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Feeding Ecology of the Snake Community of the Red Hills Region Relative to Management for Northern Bobwhite: Assessing the Diet of Snakes Using Stable Isotopes

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Snakes can respond to variation in resource availability through changes in diet and species interactions. For many species, however, patterns of variation in diet are poorly known. Making use of novel ^{13}C, through the provision of supplemental food in the form of milo (*Sorghum* spp.) for Northern Bobwhite (*Colinus virginianus*), we used carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope analyses to evaluate the specificity of mammalian and herpetofaunal diet, trophic niche width, and trophic niche overlap between five snake species in the Red Hills of the southeastern United States. Most mammalian prey and Northern Bobwhite were characterized by different δ^{13}C values in the milo- and non-milo-treated areas. Using milo to reflect dietary patterns we determined that Coachwhip (*Masticophis flagellum*), Cottonmouth (*Agkistrodon piscivorus*), and Eastern Rat Snake (*Pantherophis alleghaniensis*) were dietary generalists, revealed by larger isotopic range and lack of correspondence between isotopic values and those of distinct prey groups. In contrast, Black Racer (*Coluber constrictor*) and Corn Snake (*Pantherophis guttatus*) were dietary specialists. Black Racers fed largely on herpetofauna, and δ^{13}C values of Corn Snake revealed a gradual ontogenetic/size-related diet shift from herpetofauna to a mammalian-based diet. There was considerable overlap of isotopic trophic niche between snake species, except for the Cottonmouth with δ^{15}N values higher than other sampled species. These findings provide novel insight into snake trophic ecology.

ALL organisms are embedded within food webs, with the trophic links identifying the pathways of energy and matter transfer in ecosystems (Paine, 1988). Thus, food web studies can reveal a substantial amount of information on the pathways and mechanisms of energy flow, biodiversity, predator–prey interactions and community structure. Elucidating food web structure is one of the important first steps in understanding the organization of ecological communities (Schoenly and Cohen, 1991), for developing population and community models (Nakazawa, 2011), and developing wildlife management plans that consider whole ecosystem approaches.

Food webs, however, are complex, dynamic, and sensitive to environmental change. As a consequence, food webs are often not easily quantified (Finlay, 2001). Animals may shift their habitat use in response to resource availability, often in ways that may not be easily apparent (Willson et al., 2010). As obligate predators, snakes are tightly coupled to their resource environment (Bronikowski and Arnold, 1999; Beaupre and Douglas, 2009) and can respond relatively quickly to variation in resource availability (Matthews et al., 2002) with short- and long-term changes carrying the potential to influence population structure, movement patterns, demographics, and species interactions within snake communities (King and Duvall, 1990; Beaupre and Douglas, 2009; Miller et al., 2011).

The supplemental feeding of wildlife is a commonly used management tool for species expected to be influenced by low resource availability (Guthrey et al., 2004; Putnam and Staines, 2004). Provisioning resources for target wildlife can help enhance desired attributes of these populations yet this management action can also carry unintentional consequences for other members of targeted ecological communities. For example, it is known that supplemental feeding for game species such as Northern Bobwhite (*Colinus virginianus*) can influence the spatial distribution and demographics of other ecological community members (Morris et al., 2010), with most studies focused on either mammalian (Doonan and Slade, 1995; Godbois et al., 2004) or avian (Turner et al., 2008) species. Based on results from several of these studies, it is also expected that the provision of supplemental feeding for Northern Bobwhite can also influence herpetofaunal community dynamics. However, the influence of supplemental feeding on snake trophic ecology has yet to be investigated.

Obtaining reliable information on snake trophic dynamics can be hindered by several factors. Snakes often feed infrequently, consuming large prey that is digested slowly and efficiently, including both soft and hard tissues (Cundall and Greene, 2000). Thus, diet studies incorporating regurgitation techniques, or fecal analysis, can incur bias and deliver unreliable estimates (Stickel et al., 1980; Kephart and Arnold, 1982; Cook, 1983; Houston and Shine, 1993; Santos et al., 2000). An additional bias of diets that are assessed from trapped snakes comes from the prey items that may be consumed while snakes are caught in traps (Houston and Shine, 1993).

Stable isotope analysis provides an alternative method for assessing diet choice and the trophic ecology of snakes (e.g., Post, 2002; Smith et al., 2002; Willson et al., 2010), and has the advantage over stomach content data because isotope analysis 1) can integrate diet over a longer period, 2) reflects the assimilated components of the diet, and 3) provides data even when stomachs are empty (Tieszen et al., 1979). Furthermore, stable isotope analysis can expand on inference based on species-specific differences in habitat use, accounting for seasonal and spatial dietary patterns or

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contrasts (Ford et al., 1991; Willson et al., 2010) and can often be determined non-lethally using biopsied tissue samples.

To address the lack of information on snake feeding ecology, and the influence of supplemental feeding management, $\delta^{13}C$ and $\delta^{15}N$ were quantified in five species of snakes and potential prey from two regions of the coastal plain of the southeastern United States. The unique chemical attributes of milo (i.e., a C4 plant with ^{13}C enriched $\delta^{13}C$ values), which is used as a supplemental feed for Northern Bobwhite, provided an opportunity to study the feeding ecology of snakes and the relative importance of mammalian vs. herpetofauna in the diet of these five species. We hypothesize that the unique chemical attributes of milo will be most notable in Northern Bobwhite, granivorous small mammals, and in higher order consumers that prey on these species. Alternatively, the isotopic qualities of milo will be less notable in tissues sampled from species which do not directly, or indirectly, consume milo. Collectively, by quantifying the presence of milo among different snake species and their prey this study provides information on the relative diet composition of each studied snake species, with some gender and size specific information, and the impacts of supplemental food provisioning for Northern Bobwhite on the diets (trophic niche, foraging ecology, etc.) of these snake species.

MATERIALS AND METHODS

Study site and sampling.—Field sampling was conducted at the Tall Timbers Research Station (TTRS) and the Pebble Hill Plantation (PH), both located in Red Hills region of northern Florida and southern Georgia. TTRS covers approximately 1,500 ha in Leon County, Florida. Forest communities of TTRS are dominated by upland longleaf (*Pinus palustris*), shortleaf (*P. echinata*), and loblolly pine (*P. taeda*). Bottomland hardwoods interspersed across this upland landscape comprise oaks (*Quercus* spp.), hickories (*Carya* spp.), sweet gum (*Liquidambar styraciflua*), and black gum (*Nyssa sylvatica*). PH includes approximately 1,250 ha in Thomas and Grady counties in Georgia comprising similar upland and bottomland forest communities to those found at TTRS. Both TTRS and PH are intensively managed using annual prescribed burning and extensive mechanical techniques to maintain open savannah-like habitat in upland pine forests. Both sites are actively managed for Northern Bobwhite, actions which include meso-mammalian predator control and supplemental feeding (milo [*Sorghum* spp.]). Application of supplemental feed was made to half of each study site on a weekly basis. The other half of each study site was unfed, serving as a control area.

All samples were collected in May and October of 2004 at TTRS and PH. Snakes were captured using drift fence arrays consisting of four 15-meter silt fences radiating from a central point, with four hardware cloth funnel traps at each respective end and one wooden box trap in the middle to maximize captures (Greenberg et al., 1994). Snake traps at TTRS were on average 720 m from the feed line and 1,037 m from the feed line at PH. Additionally, treatment and control areas at TTRS and PH were separated by a road. Although this road is not a physical barrier to movement, it is a potential deterrent to the movement of our target species. Upon capture, target species (all non-venomous snakes with the exception of Cottonmouths captured at TTRS) were weighed, measured (snout–vent length [SVL]), and sexed by probing for hemipenes. Muscle tissue samples were collected for stable isotope analysis, non-destructively, from captured snakes by taking a small biopsy lateral to the dorsal midline of the snake and caudal to three quarters of the snake's body length. The area was first cleaned with rubbing alcohol, a small incision (1 cm) was made in the epidermis of the snake, and a 3 mm biopsy punch (Anthony Products International) was used to extract a small amount of muscle tissue (10–20 mg). The tissue sample was then placed in a micro-tube and stored at –20°C until analysis.

Prey samples were collected in the snake traps or opportunistically from within the different treatment areas. Small rodent traps (8 × 9 × 23 cm; HB Sherman Trap Company, Tallahassee, FL) were also placed near snake traps for two days in May and October to augment sample numbers.

Stable isotope analysis.—All stable isotope analysis was performed at the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia (UGA) or the Chemical Tracer Laboratory, University of Windsor. Details about the analytical methods can be found on the UGA website (www.uga.edu/sisbl); methods were essentially identical between the labs. Briefly: tissue samples were washed with distilled water, freeze dried, ground to a fine powder using a ball mill, and sealed into tin capsules. Tin capsules were combusted into the simple gases N_2 or CO_2 on an elemental analyzer placed at the front end of an isotope ratio mass spectrometer (IRMS). The IRMS can separate the isotope ratios of N and C in N_2 and CO_2, respectively. Because the sensitivity of the IRMS can vary, gases from the samples were compared to standard reference materials for stable isotopes (air for N_2 and PeeDee Belemnite for CO_2). Stable isotope abundances are expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] * 1000,$$

where X is ^{13}C or ^{15}N, and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Stable isotope values did not differ between sample runs or across laboratories (Ecology Laboratory, UGA and Chemical Tracers Laboratory, GLIER). Stable isotope values of internal standards (NIST standards ammonium sulfide and sucrose) showed excellent accuracy, falling within 0.1 of the accepted values for $\delta^{13}C$ and $\delta^{15}N$. The precision of the isotopic analyses were better than ±0.15 for $\delta^{15}N$ and 0.2 for $\delta^{13}C$ for standards and triplicate analysis of every twelfth sample, variation established as acceptable for stable isotope analysis.

Statistical analysis.—Multivariate analysis of variance (MANOVA) was used to test for the effects of site and season × site interactions on isotope values of each species. For this analysis we considered samples as collected from two different seasons; those collected in May 2004 were coded as one season and those in October 2004 as a different season. Following MANOVA, we applied ANOVA tests to identify differences in $\delta^{13}C$ and $\delta^{15}N$ among seasons and sampling locations. Linear regressions were used to examine relationships between $\delta^{13}C$ and $\delta^{15}N$ and snake total length for each species followed by likelihood ratio tests, comparing the fit of the more-parameterized model that included treatment to the less-parameterized model that did not.

Table 1. Stable isotope values (mean ± 1 SE; unless $n < 3$) of mammals, herpetofauna, and Northern Bobwhite eggs collected at Pebble Hill Plantation and Tall Timbers Research Station, in southern Georgia and northern Florida, during May and October 2004. Milo-treated indicates that milo was available as a supplemental food source for Northern Bobwhite, as part of normal management practices at these sites. Blank cells reflect no individuals of that species were collected. Scientific names appear below table.

	Milo treated			Not milo treated		
Common name	*n*	δ^{13}C	δ^{15}N	*n*	δ^{13}C	δ^{15}N
Milo	5	2.40±0.42	−12.36±0.29			
Pebble Hill, May						
Cotton Mouse	8	−22.52±3.21	3.1±1.02	6	−24.47±0.71	3.89±1.12
Cotton Rat	8	−19.0±4.77	2.92±0.78	3	−23.84±2.57	2.93±0.4
Eastern Wood Rat	1	−26.03	1.56			
Least Shrew	2	(−23.3, −21.58)	(5.56, 7.07)			
Eastern Spadefoot Toad	4	−23.47±0.51	3.87±0.42	1	−22.19	4.99
Eastern Fence Lizard	3	−23.91±0.54	3.12±0.52	2	(−24.2, −23.08)	(3.13, 3.48)
Northern Green Anole	1	−24.5	2.77	1	−24.47	3.35
Southeastern Five-lined Skink	3	−22.29±1.54	3.78±0.63	1	−24.2	3.3
Six-lined Racerunner				3	−23.93±0.13	3.38±0.37
Southern Toad	3	−23.91±0.37	3.58±0.26	1	−23.72	4.42
Northern Bobwhite (eggs)	5	−18.1±0.1	4.8±0.1	5	−26.6±0.50	4.4±0.20
Tall Timbers, May						
Cotton Mouse	8	−18.72±4.08	5.26±1.49	7	−22.83±3.45	4.74±0.85
Cotton Rat	6	−17.07±1.82	4.33±0.58	6	−24.02±1.93	3.53±1.62
Eastern Wood Rat	1	−26.34	2.55	1	−25.2	4.07
Least Shrew	3	−23.54±0.28	5.55±0.15	1	−23.69	4.38
Broad-headed skink	4	−23.82±1.16	3.52±0.29	1	−24.65	3.54
Green Anole	2	(−24.84, −24.36)	(3.44, 3.57)	3	−24.46±0.62	4.06±0.56
Six-lined Racerunner	5	−23.67±0.75	2.87±0.47			
Southeastern Five-lined Skink	3	−23.53±1.14	2.76±0.51			
Northern Bobwhite (eggs)	5	−19.0±0.08	5.8±0.1	5	−24.10±0.10	4.5±0.10
Pebble Hill, October						
Cotton Mouse	1	−17.24	5.5	2	(−24.27, −23.87)	(3.88, 4.45)
Cotton Rat	2	(−15.57, −15.03)	(4.79, 5.12)	6	−23.35±2.65	3.04±0.9
Eastern Fence Lizard	2	(−24.14, −23.92)	(2.9, 2.9)			
Green Anole				3	−24.78±0.25	3.47±0.17
Tall Timbers, October						
Cotton Mouse	3	−22.75±0.17	3.43±1.07			
Cotton Rat	5	−18.27±2.14	4.62±0.83	6	−24.61 ±1.16	3.16±1.4
Least Shrew	2	(−23.51, −24.01)	(4.81, 5.20)	2	(−24.39, −24.60)	(4.49, 5.28)
Eastern Fence Lizard	3	−24.00±0.45	2.99±0.06	1	−24.7	2.67
Eastern Spadefoot Toad				1	−23.34	4.04
Green Anole	2	(−24.93, −24.54)	(2.75, 3.4)	5	−24.94 ±2.07	3.61±0.51

Cotton Mouse (*Peromyscus gossypinus*), Cotton Rat (*Sigmodon hispidus*), Eastern Wood Rat (*Neotoma floridana*), Least Shrew (*Cryptotis parva*), Broad-headed Skink (*Plestiodon laticeps*), Eastern Spadefoot Toad (*Scaphiopus holbrookii*), Eastern Fence Lizard (*Sceloporus undulatus*), Northern Green Anole (*Anolis carolinensis*), Southeastern Five-lined Skink (*Plestiodon inexpectatus*), Six-lined Racerunner (*Aspidoscelis sexlineatus*), Southern Toad (*Anaxyrus terrestris*), Northern Bobwhite (*Colinus virginianus*), Milo (*Sorghum* spp.)

Because the turnover rate of stable isotopes in snake tissues is slow (Pilgrim, 2007; Fisk et al., 2009), any species that has a diet that varies seasonally or ontogenetically may not have isotope values that are in equilibrium with its diet. We therefore used the isotopic values as a relative metric of dietary differences within and between species as well as trophic niche characteristics among species (Willson et al., 2010). Isotopic niche spaces were estimated using the stable isotope Bayesian ellipses in R (SIBER; Parnell et al., 2008, 2010), where size-corrected standard ellipse areas (SEA_c) were calculated using δ^{13}C and δ^{15}N residual values from the linear models evaluated for each snake species (Jackson et al., 2011). To further evaluate how isotope ratios changed within species relative to supplemental feeding, we compared the overlap of standard ellipses within species between treatments.

All statistical tests were performed using the R statistical computing package (Version 2.11.1; R Development Core Team, 2011). All analyses were considered statistically significant at $P < 0.05$.

RESULTS

Prey species.—Supplemental feeding with milo for Northern Bobwhite has a significant influence on δ^{13}C values measured in this species' eggs and in most small mammal species (Table 1, Fig. 1). In general and considering the

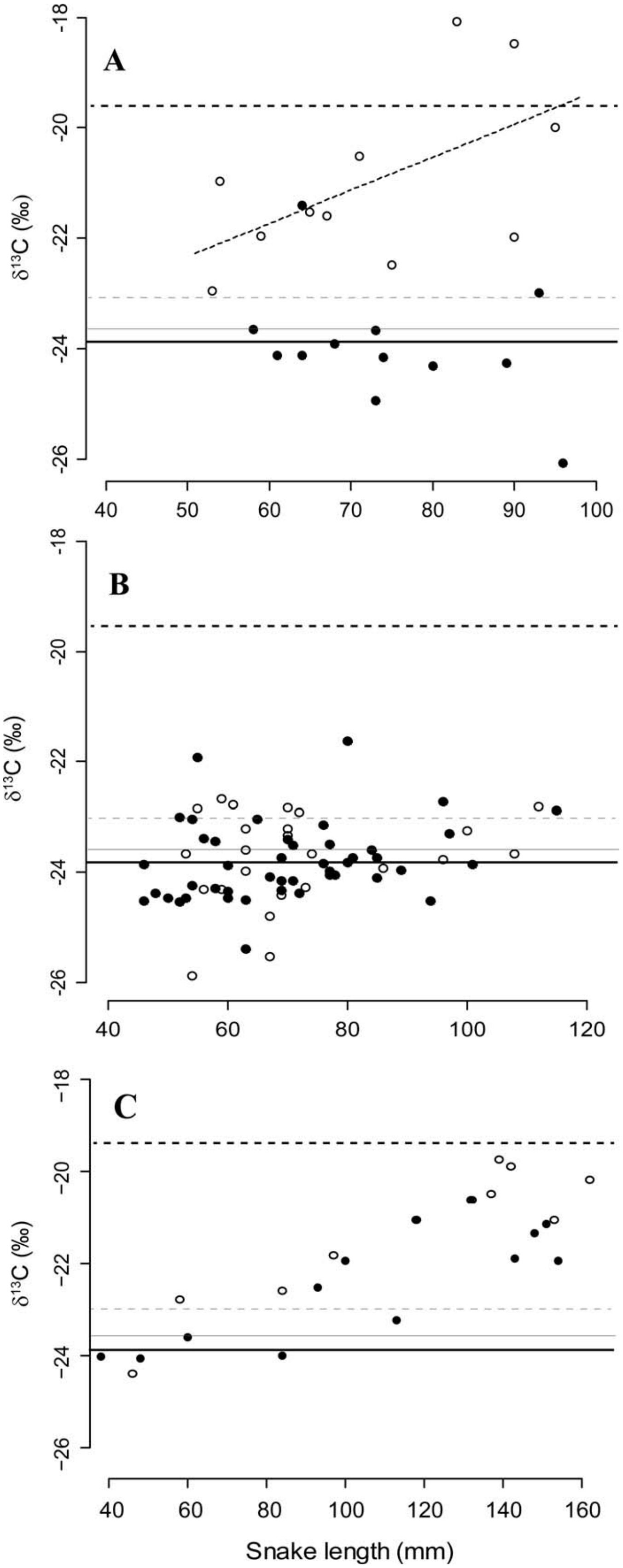

Fig. 1. Relationship between snake length and δ^{13}C for (A) Corn Snake, (B) Black Racer, and (C) Coachwhip in milo-treated (open circles) and untreated (black filled circles) areas in the Red Hills region of Florida and Georgia. Dashed lines represent mean δ^{13}C values from small mammals (black) and herpetofauna (gray) sampled from milo-treated sites. Solid lines represent sample means of mammals (gray) and herpetofauna (black) sampled from non-milo treatment sites.

influence of milo supplementation, stable isotope values in probable snake prey species were consistent between Pebble Hill and Tall Timbers, indicating similar ecosystem and food web characteristics. For Cotton Mouse (*Peromyscus gossypinus*) and Cotton Rat (*Sigmodon hispidus*), δ^{13}C did not differ between sampling date or location ($P > 0.06$), but had significantly higher δ^{13}C values in the milo-treated versus non-treated areas (Cotton Mouse: $t_{33} = 2.57$, $P = 0.015$; Cotton Rat: $t_{38} = 6.14$, $P < 0.001$). Isotopic values in herpetofauna did not differ between milo-treated and untreated areas, indicating that the milo is not incorporated by these organisms (Northern Green Anole [*Anolis carolinensis*]: $t_{15} = 0.19$, $P = 0.85$; Eastern Fence Lizard [*Sceloporus undulatus*]: $t_{11} = 0.26$, $P = 0.8$). Comparing isotopic values between pooled species of small mammals with those of herpetofauna from milo-treated sites showed significantly higher δ^{13}C values in small mammals (mean mammals: -23.42; mean herpetofauna: -19.78; $t_{45.79} = -6.4$, $P < 0.001$), a relationship that was not apparent in the non-milo treatment ($t_{61.15} = -0.79$, $P = 0.44$). There was no statistical evidence of higher δ^{15}N values for small mammals sampled from the milo- versus non-milo-treated plots ($t_{123.5} = -1.16$, $P = 0.24$).

Snakes.—Over the course of the sampling period in May and October 2004, 124 snakes were captured and biopsied. The majority of these captures (91%) comprised five species: 1) Black Racer (*Coluber constrictor*), 2) Coachwhip (*Masticophis flagellum*), 3) Corn Snake (*Pantherophis guttatus*), 4) Eastern Rat Snake (*Pantherophis alleghaniensis*), and 5) Cottonmouth (*Agkistrodon piscivorus*; Table 2).

MANOVA using both δ^{13}C and δ^{15}N for snakes sampled from the non-milo treatment revealed significant differences between species (Wilk's L = 0.44, Approx. $F_{8,136} = 8.5$, $P < 0.001$) but no significant interaction between species and season (Wilk's L = 0.88, Approx. $F_{8,126} = 1.03$, $P = 0.42$), or between species and study area (PH vs. TTRS; Wilk's L = 0.87, Approx. $F_{6,128} = 1.49$, $P = 0.19$). There was limited evidence that isotopic values differed by sex within species (Wilk's L = 0.81, Approx. $F_{8,126} = 1.75$, $P = 0.09$). This relationship was likely driven by differences in δ^{15}N related to sex in Cottonmouths where the δ^{15}N values of females were significantly higher than that of males ($t_{8.91} = 2.52$, $P = 0.03$), although length did not differ between sexes ($t_{8.6} = -1.49$, $P = 0.17$). Comparison of the δ^{13}C and δ^{15}N values of snake muscle collected from the milo treatment also revealed differences between species (Wilk's L = 0.42, Approx. $F_{8,90} = 6.1$, $P < 0.001$), but not between species and season (Wilk's L = 0.86, Approx. $F_{8,80} = 0.80$, $P = 0.60$) or between species and site (Wilk's L = 0.95, Approx. $F_{6,82} = 0.33$, $P = 0.92$). Like the non-milo treatment, there was no evidence from the milo treatment that isotopic values differed by sex within species (Wilk's L = 0.81, Approx. $F_{6,82} = 1.53$, $P = 0.18$).

Corn Snakes from milo-treated sites exhibited a positive relationship between tissue δ^{13}C and length ($y \sim -21.38 + 0.09 * \text{length}$ (mm): $t_1 = 2.45$, $P = 0.02$), while individuals sampled from the non-milo-treated sites did not ($t_1 = -1.2$, $P = 0.24$; Fig. 1). There was no evidence of a relationship between the δ^{15}N values and treatment or length for this species ($F_{3,19} = 0.25$, $P = 0.86$). The δ^{13}C values of Black Racers did not differ between treatments ($X^2 = 0.48$, $P = 0.49$) or with snake length ($F_{3,68} = 1.03$, $P = 0.38$; Fig. 1). Black Racer δ^{15}N values differed between treatments ($F_{168} =$

Table 2. Stable isotope values (mean ± 1 SE) in snakes collected at Tall Timbers Research Station and Pebble Hill Plantation, in northern Florida and southern Georgia during 2004. Milo treated refers to the area where the sample was collected was fed with milo following normal management practices on plantations managed for Northern Bobwhite.

	Milo treated					Not milo treated				
Species	*n*	Mass	Length	δ¹⁵N	δ¹³C	*n*	Mass	Length	δ¹⁵N	δ¹³C
Tall Timbers										
Black Racer	11	144.3±138.3	73.5±17.4	4.4±0.6	−23.6±0.8	25	145.6±145.0	68.8±16.6	4.7± 0.7	−24.0±0.9
Coachwhip	4	727±416.5	136.0±60.1	6.1±0.9	−20.9±2.4	3	109.3±52.8	67.0±23.3	5.2±0.8	−23.39±0.3
Corn Snake	6	105.3±125.4	64.5±11.5	5.0±0.4	−21.0 ±1.7	8	128.6±41.9	68.9±7.5	4.6±0.7	−23.8±1.0
Cottonmouth	4	451.5±508.6	63.5±21	5.7±0.5	−21.4±2.3	5	469.8±640.7	54.8±29.8	6.8±0.8	−23.2±2.1
Eastern Rat Snake	13	298.0±226.3	90.3±34.1	5.1±0.7	−22.0±2.0	13	222.5± 212.0	81.8±24.6	5.3± 0.9	−23.6±1.1
Pebble Hill										
Black Racer	13	130.8±110.1	72.3±16.7	4.3±0.4	−23.7±0.9	23	139.7± 68.6	70.2±14.6	3.7±0.7	−23.9±0.6
Coachwhip	8	354.5±206.6	115.0±33.3	4.8±0.9	−21.6±1.7	10	436.2±248.9	118.1±36.5	5.3±0.9	−22.1±1.2
Corn Snake	5	254.6±125.4	83.0±12.6	4.5±0.4	−20.9±1.6	4	202.3±93.6	85.5±14.6	4.7±0.4	−24.4±1.3
Eastern Rat Snake	4	250.2±187.2	93.6±25.1	4.6±0.4	−22.5±1.7	5	460.8±182.4	117±17.4	4.9±0.6	−22.5±1.0
Cottonmouth	3	579±155.3	145±12.1	5.3±1.5	−20.9±0.8	0				

Black Racer (*Coluber constrictor*), Coachwhip (*Masticophis flagellum*), Corn Snake (*Pantherophis guttatus*), Cottonmouth (*Agkistrodon piscivorus*), Eastern Rat Snake (*Pantherophis alleghaniensis*)

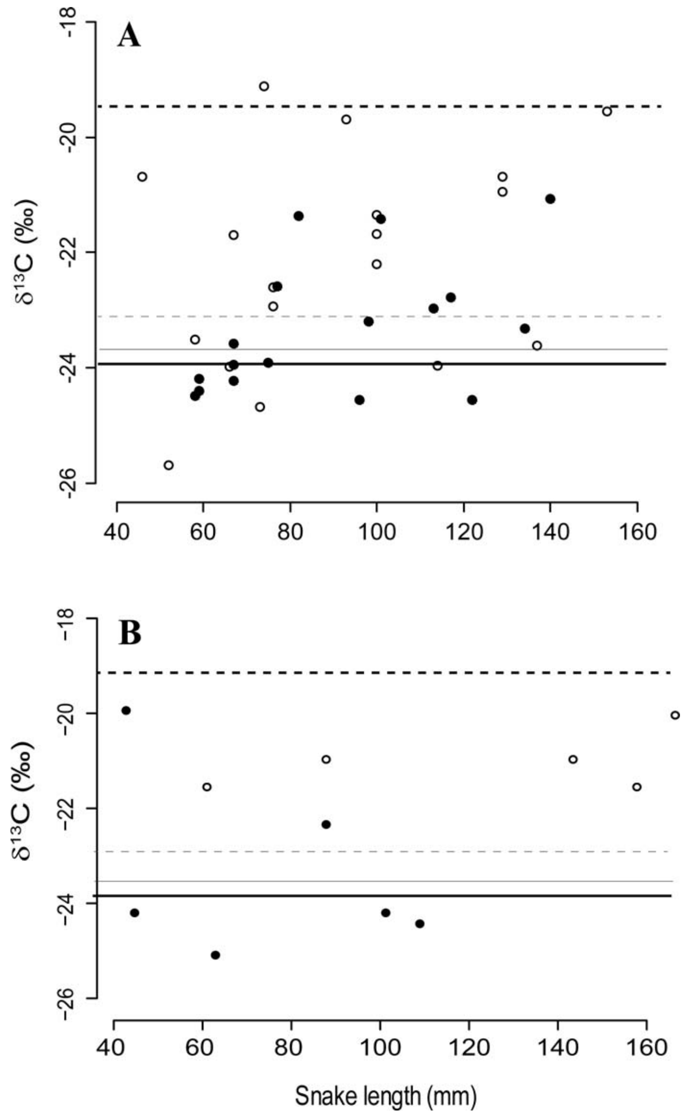

Fig. 2. Relationship between snake length and δ¹³C for (A) Eastern Rat Snake and (B) Cottonmouth in milo-treated (open circles) and untreated (black filled circles) areas in the Red Hills region of Florida and Georgia. Dashed lines represent mean δ¹³C values from small mammals (gray) and herpetofauna (black) sampled from milo-treated sites. Solid lines represent sample means of mammals (gray) and herpetofauna (black) sampled from non-milo treatment sites.

8.60, $P = 0.005$), with lower values in the milo-treated areas ($t_1 = -3.10$, $P < 0.01$). On average, the δ¹³C values of Coachwhips were higher in milo treatment areas ($t_1 = 2.74$, $P = 0.01$) and increased with snake length ($t_1 = 8.42$, $P < 0.001$), although the slope of this relationship did not differ between treatments ($X^2 = 1.31$, $P = 0.26$; Fig. 1). There were no relationships between δ¹⁵N values, treatment, or snake length evident for Coachwhips ($F_{3,20} = 0.38$, $P = 0.76$). Eastern Rat Snakes showed an enrichment in δ¹³C in the milo treatment ($t_1 = 2.33$, $P = 0.03$) and a consistent δ¹³C increase with snake length within treatments ($F_{1,33} = 4.78$, $P = 0.04$; Fig. 2). There was no relationship between δ¹⁵N values, treatment, or length for this species ($F_{3,31} = 1.58$, $P = 0.21$). Neither the δ¹³C nor δ¹⁵N values of Cottonmouths differed between treatments or in relation to snake length ($P > 0.05$; Fig. 2).

For samples collected from non-milo-treated areas, the lowest δ¹³C value was measured in Corn Snakes (−26.07‰),

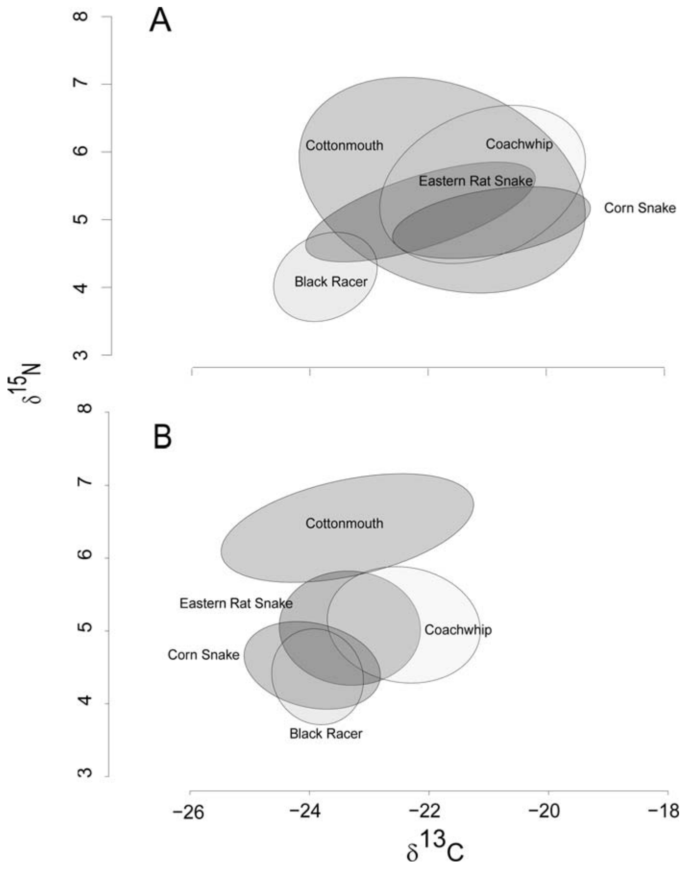

Fig. 3. Stable isotope Bayesian ellipses adjusted for sample size (SEA_c) drawn using carbon and nitrogen values for snake species sampled from study sites (A) where milo was provided and (B) where milo was not provided. Ellipses reflect 95% credible areas. The proportion of ellipse overlap between species appears in Table 3.

with the lowest $\delta^{15}N$ value in Black Racers (3.32‰). Cottonmouths showed the highest $\delta^{13}C$ and $\delta^{15}N$ values of any species sampled in this treatment (−19.94‰, 5.76‰, $\delta^{13}C$ and $\delta^{15}N$, respectively). For the milo treatment, the lowest $\delta^{13}C$ value was measured for Black Racers (−25.87‰) while the highest $\delta^{13}C$ values were measured in Corn Snakes (−18.07‰). The lowest $\delta^{15}N$ value measured in the milo treatment came from Black Racers (3.15‰) and the highest from Cottonmouths (6.88‰). Mean $\delta^{13}C$ values of Black Racers were most similar to sampled herpetofauna (−23.87‰ vs. −23.81‰; Fig. 1). Mean $\delta^{13}C$ values of Cottonmouths were more closely related to those of small mammals in the milo treatments (−21.78‰ for Cottonmouths vs. −21.44‰ for small mammals; Fig. 2). Corn Snakes showed the largest difference in $\delta^{13}C$ between milo and non-milo treatments. Within the milo treatment Corn Snakes $\delta^{13}C$ increased with age/size (Fig. 1).

Overlap of standard ellipses among species was greatest for the Eastern Rat Snake and least for the Cottonmouth (Table 3; Fig. 3). Of the five species examined, two species, the Black Racer and Corn Snake, had smaller isotopic niche spaces in the milo-treated sites (Table 4). Black Racers had the smallest isotopic niche breadth while Cottonmouths had the widest. Difference in isotopic niche space between milo- and non-milo-treated sites was least for Coachwhips, followed by Eastern Rat Snakes. Cottonmouths showed the greatest difference in niche space between milo-treated and untreated sites (Table 4). Overlap of ellipses within species, comparing milo-treated with non-milo-treated sites, revealed that Corn Snake and Cottonmouth shared no ellipse space between treatments, while Black Racer, Coachwhip, and Eastern Rat Snake shared isotope niche space between treatments (Table 3). Overlap of standard ellipses within species, between treatments indicated that within species, Corn Snake and Cottonmouth shared no ellipse space. Between treatments Black Racer, Coachwhip, and Eastern Rat Snake all shared ellipse space, within species (Table 3).

Table 3. Proportion of overlap in standard ellipse area between species sampled from the milo and not milo treated study sites. Ellipse areas calculated using size-corrected *standard ellipse areas* (SEA_c). Values for each species reflect the species listed in the column name relative to the species given in the row label. Values in bottom row are proportions of ellipse space shared by like species between treatments.

	Milo treated				
Species	Black Racer	Coachwhip	Corn Snake	Cottonmouth	Eastern Rat Snake
Black Racer	–	0	0	< 0.01	< 0.01
Coachwhip	0	–	26.73	38.09	29.85
Corn Snake	0	63.62	–	20.22	21.37
Cottonmouth	< 0.01	88.08	92.11	–	23.76
Eastern Rat Snake	< 0.01	54.43	16.37	82.92	–
	Not milo treated				
Species	Black Racer	Coachwhip	Corn Snake	Cottonmouth	Eastern Rat Snake
Black Racer	–	6.04	86.74	0	45.0
Coachwhip	2.99	–	8.1	0.22	51.45
Corn Snake	65.38	12.32	–	0	48.29
Cottonmouth	0	0.16	0	–	1.45
Eastern Rat Snake	24.48	56.53	34.86	2.22	–
Proportion shared between treatments	34.14	64.18	0	0	31.83

Table 4. Sample size-corrected standard ellipse areas (95%, SEA_c) calculated using $\delta^{13}C$ and $\delta^{15}N$ residual values from the linear models evaluated for each snake species (Jackson et al., 2011). Milo treated and Not milo treated reflects whether milo was available as a food supplement within study area.

	Milo treated		Not milo treated	
Species	*n*	SEA_c	*n*	SEA_c
Black Racer	24	1.26	48	1.58
Coachwhip	12	2.27	13	1.94
Corn Snake	11	1.78	12	2.04
Cottonmouth	7	4.84	5	2.38
Eastern Rat Snake	17	3.11	18	2.78

DISCUSSION

The results of this study demonstrate that the influence of seeding of milo for the management of Northern Bobwhite varies depending on the community members examined. Not surprisingly, small mammals and quail utilized the milo but herpetofauna (small reptiles and amphibians) did not. This difference allowed an assessment of the feeding ecology of the snakes and their trophic interactions, including trophic niche space and overlap. Herein, the influence of milo on the terrestrial food web is limited to species that utilize seeds or grain. Prey species that consume seeds and vegetation (e.g., Cotton Mouse, Cotton Rat, and Northern Bobwhite) utilized the milo in their diets, reflected by $\delta^{13}C$ values approximate to and $\delta^{15}N$ above that of milo. For those herpetofaunal species assessed, there was no evidence that milo contributed significantly to their diet based on $\delta^{13}C$. As consumers of granivorous birds and mammals, Corn Snakes, Coachwhips, and Cottonmouths all demonstrated at least some higher $\delta^{13}C$ values (closer to milo) in the milo-treated areas. The $\delta^{13}C$ values of Black Racers, a species appearing to prey largely on herpetofauna within our study area, and the Eastern Rat Snake, a generalist predator, did not change appreciably between treatments.

Among the species examined, Corn Snakes showed the largest difference in $\delta^{13}C$ values between milo and non-milo treatment areas, suggesting that they consume and assimilate a high proportion of granivorous mammals and birds, relative to other prey. However, $\delta^{13}C$ values measured for this species increased with age/size, reflecting an ontogenetic diet shift in which young Corn Snakes fed heavily on herpetofauna, then switched to a more mammal- or avian-based diet with age. This ontogenetic diet shift in Corn Snakes, while often reported, has not been clearly documented in the scientific literature. Higher $\delta^{13}C$ values in Coachwhips relative to length showed no difference between the milo-treated and untreated sites. This species also showed the greatest proportion of overlap in ellipses between treatments. These results likely reflect a similar diet in each treatment area. Few studies have quantified this species' trophic patterns (Halstead et al., 2008), yet the absence of a detectable change in carbon and nitrogen relative to milo supplementation supports the finding of Hamilton and Pollack (1956) that large snakes continue to use the same food items as smaller juveniles, including insects and arthropods, small mammals, reptiles, amphibians, and birds (Beane, 2013). Absence of differences in the $\delta^{13}C$ of Coachwhip between treatments could reflect that larger Coachwhips incorporate a greater proportion of prey that are more enriched in $\delta^{13}C$, prey that rely on sources of plant matter other than milo, that are available in both milo-treated and non-milo-treated areas.

For Black Racers, consistent $\delta^{13}C$ values between sites and across sized coupled with the pattern of enriched $\delta^{15}N$ with length likely reflects a size-related shift from insects and smaller herpetofauna (such as small lizards, Hamilton and Pollack, 1956) to larger prey items occupying a higher trophic level (Halstead et al., 2008). Evidence from the milo-treated and untreated areas indicates that the Eastern Rat Snake is also a dietary generalist, or alternatively, as an arboreal predator, may have consumed prey that did not consume milo. Although carbon isotope values of Eastern Rat Snakes were more enriched in the milo treatment, reflecting incorporation of mammalian and/or avian prey, there was no definable relationship between carbon isotope values and snake length in either treatment. Juvenile and adult rat snakes consume small mammals; whereas, younger, smaller snakes also consume reptiles and amphibians, but older, larger individuals tend to rely on mammals, birds and bird eggs (Hamilton and Pollack, 1956; Fitch, 1963; Jackson, 1974).

Based on $\delta^{15}N$ values Cottonmouths held the highest trophic position and the broadest prey base as associated with the largest isotopic niche widths. Of the species examined, Cottonmouths were the only species whose isotopic values suggested sexual dietary dimorphism with higher $\delta^{15}N$ values among females. Male Cottonmouths tend to be larger than females yet we did not find significant length differences between the sexes. This is consistent with the findings of previous studies in suggesting that it is unlikely that male and female Cottonmouths express ontogenetic shifts in prey (Vincent et al., 2004a). Head morphology differs between sexes, facilitating consumption of differently sized or shaped prey (Vincent et al., 2004b). For instance, Vincent et al. (2004b) found male Cottonmouths may consume a higher frequency of fish, arthropods, and mammals while Vincent et al. (2004a) comments on the preferential consumption of fish by male Cottonmouths. As fish were not included in the present analysis our data do not allow us to tease apart the contribution of fish to male and female Cottonmouths. Further study addressing differences in the contributions derived from terrestrial vs. aquatic sources, for this and other snake species, would be of interest.

The observed differences in snake carbon stable isotope values between the milo- and non-milo-treated areas suggest one of two possibilities: 1) snake species did not feed differently between treatments but the isotope values in prey differed by treatment, or 2) snakes focused on prey items that differed in abundance by treatment, changing their feeding ecology to match prey availability. In either case, the presence of milo helped to reveal the feeding ecology of snake species. Three of the five species examined were characterized by greater isotopic niche breadth in milo-treated areas. Only two species, the Black Racer and Corn Snake, had smaller isotopic niche breadth in the milo treatments, which might suggest focusing on a more abundant prey within milo treatments. As the addition of milo may have increased the breadth of isotope values in the community, it remains uncertain whether the observed differences in niche space reflect distinct diets, variation in isotope values within the ecosystem, or some combination of both.

Although not resolved (Luiselli, 2006), evidence suggests that snake diet can relate to prey availability, competition between species, sex- and age-based size differences, interspecific competition within the community and population-specific preferences (Fitch, 1982; Halstead et al., 2008). When competitors are present there is a tendency for some snake species to shift from a general diet toward a more specialized diet (Mori and Vincent, 2008; Willson et al., 2010). Although we did not collect information on snake abundance or density, the restricted niche breadth of the two species (Corn Snake and Black Racer) may provide evidence of such competition. Typically considered a generalist forager, the ^{13}C isotopic values of the Black Racers indicated minimum contributions from mammalian prey; rather, they appeared to feed largely on herpetofauna. Concomitant with an increase in Corn Snake size, feeding switched from one consisting largely of herpetofauna to a more mammalian-based diet. Furthermore, Black Racers and Corn Snakes both exhibited smaller niches in the milo-treated sites, further supporting specialist feeding by these species within the milo treatment.

Although the results of this study expand our working knowledge of community interactions, more research is needed to assess the impact of supplemental feeding at the community level in these systems. Such research could be married with these stable isotope results to give an ecosystem approach to assessing the influence of supplemental feeding in southeastern ecosystems while also providing novel insight into snake ecology and predator–prey dynamics.

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