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RESEARCH ARTICLE

Introducing Fecal Stable Isotope Analysis in Primate Weaning Studies

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This research investigates the potential of a new, noninvasive method for determining age of weaning among primates using stable carbon and nitrogen isotope ratios in feces. Analysis of stable isotope ratios in body tissues is a well-established method in archeology and ecology for reconstructing diet. This is the first study to investigate weaning in primates using fecal stable isotope ratios. Diets of a single François' langur (*Trachypithecus francoisi*) mother–infant pair at the Toledo Zoo are reconstructed using this technique to track changes in infant suckling behavior over the weaning period. Stable isotope ratios in feces are sampled instead of more traditional samples such as bone or hair to enable daily, noninvasive snapshots of weaning status. Isotopic assessments of weaning status are compared to visual assessments to identify any discordance between the two. Three measurements documented the transition from breast milk to solid foods: stable carbon isotope ratios ($\delta^{13}C$), stable nitrogen isotope ratios ($\delta^{15}N$), and nitrogen content of feces (%N). It appears that solid foods were introduced at approximately 2 months of infant age, but that nursing continued into the 12th month, when sample collection ceased. Stable isotope data exposed a much longer weaning period than what was expected based on previously published data for captive langurs, and clarified visual estimates of weaning status. This reflects the method's sensitivity to suckling at night and ability to distinguish actual nursing from comfort nursing. After testing this method with zoo animals, it can readily be applied among wild populations. An isotopic approach to weaning provides a new, accurate, and biologically meaningful assessment of interbirth intervals, and facilitates a better understanding of mother–infant interactions. Both of these outcomes are critical for developing successful conservation strategies for captive and wild primates. Am. J. Primatol. 74:926–939, 2012.

Key words: nursing; lactation; carbon; nitrogen; François' langur

INTRODUCTION

Identifying the age at which primate infants are fully weaned is an obstacle for field primatologists relying solely on behavioral observations [e.g., Jay, 1965; Lee, 1996; Rajpurohit & Mohnot, 1990]. It is difficult to observe weaning among arboreal or poorly habituated groups, and because researchers are limited to diurnal observations they are unable to detect suckling at night [Lee, 1996], and cannot account for the actual rate of milk transfer [Martin, 1984]. Additionally, regardless of how long an infant appears to suckle, the behavior may be "comfort nursing" in which an infant makes contact with its mother's nipple months after weaning takes place as a means of reassurance rather than sustenance [Jay, 1965; Lee, 1987]. Although comfort nursing is physiologically meaningless, actual nursing has critical physiological, behavioral, and methodological ramifications in primate studies. Delaying the mother's return to sexual receptivity [Charnov, 1991; Lee, 1996; Pagel & Harvey, 2002], affecting an infant's vulnerability to infanticide [Crockett & Sekulic, 1984], and distinguishing infants from juveniles in groups are some of these. Because interbirth intervals are considered when developing population viability analyses, precise assessments of weaning are vital for managing effective conservation strategies [Caro, 1999].

Weaning plays a critical role in primate group interactions and reproductive strategies [Charnov, 1991]. Because a mother resumes menstruation as weaning progresses, age at weaning determines the length of interbirth intervals and the number of offspring a female can bear in her lifetime [Lee, 1987]. Weaning represents a genetic conflict of interests

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between mother and infant who are both attempting to maximize their evolutionary fitness [Martin, 1984; Trivers, 1974]. Mothers are 50% related to all their infants, whereas infants are 100% related to themselves and, excepting the case of monozygotic multiple births, 50% or 25% related to their siblings. Because of this, it is in the infant's best interest to prolong nursing, while mothers should be motivated to wean their young as early as possible in the interest of reproducing again. This idea remains controversial [Godfrey, 1995; Maestripieri, 2002]. One reason why explaining parent–offspring conflict is difficult is the lack of a precise measurement of infant weaning status [Martin, 1984]. Proxy indicators of weaning such as time spent on the mother's ventral surface, the transition from riding to walking, mother–infant conflicts, and amount of solid foods in the infant's diet provide the nearest visual estimates of early life history [Rajpurohit & Mohnot, 1990; Rhine et al., 1984; Zhao et al., 2008]. This study uses a new technique—assessment of stable carbon and nitrogen isotope ratios in feces—to determine daily snapshots of weaning status among François' langurs (*Trachypithecus francoisi*) at the Toledo Zoo in Ohio.

Isotopes are atoms of the same element with the same number of protons, but different numbers of neutrons. Stable isotope analysis in anthropology and ecology is based on the principle "you are what you eat": stable isotope ratios of foods are reflected in tissues of their consumers. Stable carbon and nitrogen isotopes show trophic-level enrichment patterns, revealing relative reliance on plant vs. animal foods [Chisholm, 1989; DeNiro & Epstein, 1981; Fuller et al., 2004; Minawaga & Wada, 1984; Schoeninger, 1989].

Stable isotope analyses using both hair and feces have been applied to studies that estimate primate diet [gorilla (*Gorilla gorilla*): Blumenthal et al., 2011; baboon (*Papio ursinis*): Codron et al., 2006; ring-tailed lemur (*Lemur catta*): Loudon et al., 2007; bonobo (*Pan paniscus*): Oelze et al., 2011; Sponheimer et al., 2006; howler monkey (*Alouatta palliata*), spider monkey (*Ateles geoffroyi*), muriqi (*Brachyteles arachnoids*), and capuchin (*Cebus capucinus*): Schoeninger et al., 1997; galago (*Galago garnettii* and *G. zanzibaricus*) and white-footed sportive lemur (*Lepilemur leucopus*): Schoeninger et al., 1998; chimpanzee (*Pan troglodytes*): Schoeninger et al., 1999]. Beyond diet reconstructions, stable isotope ratios have seen limited applications to studying primate nutritional stress [bonobo (*P. paniscus*): Deschner et al., 2012; human (*Homo sapiens*): Fuller et al., 2004, 2005, 2006], and have untapped potential in nonhuman primate weaning studies.

Throughout weaning, an infant's diet gradually shifts from 100% mother's milk to 100% solid foods. The transition can be monitored with stable carbon and nitrogen isotope ratios of body tissues. Stable isotope ratios are commonly used by archaeologists to investigate weaning in past human populations using bone and teeth [Herring et al., 1998; Richards et al., 2002], and have been used to monitor the transition among living humans using hair and fingernails [Fogel et al., 1989; Fuller et al., 2006]. Some research has used strontium stable isotopes in hard tissues (teeth) to study weaning stress in primates [Dirks et al., 2010]. Outside anthropology, ecologists have used stable isotope signatures to study weaning in mammals, including isotopic analyses of bone collagen to track the durations of lactation and weaning among seals [Newsome et al., 2006], of hair to investigate lactation schedules and breeding tactics of meerkats [Dalerum et al., 2007], and of blood components to identify a weaning signal in 14 other species of mammals [Habran et al., 2010; Jenkins et al., 2001; Kurle, 2002; Polischuk et al., 2001]. In these studies, researchers observe differences in stable isotope ratios between the mother and infant when the infant is suckling (trophic-level effects), differences that decrease throughout or after weaning until the stable isotope ratios of mother and infant are indistinguishable and the pair consumes the same diet [but see exceptions in Jenkins et al., 2001].

This is the first study to use stable isotope ratios in feces to track weaning in mammals. Samples used in previous isotopic studies of weaning—bone, hair, and blood—are difficult or unethical to collect from free-ranging primates, many of which are endangered, and collection can be stressful even for captive animals. Furthermore, these tissue types are formed over periods of weeks to years, representing a time-average of diet rather than the precise life-history events represented by excretion products. Feces are noninvasive to collect, and are synthesized quickly [Codron et al., 2006; Hwang et al., 2007; Sponheimer et al., 2003a], allowing weaning to be tracked in day-to-day increments. The rate at which feces are synthesized from diet and excreted varies between species and depends on digestive physiology [Sponheimer et al., 2003a]. For example, ruminants store food in their digestive tracts for relatively long periods of time during which semidigested cuds are regurgitated and chewed, meaning that ruminant feces do not represent the most recent meals. Sponheimer et al. [2003a] report that a switch from C_3 to C_4 fodder took 200 hr to be fully reflected in feces of a ruminant alpaca, compared to only 60 hr reported for a nonruminant horse. Langurs are nonruminant foregut fermenters with also the capacity to ferment digesta in their hindguts [Caton, 1999; Van Soest, 1994, p 57–76]. This digestive anatomy, uncommon among primates, enables langurs to extract energy from structural polysaccharides, such as cellulose from leaves. In a study of feces retention times between two langur species, *Trachypithecus obscurus* and *Trachypithecus cristatus*, the mean retention time of food in the digestive tract

was 2.5 days [Caton, 1999]. This is slow compared to cercopithecines, whose mean retention time is just 1.5 days. Slower digestion in colobines reflects their unique dual-chamber fermenting anatomy. Diet information from a colobine fecal sample is therefore more blurred than that from other primate species. Nevertheless, a 60-hr snapshot of weaning status is drastically more precise than what is possible using other methods.

Predicted Values

To monitor the weaning process, three measurements are considered: stable carbon isotope ratios ($\delta^{13}C$), stable nitrogen isotope ratios ($\delta^{15}N$), and nitrogen content of feces (%N). Stable isotope values are expressed as delta ratios (δ) of the heavier to the lighter isotope in the sample in relation to the ratio of the heavier to the lighter isotope in a standard substance (Vienna Pee Dee Belemnite for $\delta^{13}C$ and AIR for $\delta^{15}N$) using permil (‰) values.

$\delta^{13}C$ ratios are useful for measuring the vegetal component of diet, including the amount and type (e.g., grasses; leaves) of plants consumed. In this study, $\delta^{13}C$ data are used to monitor (1) the mother's largely vegetarian diet, and (2) the introduction of carbon-rich plant foods to the infant's diet, distinguishing between mouthing of solid foods and actual consumption of those foods. $\delta^{15}N$ ratios detect an animal's trophic position (i.e., a suckling vs. a weaned infant), and are of primary interest here. %N measures crude protein content and also monitors the reduction of breast milk in the diet. Stable carbon and nitrogen isotopes in feces represent the same isotope pool that is retained in the body for tissue formation after excretion of urea, meaning feces should show trophic-level enrichment patterns similar to hair, collagen, and nails [Codron et al., 2005].

Stable nitrogen isotope ratios in animal tissues reflect trophic position in the local food web. This is because, as nitrogen from food sources is ingested and incorporated into consumer tissues, the lighter isotope ^{14}N breaks down more readily than the heavier isotope ^{15}N, and is excreted with urea [Steele & Daniel, 1978]. As a consequence, urea is isotopically "light," while remaining $\delta^{15}N$ ratios of consumer tissues such as bone, hair and milk are approximately 3‰ higher than those of food sources [Minawaga & Wada, 1984]. Because most plants exhibit $\delta^{15}N$ ratios near 2–3‰ [Nardoto, 2006], it is predicted that the langur mother investigated in this study will have body tissues with a $\delta^{15}N$ ratio of approximately 5–6‰. No reports of stable isotope values of nonhuman primate milk could be found in previously published literature, but among humans, it has previously been reported that the $\delta^{15}N$ value of human breast milk protein (the milk component primarily used for infant tissue-building) is similar to that of body tissues [Fuller, 2003].

Studies of small mammals demonstrate that fecal $\delta^{15}N$ is enriched by approximately +2.5‰ compared to $\delta^{15}N$ of diet [Hwang et al., 2007]. This diet–feces enrichment is similar in llamas (+3.0‰) [Sponheimer et al., 2003c]. Feces of nursing primate infants are therefore expected to exhibit $\delta^{15}N$ values approximately 2.5‰ higher than $\delta^{15}N$ of their mothers' milk (milk having an expected value of 5–6‰). That is, feces from a suckling infant should exhibit $\delta^{15}N$ ratios of 7.5–8.5‰. Feces of the mother should be 2.5‰ higher than that of *her* available plant foods (having values near 2–3‰), or 4.5–5.5‰. As weaning progresses, it is predicted that this 3‰ difference between feces of infant and mother will gradually decline, and finally disappear as the pair eventually consume the same foods.

Stable carbon isotope ratios also differ systematically between diet and consumer. Between plants and consumer bone collagen, there is a positive ^{13}C enrichment of approximately 5‰ due to metabolic fractionation [Schoeninger & DeNiro, 1984]. Because nearly all foods provided to langurs at the Toledo Zoo are C_3 plants exhibiting $\delta^{13}C$ values of around −26.0‰ [Nardoto, 2006; Schoeller et al., 1986; Schoeninger et al., 1998], $\delta^{13}C$ values of the mother's collagen are predicted to be approximately −21.0‰. Collagen and milk protein have similar $\delta^{13}C$ values. Fuller [2003] reports that milk protein $\delta^{13}C$ is approximately 1–2‰ lower than fingernail $\delta^{13}C$ from the same mother. Similarly, collagen $\delta^{13}C$ is approximately 1.4‰ lower than fingernail $\delta^{13}C$ [O'Connell et al., 2001]. Thus, the suckling infant's diet has an expected $\delta^{13}C$ value similar to its mother's predicted collagen value of −21.0‰.

Most controlled experiments investigating the relationship between diet and feces $\delta^{13}C$ have been conducted on large-bodied foregut fermenters such as llama. Their fecal $\delta^{13}C$ values tend to be approximately 0.3–1.3‰ lower than diet [Sponheimer et al., 2003a]. Controlled feeding experiments suggest the diet–feces space is smaller for C_3 diets (−0.6‰) than for C_4 diets (−1.0‰) [Sponheimer et al., 2003a]. Larger diet–feces differences of up to −5.9‰ are reported for smaller mammals that use hindgut fermentation, such as chipmunk and vole [Hwang et al., 2007]. Neither large foregut ruminants nor small hindgut fermenters present an ideal model for langurs, which are medium-bodied, nonruminant foregut fermenters [Caton, 1999]. For this study, the $\delta^{13}C$ diet–feces space of −3.1‰ will be used because it was the mean value determined experimentally by Hwang et al. [2007] to be appropriate for small-bodied animals fed mixed C_3/C_4 diets, which the langurs at the Toledo Zoo consume. With this −3.1‰ diet–feces spacing value in mind, feces of the mother are predicted to exhibit $\delta^{13}C$ values around −29.1‰. Feces of the nursing infant are expected to exhibit $\delta^{13}C$ values 3.1‰ lighter than breast milk, or −24.1‰. The expected 5‰ $\delta^{13}C$

difference between feces of mother and infant should gradually decline through weaning as mother and infant come to consume the same foods.

$\delta^{15}N$ primarily reflects the major calorie sources in diet. If an infant meets 100% of its caloric needs from nursing, its $\delta^{15}N$ ratio will not necessarily reflect minor amounts of nitrogen-poor plant foods added on top of milk consumption. Plant foods, which are carbon-rich, have a stronger effect in diluting the carbon pool available to the infant, making $\delta^{13}C$ more sensitive to the introduction of plant foods in diet [Fuller et al., 2006]. Although $\delta^{15}N$ ratios might show little change with the introduction of solid foods, $\delta^{13}C$ ratios may show an earlier and more rapid decline, as has been documented among fingernails of human infants [Fuller et al., 2006]. The addition to solid foods is significant as it exposes the infant to a new pathogen environment, and reduces the lactation stress on a female who may then return to estrus. $\delta^{13}C$ will be used as a secondary source of information on weaning.

Nitrogen content (%N) in feces is another useful measure that represents crude protein content of diet. In previous studies of primates, nitrogen content was used to assess diet and habitat quality [Chapman et al., 2005; Codron et al., 2006]. %N values are produced in the process of stable isotope analyses and in the present study will be a third measurement used to detect consumption of breast milk, which among Old World monkeys (baboons, talapoin monkeys, rhesus macaques) contains approximately 16 mg/ml protein [Buss, 1968; Buss & Cooper, 1970; Kunz & Lönnerdal, 1993]. It is expected that the infant will exhibit higher %N than its mother while subsisting off breast milk and that %N will gradually decline as the infant is weaned onto less nitrogen-rich plant foods. Nutritional status of mothers is an important factor influencing the weaning process [Lee, 1987]. In this study, %N data of the mother while she is pregnant, lactating, and nonlactating are investigated to examine the crude protein content of her diet and subsequently her nutritional status throughout these phases.

The first goal of this research is to investigate the isotopic relationships between feces and diet in a captive mother–infant François' langur dyad. Data on the diet–feces relationship used as an interpretive baseline for this study are from small mammal studies including primarily rodents; there is currently no primate-specific experimental stable isotope spacing data relating diet to feces. Once this relationship is assessed among captive animals with semicontrolled diets, the method can be applied to other mother–infant pairs in both captive and wild groups, and will require fewer fecal samples to track weaning. The second goal of this research is to evaluate the different types of information provided by stable carbon and nitrogen isotopes in relation to the weaning process. A final goal of this study is to evaluate isotopically the reliability of observational data in assessing weaning age of the François langur infant. The extent of any incongruence between observational and chemical methods can be considered toward a more precise assessment of weaning. By investigating François' langurs, which are highly endangered, this study provides a new perspective on weaning behavior and interbirth intervals for this species that will help improve species survival plans and population viability analyses. Based on the above discussions, the following hypotheses are tested:

(1) Feces of the mother will exhibit $\delta^{13}C$ values of approximately $-29.1‰$.
(2) Feces of the suckling infant will exhibit $\delta^{13}C$ values of approximately $-24.1‰$ during the first month after birth.
(3) The expected $\delta^{13}C$ difference between feces of the mother and of the suckling infant will decrease and disappear as weaning proceeds.
(4) Feces of the mother will exhibit $\delta^{15}N$ values of 4.5–5.5‰.
(5) Feces of the infant will exhibit $\delta^{15}N$ values of 7.5–8.5‰ during the first month of age.
(6) The expected $\delta^{15}N$ difference between feces of the mother and of the suckling infant will decrease and disappear as weaning proceeds.
(7) $\delta^{13}C$ values of infant feces will decline earlier and more rapidly than $\delta^{15}N$ values.

METHODS

Materials

The protocol for this study adheres to the legal requirements of the United States and the specific principles of the American Society of Primatologists for the ethical treatment of animals, and met the approval of the Toledo Zoo Animal Care and Management Committee and the Executive Director.

In this pilot study, fecal stable isotope analysis is applied to a captive mother and infant François' langur dyad at the Toledo Zoo and Aquarium. François' langurs are endangered primates that occupy subtropical limestone hill regions of China and Vietnam [Li et al., 2007]. Historically their range was much larger but due to habitat destruction and hunting, in the last few decades numbers have dramatically declined [Hu & Wei, 2002; Li et al., 2007; Zhou et al., 2007]. In Guangxi Province, China, which in the 1980s contained the largest global population of François' langurs (4,000–5,000 individuals), the total number estimated in 2002–2003 was just 307 individuals [Li et al., 2007]. Conservation measures will be critical for the survival of this species in the wild but have only recently been implemented in Guangxi Province [Hu & Wei, 2002; Li et al., 2007]. Wild François' langurs feed primarily on leaves, along with fruit and seeds [Hu, 2011]. François' langurs are relatively understudied, partially owing to their

inaccessible rocky limestone forest habitat. Recent captive breeding successes at zoos (e.g., the Taronga Zoo in Australia and US zoos in Toledo, Ohio, Evansville, Indiana and Omaha, Nebraska) are providing valuable new information on care and reproductive management among this species.

In the wild, the weaning period of Francois' langurs has been estimated at 19–21 months [Zhao et al., 2008]. According to a publication by Y.M. Lai in 1987 [as cited by Zhao et al., 2008], the duration of nursing is shorter in captivity, on the order of just 6 months. The difference, if accurate, is likely due to higher nutritional quality of captive animal diets, which are based on vegetables and fruits. In the wild, Hanuman langur infants (*Trachypithecus entellus*) are completely dependent on their mothers for at least 1 month after parturition [Jay, 1965]. Solid foods are part of the wild Hanuman langur infant diet around 3 months of age [Rajpurohit & Mohnot, 1990]. Between 4 and 10 months, weaning begins as the mother begins to reject her offspring [Jay, 1965; Rajpurohit & Mohnot, 1990]. This intensifies at 11 months of age until weaning is complete by 15 months [Jay, 1965; Rajpurohit & Mohnot, 1990]. A shorter timeline is expected as the framework of the present study, which involves captive langurs.

During the sample collection period, the François' langur group at the Toledo Zoo comprised four individuals: one adult female, one adult male, one juvenile male, and one infant. The infant, a male, was born on August 25, 2010. The infant's birth was anticipated because the Toledo Zoo conducts routine ultrasounds, which detected pregnancy at an early stage in March 2010. Gestation lasted an estimated 6 months. Following birth, the mother received Depo-Provera® shots as a method of birth control and a melengestrol acetate implant was inserted in mid-February, neither of which disrupted lactation.

Sample Collection, Preparation, and Analysis

Feces were collected by zookeepers from the exhibit area shortly following deposition during routine cleanings while the animals were not present. The mother was fed oatmeal cookies containing green food dye so that her feces could be distinguished from the rest of the group. Food dye is not expected to significantly alter bulk fecal stable isotope values, and as a constant ingredient in the mother's diet throughout the study, should not affect the transitions of interest here. Feces of the infant were easily identifiable throughout the collection period, being smaller and often pelleted, and no labeling was required. Samples were collected by zookeepers at different frequencies over the course of three research phases between July 2010 and September 2011. During Phase 1, commencing 1 month before expected parturition, feces of the mother were sampled eight times to establish a preparturition dietary baseline. During Phase 2, when the infant is exclusively nursing, samples were to be collected twice per week from both mother and infant. This phase lasted from late August through November, when the infant appeared to obtain virtually all its calories from breast milk (see also Zhao et al. [2008] for wild animal data). However, it was not until September 14, 2010 (3 weeks of infant age) that the first infant sample could be collected. Prior to this, defecation occurred while clinging to the mother, which the mother would clean. Phase 3 began in December when the infant was observed eating considerably more solid foods and spending more time apart from the mother. During Phase 3 samples were collected twice weekly from the mother and daily from the infant. Phase 3 was initially expected to last through 6 months of infant age, as this was the weaning period expected for captive langurs, based on a 1987 report by Y.M. Lai [as cited by Zhao et al., 2008].

Diets of the Toledo Zoo langurs comprise (by food weight) approximately 45% vegetables, 45% leafy greens, and 10% primate biscuit. Periodic enrichment items include fruit, peanuts, browse, and pasta, which are offered once or twice a week. Such a diet is estimated to have a $\delta^{13}C$ value of approximately $-26‰$ and a $\delta^{15}N$ value of 2–3‰. To calculate a dietary baseline, a number of foods were analyzed, including tomato, green pepper, carrot, broccoli, zucchini, green beans, eggplant, turnip, turnip greens, and a primate "Leafeater" biscuit. These food samples were taken by zookeepers from among the foods offered to the animals during several days in August, September, and October 2011. Food samples were frozen, freeze-dried, and homogenized in a mortar/pestle.

Fecal sample preparation followed previously established protocol [Codron et al., 2006]. Fecal samples were stored frozen at the zoo in plastic collection bags until they could be transported The Ohio State University and oven-dried for several days at 60–80°C. Dried feces were homogenized with a mortar and pestle so that the powdered sample could pass through a 0.5-mm mesh sieve. An effort was made to remove undigested food particles and hair from the sample while grinding.

Subsamples weighing between 1.3 and 1.5 mg for feces and 2.4 and 2.5 mg for foods were weighed into tin capsules at The Ohio State University Stable Isotope Biogeochemistry Laboratory. Subsamples were analyzed on a Costech Elemental Analyzer coupled to a Finnigan Delta IV Plus stable isotope ratio mass spectrometer under continuous flow using a CONFLO III interface. Stable carbon ($\delta^{13}C$ = permil deviation of the ratio of ^{13}C:^{12}C relative to the Vienna Peedee Belmenite Limestone standard) and stable nitrogen ($\delta^{15}N$ = permil deviation of ^{15}N:^{14}N relative to air) isotope measurements were made where the average standard deviation of repeated measurements of the USGS24, IAEA-N1, and

IAEA-N2 standards were 0.05‰ for $\delta^{13}C$ and 0.13‰ for $\delta^{15}N$. Data are reported to the nearest 0.1‰, accordingly. Approximately 10% of all samples were run in duplicate. Both stable carbon and nitrogen isotopes are reported in relation to the accepted standards according to the equation $\delta = ([R_{sample} - R_{standard} - 1] \times 1{,}000)$.

In addition to stable isotope ratios, the mass spectrometer measures overall carbon and nitrogen content (%C and %N) of the samples. From these measurements, carbon to nitrogen (C:N) ratios were calculated according to the equation C:N = (%C/12)/(%N/14). The C:N ratios of samples can detect inclusion of unwanted substances with %C or %N values that differ from feces, and are used to investigate sample integrity, and also reflect changes in %N with varying protein content of the diet.

Descriptive statistics are presented hereafter as means and standard deviations. Pearson's correlations are used to explore any relationships between C:N ratios and stable isotope values. Both r values and associated P values are reported, and P values are considered significant at the 0.05 level. Other inferential statistics are not used on the basis of the small sample used for this pilot study.

RESULTS

Foods

Stable isotope signatures of foods are highly variable, especially for nitrogen (Table I). Most $\delta^{15}N$ values are low with the exceptions of a green pepper and turnip greens. The range of $\delta^{15}N$ values is 0.4‰ (green bean, a low-^{15}N legume) to 12.0‰ (turnip greens; green pepper). The range of $\delta^{13}C$ values is smaller, from −30.9‰ (turnip greens) to −22.4‰ ("Leafeater" biscuit, containing ground corn, a plant enriched in ^{13}C). Three tomatoes and three types of lettuce were assayed and reveal that the stable isotope values of even a single food type vary widely.

TABLE I. Food Stable Isotope Values

Food item	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Broccoli	3.3	−28.3
Turnip	0.9	−27.9
Turnip greens	12.0	−30.9
Zucchini	6.4	−26.8
Eggplant	5.5	−26.8
Green bean	0.4	−28.0
Tomatoes		
Regular	3.0	−27.8
Roma	0.9	−27.9
Roma	2.0	−28.0
Green pepper	12.0	−29.3
Carrot	1.3	−26.7
Lettuce		
Iceberg	4.8	−27.6
Romaine	3.4	−27.5
Leaf	1.2	−29.4
Leafeater biscuit	2.9	−22.4
Mean ± SD	4.0 ± 3.7	−27.7 ± 1.8

Stable nitrogen ($\delta^{15}N$) and stable carbon ($\delta^{13}C$) isotope values of a subsample of foods routinely fed to Toledo Zoo langurs. Each food listed was analyzed once.

Integrity of Data

Duplicate analyses of fecal samples were performed to investigate the extent of within-sample variation. One triplicate and five duplicate samples were analyzed from the mother, and eight duplicate samples were analyzed from the infant. In general, replicability was very high. Other than one sample from the mother (the $\delta^{15}N$ values of its duplicates differing by 0.7‰) all replicate isotope analyses were within 0.3‰ for $\delta^{15}N$, 0.4‰ for $\delta^{13}C$, and 0.5 for %N. The maximum and mean differences between each set of replicate samples are presented in Table II.

Possible "contamination" of samples by microbes or undigested foods is investigated by comparing C:N ratios to stable isotope values of feces. There is no relationship between C:N ratios and either $\delta^{13}C$ ($r = -0.289$, df = 223, $P < 0.001$, $N = 225$) or $\delta^{15}N$ ($r = -0.161$, df = 223, $P = 0.016$, $N = 225$; Fig. 1). Fecal C:N ratios of the mother range from 10.7 to 17.9. Those of the infant range from 7.0 to 14.8, with the lowest values occurring during the first 3 months.

The integrity of only one sample is suspect on the basis of questionable %C and %N data. The C:N ratio for this infant sample from October 19, 2011 was extremely high at 60.7, indicating that the substance analyzed was not characteristic of most feces, perhaps containing a disproportionate amount of undigested high-carbon fatty acids from breast milk. This sample's stable isotope values were still within the overall range of variation for the infant, although the $\delta^{13}C$ value was slightly lower than the other values from October 2010. Macroscopically, this sample was clearly unlike the others in having a greasy texture and appearance. This sample appears in Figure 1 but will be excluded from further discussion and does not appear in the remaining figures.

Fecal Samples

Mean fecal $\delta^{15}N$ and $\delta^{13}C$ values and standard deviations for each calendar month are presented in Table III. Figure 2 displays both the mother's and the infant's fecal $\delta^{15}N$, $\delta^{13}C$, and %N values, and represent all 143 days for which samples were analyzed. Figure 3 displays similar information using just one set of data points each, which represent the *difference* between the mother's and the infant's $\delta^{15}N$, $\delta^{13}C$, and %N values. These values were calculated by subtracting the mother's from the infant's values, and, unlike in Figure 2, only days when both the mother and infant were sampled are represented

TABLE II. Replicate Analyses

	δ^{15}N (‰)		δ^{13}C (‰)		N content (%)	
	Maximum difference	Mean difference	Maximum difference	Mean difference	Maximum difference	Mean difference
Mother	0.7	0.2	0.3	0.1	0.3	0.1
Infant	0.3	0.2	0.4	0.1	0.5	0.1

Stable nitrogen (δ^{15}N) and stable carbon (δ^{13}C) isotope and nitrogen/protein content (%N) results from replicate analyses of feces from mother and infant, showing both the mean and the maximum differences obtained from repeated analyses.

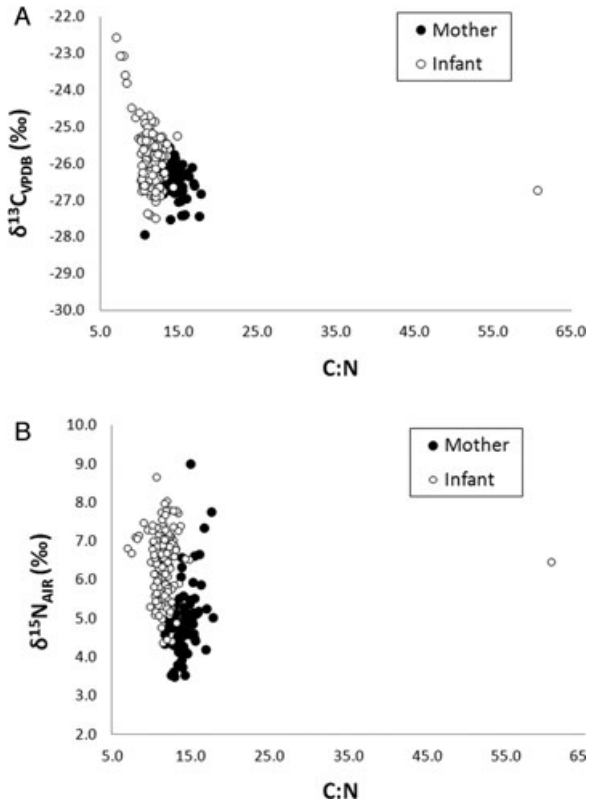

Fig. 1. The relationship between the carbon to nitrogen (C:N) ratios and stable carbon isotope ratios (δ^{13}C) for all mother and infant samples (A), and the relationship between the carbon to nitrogen ratios and stable nitrogen isotope ratios (δ^{15}N) for all mother and infant samples (B).

($n = 82$). This normalizes the data for fluctuations in stable isotope ratios that are merely due to day-to-day variations in the mother's diet, and emphasizes the variation that is due to nursing. These infant–mother values ($\Delta^{15}N_{infant-mother}$) also appear in Table III.

The mean fecal δ^{15}N value of the mother is 5.0 ± 0.9‰ ($n = 82$) with 90% of her δ^{15}N values falling between 4.0‰ and 5.5‰. The mean fecal δ^{15}N value of the infant is 6.4 ± 0.8‰ ($n = 143$). As shown by Figures 2A and 3A, many ($n = 22$) of the infant's fecal δ^{15}N values are more than 2‰ higher than those of the mother. The maximum enrichment of infant fecal ^{15}N above the mother was 3.5‰ (October 26, 2010).

The mean δ^{13}C value of the mother was −26.4 ± 0.5‰. The overall mean δ^{13}C value of the infant was −25.9 ± 0.8‰, but prior to the second month, when the infant was exclusively suckling, the mean δ^{13}C value was −23.7 ± 1.2‰ ($n = 6$). For the first 3 weeks of sample collection and the first 6 weeks after birth, the infant fecal δ^{13}C values are consistently higher than its mother (Figs. 2B and 3B). Excepting one low-^{13}C outlier, which may contain undigested fatty acids, infant δ^{13}C values are on average 3.1‰ higher than mother values. On October 8, 2010 (approximately 6 weeks), there is a change in the infant δ^{13}C: after this date, infant values are lower. Starting on December 17, 2010 (approximately 4 months), the δ^{13}C values of infant and mother become similar.

The mean fecal %N value of the mother was 3.5 ± 0.4%, and of the infant was 4.5 ± 0.8%. During October and November 2010, the infant's fecal values are up to 3.4% higher than its mother, but after 3 months of age the infant's fecal %N drops to within 1% of its mother (either higher or lower; Figs. 2C and 3C).

DISCUSSION

Data Concerning Foods and Diet–Feces Spacing

The stable isotope values of foods in this study vary widely, which can result from different production strategies (e.g., fertilization techniques [Bogaard et al., 2007; Meints et al., 1975]). Consequently, daily fluctuations in the mother's δ^{15}N values can be expected, and attributed to variations in the stable isotope signatures of foods consumed. Fluctuations in a suckling infant's δ^{15}N values should approximately match those of its mother, but more detached variation can be expected when the infant is weaning.

Different foods contribute more or less to the bulk stable isotope signature of the langur diet depending on how much (fresh food weight in kg) is offered each day. By considering how many kg of each type of food is provided for the animals daily

TABLE III. Stable Isotope Results for Mother and Infant

	Mother				Infant				$\Delta^{15}N_{infant-mother}$ (‰)
Month	*n*	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	%N	*n*	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	%N	
July 2010	2	4.5 ± 0.1	−27.4 ± 0.0	2.9 ± 0.0	0				
August 2010	3	5.4 ± 0.6	−26.9 ± 0.4	3.1 ± 0.2	0				
September 2010	1	5.0	−26.3	3.3	4	6.7 ± 0.6	−23.9 ± 1.6	6.2 ± 1.6	1.7
October 2010	5	5.2 ± 0.7	−26.6 ± 0.2	3.3 ± 0.3	8	6.8 ± 0.8	−24.8 ± 1.0	5.4 ± 1.0	1.6
November 2010	6	5.4 ± 1.1	−26.6 ± 0.4	3.3 ± 0.3	11	7.2 ± 0.7	−25.0 ± 0.4	4.4 ± 0.9	1.8
December 2010	7	4.4 ± 0.5	−26.3 ± 0.4	4.0 ± 0.2	22	6.6 ± 0.6	−25.7 ± 0.3	4.6 ± 0.4	2.1
January 2011	8	4.9 ± 0.7	−26.2 ± 0.5	3.8 ± 0.3	23	7.1 ± 0.6	−25.9 ± 0.3	4.4 ± 0.6	2.2
February 2011	13	4.8 ± 0.5	−26.2 ± 0.3	3.7 ± 0.3	19	6.6 ± 0.6	−26.0 ± 0.4	4.9 ± 0.3	1.7
March 2011	9	4.8 ± 0.5	−26.4 ± 6.0	3.3 ± 0.3	13	6.0 ± 0.7	−26.5 ± 0.4	3.8 ± 1.0	1.2
April 2011	7	4.8 ± 0.9	−26.0 ± 0.3	3.3 ± 0.4	15	5.7 ± 0.8	−26.4 ± 0.3	4.0 ± 0.7	0.9
May 2011	8	4.4 ± 0.9	−26.6 ± 0.6	3.5 ± 0.3	13	5.6 ± 0.6	−26.7 ± 0.3	4.4 ± 0.5	1.3
June 2011	2	6.6 ± 3.3	−26.8 ± 0.3	3.3 ± 0.1	2	5.5 ± 0.4	−26.9 ± 0.0	4.0 ± 0.1	1.1
July 2011	3	5.9 ± 0.9	−26.9 ± 0.5	3.6 ± 0.3	3	6.3 ± 0.5	−26.8 ± 0.7	4.4 ± 0.0	0.4
August 2011	1	7.3	−26.1	2.9	2	5.4 ± 0.3	−26.0 ± 0.4	4.0 ± 0.7	1.9

Stable nitrogen ($\delta^{15}N$) and stable carbon ($\delta^{13}C$) isotope values and nitrogen/protein content (%N) of feces from the mother and her infant. Values are grouped by month; sample sizes are shown for each month. The average monthly difference between mother and infant $\delta^{15}N$ fecal values are shown in the right-most column ($\Delta^{15}N$).

and "weighting" each item's isotopic contribution to the bulk diet accordingly, it was possible to calculate ranges of bulk diet values for six hypothetical diets containing various combinations of foods. The $\delta^{13}C$ range for hypothetical diets was −23.5‰ to −24.6‰ and the $\delta^{15}N$ range was 3.8–4.3‰. Both ranges are slightly above what was expected based on values of modern foods [Nardoto, 2006]. Higher $\delta^{13}C$ values of the diet explain why mother fecal $\delta^{13}C$ values are approximately 3‰ higher than expected. Above-expected dietary $\delta^{15}N$ may be because of the impact of the high-^{15}N green pepper analyzed here on the calculations, as peppers are fed to the langurs every day and this high value consequently contributes to every one of the calculated diets. In light of the wide $\delta^{15}N$ variation of tomatoes and lettuce reported here, it is entirely possible that other green peppers might exhibit lower $\delta^{15}N$ values, which would lower the bulk average of actual diets by 1–2‰. For $\delta^{13}C$, higher than expected values calculated for bulk diets are due to the primate biscuit that constitutes approximately 10-12% by weight of the daily diet and has a relatively high $\delta^{13}C$ ratio (−22.4‰) attributable to corn as an ingredient. Wild animal diets would likely more consistently reflect the predicted $\delta^{15}N$ and $\delta^{13}C$ values, although wild plants also vary in their stable isotope values and should be measured [Schoeninger, 2010].

In terms of the diet–feces spacing relationships, based on the mother's fecal stable isotope values and using the calculated diet values, $\delta^{13}C$ of feces was approximately 2–3‰ lower than $\delta^{13}C$ of diet, more consistent with the values reported by Hwang et al. [2007] for small rodents than those reported by Sponheimer et al. [2003a] for large-bodied animals. Fecal $\delta^{15}N$ of the mother was approximately 1‰ higher than that of the calculated diet, which is a lower diet–feces spacing than previously reported for either rodents or large-bodied herbivores [Hwang et al., 2007; Sponheimer et al., 2003c]. As previously mentioned, this is possibly due to the high-^{15}N green pepper, which raises calculated diet values somewhat thereby reducing the diet–feces spacing.

Relationship Between Mother and Infant

$\delta^{15}N$ and the persistence of suckling

It appeared to zookeepers that by the fifth month (January 2011) the infant was suckling very little. Indeed, the first day that the infant's fecal $\delta^{15}N$ value is nearly identical to its mother's is January 8, 2011. Yet according to the stable isotope data, a considerable amount of the infant's calories were still derived from suckling on many days in January 2011: some of the infant's fecal $\delta^{15}N$ values are still nearly 2‰ higher than its mother, consistent with one trophic position. Either what appeared to be comfort nursing after January 2011 was in fact actual nursing, or nursing continued for a prolonged period at night (or both). The infant continued to suckle throughout the intended 6-month sample collection period, exhibiting fecal $\delta^{15}N$ values 1–3‰ higher than its mother. Because of this, the sample schedule was extended in an effort to capture the cessation of suckling. Beginning in April 2011 (month 8), values of the infant begin to drop below those of the mother (Figs. 2A; 3A), an indication of nutritional independence for some days. Two-thirds of the infant's fecal $\delta^{15}N$ values are still higher than the mother's after the initial drop in April 2011, and even 1 year after birth, the infant's $\delta^{15}N$ values are elevated, indicating the persistence

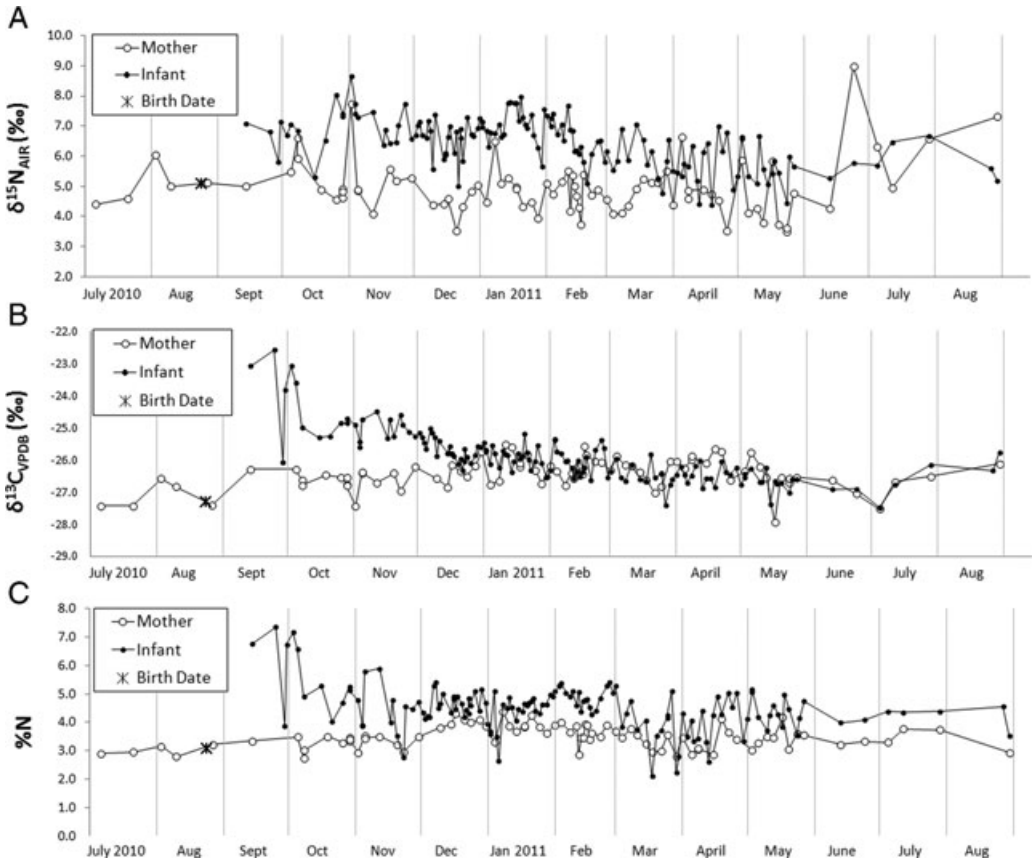

Fig. 2. Comparing stable nitrogen isotope values ($\delta^{15}N$) (A), stable carbon isotope ratios ($\delta^{13}C$) (B), and percent nitrogen content in feces (%N) (C) of all mother and infant fecal samples through time.

of suckling. The infant could be seen suckling as late as October 2011 (14 months after birth). Stable isotope data suggest this was for physiological reasons and not comfort nursing.

Numerous fluctuations in fecal $\delta^{15}N$ of the mother throughout the sample period are not surprising, considering feces are synthesized quickly and fecal stable isotope values are barely "smoothed out" by time averaging. Similar day-to-day variation has been reported among bonobos using stable isotope values in urine [Deschner et al., 2012]. Although physiology likely plays a role in this, the fluctuations are probably primarily due to differences in the isotopic signatures of foods offered each day. Animals may have consumed high-^{15}N turnip greens one day and low-^{15}N green beans another. As evidenced by the variable stable isotope signatures of multiple tomatoes and lettuce in this study, even one food type can produce a considerable range of stable isotope variation among animals. The fact that feces represent a snapshot of approximately 60 hr helps smooth some of this variation. Because diets of wild primates are less heterogeneous day-to-day than foods produced using modern agricultural techniques, their stable isotope signatures would be less variable and easier to interpret. Zoo animals were chosen for this pilot study in part because of their controlled diets, yet although a precise "menu" of foods can be known, the isotopic variability of these commercially produced foods is more uncertain than what may be expected for wild diets.

A final observation regarding $\delta^{15}N$ is that at the end of the sample period (June–August 2011), the mother's values are unexpectedly high (as much as 3.2‰ higher than the infant). The only change to animals' diets during this time was addition of more browse (mulberry, willow, and grapevine), of which langurs will eat the leaves and bark. Within plants, leaf $\delta^{15}N$ can indeed be higher than $\delta^{15}N$ of other roots and stems due to patterns of nitrogen assimilation from nitrate [Evans, 2001]. Yet browse was also available during the previous summer and no pattern of $\delta^{15}N$ enrichment in the mothers' feces is observed during this time (Fig. 2A). Also, the infant's $\delta^{15}N$ values are not as high as the mother's during this time though they had access to the same foods, cautioning against a dietary explanation for the enrichment. It is nevertheless possible that mother and infant simply chose to eat different foods (different amounts of browse or high-^{15}N vegetables).

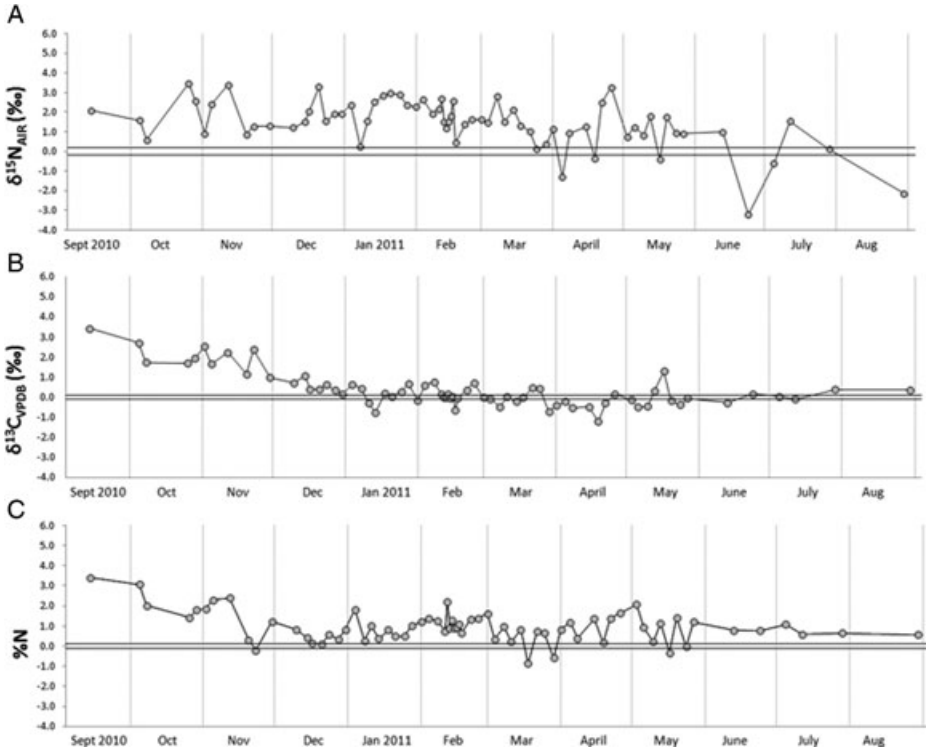

Fig. 3. The difference between mother and infant stable nitrogen isotope values ($\Delta^{15}N$) (A), stable carbon isotope values ($\Delta^{13}C$) (B), and infant nitrogen content in feces (%N) (C) plotted through time. Horizontal lines represent a margin of error which is ± the mean difference obtained from replicate analyses of the same samples (Table II).

If diet differences do not fully explain ^{15}N enrichment of the mother, there may be physiological explanations. Sponheimer et al. [2003b; 2003c] noted that herbivores fed high-protein diets in controlled feeding studies exhibit higher $\delta^{15}N$ ratios in hair than those fed lower protein diets. The authors attribute this to the fact that of the two major pathways for nitrogen efflux, urine, and feces, urine production causes fractionation such that urea is isotopically depleted, and remaining body pool nitrogen is enriched. When animals have an excess of protein in their diets, the excess is lost through urine, not through feces. The result of this is that on high protein diets, relatively more nitrogen is lost through urine, leading to relatively more isotopic fractionation and higher $\delta^{15}N$ values in body tissues [Sponheimer et al., 2003b; 2003c]. High protein in the mothers' diet (possibly through eating browse), or any other physiological condition leading to increased bioavailability of protein, could instigate this metabolic response and leave mothers' tissues ^{15}N-enriched. However, %N of the mother does not increase alongside $\delta^{15}N$ (Figs. 2A and C), as would be expected if crude protein in her diet was higher.

Alternatively, during periods of nutritional stress (negative nitrogen balance), $\delta^{15}N$ values are higher due to the necessary catabolism of an animal's own tissues [Deschner et al., 2012; Fuller et al., 2004, 2005; Hobson et al., 1993]. Increased $\delta^{15}N$ ratios of the mother could potentially correspond to negative nitrogen balance, although she was not known to experience nutritional stress during the sample period. Although the reason for the mother's elevated $\delta^{15}N$ ratios are not fully understood at present, these issues underscore the complexity of stable nitrogen isotopes in biological systems, and warrant further investigation with a greater number of individuals. From the elevated $\delta^{15}N$ values of the mother in June, July, and August 2011, it at least is apparent that the infant is no longer at a consistently higher trophic position compared to its mother from month 11 onwards, although some suckling seems to have persisted.

$\delta^{13}C$ and the addition of solid foods

The stable carbon isotope ratio reflects carbon-rich plants in the diet and is more sensitive than $\delta^{15}N$ to the addition of solid foods. According to the stable isotope data, the infant ate very few plant foods during the first 6 weeks after birth. Zookeepers noted that in the first month of sample collections the infant was mouthing foods such as broccoli and lettuce, but the stable isotope data do not suggest these foods were actually consumed. After 6 weeks of age (October 8, 2010), it appears that solid foods began to contribute considerably more to the infant's diet. By 24 weeks of age, the solid food diets of mother and infant are isotopically indistinguishable. Infant $\delta^{13}C$ values

decline more rapidly than do infant $\delta^{15}N$ values as weaning progresses, as has been reported elsewhere for human infants [Fuller et al., 2006]. This supports the idea that while $\delta^{13}C$ values reflect overall diet, including dietary energy sources, $\delta^{15}N$ values more closely track the major protein sources in diet.

These data demonstrate a trophic enrichment in $\delta^{13}C$ between mother and infant. The maximum trophic enrichment of infant fecal ^{13}C in the present study is +3.5‰, somewhat less than the expected +5‰. It should be borne in mind that during the first 3 weeks following birth, no samples could be collected from the infant, and thus the first available datum may not represent a pure-suckling diet—that is, solid foods may have been introduced undetected prior to September 14, 2010. However, no undigested solid foods were identified in feces during September and October 2010. It may also be the case that langur milk protein is slightly depleted in ^{13}C compared to human milk protein which was used to build the prediction model [Fuller, 2003], lessening the predicted mother–infant $\delta^{13}C$ spacing.

Nevertheless, the 3.5‰ mother–infant $\delta^{13}C$ space is greater than what is previously reported for a single trophic position. When $\delta^{13}C$ values from collagen are used in archeology for diet reconstructions, a ^{13}C enrichment of approximately +1‰ has been observed between prey and predator (referred to as the "carnivore effect") [Schoeninger & DeNiro, 1984]. One explanation for the carnivore effect is that $\delta^{13}C$ from the flesh of an animal is up to 4‰ lower than $\delta^{13}C$ from bone collagen of the same animal, yet the diet–tissue spacing value for $\delta^{13}C$ is +5‰, leaving the carnivore's collagen 1‰ higher than collagen of its prey [Schoeninger, 1989]. However, Fuller [2006] observed that fingernails of suckling human infants are 1‰ higher than fingernails of mothers, yet, unlike the case with flesh and collagen, breast milk protein $\delta^{13}C$ is just 1–2‰ lower than fingernail $\delta^{13}C$ from the same individual [Fuller, 2003]: consequently, a fingernail-to-fingernail difference of 3–4‰ could be expected between mother and infant, yet the observed diet–tissue space is lower, evoking the need for other explanations. The reason why $\delta^{13}C$ trophic enrichment in the present study is greater than previously reported for collagen and fingernails is uncertain. At present, few controlled feeding experiments are available to inform on the $\delta^{13}C$ carnivore effect. It is likely that more detailed experimental data will make important contributions to understanding this research area in the future.

It has been reported elsewhere that $\delta^{13}C$ values do not show trophic enrichment in the tissues (blood) of nursing infants because trophic enrichment is "canceled out" by the ^{13}C-depletion of milk when its lipid component is considered [Jenkins et al., 2001]. These results are not replicated here with langurs. Interspecific $\delta^{13}C$ values of breast milk may be at the root of this, with langurs exhibiting higher milk values than those animals studied by Jenkins et al. [2001]. It is worth noting that as lactation proceeds, crude protein content of milk decreases while lipid concentration increases [Buss, 1968]. Lipids are relatively depleted in ^{13}C [DeNiro & Epstein, 1977; Fuller, 2003], and potentially, this phenomenon could contribute to the decline in infant $\delta^{13}C$ values as weaning proceeds, independent of the introduction of solid foods or a trophic-level effect. It would not, however, affect initial trophic enrichment in the early months. Measuring the isotopic composition of breast milk throughout lactation would be very useful for future weaning studies incorporating $\delta^{13}C$ data.

Day-to-day fluctuations represent more or less consumption of solid foods, but are also probably exaggerated: when consuming solid foods, undigested particles make their way into an animal's feces [Codron et al., 2011]. Many of these are removed in sample preparation during sieving, but some particles of food are present in the final sample. Whole (albeit small) particles are detected in the mass spectrometer and are overrepresented compared to the diluted signature that would be obtained were the particles fully digested. The particles represent what was *not* used for nutrition. This is less problematic than it may seem because the stable isotope signatures of undigested parts of foods (e.g., stems) do not differ greatly from the digested parts (e.g., leaves) [e.g., Codron & Codron, 2009; Codron et al., 2005] and still reflect the ingestion of solid foods. Presence of undigested food particles influences $\delta^{13}C$ more strongly than $\delta^{15}N$ because plant matter has high carbon and low nitrogen content. Also for this reason, "contaminated" samples potentially could be identified by examining C:N ratios and removing samples with anomalously high values.

%N and the crude protein content in diet

Fecal %N values of the mother do not vary widely, reflecting the relatively homogeneous protein content of her vegetal diet. Fecal %N values of the infant show broad day-to-day fluctuations, reflecting variations in dependence on milk vs. plant foods. The crude protein content of infant feces is high during the first 3 months of age due to breastfeeding, and drops considerably afterwards, reflecting the gradual addition of low-protein plant foods in the diet. However, even during the final months of sample collection, the infant exhibits consistently higher fecal %N than its mother, suggesting a higher protein diet indicative of persistent suckling through the 12th month.

Revisiting the Original Hypotheses and Future Directions

Fecal $\delta^{13}C$, $\delta^{15}N$, and %N values of the François langurs investigated here are indicative of nursing, a

time when the infant is a trophic position higher than its mother, and of weaning, the gradual decrease in suckling. Hypotheses 1 and 2 predicted that the mother and suckling infant would exhibit $\delta^{13}C$ values of −29.1‰ and −24.1‰, respectively. Contrary to these predictions, both the mother and infant exhibited higher values, which may be attributed to the higher than expected dietary baseline $\delta^{13}C$ measured here. Hypotheses 3 predicted a gradual decline in infant fecal $\delta^{13}C$ to maternal values, and is supported. Together, these results point to the utility of the $\delta^{13}C$ ratio in feces to record trophic-level enrichment patterns during weaning.

Hypotheses 4 and 5, which predict mother and suckling infant fecal $\delta^{15}N$ values of 4.5–5–5‰ and 7.5–8.5‰, respectively, are supported. This indicates the utility of fecal $\delta^{15}N$ values to record a trophic effect from suckling. Hypothesis 5, which predicted that $\delta^{15}N$ values would decline throughout the course of the study, is only partially supported. Judging from the $\delta^{15}N$ data, weaning was not complete when sample collection ended: infant fecal $\delta^{15}N$ values do not consistently reach maternal values. However, changes in the infant–mother $\delta^{15}N$ values through time (particularly before and after March 2011) are likely indicative of weaning. Hypothesis 7 predicted fecal $\delta^{13}C$ values of the infant would decline earlier and more sharply than $\delta^{15}N$ values, which is supported, indicating these measurements are useful for recording two different, complementary types of information about the weaning process.

These data refine visual estimations because stable isotope signatures permit detection of suckling at night, distinguish between actual nursing and comfort nursing, reflect actual rate of milk transfer, and distinguish between mouthing of solid foods and actual consumption of solid foods. Zookeepers witnessed mouthing of solid foods prior to 6 weeks, but stable isotope data suggest solid foods may not actually have been consumed until after 6 weeks. By 16 weeks of age, the infant obtained most of its calories from solid foods. Through visual observation, nursing appeared to be greatly reduced by 20 weeks, but $\delta^{15}N$ data suggest that the infant still met a significant amount of its dietary needs through nursing at that time, and continued to nurse as late as 1 year after birth, which is when sample collection ceased. The infant was still observed to nurse at 12 months of age, when sample collection ceased.

In this case, the infant's observed nursing appears to have been for dietary purposes and not merely comfort nursing, as is frequently suspected. It was expected that the weaning period for these captive langurs would be much shorter than 12+ months due to more regular access to highly nutritious foods. However, the actual weaning period for these animals was much longer, and may be comparable to weaning periods reported from the wild [e.g., Poirier, 1972]. Reported weaning periods from the wild may also prove to be underestimations when an isotopic approach is applied as an independent source of information.

Application of this method in the wild is straightforward so long as feces can be collected from mothers and infants, and the individuals depositing feces can be identified. The primary goal of this study was to monitor day-to-day changes in weaning status, which required a large number of samples. Fewer samples need be collected to apply this method in the future, and the number collected will depend on the questions asked. Because of the substantial daily fluctuations observed here, it is recommended that samples be collected in sequences of 3 days at intervals of interest to the researchers (weekly, monthly, seasonally) to determine the extent of an infant's breastfeeding at a given time. Colobines, with their unusual digestive morphology, give fecal samples that record foods consumed over the past approximately 2.5 days. Feces from virtually all other primate taxa will yield finer chronological resolutions, which may increase apparent daily variations, but will also refine the method's precision. A dietary baseline should be estimated in the wild, either by measuring stable isotope ratios in available foods, or by measuring fecal stable isotope ratios of other nonlactating, nonsuckling group members, who integrate the signals of the foods consumed and serve as a proxy indicator of the overall stable isotope environment [Bump et al., 2007]. In addition to use in the wild, this method has useful applications in other captive studies; for example, isotopic labeling of foods to reveal allonursing in groups containing more than one lactating female, and monitoring nutritional stress and food partitioning during the introduction of new group members.

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