LAND USE AND EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*) POPULATIONS IN THREE HIWASSEE RIVER TRIBUTARY WATERSHEDS

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In dedication to my father and sister who always supported me in my goals and nurtured me throughout my life.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

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The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a cryptic, long-lived, species in the family Cryptobranchidae. Declines in populations of many aquatic species, including *C. a. alleganiensis* may be related to changes in the streamside and watershed physical characteristics. This study examined the potential link between changes in substrate condition (fine sediment accumulation) and differences in *C. a. alleganiensis* length frequency, mass, tail circumference, and tail fin height within three tributaries to the Hiwassee River in North Carolina. Changes in these characteristics will indicate which habitats support the healthiest *C. a. alleganiensis* populations. I characterized the substrate within the three streams and sampled *C. a. alleganiensis* populations from the three streams. Snout-vent length frequencies were not significantly different among streams. Mean mass, mass:snout-vent length, and mass adjusted for snout-vent length in *C. a. alleganiensis* populations within Tusquitee Creek were larger when compared to Fires Creek, but not Brasstown Creek. The larger *C. a. alleganiensis* observed within Tusquitee Creek were thought to be the result of the compounding influences of stream reach position, sediment accumulation, point discharges, and other associated variables.

INTRODUCTION

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) is one of three extant species in the salamander family Cryptobranchidae: *Andrias davidianus*, the Chinese giant salamander, *A. japonicas*, the Japanese giant salamander, and the hellbender, *Cryptobranchus alleganiensis,* within the United States. Both Asiatic salamanders are protected within their respective countries. The hellbender has two subspecies, the Eastern hellbender (*alleganiensis*) and the Ozark hellbender (*bishopi*) (Phillips and Humphries 2005). The Eastern subspecies currently occurs from southern New York south to northern Georgia and west to central Missouri (Petranka 1998, Bartlett and Bartlett 2006). All states within the range list *C. a. alleganiensis* as a Species of Concern, a Critically Imperiled species, or a Locally Rare species (Natureserve 2011). The US Fish and Wildlife Service currently lists the Ozark subspecies as Endangered (US FWS, 2011). In Western North Carolina, observations prior to 2007 were limited to isolated observations ranging back to 1918 (Lori Williams NCWRC, personal communication 2007).

Cryptobranchus a. alleganiensis is completely aquatic and inhabits fast-flowing, clean rivers with low silt load and an abundance of large rocks (Nickerson and Mays 1973, Routman and others 1994, Humphries and Pauley 2005). In Western North Carolina, *C. a. alleganiensis* occurs in riffle with water depth less than 1 meter at baseflow, in areas with an abundance of boulders or cobbles and gravel (Ball 2001). *Cryptobranchus a. alleganiensis* breeds from August to September. Males build nests, usually under a large rock or other stable debris, with the entrance facing downstream. Multiple female *C. a. alleganiensis* will then enter the nest and deposit a series of marble-sized eggs in a rosary formation, very similar to a string of beads. The male then enters and spreads milt on the eggs. Over the course of one breeding season each nest may hold as many as 1900 eggs. Incubation lasts for 6-8 weeks (Nickerson and Mays 1973). *Cryptobranchus a. alleganiensis* are extremely territorial. Individual home ranges are between 346 m^2 and 198 m^2 (Nickerson and Mays 1973, Humphries and Pauley 2005).

At hatching, *C. a. alleganiensis* larvae average ~30 mm in length and have filamentous gills (Phillips and Humphries 2005). *Cryptobranchus a. alleganiensis* larvae undergo incomplete metamorphosis at about 18 months of age when they lose external gills but retain some larval characteristics, such as gill slits and the absence of eyelids. Age at sexual maturity varies from 4-8 years (Nickerson and Mays 1973, Petranka 1998). *Cryptobranchus a. alleganiensis* are long-lived, reaching at least 29 years in captivity. Lifespan in the wild is not known, but estimates place maximum age at 30-50+ years (Nickerson and Mays 1973, Phillips and Humphries 2005). Individuals range in size from 30-74 cm in length and vary in color from gray to olive brown, often having a mottled pattern (Behler and King 1979). The head is dorso-ventrally flattened; the torso is stout, ending in a rudder-like muscular tail. The skin is the primary respiratory organ, although vestigial lungs are present (Guimond and Hutchison 1973).

Cryptobranchus a. alleganiensis are primarily carnivorous, feeding on crayfish, fish, and various invertebrates; however, they also scavenge, evident from their frequent encounters with anglers (Nickerson and Mays 1973). Crayfish compose as much as 90%

of their diet (Humphries and Pauley 2005, Petranka 1998). Feeding and all other activities are predominantly nocturnal (Nickerson and Mays 1973), although in some streams diurnal activities are common (Jeff Humphries, Post Doc, Clemson University pers. comm. 2007).

Declines in populations of many aquatic species, including *C. a. alleganiensis* are associated with damming, increased siltation, stream channelization, riparian deforestation, and a variety of agricultural and industrial pollutants (Nickerson and Mays 1973, Resh and others 1988, Allen and others 1997, Petranka 1998, Nickerson and others 2002, and Wheeler and others 2002). Wheeler and others (2002) noted an average of 77% decline in both Midwest *C. alleganiensis* subspecies over a 20+ year study.

Changes in the streamside and watershed physical characteristics adversely affect many aquatic species. These alterations induce changes in the bioenergetics and hydraulic characteristics of a stream reach. For example, the removal of riparian vegetation decreases the amount of coarse organic inputs to the stream system while simultaneously increasing the hydrologic inputs. Riparian vegetation removal affects stream systems by increasing light levels, stream temperature, sediment deposition, nutrient inputs, and organic inputs (Peterjohn and Correll 1984). A decrease in riparian woody debris alters habitat structure and can lead to changes in species composition. Both crop and animal agricultural operations are non-point sources for nutrient enrichment and sedimentation of aquatic systems (Resh and others 1988, Allen and others 1997). Nutrient enrichment increases primary productivity and favors algal growth, sometimes leading to depletion of dissolved oxygen and causing shifts in the biotic composition (Smith and others1999 and Allen 2004). The extent of agricultural uses within a catchment has been noted as a good predictor of stream condition (Allen and others 1997). Percent non-forest, paved road density/length, and building number/density all negatively impact stream systems (Bolstad and Swank 1997).

Sedimentation alone has many effects on stream systems, specifically, increased turbidity, scouring of biofilms, and sediment deposition. These effects lead to a decrease in primary productivity of the system by abrading aufwuchs, decreasing light penetration, and causing bottom-up effects leading to alterations in the upper tiers of the food web. Increased sediment input tends to homogenize stream depths by filling pools and further decreasing benthic habitats by filling interstitial spaces within the substrate (Henley and others 2000 and Allen 2004). Larval *C. a. alleganiensis* are known to utilize the interstices of cobbles and gravels as cover making sedimentation a threat to the survival of the smaller size classes (Nickerson and Mays 1973). Depending on the level of sediment accumulation, the loss of the larger cover habitats to embedding may also affect juvenile and adult *C. a. alleganiensis*.

Siltation causes changes in habitat structure, affecting community structure and the demographics of some species (Henley and others 2000). Presumably, one may observe these effects within specific body measurements of specific animals within the community.

In controlled experiments, high food availability resulted in increases in body size, lipid levels, and larger clutch size in *Ambystoma opacum*, suggesting an increased fitness over individuals in medium and low food treatments (Scott and Fore 1995).

Similarly, observations of *C. a. alleganiensis* from stocked trout streams versus non-stocked streams indicate that *C. a. alleganiensis* may exhibit larger bodies, tails, and tail fins in stocked waters due to the presence of additional forage base from the cleaning of fish and discarded bait. Additional accounts of *C. a. alleganiensis* robbing stringers of fish support this hypothesis (Jeff Humphries, Post Doc, Clemson University, personal comm. 2007).

In amphibians, lipids are the primary energy reserve. Amphibians do not store lipids as a subcutaneous layer, but in specific areas throughout the body and organs (Fitzpatrick 1976 and Pond 1978). Concentrations of fat bodies tend to occur in the abdomen, tail, and in soft tissues, specifically in urodelids. Previous studies reported the size of fat bodies was closely related to the maintenance and size of gonads (Rose 1967, Fitzpatrick 1973, Fitzpatrick 1976, Jorgensen 1992).

Little is known about growth, fitness, fecundity, or survivorship of *C. a. alleganiensis* and how habitat alterations due to logging, development, and other anthropogenic activities may affect these metrics. Also, there is little direct evidence of the effects of siltation on hellbender populations. This study examined the potential link between changes in substrate condition (siltation) and altered hellbender population biometrics within three tributaries to the Hiwassee River in North Carolina. This study was designed under the assumption that increases in mass, tail circumference, and tail height are indicative of increased fitness of *C. a. alleganiensis*.

I developed the following objectives to investigate the possible effects of siltation on *C. a. alleganiensis* population biometrics:

1. Characterize the habitat types available for *C. a. alleganiensis* with regard to streamside condition and distribution of particle sizes.
2. Identify correlations between habitat characteristics and *C. a. alleganiensis* population characteristics, specifically length-frequency distributions, hellbender density, snout-vent length, mass, tail circumference, and tail height.

These objectives lead to five hypotheses:

1. With increased sedimentation, the length frequencies observed within hellbender populations should become skewed toward larger size classes. As streams become more sedimented, cover rocks and components of larval *C. a. alleganiensis* habitat become covered or otherwise disturbed, decreasing either larval survival or abundance, and resulting in an observable skew in the population structure toward larger individuals.
2. As streams become more sedimented, hellbender population density should decrease. Similar to the effects of sedimentation on *C. a. alleganiensis* length frequency, the lowered recruitment or decreases in the number of suitable cover objects should lower the total numbers of individuals in a stream reach.
3. Mean *C. a. alleganiensis* mass, adjusted for body length, should decrease with increases in stream alteration. If increases in fine sediments are detrimental *C. a. alleganiensis* prey habitat or stream productivity, then streams with increased sediment loads would have lowered prey availability. The reduced prey density will result in *C. a. alleganiensis* having a smaller mass relative to snout-vent length.

4. The mean tail circumference and tail height relative to body mass of *C. a. alleganiensis* should decrease with increasing stream alteration. If increases fine sediments are detrimental to *C. a. alleganiensis* prey habitat or stream productivity, then streams with increased sediment loads would have lowered prey availability. The reduced prey density will result in *C. a. alleganiensis* having smaller tail circumference and tail height relative to mass.
5. The mean tail circumference and tail height relative to snout-vent length of *C. a. alleganiensis* should decrease with increasing stream alteration. If fine sediments are detrimental to *C. a. alleganiensis* prey habitat or stream productivity, then streams with increased sediment loads would have lowered prey availability. The reduced prey density will result in *C. a. alleganiensis* having smaller tail circumference and tail height relative to snout-vent length.

MATERIALS AND METHODS

Study Area

I sampled within three, fourth-order tributaries to the Hiwassee River, Clay County, North Carolina (Figure 1). I chose these three streams based upon their close proximity to one another, similar geology, and differences in North Carolina Division of Water Quality (DWQ) designation.

In the Upper Hiwassee River basin (subbasin 01), 13.7 percent of the basin is used for cultivated crop or pasture. An additional 2.5 percent is urban land use (NCDENR 2005).

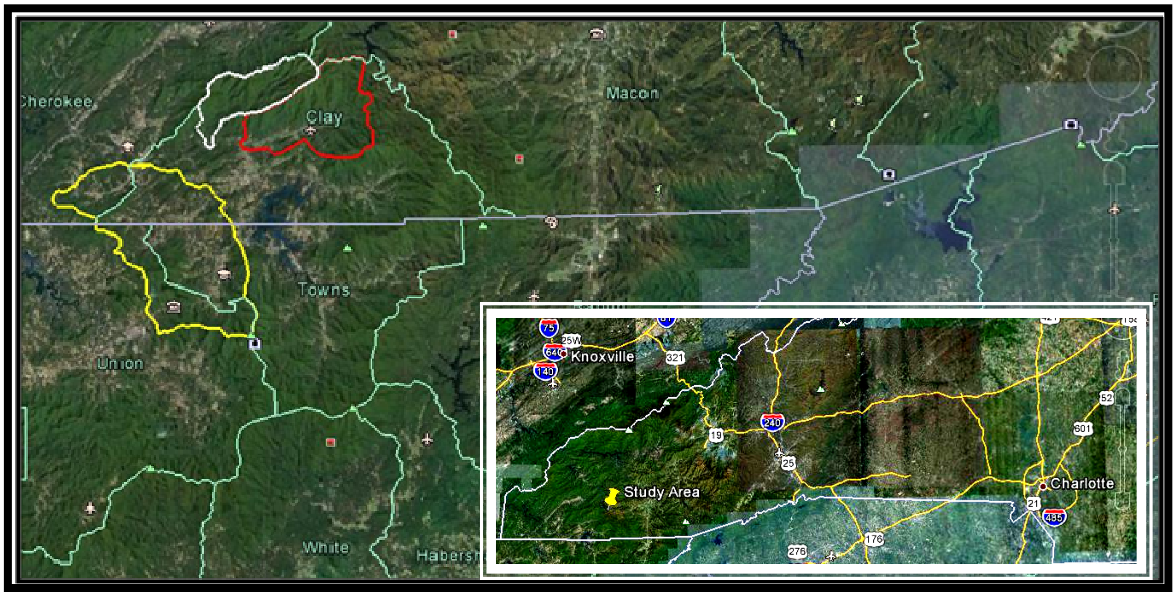

Figure 1. General study area location, Clay County, NC (Google Earth 2012).

In the Coweeta Creek basin, similar to the Hiwassee drainage, land disturbing activities are concentrated in the downstream and riparian areas (Bolstad and Swank

1997, Scott 2001). Historically and still today, land disturbing activities still occur in the riparian lower gradient areas, likely due to the ease in accessibility and relationship to a water source, specifically for agricultural operations.

Brasstown Creek was the largest stream in the study in terms of watershed area. This stream originates in north Georgia and flows north-northwest into North Carolina where it converges with the Hiwassee River less than one kilometer north of Brasstown, North Carolina (Figure 1). Brasstown Creek was listed as 303D in 1998 due to sedimentation and water quality issues (NC DENR 2010c). This stream has a DWQ designation of Water Source IV (WS IV) with no subclassification (NC DENRc, NC DENRd). The WS IV classification indicates that the waters occur in moderately developed watersheds with a water source downstream. The dominant underlying geology of this stream is Biotite Gneiss (ZYbn) in the headwaters (Weiner and Merschat 1992, Appendix A, Figure A2). The midreaches of this stream are Great Smoky undivided (Zgs) and Wehutty Formation (Zwe). The lower reaches including the study sites are Tusquitte Quartzite and the Nantahala Formation (Znt), the Brasstown Formation (Zb), the Mineral Bluff Formation and Nottely Quartzite (Zmb), and the Andrews Formation and Murphy Marble (Zma) (Weiner and Merschat 1992, Appendix A, Figure A2).

Tusquitee Creek was the second largest stream in the study. Tusquitee Creek originates near Nantahala Lake and flows southwest between the Vineyard and Tusquitee Mountains where it converges with the Hiwassee River approximately two and a half kilometers north of Hayesville, North Carolina (Figure 1). Tusquitee Creek is classified as a WS IV, with trout water (Tr) and high quality water (HQW) subclassification (NC DENRc, NC DENRd). The dominant underlying geology of this stream is the Dean Formation (Zd), Biotite Gneiss (ZYbn) and the Ammons Formation (Zam) in the headwaters. The mid and lower reaches of this stream, including the study sites, are primarily the Dean Formation (Zd) and secondarily Tusquitee Quartzite, the Nantahala Formation (Znt), and the Ammons Formation (Zam) (Weiner and Merschat 1992 and Appendix A, Figure A2).

Fires Creek was the smallest stream in the study. Fires Creek also originates near Nantahala Lake and flows southwest between the Valley River and Tusquitee Mountains were it converges with the Hiwassee River five kilometers northwest of Hayesville, North Carolina (Figure 1). Fires Creek is classified as a WS IV, with a Tr, and outstanding resource water (ORW) subclassification (NC DENRc, NC DENRd). The underlying geology of this stream, including the study sites, is primarily the Brasstown Formation (Zb) and secondarily Tusquitee Quartzite, with the Nantahala Formation (Znt) in the extreme headwaters (Weiner and Merschat 1992 and Appendix A, Figure A2).

Stream Characterization

I selected three sites in each of the three streams for study. Each site consisted of at least one riffle, one pool, and one run. Sites were located in the lower reaches of each stream system. The most downstream site in each stream was located within two miles of the Hiwassee River. Subsequent sites were distributed upstream. The total area of each site was 2000 square meters, comprising approximately 10 hellbender home ranges (Humphries and Pauley 2005).

At each of the nine sites I established an area-constrained grid with orange flags placed every 10 meters on each bank. These flags were used to locate hellbenders captured within each site and as a point of reference for other observations made during the study.

I conducted pebble counts at each site by the zig-zag method to characterize the particle size distribution at each site (Bevenger and King 1995). The transects originated at the downstream limit of each site and crossed the channel in 10 meter increments ending at the uppermost limit of the site. Particles were sampled blindly at one meter intervals along the zig-zag transect selecting the first particle touched for measurements. Size classes were defined using calipers to measure the intermediate axis using the Modified Wentworth Scale (Bain and Stevenson 1999). For particle size classes to small for measurement with calipers a © 1984 W.F. McCollough Sand-gage was used to quantify particles < 2 mm by feel. During the pebble count procedure, I visually estimated embeddedness twice along each cross-channel transect using a modification of the method discussed in Bain and Stevenson (1999). An embeddedness value was assigned for both the left ascending and right ascending portion of the stream at each crossing. I assigned embeddedness categories in increments of 10 percent with 10 possible scores (0, 10, 20, 30.... percent, etc.). A total of 30 estimates were recorded at each site. I organized pebble counts into frequency distributions and cumulative percentage graphs of particle sizes, using The Reference Reach Spreadsheet Version 2.1L

(Mecklenburg 1999). Differences in embeddedness estimates among the three streams were analyzed using a Chi Square test of homogeneity.

I separately recorded the intermediate diameter of ten boulders greater than 500 mm for analysis of differences in available cover habitat among streams. I used SAS version 9.0, SAS Institute Inc. Cary, NC, for analysis of variance.

I measured temperature, conductivity, dissolved oxygen, and pH at baseflow four times during the sampling season using a YSI Corporation 650 MDS multi-parameter display and 600R sonde. Measure measurements were taken within the thalweg at the most downstream site in each basin. Tusquitee Creek was only measured on three occasions. These measurements were used to evaluate water chemistry throughout the *C. a. alleganiensis* survey efforts. Measurements of water quality data were compared to North Carolina Department of Environment and Natural Resources (NCDENR) data to evaluate stream chemistry at baseflow.

Salamander Surveys

I surveyed each site monthly for juvenile and adult *C. a. alleganiensis* four times between April and September 2008 by area constrained cover search. During cover searches, all movable cover objects with an intermediate diameter greater than or equal to 500 mm were searched. I avoided cover objects with an intermediate axis measurement less than 500 mm to avoid disturbing potential habitat for larval *C. a. alleganiensis.* Cover objects were raised either by hand or using a pevee and were manually searched. When I captured a salamander, I transferred it to a nylon net bag and noted the location of capture. The capture location was recorded to ensure each salamander was returned to its original cover object. Each animal was measured for snout-vent length, tail circumference just posterior to the rear legs, maximum tail height, and mass (Figure 2). Sex was recorded during the breeding season when male cloacas were fully swollen.

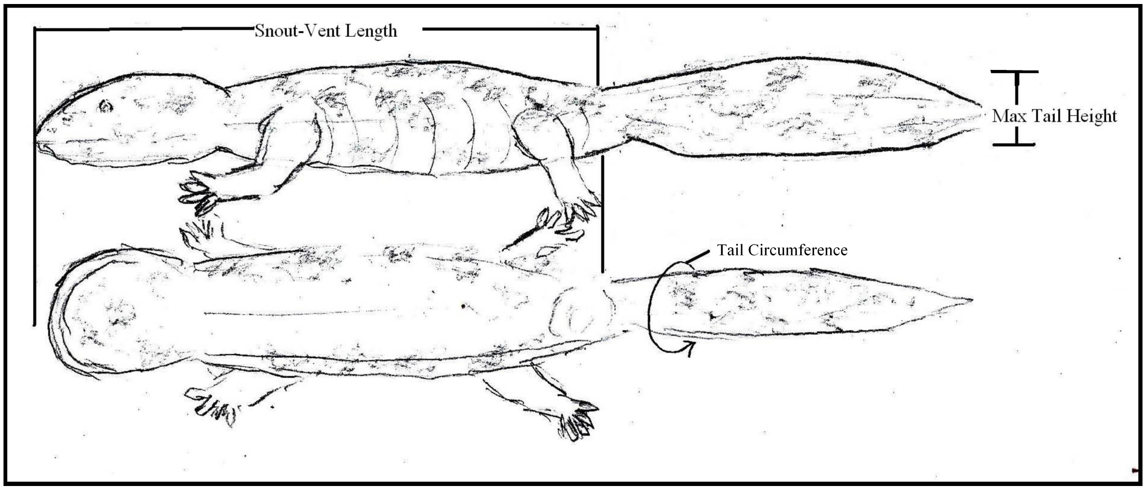

Figure 2. Sketch of *Cryptobranchus a. alleganiensis* body metrics and general body form.

After all physical data were collected, an individually numbered Biomark© Radio Frequency Identification (RFID) tag was scanned and its individual number recorded. I then inserted the tag at the dorsal origin of the hellbender's tail. Hellbenders were released within 10 minutes of capture at the site of capture. In successive surveys, all individuals were scanned for previously implanted RFID tags, prior to processing. Individuals with no previous RFID were tagged and the RFID number recorded before release. Sampling protocols and methods adhered to the guidelines for use of live amphibians and reptiles in field and laboratory research (Beaupre and others 2004).

Length frequency distributions of *C. a. alleganiensis* were compared among streams with Fisher's Exact test using R-statistical package (R Development Core Team 2011). I performed a logarithmic transformation of *Cryptobranchus a. alleganiensis* biometrics to correct for differences in variation with increasing mean values. I used analysis of variance to assess any differences in biometrics among streams. Hellbender biometrics were further analyzed by first correcting for differences among individuals in both snout-vent length and mass using two approaches, ratios and analysis of covariance. These ratios were intended to correct for the effect of both mass and snout-vent length on the remaining biometrics. I divided tail height, tail circumference, and mass by snout-vent length for each individual hellbender captured during the study. Similarly, I divided tail height and tail circumference by mass. These ratios were log-transformed to correct for differences in variance with increasing means. Biometrics of individuals that were captured on multiple occasions were included only once in the statistical tests. Analysis of variance was used to assess any differences in means among streams. Where salamander biometrics differed among streams, a Sheffe's Test was used to test which streams differed. I used multiple regression analysis to test for differences among streams in the relationship between *C. a. alleganiensis* snout-vent length and tail circumference, tail height, and mass, and between mass and tail height and tail circumference. I calculated the residuals from the regressions of these relationships and plotted them to assess the ability of the model to accurately describe the relationship between mass and snout-vent length. The residuals were also tested for differences among streams using analysis of variance. I used SAS version 9.0, SAS Institute Inc., for analysis of variance and analysis of covariance.

Crayfish Surveys

In order to augment the biometric data collected for *C. a. alleganiensis*, crayfish populations were sampled to assess food availability. Crayfish sampling consisted of three efforts at each site over an interval of four to five weeks between July and November of 2009. I used five baited minnow traps per trapping event. Each trap was placed at a random distance upstream from the start of the site and from the left ascending bank. Traps were staked and weighted with rocks and baited with six grams of chicken liver. Traps were fished overnight for a minimum of 12 hours. Captured crayfish were counted and total carapace length and live mass was recorded for each individual. Following data collection, crayfish were released at the site of capture.

Analysis of variance and analysis of covariance were used to examine differences among streams in crayfish total carapace length, crayfish mass, total carapace length and mass corrected by total carapace length. I compared the relationship between crayfish catch per unit effort and *C. a. alleganiensis* catch per unit effort among streams using multiple regression analysis. While crayfish biometrics are presented here untransformed, for ease of interpretation, statistical analysis was conducted on log natural transformed data, with the exception of crayfish catch per unit effort (CCPUE). I used SAS version 9.0, SAS Institute Inc., for analysis of variance and analysis of covariance.

RESULTS

Stream Characterization

Brasstown Creek exhibited the highest median embeddedness, largest watershed area, and lowest D_{50} (median particle diameter) values of all the streams, 30 %, 215 km^2, and 9.33 mm respectively. Mean stream width at the Brasstown Creek sites was 14.04 ± 0.72 meters wide. Average boulder intermediate diameter was 526.2 ± 65.7 mm (Table 1 and Appendix A; Table A1, Appendix A, Figure A2).

Water temperature in Brasstown Creek ranged from 22.86 to 12.35 ^{o}C from July 2011-December 2011 (Table 2). Dissolved oxygen levels ranged from 8.26 to 9.51 mg/l, fluctuating with changes in temperature. Conductivity and pH values ranged from 40 to 44 µS/cm and 6.96 to 7.26, respectively (Table 2).

Tusquitee Creek exhibited intermediate embeddedness, watershed area and D_{50} values, 20%, 111 km^2 and 57.15 mm, respectively. Sites within this stream averaged 13.07 ± 0.67 meters wide. Mean boulder diameter was 511.5 ± 55.8 mm (Table 1 and Appendix A; Table A1, Appendix A, Figure A3).

Water temperature in Tusquitee Creek ranged from 22.21 to 12.14 ^{o}C (Table 2). Dissolved oxygen levels ranged from 8.6 to 9.96 mg/l. Conductivity and pH values ranged from 15 to 22 µS/cm and 6.65 to 7.2, respectively (Table 2).

Fires Creek exhibited the lowest median embeddedness, smallest watershed area and highest D_{50} values, 10%, 60 km^2 and 80.45 mm respectively. Mean stream width at the sites within Fires Creek was 11.49 ± 0.72 meters, a statistically less both Tusquitee and Brasstown Creeks ($F_{(2,137)}$ = 13.2, P = <0.0001). Average boulder diameter was 526.9 ± 74.4 mm (Table 1 and Appendix A; Table A1, Appendix A, Figure A4).

Table 1. Summary of the watershed characteristics of Brasstown Creek, Tusquittee Creek and Fires Creek, Clay County, NC

		Watershed	
Metric	Brasstown Creek	Tusquittee Creek	Fires Creek
Watershed area (sq km)	215	111	60
Watershed Slope	0.005	0.025	0.025
Stream order at study sites	4	4	4
Avg stream width (m ± 2se)	14.04 ± 0.72	13.07 ± 0.67	11.49 ± 0.72
Present land use	Agricultural	Agricultural	Timber production
Past land use (50 ybp)	Agricultural	Agricultural	Timber production
Past land use (100 ybp)	Agricultural	Agricultural	Timber production
Underlying geologic feature	Murphy Belt-Great Smoky Group	Murphy Belt-Great Smoky Group	Murphy Belt-Great Smoky Group
Underlying geology Map Unit (ordered by area)*	ZYbn, Zgs, Zwe, Znt, Zb, Zmb, Zma	Zd, Zam, Znt	Zb, Znt
D_{50} calculated (mm)	9.3	57.2	80.5
D_{84} calculated (mm)	79.3	246.3	330.8
Boulder diameter (mm ± 2se)*	526.2 ± 65.7	511.5 ± 55.8	526.9 ± 74.4
Median Embeddedness (%)	30	20	10
DWQ Designation**	WS-IV, no sub-class	WS-IV, (Tr) Trout water, (HQW) High Quality Water	WS-IV, (Tr) Trout water, (ORW) Outstanding Resource Water

* Data acquired from (Wiener, L.S., and Merschat, C.E., 1992)
** Data acquired from (North Carolina Divison of Water Quality 2010)
Additional information for geologic map units and stream metrics are available in Appendix A, Table A2, Table A3 and Figure A2.

Water temperature in Fires Creek ranged from 23.55 to 11.96 ^{o}C (Table 2). Dissolved oxygen levels ranged from 7.91 to 9.79 mg/l. Conductivity and pH values ranged from 10 to 14 µS/cm and 6.25 to 7.06, respectively (Table 2).

Table 2. Summary of physical water quality samples collected from Brasstown Tusquittee, and Fires Creeks, Clay County, NC

Stream	Date	T (C)	DO (mg/L)	pH (SU)	Cond (µS/cm)
Brasstown Creek	7/12/2008	22.17	8.53	7.26	42
	8/30/2008	22.86	8.29	7.1	43
	9/14/2008	21.31	8.26	7.01	44
	12/20/2008	12.35	9.51	6.96	40
Tusquittee Creek	7/12/2008	22.21	8.78	7.2	15
	8/30/2008	18.18	8.6	6.68	22
	9/14/2008	-	-	-	-
	12/20/2008	12.14	9.96	6.65	15
Fires Creek	7/12/2008	23.55	7.91	6.25	14
	8/30/2008	18.92	8.6	6.64	13
	9/14/2008	20.82	7.99	7.01	14
	12/20/2008	11.96	9.79	7.06	10

D_{50} values were lowest in Brasstown Creek indicating a higher percentage of fine sands and silts in the substrate. The highest D_{50} values were measured from Fires Creek in which the substrate is much coarser and dominated by the gravel and cobble size classes. Conversely, the highest embeddedness values ($\chi^2 = 155$, df = 8, P = 0.0001) and conductivities were recorded from Brasstown and the lowest from Fires Creek (Table 1 and Figure 1).

Salamander Surveys

Forty-eight different hellbenders were observed at least once during the study. Fourteen were captured from Brasstown Creek, sixteen from Tusquitee Creek, and eighteen from Fires Creek. Recapture rates of *C. a. alleganiensis* during the study were too low for analysis. Five recaptures of four individuals were recorded from Brasstown Creek, five recaptures of five individuals from Tusquitee Creek, and three recaptures of three individuals from Fires Creek.

There was no significant difference in hellbender snout-vent length frequency distribution, mean snout-vent length, or catch per unit effort among the three streams (Figure 2; $\chi^2 = 4.88$, df = 10, P = >0.8 and Appendix B; Table B1).

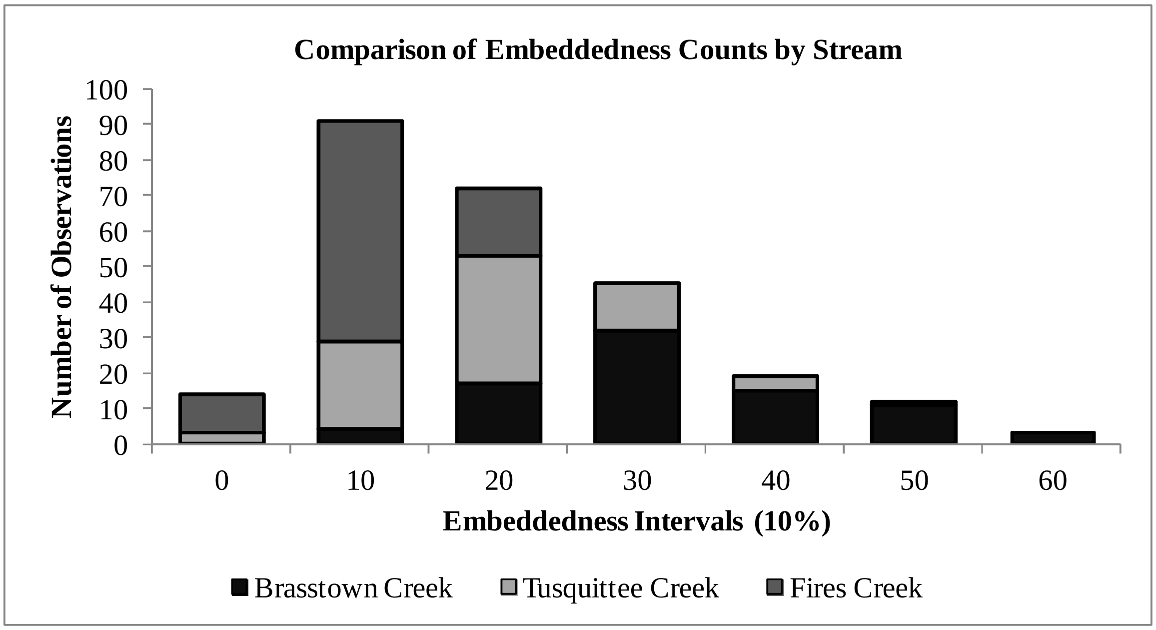

Figure 3. Comparison of embeddedness counts within Brasstown, Tusquitee, and Fires Creeks, Clay County, NC.

Cryptobranchus a. alleganiensis collected from Tusquitee Creek were more massive than those collected from Fires Creek but not from Brasstown Creek (Figure 3; $F_{2,44} = 3.14$, P = 0.05). Mass corrected by snout-vent length showed a similar relationship to that observed for mass. The mass:snout-vent length relationship of hellbenders collected in Tusquitee Creek was significantly different from those collected from Fires Creek but not from Brasstown Creek. (Figure 4; $F_{2,43} = 4.91$, P = 0.01).

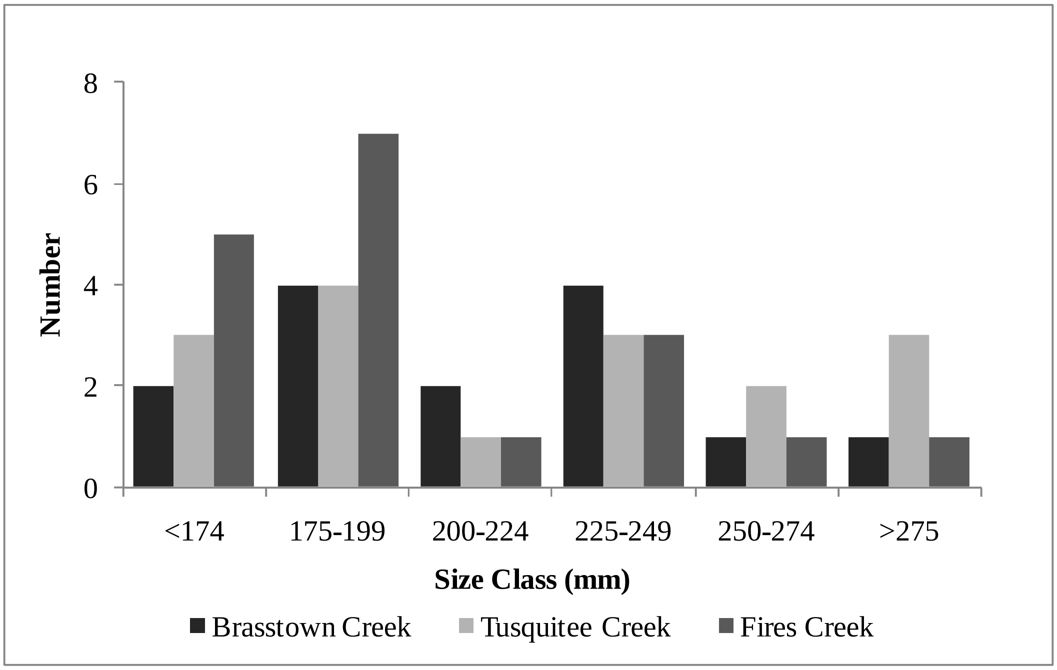

Figure 4. Comparison of *C. a. alleganiensis* snout-vent length frequency distributions among Brasstown, Tusquitee, and Fires Creeks, Clay County, NC.

The slope of the mass-snout-vent length relationship was not significantly different among streams, (Appendix B; Table B2. $F_{2,40} = 1.38$, $P = >0.34$). The overall size of hellbenders per unit length differed among streams (Figure 5; $F_{2,42} = 9.24$, $P = 0.0005$).

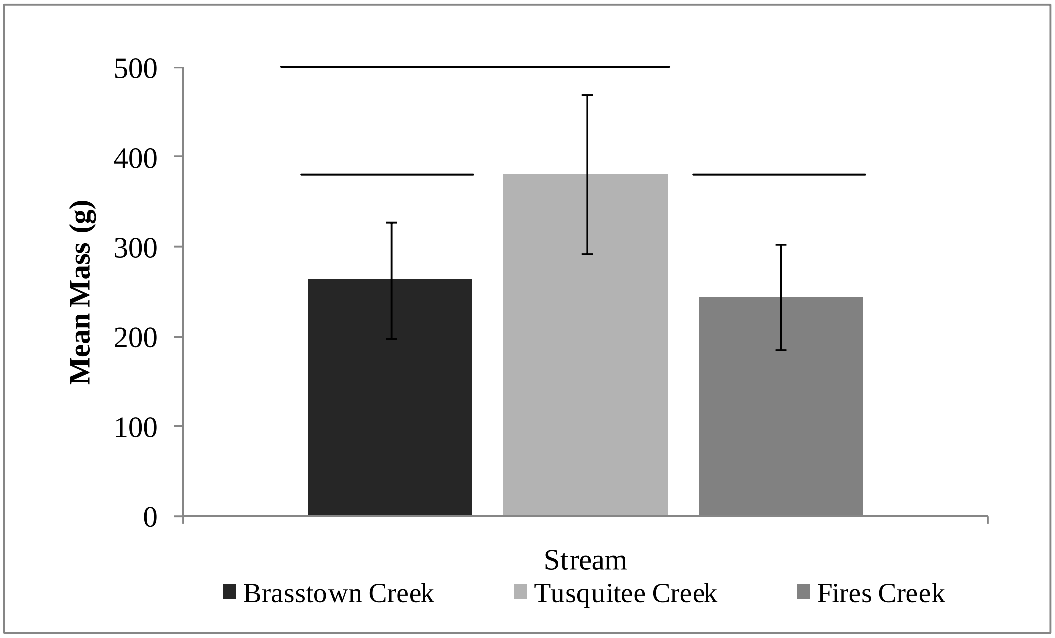

Figure 5. Mean mass of *C. a. alleganiensis* observed within Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. Error bars represent the 95% confidence interval. Scheffe's test results depicted by lines above like means.

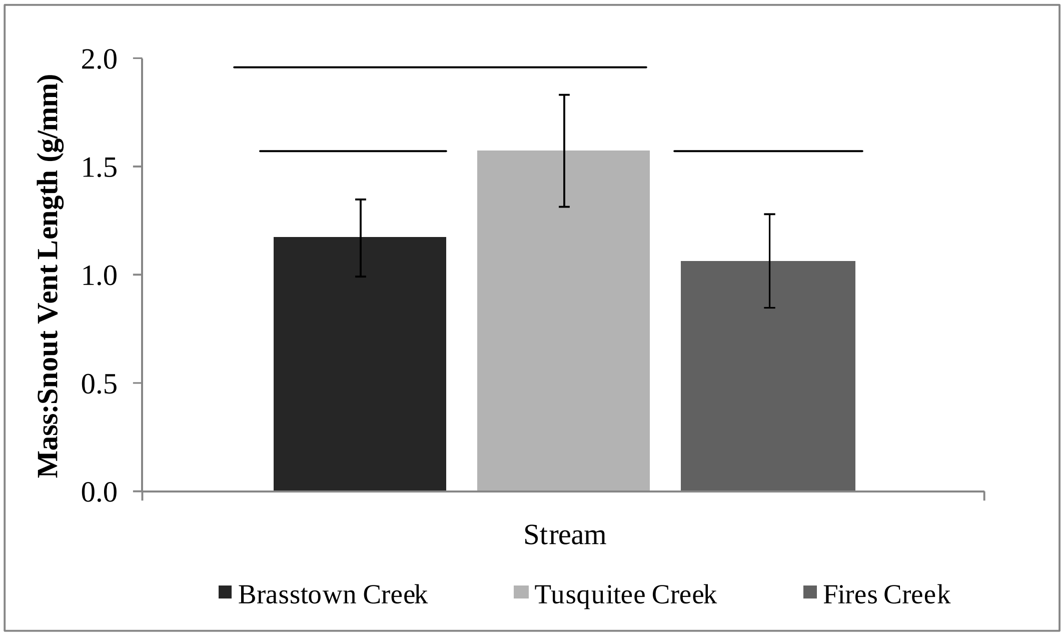

Figure 6. Mean mass corrected by snout-vent length of *C. a. alleganiensis* observed within Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. Error bars represent the 95% confidence interval. Scheffe's test results depicted by lines above like means.

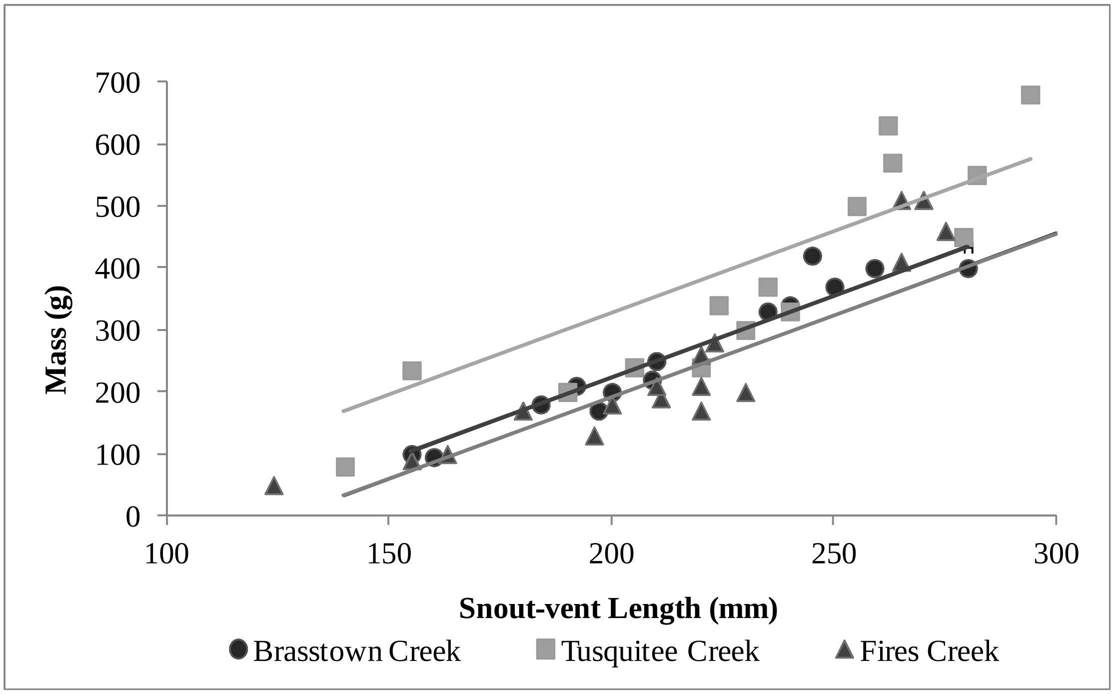

Figure 7. Mass vs. snout-vent length of *C. a. alleganiensis* observed within Brasstown, Tusquitee, and Fires Creeks, Clay County, NC, assuming equal slopes.

An analysis of variance performed on the residuals from the mass vs. snout-vent length regression indicated a similar relationship, with Tusquitee Creek deviating significantly from Fires Creek but not from Brasstown Creek (Figure 6; $F_{2,43} = 9.04$, P = 0.0005).

A significant difference in hellbender mean maximum tail height was noted only when corrected by mass (Figure 7; $F_{2,44}= 3.43$, P = 0.04 and Appendix B; Table B1). The tail height:mass ratio was lowest for Tusquitee Creek, the creek where average mass was highest. Conversely, results from analysis of covariance indicated that no significant differences existed among streams in slope, or intercept assuming equal slopes, of the tail height-mass relationship (Appendix B; Table B3. $F_{2,41} = 0.74$, P= >0.4, $F_{2,41} = 0.25$, P = >0.7, respectively).

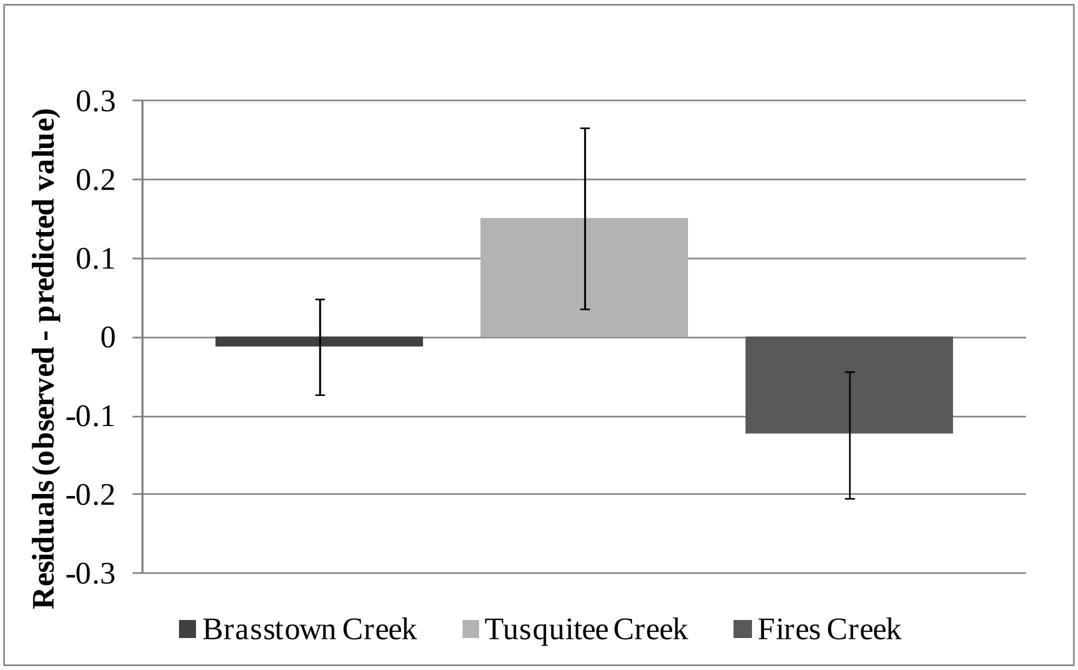

Figure 8. Mean residual mass calculated from mass vs.snout-vent length regression of *C. a. alleganiensis* observed at all sites within Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. Error bars represent the 95% confidence interval.

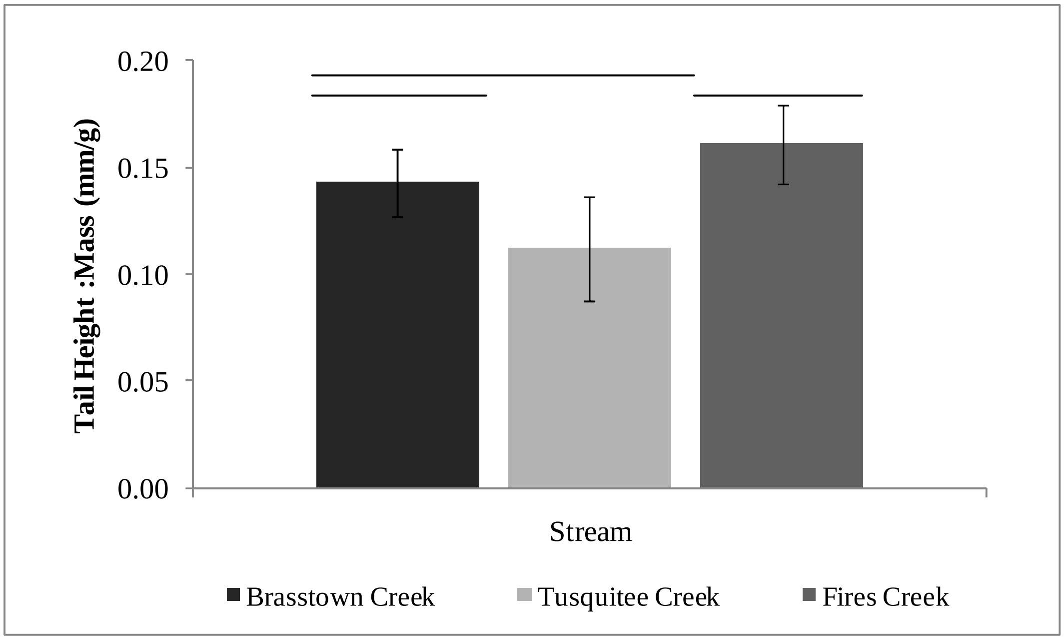

Figure 9. Mean tail height corrected by mass of *C. a. alleganiensis* observed within Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. Error bars represent the 95% confidence interval. Scheffe's test results depicted by lines above like means.

Mean tail circumference of hellbenders within Tusquitee Creek was marginally higher than that of both Brasstown and Fires Creeks (Figure 8; $F_{2,45} = 2.53$, $P = 0.09$). Tail circumference corrected by or compared with mass or snout-vent length was not significantly different among streams (All $P > 0.2$; Appendix B; Tables B1, B4, and B5).

Crayfish Surveys

I collected 43 crayfish during 134 trap nights: 10 from Brasstown Creek, 17 from Tusquitee Creek, and 16 from Fires Creek. Two species were represented during the study, *Cambarus (Cambarus) bartonii* (Fabricius) and *Cambarus (Cambarus) sp. A* (Cooper 2004, Hobbs and Peters 1977).

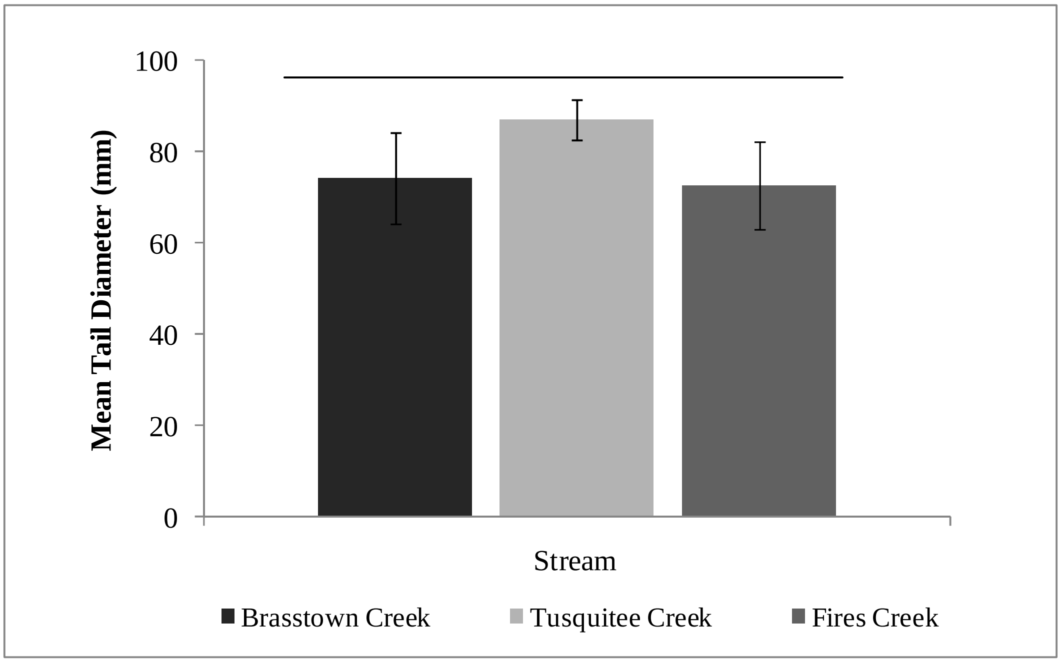

Figure 10. Mean tail circumference of *C. a. alleganiensis* observed within Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. Error bars represent the 95% confidence interval. Scheffe's test results depicted by a line above like means.

Crayfish total carapace length, mass, and catch per unit effort did not differ significantly among the three streams (Table 3).

The slope of the relationship between crayfish total carapace length and mass was significantly different at Fires Creek when compared to Brasstown and Tusquitee Creeks. The slope of the regression was lowest in the crayfish collected from Fires Creek compared to both Brasstown and Tusquitee Creeks (Figure 9; $F_{5,37} = 145.9$, $P = 0.0006$).

Table 3. Summary of the biometrics and catch rates of crayfish within Brasstown Creek, Tusquitee Creek and Fires Creek study areas, Clay County, NC

	Means by Stream (± 95% CI)		
Total Carapace Length (mm)	Brasstown	Tusquitee	Fires
	28.9 ± 3.46	27.76 ± 2.62	30.12 ± 2.8
$F_{(2,40)} = 0.57$ p = >0.5			
Mass (g)	Brasstown	Tusquitee	Fires
	9.22 ± 3.16	8.25 ± 2.36	9.56 ± 1.82
$F_{(2,40)} = 0.50$ p = >0.6			
Catch/unit effort (crayfish/trap night)	Brasstown	Tusquitee	Fires
	0.22 ± 0.17	0.38 ± 0.04	0.36 ± 0.28
$F_{(2,40)} = 0.64$ p = >0.5			

I observed no significant relationship between *C. a. alleganiensis* catch per unit effort (HCPUE) and crayfish catch per unit effort (CCPUE) (Appendix C; Table C1; $F_{3,7} = 0.15$, $P = 0.8$).

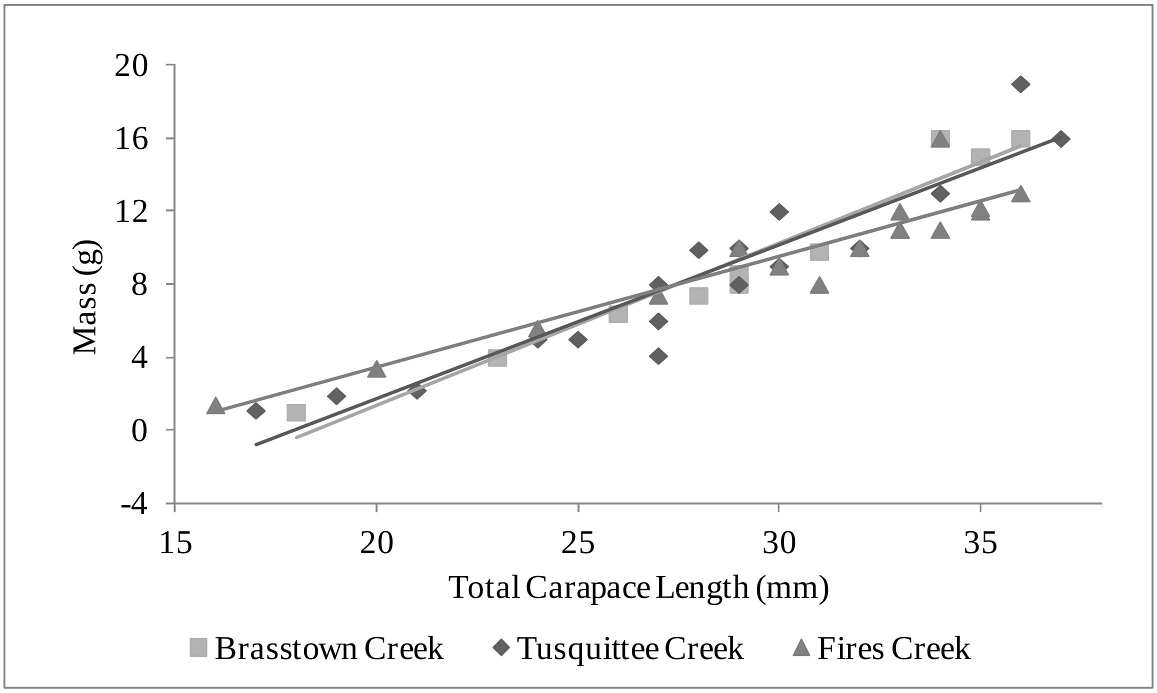

Figure 11. Comparison of slopes of mass vs. total carapace length of crayfish observed within Brasstown, Tusquitee, and Fires Creeks, Clay County, NC.

DISCUSSION

Stream Characterization

The three streams differed in watershed area. The Brasstown catchment was the largest, followed by Tusquitee, and Fires Creek (Table 1). The difference in watershed area was not translated to stream widths in all cases. Brasstown Creek and Tusquitee Creeks were not significantly different from each other, and Fires Creek was approximately one meter narrower.

While all three streams are fourth order within the study areas, the differences in the watershed areas may be driving differences in the functionality of the streams. Of the three streams, Fires Creek is the least disturbed and is representative of a typical headwater stream. Fires Creek is a closed canopy, low productivity, cold-water stream with naturally low biological diversity and is classified as outstanding resource waters (Lorie Stroup, USDAFS, Personal Communication 2009, NCDENR 2010b).

Brasstown Creek, unlike Fires Creek, is a heavily urbanized stream in its headwaters. One National Pollution Discharge Elimination System (NPDES) wastewater treatment discharge is present in Young Harris, Georgia (NCDENR 2010b). This discharge represents an input of 0.24 million gallons per day (MGD) or approximately 0.01 cubic meters per second (CMS) of treated effluent into the system. Much of Brasstown Creek is an open canopy system with a history of sediment inputs, turbidity, and agricultural impacts. Brasstown Creek is in flux between a cool and warm-water system (NCDENR 2005). Brasstown Creek likely exhibits higher productivity relative to the other streams in the study. Physical water quality parameters measured during the study in conjunction with the North Carolina Division of Water Quality's 2005 Basinwide Assessment Report, support the assumption that an increased nutrient load may be causing an increase in productivity and species diversity within this watershed. Species richness of both fishes and benthic organisms was comparable with that found in Fires Creek (NCDENR 2010a, NCDENR 2010b).

Tusquitee Creek is intermediate in terms of watershed area and land use between both Fires and Brasstown Creeks. Tusquitee Creek has two minor NPDES discharges and drains a significant area of agricultural land (NCDENR 2012e). This stream is a cool-cold water system with relatively low fish species richness compared to both Fires and Brasstown Creeks (Lorie Stroup, Personal Communication 2009, NCDENR 2010b). Results from the NCDENR study with regard to the fishery were based upon sampling in one location and may not provide an accurate representation of fish species richness. Benthic macroinvertebrate species richness was similar among all streams (NCDENR 2005, NCDENR 2010a, and NCDENR 2010b).

All streams are underlain by a portion of the Tusquitee Quartzite and Nantahala Formation (Znt) map unit. The streams differed in three map units (Zam), (Zd), and (Zb). These differences in map units appear inconsequential, as many of the geologic units in the survey area were composed of schists or slates. Shists and slates are structurally characterized by cleavage and produce large slabs under weathering (USGS 2010). The schist and slate components of the underlying geology are the most likely source of suitable cover habitat for *C. a. alleganiensis* within these drainages.

Salamander Surveys

All three streams show a similar pattern in hellbender length frequency. In each stream, the majority of *C. a. alleganiensis* were grouped around the 175-199 mm and 225-249 mm length classes. This type of pattern usually suggests multiple year classes, an indication of past successful reproduction years. The more size classes observed within a sample, the healthier the population is thought to be. In this case, the small sample size limits is what can be inferred from the pattern.

Results from the study did not support the original hypotheses on the biometrics of *C. a. alleganiensis* among the three streams. *Cryptobranchus a. alleganiensis* length frequencies did not differ among streams. *Cryptobranchus a. alleganiensis* biometrics did not follow a progression with increased stream sedimentation, although a reoccurring pattern was observed throughout the study. Results routinely indicate differences in the *C. a. alleganiensis* population biometrics in Tusquitee Creek, especially when compared with Fires Creek populations. Specifically, tail circumference, mass, and mass corrected by and compared to snout-vent length deviated among these streams. All metrics of individuals collected from the *C. a. alleganiensis* population within Tusquitee Creek was significantly larger than those collected from Fires Creeks but not Brasstown Creek. One exception was noted when tail height was corrected by mass. Tail height was noted to be significantly less in Tusquitee Creek when corrected by mass, although the relationship was inverse to that hypothesized. Tail height as a stand-alone metric was not significantly different between streams indicating that tail height is not a reliable indicator of *C. a. alleganiensis* body condition or habitat condition as predicted. While multiple body metrics were shown to be significantly different between streams, the major driver for these differences is body mass of individual *C. a. alleganiensis*.

In general, increases in body mass are explained by an excess of caloric intake when compared with an individual's energy consumption and losses (Benke and others1988 and Benke 2009). In salamanders, it has been shown that increases in body metrics are the result (Scott and Fore 1995). Generally, increases in food availability or a decrease in competition for available resources is the driving factor behind increases in body mass. In other salamander species, increases in body metrics have been assumed indicative of increased fitness (Scott and Fore 1995), or related to reproduction efficiency (Fitzpatrick 1973). The factors responsible for increased tail circumference are equivalent to those for salamander mass, as the tail is a documented lipid storage site (Fitzpatrick 1976 and Jorgensen 1992).

Crayfish Surveys

Crayfish mass and total carapace length were not significantly different among streams. In contrast, the relationship between mass and total carapace length was significantly different for crayfish in Fires Creek compared to both Brasstown and Tusquitee Creeks. Crayfish in Fires Creek exhibited a slower increase of mass per unit length throughout the measured size range, suggesting a slower growth of individual crayfish. These slower growth rates are consistent with a lower productivity stream such as Fires Creek.

Predator-prey interactions have been linked to altered growth rates of prey species (Turner 2004, Arthur and others 2004, Brodin and Johansson 2004). Avoidance of predators has been related to reduce foraging time/efficiency and slower growth rates (Ball and Baker 1996). In other cases, predator interactions lead to increased growth rates due to reductions in prey population sizes (Nystrom and Abjornsson 2000, Peckarsky and others 2008). Crayfish typically fill the role of a predator species, specifically, in interaction with amphibians (Nystrom and Abjornsson 2000, Walls and others2002). Few studies have directly documented the effects of amphibian predation on crayfish. Crayfish, in the context of this study, are prey; however, crayfish could eat larval hellbenders. A study by Hill and Lodge (1995) showed that in the presence of a predatory fish, crayfish had reduced feeding rates and overall survival. Presumably, the slower growth rate of crayfish observed in Fires Creek may be explained by the lower productivity of the stream system.

Conclusions

The similarity in *C. a. alleganiensis* biometrics between Fires Creek and Brasstown Creek suggest those biometrics are related to multiple aspects of the stream systems. The increased sediment levels within Brasstown Creek may offset the bottom up effect of increased nutrient inputs by limiting the establishment of aufwuchs, thereby, decreasing the overall productivity of the system. Conversely, Fires Creek is naturally a low productivity system and more comparable to Brasstown in terms of productivity and *C. a. alleganiensis* biometrics.

Tusquitee Creek, being the intermediate stream in terms of watershed size and upstream impacts, may be experiencing an optimum mix of good water quality and disturbance, meaning that the level of disturbance is not so severe that it greatly impacts habitat, decreases productivity, or effects water quality. The intermediate disturbance hypothesis, suggested by Fox and Connell (1979), implies that disturbance at certain levels or cycles increases habitat heterogeneity and species richness. Moderate increases in sediment levels would add some nutrients to the stream in the form of organics and, in agricultural areas, nitrogen and phosphorus. The lower watershed in Tusquitee Creek has a considerable amount of agricultural lands but does not exhibit excessive levels of fine sediments.

Another approach to explaining the results noted within Tusquitee Creek is based upon the River Continuum Concept Vannote and others (1980). This approach focuses on the stream characteristics as a function of stream order. The larger the stream, the greater productivity and species richness based on a more fine organic material inputs, higher water temperature, and sunlight penetration. Tusquitee Creek, through disturbance within the riparian buffer and agricultural inputs, may function more like a larger order stream with higher productivity than would have occurred naturally. Fires Creek being a relatively undisturbed watershed functions as described by Vannote and others (1980). This concept deals with the natural change of characteristics of a watershed from headwaters to terminus and may not as applicable in describing streams with a history of extensive levels of disturbance such as Brasstown Creek.

Finally, the geographical position of the study areas within the three stream basins may be the most significant variable on the results of the study. All three study areas were located at approximately the same distance upstream from the Hiwassee River. For each stream, the additive impact of “upstream watershed area” on the habitat characteristics may be the most important variable. The effect of “upstream watershed area" is a gross simplification of a myriad of processes and variables effecting stream systems. In this case, the suite habitat variables found in the lower reaches of a watershed of similar size to Tusquitee Creek may be the major factor in the body condition of *C. a. alleganiensis*. Considering that both Brasstown and Fires Creek were insignificantly different in *C. a. alleganiensis* biometrics, it would be reasonable to make this loose correlation.

Whether or not the differences noted in *C. a. alleganiensis* biometrics and crayfish biometrics are related to anthropogenic influences, stream productivity, or study site position within the three streams, the fact remains that there are some significant differences among the three streams in regards to *C. a. alleganiensis* and crayfish.

Further Research

The major issue with studying a cryptic animal such as *C. a. alleganiensis* is that small sample sizes often limit clarity of the results. This scenario was especially true in this study. Interpretations were based on measurements from 48 individual hellbenders and 43 crayfish. Future studies should focus on single stream systems with additional and larger sites. Additionally, larval life stages were not considered during this study. Ultimately, the future of *C. a. alleganiensis* populations are dependent upon recruitment and survivorship of the next generation, and presently little is known about the larval life stage. Finally, future work should better investigate the effects of watershed position, sedimentation, and anthropogenic effects on *C. a. alleganiensis* habitat variables, specifically, food resources, cover habitat, and water chemistry.

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APPENDIX A

Table A1. Results from analysis of variance (ANOVA) of stream metrics, Brasstown, Tusquitee, and Fires Creeks, Clay County, NC.

	Means by Stream (± 95% CI)		
Metric	Brasstown	Tusquitee	Fires
Boulder Diameter (mm)	526.2 ± 65.7	511.57 ± 55.8	526.8 ± 74.4
$F_{2,87} = 0.07$ P = >0.9			
Width (m)	11.49 ± 0.72	13.07 ± 0.67	14.04 ± 0.72
$F_{2,137} = 13.2$ P = <0.0001			

Table A2. Summary of the underlying geology of the study sites located on Brasstown, Tusquitee and Fires Creeks, Clay County, NC.

Map unit	
	Murphy Belt Rocks
Zmb	Mineral Bluff Formation and Nottely Formation
Zma	Andrews Formation and Murphy Marble
Zb	Brasstown Formation
Znt	Tusquitee Quartzite and Nantahala Formation
	Great Smoky Group (Ocoee Supergroup)
Zgs	Great Smoky undivided
Zd	Dean Formation
Zam	Ammons Formation
Zwe	Wehutty Formation
	Basement Rocks
Zybn	Biotite Gneiss

Wiener, L.S., and Merschat, C.E., 1992; Geologic map of Southwestern North Carolina Including Adjoining Southeastern Tennessee and Northern Georgia.

Table A3. Summary of the watershed characteristics of sites within Brasstown Creek, creek and Fires Creek. Clay County, NC

	Site		
Metric	Brasstown Site 1	Brasstown Site 2	Brasstown Site 3
Watershed area (sq km)	215	190	190
Stream order at study sites	4	4	4
Avg stream width (m ± 2se)	13.7 ± 0.8	15.2 ± 1.2	13.0 ± 1.5
Slope	0.01	0.01	0.007
Cross Sectional Area (m^2)	20.9	-	-
Mean Depth (m)	1.2	-	-
Max Depth (m)	2.2	-	-
D_{50} calculated (mm)	9.8	6.0	0.4
Boulder diameter (mm ± 2se)	550 ± 135	461.5 ± 69.8	567 ± 122.3

	Tusquittee Site 1	Tusquittee Site 2	Tusquittee Site 3
Watershed area (sq km)	108	105	101
Stream order at study sites	4	4	4
Avg stream width (m ± 2se)	14.2 ± 1.1	14.0 ± 0.8	11.0 ± 0.8
Slope	0.01	0.008	0.03
Cross Sectional Area (m^2)	11.8	-	-
Mean Depth (m)	0.7	-	-
Max Depth (m)	0.9	-	-
D_{50} calculated (mm)	57.0	62.6	82.8
Boulder diameter (mm ± 2se)	564.2 ± 98.3	449.7 ± 107.2	520.8 ± 114.7

	Fires Site 1	Fires Site 2	Fires Site 3
Watershed area (sq km)	57	54	50
Stream order at study sites	4	4	4
Avg stream width (m ± 2se)	12.5 ± 0.8	9.3 ± 0.5	12.6 ± 1.4
Slope	0.002	0.008	0.003
Cross Sectional Area (m^2)	8.8	-	-
Mean Depth (m)	0.6	-	-
Max Depth (m)	0.8	-	-
D_{50} calculated (mm)	120.0	88.0	72.0
Boulder diameter (mm ± 2se)	478.1 ± 83.8	657.9 ± 160.4	444.3 ± 93

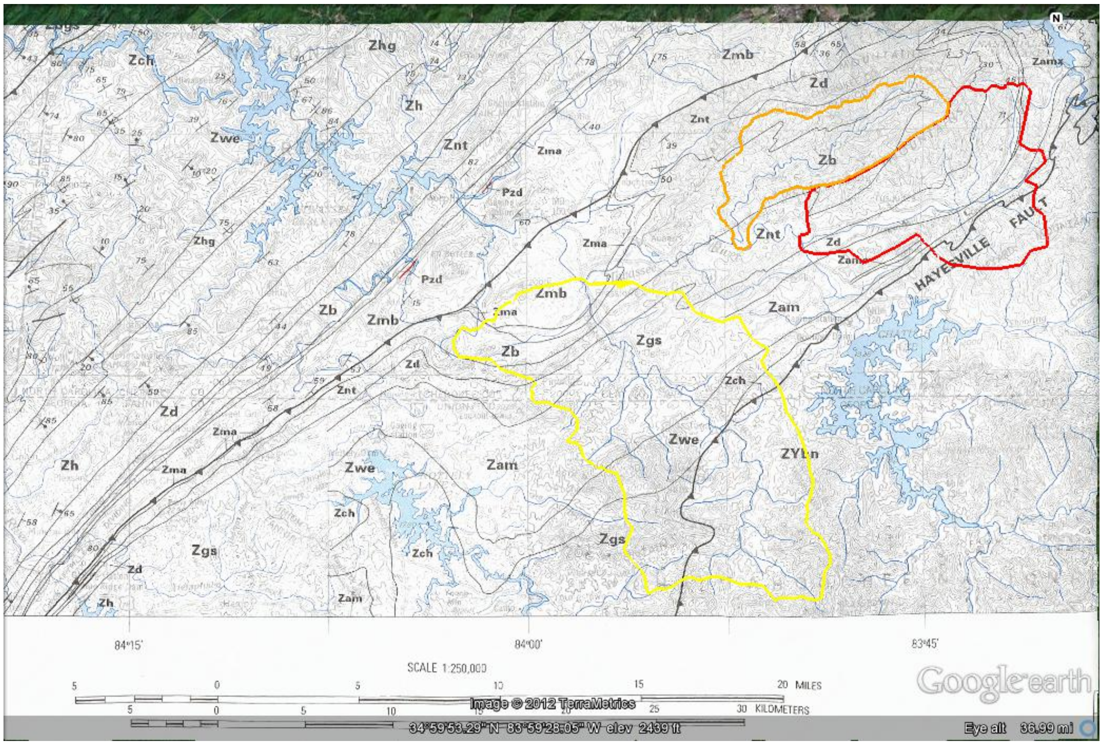


Figure A2. Geologic map and study area watersheds. From: Goggle Earth 2012 and Wiener and Merschat 1992)

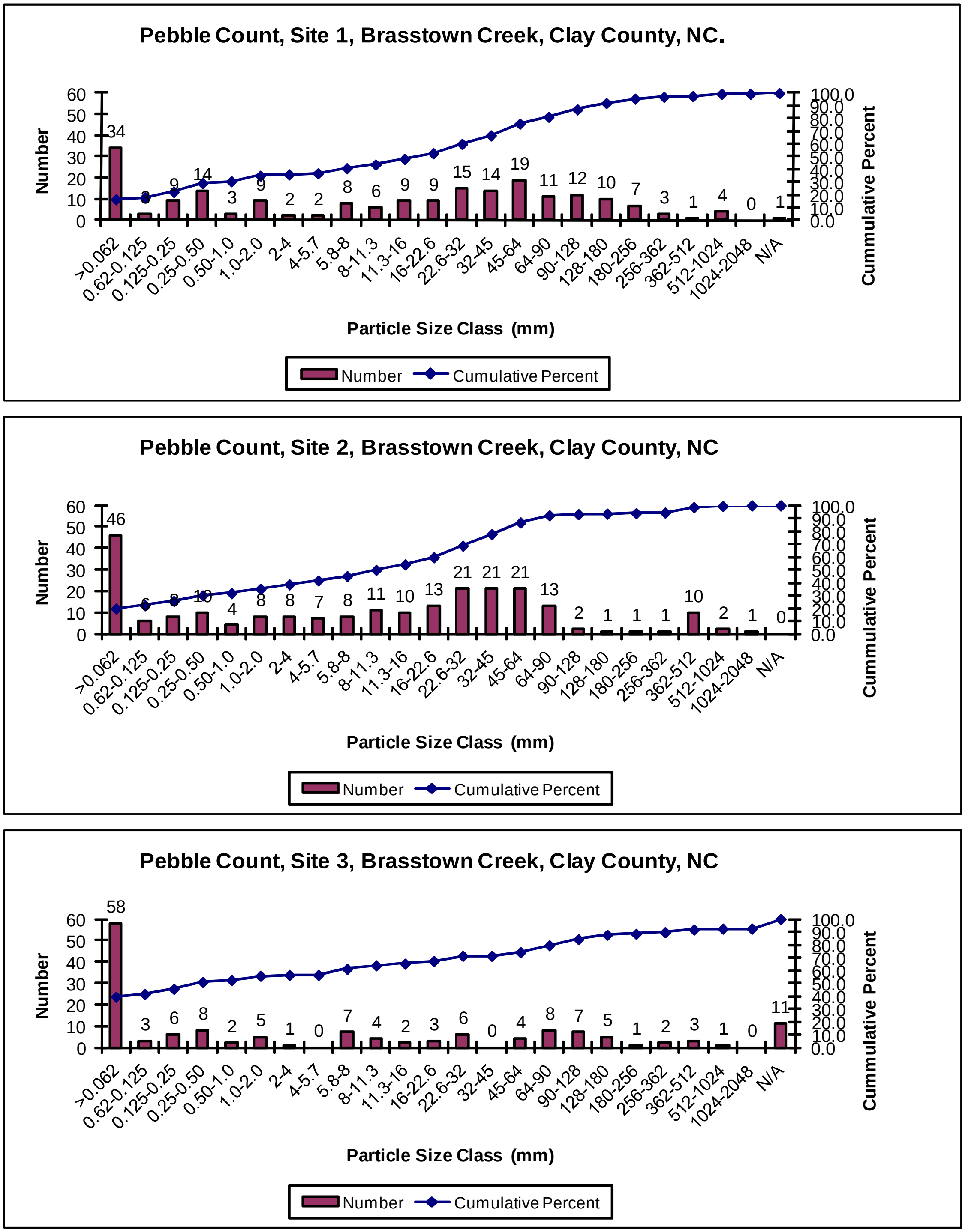
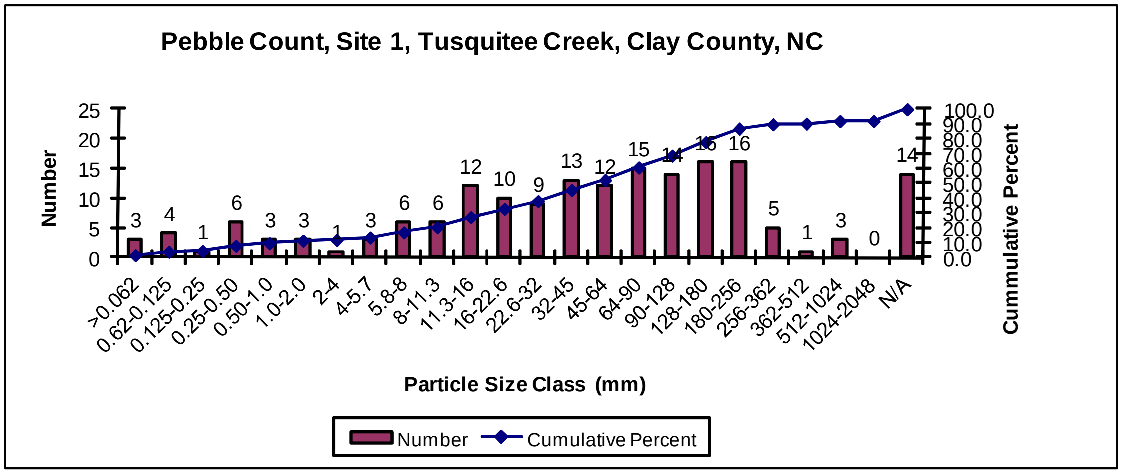
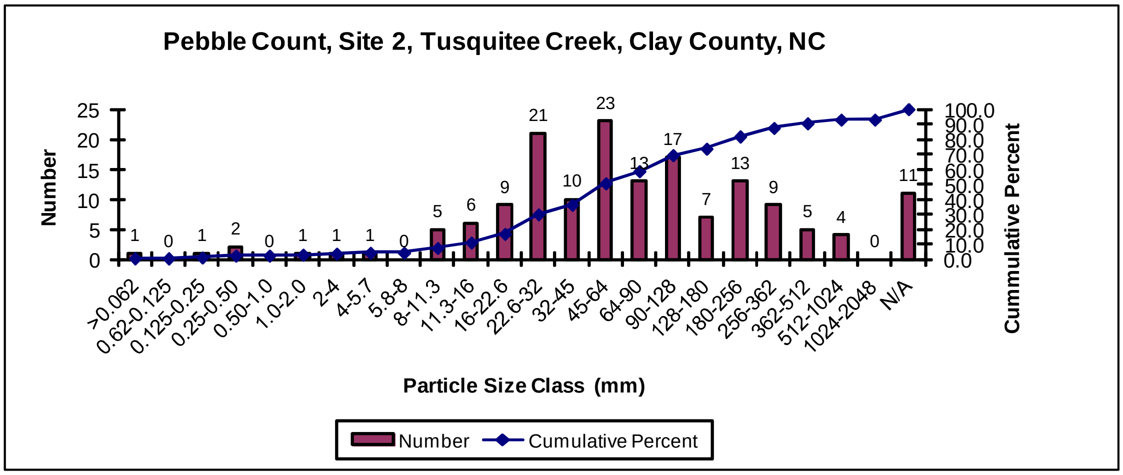


Figure A2. Pebble counts, Brasstown Creek. Sites ordered from downstream to upstream.





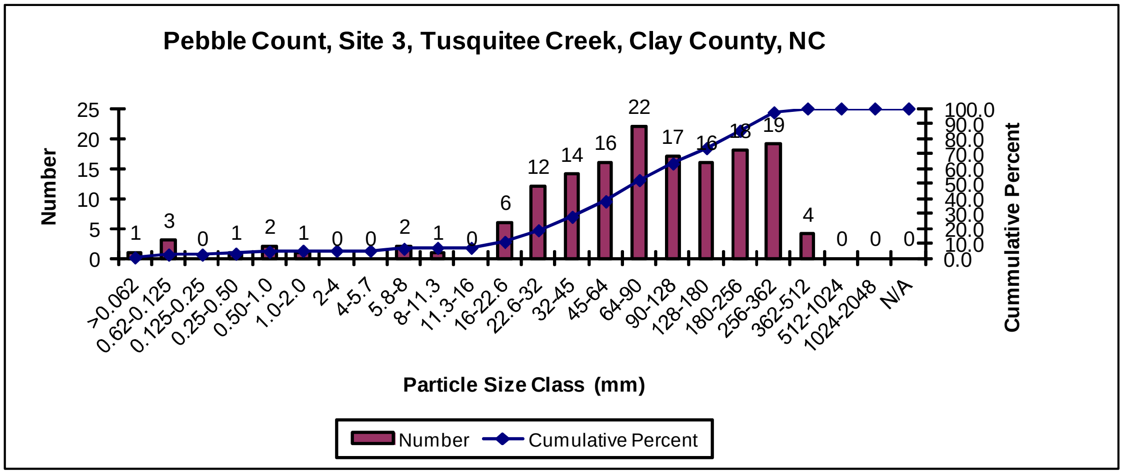
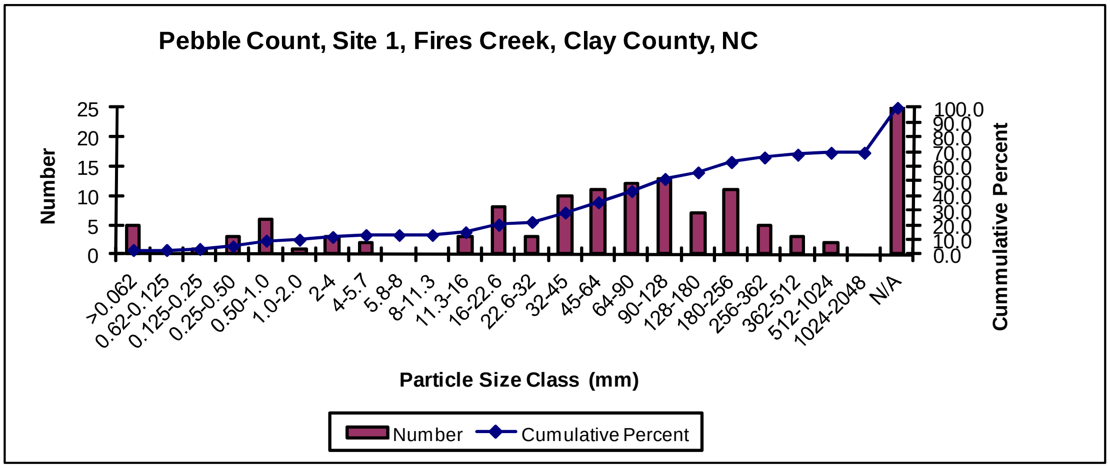
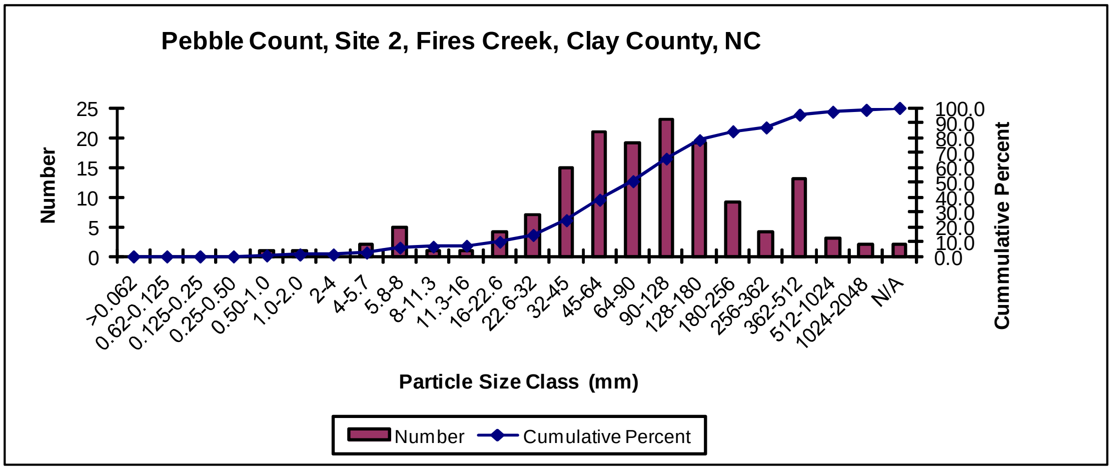


Figure A3. Pebble counts, Tusquitee Creek. Sites ordered from downstream to upstream.


Pebble Count, Site 1, Fires Creek, Clay County, NC


Pebble Count, Site 2, Fires Creek, Clay County, NC

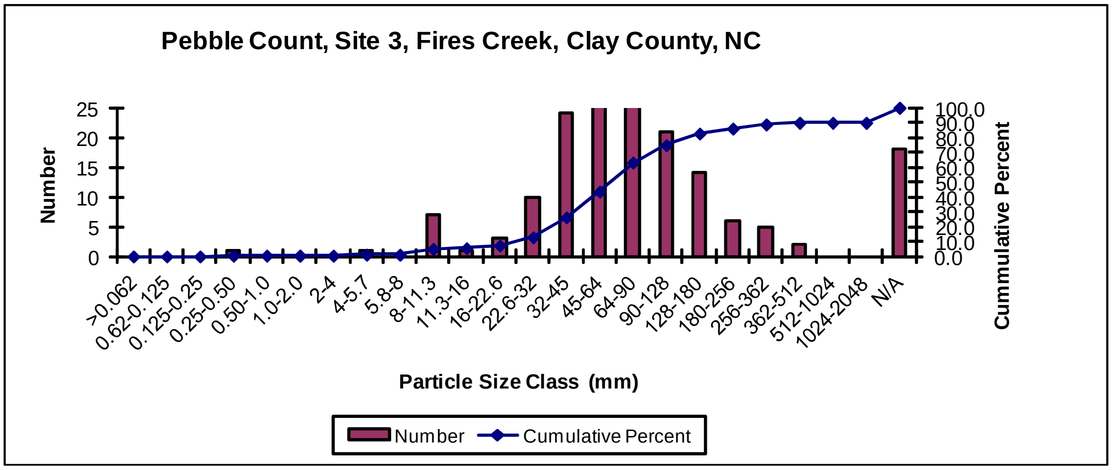

Pebble Count, Site 3, Fires Creek, Clay County, NC

Figure A4. Pebble counts, Fires Creek. Sites ordered from downstream to upstream.

APPENDIX B

Table B1. Results from analysis of variance (ANOVA) of *C. a. alleganiensis* biometrics collected from Brasstown, Tusquitee, and Fires Creeks, Clay County, NC.

	Means by Stream (± 95% CI)		
Biometric	Brasstown	Tusquitee	Fires
snout-vent length (mm) $F_{2,44}$ = 0.68 P = 0.5105	215.43 ± 19.5	230.38 ± 21.3	213.35 ± 20.02
mass (g) $F_{2,44}$ = 3.14 P = 0.0529	263.21 ± 58.6	381.00 ± 88.69	244.44 ± 64.87
tail circumference (mm) $F_{2,45}$ = 2.53 P = 0.0912	74.21 ± 9.96	87.00 ± 6.57	72.67 ± 9.43
tail height (mm) $F_{2,45}$ = 2.22 P = 0.1205	32.29 ± 3.11	37.31 ± 4.44	31.61 ± 3.83
mass:snout-vent length (g/mm) $F_{2,43}$ = 4.91 P = 0.0120	1.17 ± 0.18	1.57 ± 0.26	1.06 ± 0.22
tail height:snout-vent length (mm/mm) $F_{2,44}$ = 1.88 P = 0.1649	0.15 ± 0.01	0.16 ± 0.02	0.15 ± 0.01
tail height:mass (mm/g) $F_{2,44}$ = 3.43 P = 0.0413	0.14 ± 0.03	0.11 ± 0.02	0.16 ± 0.04
tail circumference:mass (mm/g) $F_{2,44}$ = 1.41 P = 0.2556	0.32 ± 0.074	0.28 ± 0.08	0.39 ± 0.1
tail circumference:snout-vent length (mm/mm) $F_{2,44}$ = 1.59 P = 0.2157	0.35 ± 0.04	0.38 ± 0.03	0.34 ± 0.04
hellbender catch/unit effort (hellbenders/survey) $F_{2,33}$ = 0.09 P = 0.9125	1.54 ± 0.87	1.75 ± 1.06	1.83 ± 0.41
hellbender residual mass (g) calculated from mass vs. snout-vent length regression $F_{2,43}$ = 9.24 P = 0.0005	-0.01 ± 0.06	0.15 ± 0.12	-0.12 ± 0.08

*Untransformed means and confidence intervals are listed for ease of interpretation.

Table B2. Results from analysis of covariance (ANCOVA) for *C.a. alleganiensis* ln mass vs. ln snout-vent length, in Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. (Lower section values based upon assumption of equal slopes)

Source	df	Type III SS	MS	F	P
Stream	2	0.09230243	0.04615122	1.38	0.264
Ln sl	1	11.73027247	11.73027247	350.03	<.0001
Ln sl X stream	2	0.07473008	0.03736504	1.11	0.3379
Error	40	1.34047438	0.03351186		
Source	df	Type III SS	MS	F	P
Stream	2	0.62241059	0.31120529	9.24	0.0005
Ln sl	1	12.51340741	12.51340741	371.37	<0.0001
Error	42	1.41520446	0.03369534		

Table B3. Results from analysis of covariance (ANCOVA) for *C.a. alleganiensis* ln tail height vs. ln mass vs., in Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. (Lower section values based upon assumption of equal slopes)

Source	df	Type III SS	MS	F	P
Stream	2	0.02230041	0.01115021	0.73	0.4875
Ln mass	1	1.4991627	1.4991627	98.31	<0.0001
Ln mass X stream	2	0.02244816	0.01122408	0.74	0.4852
Error	41	0.62520394	0.01524888		
Source	df	Type III SS	MS	F	P
Stream	2	0.00751776	0.00375888	0.25	0.7803
Ln mass	1	1.74110702	1.74110702	115.6	<0.0001
Error	43	0.64765211	0.01506168		

Table B4. Results from analysis of covariance (ANCOVA) for *C. a. alleganiensis* ln tail circumference vs. ln mass in Brasstown, Tusquitee, and Fires Creeks, Clay County, NC (Lower section values based on assumption of equal slopes).

Source	df	Type III SS	MS	F	P
Stream	2	0.13725561	0.0686278	0.97	0.3886
Ln mass	1	1.11565169	1.11565169	15.73	0.0003
Ln mass X stream	2	0.12885227	0.06442613	0.91	0.4112
Error	41	2.90869425	0.07094376		
Source	df	Type III SS	MS	F	P
Stream	2	0.05023382	0.02511691	0.36	0.7028
Ln mass	1	1.03229519	1.03229519	14.61	0.0004
Error	43	3.03754651	0.07064062		

Table B5. Results from analysis of covariance (ANCOVA) for *C. a. alleganiensis* ln tail circumference vs. ln snout-vent length, in Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. (Lower section values based on assumption of equal slopes).

Source	df	Type III SS	MS	F	P
Stream	2	0.06973941	0.0348697	0.48	0.6217
Ln sl	1	1.07906403	1.07906403	14.88	0.0004
Ln sl X stream	2	0.06210953	0.03105476	0.43	0.6545
Error	41	2.9727025	0.07250494		
Source	df	Type III SS	MS	F	P
Stream	2	0.27210441	0.1360522	1.93	0.1578
Ln sl	1	1.05147477	1.05147477	14.9	0.0004
Error	43	3.03481202	0.07057702		

Table B6. Results from analysis of covariance (ANCOVA) for *C.a. alleganiensis* ln tail height vs. ln snout-vent length, in Brasstown, Tusquitee, and Fires Creeks, Clay County, NC. (Lower section values based upon assumption of equal slopes)

Source	df	Type III SS	MS	F	P
Stream	2	0.01967818	0.00983909	0.47	0.6309
Ln sl	1	1.50261585	1.50261585	71.15	<0.0001
Ln sl X stream	2	0.0189558	0.0094779	0.45	0.6415
Error	41	0.86588113	0.02111905		

Source	df	Type III SS	MS	F	P
Stream	2	0.07677687	0.03838843	1.87	0.1671
Ln sl	1	1.68447475	1.68447475	81.86	<0.0001
Error	43	0.88483693	0.0205776		

APPENDIX C

Table C1. Results from analysis of covariance (ANCOVA) for crayfish catch per unit effort (CCPUE) vs. *C. a. alleganiensis* catch per unit effort (CPUE), in Brasstown, Tusquitee, and Fires Creeks, Clay County, NC

Source	df	Type III SS	MS	F	P
Stream	2	2.52855191	1.26427595	3.35	0.1718
CCPUE	1	0.65073761	0.65073761	1.73	0.2803
CCPUE X stream	2	2.41528958	1.20764479	3.2	0.1801
Error	3	1.13085492	0.37695164		
Source	df	Type III SS	MS	F	P
Stream	2	0.2159996	0.1079998	0.15	0.8626
CPUE	1	0.08033024	0.08033024	0.11	0.7501
Error	5	3.5461445	0.7092289		