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Effectiveness of manual palpation in the Northern Water Snake, Nerodia sipedon sipedon, as a method to extract gut contents for dietary studies

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Effectiveness of manual palpation in the Northern Water Snake, *Nerodia sipedon sipedon*, as a method to extract gut contents for dietary studies.

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By

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ABSTRACT

Analysis of manual palpation as a method to extract gut contents in the Common Water Snake (*Nerodia sipedon sipedon*)

The Common Water Snake, *Nerodia sipedon sipedon*, is one of six subspecies, and the only species of *Nerodia* to inhabit West Virginia. Because it is abundant and information on the species is lacking within the state, it was used as my study organism. The effectiveness of manual palpation, the most commonly used method in snake diet related studies, was tested against museum dissection by comparing presence and absence of gut contents. Nineteen individuals of 76 manually palpated collected snakes were compared to 6 individuals of 37 dissected snakes using a Pearson chi-square analysis. Results suggested manual palpation is an effective method to obtain diet data, X^2 (1, N=135) = 0.7296, p= 0.05. In addition, morphological data were collected from field specimens and used to determine the best predictor of sex, as well as to obtain information on average snout-vent length, total length, and mass of *N. s. sipedon* in West Virginia. Field notes of cover object use, behavior, and other herpetofauna from the field site were also reported. Data collected from this study will provide information on the effectiveness of manual palpation and information on the natural history of *N. s. sipedon* in West Virginia.

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Chapter One

Study organism

Taxonomy

The Common Water Snake, *Nerodia s. sipedon* (Linnaeus 1758), is in the Colubridae family. Colubrid snakes encompass the largest family of living snakes and include 300 genera and more than 1,400 species in all continents except Antarctica (Ernst 1989). The family Colubridae includes 23 genera, 50 species, and 95 subspecies within the United States (Ernst 1989). Few characteristics are shared by the 19 West Virginia colubrids, however, their head is as wide or wider than their neck with large regularly arranged scales (Behler 1979). The genus *Nerodia* (Gr. *neros*, a swimmer; Gr. *ode*, a thing like), within the subfamily Natricinae, contains the North American water snakes and the salt marsh snakes (Hulse, et al. 2001). Nine species are contained within this genus. *Nerodia* are characterized by their medium-sized to large, heavy body, and strongly keeled dorsal scales (Ernst and Barbour 1989; Hulse, et al. 2001; Mitchell 1994).

Description

Nerodia s. sipedon, is a moderate-sized, heavy-bodied snake species (Hulse, McCoy, and Censky 2001; Gibbons and Dorcas 2004). They may exceed 1,219 mm in length. However, size varies among the species with longitudinal variation. They are sexually dimorphic with the females of the species being longer and weighing more than the males (Weatherhead et al. 1995). In Virginia, adult females averaged longer (SVL ave. =774.3± 151.9 mm, 505-1,294 mm, n = 142) and had a shorter tail length (21.3±2.2%, 12.9-26.6, n=112) than males (SVL ave. =572.7±98.6 mm, 403-915, n=119, TL ave.= 24.5± 2.2%, 12.8-29.1, n=110) (Mitchell 1994).

Their dorsal and ventral coloration and pattern are highly variable. They range from a tan, olive, light gray, to dark gray background with dorsal crossbands that range in coloration from light brown, red, to dark gray (Behler 1979). The dorsal crossbands are widest in the vertebral area and then narrow as they approach the ventrals (Barbour 1971; Gibbons 2004, Hulse et al. 2001) which helps to distinguish them from the venomous Northern Copperhead, *Agkistrodon contortrix mokasen*, which has dorsal hourglass markings. Older individuals and individuals that are in the process of a shed appear lighter and uniform in color. The ventral coloration is typically a yellow-brown and sometimes, red, and marked with darker crescent-shaped markings.

Dorsal scales are strongly keeled and occur from 21 to 23 rows at their midbody. They have a divided anal plate, and their sub-caudal scales are in two rows (Hulse, McCoy, and Censky 2001).

Natural History

Nerodia s. sipedon are associated with any body of water and surrounding habitat, including man-made impoundments, streams, ponds, rivers, and lakes (Gibbons 2004). They bask along the bank and on overhanging vegetation in trees and shrubs. When encountered they typically seek refuge in the aquatic environment, or immediately bite at the intruder. They have an aggressive temperament and cause extensive bleeding after a bite due to saliva containing anti-coagulant properties (Silberhorn 2001). They release a foul-smelling musk to deter predators from ensuing capture.

Nerodia s. sipedon are voracious predators and generalist feeders, they do not have a prey preference and consume anything that can fit into their mouth. During the colder months of their active period they forage in the daytime and transition to nighttime foraging as temperatures increase. Documented diversity of prey species consumed by *N. sipedon* is greater than any other water snake and includes more than 80 species and 47 genera in 19 different families of fishes, more than 30 species and 16 genera in 10 families of amphibians, 2 species of small mammals, and a variety of invertebrates (Gibbons and Dorcas 2004). Although crayfish are ubiquitous with their habitat, they do not feed on them like other snakes (i.e. *Regina septemvittata*). Fish are the most abundant taxa in dietary studies (Uhler et al.1939, Gibbons 2001, King 1993) and increase in size as snake mass increases (King 1993).

Reproduction of *Nerodia* has been well studied. They come out of their hibernacula (i.e. man-made infrastructures, crayfish burrows, rock crevices) in late spring to early summer based on their range. Then, they ensue courtship which has been documented as 1 female and up to 5 males (Mushinsky 1979). Males use their reproductive copulatory organs, hemipenes, to anchor into the female's vent and insert sperm. Documentation by Weatherhead et al. (1995) has shown that males reach sexual maturity at approximately 375 mm SVL at 35 months, and females reach sexual maturity at approximately 550 mm SVL at 32 months. However, females observed in mating aggregations were approximately 48 months old (Weatherhead, et al. 1995); they delay reproduction by one year after sexual maturity occurs. *Nerodia s. sipedon* are ovoviviparous and are able to give birth to up to 80 young (Loughman, pers. com.) in late summer to early fall. At birth, neonates range from 127 mm to 152.4 mm (Cruz, unpubl. data).

Distribution and Status

Nerodia s. sipedon occurs in 30 states throughout the United States. The species ranges from extreme southern Quebec and southern Ontario, to coastal Maine, south to northern South Carolina, Georgia and Alabama, and west to Nebraska, Kansas, eastern Colorado and northeastern Oklahoma (Conant and Collins 1989; Ernst and Barbour 1989). Because *N. s.*

sipedon persists across the United States and is considered common, the species does not have a state or federal listing. Population numbers are abundant throughout the total range.

Threats and Conservation

Nerodia s. sipedon is not listed as a species in imperil or a species sensitive to habitat alteration (Gibbons 2004). In many cases, habitat alteration and accumulation of riffraff enhance habitat for the species and create sites for basking and cover from predation. However, the species should be monitored for population decline due to long-term anthropogenic impacts and persecution by humans (King 1939 and Gibbons et al. 2000). In addition, an appreciation for snakes should be encouraged and reinforced in zoos (Burghardt et al. 2009) and featured in public media (King, et al. 2006) to increase understanding of the taxa and reduce non-purposeful and purposeful killings by the public.

Chapter Two

Effectiveness of Manual Palpation

Abstract

Dietary studies have valuable implications to natural history, conservation, and management of species. Four known methods are used to determine the presence, absence, and to identify gut contents for dietary studies and include: stable isotope analysis, fecal analysis, gut flushing, and manual palpation. I tested the effectiveness of the latter method in the Common Water Snake, *Nerodia sipedon sipedon*. Ninety-five individuals were captured using visual encounter surveys (VES) and manually palpated for presence/absence of gut contents. Thirty-seven specimens from the Marshall University Herpetology Museum were dissected and the number of individuals with gut content presence/absence was compared to the manually palpated individuals. Based on the data collected, a Pearson chi-square test was conducted resulting in failure to accept the null hypothesis, X^2 (1, N=135) = 0.7296, p= 0.05. The results suggest manual palpation is a more effective method than dissection of museum specimens and result in significantly different numbers of gut content samples. The study provides the first quantitative information regarding the effectiveness of methodologies used in dietary studies and the most frequently used in snake studies.

Introduction

Diet is directly related to habitat selection, energetics, reproduction, and ultimately the survival of a species (Mcdonald et al. 2012), and so understanding food selection is an important part of the natural history of a species and an essential component of conservation and management planning (Beaupre 2002). Diet of a species can also provide insight into ecosystem integrity (Maurer 1993) by the presence or absence of generalist, specialist, and native and non-native prey species in gut content samples. In addition, dietary studies provide information to dietary shifts at a localized to landscape level by comparing prey preference and abundance of prey items over time. Because dietary studies are important to the ecology and conservation of species and environments, it is important to study and analyze the effectiveness of the methodologies of extracting and identifying gut contents.

Five techniques are abundant in wildlife literature to obtain and identify gut contents in dietary studies. Microsatellite analysis, stable isotope analysis, dissections of museum specimens, gut flushing, and manual palpation are useful, but use of each method is dependent on study objectives and available resources. Microsatellite analysis is accompanied with dietary sources, such as stomach contents, scat, and regurgitant (Oyler-McCance et al. 1999). Genetic markers are used to identify dietary samples, but must have an appropriate level of resolution and include a large array of species so as to not bias the results. Genetic analysis has been used in studies to identify predation on imperiled wildlife (Banks et al. 2003), foraging ecology (Clare et al. 2009), and spatial and temporal variations in diet (Fedriani and Kohn 2001, Prugh et al. 2008). Stable isotope analyses can trace the relative amounts of ecologically important elements (i.e. $C^{13:}C^{12}$ and N^{15}:N^{14}) in living organisms (Ehleringer and Rundel 1989, Gannes et al. 1998). Because stable isotopes of prey can become transferred to consumer tissues, studies using stable isotope analysis can help draw inferences of trophic level interactions (Dorcas and Willson 2009). Dissection of museum specimens is useful and has provided information on dietary composition (Greene 1986), and predator-size and prey-size relationships (James, et al. 1992, Shine 1977, 1987, Seib 1984). However, using this method alone can lead to snapshots of diet composition of individuals, and neglect information on localized populations. Gut flushing is a nonlethal method (Kamler and Pope 2001) that is conducted through use of water pumping devices (Leglar 1977) to extract gut contents. This method has been most commonly used in fish, amphibian, and avian studies. Manual palpation is a commonly used method and is the process in which an animal is stimulated to regurgitate a recently digested meal (Mushinsky and Hebrard 1977, Fitch 1987). For example, Jones et al. (2001) used this method to examine dietary shifts and the effects native species can have on an invasive species population. Because this method requires identifiable prey items, many individuals may be needed to obtain an adequate sample size. In addition, this method may be difficult to use on species that eat infrequently (Dorcas and Wilson 2009). Combinations of the various techniques can alleviate bias and strengthen the results of dietary studies.

Reptile populations, specifically snake populations, are becoming a growing concern to biologists. The number of declining species is beginning to reach those of the worldwide amphibian decline, and signs of population stability for many species are unseen (Gibbons et al. 2011). Similar to amphibians, snakes prove to be valuable as indicators of habitat quality, via environmental toxicity studies. Studies focusing on population numbers and diet can contribute information to shifts in trophic level interactions as well. Diet is directly related to the ultimate survival of a species and can provide a great deal of information about ecosystem health. It is important that methodologies of studying diet of snakes are effective.

Manual palpation is the most commonly used method in snake diet related studies. It requires little equipment and financial resources, making it efficient to use in field projects. However, little research has been done to examine the effectiveness of manual palpation on the ability to obtain identifiable gut contents for dietary studies. I was interested in examining the effectiveness of this commonly used method. This information is important because it will provide researchers with information regarding the validity of the methodology and previously published data, and assist in encouraging a different methodology to be used if it is invalid. This information will provide insight into whether or not this method should be continued to be used, or should be coupled with another method.

Nerodia s. sipedon, is a subspecies of *N. sipedon*, and has a large range throughout the United States. Its high population numbers across its range make it an ideal study subject. In addition, it is within the same genus as the federally threatened Copperbelly Water Snake, *Nerodia erthrogaster neglecta*, and the recently delisted Lake Erie Water Snake, *Nerodia s. insularum*. It makes it important to study the effectiveness of manual palpation to ensure the most accurate data for an increasing number of declining reptiles so researchers are able to understand declines, conservation, and management.

Methods

Study Site

This study was conducted on North Fork Short Creek in Ohio County, near Wheeling, West Virginia. The stream is 10-km long, and third order stream that flows into the Ohio River. Although snakes could be found throughout the stream, the study transect was 4-km long and included an urban neighborhood, cow pasture, natural areas, and the Short Creek Landfill. Aquatic habitats in the study area were characterized by lotic conditions with varying riffles, runs, and pools, and a limestone and gravel bottom. The surrounding terrestrial environment was characterized by oak, maple, poplar trees, and Japanese knotweed.

Snake Survey Protocol

Active capture methods were used to locate and capture snakes and included: visual encounter surveys (VES) (Campbell and Christman 1982) and turning natural and artificial cover objects (Fitch 1992). Visual encounter surveys involved opportunistically searching for snakes while they were foraging, basking, and hiding under cover objects. Cover objects were not placed along the stream bank within the transect area prior to surveying. VES were conducted 2 meters up both stream banks. The 4-km transect was surveyed every other weekend during the snakes' active period, from 11 May 2012 to 15 September 2012.

Upon capture, a Passive Integrated Transponder (PIT) tag was inserted into each snake subcutaneously with a large-bore needle (Dorcas and Wilson 2009) towards the posterior portion of the snake's body near the seventh ventral scale. The PIT tags provided each snake with an unambiguous identification number. The tags were scanned using a Biomark 601 passive integrated transponder (PIT) scanner and the snake's number was recorded. Each snake was scanned after the first survey day to determine presence or absence of a PIT tag.

Diet of snakes was determined by stimulating snakes to regurgitate ingested prey by manual palpation (Mushinsky and Hebrard 1977, Fitch 1987). Manual palpation is when the ventral side of the snake is massaged towards the cephalic region until a regurgitation response is induced. All snakes were manually palpated in the absence and presence of a noticeable food bolus. Snakes were stimulated to regurgitate twice within the active season to reduce stress upon the individual. Gut contents were stored in vials of 70% ethanol solution for future identification.

Museum Dissection Protocol

Thirty-seven *N. s. sipedon* from the Marshall University Herpetology Museum were dissected for presence and absence of gut contents. All snakes were collected in the months that were sampled during the 2012 survey period, 11 May to 15 September. Specimens were dissected and the presence and absence of gut contents were noted. Identifiable gut contents were stored in vials of 70% ethanol solution for future studies.

Manual Palpation Analysis

I performed a pearson chi-square test to compare the relationship between the number of gut contents present in the *N. s. sipedon* surveyed in the field and the number of gut contents that were found present in the Marshall University Herpetology Museum samples.

Results

I captured ninety-six *N. s. sipedon* including recaptures and road kills. Twenty-five percent of total individuals were captured in May, 24% were captured in June, 8% were captured in July, 33% were captured in August, and 9% were captured in September. After September, I did not manually palpate snakes to ensure over-winter survival. Nineteen individuals showed a presence of regurgitated prey items, all of which were fish species. Thirty-seven individuals from the Marshall University Herpetology Museum were dissected and 6 individuals showed presence of gut contents. The museum samples consisted of fish, and amphibian tadpoles and froglets. In addition, several ants were observed in one individual also obtaining fish in its gut.

The Pearson chi-square test was performed to determine if the presence of prey items were equally detected among the individuals collected in the field and the museum specimens. The results suggest there is a significant difference between the proportion of individuals that obtained gut contents in the field and those having gut contents present in museum specimens, X^2 (1, N=135) = 0.7296, p= 0.05.

Discussion

I captured ninety-six *N. s. sipedon* within the 2012 survey period, 11 May to 15 September. Percent total captures varied among months during the active season. The highest percent captures occurred in May and June when snakes were emerging, foraging, and breeding, and in August when snakes were foraging before they returned to hibernacula. The lowest capture rates occurred in July when snakes migrated to more secretive areas to thermoregulate due to increased temperatures, and in September. However, May and September sampling efforts were abbreviated.

The *Nerodia s. sipedon* captured at North Fork Short Creek with gut contents present contained 100% fish species. Although, literature suggests fish comprise a majority of *Nerodia s. sipedon diet*, they are generalist species and will consume other prey items (Uhler, et al. 1938. Amphibians were also in abundance along the creek banks; however, they were non-existent in the gut of captured snakes. In addition, the museum dissected specimens contained frog tadpoles and froglets. Ants were also present in the gut of a dissected individual indicating *N. s. sipedon* consume carrion.

The study indicates that a significant difference exists between the proportion of gut contents present in manually palpated individuals from North Fork Short Creek and the number of gut contents present in the dissected museum specimens from Marshall University. The study suggests that manual palpation, the most commonly used method in snake diet-related studies, is an effective method when compared to dissection. It can be assumed that past studies that have used the manual palpation yielded valid data regarding diet composition, dietary shifts, and body condition indices, etc.

Manual palpation is the most efficient method used in snake diet-related studies. It requires little resources (i.e. funds and preserved specimens) and provides an immediate snapshot of a population's diet, which is becoming increasingly important due to reptile declines. Localized populations are more likely to decline or become extirpated as a result of habitat fragmentation due to urban sprawl. As a result, sufficient data from dietary studies is becoming increasingly important to identify the dietary requirements and the loss of prey items of snakes and other wildlife.

Future studies

This study was designed to discover the effectiveness of manual palpation when compared to museum dissections. However, three other methods are used in wildlife literature and include: microsatellite analysis, stable isotope analysis, and gut flushing. Microsatellite analysis may offer a more in-depth examination of diet and provide information on gut contents that have been fully digested. In addition, stable isotope analysis may provide a more accurate description of dietary shifts over time. Although the three methods are more time and resource dependent, they also should be tested for their effectiveness and ability to provide sufficient data based on study objectives.

Chapter Three

Mensural Data

Abstract

Morphological characteristics are related to an animal's habitat, available food resources, and sex. In addition, comparing intraspecific morphological characteristics among differing populations can provide insight into health of a total population. The Common Water Snake, *Nerodia sipedon sipedon*, was captured in North Fork Creek, Wheeling, WV, and measured for cranial width, cranial length, mass, snout-vent length, and total length. Morphological characteristics were compared with each other and between male and female snakes. Results suggested cranial width was the best predictor of snake snout-vent length among neonate, juvenile, and adult snakes. In addition, mass is a better predictor of sex than tail length in adult snakes. Mean average mass, snout-vent length, and total length were also calculated for adult male and female snakes, which can be added to the West Virginia Biological Survey database. This study provides baseline data for future morphological studies on *N. s. sipedon* in West Virginia.

Introduction

Several factors or determinants can affect the morphological characteristics of a snake species. Morphology can vary as a result of habitat type, available resources, intraspecific and interspecific competition, and sex of an individual. Studying intraspecific morphological differences and similarities can ultimately provide insight into habitat conditions, and the health of an individual or population.

Nerodia s. sipedon is an aquatic species and has adapted morphological characteristics similar to other species that inhabit aquatic environments. They have narrow heads which help to reduce hydrodynamic drag (Hibbits and Fitzgerald 2005), short tails, and an ovoviviparous reproductive strategy to improve maneuverability within the aquatic environment. They are also one of the larger and heavier aquatic snakes in North America. Contrary to aquatic species, arboreal species have elongated bodies, low body masses, small heads, and pointed snouts (Lillywhite and Henderson 1993). Terrestrial species have variable morphological characteristics similar to aquatic and arboreal species.

Many species can also have characteristics differing between the male and female sex, also known as sexual dimorphism. Sexual dimorphism is when conspecifics have distinguishing coloration, patterning, internal reproductive organization, and difference in morphology or size that helps to distinguish sex of an individual. Difference in coloration and patterning is rarely seen among snake species of different sexes, however, sexual size dimorphism (SSD) is more (Shine 1993). Females grow larger than conspecific males in about two-thirds of all snake species (Shine 1978; 1993). However, species that are known to have male-male combat during reproductive events are those species with males are larger than females. Species in higher latitudes show a higher degree of sexual dimorphism than those in lower elevations (Shine 1993).

The primary objective of this study was to gather morphological data of *N. s. sipedon* in West Virginia, report the observational data so it can be used in the West Virginia Biological Survey database, and compare the data to *N. s. sipedon* in states surrounding West Virginia. Comparing morphological data from different states and biotic factors can provide insight into the conditions of the different populations.

Methods

Study Site

This study was conducted in the Northern Panhandle in Wheeling, West Virginia (USA) in Ohio County on snakes living in North Fork Short Creek, a 10-km long, and 3rd order stream that flows into the Ohio River. Although snakes could be found throughout the stream, the study transect was 4-km long and included an urban neighborhood, cow pasture, natural areas, and the Short Creek Landfill. Aquatic habitats in the study area were characterized by lotic conditions with varying riffles, runs, and pools, and a limestone and gravel bottom. The surrounding terrestrial environment was characterized by oaks, maples, poplars, and Japanese knotweed.

Snake Survey Protocol and Analysis

I active capture methods to locate and capture snakes and included: visual encounter surveys (VES) (Campbell and Christman 1982) and turning natural and artificial cover objects (Fitch 1992, Grant et al. 1992). Visual encounter surveys involved opportunistically searching for snakes while they were foraging, basking, and hiding under cover objects. Cover objects were not placed along the stream bank within the transect area prior to surveying. VES were conducted 2 meters up the stream bank. The 4-km transect was surveyed every other weekend during the snakes active period from 11 May 2012 to 15 September 2012.

Upon capture, a Passive Integrated Transponder (PIT) tag was inserted into each snake subcutaneously with a large-bore needle (Dorcas and Wilson 2009) towards the posterior portion of the snake's body near the seventh ventral scale. The PIT tags provided each snake with an unambiguous identification number. The tags were scanned using a Biomark 601 passive integrated transponder (PIT) scanner and the snake's number was recorded. Each snake was scanned after the first survey day to determine presence or absence of a PIT tag.

Five different morphological measurements were taken on each snake and included: cranial width (cw), cranial length (cl), snout-to-vent length (svl), tail length (tl), and mass. The means were calculated for svl, total length (ToL), mean proportion of tl to svl and ToL, and mass was reported with ± standard error. Microsoft Excel was used to compare mass (mg), cranial width (mm), and cranial length (mm) as a function of snout-vent length (mm). Tail length (mm) and mass (mg) were also reported as a function of snout-vent length (mm) among males and females.

Results

Over the course of the study, I captured 96 *Nerodia s. sipedon*, which included 29 females, 26 males, and 45 juveniles and neonates. Five individuals were recaptured. Thus, the total number of captures did not define the total population for the 4 km transect.

Sexual Size Dimorphism

Female *Nerodia s. sipedon* were larger on average than the males for snout-vent length, total length, and mass within North Fork Short Creek, WV. Females had an average snout-vent length of 570±45.8 mm (n=29), average total length of 707±30.9 mm (n=25), and an average mass of 1,654±243.5 (n=25). Whereas, males had a snout-vent length of 450±21.0 mm (n=26), average total length of 463.9±25.8 (n=26), and an average mass of 737±86.6 (n=20).

Females have an overall larger svl, total length ToL, and mass than males. Females grow longer, and as a result have a higher body mass (y=8.478x-3213.5, R^2=0.8223) than males (y=3.3572x-871.11, R^2=0.7688) (Fig. 5); however, at any given snout-vent length, male mass falls within the range of female mass creating an exponential relationship (Fig. 1). Mass increases as a function of snout-vent length for both sexes of *N. s. sipedon* (n=90, R^2=0.7437).

Males had an overall longer tail length than females (Fig. 4), but was not the best predictor of sex (male R^2=0.5866 and female R^2=0.3013) as suggested in other studies (King 1989). Mass was a better predictor of sex than tail length (males R^2=0.7688 and females R^2=0.8223). However, tail length has been shown to be a good predictor of sex in studies of natricine species (King 1989).

Morphological Relationships

A positive relationship exists among the morphological characteristics of *N. s. sipedon*. The linear correlation between cw and svl was the weakest (Fig. 2) (R^2=0.6592) and cranial length and snout-vent length had the strongest linear correlation (Fig. 3) (R^2=0.8248). Mass and snout-vent length had a positive, exponential correlation (Fig. 1) (R^2=0.7437).

Discussion

In this survey, morphological measurements were taken to determine the mean snout-vent length, total length, and mass for male and female *N. s. sipedon* in West Virginia, to compare the measurements with snakes in surrounding states of Pennsylvania and Virginia (Table 6), and to examine the relationships between the morphological characteristics. Females were larger than males and had a higher mean snout-vent length, total length, and mass. The data collected were similar to data from Pennsylvania and Virginia, as well as literature found relating to sexual dimorphism of *Nerodia*. In addition, a positive correlation existed among male and female snout-vent length and tail length, and snout-vent length and mass. Mass was a better predictor of sex than tail length which was in contrast to the literature of natricine species (King 1989). Male and female mass increased exponentially; however, male mass fell within the exponential growth curve of female mass.

The morphological characteristics of the 90 adults, juveniles, and neonates were compared and positive relationships existed among them. Snout-vent length and cranial length had the strongest positive correlation and snout-vent length and cranial width had the weakest correlation.

Future studies

This study generated information for the West Virginia Biological Survey database and provides baseline information for future work conducted on the morphological characteristics of *N. s. sipedon* in West Virginia. Future studies to be conducted should analyze the similarities and differences of body condition, size, and structure of *N. s. sipedon* in differing aquatic habitats. Because this species is a habitat generalist and lives within small creeks to large rivers and lakes, the mass and morphological characteristics may vary as a result of varying habitat size. A study coupled with available food resources, natural cover objects, and percentage of canopy cover can also suggest the type of habitat that is most preferred across all available aquatic habitats. In addition, it can also suggest which habitats are more likely to support populations of this species, which is important due to urban sprawl. Other studies should compare current and past population numbers, presence and absence of *N. s. sipedon*, as well as abiotic and biotic factors among sites throughout West Virginia to determine factors that affect population numbers of a "common" species. Information such as this is vital for the long-term conservation of the species.

Chapter Four

Field Notes

Cover Objects

It is not uncommon for snakes to aggregate under cover objects. Cover objects offer protection from predators and sites of thermoregulation. Common cover object sites include: rocks, logs, shrub and tree foliage, and riffraff. Riffraff are piles of unwanted debris from humans made of garbage materials and substrate.

I found *Nerodia s. sipedon* throughout the creek under cover objects, however, a few sites among the creek served as areas of higher density aggregations. The site deemed "the cow pasture" had a south-facing bank with a 6-m long stretch of large, flat rock. Many snakes were captured underneath the rocks in aggregations throughout the season. Other areas of high captures were underneath riffraff created from a tire and black mat on the south-facing bank. In addition, other *N. s. sipedon* were found under a large mat that laid upon an elevated portion of stream in the shade. In many cases, human impact has negative impacts on animal population and behavior. In the instance of North Fork Short Creek, many snakes appeared to benefit from human impact and the amount of unwanted materials that washed into the creek created habitat for the snakes. The observations regarding the use of the riffraff suggest that some species benefit from anthropogenic effects on "natural" habitat. Future studies should be targeted on the benefits of human-made or synthetic habitats, and whether they are able to sustain not only common species, but imperiled species as well.

Basking Behavior

All reptiles, including snakes, are both ectothermic and poikilothermic. Ectothermic organisms rely on external sources to regulate internal bodily temperatures. Poikilothermic organism's internal bodily temperature varies with the temperature of the external surrounding environment. Thermal ecology examines how environmental thermal variation affects the distribution of species or abundance of organisms based on the preferred bodily temperature (T_b). Many studies have been focused on the preferred conditions for ectothermic and poikilothermic individuals.

From late May to mid-July, *N. s. sipedon* were observed basking along the stream bank atop rocks and riffraff. As the field season progressed, more snakes basked in the shade, on the stems of the Japanese knotweed, specifically from late July through August when temperatures increased. All snakes found within the Japanese knotweed were adults. However, observations could be a result of observer bias due to size of snakes and detectability among flora. I speculate that the *N. s. sipedon* moving from ground-basking sites to arboreal basking sites is the result of an increase in temperature and an attempt to achieve preferred T_b. Evapotranspiration from plants, such as the Japanese knotweed, causes a decrease in microhabitat temperatures leading to a microclimate suitable for an ideal T_b of *N. s. sipedon*. Further studies should be conducted to examine the microhabitat conditions that are conducive to this species thermal regulatory need beyond those that are observed during months of high detectability.

Feeding Behavior

One female was observed feeding within the creek in early June. The female positioned herself on the bottom of the creek. As fish swam in front of her, the first half of her body swayed laterally and followed the direction of the swimming fishes with its mouth open. After swaying laterally several times she captured a large creek chub and swam with it to the creek bank. It seemed as though she was waiting for it to die before swallowing it. The snake was captured and recorded, and the fish was placed in a 70% ethanol solution.

The feeding behavior of the female *N. s. sipedon* was typical of the feeding behavior of *Nerodia rhombifer*, the Diamondback Watersnake. It has been documented by Kofron and Dixon (1980) making similar lateral undulations while following prey. They also observed the species entrapping the fish in the center of its body, and then searching for the prey with a gaped mouth until prey was captured. Using a gaped mouth to search for fish suggests that these snakes either do not rely on chemoreception during foraging, have poor visual acuity, or have developed this behavioral adaptation as result of turbid conditions (Gibbons and Dorcas 2004).

Behavior Upon Capture

Literature states that *Nerodia* are the most aggressive watersnakes and will immediately bite upon capture. However, many snakes that I captured during the field season did not strike and allowed me to hold them with little resistance. From personal experience with *N. s. insularum* and *N. s. sipedon*, the more docile behavior of *N. s. sipedon* is likely to be associated with the Wheeling locale, or is the result of another unobserved variable.

Other Herpetofauna

Throughout the field season, 104 *N. s. sipedon* were captured, 5 of them were recaptures, resulting in 99 individuals. North Fork Short Creek also had an abundance of other herpetofauna. One hundred and seventeen Queensnakes, *Regina septemvittata*, were captured as well as Eastern Garter Snake, *Thamnophis s. sirtalis*, and Eastern Milksnake, *Lampropeltis t. triangulum*. Other species that were observed included: a juvenile Snapping Turtle, *Chelydra serpentina*, a River Cooter, *Pseudemys c. concinna*, Green Frogs, *Lithobates c. melonata*, American Bullfrogs, *Lithobates catesbeianus*, Pickerel Frogs, *Lithobates palustris*, Northern Two-lined Salamanders, *Eurycea bislineata*, Northern Dusky Salamanders, *Desmognathus fuscus*, and Long-tailed Salamanders, *Eurycea l. longicauda.* Despite the evident human impact on the stream, observational data of the number and age class variation suggests that the habitat is suitable for a variety of reptiles and amphibian species.

Table 1 Snout-vent length (mm) data of *Nerodia s. sipedon* in West Virginia, Virginia, and Pennsylvania.			
	West Virginia	**Virginia**	**Pennsylvania**
Male	450 ± 21.0 n=26	572.7 ± 98.6 n=119	445 ± 13.4 n=44
Female	570 ± 25.8 n= 29	774.3 ± 151.9 n=142	700 ± 16.5 n=28

Table 2 Total length (mm) of *Nerodia s. sipedon* in West Virginia, Virginia, and Pennsylvania.			
	West Virginia	**Virginia**	**Pennsylvania**
Male	463.9 ± 25.8 n=26	---	575 ± 19 n=33
Female	707 ± 30.9 n=25	---	907 ± 22.4 n=21

Table 3 Mass (mg) of *Nerodia s. sipedon* in West Virginia, Virginia, and Pennsylvania.			
	West Virginia	**Virginia**	**Pennsylvania**
Male	737 ± 86.6 n=20	1,960 ± 1,113 n=39	---
Female	1,654 ± 243.5 n=25	4,542 ± 2,396 n=52	---

Table 4 Mensural data of *Nerodia s. sipedon* in West Virginia, Virginia, and Pennsylvania.			
	SVL (mm)	**ToL (mm)**	**Mass (mg)**
West Virginia			
Male	450 ± #, n=25	463.9 ± #, n=25	737 ± #, n=19
Female	570 ± #, n= 29	707 ± #, n=25	1,654 ± #, n=25
Virginia			
Male	572.7 ± 98.6, n=119	---	1,960 ± 1,113, n=39
Female	774.3 ± 151.9, n=142	---	4,542 ± 2,396, n=52
Pennsylvania			
Male	445 ± 13.4, n=44	575 ± 19, n=33	**---**
Female	700 ± 16.5, n=28	907 ± 22.4, n=21	**---**

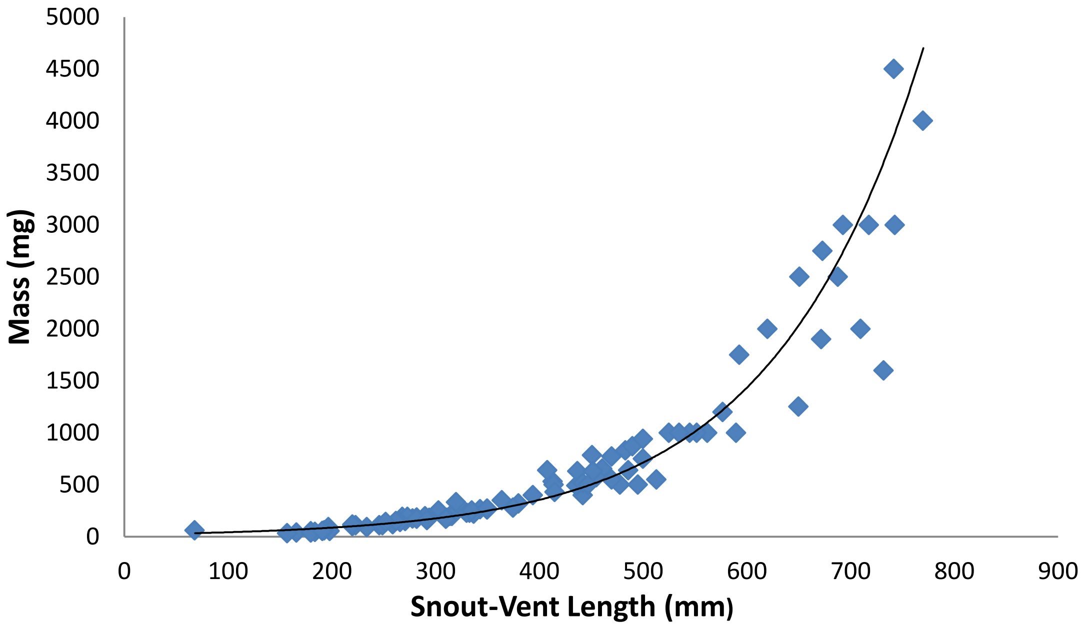

Figure 1 Mass (mg) as a function of snout-vent length (mm) in 90 *Nerodia s. sipedon*. The regression equation is y= 4.7133x-1193.2. R^2= 0.7437.

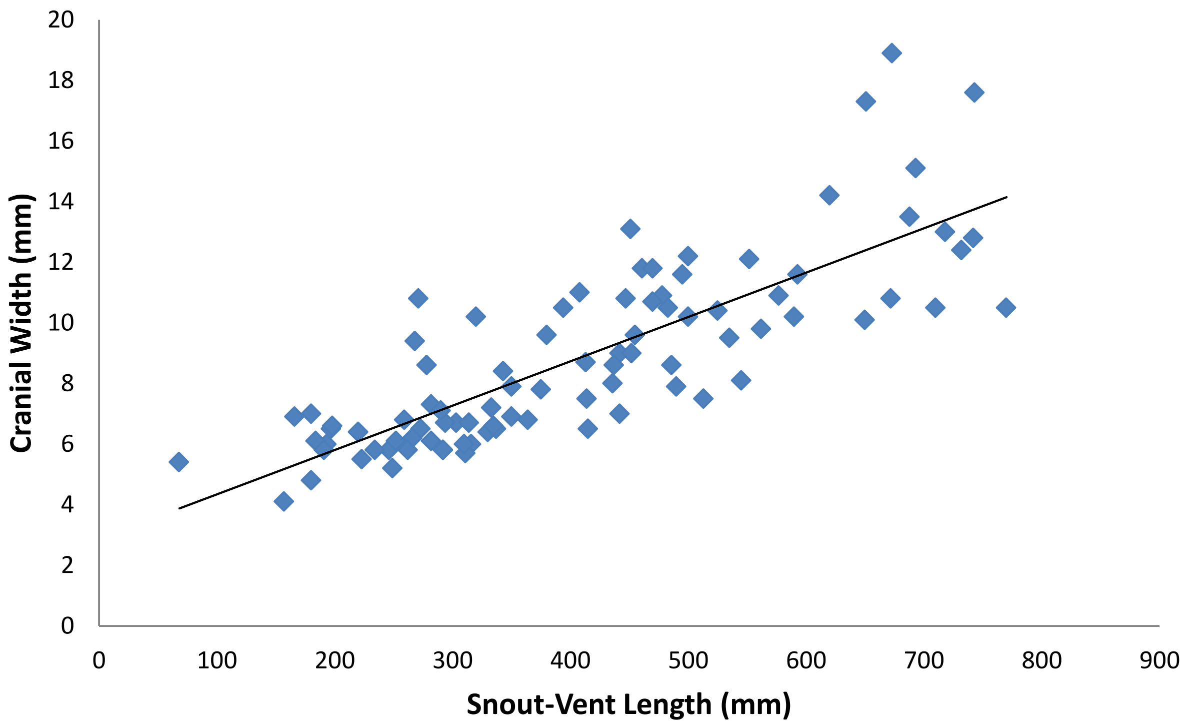

Figure 2 Cranial width (mm) as a function of snouth-vent length (mm) in 90 *Nerodia sipedon sipedon*. The regression equation is y= 0.0146x + 2.8769. R^2= 0.6592.

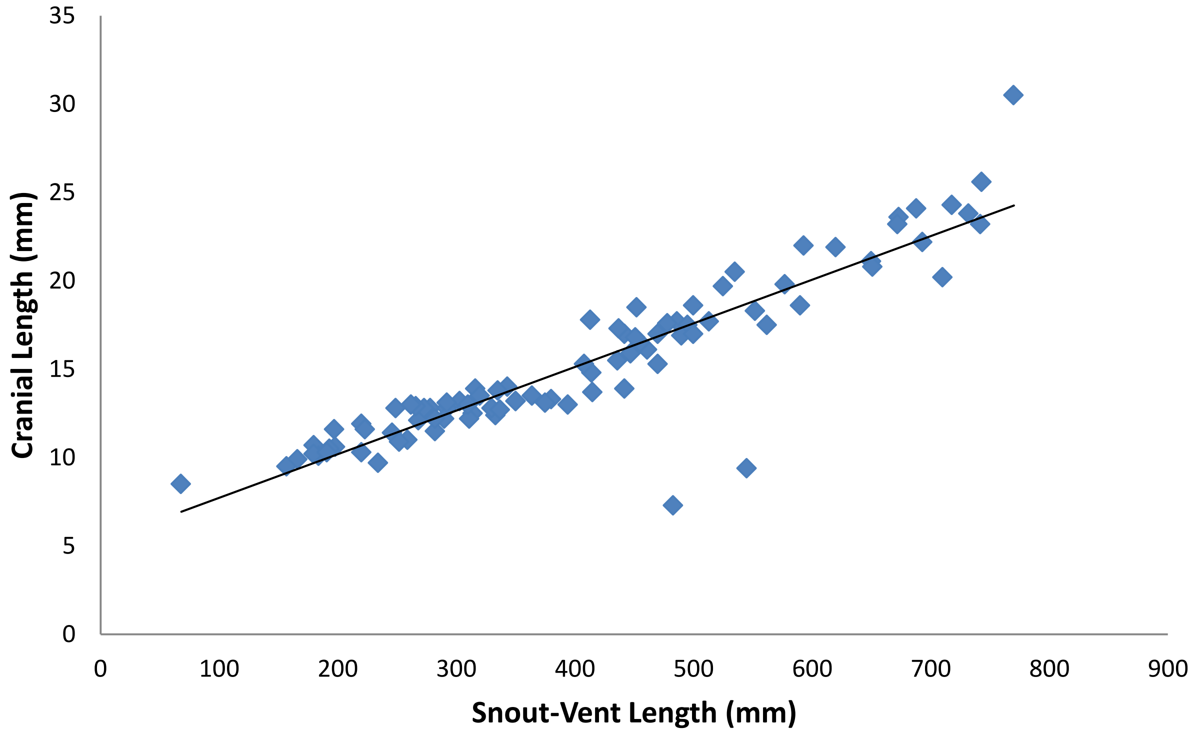

Figure 3 Cranial length (mm) as a function of snout-vent length (mm) in 90 *Nerodia s. sipedon*. The regression equation is y= 0.0249x + 5.2516. R^2= 0.8248.

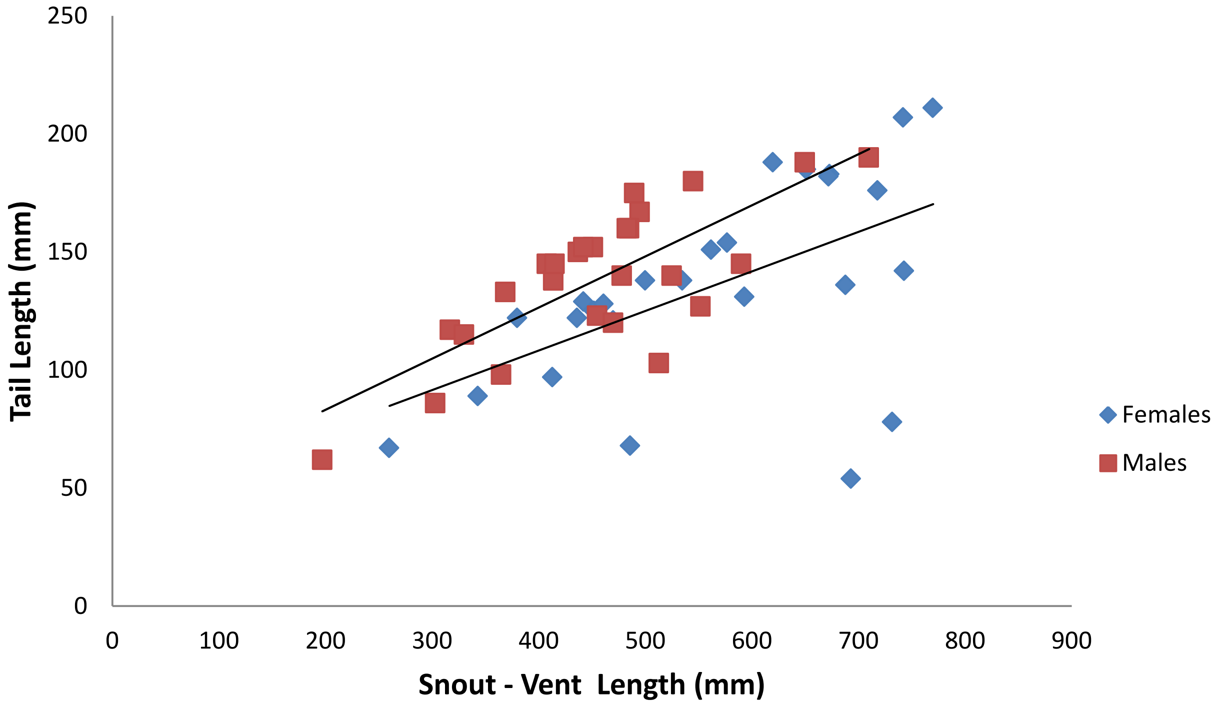

Figure 4 Tail length (mm) as a function of snout-vent length (mm) in 50 *Nerodia s. sipedon* (25 males, 25 females). The regression equation for males is y=0.2164x + 39.938. R^2= 0.5866. The regression line for females is y= 0.1676x + 41.294. R^2= 0.3013. Note that most males have longer tail length than females.

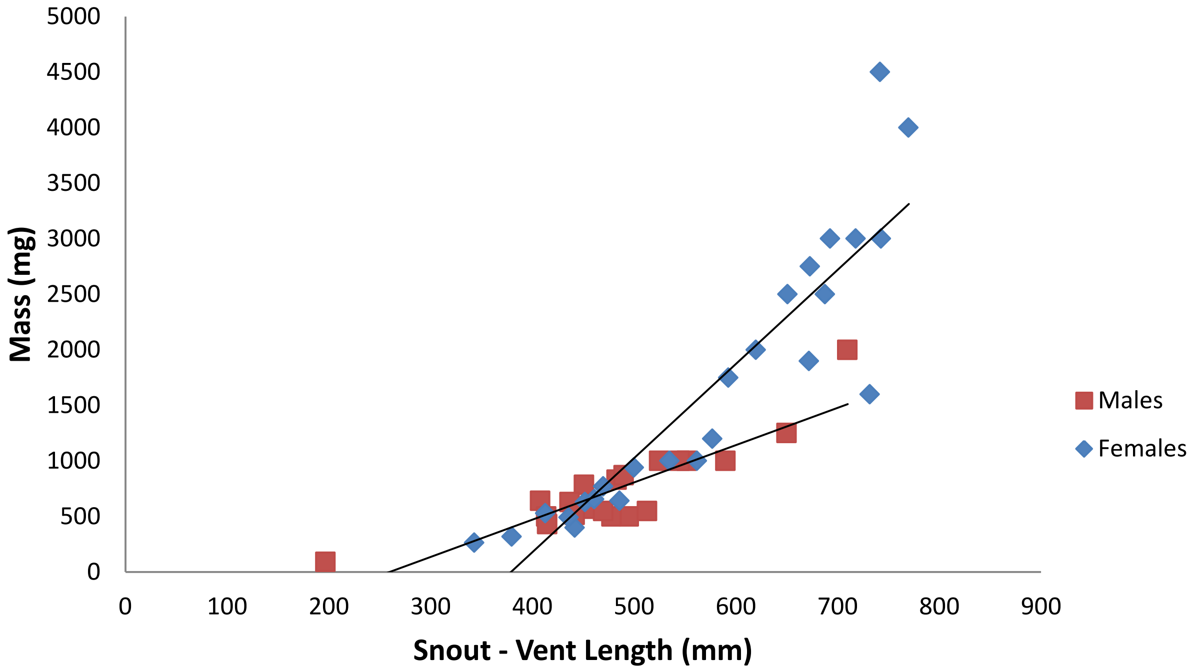

Figure 5 Mass (mg) as a function of snout-vent length (mm) in 45 *Nerodia s. sipedon* (20 males, 25 females). The regression equation for the males is y=3.3572x - 871.11. R^2=0.7688. The regression equation for the females is y=8.478x - 3213.5. R^2=0.8223. Note that most females have a larger body mass as a function of snout-vent length.

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