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Dietary resource utilization patterns and head morphology among three sympatric watersnake species.

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DIETARY RESOURCE UTILIZATION PATTERNS AND HEAD MORPHOLOGY AMONG THREE SYMPATRIC WATERSNAKE SPECIES

By

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B.S., Murray State University, 1997
M.S., University of Nebraska, 2001

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A Dissertation Approved on

October 19, 2016

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DEDICATION

To Jen

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ABSTRACT

DIETARY RESOURCE UTILIZATION PATTERNS AND HEAD MORPHOLOGY AMONG THREE SYMPATRIC WATERSNAKE SPECIES

Micah W. Perkins

October 19, 2016

The coexistence of similar species may be related to a variety of resource utilization differences including resource interactions. Dietary resource utilization variation may be the most important difference allowing for the coexistence of sympatric snake species. Many watersnakes (*Nerodia* spp.) live in sympatry and use similar aquatic habitats feeding mainly on fishes and amphibians. While these sympatric watersnakes may have different general foraging patterns, snake diet may be affected by a variety of factors including snake size, sex and seasonal changes in prey populations. Therefore, I initiated an investigation to understand the coexistence of sympatric plain-bellied (*N. erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes by addressing their dietary resource utilization patterns. I incorporated seasonal factors and intraspecific differences, and I also complemented traditional gut content analyses with stable isotope techniques. Also, since snakes swallow their prey whole and are gape-limited predators, I connected diet to watersnake head morphology. Results indicated that northern watersnakes ate fish families according to their availability except for the avoidance of Aphredoderidae. I also determined that northern watersnakes had smaller head sizes and a diet closer to the piscivorous diamondback watersnake but with a larger anuran component. There were sex differences in snake head size and all three species had different head shapes relating to diet. In addition, gut contents were determined from 60 individual watersnakes in 2013 and 118 in 2014 with plain-bellieds feeding mainly on anurans, diamondbacks on fishes, and northern watersnakes feeding mostly on fishes but with a higher anuran component than diamondback watersnakes. Season affected dietary overlap with each watersnake species having reduced overlap for a different season. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses provided long-term dietary information from 333 individual watersnakes with diamondback watersnakes feeding at higher trophic levels while plain-bellied watersnakes fed more from terrestrial prey sources. The application of stable isotope techniques helped to demonstrate shifts in dietary resource utilization relating to snake size. I have provided detailed trophic information beyond general watersnake dietary descriptions. This research has allowed me to reveal a complex foraging system affected by a variety of factors allowing for the coexistence of sympatric plain-bellied, diamondback and northern watersnakes.

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CHAPTER I

INTRODUCTION TO THE DISSERTATION

Understanding how similar species coexist is a fundamental ecological question. Theory predicts the most highly competitive species will drive others to extinction (Hardin 1960), but there are instances where seemingly similar species persist in the same area. Such species coexistence is often dependent on resource partitioning or differences in resource utilization (MacArthur 1958, Pianka 1973). Resource utilization differences can involve various factors including dietary, spatial or temporal (Pianka 1973, Vitt 2001).

Additional research is needed to investigate the comparative ecology of coexisting watersnakes (Himes 2003*b*), specifics involving watersnake foraging ecology (Gibbons and Dorcas 2004), and the ecological importance of reptiles in wetland systems (Laubhan et al. 2005). Many watersnake species overlap in distribution (Ernst and Ernst 2003), utilize similar aquatic habitats (Hebrard and Mushinsky 1978, Mushinsky et al. 1980) and feed mainly on amphibians and fishes (Gibbons and Dorcas 2004). While sympatric snakes may differ in few or many resources, dietary may be the most important resource utilization difference (Toft 1985, Vitt 2001, Goodyear and Pianka 2008). Therefore, I chose to investigate the coexistence of sympatric watersnakes (*Nerodia* spp.) addressing dietary resource utilization.

I had a unique opportunity to study high densities of plain-bellied (*Nerodia* *erythrogaster*), diamondback (*N. rhombifer*), and northern (*N. sipedon*) watersnakes occupying similar wetland habitats. I investigated the diet ecology and head morphology of the three sympatric watersnake species to understand their coexistence. I used traditional gut content analyses complemented with stable isotope techniques to investigate snake diet. I also related snake diet to snake head metrics to investigate the relationship between foraging and head morphology.

In Chapter 2, I investigated northern watersnake selection of fish prey. The northern watersnake has the largest distribution of any watersnake in the genus *Nerodia* and the most diverse diet (Gibbons and Dorcas 2004). While studies have addressed the diet of this ubiquitous, opportunistic forager, research has not addressed whether the northern watersnake is selecting or avoiding specific fish prey. I investigated individual fish families and fish length involving northern watersnake diet selection and demonstrated the avoidance of one fish family by northern watersnakes.

Since snakes swallow their prey whole and are gape-limited predators, I related head morphology to diet for plain-bellied, diamondback and northern watersnakes in Chapter 3. Resource utilization differences can relate to variation in morphology (Pianka 1973, Morin 1999) and watersnake species can overlap in diet but focus on different prey taxa. I addressed whether dietary differences are related to variation in snake head morphology. I incorporated the factor of snake sex as female watersnakes reach larger sizes than conspecific males (Gibbons and Dorcas 2004).

In Chapter 4, I examined dietary resource utilization among the three watersnake species using gut content analyses. While interspecific dietary differences may exist, watersnake foraging ecology is more complex than realized, with differences in snake sex, snake size (ontogeny), and seasonal prey availability. Such factors have rarely been examined in conjunction with watersnake species dietary resource utilization. I applied descriptive statistics and a model comparison approach to identify specific patterns and to demonstrate the various dietary factors allowing for watersnake coexistence.

Analyzing the gut contents of an individual animal only provides information about a single meal. To investigate watersnake diet over the long term, I completed a stable isotope study for Chapter 5. A stable isotope analysis provides additional dietary information that would be unavailable in a gut content analysis (Stewart et al. 2003), and in combination with traditional analyses, stable isotopes can provide information on how sympatric species partition their diets within a complex system (Willson et al. 2010). Such a stable isotope analysis has not been performed for these three watersnakes individually or in sympatry. To the best of my knowledge, this dissertation involves the largest stable isotope study on snakes ($N = 333$ individual snakes). Stable isotope analysis techniques allowed me to identify where an individual watersnake is performing the majority of its foraging (aquatic or terrestrial) and at what trophic level.

My dissertation provides information beyond the general dietary descriptions of plain-bellied, diamondback and northern watersnakes. This research has allowed me to understand coexistence of similar watersnake species by studying interspecific and intraspecific dietary factors, including interactions. I demonstrate that species coexistence can involve a complex foraging system affected by species, sex, size and seasonal dynamics.

CHAPTER II

NORTHERN WATERSNAKE (NERODIA SIPEDON) SELECTION OF FISH PREY

SUMMARY

The northern watersnake (*Nerodia sipedon*) is found in a wide variety of aquatic habitats throughout North America. Northern watersnakes use several different foraging strategies and have diverse diets, but populations are often mostly piscivorous. Although previous studies have examined the diet of this species, research has not addressed whether the northern watersnake is preferentially selecting or avoiding particular fish as prey. In this study, I sampled snake stomach contents and used Chesson's alpha index (α_i) to investigate whether northern watersnakes are eating different fish families in proportion to their availability in the habitat or are preferentially selecting or avoiding specific fish families. The northern watersnake fed on fish prey from six families in 2013 (N = 15) and 2014 (N = 36). Five of those fish families were eaten in proportion to their availability, but Aphredoderidae, the pirate perch (*Aphredoderus sayanus*) family, was avoided by northern watersnakes. This is the first study testing prey preferences in the northern watersnake.

INTRODUCTION

The northern watersnake (*Nerodia sipedon*) is widely considered to be a generalist species. It has the largest range of any watersnake in North America (Gibbons and Dorcas 2004), stretching across portions of the Great Plains, the majority of the eastern United States, southeastern Ontario (Gibbons and Dorcas 2004) and southern Quebec (Ernst and Ernst 2003). It can be found almost in any freshwater habitat type (Ernst and Ernst 2003, Gibbons and Dorcas 2004), including fish hatcheries (Bauman and Metter 1975), streams (Cecala et al. 2010), lakes of various sizes (Zelnick 1966, King 1993) and a variety of wetlands (Roe et al. 2003). It also uses a variety of foraging strategies including active foraging, ambush and tongue-flick probing (Meyer 1992, Balent and Andreadis 1998). Not surprisingly, this species has the most diverse diet of any Northern American watersnake, preying on fishes, amphibians, arthropods, mollusks, annelids, and even small mammals (Ernst and Ernst 2003, Gibbons and Dorcas 2004).

Previous studies have suggested that dietary differences across watersnake populations may be the result of differences in prey availability (Ernst and Ernst 2003, Bowen 2004) and within populations, the northern watersnake's diet can change over time in response to changes in prey abundance. For example, as amphibian populations declined, one northern watersnake population shifted from a heavily amphibian-based diet to feeding only on fishes (Meyer 1992, Carbone 1993). Similarly, a population of the northern subspecies, the Lake Erie watersnake (*N. s. insularum*), altered its feeding patterns over time, changing the proportions of amphibians versus fishes in its diet according to relative prey abundance (King et al. 1999*b*, King et al. 2006).

Fishes may be the northern watersnake's most common prey (Ernst and Ernst 2003, Himes 2003*a*, Gibbons and Dorcas 2004). In previous studies, the percentage of northern watersnake diet comprised by fishes ranged from 48–92% but was generally well above 50% (48%: Roe et al. 2004, 65%: this study, 78%: Zelnick 1966, 90%: Lacy

1995, and 92%: King 1986). An early study showed that a northern watersnake population in Maryland, USA had an innate preference for fish prey with 80% of individuals selecting fish over anurans and earthworms (Dix 1968). Previous research has not addressed whether northern watersnakes are eating fish according to their availability or if whether this species prefers or avoids specific fish. Roe et al. (2004) and Gibbons and Dorcas (2004) suggested that the northern watersnake may be eating prey according to their availability but this hypothesis has not been tested.

Watersnake diet can be influenced by other factors including prey size and shape. As snakes increase in size, smaller prey will often decrease in diet (Plummer and Goy 1984, Arnold 2001, Bowen 2004), and northern watersnake length and mass can correlate with prey size (King 1993). Prey shape along with prey size can also affect snake foraging (Voris and Voris 1983, Vincent et al. 2006*b*). With these in mind, fishes of various sizes and shapes may be important in prey preference or avoidance. This study investigates whether particular fish families are selected for, avoided or eaten in proportion to their relative abundance by northern watersnakes considering fish size and shape.

METHODS

The study site was a 100-hectare section of the Sloughs Wildlife Management Area (Henderson County, Kentucky, USA), which is managed by the Kentucky Department of Fish and Wildlife Resources. This section is located 2 kilometers southeast of the Ohio River and is known as Hardy Slough/Muddy Slough. Habitat types included moist soil units (shallow wetlands managed for wintering waterfowl), scrub-shrub wetlands and palustrine forest. Dominant plants were water primrose (*Ludwigia* sp.), smartweed (*Polygonum* sp.), water lily (*Nuphar* sp.) cattail (*Typha* sp.), buttonbush (*Cephalanthus occidentalis*), black willow (*Salix nigra*) and hackberry (*Celtis occidentalis*). The study period was divided into spring (April–May), early summer (June–July), and late summer (August–September) in 2013 and 2014.

I captured northern watersnakes using a variety of methods including hand capture, cover board placement, stand-alone aquatic funnel traps, and drift fence arrays (terrestrial and aquatic) with funnel traps. For each captured watersnake, I measured snout-vent length (SVL) in millimeters and used cloacal probing to determine sex. I marked snakes with both subcutaneous pit tags (Gibbons and Andrews 2004) and ventral scale-clip patterns (Plummer and Ferner 2012) to enable me to identify any recaptures. In order to determine snake diet, I used gentle palpation to force northern watersnakes to regurgitate gut contents (Kofron 1978, Fitch 2001). I measured the standard length of fishes found in snake gut contents and identified them to family because partial digestion of some prey items prevented more specific identification. Each snake was released at its capture location. All animal capture, handling and processing activities were approved by the University of Louisville Institutional Animal Care and Use Committee (IACUC Protocol: #13037).

For each of the 3 seasons in 2013 and 2014, I calculated the proportion of each fish family in the diet of the northern watersnakes by summing the number of fishes in a given family across all snake stomach contents in a season over the total number of fishes found in all snakes for that season. For snake diet, this resulted in fish family proportions for each season and a mean proportion for all fish families over the two-year study.

To determine prey availability, I placed an average of 36.3 (SD 16.4) aquatic funnel traps opened for two days and nights (~48 hours) each week in each of the three seasons in 2013 and 2014 removing fishes after day 1 for each 48-hour sampling period. Each trap had 25% of the trap above the waterline to prevent the drowning of non-target animals. Trap nights per season equaled the number of traps multiplied by the number of days each trap was out for a given season. If a snake was found in a given prey trap, that trap was not included in the count of trap nights and any prey in the trap were similarly ignored. I identified captured fishes to family and I measured standard length, body depth and body width of each fish. I determined prey availability for each fish family in each season by using the number captured per trap night per season and I determined a mean number per trap night for each fish family for the entire two-year study. Relative prey availability was determined for each fish family for the 3 seasons in each year by summing the mean number of fishes in a given family captured per trap night in a season over the total mean number of fishes captured per trap night for that season. This resulted in proportions of captured fish in each family for each season and a mean proportion for all fish families captured over the two-year study for available prey.

Chesson’s alpha selection index ($\alpha_i = (r_i/n_i)/\Sigma(r_j/n_j)$) was used to determine whether snakes were preferentially selecting or avoiding particular fish families (Chesson 1978, Lawson et al. 1998). Chesson’s alpha values were determined for each fish family for each season in 2013 and 2014, which were used to determine a mean value for each family over the two-year study (Pattinson et al. 2003). Chesson’s alpha selection index values were scaled from -1 to 1 ($((\alpha_i/(\alpha_i + \Sigma_{j \neq i}\, \alpha_i/(m - 1)) \cdot 2) - 1)$, with 0 indicating no selection, positive values indicating selection and negative values indicating avoidance

(Chesson 1983). To assess whether any selection or avoidance was significant, 95% confidence intervals were calculated for each scaled Chesson's alpha selection index value for each fish family (Pattinson et al. 2003).

To further investigate the effect of fish size and shape on northern watersnake diet, I used a general linear model to determine if standard fish length was related to the ratio of fish body width to body depth. I incorporated fishes from northern watersnake diet and captured available prey. Slopes between northern watersnake diet and captured available prey were tested to determine differences.

RESULTS

In 2013, 72 individual northern watersnakes were captured, with 15 having fishes in regurgitated gut contents. These 15 snakes had a mean SVL of 557 mm (35.27 SE; range 327–729 mm). In 2014, 114 new individuals were captured, with a total of 36 snakes having fishes in gut contents. These 36 snakes had a mean SVL of 525 mm (21.01 SE; range 275–794 mm). In 2014, there were also 8 recaptures from 2013 but recaptured individuals with gut contents in 2014 did not have fish in 2013. A total of 3 individuals were caught twice within years (1 in 2013 and 2 in 2014) and regurgitated fishes both times. In all 3 individuals, fishes in the 2 gut content samples were from different families. These diet data from the recaptures were included in the analyses.

In 1,364 trap days, I captured fishes belonging to 8 fish families, with 349 fishes captured in 2013 and 592 fishes captured in 2014 (Table 1). Amiidae comprised 22.8% of these available prey, followed by Centrarchidae (20.1%), Poeciliidae (18.1%), Aphredoderidae (15.4%), Esocidae (14.6%), Cyprinidae (5.6%), Lepisosteidae (1.7%), and Elassomatidae (1.7%). Fishes belonging to six different families were found in the gut contents of northern watersnakes (Table 2). Esocidae made up 28.9% of the fishes in gut contents, followed by Amiidae (25.4%), Centrarchidae (17.4%), Poeciliidae (11.8%), Aphredoderidae (10.5%) and Cyprinidae (6.0%). Lepisosteidae and Elassomatidae were not found in northern watersnake diet.

Esocidae and Amiidae together comprise 37.4% of available prey but 54.3% of snake diet, with both families present in higher mean proportions in snake diet than in the fishes available in the habitat (Figure 1). Aphredoderidae, Poeciliidae, and Centrarchidae all had lower proportions in snake diet than in the prey population while Cyprinidae was nearly equal for snake diet (6.0%) and prey availability (5.6%).

Scaled Chesson's alpha selection values were above 0 for fishes in Amiidae and Esocidae, but 95% confidence intervals included 0 and thus indicated that watersnakes were not preferentially selecting prey from these families (Figure 2). Scaled Chesson's alpha selection values for Cyprinidae, Poeciliidae, and Centrarchidae were below 0, but again the 95% confidence intervals included 0, indicating no significant avoidance of these groups by watersnakes. However, northern watersnakes avoided Aphredoderidae.

Northern watersnakes did not appear to select prey by size, at least within the fish families on which they fed. Esocidae was the only fish family that had a longer average standard length in snake diet (94.46 mm) than in captured available prey (90.00 mm), but this difference was not significant ($F_{1,60} = 0.45$, $P = 0.51$). Except for Elassomatidae, which was not fed upon by the snakes, the two families of Aphredoderidae and Poeciliidae had the smallest average length of the potentially available fishes in this study, and fish in those two families also had the shortest average standard lengths of the prey in snake gut contents.

Fish body shape may play some role in determining how large an individual snake is capable of swallowing. Fishes in Amiidae and Esocidae had the longest average standard lengths of the fishes found in snake gut contents and ratios of body width to body depth for fishes in those two families were closer to 1 than were those ratios in the other 4 fish families found in captured prey (Table 2). The general linear model analysis results indicated the ratio of fish body width to body depth model was significant ($F_{3,8}$ = 5.25, P = 0.027, R^2 = 0.663). Fishes with longer standard lengths had higher ratios of body width to body depth (F_1 = 14.08, P = 0.006) indicating that larger fish had more tubular shapes. However, fish found in snake diet and captured available prey had similar slopes (F_1 = 0.53, P = 0.489) (Figure 3).

DISCUSSION

Northern watersnakes did not preferentially select their fish prey from any particular family, instead taking prey from most fish families in proportion to their relative abundance. The two least common fish families at the study site, Lepisosteidae and Elassomatidae, were not included in the diet of the northern watersnakes. Research has suggested that northern watersnakes are not preferentially preying on specific prey species (Gibbons and Dorcas 2004, Roe et al. 2004); this study provides a test for this hypothesis and demonstrates that northern watersnakes are likely to be eating most fish species as they encounter them.

Northern watersnakes did however prey on the family Aphredoderidae significantly less frequently than expected based on its relative abundance. The pirate perch (*Aphredoderus sayanus*) is the sole species in this family, and among congeners of the northern watersnake, the pirate perch has previously only been recorded in the diet of banded (*N. fasciata*) and brown (*N. taxispilota*) watersnakes (Ernst and Ernst 2003, Gibbons and Dorcas 2004). Aphredoderidae in this study had the smallest average standard length of the six fish families eaten by northern watersnakes. Aphredoderidae was similar in availability (15.4%) to the fish family most often preyed upon by northern watersnakes, Esocidae (14.6%).

Resetarits and Binckley (2013) indicated that the pirate perch contains chemical camouflage, making the fish cryptic to a wide variety of pirate perch prey. This chemical masking may both help the pirate perch in foraging and help it avoid predation (Resetarits and Binckley 2013) and one study on eastern ribbon snakes (*Thamnophis sauritus*) found that ribbon snakes did not eat pirate perch even though pirate perch were very abundant (Langford et al. 2011). Northern watersnakes use both olfaction and vision when foraging (Drummond 1985, Balent and Andreadis 1998) and although northern watersnakes can successfully forage using only chemical cues (Gove and Burghardt 1975), prey capture success increases when northern watersnakes use both olfaction and vision (Drummond 1979). The pirate perch forages mostly at night (Froese and Pauly 2016) and Ernst and Ernst (2003) indicated that 1800 to 2400 hours may be particularly important for northern watersnake foraging but that easily captured prey will be taken during the day. The northern watersnake may thus be at a disadvantage for encountering and capturing pirate perch if the fish is foraging at night and chemical cues may not assist vision for foraging. Future research will need to determine if the pirate perch’s chemical camouflage can be sensed by northern watersnakes.

Esocidae and Amiidae likely play an important role in northern watersnake diet. Together, they constituted over 50% of the prey items taken in this study. Further, the proportions of Esocidae and Amiidae in the snake diet were greater than available proportions and mean selection indices were greater than zero. These two fish families also had the largest mean standard lengths of the fishes in the snake diet, suggesting these two groups provide a significant proportion of the calories consumed by the watersnakes at this site. Members of the Esocidae family have previously been reported as northern watersnake prey (Ernst and Ernst 2003, Gibbons and Dorcas 2004). In this study, Esocidae had the highest selection index and was the only fish family in snake gut contents with an average standard length greater than available prey. Esocidae was the fifth most abundant available fish family but represented the largest proportion of the northern watersnake diet. Amiidae had not been previously recorded as being northern watersnake prey (Ernst and Ernst 2003, Gibbons and Dorcas 2004), but it was the most abundant available fish in this study. Local northern watersnakes may have had a dietary shift to this prey, given that watersnakes are known to adjust their diet to include abundant prey types (Roe et al. 2004, King et al. 2006).

Both fish size and shape are known to affect snake foraging (Voris and Voris 1983) and the body shape of the fishes in the Esocidae and Amiidae families may have facilitated the capture and consumption of relatively large individuals by the watersnakes. Fishes in these families had ratios of body width to depth closer to 1 than did fishes in the other families with Amiidae having an average value of 1.02. Esocidae and Amiidae have very similar shapes, with Esocidae being saggitiform (arrow-like or tubular) and Amiidae being cylindrical. Given that these two fish families were on average the largest fish by length eaten by northern watersnakes in this study, bodies with a relatively circular cross-section instead of being either relatively deep-bodied or dorso-ventrally flattened may allow for easier swallowing and ingestion by northern watersnakes and other gape-limited predators. Research with the banded watersnake, indicated that prey with greater height or width resulted in more skull movements for ingestion and difficulties with prey movement through the snake digestive tract (Vincent et al. 2006*b*). Not surprisingly, and similarly to the northern watersnake in this study, banded watersnakes tend to eat primarily fusiform fishes (Mushinsky et al. 1982, Vincent et al. 2007), and they also prefer tubular-bodied salamanders over tall, narrow Centrarchidae fish (Wilson and Hopkins 2011). Miller and Mushinsky (1990) demonstrated that fusiform-shaped fish were preferred by older mangrove watersnakes (*N. f. compressicauda*). Larger, tubular fish that may be easier to swallow such as Esocidae and Amiidae may be important because larger northern watersnakes may often be feeding only on large prey (King 1993, Bowen 2004). While northern watersnakes did not select fish with greater ratio of fish body width to body depth over what is available in the habitat, larger fish with more tubular shapes may be important for foraging northern watersnakes.

The northern watersnake has a diverse diet across its distribution and dietary flexibility within populations. In general, we would predict that northern watersnakes will be eating fishes according to their abundance and availability in the habitat unless ecological or behavioral factors alter the probability of some fish from being encountered and captured. Some northern watersnake populations are of conservation concern (King et al. 2006) but many localities have large populations of northern watersnakes (Bauman and Metter 1975, Laurent and Kingsbury 2003, Gibbons and Dorcas 2004) and as long as there is prey present, the adaptable northern watersnake will likely be present in aquatic areas throughout the species’ range.

Table 1. Mean number of fishes captured per trap night for eight fish families by sampling for available prey using aquatic funnel traps.

Fish family	Mean number of fish per trap night (SE)
Lepisosteidae	0.006 (0.004)
Amiidae	0.430 (0.364)
Cyprinidae	0.041 (0.026)
Esocidae	0.061 (0.010)
Aphredoderidae	0.083 (0.028)
Poeciliidae	0.172 (0.105)
Centrarchidae	0.145 (0.053)
Elassomatidae	0.009 (0.009)

Table 2. Mean standard length in mm (SE) for eight fish families found in northern watersnake (*Nerodia sipedon*) diet and captured available prey. Ratio of body width to body depth data are based on available prey.

	Snake Diet		Available Prey		
Fish family	*N*	Mean standard length (SE)	*N*	Mean standard length (SE)	Ratio of body width to body depth (SE)
Lepisosteidae	0	—	13	364.51 mm (48.96)	1.01 (0.03)
Amiidae	6	86.08 mm (11.12)	32	162.85 mm (20.78)	1.02 (0.16)
Cyprinidae	8	50.58 mm (5.80)	18	62.86 mm (4.35)	0.52 (0.02)
Esocidae	21	94.46 mm (5.29)	41	90.00 mm (3.95)	0.76 (0.02)
Aphredoderidae	11	32.16 mm (1.16)	33	51.08 mm (3.09)	0.59 (0.04)
Poeciliidae	11	35.55 mm (1.02)	19	36.73 mm (0.77)	0.54 (0.33)
Centrarchidae	13	47.67 mm (5.58)	63	64.94 mm (3.78)	0.38 (0.01)
Elassomatidae	0	—	3	31.15 mm (1.30)	0.44 (0.03)

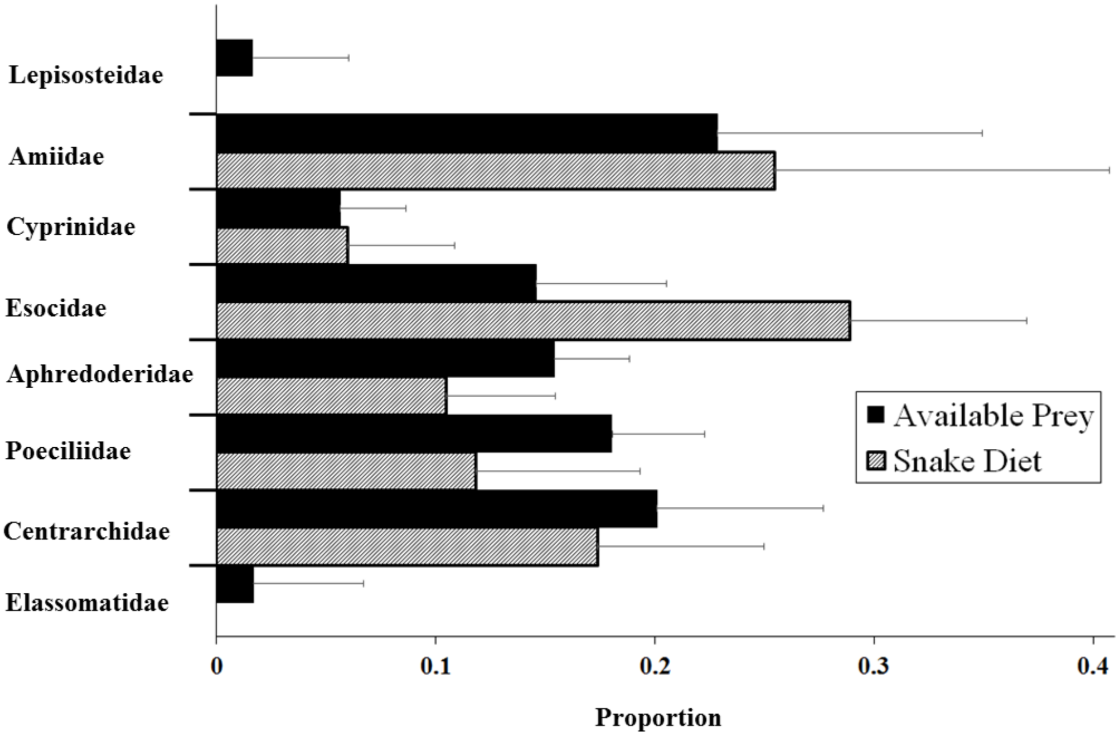

Figure 1. Mean proportions of available prey (number/trap night) and prey in gut contents (number in gut contents) of northern watersnakes (*Nerodia sipedon*) for eight fish families. Error bars indicate standard errors.

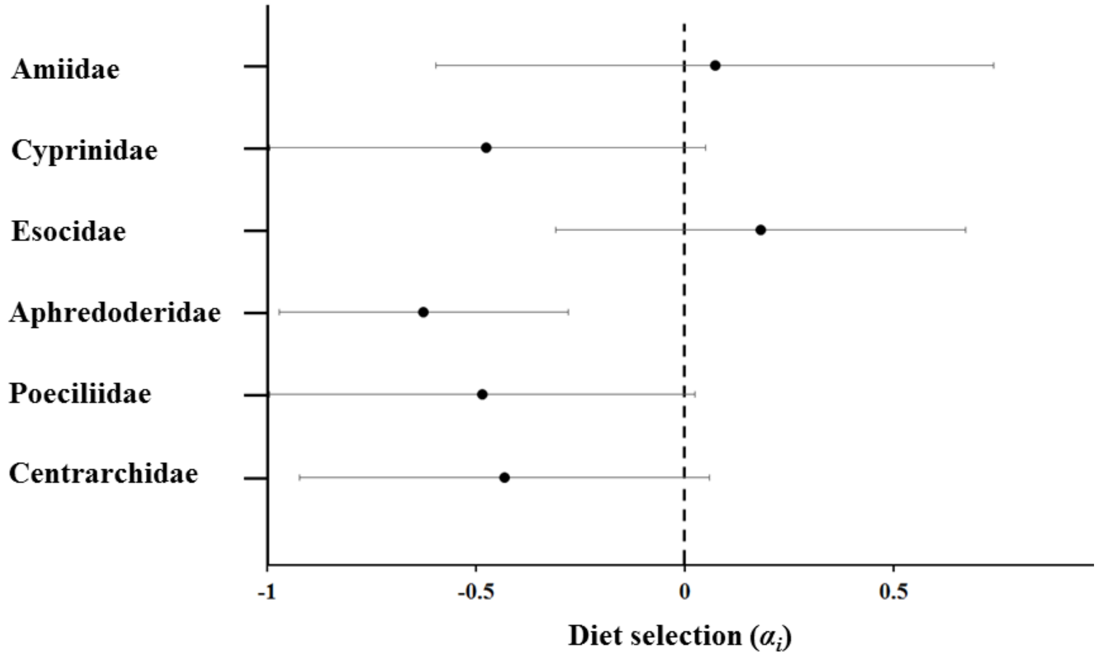

Figure 2. Northern watersnake (*Nerodia sipedon*) diet selection for 6 fish families indicated by scaled Chesson's alpha selection index (α_i). Error bars indicate 95% confidence intervals.

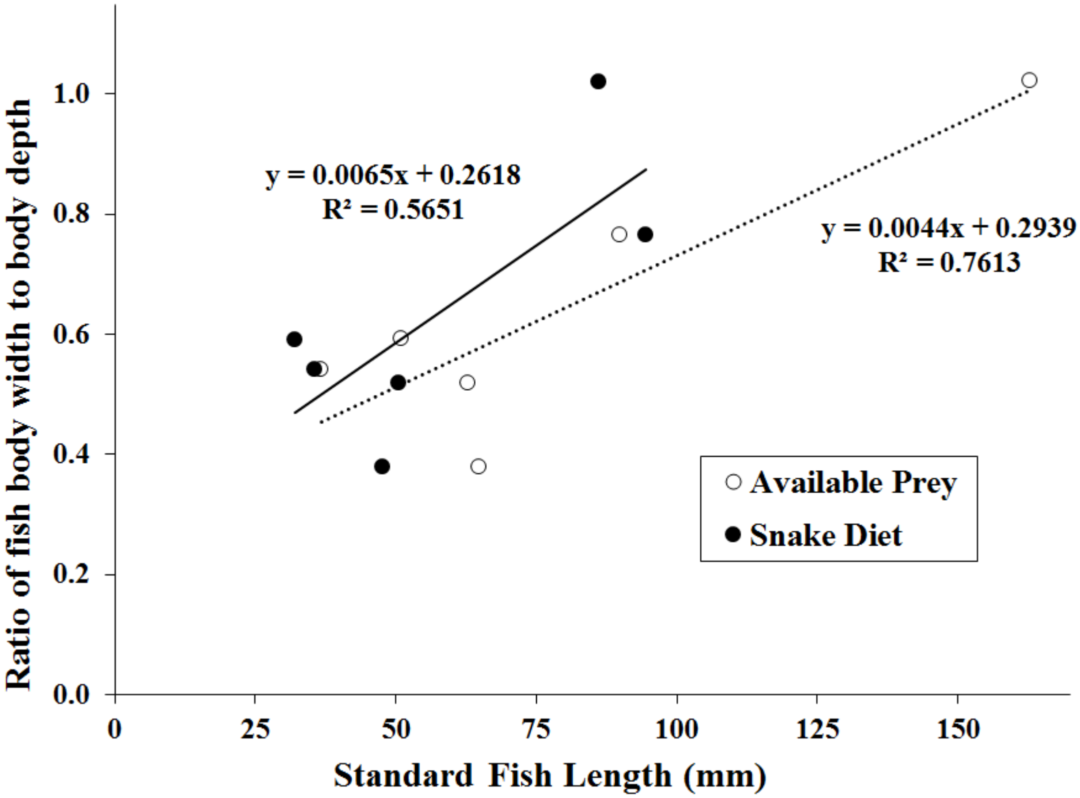

Figure 3. Ratio of fish body width to body depth regressed against standard fish length (mm) for 6 fish families. The dark line indicates snake diet while the dotted line relates to available prey.

CHAPTER III

THE RELATIONSHIP OF HEAD MORPHOLOGY AND DIET AMONG THREE SYMPATRIC WATERSNAKE SPECIES

SUMMARY

Morphological differences of sympatric species may relate to dietary resource utilization. One such example is snake head morphology since snakes swallow their prey whole and are gape-limited predators. Plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*), and northern watersnakes (*N. sipedon*) inhabit similar wetlands with all three species feeding on amphibians and fishes. I investigated the relationship of watersnake head morphology and sex with diet of these congeneric watersnake species. The northern watersnake had smaller head sizes and a diet closer to the piscivorous diamondback watersnake but with a larger anuran component. The narrower heads and decreased interocular distances of plain-bellied watersnakes were likely a benefit for foraging on and swallowing of anurans. The diamondback watersnake with a wider head and decreased interocular distance may aid in feeding on fishes. The northern watersnake had an intermediate head shape and was found to feed on anurans but mostly fish. Head morphology can be used to help in the understanding of dietary resource utilization among sympatric snake species.

INTRODUCTION

Morphology relates to dietary resource utilization, feeding behavior and foraging strategies throughout vertebrate taxa (Ricklefs and Travis 1980, Milton 1981, Verwaijen et al. 2002, Donadio and Buskirk 2006, Kahilainen and Østbye 2006, Ledon-Rettig et al. 2008). As similar species live in sympatry feeding on different resources, the investigation of morphological differences can help in the understanding of species coexistence.

Morphology involving the head and skull is important when discussing diet especially for snakes because they swallow their prey whole and are gape-limited predators. Many studies have demonstrated the relationship between snake head or skull morphology and snake diet (Greene 1983, Dwyer and Kaiser 1997, Meik et al. 2012) with prey size and shape being important factors (Voris and Voris 1983, Vincent et al. 2006*b*). Foraging activity (Hibbits and Fitzgerald 2005, Herrel et al. 2008) and dietary specificity (Mori and Vincent 2008) have been argued to correspond with head morphology in snakes. Sexual dimorphism and its effect upon head morphology may explain dietary differences observed between males and females (Camilleri and Shine 1990, Shine 1991*a*, Vincent et al. 2004*a, b*, Meik et al. 2012). Moreover, closely-related snake species living in sympatry can have differences in head morphology presumably due to differing diets (Henderson, et al. 2013, López et al. 2013).

The natricine plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes have overlapping ranges, diets and habitat similarities (Laurent and Kingsbury 2003, Gibbons and Dorcas 2004, Marshall 2008). Although the overall diets of these species broadly coincide with all three feeding primarily on amphibians and fishes, the species do partition dietary resources (Mushinsky and Hebrard 1977*a*, Luiselli 2006). Plain-bellied watersnakes eat mostly amphibians (Preston 1970, Mushinsky and Hebrard 1977*a*, Roe et al. 2004) while diamondback watersnakes eat mainly fishes (Hess and Klimstra 1975, Kofron 1978, Savitsky 1989). The northern watersnake is considered to have the broadest diet of any watersnake in North America (Ernst and Ernst 2003, Gibbons and Dorcas 2004). Fishes are often the northern watersnake's main prey group (Lacy 1995, Himes 2003*a*, Gibbons and Dorcas 2004), but some populations have an elevated anuran component along with fishes (Meyer 1992, Roe et al. 2004) and some populations feed more on amphibians than fishes (King 1986, Bowen 2004). A review found that the percentage of the diet comprised by amphibians ranged from 4 to 53% across northern watersnake populations (King 1986).

I examined if differences in head morphology in these three congeneric watersnakes are related to differences in diet across species and between sexes. With plain-bellied watersnakes heavily reliant on anurans and diamondback watersnakes concentrating on fish, these dissimilarities may possibly relate to head morphological differences between these two watersnake species. Natricine snake research has indicated that fish-eating snakes may have narrower heads while wider heads were more important for frog capture (Brecko et al. 2011). Conversely, natricine snakes that ate frogs had smaller heads than fish-eating individuals (Vincent et al. 2007). Savitsky (1983) indicated that piscivorous adaptations in snakes did not include constrained head widths. To understand these contradictions, my research examined the relationship between watersnake head morphology and diet. In addition, with northern watersnake diet varying across its range, the head morphology of this species may similarly vary and thus may more closely resemble either the more anurophagous plain-bellied watersnake or the piscivorous diamondback watersnake depending on its local diet. Furthermore, female adult watersnakes reach larger lengths and mass than males (King et al. 1999*a*, Gibbons and Dorcas 2004). Watersnake sex could differ in head morphology alongside body size with these head morphological differences potentially allowing for sexes to differ in diet. To address these questions, my research investigates head morphology and diet from sympatric plain-bellied, diamondback and northern watersnakes.

METHODS

The study site was a 100-ha section of Sloughs Wildlife Management Area (Henderson County, Kentucky, USA), which is managed by the Kentucky Department of Fish and Wildlife Resources. This section is located 2 kilometers southeast of the Ohio River and is known as Hardy Slough/Muddy Slough. Habitat types included moist soil units (shallow wetlands managed for wintering waterfowl), scrub-shrub wetlands and palustrine forest. Dominant plants were water primrose (*Ludwigia* sp.), smartweed (*Polygonum* sp.), water lily (*Nuphar* sp.) cattail (*Typha* sp.), buttonbush (*Cephalanthus occidentalis*), black willow (*Salix nigra*) and hackberry (*Celtis occidentalis*). The study period for snake morphology metrics was from April to September 2014 while snake diet data were obtained from April to September in both 2013 and 2014.

I captured snakes by a variety of methods including hand capture, cover board placement, stand-alone aquatic funnel traps, and drift fence arrays (terrestrial and aquatic) with funnel traps. For each captured watersnake, I measured snout-vent length (SVL) and determined sex by cloacal probing. I used digital calipers to measure (± 0.01 mm) morphological features of the head that are known to be important factors in prey capture, feeding and foraging (Vincent et al. 2004*a*, Brecko et al. 2011, López et al. 2013). These morphological features included maximum head width and head length, which was measured as the distance from the center of the rostral scale to the midpoint between the posterior edges of the parietal scales. To assess snake jaw dimensions, I measured tip width as the distance between the outer edges of the rostral scale and jaw length as the distance from the center of rostral scale to the posterior edge of the most posterior supralabial scale. I included two morphological features important for watersnake foraging in the analysis: interocular distance (the distance between the distal edges of the two supraocular scales) and eye position (the posterior edge of the preocular scale to the center of the rostral scale).

I marked snakes with subcutaneous pit tags (Gibbons and Andrews 2004) and ventral-scale clip patterns (Plummer and Ferner 2012) to identify any recaptures. To determine watersnake diet, I used gentle palpation to force watersnakes to regurgitate their gut contents (Kofron 1978, Fitch 2001). I identified snake gut contents to fish, Caudata, Anura (tadpole/metamorph) or Anura (froglet/adult). Anuran metamorphs are tadpoles with well-defined legs that are beginning to show adult traits and froglets are recently metamorphosed frogs with no tadpole traits (McDiarmid and Altig 1999). The volume (ml) of each prey item was recorded. I released each snake at its capture location. All animal capture, handling and processing activities were approved by the University of Louisville Institutional Animal Care and Use Committee (IACUC Protocol: #13037).

To analyze diet, I incorporated three quantitative values into an Index of Relative Importance (IRI) using the equation $IRI = (\%N + \%V) \cdot (\%F)$ (Pinkas et al. 1971, Franks and Vanderkooy 2000, Vaudo and Heithaus 2011). Here, %N is the numerical prey category percentage pooling all individual snakes (numeric prey percentage). The total volume percentage of all prey from that category that were found in stomachs of all snakes (volume prey percentage) was %V and %F is the percentage of individual snakes that had eaten from a prey type category (occurrence percentage). To compare IRI values from different prey groups, each prey category (IRI_i) was converted to a percentage ($\%IRI_i$) by dividing the specific prey category (IRI_i) by the sum of IRI_i values using the following equation: $\%IRI_i = 100 \cdot IRI_i / \sum IRI_i$ (Cortés 1997, Kinney et al. 2011). Prey category percentages ($\%IRI_i$) sum to 100 with higher values indicating greater importance in watersnake diet. My %IRI analysis was performed using 4 prey categories (fishes, Caudata, Anura (tadpole/metamorph) and Anura (froglet/adult).

Before head morphology analyses, I $\log_{10}$ transformed SVL and all head metrics to meet assumptions of normality and homoscedasticity (Sokal and Rohlf 2011). I determined swallowing gape for each individual watersnake by incorporating head width and jaw length into a gape index ($(\text{head width} \cdot \text{jaw length} \cdot \pi) \cdot 0.25$) (King 2002, Vincent et al. 2007). Snout-vent length, species and sex were the explanatory variables in a general linear model with gape index being the response variable and resulting regression slopes were tested to determine significant differences among watersnake species and between sexes.

I individually regressed each of the 6 head metrics against SVL, which yielded size-adjusted head metric residuals to eliminate the effect of larger snakes having larger heads in analyses (Vincent et al. 2004*a*, Hibbits and Fitzgerald 2005, Vincent et al. 2006*a*, Brecko et al. 2011, Hampton 2011). To investigate the differences in head morphology among watersnake species and sex, a principal component analysis (PCA) was performed on the resulting head metric residuals. Scree plot analysis and latent root criterion were used to select principal components for further analysis (McGarigal et al. 2000, Brecko et al. 2011). I investigated component factor loading scores in the selected principal components to determine the importance of particular head metrics. To examine the effect of ontogeny on head morphology, I regressed selected principal components against SVL (Vincent et al. 2004*b*). Selected components from PCA were used as dependent variables in a multivariate analysis of variance (MANOVA) along with follow-up univariate analysis of variance (ANOVA) F-tests with species, sex and species-sex interaction as independent variables. Follow-up least squares mean tests were used to determine individual group differences. I performed all statistical analyses using SAS software (SAS Institute 2000) and I considered tests to be statistically significant at $\alpha = 0.05$.

RESULTS

In 2014, head measurements were obtained for a total of 275 individual watersnakes, including 74 plain-bellied, 79 diamondback and 122 northern watersnakes (Table 3, Table 4). Based on 2013 and 2014 data, froglet/adult anurans made up most (%IRI = 78.3%) of plain-bellied watersnake diet (Table 5). Northern (%IRI = 87.3%) and diamondback (94.5%) watersnakes fed predominantly on fishes, but northern watersnakes fed more on froglet/adult anurans, as indicated by an %IRI value that was 9.3% higher. Female diamondback watersnakes had a %IRI value for fishes that was 9.1% higher when compared to male diamondback watersnakes.

The gape index general linear model was significant ($F_{11,263} = 427.83$, $P < 0.0001$), with SVL having a significant positive relationship with gape index ($F_1 =$

4356.47, $P < 0.001$) but when body size (SVL) was included, slopes did not differ among species ($F_2 = 0.87$, $P = 0.42$) or with species-sex interaction ($F_2 = 2.20$, $P = 0.11$) (Figure 4). In the principal component analysis, the first two principal components (PC1 and PC2) together explained 62.5% of the variance in head dimensions (Figure 5) and were selected for further analysis. The component factor loadings for PC1 from all 6 head metrics had high positive values (>0.510) demonstrating that PC1 is an indicator of head size (Table 6) (Vincent et al. 2004*a*). For PC2, head length and interocular distance both had high positive loading values (>0.493) while tip and head width both had high negative loading values (<-0.440) indicating that PC2 is an indicator of head shape (Figure 6). Regression analyses of snout-vent length against PC1 ($R^2 = -0.004$; $P > 1.00$) and PC2 ($R^2 = -0.004$; $P > 1.00$) were not significant for snakes overall, indicating that there were no ontogenetic effects on head morphology. Resulting R^2 values ranged from -0.004 to 0.083 when investigating individual watersnake species ontogeny for each principal component.

The MANOVA on PC1 and PC2 was significant for species (Wilks' lambda = 0.56, $F_{4,536} = 44.37$, $P < 0.0001$) and sex (Wilks' lambda = 0.91, $F_{2,268} = 13.79$, $P < 0.0001$) and on the border of significance for species-sex interaction (Wilks' lambda = 0.97, $F_{4,536} = 2.25$, $P = 0.06$). A follow-up univariate (ANOVA) F-test on PC1 was significant overall ($F_{5,269} = 16.39$, $P < 0.0001$), with significant species ($F_2 = 23.31$, $P < 0.0001$), sex ($F_1 = 26.28$, $P < 0.0001$) and species-sex interaction ($F_2 = 4.52$, $P = 0.01$) effects. Follow-up least squares means tests on PC1 indicated that northern watersnakes had lower PCA1 values than both diamondback and plain-bellied watersnakes ($P < 0.001$), and there was no difference in PC1 values between plain-bellied and diamondback watersnakes ($P = 0.193$) (Table 7, Figure 7). Follow-up least squares mean tests on sex within species on PC1 demonstrated that females had higher PC1 values than males in diamondback ($P < 0.001$) and northern watersnakes ($P = 0.004$) but not in plain-bellied watersnakes ($P = 0.372$). Follow-up least squares mean tests on sex among species on PC1 showed that female diamondback watersnakes had higher PC1 values than all sexes of plain-bellied and northern ($P < 0.002$) watersnakes. In addition, female plain-bellied watersnakes had higher PC1 values than diamondback males and both northern watersnake sexes ($P < 0.045$). Northern watersnake males had lower PC1 values than males of both diamondback and plain-bellied watersnakes ($P < 0.012$), but diamondback male PC1 values were not different than male plain-bellied ($P = 0.279$). Female northern watersnake PC1 values did not differ significantly from those of males of both plain-bellied and diamondback watersnakes ($P > 0.162$). A follow-up univariate (ANOVA) F-test on PCA2 resulted in overall significance ($F_{5,269} = 29.42$, $P < 0.0001$), with significant species effects ($F_2 = 72.98$, $P < 0.0001$) but no significant sex ($F_1 = 0.2947$, $P = 0.30$) or species-sex interaction ($F_2 = 0.01$, $P = 0.99$) effects. Least squares means tests on PCA2 indicated significant differences ($P < 0.0001$) among all watersnake species.

DISCUSSION

Species- and sex-specific effects in watersnake head morphology appear to relate with differences in diet and foraging. Plain-bellied watersnakes were mainly anurophagous and diamondback watersnakes primarily piscivorous. Northern watersnakes with smaller head sizes and intermediate head shapes had a diet between the two conspecifics but closer to the fish-eating diamondback watersnakes.

Gape indices had strong effects on maximum prey size suggesting that larger prey could increase in the diet with increased snake size. Watersnake gape indices did not vary across species but did increase with SVL. Plain-bellied and diamondback watersnakes obtain larger sizes (SVL) than northern watersnakes (Shine 1991*a*, Gibbons and Dorcas 2004, this study), which result in larger gapes for the largest individuals. Further, plain-bellied and diamondback watersnakes had relatively larger heads than northern watersnakes. Larger head sizes and the longest individuals having larger gape indices can potentially allow the plain-bellied and diamondbacks to ingest larger prey unavailable to northern watersnakes. However, dietary resource overlap is likely low as these two larger species differed strongly in the majority of prey consumed.

Head shape differed significantly among the three watersnake species after effects of body size were removed for analysis. Plain-bellied watersnakes had long, narrow heads and widely spaced eyes. On the other hand, diamondback watersnakes had broad, short heads and closely spaced eyes. Northern watersnakes' heads were intermediate in shape. An earlier study that did not remove head size, similarly found that plain-bellied watersnakes had narrower, longer heads, smaller head volume and increased ocular distance when compared to diamondback watersnakes (Herrel et al. 2008).

In this system, plain-bellied watersnakes with narrow, longer heads may be important for the capture and eating of anurans even though studies have made conflicting conclusions about the benefits of different head shapes. Hampton (2011) demonstrated that wider heads may be important for frog capture and Brecko et al. (2011) indicated that snakes that ate frogs had wider heads. Conversely and controlling for snake size, banded watersnakes (*N. fasciata*) that ate frogs had smaller heads than fish-eating individuals (Vincent et al. 2007). If anurans and fish are of the same mass, anurans may be narrower (Hampton 2013), which supports that plain-bellied watersnakes would have narrower heads when compared to the wider diamondback heads. In this study system, a larger number of small froglets are highly abundant (3.2 mean per trap night in early summer) and very small with a mean 37.9 mm SVL (SD 10.2) (Chapter 4). A wider head may not be necessary to capture and eat these numerous froglets.

Plain-bellied watersnakes are more terrestrial than any of the other watersnake species (Gibbons and Dorcas 2004) and foraging in a mostly 2-dimensional environment may require a narrower head for foraging, dispersal, etc. A head that is somewhat narrower may be beneficial for going through small spaces, crevices and holes in terrestrial environments. Shine (1991*b*) indicated that larger-bodied snakes in search of prey were not able to enter small crevices. A narrow head for movement and large overall head size for swallowing large anurans may be in balance for plain-bellied watersnakes.

In this system, a wider head and tip appear to be important for capturing and ingesting fish for diamondback watersnakes. While searching for prey in aquatic habitats, diamondback watersnakes perform open-mouth foraging (Savitsky 1989), which with a wide head may be beneficial for catching fast fish. Also fish that are large in size, very wide or increased body depth may be difficult to swallow. A wider head, which is involved in swallowing gape may be important for capturing such large, wide or deep prey. Wilson and Hopkins (2011) indicated that banded watersnakes preferred “tubular-shaped” salamanders over tall, narrow Centrarchidae fish. Highly reliant on fish, diamondback watersnakes would likely not switch to more tubular salamander prey but would require a wider head to ingest prey larger in size or prey with increased body depth or width. Northern watersnakes with head width and tips between narrow and wide may be beneficial for their diet in between plain-bellied and diamondback watersnakes foraging on fishes and anurans.

The anurophagous plain-bellied watersnake had a greater interocular distance than the piscivorous diamondback watersnake, which could be due to temporal pattern of activity, habitat use, or prey preferences. Plain-bellied watersnakes are more diurnal and terrestrial than diamondback watersnakes (Preston 1970). Increased interocular distance may be beneficial for plain-bellied watersnakes for their diurnal activity and frog capture in terrestrial environments. Eyes further apart may also help in predator detection in terrestrial environments where the plain-bellied may be vulnerable when unable to readily escape into aquatic habitats. Individuals of the dice snake (*Natrix tessellata*), an old-world natricine watersnake, that ate frogs had increased interocular distance over individuals that ate fish (Brecko et al. 2011).

Conversely, diamondbacks are much more nocturnal (Mushinsky and Hebrard 1977*b*, Gibbons and Dorcas 2004) and aquatic (Gibbons and Dorcas 2004). Diamondbacks use vision when searching for prey but vision may not be as important in eutrophic water and water high in sedimentation (Savitsky 1989). My system involves murky slough habitats. Also, a decreased interocular distance of diamondback watersnakes would place their eyes more dorsally, and when searching for prey in aquatic habitats, prey above the snake may be more easily seen based on silhouette if the snake is foraging below the prey. Diamondback watersnakes approach fish underwater, wait for fish to swim above and are then very successful in fish capture when pursuing from below (Savitsky 1989). In addition, the contrast of fish versus its background is a very important factor in the capture of prey by diamondback watersnakes (Czaplicki and Porter 1974), which may offer support for diamondback eyes to be more dorsal when foraging underwater below prey. A decreased interocular distance may be beneficial for aquatic foraging by the piscivorous diamondback watersnakes. The northern watersnake with intermediate levels of interocular distance may be beneficial for their diet in between plain-bellied and diamondback watersnakes foraging on fishes and anurans.

Northern watersnake diet was more similar to the piscivorous diamondback than the anurophagous plain-bellied watersnake but which conspecific's head morphology is closer to the northern? Northern watersnakes differed from plain-bellied and diamondback watersnakes on both principal components with smaller head sizes and intermediate head shapes. To answer my question, it appears that northern watersnakes differ from both plain-bellied and diamondback watersnakes in terms of overall head morphology.

Northern watersnakes with intermediate head shapes support their diet in between plain-bellied and diamondback watersnakes. Such intermediate head morphology and diet may allow northern watersnakes to coexist with the other two larger species. Also potentially allowing a smaller watersnake with a smaller head size to coexist, northern watersnakes have the ability to switch prey with changing prey levels (King et al. 1999*b*, King et al. 2006), which may also relate to the intermediate northern watersnake head shape. In addition, Himes (2003*b*) indicated that northern watersnakes may not be competing with diamondback watersnakes if adequate prey is available, and northern watersnakes may be better competitors than diamondback watersnakes. The northern watersnake appears to do very well in my system with 122 individuals captured for head morphology metrics compared to 74 plain-bellied and 79 diamondback watersnakes.

Watersnake sex along with species had varying effects on head morphology. Watersnake gape indices did increase with snake size but did not vary with sex. Female watersnakes obtain longer SVLs than male watersnakes (Shine 1991*a*, Gibbons and Dorcas 2004, this study) resulting in higher gape indices for the longest female snakes. The longest female watersnake captured was 984 mm SVL while the longest male was 742 mm SVL. PC1, an indicator of head size, demonstrated that both diamondback and northern female watersnakes had larger head sizes than their male counterparts within species. For both species, females foraged more on fishes than males with diamondback sex having a larger %IRI fish difference of 9.1%. In support, the biggest difference within species with regard to sex head size morphology was for the diamondback watersnake. Similarly with snake size removed, female diamondback watersnakes will eat larger prey than male diamondbacks (Mushinsky et al. 1982). Female diamondback watersnakes also had a larger head size than all sexes of plain-bellied and northern watersnakes possibly resulting in the female diamondbacks eating larger fish, which may be unavailable to all others. In my research, the mean volume (18.8 ml) of individual fish prey for female diamondback watersnakes was greater ($F_{1,164} = 6.64$, $P = 0.01$) than the mean volume (5.31 ml) of individual fish prey for all other snakes including diamondback males and both sexes of the two conspecifics.

Plain-bellied watersnake sex did not affect head size with both sexes foraging mainly on anurans but differing according to anuran life stage. Females fed 14.2%IRI higher on froglet/adult anurans while males fed 22.1%IRI higher on tadpole/metamorph anurans. Conversely, Mushinsky et al. (1982) indicated sex did not affect plain-bellied watersnake diet and anuran prey sizes allowing dietary overlap between the sexes of plain-bellied watersnakes. However, this 1982 study did not consider different anuran life stages. While plain-bellied watersnake sexes have similar head sizes and have considerable dietary overlap, there appears to be some differences in diet relating to plain-bellied sexes feeding on different anuran life stages.

Male northern watersnakes had a smaller head size than all other snakes. While male northern watersnakes may be the smallest snakes and have the smallest head sizes, they may be able to coexist with the other predominantly fish-eaters, female northern and both diamondback sexes, by male northern watersnakes having foraging differences. Male northern watersnakes had the lowest fish %IRI (81.7%) and highest Anuran %IRI (17.5%) of the sexes of the two mainly piscivorous watersnake species.

Gut content data and morphology may provide evidence that female northern and male diamondback watersnakes could potentially have much dietary overlap. These two opposite sexes from different species fed on similar levels of fishes and anurans with only 0.5% IRI difference for fishes and 4.4% IRI difference for froglet/adult anurans. Regarding morphology, PC1 values indicated head sizes were very close with female northern being -0.184, and -0.158 for male diamondback watersnakes. The mean lengths of the two were similar with northern female being 551 mm SVL and male diamondback being 491 mm SVL, and indicated previously, similar-sized (SVL) snakes have similar gape indices. With similar diets, gapes, head size and mean snout-vent lengths, the highest potential for competition would be between female northern and male diamondback watersnakes. On the other hand, head shape differences (PC2) between these two piscivorous species may provide some evidence for dietary partitioning that could relieve competition between female northern and male diamondbacks.

Head shape for all three watersnake species did not differ according to sex. Similar head shapes for sexes within species provides support that watersnake sexes are not dietary specialists. There were dietary differences relating to watersnake sex and sexual dimorphism did play a role for head size for diamondback and northern watersnakes, but there is not enough evidence to indicate that sexes are specializing on specific prey. If such dietary specialization occurred by sex with the dietary exclusion of the opposite conspecific sex’s preferred prey, sexual dimorphism involving head shape would likely occur (Camilleri and Shine 1990).

Plain-bellied, diamondback and northern watersnakes have dietary differences due to species and sex factors. Such various factors on diet can relate to head morphology and can be used to help understand the coexistence among sympatric species. Overall, snake head morphology may provide a window into the diet and foraging strategies of watersnakes.

Table 3. Snout-vent length (SVL) mean and range (mm) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

	N	SVL Mean (SE)	SVL Range
Plain-bellied	74	620 (22.9)	302–984
Female	40	675 (34.3)	302–984
Male	34	556 (25.8)	319–742
Diamondback	79	512 (19.18)	212–900
Female	46	528 (29.2)	212–900
Male	33	491 (20.7)	313–718
Northern	122	508 (11.2)	271–794
Female	68	551 (16.0)	271–794
Male	54	456 (12.0)	275–601

Table 4. Mean $\log_{10}$ snout-vent length and $\log_{10}$ mean head morphology metrics in mm (SE) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

Group	*N*	Snout-vent length	Tip	Head width	Jaw length	Head length	Interocular distance	Eye position
Plain-bellied	74	2.768 (0.018)	0.659 (0.014)	1.215 (0.017)	1.406 (0.014)	1.333 (0.012)	0.934 (0.011)	0.939 (0.013)
Female	40	2.802 (0.026)	0.679 (0.020)	1.257 (0.024)	1.437 (0.020)	1.357 (0.018)	0.956 (0.016)	0.966 (0.019)
Male	34	2.728 (0.022)	0.636 (0.017)	1.165 (0.022)	1.369 (0.017)	1.304 (0.015)	0.907 (0.014)	0.908 (0.017)
Diamondback	79	2.691 (0.016)	0.631 (0.013)	1.189 (0.016)	1.351 (0.012)	1.262 (0.011)	0.847 (0.010)	0.898 (0.013)
Female	46	2.693 (0.024)	0.647 (0.019)	1.209 (0.024)	1.370 (0.018)	1.271 (0.017)	0.857 (0.016)	0.908 (0.019)
Male	33	2.678 (0.018)	0.607 (0.016)	1.161 (0.018)	1.324 (0.013)	1.249 (0.013)	0.833 (0.011)	0.885 (0.018)
Northern	122	2.692 (0.010)	0.615 (0.009)	1.158 (0.011)	1.326 (0.008)	1.261 (0.008)	0.858 (0.008)	0.876 (0.009)
Female	68	2.726 (0.014)	0.639 (0.012)	1.204 (0.016)	1.358 (0.012)	1.286 (0.010)	0.884 (0.010)	0.906 (0.012)
Male	54	2.650 (0.012)	0.585 (0.012)	1.101 (0.012)	1.285 (0.010)	1.230 (0.009)	0.824 (0.009)	0.837 (0.010)

Table 5. The Index of Relative Importance (%IRI) for fishes, Caudata, Anurans (tadpole/metamorph) and Anurans (froglet/adult) based on snake gut content data for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

	N	Fishes	Caudata	Anura (Tadpole/ Metamorph)	Anura (Froglet/ Adult)
Plain-bellied	63	14.4	0.4	6.9	78.3
Female	37	13.7	0.8	0.2	85.3
Male	26	6.6	0.0	22.3	71.1
Diamondback	39	94.5	1.7	2.1	1.7
Female	20	97.9	0.5	1.1	0.5
Male	19	88.8	6.1	1.2	3.9
Northern	74	87.3	0.3	1.4	11.0
Female	41	89.3	0.6	1.8	8.3
Male	33	81.7	0.0	0.8	17.5

Table 6. Component factor loadings (PC1 and PC2) from a principal component analysis for 6 $\log_{10}$ head morphology metrics for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

	Tip	Head width	Jaw length	Head length	Interocular distance	Eye position
Principal component 1	0.582	0.571	0.836	0.673	0.510	0.705
Principal component 2	-0.440	-0.570	0.046	0.493	0.625	-0.153

Table 7. Mean principal component values (PCA1 and PCA2) for 6 log$_{10}$ head morphology metrics for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

	PCA1		PCA2	
Group	Mean	SE	Mean	SE
Plain-bellied	0.176*	0.092	0.890*	0.086
Female	0.260	0.118	0.851	0.113
Male	0.076	0.143	0.935	0.135
Diamondback	0.441$^{>}$	0.110	-0.678*	0.091
Female	0.870^{+}	0.131	-0.727	0.102
Male	-0.158^{+}	0.135	-0.610	0.167
Northern	-0.392*$^{>}$	0.089	-0.101*	0.076
Female	-0.184^	0.113	-0.148	0.109
Male	-0.654^	0.135	-0.041	0.105

*$^{>}$ species were significantly different within component.
$^{+}$ ^ sexes within species were significantly different within component.

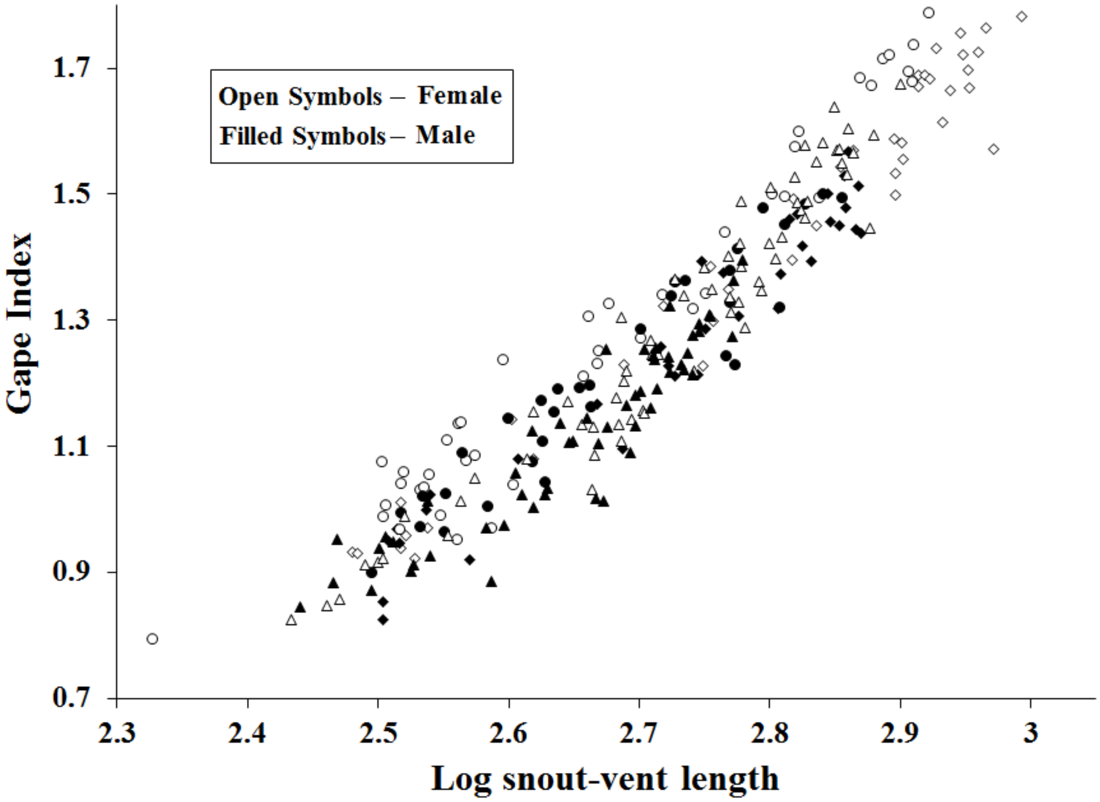

Figure 4. Gape index versus $\log_{10}$ snout-vent length (mm) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes. Diamond symbols represent plain-bellied, circle symbols represent diamondback, and triangle symbols represent northern watersnakes.

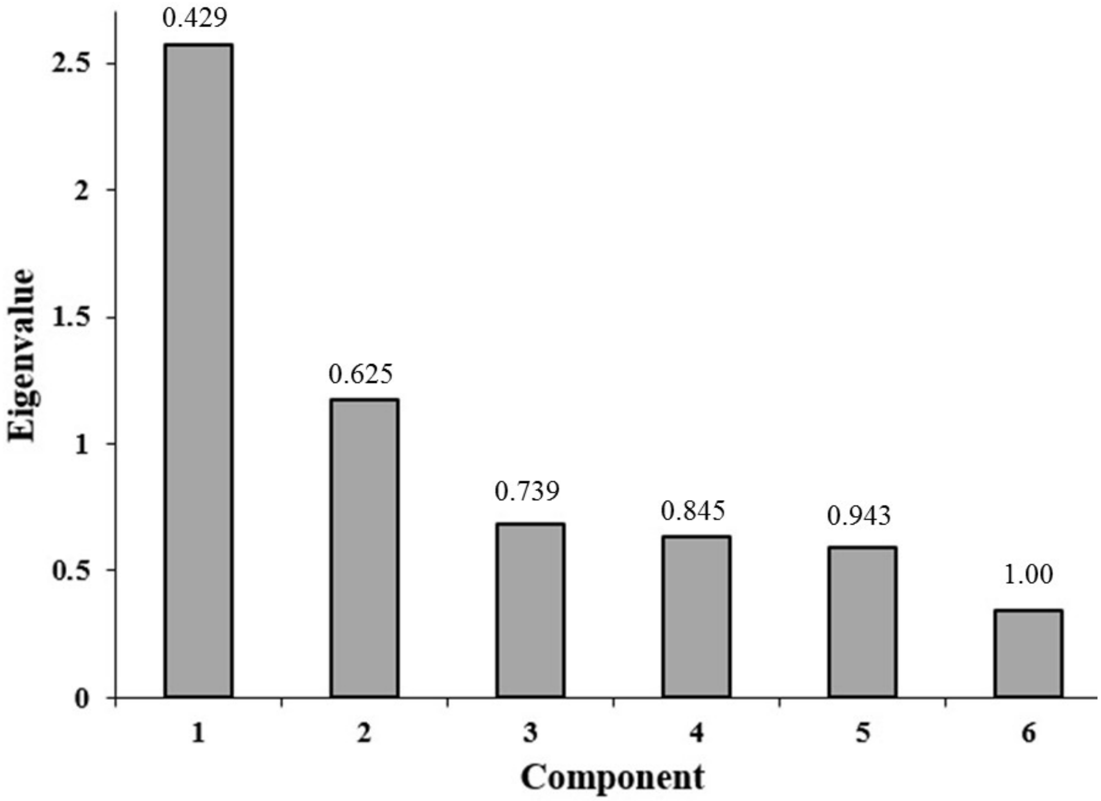

Figure 5. Principal component analysis scree plot for 6 $\log_{10}$ head morphology metrics (mm) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*). The numbers above each bar indicate the cumulative variance explained by each successive component.

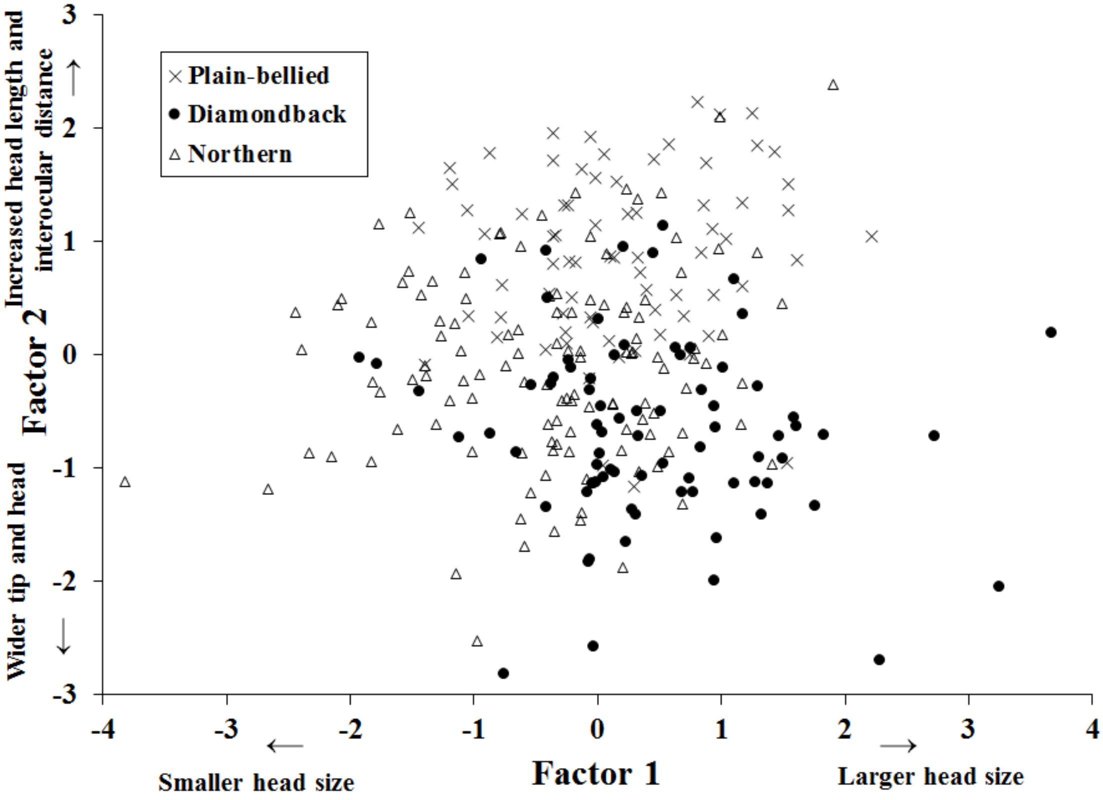

Figure 6. Scatter plot of the component factor loadings from the first two principal components for 6 $\log_{10}$ head morphology metrics (mm) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes. Each symbol represents an individual snake.

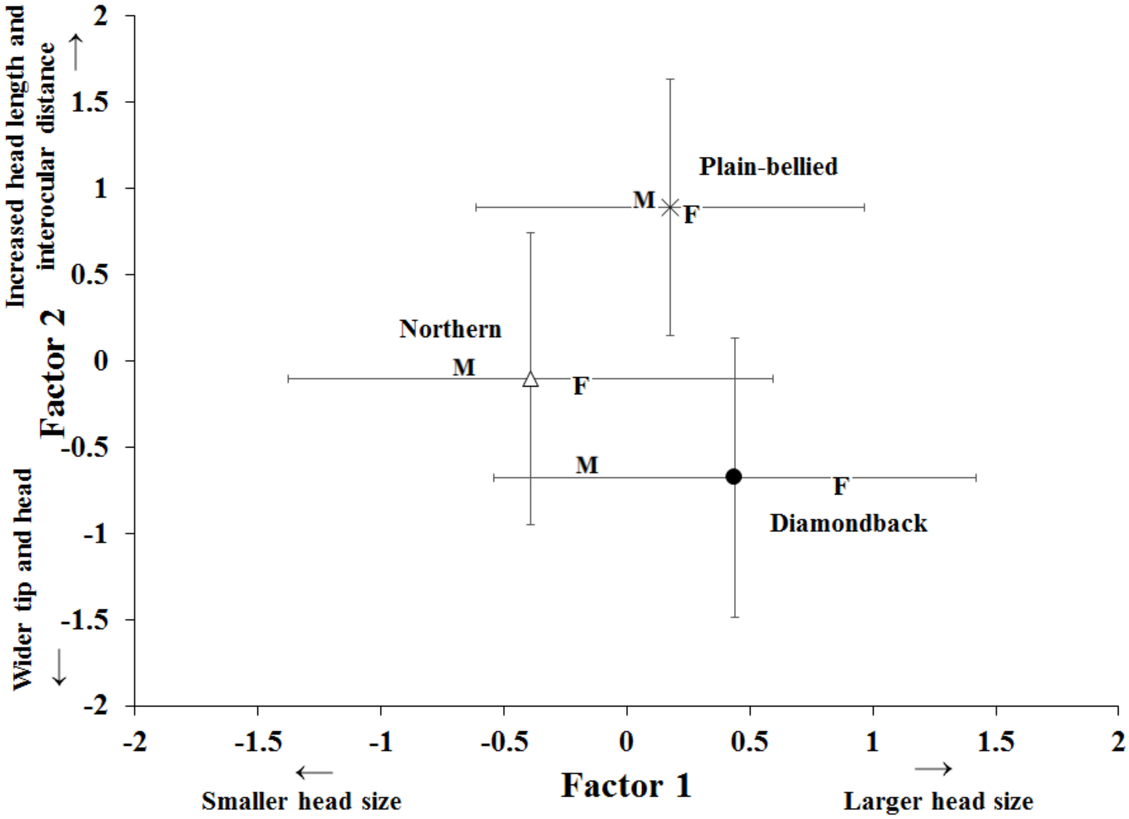

Figure 7. Mean component factor loadings from the first two principal components for 6 $\log_{10}$ head morphology metrics (mm) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes. Error bars represent 1 standard deviation around each species mean. The symbols F (female) and M (male) represent the mean component factor loadings (both principal components) according to sex for each watersnake species.

CHAPTER IV

THE FORAGING ECOLOGY OF THREE SYMPATRIC WATERSNAKE SPECIES

SUMMARY

Similar species may be able to coexist because of microhabitat, landscape or temporal resource utilization differences but variation in dietary resource utilization may be the most important in affecting the coexistence of sympatric snake species. Watersnakes (*Nerodia* spp.) often inhabit similar wetland habitats feeding mostly on fishes and amphibians. Dietary differences among such sympatric watersnake species may be complicated by seasonal changes in prey populations and a variety of intraspecific factors. To understand watersnake coexistence, I investigated dietary resource utilization among sympatric plain-bellied (*N. erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes. Gut contents were determined from 60 individual watersnakes in 2013 and 118 in 2014. Plain-bellieds fed mostly on anurans, diamondbacks on fishes, and northern watersnakes on fishes with an elevated anuran component. Season affected dietary overlap with each watersnake species having reduced overlap for a different season. While plain-bellied watersnakes ate mainly anurans, the smallest and largest plain-bellied watersnakes were more likely to have gut contents containing fish. Dietary resource utilization was affected by various factors resulting in a complicated dynamic foraging system likely allowing for coexistence of sympatric watersnake species.

INTRODUCTION

When seemingly similar species are sympatric, theory predicts the mostly highly competitive species will drive others to extinction (Hardin 1960). However, when similar species persist in the same area and overlap in resource utilization, coexistence is dependent on resource partitioning or resource utilization differences (MacArthur 1958, Pianka 1974, Wieland and Bazzaz 1975). Such differences in resource utilization can involve various factors including dietary, spatial, or temporal (Pianka 1973, Vitt 2001).

Watersnakes of the genus *Nerodia* are distributed throughout the eastern United States with many species overlapping in distribution (Ernst and Ernst 2003), utilizing similar aquatic habitats (Hebrard and Mushinsky 1978, Mushinsky et al. 1980) and feeding mostly on amphibians and fishes (Gibbons and Dorcas 2004). Co-existing watersnakes can differ in resource utilization by time of activity (Mushinsky and Hebrard 1977*b*, Hebrard and Mushinsky 1978), utilizing different focal areas across landscapes (Roe et al. 2003, Roe et al. 2004, Marshall 2008) and microhabitat differences in local aquatic areas (Hebrard and Mushinsky 1978, Laurent and Kingsbury 2003). While coexisting watersnakes may differ in few or many resources, dietary may be the most important resource utilization difference involving sympatric snake species (Henderson 1974, Brown and Parker 1982, Toft 1985, Vitt 2001, Goodyear and Pianka 2008).

Plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern watersnakes (*N. sipedon*) are sympatric in areas of the eastern United States and have considerable dietary overlap in various prey items including tadpoles, adult frogs, salamanders and a diversity of fish (Mushinsky and Hebrard 1977*a*, Mushinsky et al. 1982, Gibbons and Dorcas 2004). In many habitats, plain-bellied watersnakes may be eating mainly amphibians (Preston 1970, Mushinsky and Hebrard 1977*a*, Roe et al. 2004) while diamondback watersnakes may focus on fishes (Hess and Klimstra 1975, Kofron 1978, Savitzky 1989). In addition, the northern watersnake eats a large variety of prey but fish may be the primary diet item (Ernst and Ernst 2003, Himes 2003*a*, Gibbons and Dorcas 2004). While general diet descriptions have been identified, dietary analyses have not addressed all three watersnakes in sympatry.

Also, various factors can potentially affect the diets of sympatric plain-bellied, diamondback and northern watersnakes. Ontogenetic changes can complicate watersnake foraging ecology (Luiselli 2006), which may be related to both watersnake size and age (Mushinsky and Lotz 1980). The effect of age on watersnake diet has rarely been addressed by researchers (Lacy 1995). Moreover with female watersnakes reaching larger sizes than conspecific males, larger females may be able to swallow larger prey resulting in dietary differences due to sex (Mushinsky et al. 1982).

The availability of watersnake prey can also change with season, which has been greatly unexplored in watersnakes (Willson et al. 2010). Habitat changes such as flooding have been demonstrated to affect prey availability resulting in changes in dietary overlap among watersnakes (Hampton and Ford 2007). Changing prey levels may be important for watersnake dietary resource utilization as northern watersnake diet can change with changes in prey populations (Carbone 1993, King et al. 1999*b*, King et al. 2006).

With these in mind, watersnake dietary resource utilization and foraging differences may be complicated by many factors. To understand coexistence and address the question of diet affected by various factors, I investigated dietary resource utilization among plain-bellied, diamondback and northern watersnakes incorporating differences in species, season, ontogeny and sex.

METHODS

The study site was a 100-hectare section of the Sloughs Wildlife Management Area (Henderson County, Kentucky, USA), which is managed by the Kentucky Department of Fish & Wildlife Resources. This section, located 2 kilometers southeast of the Ohio River, is known as Hardy Slough/Muddy Slough, and has high densities of the three target watersnake species (Laurent and Kingsbury 2003). Habitat types included moist soil units (shallow wetlands managed for wintering waterfowl), scrub-shrub wetlands and palustrine forest. Dominant plants were water primrose (*Ludwigia* sp.), smartweed (*Polygonum* sp.), water lily (*Nuphar* sp.) cattail (*Typha* sp.), buttonbush (*Cephalanthus occidentalis*), black willow (*Salix nigra*) and hackberry (C*eltis occidentalis*). The study period was from April through September in 2013 and 2014.

In order to determine prey availability, I sampled potential snake prey weekly. To define three ecologically relevant seasons, I used changes in the capture rates for anurans at different life stages: tadpole, metamorph, froglet and frog. Anuran metamorphs are tadpoles with well-defined legs that are beginning to show adult traits and froglets are recently metamorphosed frogs with no tadpole traits (McDiarmid and Altig 1999). The spring season started in April and continued until there was a noticeable drop-off (90% decrease) in the number of captured anuran tadpoles and metamorphs. The early summer season then began and continued until the capture rate of anuran froglets noticeably (90% decrease) declined. Late summer then began. Spring was from April through May 29 in 2013 and from April through May 27 in 2014. Early summer was May 30 to July 23 in

2013 and May 28 to July 24 in 2014. Late summer ran from the day after early summer ended through September for both years of the study.

Aquatic sampling methods for potential snake prey included stand-alone aquatic funnel traps, aquatic hoop traps and aquatic drift fence arrays with aquatic funnel traps. All aquatic funnel traps had ~25% of the trap above the water line to prevent the drowning of non-target animals. Terrestrial sampling methods for potential snake prey included terrestrial drift fences associated with terrestrial funnel traps and pit-fall traps (5-gallon buckets). I built all drift fences from silt fence material and wooden stakes, and all funnel traps (aquatic and terrestrial) had 0.64 cm mesh size and circular openings that ranged from 3.8 cm to 10 cm in diameter. I identified and recorded captured potential prey and I determined the number of trap nights (number of traps multiplied by the number of days the traps were open) for each season. Captured anurans were recorded as tadpoles, metamorphs, froglets or adults.

I captured snakes by a variety of methods including hand capture, cover board placement, stand-alone aquatic funnel traps and drift fence arrays (terrestrial and aquatic) with funnel traps. For each captured watersnake, I measured snout-vent length (SVL) in millimeters and determined sex by cloacal probing. To determine recaptures, snakes were marked with both subcutaneous pit tags (Gibbons and Andrews 2004) and ventral scale-clip patterns (Plummer and Ferner 2012).

To determine their diet, I forced watersnakes by gentle palpation to regurgitate their gut contents (Kofron 1978, Fitch 2001). I identified snake gut contents only to family because partial digestion of some prey items prevented identifying them to lower classification. In addition, volume (ml) and length of each prey item were recorded and life stage was recorded for amphibian prey. I released each snake at its capture location. All animal capture, handling and processing activities were approved the University of Louisville Institutional Animal Care and Use Committee (IACUC Protocol: #13037).

To investigate the importance of specific prey, I incorporated three quantitative values into an Index of Relative Importance (IRI) using the equation $\mathrm{IRI} = (\%N + \%V) \cdot (\%F)$ (Pinkas et al. 1971, Franks and Vanderkooy 2000, Vaudo and Heithaus 2011). Here, %N is the numerical prey category percentage pooling all individual snakes (numeric prey percentage). The total volume percentage of all prey from that category that were found in stomachs of all snakes (volume prey percentage) was %V, and %F is the percentage of individual snakes that had eaten from a prey type category (occurrence percentage). To compare IRI values from different prey groups within an analysis, each prey category (IRI_i) was converted to a percentage ($\%\mathrm{IRI}_i$) by dividing the specific prey category (IRI_i) by the sum of IRI_i values using the following equation: $\%\mathrm{IRI}_i = 100 \cdot \mathrm{IRI}_i / \Sigma \mathrm{IRI}_i$ (Cortés 1997, Kinney et al. 2011). Involving a specific %IRI analysis, prey category percentages ($\%\mathrm{IRI}_i$) will sum to 1 with higher values indicating greater importance in watersnake diet. One %IRI analysis was performed using 6 prey categories (crayfish, fish, siren, mole salamander, anuran tadpole/metamorph and anuran froglet/adult). A second %IRI analysis was performed on 12 taxonomic families of ingested prey including 1 crayfish family (Cambaridae), 7 fish families (Lepisosteidae, Amiidae, Cyprinidae, Esocidae, Aphredoderidae, Poeciliidae, Centrarchidae) and 4 amphibian families (Sirenidae, Ambystomatidae, Hylidae and Ranidae).

To further analyze watersnake diet, I used a model comparison approach utilizing logistic regression (Hosmer and Lemeshow 2000) and information theoretic methods

(Burnham and Anderson 2002). I estimated the probability of watersnake gut contents containing a particular diet item. Diet items, analyzed separately, were fish, tadpole/metamorph anuran or froglet/adult anuran. The binary response variable was the presence of a particular diet item (0, not present; 1, present). To understand overall watersnake diet in this system and differences among watersnakes species, I analyzed the three watersnake species together using snake species, year, sex, SVL and season as predictor variables. Seasons were converted to numeric values (spring = 1; early summer = 2; late summer = 3) for this analysis. I built 32 *a priori* candidate models using relevant interactions and higher order functions. Second, I separately analyzed each watersnake species using the same predictor variables minus snake species (18 models).

I identified parsimonious models using resulting AIC_c (Akaike's Information Criterion for small sample size) and Akaike weights (w_i) from models $\leq 2\Delta i\ AIC_c$ (Anderson et al. 2000, Burnham and Anderson 2002). I determined predictor variable importance by calculating parameter estimates, unconditional standard errors and sum of Akaike weights (Σw_i) for predictor variables from models $\leq 2\Delta i\ AIC_c$ (Burnham and Anderson 2002). To illustrate importance, I plotted the estimated probability in diet versus selected predictor variables.

To help further understand modeling results I used Simpson's Inverse Diversity Index ($D = 1/\Sigma p_i^2$) to investigate diversity of snake prey. Simpson's Inverse Diversity Index (D) uses prey category proportions, with resulting higher D values indicating increased diversity of ingested prey (Pianka 1973, Feinsinger et al. 1981, Hadi et al. 2012). Prey categories used for this index were the 12 previously mentioned taxonomic families of ingested prey.

I also used a Proportional Similarity Index (PSI) to determine dietary overlap relating to season by comparing snake groups using the following equation: $PSI = 1 - 0.5 \cdot \Sigma|p_{ij} - q_{ij}|$ (Schoener 1968, Lanszki et al. 1999). The values of a PSI, which compares two groups, range from 0 to 1 with a value of 1 indicating complete overlap in diet and a zero value indicating no overlap in diet. Wallace (1981) indicated that PSI values >0.60 may be biologically meaningful. The PSI analysis was performed on the 12 previously mentioned taxonomic families of ingested prey.

RESULTS

In 2013, there were 446 trap nights for aquatic and 589 trap nights for terrestrial sampling of potential prey. In 2014, there were 918 aquatic and 886 terrestrial. Because the capture rates of anuran tadpoles/metamorphs and froglets were used to define seasons (see Methods), the mean number of anuran tadpoles/metamorphs captured per trap night was high during spring in both 2013 and 2014 (Figure 8). Anuran froglets were absent in spring, had high capture rates in early summer and disappeared in late summer in both years (Figure 9). In spring 2013, aquatic traps captured a high mean number of fishes per trap night, which was the result of two traps capturing large broods of bowfin (*Amia calva*) early in the season. Adult frogs were captured at low rates during all seasons, likely because they could readily jump out of terrestrial pitfall traps.

In addition to the 3 target watersnake species, brown snakes (*Storeria dekayi*), eastern garter snakes (*Thamnophis sirtalis*), eastern ribbon snakes (*Thamnophis sauritus*), black kingsnakes (*Lampropeltis nigra*) and rat snakes (*Pantherophis obsoletus*) were captured in this study. In 2013, a total of 196 individual watersnakes were captured, which included 76 plain-bellied, 48 diamondback and 72 northern watersnakes (Table 8).

Four plain-bellied, 2 diamondback and 5 northern watersnakes were recaptured in 2013. In 2013, 27 plain-bellied, 11 diamondback and 22 northern watersnakes had gut contents that could be palpated and regurgitated. In 2014, 266 individual watersnakes were captured for the first time, including 74 plain-bellied, 78 diamondback and 114 northern watersnakes. In addition, 11 snakes first captured in 2013 were recaptured in 2014 (1 plain-bellied, 2 diamondback and 8 northern), and 24 snakes first captured in 2014 were recaptured that year (7 plain-bellied, 3 diamondback and 14 northern). In 2014, 36 plain-bellied, 28 diamondback and 54 northern watersnakes had gut contents that could be palpated and regurgitated. Of the recaptures, only 2 northern watersnakes had gut contents both times they were captured in 2013 and in 2014.

Fishes comprised the majority of diamondback (%IRI = 95.2%) and northern (86.9%) watersnake diet compared to plain-bellied (13.8%) watersnakes (Table 9). Plain-bellied watersnakes fed mostly (%IRI = 79.1%) on froglet/adult anurans with northern being 11.5% and diamondback watersnakes 1.6%. Ranidae was the prey family with the highest %IRI for all three watersnake species with plain-bellied being 96.5%, northern 49.4% and diamondback 32.5%. Almost half of the overall diamondback diet consisted of 2 fish families, Amiidae and Esocidae (%IRI sum 46.9%). The most important overall fish family for northern watersnakes was Esocidae (%IRI = 25.0%).

The probability of overall watersnake gut contents containing a particular item was affected by various factors (Table 10). The probability of containing fish was determined by snake species and season. Plain-bellied watersnake gut contents were less likely to contain fish when compared to diamondback and northern watersnakes (Figure 10), and watersnakes overall fed more on fish with seasonal changes from spring to early summer to late summer. The opposite pattern was found for tadpole/metamorph anurans with watersnakes feeding on fewer with increased season. Plain-bellied watersnakes were more likely to feed on froglet/adult anurans compared to diamondback and northern watersnakes, and watersnakes overall fed on fewer froglet/adult anurans in 2014.

A few different factors were important in affecting plain-bellied watersnake diet (Table 11). The probability of plain-bellied watersnake gut contents containing fish was higher for males and also higher in 2014. The probability of fish in plain-bellied watersnake gut contents had a negative quadratic relationship relating to SVL with higher probabilities for the smallest and largest snakes but at low levels for medium-sized snakes (Figure 11). Similar to watersnakes overall, plain-bellied watersnakes fed on fewer tadpole/metamorph anurans with the progression of the seasons (Figure 12). Plain-bellied watersnakes fed at higher levels of froglet/adult anurans with seasonal changes but on fewer froglet/adult anurans in 2014.

Diamondback watersnake diet was affected by various factors (Table 12). Females were more likely than males to have gut contents containing fish, and fish increased in diamondback diet through the seasons. Season negatively affected the probability of gut contents containing tadpole/metamorph anurans. The only factor affecting froglet/adult anurans for diamondbacks was year with lower levels in 2014.

Year and seasonal factors were important in determining the probability of gut contents containing specific dietary items for northern watersnakes (Table 13). Similar to diamondbacks, northern watersnakes fed more on fish with seasonal changes. The probability of gut contents containing tadpole/metamorph anurans increased with year with more being fed on in 2014. The probability of froglet/adult anurans found in northern watersnakes was only affected by season with a strong evident quadratic relationship. Northern watersnakes did not feed on froglet/adult anurans in the spring season but fed at a high levels in early summer and had a sharp drop off in late summer.

Plain-bellied watersnakes had lower diversity (*D*) values for all snake groupings (Table 14) indicating a narrow plain-bellied watersnake diet. The smallest (<450 mm SVL) and largest (>850 mm SVL) plain-bellied watersnakes were the only plain-bellied groups to have *D* values over 1.778. Diamondback and northern watersnakes had more diverse diets involving a variety of prey families with the majority being fishes. Plain-bellied watersnake prey diversity was similar across seasons while diamondback watersnake prey diversity increased with season. Northern watersnake prey diversity varied seasonally with a drop in early summer and with late summer being the highest.

Seasonal dietary overlap investigating prey families had varying results (Table 15). Plain-bellied watersnake diets were similar through the seasons with PSI values ranging from 0.800 to 0.828 while diamondback diet varied by season with the least amount of dietary overlap between spring and late summer (PSI = 0.267). Dietary overlap among watersnake species demonstrated that plain-bellied and diamondback watersnakes had high overlap in spring (PSI = 0.700) while plain-bellied and northern watersnakes had somewhat high dietary overlap in early summer (0.615). Diamondback and northern watersnakes had very high dietary overlap in late summer (PSI = 0.819).

DISCUSSION

The overall diets of the three watersnake species followed general expectations based on previous studies. Plain-bellied watersnakes had a narrow diet focused on anurans with others indicating similar results of 65 to 97% on anurans (Mushinsky and

Hebrard 1977*a*, Byrd et al. 1988, Roe et al. 2004). Diamondback watersnakes predominantly ate fishes. Similarly, fish in diamondback diet can range from 89 to 98.5% (Preston 1970, Hess and Klimstra 1975, Mushinsky and Hebrard 1977*a*). Northern watersnakes have a very diverse diet but fish may be the main prey item (Lacy 1995, Himes 2003*a*, Gibbons and Dorcas 2004). This research supported a mostly piscivorous diet for northern watersnakes but with a higher anuran component than was found in diamondbacks. In support, northern watersnakes may spend less time in aquatic habitats than diamondback watersnakes (Savitsky 1989). Also similar to other research, salamanders and crayfish were mostly minor diet items for these three watersnake species (Ernst and Ernst 2003, Gibbons and Dorcas 2004).

Seasonal prey factors were very important in this foraging system resulting in changes in both dietary composition and overlap for all three watersnake species. The spring season provided a large number of tadpole/metamorph anurans. While plain-bellied watersnakes eat mainly adult frogs and diamondback watersnakes are mostly piscivorous (Preston 1970), this intermittent tadpole/metamorph stage was an important spring resource for both snake species with this resource decreasing with season. Preston (1970) indicated that 11% of diamondback watersnake diet consisted of tadpoles, and Byrd et al. (1988) found that diamondback watersnakes ate more anurans in early spring when anurans were higher in number. Diamondback watersnakes are often found close to water and in areas with a high amount of open water (Savitsky 1989, Dorcas and Gibbons 2004), which supports feeding on the aquatic tadpole/metamorph anurans. Also, the spring prey diversity value for diamondbacks was at its lowest level likely indicating the importance of tadpole/metamorph anurans with their piscivorous diet. Such similar spring foraging by plain-bellied and diamondback watersnakes resulted in elevated overlap (PSI = 0.700) but about half of all plain-bellieds had gut contents containing froglet/adult anurans and about half of diamondbacks had fishes. Since tadpole/metamorph anurans were not the main prey type for either watersnake, such spring overlap would not likely affect coexistence by itself. This spring anuran resource pulse was not important for northern watersnakes with the probability of tadpole/metamorph anurans in their diet being affected only by year. Conversely, Zelnick (1966) indicated that 65% of amphibians eaten by northern watersnakes were tadpoles.

Seasonal prey factors affecting diet and overlap continued into early summer. The elevated tadpole/metamorph anurans of spring resulted in a large number of froglets in early summer. Plain-bellied watersnakes forage mainly on froglet/adult anurans and their estimated probability of occurrence in plain-bellied diet increased from 50.9% in spring to 65.8% in early summer. Also, the opportunistic northern watersnake took advantage of this early summer resource pulse going from not feeding on this prey in spring to having a 47.2% estimated probability of foraging on froglet/adult anurans in early summer. Northern watersnakes have a varied diet in which they may eat readily available prey (Gibbons and Dorcas 2004, Roe et al. 2004). In support, northern watersnake prey diversity was at its lowest seasonal level in early summer when northern watersnakes fed more on froglet/adult anurans. Plain-bellied and northern watersnakes had their highest dietary overlap (PSI = 0.615) during early summer. Compared to anurophagous plain-bellied watersnakes, northern watersnakes have a broader diet eating more fish along with anurans (Roe et al. 2004, Gibbons and Dorcas 2004). My research indicated a 64.7% estimated probability of northern watersnakes containing fish in early summer. Therefore, slightly less than two-thirds dietary overlap levels for plain-bellied and northern watersnakes for early summer would not likely affect coexistence by itself.

The late summer season also continued the pattern of season affecting watersnake diet. Tadpole/metamorph anurans were at low levels in the environment and were fed on very little. Also in late summer, froglet/adult anurans were at their highest level in plain-bellied diet (78.1% estimated probability) but dropped to 7.1% for northern watersnakes. Diamondback and northern watersnakes feed mainly on fishes and this prey increased in their diets through the seasons with both species feeding at similar high levels in late summer. In support, both piscivorous snakes had their highest prey diversity values in late summer indicating feeding on a high diversity of fish. Diamondback and northern watersnakes had high dietary overlap only in this late summer period with PSI = 0.819. Such increased dietary overlap could result in potential competition in late summer. However, coexistence may be facilitated by an interaction of diet and habitat. Diamondback watersnakes could be feeding more in deeper aquatic habitats while northern watersnakes could be feeding more along the water's edge (Himes 2003*b*).

Seasonal factors greatly impacted prey resources, watersnake foraging and dietary overlap in this system. Tadpole/metamorph anuran levels in spring were utilized by both plain-bellied and diamondback watersnakes while froglet/adult anurans in early summer were important for plain-bellied and northern watersnakes. In late summer, diamondback and northern watersnakes had much dietary overlap feeding on a diversity of fishes. Such seasonal prey pulses could reduce potential competition (Willson et al. 2010). Each season had a different watersnake species pair having high overlap with each watersnake species having a unique season with reduced overlap, which also likely complemented coexistence. Spring had northern watersnakes with reduced overlap, early summer for diamondback watersnakes and late summer for plain-bellied watersnakes.

Snake sex was only important in affecting the probability of fish in watersnake diet. Female diamondbacks were more likely to feed on fish than conspecific males. Adult female watersnakes obtain larger lengths and mass than males (King et al. 1999*a*, Gibbons and Dorcas 2004), and Mushinsky et al. (1982) indicated that female diamondback watersnakes will also eat larger and different prey types. With these in mind, female diamondbacks may be more likely to feed on larger fish that may be unavailable to smaller males. Snake sex also affected the probability of gut contents containing fish for plain-bellied watersnakes with males having an elevated level of probability in diet. This plain-bellied sex effect feeding on fish may be on the border of being meaningful as it was the fourth most important variable affecting fish probability with the smallest sum of Akaike weights ($\Sigma w_i = 0.427$). Demonstrating opposite findings, fish may be more important for plain-bellied females (%IRI = 13.7) with males being %IRI = 6.6% (Chapter 3). While logistic regression modeling investigated the probability of occurrence, %IRI incorporates prey volume and numerical prey percentage along with occurrence. Mushinsky et al. (1982) found that plain-bellied watersnake diet did not vary with sex. More research is needed to understand the effect of sex on fish in plain-bellied watersnake diets.

The impact of snake size (SVL) was only important in affecting the probability of fish in the gut contents of plain-bellied watersnakes. Fishes were meaningful only for the smallest and largest plain-bellieds, which is further supported by prey diversity indices (*D*) being at the highest levels for the smallest and the largest plain-bellied watersnakes.

Such elevated *D* values for the smallest and largest snakes indicate the addition of other prey, likely fishes, to the anurophagous plain-bellied diet. Similarly, Mushinsky et al. (1982) demonstrated that 85% of plain-bellied watersnakes <500 mm SVL fed on fish, 13.4% of 500–999 mm SVL, and 50% of >1000 mm SVL. Also in this 1982 study, plain-bellied watersnakes shifted from a diet predominantly of fish to anurans when snakes reached 500 mm SVL. In addition, plain-bellied watersnake chemoreception may be focused on fish until snakes reach 8 or 9 months old then keying in on anurans (Mushinsky and Lotz 1980). My research did not directly indicate that fishes were the primary dietary items for small-plain-bellied watersnakes, but I did demonstrate that the smallest individuals (276 mm SVL) in this research had the highest estimated probability (65%) of having gut contents containing fish and similarly decreasing. However, I did demonstrate that fish probability in plain-bellied watersnake diet began to increase at 650 mm SVL. While amphibian life stages vary with season, fish may be a reliable resource for watersnakes (Roe et al. 2004). Therefore, anurophagous plain-bellied watersnakes may forage on readily available, abundant fish when snakes are small or large.

Similar watersnake species may be able to live in sympatry because of differences in the utilization of microhabitat, landscape or temporal resources but dietary differences are likely to be one of the most important in allowing for coexistence. Watersnake dietary utilization affected by species, season, sex and ontogeny result in a complicated dynamic foraging system, which supports the importance of investigating beyond general diet descriptions for species. The coexistence of closely related species may be facilitated by adequate prey levels and changes in such prey levels or the absence of certain prey groups could greatly affect foraging systems and coexistence.

Table 8. Snout-vent length (SVL) mean and range (mm), and mass (grams) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N*. *rhombifer*) and northern (*N*. *sipedon*) watersnakes.

	2013					2014				
	N	SVL		Mass		*N*	SVL		Mass	
		Mean (SE)	Range	Mean (SE)	Range		Mean (SE)	Range	Mean (SE)	Range
Plain-bellied	76	596.96 (20.510)	276–947	176.76 (16.96)	19–701	74*	614.42 (23.42)	252–984	225.05 (21.69)	9–750
Female	44	608.61 (31.46)	276–947	198.00 (27.16)	19–701	40	674.90 (34.27)	302–984	293.68 (33.99)	15–750
Male	32	580.93 (22.71)	362–880	147.56 (14.10)	29–293	33	552.09 (26.20)	319–742	148.42 (17.12)	19–297
Diamondback	48	607.48 (32.99)	282–1027	301.37 (43.58)	20–1092	78*	501.98 (18.90)	213–900	53.08 (19.05)	7–792
Female	28	674.44 (47.74)	317–1027	414.20 (66.02)	23–1092	45	520.07 (28.71)	213–900	186.64 (30.43)	9–792
Male	20	513.73 (33.70)	282–718	149.04 (25.04)	20–366	32	484.92 (20.51)	313–718	110.44 (14.79)	24–327
Northern	72	488.25 (16.16)	207–791	101.77 (11.18)	8–435	114	500.93 (11.51)	271–794	114.03 (8.66)	13–409
Female	33	577.56 (24.11)	327–791	156.67 (20.07)	23–435	63	541.13 (16.68)	271–794	147.79 (13.71)	13–409
Male	39	412.68 (12.60)	207–564	55.32 (4.53)	8–111	51	451.26 (12.41)	275–601	72.31 (5.44)	17–155

*total number does not match the sum of sexes because there was 1 snake with an unknown sex.

Table 9. The Index of Relative Importance (%IRI) for crayfish, fish, sirens, mole salamanders, Anurans (tadpole/metamorph), and Anurans (froglet/adult), and for crayfish, fish and amphibian families based on snake gut content data for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

Snake Group	*N*	Crayfish	Fish	Siren	Mole Salamander	Anura (Tadpole/ Metamorph)	Anura (Froglet/ Adult)
Plain-bellied	63	0.0	13.8	0.1	0.0	7.0	79.1
Diamondback	39	0.0	95.2	1.1	0.0	2.1	1.6
Northern	74	0.0	86.9	0.1	0.0	1.5	11.5

Snake Group	*N*	Cambaridae	Lepisosteidae	Amiidae	Cyprinidae	Esocidae	Aphredoderidae	Poeciliidae	Centrarchidae	Sirenidae	Ambystomatidae	Hylidae	Ranidae
Plain-bellied	63	0.1	0.0	2.0	0.0	0.4	0.1	0.6	0.0	0.1	0.1	0.1	96.5
Diamondback	39	0.0	6.3	24.7	3.5	22.2	6.0	0.1	0.7	3.9	0.1	0.0	32.5
Northern	74	0.1	0.0	5.0	3.7	25.0	3.0	4.0	9.5	0.2	0.1	0.0	49.4

Table 10. Logistic regression models ($\leq 2\ \Delta_i$ AIC$_c$) testing the probability of gut contents containing a particular diet item (fish, tadpole/metamorph anuran or froglet/adult anuran) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes with resulting average parameter estimates, unconditional standard errors and sum of Akaike weights of predictor variables found in $\leq 2\ \Delta_i$ AIC$_c$ models.

Models [a]	K_i	AIC$_c$	Δ_i	w_i
Probability of fish				
Snake species	3	192.48	0.00	0.448
Snake species, season, year	4	193.61	1.13	0.254
Probability of tadpole/metamorph anuran				
Season	2	133.41	0.00	0.241
Season, year	3	134.26	0.85	0.158
Season, sex	3	135.33	1.92	0.092
Season, SVL	3	135.35	1.94	0.091
Probability of froglet/adult anuran				
Snake species, year	3	187.89	0.00	0.334
Snake species, year, SVL	4	189.37	1.48	0.159
Snake species, year, sex	4	189.88	1.99	0.123

[a] K_i = number of model parameters. AIC$_c$ = Akaike information criterion corrected for small sample size. Δ_i = distance of model from the best model (Δ_i = AIC$_{c,\,i}$ – minAIC$_c$). w_i = the estimated probability of being the best model (Akaike weight).

Variables [b]	Estimate	SE	Σw_i
Probability of fish			
Snake species (plain-bellied)	-2.2247	0.4364	0.967
Season	0.4545	0.2383	0.735
Year	0.1339	0.1642	0.367
Probability of tadpole/metamorph anuran			
Season	-0.5768	0.2957	0.828
Year	0.2567	0.2738	0.426
SVL	0.0003	0.0006	0.288
Sex (Female)	0.1085	0.1944	0.263
Probability of froglet/adult anuran			
Snake species (plain-bellied)	1.6325	0.4415	0.999
Year	-0.6149	0.3253	0.758
SVL	-0.0002	0.0003	0.256
Sex (Male)	0.0169	0.0856	0.204

[b] Σw_i = sum of Akaike weights indicating variable importance with higher values having more support.

Table 11. Logistic regression models ($\leq 2\ \Delta_i$ AIC$_c$) testing the probability of gut contents containing a particular diet item (fish, tadpole/metamorph anuran or froglet/adult anuran) for plain-bellied (*Nerodia erythrogaster*) watersnakes with resulting average parameter estimates, unconditional standard errors and sum of Akaike weights of predictor variables found in $\leq 2\ \Delta_i$ AIC$_c$ models.

Models [a]	K_i	AIC$_c$	Δ_i	w_i
Probability of fish				
SVL, SVL*SVL, year	4	60.83	0.00	0.168
SVL, SVL*SVL, year, sex	5	60.85	0.02	0.167
SVL, SVL*SVL	3	60.86	0.03	0.166
Year	2	62.47	1.64	0.074
Sex	2	62.57	1.74	0.070
Probability of tadpole/metamorph anuran				
Season	2	45.03	0.00	0.209
Sex	2	46.67	1.64	0.092
Year, season	3	46.73	1.70	0.089
SVL	2	46.77	1.74	0.088
Sex, season	3	46.86	1.83	0.084
Probability of froglet/adult anuran				
Year	2	77.03	0.00	0.202
Season	2	77.94	0.91	0.128
Year, season	3	78.21	1.18	0.112
Year, sex	3	78.66	1.63	0.089

Variables [b]	Estimate	SE	Σw_i
Probability of fish			
SVL	-0.0161	0.0127	0.649
Year	0.5404	0.4906	0.551
SVL*SVL	$1.2 \cdot 10^{-5}$	$8.0 \cdot 10^{-6}$	0.501
Sex (Male)	0.5663	0.5213	0.427
Probability of tadpole/metamorph anuran			
Season	-0.5054	0.4214	0.575
Sex (Male)	0.1006	0.6821	0.423
SVL	0.0009	0.0013	0.397
Year	-0.1246	0.2925	0.308
Probability of froglet/adult anuran			
Year	-0.6043	0.4465	0.597
Season	0.2451	0.2377	0.465
Sex (Female)	0.3300	0.3906	0.372

[a] K_i = number of model parameters. AIC$_c$ = Akaike information criterion corrected for small sample size. Δ_i = distance of model from the best model (Δ_i = AIC$_{c,i}$ – minAIC$_c$). w_i = the estimated probability of being the best model (Akaike weight).

[b] Σw_i = sum of Akaike weights indicating variable importance with higher values having more support.

Table 12. Logistic regression models ($\leq 2\ \Delta_i$ AIC$_c$) testing the probability of gut contents containing a particular diet item (fish, tadpole/metamorph anuran or froglet/adult anuran) for diamondback (*Nerodia rhombifer*) watersnakes with resulting average parameter estimates, unconditional standard errors and sum of Akaike weights of predictor variables found in $\leq 2\ \Delta_i$ AIC$_c$ models.

Models [a]	K_i	AIC$_c$	Δ_i	w_i
Probability of fish				
Sex	2	44.98	0.00	0.154
Season	2	45.12	0.14	0.144
Sex, season	3	45.25	0.27	0.135
Year	2	46.41	1.43	0.075
Year, season	3	46.56	1.58	0.070
Year, sex	3	46.89	1.91	0.059
Sex, SVL	3	46.95	1.97	0.058
Probability of tadpole/metamorph anuran				
Season	2	35.26	0.00	0.207
SVL	2	36.02	0.76	0.142
Year	2	36.73	1.47	0.099
Year, Season	3	37.03	1.77	0.085
Sex	2	37.14	1.88	0.081
Probability of froglet/adult anuran				
Year	2	25.12	0.00	0.276

Variables [b]	Estimate	SE	Σw_i
Probability of fish			
Sex (Female)	0.6994	0.6247	0.560
Season	0.4525	0.3813	0.532
Year	0.2430	0.3205	0.334
SVL	-0.0002	0.0010	0.313
Probability of tadpole/metamorph anuran			
Season	-0.4487	0.3672	0.519
SVL	0.0010	0.0012	0.409
Year	0.3211	0.4875	0.365
Sex (Male)	0.0111	0.2951	0.272
Probability of froglet/adult anuran			
Year	-1.6676	1.0094	0.694

[a] K_i = number of model parameters. AIC$_c$ = Akaike information criterion corrected for small sample size. Δ_i = distance of model from the best model (Δ_i = AIC$_{c,i}$ – minAIC$_c$). w_i = the estimated probability of being the best model (Akaike weight).

[b] Σw_i = sum of Akaike weights indicating variable importance with higher values having more support.

Table 13. Logistic regression models ($\leq 2\ \Delta_i\ \mathrm{AIC_c}$) testing the probability of gut contents containing a particular diet item (fish, tadpole/metamorph anuran or froglet/adult anuran) for northern (*Nerodia sipedon*) watersnakes with resulting average parameter estimates, unconditional standard errors and sum of Akaike weights of predictor variables found in $\leq 2\ \Delta_i\ \mathrm{AIC_c}$ models.

Models [a]	K_i	$\mathrm{AIC_c}$	Δ_i	w_i
Probability of fish				
Season	2	81.53	0.00	0.328
Year, season	3	82.90	1.37	0.166
SVL, season	3	83.22	1.69	0.141
Sex, season	3	83.40	1.87	0.129
Probability of tadpole/metamorph anuran				
Year	2	59.36	0.00	0.164
Year, season	3	60.26	0.90	0.105
Year, sex	3	60.31	0.95	0.102
Year, sex, season	4	60.62	1.26	0.088
Sex	2	60.80	1.44	0.080
Season	2	60.82	1.46	0.079
Probability of froglet/adult anuran				
Season, season*season	3	70.56	0.00	0.995

Variables [b]	Estimate	SE	Σw_i
Probability of fish			
Season	1.2600	0.4609	0.973
Year	-0.1775	0.2387	0.324
SVL	0.0004	0.0008	0.305
Sex (Female)	0.0722	0.2089	0.283
Probability of tadpole/metamorph anuran			
Year	0.962	0.775	0.620
Sex (Female)	0.413	0.479	0.461
Season	-0.281	0.286	0.443
Probability of froglet/adult anuran			
Season	66.4948	1.0456	0.998
Season*season	-13.7877	0.0659	0.995

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[a] K_i = number of model parameters. $\mathrm{AIC_c}$ = Akaike information criterion corrected for small sample size. Δ_i = distance of model from the best model ($\Delta_i = \mathrm{AIC}_{c,\,i} - \mathrm{minAIC}_c$). w_i = the estimated probability of being the best model (Akaike weight).

[b] Σw_i = sum of Akaike weights indicating variable importance with higher values having more support.

Table 14. Simpson's Inverse Diversity Index (*D*) based on prey family data found in snake gut contents for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

	Plain-bellied		Diamondback		Northern	
Snake Group	*N*	*D*	*N*	*D*	*N* *	*D*
Species	63	1.778	39	6.485	74	5.942
Season						
Spring	16	1.772	7	2.909	7	4.765
Early Summer	26	1.765	19	4.738	40	3.917
Late Summer	21	1.724	13	5.000	29	6.218
SVL						
<450 mm	17	2.513	18	5.882	21	4.642
450–650 mm	21	1.111	13	5.143	41	5.150
650–850 mm	16	1.730	4	2.571	12	6.737
>850 mm	9	2.200	4	4.000	0	—

*total number of northern watersnakes according to season resulted in 76 individuals because 2 individual snakes had gut contents when initially captured and also when recaptured.

Table 15. Proportional Similarity Index (PSI) values comparing prey families based on snake gut content data within and across snake species for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

Within Snake Species	Plain-bellied	Diamondback	Northern
Season			
Spring / Early Summer	0.807	0.412	0.444
Spring / Late Summer	0.800	0.267	0.451
Early Summer / Late Summer	0.828	0.502	0.578

Across Snake Species	Plain-bellied / Diamondback	Plain-bellied/ Northern	Diamondback/Northern
Season			
Spring / Spring	0.700	0.422	0.583
Early Summer / Early Summer	0.399	0.615	0.458
Late Summer / Late Summer	0.300	0.300	0.819

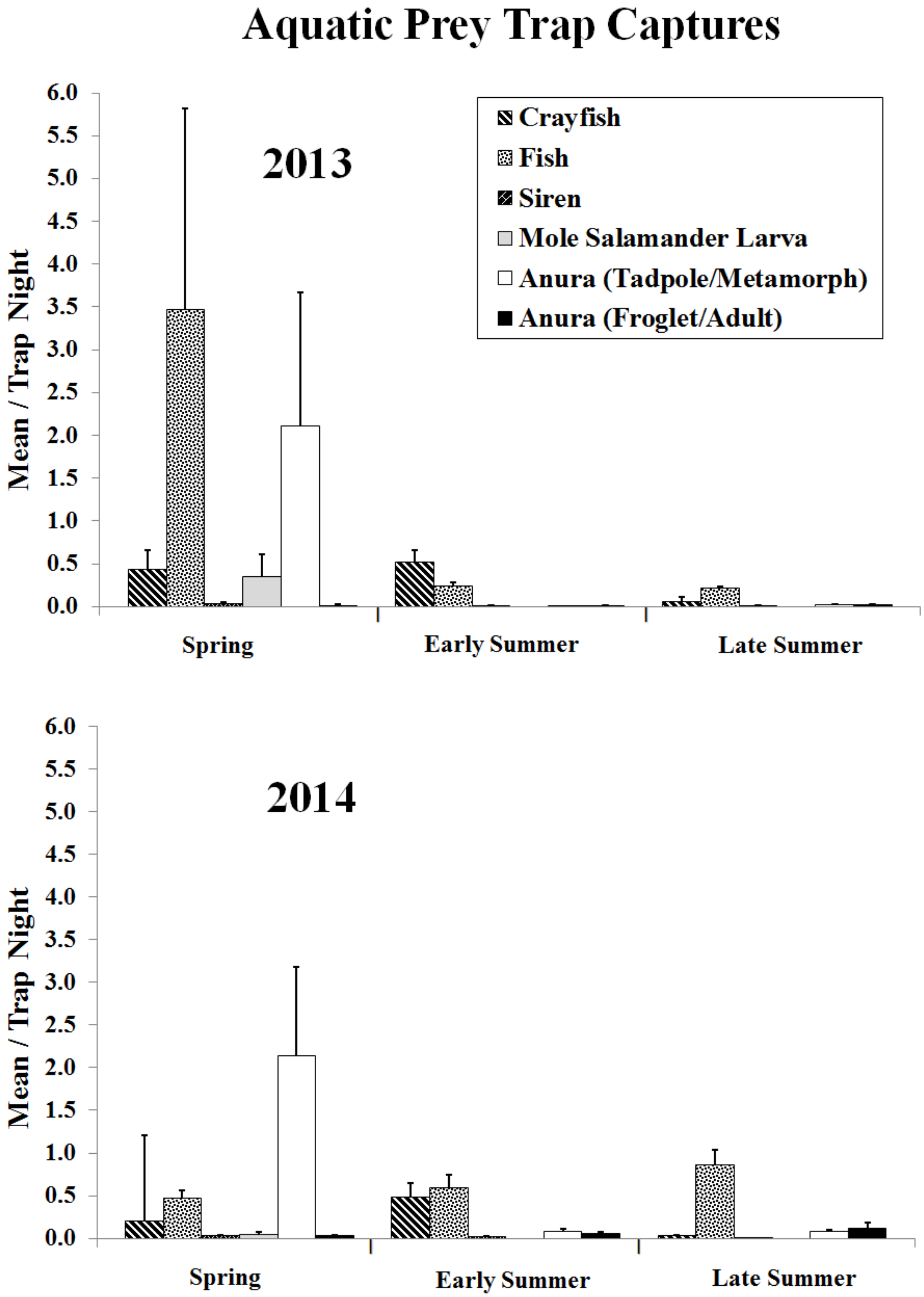

Figure 8. Mean number of aquatic prey captured per trap night by season. Error bars indicate 1 standard error.

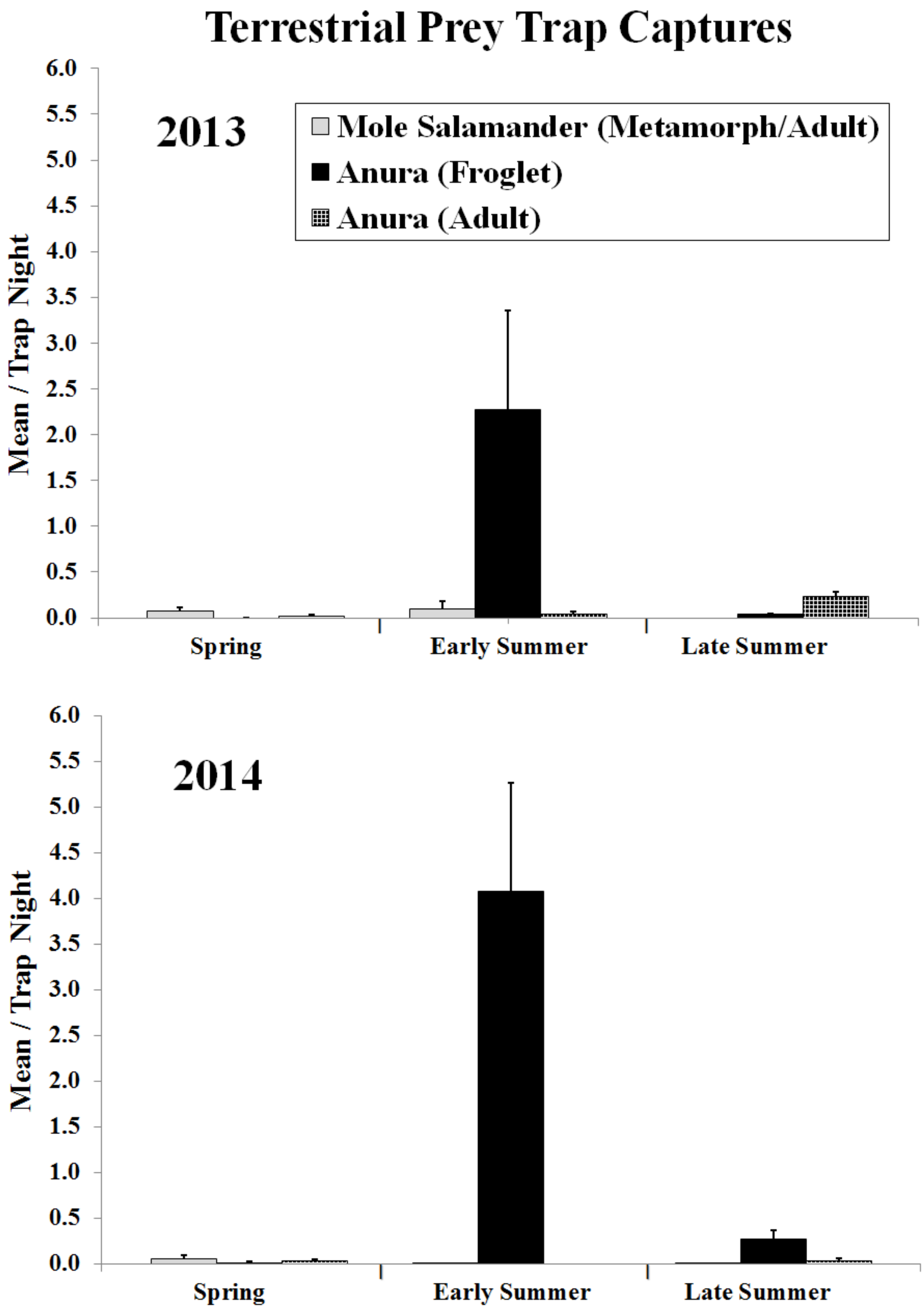

Figure 9. Mean number of terrestrial prey captured per trap night by season. Error bars indicate 1 standard error.

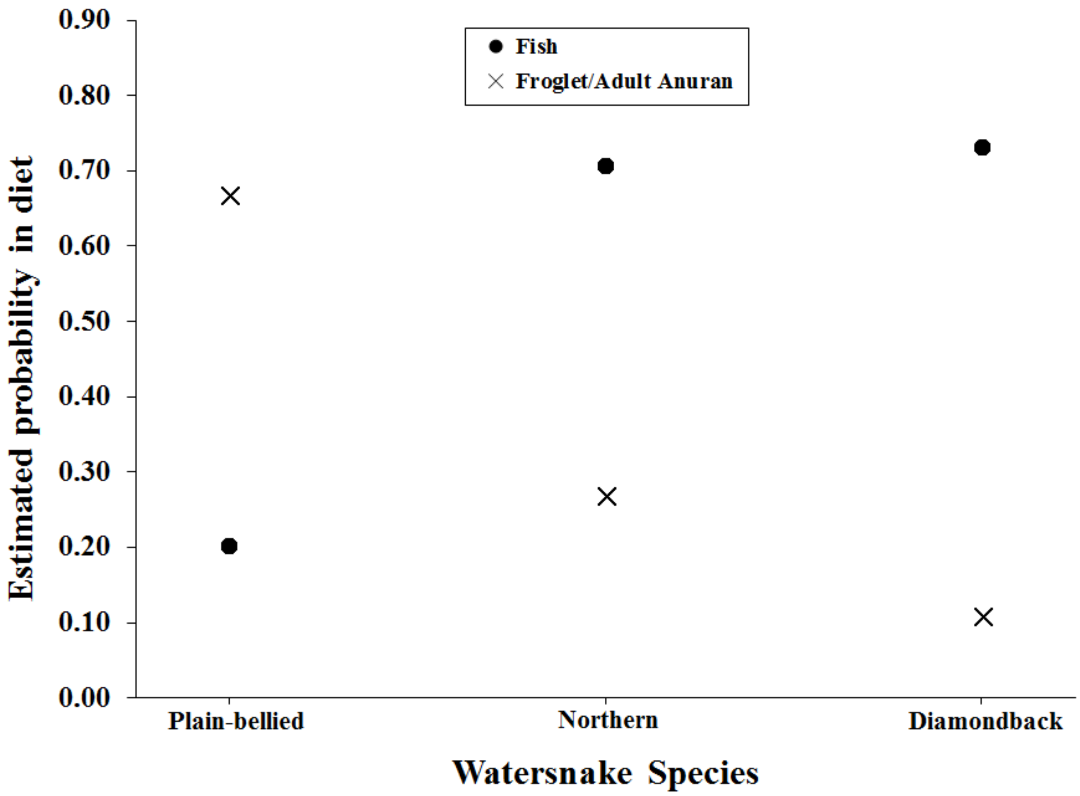

Figure 10. Estimated probability of gut contents containing fish or froglet/adult anurans according to watersnake species for plain-bellied (*Nerodia erythrogaster*), northern (*N. sipedon*) and diamondback (*N. rhombifer*) watersnakes using logistic regression models.

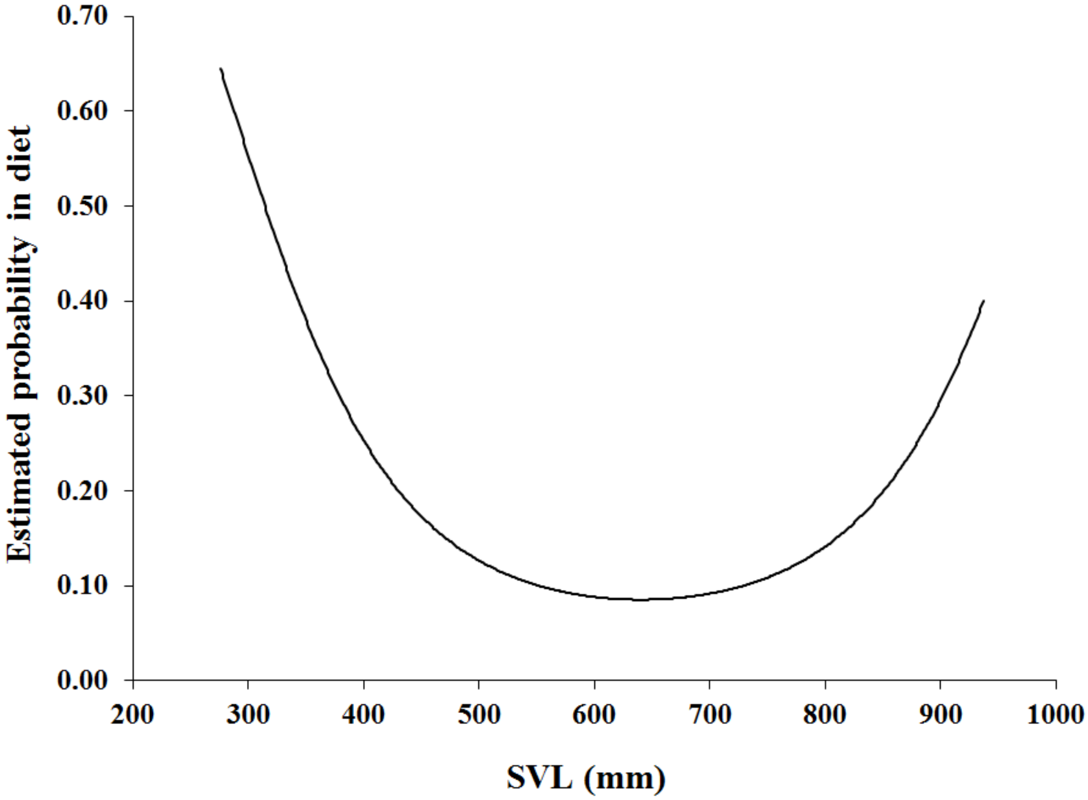

Figure 11. Estimated probability of gut contents containing fish according to snake length (SVL) for plain-bellied watersnakes (*Nerodia erythrogaster*) using logistic regression.

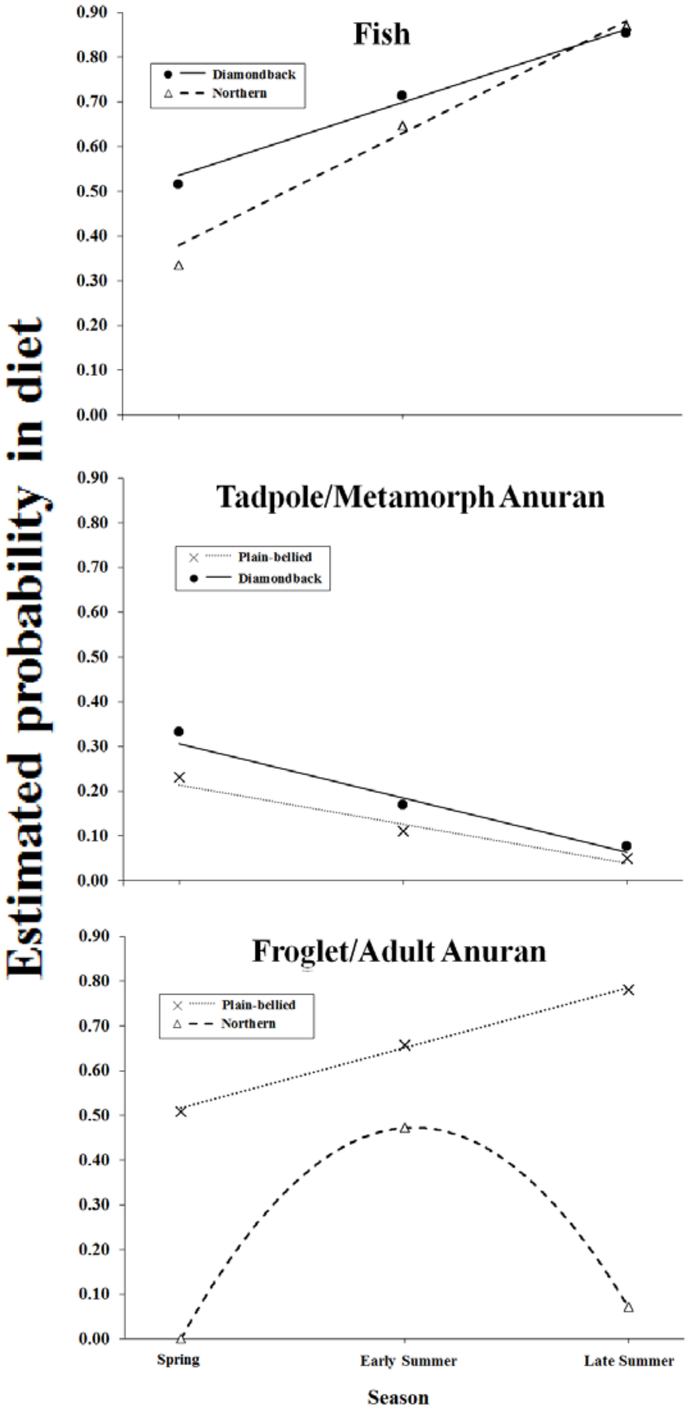

Figure 12. Estimated probability of gut contents containing a particular diet item (fish, tadpole/metamorph anuran or froglet/adult anuran) according to season for plain-bellied (*Nerodia erythrogaster*), northern (*N. sipedon*) and diamondback (*N. rhombifer*) watersnakes using logistic regression models.

CHAPTER V

TROPHIC NICHE ECOLOGY OF THREE SYMPATRIC WATERSNAKE SPECIES REVEALED BY STABLE ISOTOPE ANALYSIS

SUMMARY

Ecologically similar, sympatric species can coexist by variation in temporal, spatial or trophic niches but many studies do not include intraspecific factors when attempting to understand species coexistence. For coexisting watersnakes (*Nerodia* spp.), species are often found together in similar aquatic habitats feeding mainly on amphibians and fishes, and trophic factors may play the biggest role in differential niches. While trophic niche ecology may vary among sympatric watersnake species, diet may also be affected by intraspecific factors such as snake size and sex, resulting in a complex foraging system. Investigations of watersnake diet and sympatric niche partitioning have analyzed gut contents but this limits dietary information to a single meal. Stable isotope analyses, on the other hand, provide long term dietary information and may better elucidate the complex dietary relationships within and among species. I completed a stable isotope analysis investigating trophic niche ecology incorporating intraspecific variation to understand the coexistence of sympatric plain-bellied (*N. erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes. Stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) were obtained from 333 watersnakes and 299 potential snake prey items in 2013 and 2014. Diamondback watersnakes fed at higher trophic levels while plain-bellied watersnakes fed more from terrestrial prey sources. As both plain-bellied and diamondback watersnakes increased in size, $\delta^{13}C$ variance decreased offering additional support for each snake species narrowing in on its respective prey with terrestrial anurans for plain-bellied and fishes for diamondback watersnakes. The northern watersnake had an intermediate diet affected by ontogeny and sex. Snake species, sex and size had varying effects on trophic niche overlap, width and position resulting in a complicated trophic system likely allowing for coexistence. Stable isotope analyses revealed how similar species may live in sympatry because of the interaction of interspecific and intraspecific factors.

INTRODUCTION

Understanding the coexistence of similar species is a fundamental question in ecology. Competitive exclusion or displacement will occur if similar sympatric species utilize similar resources at high levels (Gause 1932, Hardin 1960, Abrams 1983). To avoid competitive exclusion, similar species will differ in resource utilization or partition their niches (MacArthur 1958, Toft 1985). Such niche partitioning occurs across a broad assembly of species and ecosystems (Schoener 1974, Morin 1999).

Niche partitioning can involve differentiation of the spatial, temporal or trophic niches (Schoener 1968, Pianka 1973, Pianka 1975, Stewart et al. 2003). These three niche partitioning types can be further divided into habitat scale, temporal scale, food type and food size (Schoener 1974, Toft 1985). Spatial or habitat partitioning includes classic examples such as MacArthur’s warblers (MacArthur 1958) and the coexistence of three successional annuals with root systems at different soil depths (Wieland and Bazzaz

1975). Temporally partitioning their rocky desert habitats, two desert spiny mice (*Acomys* spp.) would not co-occur if both were diurnal (Shkolnik 1971, Kronfeld-Schor et al. 2001). Spatial, temporal and trophic niche partitioning are not necessarily mutually exclusive and coexisting species can partition a variety of niche dimensions (Schoener 1974, Butt and Tahir 2010, Hadi et al. 2012). While niche differentiation among species promotes existence, many partitioning studies do not include intraspecific characteristics or intraspecific variability (Hirai and Matsui 2002, Bolnick et al. 2011, Zhao et al. 2014).

Intraspecific variability should be included when investigating niche partitioning as intraspecific factors can help facilitate coexistence by reducing overlap among potentially competing species (Wilbur 1980, Lichstein et al. 2007, Lasky et al. 2014). Life stage, sex and size are some intraspecific factors that could be important in affecting species coexistence and help in the understanding overall niche partitioning. For example, sympatric tropical piscivorous fish have juvenile stages primarily feeding on invertebrates while adults eat mainly fish, with species differing at the age of dietary change (Winemiller 1989). In addition, male and females of sympatric diving seabirds differ in both time of foraging and food type likely benefitting seabird coexistence (Bearhop et al. 2006). Also, two sympatric frog species partitioning food resources involves one species having ontogenetic dietary change with froglet body size (Hirai and Matsui 2002). Such intraspecific factors can have a large effect on a species' niche. Polis (1984) went as far to state that intraspecific partitioning could result in age groups existing as "ecological species." If intraspecific factors are included in interspecific niche variation, we can provide an even better picture of how similar species coexist.

To further our understanding of the complexity of niche partitioning, a population of coexisting watersnakes (*Nerodia* spp.) would be a model system to investigate. Watersnakes are distributed throughout the eastern United States with many species overlapping in distribution (Ernst and Ernst 2003) and habitat use (Hebrard and Mushinsky 1978, Mushinsky et al. 1980). Niche partitioning can occur among coexisting watersnakes through temporal differences in foraging (Mushinsky and Hebrard 1977*b*, Hebrard and Mushinsky 1978), utilizing different focal areas across landscapes (Roe et al. 2003, Roe et al. 2004, Marshall 2008) or partitioning microhabitats in local aquatic areas (Hebrard and Mushinsky 1978, Laurent and Kingsbury 2003). However, differences in trophic niches may be the most important means of niche partitioning for sympatric snake species (Henderson 1974, Brown and Parker 1982, Toft 1985, Vitt 2001, Goodyear and Pianka 2008).

As is the case for many taxa, trophic niche variation among watersnake species can be complicated by intraspecific factors. Watersnake foraging ecology varies ontogenetically (Luiselli 2006), with age-related changes in chemoreceptive responses (Mushinsky and Lotz 1980), foraging behavior (Savitsky and Burghardt 2000) and diet (Mushinsky et al. 1982, Plummer and Goy 1984). Sex could also affect watersnake trophic niche utilization. With female watersnakes reaching larger sizes than conspecific males, larger females may be able to swallow larger prey resulting in dietary differences between sexes (Mushinsky et al. 1982). In addition, dietary studies involving snakes has mostly focused on adult individuals (Mushinsky 2001). The study of intraspecific along with interspecific factors for watersnake niche partitioning could help to understand the complex nature of trophic niche utilization.

Gut content analyses have been used to understand watersnake diet (Bauman and Metter 1975, Plummer and Goy 1984, King et al. 1999*b*) and to investigate trophic niche utilization among sympatric watersnake species (Mushinsky et al. 1977*a*, 1982). Although this research has provided valuable information, gut contents provide short-term information involving only a single meal and have inherent biases (Bearhop et al 1999, 2004). Conversely, stable isotope analyses provide long-term diet data (Bearhop et al. 2004, Boecklen et al. 2011), additional dietary information not available from gut content studies (Stewart et al. 2003) and information about trophic niche shifts (Newsome et al. 2007, Brischoux et al. 2011). Stable isotopes can also help determine where an animal is foraging (Newsome et al. 2007, Trakimas et al. 2011). Lower $\delta^{13}C$ values indicate carbon sources from an aquatic environment (feeding on aquatic prey); higher $\delta^{13}C$ values indicate carbon sources from a terrestrial environment (feeding on terrestrial prey) (Rau 1980; Rasmussen 2010; Trakimas et al. 2011). Stable isotopes can also be used to determine the trophic level at which an animal is feeding (Gannes et al. 1997, Post 2002) with higher $\delta^{15}N$ values indicating higher trophic levels (Peterson and Fry 1987; Gaines et al. 2002).

Considering these factors, I have an ideal system of coexisting watersnakes to tease out the details of interspecific and intraspecific trophic niche variation. To understand the coexistence of sympatric plain-bellied (*N. erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes, I completed a stable isotope analysis investigating three aspects of the trophic niche: trophic niche overlap (prey similarity), trophic niche width (prey variety) and trophic niche position (prey types) (Hammerschlag-Peyer et al. 2011, Vaudo and Heithaus 2011, Comas et al. 2014). I also incorporated the effects of sex and ontogeny to address intraspecific variation in the trophic niche.

METHODS

Study Site

I performed this study from April to September in 2013 and 2014 on a 100-hectare section of the Sloughs Wildlife Management Area (Henderson County, Kentucky, USA). This section, located about 2 kilometers southeast of the Ohio River, is known as Hardy Slough/Muddy Slough and has high densities of the 3 target watersnake species (Laurent and Kingsbury 2003). Habitats in the sloughs include shallow wetlands managed for wintering waterfowl, scrub-shrub wetlands and palustrine forest. Dominant plants were water primrose (*Ludwigia* sp.), smartweed (*Polygonum* sp.), water lily (*Nuphar* sp.) cattail (*Typha* sp.), buttonbush (*Cephalanthus occidentalis*), black willow (*Salix nigra*) and hackberry (*Celtis occidentalis*).

Snake and Prey Sampling

I captured snakes by a variety of methods including hand capture, cover board placement, stand-alone aquatic funnel traps and drift fence arrays (terrestrial and aquatic) with funnel traps. For each captured watersnake, I measured snout-vent length (SVL) and determined sex by cloacal probing. Snakes were individually marked with both subcutaneous pit tags (Gibbons and Andrews 2004) and with unique ventral scale-clip patterns (Plummer and Ferner 2012) enabling me to identify recaptures. The scale clips were also used for stable isotopes. Each watersnake was released at its capture location. All animal capture, handling and processing activities were approved by the University of Louisville Institutional Animal Care and Use Committee (IACUC Protocol: #13037).

During the same period as snake capture, I sampled snake prey in order to determine prey availability and to obtain prey stable isotope samples. Prey sampling occurred weekly and traps were open for two days and nights (~48 hours). To sample aquatic prey, I used stand-alone aquatic funnel traps, aquatic hoop traps and aquatic drift fence arrays with aquatic funnel traps. All aquatic funnel traps had ~25% of the trap above water to prevent the drowning of non-target animals. I sampled terrestrial prey using drift fences associated with funnel traps and pit-fall traps (5-gallon buckets). All drift fences were built from silt fence material and wooden stakes, and all funnel traps (aquatic and terrestrial) had a mesh size of 0.64 cm and circular openings that ranged from 3.8 cm to 10 cm in diameter. Prey items that I captured and recorded included amphibians, fish and crayfish. Captured anurans were recorded as tadpoles, metamorphs (i.e., tadpoles that had well-defined legs and beginning to show adult traits), froglets (recently metamorphosed frogs with no tadpole traits) or adults (McDiarmid and Altig 1999).

Stable Isotope Analysis

To perform stable isotope analysis on snakes, I used scale clips from 2013 and 2014 with each sample being from a different individual watersnake. Neonate snakes retain maternal isotope signatures, and thus stable isotopic values in neonate snakes can in part reflect maternal diet rather than their own (Pilgrim 2007). To prevent sampling watersnakes that might retain maternal isotopic signatures, I obtained and analyzed stable isotopes only from watersnakes ≥275 mm SVL. For comparison, a neonate northern watersnake from this study was 179 mm SVL and a neonate diamondback watersnake was 202 mm SVL. Elevated stable isotope values in the smallest snakes would indicate the retention of maternal isotope signatures, but no elevation was found when stable isotope values were plotted against snake SVL.

I obtained tissue samples for stable isotope analyses from a subset (20%) of captured potential prey animals in 2013 and 2014. These samples included whole bodies of anuran tadpoles, anuran metamorphs and fish <50 mm in standard length (Sanderson et al. 2009, Schielke and Post 2010, Trakimas et al. 2011); toe clips of the tip of the longest toe on a hind foot for froglets and adult frogs (Trakimas et al. 2011); and a caudal fin sample (~7 mm diameter) for fishes ≥50 mm in standard length (Kelly et al. 2006, Sanderson et al. 2009). Salamander tissues were sampled using a small clip (~3mm) of the distal point of the tail (Milanovich and Maerz 2012), and stable isotopes in crayfish, which were sampled only in 2014, were analyzed from a 3 mm sample of the uropod exoskeleton (Hollows et al. 2002).

I placed all snake and prey samples for stable isotope analyses in a freezer at -80° Celsius then I dried them in a drying oven for 48 hours at 60° Celsius. Samples were then stored in plastic vials in darkness. If necessary, I homogenized each individual sample by grinding it with mortar and pestle. I did not extract lipids from samples before stable isotope analysis because carbon to nitrogen ratios were 3.16–3.18 (Post et al. 2007, Young et al. 2010*a*, Tronquart et al. 2012) and in addition, Steinitz et al. (2016) found lipid extraction did not affect stable isotope values in a squamate (*Cyclura* spp.). Prepared samples were weighed (1.24 mg ± 5.8 SD) and placed into individual 3.5 x 5 mm tin capsules.

All stable isotope samples were analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values at the University of California, Davis Stable Isotope Facility using an isotope ratio mass spectrometer with an elemental analyzer. Stable isotope values were expressed in standard delta notation (δ) in parts per thousand (‰), where δX = (R_{sample}/$R_{standard}$ –1) · 1000, with, R_{sample} and $R_{standard}$ being the molar ratios of C^{13}/C^{12} and N^{15}/N^{14} of the sample and the standard reference material. The standard reference material was Vienna PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen.

Trophic discrimination (enrichment) factors are necessary to accurately determine resource use with stable isotopes. Trophic discrimination factors are the difference (‰) between consumer and source stable isotope values (Phillips and Gregg 2001, Caut et al. 2009) and are applied to address differences in isotope ratio between consumers and resources caused by absorption of prey (Parnell et al. 2010, Parnell and Jackson 2011). Many stable isotope diet and food web studies utilize general trophic discrimination factors such as 1.0 $\delta^{13}C$ ‰ and 3.0 $\delta^{15}N$ ‰ established by Peterson and Fry (1987) and 0.4 $\delta^{13}C$ ‰ and 3.4 $\delta^{15}N$ ‰ established by Post (2002), but trophic discrimination factors are often taxon-specific (Caut et al. 2009, Martínez del Rio et al. 2009, Warne et al. 2010). However, trophic discrimination factors for snakes have not been established (Pilgrim 2005, Chiucchi 2011). For this study, trophic discrimination factors of 0.17 $\delta^{13}C$ ‰ (SE ±0.03) and 2.8 $\delta^{15}N$ ‰ (SE ±0.11) from the green sea turtle, *Chelonia mydas,* (Seminoff et al. 2006) were used, as it is the most closely related reptile for which we had discrimination information on skin. All reported watersnake stable isotope results for this study have been first corrected by subtracting these trophic discrimination factors from original watersnake stable isotope data.

Statistics

I used $\delta^{13}C$ and $\delta^{15}N$ values to determine trophic niche overlap (prey similarity) among species, sex and snake sizes. To assess the effect of snake size on diet, I divided snakes into 4 different size classes (<450 mm SVL, 450–650 mm SVL, 650–850 mm SVL and >850 mm SVL). Northern watersnakes were not included in the largest size class because all captured northern watersnakes were <850 mm SVL. I used linear mixed models with $\delta^{13}C$ or $\delta^{15}N$ as a dependent variable, year as a random factor and watersnake species, sex, snake size classes and all possible interactions as independent variables. Follow-up Tukey-Kramer multiple comparison tests were utilized when main effects were significant. Also, I performed regression analyses for $\delta^{13}C$ and $\delta^{15}N$ versus the continuous variable of SVL (mm) for each watersnake species. For a given isotope, regression lines were tested to determine slope differences. In this study, I utilized SVL both as continuous and size class predictor variables in different analyses. Both were necessary as the continuous variable more precisely determined the relationship with isotopic change but size classes provided the ability to compare variances, compare group means, portray a visual of ontogenetic change in isotopic bi-plots and the ability to address ontogenetic changes for stable isotope mixing model mean proportions.

To examine trophic niche width (prey variety) across watersnake species, sex and size, I investigated the variance of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. Higher stable isotope variance levels will indicate larger trophic niche widths (Bearhop et al. 2004, Fink et al. 2012). Levene’s test for homogeneity of variance and Tukey-type multiple comparison tests were utilized to test for differences in variance among watersnake species and sex (Willson et al. 2010, Zar 2010, Fink et al. 2012). Rather than testing all possible combinations of snake size class within and among watersnake species resulting in increasing the probability of Type I error, I investigated variance due to size only within watersnake species resulting in only 6 tests. I divided watersnakes into 100-mm size classes (300–400 mm SVL, 400–500 mm SVL, etc.) and then compared stable isotope variance for those classes within species. Regression analyses were performed on $\delta^{13}C$ and $\delta^{15}N$ separately to investigate stable isotope variance among the size classes within watersnake species.

Stable isotope mixing models are used to identify the proportions of prey in consumer diets and help to determine and compare trophic niche position (prey types) (Phillips et al. 2005, Martinez del Rio et al. 2009). Mixing models incorporate stable isotope values for individual snakes (consumer data), trophic discrimination factors with standard deviations and stable isotope mean and standard deviation values from potential snake prey (source data) groups (Phillips et al. 2014). An important assumption in using these mixing models is that isotope values are in equilibrium, i.e., do not vary across time, for either the prey or the snake species (Harvey et al. 2002, Xia et al. 2013). To determine whether isotope values varied by year in the prey, I tested the effect of year separately for $\delta^{13}C$ and $\delta^{15}N$ for the 6 prey stable isotope groups to determine if year had a factor on potential snake prey stable isotopes. In many studies, equilibrium has not been achieved in the consumer (Carleton et al. 2005, Sweeting et al. 2007, Fink et al. 2012, Murray and Wolf 2013). As turnover for snake tissue stable isotopes may occur over long periods, snake stable isotope levels may not reach equilibrium (Pilgrim 2007, Fisk et al. 2009). However, watersnake gut content data ($N = 176$) from this study population

(Chapter 4) during the same sample period and a large amount of watersnake diet literature were used to validate mixing model results.

For the mixing models, I included the snake prey groups and snake groups (species, sex, size classes), and results were reported in mean proportion of each resource for each of the snake groups, along with 95% credible intervals (Bayesian statistics) (Parnell et al. 2010). Credible intervals indicate a confidence (95% in this case) that the true mean is contained in the interval (Jackson et al. 2011, Hopkins and Ferguson 2012). The mixing model stable isotope analysis was completed using Stable Isotope Analysis in R (SIAR v 4.) (Parnell et al. 2010, Parnell and Jackson 2011). All other statistical analyses were performed using SAS software (SAS Institute 2000), and statistical tests were considered to be significant at $\alpha = 0.05$. If data did not meet assumptions for parametric analyses, I performed square root transformations on dependent variables.

RESULTS

Snake stable isotope data were analyzed from a total of 333 individual watersnakes (163 in 2013 and 170 in 2014), which included 116 plain-bellied, 106 diamondback and 111 northern watersnakes. The trophic niche overlap (prey similarity) model for $\delta^{13}C$ was significant ($F_{20,312} = 15.98$, $P < 0.0001$) with watersnake species ($F_2 = 76.33$, $P < 0.0001$) and size class ($F_3 = 22.47$, $P < 0.0001$) effects (Figure 13). However, none of year ($F_1 = 0.03$, $P = 0.870$), sex ($F_1 = 0.32$, $P = 0.571$) or any interactions ($P \geq 0.104$) had a significant effect on $\delta^{13}C$. Tukey-Kramer multiple comparison tests showed that plain-bellied watersnakes ($\delta^{13}C$ -27.23 $\pm$ SE 0.12) had greater $\delta^{13}C$ values than both diamondback ($\delta^{13}C$ -29.05 $\pm$ SE 0.11) and northern watersnakes ($\delta^{13}C$ -28.76 $\pm$ SE 0.11), with no other species differences. Tukey-Kramer multiple comparison tests also showed that all watersnake size classes were different, with larger size classes having larger $\delta^{13}C$ values (Figure 14).

Potential snake prey stable isotope data were analyzed from a total of 299 individual animals (154 in 2013 and 145 in 2014), and stable isotope cluster patterns resulted in 6 potential snake prey isotope groups. These groups were aquatic salamanders, tadpole/metamorph anurans, froglet/adult anurans, crayfish, Lepisosteidae and all other fishes. From this point on, the non-lepisosteid fish will be referred to simply as fish. Investigation of residuals demonstrated that $\delta^{13}C$ and $\delta^{15}N$ values were both normally distributed, but Levene's test for homogeneity of variance for watersnake species was significant for $\delta^{15}N$ ($F_{2,330} = 5.50$, $P = 0.004$). Accordingly, the square roots of $\delta^{15}N$ values were used for analyses of trophic niche overlap.

The overall trophic niche overlap model for $\delta^{15}N$ was significant ($F_{20,312} = 18.93$, $P < 0.0001$) with significant effects for watersnake species ($F_2 = 32.19$, $P < 0.0001$), sex ($F_1 = 4.06$, $P = 0.045$) and size class ($F_3 = 69.21$, $P < 0.0001$) effects. However, there were no year ($F_1 = 0.01$, $P = 0.905$) or interaction effects ($F_1 = 0.32$, $P \geq 0.493$) on $\delta^{15}N$. Tukey-Kramer multiple comparison tests indicated that diamondback watersnakes ($\delta^{15}N$ 7.82 ± SE 0.17) had higher $\delta^{15}N$ values than both northern ($\delta^{15}N$ 6.72 ± SE 0.16) and plain-bellied ($\delta^{15}N$ 6.69 ± SE 0.13) watersnakes, but showed no other species differences. Female watersnakes ($\delta^{15}N$ 7.33 ± SE 0.13) had greater $\delta^{15}N$ values than males ($\delta^{15}N$ 6.72 ± SE 0.12). Tukey-Kramer multiple comparison tests indicated that there was no significant difference in $\delta^{15}N$ values between snakes in the two largest size classes (>850 mm and 650–850 mm SVL). However, all other size class comparisons showed significant difference, with larger snakes having greater $\delta^{15}N$ values.

Both $\delta^{13}C$ and $\delta^{15}N$ had significant positive relationships with SVL (continuous variable) in all three watersnake species ($\delta^{13}C$: plain-bellied $F_{1,114} = 33.28$, $P < 0.0001$; diamondback $F_{1,104} = 48.86$, $P < 0.0001$; northern $F_{1,109} = 26.98$, $P < 0.0001$) ($\delta^{15}N$: plain-bellied $F_{1,114} = 122.93$, $P < 0.0001$; diamondback $F_{1,104} = 113.54$, $P < 0.0001$; northern $F_{1,109} = 77.64$, $P < 0.0001$) (Figure 15). There was no difference in the relationship of $\delta^{13}C$ values and SVL across watersnake species ($F_2 = 0.24$ $P = 0.790$) but there was a significant interaction between SVL and watersnake species ($F_2 = 3.65$, $P = 0.027$) for $\delta^{15}N$. Regarding $\delta^{15}N$, diamondback slope was similar to plain-bellied ($F_1 = 0.89$, $P = 0.347$) but was approaching significance ($F_1 = 3.72$, $P = 0.055$) for having a different slope than northern watersnakes. Slopes of $\delta^{15}N$ differed ($F_1 = 7.28$, $P = 0.007$) between plain-bellied and northern watersnakes.

Prey variety measured by trophic niche width (variance) had varying results. There was no difference in $\delta^{13}C$ variance for the three watersnake species (Levene's test for homogeneity of variance: $\delta^{13}C$, $F_{2,330} = 2.03$, $P = 0.133$) (Table 16). However, variance of $\delta^{15}N$ differed across species ($\delta^{15}N$, $F_{2,330} = 5.50$, $P = 0.005$) with Tukey-Type multiple comparison tests indicating that diamondback watersnakes had a larger $\delta^{15}N$ variance than plain-bellied watersnakes ($q = 3.43$, $q_{0.05,3} = 3.314$, $P < 0.05$). Within species, sex did not affect $\delta^{13}C$ variance for plain-bellied ($F_{1,114} = 0.03$, $P = 0.857$) or diamondback ($F_{1,104} = 0.04$, $P = 0.846$), but values approached significance ($F_{1,109} = 3.67$, $P = 0.058$) for northern watersnakes (males: $s^2 = 1.62$; females: $s^2 = 0.92$). The variance of $\delta^{15}N$ was not affected by sex for plain-bellied ($F_{1,114} = 0.72$, $P = 0.397$) or diamondback ($F_{1,104} = 1.67$, $P = 0.199$). However, female ($s^2 = 3.25$) northern watersnakes had greater $\delta^{15}N$ variance than male ($s^2 = 1.65$) northern watersnakes ($F_{1,109} = 11.24$, $P = 0.001$).

Snake size class (100 mm SVL groups) affected trophic niche width (variance). $\delta^{13}C$ variance decreased as plain-bellied ($F_{1,5} = 7.46$, $P = 0.041$) and diamondback ($F_{1,6} = 15.94$, $P = 0.007$) watersnakes increased in size class, but no relationship was found with northern watersnake size class ($F_{1,3} = 1.10$, $P = 0.371$) (Figure 16). Variance of $\delta^{15}N$ was not related to size class of plain-bellied watersnakes ($F_{1,5} = 2.07$, $P = 0.210$) but decreased with increased northern watersnake size class ($F_{1,3} = 29.55$, $P = 0.012$). For diamondback watersnakes, the linear regression involving $\delta^{15}N$ and snake size class was not significant ($F_{1,6} = 1.33$, $P = 0.083$, $R^2 = 0.42$), but the quadratic regression was significant and a better fit for the data ($F_{2,5} = 6.10$, $P = 0.046$, $R^2 = 0.71$), with a significant linear term ($F_1 = 7.22$, $P = 0.044$) and the quadratic term approaching significance ($F_1 = 4.99$, $P = 0.076$).

In general, year had little influence on $\delta^{13}C$ and $\delta^{15}N$ values in potential snake prey. Year had no significant effect on either $\delta^{13}C$ or $\delta^{15}N$ in fishes, salamanders or anurans ($\delta^{13}C$: fish $F_{1,97} = 0.44$, $P = 0.509$; aquatic salamanders $F_{1,33} = 1.94$, $P = 0.173$; tadpole/metamorph anurans $F_{1,65} = 0.06$, $P = 0.800$; froglet/adult anurans $F_{1,73} = 2.36$, $P = 0.129$) ($\delta^{15}N$: fish $F_{1,97} = 2.75$, $P = 0.101$; aquatic salamanders $F_{1,33} = 3.49$, $P = 0.071$; tadpole/metamorph anurans $F_{1,65} = 0.70$, $P = 0.405$; froglet/adult anurans $F_{1,73} = 3.27$, $P = 0.075$). The effect of year on stable isotope values could not be tested on crayfish, which were sampled only in 2014. In Lepisosteidae, year had no significant effect on $\delta^{15}N$ ($F_{1,11} = 0.11$, $P = 0.751$) but did significantly affect $\delta^{13}C$ values ($F_{1,11} = 11.06$, $P =$

0.007); however this could be a result of small sample sizes, given that there were only 10 tissues samples in 2013 and 3 in 2014.

Mixing models indicated that snake species affected trophic niche position (prey type) (Table 17). When considering credible intervals (CI), the only interspecific difference was that plain-bellied watersnakes fed more on froglet/adult anurans than did diamondback watersnakes (Figure 17). All other prey groups overlapped when considering only watersnake species. Trophic niche position was not affected by watersnake sex, with little variation for plain-bellied and diamondback watersnakes. Although not significant according to credible intervals, each of the 6 potential prey groups differed to some degree with northern watersnake sex. Northern watersnake males had elevated levels of tadpole/metamorph anurans and crayfish while females had elevated levels of fish, Lepisosteidae, aquatic salamanders and froglet/adult anurans.

Snake size class also affected trophic niche position results. For all watersnake species, general trends showed the importance of crayfish and tadpole/metamorph anurans decreasing with snake size, the importance of froglet/adult anurans and Lepisosteidae increasing with snake size and the proportion of fish and aquatic salamanders varying little (Figure 18). Plain-bellied watersnakes that were <450 mm in SVL fed on more crayfish than snakes >650 mm in SVL, and snakes in the 450–650 mm SVL size class fed on more crayfish compared to snakes with SVL >850 mm (Figure 19). Plain-bellied watersnakes <450 mm in SVL also fed less on froglet/adult anurans than plain-bellieds >650 mm in SVL. Both diamondback and northern watersnakes <450 mm in SVL ate fewer Lepisosteidae and more tadpole/metamorph anurans than conspecifics >650 mm SVL.

The following trophic niche position results across watersnake species for size classes are summarized according to prey group. Credible intervals overlapped among watersnakes species across the same size classes for fish, Lepisosteidae, aquatic salamanders and tadpole/metamorph anurans. On the border of significance, diamondback watersnakes >650 mm SVL fed more on Lepisosteidae than similar-sized plain-bellied watersnakes. The smallest plain-bellied snakes (<450 mm SVL) fed significantly more on crayfish than did similar-sized diamondbacks. Plain-bellied watersnakes >450 mm SVL fed more on froglet/adult anurans than did diamondback watersnakes >450 mm SVL; Plain-bellieds 650–850 mm SVL also fed more on froglet/adult anurans than did northern watersnakes of the same size class.

DISCUSSION

Stable isotope analyses revealed that both interspecific and intraspecific factors affected trophic niche ecology and likely allow for the coexistence of these three sympatric watersnake species. Plain-bellied, diamondback and northern watersnakes all eat amphibians and fishes, but may vary in trophic niche overlap, width or position. Additionally, intraspecific factors likely affect coexistence, with females and larger snakes feeding at higher trophic levels and on more terrestrial prey. While many factors may also contribute to watersnake coexistence, including landscape effects (Steen et al. 2014), microhabitat differences (Laurent and Kingsbury 2003) and interactions of temporal, spatial and dietary effects (Vitt 2001, Durso et al. 2013), differences in diet likely best explain coexistence as sympatric North American watersnakes commonly partition their trophic niches (Luiselli and Rugiero 1991, Luiselli 2006).

Interspecific Differences

Interspecific differences affected trophic niche overlap. Plain-bellied watersnakes had the highest δ^{13}C levels, indicating the importance of terrestrial prey. Such terrestrial prey were adult anurans, which make up the majority plain-bellied watersnake diet (Preston 1970, Mushinsky and Hebrard 1977*a*, Roe et al. 2004). The high δ^{15}N values for diamondback watersnakes demonstrated feeding at higher trophic levels. In this study, fishes (δ^{15}N 9.15 ± SE 0.14), Lepisosteidae (δ^{15}N 12.61 ± SE 0.45) and aquatic salamanders (δ^{15}N 8.23 ± SE 0.37) had high δ^{15}N, and these 3 prey groups made up 54% of diamondback diet according to mixing models. Diamondback watersnakes are highly aquatic and eat mostly fish (Mushinsky et al. 1982, Gibbons and Dorcas 2004), which offers support for foraging on these aquatic prey at high trophic levels.

Northern watersnakes had similar δ^{13}C values to those of diamondbacks and similar δ^{15}N values to those of plain-bellied watersnakes. The northern watersnake has the most diverse diet of any watersnake in North America, feeding mainly on amphibians and fish (Ernst and Ernst 2003, Gibbons and Dorcas 2004), and it may be opportunistically feeding on readily available prey (Roe et al. 2004, Gibbons and Dorcas 2004). The northern watersnake, with a generalist diet between the piscivorous diamondback and the anurophagous plain-bellied, likely coexists by differing with each congener in alternate stable isotope dimensions.

Trophic niche width had different results for each isotope. Variance of δ^{13}C was similar among watersnake species indicating a similar prey variety width. However, trophic niche width measured by δ^{15}N variance did vary among watersnake species, with diamondbacks having slightly greater trophic niche width than the plain-bellieds and northern watersnakes intermediate but not significantly different from either. The high trophic niche width of diamondback watersnakes is not surprising, given that they fed on tadpole/metamorph anurans that feed on algae or detritus, which are at a low trophic level (Altig et al. 2007), but also on Lepisosteidae, which are predatory fish and thus at a high trophic level (Zeug and Winemiller 2008, Fletcher et al. 2015). Prey items from these two divergent trophic levels were 37% of diamondback diet compared to 14% for plain-bellied watersnakes.

Interspecific differences had very little effect on trophic niche position. The only difference was plain-bellieds feeding more on froglet/adult anurans than diamondback watersnakes providing additional support for high levels of adult anurans in plain-bellied watersnake diet. With this being the only trophic niche position difference, this offers further support of factors beyond interspecific allowing for watersnake coexistence. Such factors allowing for species coexistence can be intraspecific (Lichstein et al. 2007), which can blur species niche differences and decrease the effect of species niche partitioning (Lasky et al. 2014).

Sex

Watersnake sex affected trophic niche overlap. Across species, female watersnakes fed at higher trophic levels ($\delta^{15}N$) than males. Mushinsky et al. (1982) demonstrated that diets differed between sexes for piscivorous but not anurophagous watersnakes. While I did not demonstrate an interaction of sex and watersnake species affecting $\delta^{15}N$, dietary differences relating to sex could be associated with various factors. Female watersnakes obtain larger sizes than males and larger prey at higher trophic levels may only be available for larger females. Also since snakes drop smaller prey from their diets as they increase in size (Plummer and Goy 1984, Arnold 2001, Bowen 2004), males may be eating prey that larger females remove from their diets. Conversely, gravid female watersnakes may be selecting different prey to aid in embryo development. Gravid female watersnakes may alter their behavior to increase embryogenesis (Brown and Weatherhead 2000).

Trophic niche width varied with sex only for northern watersnakes. Male northern watersnakes had larger (marginally significant) δ^{13}C values, likely because males fed on a larger variety of aquatic and terrestrial prey. Female northern watersnakes had a greater range of δ^{15}N values and hence foraged over a larger variety of trophic levels. Additionally, the northern watersnake was the only species to have all prey groups indicating some hint of variation (not significant) for trophic niche position relating to sex. Northern watersnake sexes overlapped in diet in this study, but previous work has shown that males and females may feed on different prey (Lacy 1995) and use different habitats, particularly when females are gravid (Pattishall and Cundall 2009, Neuman-Lee et al. 2013). My research has indicated differences in trophic niche width regarding both isotope axes for northern watersnake sexes, which could be related to dietary and habitat variation. Such sex differences could help to explain how this generalist may be able to coexist with watersnakes feeding more on specific prey and could offer some support for a small degree of niche differentiation due to northern watersnake sex.

The effect of sex had varying effects on watersnake trophic niche ecology. Intraspecific competition can be reduced if sexes have foraging differences (González-Solís et al. 2008), which could also have effects on niche partitioning among species. Though across a variety of taxa, sex may not result in dietary differences (Ben-David et al. 1996, Young et al. 2010*b*, Hamilton et al. 2012, Bianchi et al. 2014). However if evident sexual dimorphism factors are present in species, sex may often result in ecological differences affecting niche ecology (Shine 1989, Tucker et al. 1995, Verwaijen et al. 2002, Bolnick et al. 2003).

Snake Size

The trophic niche ecology of sympatric plain-bellied, diamondback and northern watersnakes was greatly affected by snake size (SVL). As snakes increased in size, all three species incorporated more terrestrial prey in their diets ($\delta^{13}C$), suggesting they shift to using terrestrial habitats more frequently or else hunt for frogs along the water's edge. Northern watersnakes may eat adult anurans only when snakes reach the juvenile or adult stage (Lacy 1995). Northern watersnakes appeared to have a trophic position shift to feed more on froglet/adult anurans when snakes reached 450 mm SVL. In addition, plain-bellied watersnakes may switch from fish to adult anurans when snakes reach 500 mm SVL (Mushinsky et al. 1982). This fish to adult anuran trophic shift may be an innate chemical response (Mushinsky and Lotz 1980). I did not find a shift from fish to adult anurans with increased plain-bellied size but both mixing model results and decreased $\delta^{13}C$ variance further demonstrated a trophic level shift with larger plain-belied watersnakes focusing their feeding on froglet/adult anurans.

All three snakes also foraged at higher trophic levels ($\delta^{15}N$) with increased snake size, which could indicate feeding on larger or different prey. Watersnakes may be dropping small tadpole/metamorph anurans from their diets as snakes increase in size and focus on larger prey found at higher trophic levels. Diamondback watersnakes shift from smaller to larger fish as snakes increase in size (Kofron 1978, Mushinsky et al. 1982, Plummer and Goy 1984). Feeding on larger fish could be a trophic shift as larger fishes may be at higher trophic levels (Gu et al. 1996, Fry et al. 1999, Jennings et al. 2001). Diamondback watersnakes had ontogenetic changes in trophic niche overlap, position and a large reduction in both δ^{13}C and δ^{15}N variance (trophic niche width) indicating a trophic niche shift with larger diamondbacks focusing in on Lepisosteidae and other fishes at the highest prey trophic levels. Similarly, many animal taxa may have the largest individuals narrowing in on specific prey (Scharf et al. 2000). Lepisosteidae held the highest prey trophic level in my system and in other studies (Zeug and Winemiller 2008, Fletcher et al. 2015). The Lepisosteidae family was represented by the spotted gar (*Lepisosteus oculatus*) in my research, and spotted gar are “apex predators” feeding on a diversity of prey (Zeug and Winemiller 2008). In addition, spotted gar forage mostly at night (Snedden et al. 1999) and diamondback watersnakes are mostly nocturnal (Mushinsky and Hebrard 1977*b*, Gibbons and Dorcas 2004), which could be a factor for large diamondbacks feeding on this high trophic level fish.

In regards to trophic levels, small northern watersnakes had similar δ^{15}N values with similar-sized plain-bellied watersnakes but had a larger rate of increase with snake size resulting with the largest northern watersnakes having similar δ^{15}N values with similar-sized diamondbacks. Northern watersnakes have the ability to switch prey based on availability (King 1993, King et al. 1999*b*, King et al. 2006) and juvenile watersnakes have a variety of foraging strategies (Balent and Andreadis 1998). Therefore, northern watersnakes could possibly quickly alter their diets as snakes increased in size. With this in mind, the largest northern watersnakes (650–850 mm SVL) may have increased competition with similar-sized diamondbacks as these two groups had very similar trophic niche positions, much trophic niche overlap and similar trophic niche width (δ^{13}C variance). Also, trophic niche width (δ^{15}N variance) had a sharp decrease with increased northern watersnake size, which is likely due to large northern watersnakes dropping smaller prey at lower trophic levels from their diet. Himes (2003*a*) found that fishes were the main diet item for large northern watersnakes, and in my study fishes were at higher trophic levels and many fishes were likely larger in size than the lower trophic level tadpole/metamorph anurans.

Snake size had a strong impact on the trophic niche ecology of sympatric watersnakes. Across a variety of taxa, many species have ontogenetic dietary shifts (Werner and Gilliam 1984, Ross 1986, Hirai and Matusui 2002, Wallace and Leslie 2008). Such dietary changes with species size can have important impacts on niche partitioning. Similarly, age group dietary differences can increase species niche width and have ages exist as “ecological species” (Polis 1984). Such intraspecific variation can make it difficult to identify species as discrete units (Bolnick et al. 2003) with individuals at specific ages occupying only a section of a species’ niche (Zhao et al. 2014). With these in mind, ontogenetic changes relating to size, age and diet can have important impacts on trophic niche ecology, niche partitioning and coexistence of similar, sympatric species.

The investigation of trophic niche position indicated that crayfish were important in the diets for all three species especially for plain-bellied watersnakes <650 mm SVL. Crayfish were very abundant in this system but were rarely found in watersnake gut contents (Chapter 4). Crayfish are considered minor dietary items for all three watersnake species (Mushinsky and Hebrard 1977*a*, Fontenot et al. 1993, Gibbons and Dorcas 2004) yet crayfish in watersnake diet may be more important than realized because molting crayfish may be quickly digested and underestimated in watersnake gut content studies (Fontenot et al. 1993). Moreover, it is possible that young crayfish are important in the diets of smaller watersnakes. Cecala et al. (2010) indicated that 2 out 5 juvenile northern watersnakes had gut contents containing crayfish, and crayfish in watersnake diet may be related to snake ontogeny (Fontenot et al. 1993). Young crayfish grow quickly and can molt 11 to 14 times in the first few months (Reynolds 2002, Taylor and Schuster 2004), and crayfish in the process of molting have reduced mobility, soft exoskeletons and are very susceptible to predation (Taylor and Schuster 2004). Young watersnakes could possibly be feeding on these abundant, young small crayfish molting at high levels. Conversely, mean crayfish $\delta^{13}C$ values were intermediate between tadpole/metamorph anurans and froglet/adult anurans. It is possible that watersnakes feeding on equal amounts of these two prey, may fall near crayfish in isotopic space, thus resulting in a large crayfish signal. As a result, while crayfish may be in their diets, the stable isotope analysis may be placing too much importance on crayfish in watersnake diets. Additional research is needed to investigate crayfish in watersnake diets.

Summary

Applying stable isotope techniques to the study of watersnake trophic niche ecology revealed information not available from gut content investigations. Plain-bellied, diamondback and northern watersnakes can forage on the same prey but have interspecific differences in trophic niche ecology. Such interspecific differences could result in lower levels of interspecific competition resulting in age groups within species widening their resource use (Polis 1984). It is important to investigate all age groups as many resource partitioning studies often only include adults and later life stages (Ross 1986).

Often overlooked in community ecology, intraspecific factors can have large effects on the niches of species and help facilitate coexistence (Violle et al. 2012). Ontogeny is one intraspecific factor having a strong impact in this system. Ontogenetic dietary differences may reduce competition among sympatric watersnakes (Himes 2003*b*). As plain-bellied and diamondback watersnakes increased in size, these two species had ontogenetic changes and focused in on their respective prey. The diet of the northern watersnake overlaps with both congenerics, and the northern watersnake is likely able to coexist because of its varied diet, ontogenetic changes and dietary differences between northern watersnake sexes. The complex nature of watersnake trophic niche ecology is dynamic with ontogenetic and sex effects. These intraspecific factors along with interspecific dietary differences likely interacted to allow the coexistence of watersnake species.

Indicating that similar, sympatric species can coexist because of interspecific niche partitioning is a great oversimplification of biological communities. While species can partition the spatial, temporal or trophic niches, various intraspecific variables need to be considered. Sympatric species can have trophic shifts related to ontogeny resulting in a community of complex interactions (Werner and Gilliam 1984). When considering a myriad of factors affecting niche partitioning or niche variation, we can further reveal how similar species live in sympatry.

Table 16. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) summary statistics for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

Snake Group	*N*	$\delta^{13}C$	SE	s^2	$\delta^{15}N$	SE	s^2
Plain-bellied Watersnake	116	-27.23	0.12	1.65	6.69	0.13	1.91
Sex							
Female	65	-27.19	0.16	1.69	6.84	0.18	2.07
Male	51	-27.28	0.18	1.62	6.50	0.18	1.67
Size Class							
<450 mm SVL	29	-28.14	0.26	2.02	5.47	0.17	0.87
450–650 mm SVL	40	-27.23	0.20	1.54	6.21	0.15	0.92
650–850 mm SVL	27	-26.85	0.17	0.75	7.53	0.18	0.88
>850 mm SVL	20	-26.44	0.19	0.70	8.29	0.19	0.73
Diamondback Watersnake	106	-29.05	0.11	1.18	7.82	0.17	3.03
Sex							
Female	59	-28.88	0.14	1.13	8.09	0.24	3.29
Male	47	-29.27	0.16	1.19	7.48	0.23	2.56
Size Class							
<450 mm SVL	39	-29.69	0.16	1.00	6.40	0.17	1.18
450–650 mm SVL	28	-29.21	0.20	1.07	7.66	0.29	2.29
650–850 mm SVL	27	-28.29	0.16	0.70	9.11	0.22	1.27
>850 mm SVL	12	-28.37	0.09	0.10	9.92	0.10	0.13
Northern Watersnake	111	-28.76	0.11	1.25	6.72	0.16	2.67
Sex							
Female	59	-28.64	0.12	0.92	7.13	0.23	3.25
Male	52	-28.90	0.18	1.62	6.26	0.18	1.65
Size Class							
<450 mm SVL	37	-29.49	0.16	0.95	5.71	0.23	1.90
450–650 mm SVL	50	-28.51	0.15	1.11	6.57	0.19	1.74
650–850 mm SVL	24	-28.16	0.18	0.76	8.60	0.17	0.69

Table 17. Stable isotope mixing model mean proportions (± SD) of each prey group for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

Snake Group	*N*	Crayfish	Fish	Lepisosteidae	Aquatic Salamander	Anuran (Tadpole/ Metamorph)	Anuran (Froglet/ Adult)
Plain-bellied Watersnake	116	0.299 (0.097)	0.057 (0.044)	0.061 (0.043)	0.073 (0.057)	0.082 (0.065)	0.428 (0.074)
Sex							
Female	65	0.263 (0.100)	0.063 (0.048)	0.068 (0.047)	0.081 (0.062)	0.082 (0.064)	0.443 (0.080)
Male	51	0.338 (0.097)	0.057 (0.044)	0.055 (0.040)	0.075 (0.057)	0.083 (0.065)	0.392 (0.078)
Size Class							
<450 mm SVL	29	0.519 (0.086)	0.051 (0.041)	0.032 (0.026)	0.070 (0.054)	0.174 (0.082)	0.154 (0.068)
450–650 mm SVL	40	0.400 (0.080)	0.047 (0.037)	0.041 (0.031)	0.064 (0.049)	0.077 (0.057)	0.371 (0.070)
650–850 mm SVL	27	0.146 (0.074)	0.070 (0.053)	0.104 (0.054)	0.098 (0.069)	0.057 (0.042)	0.525 (0.070)
>850 mm SVL	20	0.077 (0.052)	0.060 (0.047)	0.190 (0.053)	0.070 (0.053)	0.036 (0.029)	0.567 (0.065)
Diamondback Watersnake	106	0.159 (0.081)	0.187 (0.099)	0.179 (0.070)	0.176 (0.103)	0.193 (0.082)	0.106 (0.062)
Sex							
Female	59	0.144 (0.082)	0.184 (0.092)	0.202 (0.069)	0.176 (0.099)	0.163 (0.079)	0.131 (0.064)
Male	47	0.166 (0.080)	0.198 (0.098)	0.129 (0.068)	0.192 (0.110)	0.224 (0.082)	0.091 (0.059)
Size Class							
<450 mm SVL	39	0.209 (0.070)	0.140 (0.082)	0.043 (0.034)	0.194 (0.102)	0.355 (0.073)	0.059 (0.043)
450–650 mm SVL	28	0.155 (0.079)	0.195 (0.093)	0.148 (0.070)	0.194 (0.103)	0.207 (0.078)	0.101 (0.061)
650–850 mm SVL	27	0.070 (0.051)	0.171 (0.086)	0.288 (0.059)	0.188 (0.098)	0.064 (0.047)	0.219 (0.056)
>850 mm SVL	12	0.048 (0.038)	0.211 (0.086)	0.377 (0.060)	0.140 (0.079)	0.051 (0.039)	0.173 (0.062)
Northern Watersnake	111	0.256 (0.096)	0.128 (0.083)	0.072 (0.050)	0.153 (0.101)	0.223 (0.091)	0.168 (0.071)
Sex							
Female	59	0.199 (0.077)	0.145 (0.081)	0.096 (0.057)	0.167 (0.093)	0.191 (0.073)	0.202 (0.059)
Male	52	0.320 (0.105)	0.101 (0.073)	0.055 (0.042)	0.142 (0.100)	0.258 (0.101)	0.124 (0.074)
Size Class							
<450 mmm SVL	37	0.296 (0.071)	0.094 (0.062)	0.032 (0.026)	0.141 (0.082)	0.370 (0.073)	0.067 (0.045)
450–650 mm SVL	50	0.264 (0.088)	0.103 (0.072)	0.060 (0.043)	0.145 (0.094)	0.214 (0.083)	0.214 (0.068)
650–850 mm SVL	24	0.105 (0.063)	0.160 (0.085)	0.244 (0.059)	0.163 (0.087)	0.079 (0.055)	0.249 (0.061)

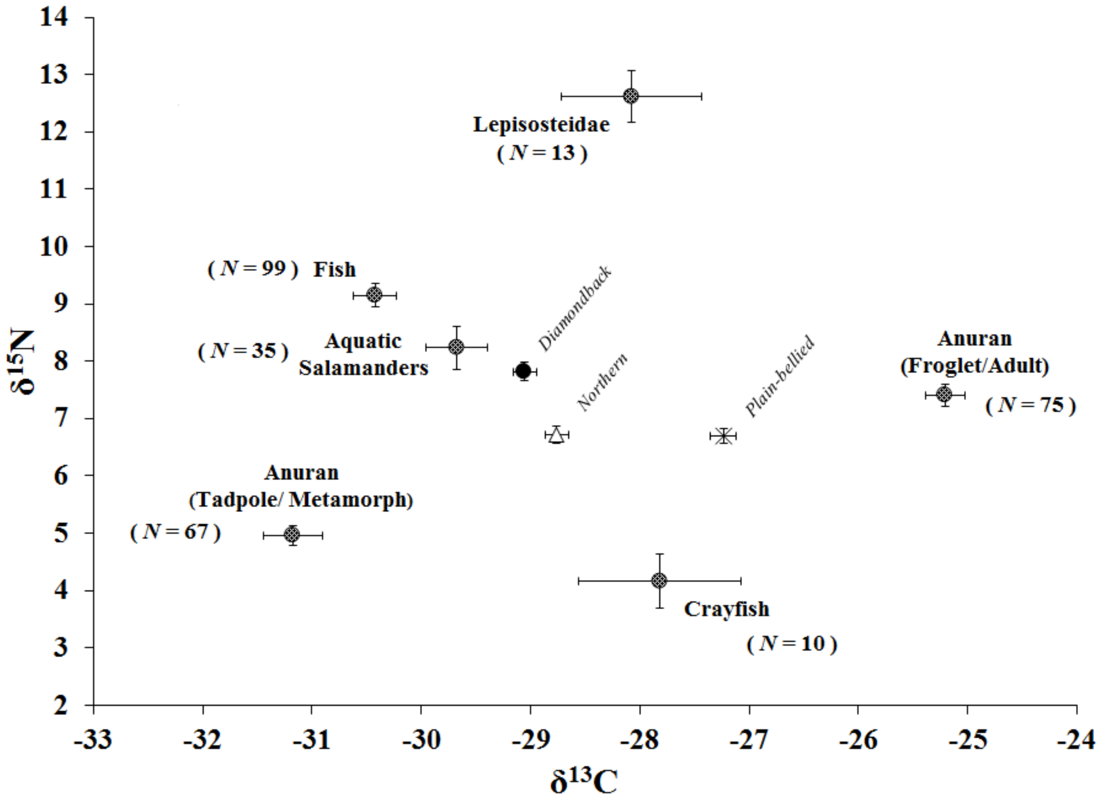

Figure 13. Mean potential snake prey stable isotope groups and mean snake stable isotope values for plain-bellied, diamondback and northern watersnakes. Error bars represent ± 1 SE.

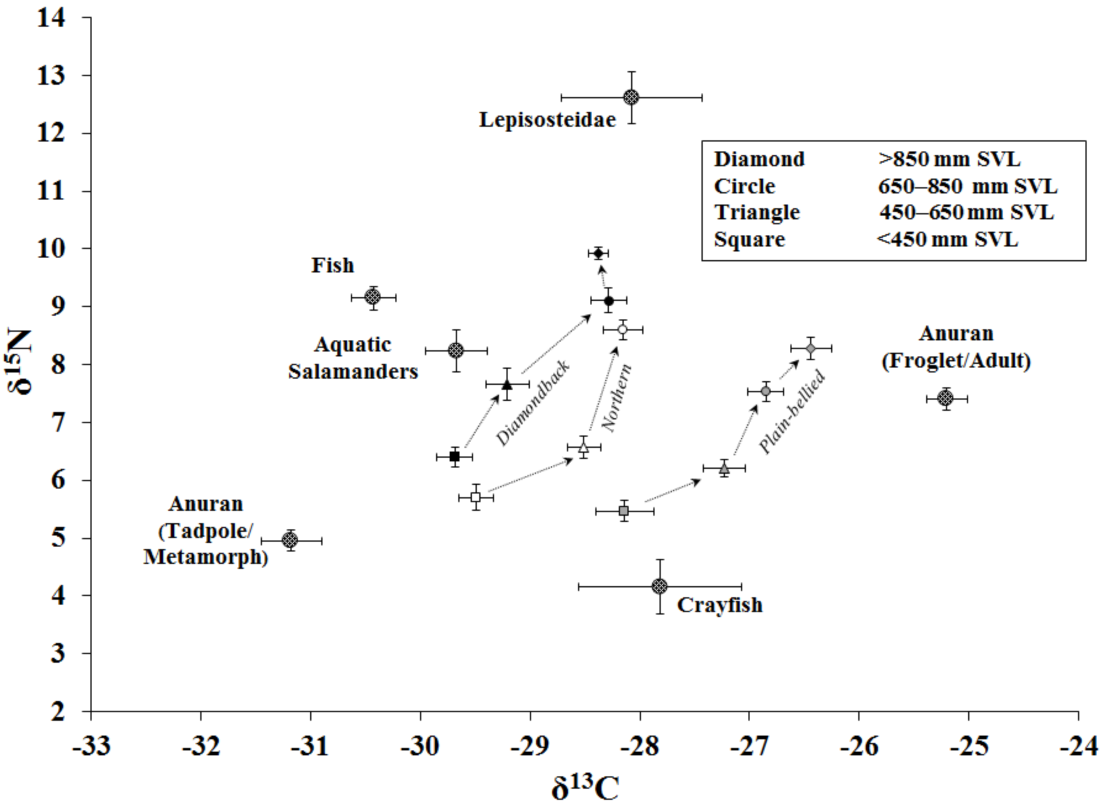

Figure 14. Mean potential snake prey stable isotope groups and mean snake stable isotope values for plain-bellied (gray symbols), diamondback (black symbols) and northern (white symbols) watersnakes based on snake size class. Arrows indicate the increase in size class for each watersnake species. Error bars represent ± 1 SE. SVL is snout-vent length (mm).

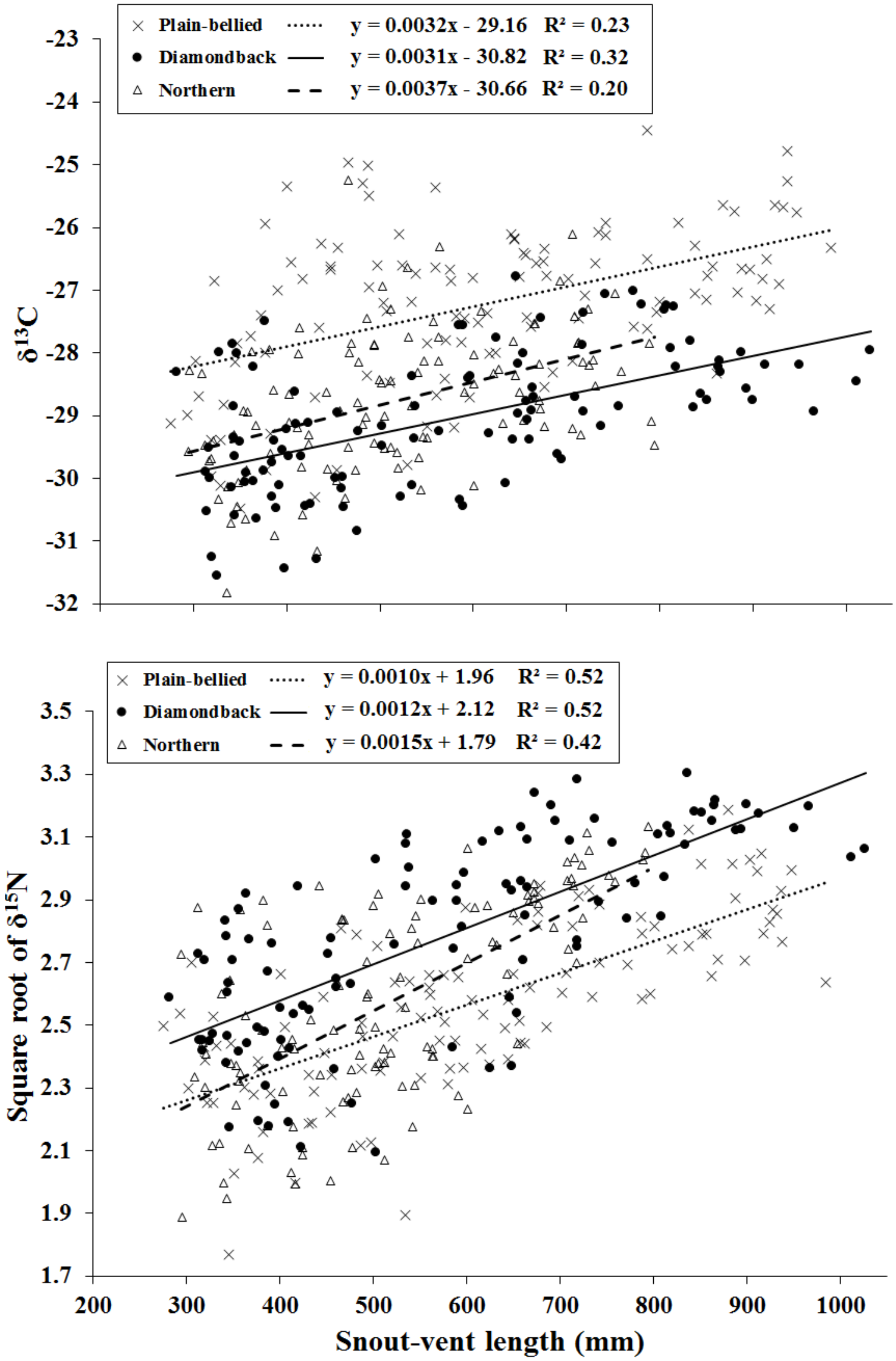

Figure 15. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values versus snake snout-vent length (mm) for plain-bellied, diamondback and northern watersnakes.

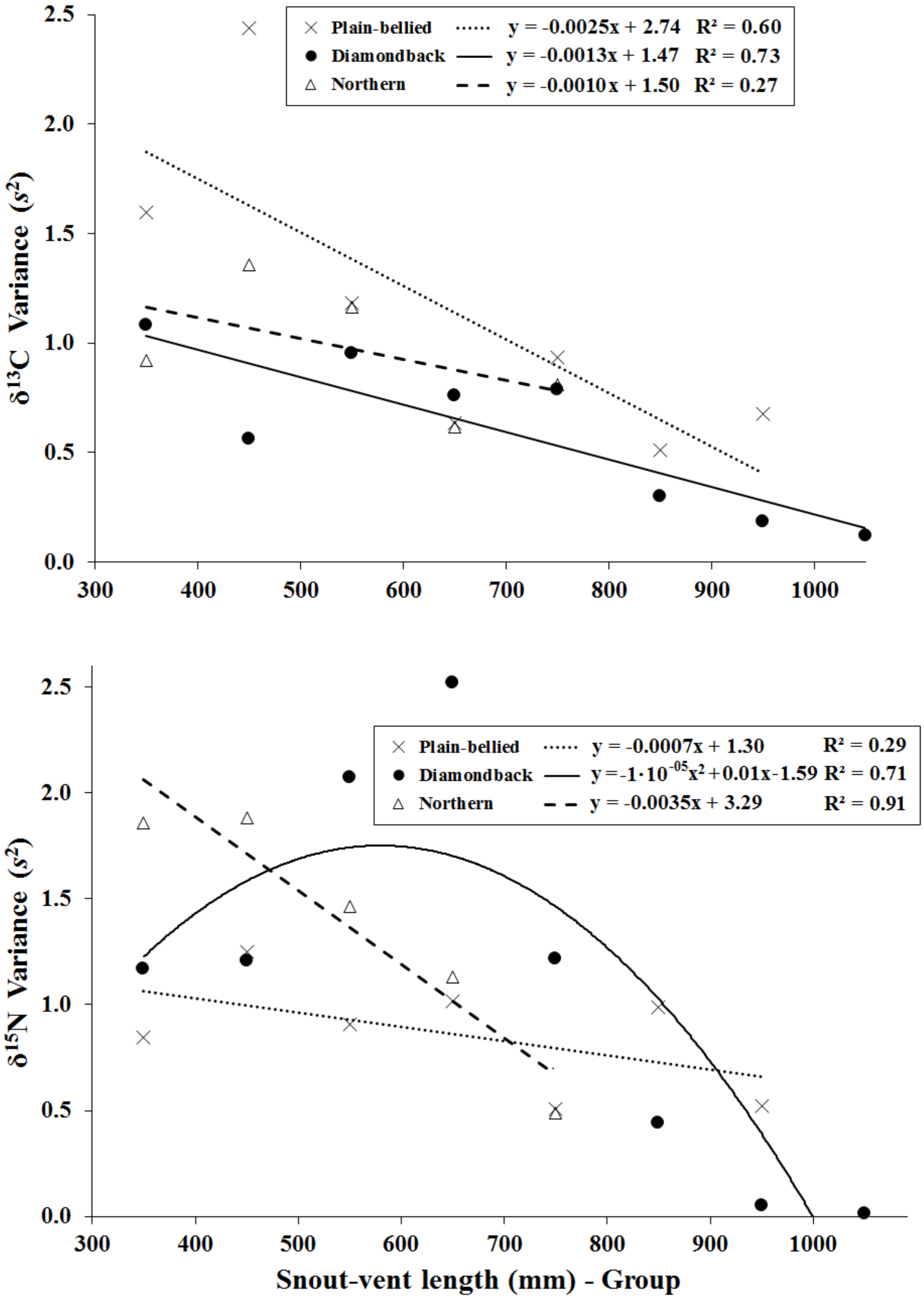

Figure 16. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) variance versus snake snout-vent length (100 mm groups) for plain-bellied, diamondback and northern watersnakes.

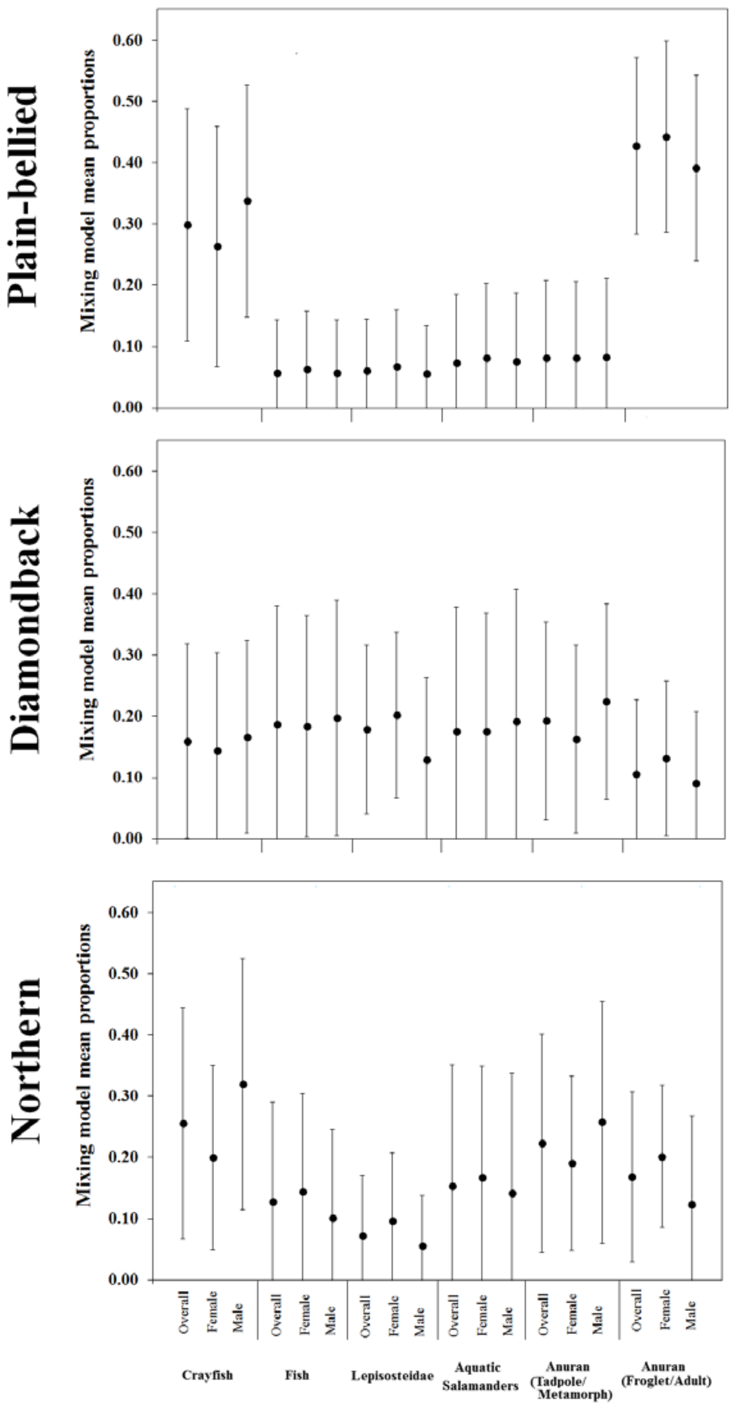

Figure 17. Stable isotope mixing model mean proportions with 95% credible intervals (error bars) of each prey group for snake species overall and sex for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

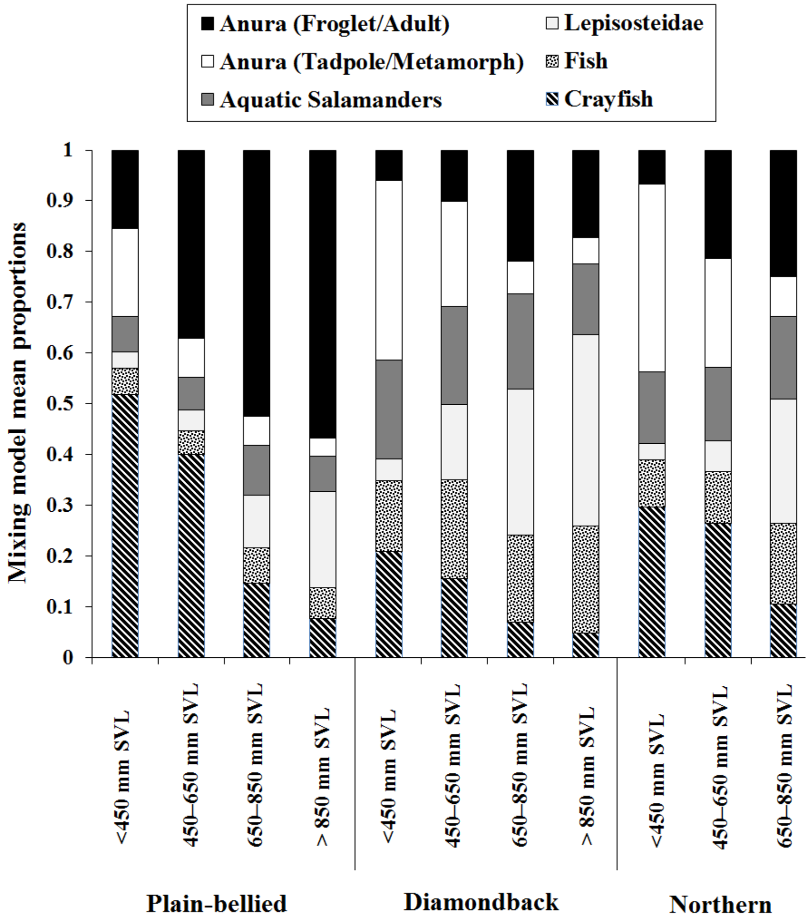

Figure 18. Stable isotope mixing model mean proportions of each prey group for snake size class (SVL mm) for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

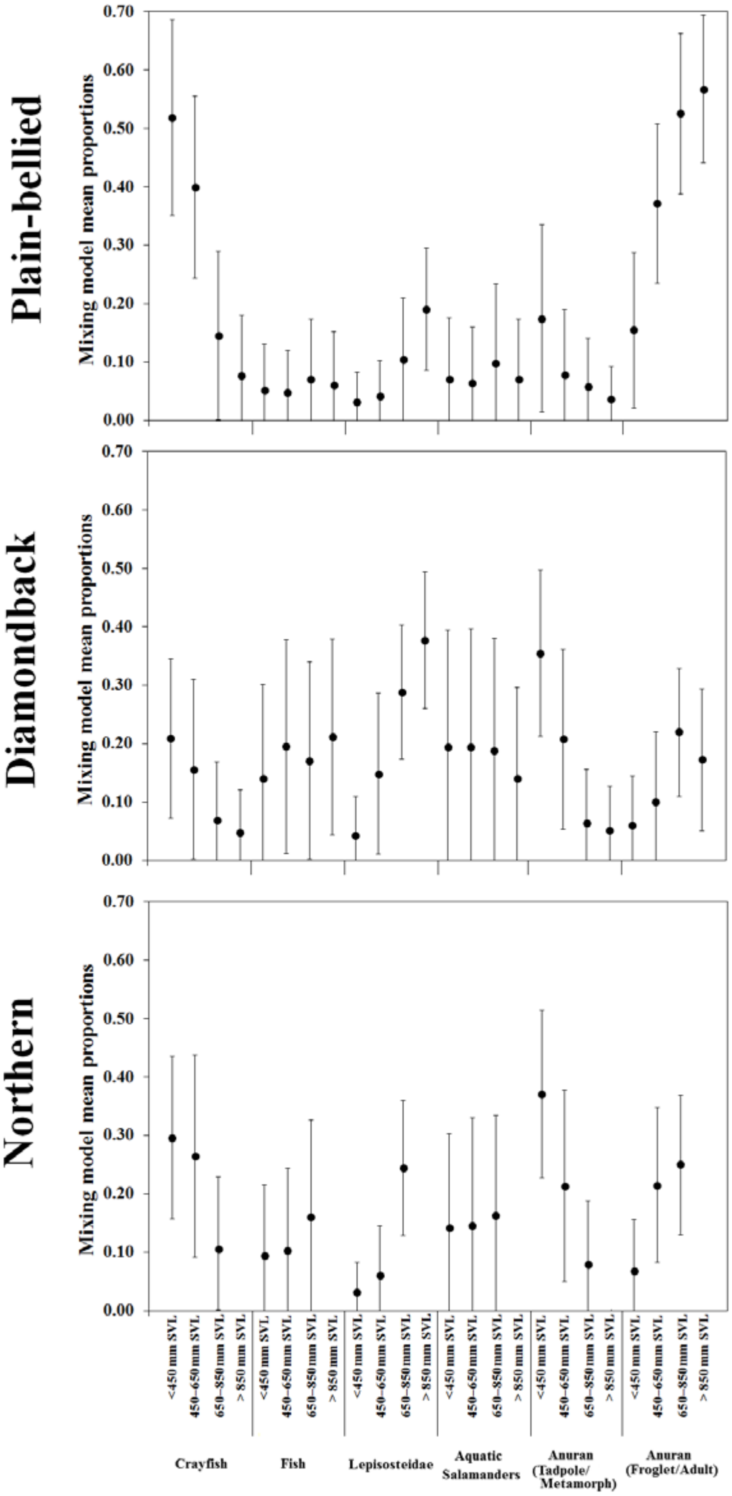

Figure 19. Stable isotope mixing model mean proportions with 95% credible intervals (error bars) of each prey group for snake size class for plain-bellied (*Nerodia erythrogaster*), diamondback (*N. rhombifer*) and northern (*N. sipedon*) watersnakes.

CHAPTER VI

SUMMARY AND FUTURE DIRECTIONS

This dissertation demonstrates how closely related species can coexist in a complex foraging system. Sympatric species may co-occur because of macrohabitat overlap, temporal variation or differences in microhabitat selection. However, dietary resource utilization differences are important to understand for sympatric watersnakes.

This research is the first to test prey selection by the northern watersnake (*Nerodia sipedon*) and demonstrate that this ubiquitous, opportunistic watersnake is avoiding a particular fish. Even though northern, diamondback (*N. rhombifer*) and plain-bellied (*N. erythrogaster*) watersnakes have dietary overlap, I related watersnake head size and shape characteristics to foraging and dietary variation. Also, snake gut content results helped to further the understanding of the complicated nature of watersnake foraging with species, season, sex and snake size effects. Such a variety of factors affect diet and likely allow for sympatry. This research was also the first to determine that seasonal prey differences allowed for each individual watersnake species to have low dietary overlap in a different unique season.

Traditional gut content results were complimented by long-term dietary information from stable isotopes analyses. Stable isotopes helped to determine where watersnakes were performing the majority of their foraging and at what trophic level. Such a stable isotope analysis has not been performed for these three watersnakes individually or in sympatry. To the best of my knowledge, this dissertation involves the largest stable isotope study on snakes (*N* = 333).

The several analysis methods in this dissertation provided a variety of watersnake dietary resource utilization information such as the importance of tadpole/metamorph anurans for diamondback watersnakes and eating of crayfish by plain-bellied watersnakes. There were some differences in the findings from gut content and stable isotope analyses but the combination of these two methods help to understand this complicated foraging system. General trends are evident supporting that plain-bellied watersnakes forage mostly on anurans, diamondback watersnakes foraging mostly on fishes and northern watersnakes having an intermediate diet but closer to diamondback watersnakes. My research supports that watersnake foraging is much more complex than previously realized.

This dissertation sets a strong foundation for future work involving watersnake dietary resource utilization. Prospective research can add additional study sites while including different wetland habitats and investigating from a landscape level. As this foraging system is dynamic with changing prey levels, researchers could address effects of flooding and drought on prey populations and how these changes could affect watersnake coexistence and abundance. Watersnake species likely can coexist with adequate prey levels but studies could address areas with reduced prey, such as fishless ponds, to determine effects on watersnakes. Incorporating a variety of factors beyond trophic can help to better understand how watersnake species live in sympatry.

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