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Dietary Preference of the Queensnake (*Regina septemvittata*)

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DIETARY PREFERENCE OF THE QUEENSNAKE (*Regina Septemvittata*)

A thesis submitted to
the Graduate College of
Marshall University

In partial fulfillment of
the requirements for the degree of
Master of Science
in Biology

by

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Marshall University
July 2013

ABSTRACT

Dietary Preference of the Queensnake (*Regina septemvittata*)

The Queensnake (*Regina septemvittata*) is a small secretive water snake found throughout the eastern United States. Once common, their numbers have declined to the extent that they are now threatened throughout most of their range, largely the result of pollutant-based reduction in prey species. These snakes are assumed to eat molted crayfish exclusively. For some common crayfish species, molting happens only twice a summer during a two-week period. It has not been documented if Queensnakes eat anything besides crayfish on a regular basis. The purpose of this study was to determine the prey preference of Queensnakes with particular focus on crayfish species. Because Queensnakes are considered to be dietary specialists, they are at great risk of becoming extirpated should their food source diminish and are therefore effective bio-indicators for the streams where they live. Data collected from this study will better enable biologists to determine what habitats and prey items are required to conserve this species.

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I would first like to thank Dr. Pauley for being an excellent advisor. He gave me excellent guidance and helped me gain confidence in myself as a scientist. He also was extremely patient with me and put up with all of my wild questions.

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Next I would like to thank Marcie Cruz for being my right hand woman in the field. She helped with capturing snakes, recording data, and PIT tagging the snakes. The amount of data collected could not have been accomplished without her. I would also like to thank Amy Fiedler for helping in the field, as well as Chris Bowers, Abby Sinclair, and Derek Breakfield; and Kelli Herrick (Grandma) for helping me with statistics.

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Chapter 1

Dietary Preference of the Queensnake (*Regina septemvittata*) in West Virginia

Abstract

The Queensnake (*Regina septemvittata*) is the smallest water snake in West Virginia. They are found in wet habitats with rocky bottoms that provide cover for both snake and prey, which is predominantly molting crayfish. However, it has not been determined whether these snakes prefer one species of crayfish over another. Two species of crayfish occur at North Fork Short Creek in Wheeling West Virginia: *Cambarus carinirostris* (Rock Crayfish) and *Orconectes obscurus* (Allegheny Crayfish). *Orconectes obscurus* undergo two synchronous molts biannually during the summer months, whereas *C. carinirostris* molts randomly throughout the summer. Crayfish populations were sampled at the North Fork Short Creek site using seine hauls throughout the summer of 2012, resulting in a capture of 120 *O. obscurus* and 33 *C. carinirostris*. At the same site, 120 Queensnakes were captured, and stomach contents were obtained from 34 snakes yielding 36 crayfish. Of those 36, 18 were *C. carinirostris*, 11 were *O. obscurus*, and 7 were unidentifiable. A chi square analysis was conducted to determine whether the ratio of species in the gut contents represented the ratio of population sizes estimated in the field. The calculated X^2 (28.1) exceeded the expected value for $\alpha=0.05$ (3.84), suggesting a strong preference for *C. carinirostris* as prey.

Introduction

The Queensnake is the smallest water snake in West Virginia, averaging between 30 and 35 centimeters in length. The largest one ever recorded was 94 centimeters (Gibbons & Dorcas, 2004). Queensnakes are secretive and docile like the other species of *Regina* (Smith & Huheey, 1960). Their backs are dark brown with two yellow stripes running along the side (Gibbons & Dorcas, 2004). They have four stripes on the ventral surface, making Queensnakes the only species of North American water snake with four ventral stripes (Gibbons & Dorcas, 2004). Unlike a lot of species of reptiles and amphibians, there are no recognized subspecies, but there are some slight color variations depending on where in the snake's range it is found (Conant, 1958). Fossil records of this species date back to the late Pleistocene (Holman 2000), indicating that this is a very successful species of snake. The range of the Queensnake includes most of the non-coastal areas of the Atlantic states from New York to the Western Panhandle of Florida to as far west as Arkansas (Gibbons & Dorcas, 2004). Queensnakes are now endangered in New York and Wisconsin (Gibbons & Dorcas, 2004), and they have been reported to be extirpated from Missouri (Johnson, 1992). The activity period of these snakes is between April and May, depending on range, and October and November, depending on range and temperature (Conant, 1938).

The habitat for Queensnakes is usually rivers and streams with a rocky bottom, which provides cover for their preferred food, crayfish. However, there have been records of them living in freshwater marshes, lakes, and other aquatic areas with crayfish (Duellman 1947; Ernst & Barbour 1989). Queensnakes prefer to have overhanging vegetation and brush piles near the banks for basking and cover objects. They are often seen basking in trees, and when approached they drop down into the water. Queensnakes move faster in the water than land at all

temperatures (Finkler & Claussen, 1999), and therefore are always quick to drop into water when a human or predator startles them. These snakes have a much longer tail than other members of *Regina*, and this is speculated to be because of its arboreal basking habits (Rossman, 1963).

Queensnakes become sexually mature in two to three years (Branson & Baker 1974). They mate in the spring as they emerge from hibernacula (i.e. muskrat burrows, crayfish burrows, and deep crevices in rocky sections of a stream (Hulse et al, 2001)). They are viviparous, meaning they give birth to live young. They birth 5-23 young between June and October depending where in their range they occur. Neonates average six inches in length (Gibbons & Dorcas, 2004).

Because this species is not very large, it is susceptible to predation. Animals documented to prey on Queensnakes are large fishes (Wood, 1949), hellbenders (*Cryptobranchus a. alleghaniensis*) (Branson & Baker 1974), and black racers (*Coluber c.constrictor*) (Palmer & Braswell, 1995). The primary defense of Queensnakes is to flee, and if caught, they will release a foul smelling musk all over the predator. They rarely bite when handled, but it has been documented that individuals over two feet in length may bite (Gibbons & Dorcas, 2004).

The Queensnake's diet is comprised mainly of crayfish, specifically soft molted crayfish. Wood (1949) found that 42 of 43 disgorged food items were crayfish, and Brown (1979) found 63 of 63 disgorged food items to be crayfish. There have been other records of different prey items in Queensnakes, such as dragon fly nymphs (Raney & Roecker, 1947), darters (Wood, 1949), and a single snail (Adler & Tilley, 1960). Based on these previous studies I determined I would need a large population of Queensnakes from which to collect stomach contents, as well as a large population of crayfish to determine the population dynamics between crayfish and Queensnakes.

Over 50% of the United States freshwater crayfishes are endangered (Taylor et al, 1996). The contributing factors include limited natural range, habitat alteration, and the introduction of non-native species (Taylor et al, 1996). Crayfish are essential to the environment. They serve as predators, bio-processors of vegetation and carrion, and they are a food source for fishes and other terrestrial organisms (Taylor et al, 2007). Crayfish are economically important, supporting bait fisheries as well as the human food fishery (Taylor et al, 2007). Queensnakes, like crayfish, serve as bio-indicators to the health of aquatic ecosystems. By monitoring Queensnake populations, biologists can determine the health of crayfish populations within streams that Queensnakes inhabit.

A population of Queensnakes in North Fork Short Creek in Wheeling, West Virginia, coexists with two species of crayfish, *O. obscurus* and *C. carinirostris*. *Orconectes obscurus* molts synchronously twice a summer during the same two-week period and is the most common species in the stream, whereas the *C. carinirostris* molts individually throughout the summer. The main objectives of this study are as follows:

Objectives

1. Determine if Queensnakes prefer one species of crayfish over the other as prey
2. If Queensnakes prefer *O. obscurus*, do the mass molts correlate with the phenology of the snake (emerging from hibernacula, breeding, and birthing of young)

Because Queensnakes are dietary specialists, they have the potential to be eradicated quickly if their habitats are altered through pollution or destroyed by developments. If crayfish start dying off due to polluted waters, Queensnakes may not be able to adapt quickly enough to a different food source, and may therefore also die off. Queensnakes, like most other herpetofauna, are great bio-indicators on what humans are doing to the environment.

Methods

Study Site

This study was conducted on a 4 km stretch of North Fork Short Creek in Wheeling, West Virginia less than a mile from West Liberty University in Ohio County. The stream has been greatly disturbed by human presence. The stream flows through cow pastures, neighborhoods, and a landfill. Species of riparian vegetation are variable throughout the stream. In the cow pasture, vegetation consists of grass. Farther downstream the banks become thick with Japanese Knotweed. Farther downstream there is a deciduous forest, composed of maples, oaks, and sycamore. Other areas of the stream do not have riparian vegetation but rock retaining walls. There is also an abundance of human made debris in and around the stream, including tarps, mats, wooden boards, plastic bags, bottles, and other trash. There is a great diversity of wildlife in the stream. Multiple species of fish, birds, amphibians, and two species of crayfish are prevalent.

Field Sampling

Queensnakes were collected by hand every other weekend from May 2012 to September 2012. I collected every other weekend to create as little stress as possible on the snakes in this area. This distance was walked entirely twice a weekend. Once a snake was captured, basic morphometrics were taken on the snake, including snout-vent length (SVL), tail length (TL), cranial width (CW), cranial length (CL), and weight (g). Regurgitation of each snake's digestive tract was done by massaging the bolus in the stomach toward the head until the stomach contents came out. The stomach contents were then preserved in 70% ethanol to await species identification. Last, if the snake was large enough, a PIT tag was inserted into the animal's body

cavity giving it a personal identification number for use in mark and recapture information. Snakes were released unharmed where they were collected.

The stream was sampled for crayfish using seine hauls once a month. I conducted 10 seine hauls in a riffle which is a shallow stretch of stream with high water flow, 10 in a run which is a straight stretch of stream that is deeper than a riffle and not as high flow, and 10 in a hole which is a deep pocket of water with little or no water flow, for a total of 30 seine hauls in a month. Seining was conducted moving upstream to avoid collecting crayfish from previous spots that I may have missed and caused them to float downstream. Once collected crayfish were humanely euthanized in 70% ethanol (Loughman et al. 2009) and brought to the lab to be identified to species. This helped give me an estimation of the ratio of population sizes in the creek.

Data Analysis

A chi square analysis was conducted to determine whether the ratio of species in the gut contents represented the ratio of population sizes estimated in the field; this would help determine if snakes were choosing one species of prey over the other. Another chi square analysis was also conducted to see if the assumed population dynamic ratio of 1:1 existed between *O. obscurus* and *C. carinirostris* throughout the summer.

Results and Discussion

Throughout the summer I collected 120 different Queensnakes, with only three recaptures, suggesting the population is very large and healthy, or snakes were dispersing after being captured and new ones were migrating in taking their place. Out of the 120 Queensnakes captured, 17 were females, 47 were males, and 56 were juveniles. This resulted in a male to

female sex ratio of 2.76:1. Out of the 120 snakes, stomach contents were obtained from 34, which ended up resulting in a total of 36 stomach contents to be identified. There are more stomach contents than snakes because 2 different Queensnakes regurgitated 2 crayfish. Originally I was told Queensnakes would regurgitate on their own if you simply handle them too much and stress them out. This turned out to not work in this particular population of Queensnakes, so I turned to massaging the bolus to obtain stomach contents. Of the 36 stomach contents, 18 were *C. carinirostirs*, 11 were *O. obscurus*, and 7 were unidentifiable.

I collected 153 crayfish from May to September. One hundred and twenty of them were *O. obscurus*, and 33 were *C. carinirostris*. This resulted in an overall species ratio of 3.63:1 throughout the entire summer. However, chi square analyses showed a shift in the populations throughout the summer. Chi square analysis was conducted for each month; with the assumption that the ratio between species of crayfish in the stream was 1:1. Calculated X^2 values for May and June were lower than the expected ($X^2_{\text{tab}}=3.84$, $\alpha=0.05$), suggesting no species preference in prey. Calculated X^2 for July, August, and September exceeded the critical value ($X^2_{\text{tab}}=3.84$, $\alpha=0.05$) suggesting a population shift to a higher ratio of *O. obscurus* to *C. carinirostris*. This could possibly be the result of the dry summer in 2012. When the *C. carinirostris* were collected, they were fossorial, while *O. obscurus* were in permanent bodies of water and are historically considered non-burrowing crayfish (Berrill & Chenoweth, 1982). Water levels dropped toward the middle of the summer possibly causing *C. carinirostris* to bury deeper; therefore, making them harder to collect.

The results of chi-square analysis between the ratio of species in the gut contents and the estimated population sizes in the field rejected my original hypothesis that Queensnakes will gorge themselves during the *O. obscurus* synchronous molts. The calculated X^2 (28.1) exceeded

the expected value for $\alpha = 0.05$ (3.84), suggesting that Queensnakes have strong preference for *C. carinirostris* as prey. This could possibly be explained by looking at the defense habits of the two crayfish in the creek. *Orconectes obscurus* is much faster than *C. carinirostris*, and when disturbed took off quickly and were difficult to capture. Being much slower, *C. carinirostris* seemed to duck their heads and back into a corner under the rock where they were found and were ready to defend themselves when disturbed. This would make *C. carinirostris* much easier prey than the faster *O. obscurus*. So it may not be that Queensnakes are actively pursuing one species over the other; they may just be eating whatever molted crayfish they can catch, and *C. carinirostris* seem to be easier to catch than *O. obscurus*. Future work on this study and Queensnakes should look at the different crayfish genera in the streams where Queensnakes occur. Perhaps these snakes need habitats rich with *Cambarus* species, which seem to be bulkier and slower and easier to catch, to really thrive.

Species	# collected	Ratio	Expected #
O.o.	120	3.63	22.75
C.c.	33	1	6.25

Table 1- The ratio of *O. obscurus* (O.o.) to *C. carinirostris* (C.c.) based on seining data. Using that ratio, the expected results were calculated from the 29 identifiable stomach contents.

Species	Observed	Expected	Obs-Exp	(O-E) ²	(O-E) ² /E
O.o.	11	22.75	-11.745	137.94	6.06
C.c.	18	6.25	11.745	137.94	22.05
Total	29	29	0	275.89	28.1

Table 2- Chi-square analysis. Calculated value was 28.1, which exceeded the tabular value of 3.84 suggesting a preference as *C. carinirostris* (C.c.) as prey.

Chapter 2

Body Mass Index (BMI) fluctuations of the Queensnake (*Regina septemvittata*) throughout a summer in a West Virginia stream

Abstract

The Queensnake is a dietary specialist, specializing in eating molted crayfish. In North Fork Short Creek, near Wheeling West Virginia, Ohio County, two species of crayfish occur with the Queensnake population. The most abundant species in the stream is *Orconectes obscurus* (Allegheny Crayfish). This species undergoes two synchronous molts twice summer, one at the end of May and the other at the end of July. The least common crayfish in the stream is the Rock Crayfish (*Cambarus carinirostris*). This species molts randomly throughout the summer. Queensnakes were collected throughout the summer of 2012, and a regression analyses was used to examine mass versus snout-vent length (SVL) for 54 juveniles and 47 males. Student residuals were obtained from the regression, which gave body mass index (BMI) for each individual Queensnake. Numbers less than zero were below average BMI, zero was average BMI, and above zero was an above average BMI. To see if there was a change in BMI in the Queensnake population as the summer progressed, an analysis of variance test (ANOVA) was used between the student residuals and the months the Queensnakes were captured. I hypothesized that the BMI for the Queensnake population would go up during the mass molts of *O. obscurus*, and drop in between the mass molts. A statistical significance was found between July-June and July-May for juvenile Queensnakes, with July having the higher BMI, and statistical significance was found between July-August and July-September, with July having the highest BMI.

Introduction

The body mass index (BMI) of animals refers to their energetic state. A higher BMI means that an animal has more energy reserves than an animal with a lower BMI (Schulte-Hostedde et al. 2005). More energy reserves allow an animal to fast longer during harsh times (Millar & Hickling 1990). To calculate BMI for snakes, body mass and snout vent length (SVL) need to be collected. Running a regression analysis between body mass and SVL will produce student residuals, which are used for determining the BMI for a particular snake. Student residuals will fall between a range of -2 and 2. Any residual below zero shows that the snake has below average BMI. A student residual of zero shows that the snake has an average BMI, and any residual above zero shows that that particular snake has an above average BMI.

A population of Queensnakes (*Regina septemvittata*) in Wheeling West Virginia was studied throughout the summer of 2012 to determine if the average BMI for this particular population fluctuated throughout the summer, based upon the phenology of molting crayfish. The most prevalent species of crayfish in the creek, *Orconectes obscurus* (Allegheny Crayfish), undergoes two mass synchronous molts each summer; once at the end of May and beginning of June and one at the end of July beginning of August. I hypothesized that the Queensnake population's average BMI would increase to above average at the end of each molt, and drop below average between the mass molts.

Methods

Study Site

This study was conducted on a 4 kilometer stretch of North Fork Short Creek in Wheeling West Virginia less than a mile from West Liberty University in Ohio County. The stream has been greatly disturbed from human presence. The stream flows through cow

pastures, neighborhoods, and a landfill. Species of riparian vegetation are variable throughout the stream. In the cow pasture vegetation consists of grasses. Farther downstream the banks are covered with Japanese knotweed. Farther downstream there is deciduous forest, with maples, oaks, and sycamore. Other areas of the stream do not have riparian vegetation but rock retaining walls. There is also an abundance of human made debris in and around the stream, including tarps, mats, wooden boards, plastic bags, bottles, and other trash. There is also a great diversity of wildlife in the stream. Multiple species of fishes, birds, amphibians, and two species of crayfish are prevalent.

Field Sampling

Queensnakes were collected by hand every other weekend from May 2012 to September 2012. I collected every other weekend to create as little stress as possible on the snakes in this area. The kilometer stretch was walked entirely twice a weekend. Once a snake was captured, basic morphometrics were taken on the snake, including snout-vent length (SVL), tail length (TL), cranial width (CW), cranial length (CL), and weight (g). Last, if the snake was large enough, a PIT tag was inserted into the animal's body cavity giving it a personal identification number for use in mark and recapture information. Snakes were released unharmed where they were collected.

The stream was sampled for crayfish using seine hauls once a month. I conducted 10 seine hauls in a riffle which is a shallow stretch of stream with high water flow, 10 in a run which is a straight stretch of stream that is deeper than a riffle and not as high flow, and 10 in a hole which is a deep pocket of water without with little or no water flow, for a total of 30 seine hauls in a month. Seining was conducted moving upstream so as to not collect crayfish from previous spots that I may have missed and caused them to float downstream. Once collected

crayfish were humanely euthanized in 70% ethanol (Loughman et al. 2009) and brought back to the lab to be identified to species. This helped give me an estimation of the ratio of population sizes in the creek.

Data Analysis

A regression analysis using the program SAS was conducted between mass and SVL, for juvenile Queensnakes and adult male Queensnakes. Female Queensnakes were omitted because they were gravid and do not eat while they are gravid. Student residuals were obtained from the regression analysis to give values to rate individual Queensnake body mass index (BMI). Once this was completed an analysis of variance test (ANOVA) was conducted between body mass conditions of individual juvenile and male Queensnakes and the month that they were caught, to determine if there was a change in average Queensnake BMI by month for this population of Queensnakes.

Results and Discussion

There was a statistical significance at the .05 level between the months of July-August and July-September for males (Figure 1). There was also a statistical significance at the .05 level for the juveniles between the months of July-May and July-June (Figure 2). For both males and juveniles July had the highest average BMI. This suggests two things. The first possibility is that Queensnakes rely on the second mass molt of *O. obscurus* to spike their BMI before entering their hibernacula. However, based on my dietary preference study, where I found Queensnakes prefer *C. carinirostris* over *O. obscurus*, I doubt this is the reason why BMI levels are so high compared to other months. In order to accept that hypothesis, I would need to see statistical significance between May and other months, with May and August having the highest

BMI average due to the timing of the synchronous molts of *O. obscurus*. Male Queensnakes did have the highest BMI average during May and July (Figure 1) but not high enough to be statistically significant. Juveniles had their highest BMI averages during July and August (Figure 2), which does not coincide with the synchronous molts of *O. obscurus*. *Orconectes obscurus* undergo their synchronous molts during the beginning of May and end of July. If Queensnakes were depending on these synchronous molts through the year, it would be expected that their BMI would be higher in the beginning of June and beginning of August. These results show Queensnakes do not depend on the *O. obscurus* synchronous molts for survival. These snakes have no preference over which species of crayfish they eat. However, *O. obscurus* are thinner, faster, and more apt to flee than *C. carinirostris*, which is bulkier, slower, and more likely to stay and defend itself. This might make *C. carinirostris* easier to capture, which could partly explain why the Queensnake does not depend on the synchronous molts of *O. obscurus*.

Conclusion

Based upon my dietary preference study and looking at Queensnakes' monthly BMI's throughout the summer, I conclude that the survival of the Queensnake is not dependent on the synchronous molts of *O. obscurus*. Instead, Queensnakes rely on the availability of prey throughout the summer, not just two specific molting events.

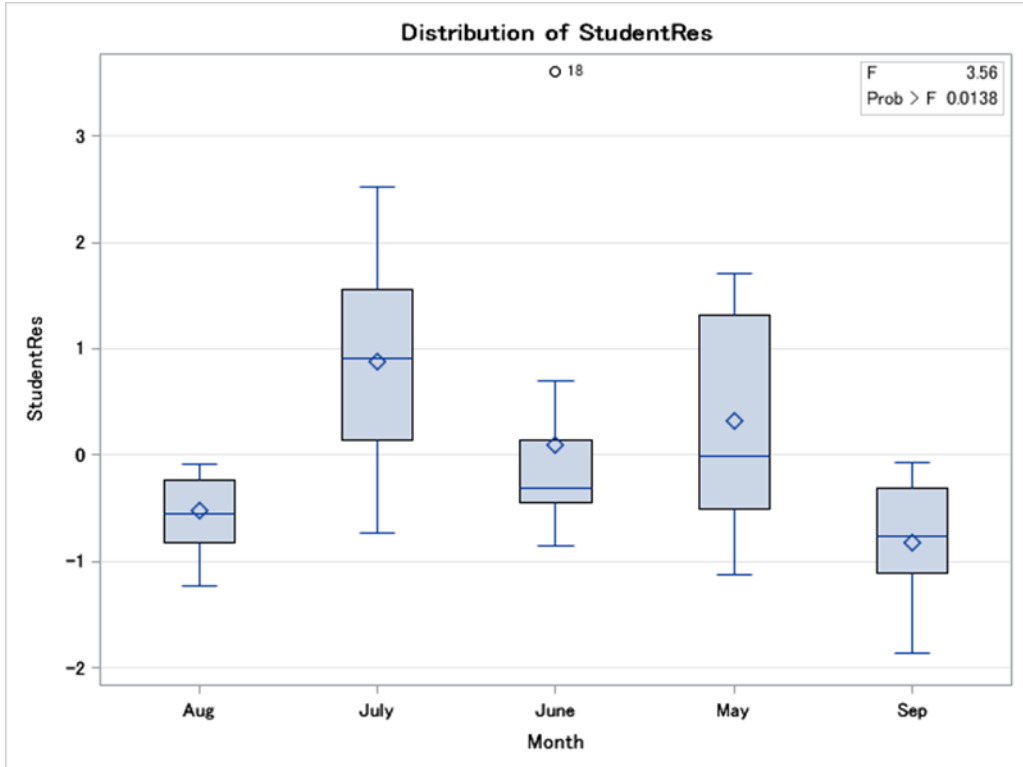


Figure 1- ANOVA between BMI for male Queensnakes and months Queensnakes were captured.

Comparisons significant at the 0.05 level are indicated by ***.				
Month Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
July – May	0.5632	-0.7713	1.8977	
July – June	0.7932	-0.5241	2.1105	
July – Aug	1.4111	0.0565	2.7656	***
July – Sep	1.7082	0.0921	3.3244	***
May – July	-0.5632	-1.8977	0.7713	
May – June	0.2300	-0.8384	1.2985	
May – Aug	0.8479	-0.2662	1.9620	
May – Sep	1.1451	-0.2756	2.5657	
June – July	-0.7932	-2.1105	0.5241	
June – May	-0.2300	-1.2985	0.8384	
June – Aug	0.6179	-0.4755	1.7113	
June – Sep	0.9150	-0.4895	2.3196	
Aug – July	-1.4111	-2.7656	-0.0565	***
Aug – May	-0.8479	-1.9620	0.2662	
Aug – June	-0.6179	-1.7113	0.4755	
Aug – Sep	0.2972	-1.1424	1.7367	
Sep – July	-1.7082	-3.3244	-0.0921	***
Sep – May	-1.1451	-2.5657	0.2756	
Sep – June	-0.9150	-2.3196	0.4895	
Sep – Aug	-0.2972	-1.7367	1.1424	

Table 3- Tukey honest significance test between BMI for male Queensnakes and months captured.

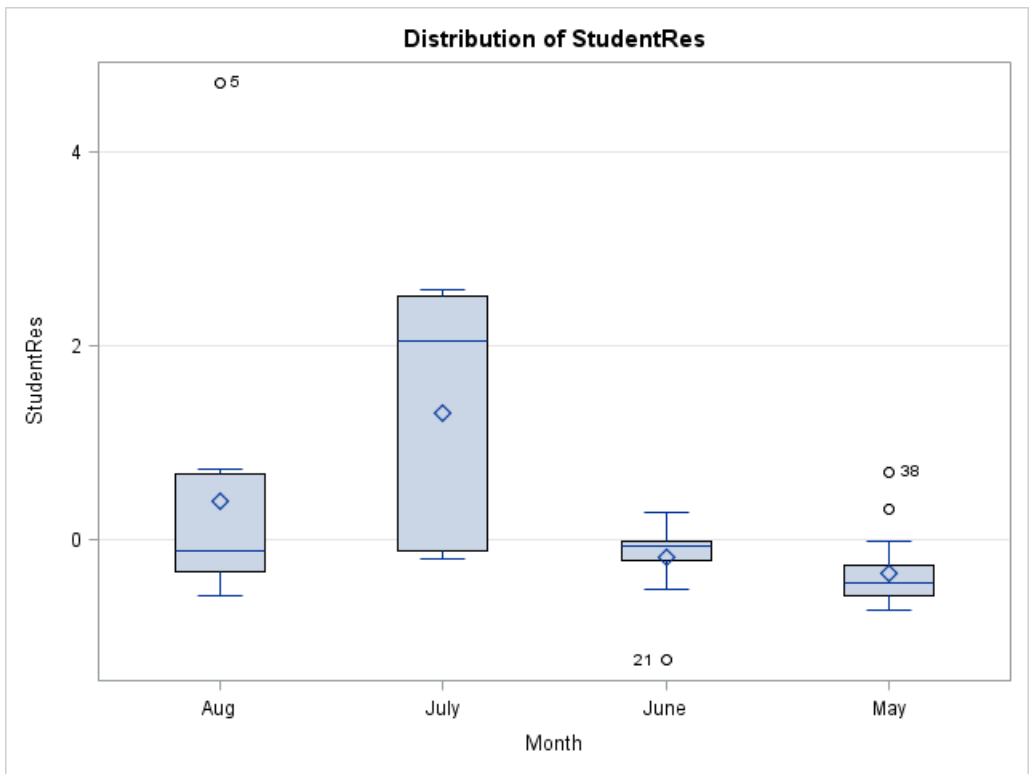


Figure 2- ANOVA between BMI for juvenile Queensnakes and months Queensnakes were captured.

Comparisons significant at the 0.05 level are indicated by ***.				
Month Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
July - Aug	0.9179	-0.1678	2.0037	
July - June	1.4936	0.3739	2.6134	***
July - May	1.6584	0.6587	2.6581	***
Aug - July	-0.9179	-2.0037	0.1678	
Aug - June	0.5757	-0.3731	1.5245	
Aug - May	0.7405	-0.0631	1.5441	
June - July	-1.4936	-2.6134	-0.3739	***
June - Aug	-0.5757	-1.5245	0.3731	
June - May	0.1648	-0.6842	1.0138	
May - July	-1.6584	-2.6581	-0.6587	***
May - Aug	-0.7405	-1.5441	0.0631	
May - June	-0.1648	-1.0138	0.6842	

Table 4- Tukey honest significance test between BMI for juvenile Queensnakes and months captured.

Chapter 3

Field Notes on North Fork Short Creek, Wheeling West Virginia

Cover Objects

Queensnakes were captured throughout the entire summer of 2012. However, areas where they were found changed as the summer progressed. From May to June, most snakes were captured under cover objects. Most cover objects were flat rocks in the sunlight. Others were under litter, such as old tires and black tarps. I believe the snakes choose these types of cover objects when the air temperature was cool. These types of cover objects could be used as a heat source because these animals are ectothermic and need heat to regulate their body temperature in cooler conditions. As the summer progresses, air temperature during July, August, and the beginning of September were really hot, and the spots on creek where snakes were always found became areas that did not have a lot of snakes. The new areas that I had high capture rates were then cover objects in the shade, or on the banks with shallow water underneath. Occasionally, I would find snakes basking in the Japanese knotweed, but even those basking areas were slightly shaded. However, there was one spot that yielded a snake every weekend. That was an old black tarp draped over a large boulder underneath a large sycamore tree. I believe this place remained a consistent location for high capture rates because the temperature probably did not fluctuate much throughout the year. This tarp was not as exposed to the sun as much as other areas along the creek bank. Based on my observations this summer, I would recommend that future researchers that want to attract Queensnakes place cover objects along the habitat that include thin black rubber mats, tarps, and other flat heat holding objects. I would recommend placing the cover objects in partial sun, because that should help in the capture of Queensnakes throughout the season.

Behavior

It has been assumed that Queensnakes are fragile, docile animals that when confronted and grabbed attempt to flee (Smith & Huheey, 1960). It has been stated that they rarely strike (Gibbons & Dorcas, 2004). I found the opposite. When the air temperature reached approximately 75 F°, adult Queensnakes, especially large females, would puff out their throats and strike repeatedly. I gave them opportunities to inflict a bite, but only one really latched on to my hand. It seems that these were mock strikes, similar to those of the Eastern Hognose Snake. The bite that I did receive I barely felt, and it did not break the skin.

Another behavioral “theory” that I believe I disproved is that when these snakes are stressed, they will regurgitate their stomach contents (Loughman Z. 2012, May 11, personal interview). The first weekend I captured 47 Queensnakes, and received stomach contents from none of these. I would hold them upside, gently squeeze them, shake their tail, and do everything I could to stress them out so that they would regurgitate. They would not do so. Quite a few of these snakes had what I believed was a bolus and was surprised that they did not regurgitate it. It took gentle massaging of the bolus to cause the Queensnakes to release their stomach contents. I believe this shows just how stress tolerant these snakes are.

Reproduction

Queensnakes drop their young at the end of August. In order to conduct a study of Queensnake reproduction, a minimum of 10 gravid females were needed. However, only one was able to be collected. Upon capture, the gravid female’s pre-birth weight was 83 grams. The gravid female was brought back to the lab and placed in a breathable container filled with mulch substrate. On August 20, 2012, at 21:15, she dropped 9 young. As soon as the neonates were born, they pushed through the amniotic sac and shed their skin. Average neonate weight was

2.9 grams, average snout-vent length (SVL) was 16.1 cm, average tail length (TL) was 4.9 cm, average cranial width (CW) was 4.1 mm, and the average cranial length (CL) was 8.4 mm. Post birth weight for the female was 38 grams, which is a 55% decrease in weight from the pre-birth weight of 83 grams.

Mark and Recapture

I captured 120 individual Queensnakes along the 4 km stretch of North Fork Short Creek. The 66 adult snakes had a PIT tag inserted underneath their dermis. PIT tags cause minimal discomfort to animals while being inserted (Gibbons & Andrews, 2004). Throughout the summer of 2012, I only had 3 recaptures. This suggests three possibilities. The first is that snakes were disturbed and left the area after being captured, the snakes were losing their PIT tags, or the Queensnake population at North Fork Short Creek is large.

Morphometric comparisons

Queensnakes are medium-sized snakes. The largest snake captured was 572 mm. The Queensnake in West Virginia showed sexual dimorphism in body size. Mature males were smaller in SVL, with an average SVL of 385 mm. Mature females were larger, with an average SVL of 499 mm. Males have longer tails than females relative to their SVL. The mean tail length for males is 31% of the SVL and 23% of total length, while mean tail length for females is 27% of SVL and 21% of total length.

Mature males in West Virginia were smaller, on average, compared to Pennsylvania (Hulse et al. 2001) and Virginia (Mitchell, 1994) Queensnakes (Table 7). Mature females in West Virginia had a larger average SVL compared to mature females in Pennsylvania and Virginia. However, Virginia had a much larger average tail length (TL) than West Virginia and Pennsylvania (Table 8). All mature females in West Virginia, Virginia, and Pennsylvania are

larger on average than mature males. But, females in Virginia and Pennsylvania had much larger average TL than the West Virginia mature males. Sexual dimorphism occurred in all populations, with males having longer tails than the females, but it seems sexual dimorphism may not occur between different populations, as the West Virginia mature males had much shorter TL than both Virginia and Pennsylvania females (Tables 7&8).

	SVL (mm)	TL (mm)	CL (mm)	CW (mm)	Weight (g)
Male n=47	385	118	12.4	6.7	29.8
Female n=17	499	133	14.7	8.4	66.1

Table 5- Average morphometric measurements for Queensnakes captured in WV

	SVL (mm)	TL (mm)	CL (mm)	CW (mm)	Weight (g)
Male n=47	246	71	5	4.3	58.6
Female n=17	152	80	4.4	6.1	65

Table 6- Ranges of the morphometric measurements for Queensnakes captured in WV

State	SVL (mm)	TL (mm)
WV	385	118
VA	412.3	269
PA	405.5	134.1

Table 7- Average SVL and TL for mature males in three states, WV, VA, and PA.

State	SVL (mm)	TL (mm)
WV	499	133
VA	440.9	245
PA	490.3	140.2

Table 8- Average SVL and TL for mature females in three states, WV, VA, and PA.

Other herpetofauna in the stream

In addition to having an abundance of Queensnakes, North Fork Short Creek has many other species of herpetofauna. The Common Watersnake (*Nerodia s. sipedon*) was just as abundant as the Queensnake. I helped capture over 100 individuals of this species. Common Watersnakes were probably abundant due to great food availability of the stream. There were

many fishes in the stream, the Common Watersnakes' favorite prey. Other species of snakes included a population of Eastern Gartersnakes (*Thamnophis s. sirtalis*), and Eastern Milksnakes (*Lampropeltis t. triangulum*).

North Fork Short Creek had other herpetofauna besides snakes. My assistants and I captured a few different species of testudines, including one juvenile Snapping Turtle (*Chelydra s. serpentina*), and a turtle that we could not identify. We also found a variety of amphibians, including Northern Green Frogs (*Lithobates clamitans*), American Bullfrogs (*Lithobates catesbeiana*), Pickerel Frogs (*Lithobates palustris*), Northern Two-lined Salamanders (*Eurycea bislineata*), Northern Dusky Salamanders (*Desmognathus fuscus*), and Long Tailed Salamanders (*Eurycea l. longicauda*).

Conclusion

For as much human impact that this stream has had, I believe it is healthy. Reptiles and amphibians are used worldwide as bio indicators, and the biodiversity and abundance of species in this stream is large and healthy. I would recommend this area to future students looking to conduct research on Queensnakes, Common Watersnakes, Eastern Gartersnakes, or any of the amphibians listed above.

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Appendix



Office of Research Integrity

April 9, 2013

Timothy J. Brust
616 Hal Greer Blvd. Apt. 1
Huntington, WV 25701

Dear Mr. Brust:

This letter is in response to the submitted thesis abstract titled “Dietary Preference of the Queensnake (*Regina septemvittata*).” After assessing the abstract it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocol #498. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
Director
Office of Research Integrity

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