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Trophic Interactions in a Semiaquatic Snake Community: Insights into the Structure of a Floodplain Food Web

Cynthia M. Carter
Eastern Illinois University
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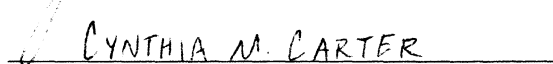RE: Preservation, Reproduction, and Distribution of Thesis Research

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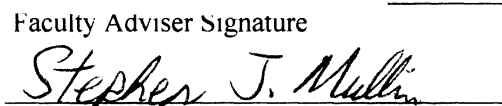
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Trophic interactions in a semiaquatic snake community:

Insights into the structure of a floodplain food web.

BY

Cynthia M. Carter

THESIS

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ABSTRACT

Food webs provide a useful conceptual framework for evaluating the relationships that exist within ecological systems. Characterizing the interactions within these webs can improve our understanding of how communities are structured and what mechanisms stabilize them. Untangling these interactions can be an intractable problem in complex systems and insights gained from conventional methods are often accompanied by inherent sources of bias. This study used stable isotope analysis, an alternative to traditional methods, to investigate the roles and relative contributions of consumers at the top of a food web to community structure and stability. I compared the niche parameters of five syntopic semi-aquatic snake species using the ratios of naturally occurring carbon and nitrogen isotopes to determine their relative trophic positions and estimate the contributions of potential prey sources to their diets. Analyses using Bayesian mixing models revealed evidence of niche partitioning among consumer groups and indicated that competitive dynamics have helped to shape the structure of this community. I identified ontogenetic differences in the trophic niches occupied by distinct age classes from three consumer species. I also detected temporal shifts in trophic structure that might be the result of intra-annual variation in resource availability. While competition appears to play a role in structuring this community, the trophic niches occupied by consumer groups seem to be somewhat plastic. Temporal shifts in resource availability have the potential to influence not only the relationships among competing consumers, but also their interactions with prey groups. Future research should examine how periodic fluctuations in prey abundance influences the connectivity, and by extension the stability, of this community.

DEDICATION

For MEBC, SLC, and KRLN

ACKNOWLEDGMENTS

"Working hard is not just effort– it's learning. It's trying new things, things that interest you that you might be scared to do or think you won't be able to do. Learning more is all about taking chances."

– Dolly Parton

Dr. Stephen Mullin and I took a chance when we developed this project. At the time, there were plenty of reasons why it might have been smarter to take things in a different direction. Our ideas were logistically challenging and the risk that we might not succeed was very real. But my fear of falling flat on my face, and Steve's practical nature were superseded by our fascination with the critters and questions at the heart of this research. I am incredibly grateful for his unwavering encouragement of my enthusiasm. I am also thankful for the support and guidance of my committee members, Dr. Zhiwei Liu and Dr. Robert Colombo. Collectively, they have taught over half of the courses I have taken as a graduate student and have both added new dimensions to my research interests. Their counsel has been priceless.

There is not doubt that this research could not have happened without my collaborators at Mingo National Wildlife Refuge. The entire staff provided me with invaluable assistance throughout my project. I am particularly grateful for the support of Ben Mense, Brad Pendly, and Lindsey Landowski. I felt more at home while at Mingo than I ever did in Charleston, and the idea of concluding my work there is truly heartbreaking. This research also would not have been possible without permission from the U.S. Fish and Wildlife Service and the Missouri Department of Conservation and, perhaps more importantly, funding from Eastern Illinois University in the form of a graduate assistantship, a Graduate

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INTRODUCTION

Factors that shape and maintain ecological systems have long been the focus of ecological investigations (Darwin 1859, Elton 1927, Odum 1953). Trophic webs provide one of the most useful conceptual frameworks for evaluating the interactions within communities and determining how they influence characteristics like structure and resilience (Dunne 2006, Layman et al. 2015, Lindeman 1942, MacArthur 1955, Paine 1980). Ongoing anthropogenic landscape alterations and attendant declines of biological diversity highlight the need for studies examining the mechanisms that stabilize these webs in order to predict how niche parameters might respond to shifts in community composition (Hooper et al. 2005, Rooney et al. 2006).

The complexity of interactions within food webs, including pathways of energy flow, has been proposed as an important factor contributing to the stability of communities (MacArthur 1955, Odum 1953). Under this paradigm, stability is hypothesized to increase with the number of connections in a food web, and fluctuations in the populations of well-connected species should be less likely to impact the rest of the community than those with limited, strong connections (Dunne et al. 2002, MacArthur 1955). Simplified food chains that include species with highly specialized diets, and therefore strong interactions, are thought to be susceptible to disturbances that might produce trophic

cascades (Finke and Denno 2004). Conversely, highly connected generalist species should help to stabilize food webs because the network of pathways that pass through them dampen their impacts on other species, and because their populations tend to be resilient to changes in resource availability (McCann et al. 1998, McKinney 1997, Purvis et al. 2000).

Similarity in resource use among species is another aspect of community structure that might contribute to stability. Interspecific competition for resources is widely viewed as an important factor shaping communities, though its precise role has been a subject of debate among ecologists (Connell 1983, Elton 1946, Hardin 1960, Schoener 1982, Tilman 1982). Niche partitioning (low overlap) among consumers might reflect strong competitive interactions among species for limited resources (Pianka 1974). Alternatively, trophic redundancy (high overlap), which occurs when multiple consumers share a trophic niche, might reflect relaxed competition among consumers, and can influence community stability by improving resilience to disturbances (Johnson 2000, Lawton and Brown 1993).

Unfortunately, untangling the interactions that occur within food webs is often challenging, particularly in complex systems. Dietary analysis is a common approach used to characterize niche parameters. Conventional methods of obtaining dietary information are often accompanied by logistical and

conceptual constraints, and are limited in the amount of information they provide (Bearhop et al. 2004, Newsome et al. 2007). For example, analyses of gut and fecal content provide data on diet composition that range over brief temporal scales and often do not accurately reflect proportional contributions of prey items (Votier et al. 2003). These methods also require that partially or fully digested prey items be identified accurately, and assume that all ingested items are equally assimilated by consumers (Bearhop et al. 2004).

Stable isotope analysis (SIA) has become an increasingly popular alternative approach to food web studies and is not limited by the same constraints as conventional methods (Bearhop et al. 2004, Layman et al. 2012). This technique offers insight into the trophic structure of communities by comparing the relative abundance of naturally occurring carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes in consumer tissues. Because the ratios of these isotopes are predictably altered as they move up the food chain, they can be used to estimate the position of consumers relative to other organisms in a trophic web. Carbon isotope ratios are influenced by the photosynthetic pathways of primary producers and, because they are largely conserved throughout food webs, provide information about the source of carbon nutrients (Peterson and Fry 1987). Nitrogen isotope ratios can be used to identify the trophic level of a consumer because the heavier isotope (^{15}N) is preferentially

incorporated each time nutrients from ingested tissues are assimilated (Peterson and Fry 1987, Post 2002). Collectively, this information characterizes the "isotopic niche" occupied by an organism (Newsome et al. 2007), which can be used to examine aspects of the more intractable ecological niche and hereafter is used interchangeably with the term "trophic niche." The rate at which isotopic information is incorporated varies among types of tissues, and therefore comparisons of multiple tissues can provide dietary information at different time scales (Phillips and Eldridge 2006, Newsome et al. 2007). The ability to conduct SIA using multiple tissues and time frames also makes this approach less susceptible to temporal bias in sample collection than traditional methods (MacNeil et al. 2005, Pinnegar and Polunin 1999). The applications of SIA to food web studies offer quantitative insight into factors influencing community dynamics and can reveal pathways of energy transfer (Parnell et al. 2010, Semmens et al. 2009).

Characterizing the complex interactions that shape communities is an essential step towards understanding the mechanisms that help to stabilize them against disturbances. Using SIA, I examined the niche parameters of consumers in a semi-aquatic community in the Mississippi Alluvial Valley. Of particular interest were the relative contributions of top predators, specifically snake species, to trophic dynamics and the degree of dietary overlap among them. I

estimated the contributions of candidate prey species to predator diets,

quantified the trophic niche occupied by each species and considered how

niche parameters might respond to shifts in community composition. I also

examined how trophic niche dimensions varied among age classes and across

time. This research is unique from prior studies of semi-aquatic food webs in its

taxonomic scope, geographic location, and analytical approach to examining

trophic interactions.

Semi-aquatic snake assemblages have been shown to exhibit low dietary

overlap among species and, therefore, strong niche partitioning (Camp et al.

1980, Durso et al. 2013, Luiselli 2006, Mushinsky and Hebrard 1977, Willson et

al. 2010). I predicted the snakes in this community would occupy distinct trophic

niches with minimal overlap among species. Because floodplains are dynamic

systems that experience seasonal shifts in productivity (Junk et al. 1989, Tockner

and Stanford 2002), I also expected that consumer niche dimensions would vary

among tissue types, particularly for generalist species, to reflect temporal

variation in resource use (Willson et al. 2010). Snakes are gape-limited

predators, meaning that the dimensions of their mouths limit the maximum prey

size that can be consumed by a snake of a given body size. Thus, the diets of

juvenile snakes are restricted to smaller prey items when compared to adult

conspecifics. Prey size does not necessarily scale up with predator growth,

however, meaning adult snakes have the potential to consume both large and small prey (Andreadis and Burghardt 2005, Gibbons and Dorcas 2004). I predict that the snake species in this community will exhibit ontogenetic diet shifts, and that differences in trophic position between age classes will be more pronounced in species where adult diets are specialized (Mushinsky et al. 1982, Werner and Gilliam 1984).

METHODS

Study Site

The southeastern lowland region of Missouri encompasses the northernmost extent of one of the most imperiled ecosystems in the United States -- the Mississippi Alluvial Valley. This system has experienced unparalleled declines of bottomland hardwood forests and substantial alterations to its hydrologic regime over the last 250 years as a result of deforestation, agricultural conversion, and the construction of river-engineering structures (Alexander et al. 2012, Gardiner and Oliver 2005, Noss et al. 1995). In 1983 it was estimated that only two percent of pre-settlement bottomland hardwood forests remained in southeastern Missouri (Vaught and Bowmaster 1983). The dynamic nature of this floodplain system once promoted high levels of regional biodiversity. However, anthropogenic habitat alteration and the suppression of seasonal flooding have altered the suite of ecological processes that maintained this diversity, and both terrestrial and aquatic communities have experienced marked declines in richness and abundance (Baker et al. 1991, Ward and Stanford 1995).

Mingo National Wildlife Refuge (MNWR) in Stoddard and Wayne counties contains the single largest intact area of bottomland hardwood forests remaining in Missouri. Situated at the junction of the Mississippi Lowlands and

the Ozark Plateau, MNWR spans 8,378 ha, including 6,070 ha of bottomland

hardwood forests and 1,416 ha of marshland (USFWS 2006). Prior to its

acquisition by the U.S. Fish and Wildlife Service (USFWS) in 1944, much of the

swamp was drained and deforested; however, targeted restoration and

management efforts in the intervening years have restored MNWR as a regional

wildlife stronghold (USFWS 2006). The hydrology of this site is manipulated to

mimic naturally-occurring seasonal floods that have been largely suppressed by

the construction of levees. These floods play a critical role in the life cycles of a

wide range of floodplain species (Heitmeyer et al. 1989).

Focal Predators

Trophic niche dimensions, including diet, were characterized for five

species of semi-aquatic snakes, belong to three subfamilies within two families,

in this community. *Farancia abacura* (Colubridae: Xenodontinae; Red-bellied

Mudsnakes) is an a species of conservation concern in Missouri belonging to a

subfamily whose ecology and natural history are poorly understood, presumably

because of their secretive nature. The limited, and largely outdated, literature on

this species (e.g., Dabney 1919, Dundee et al. 1989, Meade 1946, Reynolds and

Solberg 1942, Semlitsch et al. 1988) indicates that they are dietary specialists,

feeding primarily on elongate amphibians (e.g., *Amphiuma* and *Siren*).

Agkistrodon piscivorus (Viperidae: Crotalinae; Western Cottonmouths) are opportunistic venomous predators known to consume a wide range of aquatic and terrestrial prey species, particularly fish and amphibians (Gloyd and Conant 1990, Savitzky 1992). The colubrid subfamily Natricinae is represented by three species, *Nerodia fasciata* (Southern Watersnakes), *N. rhombifer* (Diamond-backed Watersnakes), and *Thamnophis proximus* (Western Ribbonsnakes). *Nerodia fasciata* is a mid-sized watersnake known to commonly consume fish and amphibians, and exhibits ontogenetic diet shifts (Gibbons and Dorcas 2004, Mushinsky et al. 1982). The natural history of *N. rhombifer* is similar to its congener, except that it depredates primarily fish species and, to a lesser extent, amphibians (Ernst and Ernst 2003, Gibbons and Dorcas 2004). *Thamnophis proximus* is the smallest of the focal consumers examined in this study, and also the most terrestrial. The diet of *T. proximus* predominately consists of anuran prey items; however they have been documented to consume fish, salamanders, and invertebrates (Hampton 2008, Rossman 1996).

Field Methods and Sample Collection

Snakes and candidate prey species were captured contemporaneously at MNWR between September 2013 and October 2014. I focused my sampling efforts on areas of the refuge where the target species had been previously

observed; however, surveys were conducted in nearly all habitats capable of supporting these taxa. I employed both active and passive trapping methods including the use of aquatic drift fences, rubber cover mats, minnow traps, rectangular funnel traps, aquatic barrel traps containing mesh funnels, hoop nets, seines, dip nets, and visual encounter surveys (Dodd 2010, McDiarmid et al. 2012). I conducted surveys at varies times, both during the day and at night, and in a wide range of weather conditions to facilitate comprehensive sampling of the community.

Upon capture I recorded the time and location of collection for specimens of all target species. For all snakes, I recorded the sex, snout-vent length (SVL; ±1 mm), tail length (TL; ±1 mm), and mass (±0.1 g). I collected tissue from the ventral scales (approximately 5-10 scale clips) and blood from the subcaudal vein (0.2–0.5 ml) from each individual snake, with the volume and method of tissue collection (e.g., needle gauge) selected based on the size of the individual. Immediately after collection, I separated red blood cells (RBC) from plasma by centrifuging samples at 1,300 rpm for approximately 10 minutes. Scale and blood tissues were stored in individual tin capsules and 1.5-ml microcentrifuge tubes, respectively, at -20°C to prevent sample degradation until they could be transported to a lab facility for SIA. I assigned each individual snake a unique identification number, which was branded onto their ventral

scales using a medical cautery unit (Winne et al. 2006) for future identification. Additional observations regarding body condition, abnormal characters, or the presence of stomach contents were recorded. Snakes were subsequently released at their site of capture.

Candidate prey species were promptly euthanized and stored whole in individual aluminum foil packages at -20°C. All vertebrates encompassed by this research were handled in accordance with an approved protocol (#13-006) from the Eastern Illinois University Institutional Animal Care and Use Committee.

Stable Isotope Analysis

I dispensed 10 μl of each plasma and RBC sample into individual, pre-weighed tin capsules. I freeze dried all snake tissue samples for 48 hours to ensure complete dehydration. Water was removed from whole prey samples using either a freeze drier or a drying oven until a stable dry mass was achieved (5-7 days or 48 hours at 40°C, respectively). Mass-related detection thresholds of the equipment used for SIA constrained the amount of each individual prey item that could be analyzed. This restriction required that I obtain subsamples of large prey items. To ensure all subsamples would be representative of the whole organism, I ground each prey item into a homogenous matrix using a cryogenic grinding mill (Spex SamplePrep 6870 Freezer/Mill with liquid nitrogen). Samples

too large to fit into the mill were first pulverized using a 1000 watt quad-blade blender (Ninja NJ600 Professional Blender) and the resulting mixture was ground in the mill. Homogenized samples were transferred to separate glass vials and stored at room temperature. I weighed out 0.06–1.2 mg of each prey and snake scale sample and placed them into individual tin capsules. All tin sample capsules (prey, scale, blood, and plasma) were crimped closed and compressed to achieve uniform capsule dimensions. Samples were processed by the Odum School of Ecology's Analytical Chemistry Laboratory at the University of Georgia (Athens, GA) using a Carlo Erba CHN elemental analyzer and a Thermo Finnigan Delta C mass spectrometer. The ratios of ^{12}C to ^{13}C and of ^{14}N to ^{15}N stable isotopes were measured against Pee Dee Belemnite (PDB) and atmospheric air standards, respectively, and reported in delta (δ) notation as parts per thousand (‰).

Statistical Analyses

The size distributions for each snake species were divided into three size classes (juvenile, subadult, and adult) in order to examine any ontogenetic shifts in diet. The tissues of neonate snakes have been demonstrated to retain isotopic signatures transferred from their mothers, and therefore isotopic analyses of particularly young snakes might not necessarily reflect the range of resources

they have consumed since birth or hatching (Pilgrim 2007). To determine if any of the juvenile snakes collected during this study exhibited maternal signatures I generated scatter plots illustrating the relationship between subject SVL and nitrogen values for each species. I classified small snakes with elevated isotopic nitrogen signatures (relative to conspecifics in larger size classes), as possessing residual maternal signals. These individuals were censored from analyses of isotopic data to ensure they would not obscure patterns driven by the prey actually consumed by snakes.

All statistical analyses were performed in the R environment (R Core Team 2015). I used analyses of variance (ANOVA) to examine the effects of various factors on both $\delta^{13}C$ and $\delta^{15}N$ which, if significant and not properly accounted for, could confound consumer diet models. When ANOVAs revealed significant effects, Tukey pair-wise comparisons were made post-hoc to evaluate differences between groups. I performed ANOVAs to test for isotopic differences between predator tissue types (RBC, plasma, and scale) as a function of size class, sex, and collection season for each snake species. I also used ANOVAs to detect isotopic differences between prey species. The results of these analyses guided my decisions regarding the structure of both consumer and prey datasets used in subsequent models, such that variance was maximized between groups and minimized within them.

I modeled consumer diets using a mixing model software application, Stable Isotope Analysis in R (SIAR v4.1.2; Parnell et al. 2010) SIAR uses a Bayesian approach to estimate the proportional contributions of prey sources to consumer diets and provides the estimates in the form of probability distributions. These models account for variability in the isotopic composition of both consumers (isotopic values reported for individuals of each snake species) and sources (means and standard deviations of isotopic values reported for groups of prey species). SIAR also allows for the incorporation of trophic enrichment factors (TEF; mean ± 1 SD) for source groups to correct for isotopic fractionation that occurs when source tissues are assimilated by predators. I used the TEF values supplied by the developers of SIAR, which closely mirror the average fractionation values reported in the literature (1.63 ± 0.63 ‰ for δ^{13}C and 3.54 ± 0.74 ‰ for δ^{15}N; Peterson and Fry 1987). I set each model to run for 1,000,000 iterations, with a burn in of 400,000 iterations.

The isotopic niches of consumer groups were modeled using the Stable Isotope Bayesian Ellipses in R (SIBER) routines within SIAR (Jackson et al. 2011). Total niche area was calculated for each consumer group using convex hull areas (TA), which samples all individuals within each group. Because this metric is sensitive to outliers and can over-estimate niche breadth, core niche area was calculated using Bayesian standard ellipse areas correct for sample size (SEAc).

RESULTS

Consumer Groups

During Fall (mid September – early November) of 2013, and Spring (April –

May), Summer (June – July), and Fall (late August – early November) of 2014, I

captured 162 individual snakes across three seasons (n = 45 in Fall, 68 in Spring,

and 40 in Summer). A total of 446 tissue samples (n = 135 of plasma, 159 of

RBC, and 152 of scale) were collected for SIA. Of these, 13 plasma samples

collected from juvenile snakes were considered to possess residual maternal

signatures and were censored from further analyses. Because RBC and scale

tissues integrate dietary information over longer time scales than plasma (Fisk et

al. 2009), and because the age of juvenile snakes is difficult to estimate

accurately, I excluded blood and scale samples collected from juvenile size

classes to prevent residual maternal signals in those tissues from influencing

interpretations of dietary content.

The $\delta^{15}N$ signatures of plasma tissue were similar between sexes for all

species (ANOVA, p > 0.14); corresponding $\delta^{13}C$ values were also similar to one

another (p > 0.13), with the exception of N. fasciata (ANOVA, $F_{1,51}$ = 1.71, p =

0.04) wherein males were enriched in $\delta^{13}C$ and depleted in $\delta^{15}N$ relative to

females. Isotopic differences were detected between size classes of F. abacura

($\delta^{15}N$: $F_{1,21}$ = 75.80, p < 0.001; $\delta^{13}C$: $F_{1,21}$ = 7.81, p = 0.01), N. fasciata ($\delta^{15}N$: $F_{1,51}$ =

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27.00, $p < 0.001$; $\delta^{13}C$: $F_{1,51} = 15.24$, $p < 0.001$), and *T. proximus* ($\delta^{15}N$: $F_{1,9} = 28.98$, $p < 0.001$; $\delta^{13}C$: $F_{1,9} = 14.17$, $p < 0.005$), with adult plasma exhibiting enriched $\delta^{13}C$ and $\delta^{15}N$ values relative to that of juveniles for each species (Table 1). Because the isotopic spaces occupied by each of these age classes were distinct, I treated them as independent groups in all future analyses.

I was unable to discern any ontogenetic differences in the isotopic signatures of *A. piscivorus* ($\delta^{15}N$: $F_{1,13} = 2.59$, $p = 0.13$; $\delta^{13}C$: $F_{1,13} = 3.38$, $p = 0.09$), *N. rhombifer* ($\delta^{15}N$: $F_{1,10} = 1.86$, $p = 0.20$; $\delta^{13}C$: $F_{1,10} = 0.09$, $p = 0.77$); therefore, I did not separate either of these species by life-history stage (Table 1). Juvenile *N. fasciata* were the only consumers that exhibited seasonal variation in the isotopic composition among plasma samples ($p > 0.08$ for either element in all other species). Juvenile and adult *T. proximus* were excluded from tests of seasonal variation because of small sample sizes. I detected a decrease in $\delta^{13}C$ between Spring and Summer for juvenile *N. fasciata* ($F_{2,37} = 23.16$, $p < 0.001$; Tukey's post-hoc, $p = 0.006$) and an increase in $\delta^{13}C$ between the summer and Fall seasons (Turkey's post-hoc, $p < 0.001$). Juvenile *N. fasciata* also exhibited a seasonal shift in $\delta^{15}N$, with Fall samples being enriched relative to those collected in the summer ($F_{2,37} = 18.65$, $p < 0.001$; Tukey's post hoc, $p = 0.002$).

Agkistrodon piscivorus was the only species whose $\delta^{15}N$ values differed between tissue types, with scale being more enriched than RBC tissues (Table 3:

$F_{2,49}$ = 3.41, p = 0.04; Tukey's post-hoc, p = 0.03). The only group whose tissues differed in their δ^{13}C composition was adult *F. abacura*, wherein plasma was depleted in nitrogen relative to RBC tissue (Table 3: $F_{2,22}$ = 4.83, p = 0.02; Tukey's post-hoc, p = 0.02). In plasma samples, all juvenile groups were depleted in nitrogen isotopes relative to adults (Figure 1A). Juvenile *N. fasciata* exhibited the widest range of δ^{15}N values, while the narrowest range was encompassed by adult *T. proximus*. The widest range of δ^{13}C values occurred in *N. rhombifer*, and the most narrow, in juvenile *T. proximus*.

Prey groups

Under the same temporal and spatial conditions used for snake captures, I collected 290 individual candidate prey items belonging to 21 species. Of these individuals, 41 were collected in Spring, 167 in Summer, and 82 in Fall. Prey species were classified into seven distinct groups with minimal overlap between groups (Table 2). Adult aquatic salamanders were the most ^{15}N enriched of all prey groups, with sirens exhibiting depleted ^{15}N and enriched ^{13}C values relative to amphiumas. Anuran samples aggregated into two groups, with *Acris blanchardii*, *Anaxyrus americanus*, *A. fowleri*, and *Hyla versicolor* making up the most ^{13}C enriched group compared to other prey taxa. *Lithobates catesbeianus*, *L. clamitans*, *L. sphenocephalus* and *H. cinerea* made up the other anuran

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group, which was relatively ^{15}N enriched and ^{13}C depleted. Ranid tadpoles were the group of prey taxa having the most depleted values in both ^{13}C and ^{15}N. All fish species were combined to form a single group that was slightly depleted in ^{15}N relative to siren, and slightly enriched in ^{13}C relative to ranid tadpoles. Larval and juvenile aquatic salamanders were combined to form a single group, which had enriched ^{13}C and ^{15}N values relative to ranid tadpoles.

SIAR Models

Mixing models based on plasma tissue showed that juvenile *F. abacura* and *N. fasciata* were the only groups where a single prey source was estimated to contribute more than 50% of their diet. For both species, ranid tadpoles were the largest estimated contributor (74% and 82%, respectively; Figure 3). The second largest contributors to these two groups were fish (10% in juvenile *F. abacura*) and terrestrial frogs (7% in juvenile *N. fasciata*). The diets of adult *N. fasciata* included the same major contributors as their juvenile conspecifics, though in different proportions (37% ranid tadpoles, 22% terrestrial anurans; Figure 2). Ranid tadpoles and terrestrial anurans made up the majority of adult *N. fasciata* diets (37% and 22%, respectively; Figure 2). Adult *F. abacura* diets were less specialized those of juveniles—fish were the largest single contributor (22%) followed by amphiumas (18%), siren (17%), juvenile salamanders (14%)

and ranid tadpoles (14%). The opposite was true for *T. proximus*, with juveniles

having more varied diets than adults. The largest contributors to juvenile *T.*

proximus diets were ranid tadpoles (19%), terrestrial anurans (18%), aquatic

anurans (17%), and juvenile salamanders (16%; Figure 3). Adult *T. proximus* were

estimated to primarily consume aquatic and terrestrial anurans (19% and 25%;

Figure 2). The prey groups estimated to comprise the largest proportion of *N.*

rhombifer diets were ranid tadpoles (25%), juvenile salamanders (19%), and fish

(18%; Figure 2). *Agkistrodon piscivorus* diets were similar in composition to

those of *N. rhombifer*, with ranid tadpoles (22%), juvenile salamanders (18%) and

fish (14%) making up relatively large proportions (Figure 2). However, the diets

of *A. piscivorus* also included aquatic (15%) and terrestrial (12%) anurans.

Estimates of diet based on plasma were largely supported by models

generated using RBC and scale tissues (Figures 2 and 3). Of all consumer

groups, *N. rhombifer* and adult *N. fasciata* dietary proportions were the most

consistent across tissue types (Figure 2). Adult *F. abacura* dietary estimates also

lacked much variability. Proportions of juvenile salamanders in the diet of adult

F. abacura decreased in scale tissue models, however, and estimates of ranid

tadpole contributions decreased for both RBC and scale (Figure 2). The

proportions of terrestrial anurans estimated to contribute to adult *T. proximus*

diet were similar between plasma and scale tissue; however, estimates derived

from RBC tissue were nearly twice as high (48%; Figure 2). *Agkistrodon piscivorus* showed the greatest variability in dietary proportions across tissues. Fish and juvenile aquatic salamander estimates for this snake species were lower for RBC and scale tissues (RBC 2% and 6%, scale 2% and 4%, respectively). Estimates of amphiuma and siren contributions were much larger for scale tissue than for either plasma or RBC (13% and 17%, respectively; Figure 2).

SIBER Models

Plots generated in SIBER displayed convex hulls (TA) and standard ellipse areas (SEAc) of consumer groups, showing the extent of isotopic space and the core isotope niche, respectively, occupied by each group (Figure 4). The TA of each group overlapped with that of at least one other group, regardless of the tissue type used in the model (Figure 4A). In models generated using plasma tissue, neither adult *F. abacura*, juvenile *F. abacura*, nor adult *T. proximus* exhibited any overlap in their SEAc with any other group (Figure 3B). Models run using RBC and scale tissue showed overlap in the SEAc of adults of each species with that of at least one other group (Figure 4B). The consumer groups with the largest SEAc were juvenile *N. fasciata* (plasma), adult *N. fasciata* (RBC), and adult *F. abacura* (scale). The smallest SEAc for all tissues belonged to adult *T. proximus.*

DISCUSSION

Isotopic niche width, position, and overlap of consumer tissues varied

widely across this community. When considered along with dietary estimates,

these niche parameters revealed distinct differences in the roles that consumers

occupy within the community structure. Consumer groups with overlapping core

niche areas were often estimated to have similar prey groups contributing to

their diets; however, the proportional contributions of these prey were always

distinctly different. The opposite was also true — species with similar diet

composition were shown to occupy discrete regions of isotopic space.

Ontogenetic shifts in diet and niche parameters were identified in several

consumer species where different age classes occupy distinct trophic niches.

Comparisons of diet and core niche area among tissue types of adult consumers

revealed patterns in resource use indicating that temporal variation in prey

availability might be an important factor influencing trophic interactions in this

community.

Models using plasma tissue showed that juvenile and adult snakes occupy

distinct trophic levels, with juveniles tending to be depleted in both $\delta^{15}N$ and

$\delta^{13}C$ relative to adult conspecifics, and with no overlap in SEAc between age

classes. Of all groups included in this model, juvenile *F. abacura* and *N. fasciata*

exhibit the highest degree of dietary specialization, with tadpoles estimated to

make up over one-third of their respective diets. In addition to ranid tadpoles,

mixing models also estimated that juvenile *F. abacura* diets included fish. This

finding is further supported by observations of both tadpoles and mosquito fish

(Gambusia affinis) in the regurgitated contents of captured specimens during

this study. Very little is known about the ecology of juvenile *F. abacura*, and at

the time of this study I could find no diet records or feeding observations of wild

individuals in the published literature, though several anecdotal reports

document largely unsuccessful attempts to feed captive individuals (e.g.,

Goldstein 1941, Martin 1998, Meade 1934, Reynolds and Solberg 1942);

therefore, it is unclear whether or not the pattern of resource use in this

community is unique. The SEAc of juvenile *F. abacura* was slightly smaller than

that of adult conspecifics. In contrast, juveniles had a much larger TA than

adults; however, the size of the juvenile TA appears to have been influenced by

a single outlying point located within the SEAc of adults. The SVL of this outlying

individual approached the upper size limit used to define the juvenile size class,

and this might explain the similarity of this individual's trophic position to that of

adults. The isotopic niche of adult *F. abacura* spanned a larger range of $\delta^{13}C$

values than that of juveniles. This indicates that they were assimilating nutrients

from a wider variety of sources, a notion that was supported by the more-

generalized diet estimated for this group by mixing models. The relative width

of adult *F. abacura*'s isotopic niche and the large, nearly equal, estimated

contributions of fish, sirens, and amphiumas to their diet provides evidence that

this species is less specialized than previously thought (e.g., Buck 1946, Dundee

et al. 1989, Durso et al. 2013, Martin 1998, Neill 1964). The lack of individuals

occupying the isotopic space between adult and juvenile ellipse areas indicates

that ontogenetic diet shifts in this species are driven by the ability of adults to

consume prey sources not accessible to juveniles (i.e., amphiuma and siren).

Additional sampling of intermediate sized individuals is needed to evaluate the

exact nature of this shift. Ontogenetic differences in the skull morphology of *F.*

abacura supports the idea that prey handling abilities differ among size classes

in this species (see Hampton and Kalmus 2014).

As with *F. abacura*, the SEAc of *N. fasciata* was similar for both age classes,

though the ellipse representing juveniles was slightly smaller than that of adults.

In contrast, the TA represented by juvenile *N. fasciata* was nearly six times larger

than that of adults (and was the largest among all species). While inter-individual

differences in diet or variability in the $\delta^{13}C$ content of their primary prey could

explain this high degree of variation, seasonal shifts in resource use (e.g.,

increased consumption of aquatic anurans during Spring) might also contribute

to their relatively large isotopic niche. Model estimates of adult *N. fasciata*

revealed a more generalist strategy, however, with the two largest dietary

contributors (tadpoles and terrestrial anurans) being the same for both age classes. These groups shared an overlap in the range of $\delta^{13}C$ values, which indicates that some portion of their diets come from the same basal resources. The SEAc of adult *N. fasciata* also included values more enriched in $\delta^{13}C$ than those of juveniles, which might reflect their use of a larger foraging area that includes a greater diversity of resource pools (Hammerschlag-Peyer et al. 2011). *Nerodia fasciata* appears to incorporate resources from both terrestrial and aquatic systems and might couple distinct paths of energy flow through this trophic web; however, this effect might be diminished by the relatedness of these prey groups (Rooney et al. 2006). These findings differ slightly from the common perception that *N. fasciata* is a generalist species that experiences an ontogenetic diet shift away from fish and towards anurans with growth (Gibbons and Dorcas 2004, Mushinsky et al. 1982). I found no evidence that juvenile *N. fasciata* were consuming fish species during the course of this study, but did observe both ranid tadpoles and Blanchard's Cricket Frogs (*Acris blanchardii*) in the regurgitated stomach contents of captured individuals.

Of the three species with separate size classes, juvenile and adult *T. proximus* had the most similar diet compositions but the largest discrepancy in the sizes of SEAc, with the ellipses of adult *T. proximus* being the smallest among all consumer groups. Estimates of diet based on plasma indicated that

anuran species (both aquatic and terrestrial groups) were an important prey resource to *T. proximus* regardless of age class. Juvenile diets incorporated greater proportions of aquatic prey (fish, ranid tadpoles, and juvenile salamanders) than adults, which might account for the differences in the $\delta^{13}C$ values and SEAc between age classes. These two groups contained the smallest sample sizes of all eight focal consumers in the plasma model (*T. proximus*, n=6 juveniles, 5 adults). My conclusions about the ecology of *T. proximus* are limited by the relatively small sample size. Additional sampling of this population is needed to confirm the patterns in resource use observed here, and to determine what factors are driving ontogenetic shifts in the diet of this species.

Plasma based models of *Agkistrodon piscivorus* and *N. rhombifer* showed a high degree of overlap of SEAc between these species. Similarities in their $\delta^{15}N$ ranges indicate that these species occupy the same trophic level. Both species are centrally positioned within the range of carbon values exhibited by members of this food web, which supports the inference that both are generalist consumers. *Nerodia rhombifer* encompassed a wider range of $\delta^{13}C$ values than any other group in this model, including *A. piscivorus*, which is consistent with a diet that incorporates multiple sources of dietary carbon. Mixing models estimated that both *N. rhombifer* and *A. piscivorus* consumed prey groups from both terrestrial and aquatic habitats, albeit in differing proportions. This

conclusion is further supported by observations of *A. piscivorus* consuming

leopard frogs *(Lithobates sphenocephalus)* and by the presence of ranid

tadpoles, juvenile bowfin (*Amia calva*) and flier sunfish (*Centrarchus*

macropterus) in the regurgitated stomach contents of *N. rhombifer*. The high

degree of niche overlap between these two species provides some evidence

that they might compete for resources; however, the apparent integration of

multiple energy flow pathways by *N. rhombifer* probably dampens the intensity

of that competition (Layman et al. 2007). *Nerodia rhombifer* might play an

important role in stabilizing the trophic structure of this community by coupling

distinct food chains (Rooney et al. 2006).

Isotopic comparisons of multiple tissues improved the resolution at which

trophic relationships of adults of the consumer groups could be examined.

Differences in the assimilation rates of these tissues provided the opportunity to

examine the dynamics in trophic structure and identify temporal shifts in isotopic

niche dimensions (MacNeil et al. 2005, Reich et al. 2008). Examinations of

models using RBC and scale tissues showed an expansion the isotopic niche of

adult *F. abacura* (δ^{15}N), relative to plasma models. Increased variability of δ^{15}N

values in less metabolically-active tissues indicates that the diversity of prey

consumed by adult *F. abacura* fluctuates over time; however, little deviation in

their δ^{13}C range demonstrates consistent use of resource pools. For all three

tissues, mixing models estimated that amphiumas, sirens, and fish were major contributors to the diet of adult *F. abacura*, but only plasma and RBC models showed contributions from ranid tadpoles and juvenile salamanders. It is likely that the trophic niche of adult *F. abacura* fluctuates seasonally as a function of prey availability even though the consumption of primary prey sources was relatively consistent over time at this site.

The ranges of both $\delta^{15}N$ and $\delta^{13}C$ values shifted among tissues of adult *N. fasciata*. SEAc derived from RBC and scale tissues were more depleted in $\delta^{13}C$ than those from plasma, showing shifts in the basal resource pools accessed by this species over time, perhaps the result of seasonal differences in habitat use or prey availability. The range of $\delta^{15}N$ values in RBC tissue was much wider than in scale or plasma, and included more depleted values. Relative consistency in the range of $\delta^{15}N$ values in scale and plasma tissues shows that adult *N. fasciata* are largely consuming the same kinds of prey over long time scales; however, deviations in the values of RBC tissues indicate that there might have been a temporary fluctuation in the composition of their diet at an intermediate time interval. Mixing models showed some fluctuations in the estimated contributions of tadpoles and aquatic anurans across tissues, supporting the notion that types of prey consumed are consistent across time, but their relative contributions might periodically fluctuate.

The SEAc of adult *T. proximus* were the most variable among tissues, showing dramatic differences in both the shape and position of their isotopic niche. The range of δ^{15}N values encompassed by their SEAc was similar for both plasma and scale tissues, while the ellipse for RBC tissues included more depleted values, indicating a shift in diet to include higher proportions of prey species that occupy a lower trophic level. Both ellipses for RBC and scale tissues showed a shift in δ^{13}C composition toward more depleted values, with scale tissue showing the most extreme change, indicating a change in the carbon source of their consumed nutrients. Mixing models for *T. proximus* showed that the estimated contribution of ranid tadpoles was highest for scale tissues, and the contribution of terrestrial anurans was highest for RBC tissues. Despite my concerns regarding sample size for this species, consistency in the composition of diet estimated for adult *T. proximus* across all tissues is still informative. As with plasma, more RBC and scale samples are needed to determine whether or not the isotopic niche of this species changes over time.

As was the case with adult *N. fasciata*, the isotopic niche estimates for *N. rhombifer* from RBC and scale tissues were more depleted in δ^{13}C than those from plasma, similarly indicating seasonal shifts in habitat use or prey consumption. The range of δ^{15}N values encompassed by SEAc were slightly more enriched for scale and more depleted for RBC tissues, relative to plasma,

though the magnitudes of their $\delta^{15}N$ ranges (i.e., ellipse heights) are similar. This pattern is supported by the dietary estimates of mixing models for this species, which show that, relative to plasma, scale models estimate larger contributions of prey from higher trophic levels and RBC models estimate larger contributions of prey from lower trophic levels.

Differences in the position of SEAc of *Agkistrodon piscivorus* tissues mirrored those observed for *N. rhombifer*. Model estimates for *A. piscivorus* had the greatest amount of variation between tissues of all tested consumer groups. It appears that the diet composition and trophic position of this species varies appreciably over time. Terrestrial and aquatic anurans and ranid tadpoles are the only prey groups that were consistently estimated to contribute substantially to the diet of *A. piscivorus*, while the importance of salamander groups and fish fluctuates between tissues. These differences might reflect changes in the availability of certain prey over time, a generalist foraging strategy, or a combination of these factors.

Conclusions

Collectively, the mixing models provide evidence that competition among these semi-aquatic snake groups has helped to shape the structure of this community. Although there is considerable overlap in the types of prey being

consumed by these species, their relative contributions to snake diets are largely

dissimilar. These differences in resource use support the prediction that

consumer niches have been partitioned in this trophic web to minimize overlap

among species. I also found evidence of ontogenetic diet shifts in the trophic

niche of *F. abacura*, *N. fasciata*, and to a lesser degree, *T. proximus*. Other than

juvenile *F. abacura* and *N. fasciata*, none of the consumer groups examined in

this study showed dietary specialization on a single prey group. For adult

species, the estimated proportions of prey groups contributing to their diets

appear to shift over time. Consistent sampling of all species across seasons is

needed in order to determine the precise factors precipitating these changes.

Increasing the sample sizes for each season might also reduce variance in the

consumer groups and reveal seasonal differences in diet not detected in these

analyses. I encourage future investigators to consider incorporating measures of

seasonal variation in prey abundance into studies of food web dynamics (cf.

Willson et al. 2010). Additionally, determining the precise rate of isotopic

turnover for different consumer tissues would provide a more detailed view of

temporal dietary shifts. Fluctuations in the values of carbon sources might also

have influenced the results of my analyses. The use of tissues with slower

metabolic turnover rates (RBC and scale) that incorporate dietary information

over longer periods of time, however, make it unlikely that any differences had

biologically-relevant impacts on my interpretations (Heithaus et al. 2013).

Additional laboratory experiments are needed to empirically determine the precise trophic enrichment factors of $\delta^{13}C$ and $\delta^{15}N$ in each of the snake species used in this study. Despite its limitations, stable isotope analysis provides useful insights into the ecological role of these consumer and prey groups and the trophic interactions among them. Continued monitoring of this community is recommended given the dwindling availability bottomland forest habitat in this region and the continued alteration of the surrounding floodplain. Additional sampling during periods of unfavorable environmental conditions would provide added insight into the response of this community to shifting conditions its susceptibility to changing conditions (Willson et al. 2006). A clear understanding of how this community responds to stressors will aid management and conservation agencies in their continued efforts to preserve it.

TABLES

Table 1. Morphological measurements — snout-vent length (SVL) and tail length (TL) — and results from separate analyses of variance assessing differences in stable isotope ratios of nitrogen (δ^{15}N) and carbon (δ^{13}C) for juvenile and adult age classes of five species of snakes from Mingo National Wildlife Refuge (Stoddard and Wayne Counties, Missouri, USA) during 2013 and 2014. Species abbreviations used in tables and figures are listed in the second column. All values reported as means ± 1 SE. Sample sizes are given in parentheses. Within species, values not sharing at least one superscript for a particular isotope are statistically different ($p < 0.05$) as detected by Tukey post-hoc tests.

Species	Code	Age Class	SVL (cm)	TL (cm)	δ^{15}N	δ^{13}C
Farancia abacura	FAAB	Juvenile (14)	28.33 ± 1.99	4.22 ± 0.40	7.02 ± 0.20[A]	-30.51 ± 0.16[A]
		Adult (9)	88.91 ± 5.4	14.18 ± 0.59	9.5 ± 0.17[B]	-29.38 ± 0.44[B]
Agkistrodon piscivorus	AGPI	Juvenile (7)	53.6 ± 1.28	8.61 ± 0.48	8.55 ± 0.25[A]	-26.95 ± 0.39[A]
		Adult (9)	77.19 ± 2.18	13.38 ± 0.28	9.05 ± 0.24[A]	-28.19 ± 0.41[A]
Nerodia fasciata	NEFA	Juvenile (40)	23.83 ± 0.43	7.02 ± 0.12	6.16 ± 0.12[A]	-28.93 ± 0.21[A]
		Adult (13)	54.37 ± 4.44	14.82 ± 1.27	7.45 ± 0.25[B]	-27.27 ± 0.35[B]
Nerodia rhombifer	NERH	Juvenile (2)	49.00 ± 7.00	12.00 ± 1.00	8.30 ± 0.31[A]	-27.58 ± 0.38[A]
		Adult (9)	80.28 ± 3.14	18.50 ± 2.30	8.78 ± 0.24[A]	-27.83 ± 0.61[A]
Thamnophis proximus	THPR	Juvenile (6)	32.03 ± 2.44	13.35 ± 1.13	5.88 ± 0.41[A]	-26.95 ± 0.25[A]
		Adult (5)	57.92 ± 4.42	21.14 ± 0.99	8.67 ± 0.29[B]	-25.47 ± 0.30[B]

Table 2. Composition of prey source groups used in stable isotope mixing models. Sample sizes are given in parentheses for both groups and individual species. Source group abbreviations used in tables and figures are listed in the second column.

Group Name	Code	Species
Aquatic anurans (39)	AA	*Hyla cinerea* (10)
		Lithobates clamitans (8)
		Lithobates catesbeianus (3)
		Lithobates sphenocephalus (18)
Terrestrial anurans (37)	AT	*Acris blanchardi* (12)
		Anaxyrus americanus (6)
		Anaxyrus fowleri (10)
		Hyla versicolor (9)
Fish (119)	F	*Ameiurus melas* (11)
		Aphredoderus sayanus (28)
		Centrarchus macropterus (10)
		Elassoma zonatum (9)
		Esox americanus (13)
		Fundulus dispar (12)
		Gambusia affinis (6)
		Lepomis symmetricus (22)
		Lepomis gulosus (8)
Amphiumas (8)	A	*Amphiuma tridactylum* (8)
Adult Sirens (5)	S	*Siren intermedia* (5)
Juvenile salamanders (20)	JS	*Ambystoma maculatum larva* (7)
		Juvenile *Siren intermedia* (13)
Ranid tadpoles (62)	T	Larval *Lithobates sp.* (62)

Table 3. Results from separate analyses of variance assessing differences in stable isotope ratios (mean ± SE) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) among tissue types collected from six species of snakes at Mingo National Wildlife Refuge (Stoddard and Wayne Counties, Missouri, USA) between 2013 and 2014. Species abbreviations are defined in Table 1. Sample sizes are noted in parentheses for each tissue type. Within each species group, values sharing the same superscripts for a particular isotope are statistically similar as detected by Tukey post-hoc pair-wise comparisons

| Species	Age Class	$\delta^{15}N$			$\delta^{13}C$		
		Plasma	RBC	Scale	Plasma	RBC	Scale
FAAB	Juvenile	7.04 ± 0.18 (15)	–	–	-30.50 ± 0.15 (15)	–	–
	Adult			10.24 ± 0.31 (7)[A]	-29.38 ± 0.44 (9)[A]	-27.55 ± 0.44 (9)[B]	-27.78 ± 0.54 (7)[A,B]
AGPI	All	8.84 ± 0.17 (18)[A,B]	8.51 ± 0.20 (18)[A]	9.24 ± 0.21 (16)[B]	-27.72 ± 0.32 (18)[A]	-26.89 ± 0.30 (18)[A,B]	-26.59 ± 0.33 (16)[B]
NEFA	Juvenile	6.16 ± 0.12 (40)	–	–	-28.93 ± 0.21 (40)	–	–
	Adult	7.45 ± 0.25 (13)[A]	7.33 ± 0.29 (17)[A]	7.94 ± 0.25 (17)[A]	-27.27 ± 0.35 (13)[A]	-27.14 ± 0.32 (17)[A]	-27.26 ± 0.29 (17)[A]
NERH	All	8.63 ± 0.20 (12)[A]	8.31 ± 0.22 (14)[A]	8.98 ± 0.24 (14)[A]	-27.91 ± 0.47 (12)[A]	-28.05 ± 0.40 (14)[A]	-28.00 ± 0.32 (14)[A]
THPR	Juvenile	5.86 ± 0.41 (6)	–	–	-26.95 ± 0.25 (6)	–	–
	Adult	8.67 ± 0.29 (5)[A]	7.93 ± 0.24 (7)[A]	8.62 ± 0.18 (6)[A]	-25.47 ± 0.30 (5)[A]	-25.30 ± 0.26 (7)[A]	-25.83 ± 0.34 (6)[A]
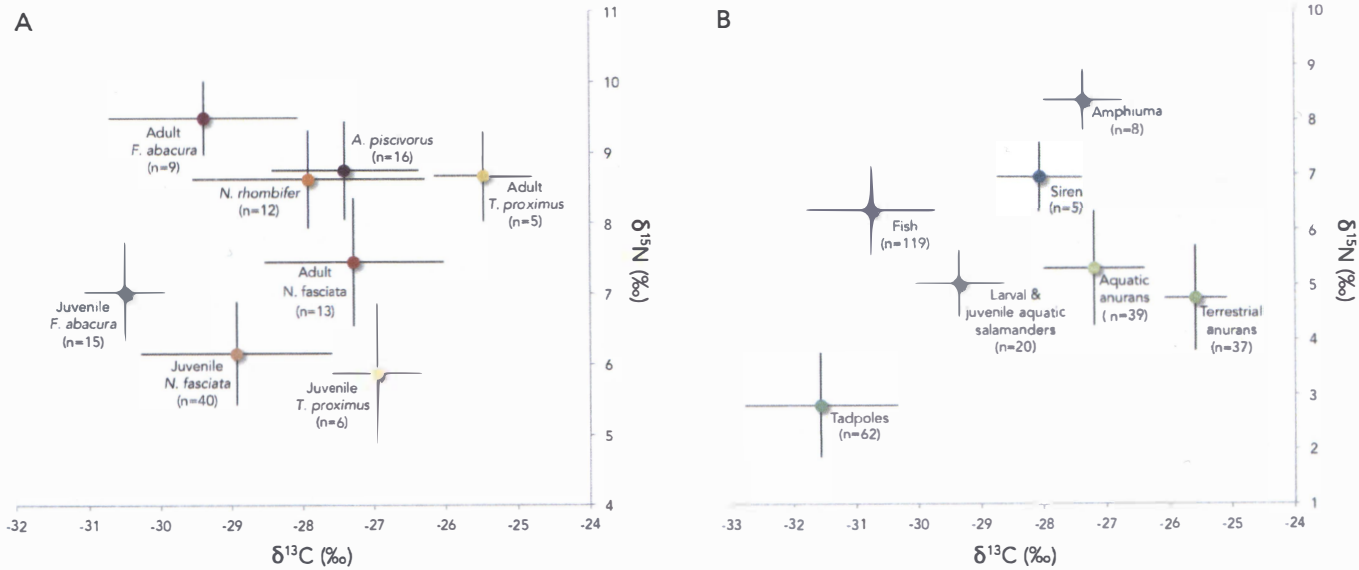
Figure 1. Isotopic values (mean ± SD) of plasma tissue from snake species (A) and of homogenized subsamples taken from the whole

body specimens of candidate prey species (B) from Mingo National Wildlife Refuge (Stoddard and Wayne Counties, Missouri, USA)
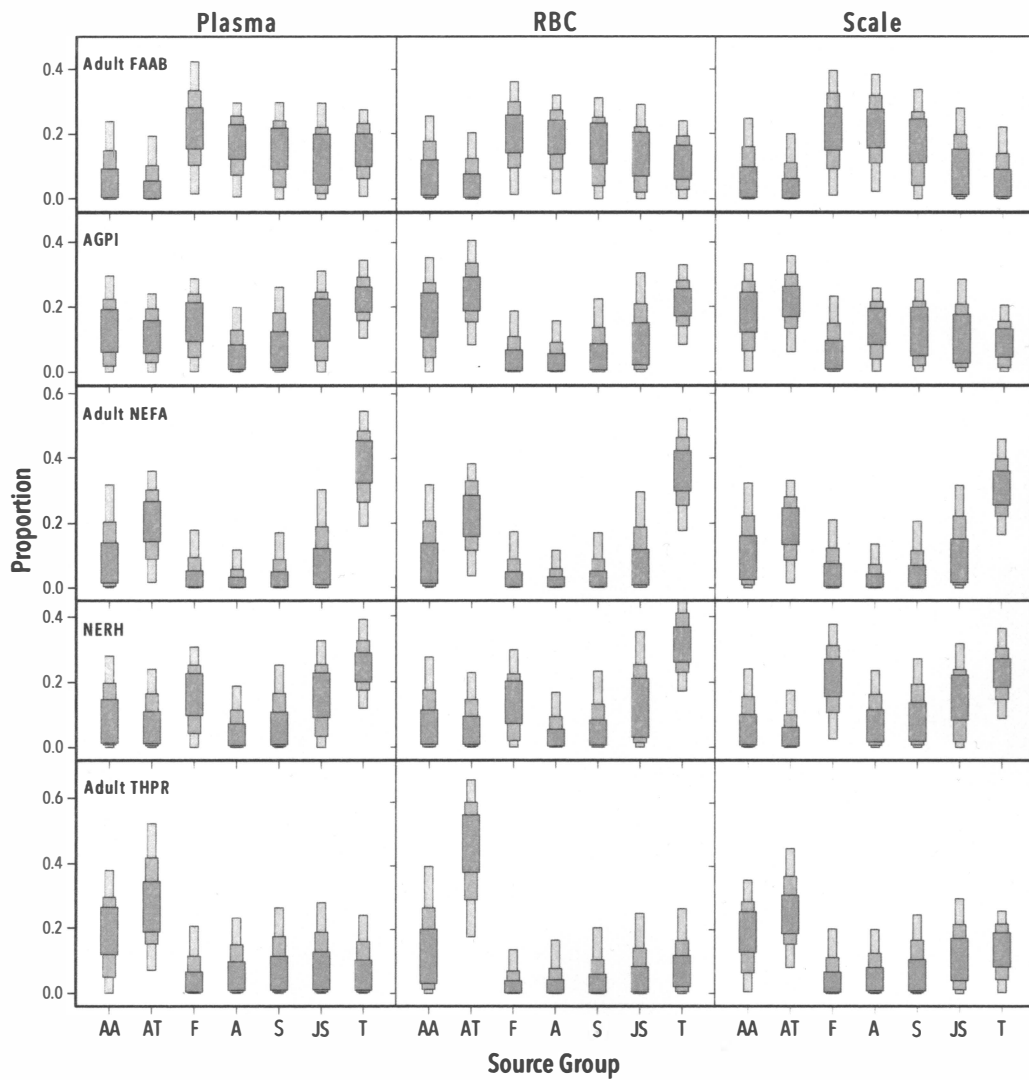
during 2013 and 2014

Figure 2. Estimated proportional contributions of prey source groups to adult snake diets represented as 95%, 75%, and 50% credibility intervals (represented by progressively lighter shading and decreased bar width). Estimates are shown for all tested consumer tissues. See Table 1 for snake species abbreviations and Table 3 for prey group abbreviations.
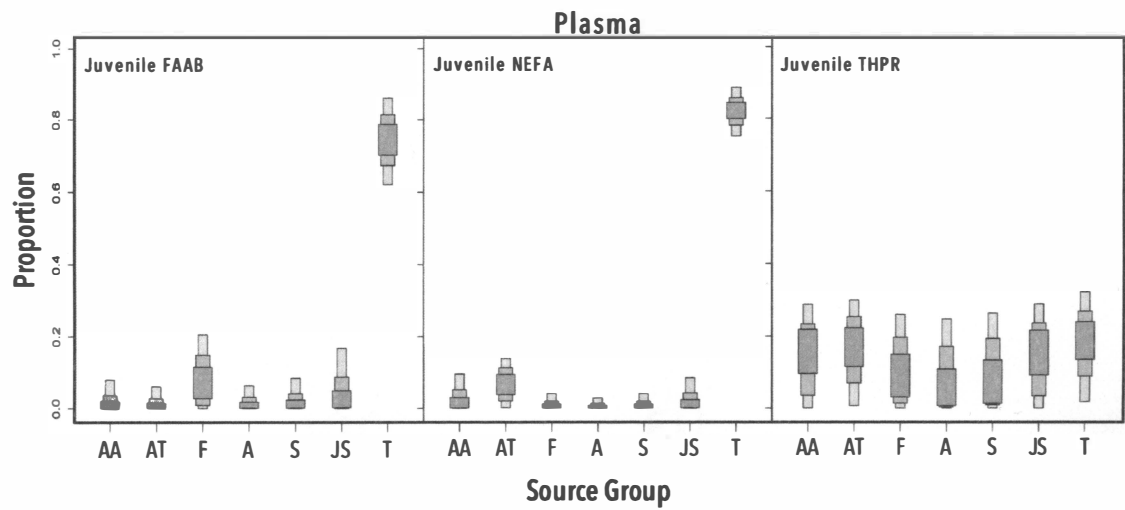
Figure 3. Estimated proportional contributions of prey source groups to juvenile snake diets represented as 95%, 75%, and 50% credibility intervals (represented by progressively lighter shading and decreased bar width). Estimates are shown for plasma tissue. See Table 1 for snake species abbreviations and Table 3 for prey group abbreviations.
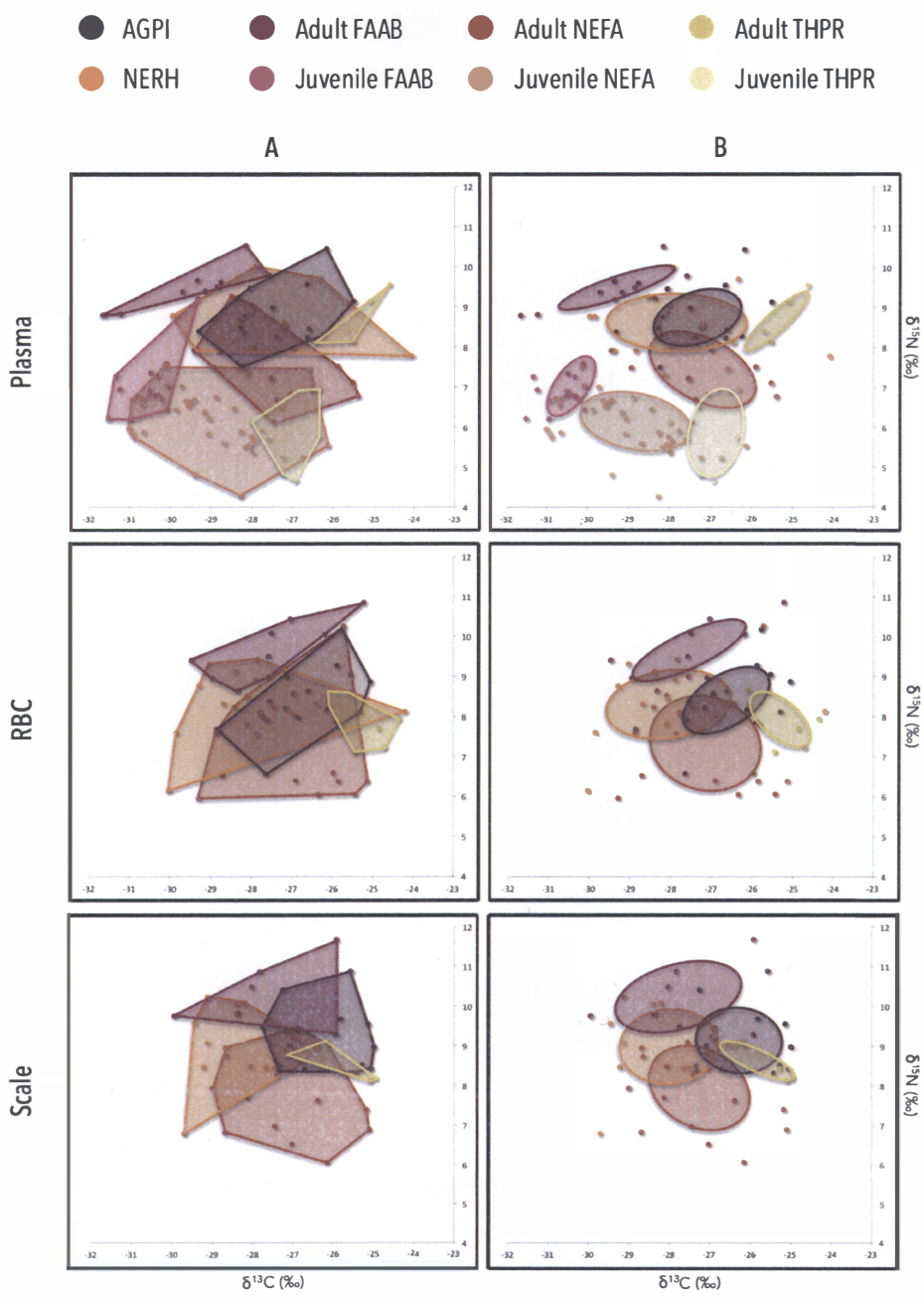
Figure 4. Isotopic composition plotted for plasma, red blood cell, and scale tissues from each sampled snake consumer group. Convex hulls depicting isotopic niche width (A) and standard ellipse areas representing core isotopic niche (B) were calculated using the stable isotope mixing models.

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