were then weighed and folded into tin capsules for stable isotope analysis. Stable isotope ratios were then used to confirm that hair from suckling infants can be identified and distinguished from adult female hair samples. In the following sections I will detail how we processed and analyzed the hair so that other researchers have all the necessary information to repeat these methods and apply them themselves in future studies.

2.4.1 Hair storage and preparation

There were two separate processing times for the hair samples. First, in June 2018, Dr. Badescu and I processed and analyzed the first set of hairs collected from Dr. Badescu's field season in 2013 and 2014. There were a total of 77 hair strands in the first set of hairs from both infants and mothers. Second, in June 2019, I processed and analyzed the second set of hair strands that contained hair strands from the second field season in 2018. The second set of hair strands also contained hair strands from Dr. Badescu's field season in 2013 and 2014 that were found after June 2018. There were a total of 92 hair strands from infants, mothers, and adult males in the second set of hair samples that were processed and analyzed in June 2019.

Hair samples were processed at the Archaeology Preparation Lab in the Department of Anthropology and Archaeology, University of Calgary. A total of 169 hair strands from infants aged 0-5 (Table 1) and their mothers were used. Additionally, a total of 28 adult male hair strands were used. Hair strands were removed from the tin foil and separated from the feces (for the hair samples that were stuck to feces). Hair strands were then placed in glass vials that were labeled with an ID number that matched to the corresponding fecal sample (Oezle 2015). Strands found in the same fecal sample were separated into individual containers but were labeled as the same ID number. Hairs that could not be initially separated without possibly damaging the sample were kept together until they were later cleaned in an ultrasonic bath with double distilled water during the initial cleaning process.

2.4.2 Initial cleaning

After all the hairs were separated and labeled, they underwent the initial cleaning process using an ultrasonic bath of double distilled water. This initial cleaning was used to clean all the hairs of dirt and debris, and for those hairs that were initially stuck to fecal matter, the first ultrasonic bath was used to separate the hair from larger clumps of feces and other possible hair strands. Each sample was placed in a glass vial. The vial was then filled with enough double-distilled water to completely submerge the hair and the top was sealed with parafilm. We covered the vials with parafilm to prevent the hairs from being pushed out of the vials by the sonicator's vibrations. All hair strands were sonicated in double-distilled water for 30 minutes inside the ultrasonic bath. The heat and vibrations from the ultrasonic bath helped remove excess dirt, fecal matter, and skin from the hairs. This also allowed us to separate hair strands that were held together by dirt and fecal matter. The initial cleaning sonicated the hair in double distilled water and was later followed by a second cleaning that sonicated the hair strands in a 90% ethanol solution to remove oils and lipids from the hair strands (See Table 2 step 5). Between each step throughout the processing, hairs were kept in a freezer (Oelze 2015; Jorkov & Groke 2016).

2.4.3 Weighing the hairs

In the first lab session (2018) at the Archaeology Prep Lab we decided to weigh each hair strand from the first set of samples (N = 77) before the second cleaning to record a pre-clean weight. We weighed each of the hairs using a microbalance that could precisely weigh a single hair strand to the nearest microgram (μg) (Oelze 2015). Each hair was then weighed a second and final time after the second cleaning in an ethanol ultrasonic bath to give us a post-cleaning weight. In the second lab session (2019) at the Archaeology Prep lab we decided to not include a pre-clean weight with the second set of samples (N = 92) as we did not think this step was necessary. The post-cleaning weight, after the two ultrasonic baths, was more valuable since that information was necessary for the stable isotope analyses when it is entered in the Isotope Ratio Mass Spectrometer and Elemental Analyzer. It also provided a more accurate measurement of the hair strand after oils and lipids were removed (See section 2.4.5, Final cleaning and removal of lipids).

2.4.4 Under a microscope

One method to visually distinguish infant from maternal hair is to use the differences

between hairs in length and diameter (Oezle 2015). Bonobo infant hair was found to be ~20 μm thinner compared to adult maternal hair but even infant hairs showed a variation between hairs of ~60 μm based on the hair strands chosen (Oelze 2015). With the first set of hair samples, we calculated the hair diameter perpendicular to the estimated center of the shaft using a stereomicroscope (Hund Microscope H600 Polarized with camera adapter) at 10X magnification. We then noticed that the diameter could vary in different areas across the strand of hair especially near the end of the strand where the hair narrows. We decided a more accurate way of measuring strand diameter would be to calculate the hair diameter at three different locations perpendicular to the shaft of the hair strand using a stereomicroscope at 10X magnification (Oelze 2015). For the second set of samples, we collected the diameter in the middle, near the root, and near the end of each hair strand. The average of these 3 diameters was then calculated and recorded as the diameter of that hair strand.

After the diameter was recorded, we located the root of the hair under a microscope. We then determined the growth phase of the hair: if the root was telogen, anagen, catagen, unknown, or missing if the hair strand was torn and did not include the root. Most of the hair strands (95%) collected were found to be in the telogen phase which is important to know if a study's main goal is to sequence the hair and pinpoint diet within a specific timeframe (See section 2.4.6, Stable Isotope Analysis of Hair Keratin below; Oelze 2015; Jorkov & Grocke 2016). We collected photographs of each diameter measurement (at all 3 points) along the shaft of the hair under the microscope, as well as the root (Figure 1).

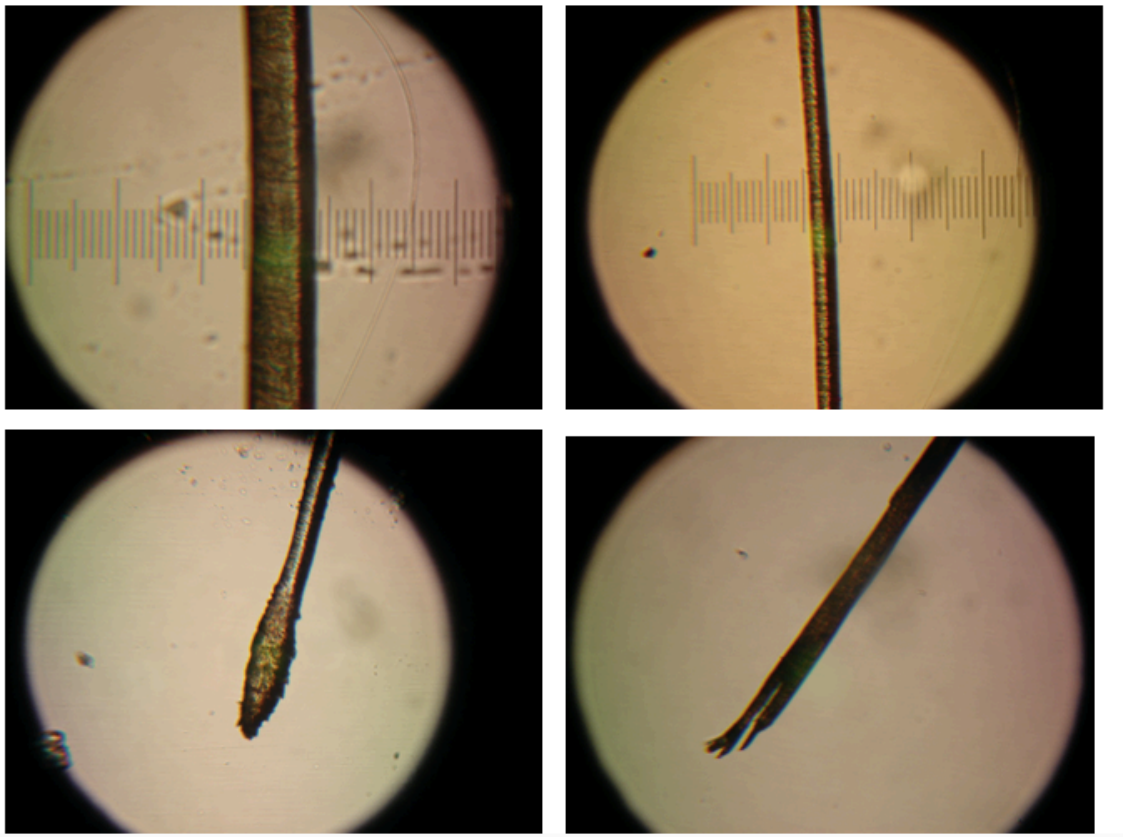


Figure 1. Measurement of an adult hair strand diameter (top left); measurement of an infant strand diameter (top right). Root of hair strand in telogen phase (bottom left); broken hair strand with no root (bottom right). All photos are a close-up image under a stereomicroscope at 10X magnification.

After the average diameter was recorded for each hair strand, we placed the hair strand on a sheet of paper and used a ruler to measure the length of each hair strand. Throughout this process, we used a set of tweezers to handle the hairs. When working with tweezers it was important to choose a pair that was not too sharp as that could have easily split the hair. We also decided not to use plastic gloves because the electrostatic charge made it much harder to control the hair (Oelze 2015). We also decided to work on a white surface as lost hairs were much easier to find on a white surface compared to one that was darker and hid the hair strands. We chose tweezers that were slightly dull and made sure not to touch the hairs when unnecessary to keep oils created by our hands off the hairs. We took photographs on an iPhone of each hair beside a ruler (cm) and recorded the lengths.

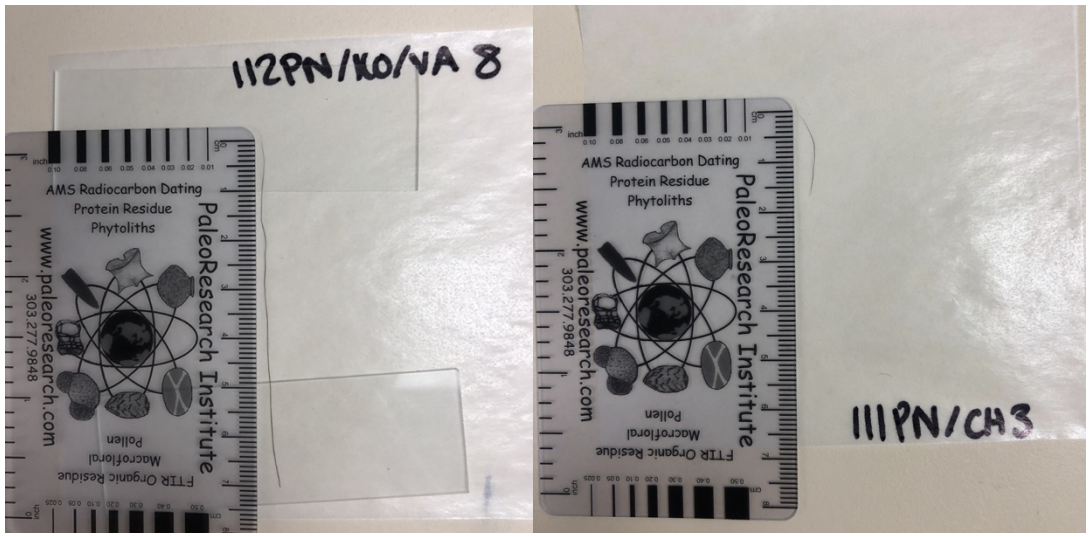


Figure 2. The length (cm) of an adult mother's hair strand (left) compared to the length of her infant's hair strand (right). Stable isotope analysis confirmed that the left hair is an adult female and the right strand belongs to an infant.

2.4.5 Final Cleaning and Removal of Lipids

When a hair strand emerges from the dermis, it is coated in lipids produced by the sebaceous and apocrine glands (Harkey 1993). Thus, besides sweat, the skin can contaminate hairs by coating them in oily and waxy lipids that surround the hair keratin matrix (Harkey 1993). Lipids can influence stable isotope ratios because the adipose tissues are depleted in $\delta^{13}C$ compared to proteins and carbohydrates. Therefore, oil and lipids on the hair strand will cause a more negative $\delta^{13}C$ value and will not reflect a true measure of the isotopic ratio found in the hair keratin (Rioux et al. 2019). When extracting lipids from caribou hair, the result showed a significant increase in both $\delta^{13}C$ and $\delta^{15}N$ values compared to hair strands that did not undergo lipid extraction (Rioux et al. 2019). Therefore, dirt contaminants and lipids need to be extracted and cleaned from the hair strand to measure the true isotope ratios of hair protein (Harkey 1993, Oezle 2015, Schoeninger 1999l; L'Herault et al. 2018; Dalerum et al. 2007).

We removed lipids from hairs by bathing each hair in a 90% ethanol solution (L'Herault et al. 2018) rather than a mixture of methanol/chloroform to extract the lipids (Oezle 2015; Schoeninger 1999l; Jorkov & Grocke 2016; Dalerum et al. 2007; Rioux et al. 2019). We chose to use a less aggressive solution of ethanol instead of methanol/chloroform because we wanted to ensure the thin infant hairs would not be destroyed by more abrasive chemicals. Similar to the first

round of cleaning, the hair strands were placed in labeled glass beakers and covered with the 90% ethanol solution. They were then covered with parafilm, placed in the ultrasonic bath, and were sonicated for 30 minutes (Oelze 2015). Once out of this ethanol bath, we placed each hair strand in a clean kim-wipe and gently rubbed the hair strand with the kim wipe to mechanically remove any left-over lipids or debris.

To remove the ethanol solution from the hair strands following the ultrasonic bath we prepared a filtering apparatus. To do this, we placed a funnel inside a beaker and then used a piece of filter paper on top to prevent the hair from falling down the funnel. Each hair strand was placed into the funnel with filter paper and rinsed three times with double distilled water (Oezle 2015, Schoeninger 1999l; L'Herault et al. 2018; Dalerum et al. 2007; Jorkov & Grocke 2016). The hair was repeatedly soaked and rinsed to ensure the ethanol would rinse off and drain. The runoff ethanol solution was then disposed of in the proper waste container. While the hairs were being bathed and cleaned, the glass jar that belonged to each sample was cleaned with an air duster that sprayed air into the container removing any dust or small debris particles left inside. We then dried each hair strand with kim-wipes and wrapped it in a clean dry kim-wipe before placing it back into the cleaned glass container.

2.4.5 Stable isotope analysis of hair keratin

The hair samples were weighed and prepared for stable nitrogen and carbon isotope analyses at the Isotope Sciences Lab (ISL), University of Calgary. Dull tweezers were used to handle all the hairs to ensure that we did not touch the hairs and leave any residue of oils or lipids from our hands. The ideal sample weight is approximately 0.5 mg but $\delta^{13}C$ and $\delta^{15}N$ values can be read in hair keratin with a sample weight as low as 0.3 mg of hair tissue (Oezle 2015; Jorkov & Grocke 2016), and even 0.1 mg based on our results. If hair strands were less than 0.1 mg and came from samples that included multiple strands collected at the same time and we knew for certain that they were from the same individual, then these hair strands were combined into the same tin capsule to increase the likelihood that the sample's $\delta^{13}C$ and $\delta^{15}N$ values would be read. Most hair strands (96%) weighed over 0.1 mg and so each sample consisted of that one full hair strand.

Short hairs, especially infant hairs, were folded into clean tin capsules (Jorkov & Grocke

2016). Longer hairs, especially those that belonged to females or males would sometimes bounce out of the capsule when folded, so I decided to cut the hair into pieces right above the capsule so they would fall in. Hairs that were cut into pieces and placed in the capsule were weighed again after being rolled in the capsule to ensure none of the hair strand was missing. Between each hair sample, I made sure to wipe down the tweezers and microbalance with ethanol to reduce the risk of cross-contamination.

Cutting up and sequencing of hair samples would allow one to assess temporal variation in hair isotope values (Oezle 2015; Williamson & Katzenberg 2012). This is because hair can then be sectioned equally into sequences to provide a recent feeding history of an animal and reveal what that individual was eating in different periods of time (based on the growth rate for that species) while that hair strand is growing (Holá et al. 2015; Oelze 2015). The downside of this method is that you need several hair strands to give you enough material to detect a nursing signal using stable isotopes. Selected hairs need roots in the same hair growth phase (e.g. telogen), be of similar length and thickness, and would require several hair strands from the same infant, collected within a small time-frame (Oelze 2015). A homogenous sample would need to consist of all hair strands cut and sequenced evenly across the same point to pinpoint the same time frame you want to analyze within each hair strand (Oelze 2015). Since we did not have several hairs from the same infant collected within the same time frame for every chimpanzee infant and mother, we did not cut up and sequence hair strands. Rather, we chose to use the entire hair strand to determine an average isotope value for a single individual for a longer period of at least 5–6 subsequent months (Oezle 2015). This method also allowed us to include hairs that had a root in an alternate phase (N=8) other than telogen or hairs that did not include a root (N=27). Hair growth for chimpanzees is approximately 1 cm per month (this is similar to human hair growth) (Oelze 2015). For example, if a hair strand with a telogen root was collected and we measured that this hair strand is 4 cm long, that hair strand would likely give us an amalgamation for the individual's diet over the 4 month period prior to the date of hair collection. However, hair strands in the telogen phase are metabolically inactive and rest until they are shed (Oelze 2015). These naturally shed hair strands in the resting (telogen) phase are likely to cause a delay in the isotopic response. In humans, the telogen growth stage lasts about 10 weeks (Oezle 2015) and may not absorb the most recent dietary isotopic signal incorporated in a physiologically active hair strand (hair in the anagen phase) (Oelze 2015). Therefore, there is a period of up to 10 weeks that the hair strand can remain on the body

in the resting phase without any growth and, thus, does not reflect stable isotopes from the food during the time it is resting (Oelze 2015). This means that in the results, the telogen hair samples collected need to account for this possible 10 weeks of dormancy of the hairs. Therefore, the results should be interpreted with a possible 2.5 month grace period of the infant's age from the date that hair strand was collected i.e. the hair strand can belong to the infant when they were 2.5 months younger from the date of data collection.

To present the results, I used the current age of the infant when the hair strand was collected and then I interpreted the results taking into account that the isotopic values from this hair strand could be representation of the infant from the age it is now to 2 to 2.5 months younger. Calculating a new date based on hair growth may introduce alternate inaccuracies, and because hair growth rates in chimpanzees is uncertain and may not be exactly 1 cm per month, the date when the hair was collected with a 2.5 month grace period is more reliable. Therefore, the results were interpreted knowing that the hair strand is representing the diet of the infant at its maximum oldest age,When categorizing infants into age groups, I took this into account and I placesInfants in the age group that would encompass this 2.5 month grace period.

We used a Costech Elemental Analyzer coupled to a Thermo Finnigan Delta PLUS XL stable isotope ratio mass spectrometer under continuous flow using a CONFLO III interface (Badescu et al. 2017). We measured stable carbon isotopes as the permil (‰) deviation of ^{13}C:^{12}C relative to Vienna Pee Dee Belemnite Limestone standard ($\delta^{13}C=[(^{13}C/^{12}C_{\text{ samples}} \div {}^{13}C/^{12}C_{\text{ standard}}) - 1] \times (1{,}000)$) and stable nitrogen isotopes as the permil deviation of ^{15}N:^{14}N relative to AIR ($\delta^{15}N=[(^{15}N/^{14}N_{\text{ samples}} \div {}^{15}N/^{14}N_{\text{ standard}}) - 1] \times (1{,}000)$). The Elemental Analyzer provides data on carbon (%C) and nitrogen (%N) content of the samples. The average standard deviation of repeated measurements of the USGS24, IAEA-N1, and IAEA-N2 standards were 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ (Badescu et al. 2017; Badescu et al. 2020).

2.6 Data sorting and analysis

We applied $\delta^{13}C$ and $\delta^{15}N$ analyses to keratin of 169 hair strands from infants, adult females, and adult male chimpanzees at Ngogo field site. Out of the total 169 hair strands, 74 belonged to infant chimpanzees, 67 belonged to adult female mothers, and 28 to adult males.

We attempted to use two methods to discern infant from maternal hairs. First, we used measurements of hair strand diameter and length, as presumably, maternal hairs would have a greater diameter and length than the hairs of her infant (Oelze 2015). This visual technique posed some challenges (see section 3.2 Results below and Discussion) and may not have allowed us to identify which hairs came from mothers versus their infants. Second, we relied on the stable isotope values themselves, post-analysis, to discern which hairs were most likely from the mother versus her infant. In order to identify infant from adult maternal hair in the analysis, we relied on hair strands from a clump containing a set of multiple hair strands that were collected at the same time. When several hairs were collected after a mother-infant pair groomed on the ground, for example, we would be able to assume that out of those several hairs found, some could be the mother's and others could be the infant's. After the stable isotope analyses, we predicted that certain hair strands from the set belonged to the mother because they were more depleted in the heavy nitrogen and carbon isotopes and showed lower $\delta^{13}C$ and $\delta^{15}N$ values compared to the hairs that were assumed to be from the infant. Strands from the same hair clump that were more enriched in the heavy carbon and nitrogen isotopes and showed higher $\delta^{13}C$ and $\delta^{15}N$ values were therefore assumed to belong to the infant. Out of the total sample (N=169), 51 hair strands from 9 mother-infant pairs came from several samples that had several hairs that we collected at once (i.e. a clump) (Table 3). When comparing the stable isotope values of these samples with those from hair samples found stuck to the tops of fecal matter, we could see differences in stable isotope values. We determined that the hairs collected in a clump presented a more reliable sample to discern infant feeding transitions, and we discuss these further in the Results and Discussion sections.

Table 3. The total number of hair strands that came from a sample that contained multiple hair strands (i.e. clumps) broken down into age categories. The total number of hair clumps, hair strands, and infant-mother dyads for each infant age category based on the results from isotopic analysis.

Infant age category (years)	Total number of hair clumps (Sample IDs)	Total number of infant hair strands in clump	Total number of mother hair strands in clump	Total number of hair strands within the hair clump	Number of infant-mother dyads
Infants $\leq$ 1.5 years old	4	6	8	14	4

Infants > 1.5 and 2	4	17	12	29	2
Infants > 2 and 3	2	2	2	4	1
Infants > 3 years old	2	2	2	4	2
Total	**12**	**27**	**24**	**51**	**9**

Of the 51 samples that included multiple hair clumps, 6 belonged to infants ≤1.5 years old, 17 belonged to infants > 1.5-2 years old, 2 belonged to infants > 2-3 years old, and 2 belonged to infants > 3 years old. Of the 51 samples, 27 belonged to their adult mothers; 8 belonged to mothers of infants ≤1.5 years old, 12 belonged to mothers of infants > 1.5-2 years old, 2 belonged to mother of infants > 2-3 years old, and 2 belonged to mothers of infants > 3 years old.

Having samples from both the infant and their mother was needed, as the mother's isotopic values of stable carbon and nitrogen created a dietary baseline that we used to identify if the infant's values signified that they were enriched in the heavy isotopes (Badescu et al. 2017; Oelze 2015). Suckling infants are enriched in ^{13}C and ^{15}N and appear on a higher trophic level due to the consumption of maternal milk, which is an animal product. The maternal values reflect a mostly vegetarian diet and are a trophic level below her suckling infant (Tsutaya & Yoneda 2015). The mother's $\delta^{13}C$ and $\delta^{15}N$ values are subtracted from the infant's $\delta^{13}C$ and $\delta^{15}N$ values and it is this value (the difference) that decreases and approaches zero as the infants are progressively weaned and start to show a value similar to their mothers (approaching the dietary baseline). The difference between the mother and infants $\delta^{13}C$ and $\delta^{15}N$ values are therefore needed to identify if there is a nursing signal in the infant hair sample, as well as to determine whether the infant has begun consuming plant foods (Badescu et al 2017; Reitsema 2012; Oelze 2015).

Chapter 3 - Results

3.1 Hair organization and distinguishing between maternal and infant hair strands

I will first provide a more concise summary of the sample and methods applied. The total sample consisted of 169 hair strands including hair from infants (N=74), mothers (N=67), and adult male chimpanzees (N=28) at the Ngogo field site. I measured the length and diameter from each hair strand from all 169 hair stands. I conducted stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses to keratin from each hair strand, and included both entire hair strands (N= 142) and hair strands that were broken either on the end or the root side of the hair (N=27). Study infants were then categorized into the 4 following age groups based on known or estimated birth dates: ≤ 1.5 years, > 1.5-2 years, > 2-3 years, and > 3 years. I also had a category for maternal hair strands and another category for adult male hair strands.

Out of the 169 hair strands, 51 hair strands from both infants (N=27 hairs), and their mothers (N=24 hairs), came from samples that included a set of multiple hair strands collected together in clumps at the same time. The remaining hair samples (N=90 hairs) were single hair strands collected on their own (N infants = 47 hairs; N mothers = 43 hairs; N adult males = 28 hairs). I separated the single hair strands from the clumps because I was most certain of the origin of the clumps based on how they were collected (i.e. found on substrates like the ground or logs after resting or grooming), and thus these hairs provided a more reliable sample than the hairs collected as single strands from feces, especially the hair strands collected in 2013 and 2014 that were processed in June 2018 (N=77).

I will now present results on how I distinguished between maternal and infant hairs. I will present the stable isotope analyses for the hairs collected in clumps separately (N=51), followed by stable isotope analyses for all the single hair samples (N=169). To conclude, I will compare these two data sets (i.e. the results from the hair clumps compared to all the single hair strands), stating the differences and similarities in the stable isotope values and overall trends between mother and infant hair strands.

Table 4. Hair strands that came from a sample that contained multiple hair strands. Each sub-set belongs to same sample ID, but within each subset, hairs can either belong to the mother or

infant, as determined from results of isotopic analysis. Infants are distributed into 4 infant categories with information regarding the sample ID and the mother-infant pairs found in each age group.

Infant Age Category (years)	Sample ID	Collection Year	Total hairs in sub-set	Number of infant hairs identified in sub-set	Number of maternal hairs identified in sub-set	Mother infant pairs
Infants ≤ 1.5 years old	346HN	2013-2014	4	2	2	HN-FZ
	491KK	2013-2014	3	2	1	KK-CN
	591 NI	2013-2014	5	1	4	NI-SA
	617DE	2018	2	1	1	DE-KD
Infants > 1.5 and 2	112PNKOVACH	2018	17	15	2	CH-PN
	111PNCH	2018	2	1	1	CH-PN
	110PNKOVACH	2018	7	0	7	CH-PN
	523KO	2013-2014	3	1	2	KO-PN
Infants > 2 and 3	40TO	2013-2014	2	1	1	TO-CA
	788TO		2	1	1	
Infants > 3 years old	480KA	2013-2014	2	1	1	KA-PZ
			2	1	1	
	499HD	2013-2014				HD-MF

All the hair strands were observed under the microscope to try and visually distinguish infant from maternal hairs and to view each hair strand's morphological properties (diameter, length). We relied on the 51 hairs collected in clumps in the data analysis to determine if the hair strand belonged to a mother or an infant using these properties since the origin of the hair was most reliable. This visual technique posed some challenges (see 3.2 Results below and Discussion) and may not have allowed us to identify which hairs came from mothers versus their infants. Next, we relied on the stable isotope values themselves, post-analysis, to discern which hairs were most likely from the mother versus her infant. Hair strands from a set of multiple that were collected at the same time allowed us to see that certain hair strands from the set belonged to the mother because these were depleted in the heavy nitrogen and carbon isotopes and showed lower $\delta^{13}C$ and $\delta^{15}N$ values. Out of the hair strands collected together, the maternal strands and the infant strands varied isotopically from one another, either considerably or less-so depending on the age of the infant (Table 5). However, when multiple hairs from the mother and/or multiple hairs from the infant were collected together as a group (i.e. a clump), the maternal hairs and the infant hairs

showed little to no isotopic variation within-category. That is, the hairs identified as coming from the mother were nearly identical in their isotopic values, while the hairs identified as the infant's, were also, isotopically similar (global mean average and STDV for $\delta^{13}C$ and $\delta^{15}N$, respectively, for infant hair clumps 8.3‰ +/- 0.24 and -23.6‰ +/- 0.31, Table 5, and global mean average and STDV for $\delta^{13}C$ and $\delta^{15}N$, respectively, for maternal hair clumps 6.8‰ +/- 0.21 and -23.5‰ +/- 0.19, Table 6).

Table 5. Mean and standard deviation of **infant** hair strands that were collected in a set of multiple categorized by infant age. The global mean and global standard deviation of all infant age categories.

Infant Age Category (years)	**Stable nitrogen isotope ratio ($\delta^{15}N$) in permil (‰)**		**Stable carbon isotope ratio ($\delta^{13}C$) in permil (‰)**	
	Mean	**Standard deviation**	**Mean**	**Standard deviation**
Infants ≤ 1.5 years old	8.6	0.24	-23.2	0.40
Infants > 1.5-2 years old	9.2	0.06	-22.2	0.07
Infants > 2-3 years old	7.9	0.45	-23.3	0.63
Infants > 3 years old	7.4	0.21	-23.8	0.14
Global mean	8.3	0.24	-23.6	0.31

Table 6. Mean and standard deviation of **maternal** hair strands that were collected in a set of multiple and categorized by the age of their infant. The global mean and global standard deviation of all maternal hair strand from all infant age categories.

Mothers in Infant Age Category (years)	**Stable nitrogen isotope ratio ($\delta^{15}N$) in permil (‰)**		**Stable carbon isotope ratio ($\delta^{13}C$) in permil (‰)**	
	Mean	**Standard deviation**	**Mean**	**Standard deviation**
Mothers of infants ≤ 1.5 years old	6.8	0.18	-23.8	0.16
Mothers of infants 1.5-2	6.6	0.08	-23.6	0.07
Mothers of infants 2-3	7.0	0.21	-23.3	0.36

years old Mothers of infants > 3 years old	6.7	0.27	-23.4	0.18
Global mean	6.8	0.21	-23.5	0.19

Using this post-analysis reasoning, we predicted that multiple hairs belonged to two separate individuals, and within the context in which they were collected (i.e. off a specific substrate after a mother-infant had groomed or rested for some time), we could deduce that the enriched hairs came from the infant and the depleted hairs came from the mother.

3.2 Length and diameter of infant and maternal hairs

The length and diameter of 51 hair strands, from 9 mother-infant pairs, were used in this part of the analysis to try and visually distinguish maternal from infant hairs. The hairs used to compare length and diameter were complete hairs from mothers and infants belonging to a set of multiple collected at the same time (see section 3.1 above). Complete male hair strands (N=28) were also measured and used as a reference.

3.2.1 Length and diameter of samples in individual infant age groups

Hair length and diameter varied within each age category and there was a high degree of overlap between infant and maternal strands. Each age group varied in the number of both infant and maternal hairs used in the analyses. The length (cm) and diameter (μm) of each hair strand within each age class used to calculate the means of both the length and diameters are illustrated in the figures below (Figure 3 to Figure 6). Each point represents an independent strand of hair from the infant or mother. Males were included as a reference point (N=28).

3.2.1.1 Infants in age class ≤1.5 years old

There were 6 samples that belonged to infants between the age of 0 and ≤ 1.5 years old. Figure 1 illustrates each hair strand as a data point, containing the length (cm) and diameter (μm) of each hair strand. The two hair strands that represent the lowest diameter in this age group belonged to infants and have a mean diameter of 0.050 μm and 0.055 μm. Infant hairs in this age

group represented the 3 longest lengths, excluding male hair strands, with lengths of 5.6 cm, 4.9 cm, and 4.6 cm. The greatest diameter from this age group is 0.100 µm and found in two hair strands, one infant hair strand and one maternal hair strand.

The range in this data set is from 0.05 µm to 0.09 µm (diameter) and from 1.7 cm to 5.6 cm (length) for infant hair strands. The range is from 0.06 µm to 0.10 µm (diameter) and from 1.8 cm to 4.2 cm (length) for maternal hair strands. This does not include any male hair strands. The greatest difference in diameter between a maternal and an infant hair strand was a diameter of 0.05 µm, however, this did not come from an infant-mother pair, but rather, these came from and infant and mother in two different pairs.

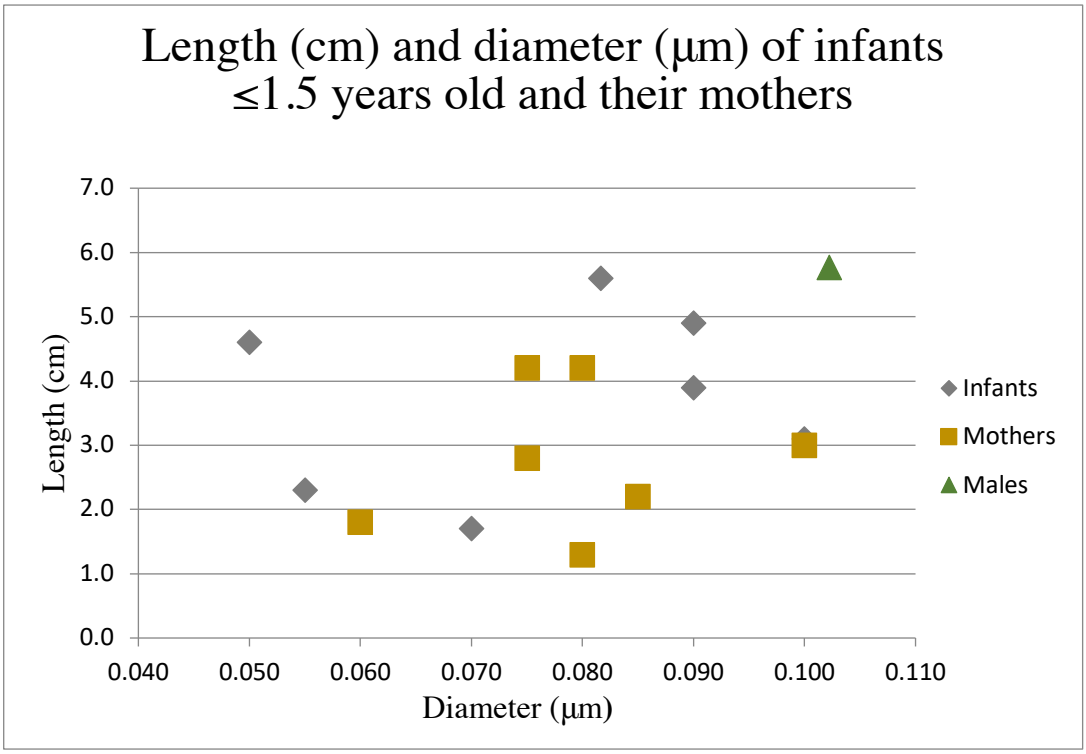


Figure 3. Length and diameter of individual infant and maternal hair strands in the age group <1.5 years old. Diameter represents an average diameter for each individual that was taken from 3 points along each hair strand. Males are represented by the orange triangle stating mean diameter of all male hair strands (n=28) and used as a reference.

3.2.1.2 Infants in the age class > 1.5 to 2 years old

There were 17 infant samples and 11 maternal samples that belonged to infants between the age of 1.5 and 2 years old (Figure 4). This age group had the largest number of infant hair strands, however, all but one pair of hair strands belonged to the same infant-mother pair, CH-PN.

The two hair strands with the lowest diameter in this age group belonged to infants and had a mean diameter of 0.055 µm and 0.058 µm. Infant hairs represented the 3 longest lengths, excluding male hair strands, with lengths of 8.2 cm, 7.9 cm, and 7.6 cm. The hair with the greatest diameter belonged to infant CH, with a diameter of 0.095 µm. This diameter was smaller than the mean diameter of the male hair strands (0.102 µm). Both infant and maternal hairs in this set contained strands longer than male hairs, with the longest hair being 8.2 cm from an infant. The range in this data set was from 0.055 µm to 0.095 µm (diameter) and from 1.8 cm to 8.2 cm (length) for infant hair strands. The range was from 0.065 µm to 0.088 µm (diameter) and from 1.4 cm to 7.2 cm (length) for maternal hair strands. The greatest difference in diameter between a maternal and an infant hair was a diameter of 0.033 µm from the infant-mother pair CH-PN.

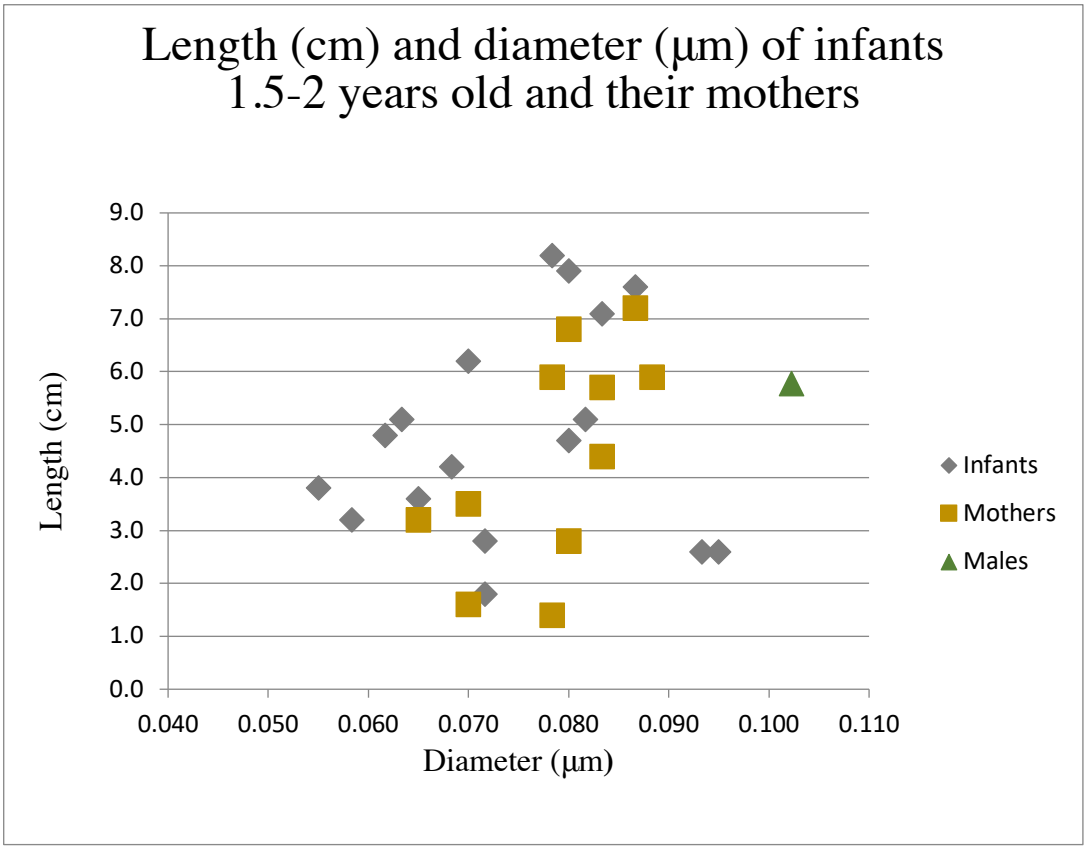


Figure 4. Length and diameter of individual infant and maternal hair strands in the age group 1.5-2 years old. Diameter represents an average diameter for each individual that was taken from 3 points along each hair strand. Males are represented by the orange triangle stating mean diameter of all male hair strands (N=28) and used as a reference.

3.2.1.3 Infants in the age class >2 to 3 years old

There were 2 infant samples and 2 maternal samples that belonged to infants between 2 and 3 years old (Figure 5). Both hair strand sets belonged to the same infant-mother pair, TO-CA. Both infant strand diameters were greater than the adult female hairs, with a diameter of 0.08 µm and 0.11 µm. TO was a male infant and had surpassed the mean diameter of adult male hairs (0.102 µm). The maternal hair strand (CA) had the longest hair in this sample, with a length of 3.5 cm. The range in this data set was from 0.05 µm to 0.11 µm (diameter) and from 1.7 cm to 3.0 cm (length) for infant hair strands. The range was from 0.06 µm to 0.067 µm (diameter) and from 2.0 cm to 3.5 cm (length) for maternal hair strands. The greatest difference in diameter between a maternal and an infant hair was a diameter of 0.05 µm, however, this did not come from the same hair clump. Instead, this infant hair belonged to one of the CA-TO hair clump, while the maternal strand belonging to CA came from the other clump. The greatest difference in diameter was 0.049 µm from a mother-infant pair, but in this case the infant's hair strand was thicker than his mother's hair strand.

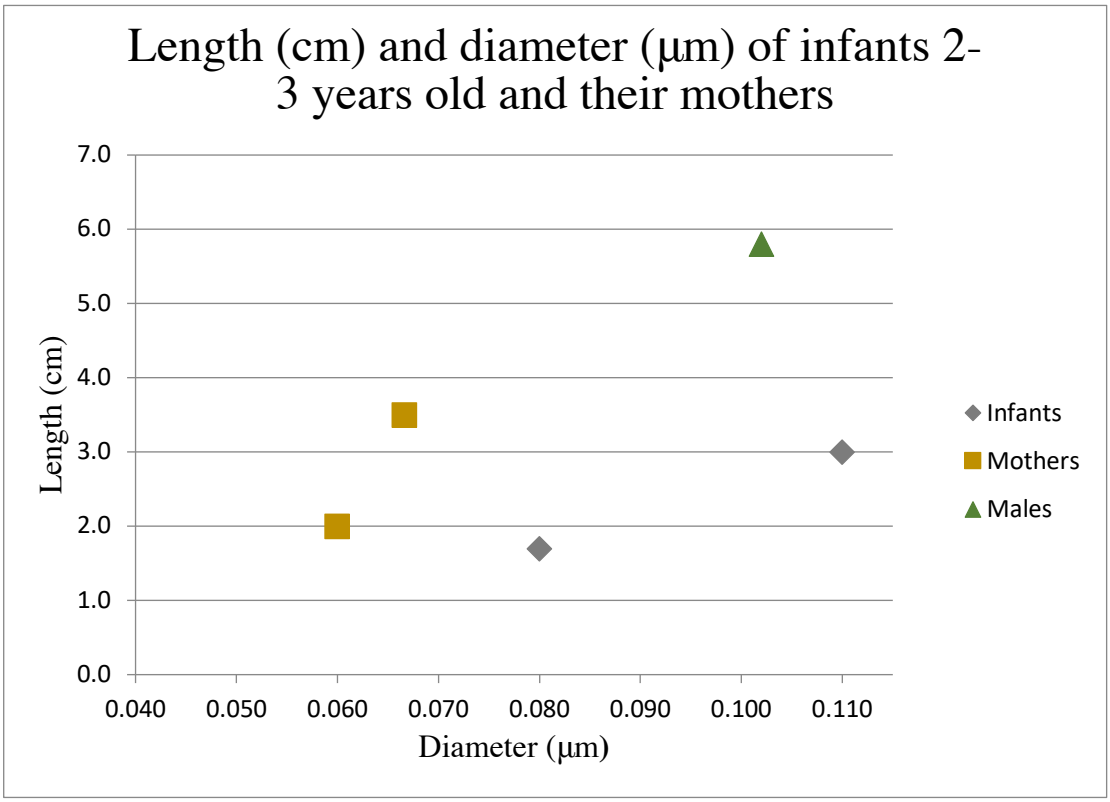


Figure 5. Length and diameter of individual infant and maternal hair strands in the age group 2-3 years old. Diameter represents an average diameter for each individual that was taken from 3 points along each hair strand. Males are represented by the orange triangle stating mean diameter of all

male hair strands (N=28) and used as a reference.

3.2.1.4 Infants in age class > 3 years old

There were 2 infant samples and 2 maternal samples that belonged to infants over 3 years old (Figure 6). The mean diameters of both infant hairs were the same as their corresponding maternal mean diameters (0.08 µm and 0.087 µm). The diameters from the infant and mother hair strands did not surpass the diameter of males (0.102 cm). The longest hair strand, with a length of 8.1 cm, belonged to an adult female and was 4.6 cm longer than the longest infant hair in this data set. The range in this data set was from 0.08 µm to 0.087 µm (diameter) and from 2.0 cm to 3.5 cm (length) for infant hair strands. The range was from 0.08 µm to 0.087 µm (diameter) and from 2.4 cm to 8.1 cm (length) for maternal hair strands. The greatest difference in diameter between a maternal and an infant hair was a diameter of 0.02 µm, however, this was not from an infant-mother pair.

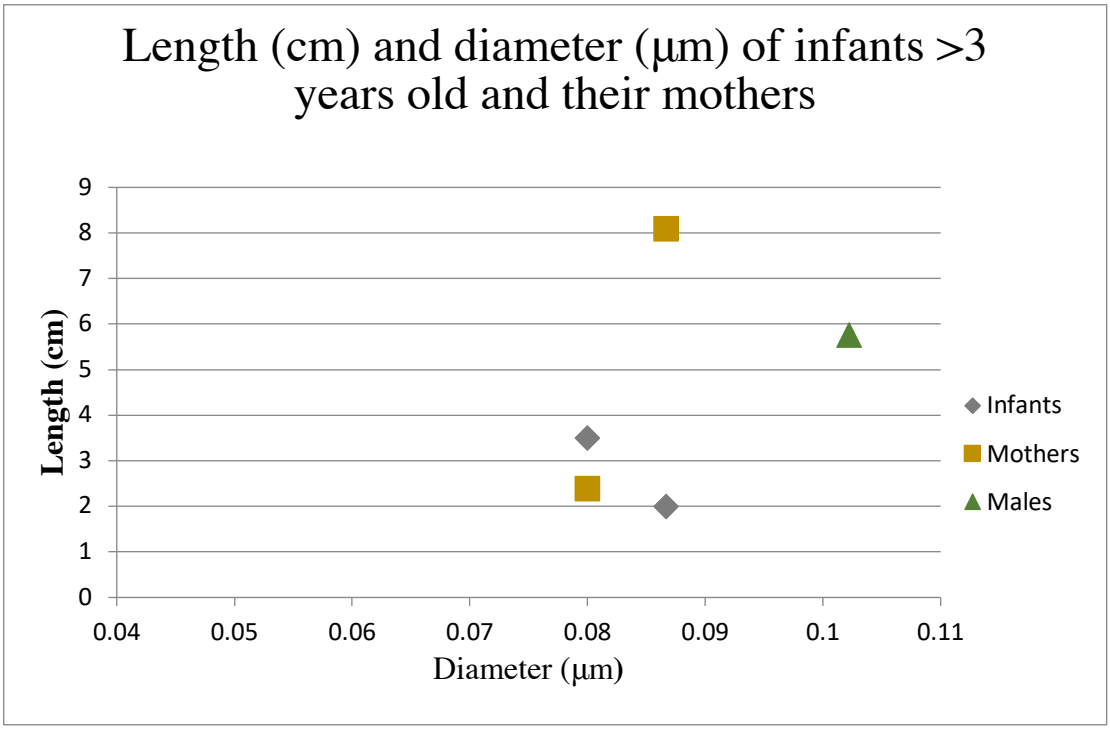


Figure 6. Length and diameter of individual infant and maternal hair strands in the age group >3 years old. Diameter represents an average diameter for each individual that was taken from 3 points along each hair strand. Males are represented by the orange triangle stating mean diameter of all male hair strands (N=28) and used as a reference.

3.2.2 Mean length and diameter of infant and maternal hairs from all age groups

Infants under the age of 1.5 years old (N=4) had an average mean diameter of 0.077 μm ± 0.01 μm and length of 3.8 cm ± 0.54 cm (Figure 7). This is thinner and longer than their corresponding mothers that had a mean diameter of 0.079 μm ± 0.001μm and a length of 2.8 cm ± 0.42 cm. Infants between the ages of 1.5 and 2 (N=16) were the thinnest out of all age categories with a mean diameter of 0.074 μm ± 0.02 μm and a mean length of 4.8 cm ± 0.49 cm. Their mothers had a mean diameter of 0.078 μm ± 0.002 μm and a mean length of 4.4 cm ± 0.61 cm making them slightly shorter that the infant hairs. Infants over the age of 2 (N=2) had the greatest overall mean diameter of 0.095 μm ± 0.02 μm, but had the average shortest hair strands at 2.4 cm ± 0.65 cm. Their corresponding mothers had a mean diameter of 0.063 μm ± 0.003 μm and an average length of 2.8 cm. Infants over 3 years old (N=2) had an average diameter of 0.83 μm ± 0.03 μm, the same as their mothers, but appeared to be on average 2.5 cm shorter, with an average length of 2.8 cm ± 0.75 cm.

When comparing all infant hair strands to maternal hair strands, on average, infant hair strands showed a mean diameter of 0.082 μm ± 0.005 μm. This was thicker than the female hair strands that together had a mean diameter of 0.075 μm ± 0.004 μm. Infant hair strands were however, on average, shorter than all the maternal hair strands. The maternal strands had a mean length of 3.8 cm ± 0.62 cm, while the infant hair strands had a mean of 3.4 cm ± 0.05 cm. There was a high degree of overlap in the thickness and length of maternal hairs with those of infant hairs in all age categories.

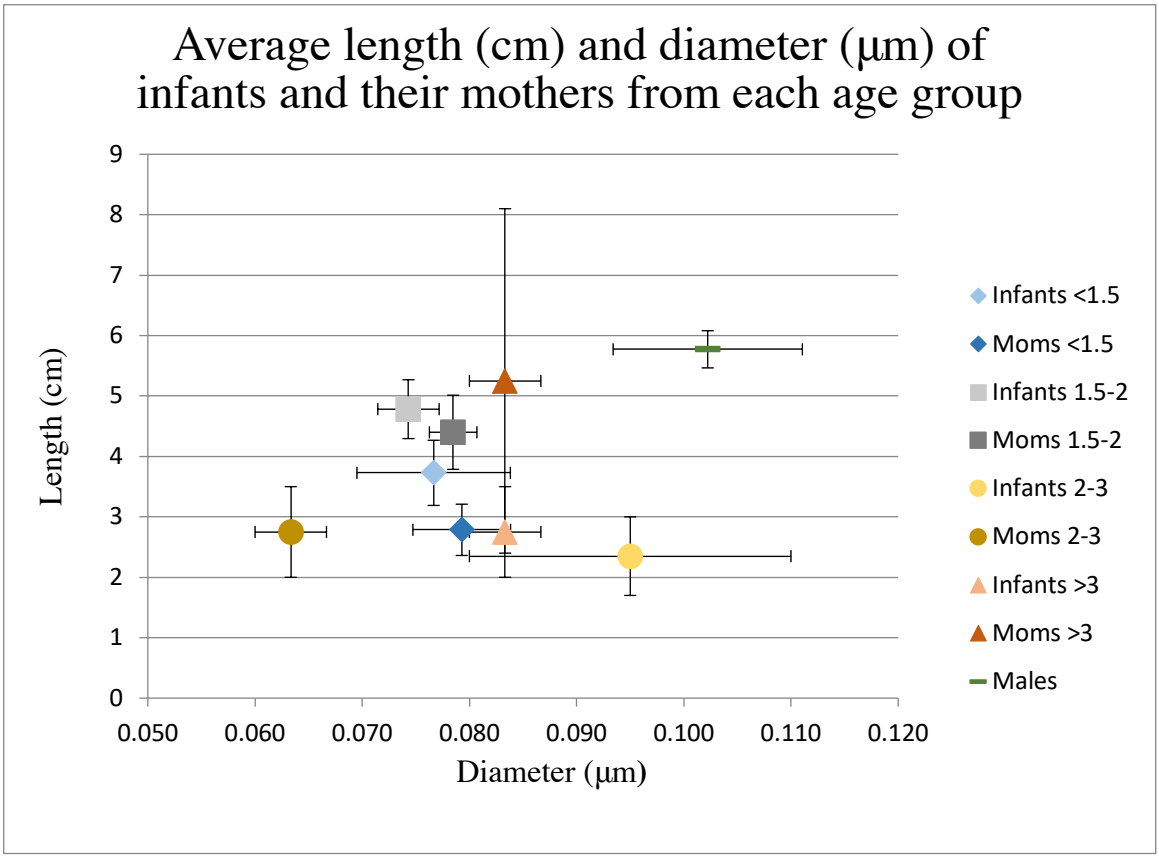


Figure 7. Mean length (cm) and diameter (µm) of infant hairs in different age groups and their corresponding mothers. Infants and corresponding maternal hairs for that age group are represented by the same shape and colour but a shade darker. Males are represented by the orange line. Black lines represent standard error of the mean.

The length (cm) and diameter (µm) of hair strands did not show differences that could alone be used to distinguish infant from maternal hairs. Although maternal and infant hair showed a high degree of overlap, making it hard to distinguish the hairs, on average, infant hair proved to be thinner and shorter to that of male hair. Male hair strands (N=28) had a mean diameter of 0.102 µm ± 0.009 µm and on average were 5.8 cm ± 0.31 cm longer than the infants. Thus, infant (and maternal) hairs can be distinguished from adult male chimpanzee hairs by strand diameter (~20 µm lower for infants) and length.

3.3 Stable isotope analysis of hairs from hair clumps

3.3.1 Stable nitrogen isotopes ($\delta^{15}N$)

The ratio of stable nitrogen isotopes ($\delta^{15}N$) in the diet decreased with increasing infant age (infants > 1.5 years old, 8.6 permil, ‰; infants > 1.5-2, 9.2 ‰; infants > 2 -3, 7.9 ‰; infants > 3, 7.4 ‰; Table 5 and Figure 8). The mean $\delta^{15}N$ of infant hair strands from all age categories combined (N=22 infant hairs) was 8.3 ‰ with a standard deviation of ± 0.24 ‰ (Range: 7.4 - 8.6 ‰). Infants ≤ 1.5 had a $\delta^{15}N$ value of 8.6 ‰ ± 0.24 ‰. Infants between > 1.5 and 2 years old showed the greatest proportion of $\delta^{15}N$ in their hair keratin of 9.2 ‰ ± 0.06 ‰. This was the only value that deviated from the downward trend (Figure 8), and could be attributed to the same infant, CH,who was 1.5 years old and represented most of the hair strands within the age category of 1.5 to 2 years old in both graphs. Infants > 2.5-3 and > 3 years old appeared to be less enriched in the heavy nitrogen isotope, with values of 7.9 ‰ ± 0.45 ‰ and 7.4 ‰ ± 0.21 ‰ respectively.

The values of $\delta^{15}N$ found in maternal hair keratin (N=25 maternal hairs) stayed generally consistent and had a mean $\delta^{15}N$ value of 6.8 ‰ ± 0.31 ‰ (Range: 6.6 - 7.0 ‰). Adult male hair strands (N=28 male hairs) had a $\delta^{15}N$ value of 7.3 ‰ ± 0.04 ‰ (Range: 6.8 - 7.8 ‰).

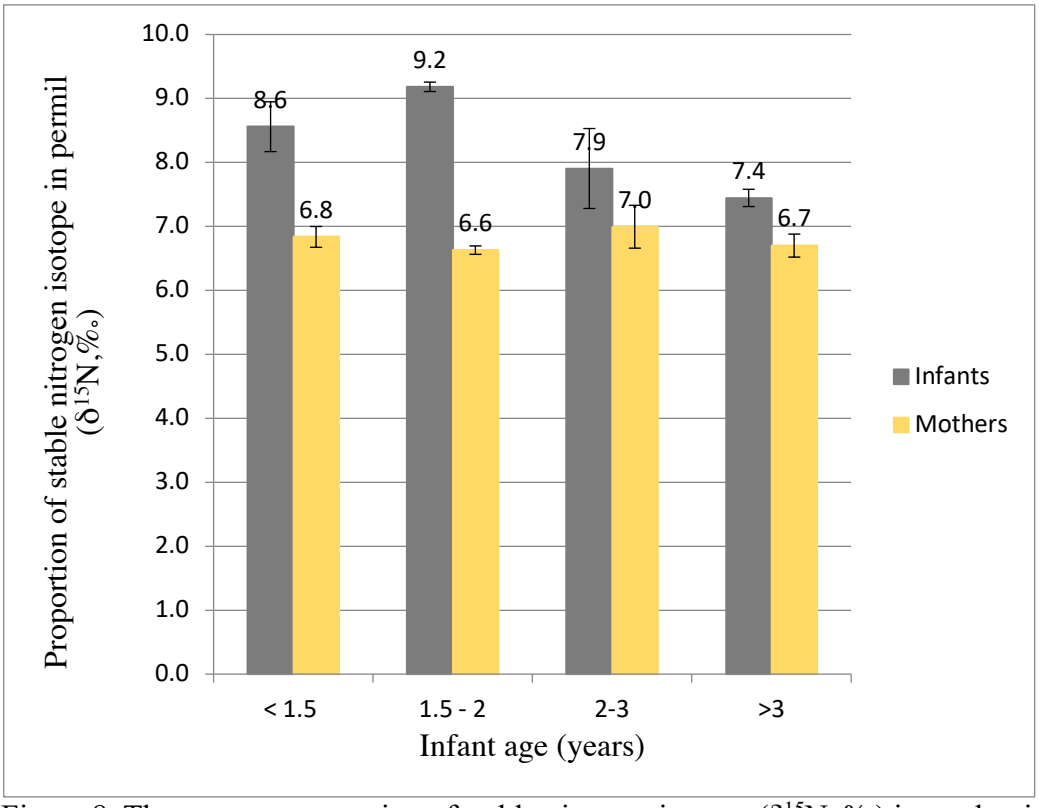


Figure 8. The average proportion of stable nitrogen isotope ($\delta^{15}N$, ‰) in mother infant pairs found in hair keratin distributed by infant age group. Age groups of infants distributed across 4 categories. Vertical bars show standard error.

3.3.2 Stable carbon isotopes ($\delta^{13}C$)

The stable carbon isotope ($\delta^{13}C$) ratio in the infant diet generally decreased (became more negative) with increasing infant age (infants ≤ 1.5 years old, -23.2 ‰; infants > 1.5-2, -22.2 ‰; infants > 2-3, -23.3 ‰; infants > 3, -23.8 ‰; Figure 9, Table 5 shows standard deviation). The mean stable carbon isotope ratio of infant hair strands from the four age categories together (N=22 infant hairs) is -23.6 ‰ ± 0.31 ‰ (Range: -23.8 - -22.2 ‰). Infants ≤ 1.5 had a $\delta^{13}C$ value of -23.2 ‰ ± 0.40 ‰. Infants between the ages of 1.5 and 2 years old showed the greatest ratio of $\delta^{13}C$ with a value of -22.2 ‰ ± 0.07 ‰ in their hair keratin. This value was the only one that did not follow the downwards trend. This was similar to the stable nitrogen isotopes for this age group, which were also higher than the previous age group of younger infants. Infants ≤1.5 years old were enriched in the heavy carbon isotope compared to their mothers with a $\delta^{13}C$ value of -23.2 ‰ ± 0.40 ‰. Infants between 1.5 and 2 years old were even more enriched in the stable carbon isotope compared to infants ≤1.5 years old, with a $\delta^{13}C$ value of -22.2 ‰ ± 0.07 ‰. Infants between 2.5 to >3 years were less enriched in the heavy carbon isotope, as they had a more negative $\delta^{13}C$ value of -23.3 ‰ ± 0.14 ‰. Infants > 3 years old had the lowest $\delta^{13}C$ ratio of -23.8 ‰ ± 0.14 ‰ in their hair keratin.

The values of $\delta^{13}C$ found in maternal hair keratin (N=25 maternal hairs) stayed consistent and had a mean $\delta^{13}C$ value of -23.5 ‰ ± 0.19 ‰ (Range: -23.8 - -23.4 ‰). Adult male hair strands (N=28 male hairs) had a $\delta^{13}C$ value of -23.7 ‰ ± 0.04 ‰ (Range: -24.1 - -23.1 ‰).

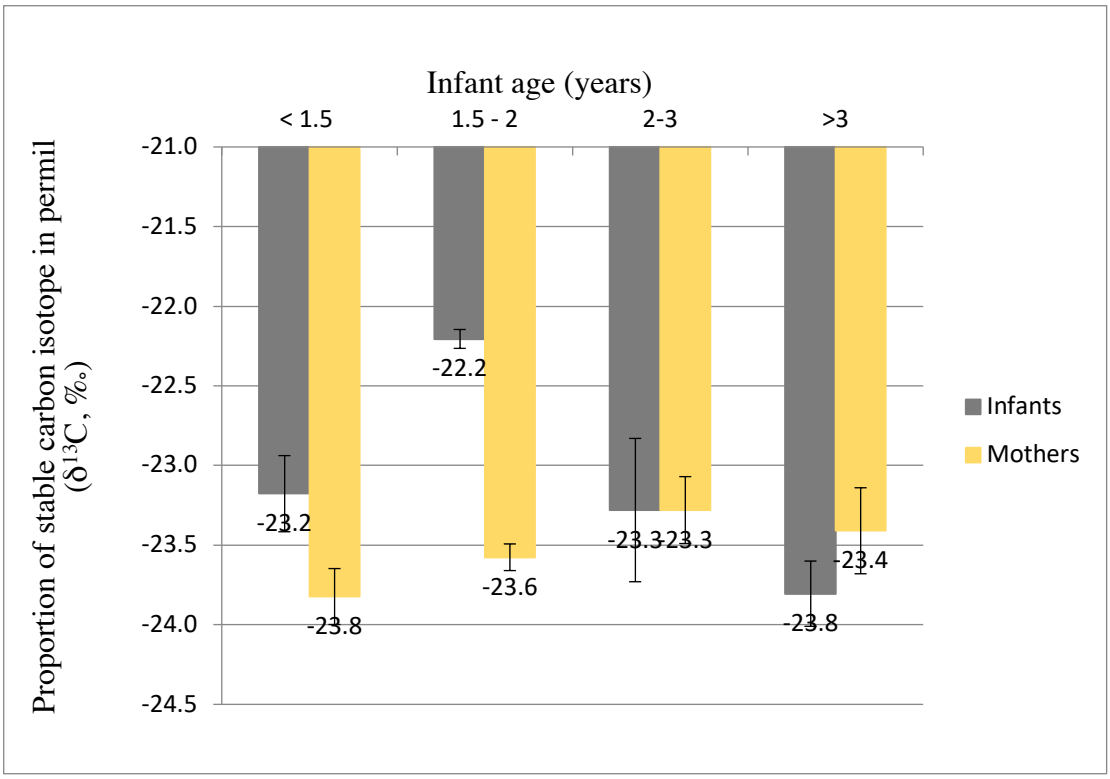


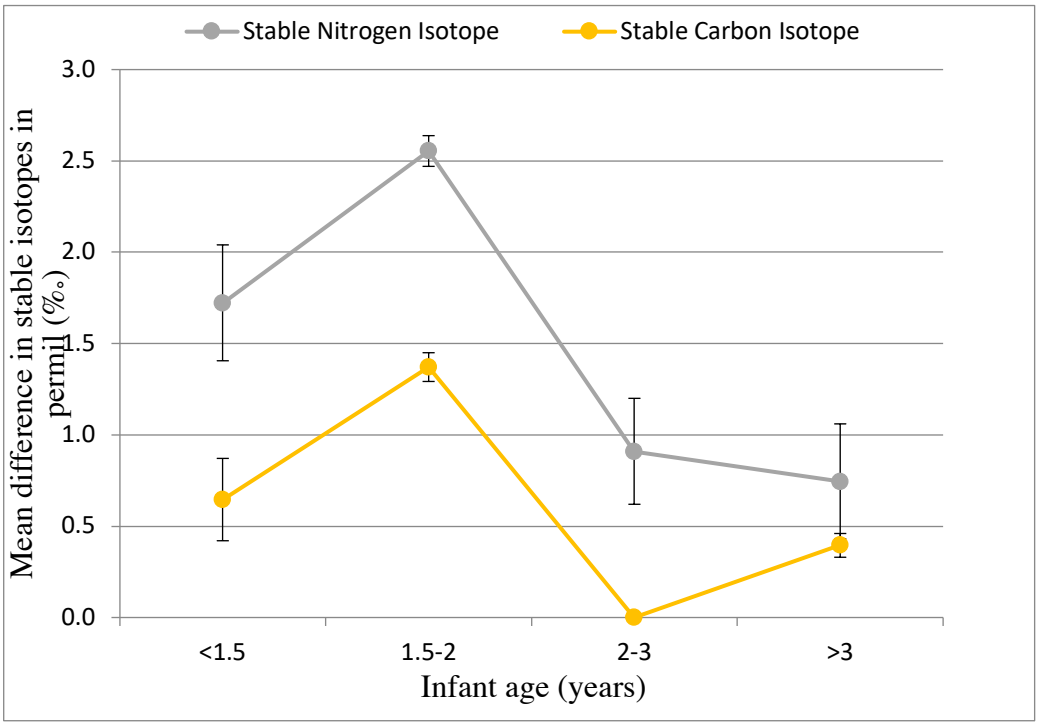
Figure 9. The average proportion of stable carbon isotope ($\delta^{13}C$, ‰) in mother-infant pairs found in hair keratin, distributed by different infant age groups. Age groups of infants distributed across 4 categories. Vertical bars show standard error.

3.3.3 Mean difference of stable isotopes found in hair keratin between infant and maternal hair strands

3.3.3.1 Mean difference Stable Nitrogen Isotopes ($\delta^{15}N$)

Infants ≤1.5 years old showed mean $\delta^{15}N$ values of 1.5 ‰ greater than their mothers (Figure 10A). Infants aged 1.5 to 2 years old were most enriched in the heavy nitrogen isotope out of all infant categories, and showed even greater mean differences in stable isotopes than their mothers with mean $\delta^{15}N$ values 2.6 ‰ greater than their mothers. Infants between the ages of > 2 to 3 years old showed a mean $\delta^{15}N$ value of 0.09 ‰ greater than their mothers. Infants > 3 years old showed the lowest mean difference in $\delta^{15}N$ ratios compared to their mothers and had a mean $\delta^{15}N$ value 0.07 ‰ greater than their mothers. This trend was similarly seen when infants were broken down into six age groups (Figure 10B), so even though the sample sizes for certain age categories were small, when broken down into 6 groups, the decreasing trend in the differences between mother-infant stable nitrogen isotopes was still clear. Overall, Figure 10A and 10B

illustrate that as infants got older, the mean difference between mother-infant $\delta^{15}N$ ratios decreased. Although infants showed a steady decrease in the heavy nitrogen isotope as they aged, infants > 3 years old still had mean $\delta^{15}N$ ratios that were not identical to their mothers, as they showed an elevation in their $\delta^{15}N$ of 0.7 ‰ higher than their mothers. However, it should be noted that infants over the age of 3 years old closely resembled $\delta^{15}N$ values of adult male hair strands, as infants showed a difference of only 0.1 ‰ higher in their $\delta^{15}N$ values compared to the adult male hairs.



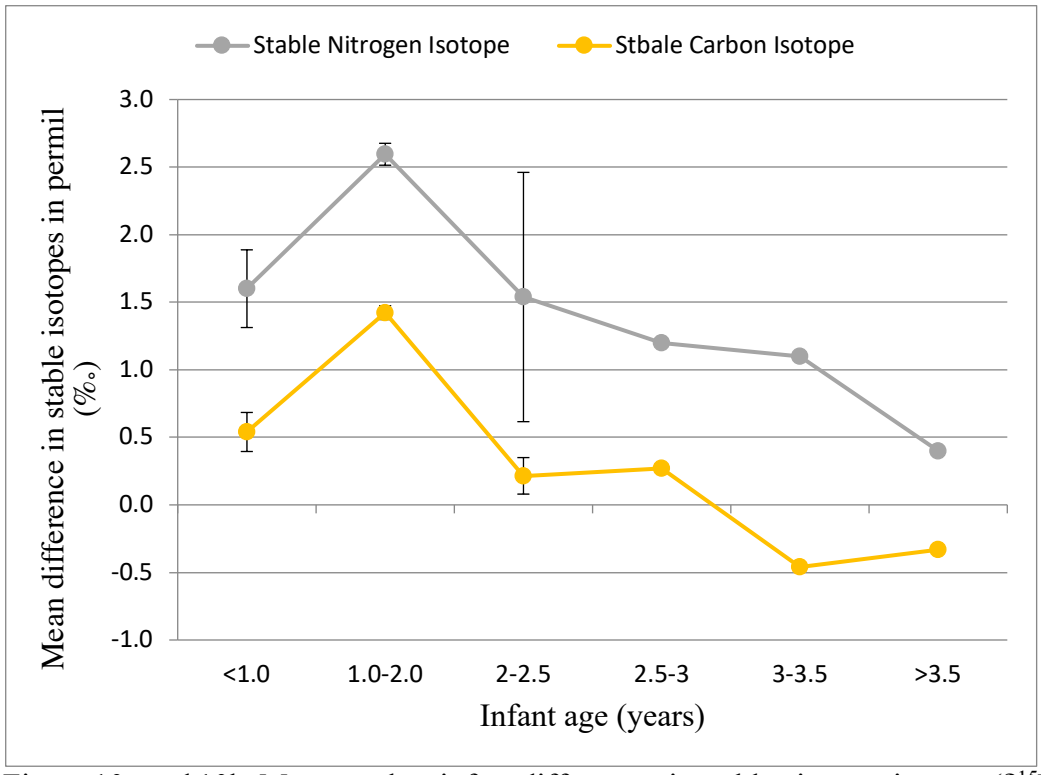


Figure 10a and 10b. Mean mother-infant differences in stable nitrogen isotope ($\delta^{15}N$, ‰) and stable carbon isotope ($\delta^{13}C$, ‰). Age groups of infants distributed across 4 categories (A, top) and age group of infants distributed into 6 age categories (B, bottom). Vertical bars show standard error.

3.3.3.2 Mean difference stable carbon isotopes ($\delta^{13}C$)

Infants ≤1.5 years old were enriched in the heavy carbon isotope compared to their mothers and showed a mean difference in their $\delta^{13}C$ value of 0.6 ‰ greater than their mothers (Figure 8A). Infants 1.5 to 2 years old showed even greater mean differences in stable carbon isotopes than their mothers, with a $\delta^{13}C$ value 1.4 ‰ greater than their mothers. Infants between 2 and 3 years old showed identical $\delta^{13}C$ values to their corresponding mothers, with a difference of 0 ‰ between mother and infant $\delta^{13}C$ ratios at this age group. Infants >3 years old were depleted in the heavy carbon isotope, and showed a mean $\delta^{13}C$ value 0.4 ‰ lower than their mothers. Rather, infants > 3 years old showed mean $\delta^{13}C$ ratios that were more similar to adult male hair strands (0.1 ‰ lower). These trends were similar when infants were broken down into six age groups (Figure 10B), so even though the sample sizes for certain age categories were small, when broken down into 6 groups, the decreasing trend in the differences between mother-infant stable carbon isotopes was still observable.

3.4 Analysis of individual hair strands (hair clumps removed)

Figures 11 to 13 include all individual hair strands that did not belong to a clump of hair (N=118) that were processed and analyzed during both lab sessions (June 2018 and June 2019). This included infant (N=47 hairs), female mothers (N=43 hairs), and adult male (N=28 hairs) hair strands. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses were applied to keratin from each hair strand and categorized by age group in the same way as the clumps of hair. The mean $\delta^{15}N$ value of 6.8‰ ± 0.07‰ and $\delta^{13}C$ value of -23.7 ‰ ± 0.07 ‰ represented all female values based on the mean $\delta^{15}N$ and $\delta^{13}C$ values from all single, adult female hairs. This was used as a comparison against all infant isotopic values that were categorized by age group. So here, instead of matching infant hairs with a corresponding maternal hair, we compared values of each infant hair to the average adult female values of all single maternal hairs.

Table 7. All individual hair strands used in stable isotope analysis with the samples from the hair clumps removed. Infant samples are distributed based on their age when the sample was collected. The mean $\delta^{15}N$ and $\delta^{13}C$ was calculated using all female hair strands from the single hairs.

Infant age category	**Total number of hair strands**	**Stable nitrogen isotope ratio ($\delta^{15}N$) in permil (‰)**	**Stable carbon isotope ratio ($\delta^{13}C$) in permil (‰)**
Infants younger than 1.5 years old	13	8.6	-23.2
Infants between the age of 1.5 and 2 years old	3	7.1	-23.5
Infants between the age of 2 and 3 years old	11	7.6	-23.3
Infants older than 3 years old	20	7.8	-23.2
Female maternal adult	43	6.8	-23.6
Male adult	28	7.3	-23.7
Total Hairs	118		

3.4.1 Stable nitrogen isotope ($\delta^{15}N$)

When taking into account all hair strands with the strands from the clumps removed, the ratio of stable nitrogen isotopes in the diet slightly decreased with increasing infant age (Figure 11). The mean stable nitrogen isotope ratio of infant hair strands from all four age categories combined (N=47 hairs) is 7.6 ‰ ± 0.18 ‰ (Range: 6.3 - 10. ‰). Infants ≤ 1.5 years old (N=13 hairs) showed the greatest mean ratio of stable nitrogen with an isotopic value of 8.0‰ (standard deviation 0.30 ‰). Infants between the age of 1.5 and 2 years old (N=3 hairs) showed the lowest ratio of stable nitrogen isotope ($\delta^{15}N$) in their hair keratin with a mean isotopic value of 7.1 ‰± 0.19 ‰. Infants between the age of 2 and 3 years old (N=11 hairs) appeared to be the least enriched inthe heavy nitrogen isotope with a mean $\delta^{15}N$ value of 7.6 ‰ ± 0.30 ‰, but this value increased to 7.8 ‰± 0.27 ‰ for infants >3 years (N=20).

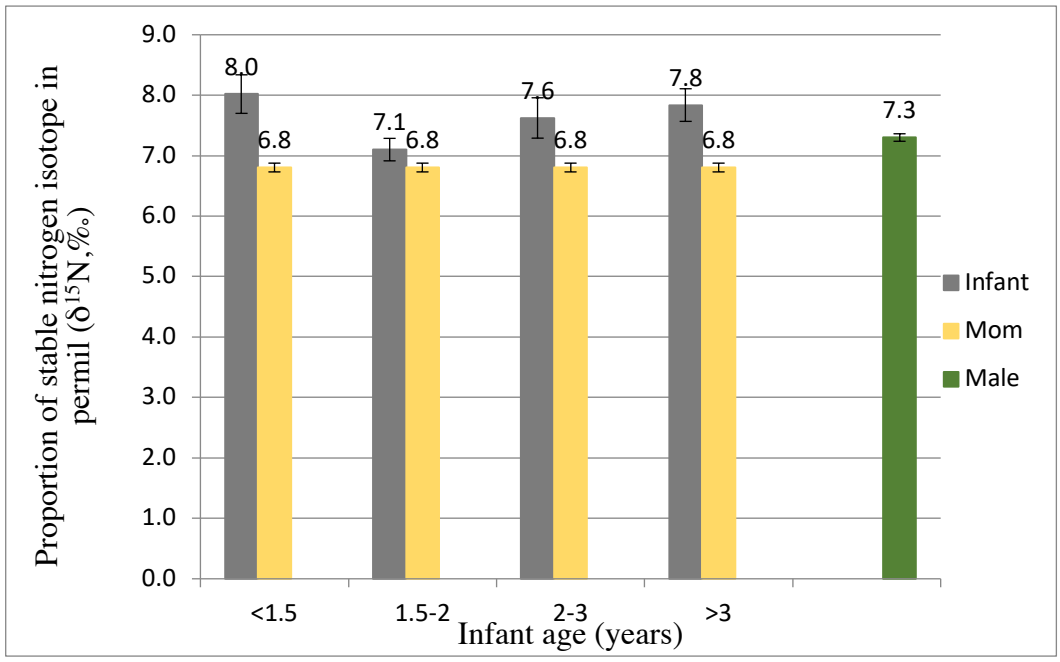


Figure 11. The average proportion of stable nitrogen isotope ($\delta^{15}N$, ‰) found in infants, female mother, and male hair keratin in sample with clumps removed (N=118). Infants show increased isotopic values compared to adult female mothers and male chimpanzee hair strands. Vertical bars show standard error.

3.4.2 Stable carbon isotope ($\delta^{13}C$)

When taking into account all hair strands (the hair clumps not included), the proportion of stable carbon ($\delta^{13}C$) in the diet did not follow a trend, but slightly decreased and then increased with increasing infant age (Figure 12). The mean stable nitrogen isotope ratio of infant hair strands

from all four age categories combined (N=47 hairs) is -23.3 ‰ ± 0.08 ‰ (Range: 24.1 - -22.8 ‰). Infants ≤ 1.5 years old (N=13 hairs), as well as infants > 3 (N=20 hairs) showed the greatest ratio of stable carbon isotope ($\delta^{13}C$) in their hair keratin with a mean value of -23.2 ‰ ± 0.16 ‰ and -23.2 ‰ ± 0.15 ‰, respectively. Infants between 1.5 and 2 years old (N=3 hairs) showed the lowest ratio of stable carbon with an isotopic value of -23.5 ‰ ± 0.34 ‰. Infants between the age of 2 and 3 years old (N=11 hairs) showed a mean $\delta^{13}C$ value of -23.3 ‰ ± 0.15 ‰.

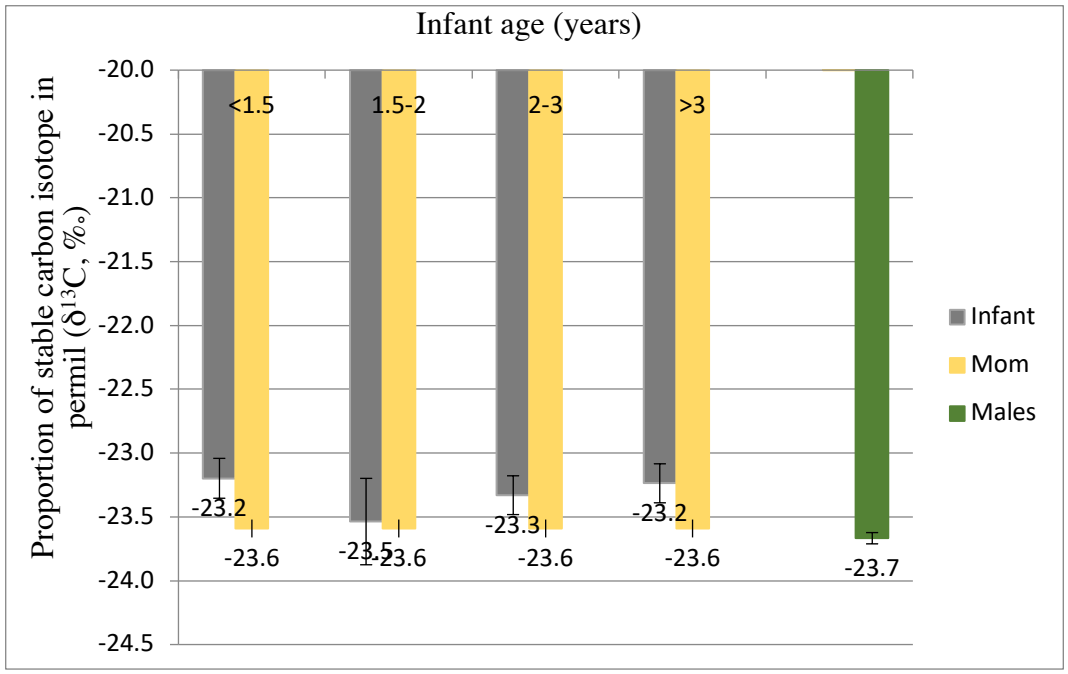


Figure 12. The average proportion of stable carbon isotope ($\delta^{13}C$) found in infants, female mother, and male hair keratin in sample with clumps removed (N=118). Infants show increased isotopic values compared to adult female mothers and male chimpanzee hair strands. Vertical bars show standard error.

3.4.3 Mean difference of stable isotopes found in hair keratin between all infant and maternal hair strands

3.4.3.1 Mean difference in stable nitrogen isotopes ($\delta^{15}N$)

Infants ≤ 1.5 years old showed a mean $\delta^{15}N$ value 1.3 ‰ ± 0.30 ‰ greater than their mothers (Figure 14). Infants between 1.5 to 2 years old showed the smallest difference in stable nitrogen isotopes, with a mean $\delta^{15}N$ value 0.1 ‰ ± 0.04 ‰ greater than their mothers. This group of infants showed the least difference in nitrogen isotopic ratios compared to their mothers and resembled $\delta^{15}N$ values the most similar to adult female mothers out of all infant age groups. Infants

between the ages of 2 and 3 showed mean $\delta^{15}N$ values 0.8 ‰ ± 0.27 ‰greater than their mothers. Infants > 3 years old had mean $\delta^{15}N$ values 1.2 ‰ ± 0.23 ‰ greater than their mothers. Thus, their values did not closely resemble the mean proportion of stable nitrogen isotopes found in their mothers' hair keratin and remained higher. This means infants 3 years and older were still enriched in the heavy nitrogen isotope compared to their mothers. Therefore, there was not a clear downward trend in mother-infant $\delta^{15}N$ differences to show that as infants aged the difference between infant and maternal values became smaller.

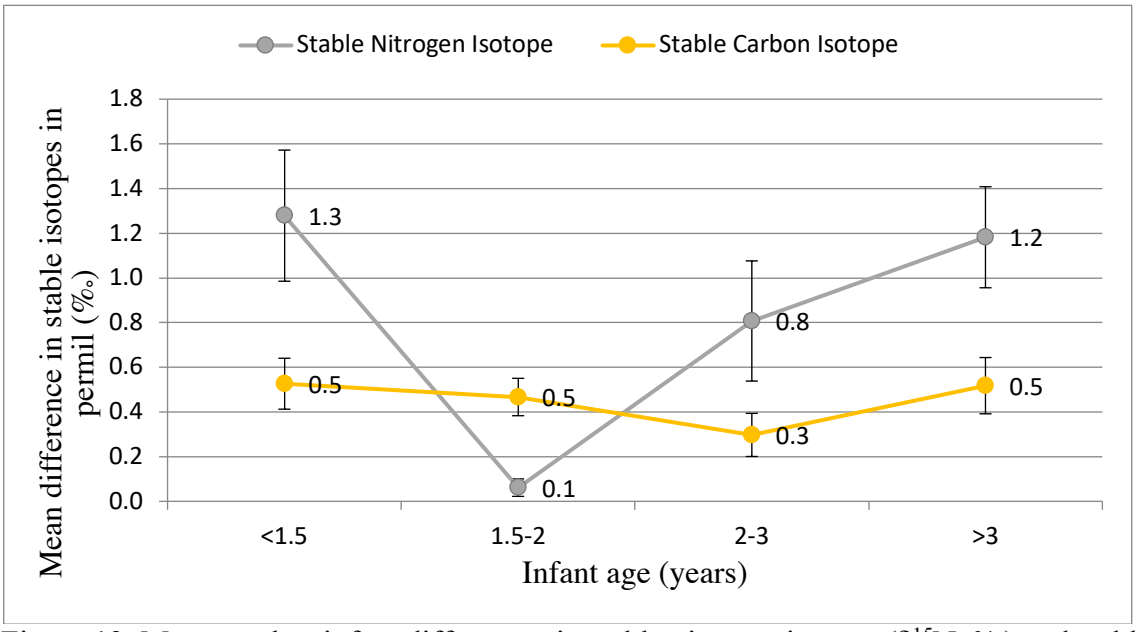


Figure 13. Mean mother-infant differences in stable nitrogen isotope ($\delta^{15}N$, ‰) and stable carbon isotope ($\delta^{13}C$, ‰) of all single hair strands. Vertical bars show standard error.

3.4.3.2 Mean difference in stable carbon isotopes ($\delta^{13}C$)

Across the 4 infant age groups, the difference in infant and maternal mean $\delta^{13}C$ values stayed consistent. Infants ≤1.5 years old, infants between 1.5 and 2 years old, and infants >3 years old showed a mean $\delta^{13}C$ value 0.5 ‰ (standard deviation respectively ± 0.11 ‰, ± 0.08 ‰, ± 0.13 ‰) greater than their mothers (Figure 14). Infants between the ages of 2 and 3 showed mean $\delta^{13}C$ values 0.3 ‰ ± 0.0 ‰ greater than their mothers. This age group of infants showed $\delta^{13}C$ isotopic ratios compared to their mothers most similar to adult female mothers out of all infant age groups, with the smallest difference between infant and maternal values. Infants ≤1.5 years old, between 2 and 3, and infants > 3 were slightly more enriched in the heavy carbon isotope compared to infants between 2 and 3. However, infants > 3 years old still remained enriched in ^{13}C compared

to their mothers, since the difference between infant and maternal values was not zero. The $\delta^{13}C$ values were still high relative to what was found in adult female and male hair strands. Overall, there was not a steady decrease in the stable carbon isotopic values as infants increased in age.

3.5 Analysis of hair clumps compared to single hair strands

3.5.1 Comparing $\delta^{15}N$ values and trends in all data sets

Overall, the infant hair strands that came from hair clumps (N=27 infant hairs) showed greater $\delta^{15}N$ values across all age groups compared to the mean $\delta^{15}N$ values from the data set containing only single hair strands (N= infant 47 hairs). The global mean of maternal hair strands from both data sets was 6.8 ‰. The hair clumps best showed an overall decrease of the stable nitrogen isotope in infants ≤ 1.5 to infants >3 years old, especially between the first two age groups and the third infant age group, resulting in a steady decrease in stable nitrogen isotopes in infants > 2 years old (8.6 ‰, 9.2 ‰, 7.9 ‰, 7.4 ‰). When only looking at the data set with the single hair strands, the $\delta^{15}N$ values initially decreased and then continued to increase (8.0 ‰, 7.1 ‰, 7.6 ‰, 7.8 ‰) between age groups.

The data set containing hair clumps showed that infants were overall more enriched in ^{15}N compared to their mothers until the last infant age group (infants > 3 years old). The hair clumps of infants > 3 years old had a mean $\delta^{15}N$ value of 7.4 ‰, which is lower than the $\delta^{15}N$ value from the set containing single hair strands (7.8 ‰).

Both the hair clumps and single hair data sets were similar in showing that infants > 3 years old did not approach a $\delta^{15}N$ value that resembled their mothers. The hair clumps, however, had a much smaller difference between the oldest infant age group (infants > 3) and their mothers with a mean $\delta^{15}N$ difference 0.7 ‰. When looking at the single hair strands, the mean $\delta^{15}N$ difference between the oldest infants and their mothers was still 1.2 ‰ higher. Overall, the differences in mean $\delta^{15}N$ values between infants and mothers were greater in the data from hair clumps across all age groups, but then showed the greatest drop in $\delta^{15}N$ values around 3 years of age.

The single hair strands had the lowest difference between infants and mothers (0.1‰) and this was found between mothers and infants 1.5 to 2 years old. In the hair clumps, this infant age group had values 2.6 ‰ greater than their mothers. Although the $\delta^{15}N$ values from the single hairs

closely resembled their mothers in this age group, this difference increased in the next infant age group and became more similar to the results found in hair clumps (0.8 ‰ and 0.9 ‰ respectively).

3.5.2 Comparing $\delta^{13}C$ values and trends in all data sets

The infant hair strands that came from hair clumps (N=27 hairs) showed similar $\delta^{13}C$ values across all age groups compared to the mean $\delta^{13}C$ values from the set containing only single hair strands (N=47 hairs). The only exception was for infants between 1.5 and 2 years old. In this age category the single strands showed mean $\delta^{13}C$ values much lower (-23.5 ‰) compared to infant hair strands from the hair clumps (-22.2 ‰). Infants ≤ 1.5 years old in the hair clumps reflected mean $\delta^{13}C$ values that were identical to infants ≤ 1.5 years old in the single hair set. In both these data sets, infants were enriched in ^{13}C compared to their mothers. In the data from hair clumps, infants were even more enriched than their mothers in ^{13}C between 1.5 and 2 years old, but in set of single hair strands, the infants' mean $\delta^{13}C$ values decreased and resembled $\delta^{13}C$ values more similar to their mothers.

Although the hair clumps showed an overall slight decrease from infants ≤ 1.5 to infants >3 years old in mean $\delta^{13}C$ values, the difference between mothers and infants fluctuated from decreasing to increasing values between age groups. The set containing single infant hair strands that were not from the hair clumps stayed the most consistent. In set containing only the single hairs, the difference between infant and mother mean $\delta^{13}C$ values remained at 0.5 ‰ with the exception of infants between 2 and 3 years old. Infants in this age group had mean $\delta^{13}C$ values 0.3 ‰ greater than their mothers.

One main difference between the data sets was that that infants > 3 years old from the hair clumps had mean $\delta^{13}C$ isotopic values even lower (i.e. more negative) than their mothers. This means that in the data set containing only hairs clumps, infants > 3 years old were even more depleted in heavy carbon compared to their mothers, resulting in a more negative $\delta^{13}C$ isotopic value. This was not found in the data sets containing only the single hair stands, as infants > 3 years old remained enriched in heavy carbon compared to their mothers.

Overall, when comparing the results of the hair clumps to the individual hair strands, the hair clumps were overall more reliable in showing the progression of infant dietary intake through nutritional development and proved to be more reliable in answering my research objectives.

CHAPTER 4 - DISCUSSION

4.1 Summary of key results

Overall, we could distinguish maternal from infant hair and detect mother-infant trophic level differences using stable isotope analysis of fallen hair from wild chimpanzees. We could not distinguish infant from maternal hair strands using only visual techniques, as the length (cm) and diameter (μm) of hair strands did not show great enough and consistent differences that can alone be used to distinguish the hairs. Although maternal and infant hair strands showed a high degree of overlap in length and diameter, which made it hard to distinguish the hairs, on average, infant and maternal hair proved to be thinner and shorter to that of adult male hair. Thus, the results indicated that infant and maternal hair could be distinguished from adult male chimpanzee hair by strand diameter (~20 μm lower for infants). The lengths of infant and maternal hairs overlapped greatly, and were not a good indicator of whether the hair belonged to the mother or the infant. Hairs that came from clumps that belonged to an infant-mother pair showed that infant hairs can be as long if not longer than their mothers.

The results showed that infants ≤ 2 years old had the greatest ratio of stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$). Infants between 1.5 and 2 years old had greater average ratios of $\delta^{13}C$ (-22.2 permil, ‰) and $\delta^{15}N$ (9.2 ‰) than infants ≤ 1.5 years old ($\delta^{13}C$ mean value of -23.2 ‰, $\delta^{15}N$ mean value of 8.6 ‰). Infants > 2 years old appeared to be less enriched in the heavy nitrogen isotope, and this was followed by a steady decrease of $\delta^{15}N$ throughout infancy. Although infants showed a steady decrease in $\delta^{15}N$ as they aged, infants > 3 years old had mean $\delta^{15}N$ ratios that remained somewhat higher than their mother (0.07 ‰ higher than mothers). However, infants over the age of 3 years old showed $\delta^{15}N$ values that closely the $\delta^{15}N$ values of adult male hair strands. Overall, as infants aged, the difference between the mean $\delta^{15}N$ isotopic ratios of infant and maternal hair strands decreased.

The results indicated that as infants got older, the mean difference between mother-infant $\delta^{13}C$ ratios decreased, although this pattern was less clear than for $\delta^{15}N$ mother-infant differences. Hair strands found in clumps from infants > 3 years old showed mean $\delta^{13}C$ ratios that mirrored their adult mothers, but then became even more depleted in the heavy carbon isotope compared to their mothers. When looking at the single hair strands that did not contain strands from the hair clumps, infants > 3 years old remained enriched in the heavy carbon isotope rather than being more

depleted than their mothers, which differs from the clumps of hairs. The single hair strands compared to the hair clumps showed a higher degree of variation in $\delta^{13}C$ values, and the decreasing trend in mother-infant $\delta^{13}C$ differences as infants aged was less clear. As infants approached the weaned event, which generally occurs between 4 and 5 years old (Badescu et al. 2017; Bray et al. 2017), infants' mean $\delta^{15}N$ and $\delta^{13}C$ values should closely resemble the values of their mothers. However, our data did not have a great enough sample size and did not include any hairs for infants between 4 and 5 years old, and we were thus not able to hone in on the weaned event to establish an average weaned age using hair in this community of chimpanzees. I was, however, able to use the hair keratin stable isotope results to establish the ages at which the weaning process appeared to begin, and when infants may have started to rely on adult foods in this community of chimpanzees, and these findings will be discussed in detail below (Section 4.5).

4.2 Distinguishing infant from maternal hair

My first goal was to establish a protocol to differentiate between chimpanzee infant and adult hair strands. To assess mother-infant trophic levels, as well as in any potential future studies aiming to reconstruct primate infant diets, infant and maternal hair strands need to be separated with care (Oelze 2015). To distinguish infant from maternal hair, we used two techniques.

4.2.1 Length and diameter

The first technique I used to discern infant and adult hairs involved using the measurements of hair strand diameter (μm) and length (cm). Presumably, maternal hairs would have a greater diameter and length than the hairs of her infant, as has been found in wild bonobos (Oelze 2015). However, this visual technique posed some challenges and did not allow me to identify which hairs came from mothers versus their infants due to how varied each hair strand was in its measurements. Hair strands can vary in structure (e.g. the diameter and length), in colour, and in other properties such as softness or rigidity, depending on what part of the body it sheds from (Personal observation). For example, infants can have light or white tufts of hair on the lower part of their body (on the rump) that are thin and fragile, but older adults can also have thin and white hairs that are noticeable with age. The chimpanzees at Ngogo showed a variety of different physical traits to help us distinguish individuals from one another including variation in hair colour

such as blonde or reddish hair. On average, chimpanzee infant hairs should have a thinner diameter (Oelze 2015) from adult chimpanzees, but based on our results, adults often also had fully intact hair strands (with the root attached) that were thin and short.

Furthermore, hair strands that were found stuck to feces may not have belonged to the chimpanzee individual who deposited the fecal sample, but rather a different individual. Hair found on top of a fecal sample could have fallen on it from another individual who was feeding or resting in the vicinity of the individual who made the fecal sample. Therefore, it is possible that the hair strand could have been shed on the fecal sample and it could be mistakenly assumed it was from the individual who the fecal sample belonged to. This is why we decided to split the data in two sections – the clumps of hair versus the single individual hair strands.

Research aimed at reconstructing primate infant diets need to be precise about how hairs are collected, and future studies should aim to collect hair from fresh nests (Oelze, 2015) or on substrates right after the individual has left. Although infant and maternal hairs cannot be distinguished from strand mean diameter and length independently in wild chimpanzees, measuring these physical characteristics can still be used as a tool alongside isotope analysis. In the future, if there is a way to establish which body part each hair fell from, then it might be possible to account for some of the great intra-individual variation we found in hair strand length and diameter; this could help to better establish the range in values coming from infants versus mothers, based on which part of the body the hair originated from. This might be possible if one could observe which places on the body mothers and infants were grooming on, prior to collecting the hair from the substrate they were on after the mother-infant pair left. If the mother and infant were mostly grooming around the face and chest, for instance, then we could predict that most likely the hairs found on the ground after the grooming session came from the individuals' faces and chests.

4.2.2 Stable isotope analysis

Our second method to differentiate between infant and adult hairs relied on the stable isotope values themselves, post-analysis, to discern between which hairs were most likely from the mother versus her infant. We obtained isotope data from 74 potential infant hair samples, 67 potential mother samples, and 28 adult male samples (Appendix Table 1). Out of the 169 hair strands, the 51 hair strands from both infants and their mothers came from samples that included

a set of multiple hair strands collected together in clumps at the same time yielded acceptable atomic $\delta^{13}C$ and $\delta^{15}N$ ratios that were in line with what we expected to see in terms of dietary intake for infants and adults. From these ratios we predicted that certain hair strands from the set belonged to the mother because they were more depleted in the heavy nitrogen and carbon isotopes and showed lower $\delta^{13}C$ and $\delta^{15}N$ values compared to the hairs that were assumed to be from the infant. Hairs that were identified as the mother's or as the infant's from the clumps of hair, showed little intra-individual variation compared to hairs collected individually. In our stable isotope results it was clear that within the clump of hair two individuals were represented within that sample. In the clump there were often 2 or 3 hairs that were from one individual, the mother for example, with isotopic values that were very similar for these hairs. In the same clump it was clear that the other 2 or 3 hairs belonged to another individual, the infant, with isotopic values that were different from the first individual (the mother), but also had little intra-individual variation between them. For example, the clump of hair (112PNCH) that belonged to Penelope and her infant contained 16 hair strands. Out of the clump, 2 hair strands showed $\delta^{15}N$ values of 6.0‰ and 6.2‰, and likely belonged to the mother. The hairs assumed to be from her infant had values higher than this, with $\delta^{15}N$ values of approximately 9.0‰.

It is unlikely that two hair strands from the same individual would have different isotopic signatures with a difference of 1 ‰ or 2 ‰ in this community of chimpanzees. Hair strands that are isotopically different are not from the same individual since hair strands collected a few months apart would result in little variation from the same individual. The Ngogo chimpanzee diet has little seasonal variation and although adult chimpanzees feed within small sub-groups, they often consume the same food items day by day (Potts and Lwanga 2013). However, even if adults happened to be consuming different types of ripe fruit, this would still not explain the higher $\delta^{15}N$ values themselves as these values are a better indicator of trophic level position and signify more protein rich foods (Reitsema 2012; Tsutaya & Yoneda 2015). Female adults do not consume as much animal protein compared to male chimpanzees, and the small traces they do eat would not be enough to show up in their $\delta^{15}N$ values (Watts & Mitani 1999; Watts & Mitani 2002)). Similarly, it is rare that infant chimpanzees consuming milk protein through suckling would fluctuate between trophic levels in a few months time. It is unlikely that an infant would show cyclical nursing patterns across several months, and once nursing decreases, it is difficult for the mother to increase her lactation effort again. This is because once an infant is no longer suckling

as frequently, the lack of nipple stimulation would compromise lactation, and it would be difficult for the female to produce again the same, higher amount of milk as before (Gomendio 1989; Gomendio 1991).

I aimed to establish methods for hair collection of non-human primates for future studies, and my study demonstrated how hair strands found on substrates rather than stuck to feces increased the chances of reliably identifying which hair strand belonged to which individual. This was most apparent in the 28 male hair strands that we collected. Adult male chimpanzees were easier to accurately collect hairs from because they regularly groomed one another on the ground and adult males were usually easier to follow and observe as they were more habituated to observers than adult females and infants. Hairs were present in much larger clumps (as many as 13 at a time) and were held together by fresh skin which allowed us to clearly identify when multiple hairs in a larger clump came from one particular male versus another that he was grooming with, for example. Oelze (2015) sampled hair strands non-invasively from fresh nests created by free-ranging bonobos. Our study did not contain any hair strands from nests due to the nests being too high up for us to reach and we did not have the right equipment to climb trees to retrieve the hairs. In the future, hair isotope studies should incorporate hair collection from nests, in addition to collection from substrates after resting or grooming, to increase sample sizes of mother-infant hairs and to increase the reliability of the methods.

We determined that the hairs collected as clumps from the ground after mothers and infant groomed or rested should be used to assess the second goal of my study, which was to evaluate mother-infant trophic-level differences and identify infant feeding transitions, including when infants started to rely more regularly on solid foods and to evaluate the weaning process using hair keratin stable isotopes. I found that the stable nitrogen ($\delta^{15}N$) and stable carbon ($\delta^{13}C$) isotopic values from the hair strands coming from clumps were overall more consistent with past studies that evaluated mother-infant trophic levels in primates to assess the relative contribution of maternal milk in infant diets (e.g. Tsutaya & Yoneda 2015; Badescu et al. 2017; Reitsema 2012; Bray et al. 2017; Fuller et al 2006; Fahy et al. 2014).

4.3 Assessing infant-mother trophic levels

The second goal of my thesis was to evaluate mother-infant trophic-level differences using

stable isotopes found in hair keratin, and through these differences, track infant feeding development.

To interpret the results, I used the age of the infant at the date when the hair strand was collected and took into account that the hair strand showed a maximum age for the infant, with a possible 10 weeks of error in this age.This is because hair strands can remain on the body in the telogen, resting phase without any growth for 10 weeks before it is shed (Oelze 2015). In my results, all of the infant samples from the clumps of hair still belonged to the same infant age category when accounting for the possible 10 weeks of error. For example, 3 infants ≤1.5 years old were 10 months old when their hair strands were collected. If we account for this time period, assuming that the hair strand was on their body and did not shed until the 10 weeks was over, then that hair strand would represent the infant's diet when it was 7-8 months old. When I subtracted 2.5 months from the age of all the infants in the sample from clumps, it did not change what age group they belonged to and therefore the results from their hair strands still reflected stable isotopic values for their age group.

Due to hair growth and how hair keratin stores dietary information over time, it is also important to note that each hair strand collected reflects approximately 6 months of dietary information on the infant before it was shed (Oelze 2015). We decided not to sequence the hair into sections corresponding to distinct time frames because we did not collect enough hairs from each individual at a specific time period. Thus, we would not have had a big enough sample to analyze sequences along the hair for the analyses. Instead, the stable isotope values in our results represented an average of the food items consumed by the infant over the entire period that the hair was growing. Therefore, infant hair strands contained stable isotope data of the food items that were consumed in the last few to several months (depending on the length of the hair) before the hair strand was shed (Oelze 2015). Although we considered the possible 10 weeks of error before the hair strand was shed, it is possible that the infants' hair strands showed an average of their diet, including their diet several months prior to the date the hair was collected. I still decided to use the current age of the infant when the hair strand was collected because hair growth rates are unknown in wild primates (Oezle 2015), and I did not want to add more uncertainty and possible room for error.

4.4 Stable nitrogen isotope ratios ($\delta^{15}N$): suckling and weaning

4.4.1 Infants ≤1.5 and >1.5 to 2 years old

The average $\delta^{15}N$ ratios from infant hair strands indicated that as infants got older there was a depletion of ^{15}N, and the average $\delta^{15}N$ difference between chimpanzee infants and their mothers decreased. The average $\delta^{15}N$ difference between the youngest group of infants, ≤1.5 years old, and their mothers was smaller than the average of slightly older infants between 1.5 and 2 years old. Infants ≤1.5 years old showed a 1.5 ‰ elevation of mean $\delta^{15}N$ in their hair strands compared to their mothers, while infants between 1.5 and 2 years old showed a 2.6 ‰ elevation compared to their mothers. Therefore, this study showed that infants ≤2 years old have the greatest proportion of stable nitrogen isotopes ($\delta^{15}N$) in their hair keratin, and hence have the greatest proportion of maternal milk in their overall diet compared to other age categories. Indeed, observational and isotopic data in feces and tooth enamel indicate that the diets of chimpanzee infants in the wild up to 2 years old can primarily be made up of maternal milk (Fahy et al. 2014;). However, when taking into account the possible 6 month range in the ages in which we can interpret these data, as the hair keratin stable isotope values reveal what infants ate within several months before the hair was collected, it may be more accurate to say that the maximum enrichment is shown by infants up to 2 years old minus 6 months, so at approximately 1.5 years old.

Our results are consistent with Oelze (2015) who collected hair strands of free-ranging infant and mother bonobos (*Pan paniscus*) at Salonga National Park, Democratic Republic of Congo, in that both of our results detected a strong nursing signal in infants between 1 and 2 years old. However, we found a greater difference in the keratin stable nitrogen isotope of slightly older chimpanzee infants (between 1.5 and 2 years old). Compared to the elevations in keratin stable nitrogen isotopes of infants compared to their mothers in our study (1.5 ‰:≤1.5 years old, 2.6 ‰:> 1.5 and ≤ 2 years old), Oelze (2015) found a 1.2 ‰ elevation in the bonobo infants compared to their mothers for infants >1 and <2 years old. When we look at the base values in keratin stable nitrogen isotopes for infants and mothers, we see that bonobo infants between 1 and 2 years old showed a mean $\delta^{15}N$ value of 9.7 ‰ in their hair keratin, which is similar to chimpanzees at Ngogo of the same age ($\delta^{15}N$ value of 9.2 ‰). However, bonobo mothers had a $\delta^{15}N$ value of 8.5 ‰ (Oelze 2015) in their hair keratin, which is higher than Ngogo chimpanzee females that showed an average $\delta^{15}N$ value of 6.8 ‰. Thus, the higher difference in $\delta^{15}N$ values between infant and mothers that we see in chimpanzees compared to

bonobos is most likely driven by the fact that adults have different diets, with the bonobo mothers consuming a diet that is more enriched in ^{15}N compared to the diet of chimpanzee females.

Both chimpanzees and bonobos are frugivorous and rely on different types of ripe fruit as their predominant food source (Watts et al. 2012; Carlson & Crowley 2016; Serckx et al. 2015) Bonobos both prefer and rely on fruit coming from mature forest and their fallback foods often consist of different fruit species from forest edges (Serckx et al. 2015). Fruit makes up the largest diet component of chimpanzees at Ngogo, and the most important food species at Ngogo is a fig, *Ficus mucuso* (Watts et al. 2012). Chimpanzees at Ngogo also eat a variety of leaves, pith, stems, and flowers (Carlson & Crowley 2016). The chimpanzee diet at Ngogo is mostly made up of C_3 plant foods with a mean $\delta^{13}C$ of -27.7 ± 2.6 ‰ and a mean $\delta^{15}N$ of 4.5 ± 1.8 ‰ (Carlson et al. 2011). *Ficus mucuso*, quantitatively the most important food at Ngogo field site and often consumed by the chimpanzees, had a mean $\delta^{13}C$ -26.9 ± 0.3‰, and a mean $\delta^{15}N$ of 4.1 ± 0.9 ‰ (Carlson et al. 2011). At Salonga National Park, free-ranging adult bonobos showed $\delta^{15}N$ values between 7.7-9.1 ‰ with a mean of 8.4 ‰ in their hair strands (Oelze et al. 2011). This is even higher than the $\delta^{15}N$ values from the male chimpanzee hair strands at Ngogo (mean $\delta^{15}N$ value of 7.3‰). This is because the five most important food sources at Salonga had a mean $\delta^{15}N$ value of 8.4 ‰ and a mean $\delta^{13}C$ of -25.8 ‰. The food items showed a wide range of $\delta^{13}C$ values between -36.2 ‰ to -24.5 ‰ and $\delta^{15}N$ values between 2.6 ‰ to 9.3 ‰ (Oelze et al. 2011). Thus, the bonobo diet at Salonga is overall more enriched in ^{15}N than that of the chimpanzees at Ngogo. Unlike the Ngogo chimpanzees, there is no significant difference between female and male bonobo stable isotope ratios at Salonga National Park.

Our study was not consistent with Oelze (2015) in that the youngest group of infants at Ngogo (infants ≤1.5 years old) showed a clear nursing signal whereas the youngest bonobo infants did not. Oelze (2015), however, only had hair samples of newborns less than 1 month old, while our samples from the youngest age group contained infants greater than 1 month old. The youngest infant in our samples was 2 months old, and showed a 1 ‰ elevation in $\delta^{15}N$ above its mother, which was not similar to Oelze's study. Bonobo infants <6 months old had a mean $\delta^{15}N$ of 8.4 ‰, while the mothers had a mean of 8.5 ‰, thus, mothers showed a 0.1 ‰ elevation in $\delta^{15}N$ compared to the infants in this age group. It could be that a clear nursing signal in hair tissue is not easily detected prior to 1 month after birth in apes, which could be similar to another fast-turnover tissue,

feces (see next paragraph below where I compare results of this study with a previous study on fecal stable isotopes at Ngogo).

My finding that average mother-infant $\delta^{15}N$ differences of infants ≤1.5 years old were smaller than the average of slightly older infants (>1.5 to 2 years old) is consistent with a similar pattern found using fecal stable nitrogen isotope data of mother-infant pairs of chimpanzees at Ngogo (Badescu et al. 2017). However, this similar pattern was apparent when comparing the fecal stable nitrogen isotopes of newborns <6 months old to infants between 6 months and 1 year old, which are younger age categories than in my study. In my study we had only 1 infant (KK) that was 2 months old.

I did not have enough hair samples to categorize infants into smaller age groups, which would have allowed me to target a smaller time period within their development. Indeed, our youngest age group contained all infants ≤1.5 years old, and we were not able to break this down into even younger categories. Infants at Ngogo showed a mean maximum elevation in their fecal $\delta^{15}N$ above their mother of 2 ‰ (Badescu et al. 2017) while our study showed a maximum 2.6 ‰ elevation of mean hair keratin $\delta^{15}N$ between infants and their mothers. However, when we compared our data from single hair strands (N=118), the maximum elevation was 2.1 ‰, which was more consistent with data from mean fecal $\delta^{15}N$ ratios at Ngogo (Badescu et al. 2017). Infants showing $\delta^{15}N$ values nearly 2 ‰ higher than their mother is consistent with one trophic position and indicates exclusive suckling (Reitsema 2012). Human babies that are exclusively breastfed also show $\delta^{15}N$ values on average 2 ‰ to 3 ‰ higher than their mothers in their tissues (Fahy et al., 2014; Fuller et al. 2017). Our results are therefore in line with studies of human babies that used stable isotopes sourced from both collagen and keratin of various body tissues including bone, fingernails, blood, and hair strands (Tsutaya & Yoneda 2015; Fuller et el. 2006).

My results indicated that the relative contribution of milk in the diet started to decrease after 2 years of age in chimpanzees, using stable nitrogen isotopes sourced from hair keratin. This differs from results found when sourcing the stable nitrogen isotope from feces (Badescu et al. 2017). Badescu et al. (2017) found that the relative contribution of milk in the diet started to decrease at 1 year of age at Ngogo. However, the results reported here in the $\delta^{15}N$ values of hair keratin found in infant chimpanzees at Ngogo less than two years old (infants ≤1.5 and infants > 1.5 and <2 years old) are similar to chimpanzees at Taï, Cote D'Ivoire, as determined from stable

nitrogen isotope ratio differences in dentine (Fahy et al. 2014). Chimpanzees at Taï, Cote D'Ivoire also showed no isotopic evidence of the weaning process (i.e. a marked decrease in the contribution of milk in the diet) prior to 2 years of age (Fahy et al., 2014). My findings indicate that infants did not undergo a depletion in the heavy nitrogen isotope until after 2 years of age in their hair keratin similar to dentine stable nitrogen (Fahy et al., 2014). This means that the weaning process did not begin until infants were approximately 2 years old. However, when taking into account the possible 6 month range in the ages in which we can interpret these data, it may be more accurate to say that the weaning process began for infants between 1.5 and 2 years old.

One limitation of using fecal stable isotopes is that fecal stable nitrogen detects day-to-day changes in diet and therefore can show high daily variation in nursing patterns and $\delta^{15}N$ values (Reitsema 2012). Similar to stable nitrogen sourced from dentine, hair reflects dietary intake over several weeks or months and shows less variation in isotopic values, albeit over a less precise period of time (Reitsema 2012; Fahy et al. 2014; Badescu et al. 2017; Tsutaya & Yoneda 2015; Oelze 2015). Compared to fecal stable isotope results, stable nitrogen and stable carbon isotopes in hair keratin could better indicate at what age the relative contribution of milk in the diet starts to decrease for chimpanzee infants at Ngogo. Close to 600 fecal samples would be needed to investigate at what age the relative contribution of milk decreases and solid food increases in the infant diet. Compared to my study, Badescu et al. (2017) had more fecal samples that could be separated into more distinct age categories throughout infancy. Although Badescu et al. (2017) found that the relative contribution of milk in the diet starts to decrease at approximately 1 year old based on stable isotopes sourced from feces, my data might be more indicative of the transition to the consumption and reliance on solid foods in the infant diet near 2 years of age (see below section 4.6, Hair isotopic indicators of infant feeding transitions).

4.4.2 Infants >2 to >3 years old

Infants >2 to ≤3 years old appeared to be less enriched in the heavy nitrogen isotope, with an elevation of 0.9 ‰, which indicated that while these infants were still relying on maternal milk, the proportion of milk to solid food in their diet was likely lower, as these older infants were consuming more and more solid plant foods (Badescu et al. 2017; Reitsema 2012). There were no immature bonobos in this age group to compare $\delta^{15}N$ values with from Oelze's (2015) study.

Bonobo infants between 3 and 4 years old showed an elevation of 0.3 ‰ between infant and maternal hair strands (Oelze 2015). Compared to bonobos, chimpanzees at Ngogo over 3 years old showed a stronger nursing signal indicating infants were still enriched in the heavy nitrogen isotope at this age. This suggests that infants > 3 years old were likely in the weaning process and maternal milk still constituted a proportion of their diet (Badescu et al. 2017; Reitsema 2012; Bray et al. 2017), especially when taking into account the possible 6 month range in the ages during the period of hair growth. In the future, more hair samples from several categories (i.e. 3-4, 4-5, 5-6, 6-7) of infants should be collected to better discern when exactly a milk signal can – or can no longer – be detected.

Although chimpanzee infants over the age of 3 years old did not show $\delta^{15}N$ values that were identical to their mothers, they did closely resemble $\delta^{15}N$ values of male hair strands (only a 0.1‰ difference between infants and males). This similarity could be due to the animal protein that the males at Ngogo consume from hunting monkeys (e.g. colobus monkeys). During my field season we witnessed multiple hunting trips executed by the males, and they often ate animal material, much more than females in the group. Indeed, studies have shown that females seldom participate in hunts and while they do beg for meat from the adult male hunters, they often only receive scraps of bone or cartilage, if anything (Watts & Mitani 1999; Watts & Mitani 2002). This could explain the higher ratio of $\delta^{15}N$ in the male chimpanzee diet compared to adult females at Ngogo. Another way to explain the higher $\delta^{15}N$ in the adult male diet at Ngogo is that adult males have access to better quality food items that are higher in protein compared to other individuals in the group, such as new leaves which are high in protein and thus in nitrogen content. It is also possible that males are selecting foods richer in nitrogen since they consistently have first choice over females while foraging and this could also lead to a higher $\delta^{15}N$ value. Similarly, we might be able to infer that recently weaned chimpanzees might be able to consume certain "weanling foods" such as new, young leaves or other food stuffs that are higher in protein, and this might explain some of the elevated $\delta^{15}N$ values of infants above their mothers of the oldest age category of infants > 3 years old.

In summary, similar to stable nitrogen in fecal samples from Ngogo (Badescu et al. 2017), the difference between mean $\delta^{15}N$ between chimpanzee infants and their mothers decreased gradually with age. Stable nitrogen isotopes in hair keratin from the Ngogo chimpanzees still showed that infants over 3 years old were enriched in the heavy nitrogen isotope. The keratin stable

nitrogen isotope results thus suggest that weaning is a gradual process in chimpanzees that may start around 1.5 or 2 years old, and continues to progress over a few years as the proportion of maternal milk gradually decreases and the infants' diets become more vegetarian and resemble their mothers' diets (Reitsema 2012; Badescu et al. 2017; Bray et al. 2014). Once the weaning process is complete and the infant is no longer consuming milk, the $\delta^{15}N$ values of the infants should mirror their mothers. A slow rather than abrupt weaning process was also seen in the stable isotope results of the Taï chimpanzees (Fahy et al., 2014) and in both chimpanzee communities at Kanyawara (Bray et al. 2014) and Gombe (Londsdorf et al. 2014) based on behavioral observations of changes in nursing patterns. Fecal stable isotopes at Ngogo indicated that most chimpanzee infants completed the weaning process at approximately 4 to 4.5 years old (Badescu et al. 2017). We did not have any infants over 4 years old in our sample to confirm this age at the weaned event using hair keratin stable isotopes. Behavioural observations revealed that infants at Ngogo continue to make nipple contacts without milk transfer (comfort nursing) for up to 2 years after being physiologically weaned, when they are no longer consuming maternal milk (Badescu et al. 2017). Relying on observational data alone to establish weaned ages could result in unreliable and delayed weaning ages due to the presence of comfort nursing (Badescu et al. 2017; Motsumoto 2017; Londsdorf et al. 2014), which highlights the advantages of using isotopic measures, whether in feces or hair keratin, to more precisely pinpoint when nutritive suckling, with milk transfer, ends and to physiologically track weaning in wild primate infants.

4.5 Stable carbon isotope ratios ($\delta^{13}C$): ingestion of solid foods

4.5.1 Infants ≤1.5 and < 1.5 to 2 years old

The mean $\delta^{13}C$ ratios from infant hair strands indicated that as infants got older there was a depletion in ^{13}C and the mean $\delta^{13}C$ difference between chimpanzee infants and their mothers decreased. The difference between mother and infant $\delta^{13}C$ values is greater for infants ≤1.5 and infants <1.5 to 2 compared to the difference in $\delta^{13}C$ values for infants > 2 years old. Thus, it is easier to see the depletion in the heavy carbon isotope in infants > 2 years old. Overall, mean mother-infant differences in $\delta^{13}C$ ratios declined with infant age, which suggests that the process of transitional feeding is gradual, but starts when infants begin to incorporate solid food in their diet at a young age, possibly at just several months old as indicated by observations of infant feeding (Badescu et al. 2017; Bray et al. 2017). Interestingly, my results showing average $\delta^{13}C$

differences between younger infants and their mothers that were smaller than the averages of slightly older infants is consistent with bonobo hair strands (Oelze 2015). The highest mean $\delta^{13}C$ difference between infants and mothers at Ngogo were in the age category of 1.5 to 2 years old, having a maximum elevation of 1.4 ‰ greater than their mothers. Similarly, Oelze (2015) found that bonobos between 1 and 2 years old showed a maximum mean elevation of 1.0‰ between infant and maternal $\delta^{13}C$ ratios.

This is also consistent with fecal stable carbon isotope data from Ngogo chimpanzees (Badescu et al. 2017). Fecal stable carbon isotopes at Ngogo ranged from 0.4 ‰ to 0.9 ‰ and the highest mean $\delta^{13}C$ difference between infants and their mother was seen in infants between 1 and 1.5 years old. When using hair keratin to track stable carbon isotopes at Ngogo, the highest mean $\delta^{13}C$ difference between infants and their mothers was 1.4‰ in infants between 1.5 and 2 years old. Compared to Reitsema's (2012) study in a langur infant using $\delta^{13}C$ in feces to track infant nutritional development, infants at Ngogo were less enriched in stable carbon. Reitsema (2012) found a $\delta^{13}C$ difference of 3.5 ‰ between a suckling infant and its mother. However, our data was in line with the 1 ‰ enrichment expected for $\delta^{13}C$ in collagen from fingernails and hair keratin in humans (Fuller et al. 2006; Badescu et al. 2017; Tsutaya & Yoneda). In archaeology, diet reconstruction relies on an increase of 1‰ between the mean $\delta^{13}C$ values of predator and prey, and so we used an increase of 1‰ between infant and mother $\delta^{13}C$ values to indicate enrichment in the heavy carbon isotope between infants (predator) and mothers (Reitsema 2012).

Stable isotopes of carbon used to reconstruct diet are indicative of plants in infants' diets and are generally more sensitive to the addition of solid foods than stable nitrogen isotopes are sensitive to the decrease of maternal milk (Badescu et al. 2017; Fuller et al., 2006; Oelze 2015; Reitsema 2012). Behavioural observations at Ngogo confirmed that infants started to sample and ingest plant foods as early as a few months old (Badescu et al. 2017; Personal observation). Thus, when infants start to sample solid food at a young age, it could result in infant $\delta^{13}C$ values that resemble their mothers' much faster and earlier in their life compared to the $\delta^{15}N$ values (Badescu et al. 2017).

Chimpanzee infants at Taï in Cote d'Ivoire did not show any signs of transitional feeding prior to 2 years old (Fahy et al. 2014), however, this study tracked infant diet by measuring stable nitrogen extracted from dentine, a tissue with a slower turnover rate than hair keratin. The addition of solid food in early infancy may not have registered in the stable nitrogen isotopes found in

dentine due to dentine being less sensitive than stable carbon isotopes in detecting the addition of smaller amounts of solid food. Fahy et al. (2014) relied only on $\delta^{15}N$ values in dentine and did not track changes in the heavy carbon isotope. This could also explain why Fahy et al. (2014) found that the decreasing relative contribution of milk and increasing relative contribution of solid food in the infant diet was not apparent until infants were two years old.

4.5.2 Infants >2 years old to >3 years old

In line with fecal stable isotopes, infants over 2 years old showed a steady decline of stable carbon isotopes in hair keratin and showed $\delta^{13}C$ ratios that mirrored their mothers (Badescu et al. 2017). At approximately 2 years old, infants at Ngogo were likely able to eat plant foods similar to older infants. Other studies indicated that by the end of their third year, infants could eat all adult foods, including food difficult to process, although they often continued to receive considerable help from their mothers to access the most difficult to process foods, such as those with tough outer shells or tough to chew (Watts 1985; Matsumoto 2018; Badescu et al. 2020). This could explain the rapid decline in the differences between mothers and infants in their stable carbon isotopes after 2 years of age, as infants relied more and more heavily on solid plant food items and foraged more consistently with the rest of the group. The nutritional content of maternal milk can change throughout infancy, and can also be responsible for the rapid depletion in the heavy carbon isotope and $\delta^{13}C$ values in infants. Throughout lactation, the lipid concentration of milk increases as the amount of crude protein decrease, which causes a depletion of ^{13}C in the breast milk (Buss 1968). The introduction of solid foods, coupled with the depletion of ^{13}C in maternal milk, can cause infants to show a depletion in the heavy carbon isotope more rapidly (Reitsema 2012).

At Ngogo, chimpanzee infants 2 to 3 years old showed mean $\delta^{13}C$ ratios that were identical to their adult mothers, with a mean $\delta^{13}C$ difference of 0.0 ‰ between mothers and infants. This suggests that infants in this age group were likely in the weaning process and although they were still suckling and consuming protein from maternal milk, a large proportion of their diet was made up of solid plant foods (Badescu et al. 2017; Londsdorf et al. 2014; Bray et al. 2017). Surprisingly, infants at Ngogo older than 3 years old showed lower mean $\delta^{13}C$ ratios than their mothers. This result reflected a similar trend found by Oelze (2015) in bonobo hair keratin stable carbon isotopes. Bonobos between 3 and 4 years old also exhibited $\delta^{13}C$ ratios lower than their mothers, which signified that infants at this age group were more depleted in the heavy carbon isotope than their

mothers (Oelze 2015). Since stable carbon isotopes ($\delta^{13}C$) primarily reveal the sources of carbon derived from mainly plants in the diet (Badescu et al. 2017), it is possible that infants > 3 years old were targeting plant parts that were more depleted in ^{13}C. However, mothers and infants often forage with one another in the same part of the canopy, and therefore, consume the same food items (Badescu et al. 2017). It could also be possible that weanling food items eaten by infants are depleted in ^{13}C and infants are consuming more of these as they start to approach the age that they are weaned. Our sample of chimpanzee hair strands belonging to infants > 3 years old (N=2 hairs) as well as Oelze's sample of bonobo hairs (N=2 hairs) were both small and therefore more hairs are required from infants in this age group to further investigate if and why stable carbon tended to be more depleted in older infant hair strands.

4.6 Hair isotopic indicators of infant feeding transitions

An challenge in assessing infant nutritional development is determining the precise age when infants shift from relying on maternal milk to solid food. Due to an infant's tendency to sample foods rarely within the first several months after birth, I suggest that the age at which an infant habitually consumes solid food and moves toward nutritional independence may be more developmentally significant than the age at first solid food consumption (Badescu et al. 2017; Bray et al. 2017). While the age at first solid food consumption may be observed to be very early in infancy, it does not indicate when exactly solid food becomes the most important source of energy, consumed daily, and a necessity for infant growth and development in wild chimpanzees. Results from both fecal (Badescu et. al 2017) and hair keratin stable isotopes in this study showed that even young infants that relied most heavily on maternal milk exhibited low mean $\delta^{13}C$ differences compared to their mothers, which confirmed that infants started to ingest solid foods within the first few months of life. While observations and stable isotopes from feces showed that infants sampled solids at 3 months old (Badescu et al. 2017; Badescu et al. 2020), stable isotopes in hair showed that infants started regularly consuming solid foods at 2 years old. Infants between 1.5 and 2 years old had $\delta^{13}C$ value elevated by 1.5 ‰ compared to their mothers, but this difference dropped to 0 ‰ in infants 2 to 3 years old. This was also seen in the Taï chimpanzees (Fahy et al. 2014) and suggests that transitional feeding commences at 2 years old (Bray et al. 2017). This is not in line with my hypothesis that infants should start to regularly consume adult food at 1 year

old, which was a prediction based on fecal stable isotope results at Ngogo (Badescu et al. 2017). My data here showed that infants became even more enriched in the heavy nitrogen and carbon isotopes after 1 year old, between the ages of 1.5 and 2 years old, before gradually decreasing. Thus, our results of hair keratin show that chimpanzee infants do not begin to rely on adult foods until they are around 2 years old. However, we must again consider the six-month grace period of hair growth before the hair is shed. If a hair strand from a two-year-old represented an average summary of the infant's diet six months prior to when it was shed, then it is possible that infants are starting to experience a gradual decrease in milk somewhere between the age of 1.5 and 2 years old.

While fecal stable isotopes are sensitive to occasional sampling of solid food, hair stable isotopes reveal overall diet similar to stable isotopes of dentine (Fahy et al. 2014; Tsutaya & Yoneda 2015; Oelze 2015). Ape infants other than humans likely outgrow the milk energy available to them at around 1 year of age (Van Noordwijk et al., 2013), but our results in addition to a few other studies show that chimpanzee infants may not need to regularly supplement their diet until slightly later in infancy (Fahy et al. 2014; Bray et al. 2017; Matsumoto 2017). My study showed that infants underwent a significant transitional period at approximately 2 years old. Although transitional feeding began prior to two years old, it is likely that transitional feeding takes a more prominent role in infant feeding development at this older age. This is when solid food may become crucial to infants' diet and they rely on these food items to meet their growing energy needs. Matsumoto (2017) found that it was not until chimpanzees were approximately 3 years old that major feeding behaviors changed. Infant chimpanzees at Kanyawara exhibited a steady increase in the amount of time allocated towards feeding on solid food with age and the duration of foraging matched that of adult levels between 4 and 6 years old (Bray et al. 2017). Therefore, it is likely that although infants sampled adult foods at a young age, they didn't start moving towards nutritional independence until later in infancy. Since $\delta^{15}N$ values track major protein sources in the diet, the fact that infants were still enriched in the heavy nitrogen isotope past 3 years old, despite being depleted in ^{13}C, confirms that well after solid food is a staple in the diet, infants still continue to suckle to fulfill nutritional and social needs (Londsdorf et al. 2014; Badescu et al. 2017; Matsumoto 2017;).

Physiological weaning starts prior to the weaned event (cessation of nipple contact) and it is during this time that infants reduce their dependence on maternal milk, although milk still

constitutes a portion of the infant diet for several years before the weaned event occurs (Badescu et al. 2017; Matsumoto 2017; Lonsdorf et al. 2014). Studies showed that nursing patterns and the time allocated to suckling in chimpanzees were stable throughout infancy (Badescu 2017; Bray et al 2017), so it is possible that maternal milk provides the same source of nutrients to older infants as it does younger, but the older infants have outgrown the energy maternal milk provides on its own and demand more sources of energy that they obtain through the foraging of plant foods.

Similar to fecal stable isotopes, I predicted that hair stable isotopes would reveal that the weaned event occured at 4 to 5 years old (Badescu et al. 2017). As infants approach the weaned event, which generally occurs at 4.5 years (Badescu et al. 2017; Reitsema 2012; Bray et al. 2017; Fahy et al. 2014; Matsumoto 2017) the mean $\delta^{15}N$ for infants should continue to decrease until they are the same as those of their mothers. However, our data did not have any samples from infants between 4 and 5 years old to determine the weaned age using hair. Future studies should focus on collecting hair samples from older infants to detect the completion of weaning using stable isotope analysis of hair keratin.

4.7 Limitations and future directions

Due to the different techniques we used to collect hair strands from infant and adult mother chimpanzees, we expected that 51 of the 169 hair strands were the most accurate in correctly matching the hair strands to the individuals to which they belonged, which reduced our sample size. The 51 hair strands chosen as the most reliable samples belonged to only 9 infant-mother pairs (and 12 sample IDs, meaning that these were 12 separate clumps that were collected, which together yielded 51 hairs from 9 mother-infant pairs). In this set there were 26 hairs that came from the same infant-mother pair (CH-PN) during 3 separate occasions (3 sample ID's) and this pair belonged to the infant age category between 1.5 and 2 years old. We found that out of these 26 hairs, 16 belonged to the infant CH, while the remaining 10 hairs belonged to her mother PN. The second infant-mother pair in that age group was another infant of PN's (KO-PN) and was collected in the 2013 field season before CH was born. Therefore, our results from hair strands in the age category of infants between the ages of 1.5 and 2 years old belonged to the same mother and her two infants. It is also important to note that from our sample of 51 hair strands, we were only able to collect 2 infant and 2 maternal hair strands from infants between the ages of 2 and 3 years old as well as infants over 3 years old. The infant-mother pair TO-CA represents both samples in the

age group of infants between 2 and 3 years old thus, only 3 individuals represent our sample of all infants over 2 years old. Moving forward, more infants in each age category are required to better estimate accurate ages when infants shift from relying on maternal milk to solids foods and to determine the age at which an infant habitually consumes and relies on solid food. This will result in stable isotope values that can better represent all infants in that age group at Ngogo. Collecting more hair samples from newborns as well as older infants that are near the end of the weaning process, or that have been weaned, are also needed to properly track infant diet and important transitions in infant feeding development. More samples from a broader range of ages throughout infancy would help us better estimate when "weaning foods" (Reitsema 2012) constitute a greater part of the diet, which would suggest that the infant is habitually consuming and relying on these foods.

Keratinous tissues such as hair illustrate dietary intake that span several weeks and provides records of dietary signals over a longer period of time, however, using hair still has its own limitations surrounding hair growth and structure (Oelze 2014; Oelze et al. 2020; Tsutaya & Yoneda 2015; Retisema 2012). Due to its nature to grow incrementally and remain metabolically inactive before being shed, the strand of hair collected may not reflect the isotopic signatures of the new food sources prior to being shed (Oelze 2015; Oelze et al. 2020). There is also a reduced isotopic response after hair emergence due to the recycling of the body's own amino acids, which means that a true isotopic signature of recently consumed food may require several weeks before being detected in the strand of hair (Oelze 2015; Tsutaya & Yoneda 2015). Therefore, the lag time between the actual dietary change and the incorporation of isotopic and trace elemental signals into the hair keratin needs to be better understood, especially because of the higher turnover rate that softer body tissues have, like hair strands, relative to harder tissues like bone collagen or dentine. In addition to this, the hair strand collected needs to be fresh (i.e., recently shed by the individual) otherwise it can reflect isotopic signatures of food items consumed earlier in an individual's life (Oelze 2015).

Primate studies that utilize stable isotopes of hair keratin to reconstruct diet rely on human hair growth rates of approximately ~1 cm per month and use this rate as a proxy for nonhuman primate hair growth because hair growth rates are unknown in most species (Oezle 2015; Oelze et al. 2020; Tsutaya & Yoneda 2015). Future research using stable isotope analysis of hair should seek to enhance our understanding of the time lags that are required for isotopic turnover in the

body and on hair growth rates in wild primates (Oelze 2015).

4.8 Conclusion

To help mitigate observational and fecal stable isotope limitations (Badescu et al. 2017), and to better understand the weaning process and the age at which chimpanzee infants habitually consumes solid food during nutritional development, I used hair keratin stable carbon and nitrogen isotopes from wild chimpanzee infants and their mothers at Ngogo, Uganda. While transitional feeding for primate infants begins when they first consume adult food, I suggested that the age at which infants regularly consume and rely on adult food may be more developmentally significant than the age at first solid food consumption. Determining this developmentally significant period within transitional feeding is difficult to detect using observations or fecal stable isotopes (Badescu 2017; Matsumoto, 2017; Bray et al. 2014). We applied the combination of stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to hair keratin in order to try to more accurately pinpoint when infants start to rely predominantly on solid food for nutritional stability and move towards nutritional independence. From our research, we worked to develop a protocol that can be used in future studies that aim to reconstruct primate diets using stable isotopes of the keratinous tissue found in hair strands.

When compared to humans and other non-human primates, transitional feeding is a much longer period in ape development as infants gain ecological competence, learn what foods to ingest, and attain the skills needed to forage and process these food items (van Noordwijk et al. 2013; Londsdorf et al. 2014; Bray et al. 2017; Badescu et al. 2017). While fecal stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) showed that solid foods took a primary role in the infant diet at one year old (Badescu et al. 2017), hair keratin stable isotopes suggested that solid food did not become a primary nutritional source that infants relied on until about two years old. This period of transitional feeding in chimpanzees, and the age when infants start to rely on solid food is much later compared to human babies.

Complementary feeding in human babies (similar to transitional feeding in nonhuman primates) begins at 6 months old, when babies are fed specially selected and modified versions of adult foods that are easily digestible (Sellen 2007; Sellen 2009). Provisioned foods are not only given to infants by their mother to sustain their energy needs, but also other caregivers, since

humans involve alloparents to aid in caring for offspring (Hawkes et al. 1998; Sellen 2009; Sellen 2007). In humans, complimentary feeding has been shortened by several years, which has resulted in a relatively early weaning age compared to non-human primates (Sellen 2007; Sellen 2009; Hrdy 2009). The evolutionary steps that led human babies to rely on solid food items earlier in infancy, allowing them to advance rapidly through key steps of transitional feeding, need to be better understood. According to our hair stable isotope analysis of infant chimpanzees, the start of the weaning process, as well as the age solid food became an important food source in the infant diet to support growth and development, was later than predicted. If chimpanzees do not rely on solid food until two years of age, while human babies rely on solid food much earlier in development, then the key step to determining the evolution of early weaning in humans could be within this significant period when infants rely on solid foods to survive.

This relatively novel approach in field primatology can provide baseline data needed to better understand the evolution of primate life histories and can be used to determine precise ages at feeding transitions. This study can be used to help discover the key steps in our evolutionary history that allowed human infants to progress more rapidly through transitional/complementary feeding, and may shed light on the evolutionary steps that led to the human ability to wean human babies earlier in infancy compared to our ape cousins. My M.Sc thus provided basic data to reconstruct the evolutionary path that led to our own species' unique early life history patterns (Sellen 2007; Sellen 2009).

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Appendix

Table 8. All hair strands listed an independent sample ID's. Infant samples are distributed based on their age when the sample was collected. All maternal and male hairs are in their own category and represent one mean $\delta^{15}N$ value of 6.8 and $\delta^{13}C$ value for that category.

Infant age category	**Sample ID**	**Total number of hair strands**	**Stable nitrogen isotope ratio ($\delta^{15}N$) in permil (‰)**	**Stable carbon isotope ratio ($\delta^{13}C$) in permil (‰)**	**Weight (mg) of hair sample for stable isotope analysis**
Infants younger than 1.5 years old	727DE	17	8.1	-23.2	0.123
	245DD				0.207
	661LZ				0.246
	261AC				0.241
	576NI				0.478
	346HN1				0.324
	491KK3				0.279
	591NI1				0.243
	601KK				0.119
	902AC12				0.073
	617DE1				0.080
	449HE				0.032
	363HE				0.057
	491KK2				0.033
	109CFD3				0.054
	00BX				0.244
Infants between the age of 1.5 and 2 years old	235KO	23	8.9	-22.2	0.097
	523KO1				0.161
	671SE				0.167
	852KR				0.113
	112PNKOVA2				0.360
	112PNKOVA3				0.354
	112PNKOVA4				0.058
	112PNKOVA5				0.336
	112PNKOVA7				0.323
	112PNKOVA8				0.289
	112PNKOVA9				0.100
	112PNKOVA10				0.221
	112PNKOVA11				0.097

	112PNKOVA12				0.133
	112PNKOVA13				0.122
	112PNKOVA14				0.167
	112PNKOVA15				0.091
	112PNKOVA16				0.091
	111PNCH2				0.060
	111PNCH3				0.075
Infants between the age of 2 and 3 years old	88EE	11	7.6	-23.4	0.198
	122TO				0.131
	83KA				0.392
	840KO				0.181
	496FN				0.100
	40TO1				0.155
	562EL				0.171
	788TO2				0.054
	679AA				0.086
	435HL				0.607
	82RUZW				0.298
Infants older than 3 years old	644DY	23	7.8	-23.2	0.189
	365PW				0.346
	124PW				0.103
	482PO				0.107
	590B3				0.119
	765KA				0.193
	587EU				0.224
	555HT				0.219
	233KA				0.500
	178GU				0.113
	24GU				0.462
	324KA12				0.088
	352NA1				0.132
	499HD2				0.181
	580KA1				0.207
	352NA23				0.075
	227ZW				0.058
	632SS				0.077
	125SS				0.027
	533BW				0.075
	128ARFA				0.292
	539BI				0.409

	119RB				0.337
Female maternal adult	174FZ	67	6.8	-23.6	0.184
	51AE				0.254
	222NT				0.307
	851DH				0.475
	74SH				0.819
	155LN				0.888
	68AR				0.943
	216JE				0.355
	92RE				0.275
	258GD1				0.187
	258GD2				0.096
	418VT				0.637
	73NT1				0.507
	218FI				0.091
	226BH				0.058
	162SH				0.049
	816JE				0.017
	320SS				0.100
	346HN2				0.287
	346HN3				0.122
	40TO2				0.040
	491KK1				0.197
	499HD1				0.656
	523KO2				0.186
	523KO3				0.02
	580KA2				0.064
	591NI2				0.299
	591NI3				0.125
	591NI4				0.193
	617DE2				0.070
	788TO1				0.069
	385CN				0.041
	112PNKOVA1				0.389
	112PNKOVA6				0.156
	550KU1				0.402
	550KU2				0.130
	550KU3				0.042
	550KU4				0.038
	550KU5				0.111

	550KU7				0.073
	550KU8				0.028
	550KU9				0.107
	550KU10				0.024
	550KU111213				0.075
	110PNKOVA1				0.505
	110PNKOVA2				0.390
	110PNKOVA3				0.293
	110PNKOVA4				0.427
	110PNKOVA5				0.473
	110PNKOVA6				0.389
	110PNKOVA7				0.252
	111PNCH1				0.522
	16A2AT1				0.309
	16A2AT2				0.388
	18AT				0.154
	79JE				0.333
	126PN				0.468
	331RE				0.242
	71A3C				0.083
	134WHA1				0.140
	56WHA				0.138
	552BC				0.035
	43ANB3				0.107
	274JE				0.058
	43ANB1				0.108
	43ANB2				0.107
	82RUZWR1				0.173
Male adult	115HURM1	28	7.3	-23.7	0.276
	115HURM2				0.515
	115HURM4				0.304
	115HURM5				0.332
	115HURM6				0.406
	115HURM7				0.440
	115HURM3				0.341
	120MTMK1				0.301
	120MTMK3				0.223
	120MTMK4				0.455

	113WNBK1				0.450
	113WNBK2				0.457
	113WNBK3				0.342
	114GSWN2				0.457
	114GSWN3				0.364
	118REBW1				0.326
	118REBW2				0.609
	118REBW3				0.545
	118REBW4				0.523
	127BTDJ1				0.298
	127BTDJ2				0.243
	127BTDJ3				0.184
	127BTDJ4				0.654
	117GSWSSA5				0.679
	117GSWSSA2				0.449
	117GSWSSA3				0.393
	117GSWSSA4				0.349
	117GSWSSA6				0.641
Total Hairs	169				

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