UNIVERSITY OF CALIFORNIA, SAN DIEGO

Diet-tissue stable isotope (delta^{13}C and delta^{15}N) discrimination factors for multiple tissues from terrestrial reptiles (rock iguanas, *Cyclura* species)

A Thesis submitted in partial satisfaction of the requirements for the degree of Master of Science

in

Biology

by

Ronnie Steinitz

Committee in charge:

Carolyn M. Kurle, Chair
Elsa E. Cleland
David A. Holway
Stesha A. Pasachnik

2015

The Thesis of Ronnie Steinitz is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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EPIGRAPH

You cannot get through a single day without having an impact on the world around you.
What you do makes a difference, and you have to decide
what kind of difference you want to make.

-Jane Goodall

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ABSTRACT OF THE THESIS

Diet-tissue stable isotope (delta^{13}C and delta^{15}N) discrimination factors for multiple tissues from terrestrial reptiles (rock iguanas, *Cyclura* species)

by

Ronnie Steinitz

Master of Science in Biology

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Professor Carolyn M. Kurle, Chair

Trophic interactions can drive community structure; therefore, studying food webs is key in understanding ecological communities. Stable isotope analysis is a powerful tool for reconstructing foraging patterns. However, stable isotope discrimination factors (Δ^{13}C and Δ^{15}N) are needed to best use this tool.

We determined the first $\Delta^{13}C$ and $\Delta^{15}N$ values for Rock Iguanas (*Cyclura* spp.) to better understand isotope fractionation patterns in reptiles and estimate wild reptile diets. We analyzed $\Delta^{13}C$ and $\Delta^{15}N$ values between skin, blood, and scat from juvenile and adult iguanas held for over a year on a known diet and their food. We determined relationships between size/age and discrimination factors and compared isotope values from lipid- and non-lipid-extracted tissues and from scats that were treated/untreated with HCl. The $\Delta^{13}C$ and $\Delta^{15}N$ values ranged from -2.9 to +6.2‰ and from +1.7 to +7.0‰, respectively, with some differences among tissues and between juveniles and adults. The $\Delta^{13}C$ values from blood and skin differed among species, but not $\Delta^{15}N$ values. The $\Delta^{13}C$ values from blood and skin and $\Delta^{15}N$ values from blood were positively correlated with size/age. The $\Delta^{13}C$ values from scat were negatively correlated with size (not age). Treatment with HCl (scat) and lipid extraction (skin) did not affect isotope values.

Our results aid in the understanding of processes driving stable carbon and nitrogen isotope discrimination factors in reptiles. We provided estimates of $\Delta^{13}C$ and $\Delta^{15}N$ values and linear relationships between iguana size/age and discrimination factors for the best application of these values for interpreting wild reptile foraging ecology.

Introduction

The conservation and management of declining species and their habitats necessitate an understanding of the natural and anthropogenic processes driving the disappearance or persistence of a given species. Therefore, it is important to study species interactions such as foraging ecology and habitat use to assess their potential as drivers of community ecology and species decline (Chapron et al. 2008 and Palomares et al. 2010). There are many ways to study these, including long-term behavioral observations and fecal (scat) and stomach content analyses. There are drawbacks to these methods as they can be time and labor intensive (Moller 1983), resulting in small samples sizes that may not be representative of larger populations. In addition, stomach contents and fecal analyses only indicate an animal's most recent meal, precluding dietary estimations over longer temporal scales (Orr & Harvey, 2001).

The use of stable isotope analysis of tissues, along with analyses of potential dietary items, allows for a more comprehensive assessment of animal foraging ecology. Stable carbon ($^{13}C/^{12}C$ or $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) isotope analyses of predator and prey tissues offers a method whereby animal foraging ecology can be estimated over variable temporal scales with minimal disturbance, labor, and cost (Ben-David & Flaherty 2012 and Boecklen et al. 2011). The $\delta^{13}C$ values from animal tissues reveal dietary carbon sources allowing for the distinction between dietary items such as marine- or terrestrial-based primary production, plant or animal diet components, and C_3 or C_4 plants (DeNiro & Epstein, 1978), among other things. The $\delta^{15}N$ values from animal tissues largely reflect animal trophic position as the $\delta^{15}N$ values from organisms increase

predictably with increasing trophic levels (Ben-David and Flaherty 2012, Boecklen et al. 2011, and Minagawa & Wada 1984).

Stable isotope analysis can also provide a wide range of temporal data as isotopic turnover varies depending upon the protein turnover of a particular tissue (Kurle 2009). Therefore, analysis of multiple tissues from a single individual can provide dietary insights from several time periods. In addition, stable isotope mixing models can be used to estimate the proportion that isotopically distinct dietary items contribute to an animal's total diet (Semmens et al. 2009 and Semmens et al. 2013). However, the best use of these models for estimating diets of wild populations requires accurate model parameters, including reasonable estimates of the isotope discrimination or trophic enrichment factors (Boecklen et al. 2011). These are the differences in isotope values between a consumer and its dietary items and are expressed as $\Delta^{13}C = \delta^{13}C_{PredatorTissue} - \delta^{13}C_{Prey}$ and similarly for nitrogen. Discrimination factors are typically obtained from studies using captive animals held on known, consistent diets for an adequate amount of time and they can aid in the interpretation of stable isotope data from wild animals (Kurle et al. 2013).

It is frequently difficult to collect these data, as access to animal populations held on known diets is rare, so that determination of adequate discrimination factors is not common (Boecklen et al. 2011), especially for terrestrial reptiles (but see Murray & Wolf 2013 and Warne et al. 2010). Therefore, the $\Delta^{13}C$ and $\Delta^{15}N$ values of ~1.0‰ and ~3.4‰, respectively, are generally used as discrimination factors for many studies (DeNiro & Epstein 1978, 1981; Minagawa & Wada 1984; Post 2002). However, given the large degree of variation in the $\Delta^{13}C$ and $\Delta^{15}N$ values observed among taxa and even within

taxa among different tissues (Kurle 2002, Parng et al. 2013, Kurle et al. 2013, Kurle et al. 2014, Seminoff et al. 2007, Murray &Wolf 2013, Warne et al. 2010), the use of these generalized numbers in stable isotope mixing models can be flawed and lead to erroneous interpretations of the models (Bond & Diamond 2011, Phillips 2012). Reptiles, a taxon that is lacking in stable isotope data in general, and discrimination factors in particular, are the focus of our study.

Rock Iguanas (*Cyclura* spp.), found exclusively in the West Indies, are collectively threatened by habitat destruction and invasive species, among other threats, and the status of these species range from vulnerable to critically endangered (IUCN 2014). They typically inhabit subtropical dry forests, requiring sandy areas and particular soil conditions in which to burrow and lay eggs, and depend heavily on the presence of rocky crevices as retreats (Alberts 2000). They are predominantly herbivorous, consuming foliage, berries, and other fruits (Blair 1991), but can also feed opportunistically on animal material (e.g. Hines 2011). Because of their diet, rock iguanas play a key role in structuring their ecosystems. For example, Hartley et al. (2000) demonstrated that seeds passing through iguana digestive tracts sprout earlier than those not ingested, and with wide dispersal of the seeds by the iguanas (Alberts 2004), may lead to advantageous priority effects for these plant species (Fukami et al. 2005). Where they occur, Rock Iguanas are the largest, native herbivore, making them essential for maintaining native plant communities in the highly endangered tropical dry forest ecosystems they inhabit (Alberts 2000). Their threatened status combined with their important role as ecosystem engineers underscores the priority of the species-specific

management efforts and conservation actions led by the International Union for Conservation of Nature's (IUCN) Iguana Specialist Group (ISG) and the International Iguana Foundation (IIF).

To better understand the foraging related interactions between wild terrestrial reptiles in general, and *Cyclura* spp. in particular, and their communities, we established the $\Delta^{13}C$ and $\Delta^{15}N$ values between diet and scat, blood, and skin components for females and males and across different age and size classes from three captive rock iguana species. We targeted tissue samples that were obtained both invasively and opportunistically so as to maximize the applications of our data to studies of wild reptiles. Nearly 20% of all reptile species are at risk for extinction and another 20% are classified as having insufficient data to determine their status (Böhm et al. 2013). Data deficiencies and the numbers of threatened reptile species are especially high for species in tropical areas and recent research has called for increased study of these species for more effective targeting of conservation action (Böhm et al. 2013). The determination of these factors for reptiles is important for biological conservation as they may aid in interpretation of wild population data, and contribute to future management plans pertaining the ecological communities of Rock Iguanas.

Materials and Methods

Iguanas sampled

We obtained body tissues and physical measurements (weight (g) and snout-vent length (mm), SVL) from 34 individuals of captive populations from three Rock Iguana species, *Cyclura collei, C. lewisi,* and *C. pinguis* (Table 1) held at the San Diego Zoo Institute for Conservation Research (ICR) facilities in Escondido, CA. *Cyclura collei* historically ranged throughout Jamaica, and although the population was thought to be extinct in the wild in the 1990's, a trace population was found in the Hellshire Hills region of Southeastern Jamaica. *Cyclura lewisi* are native to Grand Cayman Island, and *C. pinguis* are native to Anegada, Guana, and Norman Islands, in the British Virgin Islands.

We held all individuals on a steady diet of 15 plant species (Table 2) for over 12 months; this length of time is an adequate time period for full stable isotope equilibration of the iguanas' tissue to their experimental diet (Reich et al. 2008). Iguanas were offered food once daily, Monday through Friday, and had water available *ad libitum*. ICR staff chopped food according to the size of the individual fed (adults: ~5x5cm and juveniles: ~2.5x2.5cm) and we collected dietary samples along with scat, blood, and shed (sloughed) skin from individual iguanas, over two sampling periods, spaced 6 months apart (February 2013 and August 2013) to account for potential seasonal variations in the stable isotope values from dietary items (Ehleringer, Phillips & Comstock 1992). We

sampled tissues from adult males and females from all three species and from both juveniles and adults of *C. lewisi* (Table 1).

Different species of Rock Iguanas mature at different ages, ranging from 2-7 years (Lemm, Lung & Ward 2007). However, within species there are contradicting estimates of the age at maturity (Alberts et al. 2004, Lemm, Lung & Ward 2007, J. Lemm, pers. obs.). Furthermore, as there are few long-term population studies of iguanas in the wild (but see Iverson et al. 2006), many observations come from captive individuals, and therefore the estimated age of sexual maturity may vary from the actual age in wild counterparts. It is generally accepted that *C. collei* and *C. pinguis* mature near the upper end of the 2-7 year age range, wheras *C. lewisi* can lay eggs as early as their second year (J. Lemm & S. Pasachnik, pers. obs.). The *C. collei* and *C. pinguis* individuals we sampled were all adults (all ages ≥ 7 years), whereas the *C. lewisi* consisted of 15 juveniles (born Aug. 2011; age < 2 years), and five adults (ages ≥ 2 years).

Stable isotope analysis

We collected scat samples either directly from the iguanas if they defecated when handled, or from their captive enclosure if they defecated earlier in the day, and we removed any external debris upon collection or later in the lab. We collected skin samples directly from individuals as they shed, washed the samples with dish soap, and rinsed them thoroughly with DI water. We froze all scat and skin samples at -20°C until we processed them for isotope analysis (see below). We collected approximately 200 uL

of whole blood from each individual from the ventral coccygeal vein using a butterfly needle during routine husbandry blood collection to minimize stress (Lemm, Lung & Ward 2007). We transferred the blood onto pre-combusted Whatman GF/F glass microfiber filter papers that we dried in sterile scintillation vials in a drying oven at 32°C for 48 hours (Kurle & Gudmundson 2007). We homogenized blood samples by hand using a mortar and pestle or metal spatula within a cryovial, and ~0.5 to 1.0 mg of these processed samples were weighed into 5 x 9 mm tin capsules. We did not extract lipids from blood as whole blood has very low lipid content (Bearhop et al. 2002). This was supported by the low (<4) C:N ratios observed from our stable isotope data (Post et al. 2007).

We freeze-dried scat and skin samples for 48 hours, and scat samples were further dried at 120°C for 48 hours to kill any potentially remaining bacteria. We removed any remaining undigested plant matter from the scats, extracted the fecal matrix material, then divided each scat into two subsamples. We agitated the sample targeted for $\delta^{13}C$ analysis with 0.5M HCl for 3h to remove any potential inorganic carbon as we were interested in measuring the $\delta^{13}C$ values from the organic components only, and then dried the samples at 32°C for 48 h. To test for effects of HCl on the $\delta^{13}C$ and $\delta^{15}N$ values from scat, we also analyzed subsamples of untreated scat material. We homogenized all scat samples by hand using either a mortar and pestle or metal spatula within a cryovial, and packaged ~5 mg of each scat sample into 5x9 mm tin capsules.

We cut skin samples into small pieces (<0.5x0.5mm) using surgical scissors, then divided each sample into two subsamples. We performed lipid extraction on the samples

targeted for $\delta^{13}C$ analysis via a method modified from Folch et al. (1957, Post et al. 2007, Sweeting et al. 2006). We placed skin samples in 15-ml glass centrifuge tubes, added 10 ml of petroleum ether (Dobush et al. 1985), capped the vials with perforated lids, and sonicated them for 10 min at 40Khz in a 60°C water bath. We then centrifuged the samples at 12,000 X g for 5 min, pipetted off the petroleum ether, rinsed each sample with ultra-pure water, centrifuged them again at 12,000 X g for 5 min, then removed the excess water with a pipette. We then dried all samples in a drying oven at 45°C for 72 h. Samples were re-homogenized before analysis. We analyzed subsamples from all skin samples with their lipids intact to test for the effects of lipid extraction on the stable isotope values, and ~0.5 to 1.0 mg of all skin samples were packaged into 5 x 9 mm tin capsules. Throughout the sampling periods, we collected all diet samples (Table 2), which we hand-washed, froze at -20°C for at least 48 hours, then freeze-dried for 48 hours. We homogenized samples by hand and packed ~3 mg into 5x9mm tin capsules.

All stable isotope analysis was obtained with a Carlo Erba CE1108 elemental analyzer interfaced via a ConFlo III device to a Thermo-Electron Delta Plus XP mass spectrometer at the Department of Earth and Marine Sciences, University of California, Santa Cruz. We calculated the average precision of these data as the SD of the $\delta^{13}C$ and $\delta^{15}N$ values from a set of standards (acetanilide), and it was 0.1‰ for both.

Statistical Analysis

The ICR kept all individuals on a weekly diet schedule (Table 2) for which we calculated the weighted percentage of each diet item by comparing the weekly total weight of each diet item to the weekly total weight of all food consumed by each

individual. Using this weighted percentage and the $\delta^{13}C$ and $\delta^{15}N$ values of each dietary item and the iguana tissues, we calculated the discrimination factors using the following equation:

$$\Delta X(‰) = (\delta X_{consumer}) - [(\%_{source\ 1} \times \delta X_{source\ 1}) + (\%_{source\ 2} \times \delta X_{source\ 2}) + \ldots],$$

where, $\Delta X(‰)$ is either the C or N isotope discrimination factor ($\Delta^{13}C$ or $\Delta^{15}N$, respectively), $\delta X_{consumer}$ is the $\delta^{13}C$ or $\delta^{15}N$ value of the tissues from the iguanas, $\%_{source}$ is the contribution of a specific diet item to the total diet, and δX_{source} is the $\delta^{13}C$ or $\delta^{15}N$ value of that particular diet or source item (Kurle 2002; Parng et al. 2013).

We performed all statistical tests with R, using parametric methods as all data met the assumptions for parametric tests. We used paired t-tests to evaluate the effects of acid treatment and lipid extraction on the isotope values from scat and skin samples, respectively, and to test for differences in isotope values between the two collection periods. We used t-tests too test for effects of sex, age-group, and sampling period on the stable isotope values from scat and skin, and of sex and age-group on the isotope values from blood samples. To test for relationships between the stable isotope values from all tissues and iguana age, size (SVL; mm), weight (g), and body condition index (BCI; $\frac{Weight_i}{SVL_i}$), we conducted linear regressions for all tissues using individuals for which we had age, SVL, and/or weight data for each respective analyses. For some, these measurements were not available and they were omitted from the regression analysis. We include results from regression analyses for age, size, weight, and BCI (even though they are all related) so that the results will be useable by researchers with access to any of

those variables as they work to best determine which $\Delta^{13}C$ or $\Delta^{15}N$ values would be most applicable to the age or size reptile of interest. To test for differences in the stable isotope values among species for all tissues collected and among tissues for each species, we conducted analysis of variance (ANOVA) tests followed by Tukey's pairwise comparisons (R core development team 2014). Values reported are means ± SD and significance was tested at the $\alpha = 0.05$ level.

Results

The $\delta^{13}C$ and $\delta^{15}N$ values from all tissues and dietary items collected during the two sampling periods were not different (Paired t-tests, all $p \geq 0.07$; see Supplemental Table S1). Therefore data were analyzed primarily for samples collected during the first sampling period (February 2013). Blood samples were only available from the second sampling period (August 2013), so these were used for the isotope analysis of blood. The $\delta^{13}C$ and $\delta^{15}N$ values were the same between sexes within each tissue type (t-tests, all $p \geq 0.25$; see Table S1), so females and males were grouped together for all comparisons. The $\delta^{13}C$ and $\delta^{15}N$ values were different among tissue types (scat, blood, and skin) for each species (ANOVA, all $p \leq 0.01$) and Tukey's post hoc tests confirmed that the isotope values from all tissues within each species were different except for the $\delta^{13}C$ values from blood and skin from *C. lewisi* ($p=0.26$), and the $\delta^{15}N$ values from blood and skin for *C. pinguis* ($p=0.22$) and from blood and scat for all three species ($p= 0.10$ to 0.56) and (see Table S1). The $\Delta^{13}C$ and $\Delta^{15}N$ values from all tissue types and across all species ranged from -2.9 to +6.2‰ and +1.7 to +7.0‰, respectively, for adults, and from -2.0 to +2.4‰ and +0.40 to +4.9‰, respectively, for juveniles (Table 1). We present the linear regression results between age, SVL, weight, and BCI and the $\Delta^{13}C$ and $\Delta^{15}N$ values below, however, as expected, the overall iguana BCI was positively correlated with age ($R^2_{adj}=0.9$, $F_{1,24}=204.2$, $p<0.01$) (Figure 1).

Scat

There were no differences in the $\delta^{13}C$ values from scat samples processed with and without the HCl agitation to potentially remove inorganic carbon prior to isotope

analysis (paired t-test, t=-1.0, df=37.0, p=0.4) (Table S1). We expected the acid treatment to have no effect on the δ^{15}N values, however we found that scat processed with HCl exhibited higher δ^{15}N values than those processed without HCl (5.3±1.1‰ vs. 4.8±0.7‰, respectively; paired t-test, t=3.5, df=9.0, p<0.01) (Table S1). Thus we used the δ^{13}C and δ^{15}N values from the untreated scat samples for our analysis.

The Δ^{13}C and Δ^{15}N values from scats from all of the species had high degrees of variability, ranging from -2.4±0.4‰ to -0.8±0.9‰ and 1.8±2.3‰ to 3.4±0.3‰, respectively, but none of these values were statistically different among species (ANOVA, $F_{2,8}$=3.4, p=0.09 and $F_{2,8}$=1.5, p=0.28, respectively; Tables 1 and S1). There were no differences in the Δ^{13}C and Δ^{15}N values from scat between the two age groups sampled from *C. lewisi* (adults vs. juveniles; t-tests, δ^{13}C: t=0.2, df=4.2, p=0.85; δ^{15}N: t=0.4, df=6.6, p=0.69) (Tables 1 and S1). Linear regression analysis demonstrated no correlation between age as a continuous variable and the Δ^{13}C and Δ^{15}N values from scat samples (δ^{13}C: R^2_{adj}=0.1, $F_{1,9}$=2.2, p=0.17; δ^{15}N: R^2_{adj}=-0.1, $F_{1,9}$=0.1, p=0.82; Tables 1 and S1, Figure S1a). However, linear regression analysis exhibited a negative correlation between the Δ^{13}C values from scats and body size (SVL, R^2_{adj}=0.8, $F_{1,5}$=20.6, p=0.01; Table S1 and Figure S1b), weight (R^2_{adj}=0.6, $F_{1,5}$=9.1, p=0.03; Table S1 and Figure S1c), and BCI (R^2_{adj}=0.7, $F_{1,5}$=13.9, p=0.01; Figure 1 and Table S1) for adults of all three species. There were no such relationships for the Δ^{15}N values from scats (Figure 1, Table S1).

Blood

The $\Delta^{13}C$ values from blood differed among all three species (ANOVA, $F_{2,11}=22.7$, $p<0.01$), but the $\Delta^{15}N$ values were not different ($F_{2,11}=1.8$, $p=0.21$) (Tables 1 and S1). Post hoc Tukey pairwise comparisons revealed that all three species had different mean $\Delta^{13}C$ values (all $p\leq0.02$), with *C. lewisi* as the lowest (1.5±0.3‰), then *C. collei* (2.1±0.4‰), and *C. pinguis* (2.8±0.2‰) (Tables 1 and S1). In addition, the mean $\Delta^{15}N$ values from *C. lewisi* juveniles were significantly lower (2.5±0.6‰) than those from the adults (3.4±0.6‰; t-test, $t=2.9$, $df=7.4$, $p=0.02$), but the $\Delta^{13}C$ values (1.4‰ to 1.5‰) did not differ between age groups (t-test, $t=1.0$, $df=6.3$, $p=0.38$) (Tables 1 and S1).

We found strong, positive correlations between age as a continuous variable and both the $\Delta^{13}C$ and $\Delta^{15}N$ values from blood ($R^2_{adj}=0.7$, $F_{1,12}=26.8$, $p<0.01$ and $R^2_{adj}=0.3$, $F_{1,12}=7.8$, $p=0.02$, respectively) (Table S1 and Figure S1a). The $\Delta^{13}C$ and $\Delta^{15}N$ values from blood were also significantly, positively correlated with size ($R^2_{adj}=0.3$, $F_{1,11}=6.6$, $p=0.03$ and $R^2_{adj}=0.7$, $F_{1,11}=32.0$, $p<0.01$, respectively) (Table S1 and Figure S1b), weight ($R^2_{adj}=0.6$, $F_{1,11}=17.5$, $p<0.01$ and $R^2_{adj}=0.6$, $F_{1,11}=19.1$, $p<0.01$, respectively) (Table S1 and Figure S1c), and BCI ($R^2_{adj}=0.5$, $F_{1,11}=15.0$, $p<0.01$ and $R^2_{adj}=0.6$, $F_{1,11}=21.4$, $p<0.01$, respectively) (Figure 1 and Table S1).

Skin

There were no differences in the $\delta^{13}C$ and $\delta^{15}N$ values from skin that had been lipid extracted or left intact (paired t-tests, $t=-0.1$, $df=7.0$, $p=0.90$ and $t=0.6$, $df=9.0$, $p=0.56$, respectively). Therefore, we used isotope values from samples that were not lipid

extracted for calculating the discrimination factors for skin. The $\Delta^{13}C$ values from skin varied among species (ANOVA, $F_{2,14}=8.5$, $p<0.01$). The order of $\Delta^{13}C$ values from skin was the same as for blood, *C. lewisi* (2.4±0.1‰), *C. collei* (3.7±1.2‰), and *C. pinguis* (5.2±0.9‰); however Tukey's tests demonstrated that the $\delta^{13}C$ values from skin were not significantly different between *C. lewisi* and *C. collei* ($p=0.19$). All other comparisons were significantly different ($p\leq0.04$; Tables 1 and S1). The $\Delta^{15}N$ values from skin did not differ among species (ANOVA, $F_{2,14}=0.6$, $p=0.6$, Tables 1 and S1). *C. lewisi* juveniles exhibited lower $\Delta^{13}C$and $\Delta^{15}N$ values from skin (-1.9±0.3‰ and 3.7±0.8‰, respectively), than *C. lewisi* adults (2.4±0.1 and 5.5±0.7‰, respectively) (t-test; $\Delta^{13}C$: $t=4.6$, $df=9.9$, $p<0.01$; $\Delta^{15}N$: $t=3.6$, $df=3.6$, $p=0.03$) (Tables 1 and S1). The $\Delta^{13}C$ values from skin collected from adults of all species were positively correlated with age as a continuous variable ($R^2_{adj}=0.8$, $F_{1,15}=53.9$, $p<<0.01$), but the $\Delta^{15}N$ values were not ($R^2_{adj}=0.0$, $F_{1,15}=0.8$, $p=0.37$)(Figure S1a, Table S1). In addition, size and weight were positively correlated with the $\Delta^{13}C$ values from skin from adults ($R^2_{adj}=0.5$, $F_{1,9}=10.2$, $p=0.01$; $R^2_{ad}=0.7$, $F_{1,9}=27.4$, $p<0.01$, respectively), whereas there were no relationships between size and weight and the $\Delta^{15}N$ values from skin ($R^2_{adj}=-0.1$, $F_{1,9}=0.1$, $p=0.79$; $R^2_{adj}=-0.1$, $F_{1,9}=0.1$, $p=0.80$, respectively) (Figure S1b and c, Table S1).

Discussion

Of 24 comparisons of the $\Delta^{13}C$ and $\Delta^{15}N$ values among tissues for all species (blood vs. skin vs. scat for the adults of three species and for juvenile *C. lewisi*), only six were not different: the $\Delta^{13}C$ values from blood and skin in *C. lewisi* adults, $\Delta^{15}N$ from blood and skin in *C. pinguis* adults, and $\Delta^{15}N$ from blood and scat from adults of all three species and juvenile *C. lewisi* (Table 1 and Table S1). Different tissues are composed of different amino acids and amino acids vary in their $\delta^{13}C$ and $\delta^{15}N$ values (Lorrain et al. 2009, Popp et al. 2007). Therefore, it is expected that different tissues from the same animal held on a constant diet will exhibit varying stable isotope values (Kurle et al. 2014).

The $\delta^{15}N$ (and $\Delta^{15}N$) values from blood and skin among species were the same (Table 1), however, they were significantly lower for juvenile *C. lewisi* than the adult *C. lewisi*. In addition, all among species comparisons for $\Delta^{13}C$ values from blood and skin were significantly different (Table 1 and Table S1). The $\Delta^{15}N$ values of tissues from vertebrates with higher growth rates, such as more rapidly growing hatchlings or juveniles, have been shown to be lower than those from animals that are not growing or have slowed growth (Fuller 2004, del Rio and Wolf 2005, Reich 2008, Kurle et al. 2014). This is because growing animals retain more ^{14}N via tissue deposition than they lose via excretion of waste compared with an animal that is not growing (but see Sponheimer 2003b). This could explain the lower $\Delta^{15}N$ values we observed in the juvenile *C. lewisi* vs. those from the adult *C. lewisi*.

To our knowledge, this same phenomenon has not been shown to occur for $\delta^{13}C$ (and thus $\Delta^{13}C$) values and, in fact, rapidly growing hatchling and juvenile sea turtles (loggerheads, *Caretta caretta*) have demonstrated $\delta^{13}C$ values in line with other published values from adults (Reich 2008). However, the $\Delta^{13}C$ values from blood and skin from the adults in this study increased with increasing size (Tables 1 and S1, Figures 2, S1b), which may be attributed to differential growth rates at different ages for reptiles as the youngest, and physically smallest, species (*C. lewisi*) had the lowest $\delta^{13}C$ values, followed by the mid-size *C. collei*, then the oldest, largest *C. pinguis*. While little data exists on *Cyclura* growth rates, they are known to grow continuously throughout their lives (Iverson et al. 2004), but exhibit different growth rates at different life stages, including higher growth and metabolic rates at younger ages (Alberts et al. 2004, Lemm, Lung & Ward 2007, per. comm. Lemm 2014). Each species in this study includes individuals of significantly different ages (*C. pinguis*: 23.0±6.7 yrs, *C. collei*: 12.1±6.4 yrs, and *C. lewisi*: 2.2±2.9 yrs (adults and juveniles); $p \leq 0.001$; Table 1) who may be experiencing different growth and metabolic rates specific to their ages. Interestingly, only the $\Delta^{13}C$ values from skin (and not those from blood which were the same) were lower for the juveniles than the adult *C. lewisi*. At present, we do not know why the $\Delta^{13}C$ values correlated so strongly with age/size in the adults, whereas the $\Delta^{15}N$ values did not, and the potential role of differential sizes and growth rates in determining $\Delta^{13}C$ and $\Delta^{15}N$ values deserves further study in both endotherms and ectotherms.

Finally, the $\Delta^{13}C$ values from scats were negatively correlated with iguana age, size, and weight. It is well established that excreta generally have lower $\delta^{15}N$ values than

animal tissue because the lighter isotope of nitrogen reacts more quickly than the heavier isotope, becoming incorporated into waste products. This is what leads to predictable enrichment in δ^{15}N values between trophic levels in a food web. As growing animals tend to retain more lighter isotopes, then it follows that their excreta would contain higher ratios of heavier to lighter isotopes than slower growing adults, leading to higher δ^{13}C (and thus Δ^{13}C) values for animals experiencing greater growth rates. Again, we would expect a similar pattern for the stable nitrogen isotope values from scats, but there were no relationships between the Δ^{15}N values from iguana scats and their age, size, or weight. The Δ^{13}C values from scats were all negative which is unexpected as isotopic discrimination factors between diet and animal tissues are generally positive. These negative Δ^{13}C values may be a product of the unique digestive system of the Rock Iguanas. Hwang et al. found that hindgut fermenters (such as rock iguanas) had much lower δ^{13}C values than foregut fermenters, and suggested that this is likely a result of differences in the biochemical pathways of the digestive system causing increased isotopic fractionation (2007).

In studies of wild reptile populations (green sea turtles, *Chelonia mydas*, loggerhead turtles, *Caretta caretta*, and leatherback sea turtles, *Dermochelys coriacea*), correlations of δ^{13}C and δ^{15}N (and, consequently, Δ^{13}C and Δ^{15}N) with body size, weight, or age are typically attributed to ontogenetic, or size- and age-related, shifts in diet preferences (Arthur et al. 2008, Hatase et al. 2002, Salmon et al. 2004). For example, such changes can occur as animals grow larger and are able to acquire larger prey. However, the San Diego Zoo ICR held individuals of all ages and species on a steady,

identical diet throughout our study (12+ months). Therefore, diet shifts with increasing age or size would not account for the observed isotopic changes and this should be considered when examining stable isotope values from different age and size-class individuals in the wild. In addition, it is frequently difficult to obtain $\Delta^{13}C$ and $\Delta^{15}N$ values from captive animals for use in studies estimating wild animal foraging ecology. As we demonstrate linear relationships between the $\Delta^{13}C$ values from blood, skin, and scat and iguana weight, size, and age, and the $\Delta^{15}N$ values from blood and iguana weight, size, and age, it may be possible for others to use our regressions to estimate the best $\Delta^{13}C$ and $\Delta^{15}N$ values for these tissues for use in interpreting wild iguana foraging ecology if the weights, sizes, and/or ages of the iguana species of interest are known. We report separate results for weight, size and age (Figure S1, Table S1) so others with only partial data (for example, one or some of these factors, and not all) can compare size, weight and/or age to help predict more accurately where their animals fit relative to these scales.

The treatment of scat samples with HCl was done to remove any potential inorganic carbon (such as bony or shell fragments; R. Brown Reid, personal communication, Washington University, St. Louis) before stable isotope analysis. This is to insure that organic carbon is targeted for analysis, as the $\delta^{13}C$ value from organic carbon is what reflects animal diet. Although we found that agitation of scat matrix in HCl did not significantly affect the $\delta^{13}C$ in our samples, indicating that no inorganic carbon was present, those that were treated with HCl did exhibit higher $\delta^{15}N$ values than those that were not. The acidification process could cause leaching of organic nitrogen

compounds (i.e. proteins or amino acids) (Mintenbeck et al. 2008), which could impact δ^{15}N values in the scat samples. As the acidification treatment of scats did not affect their δ^{13}C values, and it did affect the δ^{15}N values, we recommend omitting this treatment from future protocols for preparing scats from iguanas for stable isotope analysis unless the samples in question clearly contain significant amounts of materials with inorganic carbon. In addition, we recommend that the use of stable isotope values from scat in future studies be considered with caution as scat proved to be the least reliable tissue in its consistency of stable isotope values across species and age groups (see Tables 1, S1). Studies using stable isotope analysis are often performed for the purpose of reconstructing diets of wild animals and scat would be a relatively easy, accessible, and uninvasive tissue to use for such analyses. However, Hwang et al. found that δ^{15}N and δ^{13}C values from scat did not consistently reflect isotopic composition of diet, and Δ^{15}N and Δ^{13}C from scat offered evidence of great variation in a study across several mammalian fore- and hindgut fermenters (2007). These findings are in line with our scat analyses results, which had high degrees of isotopic variation as well. There may also be a representational bias as a scat sample may contain the highest proportion of remains of the least digestible dietary items, while the most digestible items would be least represented.

We treated skin from the iguanas with petroleum ether to remove potential lipids as reptile skin is known to have significant lipid concentrations that are thought to contribute to decreased cutaneous water loss (Roberts and Lillywhite 1980) and the presence of lipids in animal tissues reduces δ^{13}C values (Post et al. 2007). However, lipid

extraction had no significant effect on the $\delta^{13}C$ or $\delta^{15}N$ values from skin, indicating that it was unnecessary. This is likely because the lipid content of the iguana skin in our study was not high enough to affect the stable isotope values and this is supported by the C:N ratios from the skin samples which were less than 4.0 indicating a lipid content of less than 10% (Post et al. 2007).

In order to provide overall stable isotope discrimination factors across all three adult iguana species for use in studies employing other species, we have reported the mean $\Delta^{13}C$ and $\Delta^{15}N$ discrimination factors across all species for each tissue in Table 1. They were 4.1±1.4‰ and +5.5±0.6‰, respectively, for skin, +2.1±0.6‰ and +3.6±0.4‰, respectively, for blood, and -1.6±1.0‰ and +3.0±0.7‰, respectively, for scat (Tables 1 and S1). However, since we found relationships between discrimination factors and size, we recommend using the $\Delta^{13}C$ and $\Delta^{15}N$ factors from the species in this study that are closest in size to the wild species of interest when applying stable isotope discrimination factors to known size iguanas in the wild. In addition, where possible, we recommend utilizing our reported linear regression equations to estimate the appropriate discrimination factors for the size of the iguana species.

In conclusion, we found that age, and the metabolic and growth rates associated with specific life stages, likely play a key role in affecting $\delta^{13}C$ and $\delta^{15}N$ (and thus $\Delta^{13}C$ and $\Delta^{15}N$) values in rock iguanas, though the mechanisms causing these relationships require further study, especially for stable carbon isotopes. Tissues of varying molecular composition also varied isotopically, and $\delta^{13}C$ from scat exhibited inverse relationships

with age than from other tissues, possibly due to the digestion adaptations unique to hindgut fermenters.

This thesis, in full, will be submitted for publication of the material, as it may appear in Rapid Communications in Mass Spectrometry, (2015) Steinitz, Ronnie; Lemm, Jeffrey M; Pasachnik, Stesha A; and Kurle, Carolyn M. The thesis author was the primary investigator and author of this paper.

Figures

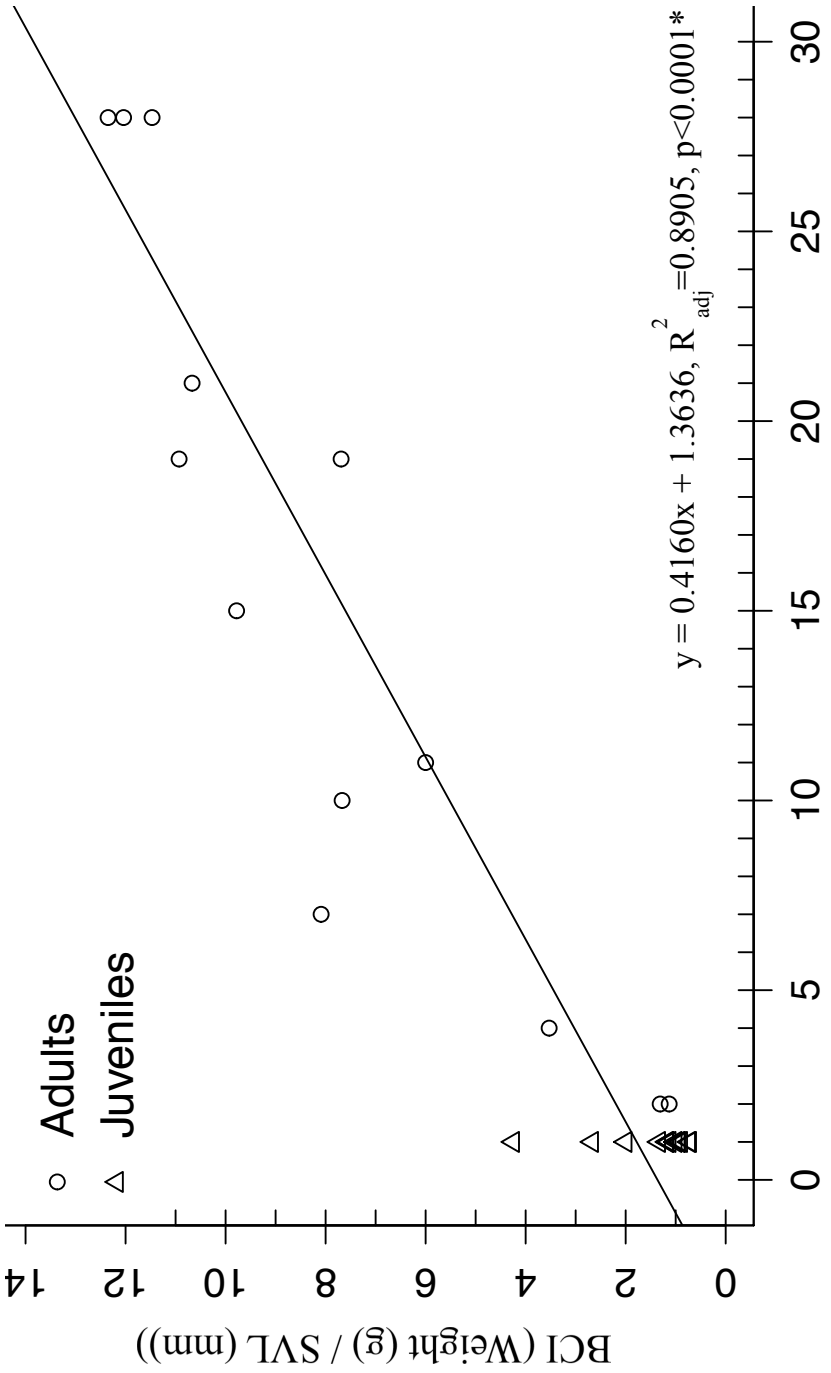
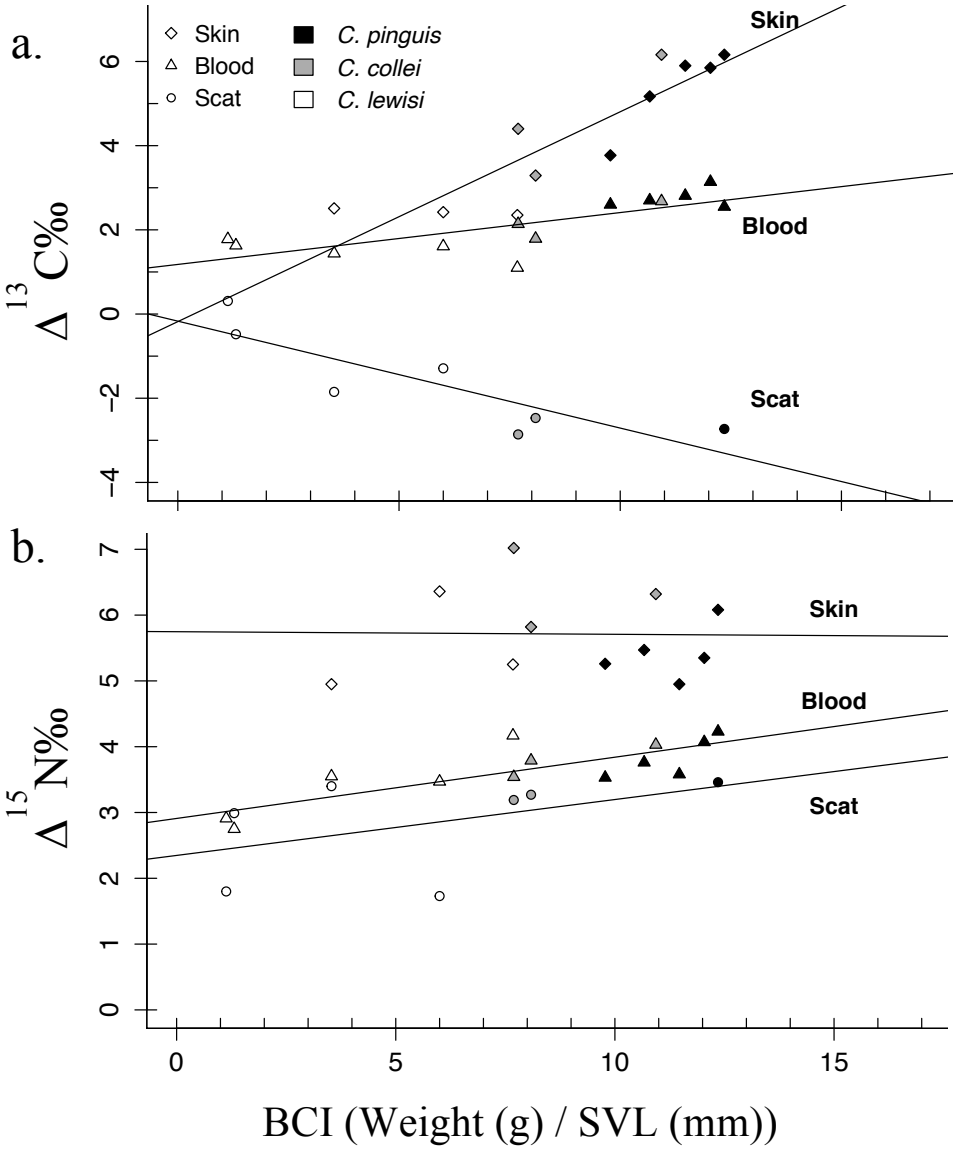


Figure 1. The linear relationship between body condition index (BCI) and age from individuals of three species of Rock Iguanas. N=26; 13 adults (◯)and 13 juveniles (△) (Supplemental Table S1)



Δ^{13}C

Skin: y=0.4985x – 0.1803, R^2_{adj}=0.8, p<0.001*

Blood: y=0.1232x + 1.1784, R^2_{adj}=0.5, p=0.01*

Scat: y=-0.2540x – 0.1687, R^2_{adj}=0.7, p=0.01*

Δ^{15}N

Skin: y=-0.0039x + 5.7477, R^2_{adj}=-0.1, p=0.96

Blood: y=0.0931x + 2.9095, R^2_{adj}=0.6, p<0.001*

Scat: y=0.0850x + 2.3474, R^2_{adj}=0.1, p=0.30

Figure 2. Linear relationships between the **a)** stable carbon (Δ^{13}C) and **b)** nitrogen (Δ^{15}N) isotope discrimination factors and simplified body condition index (BCI) from adult, captive Rock Iguanas (*Cyclura* spp.) BCI is calculated as weight (g) divided by snout ventral length (SVL; mm).

Table 1. Species, mean snout-vent-length (SVL; ±SD), mean weight (±SD), tissue type, sample number, mean stable isotope values (‰; ±SD), mean discrimination factors (‰; ±SD), and C:N ratios for tissues and diet items collected in 2013 from three Cyclura species. All animals were adults except where otherwise noted. Stable isotope values from males and females were grouped for each tissue type within each species, as there were no differences between sexes in their isotope values. See Supplemental Table S1 for more details.

Species	N	Mean Weight (Kg)	Mean SVL (mm)	Mean Age (years)	Sex (F/M)
C. lewisi[A]	5	1.4±1.3[D]	293.2±111.1[D]	5.8±4.4[H]	1/4
C. lewisi Juvenile	15	0.3±0.4[E]	187.3±51.8[E]	1.0±0.0	5/10
C. collei[B]	7	3.9±1.0[F]	433.3±367.7[F]	12.1±6.4[H]	4/3
C. pinguis[C]	7	5.5±0.9[G]	484.0±32.9[G]	23.0±6.7[H]	2/5
Adult Total[I]	19[I]	3.5±2.1[I]	398.9±112.6[I]	14.5±9.2[I]	7/12[I]

Table 1. Continued from previous page.

Species	Tissue	N	Animal		Diet		Discrimination Factors		
			$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$	C:N
C. lewisi[A]	Scat	4	-29.5±0.9	4.3±0.8	-28.6	1.8	-0.8±0.9	2.5±0.8	9.6±1.2
	Blood	5	-27.1±0.3*	5.2±0.6*	-28.6	1.8	1.5±0.3*	3.4±0.6*	3.6±0.3
	Skin	3	-26.2±0.1*	7.4±0.7*	-28.6	1.8	2.4±0.1*	5.5±0.7*	3.5±0.4
C. lewisi Juvenile	Scat	10	-29.6±0.7	4.1±1.0	-28.6	1.8	-0.9±0.7	2.3±1.0	9.1±0.8
	Blood	13	-27.3±0.2	4.4±0.6*	-28.6	1.8	1.4±0.2	2.5±0.6*	3.4±0.1
	Skin	9	-26.8±0.3*	5.6±0.8*	-28.6	1.8	1.9±0.3*	3.7±0.8*	3.3±0.1
C. collei[B]	Scat	4	-31.0±0.4	5.3±0.3	-28.6	1.8	-2.4±0.4	3.4±0.3	9.7±1.4
	Blood	4	-26.5±0.4*	5.6±0.2	-28.6	1.8	2.1±0.4*	3.7±0.2	4.3±0.8
	Skin	7	-24.9±1.2*	7.6±0.8	-28.6	1.8	3.7±1.2*	5.7±0.8	3.0±0.3
C. pinguis[C]	Scat	3	-30.1±1.2	3.6±2.3	-28.6	1.8	-1.4±1.2	1.8±2.3	6.4±4.7
	Blood	5	-25.9±0.2*	5.7±0.3	-28.6	1.8	2.8±0.2*	3.8±0.3	3.4±0.1
	Skin	7	-23.5±0.9*	7.2±0.4	-28.6	1.8	5.2±0.9*	5.4±0.4	3.3±0.1

Overall Values	$\Delta^{13}C$	$\Delta^{15}N$
Scat	-1.6±1.0	3.0±0.7
Blood	2.1±0.6	3.6±0.4
Skin	4.1±1.4	5.5±0.6

Table 1. Continued from previous page.

All among tissue comparisons within each species were statistically different (all $p \leq 0.02$), except:

A ^{13}C Blood vs. Skin: p=0.26 *B* ^{15}N Blood vs. Scat: p=0.56 *C* ^{15}N Blood vs. Scat: p=0.10
^{15}N Blood vs. Scat: p=0.21 ^{15}N Blood vs. Skin: p=0.20

* Significant differences in among species comparisons (ANOVA, Tukey's pairwise comparison). All details for the analyses can be found in Supplemental Table S1.

Weights and SVL were obtained for the following number of individuals of each species and age groups, and these were used to calculate Mean Weight and Mean SVL:

[D] 5 of 5 *C. lewisi* adults (ages 2-11)

[E] 13 of 15 *C. lewisi* juveniles (ages =1yrs)

[F] 3 of 7 *C. collei* individuals (ages 7-19yrs)

[G] 5 of 7 *C. pinguis* individuals (ages 15-28yrs)

[H] ANOVA, $F_{2,16}=12.5$, $p \leq 0.001$

[I] *Adult Total* excludes *C. lewisi* juveniles; total means including juveniles[E]: 1.9±2.2g, 293.1±137.9mm, respectively.

Table 2. The mean (±SD) stable isotope values (‰) and C:N values from dietary components fed weekly* to captive Rock Iguanas (Cyclura spp.) at the San Diego Zoo Institute for Conservation Research. The % source refers to the percentage each dietary source contributed to the overall weekly diet budget.

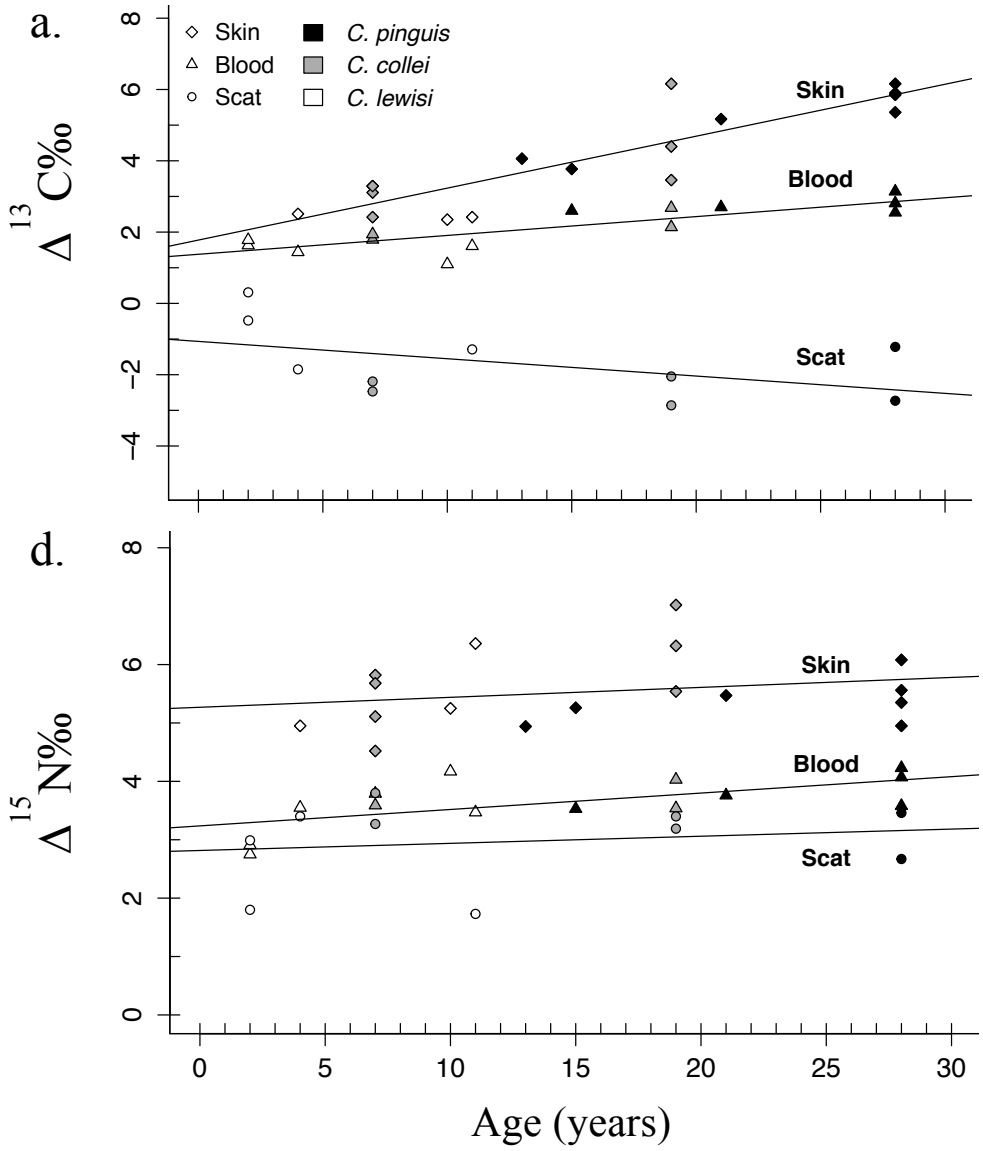
Dietary Item	Portion weight (g)	Times fed per week	Total weekly budget (g)	($\%_{source}$)	N	δ^{13}C (‰)	δ^{15}N (‰)	C:N
Dandelion Greens	125	5	625	23.19%	3	-28.75±1.10	1.62±1.41	8.64±1.22
Collard Greens	125	3	375	13.91%	2	-28.28±2.11	2.68±1.15	8.10±0.95
Green Chard	125	3	375	13.91%	2	-29.94±2.11	1.71±2.55	9.17±2.21
Mustard Greens	125	3	375	13.91%	2	-30.47±0.97	2.05±1.92	7.60±1.92
Bok Choy	125	2	250	9.28%	1	-28.49±0.0	-0.74±0.00	9.40±0.0
Escarole	125	2	250	9.28%	1	-27.58±0.0	-2.37±0.00	12.00±0.0
Kale	125	2	250	9.28%	1	-31.01±0.0	2.83±0.00	11.60±0.0
Root Veg. (variable[1])	15	5	75	2.78%	4, 4, 4[1]	-27.22±1.78	2.46±0.70	31.82±9.27
Fruit (variable[2])	15	3	45	1.67%	4, 2, 5[2]	-25.99±0.57	1.74±0.37	52.63±37.77
Zucchini[+]	15	3	45	1.67%	2	-26.14±1.13	1.31±0.16	11.73±2.08
Green Bean	15	2	30	1.11%	1	-25.63±0.0	2.79±0.00	14.40±0.0
Total weekly diet:			2695g					
Overall diet						**-28.64**	**1.84**	

*Diet amounts are reported as items offered, not consumed. This is the best representation of the amounts actually consumed.

[1] Root Vegetables: Carrot, turnip[+], yam[+], respectively

[2] Fruit: Apple[+], honeydew, papaya[+], respectively

[+] Whole item offered, including peel



Δ^{13}C

Skin: y=0.1457x + 1.7767, R^2_{adj}=0.8, p<0.0001*

Blood: y=0.0528x + 1.3778, R^2_{adj}=0.7, p<0.001*

Scat: y=-0.0486x – 1.0660, R^2_{adj}=0.1, p=0.17

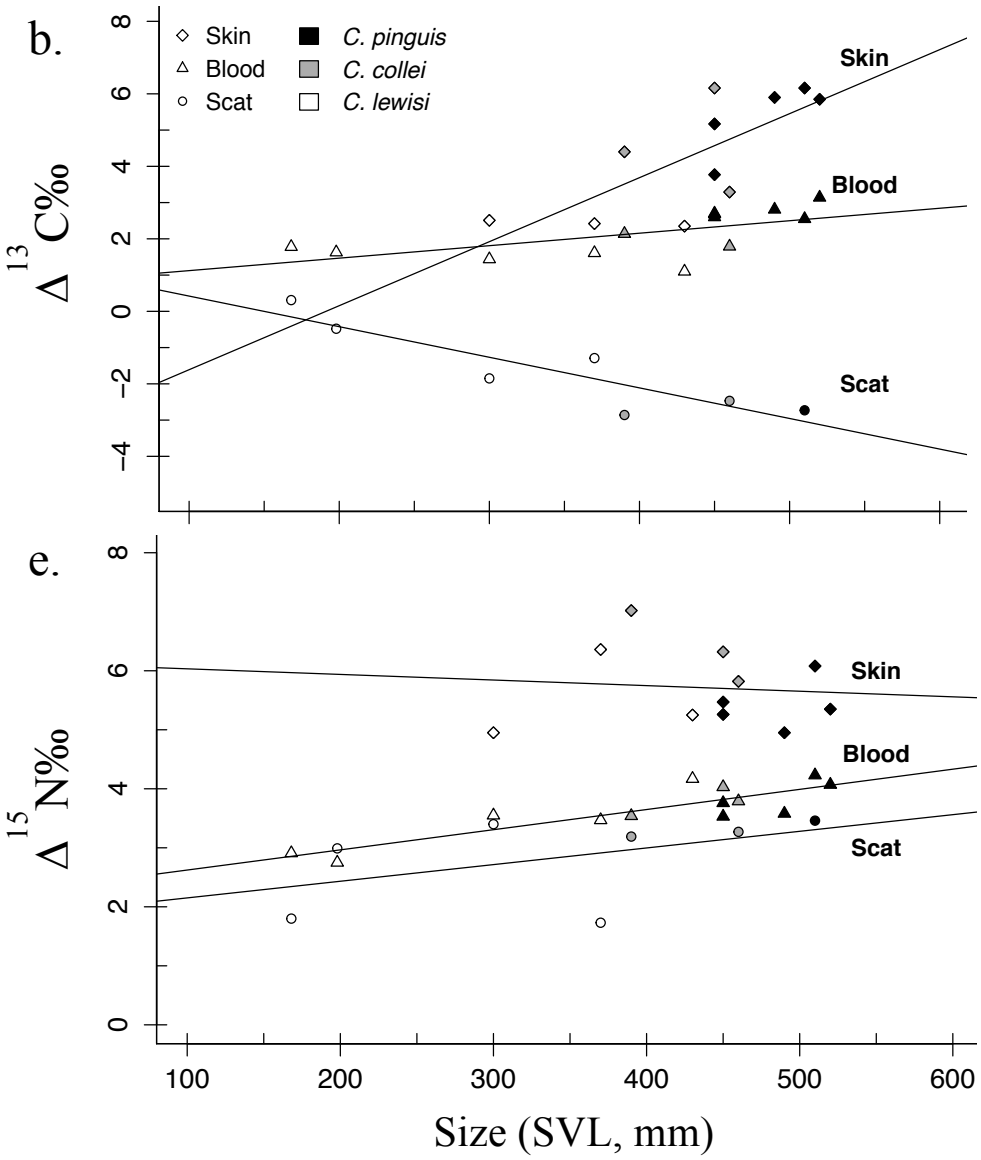
Δ^{15}N

Skin: y=0.0170x + 5.2687, R^2_{adj}=0.0, p=0.37

Blood: y=0.0281x + 3.2372, R^2_{adj}=0.3, p=0.02*

Scat: y=0.0122x + 2.8160, R^2_{adj}=-0.1, p=0.82

Supplemental Figure S1. There were significant linear relationships between the Δ^{13}C values from scat, blood, and skin from three iguana species and iguana size as measured by both a) weight (g) and b) snout-ventral length (SVL; mm), between the Δ^{13}C values from blood and skin and c) iguana age (yrs), and between the δ^{15}N values from blood and iguana d) weight, e) SVL, and f) age. No other linear relationships were significant. See Table S1 for all results.



Δ^{13}C

Skin: y=0.0177x – 3.3757, R^2_{adj}=0.5, p=0.01*

Blood: y=0.0034x + 0.7787, R^2_{adj}=0.3, p<0.03*

Scat: y=-0.0084x + 1.2651, R^2_{adj}=0.8, p=0.01*

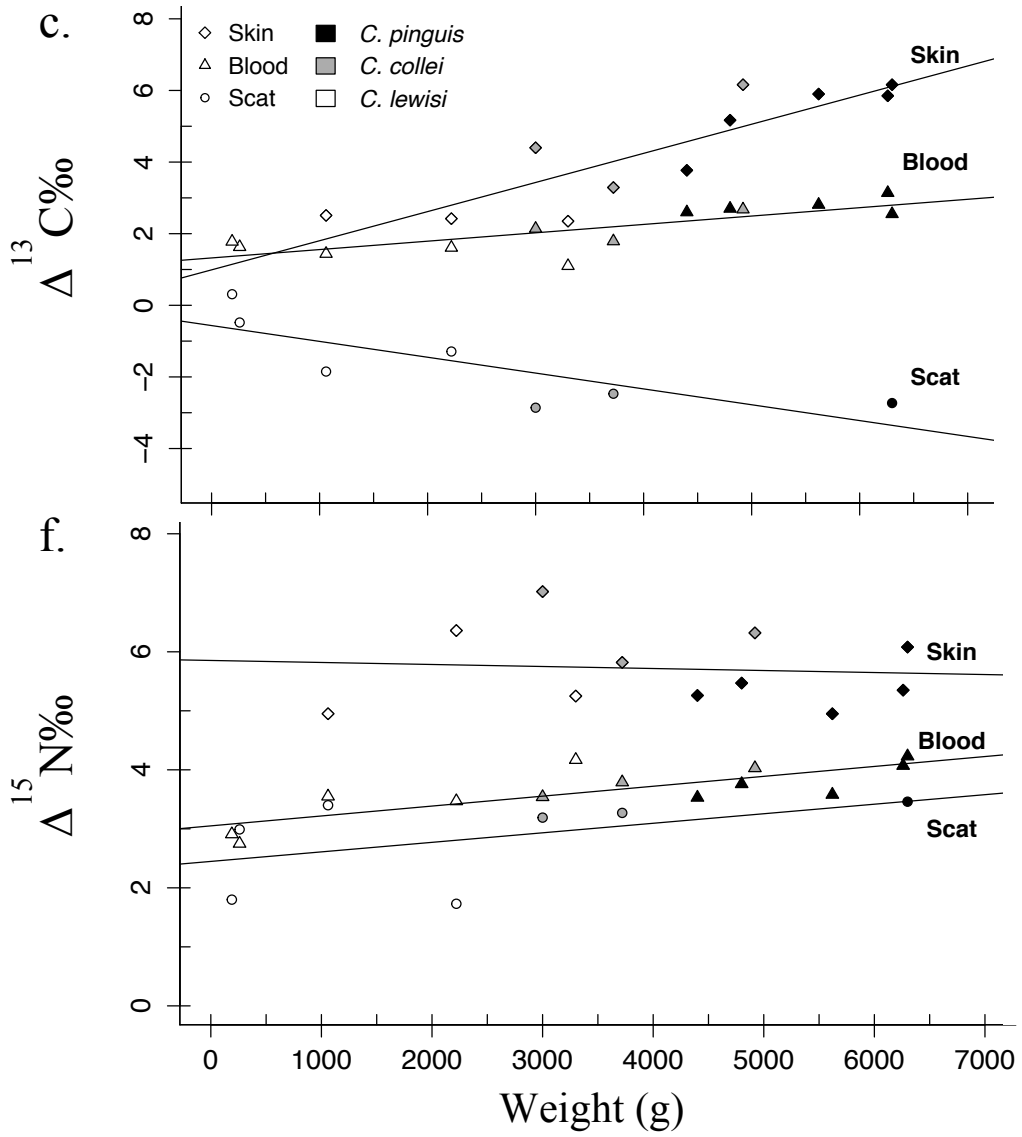
Δ^{15}N

Skin: y=0.00001x + 5.8532, R^2_{adj}=-0.1, p=0.80

Blood: y=0.0034x + 2.2810, R^2_{adj}=0.7, p<0.001*

Scat: y=0.0028x + 1.8688, R^2_{adj}=0.1, p=0.27

Supplemental Figure S1. Continued from previous page. There were significant linear relationships between the Δ^{13}C values from scat, blood, and skin from three iguana species and iguana size as measured by both a) weight (g) and b) snout-ventral length (SVL; mm), between the Δ^{13}C values from blood and skin and c) iguana age (yrs), and between the δ^{15}N values from blood and iguana d) weight, e) SVL, and f) age. No other linear relationships were significant. See Table S1 for all results.



Δ^{13}C	Δ^{15}N
Skin: y=0.0008x + 0.9898, R^2_{adj}=0.7, p<0.001*	Skin: y=0.00001x + 5.8532, R^2_{adj}=--0.1, p=0.80
Blood: y=0.0002x + 1.3229, R^2_{adj}=0.6, p<0.01*	Blood: y=0.0002x + 3.0501, R^2_{adj}=0.6, p<0.01*
Scat: y=-0.0004x – 0.5659, R^2_{adj}=0.6, p=0.03*	Scat: y=0.0002x + 2.4476, R^2_{adj}=0.1, p=0.28

Supplemental Figure S1. Continued from previous page. There were significant linear relationships between the Δ^{13}C values from scat, blood, and skin from three iguana species and iguana size as measured by both a) weight (g) and b) snout-ventral length (SVL; mm), between the Δ^{13}C values from blood and skin and c) iguana age (yrs), and between the δ^{15}N values from blood and iguana d) weight, e) SVL, and f) age. No other linear relationships were significant. See Table S1 for all results.

Supplemental Table S1. All Statistical Analyses. The statistical data from t-tests, linear regressions, ANOVAs, and Tukey post-hoc tests for differences in stable isotope values (δ^{13}C and δ^{15}N) and discrimination factors (Δ^{13}C and Δ^{15}N) (‰) for captive Rock Iguanas (*Cyclura* spp.) held on a constant diet, and their dietary items.

	Factor	Isotope	Test			p-value
Scat			Paired t-test	t	df	p
	Processing – HCl[1]	δ^{13}C		-1.0	37.0	0.35
		δ^{15}N		3.5	9.0	0.01
	Sampling Period	δ^{13}C		-1.5	8.0	0.16
		δ^{15}N		-1.8	8.0	0.11
	Sex		Simple t-test	t	df	p
		δ^{13}C		0.9	6.7	0.40
		δ^{15}N		-1.3	5.1	0.25
	Species				$F_{2,8}$	p
		Δ^{13}C	ANOVA		3.4	0.09
			C. lewisi/C. collei			0.08
			C. pinguis/C. collei			0.36
			C. pinguis/C. lewisi			0.64
		Δ^{15}N	ANOVA		1.5	0.28
			C. lewisi/C. collei			0.58
			C. pinguis/C. collei			0.26
			C. pinguis/C. lewisi			0.74
			Linear Regression	R^2_{adj}	$F_{1,9}$	p
	Age	Δ^{13}C	y=-0.0486x-1.0660	0.1	2.2	0.17
		Δ^{15}N	y=0.0122x+2.8160	-0.1	0.1	0.82
				R^2_{adj}	$F_{1,5}$	p
	BCI	Δ^{13}C	y=-0.2540x-0.1687	0.7	13.9	0.01*
		Δ^{15}N	y=0.0850x+2.3474	0.1	1.4	0.3
				R^2_{adj}	$F_{1,5}$	p
	Size (SVL)	Δ^{13}C	y=-0.0084x+1.2651	0.8	20.6	0.01*
		Δ^{15}N	y=0.0028x+1.8688	0.1	1.5	0.27
	Weight	Δ^{13}C	y=-0.0004x-0.5659	0.6	9.1	0.03*
		Δ^{15}N	y=0.0002x+2.4476	0.1	1.4	0.28
Blood			Simple t-test	t	df	p

Supplemental Table S1. Continued from previous page.

Sex	$\delta^{13}C$		-0.8	4.0	0.47
	$\delta^{15}N$		-1.4	1.3	0.34
				$F_{2,11}$	p
Species	$\Delta^{13}C$	ANOVA		22.7	<0.001*
		C. lewisi/C. collei			0.02*
		C. pinguis/C. collei			0.02*
		C. pinguis/C. lewisi			<0.001*
	$\Delta^{15}N$	ANOVA		1.8	0.21
		C. lewisi/C. collei			0.40
		C. pinguis/C. collei			0.93
		C. pinguis/C. lewisi			0.21
		Linear Equation	R^2_{adj}	$F_{1,12}$	p
Age	$\Delta^{13}C$	y=0.0528x+1.3778	0.7	26.8	<0.001*
	$\Delta^{15}N$	y=0.0281x+3.2372	0.3	7.8	0.02*
			R^2_{adj}	$F_{1,11}$	p
BCI	$\Delta^{13}C$	y=0.1232x+1.1784	0.5	15.0	<0.01*
	$\Delta^{15}N$	y=0.0931x+2.9095	0.6	21.4	<0.001*
			R^2_{adj}	$F_{1,11}$	p
Size (SVL)	$\Delta^{13}C$	y=0.0034x+0.7787	0.3	6.6	0.03*
	$\Delta^{15}N$	y=0.0034x+2.2810	0.7	32.0	<0.001*
Weight	$\Delta^{13}C$	y=0.0002x+1.3229	0.6	17.5	<0.01*
	$\Delta^{15}N$	y=0.0002x+3.0501	0.6	19.1	<0.01*
Skin		Paired t-test	t	df	p
	LE / NLE^2	$\delta^{13}C$	-0.1	7.0	0.90
		$\delta^{15}N$	0.6	9.0	0.56
	Sampling Period	$\delta^{13}C$	2.0	13.0	0.07
		$\delta^{15}N$	1.4	13.0	0.19
			t	df	p
Sex	$\delta^{13}C$	Simple t-test	-0.7	14.3	0.48
	$\delta^{15}N$		-0.4	6.9	0.72
				$F_{2,14}$	p
Species	$\Delta^{13}C$	ANOVA		8.7	<0.01*

Supplemental Table S1. Continued from previous page.

		C. lewisi/C. collei			0.18
		C. pinguis/C. collei			0.04*
		C. pinguis/C. lewisi			<0.01*
	$\Delta^{15}N$	ANOVA		0.5	0.62
		C. lewisi/C. collei			0.90
		C. pinguis/C. collei			0.60
		C. pinguis/C. lewisi			0.94
		Linear Equation	R^2_{adj}	$F_{1,15}$	p
Age	$\Delta^{13}C$	y=0.1457x+1.7767	0.8	53.9	<0.0001*
	$\Delta^{15}N$	y=0.0170x+5.2687	0.0	0.8	0.37
			R^2_{adj}	$F_{1,9}$	p
BCI	$\Delta^{13}C$	y=0.4985x-0.1803	0.8	31.9	<0.001*
	$\Delta^{15}N$	y=-0.0039x+5.7477	-0.1	0.002	0.96
			R^2_{adj}	$F_{1,9}$	p
Size (SVL)	$\Delta^{13}C$	y=0.0177x-3.3757	0.5	10.2	0.01*
	$\Delta^{15}N$	y=-0.0009x+6.1279	-0.1	0.1	0.79
Weight	$\Delta^{13}C$	y=0.0008x+0.9898	0.7	27.4	<0.001*
	$\Delta^{15}N$	y=0.00001x+5.8532	-0.1	0.1	0.80

Diet Items - Sampling Period		Paired t-test	t	df	p
Sampling Period	$\delta^{13}C$		0.5	5.0	0.62
	$\delta^{15}N$		-0.1	5.0	0.93

***C. lewisi* Juveniles - Sex**					
		Two sample t-test	t	df	p
Scat	$\delta^{13}C$		1.6	2.5	0.23
	$\delta^{15}N$		-0.9	2.3	0.45
Blood	$\delta^{13}C$		1.9	5.1	0.11
	$\delta^{15}N$		1.6	3.7	0.20
Skin	$\delta^{13}C$		2.1	1.8	0.19
	$\delta^{15}N$		2.1	2.2	0.16
		Two sample t-test	t	df	p
Size	Means		1.7	3.2	0.18

Supplemental Table S1. Continued from previous page.

Weight		Means		1.5	3.1	0.23
***C. lewisi* -Juveniles vs. adults by Tissue**						
			Two sample t-test	t	df	p
Scat	Juv. v. adults	$\Delta^{13}C$		0.2	4.2	0.85
		$\Delta^{15}N$		0.4	6.6	0.69
Blood	Juv. v. adults	$\Delta^{13}C$		1.0	6.3	0.38
		$\Delta^{15}N$		2.9	7.4	0.02*
Skin	Juv. v. adults	$\Delta^{13}C$		4.6	9.9	<0.01*
		$\Delta^{15}N$		3.6	3.6	0.03*
Tissue Types by Species						
					$F_{2,8}$	p
C. lewisi		$\delta^{13}C$	ANOVA		24.6	<0.001*
			Blood/Scat			<0.01*
			Blood/Skin			0.26
			Scat/Skin			<0.001*
		$\delta^{15}N$	ANOVA		14.8	<0.01*
			Blood/Scat			0.21
			Blood/Skin			0.01*
			Scat/Skin			<0.01*
					$F_{2,29}$	p
C. lewisi Juveniles		$\delta^{13}C$	ANOVA		124.5	<0.0001*
			Blood/Scat			0.03*
			Blood/Skin			<0.001*
			Scat/Skin			<0.001*
		$\delta^{15}N$	ANOVA		9.5	<0.001*
			Blood/Scat			0.74
			Blood/Skin			<0.01*
			Scat/Skin			<0.001*
					$F_{2,8}$	p
C. collei		$\delta^{13}C$	ANOVA		79.5	<0.0001*
			Blood/Scat			<0.001*
			Blood/Skin			<0.01*
			Scat/Skin			<0.01*

Supplemental Table S1. Continued from previous page.

		$\delta^{15}N$	ANOVA	44.5	<0.0001*
			Blood/Scat		0.56
			Blood/Skin		<0.001*
			Scat/Skin		<0.001*
C. pinguis				$F_{2,8}$	p
		$\delta^{13}C$	ANOVA	106.0	<0.0001*
			Blood/Scat		<0.001*
			Blood/Skin		<0.001*
			Scat/Skin		<0.001*
		$\delta^{15}N$	ANOVA	7.1	0.02*
			Blood/Scat		0.10
			Blood/Skin		0.22
			Scat/Skin		0.01*
Species by Tissue Type					
				$F_{2,8}$	p
Scat		$\Delta^{13}C$	ANOVA	3.3	0.09
			C. lewisi/C. collei		0.08
			C. pinguis/C. collei		0.39
			C. pinguis/C. lewisi		0.61
		$\Delta^{15}N$	ANOVA	1.5	0.29
			C. lewisi/C. collei		0.58
			C. pinguis/C. collei		0.27
			C. pinguis/C. lewisi		0.76
				$F_{2,11}$	p
Blood		$\Delta^{13}C$	ANOVA	24.6	<0.0001*
			C. lewisi/C. collei		0.02*
			C. pinguis/C. collei		0.02*
			C. pinguis/C. lewisi		<0.001*
		$\Delta^{15}N$	ANOVA	1.5	0.21
			C. lewisi/C. collei		0.41
			C. pinguis/C. collei		0.92

Supplemental Table S1. Continued from previous page.

		C. pinguis/C. lewisi			0.21
				$F_{2,14}$	p
Skin		$\Delta^{13}C$	ANOVA	8.5	<0.01*
			C. lewisi/C. collei		0.19
			C. pinguis/C. collei		0.04*
			C. pinguis/C. lewisi		<0.01*
		$\Delta^{15}N$	ANOVA	0.58	0.57
			C. lewisi/C. collei		0.88
			C. pinguis/C. collei		0.54
			C. pinguis/C. lewisi		0.94
Species by Age					
Species			ANOVA	Mean Age (yrs)	$F_{2,16}$	p
	Means		*C. pinguis*	23.0±6.7	12.5	<0.001*
			C. collei	12.1±6.4		
			C. lewisi (Total)	2.2±2.9		
			C. lewisi (Adults)	5.8±4.4		
			C. lewisi (Juveniles)	1.0±0		
			C. lewisi/C. collei		-1.8	0.21
			C.pinguis/C.collei		3.3	0.01*
			C.pinguis/C.lewisi		4.8	<0.001*
BCI by Age			Linear Equation	R^2_{adj}	$F_{1,24}$	p
BCI			y=0.4160x + 1.3636	0.9	204.2	<0.0001*

[1] Aggitated in 0.5M HCl
[2] Sonicated in petroleum ether

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