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Stable isotopes document resource partitioning and effects of forest disturbance on sympatric cheirogaleid lemurs

B. E. Crowley · M. B. Blanco · S. J. Arrigo-Nelson · M. T. Irwin

Abstract The future of Madagascar's forests and their resident lemurs is precarious. Determining how species respond to forest fragmentation is essential for management efforts. We use stable isotope biogeochemistry to investigate how disturbance affects resource partitioning between two genera of cheirogaleid lemurs (*Cheirogaleus* and *Microcebus*) from three humid forest sites: continuous and fragmented forest at Tsinjoarivo, and selectively logged forest at Ranomafana. We test three hypotheses: (H1) cheirogaleids are unaffected by forest fragmentation, (H2) species respond individually to disturbance and may exploit novel resources in fragmented habitat, and (H3) species alter their behavior to rely on the same key resource in disturbed forest. We find significant isotopic differences among species and localities. Carbon data suggest that *Microcebus* feed lower in the canopy than *Cheirogaleus* at all three localities and that sympatric *Cheirogaleus crossleyi* and *C. sibreei* feed at different canopy heights in the fragmented forest. *Microcbus* have higher nitrogen isotope values than *Cheirogaleus* at all localities, indicating more faunivory. After accounting for baseline isotope values in plants, our results provide the most support for H3. We find similar isotopic variations among localities for both genera. Small differences in carbon among localities may reflect shifts in diet or habitat use. Elevated nitrogen values for cheirogaleid lemurs in fragments may reflect increased arthropod consumption or nutritional stress. These results suggest that cheirogaleids are affected by forest disturbance in Eastern Madagascar and stress the importance of accounting for baseline isotopic differences in plants in any work comparing localities.

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Introduction

Anthropogenic forest loss and degradation are widespread phenomena that threaten forest-dwelling species with extinction. Degradation can alter the relative humidity, sun exposure, soil chemistry, nutrient cycling, vegetation structure, and species composition (Fahrig 2003; Harper et al. 2005; Kupfer et al. 2006; Laurance et al. 1998; Tabarelli et al. 1999). The consequences of such alterations on consumer species are not

uniform. Animals in fragmented habitat can experience altered interspecific and intraspecific competition, availability of food or space, predation, and disease (Kupfer et al. 2006; Laurance 1991; Swihart et al. 2003). However, while some species can be negatively impacted to the point of local extinction, others may be able to adapt or even thrive in altered habitats, either by relying more heavily on a subset of their normal food resources or by exploiting foods not consumed in pristine habitat (Gibson 2011; Laurance 1991; Nakagawa et al. 2007; Schleuning et al. 2011; Swihart et al. 2003).

Understanding the effects of forest loss and degradation is particularly relevant to the humid forests of eastern Madagascar, where more than 50 % of the original forest cover has been lost since the 1950s (Harper et al. 2007), and much of the remaining forest has been altered by human activity (Irwin et al. 2010). In this region, degraded forest, which is often but not always in isolated fragments, has lower canopy height, tree density, and species diversity and a less continuous canopy layer than intact forest (Arrigo-Nelson 2006; Brown and Gurevitch 2004; Irwin 2006). The long, narrow distribution of the rainforest corridor from Madagascar's north to far southeast combined with pervasive human settlement along its length has left it vulnerable to extensive anthropogenic disturbances. Lemurs, the endemic primates of Madagascar, play a host of vital ecological roles ranging from pollination to seed dispersal (Richard and Dewar 1991). Therefore, understanding lemur responses to these disturbances is a crucial aspect of designing effective conservation strategies.

The effects of forest degradation on lemur ecology and population health are not well documented. It is apparent that lemurs often shift their foraging strategies and habitat use in response to forest degradation and fragmentation. These patterns are consistent with ecological studies showing higher abundance of some food items (e.g., insects and fruits) near forest edges (Ganzhorn 1995; Ganzhorn et al. 1997; Irwin 2008a; Lehman et al. 2006a; Malcolm 1997). Some species, such as the frugivorous greater dwarf lemur (*Cheirogaleus major*) and red-fronted lemur (*Eulemur rufifrons*), appear to avoid forest edges in fragmented landscapes, while others, such as the insectivorous–frugivorous brown mouse lemur (*Microcebus rufus*) and the folivorous–frugivorous diademed sifaka (*Propithecus diadema*), are more frequently encountered near disturbed edges (Irwin 2008a; Lehman 2007; Lehman et al. 2006a, b). Gray-brown mouse lemurs (*Microcebus griseorufus*) living in disturbed forest are more active and eat less exudate than those living in nearby protected sites (Rasoazanabary 2011). Diademed sifakas (*P. diadema*) rely on mistletoes (genus *Bakerella*) as a dietary staple in fragmented forest (Irwin 2008b). Additional effects, such as changes in predation regime, may also be drivers of lemur abundance (Irwin et al. 2009; Peetz et al. 1992; Swihart et al. 2003), but these factors remain poorly understood (Crooks and Soulé 1999; Terborgh et al. 2001).

Further research is needed to establish firmer links between altered habitat, behavioral shifts, and the health of individuals and populations. This is not an easy task: the study of some behavioral changes, such as dietary shifts, is extremely difficult and time-intensive to document under field conditions, particularly for small or nocturnal animals. There is a very real risk that populations and species may go extinct before we are able to study them in enough detail to understand these relationships. Indirect, rapid ways of assessing behavioral shifts in response to forest degradation and fragmentation are, therefore, extremely valuable in expanding our appreciation of behavioral flexibility and physiological limits across populations and species.

Habitat alteration and associated potential changes in resource use of individual species might also have communitywide effects, particularly for species that are similar in diet and habitat use. In nature, closely related species rarely coexist unless they differ in behavior, body size, diet, or morphology (Houle 1997; Hutchinson 1959; Schreier et al. 2009; Siemers and Swift 2006). Congeneric species often reduce competition over resources by consuming different dietary items, feeding at different times of the day, or by exploiting different microhabitats such as particular heights within a forest canopy (Herrera et al. 2003; Voigt 2010). Such resource partitioning can be identified, and potentially quantified, using stable isotope biogeochemistry, since stable carbon and nitrogen isotope values in animal tissues vary as a function of both diet and habitat. Isotope data can be particularly informative for difficult to observe species such as small or nocturnal lemurs. Within a given habitat, isotope values reflect the relative contribution of plant and animal matter to an individual's diet (reviewed in Ambrose 1991; Ben-David and Flaherty 2012; Crowley 2012). Because carbon and nitrogen isotope values increase with increasing trophic level (reviewed in Ben-David and Flaherty 2012; DeNiro and Epstein 1978, 1981), a frugivorous species, such as *Cheirogaleus crossleyi* or *C. sibreei*, is expected to have fur δ^{13}C and δ^{15}N values that are ca. 2–4‰ and 2–6‰ higher than those in plants, respectively (Hyodo et al. 2010; Miron et al. 2006; Sponheimer et al. 2003a, b). A more faunivorous species, such as *Microcebus lehilahytsara*, is expected to have fur δ^{13}C and δ^{15}N values that are higher than those in sympatric herbivores (Fox-Dobbs et al. 2007; Hyodo et al. 2010).

Stable isotope data can also distinguish consumption of certain plant parts (e.g., leaves versus fruits) or functional types (e.g., CAM succulents, C_3 trees, plants with N-fixing symbiotic bacteria, and parasitic plants) provided that these groups are isotopically distinct (e.g., Blumenthal et al. 2013; Cernusak et al. 2009; Codron et al. 2007; Werner and Schimdt 2002). Plants that obtain their nitrogen directly from soil nitrate and ammonium tend to have δ^{15}N values <0‰ in moist habitats (Martinelli et al. 1999). Plants with symbiotic bacteria that fix nitrogen directly from the atmosphere (such as many legume species) have δ^{15}N values that resemble atmospheric nitrogen (0‰; e.g., Ambrose 1991; Schmidt and Stewart 2003). Hemiparasitic plants, such as xylem-tapping mistletoes, exhibit similar δ^{15}N values but substantially lower

$\delta^{13}C$ values compared to their host plants (Schulze et al. 1991). Because stable carbon and nitrogen isotope values in plants are affected by temperature, relative humidity, sun exposure, soil composition, and nutrient availability (Amundson et al. 2003; Broadmeadow et al. 1992; Ehleringer et al. 1986; Handley et al. 1999; Heaton 1999; Kapos et al. 1993; Martinelli et al. 1999), it may also be possible to differentiate animals that exploit different microhabitats such as canopy, understory, or forest edge, within a locality (Cerling et al. 2004; Hyodo et al. 2010; van der Merwe and Medina 1991). While these isotopic patterns may help identify and distinguish foraging strategies among species, they are not always straightforward. Because differences in both diet and habitat use can affect isotope values in consumer species, quantifying the relative importance of each of these factors requires detailed knowledge about a population's foraging strategies as well as abiotic and biotic environmental conditions (Flaherty and Ben-David 2010).

Disturbance in the form of logging, fire, large domesticated animals, or storm damage can influence the isotope values of plants and animals. Plants from disturbed habitats tend to have higher $\delta^{13}C$ and $\delta^{15}N$ values than those in undisturbed habitats (Cook 2001; Ehleringer et al. 1986; Evans and Belnap 1999; France 1996; Nadelhoffer and Fry 1994; Wang et al. 2007). This trend likely results from a combination of factors that affect the temperature, relative humidity, sun exposure, soil characteristics, and nutrient cycling of the disturbed habitat. Such patterns may or may not be passed on to animal consumers. Both lower and higher $\delta^{13}C$ and $\delta^{15}N$ values have been documented for mammals living in disturbed versus relatively pristine habitats (e.g., Darling and Bayne 2010; Gibson 2011; McGee and Vaughn 2003; Nakagawa et al. 2007). These contrasting trends provide evidence that consumer species may respond differently to disturbance and forest loss. Whereas some species may consume similar dietary items in disturbed and pristine habitats, others may change their foraging strategies to rely more heavily on certain foods, or use novel resources in disturbed habitats (Laurance 1991; Malcolm 1997; Nakagawa et al. 2007). Such behavioral changes have been observed for primates including lemurs (Irwin 2008b; Kelley 2011; Onderdonk and Chapman 2000; Sauther and Cuozzo 2009).

Here, we use stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values in lemur fur to investigate how forest fragmentation affects niche partitioning among sympatric mouse lemurs (*Microcebus* spp.) and dwarf lemurs (*Cheirogaleus* spp.), inhabiting three eastern rainforest localities with varying levels of disturbance (Fig. 1). Our analysis includes two species from each genus: *M. rufus* and *M. lehilahytsara*, and *C. crossleyi* and *C. sibreei*. Sympatric species of cheirogaleid lemur are thought to coexist through partitioning of dietary items (Dammhahn and Kappeler 2010; Rakotondranary et al. 2011; Thoren et al. 2011), foraging heights (Lahann 2007), and sleeping sites (Radespiel et al. 2003). While mouse lemurs are

frugivore–insectivores that regularly consume a range of fruits, arthropods, and small vertebrates, dwarf lemurs predominantly consume fruits, flowers, and nectar (Atsalis 2008; Fietz and Ganzhorn 1999; Hladik et al. 1980; Kappeler and Rasoloarison 2003; Lahann 2007; Radespiel 2006; Thoren et al. 2011).

We test three alternative hypotheses:

1. The ecological niches of these species, as measured by stable isotope values in fur, are unaffected by forest disturbance. Isotope values in fur will track those in plants at all localities.
2. Each species responds individually to disturbance and may exploit distinct, potentially novel resources in fragmented environments. Average isotope values for lemur species from fragmented habitats may, therefore, be more variable than those from continuous forest.
3. All three species have divergent diets in undisturbed forest, but rely on the same key resource in disturbed forest. Consequently, their isotope values should be distinct in intact forest, but converge in disturbed or fragmented habitat.

Methods

Study sites

We compare $\delta^{13}C$ and $\delta^{15}N$ values from three eastern rainforest localities: continuous and fragmented forests at Tsinjoarivo and a selectively logged forest at Ranomafana National Park (Fig. 1). All three of these localities are situated on the escarpment dividing the central plateau from the eastern lowlands in south-central Madagascar. Mean annual rainfall at Ranomafana is approximately 3600 mm and mean daily temperatures range from ca. 15.5-22 °C (Centre Valbio, unpublished data 2004–2009). Fur and plant samples were collected from Talatakely (21°16′12″S, 47°19′48″E, 950–1050 m). This region of the park has a mixed history of disturbance including the presence of a small village in the 1930s and 1940s. Following the relocation of the village by French colonial authorities, the forest was allowed to regenerate with little disturbance until the 1980s. It was subject to the intensive 'selective' logging of commercially valuable hardwood trees from 1986 to 1989 (Wright 1997). This area has been included within a national park and protected from disturbance since 1991.

Tsinjoarivo is situated roughly 150 km northeast of Ranomafana; the region encompasses both continuous and fragmented, degraded forests. Habitat loss and fragmentation have been occurring over the past 50 years (Irwin 2008b). We collected plant and fur samples from continuous, relatively undisturbed forest (Vatateza: 19°43′25″ S, 47°51′41″ E, 1390 m) and disturbed, fragmented forests (Mahatsinjo and
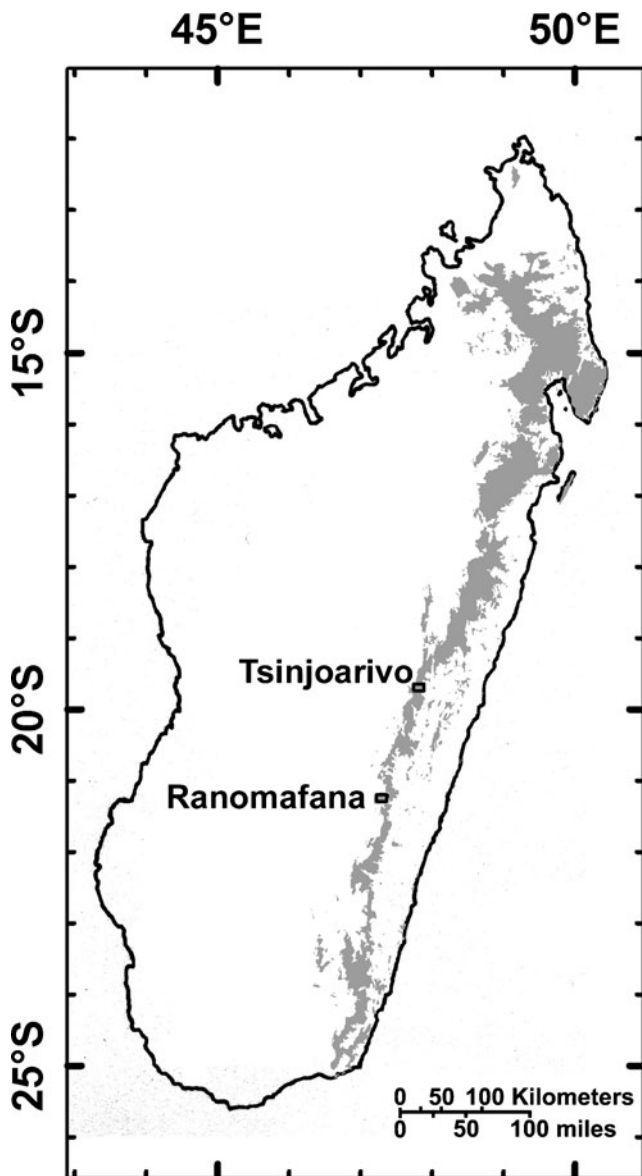
Fig. 1 Map of the study areas in Madagascar

Andasivodihazo: 19°41′15″S, 47°46′25″E, 1660 m), which lie roughly 12 km to the northwest. Permanent human populations were established in now disturbed areas in the 1980s. The fragments have experienced roughly 50 % deforestation (with cleared areas concentrated mostly in valley bottoms) and disturbance of remaining forest (through selective logging and collection of forest products). Vatateza has experienced comparatively minor levels of clearing and disturbance. The continuous forest receives an annual average of 2563 mm of rain and monthly average minimum and maximum temperatures of 8.4 and 23.3 °C, respectively (Irwin and Raharison, unpublished data in 2003–2004). Mean annual rainfall for the fragmented forests is lower (2008 mm), and the monthly average minimum and maximum temperatures are 6.6 and 26.9 °C, respectively (Irwin and Raharison, unpublished data in 2003–2004).

Species descriptions

Two species of small-bodied nocturnal cheirogaleid lemurs are found at Ranomafana (*M. rufus* and *C. crossleyi*), and three are found at Tsinjoarivo (*M. lehilahytsara*, *C. crossleyi*, and *C. sibreei*). *M. lehilahytsara* and *M. rufus* are indistinguishable by morphometric standards and can only be differentiated using genetic analyses (Blanco 2010; Kappeler et al. 2005). Both species of eastern mouse lemurs (body mass~40 g) are generally described as trophic omnivores. Their diet consists of fruits supplemented with insects (Atsalis 2008). *C. crossleyi* can be morphologically distinguished from *C. sibreei* by body mass (350 vs. 250 g, respectively), morphometrics, fur coloration, and the morphology of female genitalia (Blanco and Godfrey 2013; Blanco et al. 2009; Groeneveld et al. 2010). Both *C. sibreei* and *C. crossleyi* are thought to be primarily frugivorous, with some consumption of animal matter, as is observed in congeners (Blanco, personal observation; Lahann 2007).

Sample collection and preparation

We collected fur samples from nine *C. crossleyi* and 35 *C. sibreei* at Tsinjoarivo fragmented forest, 13 *M. lehilahytsara* and 24 *C. crossleyi* at Tsinjoarivo continuous forest, and 13 *C. crossleyi* from Ranomafana. Until recently, it was unknown that multiple species of *Cheirogaleus* live at Tsinjoarivo (Blanco et al. 2009). Whether or not *C. crossleyi* and *C. sibreei* coexist in continuous forest remains to be determined. We considered fur collected from the same animal during different years to be independent samples. We added these data to our existing published dataset (Crowley et al. 2011), which includes isotope data for mouse lemur fur from Ranomafana (43 samples) and Tsinjoarivo fragmented forest (33 samples). Detailed information about each sample, including raw isotope data, is provided in online resource 1.

Fur was collected under University of Massachusetts, Amherst IACUC protocol no. 26-17-03 (to M.B.B). Lemurs were captured between October 2004 and April 2009 (capture dates provided in online resource 1). To capture mouse lemurs, Sherman traps baited with small pieces of fresh bananas were set in pairs at ~1.5 m high and separated by a minimum of 25 m along marked trails. For dwarf lemurs, Tomahawk traps baited with fermented bananas were set at 3–10 m high and separated by a minimum of 25 m along trails. At Tsinjoarivo, traps were set close to forest edges as well as in the interior; the same applies for the continuous forest site. At Ranomafana, traps were set up along tourist trails. All traps were open around 17:00 and checked at 20:00 at Ranomafana or the following morning between 3:30–4:30 at Tsinjoarivo. Lemurs were brought back to the campsite (Tsinjoarivo) or Centre ValBio research station (Ranomafana) for data collection and released at capture sites later the same night (Ranomafana) or at dusk the same day of capture (Tsinjoarivo). At the campsite/

station, each lemur was marked with a microchip. Recaptured lemurs were identified. Fur samples were collected from the dorsal side of the base of the tail, as close to the skin as possible, using a clean pair of scissors. We then wiped fur samples clean using methanol. Cheirogaleids have continuous fur growth. Their fur ranges from ca. 1 to 2 cm in length. Based on lemur fur growth rates (Sandra Thorén personal observation), we estimate that this represents dietary information for two to three months prior to collection (Caut et al. 2008).

Published plant data exist for Ranomafana and Tsinjoarivo fragmented forest (Crowley et al. 2011). To this existing dataset, we added previously unpublished plant data from Tsinjoarivo continuous forest. Samples include leaves, fruits, seeds, and flowers from a range of canopy, understory, climbing, and hemiparasitic species. Plant material was collected at Ranomafana between December 2003 and May 2004 and at Tsinjoarivo between July 2006 and 2007. Samples were dried at <65 °C until a constant mass was reached and ground.

We weighed approximately 700 µg of fur or 5 mg of ground plant samples into tin capsules. We then combusted these samples on a Finnigan ThermoElectron Delta+XP continuous flow system connected to a Carlo Erba elemental analyzer at the University of California, Santa Cruz Stable Isotope Laboratory. The analytical precision (± 1 SD) for carbon and nitrogen was 0.09‰ and 0.08‰, respectively, based on 16 I.E. acetanilide replicates. The average difference between six fur samples run in duplicate was 0.2‰ and 0.1‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively.

Data analyses

Isotopic variability among lemurs living in different localities could reflect baseline isotopic differences among the localities or dietary differences. To test these alternatives, we examined patterns in isotope data for plants from each site, which should reflect abiotic and biotic conditions. We grouped plant samples by part (leaves, flowers, fruits/seeds) and type (canopy trees, small understory/edge trees typically <7 m height, hemiparasitic plants, and climbing plants). These categories allowed us to evaluate the isotope values for potential dietary items as well as control for baseline isotopic variability among localities. We used general linear models (GLMs) to check for differences and interactions among locality, part, and type categories. Controlling for these relationships, we then used Tukey's honestly significant difference (HSD) tests to compare means and identify homogenous subsets within each category. We tested for homogeneity of variances using Levene's test and found that our data are not homoscedastic. Attempts to log-transform or rank-transform the data were unsuccessful. We, therefore, believe that this is due to our small sample sizes. In addition to GLM tests, we confirmed results using separate Kruskal–Wallis tests for each factor for each isotope (six tests total). Although these nonparametric

tests are less powerful than the GLMs because they only consider one variable at a time, their results can be used to corroborate or contest the GLM results. We also used Kruskal–Wallis analyses to test for differences in carbon and nitrogen concentrations (atomic C:N) among localities, structure types, and plant parts.

We categorized cheirogaleids by species and location. *M. lehilahytsara*, *C. crossleyi*, and *C. sibreei* were captured in Tsinjoarivo fragmented forest, whereas only *C. crossleyi* and *M. lehilahytsara* were captured in Tsinjoarivo continuous forest. *C. crossleyi* and *M. rufus* were trapped at Ranomafana. Because *M. rufus* and *M. lehilahytsara* are morphologically and ecologically indistinguishable (Blanco 2010), we refer to both of these allopatric species as "eastern mouse lemurs" in our data analyses and discussion. Within each species, no significant differences in isotope values were found between sexes, among age groups, or between animals captured during dry and wet seasons (Mann–Whitney U-test, all $p > 0.05$). We, therefore, lumped all data for each species for subsequent analysis.

We used T-tests to compare isotope values for the two species of lemurs living at Ranomafana as well as Tsinjoarivo continuous forest (eastern mouse lemurs and Crossley's dwarf lemurs) and one-way ANOVA coupled with Tukey HSD post hoc tests to examine isotopic variability among the three cheirogaleid species at Tsinjoarivo fragmented forest. Before comparing isotope values among localities for each lemur species, we calculated the apparent fractionation (Δ) between lemurs and plants at all three localities. These values account for baseline environmental isotopic differences among localities. These values were calculated by taking the difference between the carbon or nitrogen isotope value for each lemur and the mean isotope values for plants from each habitat (e.g., Nakagawa et al. 2007). We then used one-way ANOVA coupled with post hoc tests to compare $\Delta^{13}C_{lemur-plant}$ and $\Delta^{15}N_{lemur-plant}$ values among localities for each lemur species. We tested the assumptions of homogeneity of variance using Levene's test for all lemur analyses. For any comparisons that had a sample size of <20 for one or more groups, we confirmed that results from parametric results did not differ from those obtained using nonparametric tests (e.g., Mann–Whitney U and Kruskal–Wallis tests). Significance was set at $\alpha = 0.05$ for all tests and all analyses were conducted in JMP 5.0 and SPSS 20.0.

Results

Plants

Isotope and elemental data for individual plant samples are provided in online resource 2. Our GLM results indicate that there are significant differences among plants from different localities (Ranomafana, Tsinjoarivo continuous forest and Tsinjoarivo fragmented forest; $p < 0.001$), plant parts (leaves,

flowers, and fruits/seeds; $p = 0.002$), and plant types (canopy, understory/edge, hemiparasites, and climbing plants; $p < 0.001$) for carbon, and among plants from different localities ($p < 0.001$) and plant parts ($p = 0.023$) for nitrogen (Table 1). There are no differences in $\delta^{15}N$ values among plant type categories ($p > 0.05$). There are significant site*part and site*type interactions for carbon (Table 1), and a significant site*type interaction for nitrogen (Table 2).

We note that just over 50 % of the variation in carbon isotope values and only 14.8 % of the variation in plant nitrogen isotope values is explained by the GLM models. Despite the significance of the included variables, it is clear that additional factors, such as species composition, tree density, and soil characteristics, have an effect on plant isotope values.

Kruskal–Wallis tests for each factor corroborate the GLM results (Tables 1 and 2). When run separately, there are significant differences among sites and parts for both carbon and nitrogen isotopes. There are also significant differences among plant types for carbon but not for nitrogen (Tables 1 and 2). Thus, although the data fail Levene's test for homogeneity of variances, this less powerful nonparametric alternative confirms the significance of the three main effects.

Controlling for other variables, post hoc tests indicate that carbon isotope values are lowest at Ranomafana, and they do not differ between the continuous and fragmented forest at Tsinjoarivo. Plants from Tsinjoarivo fragmented forest exhibit significantly lower $\delta^{15}N$ values than the other localities (Fig. 2). Leaves have significantly lower $\delta^{13}C$ values than flowers or fruits/seeds and significantly higher $\delta^{15}N$ values than flowers (Table 3). Canopy trees have significantly higher $\delta^{13}C$ values than any of the other types (Table 4).

There are no differences in atomic C:N ratios among localities ($\chi^2 = 0.691$, $df = 2$, $p = 0.708$; online resource 2). However, there are differences among parts and structure types (Parts: $\chi^2 = 24.655$, $df = 2$, $p < 0.0001$, Table 3; Types: $\chi^2 = 39.124$, $df = 3$, $p < 0.0001$, Table 4). Flowers and leaves have lower C:N ratios than fruits/seeds, and climbing plants have lower C:N than other types of plants.

Lemurs

Isotope data for individual lemur fur samples are provided in online resource 1. Isotopic differences among lemur populations tend to be smaller than those among plants, and the associated variances are also smaller (Fig. 2), probably because lemur fur averages the isotopic signatures of numerous individual plants from multiple species (Bump et al. 2007; Crowley et al. 2011). We find significant isotopic differences among sympatric cheirogaleid species at all three localities. Eastern mouse lemurs consistently have significantly higher $\delta^{15}N$ values and lower $\delta^{13}C$ values than Crossley's dwarf lemurs (Tables 5 and 6). At Tsinjoarivo fragmented forest where three cheirogaleid species coexist, post hoc tests reveal

unique carbon isotope values for all three species (Table 5). Sibree's dwarf lemurs have the highest $\delta^{13}C$ values, Crossley's dwarf lemurs have intermediate $\delta^{13}C$ values, and mouse lemurs have the lowest $\delta^{13}C$ values. There are no differences in $\delta^{15}N$ between $C.$ $crossleyi$ and $C.$ $sibreei$, but mouse lemurs have significantly higher $\delta^{15}N$ values than both dwarf lemur species (Table 6). We find significant differences in apparent fractionation values among localities for $Microcebus$ spp. and $C.$ $crossleyi$, (Table 7; $p < 0.0001$ for all comparisons). Patterns are similar for both species: $\Delta^{13}C_{lemur-plant}$ is largest at Ranomafana and smallest at Tsinjoarivo continuous forest, and $\Delta^{15}N_{lemur-plant}$ is larger in Tsinjoarivo fragmented forest than either Ranomafana or continuous forest (Table 7).

Discussion

Isotope data indicate that sympatric cheirogaleid lemurs have distinct niches. They also suggest that $Cheirogaleus$ and $Microcebus$ are similarly affected by fragmentation. The significance of each of these differences is discussed in detail below.

Plants

The significant interaction between site and type for both carbon and nitrogen in our GLM models likely reflects differences in forest structure among localities. Tsinjoarivo fragmented forest and Ranomafana both have a lower canopy and better-developed understory than Tsinjoarivo continuous forest. This interaction may also reflect differences in species sampled among localities. Lower carbon isotope values at Ranomafana probably reflect the higher rainfall at this locality (Kohn 2010). There are no differences in $\delta^{13}C$ values between fragmented and continuous forest at Tsinjoarivo. However, $\delta^{15}N$ values do differ among localities. Based on previous work, we had expected that the fragmented forest would exhibit higher $\delta^{15}N$ values than the undisturbed forest at Tsinjoarivo (Cook 2001; Ehleringer et al. 1986; Evans and Belnap 1999; France 1996; Nadelhoffer and Fry 1994; Wang et al. 2007). Disturbance at this locality has likely affected the sun exposure, ambient temperature and relative humidity of the forest understory, and may have affected soil moisture and nutrient cycling. We also expected that the selective logging at Ranomafana would be detectable isotopically. We do not observe either of these patterns. We find no difference between selectively logged forest at Ranomafana and the continuous forest at Tsinjoarivo. Nitrogen isotope values are significantly lower for plants from Tsinjoarivo fragmented forest. We anticipate that this pattern reflects changes in microbial activity or selective nitrogen loss of non plant-available forms of nitrogen via compaction, erosion, and leaching

Table 1 General linear model results for $\delta^{13}C$

Source	SS	df	F	P	Kruskal–Wallis P
Corrected model	563.234	26	9.774	<0.001	
Intercept	57207.607	1	25810.409	<0.001	
Site	29.578	2	6.672	<0.001	<0.001
Part	48.912	2	11.034	0.002	<0.001
Structure	50.239	3	7.555	<0.001	<0.001
Site*part	18.596	3	2.797	<0.001	
Site*type	9.526	6	0.716	0.042	
Part*type	4.601	4	0.519	0.637	
Site*part*type	14.841	6	1.116	0.722	
Error	403.395	182		0.355	
Total	183123.098	209			
Corrected total	966.629	208			

Adjusted R^2 for the corrected model is 0.523. For comparison, results from independent Kruskal–Wallis ANOVAs for each variable are also presented
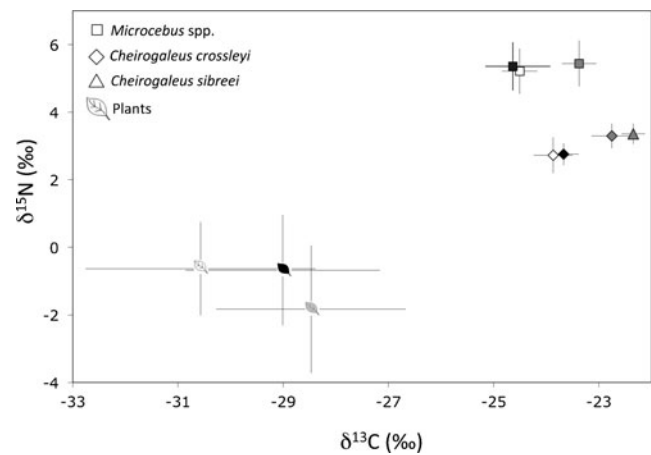

Fig. 2 Mean carbon and nitrogen isotope values ($\pm 1\sigma$) for plants and lemurs at each locality. *White symbols* indicate Ranomafana selectively logged forest, *black symbols* demarcate Tsinjoarivo continuous forest, and *grey symbols* indicate Tsinjoarivo fragmented forest

(Darling and Bayne 2010). It is also conceivable that plants growing in fragmented forest receive less nitrogen input from leaf litter and rotting wood than less disturbed forest (Darling and Bayne 2010; Huygens et al. 2008). Human extraction of forest products, such as firewood, is increased in disturbed forest. Therefore, plants from fragmented forest may be more nitrogen limited, requiring them to more efficiently recycle ^{15}N-depleted mineral forms of nitrogen such as nitrate (Amundson et al. 2003). However, because atomic C:N is indistinguishable among localities, this possibility seems unlikely. Variations in nitrogen availability should also affect the concentration of nitrogen in plant tissues (Hobbie et al. 2000).

Table 2 General linear model results for $\delta^{15}N$

Source	SS	df	F	P	Kruskal–Wallis P
Corrected model	148.769	26	2.389	<0.001	
Intercept	162.017	1	67.652	<0.001	
Site	40.446	2	8.444	<0.001	<0.001
Part	18.472	2	3.857	0.023	0.013
Structure	2.691	3	0.375	0.771	0.721
Site*part	5.005	3	0.697	0.555	
Site*type	32.359	6	2.252	0.04	
Part*type	4.747	4	0.496	0.739	
Site*part*type	16.078	6	1.119	0.353	
Error	435.866	182			
Total	770.142	209			
Corrected total	584.635	208			

Adjusted R^2 for the corrected model is 0.148. For comparison, results from independent Kruskal–Wallis ANOVAs for each variable are also presented

Nitrogen isotope values for plants from Tsinjoarivo continuous forest and Ranomafana are indistinguishable. This suggests that either (i) selective logging did not impact the abiotic or biotic conditions at Ranomafana (e.g., soil nitrogen cycling), (ii) the forest has had sufficient time to recover from any impacts that would have affected isotope values in vegetation, or (iii) Tsinjoarivo continuous forest is not pristine and may have experienced disturbance comparable to the selective logging activity at Ranomafana.

On average, leaves exhibit elevated $\delta^{13}C$ and low $\delta^{15}N$ values compared to other plant parts (Table 3). This finding is unexpected. In other studies, leaves have been reported to have lower $\delta^{13}C$ values than nonphotosynthetic parts (reviewed in Cernusak et al. 2009). Additionally, other researchers have found that leaves have slightly lower $\delta^{15}N$ values than fruits (e.g., Blumenthal et al. 2013). Leaves also have the lowest atomic C:N (although this ratio does not differ significantly from flowers; Table 3). This finding is in agreement with previous research, which has found a positive relationship between $\delta^{15}N$ values and nitrogen content in leaves (e.g., Hobbie et al. 2000). More data will be needed to test how much our results might depend on nitrogen source, environmental conditions, taxon-specific nitrogen acquisition, and mycorrhizal associations (Evans 2001; Szpak et al. 2013). Nevertheless, because these patterns are robust in our present dataset, we feel confident that they can be used to investigate dietary differences among lemur species in eastern Madagascar.

Our finding that canopy trees have significantly higher $\delta^{13}C$ values than any of the other plant types is unsurprising. An isotopic "canopy effect" attributable to variation in light, relative humidity, and respiration by soil microbes has been previously documented in forests from around the world (Broadmeadow et al. 1992; Cerling et al. 2004; Ehleringer et al. 1986; Hyodo et al. 2010; Kapos et al. 1993; van der

Table 3 Descriptive statistics for plant parts at each site

Plant part	Tsinjoarivo continuous forest			Tsinjoarivo fragmented forest			Ranomafana			All localities			
	N	$\delta^{13}C$ Mean$\pm 1\sigma$	$\delta^{15}N$ Mean$\pm 1\sigma$	N	$\delta^{13}C$ Mean$\pm 1\sigma$	$\delta^{15}N$ Mean$\pm 1\sigma$	N	$\delta^{13}C$ Mean$\pm 1\sigma$	$\delta^{15}N$ Mean$\pm 1\sigma$	N	$\delta^{13}C$ Mean$\pm 1\sigma$	$\delta^{15}N$ Mean$\pm 1\sigma$	Atomic C:N Mean$\pm 1\sigma$
Flower	8	-28.1±1.3	-1.1±1.0	5	-29.3±1.9	-3.3±1.2				13	-28.5±1.6[B]	-1.9±1.5[A]	32.9±12.1[A]
Fruit/Seed	25	-28.5±1.3	-1.0±1.9	13	-28.1±1.8	-2.5±1.9	23	-27.8±2.0	-0.8±1.7	61	-28.1±1.7[B]	-1.2±1.9[AB]	47.3±28.4[B]
Leaf	39	-29.5±2.0	-0.4±1.5	33	-28.5±1.8	-1.3±1.8	63	-31.6±1.1	-0.6±1.3	135	-30.2±2.0[A]	-0.7±1.5[B]	28.7±12.5[B]

Post hoc Tukey's honestly significant difference (HSD) tests ($\alpha = 0.05$) are presented for parts from all localities. For isotopic comparisons, these post hoc results account for other variables included in the GLM models (site and structure). Categories that share the same superscript letter are statistically indistinguishable

Merwe and Medina 1991). On average, canopy trees from this study have $\delta^{13}C$ values that are 2–3‰ higher than understory, climbing, or hemiparasitic plants (Table 4). This difference could potentially be used to distinguish lemurs that forage at different canopy heights. Lower atomic C:N in climbing plants compared to other plant types most likely relates to their rapid growth rate (Kazda and Salzer 2000). Somewhat unexpectedly, we found no isotopic differences between hemiparasites and other types. We had anticipated that the distinctive nutrient uptake of hemiparasitic plants might distinguish them from other plants. While there is an apparent trend for lower $\delta^{13}C$ values in hemiparasites, this pattern is not significant. Moreover, hemiparasites cannot be distinguished based on their atomic C:N values (Table 4). It is, therefore, unlikely that we can use isotopes to reliably distinguish consumption of the mistletoe *Bakerella*.

Lemurs

As expected, we found significant isotopic differences among lemur species both within and among localities. Carbon isotope values are consistently lower in eastern mouse lemurs than in sympatric Crossley's dwarf lemurs (Fig. 2; Table 5), suggesting that mouse lemurs may feed at lower canopy heights than dwarf lemurs at all three localities. The fact that *C. sibreei* has higher $\delta^{13}C$ values than sympatric *C. crossleyi*

in fragments suggests that the former species might consume more fruits or feed at higher canopy heights in the fragmented forest. Although differences in feeding height have not been quantified for these species at Tsinjoarivo, differences in feeding height have been proposed as a potential mechanism for niche differentiation among sympatric *Microcebus murinus*, *Cheirogaleus medius*, and *C. major* inhabiting littoral forest in southeastern Madagascar (Lahann 2007). Eastern mouse lemurs maintain $\delta^{15}N$ values that are roughly one trophic level higher than sympatric dwarf lemurs at all three localities, with a clear separation between genera even when samples from all localities are pooled (Fig. 2; Table 6). Accounting for isotopic differences among localities using apparent fractionation reveals significant differences in carbon and nitrogen among localities for both genera. Below, we revisit these results in the context of our three hypotheses.

Hypothesis 1. Cheirogaleids are unaffected by disturbance

Our results do not support this hypothesis. There are significant differences in $\Delta^{13}C_{\text{lemur-plant}}$ values among all three localities for both *Microcebus* spp. and *C. crossleyi* (Fig. 2, Table 7). Likewise, the apparent fractionation between nitrogen isotope values for lemurs and plants is significantly larger in fragmented forest for both lemur genera (Fig. 2, Table 7). These results indicate that cheirogaleid foraging may be

Table 4 Descriptive statistics for plant types at each site

Structure type	Tsinjoarivo continuous forest			Tsinjoarivo fragmented forest			Ranomafana			All localities			
	N	$\delta^{13}C$ Mean$\pm 1\sigma$	$\delta^{15}N$ Mean$\pm 1\sigma$	N	$\delta^{13}C$ Mean$\pm 1\sigma$	$\delta^{15}N$ Mean$\pm 1\sigma$	N	$\delta^{13}C$ Mean$\pm 1\sigma$	$\delta^{15}N$ Mean$\pm 1\sigma$	N	$\delta^{13}C$ Mean$\pm 1\sigma$	$\delta^{15}N$ Mean$\pm 1\sigma$	Atomic C:N Mean$\pm 1\sigma$
Canopy tree	44	-28.6±1.8	-0.7±1.6	26	-27.7±1.7	-1.6±1.6	14	-27.2±2.0	-0.1±1.2	84	-28.1±1.9[B]	-0.9±1.6[A]	35.8±22.8[A]
Climber	12	-29.1±1.7	-0.2±1.3	6	-29.4±1.4	-2.0±2.0	24	-31.1±1.7	-1.1±0.7	42	-30.3±1.9[A]	-0.8±1.3[A]	22.9±7.3[A]
Hemiparasite	8	-30.2±1.3	-1.7±2.1	10	-29.8±1.2	-2.7±1.1	23	-31.8±0.8	-0.4±1.5	41	-31.0±1.4[A]	-1.2±1.8[A]	42.8±14.7[A]
Understory tree	8	-30.1±1.5	-1.0±1.4	9	-28.8±1.5	-1.6±3.0	25	-30.9±1.6	-0.8±1.8	42	-30.3±1.8[A]	-1.0±2.0[A]	34.8±23.6[B]

Post hoc Tukey's HSD tests ($\alpha = 0.05$) are presented for structures from all localities. For isotopic comparisons, these post hoc results account for other variables included in the GLM models (site and part). Categories that share the same superscript letter are statistically indistinguishable

Table 5 Comparisons of lemur $\delta^{13}C$ values within each locality

Species	Ranomafana $\delta^{13}C$ Mean$\pm 1\sigma$ (N)	Tsinjoarivo continuous forest $\delta^{13}C$ Mean$\pm 1\sigma$ (N)	Tsinjoarivo fragmented forest $\delta^{13}C$ Mean$\pm 1\sigma$ (N)
Microcebus spp.	-24.5±0.3 (43)	-24.6±0.5 (13)	-23.4±0.3 (33)
Cheirogaleus crossleyi	-23.9±0.4 (13)	-23.7±0.3 (24)	-22.8±0.4 (9)
Cheirogaleus sibreei			-22.3±0.2 (35)
Species comparison	T=5.848 df=54 p<0.0001	T=7.267 df=35 p<0.0001	$F_{2,76}$=105.934 p<0.0001

affected by the historical logging activity at Ranomafana and strongly suggest that aspects of lemur foraging differ in the fragmented forest. Further work assessing these isotopic differences in terms of diet, nutritional ecology, and habitat use will help in assessing potential conservation implications of these patterns (Flaherty and Ben-David 2010).

Hypothesis 2. Lemur species respond differently to disturbance and may exploit novel resources in fragmented habitats

We find little support for this hypothesis. After accounting for baseline isotope values in plants, isotopic patterns among localities are similar for *Microcebus* spp. and *C. crossleyi* (Table 7). On average, $\Delta^{13}C_{lemur-plant}$ values are smallest at Tsinjoarivo continuous forest, intermediate at Tsinjoarivo fragmented forest, and largest at Ranomafana. Mean $\Delta^{15}N_{lemur-plant}$ values are significantly higher in fragmented forest for both genera. These results suggest that the two lemur genera may respond similarly to forest disturbance.

Hypothesis 3. All three lemur species rely on the same key resource in disturbed forest

We find the most support for this hypothesis. Although carbon and nitrogen isotope values for dwarf and mouse lemurs do not converge in the fragmented habitat, they do show similar patterns (Fig. 2; Table 7). Differences in $\Delta^{13}C_{lemur-plant}$ values among localities for both mouse and dwarf lemurs may indicate small shifts in feeding height or diet. For example, both genera of

lemurs may consume more fruits or flowers, or feed higher in the canopy in continuous forest than in fragmented or selectively logged forest. With our existing dataset, we cannot tease apart these alternatives (Flaherty and Ben-David 2010). It is possible that cheirogaleids rely on a resource, such as a particular species of plant, which is not isotopically distinct in fragments. It is also possible that both genera rely to varying degrees on a particular resource that we did not sample.

Little is known about the dietary preferences of dwarf or mouse lemurs at Tsinjoarivo. Dwarf lemurs have been observed to consume *Bakerella* in fragmented forest, particularly prior to hibernation, and mistletoe seeds have been identified in mouse and dwarf lemur feces at Tsinjoarivo and Ranomafana (Blanco personal observation). Because increased reliance on *Bakerella* has also been observed for sifaka (*Propithecus* spp.) inhabiting fragmented forest (Arrigo-Nelson 2006; Irwin 2008b), we had hypothesized that mistletoes might be an important resource for all lemur species in this disturbed habitat. Unfortunately, hemiparasitic *Bakerella* is not isotopically distinguishable from other plant types (Table 4). We cannot, therefore, use stable isotope data to confirm or refute increased reliance on *Bakerella* in the fragmented forest. Likewise, we cannot distinguish consumption of particular canopy or understory species. Future observations of foraging choices by mouse and dwarf lemurs may help identify other potential key resources in fragmented forest.

Patterns in nitrogen isotope data are clearer, suggesting that both cheirogaleid genera consume more animal matter, most likely arthropods, in fragmented forest. Although we did not measure isotope values in arthropods, we would expect them

Table 6 Comparisons of lemur $\delta^{15}N$ values within each locality

Species	Ranomafana $\delta^{15}N$ Mean$\pm 1\sigma$ (N)	Tsinjoarivo continuous forest $\delta^{15}N$ Mean$\pm 1\sigma$ (N)	Tsinjoarivo fragmented forest $\delta^{15}N$ Mean$\pm 1\sigma$ (N)
Microcebus spp.	5.2±0.7 (43)	5.4±0.7 (13)	5.4±0.7 (33)
Cheirogaleus crossleyi	2.7±0.5 (13)	2.8±0.3 (24)	3.3±0.4 (9)
Cheirogaleus sibreei			3.4±0.3 (35)
Species comparison	T=-12.183 df=54 p<0.0001	T=-15.262 df=35 p<0.0001	$F_{2,76}$=161.289 p<0.0001

Table 7 Apparent fractionation (Δ) between lemurs and plants at each locality

Species	Locality	N	$\Delta^{13}C_{lemur\text{-}plant}$ Mean$\pm 1\sigma$	$\Delta^{15}N_{lemur\text{-}plant}$ Mean$\pm 1\sigma$
Microcebus sp.	Ranomafana	43	6.1±2.2[A]	5.8±1.6[B]
	Tsinjoarivo continuous forest	13	4.4±1.9[C]	6.0±1.8[B]
	Tsinjoarivo fragmented forest	33	5.1±1.8[B]	7.2±2.0[A]
			$F_{2,88}=137.130$	$F_{2,88}=42.408$
			$p<0.0001$	$p<0.0001$
Cheirogaleus crossleyi	Ranomafana	13	6.7±2.2[A]	3.3±1.5[B]
	Tsinjoarivo continuous forest	24	5.3±1.8[C]	3.4±1.7[B]
	Tsinjoarivo fragmented forest	9	5.7±1.8[B]	5.1±1.9[A]
			$F_{2,45}=74.880$	$F_{2,45}=64.405$
			$p<0.0001$	$p<0.0001$
Cheirogaleus sibreei	Tsinjoarivo fragmented forest		6.1±1.8	5.2±1.9

ANOVA results comparing localities are presented for each species. Within each species, Δ values for localities that share the same superscript letter are statistically indistinguishable (Tukey's HSD test, $\alpha=0.05$)

to have higher $\delta^{15}N$ values than plant material (e.g., Herrera et al. 2001; Hyodo et al. 2010). Dammhahn et al. (2013) report mean $\delta^{13}C$ and $\delta^{15}N$ values for ground-dwelling arthropods from Tsinjoarivo fragmented forest (-24.4‰±1.03 and 2.0± 1.79‰, respectively). Although these values may not be representative for arboreal taxa, they suggest that arthropods have $\delta^{15}N$ values that are, on average, 3‰ higher than those in plants at this site. Mean $\Delta^{15}N_{lemur\text{-}plant}$ values for mouse lemurs (5.8–7.2‰) suggest that they are trophic omnivores at all three localities, although arthropod consumption may be somewhat higher in fragmented forest (Table 7). Mean $\Delta^{15}N_{lemur\text{-}plant}$ values for *C. crossleyi* exhibit a somewhat greater range among localities (3.3–5.1‰). Larger $\Delta^{15}N_{lemur\text{-}plant}$ values for dwarf lemurs from the fragmented forest (relative to continuous and selectively logged forest) most likely reflect a shift in trophic level from primarily frugivorous to omnivorous in fragmented forest. Increased reliance on arthropods has previously been inferred for omnivorous small mammals living in deteriorated forest and along forest edges (Corbin and Schmid 1995; Lehman et al. 2006a; Malcolm 1997; Nakagawa et al. 2007). For example, it has been suggested that mouse lemur density is relatively high near forest edges because arthropods are abundant in edge habitat (Corbin and Schmid 1995; Lehman et al. 2006a). Nakagawa et al. (2007) used nitrogen isotope values in small mammals to infer increased consumption of arthropods by omnivorous rats and squirrels living in degraded forest habitat. Nitrogen isotope values for these omnivorous taxa were significantly higher in degraded forest than in pristine forest. However, such trends are not uniform. Isotope data suggest that northern flying squirrels do not consume more invertebrates in secondary forest, despite an increased abundance of this prey resource (Flaherty et al. 2010). Instead, squirrels appear to avoid managed habitats altogether. In the case of eastern Madagascar, it may be that arthropod abundance is higher in the fragmented forest. Alternatively, if preferred

cheirogaleid foods (e.g., fruits) are rare or less nutritious (e.g., lower in protein: Ganzhorn et al. 2009) in the disturbed habitat, then these lemurs might need to consume more arthropods in fragmented forest regardless of whether or not they are more abundant (Balko and Underwood 2005).

The use of daily torpor or extended hibernation is critical for some cheirogaleids to survive the period of resource scarcity during the dry season. Whereas mouse lemurs undergo daily torpor and, in some cases, prolonged torpor bouts (Atsalis 2008; Dammhahn and Kappeler 2010; Kobbe and Dausmann 2009; Schmid and Ganzhorn 2009; Vuarin et al. 2013), dwarf lemurs can hibernate 4–7 months/year (Blanco et al. 2013; Blanco and Godfrey 2013; Blanco and Rahalinarivo 2010; Dausmann et al. 2005). These seasonal differences in activity may contribute to isotopic differences between *Cheirogaleus* and *Microcebus* species for two reasons. First, *Microcebus* fur should average foods consumed across all seasons, whereas *Cheirogaleus* fur should resemble the isotopic signature of rainy-season foods only. Although preliminary research suggests that there are not appreciable seasonal differences in the isotope values of plant foods (Crowley et al. 2011), future finer-scaled studies investigating how mouse lemur diets may shift seasonally will be necessary to exclude this possibility.

Second, during torpor, metabolic rate and body temperature decline, body fat is catabolized, and protein is recycled. These metabolic changes could impact fur isotope values for dwarf lemurs (Lee et al. 2012). The vast majority of the dwarf lemur samples in this study were collected in September and October, which is after individuals come out of hibernation. However, several fragment *C. crossleyi* were captured in April, just prior to going into hibernation. Contrary to expectations, we find no differences in $\delta^{13}C$ or $\delta^{15}N$ between individuals that were captured pre- and post-hibernation, despite significant differences in mass. This suggests that dwarf lemurs may not catabolize large fat or protein stores during

hibernation. Unlike the dwarf lemurs of western Madagascar, which hibernate in tree holes (Dausmann et al. 2005), *C. crossleyi* and *C. sibreei* both hibernate in underground hibernacula (Blanco et al. 2013). This enables them to maintain constant low body temperatures with infrequent arousals. Because arousals require rewarming using endogenous energy reserves, maintaining a constant low body temperature and minimizing arousals may decrease the necessity for large fat stores (Blanco et al. 2013).

Conclusions

Stable isotope data from fur provide a first step towards understanding how sympatric cheirogaleid species partition their resources, how their ecological niches may vary across sites, and how foraging may be impacted by forest fragmentation and degradation. Our results also demonstrate that accounting for baseline isotope values for plants is crucial for drawing conclusions about ecological differences among localities. Carbon isotope data suggest that both mouse lemurs and dwarf lemurs slightly modify their diet or habitat use among localities. Nitrogen isotope data suggest cheirogaleid lemurs rely more on arthropods in fragmented forests. Dwarf lemurs might be particularly adversely affected by forest fragmentation if they cannot build substantial fat stores to maintain prolonged hibernation.

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