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Baseline survey and habitat analysis of aquatic salamanders in the Pigeon River, North Carolina

Nikki J. Maxwell
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To the Graduate Council:

I am submitting herewith a thesis written by Nikki J. Maxwell entitled "Baseline survey and habitat analysis of aquatic salamanders in the Pigeon River, North Carolina." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

J. Larry Wilson, Major Professor

We have read this thesis and recommend its acceptance:

Matthew J. Gray, Lisa I. Muller

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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BASELINE SURVEY AND HABITAT ANALYSIS OF AQUATIC SALAMANDERS IN THE PIGEON RIVER, NORTH CAROLINA

A Thesis Presented for
the Master of Science Degree
The University of Tennessee, Knoxville

Nikki J. Maxwell
December 2009

DEDICATION

This thesis is dedicated to my parents, Doug and Jeanie Maxwell.
I owe my success in every aspect of life to your love and support.
Thank you for always encouraging me and embracing my love for all things science.
I love you both very much!

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ABSTRACT

The Pigeon River was severely impacted beginning in the early 1900s by a paper mill located in Canton, North Carolina. The mill discharged chemical byproducts into the Pigeon River until 1992 when the paper mill modified their processes. As a result, water quality improved but the status of salamander species in the Pigeon River was unknown. Worldwide amphibian declines over the last 20 years have drawn attention to the need for more research and a better understanding of species-specific habitat relationships. There is concern about amphibian population declines because amphibians are critical to the balance of ecosystems and are considered exceptional indicators of environmental health.

The objectives of this study were: 1) to conduct a baseline survey of salamander species composition in the Pigeon River watershed, 2) to determine if salamander populations differ above and below the Canton paper mill, and 3) to attempt to explain variance in salamander abundance, richness and diversity by comparing water quality and substrate characteristics among streams. Eight stations were examined on the Pigeon River, with four stations located above the paper mill and four stations below. We also chose three stations on each of four tributaries, Big Creek, Fines Creek, Jonathan Creek and Richland Creek. Snorkel surveys were completed in the summer of 2009. Five of eight species of stream salamanders were found that historically existed in Haywood County, NC: Eastern hellbender, Blue Ridge two-lined salamander, shovel-nosed salamander, black-bellied salamander and spring salamander. No salamanders were found in the main channel of the Pigeon River below the mill. Eastern hellbenders and Blue Ridge two-lined salamanders preferred substrates consisting of rubble and avoided bedrock. Percent rubble was the only variable retained in substrate models and was positively related to salamander abundance, richness and diversity. Conductivity, salinity, and water temperature were higher in the Pigeon River below the mill than at all other sites. Salamander abundance was explained by dissolved oxygen, pH, and stream width in water quality models. The results of this study suggest salamander abundance was negatively associated with the Pigeon River below the mill because of poor water quality and not habitat availability.

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CHAPTER 1

INTRODUCTION

Worldwide amphibian declines over the last 20 years have drawn attention to the need for more research and a better understanding of species-specific habitat relationships (Blaustein and Wake 1990; Houlahan et al. 2000). According to the International Union for Conservation of Nature (IUCN et al. 2008), 32% of amphibians are threatened or extinct (~1900 species) compared to only 24% of mammals and 12% of birds. At least 42% of amphibian populations are declining; therefore, the number of threatened or extinct animals is expected to rise (IUCN et al. 2008). Scientists have identified several factors contributing to their decline such as habitat degradation, disease, global warming, increased ultraviolet-B (UV-B) exposure, introduced species, and chemical pollution (Semlitsch 2000; Kiesecker et al. 2001; Lannoo 2005; Relyea 2005). There is concern about amphibian population declines because amphibians are critical to the balance of ecosystems and are considered exceptional indicators of environmental health (Blaustein 1994). Amphibians generally begin their lives in water as eggs and larvae and then transform to terrestrial adults. The transformation period includes structural (i.e., loss of gills) and biochemical changes that may make amphibians much more susceptible to chemical contamination (Bernake and Kohler 2008). In fact, amphibians may be the most vulnerable of all vertebrate groups to anthropogenic chemical contact (Vos et al. 2000). Amphibian eggs are especially susceptible because they lack shells or protective membranes to protect themselves from chemical contamination (Blaustein 2007).

Pigeon River History

The Pigeon River was historically a clear, cool river flowing through Haywood County, North Carolina, into the French Broad River in East Tennessee. In 1908, Champion International (now Blue Ridge Paper Products) began operation of a paper mill located directly on the Pigeon River in Canton, North Carolina. The mill used a chlorine bleaching process and discharged bleached kraft mill effluents (BKME) into the Pigeon River (Bartlett 1995). These effluents included dioxins, furans, tannins, and lignins (Bartlett 1995). The Pigeon River was thought to have had over 95 native fish species, but in 1964 the North Carolina Wildlife Resources Commission discovered the Pigeon River to be so polluted that a 32-kilometer segment in the upper portion sustained no fish (Messer 1964; Etnier and Saylor 2001). As a result of public pressure and Environmental Protection Agency regulations, the paper mill modernized their system in 1992 by replacing the chlorine bleaching process with a chlorine dioxide and oxygen delignification system. This modification reduced the use of bleaching chemicals and reduced the amount of effluents entering the Pigeon River. Since 1992, pollutants have decreased and water quality has improved (Bartlett 1995). Index of Biotic Integrity (IBI) metrics such as number of native, darter, and intolerant fish species are used to assess the health of rivers. Pigeon River IBI scores were 38 or less out of a possible 60 until 1993. Scores have improved significantly to a value of 54 in 2007 (Tennessee Valley Authority 2007). The enhancement of water quality has encouraged fish and benthic invertebrates to re-colonize, but current status of amphibians is unknown.

Paper Mill Effluents

Dioxins are an assemblage of synthetic organic chemicals containing structurally related chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibensofurans (CDFs; Denton and Arnwine 2002). The EPA has determined that dioxins are toxic at low concentrations and may cause cancer in humans (U.S. EPA 2000). Dioxins are known to bioaccumulate in the fatty tissue of fish and are assumed to do so in salamanders. Salamanders have permeable skin to allow for respiration and osmoregulation. This feature makes aquatic salamanders highly susceptible to the uptake of harmful chemicals in the water and sediment. Salamanders bioaccumulate such chemicals during the respiration process, by ingesting contaminated prey, and through cutaneous absorptions (Snodgrass et al. 2008).

Fish exposed to paper mill effluents containing endocrine disrupting chemicals have shown reproductive abnormalities, such as “suppressed male and female reproduction, reduced gonad size, and more variable fecundity” (Gagnon et al. 1995). Munkittrick et al. (1998) found paper mill effluents in Canada delayed sexual maturity in the longnose sucker (*Catostomus catostomus*) and lake whitefish (*Coregonus clupeaformis*).

Aquatic Salamanders

Salamander species diversity in the southeastern United States is considered greater than anywhere else in the world, with representatives from over 75 species of salamanders. In the southern Appalachian Mountains, three to five species of *Desmognathus* salamanders may form a community in both terrestrial and aquatic habitats (Duellman and Trueb 1994). These mountains contain high levels of salamander diversity and abundanceThese species utilize rocks for cover and as a place to deposit their eggs (Petranka 1998; Dodd 2004).

This project was the first step in attempting to understand the ecological role of aquatic salamanders in the Pigeon River watershed. This study had three objectives: 1) identify aquatic salamander species living in the Pigeon River watershed, 2) determine whether salamander abundance and diversity differed between the Pigeon River above the mill, Pigeon River below the mill, and its tributaries, and 3) identify habitat and water chemistry variables that could predict species occurrence within the Pigeon River watershed.

CHAPTER 2

METHODS

Study Area

The Pigeon River is a 5^{th} order tributary to the Tennessee River that begins in the Blue Ridge Mountains of North Carolina and flows into East Tennessee where it joins the French Broad River near Newport. River substrates include cobble, boulder, some exposed bedrock and a mixture of sand and organic matter in slower accumulation areas (Summers et al. 1991). The present study was conducted only on the Pigeon River and tributaries in Haywood County, North Carolina. Eight sampling sites were chosen on the Pigeon River with four above the paper mill (PRA) and four below the paper mill (PRB); all sites were upstream of Walters Dam (Figure 1). Sites were sampled on the Pigeon River based on availability of salamander habitats, such as riffles and an abundance of boulders (Petranka 1998; Nickerson and Krysko 2003). Three sites were chosen on each of four Pigeon River tributaries: Big Creek, Fines Creek, Jonathan Creek, and Richland Creek (Figure 1). Tributary sites were chosen systematically so the first site was at or as near as possible to the mouth of the tributary where it entered the Pigeon River. The second and third tributary sites were chosen by walking upstream from the mouth to the next available riffle habitat. All stations were separated by at least 30 meters of stream, utilized different riffles, and were separated by pools to establish independence. Eastern hellbenders rarely move 10-20 meters from their home site so 30 meters between sites was considered adequate (Peterson and Wilkinson 1996).

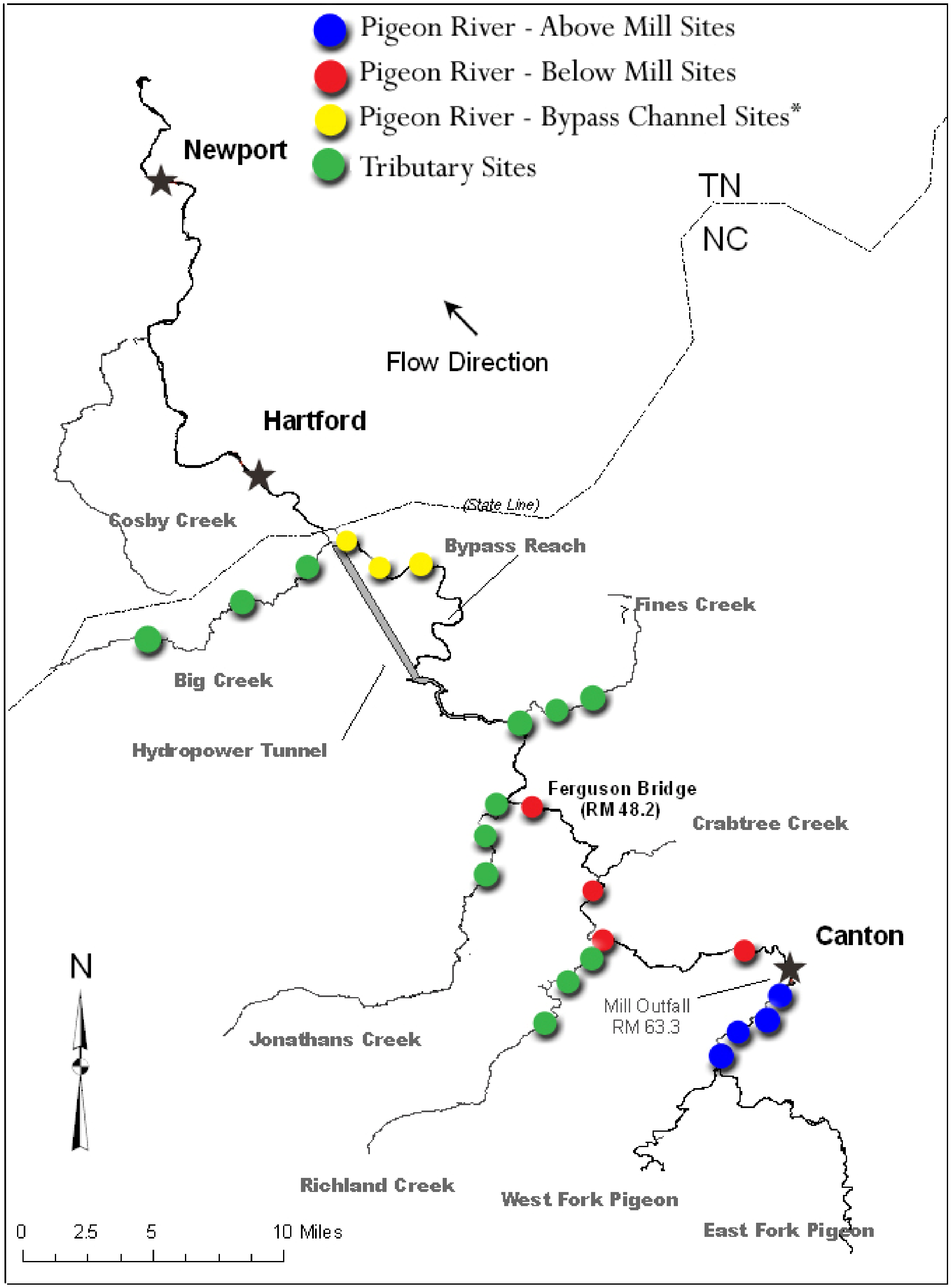

Figure 1. Map of study sites on the Pigeon River and its tributaries, North Carolina. *Bypass channel sites were searched in 2008 and not included in this study.

Sampling Methods

Historically, electroshocking was considered the most effective technique to collect large aquatic salamanders, such as the hellbender (*Cryptobranchus alleganiensis)* but this method has since been disregarded (Williams et al. 1981). Electroshocking salamanders has been shown to cause forced movement, immobilization, and unconsciousness and is discouraged because of the high potential of harming eggs and interfering with immune function (Reynolds 1983; Nickerson et al. 2002).

Field Sampling

For this study, a “station” constituted a 30-meter portion of the stream to be sampled. Each station was sampled once between May and August 2009 which corresponds with breeding seasons in Plethodontid salamanders in the southern United States (Petranka 1998). Snorkel surveys were conducted with at least three people lined up across the stream at equal distances from each other, but no more than 3.0 meters apart (Nickerson and Krysko 2003). If the stream was too wide to be adequately sampled in one pass, it was divided into sections and multiple passes were made. Snorkelers moved upstream in a zigzag pattern to ensure all rocks of suitable size (≥ 25 cm) were overturned (Figure 2). Log rollers were used for rocks that were too large or too heavy to turn by hand. Stream widths in meters and GPS locations were recorded at the beginning (0 meters) and end (30 meters) of each station with a Garmin GPSMAP 76Cx (Olathe, KS). Turbidity was recorded with a LaMotte 2020 Turbidimeter (LaMotte Company, Chestertown, MD) before snorkelers entered water to ensure they did not alter the

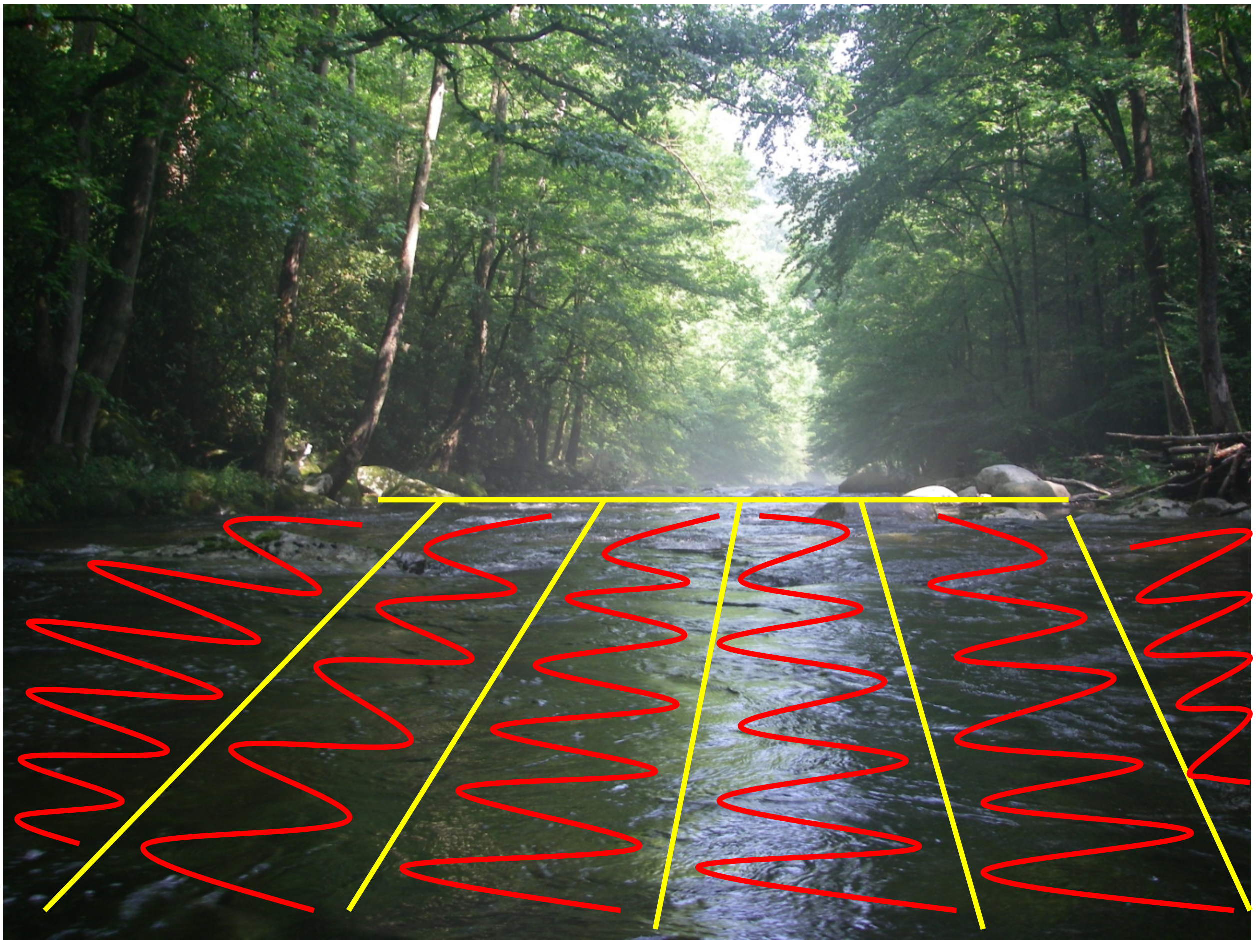

Figure 2. Schematic demonstrating snorkel technique in Big Creek, North Carolina, with yellow lines representing sections and red lines representing the snorkeler's search path upstream.

reading by stirring up sediment. Conductivity and salinity were measured with a PCSTestr 35 Multi-Parameter tester (Eutech Instruments, Vernon Hills, IL). Dissolved oxygen was measured with a YSI Model 550A [Yellow Springs Instrument (YSI), Yellow Springs, OH]. Temperature and pH were measured with a YSI Model 60. Velocity was measured with a Type AA Current Meter (Rickly Hydrological Company, Columbus, OH). All water quality measurements were obtained at the uppermost riffle at each station. Salinity and pH meters malfunctioned on two occasions so those variables could not be measured.

Snorkelers carried small plastic bottles with uniquely numbered markers made of fishing bobbers, a 1.0-ounce weighted lead sinker, and 30-pound fishing line (Figure 3). The fishing line allowed the snorkelers to easily carry the bottle around their upper arm while leaving their hands free to turn rocks. When a salamander was spotted, the snorkeler would use the bottle to capture the salamander. The bottle was pulled out of the water, inverted, and placed back in the water next to the salamander. The bottle was then tilted so that the action of the water rushing in would bring the salamander in as well. The snorkeler would place the marker under the rock so the salamander could be returned to its original location and also identify the rock to be measured. Latitude and longitude were obtained at each salamander location with a Garmin GPSMAP 76CX.

Salamanders were identified to species at the station, except for salamanders of the genus *Desmognathus*. *Desmognathus* salamanders were euthanized in MS-222 (tricaine methanesulfonate), preserved in ethanol, and taken to the University of Tennessee – Knoxville for identification. Salamanders were weighed with a Pesola spring scale (100, 300, or 600 gram

Figure 3. Snorkeler demonstrating the use of capture devices - small plastic bottles with uniquely numbered markers made of fishing bobbers, a 1.0-ounce weighted lead sinker, and 30-pound fishing line.

capacity) and measured for total length and snout-vent length using a small, modified tape measure inside a plastic case (Figure 4). This was found to be most effective for measuring very small individuals. Larger salamanders were measured with a tape measure. Salamanders were held in a water-filled plastic container until the station was completely sampled. At the conclusion of the survey, individuals were released at the location of capture.

Habitat Sampling

The longest length and greatest width were obtained for each rock that was found to have a salamander beneath. A habitat grid was constructed using ¾-inch PVC pipe and ¼-inch plastic tubing to create a 1.0-meter grid with eight equal-sized squares (Figure 5). This grid was used to measure substrate at each salamander location in the Pigeon River above the mill and Jonathan Creek by laying the center of the grid on the center of the rock under which the salamander was captured with the sides of the grid parallel to stream flow. The percentage of each type of substrate (rubble, cobble, silt, sand and bedrock) was recorded for each of the eight squares. The percentages were averaged for each salamander specific location to determine habitat used by salamanders. The grid was also used to determine overall station substrate composition. Within each station, the grid was used to measure habitat at three locations: one at the beginning of the station (0 meters), one at the middle of the station (15 meters), and one at the end of the station (30 meters). The three samples from each station were then averaged to create an overall station substrate composition, which was classified as available salamander habitat.

Figure 4. Measuring the length of a small salamander in a plastic case modified with a measuring tape.

Figure 5. PVC grid used to determine substrate composition.

Statistical Analyses

Response variables for the analyses included amphibian community variables, stream substrate and structure characteristics, and water chemistry for the Pigeon River and four of its tributaries (Big Creek, Fines Creek, Jonathan Creek, and Richland Creek). Experimental units were 30-meter transects at each station. For variables where more than one measurement was taken per station (e.g., substrate characteristics), measurements were averaged per transect prior to analysis. The total sample size was 20 stations for most analyses, except when water quality instruments did not function (n = 16-19 for these variables). Differences in the response variables among sites were analyzed using a one-way analysis-of-variance (ANOVA; Zar 2009). If the overall F-test was significant, Tukey's Honestly Significant Difference (HSD) test was used to determine if pairwise differences existed between sites (Zar 2009). For habitat preference analyses, salamander locations were the experimental units, and substrate characteristics at use (where salamanders were found) and random (available habitat for salamanders) locations within a station were compared. Because substrate characteristics along a transect within a station could not be assumed to be independent, a paired t-test was used to test for differences between use and random locations (Zar 2009). A random number table was used to determine the distance from the bank that we would travel to measure substrate at each of the three transect locations. Given that sample sizes for these analyses were relatively large (n > 10, Underwood 1997), the test for normality was not done because the Central Limit Theorem assures that the sample mean is approximately normal when sample size is large (Hogg et al.

2004). All analyses were performed using the SAS® system (SAS® Institute, Cary, North Carolina) at $\alpha = 0.05$.

The previously described analyses provided comparisons of abiotic and biotic characteristics among sites; however, there was interest in determining which substrate and water quality variables explained the most variation in salamander abundance, richness, and diversity among sites. Therefore, multiple linear regression with stepwise selection (entry and stay level of significance = 0.05) was used to identify important habitat variables for salamanders at the stations (Zar 2009). The coefficient of determination (R^2) was reported as a measure of overall model performance, and partial R^2 values and standardized regression coefficients for a measure of variable importance (Zar 2009). For models with multiple variables, the variance inflation factor (VIF) was reported, with a VIF value greater than 10 suggestive of multicollinearity (Freund and Littrell 2000).

CHAPTER 3

RESULTS

Stream salamanders were found in seven of the 20 stations searched in 2009 (Table 1). A total of 53 salamanders were captured, including 37 Blue Ridge two-lined salamanders (*Eurycea wilderae*), eight shovel-nosed salamanders (*Desmognathus marmoratus*), six Eastern hellbenders (*Cryptobranchus alleganiensis)*, one black-bellied salamander (*D. quadramaculatus*) and one spring salamander (*Gyrinophilus porphyriticus*; Figure 5). All except one Blue Ridge two-lined salamander found were larvae. Three larval hellbenders and one juvenile hellbender were found at site four on the Pigeon River above the mill. All other salamanders captured were adults.

Mean salamander abundance at Big Creek and the Pigeon River above the mill was 5-10X greater than at all other sites. Mean abundance of salamanders was 2X greater at Big Creek compared to the Pigeon River above the mill (Table 2). Mean salamander richness at Big Creek and the Pigeon River above the mill was 1-2.7X greater than at all other sites. Mean salamander richness was 1.4X greater at Big Creek compared to the Pigeon River above the mill. Differences in salamander diversity were detected among most sites. Big Creek and the Pigeon River above the mill had higher diversity than the Pigeon River below the paper mill, Jonathan Creek, Fines Creek, and Richland Creek. No salamanders were found in the Pigeon River below the mill, Fines Creek, and Richland Creek (Table 2).

Table 1. List of salamander species found in the Pigeon River above the mill, Big Creek and Jonathan Creek, May – August, 2009.

Species	Pigeon River above the mill	Big Creek	Jonathan Creek	**Total**
Blue Ridge two-lined salamander	13	22	2	37
Eastern hellbender	6	0	0	6
Shovel-nosed salamander	0	8	0	8
Black-bellied salamander	0	1	0	1
Spring salamander	0	1	0	1
Total	19	32	2	53

a) Blue Ridge two-lined salamander (*Eurycea wilderae*)

b) Eastern hellbender *(Cryptobranchus alleganiensis*)

c) Spring salamander *(Gyrinophilus porphyriticus)*

d) Shovel-nosed salamander *(Desmognathus marmoratus)*

e) Black-bellied salamander (*Desmognathus quadramaculatus*)

Figure 6. Photos of the five stream salamander species found in this study in the Pigeon River and two of its tributaries, North Carolina, May – August 2009. All photos were taken during this study, except the photo of the spring salamander (Myers et al. 2006).

Table 2. Salamander community characteristics among the Pigeon River and four of its tributaries, North Carolina, May – August 2009.

Variable[3]	Site [1,2] BC		PRA		PRB		JC		FC		RC		$F_{5,14}$	*P*
	$\overline{x}$	SE	$\overline{x}$	SE	$\overline{x}$	SE	$\overline{x}$	SE	$\overline{x}$	SE	$\overline{x}$	SE		
Abundance	10.7A	3.2	4.8AB	2.4	0B	0.0	0.7B	0.7	0.0B	0.0	0.0B	0.0	6.1	0.003
Richness	2.7A	0.7	1.3AB	0.5	0B	0.0	0.3B	0.3	0.0B	0.0	0.0B	0.0	8.3	<0.001
Diversity	0.8A	0.3	0.3AB	0.2	0B	0.0	0.0B	0.0	0.0B	0.0	0.0B	0.0	4.9	0.008

[1] BC = Big Creek, PRA = Pigeon River above paper mill, PRB = Pigeon River below paper mill, JC = Jonathan Creek, FC = Fines Creek, RC = Richland Creek.

[2] n = 3 for BC, JC, FC and RC; n = 4 for PRA and PRB.

[3] Means within rows followed by unlike letters are statistically different by Tukey's HSD test.

Rubble was the most prevalent substrate type at Big Creek with percent cover 2-6 times greater than at all other sites (Table 3). Percent cover of bedrock in the Pigeon River above the mill was 15-45% higher than at all other sites. No other significant differences were detected with respect to silt, sand and cobble (Table 3).

Conductivity and salinity in the Pigeon River below the mill were 6.6-30.2X higher than at all other sites (Table 4). Turbidity was 2.7 and 7.2X greater in Richland Creek than the Pigeon River above the mill and Big Creek, respectively. Mean water temperature in the Pigeon River below the mill was 3.8-8.1^{o}C higher than all other sites. Upper and lower site widths in both the Pigeon River above and below the mill were 1.9-4.7X greater than in all other sites. No significant differences in DO or pH were found among sites (Table 4).

Sites selected by Blue Ridge two-lined salamanders (EUWI) and Eastern hellbenders (CRAL) contained 2X and 4X more rubble than available sites, respectively (Table 5). Across species, use sites contained approximately 45% rubble. Sites avoided by CRAL and EUWI contained 7.4X and 2.2X more bedrock than use sites. Across species, avoided sites had approximately 33% bedrock (Table 5).

Percent rubble was the only variable that was retained in final substrate models (Table 6). Percent rubble was positively related to abundance, richness and diversity and explained 22-30% of variation in these response variables. The largest variation in salamander abundance in final water quality models was explained by pH (41%) with a negative relationship between abundance and pH. Stream width and dissolved oxygen explained 13% and 12% of the variation in abundance, respectively (Table 6).

Table 3. Percent cover of substrates among the Pigeon River and four of its tributaries, North Carolina, May – August 2009.

Variable	Site [1,2]												$F_{5,14}$	P
	BC		PRA		PRB		JC		FC		RC			
	$\bar{x}$ [3]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE		
Rubble	73.2A	4.7	12.6B	2.3	24.4B	8.8	35.0B	4.6	26.4B	13.7	25.3B	5.1	7.8	0.001
Cobble	25.1A	4.0	32.4A	9.2	39.5A	5.8	24.2A	4.6	38.2A	6.0	59.0A	11.6	2.6	0.073
Silt	0.0A	0.0	4.7A	2.3	1.7A	1.7	0.0A	0.0	7.8A	7.8	2.4A	2.4	0.8	0.568
Sand	0.0A	0.0	5.3A	3.5	5.5A	4.5	12.1A	1.0	11.5A	5.1	13.3A	8.3	1.2	0.353
Bedrock	1.7B	1.7	45.0A	8.6	29.0AB	8.6	28.8AB	1.5	16.1AB	3.8	0.0B	0.0	7.7	0.001

[1] BC = Big Creek, PRA = Pigeon River above paper mill, PRB = Pigeon River below paper mill, JC = Jonathan Creek, FC = Fines Creek, RC = Richland Creek.

[2] n = 3 for BC, JC, FC and RC; n = 4 for PRA and PRB.

[3] Means within rows followed by unlike letters are statistically different by Tukey's HSD test.

Table 4. Water and stream characteristics among the Pigeon River and four of its tributaries, North Carolina, May – August 2009.

Variable[1]	Site [2,3,4]													
	BC		PRA		PRB		JC		FC		RC		$F_{5,14}$	*P*
	$\bar{x}$ [5]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE		
DO	9.0A	0.1	6.8A	0.8	7.5A	0.3	8.3A	0.6	8.9A	0.2	7.7A	0.5	2.9	0.055
temp	17.8BC	0.1	20.8B	0.8	24.6A	0.5	18.6BC	0.1	16.5C	0.7	19.9BC	1.7	13.5	<0.001
pH	7.3A	0.4	7.2A	0.3	7.9A	0.1	7.6A	<0.1	7.5A	<0.1	7.3A	0.2	3.4	0.039
cond	17.6B	0.6	23.7B	0.3	532.3A	128.6	46.3B	0.4	79.8B	4.7	50.5B	2.1	10.9	<0.001
sal	23.0B	8.6	18.0B	0.5	258.5A	64.9	26.4B	0.3	37.5B	0.0	28.7B	1.2	8.8	<0.001
turbidity	0.6C	0.2	1.6BC	0.4	3.2ABC	0.6	4.2AB	0.4	ND	ND	4.3A	1.0	7.2	0.003
widthUp	13.2B	0.3	31.7A	2.6	33.8A	3.4	11.0B	1.7	8.3B	1.7	12.5B	0.7	23.2	<0.001
widthLow	14.5B	2.3	30.4A	1.2	27.7A	3.4	13.7B	1.4	6.5B	1.0	14.1B	0.9	19.9	<0.001

[1] DO = dissolved oxygen (mg/L), temp = water temperature (°C), cond = conductivity (µS), sal = salinity (ppm), turbidity = NTU, widthUp = width (m) at end of site transect, widthLow = width (m) at beginning of site transect.

[2] BC = Big Creek, PRA = Pigeon River above paper mill, PRB = Pigeon River below paper mill, JC = Jonathan Creek, FC = Fines Creek, RC = Richland Creek.

[3] n = 3 for BC, JC, FC and RC; n = 4 for PRA and PRB.

[4] ND = no data.

[5] Means within rows followed by unlike letters are statistically different by Tukey's HSD test.

Table 5. Percent composition of substrate at sites selected (use) by eastern hellbender (*Cryptobranchus alleganiensis*, CRAL) and Blue Ridge two-lined salamander (*Eurycea wilderae*, EUWI) in the Pigeon River above the paper mill, and in Jonathan Creek, North Carolina, May – August 2009.

Species[1]	Variable[2]	Site				t_{20}	*P*
		Use		Available			
		$\bar{x}$	SE	$\bar{x}$	SE		
CRAL	Rubble	56.7	8.9	12.2	0.8	5.1	0.004
	Cobble	29.6	9.0	47.2	7.4	-1.9	0.111
	Silt	0.0	0.0	0.4	0.4	-1.0	0.363
	Sand	9.2	4.7	6.0	1.9	0.7	0.534
	Bedrock	4.6	4.6	34.2	5.9	-22.5	<0.001
EUWI	Rubble	40.0	6.5	18.5	2.4	2.7	0.017
	Cobble	30.7	6.5	39.6	3.8	-1.1	0.281
	Silt	1.2	0.9	4.5	1.4	-2.6	0.021
	Sand	13.2	4.1	4.8	1.1	2.1	0.055
	Bedrock	15.0	5.4	32.5	2.3	-2.8	0.014
Overall	Rubble	44.8	5.4	16.7	1.8	4.3	<0.001
	Cobble	30.4	5.2	41.8	3.4	-1.8	0.081
	Silt	0.8	0.6	3.3	1.1	-2.6	0.017
	Sand	12.0	3.2	5.2	0.9	2.2	0.040
	Bedrock	12.0	4.2	33.0	2.3	-4.5	<0.001

Table 6. Substrate and water multiple linear regression models explaining significant variation in salamander community metrics among the Pigeon River and four of its tributaries, North Carolina, May – August 2009.

Model	Response Variable	Explanatory Variable[1,2]	Estimates		t_1	P	Partial R^2	VIF[3]
			Un-standardized	Standardized				
Substrate	Abundance	Intercept	-0.46	0.00	-0.27	0.787	NA	0.00
		RBB	0.09	0.47	2.26	0.036	0.22	0.00
	Richness	Intercept	-0.17	0.00	-0.45	0.660	NA	0.00
		RBB	0.03	0.55	2.81	0.012	0.30	0.00
	Diversity	Intercept	-0.07	0.00	-0.53	0.602	NA	0.00
		RBB	0.01	0.50	2.43	0.026	0.25	0.00
Water	Abundance	Intercept	17.13	0.00	2.62	0.024	NA	0.00
		pH	-2.95	-0.65	-3.69	0.004	0.41	1.01
		Width	0.09	0.52	2.70	0.021	0.13	1.20
		DO	0.51	0.37	1.95	0.077	0.12	1.20

CHAPTER 4

DISCUSSION

Five species of salamanders were found in this study in three of five sites: the Pigeon River above the mill, Jonathan Creek, and Big Creek. No salamanders were found in Fines Creek, Richland Creek, or in the Pigeon River below the mill. Salamander abundance, richness, and diversity were higher in Big Creek than at all other sites. Sites selected by Eastern hellbenders and Blue Ridge two-lined salamanders contained more rubble than available sites. Sites avoided by these two species contained more bedrock than use sites. Rubble was the most prevalent substrate type in Big Creek and was the only variable retained in substrate models. Bedrock was higher in the Pigeon River above the mill than at all other sites. Salamander abundance was negatively related to pH in water quality models. Conductivity, salinity, and mean water temperature were higher in the Pigeon River below the mill than at all other sites. These results suggest salamander abundance in the Pigeon River and its tributaries may be affected by poor water quality and not habitat.

Pigeon River Above vs. Below the Mill

One objective of this study was to determine whether differences existed between the Pigeon River above and below the Canton paper mill. Nineteen salamanders were found in the Pigeon River above the mill and none were found below the mill. While dissolved oxygen and pH were similar between the sections, turbidity, conductivity, salinity, and water temperature were higher below the mill.

Tributaries

No salamanders were captured in Fines Creek, most likely due to other water quality variables that were not measured in this study. There were many houses being built on the banks of the upper portion of Fines Creek during this study and I suggest construction runoff affected water quality. Only one turbidity reading was taken from Fines Creek due to meter malfunction. It could not be included in statistical analyses, but was much higher than all other sites (13.1).

Richland Creek also contained no salamanders. This stream begins at Lake Junaluska and receives most of its flow from the lake. It is likely that sediments released in the lake water contain substances that are not ideal for salamanders. A water treatment plant releases water into Richland Creek and could be a source of extra nutrients that affect water quality.

Salamander abundance was higher at Big Creek than any other site with an average of 11 salamanders per 30-meter station and 32 of the 53 salamanders found in this study were found in Big Creek. Species richness was also higher with approximately three species per station. Several water quality and habitat variables in Big Creek were much different than other sites and could explain higher salamander diversity, richness, and abundance. Big Creek had the highest percentage of rubble (73%) among all sites, which was found to be preferred salamander habitat in this study. According to habitat models in this study, rubble was the only variable predictive of salamander abundance. Big Creek also had low percentages of bedrock (2%) and silt (0%), two habitats that salamanders typically avoid. Conductivity, salinity, and turbidity were all low as well, which is an indicator of good stream health. The headwaters of Big Creek flow directly out of the Great Smoky Mountains National Park and thus are probably far less impacted than other sites in this study.

Species-specific Habitat Preference

Larval Blue Ridge two-lined salamanders (*Eurycea wilderae*) were found in sites with more rubble and sand and avoided sites containing more silt and bedrock. Larval *E. wilderae* utilize rock cover during the day for protection and become active at night searching for food (Petranka 1998). Wiltenmuth (1997) observed rock cover was abundant in streams where *E. wilderae* were found in North Carolina. Smith and Grossman (2003) found Southern two-lined salamander larvae (*E. cirrigera*) avoided silt and preferred the largest substrate available in Georgia Piedmont streams. Jollyville Plateau salamander (*E. tonkawae*) abundance was positively correlated with the mean area of rubble and cobble and the probability of the use of these substrates increased progressively with rock size (Bowles et al. 2006). Blue Ridge two-lined salamander adults typically live along the margins of streams or under cover terrestrially (Petranka 1998). In general, adults are only fully aquatic when they are breeding in streams. Breeding takes place from September-May and could explain why all but one *E. wilderae* captured in this survey were larvae (Petranka 1998).

Eastern hellbenders prefer cool, well-oxygenated water and substrates consisting of large rubble for cover (Nickerson et al. 2002). Hellbenders observed in this study preferred sites containing rubble but avoided sites containing bedrock. Six hellbenders representing three size classes (larvae, juvenile, and adult) were found in the Pigeon River above the mill. This is significant because hellbender larvae are usually difficult to locate and few studies have studied larval hellbender habitat (Nickerson and Krysko 2003). These hellbenders were located in the Pigeon River above the paper mill in a stream reach with 45% of avoided habitat (bedrock) and only 12.6% of their preferred habitat (rubble). The Pigeon River below the mill had 24.4% rubble indicating adequate habitat was available for hellbenders, but water quality potentially limited their use.

Water Quality

Water quality was very different between the Pigeon River above and below the mill. Eastern hellbender and Blue Ridge two-lined salamander abundance was negatively associated with the Pigeon River below the mill because of differences in water quality. Water temperatures in the Pigeon River below the mill were up to 8^{o}C higher (24.6^{o}C) than all other sites where these species were found. Water temperature can be very important factor in the development of eggs and the survival of salamander larvae. Mean *E. tonkawae* abundance was also negatively associated with water temperature in a recent study in Texas (Bowles et al. 2006).

Conductivity is the measure of the ability of water to conduct electricity and is directly related to the amount of total dissolved salt (TDS; Cole 1994). Sodium and chloride are two major ions that contribute to TDS and are assumed to be included in chemicals released by the Canton paper mill (Wetzel 1975). The concentration of these ions can affect the flow of water across the embryonic membrane of amphibians, preventing dissolved oxygen from reaching the inner capsule (Zug 1993; Duellman and Trueb 1994). Conductivity in the Pigeon River at the station immediately below the mill was 22.5X higher than above the mill with a mean value of 532.3 µS. Karraker et al. (2008) found survival of larval *Ambystoma maculatum* decreased with increasing conductivity from 59% (control) to 38% at 500 µS to only 11% at 3,000 µS. These authors suggested the severity of the effects of conductivity differs depending on the embryonic and larval period of the salamander.

Salinity in the Pigeon River below the mill was at least 6.8X greater than at all other sites. In order to survive, amphibians must maintain internal salinity higher, or at least equal to, their environment (Duellman and Trueb 1994). Internal electrolyte concentration varies significantly among amphibians (Duellman and Trueb 1994) and is not known for the species found in this study. There is very little documentation relating salinity to amphibians, especially salamanders. However, the levels of salinity recorded in this study were low, with the highest reading of 0.258 parts per trillion (ppt) in the Pigeon River below the mill. The salinity of seawater is usually 30-50 ppt, therefore salinity is most likely not significant at the levels found in the Pigeon River watershed.

Water Chemistry Model

Water quality models showed salamander abundance was related to pH, dissolved oxygen, and stream width. Low pH can cause developmental abnormalities and prevent hatching of amphibian embryos (Pierce 1985; Freda 1986). Low pH has also been known to cause slower growth in tadpoles (Freda and Dunson 1985). However, negative effects from pH for salamanders are usually seen at pH<4.5 (Pierce 1985). The pH range for all sites in this study was 7.3-7.9 and is not low enough to cause any developmental problems. Dissolved oxygen is essential in the development of amphibian eggs (Duellman and Trueb 1994). Low dissolved oxygen levels cause premature hatching in amphibians (Petranka et al. 1982). However, levels encountered in this study were quite high and not deemed critical. Stream widths were positively correlated with salamander abundance in this study. We standardized stations by 30 meter length, but widths were variable across sites. Thus, stations with larger widths had a larger searchable area and were more likely to contain more salamanders.

Historical Species Record vs. Species Found

According to the North Carolina Museum of Natural Sciences Research Collection Database (2009), eight salamander species have been documented in Haywood County, North Carolina, since 1940. Four of those species were found in this study in 2009: 1) the black-bellied salamander (*Desmognathus quadramaculatus*), 2) the Blue Ridge two-lined salamander (*Eurycea wilderae*), 3) the shovel-nosed salamander (*D. marmoratus*) and 4) the Eastern hellbender (*Cryptobranchus alleganiensis*). A fifth species, the seal salamander (*D. monticola*), was collected in Big Creek in 2008. Three other species documented in previous studies were not located in this survey: 1) the Allegheny Mountain dusky salamander (*D. ochrophaeus*), 2) the Northern two-lined salamander (*E. bislineata*), and 3) the red salamander (*Pseudotriton ruber*). These species have not been located since 1997, 1971, and 1980, respectively, in Haywood County, North Carolina. It is possible they were not found because of their life history characteristics.

The Allegheny Mountain dusky salamander is typically a streamside and terrestrial salamander and can be found quite far from streams, although reproduction is aquatic (Green and Pauley 1987). Larvae are usually found in seepages or slow moving parts of streams, which were not searched in this study (Petranka 1998).

Until 1987, the Blue Ridge two-lined salamander was considered a subspecies of the Northern two-lined salamander (Jacobs 1987). These two salamanders have since been declared separate species with the range of the Northern two-lined salamander extending south only to north Virginia (Sever 1999). Therefore, the Northern two-lined salamander documented in 1971 was most likely a Blue Ridge two-lined salamander.

Red salamander larvae are often found in springs and seepage areas and are rarely associated with large, quick-moving streams (Bruce 1972; Petranka 1998). It is possible they were not found in this study because of the timing of our surveys. Streams were searched from May-August and red salamanders typically remain in terrestrial sites until late summer and autumn when they move to aquatic sites to overwinter (Petranka 1998).

We also found an additional species, the spring salamander (*Gyrinophilus porphyriticus*), not recorded from Haywood County in the North Carolina Museum of Natural Sciences (NCMNS) database. The species and locations of salamanders found in this study will be sent to the NCMNS to update their research collection database.

Drought

The results of this study may have been affected by the significant drought in 2007-2008 and the subsequent changes in habitat and water levels. According to the U.S. Geological Society (2008), August 2007 had the lowest recorded monthly flows since 1932 at the Pigeon River near Canton, North Carolina, measuring station with a mean discharge rate of 61.6 cubic feet per second (cfs). Average flow for the month of August is 198 cfs. The drought continued and June and July of 2008 had the lowest recorded flows at the same station, with mean discharge rates of 82.8 and 57.7 cfs, respectively. Normal flows for June and July are 260 and 193 cfs. Low water flows potentially increase concentration of pollutants, increase water temperature, increase predation, and decrease salamander habitat. When water levels drop, eggs laid under rocks near the edge of the water may dry out thus affecting future recruitment. The effect of the 2007-2008 drought on salamander species found in this study is unknown.

The data from this study document the importance of abiotic and biotic factors on the abundance, richness and diversity of stream salamanders in the Pigeon River watershed. Many stream breeding salamanders are long-lived, exist in relatively stable populations, and tend to stay close to their home area (Welsh and Ollivier 1998). These life history behaviors make salamanders reliable indicators of diversity in stream ecosystems and their abundance can be a significant indicator of stream health (Welsh and Ollivier 1998).

CHAPTER 5

Summary and Recommendations

Snorkel surveys to determine salamander species composition in the Pigeon River and its tributaries were completed May-August 2009. Results of the study are as follows:

1) Fifty-three aquatic salamanders were found in three of six sites: Big Creek, Jonathan Creek, and the Pigeon River above the mill. No salamanders were found in Fines Creek, Richland Creek, or the Pigeon River below the mill.
2) Salamander abundance, richness, and diversity were all higher in Big Creek and the Pigeon River above the mill than all other sites.
3) Conductivity, salinity, and water temperature were higher in the Pigeon River below the mill than all other sites.
4) Eastern hellbenders and Blue Ridge two-lined salamanders preferred rubble habitat and avoided bedrock habitat.
5) Salamander abundance, richness, and diversity were positively related to percent rubble according to habitat models.
6) Salamander abundance was negatively correlated to pH and positively correlated to stream width, and dissolved oxygen according to water quality models.

The author recommends future studies to determine exact composition of chemicals at all sites, including the Pigeon River below the mill, and determine the effects of those chemicals on salamanders found in this study.

Because two tributaries contained no salamanders, the author recommends replicating the study at a later date as well as sampling other tributaries to determine whether other aquatic salamander species may be present in this watershed.

Streamside and terrestrial searches should also be done in the future to find species that may utilize the Pigeon River and its tributaries only during breeding season. These salamanders are also indicators of stream health because they lay eggs under rocks in streams and poor water quality could affect future generations.

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APPENDIX

A1. GPS coordinates of all sites on Pigeon River and four of its tributaries, North Carolina, May-August 2009.

Site[1]	Site #	Beginning Coordinates	Ending Coordinates
PRA	1	N35 30 31.4 W82 51 27.9	N35 30 33.3 W82 51 27.5
	2	N35 31 31.0 W82 50 25.3	N35 31 32.4 W82 50 24.4
	3	N35 30 26.57 W82 51 38.55	N35 30 25.8 W82 51 41.2
	4	N35 31 20.3 W82 50 56.4	N35 31 20.7 W82 50 57.3
PRB	5	N35 32 34.5 W82 51 44.3	N35 32 34.8 W82 51 45.5
	6	N35 36 52.3 W82 58 00.4	N35 36 52.3 W82 58 00.4
	7	N35 33 4.13 W82 57 16.54	N35 33 43.01 W82 57 15.48
	8	N35 33 02.6 W82 56 59.8	N35 33 01.8 W82 56 58.3
BC	1	N35 46 16.88 W83 06 01.44	N35 46 16.09 W83 06 02.17
	2	N35 46 27.85 W83 06 00.30	N35 46 27.06 W83 06 01.44
	3	N35 46 05.0 W83 06 00.9	N35 46 03.5 W83 06 03.3
FC	1	N35 39 56.3 W82 59 33.5	N35 39 57.0 W82 59 32.8
	2	N35 39 59.7 W82 59 27.5	N35 40 00.4 W82 59 28.1
	3	N35 40 06.1 W82 59 38.0	N35 40 07.1 W82 59 38.0
JC	1	N35 37 37.75 W83 00 01.83	N35 37 37.9 W83 00 03.0
	2	N35 37 36.4 W83 00 12.3	N35 37 36.4 W83 00 13.5
	3	N35 37 33.9 W83 00 22.9	N35 37 33.6 W83 00 23.8
RC	1	N35 32 59.1 W82 56 49.4	N35 32 57.6 W82 56 49.5
	2	N35 32 54.6 W82 56 47.5	N35 32 54.2 W82 56 46.3
	3	N35 32 52.3 W82 56 42.8	N35 32 51.5 W82 56 43.6

[1] BC = Big Creek, PRA = Pigeon River above paper mill, PRB = Pigeon River below paper mill, JC = Jonathan's Creek, FC = Fines Creek, RC = Richland Creek.

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