Long-Term Study on the Population Ecology of Urban Queen Snakes (*Regina septemvittata*) in Central Virginia

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Abstract

As parts of the world are becoming more urbanized, species living in urban habitat fragments are more at risk of extirpation. We conducted, in an urban environment, a 12-year (2008-2019) mark/recapture study on a Queen snake (*Regina septemvittata*) population in Rock Castle Creek, Lynchburg, VA to determine population viability. Ninety-nine individual Queen snakes were marked to determine stable population estimates (range 3 - 43 snakes) and an annual survival rate of 52%. Our research found that despite the challenges of urban living, this population is stable and similar to rural Queen snake population studies. Certain life-history traits are advantageous to urban Queen snakes' survivability, but other traits make them extremely vulnerable should habitat quality change in the future.

Long-Term Study on the Population Ecology of Urban Queen snakes (*Regina septemvittata*) in Central Virginia

Queen snakes (*Regina septemvittata*) are common to central Virginia as well as to other eastern states and their general natural histories are well known (Burst, 2013; Ernst, 2003; Gibbons & Dorcas, 2004; Mitchell, 1994). Despite their commonality, the population ecology of Queen snakes has not been thoroughly researched and no work has been done recently on their growth rates or population estimates. The most comprehensive works to which we compared our data are one-year study by Branson and Baker (1974) in Kentucky on Queen snake growth and life history characterizations in a rural stream. The other study was conducted by Ernst (2003) from 1965-1967 in southeastern Pennsylvania in a rural creek of similar size to our study site. Our study on Queen snakes is unique because the study site is in an urban setting and was conducted for 12 consecutive years. Our goal was to assess the ability of Queen snakes to handle a habitat that biologists would consider less-than-optimal and assess what factors contribute to why or why not the population is stable.

Queen snakes are a docile, non-venomous species of water snake identifiable by a dark brown body with a pale striped pattern on their venter (Gibbons and Dorcas, 2003). Queen snakes are not territorial and are often found under the same rock or structure with Northern watersnakes (*Nerodia sipedon sipedon*), another species of water snake commonly found throughout our study site. Queen snakes are found in a specific habitat type favoring shallow streams with rock bottoms, banks that provide cover, and overhanging branches for basking (Branson & Baker, 1974; Ernst, 2003).

Queen snakes prey exclusively on freshly molted crayfish (Ernst & Barbour, 1989) and are considered the most dietary selective of all water snakes (Godley et al., 1984). This highly selective diet ties the snakes' survivability to healthy population levels of crayfish which could be negatively affected by human proximity or microplastics in an urban stream (Ernst & Barbour, 1989). Crayfish are excellent bioindicators of stream health (Burst, 2013) and a decline in Queen snakes could be due to declining crayfish populations affected by water quality or acid rain (Ernst & Barbour, 1989; Mitchell, 1994). Queen snake decline could also be seen without a crayfish decline if water contaminants are interfering with crayfish molting and thus decreasing the Queen snakes' prey base (Jackrel & Reinert, 2011).

Our study stream represents a habitat fragment created by urbanization. The number and diversity of species in such fragments can often come as a surprise since these areas can be very close to large developments and roads which is true of our study site. Life in an urban environment can pose a significant threat to snake species since their populations can be at a higher risk of decline due to human predation as well as higher levels of pollution associated with urban areas. And yet, populations of wildlife, even snakes, can display a high level of adaptability and persist in these small pockets of habitat.

According to Mullin and Seigel (2009), the rate of papers done on snake natural history has been declining. Our research helps fill the literature gap by studying the population ecology (population estimates, densities, survival rates, growth rates, etc.) of Queen snakes in an urban environment over a long time period. Results will reveal the health of the stream and this snake species' ability to adapt to urban life as nearby human development has increased over the years of the study.

Materials and Methods

Study Area

Our study site was a 593 m portion of Rock Castle Creek, approximately 5 m wide, ranging from 6 - 60 cm deep. Rock Castle Creek is a urban, second-order stream that eventually flows into the James River in Lynchburg, VA (Fig. 1). At the onset of the study, the areas upstream and downstream of our study site in Rock Castle Creek were searched for snakes. The area upstream was above the source of water coming from the watershed associated with a nearby university property that adds a significant volume of water, particularly after a rainstorm event. The creek upstream of this culvert diminishes in volume and enters a series of concrete pipes. No snakes were ever found in this area. Downstream of the study site the creek enters three large metal pipes and becomes steep-sided with a lower velocity. No snakes were found there either.

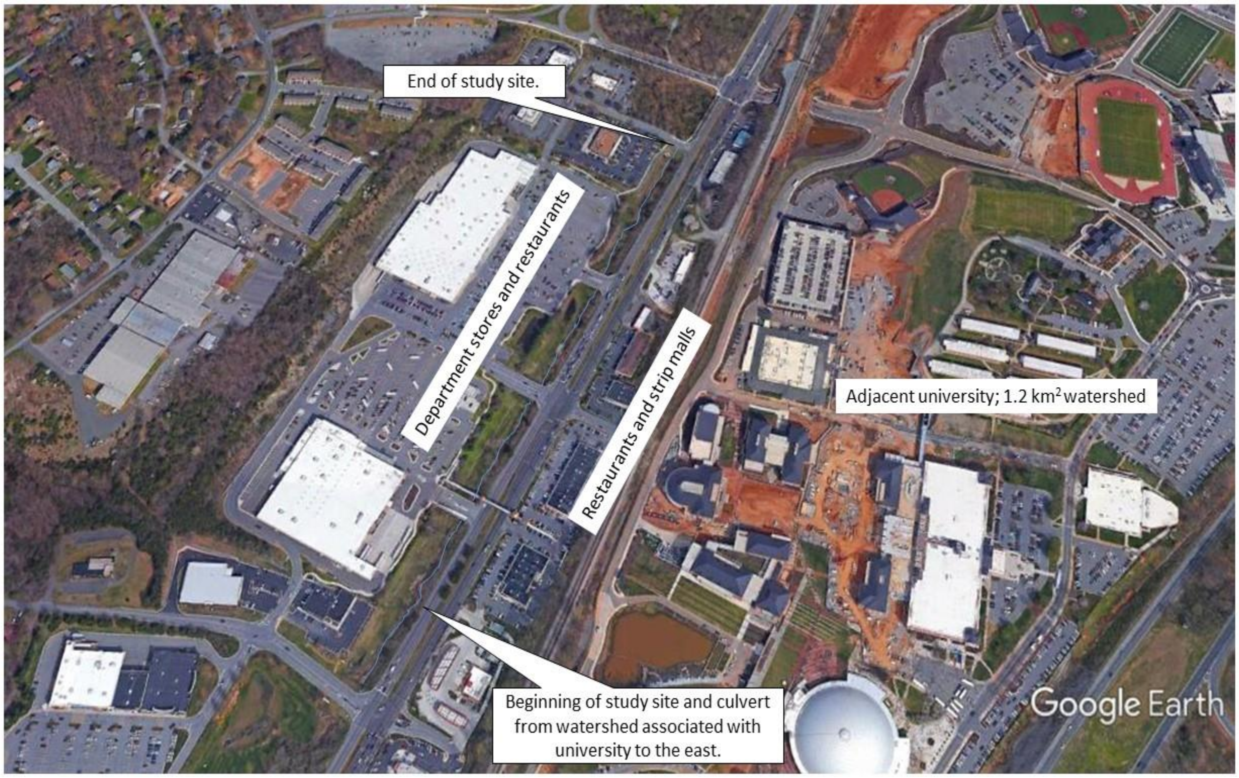

Figure 1. Arial picture of the urban study site and surrounding development. Rock Castle Creek is represented by the thin blue line to the left of the main road paralleling the university. Original image accessed on 1 August 2019 from https://www.google.com/earth.

On the south-east bank, Rock Castle Creek is bordered by Wards Rd. (main thoroughfare next to the university) approximately 8 m from the water's edge at the top of a steep bank. The north-west bank is a more gradual incline and is buffered by an approximately 40 m strip of grass before turning into major department store parking lots. The banks of the creek range from being steep for about 0.5-1 m to being very gradual and covered with flat stones. All along the creek bank, shrubs and small to medium-sized trees make excellent snake-basking spots with branches overhanging the water. The creek is mostly covered and shady, especially during the summer, but there are gaps in the trees at various places along the study site. Where the banks were gradual and in sunlight openings, we placed our black landscape fabric structures (tarp-like material) to facilitate finding snakes (Burst, 2013).

Three road bridges cross Rock Castle Creek in our study area with support bases of loose rocks (riprap) cascading down into the creek to form crevices for snake hiding spots and hibernacula. There is ample evidence of human traffic under the bridges as seen by footprints, graffiti, and people occasionally camped out. The stream is also cluttered with trash including but not limited to, bottles, plastic bags, metal pipes, toys, and food garbage.

Stream Characterization Methods

In the spring of 2019, we measured each riffle, run, and pool in our study area. For each pool, run, and riffle habitat we took three depth, velocity, substrate, and crayfish density measurements. The three locations within a habitat category where the measurements were taken were determined randomly. Our substrate categories were defined as sand, pebbles (rocks less than 6 cm), rocks larger than 6 cm with space under them (crayfish habitable), and large, immovable rocks. The substrate measurements were executed using a 0.5x0.5 m quadrat with 36 hazard points to determine percentages of different substrates within the square half meter. Crayfish were sampled using the 0.5x0.5 m quadrat placed in the water and then surrounded on three sides by a hand seine on the downstream side. We overturned and shuffled the rocks within the quadrat to force any crayfish to float/swim back into the seine where we could easily count them.

For all data, means are followed by plus or minus one standard deviation. Where appropriate, we evaluated the assumption of normality for datasets. When our data failed to meet this assumption of normal distribution, we used non-parametric methods to analyze the data. For all statistical tests, $\alpha = 0.05$.

Field Collection Methods

A mark/recapture study in Rock Castle Creek was performed from 1 May 2008 to 5 June 2019. Collectors averaged eight trips (range 2-16) to the study site per year during the active season (April to October) and were typically conducted on sunny days. Throughout the study site, we set up 7-10 structures made of black landscape fabric folded over large rocks to provide warm places for the snakes to crawl up under (Fig. 2). Additional rocks were placed on the landscape fabric to secure the structure when stream volume increased following rainfall. Our most concentrated collection efforts were in the spring and fall months (April-May and August-September) checking the structures at least once a week.

Snakes were captured by hand under structures or opportunistically (basking or swimming snakes) as we walked between structures along the study site. We marked captured snakes using 8-12 mm PIT [(Passive Integrated Transponder) AVID Identification Systems, Inc., Norco, California, USA and BIOMARK, Boise, Idaho, USA] tags. All neonates and juveniles too small to PIT tag were marked with a red sharpie which provided a temporary mark until the snake shed. Snakes that were recaptured within a year were not remeasured until they were caught during the next active season. All snakes were immediately released after processing at the same location captured.

Figure 2. Representative snake structure constructed of black landscape fabric folded over and held down by rocks for snakes to crawl into or under.

Every captured snake of any species was PIT tagged (when large enough), measured for snout-vent length (SVL) using a measuring tape and nine different-sized plastic restraining tubes (Midwest Tongs, Greenwood, Missouri, USA), sexed visually if large enough [snakes greater than 30 cm SVL], location recorded using Universal Transverse Mercator (UTM) coordinates (GPSMAP® 60CSx, Garmin, Olathe, Kansas, USA), and habitat was described unless the snake was captured under one of our tarp structures. We recorded any food regurgitated during handling and any physical abnormalities such as blisters which could be evidence of Snake Fungal Disease (SFD) caused by the fungus *Ophidiomyces ophiodiicola* (Guthrie, et al. 2016;

Lorch, et al. 2016). Methods used were approved by the Institutional Animal Care and Use Committee (IACUC 34.180215: Urban Watersnake Ecology).

Age Cohort Data Analysis

The smallest snakes we caught (16 - 17 cm SVL) were too small to tag with the older style AVID PIT tags (8-12 mm tags that were thicker than our newer 8 mm BIOMARK tags) used in the spring of 2008 through the summer of 2018. Until the fall of 2018, we could only PIT tag juvenile snakes 25 cm SVL and longer. But starting in August of 2018, we tagged juvenile snakes as small as 17 cm SVL using the thinner 8 mm BIOMARK PIT tags.

We created cohorts to determine age (one-year-olds, two-year-olds, and 3+ year-olds) from snake size using a histogram of the SVLs of all captured snakes including the recaptured SVLs. The clearest cohort groupings based on size (SVL) came from the snakes caught in the April and May months across years from which we drew our age cohorts (Wood & Duellman, 1950). Mean SVL for each age cohort was calculated from all snakes captured in April/May including snakes too small to PIT tag and all recaptures. The Mann-Whitney U test was used to compare the median SVL lengths across years of all individual female and male snakes that fell into the 3+ year-old age cohort. The mean SVL for male and female snakes was calculated from all snakes large enough to be confidently sexed (25 cm for females, 30 cm for males) and including SVL values from recaptures between years.

A chi-square test was used to compare the number of adults (24 - 36+ cm) to juveniles ($\leq$ 24 cm) snakes across years. We calculated the expected values for juveniles to be 16.4% of the total snakes found for each year based on the mean juvenile to total snakes proportion for all years. We used data only from snakes caught in April/May across years and did not include recaptures except possibly when recounting juvenile snakes which were only marked with a sharpie pen. We also ran a chi-square analysis on males versus females across all years against the expected values of a 1:1 sex ratio (Branson & Baker, 1974; Ernst, 2003).

Growth Rate Data Analysis

We calculated individual growth rates (cm/day) for each recaptured PIT tagged snake but only using the days for the duration of these snakes' active season which was from April 1st to September 30th totaling 183 days. Mean monthly growth rate for the one-year-old snakes was calculated for all snakes in that age cohort from one representative spring month (May) to the next (June; 30 days) since we couldn't analyze individual growth rates between years because these snakes were too small to PIT tag and sharpie marks are gone by the next year. Mean growth rates for two-year-old and 3+ year-old snakes for both females and males were calculated from individual recaptured snakes in their respective age cohort whether they were caught in consecutive years or had gap years between captures.

Growth rates from PIT tagged snakes were analyzed using analysis of covariance (ANCOVA) with initial capture SVL as the covariate and sex as a dummy variable (0 for females and 1 for males). Interactions between sex and SVL were assessed first for significance before fitting a reduced model that did not include the interaction term. We also ran correlations between year and annual growth rates for PIT tagged snakes (separately for male and female snakes) to test for stability of these rates over time. There are no other literature studies to which we could compare our Rock Castle snake growth/day rates (Gibbons & Dorcas, 2004). Other studies used percent change (Branson & Baker, 1974; Raney & Roecker, 1947; Wood & Duellman, 1950); therefore, we converted our growth rates to mean percent changes in growth to make comparisons between Rock Castle snake and Queen snakes from other areas. Percent changes from the one-year-old to two-year-old cohort and two-year-old to 3+ year-old cohort were calculated from the increase of mean SVL between cohorts.

Movement Measurements

We used the UTM coordinates to determine linear movement of snakes between captures. All movement between locations was calculated as straight-line distances since Rock Castle Creek is a fairly linear creek (see Fig. 1). Special attention was given to any snakes that moved to a new structure and returned to the original since this suggests a home range.

Population Estimate Data Analysis

Survival and recapture rates along with 95% confidence intervals were calculated for PIT tagged snakes over 25 cm SVL using Program MARK (http://warnercnr.colostate.edu/~gwhite/mark/mark.htm). Twenty-five cm SVL was the minimum size for the snakes used in our population estimates because earlier in the study, when we used the thicker AVID PIT tags, this was the smallest SVL snake we could safely tag. We started with the most general model to estimate these rates, which included time-varying survival and recapture rates. We then used the Akaike's information criteria to select the most parsimonious model (Cooch, E., and G. White. 2001. Using MARK – A gentle introduction, 2nd edition. http://www.phidot.org/software/mark/docs/book/).

Using the data from 2008-2019, we calculated population estimates for all PIT tagged snakes for each year using the Jolly-Seber method (Krebs, 1999; Program JOLLY (https://www.mbr-pwrc.usgs.gov/software/jolly.html). Linear density was calculated from the mean Jolly-Seber population estimates across years divided by the distance from the first Queen snake captured to the last Queen snake captured (525 m) along our study site. To compare densities to other studies, we calculated the linear density for the Ernst (2002) study by multiplying his population estimate by the percent of snakes found along his study stretch (Big Chickies Creek) and divided that study stretch length. We also calculated the number of unique (sharpie-marked juveniles were all considered unique) Queen snakes caught in 2018-2019 and them divided by 525 m to mimic Ernst's (2003) capture-number linear density.

Annual population growth rates (λ) derived from ratios of population estimates were regressed against population size. Carrying capacity was then determined from this regression model by setting λ equal to one. A simple population model, with properties dependent upon the slope of λ with population size and the equilibrium population density, was used to characterize the population data.

To evaluate whether population size could be estimated with relative density measures, we evaluated several different measures. For the first relative density measure, we calculated average relative density by dividing the total number of snakes caught in a given year by the number of trips that year. Another relative density measure was the maximum number of snakes captured for any single trip within a year. A third measure was the median number of snakes caught each year. And lastly, we took out all trips that only had two snakes or less captured (possibly due to the tarps being covered with silt from recent rainfall events which made the tarps unproductive for those trips) and then divided total snakes caught by the number of trips in that year. Each of these relative density measures was regressed against population size.

Results

Stream Characterization

Rock Castle Creek is a shallow, low gradient stream with a substrate dominated by pebbles (Table 1). Our study site was comprised of three pools, eight runs, and seven riffles. The site was dominated by runs, making up 325 m of the total length. Riffles and pools totaled to 210 m and 58 m respectively (Table 1). The mean number of crayfish increased moving from pools (0.33 crayfish/0.5 m^2) to runs (1.08 crayfish/0.5 m^2) to riffles (1.7 crayfish/0.5 m^2).

Table 1. *Habitat and crayfish characterization for the Rock Castle Creek study site.*

Habitat	Pools	Runs	Riffles
Number in stream	3	8	7
Total Length of Habitat (m)	58	325	210
Depth (cm)	33.8 $\pm$ 15.0	23.1 $\pm$ 12.5	14.0 $\pm$ 5.2
Velocity (m/s)	0.05 $\pm$ 0.07	0.11 $\pm$ 0.13	0.26 $\pm$ 1.9
Percent Pebbles (%)	60.8 $\pm$ 41.7	72.3 $\pm$ 30.9	82.7 $\pm$ 17.7
Crayfish (amount/0.5 m^2)	0.33 $\pm$ 0.5	1.08 $\pm$ 1.7	1.7 $\pm$ 1.9

Snake Community

We collected records for 408 captured snakes (including records for recaptured snakes) from 2008 to 2019 in and along the banks of Rock Castle Creek. Our records include the following number of unique snakes per species: Four *Thamnophis sirtalis* (45-50 cm SVL), two *Elaphe alleganiensis* (89-109 cm SVL), 109 *Nerodia sipedon sipedon* (17-72 cm SVL, mean SVL 28.6 cm, maximum SVL for males 54 cm and females 72 cm, three recaptures between years), and 168 *Regina septemvittata* (16-51 cm SVL, mean SVL 33.3 cm, maximum SVL for males 47 cm and females 51 cm, 28 recaptures between years). No overt, external symptoms of Snake Fungal Disease, as described by Lorch, et al. (2016), were detected in any species of snake we caught at Rock Castle Creek.

Queen Snake Population Characterization

Over the 12-year data collection period, we had 260 Queen snake captures/recaptures mostly found under rocks or other debris near the creek or in our tarp structures (89.2%). Although harder to successfully capture, the other snakes were found either basking in branches (6.9%) or swimming/foraging (3.8%). Of the 260 Queen snake captures, we identified 99 individual Queen snakes that were large enough to PIT tag. Seventy-one of these PIT tagged snakes were caught only one time and 28 were caught multiple times. Of the 28 recaptures, 15 were caught two times in consecutive years, eight were caught two times with gaps between years, and five were caught three times in consecutive years (Fig. 3). Sex ratio did not significantly deviate from a 1:1 ratio across the years of the study (x^2 = 10.5, df = 10, P = 0.399).

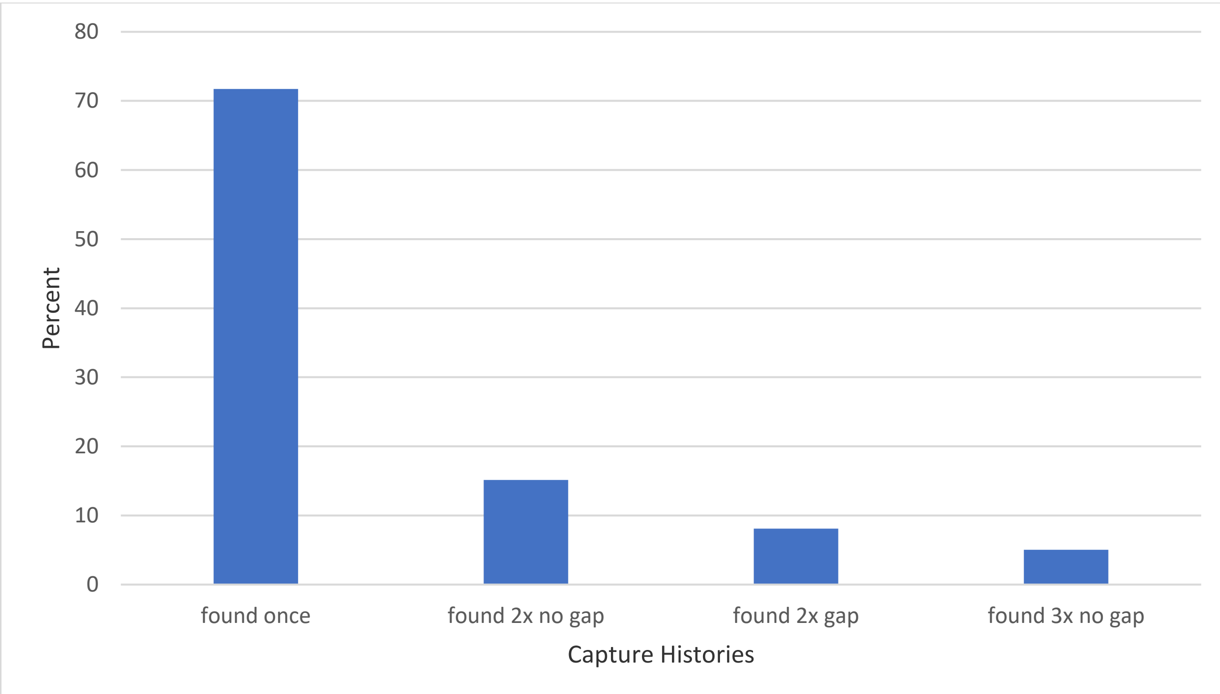

Figure 3. Capture histories for individual Queen snakes that were large enough to PIT tag.

Age Cohorts and Growth Rates

We determined three age cohorts using the SVLs from snakes captured in April and May across all years (Fig. 4). The cohort labeled "1" were the juveniles born the previous summer/fall based on typical Queen snake birth size (Branson & Baker, 1974; Ernst, 2003) and ranged from 16 - 23 cm SVL with a mean of 18.8 cm SVL. The cohort labeled "2" were snakes born the prior summer and ranged from 24 - 36 cm SVL with a mean of 31.7 cm SVL. Once year three was reached (SVL over 36 cm, mean 41.4 cm SVL), annual age cohorts could not be determined since growth slowed and cohorts blended together (labeled "3+"). The median for female 3+

cohort Queen snakes was significantly longer (43 cm SVL) than the median for the male 3+ cohort snakes (39 cm SVL; Mann-Whitney U = 305.5, n =70, P < .001). The female SVL mean was 42.6 cm (range 25 - 51) and the male SVL mean was 39.7 (range 30 - 47) and these data are comparable to Queen snake sizes reported in other studies (Table 2).

Table 2. *Comparison of means and ranges for male and female Queen snake SVLs in different study locations.*

Queen Snake Study	Location	Male SVL Range (cm)	Female SVL Range (cm)	Mean Male SVL (cm)	Mean Female SVL (cm)
Branson & Baker (1974)	KY	29.9 - 64.2	22.9 - 69.5	45.0	50.9
Ernst (2003)	PA	34.5 – 48	36.0 - 66.9	39.6	48.2
Burst (2013)	WV		Maximum - 57.2	38.5	49.9
Trauth (1991)	AR	Maximum - 44.3	Maximum - 52.8		
Mitchell (1994)	VA	30.5 - 52.2	31.8 - 55.5	41.2	44.1
Triplehorn (1949)	Ohio		Maximum - 71.6[a]		
Reichenbach (unpublished data)	Ohio	47 – 50	47 - 71	47.8	59.8
Rock Castle Creek (This study)	VA	30 – 47	25 - 51	39.7	42.6

[a] Represents one record Queen snake measurement.

The oldest Queen snake, based upon mark/recapture data, was at least seven years old. When this male snake was first caught, its SVL was 31 cm, which placed it in the two-year-old cohort (Fig. 4). Five years later it was recaptured and its SVL was then 40 cm. The adult (two-year-old and 3+ year-old cohorts) to juvenile (one-year-old cohort) ratio did not deviate significantly across years from the average ratio of 16.4% juveniles (x^2 = 13.6, df = 8, P = 0.092).

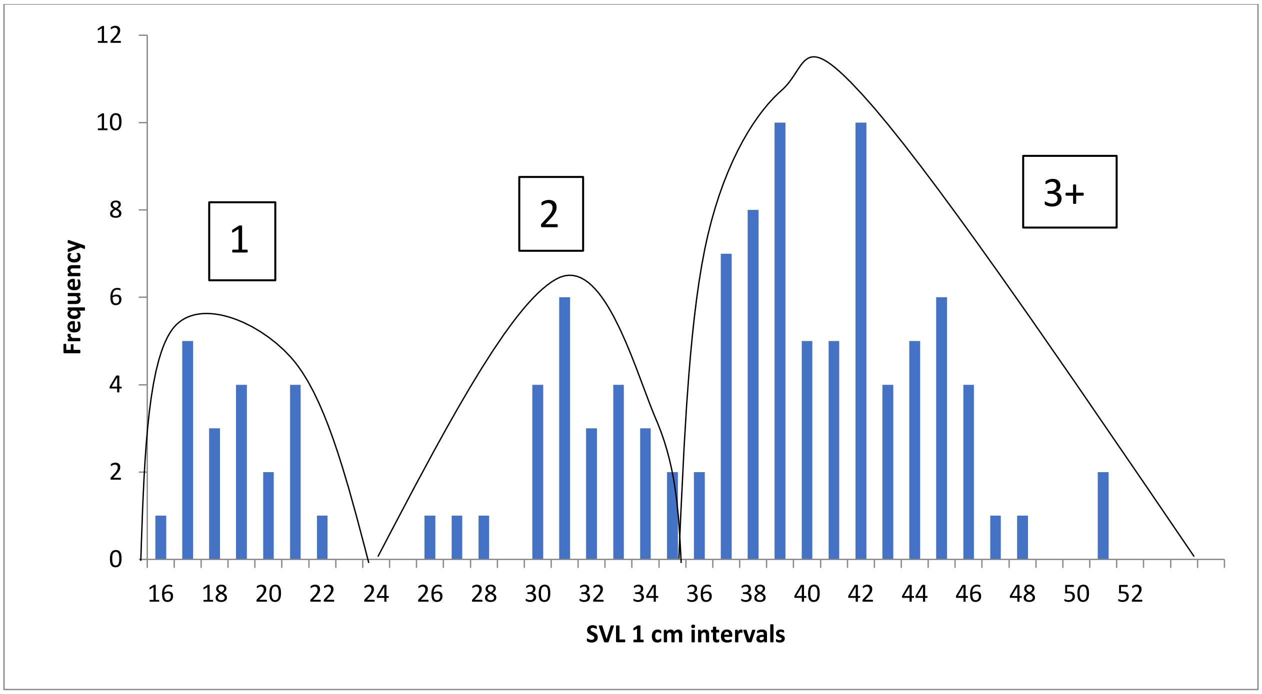

Figure 4. Snout-vent lengths (SVL) for snakes found in April and May across years used to determine age cohorts. “1” represents one-year-old, “2” - two-year-old, and “3+” - three-year-old and older snakes.

The mean growth rate for one-year-old snakes was 0.17 cm/day. We also were able to assess growth rates for 14 recaptured male and 19 recaptured female queen snakes in the two-year-old and 3+ year-old cohorts. The mean annual growth rate for female two-year-old snakes was 0.040 $\pm$ 0.01 cm/day and for female 3+ year-old snakes was 0.013 $\pm$ 0.01 cm/day. For male two-year-old snakes, the mean annual growth rate was 0.025 $\pm$ 0.01 cm/day and for male 3+ year-old snakes the growth rate was 0.0073 $\pm$ 0.008 cm/day. Growth rates declined with increasing SVL for both sexes and females grew significantly faster than males (Growth rate, mm/d) = 0.105 – 0.00224 (SVL cm) – 0.01046 (sex, where male = 1 and female = 0) (r^2 = 0.54, $F_{2,23}$ = 18.0, P<0.001, t_{SVL} = 5.82, P<0.001, t_{sex} = 2.85, P = 0.008). In the above analysis there was no interaction between sex and SVL (P = 0.36; Fig. 5). There was no temporal trend in growth rates over the years of the study for males (r = 0.453, t = 1.437, P = 0.189). However, female growth rates showed a slight positive trend over the years of the study (r = 0.534, t = 2.36, P = 0.033). Rock Castle Creek snakes increased in length from their first year of life to their second year by 68.6% and from their second year of life to their third and subsequent years by 30.6%.

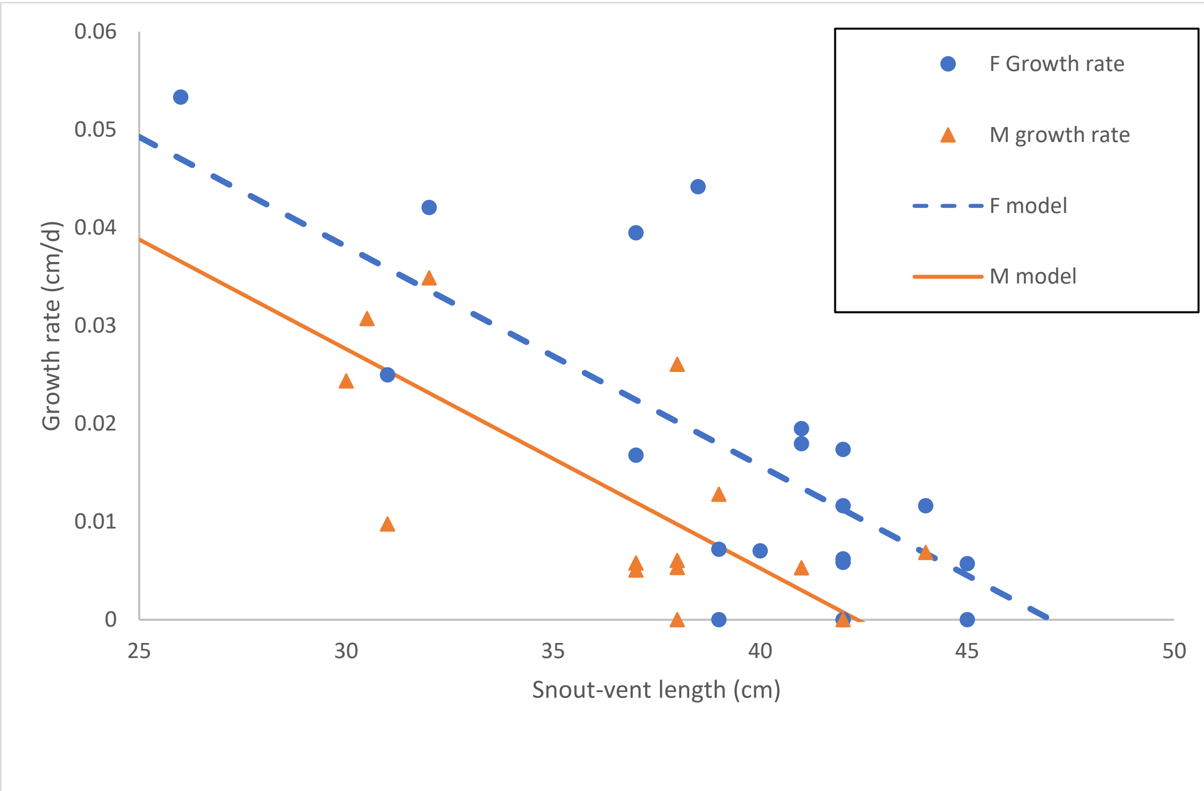

Figure 5. Growth rates (cm/d) of recaptured Queen snakes including ANCOVA model results. Dashed line and dots represent female growth rates and solid line and triangles represent male growth rates.

Movement

Within a year, few of the recaptured snakes were found under a different structure than where they were initially caught (30%) but movement between structures was more common for snakes between years (56.2%; Table 3). The mean linear distance moved by snakes in the same year was 10 m and the mean distance moved by snakes in between years was 14 m (Table 3).

Only two snakes, both recaptured between years, moved away from a location and back again, indicative of a home range. One female traveled 7 m to a new structure and then back and another male snake traveled the maximum distance recorded of 210 m (Table 3) to a new structure and back again.

Table 3. *Movements within and between years for recaptured Queen snakes.*

	Movement Within a Year (m)	**Movement Between Years (m)**
Number	60	32
Minimum	2	2
Maximum	80	210
Mean	10	14
Percent that Moved from Initial Location	30.0%	56.2%

Population Estimates

The model that held survival and recapture rate constant had the greatest amount of support based upon the Akaike Information Criterion (AICc). The next nearest model, that allowed recapture rates to vary with time, had a delta AICc of 9.1. The annual survival rate for Queen snakes was 0.52 (95% confidence intervals 0.40 - 0.63) and the annual recapture probability was 0.43 (95% confidence intervals 0.27 - 0.61). Population estimates for 2009-2019 showed an oscillating, but steady, population size averaging 24 snakes (range 3 - 43 snakes) over the 12 years of the study (Fig. 6).

Mean linear density across years for the Queen snakes was 0.046 snakes/m (Range 0.0062 - 0.0833 snakes/m). Lambda (λ) decreased linearly as population size increased in a density-dependent manner (λ= 3.34 - 0.0807 * Population Estimate; r^2 = 0.47, n = 10, F = 7.04, P = 0.029; Fig. 7). The carrying capacity for our study site was calculated at 29 snakes. A simple population model, with properties dependent upon the slope of λ with population size and the equilibrium population density, followed the oscillations seen in the annual population estimates (Fig. 8): $N_{t+1} = (1-Bz_i) N_t$ where N_{t+1} = population size next year, B = slope for lambda vs population size or 0.080731, $z_i=N_t-N_{eq}$ with $N_{eq}=29$, N_t = current population (Krebs, 2001). Since $B * N_{eq}$ is between 2 to 2.57 (0.0807*29=2.34) the population model exhibits stable limit cycles indefinitely (Krebs 2001). In addition, a relative density measure was found that could be used to predict population size with less collection effort. The maximum number of snakes caught on any single field trip for each year was significantly related to population size (Population estimate = 3.17 + 3.69(Maximum number of snakes collected on any single trip within a year); $r^2 = 0.57$, n = 11, F = 12.02, d.f. = 1,9, P = 0.007). All other relative density measures such as mean or median number of snakes collected per field trip were not significantly related to population size (P>0.05).

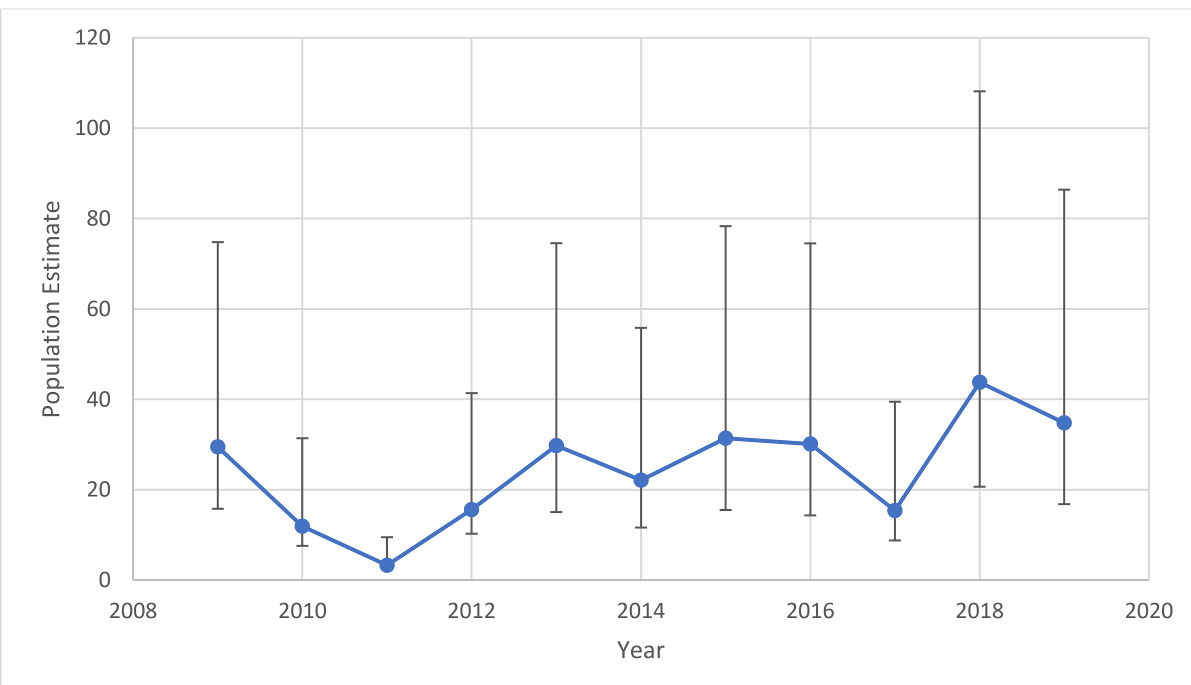

Figure 6. Population estimates for Queen snakes using the Jolly-Seber method from 2009 to 2019 with 95% confidence intervals.

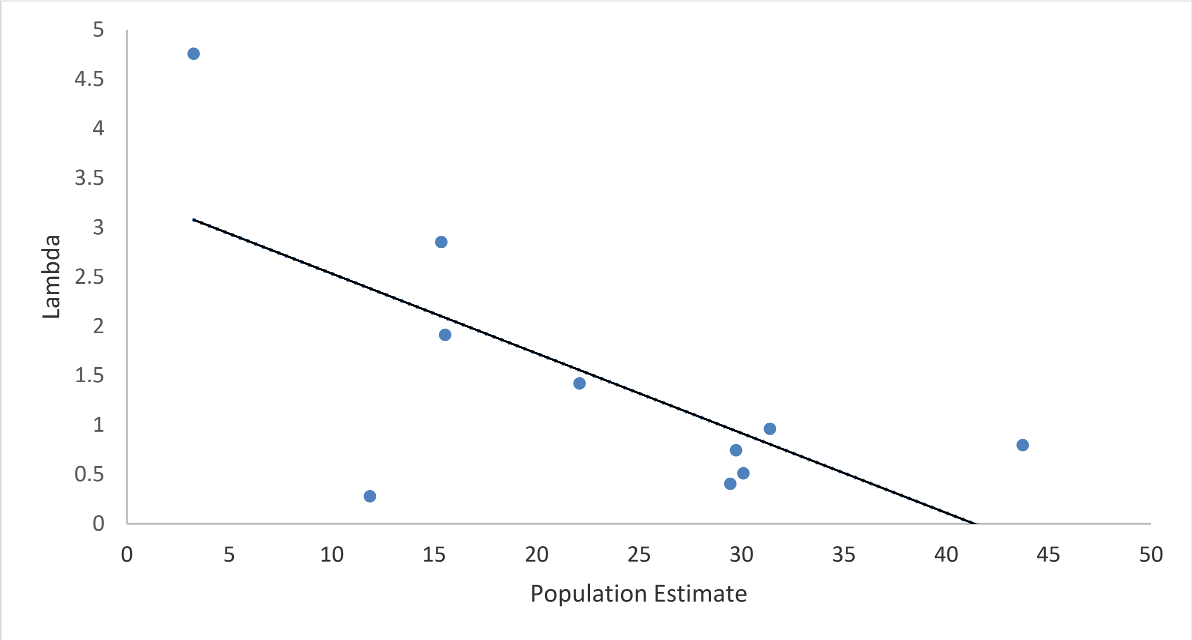

Figure 7. Lambda values relative to population estimates from 2009-2019.

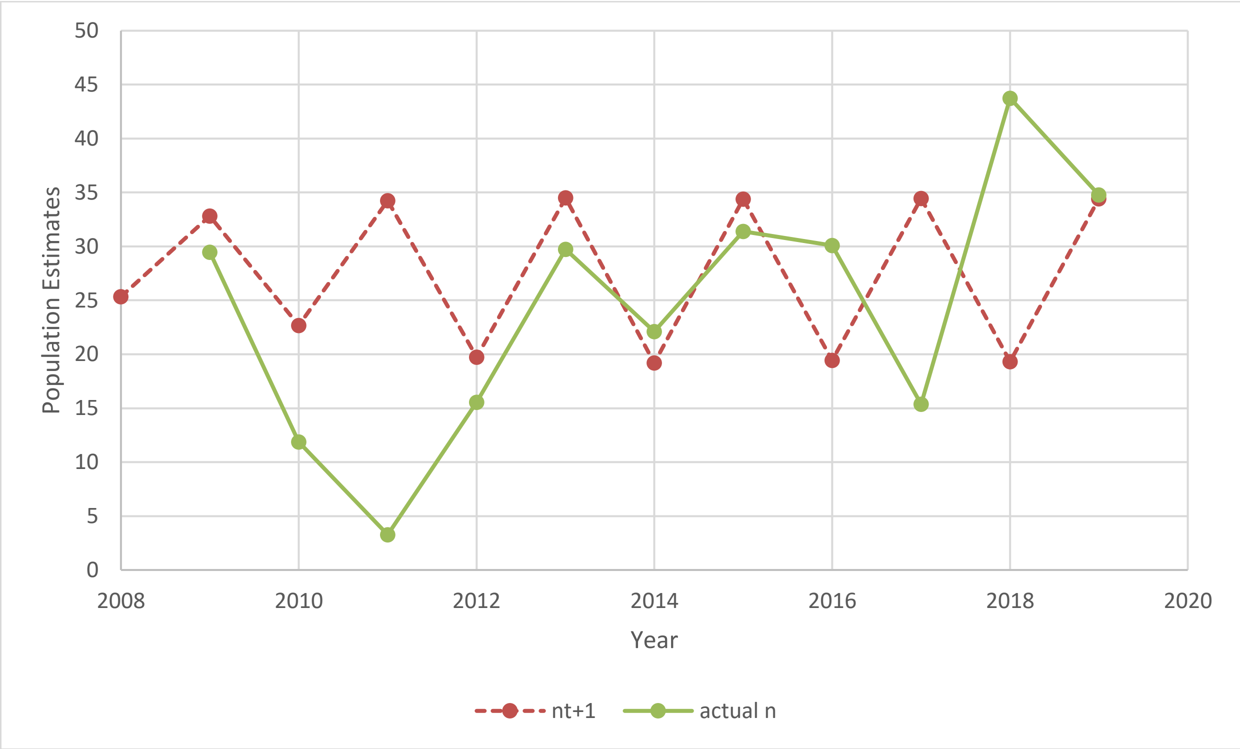

Figure 8. Population model, with properties dependent upon the slope of λ with population size and the equilibrium population density (dashed line) compared to the actual population estimates (solid line).

Discussion

Since Queen snakes only prey on molted crayfish, a healthy crayfish population is necessary for a healthy queen snake population. Rock Castle Creek has an abundance of crayfish (*Cambarus spp.*) partly due to the ideal environment of riffles found throughout 201 m of the study site (Table 1). Branson and Baker (1974) described the optimal habitat for Queen snakes as a small to medium, shallow stream, with a moderate current, and rocky banks and bottom. This description can be applied to Rock Castle Creek which also had adequate cover for snakes on the banks such as rocks and thick shrubs. Plenty of branches were overhanging the creek which also contributed to optimal nature of this creek for Queen snakes with 5.8% basking captures compared to 1.8% captures of basking snakes by Branson and Baker (1974). The reason we did not find any snakes above or below our study site is that Queen snakes require a specific habitat, described above, unlike other generalist water snakes. Although in an urban environment, we concluded that this section of Rock Castle Creek currently has the necessary elements for quality Queen snake habitat.

Our capture records are dominated by Queen snakes and Northern watersnakes since our structures were right next to the water and the other non-aquatic snake species captured were most likely present incidentally. Since these two snake species do not compete for food or predate each other (Gibbons & Dorcas, 2004) it was not uncommon to find Queen and Northern watersnakes under the same rock or structure together like other studies have found (Branson & Baker, 1974). We are unsure why there were so few recaptures between years of Northern watersnakes compared to the greater number of recaptures of Queen snakes. It may be that Northern watersnakes are more terrestrial than Queen snakes and venture further from water (Newcomer, 1974) possibly inviting a greater risk of human predation and vehicle fatalities.

Snake Fungal Disease has already been found in Northern watersnakes in Virginia (Guthrie et.al, 2016) but fortunately, no external SFD symptoms were detected on any species of snake found at Rock Castle Creek.

Female Queen snakes in Rock Castle Creek have longer SVLs, on average, than males which displays sexual dimorphism that others have noted (Branson & Baker, 1974; Ernst, 2003; Raney & Roecker, 1947; Wood, 1950). Our Queen snake SVLs (female mean = 42.6 cm, male mean = 39.7 cm) are similar to other Queen snake SVLs in other locations (Table 2). Ernst (2003) in rural Pennsylvania and Burst (2013) in an urban West Virginia stream reported male SVL means within 2 cm of our mean. However, their female means were approximately 7 cm longer than Rock Castle Creek's female means. Branson & Baker (1974) in rural Kentucky and Reichenbach's unpublished data from Lake Erie, Ohio reported SVL means and maximums greater than 8 cm longer than ours. We must be cautious contributing Rock Castle Creek's lower SVL values to the health of the stream. Other factors can influence mean size such as differences in mature SVL definitions between studies. Other influencing factors could include larger habitats (likely larger prey) like Lake Erie versus our 5 m wide creek or different environmental factors in the different locations of the Queen snake's geographic range. Our male and female maximums are similar to a small sample study (n = 7) in Arkansas (Trauth, 1991) as well as to Mitchell's work (1994) in Virginia where he reported male and female means less than 2 cm different from Rock Castle Creek's (Table 2).

Rock Castle Creek capture history percentages (Fig. 3) show that the majority of snakes were only found once which was most likely due to the species' low survival rate (0.52) and recapture probability (0.43). No survival or recapture probabilities are available in the literature to which we can compare; therefore, we compared our values to those from another common, stream-dwelling snake, the Northern watersnake. Recapture probability of juvenile Northern watersnakes (0.37) during the summer months was close to our annual rate (0.43; Cecala, 2010). In the same study (Cecala, 2010), the survival rate was much higher (0.87) than ours (0.52). The difference is mainly because their rates were monthly over just one summer while Rock Castle Creek's was an annual survival rate. Northern watersnakes had an annual survival rate closer to ours of 0.63 (95% confidence intervals 0.396 - 0.798) in Ohio and Michigan (Roe et al, 2013). Higher survival rates in Northern watersnakes in rural environments compared to Queen snakes could be due to the larger body size of Northern watersnakes.

Although this study did not focus on reproductive success or behavior directly, juveniles present in the population was used as an acceptable measure of successful reproduction (Roe et al., 2013). Our juvenile to adult ratio did not change significantly over the years of the study which suggests reproduction was stable. Our juvenile to adult ratio (0.20:1) was smaller than the ratio of Queen snakes in southwestern PA (46.3:1) where adults were defined as males greater than 20 cm SVL and females greater than 34 cm SVL (Ernst, 2003).

Our percent changes in growth (one-year-old to two-year-old snakes = 68.6%, two-year-old to 3+ year-old snakes = 30.6%) were comparable to other studies' mean percent change in Queen snakes. In Southwestern Ohio, Wood & Duellman (1950) reported approximately a 50% increase in length among Queen snakes from the neonates born in late summer compared to their next year of life. Neonates (n = 17) born in September in Western New York increased in length by 78.4% by the time they were a year older (n = 21; Raney & Roecker, 1947). Branson and Baker (1974) state that yearlings increased in mean length by 75% when compared to neonates and that second-year snakes increased by 45% from yearlings. Discrepancies in percent increase between these studies and our data were most likely due to different age class SVL cutoffs between papers and our percent change calculations do not include neonate data. All studies reported substantially decreased growth rates after passing the second full year of life which match our findings (Branson & Baker, 1974; Raney & Roecker, 1947; Wood & Duellman, 1950). The Rock Castle snake population reflects the same 1:1 sex ratio as in other rural locations (Branson & Baker, 1974; Ernst, 2003). These differ from Burst's (2013) findings of a greater male to female ratio of 2.76:1.

All the snakes we captured were within a meter of the water similar to Branson & Baker's (1974) capture radius of 3 m from the water. As with rural Kentucky snakes, we found most of our snakes under rocks/structures (Branson & Baker, 1974). The rural snakes of Branson & Baker's study (1974) also display a small home range with 11 of the 13 recaptures' movement less than 30.5 m (Range 3-137 m) from initial capture within a year. The other two recaptures had moved over 30.5 m away from their original location. However, our structures possibly created artificial homing since we concentrated our search to primarily those 7-10 locations.

In other rural areas, Queen snakes have a greater movement of 101 m linearly and 6 m away from the water (Ernst, 2003). Neither Branson & Baker (1974) nor Ernst (2003) recorded distances between years which helps explain the record distance traveled (210 m) by our one male snake between years. As in Ernst's study (2003), all Rock Castle Creek snake movement was in a linear fashion along the stream. More than half of rural snakes in southeastern Pennsylvania moved between captures within a year (Ernst, 2003) compared to 30% of snakes moving in Rock Castle Creek. Queen snakes in Rock Castle Creek do not need to move far since foraging areas, hibernacula, and thermoregulation/basking sites are all found within the stream and its immediate banks. No Queen snakes were ever found dead in the store parking lots or on the Wards Rd. adjacent to the creek.

Population estimates were stable across the 12 years. Collector effort varied between years which may have affected some of the population estimates especially in the years 2010-2012 when collection efforts were minimal (Fig. 6). We only collected at the study site five times in 2010, two times in 2011, and five again in 2012 compared to an average of eight times for the other years. The predictions from the population model follow the oscillations seen in our population estimates from 2009-2019 (Fig. 8). This indicates that the Rock Castle Queen snake population should continue to hold steady in the future barring any extreme changes to the water quality or crayfish populations. There is limited information on population estimates for Queen snakes with which to compare our data. Linear densities per year (range 0.0062 – 0.083 snakes/m) at Rock Castle Creek were lower than for the two rural study sites (1.61 snakes/m, Ernst, 2003 and 0.18 - 0.26 snakes/m, Branson & Baker, 1974). Linear density calculated from 2018-2019 data of actual snake captures was lower (0.072 snakes/meter) than Ernst's (2003, 0.593 snakes/meter).

Overall, the comparison of our Queen snakes to those from other studies (Branson & Baker, 1974; Burst, 2013; Ernst, 2003; Raney & Reocker, 1947; Wood & Duellman, 1950) indicate that our urban population is similar to those from rural areas and that Rock Castle Creek's population is stable. The similarities of our findings to the other major studies include body length and sex ratios while our linear densities were lower than those from other studies. Stability in our population is evident in the population estimates, reproductive effort and growth rates (marginal increase in females) despite our population being in an urban environment.

This long-term Queen snake population study showing stability contrasted with population declines noted worldwide in 65% of the snake species examined using long-term studies (Reading et al, 2010). Most declining species were characterized by having low fecundity, small home ranges, sit-and-wait foraging strategies, and living in an area exposed to increased human pressure (Reading et al., 2010; Reed & Shine, 2002). The other 35% of species studied showed stability and were characterized by having high fecundity, large home ranges, and were active foragers usually found in areas protected from human pressure. The Queen snake has some characteristics of those snake species that were stable – active forager (Reading et al., 2010), early maturation, and shorter generation length (Webb et al., 2002). But the Queen snake also has similar characteristics to species that have shown declines over time – small home range, occurring in an area with increasing human influence (Reading et al., 2010), habitat specialization, and prey specialization (Reed & Shine, 2002; Foufopoulos & Ives, 1999).

This stability we see in our urban population may be explained by the Queen snake's life history characteristics. Limited movement, habitat specificity, diet specialization, and small size make this species both ideally suited to an urban environment while at the same time having the potential to make this species vulnerable to extirpation. Limited movement/small home range keep Queen snakes away from roads and parking lots, decreasing road mortality which has been shown to negatively affect *Elape obsoleta* populations (Row et al., 2007). The Queen snake's small size and close ties to a creek environment make it less conspicuous to people camped out under the bridges or wandering through who may try to kill them. But this habitat specialization and small home range nature also make the Queen snake intolerant to any major events (pollution, siltation event, crayfish declines) in this small stretch of stream (Burst, 2013). Queen snakes are not adaptable to this kind of habitat quality change just like other snake species that have shown decline in long-term studies (Reading et al., 2010).

The creek has shown degradation and a decrease in water quality over time (2008-2016) according to research done by the University of Lynchburg. Declines in two indices (Index of

Biological Integrity and Ephemeroptera Plecoptera Trichoptera Index) and increases in conductivity and phosphorus levels from 2011-2016 have occurred as development of the surrounding land has continued (https://www.lynchburg.edu/academics/academic-community-centers/center-for-water-quality/stream-ecology-management/blackwater-creek-management). If environmental degradation increases to where crayfish numbers decline, this local population of Queen snakes could likely be extirpated (Roe et al., 2013). This has been seen before in a Queen snake population in southeastern PA (Ernst, 2003) when a flood swept over the dam into the snake habitat below and changes to the dam diminished the water flow, reducing the crayfish population. After this event, Northern watersnakes, a more generalized watersnake species (Gibbons & Dorcas, 2010), repopulated the habitat but no Queen snakes were ever found at the dam or in that creek again (Ernst, 2003).

Overall, it seems that Queen snakes are not sensitive to habitat loss through fragmentation as evidenced by this healthy urban population in a habitat fragment whereas a decrease in habitat *quality* could be disastrous for this population. Currently, though, this population is stable, despite being in a less than pristine environment, and this long-term study adds another example of a stable snake population to the ones examined by Reading et al. (2010).

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