UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

STABLE ISOTOPES AS INDICATORS OF BLACK-THROATED SPARROW HABITAT USE IN THE NORTHERN CHIHUAHUAN DESERT, NEW MEXICO

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

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Norman, Oklahoma
2010

STABLE ISOTOPES AS INDICATORS OF BLACK-THROATED SPARROW HABITAT USE IN THE NORTHERN CHIHUAHUAN DESERT, NEW MEXICO

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

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I dedicate this dissertation to my parents, Barbara and Jack Paritte, my brother, Nicholas Paritte, and my dog, Nuggett.

"I know all there is about birds. They live in nests. Their nests are in trees. They tweet, they fly...that's basically all they do. They eat worms."

Cohen, Age 4
Washington, OK

ACKNOWLEDGEMENTS

My tenure as a graduate student in the Department of Zoology at the University of Oklahoma has been fulfilling and rewarding. I have benefited from valuable interactions with the faculty and graduate students, and I appreciate all the help I have received in completing my dissertation. I have also enjoyed being a part of the burgeoning Ecology and Evolutionary Biology (EEB) Program, which has increased my depth of understanding of the fundamentals of ecology as an academic discipline. Participation in the EEB program through the Department of Zoology has made me a better, more well-rounded ecologist.

I would first like to thank my advisor, Dr. Jeff Kelly, for teaching me to think like a scientist. His guidance, patience, support, and constructive feedback have been invaluable during my graduate education. I would also like to thank my committee members, Dr. Brandon Bestelmeyer, Dr. Michael Engel, Dr. Michael Kaspari, and Dr. Michael Patten, for their guidance and participation in my graduate experience. Dr. Bestelmeyer provided great help with field sites and is an unending source of knowledge on desert grassland and rangeland ecology. Dr. Engel is responsible for my education in stable isotopes and has been a source of support through difficult times. He has also given me the single best piece of advice I have ever received—floss every day. Dr. Kaspari is a wealth of knowledge in ecology; I credit him with my fundamental education in ecological theory. Further, his editorial skills and advice have made me a better science writer. Dr. Patten has shaped me as a budding statistician; his support and help in learning statistical techniques have ensured the success of my research. I would also like to thank my former committee member, Dr.

Linda Wallace, who did not live to see the completion of this dissertation. Dr. Wallace shaped my knowledge of grassland plant physiology and ecology and provided me a strong female role model in science.

In addition, I would like to thank Rosemary Knapp. Her aid and support as Graduate Liaison and has made navigating the waters in and around graduate life much easier. I would also like to thank my the members of my lab for support and advice, particularly D. Max Smith, Sarah Swanson, Randy Soto, Lauren Niemann, and Eli Bridge, for help and advice. Lauren Niemann has also been a huge source of support during the ups and downs of graduate life. Thanks to the staff of the Oklahoma Biological Survey (Ranell Madding, Trina Steil, Janice Spurlock, Marsha Womack), the Department of Zoology (Carol Baylor, Robbie Stinchcomb), and the Jornada Experimental Range and Long Term Ecological Research Site (John Smith, Eddie Garcia, José Ramirez) in Las Cruces, New Mexico. Holly Marchman and Andrea Campane provided valuable help in the field. It would be impossible to list all the people that have supported me during my graduate career. Thanks to everyone who has been a part of this process; it would not have been possible without the endless support I have received.

Finally, I would like to thank my family for their support. My parents, Barbara and Jack Paritte, have always believed in me, and this has been vital to my success. Hopefully, their dreams will now come true when my journey culminates in getting a "real job."

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ABSTRACT

As humans accelerate their alteration of the landscape, it is increasingly important to understand relationships between organisms and their environment. Stable isotope analysis is an increasingly popular tool for identifying ecological processes and can be a valuable asset for understanding how landscape alteration influences species and communities. In the Chihuahuan Desert, shrub encroachment due primarily to historic overgrazing and fire suppression and exacerbated by drought is a primary manifestation of indirect human landscape alteration and has consequences for endemic biodiversity.

Shrub encroachment changes many habitat components for consumers, of which and physical structure potential energy source (*i.e.*, diet) are paramount and lack of ability to discriminate between structural and dietary components of habitat suitability has confounded general understanding of the impacts of aridland desertification on faunal communities; however, stable isotopes have the potential to delineate components of overall habitat composition that comprise consumer diet. The research detailed in my dissertation uses black-throated sparrows (*Amphispiza bilineata*) along with grasshopper species *Trimerotropis pallidipennis*, *Opeia obscura*, and *Melanoplus occidentalis* to broaden understanding of consumer use of changing environments and to further the application of stable isotopes to analysis of ecological processes.

My first chapter contributes to advancing stable isotope analysis as an accurate tool in avian ecology. Using feathers from Japanese quail (*Coturnix japonica*) obtained from the George M. Sutton Avian Research center, I explored the question,

does the method by which a feather is cleaned prior to stable isotope analysis affect its resulting stable isotope values? I applied a paired cleaning design to test the isotopic influence of the two most common cleaning agents, 2:1 chloroform:methanol solvent and dilute detergent, and not cleaning feathers. I found that different cleaning methods resulted in highly variable stable isotope ratios for hydrogen and nitrogen, but stable carbon isotope ratios were not affected. However, I further found that employing a two-step cleaning procedure, cleaning first with dilute detergent and second with 2:1 chloroform:methanol, greatly increased the precision and predictability for stable hydrogen and nitrogen values. This experiment underscores the importance of delineating standardized procedures for tissue preparation for stable isotope analyses, increases reliability of data and improves the potential for data comparison between studies.

In my second chapter, I explore the role of variation in isotopic sources in influencing consumer stable isotope ratios and how this variation affects data application toward two popular philosophies, 1) isotopic niche width, and 2) degree of individual isotopic specialization. Using stable carbon isotope data from plants, grasshoppers, and black-throated sparrows collected at the Jornada Experimental Range in Las Cruces, New Mexico, I compared means and variances of consumers to two plant isosources—C_3 and C_4 plant photosynthetic types. I found that consumers of C_3 plants had higher variation in stable carbon isotopes, primarily due to higher variation in the C_3 plant isosource. I further compared my data to simulations in which consumers used different isosources. I concluded that variance in consumer

stable carbon isotope ratios can be more clearly applied to inferences of individual specialization than to measures of a population's niche width.

In my third chapter, I use stable carbon isotope ratios to see how consumers analyzed in chapter two are using different facets of their carbonaceous habitat in the face of landscape change. My study area is located in the northern Chihuahuan Desert, an area formerly dominated by semi-arid grasslands, but now, due to alteration of grazing and fire regimes, has become dominated by shrubs. This landscape change inevitably affects the wildlife living there, so my objective was to see how shrubland habitats are used by species relative to grassland habitats, given current knowledge of species habitat use. The results of this study indicate that all species analyzed occupy habitats representative of the overall available range of habitat types from grassland to shrubland. Each grasshopper species employs a different foraging pattern: *T. pallidipennis* consumes primarily C_3 components of its habitat for food and *O. obscura* consumes primarily C_4 components, despite both occupying areas ranging from nearly 100% C_4 grass to 100% C_3 shrubs. *M. occidentalis* uses C_3 and C_4 habitat components for food proportional to their availability. Black-throated sparrows, despite frequent classification as a shrubsteppe or shrub-preferring species and frequent nesting in shrubs, consume primarily or exclusively C_4 grass-based food. This study illustrates that stable isotopes can help clarify how different components of habitats can be used for different purposes, that different species can have different consumption patterns across ecotones, and how classification of species like black-throated sparrows into particular guilds may obscure use of other habitat components.

My fourth chapter details an observed correlation between stable nitrogen and stable carbon isotope ratios in black-throated sparrows and suggests possible explanations. Stable nitrogen isotopes are typically used to indicate trophic level or nutritional stress in consumers. In this system, for all three grasshopper species analyzed in chapter 3 and both hatch-year and adult black-throated sparrows, no relationship was seen between a consumer's stable nitrogen isotope ratio and the site at which the consumer was sampled, suggesting that the type of habitat which a consumer occupies does not influence stable nitrogen isotope ratios. However, for black-throated sparrows, particularly adults, there are higher stable nitrogen isotope ratios for individuals consuming a higher proportion of C_3 carbon. Considering previous studies on stable nitrogen enrichment, trophic level, and nutritional and water stresses, the most feasible explanation for this pattern is that some black-throated sparrows are consuming higher trophic level food items containing C_3-based carbon. Further studies of gut contents and foraging behavior would help clarify this explanation. Additionally, the low trophic enrichment values seen (+2.75‰ for grasshoppers to a maximum of +3.37‰ for adult black-throated sparrows) relative to the average accepted trophic nitrogen enrichment of +3.4‰ (range 3-5‰) indicate that although this Chihuahuan Desert system is water limited, it does not exhibit overall water or nutritional-stress. This suggests that dietary differences may explain differential stable nitrogen enrichment in black-throated sparrows.

CHAPTER 1

EFFECT OF CLEANING REGIME ON STABLE ISOTOPE RATIOS OF FEATHERS IN JAPANESE QUAIL (*COTURNIX JAPONICA)*

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Abstract

Stable isotope analysis of feathers is an increasingly important source of information on diet and movement of birds. Feathers are typically cleaned with a solvent prior to analysis, but the effects of this cleaning on resulting data have not been examined critically. We conducted an experiment to determine if cleaning regime for feathers of Japanese Quail (*Coturnix japonica*) affected hydrogen (δD), carbon ($\delta^{13}C$), and nitrogen ($\delta^{15}N$) stable isotope ratios. A paired design was used to clean feathers with 2:1 chloroform:methanol or detergent. Results after initial cleaning indicate for hydrogen approximately 40‰ enrichment of 2:1 chloroform:methanol-treated feathers over other treatments but no correlation between treatments. We found a similar pattern among treatments for carbon, but the effect was on the order of 0.2‰. Nitrogen values showed no discernible correlation, but both uncleaned and 2:1 chloroform:methanol treatments on average had enriched values compared to detergent. Further, variance among samples was high for

hydrogen and nitrogen measurements. After recleaning, differences in mean hydrogen isotope ratios were no longer evident data for hydrogen and carbon became less variable, and carbon maintained its initial pattern. We suggest a standard method of cleaning feathers, first with a dilute detergent solution, then with 2:1 chloroform:methanol solvent. This study has implications for increasing repeatability of hydrogen, carbon, and nitrogen stable isotope measurements, which would increase validity inter-laboratory comparisons and the utility of large-scale projects using compiled data sets.

Introduction

Stable isotope analysis has become increasingly popular in avian ecology for tracing dietary inputs (DeNiro and Epstein 1978, 1981; Hobson et al. 1999; Wolf et al. 2002), food webs (Hobson et al. 1993; Hobson et al. 1994; Kelly 2000; Birchall et al. 2005), and movement patterns (Cherel et al. 2000; Kelly et al. 2005; Mazerolle et al. 2005; Dunn et al. 2006) of birds. The technique analyzes within-sample relative quantities of hydrogen, oxygen, carbon, nitrogen, or sulfur stable isotopes, which vary naturally due to physical, chemical, and biological phenomena.

As stable isotope approaches have been rapidly incorporated into ecology, a multitude of sample preparation methods have been employed. However, discovery of novel applications for isotopes has tended to overshadow advances in methodology. More attention has been given recently to detailing accuracy and precision of published results (Jardine and Cunjak 2005), furthering laboratory experiments to test assumptions (Gannes et al. 1997), developing correction techniques, such as those for exchangeable hydrogen (Wassenaar and Hobson 2000a, 2003), and identifying sources

of intrasample variation in stable isotope analysis in order to minimize impact on data (Wassenaar and Hobson 2006; Wunder and Norris 2008). Wunder and Norris (2008), when comparing δD error from data interpolation to analytical error, concluded that analytical error was highly influential in the incorrect assignment of birds to their geographic origin; however, their definition of analytical error only considered mass spectrometric measurement error, not error from sample preparation. Understanding the impact of various sample preparation methods on the mean and variance of stable isotope measurements is an important component of analytical error and requires controlled comparisons for each sampled tissue type.

In avian isotope studies, primarily those that estimate geographic origin, feathers are commonly sampled because they are metabolically inert once grown (Hobson and Wassenaar 1997) and are relatively unintrusive to sample (Jaspers et al. 2007). Feathers are typically cleaned with a solvent prior to analysis to remove residual dirt and oil, yet there is no standard cleaning method and effects of differences in cleaning methods on resulting data have not been examined critically. Only one attempt at cleaning comparison was found in the literature. Bensch et al. (2006), prior to analyzing feathers for an African willow warbler study, compared isotope ratios in detergent cleaned and uncleaned feathers and found no measurement difference. This comparison is significant; however, more comprehensive studies are needed to determine the most effective feather cleaning method and to move toward standard sample preparation protocol. Creating detailed cleaning protocols is a complex process, but may allow for more data consistency with the goal of obtaining the best possible estimate of the true isotope value in the tissue. If cleaning regimes

influence stable isotope ratios, developing a consistent routine with appropriate cleaning agents can increase repeatability of analyses and reduce risk for isotopic fractionation due to cleaning, thereby improving the efficacy of hydrogen, carbon, and nitrogen values in their applications and increase reliability of comparison among data sets.

Here we evaluate three common feather washing techniques to determine how they influence hydrogen, carbon, and nitrogen stable isotope ratios. From these experiments we elucidate the presence and magnitude of bias that is introduced into isotope data through cleaning agent choice and suggest which method produces the most consistent data.

Methods

We reviewed 68 publications that used feathers for stable isotope analysis (Table 1) and found reports of 14 cleaning compounds used, the two most common being a solvent of 2:1 chloroform:methanol (43%; 29 publications) and detergent (15%; 10 publications). Another eight publications (12%) either did not clean feathers before processing or did not document cleaning method. Based on this survey, we chose to use 2:1 chloroform:methanol, detergent, and no cleaning as the cleaning treatments for this study. Further, few publications mentioned comprehensive specifics of cleaning procedures (*e.g.,* duration of washing/drying, agitation method), so our experiment protocol here is based on how we clean feathers in our lab.

We obtained 17 frozen Japanese Quail (*Coturnix japonica*) of similar age, mass, and diet, from the Sutton Avian Research Center in Bartlesville, OK (36ºN, 96ºW, elevation 230m). To reduce isotopic variation seen among feathers in

individual birds, we used only the two first primary feathers from each bird, both of which should reflect isotope ratios in symmetrical patterns. Since quail only have two of any corresponding feather, comparing three cleaning treatments requires that both the left and right feathers receive two of the three treatments such that one treatment is assigned to both the left and right feathers. This duplicate treatment allows for transitive comparison of the other two treatments. If replicated treatments yield the same values from both left and right primaries (which should yield similar isotope ratios), the other two treatment values should then be comparable.

To do this, we removed left and right first primary feathers (Figure 1) and randomly assigned each feather to one of two cleaning treatments – 2:1 chloroform:methanol and detergent or detergent and no cleaning – such that each quail received both treatments. We halved feathers along the rachis.

We randomly cleaned one feather half with 1 of the 2 cleaning types in its assigned treatment and the other half with the other cleaning type. We placed feather halves cleaned in 2:1 chloroform:methanol in a 120mL sealed jar with the solvent and shook the jar for 30 seconds under a fume hood. We then removed the feather and allowed it to air dry for 24 hours under a fume hood. Similarly we cleaned the feather halves in detergent once with 1L of a 1:30 solution of Fisher® Brand Versa-Clean (catalog number 04-342) detergent:deionized water, then rinsed three times in 3-1L jars of deionized water and allowed to air dry for 24 hours under a fume hood. We changed the deionized water after every 5 feathers to prevent detergent accumulation. We left uncleaned feather halves in original condition. We then sampled feathers for δD, $\delta^{13}C$, and $\delta^{15}N$ analyses. After this first sample was taken, to further test for

solvent residue effect on isotope values and data variation we recleaned feather halves treated with the 2:1 chloroform:methanol/detergent treatment with the other treatment in its respective pair and resampled for δD, $\delta^{13}C$, and $\delta^{15}N$ analyses.

To ensure any variation in feather stable isotope ratios was not due to variation along the feather, we took δD samples from only the last 1cm of the feather and $\delta^{13}C/\delta^{15}N$ samples from next to last 1cm. One cm of feather provides enough tissue for 2 samples, allowing us to resample the same section of feather a second time after additional treatment. We used entire feathers, including rachis and barbs, for analysis. To reduce further sample variation due to isotopic variation within feather parts, we cut each sample to contain proportional quantities of the rachis and barbs.

Stable isotope analyses

Hydrogen stable isotope samples were run at the Environment Canada Stable Isotope Hydrology and Ecology Research Laboratory in Saskatoon, Saskatchewan, Canada as described in Wassenaar and Hobson (2003). $\delta^{13}C/\delta^{15}N$ samples were run at the Stable Isotope Laboratory of the Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, New Mexico.

Statistical analyses

To determine if cleaning treatments yield similar results, we performed paired t-tests to compare sample means. We also provide least squares best-fit regression lines as a descriptor of data colinearity. If treatments yield the same results, data points will fall along a 1:1 line (slope = 1, intercept = 0). We also used these tests to compare data from the detergent treatments from left and right wings of the same quail. These data should also fall along a 1:1 line if left and right wings are producing

equivalent values. For the recleaning experiment, we used the aforementioned t-tests along with Levene's test for equal variance to determine if within-sample variation decreased with recleaning.

Results

In the uncleaned/detergent treatment, uncleaned values were lighter than detergent values for δD (means, t_{14}=-2.86, p=0.013; regression, Figure 2a), but heavier than detergent means for $\delta^{15}N$ (means: t_{16}=3.37, p=0.004; Figure 2e). Uncleaned and detergent means for $\delta^{13}C$ were not different (means, t_{16}=0.64, p=0.529; regression, Figure 2c). For detergent/2:1 chloroform:methanol pairs, 2:1 chloroform:methanol values were heavier for all three elements (means: δD – t_{16}=11.91, p<0.001; $\delta^{13}C$ – t_{16}=4.018, p=0.001; $\delta^{15}N$ – t_{16}=6.66, p<0.001; regressions, δD – Figure 2b; $\delta^{13}C$ – Figure 2d; $\delta^{15}N$ – Figure 2f).

When we compared detergent treatments from left and right wings for each quail we found no differences between means (δD – t_{16}=-0.48, p=0.64; $\delta^{13}C$ – t_{16}=0.03, p=0.97; $\delta^{15}N$ – t_{16}=-0.30, p=0.77). There were 1:1 trends in δD and $\delta^{13}C$, though there was not a trend for $\delta^{15}N$ (Figure 3).

The recleaned feathers show less isotope ratio variation than those cleaned only once (Table 2, Figure 4). There were no differences between means for δD and $\delta^{13}C$ (δD – t_{16}=-0.55, p=0.59; $\delta^{13}C$ – t_{16}=0.78, p=0.44), and only a marginal difference for $\delta^{15}N$ (t_{16}=2.14, p=0.05). There were 1:1 trends for δD and $\delta^{13}C$ (Figure 4). The $\delta^{13}C$ trend is strengthened if an outlier point is excluded (y=0.60[0.19]x-7.32[3.43], where numbers in square brackets are 1SE, r^2=0.42). For $\delta^{15}N$, the linearity deviates slightly from 1:1.

Discussion

These experiments show that cleaning method affects hydrogen, carbon, and nitrogen stable isotope ratios of bird feathers. Cleaning methods are not interchangeable and cleaning only once with a given agent may not be sufficient for both precise and accurate isotope analysis. For feather cleaning prior to analysis, we propose first using detergent, then recleaning with 2:1 chloroform:methanol. Though some studies we reviewed (Hobson 1999; Wassenaar and Hobson 2000b) cleaned feathers multiple times with the same solvent, we did not test this and make no judgment about its effectiveness.

Challenges

In stable isotope analysis, absolute values of samples measured are never known, but repeated measures of homogenized samples can improve confidence in the measured isotope ratio. Cleaning agents may remove contaminants, but may also change feather isotope values either by leaving a residue with a different enough stable isotope ratio to change the measured value or by causing atom exchange, a phenomenon that involves removing atoms from the feather and replacing them with atoms from the cleaning agent. Atom exchange is most commonly seen with hydrogen (Wassenaar and Hobson 2003) and varies with pH, temperature, and solvent (Schimmelmann 1991; Campbell et al. 1995).

To infer how different cleaning treatment impacted feather isotope ratios relative to the real isotope values of the feathers, we examined the direction of isotopic shift after recleaning (Figure 5). The case in which recleaning with a second solvent shifts isotope ratios toward those of feathers cleaned only in this solvent may suggest

that solvent is driving the isotope ratio of the sample. If values shift either in the opposite direction or beyond those of the feathers cleaned only in this solvent, then it is unlikely that the solvent is primarily responsible for shifts in isotope ratios.

For hydrogen, both recleaned treatments have stable isotope ratios closer to those of the original 2:1 chloroform:methanol treatment, which is consistent with the idea that 2:1 chloroform:methanol may be driving δD values. Since both chloroform and methanol are highly volatile, potential for residue from either solvent is low. However, if we consider the known relationship between precipitation and feather δD (Hobson et al. 1999) and also that the predicted value for feather δD at Bartlesville, OK, where the quail were raised is -71±11‰ (based on water data from Bowen and Revenaugh [2003] and a standard -25‰ fractionation from water from Hobson et al. [1999]), our detergent recleaned with 2:1 chloroform:methanol (-74.12±3.77‰; Table 1) and 2:1 chloroform:methanol recleaned with detergent (-73.35±6.11‰) treatments accurately reflect the predicted value, thus supporting the notion that these treatments reflect the true isotope values of the feathers. The mean for the 2:1 chloroform:methanol only treatment (-62.06±11.12‰) is also statistically similar to the predicted value (though heavier than the recleaned treatments), but, with a variance two to three times that of the recleaned treatments, these data may be less useful for geographic assignment. Moreover, since all quail were given the same water with the same δD, regardless of the actual value, we did not expect a large δD range for quail feathers, as is seen in the recleaned treatment data. This result further suggests that some cleaning procedures can yield results with low variance.

Carbon means did not shift when recleaned. Their stability supports the notion that carbon stable isotope ratios are robust to contamination and that carbon exchange is insignificant. However, there were differences in the variance resulting from recleaning. The increased variation in the values from the feathers cleaned in 2:1 chloroform:methanol and recleaned in detergent makes us reluctant to suggest cleaning in this order. The other treatment, detergent recleaned with 2:1 chloroform:methanol, maintains variation equivalent to that seen in the two original treatments which may favor using cleaning agent in this order.

Nitrogen values for recleaned feathers are higher than both original treatments. Since neither chloroform nor methanol contains nitrogen, possibility for residue from these solvents is limited. Further, nitrogen exchange has not been documented other than with terminal metal nitrides (Woo 1993). Thus, we suggest that treatment influence related to residue or exchange from either chloroform or methanol is negligible. Detergent residue may have an effect, but since both recleaned treatments have similar means with low variance and both data sets fall outside the range of the two original treatments, we believe contamination is not a factor and the range of recleaned feather isotope ratios contains the actual feather isotope values.

Implications

In avian ecology, hydrogen stable isotopes are primarily used to assign birds to a particular geographic location. An isotopic precipitation gradient relative to latitude, altitude, and distance from the coast is well documented and transfers reliably to animal tissues (Bowen and Revenaugh 2003; Hobson et al. 1999). However, error in calculating tissue isotope ratios can alter geographic placement of birds. For the two

initial treatments in this study, SD≈22‰ and the difference between their means is about 43‰. Within a treatment, this can place a bird that belongs in Oklahoma as far north as South Dakota and southern Minnesota, or as far south as southern Texas. If you compare between treatments this range extends north into southern Canada and south to coastal Mexico. Recleaned values, which have no difference between means and SD≈8-12‰, result in a condensed geographic area within which a breeding bird may be assigned. Our Oklahoma bird would most likely still be placed in Oklahoma with these treatments, with a possible range from Kansas to north Texas.

Carbon stable isotopes are used for diet analysis, but also indicate trophic level, with 1-2‰ enrichment per trophic step. The observed ≥1‰ within-treatment variation may lead to misclassification of trophic level, for example, an herbivore may be classified as a carnivore or vice versa. Observed variation is not expected to significantly affect diet analysis unless isotopic distinction between dietary elements is small.

Nitrogen is used mainly to identify trophic level with 3-5‰ enrichment per trophic step. With an almost 2‰ difference in cleaning treatment means, cleaning method differences may misclassify an organism by one-half trophic level. If a cleaning method with higher variance is chosen, additional error may occur.

In conclusion, cleaning solvent choice does affect stable isotope ratios of bird feathers, a determination that should be considered in future experiments. This seemingly small detail in the overall scheme of a project may have serious implications for data reliability and interpretation both within and among data sets. We propose a standard two-step cleaning method using both detergent and 2:1

chloroform:methanol. Since our data suggests that the order of cleaner use may influence data variation, we specifically suggest cleaning with detergent first, then cleaning with 2:1 chloroform:methanol. This approach, however, should be considered a starting point in moving toward standardized preparation techniques for isotope samples.

Acknowledgements

The authors thank the George M. Sutton Avian Research Center for providing quail, Viorel Atudorei and Zachary Sharp for carbon and nitrogen isotope analysis, Keith A. Hobson and Leonard I. Wassenaar for hydrogen isotope analysis and insightful review, and Brandon Bestelmeyer, Michael Engel, Michael Kaspari, Linda Wallace, Adam Fudickar, Lauren Niemann, and Randy Soto for review and advice. This research was supported by a National Science Foundation Graduate Research Fellowship, the George M. Sutton Scholarship in Ornithology, and the University of Oklahoma. This work was submitted in partial fulfillment of JMP's PhD degree at the University of Oklahoma and the experiments herein comply with the all appropriate US regulations.

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Table 1. References reviewed to determine the prevalence of cleaning agents and the elements analyzed in stable isotope studies using bird feathers.

Reference	Isotopes used	Cleaning Agent
Bearhop et al. 1999	C N	0.25M sodium hydroxide and water
Bearhop et al. 2000	D C N	0.25M sodium hydroxide and water
Bearhop et al. 2001	CN	0.25M sodium hydroxide and water
Bearhop et al. 2002	C N	0.25M sodium hydroxide and water
Bearhop et al. 2006	C N	Uncleaned
Bensch et al. 2006	C N	Some uncleaned, some cleaned with detergent
Caccamise et al. 2000	C N S	Deionized water
Chamberlain et al. 1997	D C	Detergent and water
Chamberlain et al. 2000	C N	Not documented
Cherel et al. 2000.	C N	2:1 Chloroform:ether
Cherel et al. 2005a	C N	2:1 Chloroform: methanol
Cherel et al. 2005b	C N	2:1 Chloroform: methanol
Clegg et al. 2003	D	Detergent and water
Dunn et al. 2006	D	2:1 Chloroform: methanol
Graves et al. 2002	C N	2:1 Chloroform: methanol
Hebert and Wassenaar 2005	D C N S	Methanol
Hobson 1999	C N	2:1 Chloroform:methanol
Hobson and Clark 1992	C	Ether
Hobson and Wassenaar 1997	D	2:1 Chloroform:methanol
Hobson and Wassenaar 2001	D C	2:1 Chloroform:methanol
Hobson et al. 1993	N	Ether
Hobson et al. 1999	D	2:1 Chloroform:methanol
Hobson et al. 2000	C D	2:1 Chloroform:methanol
Hobson et al. 2001	D	Not documented
Hobson et al. 2003	D C N	2:1 Chloroform:methanol
Hobson et al. 2004a	D	2:1 Chloroform:methanol
Hobson et al. 2004b	D O	2:1 Chloroform:methanol
Hobson et al. 2004c	D	2:1 Chloroform:methanol
Hobson et al. 2006	D	2:1 Chloroform:methanol
Kaushal and Walsh 2002	N	Freeze-dried and stored in a dessicator

Table 1 (continued)

Kelly 2006	D	Detergent and water
Kelly et al. 2002	D	Detergent and water
Kelly et al. 2005	D	Detergent and water
Klaassen et al. 2004	C	Chloroform
Lott and Smith 2006	D	2:1 Chloroform: methanol
Lott et al. 2003	D S	Detergent and water
Mazerolle and Hobson 2005	D	2:1 Chloroform: methanol
Mazerolle et al. 2005	D	2:1 Chloroform: methanol
Meehan et al. 2001	D	Detergent and water
Meehan et al. 2003	D	Detergent and deionized water
Mehl et al. 2004	C N	2:1 Chloroform:methanol
Mituzani and Wada 1988	C N	Water
Mituzani et al. 1990	C	Water
Mituzani et al. 1991	C N	Physiological salt solution
Mizutani et al. 1992	C N	Water
Møller and Hobson 2004	D C N	2:1 chloroform:methanol
Møller et al. 2006	C N	2:1 chloroform:methanol
Neto et al. 2006	D C N	Uncleaned
Newton et al. 2006	D	2:1 Chloroform: methanol
Norris et al. 2004	D	Not documented
Ogden et al. 2004	C N	Not documented
Pain et al. 2004	D C N	0.25M sodium hydroxide and water
Paszkowski et al. 2004	C N	Distilled water
Pearson et al. 2003	C N	Ether
Podlesak et al. 2005	C	Sonication in distilled water and in petroleum ether
Romanek et al. 2000	C N	Mild detergent
Rubenstein et al. 2002	D C	Not documented
Smith and Dufty 2005	D	2:1 Chloroform: methanol
Smith et al. 2003	D	2:1 Chloroform:methanol
Smith et al. 2004	D	2:1 Chloroform:methanol
Thompson and Furness 1995	C N	Chloroform/acetone
Thompson et al. 1995	C N	Chloroform/acetone
Wassenaar and Hobson 2000	D C	2:1 Chloroform:methanol.

Table 1 (continued)

Wassenaar and Hobson 2001	D C	2:1 Chloroform:methanol
Wassenaar and Hobson 2003	D	2:1 Chloroform:methanol
Wennerberg et al. 2002	C	Chloroform
Wunder et al. 2005	D	Frozen/thawed, then 2:1 Chloroform:methanol
Yohannes et al. 2005	D C N	2:1 Chloroform: methanol

Table 2. Comparison of variance between feather halves subject to both original cleaning and recleaning with detergent and 2:1 chloroform:methanol.

Element	Treatment	Mean (‰)	Standard Deviation	Levene's Test for Equality of Variances F	Significance
H	Detergent	-105	11.62	8.2	0.007
	Detergent recleaned w/ 2:1 chloroform:methanol	-74.1	3.77		
	2:1 chloroform:methanol	-62.1	11.12	4.14	0.05
	2:1 chloroform:methanol recleaned w/ detergent	-73.4	6.11		
C	Detergent	-18.5	0.32	3.59	0.067
	Detergent recleaned w/ 2:1 chloroform:methanol	-18.2	0.22		
	2:1 chloroform:methanol	-18.3	0.33	0.12	0.733
	2:1 chloroform:methanol recleaned w/ detergent	-18.4	0.58		
N	Detergent	3.34	0.83	11.11	0.002
	Detergent recleaned w/ 2:1 chloroform:methanol	5.18	0.26		
	2:1 chloroform:methanol	4.33	0.45	5.09	0.031
	2:1 chloroform:methanol recleaned w/ detergent	5.04	0.26		

Figure 1. Schematic of treatment pairs. We assigned treatments randomly to left and right wings and feather halves.

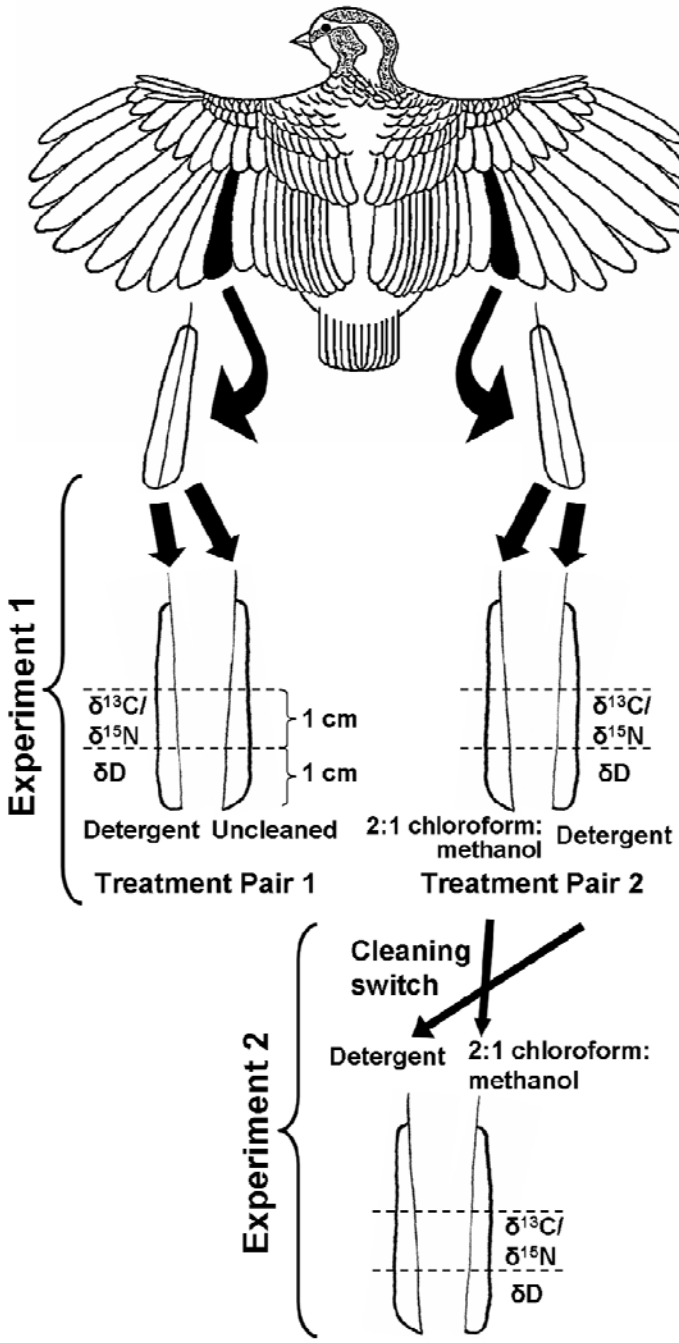


Figure 2. Stable isotope ratios of hydrogen (squares; a and b), carbon (circles; c and d), and nitrogen (triangles; e and f) for paired treatments after initial cleaning. Solid shapes represent uncleaned vs. detergent feathers, open shapes represent 2:1 chloroform:methanol feathers. Best-fit lines and equations are based on least squares. Equations include 1SE in parentheses for slope and intercept. In d) the slope is not significantly different from 1 and the y-intercept is not significantly different from zero.

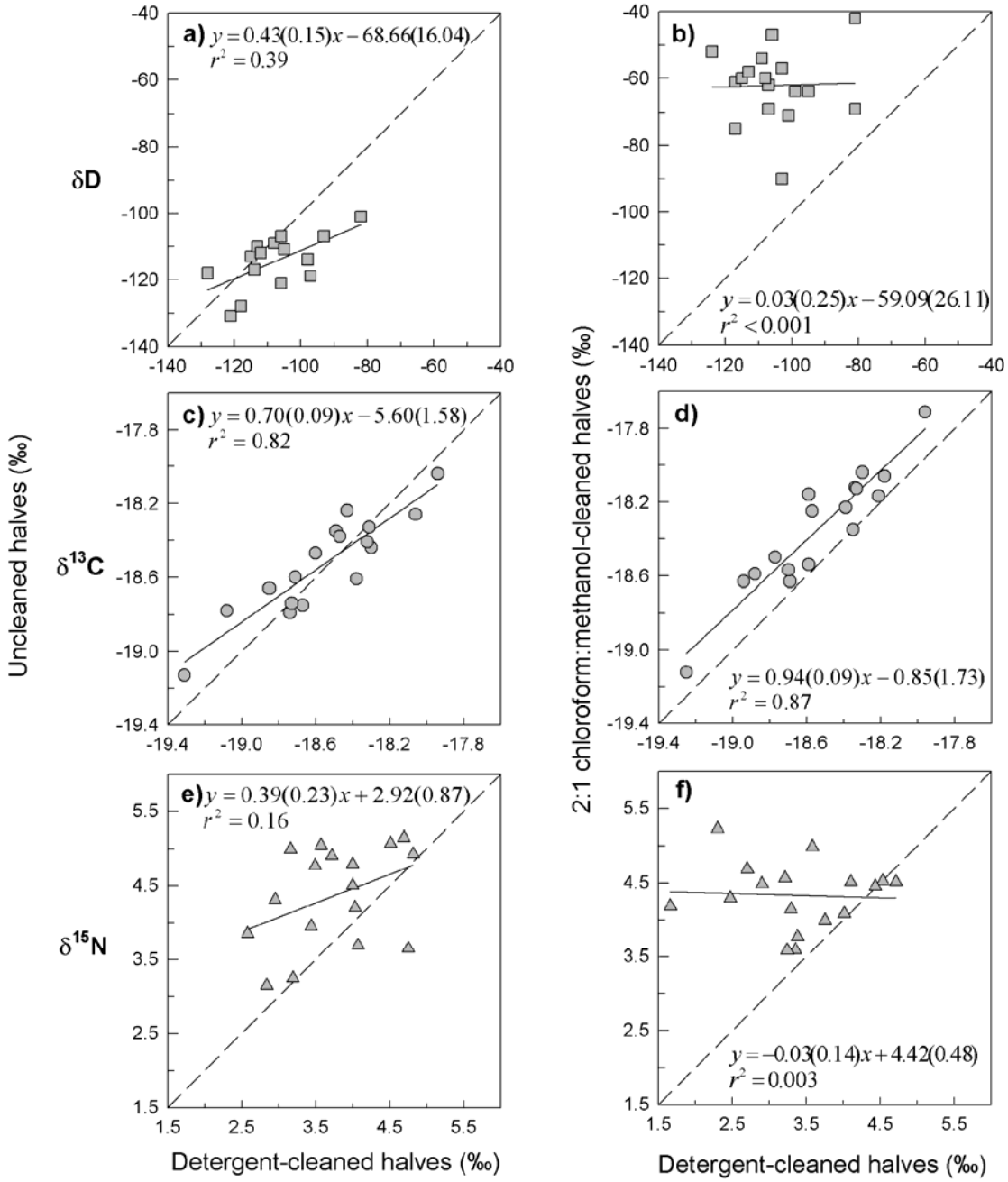


Figure 3. Comparison of detergent treatments from left and right wings of quail, for hydrogen (squares; a), carbon (circles; b), and nitrogen (triangles; c). The slope in b) is not significantly different from 1 and the y-intercepts in a) and b) are not significantly different from zero.

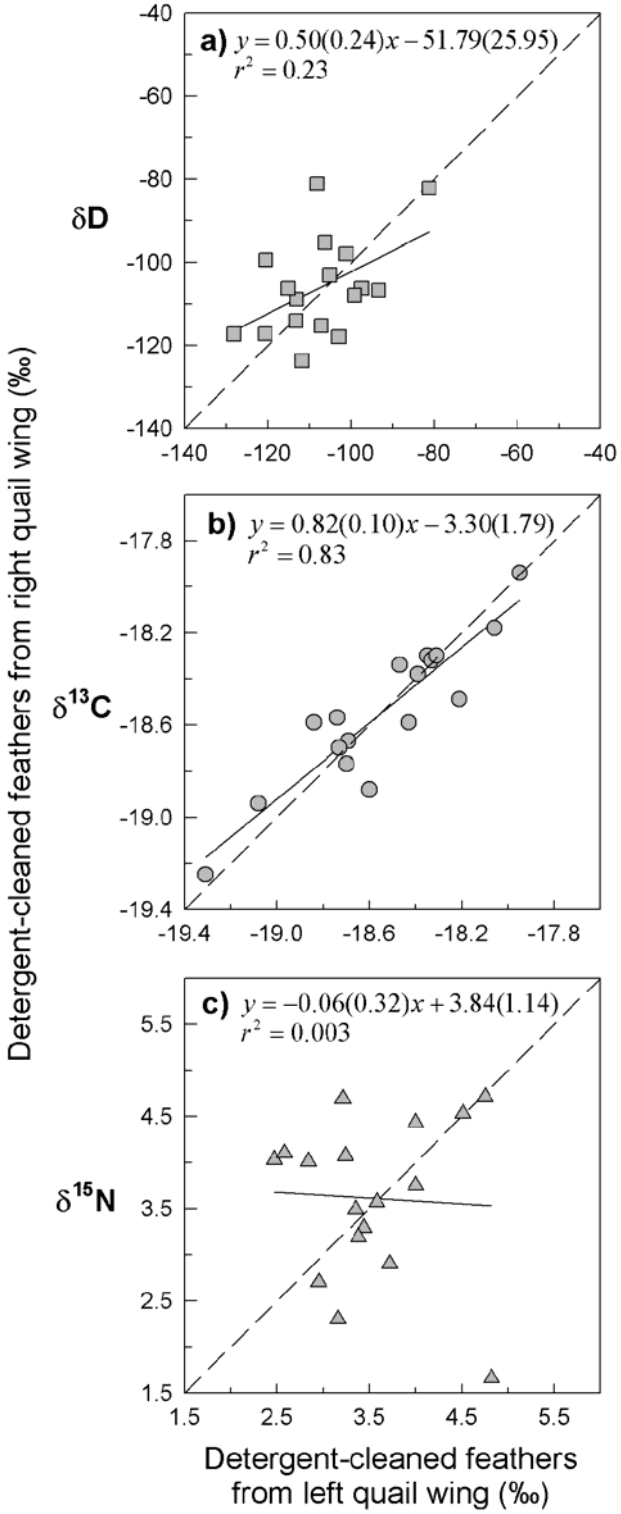


Figure 4. Comparison of stable isotope ratios for hydrogen (squares; a), carbon (circles; b), and nitrogen (triangles; c) of recleaned feather halves. In a) and b) slopes are not significantly different from 1 and y-intercepts are not significantly different from zero. It is clear that the y-value of one of the feather pairs is an outlier among all data in the experiment. We were unable to identify the source of this discrepancy.

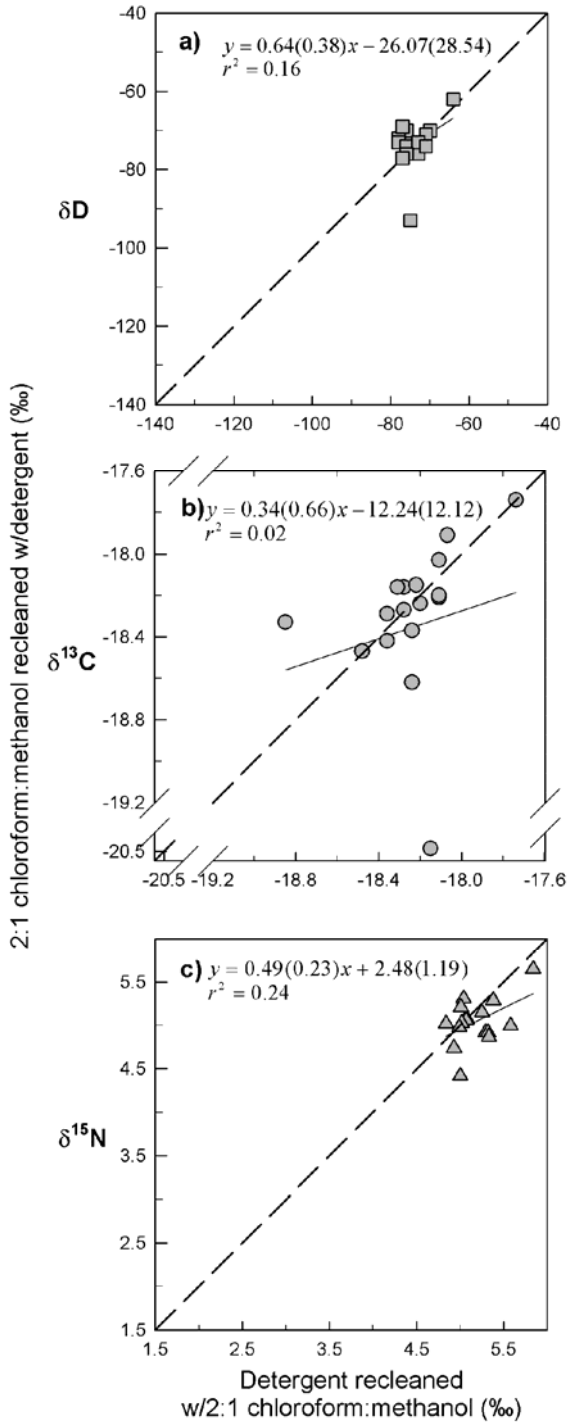
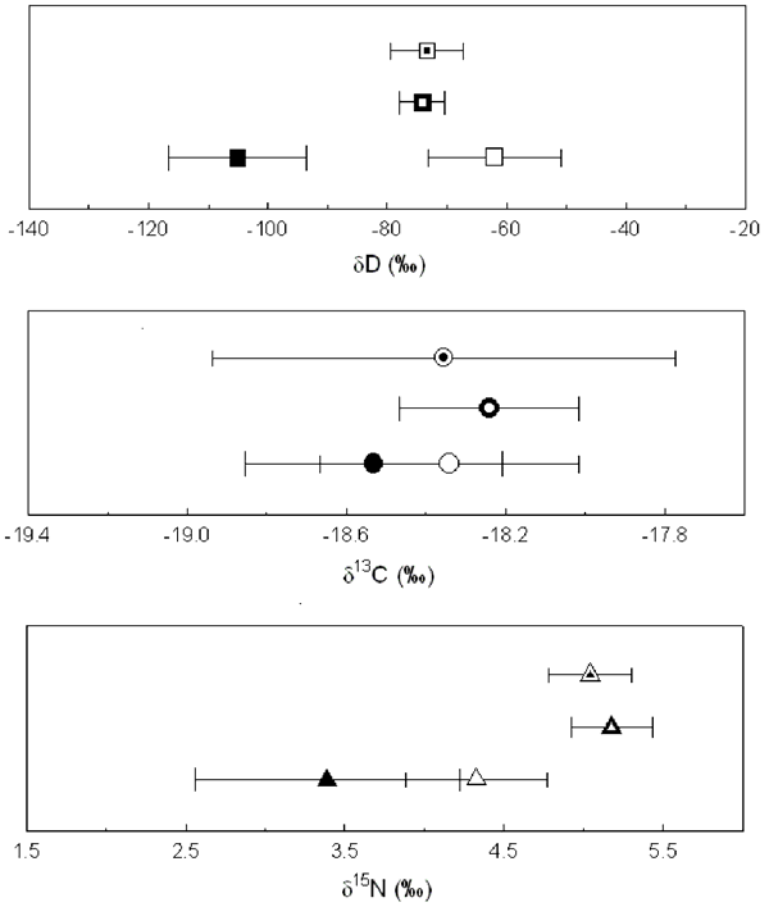


Figure 5. Mean ± 1SD for hydrogen (squares; a), carbon (circles; b), and nitrogen (triangles; c) stable isotope ratios of original detergent (dark shapes) and 2:1 chloroform:methanol (open shapes) treatments compared to the movement of the recleaned treatments, detergent recleaned in 2:1 chloroform:methanol (dark shapes with open centers) and 2:1 chloroform:methanol recleaned in detergent (open shapes with dark centers).



CHAPTER 2

ISOSOURCE VARIANCE INFLUENCES ESTIMATES OF CONSUMER ISOTOPIC NICHE AND INDIVIDUAL SPECIALIZATION

Abstract

Variation in stable isotope ratios of consumers' tissues has been assumed to reflect niche axis breadth. In contrast, variation in stable isotope ratios of food items is treated with a *ceteris paribus* assumption. If this assumption is violated, it is unclear to what degree estimates of niche breadth and individual specialization would be effected. I collected stable carbon isotope ($\delta^{13}C$) data from 28 plants, three grasshoppers, and one bird species of the Chihuahuan Desert to examine the relationship between variation in producer isotope ratios and those of consumers. Consistent with a literature review, the $\delta^{13}C$ from eight C_4 species was less variable than from 20 C_3 plants. A grasshopper species eating C_3 sources (*Trimerotropis pallidipennis*) was more variable than that one eating C_4 food sources (*Opeia obscura*); an herbivore with a mixed diet (*Melanoplus occidentalis*) showed the highest variation. Black-throated sparrows (*Amphispiza bilineata*) showed intermediate isotope ratios with intermediate variance. My results indicate that isotopic variance is a useful measure of individual specialization, but an inaccurate measure of niche axis width. In particular, variation in putative isosources may be

misinterpreted as higher niche axis breadths in consumers whereas conclusions about individual specialization remain consistent across trophic level.

Introduction

Defining niche axes transcends decades of modern ecology (Grinnell 1917, MacArthur 1958, Hutchinson 1959, Brown 1984, Pulliam 1988, Guisan and Zimmermann 2000). Isotopic niche space is a new (Muscatine et al. 1989, Hobson 1990) and controversial concept (Bearhop et al. 2004, Araujo et al. 2007, Newsome et al. 2009) which infers patterns of isosource use from a population's isotopic range. Two interpretations of variance surrounding isotopic data sets infer either 1) population niche axis width, which posits that populations with larger isotopic ranges have wider niches (Bearhop et al. 2004), or 2) degree of individual specialization within a population, which suggests that isotopic variation in a population indicates the similarity of isosource use among individuals (Bolnick et al. 2003) These two philosophies can lead to different types of conclusions from a single data set. For example, if a population has a mean stable isotope ratio with a large variance, niche width theory would suggest that the isotopic niche width of this population is large, implying that the population can use a wide range of isosources. However, individual specialization theory would suggest that there is a high degree of individual specialization in the population, which would suggest that not all individuals in the population are eating the same thing, and that high variance at the population level results from more specialized isosource use among individuals.

What influences these differing conclusions and which application of isotopic data would be more useful in the long term? Such analyses depend on controversial

assumptions, such as the scalars used for trophic enrichment (e.g., +3.4 ‰ for nitrogen and +1.1‰ for carbon; DeNiro and Epstein 1978, Post 2002, McCutchan et al. 2003). Here I evaluate the consequences of a second, *ceteris paribus* assumption that a consumer's food sources have equal variances in stable isotope ratios or that existing differences are inconsequential.

Terrestrial stable carbon isotopes ($\delta^{13}C$) are often used to identify proportion of C_3 and C_4 plant carbon in diets. Differences in isotopic fractionation during carbon fixation (Smith and Epstein 1971, Bender et al. 1973, Osmond et al. 1976, O'Leary 1988) among C_3 and C_4 photosynthetic pathways create distinct mean $\delta^{13}C$ values for C_3 (≈ -27‰ [per mil]) and C_4 (≈-13‰) plants (Evans et al. 1986, Berry 1989). C_3/C_4 photosynthetic differences should not only create different means, but also should result in greater intra- and inter-species $\delta^{13}C$ variation. Because of greater variation in stomatal CO_2 conductance in C_3 plants, they should have higher $\delta^{13}C$ variance than C_4 plants (Farquhar et al. 1989). C_3 carbon-fixing cells change ^{12}C and ^{13}C concentrations as stomata open and close, whereas C_4 bundle sheath $\delta^{13}C$ fluctuations are less pronounced; this difference should lead to lower $\delta^{13}C$ variation in C_4 plants. Re-examination of existing literature confirms both mean and variance differences in $\delta^{13}C$ values of C3 vs. C4 plants (Figure 1; Fry et al. 1978, Evans et al. 1986, O'Leary 1988, Farquhar et al. 1989, Martinelli et al. 1991). The difference in variance is rarely discussed.

In light of both differing C_3 and C_4 $\delta^{13}C$ variances and isotopic niche space controversy, I document producer $\delta^{13}C$ variation and analyze how it translates to the $\delta^{13}C$ values of primary and secondary consumers. I use a simulation to evaluate the

influence of magnitude of variance and difference between mean values using consumers with two different foraging strategies. I compare these simulations to my field data and summarize published examples of autotroph and consumer stable isotope variation and discuss consequences of trophic variance carryover for estimates of niche breadth and estimates of individual isosource specialization.

Methods

Field Data

Plant isosources and consumers. I sampled plants (*i.e.*, carbon isosources), grasshoppers, and birds on 15 sites at the Jornada Experimental Range and Long Term Ecological Research Site (Jornada LTER; +32.5º N, -106.8º W, elevation 1188 m). The Jornada LTER is located in the northern Chihuahuan Desert (Figure 2), historically a semi-arid C_4 grassland with C_3 forbs and shrubs including playas (floodplain grasslands), shrublands, and ecotones (see Wainright (2006) and Brown and Archer (1989) for further description).

Plants - I collected from 3 to14 individuals of 28 plant species in 2005 from 15 1-ha sites; in 2007 I resampled 5 of the 2005 sites and 5 other sites. I chose sites based on initial inspection of vegetation composition to ensure that the sites spanned a wide range of plant compositions and species assemblages. Not all species were collected in both years. I dried plants for 48h at 60°C, then placed 300-450µg leaf material (or green stem material in a leafless species, such as *Ephedra spp.*) into tin cups for $\delta^{13}C$ analyses. I inspected samples for debris, but did not chemically clean samples due to potential difficulty removing solvent. I chose samples primarily from

2005 clippings. To eliminate repeated sampling of the same plant I did not select the same species from the same site on the same sampling date. I used one to three samples from 2007 to verify 2005 data. If a new species was recorded in 2007, I treated it as I did the 2005 species (n=3 of 28 species).

Primary consumers – Though I collected numerous species in 2005, I analyzed 10-15 individuals of the three grasshopper species (Orthoptera: Acrididae) found at the most sites. I used sweep nets at random locations on 15 sites from June-August 2005. *Trimerotropis pallidipennis* is the most abundant New Mexico grasshopper and is found widely throughout western North American grasslands on forbs and grasses (Richman et al. 1993). *Opeia obscura* is also found throughout the North American grasslands, but its plant hosts are exclusively grasses (Richman et al. 1993). *Melanoplus occidentalis* is found throughout New Mexico, the western United States, and Canada using a mixture of forbs and grasses for food (Richman et al. 1993). Fry et al. (1978) indicate high C_3 use for *T. pallidipennis* (64% C_3 in diet; $\delta^{13}C$ = -21.0‰) and almost exclusive C_4 use for *O. obscura* (99% C_4 in diet; $\delta^{13}C$ = -13.2‰) Fry et al. did not analyze *M. occidentalis*; however, based on diet descriptions in Richman et al. (1993), I predicted this species would have $\delta^{13}C$ intermediate between C_3 and C_4 plants. For stable isotope analysis, I clipped 300-450μg of grasshopper wing, cleaned them in detergent and 2:1 chloroform:methanol solvent (Paritte and Kelly 2009), and packed them in tin capsules.

Secondary consumers – From May-August, at 12 sites in 2004 and the 15-2005 sites, I collected outer rectrices from hatch-year (HY) black-throated sparrows (*Amphispiza bilineata*; BTSPs) for $\delta^{13}C$ analysis (Bolnick et al. 2003). I cleaned

feathers in detergent and 2:1 chloroform:methanol (Paritte and Kelly 2009), removed ≈350μg of distal rachis and barbs, and packed them in tin capsules.

Stable isotope analysis. Samples were run for $\delta^{13}C$ on either a Thermo Finnigan Delta Plus XL Isotope Ratio Mass Spectrometer, interfaced to a Carlo Erba Elemental Analyzer through an open split valve (ConFlo II) at the Stable Isotope Laboratory of the Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, New Mexico, or on a Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer interfaced through an open split valve (ConFlo III) with a Costech Elemental Analyzer at the laboratory of Michael H. Engel, Department of Geology and Geophysics, University of Oklahoma, Norman, Oklahoma. All values are reported in per mil notation relative to the isotope standard Vienna Pee Dee Belemnite (vPDB).

Data Analysis. I examined $\delta^{13}C$ data for outliers (i.e., C_3 numbers for C_4 plants) and used independent samples t-tests and least-squares regression to identify trends with sampling date and site. I used Levene's test (SPSS 2004) to test homogeneity of variance of $\delta^{13}C$ values among C_3 and C_4 plant species. I used an independent-samples t-test on standard deviations of $\delta^{13}C$ values to test for equal variances with species categorized as C_3 and C_4. I used one-way ANOVAs and Tukey's HSD *post hoc* where $\delta^{13}C$ grand means and mean standard deviations were dependent variables and species was the independent variable. to determine how consumers differed in means and variances of $\delta^{13}C$ values. I interpreted data and classify C_3, C_4, and intermediate species using a trophic enrichment of zero to 1.1‰ per trophic level, the range currently accepted for $\delta^{13}C$ (DeNiro and Epstein 1978,

Post 2002, McCutchan et al. 2003). This enrichment translates into 0-1.1‰ for grasshoppers and 0-2.2‰ for BTSPs, which eat insects (primarily grasshoppers) and plant seeds (Zimmer 1983).

Data simulation

I used the BASIC programming language to model the influence of isosource variance and magnitude on means and variances of stable isotope ratios of consumer populations. I used two normally distributed isosources (means -26.8‰—C_3 and -15.27‰—C_4) to create consumer populations in which either all individuals consumed identical mixed diets ranging from 100%C_3 to 100% C_4 diets (*i.e.*, "mixed populations"), or where all individuals consumed only 100%C_3 or 100% C_4 diets and the population consisted of a range of 100% C_3 individuals to 100% C_4 individuals (*i.e.*, "target populations"). At 10% population intervals (*e.g.*, 100% C_3 and 0% C_4, 90%C_3 and 10% C_4, 80% C_3 and 20% C_4, etc.), I randomly generated $\delta^{13}C$ values for 100 consumers. For target consumers, $\delta^{13}C$ values were randomly generated from the appropriate isosource. For mixed consumers, random $\delta^{13}C$ values were generated from each isosource and consumer values were calculated proportionally from these random values. I then calculated means and standard deviations (SDs) for each population increment and diet type. I repeated this 100 times, and for each diet 10% group, calculated means, SDs, and 95% confidence intervals around means and SDs. I ran four simulations using the following isosource SDs: C_3=1, C_4=1; C_3=5, C_4=5; C_3=5, C_4=1; C_3=9, C_4=5. For simplification, I assumed no trophic fractionation in these simulations. I then performed a fifth simulation using the actual C_3 and C_4 SDs

calculated for this study – C_3=1.61, C_4=0.76 – and compared this simulation to grasshopper and BTSP $\delta^{13}C$ values.

Results

Field data

C_3 and C_4 photosynthetic groups differed as expected in mean $\delta^{13}C$ (C_3 $\delta^{13}C$=-26.84±1.61‰, C_4 $\delta^{13}C$=-15.60±0.76‰; t_{189}=-54.27, p<0.001; Figure 3) and overall C_3 variation was higher than C_4 variation ($F_{1,189}$=24.61, p<0.001). Additionally, C_3 plants (average species 1SD=1.00±0.41‰) varied more intraspecifically in $\delta^{13}C$ measurements than C_4 species (average species 1SD=0.69±0.28‰; t_{26}=-2.13, p=0.04). The $\delta^{13}C$ values of five of 28 plant species varied with time (Appendix 1): *B. eriopoda* $\delta^{13}C$ became more depleted with sample date in 2005 (y=-0.01x-13.38, r^2=0.34, p=0.05) and *S. brevifolia* (y=-0.03x-10.84, r^2=0.54, p=0.004), *C. pottsii* (t_6=3.79, p=0.01), *G. sarothrae* (t_{12}=-2.64, p=0.02), and *S. angustifolia* (t_4=2.75, p=0.05) $\delta^{13}C$ varied between years.

Of the three grasshopper species, *T. pallidipennis* had a primarily C_3 source-carbon signature and *O. obscura* had a primarily C_4 carbon signature ($\delta^{13}C$ =-23.51±1.53‰, n=15 and $\delta^{13}C$ =-15.18±1.02‰; n=15, respectively; t_{24}=-17.52, p<0.001; Fig. 3). When 0-1.1‰ trophic enrichment is considered, both species means overlap the range of means for their respective plant isosources suggesting that both species may target plants that use a single photosynthetic pathway. Similar to plants, C_3 grasshoppers (*T. pallidipennis*) had 0.51‰ greater within-species variance than C_4 grasshoppers (*O. obscura*, $F_{1,28}$=6.31, p=0.02). *M. occidentalis* had an intermediate signature ($\delta^{13}C$ =-21.47±3.51‰, n=10) with greater variance than both the *T.*

pallidipennis ($F_{1,23}$=4.76, p=0.04) and *O. obscura* ($F_{1,23}$=11.12, p=0.003). The sample distribution of *M. occidentalis* was bimodal, suggesting each individual targeted C_3 or C_4 sources rather than mixing sources, resulting in a higher population variance

Black-throated sparrows had an intermediate signature with intermediate variance (2004 $\delta^{13}C$ =-17.65±1.85‰, n=17; 2005 $\delta^{13}C$ =-17.74±2.30‰, n=53). BTSP variances were not as high as *M. occidentalis* variance because individual sparrows had mixed C_3 and C_4 diets with some variation in proportion, whereas *M. occidentalis* individuals had more targeted C3 or C4 diets, implying high variation in proportion.

Data simulation

Simulation of hypothetical primary consumer populations indicated that equal, relatively low variances among isosources provide relatively precise data (Figure 4a). Logically, populations using 100% of an isosource have variances close to that isosource regardless of diet strategy (target vs. mixed). For target populations, as the percentage of individuals consuming each isosource approaches 50%, population variance rises dramatically. In contrast, mixed population means stay similar to those of target populations, but variances slightly decrease as the diet mixing proportion approaches 50:50. Increasing the magnitude of isosource variation increases variance around dietary means and increases the overall magnitude of means, though the curve pattern remains the same (Figure 4b).

When isosource variances differ (Figure 4c and d), simulation patterns become asymmetrical. Consuming 100% of an isosource still results in variation similar to the isosource, but if one isosource has higher variance, its respective consumer variance will be higher in magnitude than consumers of the other isosource. For target

populations, dietary means are less variable closer to the lower variance isosource, but SDs are less variable toward the higher variance isosource. Mixed populations show both less variable means and SDs closer to the lower variance isosource. Similar to the equal variance simulations, increasing the magnitude of the variance pattern decreases the precision around means and SDs and increases the overall magnitude of the patterns, but maintains a similar pattern.

Field-simulation comparison

When I compare grasshopper data to the model pattern derived using the experimental plant means and variances (trophic fractionation=0 to 1.1‰; Figure 5), all three grasshoppers fall between predicted areas for 100% target and 100% mixed populations. *M. occidentalis* targets either C_3 or C_4 isosources, with about 80% of the population targeting C_3 plants. Individual *M. occidentalis* data points suggest that individuals probably target isosources close to 100%, suggesting real data may show more constricted patterns relative to model estimates. *T. pallidipennis* and *O. obscura* use mostly C_3 or C_4 isosources, respectively. According to the model estimate, *T. pallidipennis* may use more of the C_4 isosource than *O. obscura* uses of the C_3 isosource; however, both species still consume both isosources.

Discussion

Variance in $\delta^{13}C$ was greater among C_3 than among C_4 plants in a Chihuahuan Desert ecosystem. Differential variance was reflected in the diets of consumers who focused on C_3 and C_4 plants. $\delta^{13}C$ values of consumers that focused on C_3 plants varied more, not necessarily because of broader diet, but because of specialization on more isotopically variable food. Further, variance of consumers that ate both C_3 and

C_4 plants can be used to differentiate between samples comprised of individuals targeting different sources vs. samples of populations where all individuals have mixed diets. Failure to account for intraspecific isosource variation can lead to misinterpretation of variance among consumers across C_3/C_4 gradients. Similar patterns have been overlooked in the literature, spanning various trophic levels and taxonomic classifications (Figure 6).

My data are more clearly interpreted in the context of individual specialization than in the context of isotopic niche width. For example, according to individual specialization theory, since both *T. pallidipennis* and *O. obscura* means show use of both C_3 and C_4 plants and low variance relative to their predominant isosource, I would conclude that both consume primarily one isosource and may target their respective isosource with slight mixing. However, according to isotopic niche width arguments, the grasshopper *T. pallidipennis* ($\delta^{13}C$ = -23.5±1.5‰ – primarily C_3) could occupy a niche 50‰ wider than the grasshopper *O. obscura* ($\delta^{13}C$ = -15.2±1.0‰ – primarily C_4), despite both species having diet $\delta^{13}C$ values that overlap their respective target isosource and published gut content analyses that document that each species favors one photosynthetic isosource (Fry et al. 1978).

A second example of using isotopic variation to indicate more clearly individual specialization involves the grasshopper *M. occidentalis* and BTSPs. Both have intermediate $\delta^{13}C$ means, but lower BTSP variances indicate individuals maintain mixed diets, whereas *M. occidentalis* variance implies that individuals with both targeted and mixed diets. Here, isotopic variance shows a clear specialization pattern. Interpreting my data in terms of niche width, the grasshopper *M. occidentalis*

would occupy a 50-90% larger niche space than BTSPs, despite both using the same two isosources and the possibility that BTSPs are possibly eating seeds and insects, thus consuming not only two isosources, but also multiple trophic levels. Again, having a 50-90% wider niche in this system is not as understandable as the conclusion that *M. occidentalis* has greater individual specialization than do BTSPs.

Simulated data suggests that for target populations with unequal variance isosources, dietary means are less precise closer to the higher variance isosource, but variance, and thus degree of specialization, becomes more precise as the trend moves toward the higher variance isosource. Logically, precision on both axes increases as the difference between isosource means increases. Furthermore, isosource normality may influence consumer values, but to varying degrees. I performed additional simulations using continuously distributed isosources, which generally decreased the precision around means and increased the magnitude, but not precision, of SDs, compared to simulations with normally distributed isosources. Comparing simulated data to field data generally confirmed initial data analysis and agreed with conclusions derived from individual specialization theory.

In conclusion, isosources vary, often unequally, and consumer variation must be measured relative to its respective isosources. Unequal source variance is discussed in the literature for its ability to complicate calculating source proportions (Phillips and Gregg 2001, Phillips et al. 2005, Newsome et al. 2007), but is largely ignored in discussions of niche axes and individual specialization where variance plays a crucial role. Not surprisingly, C_3/C_4 variance dichotomy is not reflected in lab experiments with controlled isosources (Tieszen et al. 1983, Hobson and Clark 1992a,

b), underscoring the importance of delineating natural source variation with targeted natural experiments. This unique call for more field experiments compliments recent pleas for more laboratory isotope tests (Gannes et al. 1997, Martinez del Rio et al. 2009) and both, if designed correctly, can advance stable isotope use in ecological studies.

Acknowledgements

Thanks to Jeff Kelly, Michael H. Engel, Viorel Atudorei and Zachary Sharp for isotope analyses, to Brandon Bestelmeyer, M.H. Engel, Michael Kaspari, Michael Patten, and Linda Wallace for review and advice, Lauren Niemann for review and GIS help, and Barbara Nolen for GIS help. J.M. Paritte was supported by an NSF Graduate Research Fellowship during part of this study. Funding was provided in part by the University of Oklahoma's George Miksch Sutton Scholarship in Ornithology, M. Blanche Adams Scholarships, and college and departmental grants. This work was submitted in partial fulfillment of J.M. Paritte's Ph.D. degree at the University of Oklahoma and the experiments herein comply with the all appropriate U.S. regulations.

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Table 1. Plants identified in Jornada LTER study sites. Some species were grouped to increase sample size: four species of the family Asteraceae, three species of the family Brassicaceae, two species of the genus *Sporobolus*, and two species of the genus *Panicum*. One unidentified forb was included due to its association with *P. mutica*.

Family	Species	N	Mean±SD (‰)
Agavaceae	*Yucca elata*	6	-23.92±1.32
Asteraceae	*Acourtia nana*	6	-28.33±1.32
	Baileya multiradiata	7	-28.06±0.87
	Chrysothamnus pulchellus	3	-24.75±1.13
	Flourensia cernua	5	-26.18±0.80
	Gutierrezia sarothrae[b]	14	-27.24±1.10
	Gutierrezia sphaerocephala	3	-28.31±0.67
	Zinnia acerosa	4	-26.98±0.26
	Asteraceae, other	8	-28.30±1.26
Brassicaceae	*Brassicaceae spp.*	8	-26.38±1.07
Caesalpinaceae	*Caesalpinia jamesii*	3	-27.68±0.16
Chenopodiaceae	*Salsola tragus*	5	-14.51±0.38
Ephedraceae	*Ephedra* spp.	5	-24.84±0.67
Euphorbiaceae	*Croton potsii*[b]	8	-26.49±0.75
Fabaceae	*Janusia gracilis*	3	-27.02±1.78
	Prosopis glandulosa	13	-26.20±1.05
Hydrophyllaceae	*Nama hispidum*	6	-28.41±1.19
Malvaceae	*Sphaeralcea angustifolia*[b]	6	-28.72±0.69
Poaceae	*Aristida purpurea*	7	-14.92±0.99
	Bouteloua eriopoda[a]	13	-15.64±0.54
	Muhlenbergia porteri	6	-15.22±0.81
	Panicum spp.	3	-14.59±1.02
	Pleuraphis mutica	13	-16.15±0.38
	Scleropogon brevifolius[a]	14	-14.90±0.68
	Sporobolus spp.	7	-15.44±0.54
Polygonaceae	*Eriogonum trichopes*	4	-25.79±1.13
Solanaceae	*Solanum elaeagnifolium*	6	-26.75±1.07
Unidentified	Unidentified forb	6	-25.67±1.70

[a] Date within year significance
[b] Date between years significance

Table 2. BASIC code for computation of model values. "Target model" simulated populations of 100 individuals that consume either 100% of one or the other isosource. "Mixed model" simulated populations of 100 individuals that all consume the same predetermined proportion of the two isosources. Output is 100 population means and 1SDs for each 10% diet increment. A mean and SD for the means and a mean and SD for the SDs were then calculated from model results. For each change of variance, some variables in the model were changed. The variable **A** represents the 1SD for the C_3 isosource, **B** represents the 1SD for the C_4 isosource.

Target Model	Mixed Model
1 CLS	1 CLS
RANDOMIZE	RANDOMIZE
CLS	CLS
PRINT "100 Trials for 100% Target Consumers of C3 Carbon"	PRINT "100 Trials for Consumers of 100% C3 Carbon"
PRINT	PRINT
DIM xc3(100): DIM xc4(100): DIM ind(100)	DIM xc3(100): DIM xc4(100): DIM ind(100)
FOR j = 1 TO 100	FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0: sumc4 = 0: mean = 0: diff2 = 0: totdiff2 = 0: stdev = 0	v1 = 0: v2 = 0: w = 0: y = 0: indsum = 0: mean = 0: diff2 = 0: totdiff2 = 0: stdev = 0
FOR k = 1 TO 100	FOR k = 1 TO 100
100 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND * 2) - 1	100 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 100	w = v1 * v1 + v2 * v2: IF w > 1 THEN 100
y = SQR((-2 * LOG(w)) / w)	y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * **A** - 26.8013	xc3(k) = v1 * y * **A** - 26.8
sumc3 = sumc3 + xc3(k)	xc4(k) = v2 * y * **B** - 15.27
NEXT k	ind(k) = 1 * xc3(k) + 0 * xc4(k)
mean = sumc3 / 100	indsum = indsum + ind(k)
FOR n = 1 TO 100	NEXT k
diff2 = (xc3(n) - mean) * (xc3(n) - mean)	mean = indsum / 100
totdiff2 = diff2 + totdiff2	FOR n = 1 TO 100
NEXT n	diff2 = (ind(n) - mean) * (ind(n) - mean)
stdev = SQR(totdiff2 / 99)	totdiff2 = diff2 + totdiff2
PRINT mean; stdev,	NEXT n
NEXT j	stdev = SQR(totdiff2 / 99)
PRINT	PRINT mean; stdev,
110 INPUT "Are you ready for the next set (y or n)"; nx$	NEXT j
IF nx$ = "y" THEN 120 ELSE IF nx$ = "n" THEN 1120 ELSE 110	110 INPUT "Are you ready for the next set (y or n)"; nx$
120 CLS	IF nx$ = "y" THEN 120 ELSE
RANDOMIZE	
CLS	
PRINT "100 Trials for 90% Target Consumers of C3 Carbon, 10% C4"	
PRINT	
FOR j = 1 TO 100	
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:	

Table 2 (Continued)

```
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 90
200 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 200
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
NEXT k
FOR k = 1 TO 10
210 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 210
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 90
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 10
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
PRINT
230 INPUT "Are you ready for the next set
  (y or n)"; nx$
IF nx$ = "y" THEN 240 ELSE IF nx$ = "n"
  THEN 1120 ELSE 230
240 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 80% Target
  Consumers of C3 Carbon, 20% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 80
  IF nx$ = "n" THEN 1120
  ELSE 110
120 CLS

RANDOMIZE
CLS
PRINT "100 Trials for
  Consumers of Mixed 90% C3
  Carbon, 10% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
200 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 200
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
ind(k) = .9 * xc3(k) + .1
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
230 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 240 ELSE
  IF nx$ = "n" THEN 1120
  ELSE 230
240 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 80%
  Target Consumers of C3
  Carbon, 20% C4"
PRINT
FOR j = 1 TO 100
```

Table 2 (Continued)

```
300 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 300
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
NEXT k
FOR k = 1 TO 20
310 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 310
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 80
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 20
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
PRINT
330 INPUT "Are you ready for the next set
  (y or n)"; nx$
IF nx$ = "y" THEN 340 ELSE IF nx$ = "n"
  THEN 1120 ELSE 330
340 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 70% Target
  Consumers of C3 Carbon, 30% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 70
400 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 400
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
```

```
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
300 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 300
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
ind(k) = .8 * xc3(k) + .2
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
330 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 340 ELSE
  IF nx$ = "n" THEN 1120
  ELSE 330
340 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 70%
  Target Consumers of C3
  Carbon, 30% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
400 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 400
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
```

Table 2 (Continued)

```
NEXT k
FOR k = 1 TO 30
410 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 410
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 70
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 30
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
PRINT
430 INPUT "Are you ready for the next set
  (y or n)"; nx$
IF nx$ = "y" THEN 440 ELSE IF nx$ = "n"
  THEN 1120 ELSE 430
440 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 60% Target
  Consumers of C3 Carbon, 40% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 60
500 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 500
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
NEXT k
FOR k = 1 TO 40
510 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 510
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B -
  15.27
ind(k) = .7 * xc3(k) + .3
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
430 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 440 ELSE
  IF nx$ = "n" THEN 1120
  ELSE 430
440 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 60%
  Target Consumers of C3
  Carbon, 40% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
500 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 500
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
ind(k) = .6 * xc3(k) + .4
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
```

Table 2 (Continued)

```
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 60
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 40
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
PRINT
530 INPUT "Are you ready for the next set
  (y or n)"; nx$
IF nx$ = "y" THEN 540 ELSE IF nx$ = "n"
  THEN 1120 ELSE 530
540 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 50% Target
  Consumers of C3 Carbon, 50% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 50
600 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 600
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
NEXT k
FOR k = 1 TO 50
610 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 610
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 50
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
530 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 540 ELSE
  IF nx$ = "n" THEN 1120
  ELSE 530
540 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 50%
  Target Consumers of C3
  Carbon, 50% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
600 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 600
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
ind(k) = .5 * xc3(k) + .5
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
630 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 640 ELSE
  IF nx$ = "n" THEN 1120
  ELSE 630
```

Table 2 (Continued)

```
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 50
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
PRINT
630 INPUT "Are you ready for the next set
  (y or n)"; nx$
IF nx$ = "y" THEN 640 ELSE IF nx$ = "n"
  THEN 1120 ELSE 630
640 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 40% Target
  Consumers of C3 Carbon, 60% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 40
700 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 700
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
NEXT k
FOR k = 1 TO 60
710 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 710
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 40
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 60
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
640 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 40%
  Target Consumers of C3
  Carbon, 60% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
700 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 700
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
ind(k) = .4 * xc3(k) + .6
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
730 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 740 ELSE
  IF nx$ = "n" THEN 1120
  ELSE 730
740 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 30%
  Target Consumers of C3
  Carbon, 70% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
```

Table 2 (Continued)

```
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
730 INPUT "Are you ready for the next set
  (y or n)"; nx$
IF nx$ = "y" THEN 740 ELSE IF nx$ = "n"
  THEN 1120 ELSE 730
740 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 30% Target
  Consumers of C3 Carbon, 70% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 30
800 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 800
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
NEXT k
FOR k = 1 TO 70
810 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 810
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 30
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 70
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
830 INPUT "Are you ready for the next set
  (y or n)"; nx$
IF nx$ = "y" THEN 840 ELSE IF nx$ = "n"
  THEN 1120 ELSE 830
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
800 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 800
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
ind(k) = .3 * xc3(k) + .7
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
830 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 840 ELSE
  IF nx$ = "n" THEN 1120
  ELSE 830
840 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 20%
  Target Consumers of C3
  Carbon, 80% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
900 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 900
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
```

Table 2 (Continued)

```
840 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 20% Target
  Consumers of C3 Carbon, 80% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 20
900 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 900
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
NEXT k
FOR k = 1 TO 80
910 v1 = 2 * (RND * 2) - 1: v2 = 2 * (RND
  * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 910
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 20
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 80
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
930 INPUT "Are you ready for the next set
  (y or n)"; nx$
IF nx$ = "y" THEN 940 ELSE IF nx$ = "n"
  THEN 1120 ELSE 930
940 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 10% Target
  Consumers of C3 Carbon, 90% C4"
PRINT
ind(k) = .2 * xc3(k) + .8
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
930 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 940 ELSE
  IF nx$ = "n" THEN 1120
  ELSE 930
940 CLS

RANDOMIZE
CLS
PRINT "100 Trials for 10%
  Target Consumers of C3
  Carbon, 90% C4"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
1000 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 1000
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
ind(k) = .1 * xc3(k) + .9
  * xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)totdiff2 =
  diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
```

Table 2 (Continued)

```
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc3(n) = 0: xc4(n) = 0
NEXT n
FOR k = 1 TO 10
1000 v1 = 2 * (RND * 2) - 1: v2 = 2 *
  (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 1000
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8013
sumc3 = sumc3 + xc3(k)
NEXT k
FOR k = 1 TO 90
1010 v1 = 2 * (RND * 2) - 1: v2 = 2 *
  (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 1010
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = (sumc3 + sumc4) / 100
FOR n = 1 TO 10
diff2 = (xc3(n) - mean) * (xc3(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
FOR n = 1 TO 90
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
1030 INPUT "Are you ready for the next
  set (y or n)"; nx$
IF nx$ = "y" THEN 1040 ELSE IF nx$ = "n"
  THEN 1120 ELSE 1030
1040 CLS

CLS
RANDOMIZE
CLS
PRINT "100 Trials for 100% Target
  Consumers of C4 Carbon"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y = 0: sumc3 = 0:
  sumc4 = 0: mean = 0: diff2 = 0:
  totdiff2 = 0: stdev = 0
FOR n = 1 TO 100
xc4(n) = 0: xc4(n) = 0
PRINT mean; stdev,
NEXT j
1030 INPUT "Are you ready
  for the next set (y or
  n)"; nx$
IF nx$ = "y" THEN 1040
  ELSE IF nx$ = "n" THEN
  1120 ELSE 1030
1040 CLS

CLS
RANDOMIZE
CLS
PRINT "100 Trials for 100%
  Target Consumers of C4
  Carbon"
PRINT
FOR j = 1 TO 100
v1 = 0: v2 = 0: w = 0: y =
  0: indsum = 0: mean = 0:
  diff2 = 0: totdiff2 = 0:
  stdev = 0
FOR k = 1 TO 100
1100 v1 = 2 * (RND * 2) -
  1: v2 = 2 * (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF
  w > 1 THEN 1100
y = SQR((-2 * LOG(w)) / w)
xc3(k) = v1 * y * A - 26.8
xc4(k) = v2 * y * B -
  15.27
ind(k) = 0 * xc3(k) + 1 *
  xc4(k)
indsum = indsum + ind(k)
NEXT k
mean = indsum / 100
FOR n = 1 TO 100
diff2 = (ind(n) - mean) *
  (ind(n) - mean)
totdiff2 = diff2 +
  totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
1110 INPUT "Do you want to
  start the calculator over
  (y or n)"; nx$
IF nx$ = "y" THEN 1 ELSE
  1120
1120 PRINT "Thank you and
  good-bye!"
1130 END
```

Table 2 (Continued)

```
NEXT n
FOR k = 1 TO 100
1100 v1 = 2 * (RND * 2) - 1: v2 = 2 *
  (RND * 2) - 1
w = v1 * v1 + v2 * v2: IF w > 1 THEN 1100
y = SQR((-2 * LOG(w)) / w)
xc4(k) = v2 * y * B - 15.3452
sumc4 = sumc4 + xc4(k)
NEXT k
mean = sumc4 / 100
FOR n = 1 TO 100
diff2 = (xc4(n) - mean) * (xc4(n) - mean)
totdiff2 = diff2 + totdiff2
NEXT n
stdev = SQR(totdiff2 / 99)
PRINT mean; stdev,
NEXT j
1110 INPUT "Do you want to start the
  calculator over (y or n)"; nx$
IF nx$ = "y" THEN 1 ELSE 1120
1120 PRINT "Thank you and good-bye!"
1130 END
```

Figure 1. Modified from O'Leary (1988). Histogram showing difference in both means and variances in C_3 and C_4 plant groups. Squares are means ± 1.96 SD. Boxplots show median, 25th and 75th percentiles (box limits), 5th and 95th percentiles (whiskers), and outliers (X's).

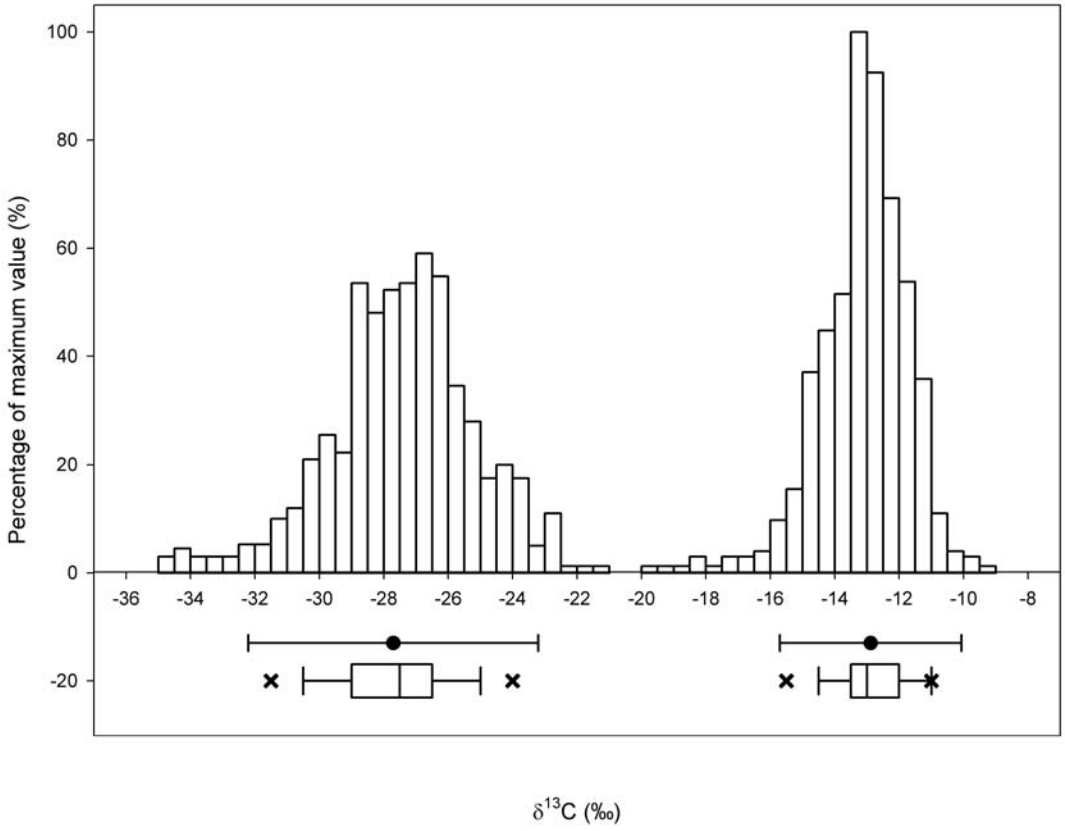


Figure 2. Map of the Jornada Experimental Range and Long Term Ecological Research (LTER) Site (inset) and the Chihuahuan Desert (shaded region) within the context of the United States of America and the United States of Mexico. Chihuahuan Desert Boundary based on an aridity index modified from Schmidt (1979) by Nolen and Monger (unpublished data). The Chihuahuan Desert boundary found within the Jornada LTER boundary is elevation based. The San Andres Mountain Range along the eastern boundary includes land that doesn't classify as Chihuahuan Desert based on this index. Data for this study was collected entirely within the Chihuahuan Desert area of the Jornada LTER.

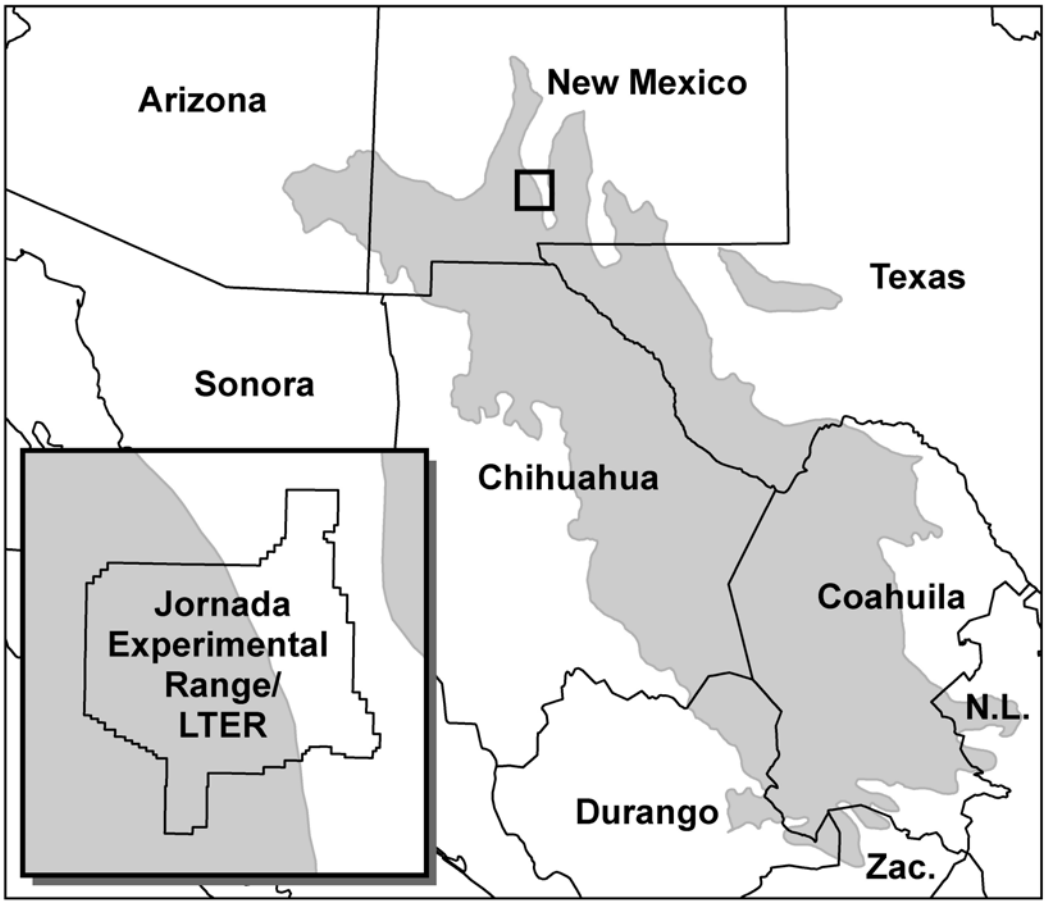


Figure 3. $\delta^{13}C$ means vs. standard deviations for Chihuahuan Desert plants (shaded ovals show 95% confidence space), grasshoppers (squares; white square=*T. pallidipennis*, black square=*M. occidentalis,* gray square=*O. obscura*) , and black-throated sparrows (triangles; black triangle=2004, white triangle=2005). Lower x-axis error bars show range in which isosource values may occur accounting for 0-1.1‰ per trophic level enrichment. Letters in parentheses show post hoc groupings of x (Tukey's HSD) and y axis (Levene's test of variance in all combinations) values in the form (x group, y group).

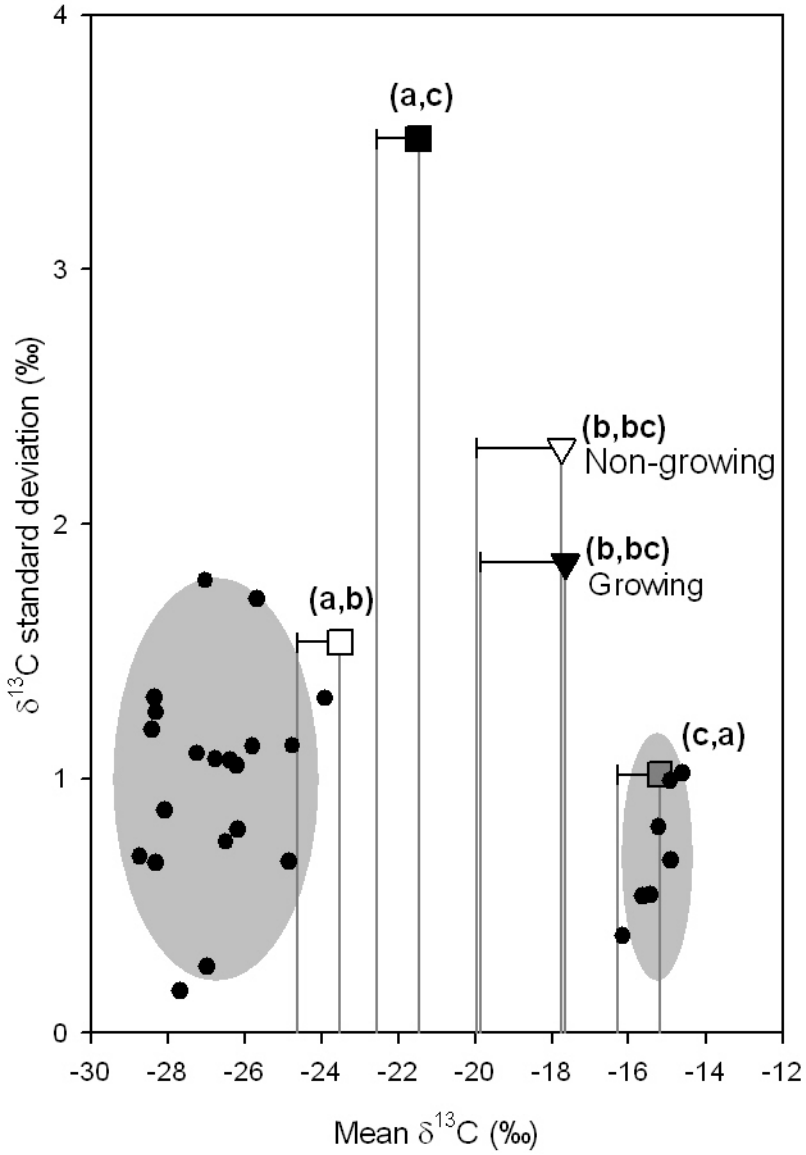


Figure 4. Simulated data sets. Graphs of means ± 1.96 SD are graphed at 10% intervals for the target diet populations (gray circles) and mixed diet populations (light red circles) with equal SDs of ±1‰ (a) and ±5‰ (b), and unequal SDs of ±5‰C_3/±1‰C_4 (c) and ±9‰C_3/±5‰C_4 (d). Differential shading of confidence ovals show degree of overlap between distributions. For simplification, no trophic level fractionation factor was used.

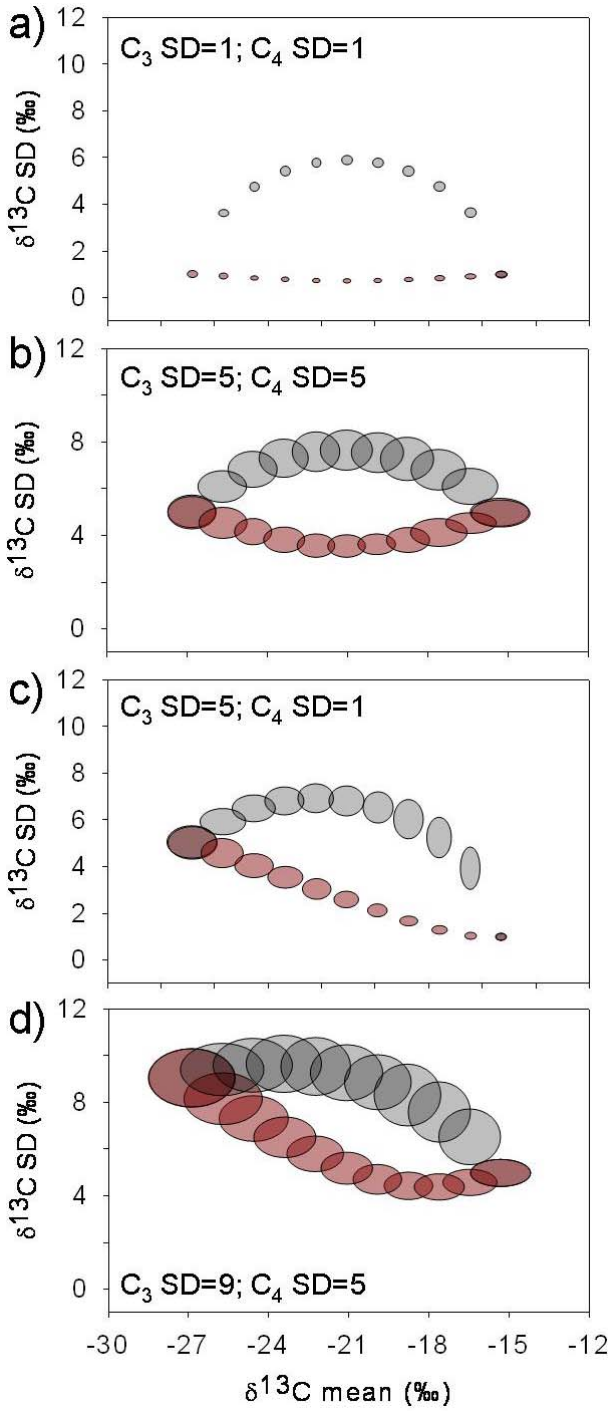


Figure 5. Simulated datasets derived using means and SDs from Chihuahuan desert plant means and SDs (C3=26.8±1.00‰ and C_4=-15.27±0.71‰) compared to the actual data from the three grasshopper species collected in this study: *T. pallidipennis* (white square), *O. obscura* (gray square), and *M. occidentalis* (white square).

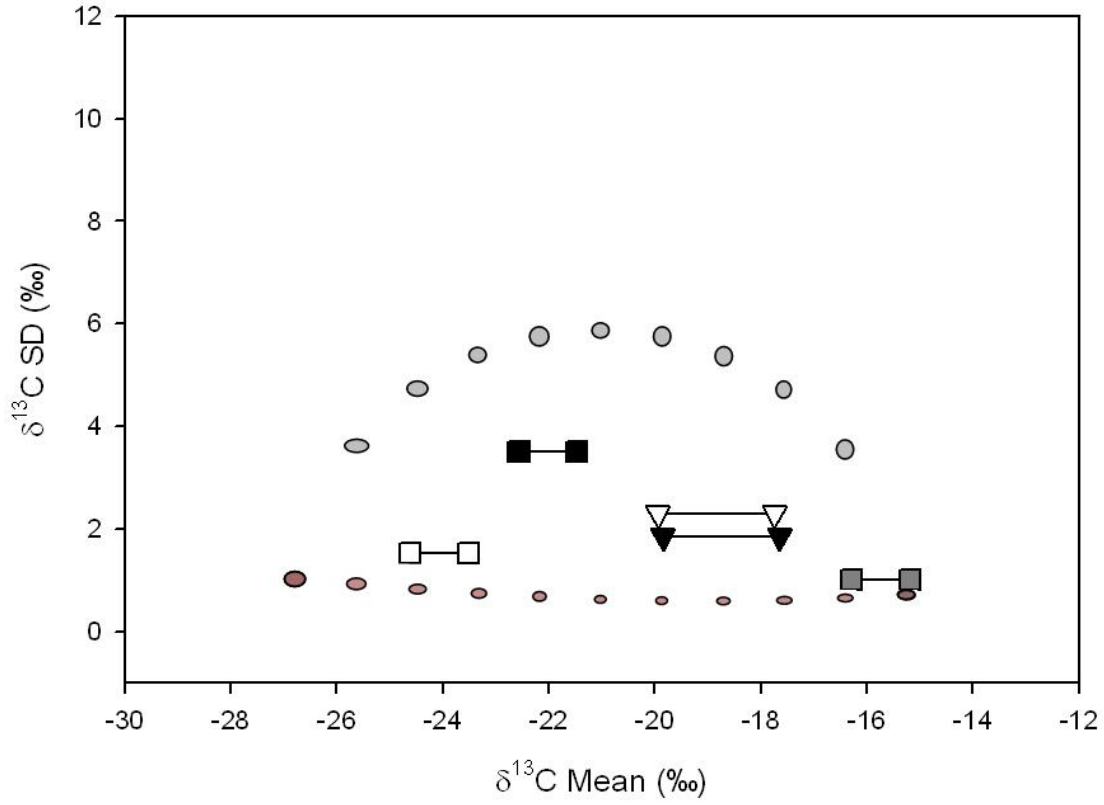
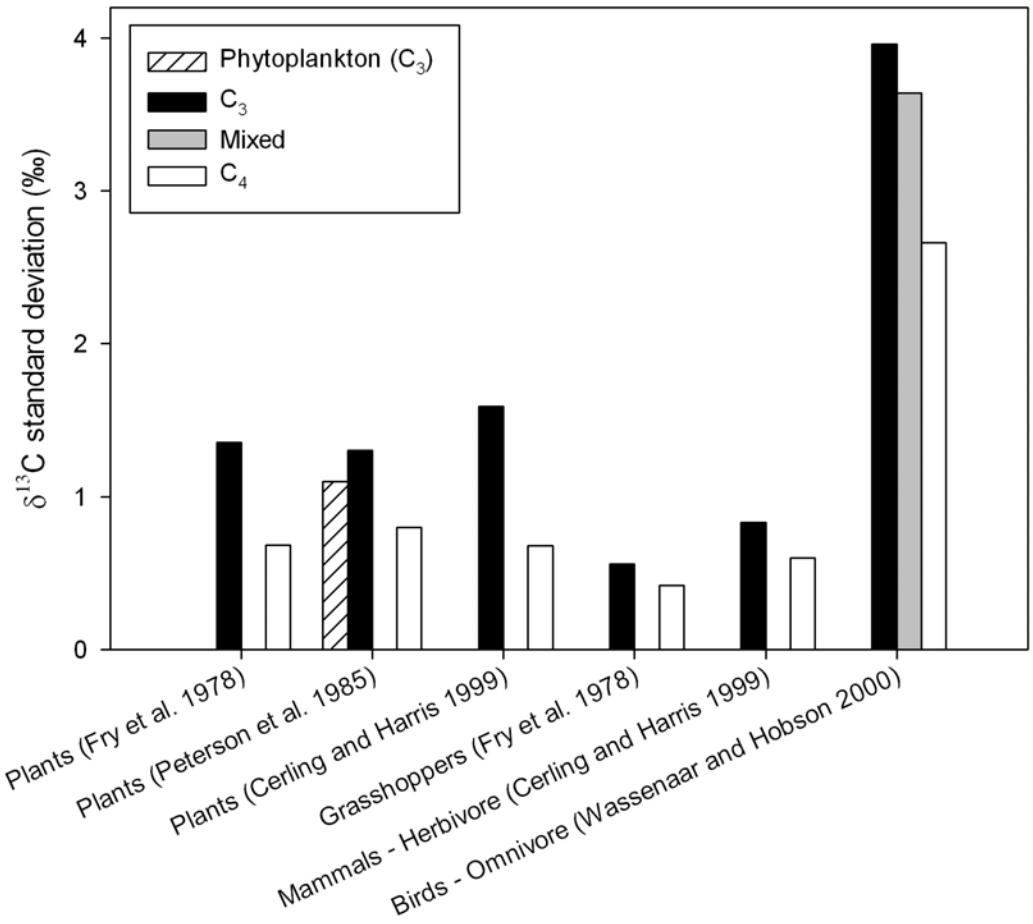


Figure 6. Data from other published sources showing C_3/C_4 variance dichotomy. Shows either SD directly from publications or, if multiple populations were sampled, pooled SD.



CHAPTER 3

STABLE ISOTOPES REVEAL THAT THE SHRUB OBLIGATE BLACK-THROATED SPARROW IS A GRASSLAND SPECIALIST IN THE SHRUB-ENCROACHED NORTHERN CHIHUAHUAN DESERT, NEW MEXICO

Abstract

To understand how consumers use their environments for different purposes, we can analyze consumer resource use relative to resource availability. In the northern Chihuahuan Desert of New Mexico, I used C_3 and C_4 plants as stable carbon isotopic sources ("isosources") to compare isosource proportions of areas inhabited by black-throated sparrows and grasshoppers to consumption of these isosources. Using biomass and stable carbon isotope ratios ($\delta^{13}C$) of plants I calculated mass-balanced $\delta^{13}C$ values for consumer habitats, which ranged from areas of nearly 100% C_3 shrubs to areas of nearly 100% C_4 grasses. I then compared these values to $\delta^{13}C$ values from black-throated sparrow feathers and blood from multiple years and $\delta^{13}C$ values from wing tissue from three species of grasshoppers. I found that each species of grasshopper employed a different consumption pattern. The grasshopper *Trimerotropis pallidipennis* consumed primarily C_3 carbon regardless of the carbon isosource composition of the area they inhabited. The grasshopper *Opeia obscura* consumed primarily C_4 carbon regardless of the carbon isosource composition of the area they inhabited. The grasshopper *Melanoplus occidentalis* consumed carbon

proportional to isosource availability. Black-throated sparrows showed patterns similar to *T. pallidipennis*, indicating high consumption of C_4 carbon in C_3 shrubland, C_4 grassland and mixed areas. This study not only shows that different species can use the same areas in different ways, but also reinforces the notion that black-throated sparrows, historically considered a species highly associated with shrubs, are dietarily tied to C_4 grasses in the Chihuahuan Desert.

Introduction

Stable isotope analysis has become a popular ecological tool because of its ability to reveal patterns in nature hidden to older methods. If resources have distinct stable isotope ratios, stable isotope analysis can quantify the proportions of these resources assimilated by consumers. Another area of inquiry is how these proportions in individual heterotrophs relate to the proportion of isotopic sources ("isosources") available in the environment. If isosources have distinct isotope ratios and a consumer uses these sources at random, the stable isotope ratio of an individual should correspond to that of the area-of-use regardless of proportions of isosources available. In contrast, differences between the isotope ratios of consumers and those expected based on the proportion of food items in the environment are evidence for selective foraging.

My objective in this study is to describe the availability of isosources across a series of vegetation ecotones and to compare the availability of these isosources to the isotope ratios found in a variety of heterotrophs. For this type of study, the northern Chihuahuan Desert of southern New Mexico is an ideal model system. Much of this area was historically desert C_4 grasslands interspersed with C_3 shrubs, but as a result

of primarily historical overgrazing, shifts in shrub seed dispersal, periodic drought, and, secondarily, fire suppression (Buffington and Herbel 1965, Brown and Archer 1989, Grover and Musick 1990), C_3 shrubs have become dominant in plant communities. The patchy nature of this transition resulted in a series of ecotones from grassland to shrubland that drives an isotopic gradient derived from the $\delta^{13}C$ differences between C_4 grasses and C_3 shrubs (Smith and Epstein 1971). I measured the physical and isotopic composition of animal habitats along this gradient and used these measurements to determine how consumers used isosources relative to their availability.

Two types of information are needed to understand how animals respond to this habitat gradient. First, on an axis from 100% of one isosource to 100% of the second isosource, where are consumers found? In the Chihuahuan Desert, do consumers rely on 100% C_3 shrubs, 100% C_4 grasses, or a mix of shrubs and grasses? This isotopic distribution information can establish whether or not consumers target a particular habitat type or range of habitat types. Second, relative to what is available where consumers found, what portion of the habitat is consumed? Do they consume resources at random, or do they target a particular source regardless of availability? These two measurements are metrics of consumer habitat use: what they choose to inhabit and what they choose to consume, which can give investigators clues to how species respond to habitat variation. Further, variation in these two metrics among species can show how coexisting consumers inhabiting areas of similar plant structure vary in dietary isosource uses. In the Chihuahuan Desert system, does a species live in all types of structural habitats, but only consume C_4 food sources, live in shrublands

and only eat C_3 food sources, or does a species live everywhere and eat everything? Further, are different consumers consuming similar or different isosources in similar habitats? Answering these questions can reveal how consumers can target habitat for both its structural and dietary components.

In the Chihuahuan Desert, I examined a relatively simple trophic chain as a model to investigate these questions. Black-throated sparrows (*Amphispiza bilineata*; BTSPs) live in semi-arid ecosystems of the southwest United States of America and northwest Mexico. According to decades of observations, BTSPs strongly associate with shrubs in these systems (Pidgeon 2001, Johnson et al. 2002). BTSPs nest primarily in shrubs, so any habitat they occupy during the breeding season typically needs at least one shrub suitable for nesting. During the breeding season, BTSPs eat mainly insects, most notably grasshoppers, by foraging in and around shrubs (Zimmer 1983, 1993, Johnson et al. 2002). Foraging behaviors, together with a higher nest density in shrublands than in grasslands, suggest that BTSPs target use of shrubby desert habitats. However, BTSP nest success can be dramatically lower in shrublands than in grasslands, so much so that shrublands can at times become a reproductive sink (Pidgeon et al. 2003). I analyzed the structural composition of BTSP areas-of-use to see where in the scheme of a grassland/shrubland habitat gradient BTSPs occur. Then, relative to BTSP areas-of-use, I examined BTSP stable carbon isotope ratios ($\delta^{13}C$) to see how assimilated stable carbon isotope ratios compare to the proportion of C_3/C_4 carbon available to individuals. Further, because BTSPs consume primarily grasshoppers during the breeding season, I analyzed commonly found grasshoppers in a similar manner to identify how grasshoppers use their respective habitat, and if this

affected the dietary carbon available to secondary consumers. Identifying the proportion of BTSP habitat used for foraging and if and how this proportion changed from grassland to shrubland will support or refute previous observations of BTSP foraging and inform understanding of BTSPs status in the changing Chihuahuan Desert environment.

Methods

Site description and Black-throated Sparrows

I performed this study at the Jornada Experimental Range and Long Term Ecological Research Site (Jornada LTER; +32.5º N, -106.8º W, elevation 1188 m; Figure 1) in the northernmost part of the Chihuahuan Desert (Wainright 2006) in 2004 and 2005. In 2004, I caught birds on four sites previously established for grassland/ecotone/shrubland (Bestelmeyer et al. 2007). Each site was organized to include subplots considered grassland, ecotone, and shrubland for 12 total subplots, four each in grassland, ecotone (i.e., mixed grass and shrub), and shrubland. I caught BTSPs using mistnets, banded all birds, collected bird measurements similar to those required by the MAPS bird banding program (DeSante and Saracco 2007), collected outer tail feathers, noted if these were currently growing or not (only growing feathers were used for $\delta^{13}C$ analysis), and collected blood samples. Feathers were stored in envelopes and blood was frozen in heparin-free capillary tubes. In 2005, I used 15 new sites identified by locating either adult and hatch-year (HY) BTSPs or adults and a nest during the breeding season, May-August. I identified 15 locations, with care to inspect areas of use that spanned the gradient of habitats on Jornada LTER from nearly 100% grass to nearly 100% shrub (no 100% grass or 100% shrub landscapes

exist at Jornada LTER). If a nest was identified, the nest location was used as the center of the site. If I saw fledged young, I flushed birds to determine coarse boundaries of their areas of use (Wiens 1969), then estimated a center for this area. I mistnetted birds as close as possible to the site's center to maximize the probability of capturing only one family group. I collected BTSP data as I did in 2004. I prepared feathers for stable isotope analysis by washing with detergent and 2:1 chloroform:methanol solvent (Paritte and Kelly 2009) and placing 300-400μg of the feather's distal end (rachis and barbs) into tin capsules. A subset of blood samples were randomly selected for analysis. Blood was vacuum sealed in glass tubes.

Primary consumers

I used sweep nets at random locations to collect arthropods on all 15 sites in 2005. I collected numerous species, but only analyzed 10-15 individuals of the three grasshopper species (Orthoptera: Acrididae) found at most sites. *Trimerotropis pallidipennis* is the most abundant grasshopper in New Mexico and is found on forbs and grasses (Richman et al. 1993). *Opeia obscura* is found exclusively on grasses (Richman et al. 1993). *Melanoplus occidentalis* uses varying plant hosts (Richman et al. 1993). All three species are common in western North American grasslands. Fry *et al.* (1978) indicated high C_3 use for *T. pallidipennis* (64% C_3 in diet; $\delta^{13}C$ = -21.0‰) and almost exclusive C_4 use for *O. obscura* (99% C_4 in diet; $\delta^{13}C$ = -13.2‰). Fry *et al.*(1978) did not analyze *M. occidentalis*; however, based on Richman et al. (1993), I predicted that this species would have $\delta^{13}C$ intermediate between the C_3 and C_4 plants. For stable isotope analysis, 300-450 μg of a grasshopper wing was clipped,

cleaned in detergent and 2:1 chloroform:methanol solvent (Paritte and Kelly 2009), and packed into tin capsules.

Plant measurements

In 2005 I measured plants from May-August in four concentric circles of areas 10 m^2, 100 m^2, 1000 m^2, and 10,000 m^2 around the center of each of the 15 sites (Figure 2). Within each of these areas, I sampled plants using a 1 m^2 circular frame placed randomly to determine if there were any scale-dependent patterns in vegetation use. I measured three-1m^2 plots in the 10m^2 area and 9-1m^2 plots in 100 m^2, 1000 m^2, and 10000 m^2. Within each 1 m^2 plot, cover was estimated for live and dead C_3 and C_4 plants at 97 points. All biomass was clipped to within 2cm of the ground. Plants were placed in paper bags for storage and transport until drying. Plants were dried for 48h in drying ovens at 55C. The plants from each plot were separated into C_3 and C_4, and then were separated into live and dead biomass. For woody biomass, if a stem seemed mostly alive, the entire stem was counted as live biomass. Only live biomass was used for analysis in this study. Live biomass was identified to species in order to confirm C_3 or C_4 classification. A total of 29 species or species groups were sampled. Russian thistle, *Salsola tragus*, was omitted from measurements; this is not thought to significantly alter results because the species was only found in 16 of 450 plots in low biomass, and its biology as an invasive wind-disperser suggests it is of low value to consumers. Each plant species was sampled for $\delta^{13}C$ analysis by placing 300-400μg of leaf tissue (or green stem in a leafless species, such as *Ephedra*) into tin capsules.

Stable isotope analysis

Grasshopper and BTSP stable isotope samples were run for $\delta^{13}C$ on either a Thermo Finnigan Delta Plus XL Isotope Ratio Mass Spectrometer, interfaced to a Carlo Erba Elemental Analyzer through an open split valve (ConFlo II) at the Stable Isotope Laboratory of the Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, New Mexico, or on a Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer interfaced through an open split valve (ConFlo III) with a Costech Elemental Analyzer at the laboratory of Michael H. Engel, School of Geology and Geophysics, University of Oklahoma, Norman, Oklahoma. All values are reported in per mil notation relative to the isotope standard Vienna Pee Dee Belemnite (vPDB). Blood samples were air dried and vacuum sealed in glass tubes, then combusted to CO_2 at 550C. The tubes were then cracked under cryogenic conditions and the CO_2 was isolated offline for analysis. Samples were run for $\delta^{13}C$ on a Delta E Isotope Ratio Mass Spectrometer.

Statistical Analysis

Biomass and cover. I performed all statistical tests using SPSS (2004). For the 10,000m^2 scale, I used biomass totals and mean $\delta^{13}C$ values for plant species to calculate mass-balanced stable isotope ratios for the 14 sites using the equation $\delta^{13}C_{site} = \sum_{i=1}^{n}\left(m_i \delta^{13}C_i \Big/ \sum_{i=1}^{n} m_i \right)$, where n is the number of plant species at each site, m_i is the biomass of each species at each site, and $\delta^{13}C_i$ is the mean $\delta^{13}C$ of each plant species. I used the resulting site $\delta^{13}C$ values to classify sites as shrubland, mixed/ecotone, or grassland. I removed one site from analysis because I only caught

one bird on it and the stable carbon isotope ratio of -20.29‰ did not clearly classify into one of the three categories. Initially, I quantified 13 plant variables. Due to the high incidence of zero data for C_3 and C_4 biomass and cover, I analyzed the frequency of presence of functional type first, then removed plots with zero data to analyze biomass and cover. I defined zero biomass as ≤1g biomass per plot and zero cover as ≤1 frame hit per plot. I then $\log_{10}(x+1)$ normalized all variables and created a correlation matrix using Spearman's rho (2004) to determine colinearity. If any variable pair showed $\rho>0.80$, I chose only one to analyze, such that the resulting suite of variables was biologically relevant (Table 1). I analyzed presence/absence data using Chi Square. I used MANOVA to analyze the effect of habitat type (shrub/ecotone/grassland) and scale on the remaining 8 variables. I used Tukey's HSD *post hoc* test to identify significant differences and calculated Hedges and Olkin's (1985) unbiased effect sizes ($d_{unbiased}$) between shrub and grass habitats.

Stable carbon isotopes. To test assumptions, I used Kolmogorov-Smirnov Z to test for normality and Levene's test for homogeneity of variance. If either of these assumptions is violated in particular cases, I present nonparametric test results. For the 2004 data, I used a one-way ANOVA with Tukey's HSD *post hoc* to determine if $\delta^{13}C$ values of BTSPs differed between habitats. For 2005 data, I used a two-way ANOVA to determine the main effects and interactions of both habitat and prey species on consumer $\delta^{13}C$. I used a one-way ANOVA with Tukey's HSD *post hoc* to compare consumer $\delta^{13}C$ to the $\delta^{13}C$ of the consumer's habitat type. If either normality or variance homogeneity assumption was violated, I replaced ANOVA with Kruskal Wallis H-test and either Tamhane's T2 (as a conservative estimate) or Games-Howell

(as a more liberal estimate) to identify *post hoc* differences. If both Tamhane's and Games-Howell indicate significant differences, I reported Tamhane's p-values. If they differ in results, I reported p-values from both tests. Because feather carbon can be considered metabolically derived from carbon in blood, I used least-squares regression to determine the relationship between feather and blood $\delta^{13}C$ values, with blood $\delta^{13}C$ as the predictor for feather $\delta^{13}C$.

Results

Plants

Mass-balanced stable carbon isotope ratios for 14 of the 15 sites sampled in 2005 fell cleanly into one of three isotopic groups: shrublands (n=4) with $\delta^{13}C$ =-27.03 to -25.48‰, mixed/ecotones (n=5) with $\delta^{13}C$ =-23.16 to -21.49‰, and grasslands (n=5) with $\delta^{13}C$ =-18.97 to -17.49‰. I used correlation matrix values to eliminate variables that were highly correlated (rho>0.80 or rho<-0.80). I condensed 13 variables to the following eight: live C_3, live C_4, and total live biomass and total C_3, total C_4, total live, total dead, and total cover.

Chi square indicated no consistent pattern among spatial scales in either presence of vegetation or cover type. As expected, presence of both C_4 (*i.e.*, grass) live biomass and C_4 live cover was more common in grasslands than in shrublands at all scales (C_4 live biomass— X^2_2=72.76, p<0.001; C_4 live cover—X^2_2=81.94, p<0.001); however, the opposite trend for presence of C_3 biomass and cover was only seen at the 100m^2 scale for C_3 biomass (X^2_2=9.21, p<0.01).

Similar to the presence data, quantified cover and biomass also showed no scale effect. Total cover was greater in grasslands than shrublands (F_2=4.02, p=0.02;

Figure 3); the trend was similar, but less dramatic, in the total live biomass (F_2=3.11, p=0.05). C_4 biomass was greater in grasslands than in shrublands (F_2=3.06, p=0.05); however, C_3 biomass was not significantly greater in shrublands than in grasslands (F_2=2.97, p=0.06), despite a large effect size. Similarly, greater C_4 cover (F_2=4.45, p=0.01) drove greater total cover measure in grasslands inhabited by BTSPs. Unbiased effect sizes confirmed that C_4 plants drive increasing biomass and cover.

Consumers

In 2004, HY BTSP feather $\delta^{13}C$ values were normally distributed with homogenous variance. Feather $\delta^{13}C$ did not differ across habitat types (Figure 4a), all with intermediate to slightly C_4 tending $\delta^{13}C$ signatures.

In 2005, both BTSP feather and blood $\delta^{13}C$ and grasshopper wing $\delta^{13}C$ were normally distributed both overall and when examined by species. $\delta^{13}C$ for all tissues combined, adult BTSP $\delta^{13}C$, BTSP blood $\delta^{13}C$, and *T. pallidipennis* $\delta^{13}C$ had homogenous variance. HY BTSP $\delta^{13}C$ (p=0.05), *O. obscura* $\delta^{13}C$ (p=0.01), and *M. occidentalis* $\delta^{13}C$ (p=0.02) did not. There were both species ($F_{5,124}$=32.48, p<0.001, Figure 5a) and habitat ($F_{2,127}$=8.01, p=0.001 Figure 5b) main effects. Habitat patterns also varied by species ($F_{9,120}$=2.18, p=0.03).

Hatch-year BTSPs had lower feather $\delta^{13}C$ values in shrublands than those in ecotones or grasslands (X^2_2=9.97, p=0.007; Figure 4b). Adult BTSPs (Figure 4c), *T. pallidipennis* (Figure 4d), *O. obscura* (Figure 4e), and *M. occidentalis* (Figure 4f) $\delta^{13}C$ did not vary between habitat types.

Relative to the calculated plant $\delta^{13}C$ (*i.e.,* source carbon $\delta^{13}C$) at an individual's capture site, each species shows a particular pattern (Figure 4, Table 2).

Both adult and HY BTSP feathers indicated primarily C_4/grass-based $\delta^{13}C$ values regardless of habitat. Hatch-year BTSPs had $\delta^{13}C$ values that were higher than expected from random habitat foraging for shrublands and mixed/ecotones, but not for grasslands. Adult BTSPs had higher than expected $\delta^{13}C$ from random habitat foraging for ecotones, but not for grasslands. Only one bird was measured in a shrubland area, but the value was higher than expected for random grassland foraging ($\delta^{13}C$=-19.05‰).

Adult and HY BTSPs had similar blood $\delta^{13}C$ values (t_{23}=-0.1, p=0.92) and blood $\delta^{13}C$ was highly correlated with feather $\delta^{13}C$ (Figure 6a; r^2=0.86, p<0.001). On average, the best fit for feather $\delta^{13}C$ was 1.54‰ higher than blood $\delta^{13}C$ values. When compared between habitat types, blood $\delta^{13}C$ shows a predominantly C_4 pattern, similar to feather $\delta^{13}C$ (Figure 6b). Blood $\delta^{13}C$ was higher in grasslands than mixed/ecotones with intermediate values in shrublands. Blood $\delta^{13}C$ values were greater than expected from random habitat foraging In both shrublands and mixed ecotones, but not in grasslands

Each grasshopper species showed a different trend when examined relative to habitat type. *T. pallidipennis* maintained a mixed, but primarily C_3 $\delta^{13}C$ signature regardless of habitat. *T. pallidipennis* $\delta^{13}C$ was similar to the shrubland and ecotone site $\delta^{13}C$ but lighter than grassland $\delta^{13}C$ values. In contrast, *O. obscura* showed a highly C_4 signature regardless of habitat. *O. obscura* $\delta^{13}C$ is marginally heavier than shrubland $\delta^{13}C$, is definitively heavier than ecotone $\delta^{13}C$, but also marginally heavier than grassland $\delta^{13}C$, suggesting that even though "grassland" sites are not 100% grass, *O. obscura* may consume closer to 100% grass than the actual habitat composition.

The non-significant shrubland result based on Tamahane's statistic is undoubtedly related to limited sample size for that category (n=4). I caught both *T. pallidipennis and O. obscura* on all 14 sites. *M. occidentalis* showed a highly variable C_4-based $\delta^{13}C$ signature in grasslands, but a more C_3 signature in mixed/ecotones and shrublands. *M. occidentalis* $\delta^{13}C$ may be slightly heavier than habitat in shrublands, but was similar to respective habitat in mixed/ecotones and grasslands. This species was found on 9 of 14 sites, but in all 3 habitat classifications.

Discussion

Black-throated sparrow habitats show a decrease in both total plant cover and biomass from grassland to shrubland, primarily driven by decreases in C_4 plants. These BTSP habitat patterns reflect recent thinking about Chihuahuan Desert shrub encroachment dynamics. Historic changes have produced large areas dominated with C_3 shrubs at the expense of C_4 grasses. Some highly resilient areas of C_4 dominance remain, with many habitats that are mixed (Grover and Musick 1990).

This study is a prime example of how stable isotope analysis can highlight the manner in which resources are used by consumers. BTSPs may use C_3 components of habitat for their structure, but they rely on C_4 habitat components for diet. Despite wide ranging composition in BTSP habitat, BTSPs maintain primarily C_4/grass-based $\delta^{13}C$ values in body tissue. Even in areas where C_3 shrubs dominate, BTSPs rely on C_4 grass productivity for food. BTSPs reliably use C_3 shrubs (with four-winged saltbush, *Atriplex canescens,* being one notable exception) for nesting, perching, shade, and territorial singing and calling (Johnson et al. 2002), which make shrubs an important structural part of a BTSP habitat. However, because BTSPs are often seen

in shrubs, BTSPs have been categorized as shrub specialists, or at the very least generalists (Delesantro 1978, Zimmer 1983, Pidgeon 2001, Johnson et al. 2002). Heavy reliance upon grass-based food webs, even in shrub dense areas, suggests that BTSPs are, in fact, dietarily tied to grasslands. Similar methods to those used here could be useful for understanding how consumers target or do not target particular isosources relative to their availability. Further research on BTSPs and other species will help confirm or redefine how species make use of their habitats for food and structure.

If BTSPs are more tied to desert grasslands than originally thought, are BTSPs falling victim to changing desert habitats? Over the past 25 years, the North American Breeding Bird Survey (BBS) documented decreased sightings of BTSPs on routes close to the Jornada LTER, which is representative of the overall trend in decreased BTSP sightings in the Chihuahuan Desert Ecoregion (Figure 7; Sauer et al. 2008). This is consistent with the large scale decrease in grass in the Chihuahuan Desert, but currently no data indicate that these decreases occurred during the period of the BBS record. Further research is needed to determine if decreased BTSP sightings is directly related to grass loss. Still, conservation of remaining intact grasslands and attempts to increase grass cover in shrub-dominated areas (Peters et al. 2006) are important conservation initiatives to ensure security of grass-based trophic systems in the Chihuahuan Desert.

Acknowledgements

Thanks to Jeff Kelly, Michael H. Engel, Viorel Atudorei and Zachary Sharp for isotope analyses and to Brandon Bestelmeyer, M.H. Engel, Michael Kaspari,

Michael Patten, and Lauren Niemann for review. J.M. Paritte was supported by an NSF Graduate Research Fellowship during part of this study. Funding was provided in part by the University of Oklahoma's George Miksch Sutton Scholarship in Ornithology, M. Blanche Adams Scholarships, and college and departmental grants. This work was submitted in partial fulfillment of J.M. Paritte's Ph.D. degree at the University of Oklahoma and the experiments herein comply with the all appropriate U.S. regulations.

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Table 1. Spearman's correlation matrix for 13 variables measured for plant plots. Data include rho, p-value, and sample size, n. If two variables had rho>0.80 or <-0.80, one variable could be eliminated from analysis.

		***C_3 Live Biomass**	***C_4 Live Biomass**	***Total Live Biomass**	*C_3 Live Cover	*C_4 Live Cover*	*C_3 Dead Cover	*C_4 Dead Cover	***Total C_3 Cover**	***Total C_4 Cover**	***Total Live Cover**	***Total Dead Cover**	*Bare Ground
***Total Cover**	rho	-0.03	0.24	0.11	0.36	0.71	0.34	0.63	0.39	0.74	0.88	0.71	-1.00
	p	0.59	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	N	308	287	426	296	238	230	233	404	306	380	319	444
*Bare Ground	rho	0.04	-0.26	-0.10	-0.35	-0.72	-0.34	-0.63	-0.39	-0.75	-0.88	-0.71	
	p	0.43	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	N	310	289	429	295	237	229	233	403	305	379	318	
***Total Dead Cover**	rho	-0.08	0.19	0.05	-0.14	0.63	0.47	0.92	0.01	0.77	0.50		
	p	0.23	0.00	0.36	0.04	0.00	0.00	0.00	0.93	0.00	0.00		
	N	217	221	307	235	228	230	234	286	245	315		
***Total Live Cover**	rho	0.01	0.29	0.17	0.50	0.76	0.14	0.48	0.40	0.68			
	p	0.85	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00			
	N	265	234	362	296	239	227	232	346	254			

(Table 1 continued)

		***C_3 Live Biomass**	***C_4 Live Biomass**	***Total Live Biomass**	*C_3 Live Cover	*C_4 Live Cover	*C_3 Dead Cover	*C_4 Dead Cover	***Total C_3 Cover**	***Total C_4 Cover**	***Total Live Cover**	***Total Dead Cover**	*Bare Ground
***Total C_4 Cover**	rho	-0.06	0.26	0.08	-0.28	0.94	0.10	0.84	-0.19				
	p	0.40	0.00	0.20	0.00	0.00	0.22	0.00	0.00				
	N	188	256	294	169	239	155	234	266				
***Total C_3 Cover**	rho	0.12	-0.09	0.03	0.87	-0.15	0.58	-0.18					
	p	0.04	0.17	0.50	0.00	0.04	0.00	0.01					
	N	287	256	388	297	204	230	200					
*C_4 Dead Cover	rho	-0.02	0.14	0.04	-0.26	0.66	0.07						
	p	0.86	0.05	0.54	0.00	0.00	0.38						
	N	139	200	223	152	217	144						
*C_3 Dead Cover	rho	0.02	0.06	0.01	0.18	0.07							
	p	0.76	0.45	0.91	0.01	0.42							
	N	160	146	221	183	144							

(Table 1 continued)

		***C_3 Live Biomass**	***C_4 Live Biomass**	***Total Live Biomass**	*C_3 Live Cover	*C_4 Live Cover	*C_3 Dead Cover	*C_4 Dead Cover	***Total C_3 Cover**	***Total C_4 Cover**	***Total Live Cover**	***Total Dead Cover**	*Bare Ground
*C_4 Live Cover	rho	-0.04	0.21	0.08	-0.23								
	p	0.64	0.00	0.24	0.00								
	N	145	203	228	155								
*C_3 Live Cover	rho	0.10	-0.01	0.09									
	p	0.12	0.93	0.12									
	N	227	163	283									
***Total Live Biomass**	rho	0.78	0.62										
	p	0.00	0.00										
	N	309	290										
***C_4 Live Biomass**	rho	0.20											
	p	0.00											
	N	196											

Note: Variables in bold were retained for data analysis. Spearman's rho values >0.80 or <-0.80 are underlined.
* Data $\log_{10}(x+1)$ transformed

Table 2. P-values for post hoc tests for consumer $\delta^{13}C$ values compared to habitat types.

Species		Shrubland	Ecotone	Grassland
HY BTSP[b]	FC	<0.001	0.002	0.95
	w/o FC	<0.001	<0.001	>0.99
Adult BTSP[a]	FC	NA	0.002	0.99
	w/o FC	NA	<0.001	0.78
All BTSP Blood[a]	FC	<0.001	<0.001	0.72
	w/o FC	<0.001	<0.001	>0.99
T. pallidipennis[a]	FC	>0.99	>0.99	<0.001
	w/o FC	0.93	>0.99	<0.002
O. obscura[b]	FC	0.32	0.002	0.24
	w/o FC	0.23	<0.001	0.02
O. obscura[c]	FC	0.02	<0.001	0.03
	w/o FC	0.02	<0.001	0.003
M. occidentalis[b]	FC	0.7	>0.99	>0.99
	w/o FC	0.24	>0.99	>0.99
M. occidentalis[c]	FC	0.07	0.67	0.99
	w/o FC	0.02	>0.99	>0.99

Note: "FC" refers to tests between observed consumer d13C values and expected (*i.e.*, site) $\delta^{13}C$ values taking into consideration possible $\delta^{13}C$ fractionation of 1.1 per trophic level "w/o FC" refers to tests between observed values and expected values without any trophic fractionation consideration. NA indicates insufficient sample site to calculate statistics.
[a]Tukey's *post hoc*
[b]Tamhane's *post hoc*
[c]Games-Howell *post hoc*

Figure 1. The Chihuahuan Desert (gray shaded area) occurs in the southwestern United States and north Central Mexico. The Jornada Experimental Range and Long-Term Ecological Research Site (inset) is in the northern Chihuahuan Desert of Las Cruces, New Mexico. The Chihuahuan Desert boundary is based upon an aridity index modified from Schmidt (Schmidt 1979) by Nolen and Monger (unpublished data). Within the Jornada LTER, the western and central portions are considered Chihuahuan Desert, but due to an elevation increase in the San Andres Mountain Range, the eastern portion does not classify as Chihuahuan Desert based on this index. Data for this study was collected within the Chihuahuan Desert portion of the Jornada LTER.

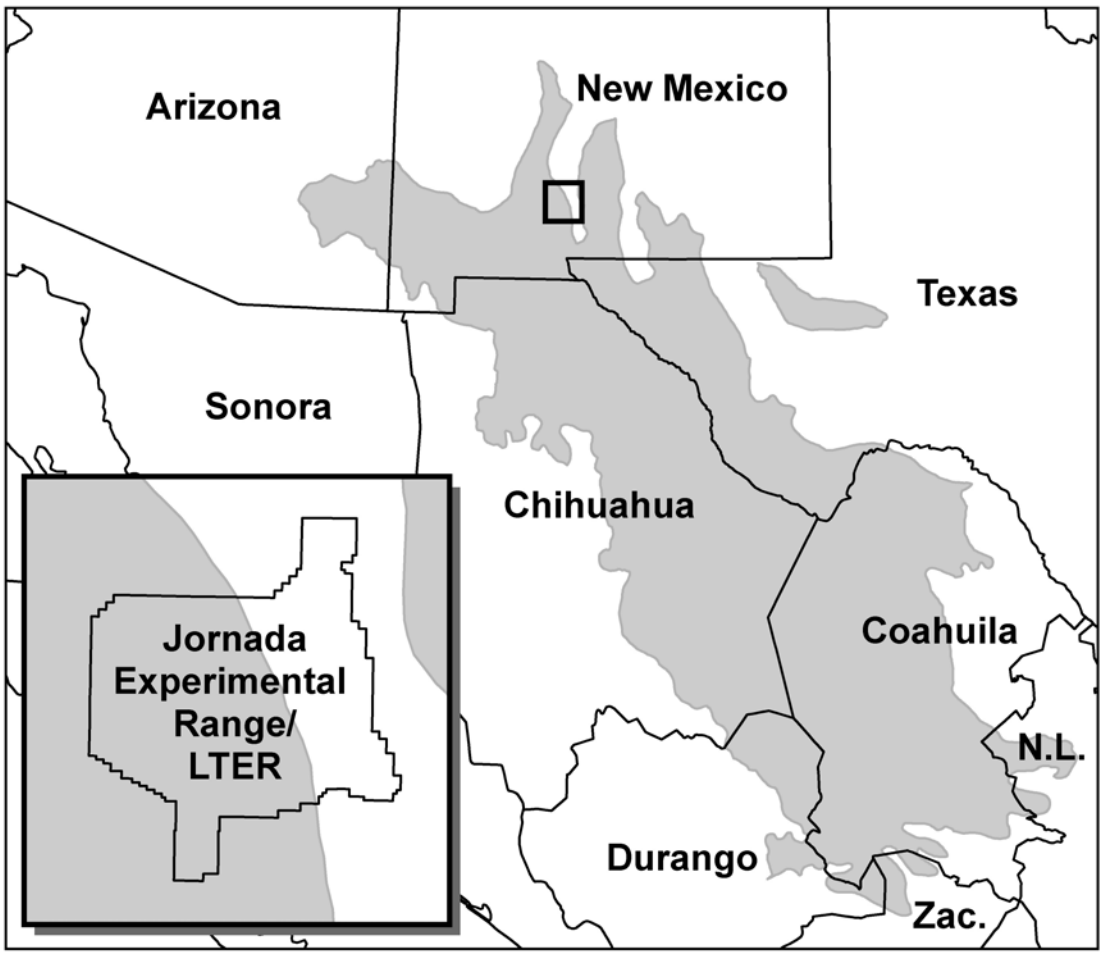


Figure 2. Schematic of BTSP use area measurement. Center point of measurement area was located at the determined central area of use or a nest, if found. Four measurement circles (concentric open circles) were delineated—10 m^2, 100 m^2, 1000 m^2, and 10,000 m^2. Small dark circles represent sample 1m^2 plots where plants were measured—3 in the 10 m^2, and 9 each in 100 m^2, 1000 m^2, and 10,000 m^2. Position of dark circles was determined randomly. Each 1m^2 plot contained 97 points on a grid where plant cover measurements were taken (enlarged circle, lower right).

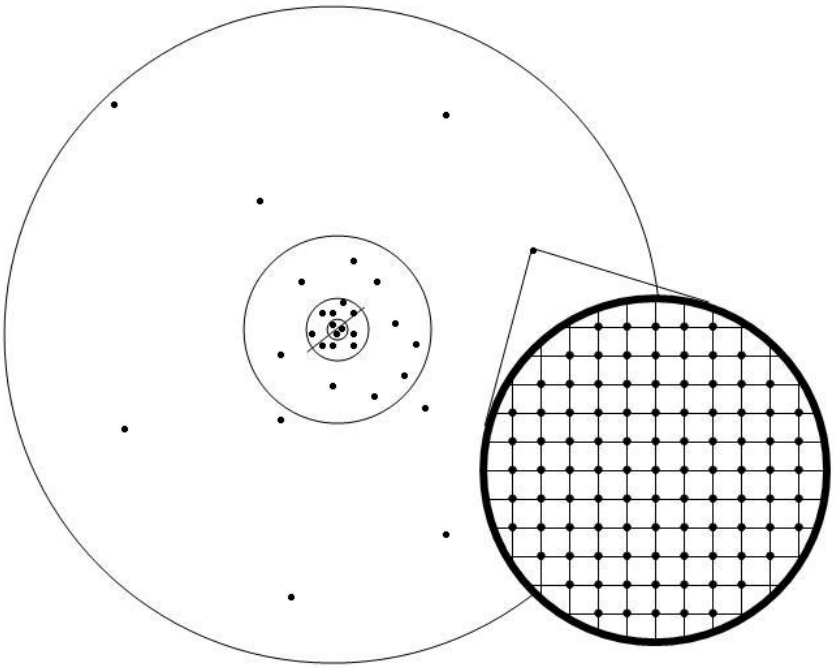


Figure 3. Means±1SE for biomass and cover measures in shrublands, mixed/ecotone areas, and grassland BTSP areas of use. Letters show Tukey's HSD *post hoc* differences. Numbers above bars indicate $d_{unbiased}$ effect sizes between shrublands and grasslands for the variables measured. Values for $d_{unbiased}$ that are greater than 0.8 represent large effect sizes (Hedges and Olkin 1985).

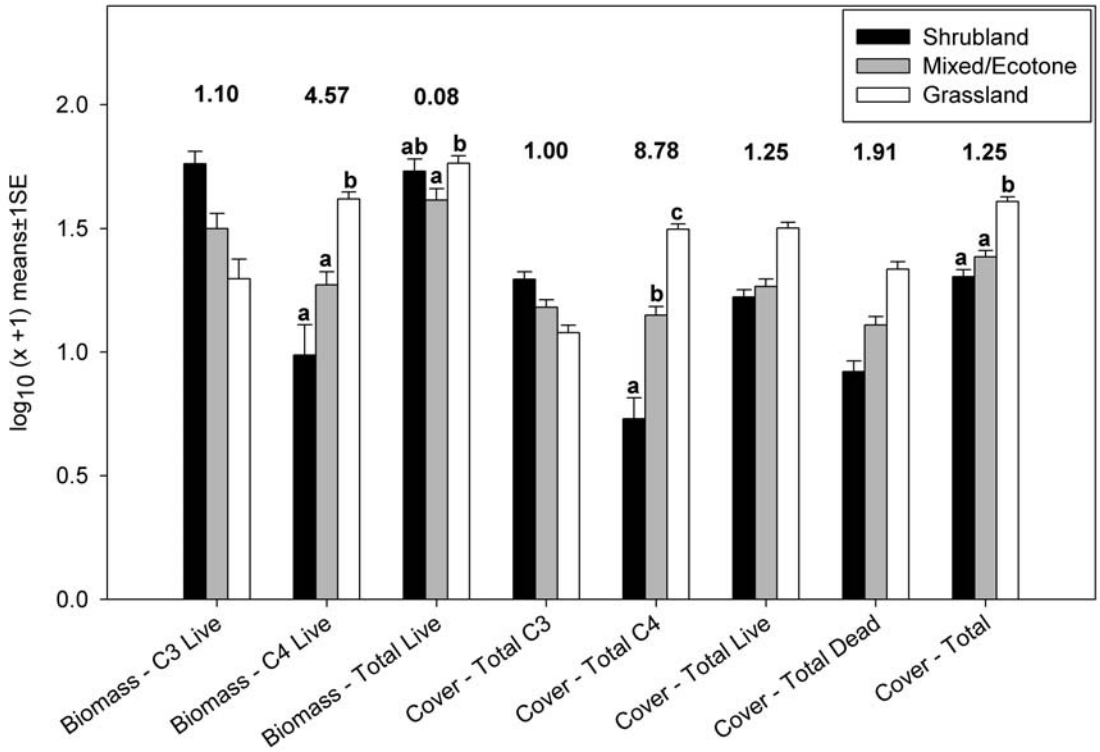


Figure 4. $\delta^{13}C$ values for 2004 HY BTSP feathers (a), 2005 HY BTSP feathers (b), 2005 adult BTSP feathers (c), *T. pallidipennis* (d), *O. obscura* (e), and *M. occidentalis* (f) compared to the $\delta^{13}C$ expected for individuals if they foraged randomly in their respective areas of use. Gray shaded areas are $\delta^{13}C$ ranges that consumers should have if tissue $\delta^{13}C$ matches use area $\delta^{13}C$. Lower bounds of dark gray area = mean plant $\delta^{13}C$ in each area of use category and upper bounds = mean plant $\delta^{13}C$ + the appropriate diet-tissue fractionation factor (1.1‰ for *T. pallidipennis*, *O. obscura*, and *M. occidentalis* and 2.2‰ for all BTSPs). Light gray areas = mean plant $\delta^{13}C$-1SD and mean plant $\delta^{13}C$ + fractionation+1SD. Letters are Tukey's HSD *post hoc* groups. Stars below gray shaded areas show significant differences from mean plant $\delta^{13}C$. Stars above gray shaded areas show significant differences from mean plant $\delta^{13}C$ + fractionation. One star (*) = Tukey's HSD, two stars (**) = Tamhane's, and three stars (***) = Games-Howell *post hoc* tests.

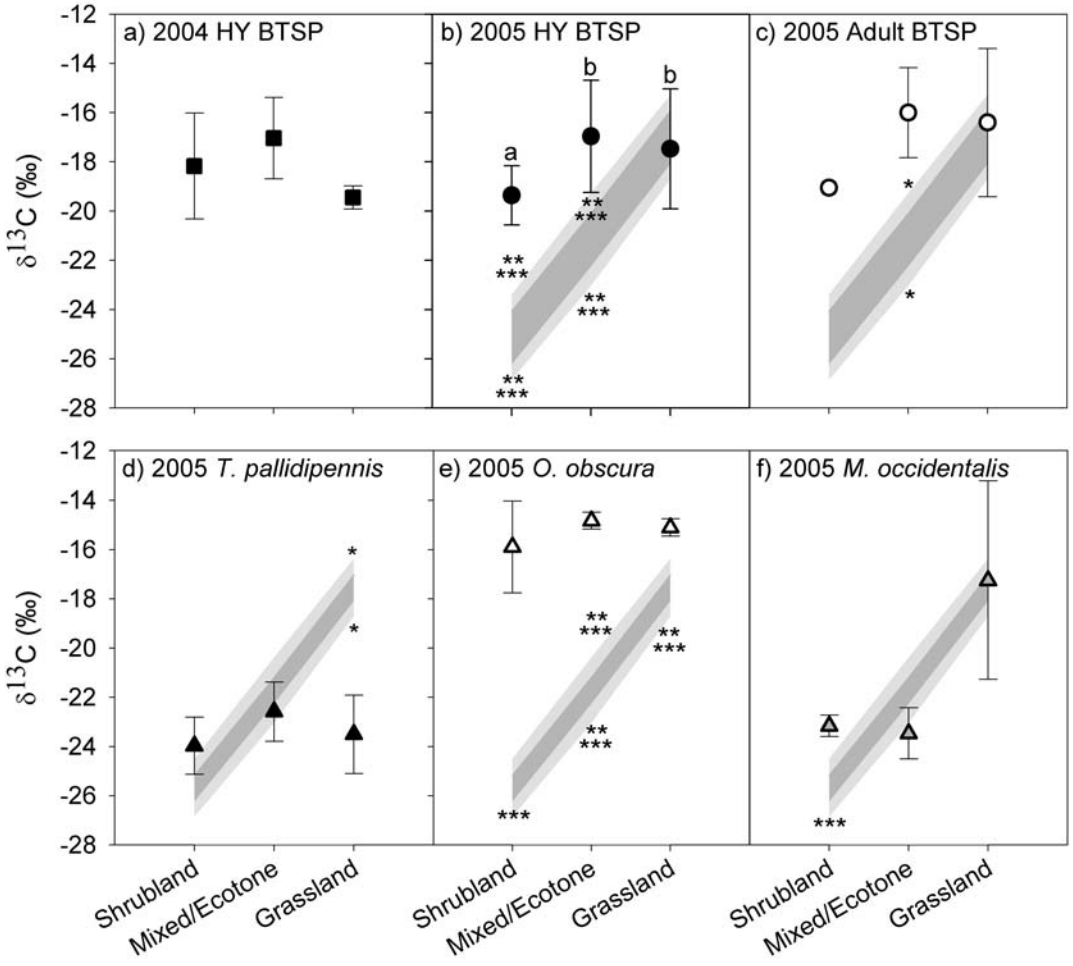


Figure 5. Boxplots for each species (a) and habitat type (b) $\delta^{13}C$. Center lines represent medians, boxes indicate 25%-75% range, whiskers represent 10%-90% range, and points indicate outliers. The species *M. occidentalis* does not show outliers due to sample size constraints (n=9). Letters indicate Tukey's HSD *post hoc* groupings.

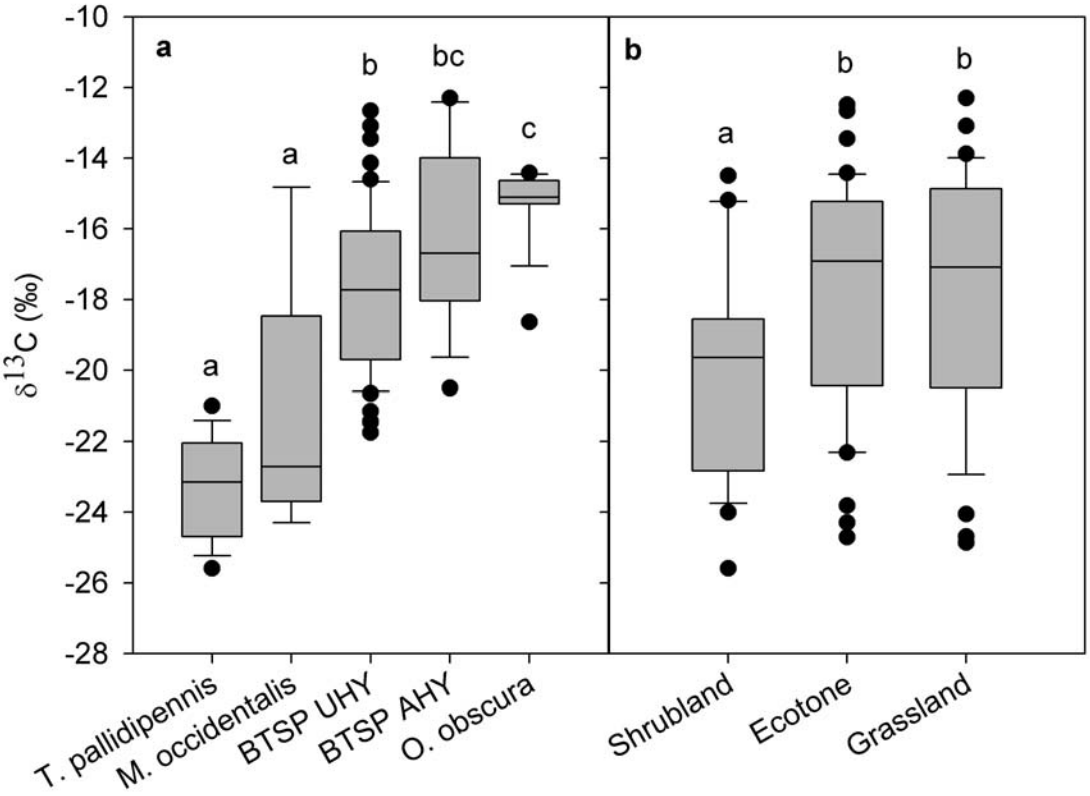


Figure 6. Regression relationship between blood $\delta^{13}C$ and feather $\delta^{13}C$ (a) and blood $\delta^{13}C$ compared to $\delta^{13}C$ values expected for individuals if they foraged randomly in their respective areas of use. Closed circles (●) are data from HY birds. Open circles (○) are data from adult birds. There was no significant difference between HY and adult blood $\delta^{13}C$. Gray shaded areas in (b) are $\delta^{13}C$ ranges that consumers should have if tissue $\delta^{13}C$ matches use area $\delta^{13}C$. Lower bounds of dark gray area equal mean plant $\delta^{13}C$ in each area of use category. Upper bounds were mean plant $\delta^{13}C$ plus a corrected fractionation factor of 2.2‰ minus the average deviation of the best fit regression line from the 1:1 line (+1.54). Stars (*) indicate significant differences between gray shaded areas and BTSP $\delta^{13}C$ values based upon Tukey's HSD *post hoc*.

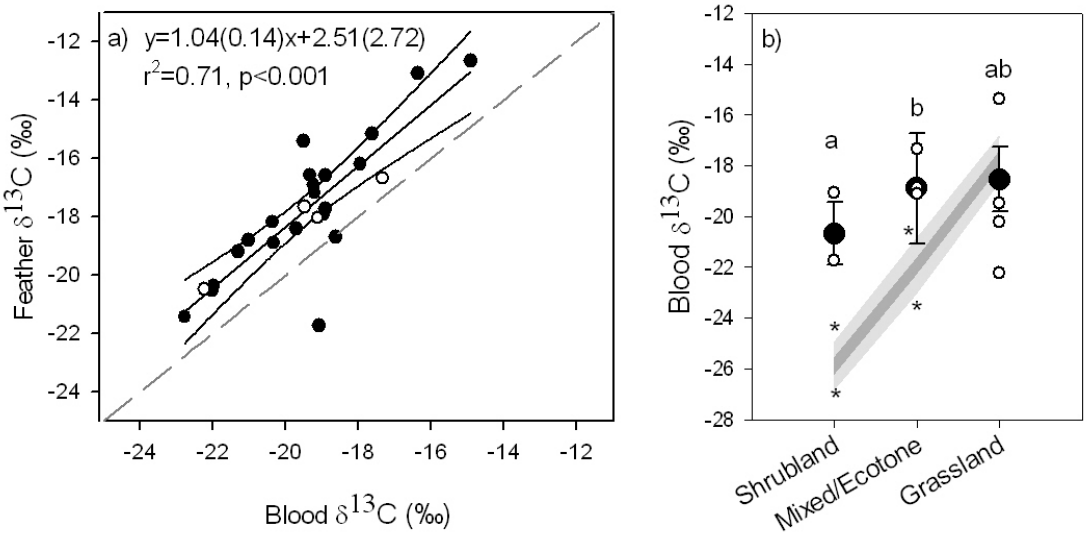
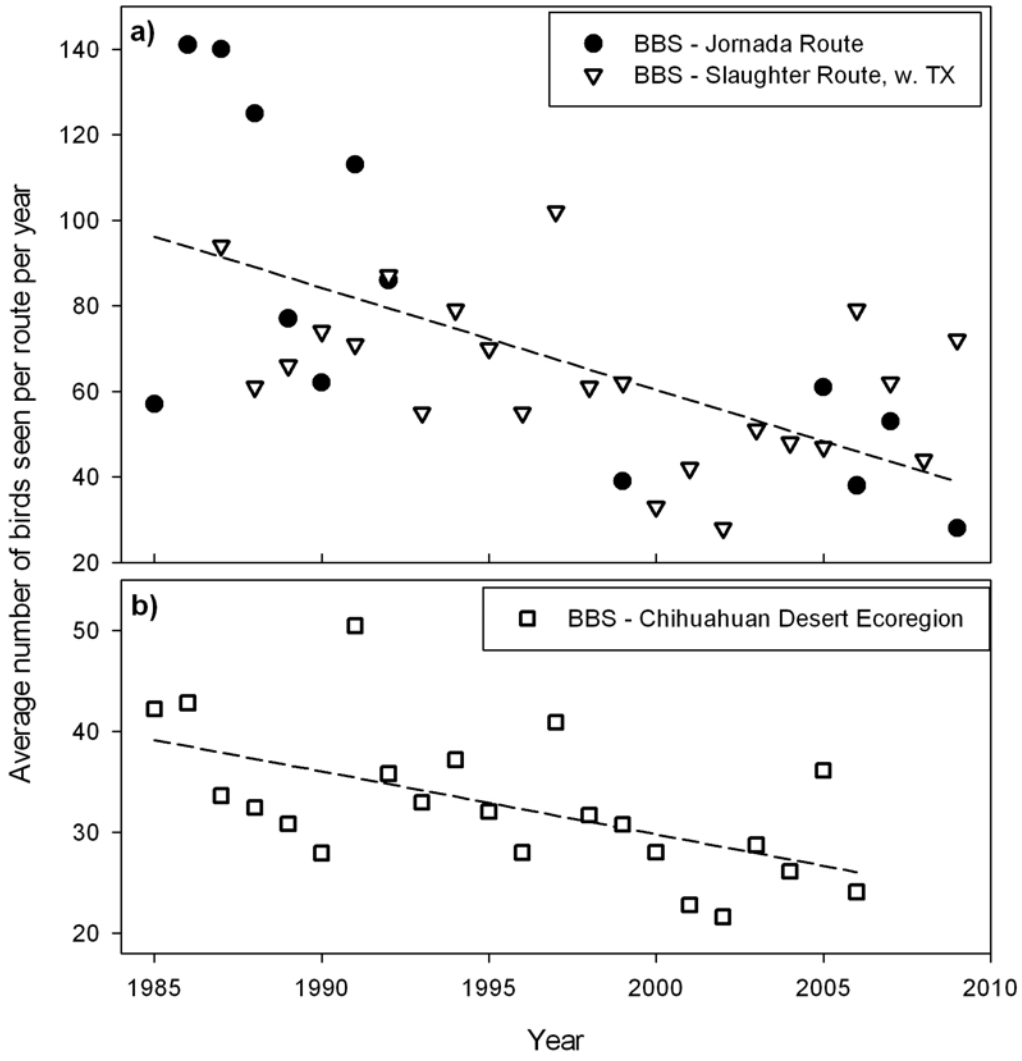


Figure 7. North American Breeding Bird Survey (BBS) data for a) the two closest routes to my study location, "Jornada," located on the Jornada LTER (dark circles), and "Slaughter," located near El Paso in west Texas (open triangles) and b) average data for all routes in the Chihuahuan Desert ecoregion (open squares) as defined by BBS. Dashed best-fit lines further underscore decreasing bird sightings.



CHAPTER 4

DIFFERENTIAL STABLE NITROGEN ISOTOPE ENRICHMENT IN BLACK-THROATED SPARROWS ACROSS AN ECOTONE IN THE NORTHERN CHIHUAHUAN DESERT, NEW MEXICO

Abstract

Stable nitrogen isotopes ($\delta^{15}N$) are commonly used to identify trophic status of consumers, but fractionation associated with nitrogen cycling and metabolism can confound data interpretation. In a Chihuahuan Desert system, I used $\delta^{13}C$ and $\delta^{15}N$ values of plants, grasshoppers, and black-throated sparrows (*Amphispiza bilineata*) to investigate whether $\delta^{15}N$ enrichment was coupled with habitat or diet of consumers. Grasshopper $\delta^{15}N$ values were 2.75‰ greater than those of plants and hatch-year black-throated sparrows $\delta^{15}N$ values were 2.36‰ greater than those of grasshoppers; both of these values are lower than the +3.4‰ per trophic level value that has been commonly reported. Adult black-throated sparrows had $\delta^{15}N$ values that were 3.37‰ heavier than those of grasshoppers. There was no significant linear relationship between the average $\delta^{13}C$ of plants at a site and the $\delta^{15}N$ values of either plants or consumers sampled at those sites; however, all consumers and plants showed significant relationships between $\delta^{15}N$ and $\delta^{13}C$ values of individuals, which indicate diet in consumers and photosynthetic type in plants. There was a negative trend between $\delta^{15}N$ values and $\delta^{13}C$ values in plants because C_3 plants had slightly higher

$\delta^{15}N$ than C_4 plants. This pattern was paralleled in grasshoppers. This relationship between $\delta^{15}N$ and $\delta^{13}C$ values became more pronounced in hatch-year black-throated sparrows; those with more C_3-based diets were slightly more enriched in $\delta^{15}N$ than grasshoppers with equivalent $\delta^{13}C$ values. Adult black-throated sparrows had the most pronounced relationship between $\delta^{15}N$ and $\delta^{13}C$ values; birds with mixed C_3/C_4 diets were on average 5.5‰ heavier than birds with mostly C_4 diets. This pattern is most likely attributable to adults with mixed diets either consuming at a higher trophic level or being exposed to increased nutritional stress. Further research is needed to distinguish these possibilities.

Introduction

Stable isotopes are useful for clarifying ecological processes. Stable nitrogen isotopes ($\delta^{15}N$) have commonly been used to identify trophic status in food webs (DeNiro and Epstein 1981), with the primary assumption that there is a constant increase in $\delta^{15}N$ of ~3.4‰ (per mil) per trophic level (Kelly 2000, Post 2002). Because nitrogen pathways are dependent on numerous ecological components, different environments with different nutrient, food, and water availabilities may diverge from this value, such that either individual food web components or entire food webs may deviate from predicted $\delta^{15}N$ values (Hobson and Clark 1992, Hobson et al. 1993).

In this study, I use the semi-arid grasslands of the northern Chihuahuan Desert as a model system to explore whether consumer species exhibit different $\delta^{15}N$ patterns across an ecological gradient of changing plant composition. The northern Chihuahuan Desert was historically C_4 grassland, but due to historical overgrazing,

shifts in shrub seed dispersal, periodic drought, and fire suppression (Buffington and Herbel 1965, Brown and Archer 1989, Grover and Musick 1990), C_3 shrubs have come to dominate much of the landscape. These phenomena have created a habitat composition gradient from grassland to shrubland, which drives a stable carbon isotope ($\delta^{13}C$) gradient derived from the $\delta^{13}C$ differences between C_4 grasses and C_3 shrubs (Smith and Epstein 1971). I use this $\delta^{13}C$ gradient to examine trophic shifts in consumers with habitat structure.

I focused on three trophic levels: desert plants as primary producers, grasshoppers as primary consumers, and black-throated sparrows (BTSPs) as secondary consumers. Grasshoppers are known to be the primary food source for BTSPs, which inhabit grassland, shrubland, and mixed environments while breeding (Zimmer 1983, 1993). Grasshoppers specialize on various plants; for this study I used three representative species that live in all three environments. *Trimerotropis pallidipennis* specializes upon shrubs and forbs. *Opeia obscura* specializes upon grasses. *Melanoplus occidentalis* varies its host plants depending upon availability in the environment (Richman et al. 1993). Personal observations confirm that these species are consumed by BTSPs, but BTSP diet is not limited to these species. Still, with this simple three-level system, I can examine trophic shifts using $\delta^{15}N$ values across a gradient of habitat structure.

Methods

Secondary consumers

In 2005, I collected feather samples from black-throated sparrows at the Jornada Experimental Range and Long Term Ecological Research Site (JER; +32.5º

N, -106.8º W, elevation 1188 m) in the northernmost part of the Chihuahuan Desert (Wainwright 2006) in 2005. Feathers were stored in envelopes. I identified 15 sites by locating either adult and hatch-year (HY) BTSPs or adults and a nest in a location during the breeding season, May-August, with care to inspect areas of use that spanned the gradient of habitats on JER from nearly 100% grass to nearly 100% shrub (no 100% grass or 100% shrub landscapes exist at JER). If a nest was identified, the nest location was used as the center point of the site. If fledged HY young were seen, I flushed birds to determine coarse boundaries of their areas of use, then estimated a center point for this area (Wiens 1969). I mistnetted birds as close as possible to the site's center point to maximize the probability of capturing only one family group. I caught BTSPs using mistnets, collected outer tail feathers, and noted if these were currently growing or not (only growing feathers were used for $\delta^{15}N$ analysis). I prepared feathers for stable isotope analysis by washing with detergent and 2:1 chloroform:methanol solvent (Paritte and Kelly 2009) and placing 300-400µg of the feather's distal end (rachis and barbs) into tin capsules.

Primary consumers

I used sweep nets at random locations to collect grasshoppers on all 15 2005 sites. For stable isotope analysis, 300-450µg of grasshopper wing was clipped, cleaned in detergent and 2:1 chloroform:methanol solvent (Paritte and Kelly 2009), and packed in tin capsules.

Plants

I clipped biomass to within 2 cm of the ground at 30 1 m^2 plots within a 1ha circle around the center of each of the 15 sites. Plants were placed in paper bags for

storage and dried for 48h in drying ovens at 55C. Live biomass was separated into C_3 and C_4 photosynthetic groups and was identified to species to confirm C_3 or C_4 classification. For woody biomass, if a stem seemed mostly alive, the entire stem was counted as live biomass. Twenty-nine species or species groups were sampled. *Salsola tragus* (Russian thistle/tumbleweed) was omitted from measurements; this is not thought to significantly alter results because the species was only found in 16 of 450 clipped plots in low biomass and its biology as an invasive wind-disperser suggests it is of low value to consumers. Each plant species was sampled for $\delta^{13}C$/ $\delta^{15}N$ analysis by initially placing 300-400 μg of leaf tissue (or green stem in a leafless species, such as *Ephedra*) into tin capsules. If more than a total of 1 kg of live biomass of a species was clipped, but 300-400 μg was too small an amount to resolve an accurate $\delta^{15}N$ value (*Bouteloua eriopoda, Gutierrizia sarothrae, Scleropogon brevifolius, Pleuraphis mutica*), new samples were run using 1.5 mg of green tissue. Only the species having >1 kg live biomass (n=6; Figure 1), accounting for 89% of the total live biomass clipped, were then used for analysis. I used live biomass totals and mean $\delta^{13}C$ values for plant species to calculate mass-balanced stable carbon isotope ratios for the 15 sites using the equation , $\delta^{13}C_{site} = \sum_{i=1}^{n}\left(m_i\delta^{13}C_i \Big/ \sum_{i=1}^{n} m_i \right)$ where n is the number of plant species at each site, m_i is the biomass of each species at each site, and $\delta^{13}C_i$ is the mean $\delta^{13}C$ of each plant species. I then used the same equation and mean $\delta^{15}N$ values for plant species to calculate mass-balanced stable nitrogen isotope ratios for each site.

Stable isotope analysis

Stable isotope samples were run for $\delta^{13}C/\delta^{15}N$ on either a Thermo Finnigan Delta Plus XL Isotope Ratio Mass Spectrometer, interfaced to a Carlo Erba Elemental Analyzer through an open split valve (ConFlo II) at the Stable Isotope Laboratory of the Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, New Mexico, or on a Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer interfaced through an open split valve (ConFlo III) with a Costech Elemental Analyzer at the laboratory of Michael H. Engel, Department of Geology and Geophysics, University of Oklahoma, Norman, Oklahoma. Stable carbon isotope ratios are reported in per mil (‰) notation relative to the standard Vienna PeeDee Belemnite (vPDB). Stable nitrogen isotope ratios are reported in per mil (‰) notation relative to the standard atmospheric nitrogen gas (N_2).

Data analysis

Data were analyzed using SPSS (2004). I inspected all data for normality using Kolmogorov-Smirnov Z tests. If assumptions were met, I used linear regression to determine if sites and plant species samples showed a $\delta^{15}N$ trend on a gradient from C_3 shrubland to C_4 grassland as represented by site-level $\delta^{13}C$ values. I used one-way ANOVA to determine if overall tissue $\delta^{15}N$ values differed among plants, grasshoppers, HY BTSPs, and adult BTSPs. I then used linear regression to determine if plant, grasshopper, HY BTSP and adult BTSP $\delta^{15}N$ showed trends relative to both (1) plant composition at the site of sample collection as represented by the composite $\delta^{13}C$ values of the plant biomass at the site and (2) $\delta^{13}C$ values of their own tissue, which in plants represents photosynthetic type (Smith and Epstein 1971) and in

consumers reflects diet composition (DeNiro and Epstein 1978). Because tissue $\delta^{13}C$ for plants and grasshoppers is bimodally distributed, tissue $\delta^{13}C$ vs. tissue $\delta^{15}N$ regressions are primarily descriptive and data are also analyzed using independent samples t-tests on tissue $\delta^{15}N$ to confirm trends. $\delta^{13}C$=-18‰ was used as the division point for plant and grasshopper groups. Plants divided clearly into C_3 and C_4 plants. One grasshopper point did not clearly fall into either of the two groups ($\delta^{13}C$=-18.64‰) and was removed for the independent-samples t-test.

Results

Stable nitrogen isotope values were normally distributed both overall (Z_{151}=0.89, p=0.41) and separately for plants (Z_{44}=0.48, p=0.97), grasshoppers (Z_{40}=0.38, p>0.99), HY BTSPs (Z_{53}=0.69, p=0.73) and adult BTSPs (Z_{14}=0.55, p=0.93). Sites showed no relationship between mass-balanced $\delta^{13}C$ and $\delta^{15}N$ values (r^2=0.18, p=0.11). Plant species had different $\delta^{15}N$ values ($F_{5,38}$=3.72, p=0.01), but $\delta^{15}N$ values for any one species did not vary across sites (Table 1; Figure 2).

Plants, grasshoppers, HY BTSPs, and adult BTSPs showed no relationship between tissue $\delta^{15}N$ and site $\delta^{13}C$ (Figure 3). However, plants on average had the lowest $\delta^{15}N$ values, grasshoppers had $\delta^{15}N$ values approximately 2.75‰ heavier than plants, HY BTSPs had $\delta^{15}N$ values approximately 2.36‰ heavier than grasshoppers, and adult BTSPs had $\delta^{15}N$ values approximately 3.37‰ heavier than grasshoppers ($F_{3,147}$=86.11, p<0.001; Table 2). HY and adult BTSP $\delta^{15}N$ values did not differ statistically.

When tissue $\delta^{15}N$ was compared to tissue $\delta^{13}C$ each trophic group shows a different trend (Figure 4). Plants show decreasing $\delta^{15}N$ with increasing $\delta^{13}C$ (r^2=0.15,

p=0.01) such that C_3 plants have slightly higher $\delta^{15}N$ values than C_4 plants (t_{42}=-2.65, p=0.01). Grasshoppers show a trend similar to plants (r^2=0.16, p=0.01; t_{37}=-2.59, p=0.01) with ~ 3‰ enrichment in $\delta^{15}N$. HY BTSPs have a slightly steeper slope, showing that HY BTSPs that consume more C3 carbon exhibit higher enrichment relative to plants than those that consume more C_4 carbon (r^2=0.24, p<0.001). This pattern becomes more evident in adult BTSPs (r^2=0.70, p<0.001), which showed about a 9‰ enrichment relative to plants in more C_3 birds, but only about a 4.6‰ enrichment in C_4 birds.

Discussion

This simple Chihuahuan Desert trophic system consisting of desert plants, grasshoppers, and black-throated sparrows showed a standard $\delta^{15}N$ trophic enrichment pattern, with plants having the lowest $\delta^{15}N$ values, herbivorous grasshoppers having slightly higher $\delta^{15}N$ values, and omnivorous to exclusively insectivorous (during nesting) BTSPs having the highest $\delta^{15}N$ values. However, in this system, trophic enrichment averaged ~2.8‰ per trophic level with high variation, not the 3.4‰ or more commonly associated with trophic enrichment (Kelly 2000). Grasshoppers showed consistent trophic enrichment across both sites and diets. However, BTSP $\delta^{15}N$ trophic enrichment varied not with plant composition in consumer habitats, but with the $\delta^{13}C$ values of consumer diets, indicating that consumers with more C_3 based diets have higher $\delta^{15}N$ than consumers with more C_4 based diets, a pattern that was most evident in adult BTSPs.

Nitrogen cycling is complex and there are many reasons why BTSPs with different dietary carbon sources may exhibit different $\delta^{15}N$ enrichment patterns.

Because enrichment seems to be linked with diet and not simply consumer habitat or location, explanations may be related to dietary intake of nitrogen or nitrogen metabolism. Three possible explanations derive from these assumptions: 1) consumers of diets containing more C_3 sources actually eat at a higher trophic level than consumers of C_4 sources, and 2) consumers of diets containing more C_3 sources are exposed to increased water stress, and 3) consumers of more C_3 sources incur increased nutritional stress.

First, the explanation that BTSPs with more C_3-based diets are eating at a higher trophic level that consumers with more C_4-based diets may be most feasible for BTSPs. Hobson and Clark (1992) showed that feeding consumers diets containing food from higher trophic levels produces higher $\delta^{15}N$ values in consumers. BTSPs eat primarily arthropods during the breeding season, and hatchling birds are fed primarily arthropods while in the nest and post-fledging. However, when BTSPs do consume seeds, the seeds eaten are primarily from C_4 grasses such as bush muhly (*Muhlenbergia porteri*) and *Panicum* spp. (Zimmer 1983, 1993). This would suggest that BTSPs with more C_4-based diets would have a higher probability of having a lower trophic status than those with C_3 diets. However, this would only be a logical conclusion if HY BTSPs were eating more C_3 seeds than adults. Consumption of C_4 grass seeds by BTSPs is common (Zimmer 1983, 1993), but significant consumption of C_3 shrub or forb seeds has not been reliably documented. Additionally, optimal foraging suggests consumers consume food sources that maximize their nutritional input over time (Fretwell and Lucas 1969, Paulissen 1987, Rosenzweig 1991). Lower prey searching time and higher encounter rates of higher trophic level C_3 arthropods

for adult birds and C_3 seeds for HY birds (who would be less effective predators (Guo et al. 2010) may increase their respective nutritional potentials. However, trophic differences may not only be limited to consumption of insects vs. seeds, but may distinguish different arthropod food sources from different trophic levels.

The second explanation, that consumers with more C_3-based diets are subject to increased water stress, is ambiguous. Lopes and Araus (2006) illustrated that water stress enriches $\delta^{15}N$ in wheat. With respect to consumers, some studies note increased $\delta^{15}N$ in some consumers and attribute this to species adaptation to a dry climate (Schoeninger and Deniro 1984, Ambrose and DeNiro 1986) or consumer occupation of a xeric habitat (Kelly 2000). Further, Sealy et al. (1987) described consumer $\delta^{15}N$ correlation with rainfall in arid areas. However, enriched $\delta^{15}N$ in more C_3-based BTSPs is tied to diet and not necessarily to habitat; thus, any explanation would have to account for C_3 consumers in areas of mostly grass having higher $\delta^{15}N$ values than those consuming C_4 carbon. Cormie and Schwarcz (1996) did link diet to enriched $\delta^{15}N$, but in their study enriched $\delta^{15}N$, which also correlated with precipitation, was more evident with increasing consumption of C_4 plants. This is opposite the trend seen in BTSPs, which showed enrichment with decreasing consumption of C_4 based food.

Third, the notion that more C_3-based consumers are more nutritionally stressed, may be feasible, but is also ambiguous. Owen-Smith (1994) showed food limitation can increase consumer foraging on less preferred food sources. If C_3- and C_4- based foods in the Chihuahuan Desert system are differentially preferred, either C_4 food limitation or the actual C_3-based food may be sources of nutritional stress.

Nutritional stress would more likely be tied to diet than to habitat, but would not explain why birds in areas with high quantities of C_4 grass (and presumably C_4 grass-based food) consume C_3 sources if they are nutritionally stressful and other sources are available.

In conclusion, it seems most likely that increased $\delta^{15}N$ in BTSPs, particularly adults, that consume more C_3-based diets is most likely attributable to consumption of a C_3 food source of a higher trophic level than typical C_4 food sources. Further studies analyzing gut contents and fledgling and adult feeding behavior in BTSPs may help clarify this possibility.

Acknowledgements

Thanks to Jeff Kelly, Michael H. Engel, Viorel Atudorei and Zachary Sharp for isotope analyses and to Brandon Bestelmeyer, M.H. Engel, Michael Kaspari, Michael Patten, and Lauren Niemann for review. J.M. Paritte was supported by an NSF Graduate Research Fellowship during part of this study. Funding was provided in part by the University of Oklahoma's George Miksch Sutton Scholarship in Ornithology, M. Blanche Adams Scholarships, and college and departmental grants. This work was submitted in partial fulfillment of J.M. Paritte's Ph.D. degree at the University of Oklahoma and the experiments herein comply with the all appropriate U.S. regulations.

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Table 1. Mean and SD $\delta^{15}N$ values for the six plant species used in data analysis. Both r^2 and p-values are for the relationship between plant tissue $\delta^{15}N$ and the $\delta^{13}C$ values of the site at which the sample was collected.

Species	Mean $\delta^{15}N$ (‰)	SD	r^2	p
B. eriopoda[a]	0.39	1.47	0.29	0.35
P. mutica[a]	1.51	1.65	0.15	0.45
S. brevifolius[ab]	2.57	1.84	0.15	0.34
P. glandulosa[ab]	2.7	2.15	0.03	0.59
G. sarothrae/G. sphaerocephala[ab]	3.04	1.01	0.23	0.23
F. cernua[b]	4.89	1.18	0.29	0.35

Note: Superscript letters [a] and [b] are Tukey's HSD groupings for species $\delta^{15}N$ main-effect.

Table 2. Mean and SD $\delta^{15}N$ values for plants, grasshoppers, HY BTSPs, and adult BTSPs. Both r^2 and p-values are for the relationship between tissue $\delta^{15}N$ and either site or tissue $\delta^{13}C$ values as indicated.

		Trophic Level $\delta^{15}N$		Site		Tissue	
Species	Mean $\delta^{15}N$ (‰)	difference (‰)	SD	r^2	p	r^2	p
Plants[a]	2.61		1.97	0.003	0.73	0.15	0.01
Grasshoppers[b]	5.36	2.75*	1.70	0.02	0.40	0.16	0.01
HY BTSPs[c]	7.72	2.36**	1.45	0.03	0.26	0.49	<0.001
Adult BTSPs[c]	8.73	3.37***	1.94	0.01	0.75	0.84	<0.001

Note: Superscript letters are Tukey's HSD groupings for species $\delta^{15}N$ main-effect.
*Grasshopppers $\delta^{15}N$-Plants $\delta^{15}N$
**HY BTSPs-Grasshopppers
***Adult BTSPs-Grasshoppers

Figure 1. Plant species found on 15 sites and their log-transformed and linear (inset) total dry live biomass. Only species with >1 kg total dry live biomass were used for mass-balanced $\delta^{15}N$ calculations for sites (black bars). Of those not used in calculations, I was measured $\delta^{15}N$ values for some (gray bars), but not others (white bars). $\delta^{15}N$ means and standard deviations are listed in Table 1.

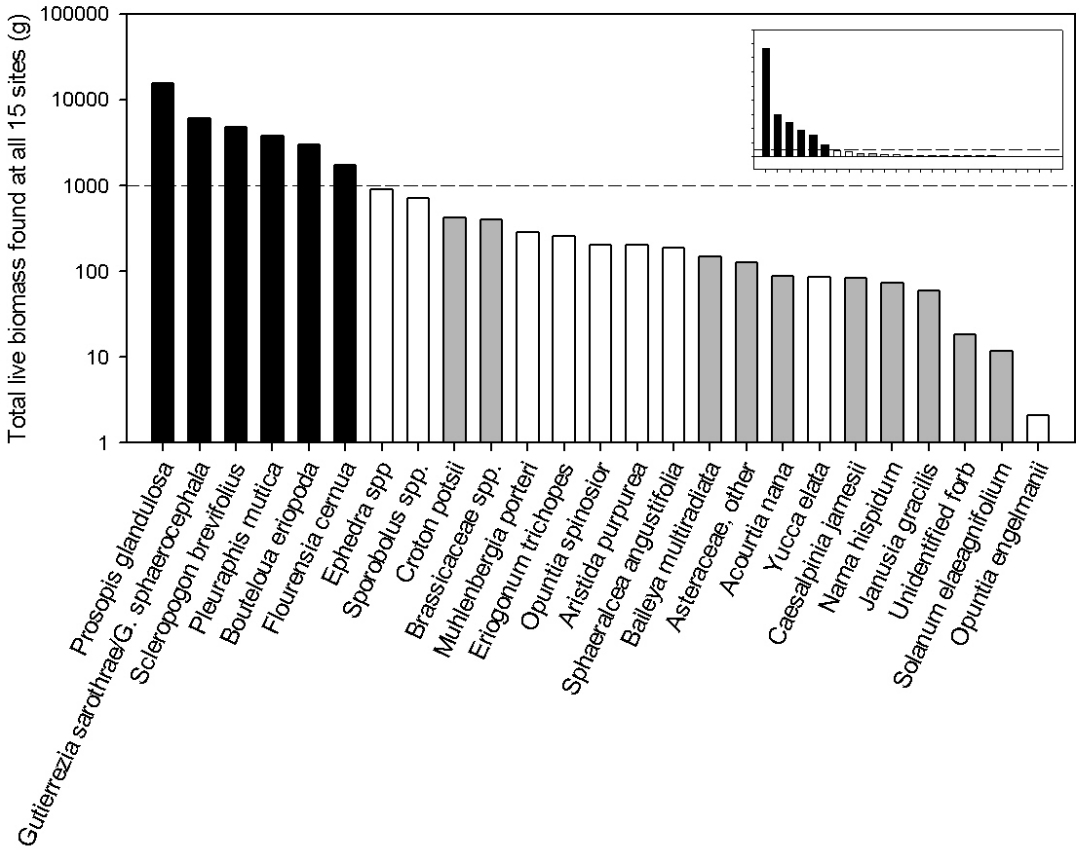

Figure 2. Mass-balanced site $\delta^{13}C$ vs. mass-balanced site $\delta^{15}N$ for 15 sites (a) and mass-balanced site $\delta^{13}C$ vs. $\delta^{15}N$ for samples taken at those sites (b). No trend was seen either within a plant species or overall. Mass-balanced site $\delta^{15}N$ values were calculated using species $\delta^{15}N$ means.

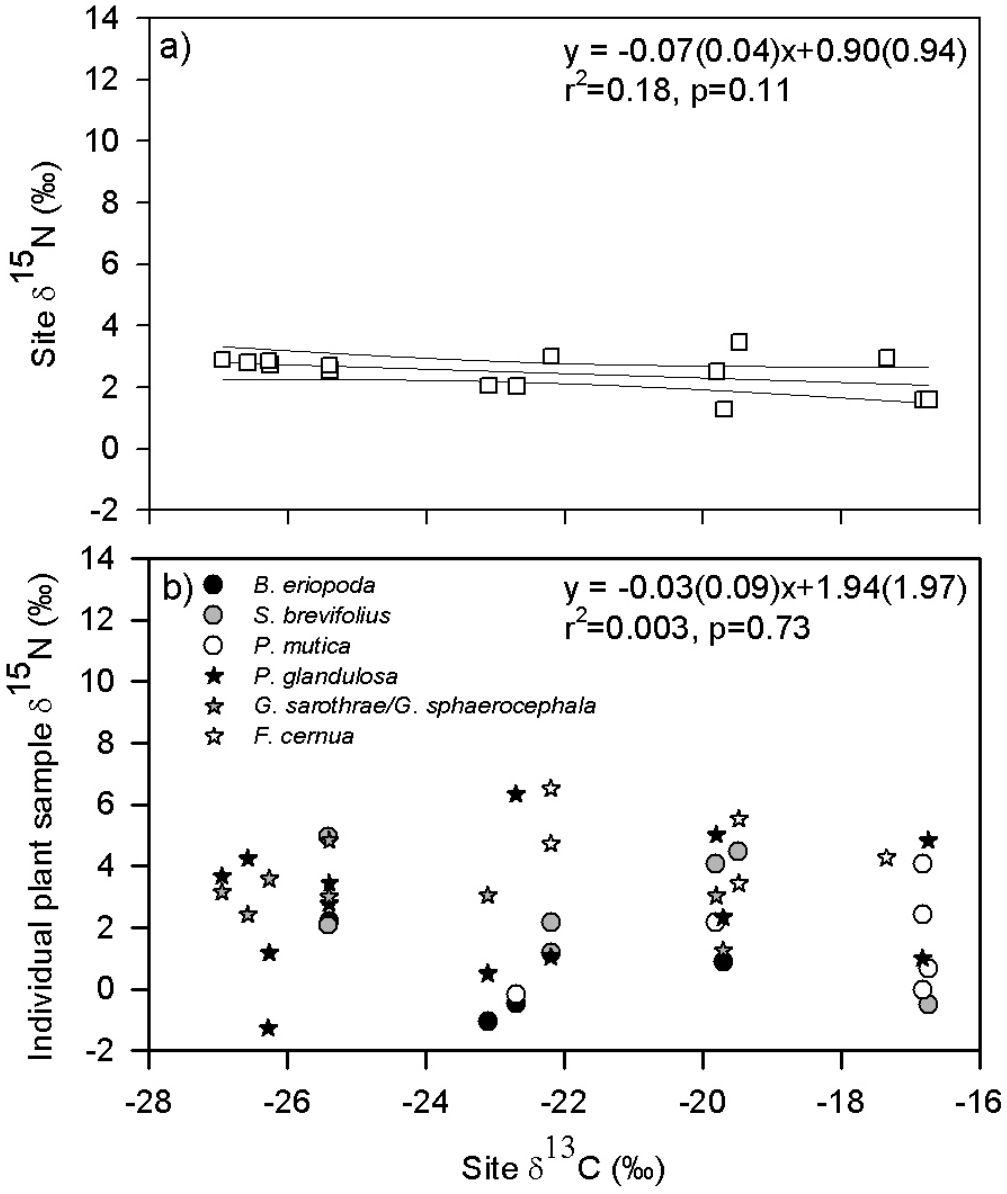

Figure 3. Mass-balanced site $\delta^{13}C$ vs. consumer tissue $\delta^{15}N$. Lines represent best fit and 95% confidence. Black lines show best fit and confidence (CI) for overall plant sample pattern in Figure 2b. Regressions (1SE in parentheses): Plants, y=-0.03(0.09)x+1.94(1.97), r^2=0.003, p=0.73; Grasshoppers, y=0.07x(0.08)+6.83(1.74), r^2=0.02, p=0.40; HY BTSPs, y=-0.06x(0.05)+6.46(1.13), r^2=0.03, p=0.26; Adult BTSPs, y=-0.06x(0.18)+7.57(3.63), r^2=0.01, p=0.75.

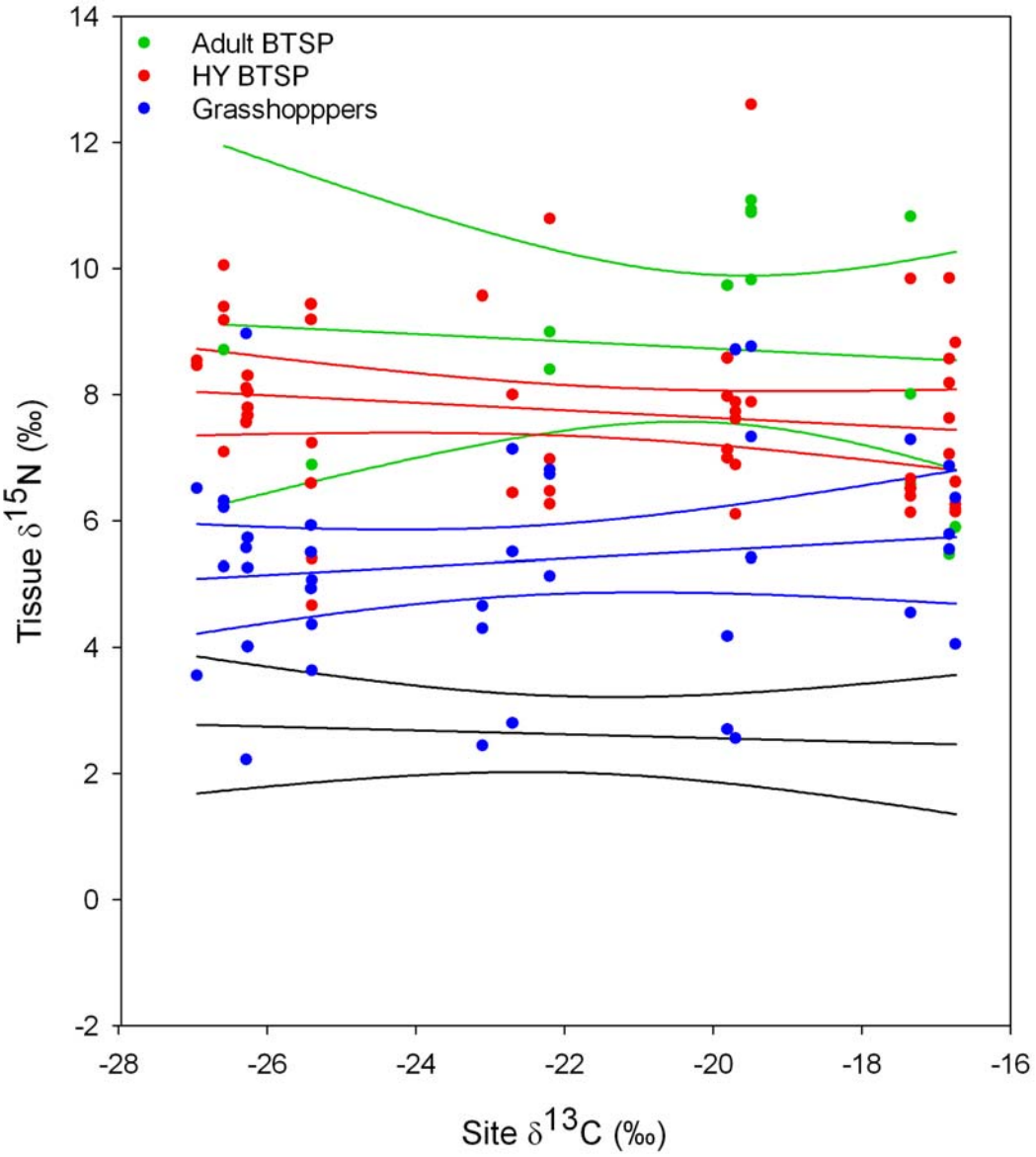

Figure 4. Plant and consumer tissue $\delta^{13}C$ vs. plant and consumer tissue $\delta^{15}N$. Lines represent best fit and 95%CI. Due to the bimodal nature of plant and grasshopper data, independent-samples t-test results are presented along with regression results. Regressions: Plants, y=-0.14(0.05)-0.36(1.12), r^2=0.15, p=0.01; Grasshoppers, y=-0.16(0.06)-2.24(1.20), r^2=0.16, p=0.01; HY BTSPs, y=-0.31(0.08)-2.28(1.38), r^2=0.24, p<0.001; Adult BTSPs, y=-0.67(0.13)-2.36(2.11), r^2=0.70, p<0.001. Independent-samples t-tests: Plants, t_{42}=-2.65, p=0.01; Grasshoppers, t_{37}=-2.59, p=0.01.