HABITAT, DISTRIBUTION, AND DIET OF THE TENNESSEE BOTTLEBRUSH CRAYFISH (*BARBICAMBARUS SIMMONSI*)

BY

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THESIS

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ABSTRACT

In order to effectively manage and conserve species, it is essential to have a basic understanding of their ecology. Unfortunately, such information is unavailable for most crayfish species, including the two members of the genus *Barbicambarus*. To obtain ecological data I conducted surveys for *Barbicambarus simmonsi* within the Shoal Creek drainage in Lawrence County, Tennessee and Lauderdale County, Alabama from Summer 2013-Spring 2014. The objectives of the first part of my study were to determine distribution, habitat use, and site occupancy of the Tennessee Bottlebrush Crayfish, *Barbicambarus simmonsi*. The distribution was increased from 3 to 14 sites across Shoal Creek. Habitat use modeling did not yield significant results, but observations indicate large flat boulders as utilized habitat. Flow was the most important covariate when determining site occupancy, but this should be interpreted with some caution, as it does not match field observations. The objectives of the second part of my study were to determine trophic position and diet of *B. simmonsi* and *B. cornutus* using stable isotope analysis and gut content analysis, and determine if there was correlation between carapace length and trophic position for each species. Crayfish for the analysis were collected during the fall of 2012 and the spring of 2013. My results indicate both species occupy a higher trophic position compared to other sympatric crayfish species. I also noted a significant positive correlation between trophic position and body size. My data suggest *Barbicambarus* species function as a predator within the stream and there is some niche separation between them and other crayfish species.

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CHAPTER 1: GENERAL INTRODUCTION

Since its inception in the 1980s, the term biodiversity has accrued a variety of definitions (Meinard et al. 2014). The widely accepted definition is that biodiversity encompasses all biological variation, ranging from large-scale biomes and ecosystems down to the level of species, genes, and behaviors (Purvis and Hector 2000). At times species richness is used as a general indicator of biodiversity in an area (Fleishman et al. 2006). Because species richness and ecosystem function are linked (Hooper et al. 2005), knowledge of both is essential for effective conservation practices. Conservation of biodiversity and ecosystem function is often hindered when basic ecological data is lacking for particular species and is exacerbated when knowledge is lacking for keystone species (Taylor et al. 2007).

Freshwater ecosystems are perhaps the most endangered ecosystem in the world, mainly due to anthropogenic effects (Dudgeon et al. 2006; Martinuzzi et al. 2014). Human water practices have led to severe degradation of freshwater systems by pollution and habitat loss, which in turn have had negative effects on biodiversity (Martinuzzi et al. 2014). Aquatic ecosystems are biologically invaluable because they support 10% of the world's species (Carrizo et al. 2013). Nowhere is the endangerment of freshwater systems more pronounced than in biologically rich regions of the world with high levels of human population centralization and growth, with one example being the southeastern United States (Martinuzzi et al. 2014). The endangerment of southeastern freshwater systems is alarming, as the area is a known hotspot for aquatic biodiversity for fishes, crayfishes, mussels, and amphibians (Taylor et al. 2007; Martinuzzi et al. 2014). Although the conservation status of fish for the southeastern United States is regularly assessed (Deacon et al. 1979; Williams et al. 1989; Warren et al. 2000), the

status of crayfish is not, and ecological data is lacking for crayfish in general (Taylor et al. 2007; Moore et al. 2013)

Among aquatic invertebrates, crayfish are a diverse group often comprising a large amount of the biomass in aquatic systems and having large impacts on the ecosystem (Momot 1995; Taylor and Soucek 2010). Crayfish are considered a keystone species because of their capacity to affect vegetation and invertebrate communities through predation and their ability alter habitat through substrate disturbance (Momot 1995; Geiger and Alcorlo 2005). Nearly two-thirds of the 600+ crayfish species occur in North America (Taylor et al. 2007), and about 3.4 new species are described each year (Moore et al. 2013). Of the 410 North American species, approximately two-thirds, mostly endemic species, occur in the southeast United States (Taylor et al. 2007). Endemic species typically have small ranges and are at higher risk to threats such as habitat loss or invasive species (Jones and Bergey 2007). Thus, it is imperative to conserve crayfish, a unique biotic resource, by broadening our knowledge of basic crayfish ecology.

The genus *Barbicambarus* is a unique genus in the eastern United States consisting of only two species. The first record of *Barbicambarus cornutus* was by Faxon in 1884 from the Green River and the species ranges within the Barren and upper Green river systems in Kentucky and Tennessee (Hobbs 1974). The first record of *Barbicambarus simmonsi* was by Taylor and Schuster in 2010 from Shoal Creek in Tennessee and Alabama. Crayfish within *Barbicambarus* are characterized by having antennae densely covered in long setae, a larger than average body size, and a strongly dorsoventrally flattened carapace (Taylor and Schuster 2010). Little is known about the ecology of *Barbicambarus* species other than anecdotal observations on habitat found in their initial descriptions. Moore et al. (2013), describe how basic life history and ecological knowledge of populations and species are essential for management and conservation.

This extends to other aquatic species. The objectives of my study were to determine the distribution, habitat use, and site occupancy of *B. simmonsi* and the trophic position and diet of both species in the genus, which will aid in the future management of these rare species. For example, Denic and Geist (2014) show functional habitat must be preserved for the pearl mussel to survive. Without functional habitat, captive breeding methods must be enlisted to preserve mussel populations (Denic and Geist 2014). Diet is also important, as the presence of preferred food items is an indicator of good habitat quality. For example, Hilderbrand et al. (1999) described how important the availability of salmon was in determining habitat quality for North American brown bears.

Initially, *B. simmonsi* was only known from two locations within Shoal Creek and one of its tributaries (Taylor and Schuster 2010). Since Taylor and Schuster (2010), no work has been done to determine the full extent of the distribution of *B. simmonsi*. I conducted presence/absence surveys along the entirety of Shoal Creek, and collected detailed habitat data. My goals were to determine distribution, habitat use, and site occupancy.

Although it has been previously assumed many crayfish are opportunistic omnivores, studies also report evidence of some species being primary predators (Momot 1995; Parkyn et al. 2001; Taylor and Soucek 2010; Thomas and Taylor 2013). Unfortunately, little is known on the diets of most crayfish species, including *B. simmonsi* and *B. cornutus.* Therefore, I conducted a study to examine the diet and trophic position of both species using gut content and stable isotope analysis. I also examined the setose structure of *Barbicambarus* antennae, which are unique to the genus and can possibly perform a function aiding in feeding behavior.

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CHAPTER 2: DISTRIBUTION AND HABITAT REQUIREMENTS OF *BARBICAMBARUS SIMMONSI*

INTRODUCTION

All organisms require certain structures or conditions in their environment for survival. Some organisms are generalists with broad habitat niches, while others are specialists with narrow habitat ranges. The distribution of specific habitat requirements is often comparable to the distribution of the species occupying them. Thus, distribution and habitat needs of a species are fundamental components of its ecology. Information on distribution is important as it allows for more effective management of native species and helps to limit introduction of non-native species (Peters et al. 2014). Habitat data is also essential for effective management, as multiple studies have shown many organisms require specific habitat in order to survive. For example, Williams et al. (1981) illustrated how hellbenders are dependent upon dissolved oxygen levels, temperature, and flow in swift water areas for survival. Denic and Geist (2014), show pearl mussels are affected by levels of fine sediment deposition. The above studies show specific ecological data is needed for effective management of specific species.

Smith et al. (1995) indicate how a thorough understanding of habitat requirements for crayfish species is essential to any kind of conservation strategy. Thus, it is alarming we lack basic ecological information for many North American species. Moore et al. (2013) estimated natural history data is lacking for 305 of 347 crayfish from the USA and Canada. With increasing levels of urbanization threatening aquatic habitats, natural history data is becoming more essential for efforts to conserve our diverse array of crayfish species within the southeastern United States (Taylor et al. 2007; Martinuzzi et al. 2014). An example of a crayfish

from this potentially affected region is the Tennessee bottlebrush crayfish, *Barbicambarus simmonsi*.

Barbicambarus simmonsi is a narrowly endemic crayfish, known only from a single stream system, Shoal Creek, a direct tributary of the Tennessee River in southern Tennessee and northern Alabama. The genus *Barbicambarus* is characterized by its larger than average body size and their unique antennae, which are densely covered in long, hair like setae (Taylor and Schuster 2010). At the beginning of my study, the only known ecological information for *B. simmosi* was it seemed to prefer large flat slab rocks within deeper parts of riffles and it was only known from 3 sites within the Shoal Creek drainage in southern Lawrence County, Tennessee and northern Lauderdale County, Alabama. The objectives of my study were to: 1) determine the distribution of *B. simmonsi* within Shoal Creek and nearby streams; 2) document habitat use; and 3) use field data to produce an updated distribution map and to model detectability and occupancy for the species.

METHODS

Study Area/Site Selection

I conducted my study in the Tennessee River drainage in southern Lawrence County, Tennessee and northern Lauderdale County, Alabama. Most of my sampling occurred at 36 sites in riffle/run sections of Shoal Creek and 1 in Factory Creek, a tributary to Shoal Creek. Three sites had recent records for *B. simmonsi*, whereas 34 sites were new. I used satellite imagery of Shoal Creek, to grossly locate reaches having wadeable riffles and runs suitable for sampling and ground truthed the reaches as sampling sites. I divided the creek into sections consisting of 8.0 stream km. Four sites, at least 1.6-stream km apart, were selected within each section using a randomization algorithm in Microsoft Excel. In addition, I sampled eight unwadeable pools. Pool

locations were recorded during my first sampling period through Shoal Creek. The eight pools sampled were randomly selected from recorded localities using the randomization algorithm in Microsoft Excel. Again, sites were not permitted to be within one mile of each other. Finally, I sampled fourteen sites in Tennessee River tributaries directly to the east and west of Shoal Creek. Within Cypress Creek and Little Cypress Creek to the west, nine sites were sampled and within Bluewater Creek to the east, five sites were sampled. Sites in Cypress Creek, Little Cypress Creek, and Bluewater Creek were not randomly selected, but were chosen wherever there was a road-stream crossing for ease of access.

Sampling Methods

I conducted my presence/absence sampling from 03 June to 26 September of 2013. At each site, I calculated sampling reach length by multiplying the stream width by 10 (adapted from Barbour et al. 1999). To reduce sampling time, I set maximum reach length at 100 m. I then divided each sampling reach into 10 m sections then conducted 3 kick sets in each using a 1.5x3 meter mesh minnow seine. Within each 10 m section, I performed kick sets on a staggering diagonal beginning with one of the downstream corners, moving to the middle and then to the opposite upstream corner (**Fig. 2.1**). Kick sets consisted of disturbing the substrate or overturning rocks within 1 m^2 of the streambed immediately upstream of the anchored seine to catch any organisms I flushed out. After each kick, I recorded the presence/absence of *B. simmonsi* and other crayfish species. All crayfish captured were counted, identified to genus, and saved live in a bucket. After sampling, all crayfish were returned to the stream.

I recorded the following habitat variables: substrate, depth, flow, distance to shore, presence/absence of vegetation, and the presence/absence of other crayfish species. I determined substrate using a metal substrate cross, where a substrate classification is made at the center and

each point of the cross. Substrate types were determined using a modified Wentworth particle size scale (Bovee and Milhous 1978) and defined as; sand (S), gravel (G), pebble (P), cobble (C), boulder (B), silt (Si), or bedrock (Bed) then given a score of sand and/silt=1, bedrock=1.5, gravel=2, pebble=3, cobble=4, and boulder=5 (Bain et al. 1985 and Litvan et al. 2010). Depth and flow were measured using a Global Water flow probe. Depth was measured in meters and flow was measured in m/sec. Recorded habitat variables were used as covariates in my subsequent occupancy modeling. Upon completing sampling for a reach, an additional 30 min (1.5 man-hours) of seining specific microhabitats (usually boulders) missed by randomized sampling was conducted to minimize the probability of recording a false absence for *B. simmonsi* at the site. All individual *B. simmonsi* captured were measured for carapace length to the nearest mm using calipers and gender was recorded by determining the presence/absence of gonopods before release at their capture points. Specimens incidentally injured or killed during sampling were vouchered for deposition in the Illinois Natural History Survey Crustacean Collection. Other crayfish species were identified to genus and returned to the stream.

Unwadable pools could not be sampled using seines because of depth and preliminary trials showed *B. simmonsi* was unresponsive to trapping. Thus, I sampled visually by SCUBA diving. At each site, I measured pool length and divided it into five equal length sections. Divers then descended to the stream bottom and a location was selected by tossing a rock over one's shoulder within each of the five sections. The diver visually surveyed for *B. simmonsi* within a 2 m radius by slowly turning over rocks. Any *B. simmonsi* captured were returned to the surface where they were sexed and measured. Specimens captured from my pool sites were vouchered for the Illinois Natural History Survey Crustacean Collection.

Cypress Creek, Little Cypress Creek, and Bluewater Creek were all sampled purely for presence/absence of *B. simmonsi* during spring of 2014. At the time these sites were sampled, the habitat use for *B. simmonsi* had been determined so sampling was targeted on large flat boulders. Sites were sampled using a 1.5x3 meter kick seine for three man-hours.

Distribution Maps

I created dot distribution maps of *B. simmonsi* using DIVA GIS (Ver. 7.5.0.0). Maps were created with a base layer outlining the United States, and two layers outlining the rivers and streams of North America. Coordinates of sites were entered into a Microsoft Excel file and then imported into DIVA GIS where they were placed as the top layer on the map.

Habitat Analysis

I consolidated all kicksets and transects to represent site-averaged measurements of depth, flow rate, distance to shore, and width. In order to convert my substrate measurements into a more continuous variable, I categorized them as follows, mean modal substrate type, mean maximum substrate type, and mean minimum substrate. Presence or absence of vegetation remained a binary variable. Using IBM® SPSS® Statistics (ver. 22.0, 2013), I performed variable reduction using Principle Components Analysis (PCA) on the continuous variables of average depth, average flow rate, average distance to shore, average stream width, mean modal substrate, mean maximum substrate, and mean minimum substrate. I retained all components with an Eigenvalue <1.00 and then used VARIMAX rotation for the factor loadings. I then performed a mixed effects binary logistic regression using the lme4 package (Bates et al. 2014) in R (R Core Team, 2014), to determine if the random effect (site nested within habitat), fixed effects (presence/absence of vegetation), and covariates (retained principle components) explained the presence/absence of *B. simmonsi*. I included repeated site-specific data for sites

where crayfish were captured multiple times. I determined which model was the best and defined my candidate set as having a cumulative sum of Akaike Weights of 0.95 using the R package MuMIn (Bartoń, 2014) The MuMIn package was also used to perform model averaging if there were multiple competing models.

Occupancy Modeling

I removed highly correlated variables based on correlation analysis using Pearson's and Spearman's rank correlation coefficients for continuous and categorical data, respectively in JMP (Ver. 9.0.0) (**Appendix A; Table A.2 and A.3).** To account for imperfect detection (Mackenzie et al. 2006), I then used single-season occupancy models in the program PRESENCE (Ver. 6.1) to estimate occupancy and detection probabilities of *B. simmonsi* using the uncorrelated habitat variables, standardized with a z-transformation, as covariates. Candidate models were ranked using Akaike's Information Criterion (AIC) and model selection was based on Akaike Weights. In the event there was no equivocal top model (*e.g.* Akaike Weights > 0.90), I performed full model averaging (Johnson and Omland 2004). Such a situation can occur when models are nested and the complexity added by covariates has little improvement on the model (Burnham and Anderson 2002; Richards 2008).

RESULTS

Distribution

I found *B. simmonsi* at 14 of 43 (33%) sites sampled, three of which were known previously (**Figure 2.2, Table 2.1**). *Barbicambarus simmonsi* was found to occur in two of the eight pool sites sampled, a habitat not previously sampled for the species. *Barbicambarus simmonsi* typically occurred in low numbers where no more than 1-5 individuals were found at a

site after extensive sampling (**Table 2.1**). Within Cypress Creek, Little Cypress Creek, and Bluewater Creek, *B. simmonsi* did not occur at any of the 14 sites sampled.

Habitat Analysis

Summary of raw habitat variables at the site level did not indicate any one covariate as good indicators of *B. simmonsi* habitat use. I found *B. simmonsi* present within a broad range of habitat measurements, which overlapped with the habitat measurements in which they were absent (**Fig. 2.2**). The species was consistently found under large flat boulders within a site, at depths between 0.20-0.60 m and in flow ranging from zero to 0.90 m/s (**Table 2.2**). There were cases, especially in the upstream half of the creek, where suitable habitat was abundant but *B. simmonsi* was not found. Habitat covariates for all kick sets are presented in **Appendix B.** Using PCA my data could be summarized by three components explaining 85.5% of the cumulative variance (**Table 2.3**). PC-1 had mean minimum substrate, mean maximum substrate, and mean modal substrate, load positively with an eigenvalue of 3.12 explaining 44.53% of variance. I termed PC-1, Substrate (**Table 2.4**). In PC-2, the average distance to shore and average stream width both loaded positively with an eigenvalue of 1.86 explaining 26.40% of variance. I termed PC-2, Stream Width (**Table 2.4**). Lastly, PC-3 had covariates for depth and flow load positively with an eigenvalue of 1.01 explaining 14.43% of variance. I termed PC-3, Depth and Flow (**Table 2.4**). Modeling yielded 12 competitive models and the top model only included the substrate component (**Table 2.5**). However, none of my models were highly weighted (W_i) so model averaging was utilized. Model averaging indicates none of the parameters are good predictors of habitat use as all parameter estimates bound zero (**Fig. 2.4**).

Occupancy Modeling

I detected *B. simmonsi* at eight of my 43 sites sampled with my standardized sampling methods (naïve occupancy = 0.1860). A candidate set of seven competitive models was developed for detectability (ΔAIC < 2, **Table 2.6**) (Burnham and Anderson 2004). I decided to use the model with the highest AIC weight and highest model likelihood (**Table 2.6**) which contained both depth and substrate. Detectability increased with increased depth and larger substrate ((β_{depth}=1.54, *SE*=0.5400; $\beta_{\text{substrate}}$=0.43, *SE*=0.27).

I developed a set of six competitive models for occupancy of *B. simmonsi* (**Table 2.7; all other occupancy models can be seen in Appendix A, Table A.1**). By summing my Akaike weights (W_i) across all models I found my most important covariate, out of my six, for predicting site occupancy was flow (flow W_i=0.74, presence/absence of other crayfish species W_i=0.33, site type W_i= 0.26 depth W_i=0.26, width W_i=0.25, substrate W_i=0.24) (Symonds and Moussalli 2010). Flow was a component of all six competitive models and occupancy probability was higher at sites with faster flows (**Table 2.7; Fig. 2.5**). However, none of my candidate models were strongly weighted (W_i), meaning no single model overwhelmingly supports the data. Thus, model averaged parameters suggest occupancy was positively related to flow (β_{FLOW}=1.56, *SE*=1.25) and stream width (β_{WIDTH}=0.03, *SE*=0.73). Occupancy was negatively related to site type (β_{SITE}=-0.04, *SE*=1.89), presence/absence of other crayfish species (β_{OS}=-0.48, *SE*=0.57), substrate type ($\beta_{SUBSTRATE}$=-0.06, *SE*=0.58), and depth (β_{SITE}=-0.04, *SE*=0.34). However, because of large standard error, none of the parameters are significant.

DISCUSSION

Distribution

My results indicate *B. simmonsi* occurs over a wider range than previously recorded within Shoal Creek, but are still endemic to a limited portion of the stream system. I captured individuals primarily in the middle and lower regions of Shoal Creek. Based on my surveys, the distribution of *B. simmonsi* in Shoal Creek extends from near the mouth of the Poplar Branch in southwestern Lawrence County, Tennessee down to the County Road 8 stream crossing in Lauderdale County, Alabama. Despite sampling, it appears *B. simmonsi* does not occur in the adjacent streams along the Tennessee/Alabama border. Although adjacent streams were not sampled as extensively, I rigorously sampled the similar habitat found in Shoal Creek. Adjacent stream sites were sampled during the spring of 2014 when the weather was uncharacteristically cold for the time of year, and was more similar to winter conditions. Multiple studies have crayfish have reduced levels of activity during periods of low temperatures and some retreat to deeper portions of their habitat or burrow into the banks, which could also explain my failure to detect any individuals (Aiken 1968; Momot and Gowing 1972; Flint 1977; Grow and Merchant 1980; Karplus et al. 1998; Bubb et al. 2002). Some sites did have what appeared to be favorable habitat that could have warrant future site visits to ensure the species is not there. Stream-road crossings were the only sites sampled as well, meaning there is potential for *B. simmonsi* to occur on less accessible sites along the streams. Areas of possible *B. simmonsi* occurrence have been shaded in green in **Figure 2.2**.

Habitat Analysis

For my habitat analysis, neither the analysis of our raw data or of our parameter estimates yielded results that were good indicators of *B. simmonsi* habitat use at the site level. This could potentially be explained by the uniformity of Shoal Creek. Habitat was similar across most of the stream leading to low habitat variability between sites. The rarity of *B. simmonsi* led to low

levels of detection and a small number of sites for which to measure positive habitat covariates, which in turn led to the development of weaker models for predicting habitat use.

Because my analysis did not yield any good predictors of habitat use I can only describe habitat use based on field observations which indicated *B. simmonsi* uses large flat boulders as habitat. My observation is consistent with the type of habitat described by Taylor and Schuster (2010) as being utilized by the specimens collected at their two sites. It is also similar to habitats used by its sister species *Barbicambarus cornutus* (Taylor and Schuster 2004). I found specimens from a variety of microhabitats spanning a wide range of flows, depths, and widths, which is potentially due to the dynamic nature of Shoal Creek, which can experience large and rapid fluctuation in flow and depth during rain events. As such, flow and depth may not play a large role in determining habitat for *B. simmonsi*. This was further supported by the presence of *B. simmonsi* in unwadable pools that have greater depth than the riffles and runs and low flow rates of 0.2000-0.4000 m/s. However, even within the pools, *B. simmonsi* use the same type of large boulder substrate. I also found the majority of *B. simmonsi* within the lower half of Shoal Creek, indicating stream width could potentially be a factor in habitat use.

Occupancy Modeling

My occupancy results indicate flow is the most important factor in determining site occupancy out of my six covariates. The species description for *B. simmonsi* described the flow at the two sites sampled to be strong in all regions, lending support to my results (Taylor and Schuster 2010). *B. cornutus* is also described as occurring in areas having current (Taylor and Schuster 2004). However, I captured *B. simmonsi* under a range of flow rates and thus the species is capable of persisting in sites with low flow.

Although my results indicate *B. simmonsi* occupancy is related to flow, they should be interpreted carefully. During my sampling, I discovered *B. simmonsi* uses large flat substrate as habitat. Because nearly every *B. simmonsi* encountered during sampling occurred underneath or near a large boulder, I would have expected occupancy to be related to substrate. In my analysis, substrate was not a significant variable because I recorded only overall substrate type at each site. The boulders *B. simmonsi* use are rarely the dominant substrate, and can be missed with my standardized sampling. Thus, a future study should include an added site covariate accounting for the presence/absence or abundance or large boulders. Given my results, the addition of a boulder covariate should yield better predictive models.

I did not find width to be an important covariate, even though we only found specimens within the bottom half (widest part) of Shoal Creek. Width may play an important factor because wider stretches of stream provide more area overall for habitat. It is more likely for a wide stretch of creek to contain more flat boulders than a narrower stretch. The presence/absence of other species was also not found to be important, which may be a result of different uses of habitat between sympatric species. Site type and depth were also found to be unimportant, likely because we found *B. simmonsi* from a wide range of depths in both pools and riffles.

Potentially, there are additional covariates explaining site occupancy of *B. simmonsi*. A study by Nolen et al. (2014) showed many local and landscape-scale variables can affect modeling, including geology and soils, which I was unable to account for. The study also found the local and landscape variables depended on the focal species being studied and the spatial scale of the modeling. If a future occupancy study was done, it could also be beneficial to look into a broader scale of environmental variables, such as geomorphology, urbanization effects, and hydrology of Shoal Creek and the surrounding land.

Conclusions

My results indicate I did not account for the appropriate covariates to explain habitat use or site occupancy using modeling, showing how difficult it can be to incorporate different sampling methods when attempting to model for a rare species. Until more efficient models can be produced, habitat specific targeted sampling is likely the most effective method in determining presence/absence of *B. simmonsi*. Future studies attempting to model occupancy or habitat for *B. simmonsi* should include a variable accounting for different size classes of boulders in order to increase the strength of models when trying to describe microhabitat. Future studies may also benefit from being designed to account for the apparent habitat specificity of *Barbicambarus* species by randomly selecting sites from a subset already determined to contain large pieces of substrate.

TABLES AND FIGURES

Table 2.1: Sites, coordinates, dates sampled, site type, and number of individuals for all sites where *B. simmonsi* were located in Shoal and Factory creeks.

Site	County, State	Date	Lat/Long	Type	No. *B. simmonsi*
Shoal Creek	Lauderdale, AL	3-Jun-13	34.95339, -87.59387	Riffle/Run	7
Shoal Creek	Lauderdale, AL	17-Aug-13	34.95339, -87.59387	Riffle/Run	6
Shoal Creek	Lawrence, TN	4-Jun-13	35.1203, -87.5089	Riffle/Run	14
Shoal Creek	Lawrence, TN	27-Mar-14	35.1203, -87.5089	Riffle/Run	2
Shoal Creek	Lawrence, TN	31-Jul-13	35.13384, -87.44829	Riffle/Run	1
Shoal Creek	Lawrence, TN	1-Aug-13	35.1066, -87.50932	Riffle/Run	2
Shoal Creek	Lawrence, TN	1-Aug-13	35.10046, -87.52119	Riffle/Run	1
Shoal Creek	Lawrence, TN	1-Aug-13	35.08008, -87.54720	Riffle/Run	4
Shoal Creek	Lawrence, TN	15-Aug-13	35.05922, -87.56850	Riffle/Run	2
Shoal Creek	Lawrence, TN	15-Aug-13	35.05085, -87.56524	Riffle/Run	6
Shoal Creek	Lawrence, TN	15-Aug-13	35.03847, -87.56725	Riffle/Run	4
Shoal Creek	Lawrence, TN	15-Aug-13	35.03237, -87.57729	Riffle/Run	1
Shoal Creek	Lauderdale, AL	16-Aug-13	35.00348, -87.57726	Riffle/Run	1
Shoal Creek	Lawrence, TN	25-Sep-13	35.04272, -87.56043	Pool	5
Shoal Creek	Lawrence, TN	25-Sep-13	35.01212, -87.57323	Pool	1
Factory Creek (Shoal Creek Trib)	Lawrence, TN	26-Sep-13	35.10119, -87.53975	Riffle/Run	4

Table 2.2: Habitat covariates at each kick set location where *B. simmonsi* were collected. Substrate measurements were recorded at each point of a substrate cross giving 5 readings. B=Boulder, C=Cobble, P=Pebble, G=Gravel, S=Sand, Si=Silt, and Bed=Bedrock. Lat/Longs are unique to the sampling reach, not the kick set location.

Site	Lat/Long	Substrate	Depth (meters)	Flow (m/s)	Site Type
Shoal Creek	34.95339, -87.59387	B,B,B,B,B	0.48	0.20	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.47	0.20	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.45	0.30	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.49	0.20	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.43	0.30	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.45	0.40	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.29	0.50	Riffle/Run
Shoal Creek	35.1203, -87.5089	B,B,G,G,S	0.56	0.10	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.33	0.20	Riffle/Run
Shoal Creek	"	B,B,G,G,G	0.39	0.30	Riffle/Run
Shoal Creek	"	B,B,G,G,G	0.39	0.30	Riffle/Run
Shoal Creek	"	B,B,G,G,G	0.39	0.30	Riffle/Run
Shoal Creek	"	B,B,B,G,G	0.30	0.20	Riffle/Run
Shoal Creek	"	B,B,G,G,G	0.29	0.20	Riffle/Run
Shoal Creek	"	B,B,B,B,G	0.50	0.10	Riffle/Run
Shoal Creek	"	B,B,G,G,G	0.50	0.40	Riffle/Run
Shoal Creek	"	B,B,G,G,G	0.50	0.40	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.50	0.40	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.33	0.20	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.33	0.20	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.33	0.20	Riffle/Run
Shoal Creek	35.13384, -87.44829	B,B,B,P,P	0.21	0.10	Riffle/Run
Shoal Creek	35.1066, -87.50932	B,B,B,G,Bed	0.40	0.50	Riffle/Run
Shoal Creek	"	B,B,Bed,Bed,Bed	0.62	0.90	Riffle/Run
Shoal Creek	35.10046, -87.52119	B,B,B,B,B	0.36	0.80	Riffle/Run
Shoal Creek	35.08008, -87.54720	B,B,B,B,B	0.27	0.90	Riffle/Run
Shoal Creek	"	B,B,B,C,G	0.39	0.90	Riffle/Run
Shoal Creek	"	B,B,C,G,G	0.40	0.80	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.37	0.90	Riffle/Run
Shoal Creek	35.05922, -87.56850	B,B,B,B,B	0.30	0.20	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.39	0.40	Riffle/Run
Shoal Creek	35.05085, -87.56524	B,B,C,C,P	0.29	0.00	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.45	0.80	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.21	0.30	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.20	0.20	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.26	0.20	Riffle/Run
Shoal Creek	"	B,B,B,G,Bed	0.52	0.30	Riffle/Run
Shoal Creek	35.03847, -87.56725	B,B,B,B,G	0.45	0.80	Riffle/Run
Shoal Creek	"	B,B,B,Bed,Bed	0.37	0.60	Riffle/Run
Shoal Creek	"	B,B,Bed,Bed,Bed	0.60	0.60	Riffle/Run
Shoal Creek	"	B,B,B,B,Bed	0.45	0.70	Riffle/Run
Shoal Creek	35.03237, -87.57729	B,B,B,P,G	0.64	0.90	Riffle/Run
Shoal Creek	35.00348, -87.57726	B,B,G,G,G	0.58	0.40	Riffle/Run
Shoal Creek	34.95339, -87.59387	B,B,G,G,Bed	0.29	0.40	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.41	0.50	Riffle/Run
Shoal Creek	"	B,B,B,B,B	0.42	0.50	Riffle/Run
Shoal Creek	"	B,B,B,Bed,Bed	0.33	0.40	Riffle/Run
Shoal Creek	"	B,B,B,G,G	0.27	0.30	Riffle/Run
Shoal Creek	"	B,B,B,G,Bed	0.20	0.40	Riffle/Run
Shoal Creek	35.04272, -87.56043	B,B,B,C,C	0.95	0.20	Pool
Shoal Creek	"	B,B,B,C,C	1.27	0.20	Pool
Shoal Creek	"	B,B,B,C,P	1.20	0.20	Pool
Shoal Creek	"	B,B,B,C,C	1.43	0.20	Pool
Shoal Creek	"	B,B,B,B,B	1.87	0.20	Pool
Shoal Creek	35.01212, -87.57323	B,B,C,C,C	1.00	0.40	Pool
Shoal Creek	35.10119, -87.53975	B,C,P,P,G	0.30	0.70	Riffle/Run

Table 2.2 cont'd

Site	Lat/Long	Substrate	Depth (meters)	Flow (m/s)	Site Type
Shoal Creek	"	B,B,B,P,G	0.29	0.90	Riffle/Run
Shoal Creek	"	B,B,B,P,P	0.49	0.60	Riffle/Run
Shoal Creek	"	dead: in the open	-	-	Riffle/Run
Shoal Creek	35.1203, -87.5089	In the open	0.31	0.30	Riffle/Run
Shoal Creek	"	B,B,B,P,G	0.31	0.30	Riffle/Run

Table 2.3: Eigenvalues and the variance explained for principal component scores for seven habitat covariates measured from 43 sites along Shoal Creek sampled for *Barbicambarus simmonsi*

Component	Total	% of Variance	Cumulative %
1	3.12	44.53	44.53
2	1.86	26.59	71.12
3	1.01	14.43	85.54
4	0.57	8.12	93.66
5	0.28	3.99	97.65
6	0.14	1.97	99.62
7	0.03	0.38	100.00

Table 2.4: Component loadings for seven habitat covariates measured from 43 sites on Shoal Creek which were sampled for *Barbicambarus simmonsi*. Scores greater than 0.50 and less than -0.50 are bolded. All values are from a PCV with a VARIMAX rotation.

	Component		
Variable	**1**	**2**	**3**
Depth	0.41	0.07	**0.79**
Flow	0.42	-0.16	**-0.73**
DistoShore	-0.21	**0.92**	-0.07
AvgWidth	0.14	**0.84**	0.35
MModSub	**0.97**	-0.07	0.15
MMaxSub	**0.91**	-0.06	0.03
MMinSub	**0.95**	-0.001	-0.10

Table 2.5: Ranking of habitat use models for *B. simmonsi.* The covariates include the intercept, principal component 1, principal component 2, principal component 3, and presence/absence of vegetation. Delta AICc is the AICc for a given model minus the AICc for the top model. K represents the number of parameters, W_i represents Akaike weights, -2*LL represents the log likelihood, and Likelihood represents the model likelihood. All 12 competitive models are presented.

Model	(Intercept)	PC1	PC2	PC3	Vegetation	ΔAICc	W_i	K	-2*LL	Likelihood
2	-1.49	0.75	NA	NA	NA	0.00	0.20	3.00	-22.05	1.00
1	-1.85	NA	NA	NA	NA	0.26	0.18	2.00	-23.33	0.88
4	-1.52	0.77	-0.49	NA	NA	1.50	0.10	4.00	-21.60	0.47
3	-1.90	NA	-0.46	NA	NA	1.79	0.08	3.00	-22.95	0.41
10	-2.11	0.89	NA	NA	+	1.98	0.08	4.00	-21.84	0.37
5	-1.91	NA	NA	-0.30	NA	2.21	0.07	3.00	-23.16	0.33
6	-1.58	0.75	NA	-0.19	NA	2.22	0.07	4.00	-21.96	0.33
9	-1.90	NA	NA	NA	+	2.55	0.06	3.00	-23.33	0.28
12	-1.96	0.87	-0.44	NA	+	3.77	0.03	5.00	-21.47	0.15
7	-1.96	NA	-0.46	-0.30	NA	3.85	0.03	4.00	-22.78	0.15
8	-1.55	0.77	-0.47	-0.16	NA	3.89	0.03	5.00	-21.54	0.14
11	-1.90	NA	-0.46	NA	+	4.19	0.02	4.00	-22.95	0.12

Table 2.6: Ranking of detection (p) models for *B. simmonsi* based on Akaike's Information Criterion (AIC). The covariates include depth, substrate, the presence/absence of vegetation (vegetation), distance from shore (distance), and flow. Delta AIC is the AIC for a given model minus the AIC for the top model. K represents the number of parameters, Wi represents Akaike weights, and -2*LL represents the log-likelihood. Likelihood represents the model likelihood. All models better than the intercept-only model are shown.

Model	ΔAIC	W_i	K	-2*LL	Likelihood
ψ(.),p(depth,substrate)	0.00	0.16	4	102.73	1.00
ψ(.),p(depth)	0.41	0.13	3	105.14	0.81
ψ(.),p(depth,substrate,vegetation)	0.99	0.10	5	101.72	0.61
ψ(.),p(distance,depth,substrate)	1.30	0.08	5	102.03	0.52
ψ(.),p(distance,depth)	1.49	0.08	4	104.22	0.47
ψ(.),p(depth,vegetation)	1.75	0.07	4	104.48	0.42
ψ(.),p(depth,flow,substrate)	2.00	0.06	5	102.73	0.37
ψ(.),p(distance,depth,substrate,vegetation)	2.22	0.05	6	100.95	0.33
ψ(.),p(depth,flow)	2.40	0.05	4	105.13	0.30
ψ(.),p(distance,depth,vegetation)	2.75	0.04	5	103.48	0.25
ψ(.),p(depth,flow,substrate,vegetation)	2.98	0.04	6	101.71	0.23
ψ(.),p(distance,depth,flow,substrate)	3.27	0.03	6	102.00	0.20
ψ(.),p(distance,depth,flow)	3.38	0.03	5	104.11	0.18
ψ(.),p(depth,flow,vegetation)	3.70	0.02	5	104.43	0.16
ψ(.),p(substrate)	4.38	0.02	3	109.11	0.11
ψ(.),p(distance,depth,flow,vegetation)	4.52	0.02	6	103.25	0.10
ψ(.),p(flow,substrate)	5.92	0.01	4	108.65	0.05
ψ(.),p(substrate,vegetation)	5.96	0.01	4	108.69	0.05
ψ(.),p(distance,substrate)	5.98	0.01	4	108.71	0.05
ψ(.),p(distance,flow,substrate)	7.26	0.004	5	107.99	0.03
ψ(.),p(flow,substrate,vegetation)	7.41	0.004	5	108.14	0.02
ψ(.),p(distance,substrate,vegetation)	7.52	0.004	5	108.25	0.02
ψ(.),p(distance,flow,substrate,vegetation)	8.69	0.002	6	107.42	0.01
ψ(.),p(.)	10.49	0.0008	2	117.22	0.01

Table 2.7: Ranking of occupancy (ψ) models for *B. simmonsi.* The covariates include flow, presence/absence of other species (OS), depth, substrate, stream width, and site type. Delta AIC is the AIC for a given model minus the AIC for the top model. K represents the number of parameters, W_i represents Akaike weights, -2*LL represents the log likelihood, and Likelihood represents the model likelihood. All models better than the intercept-only model are shown. All other models can be seen in Appendix A, Table A.1.

Model	ΔAIC	W_i	K	-2*LL	Likelihood
ψ(flow),p(depth,substrate)	0.00	0.14	5	98.37	1.00
ψ(flow,OS),p(depth,substrate)	1.18	0.08	6	97.55	0.55
ψ(flow,depth),p(depth,substrate)	1.99	0.05	6	98.36	0.37
ψ(flow,substrate),p(depth,substrate)	1.99	0.05	6	98.36	0.37
ψ(width,flow),p(depth,substrate)	2.00	0.05	6	98.37	0.37
ψ(site,flow),p(depth,substrate)	2.00	0.05	6	98.37	0.37
ψ(.),p(depth,substrate)	2.36	0.04	4	102.73	0.31

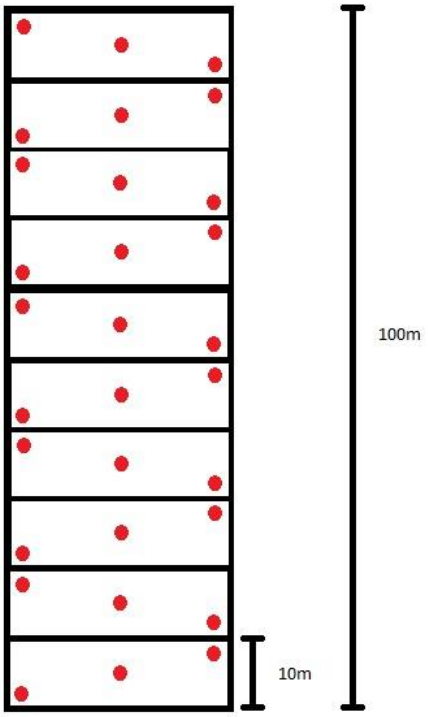

Figure 2.1: Schematic of crayfish occupancy sampling protocol within a 100m stream reach. Each red dot represents a kick set location.

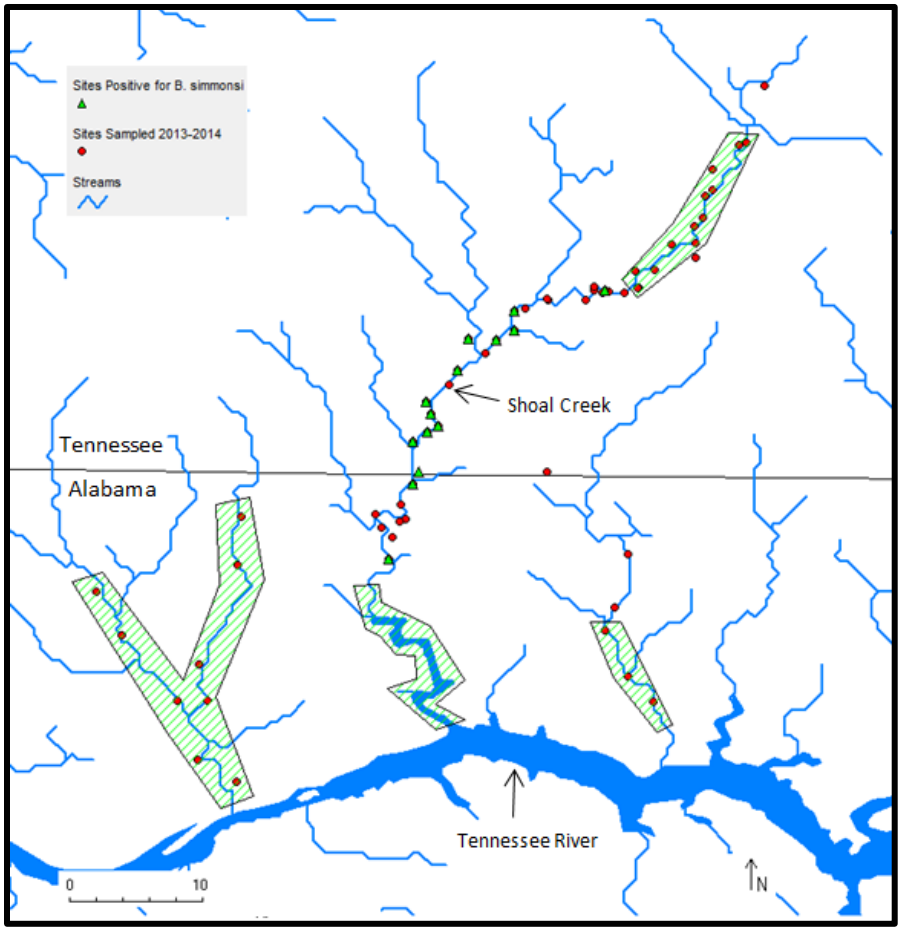

Figure 2.2: Map of sites sampled during 2013 and 2014 in the Tennessee River drainage of southern Tennessee and northern Alabama. Green dots indicate positive sites for *B. simmonsi*. Red dots indicate negative detection sites. Areas shaded in green represent areas where favorable habitat is possibly present, but *B. simmonsi* was either undetected or was not sampled for during surveys.

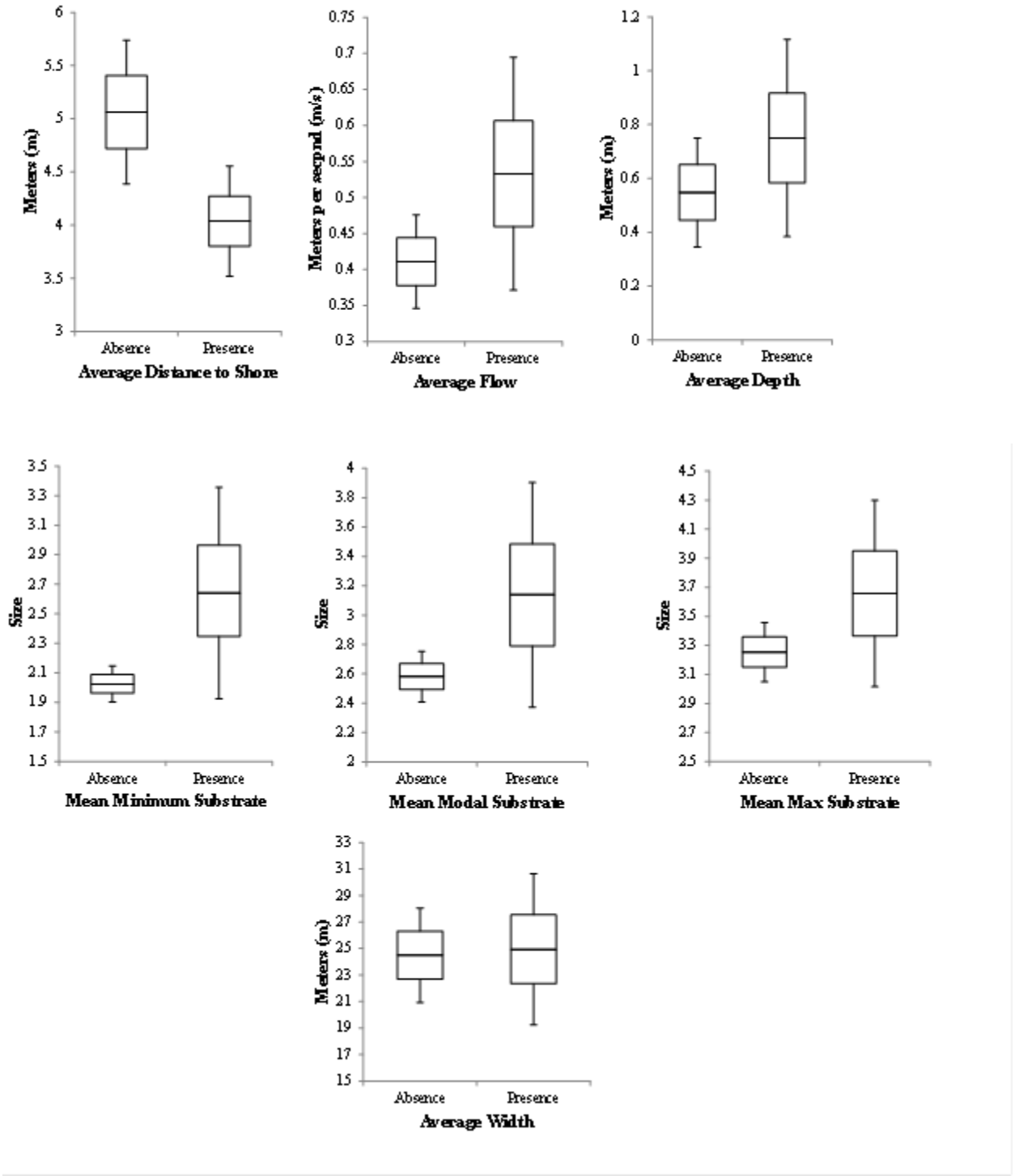

Figure 2.3: Mean measurements for all habitat covariates measured at sites where *B. simmonsi* was present and absent. (sites, n=43) Horizontal lines are means, boxes indicate plus/minus one standard error, and whiskers indicate the upper and lower confidence intervals. Note the different ranges and units of the Y-axis.

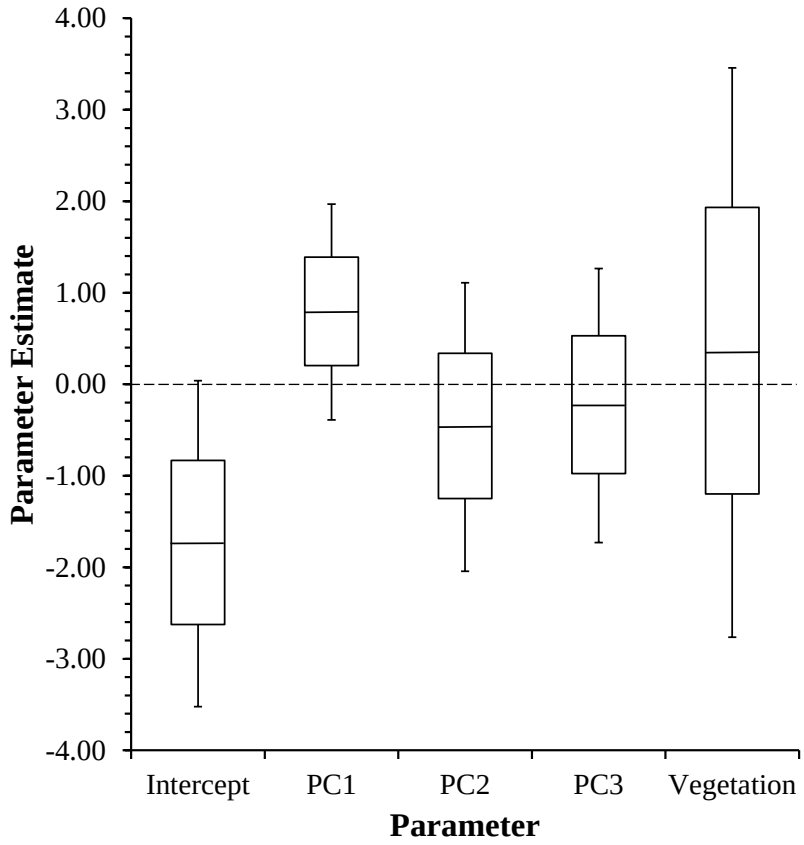

Figure 2.4: Parameter estimates for all parameters run for habitat analysis. PC1 represents substrate, PC2 represents stream width, and PC3 represents depth and flow. Horizontal lines indicate estimates, boxes indicate plus/minus one standard error, and whiskers indicate the upper and lower confidence intervals.

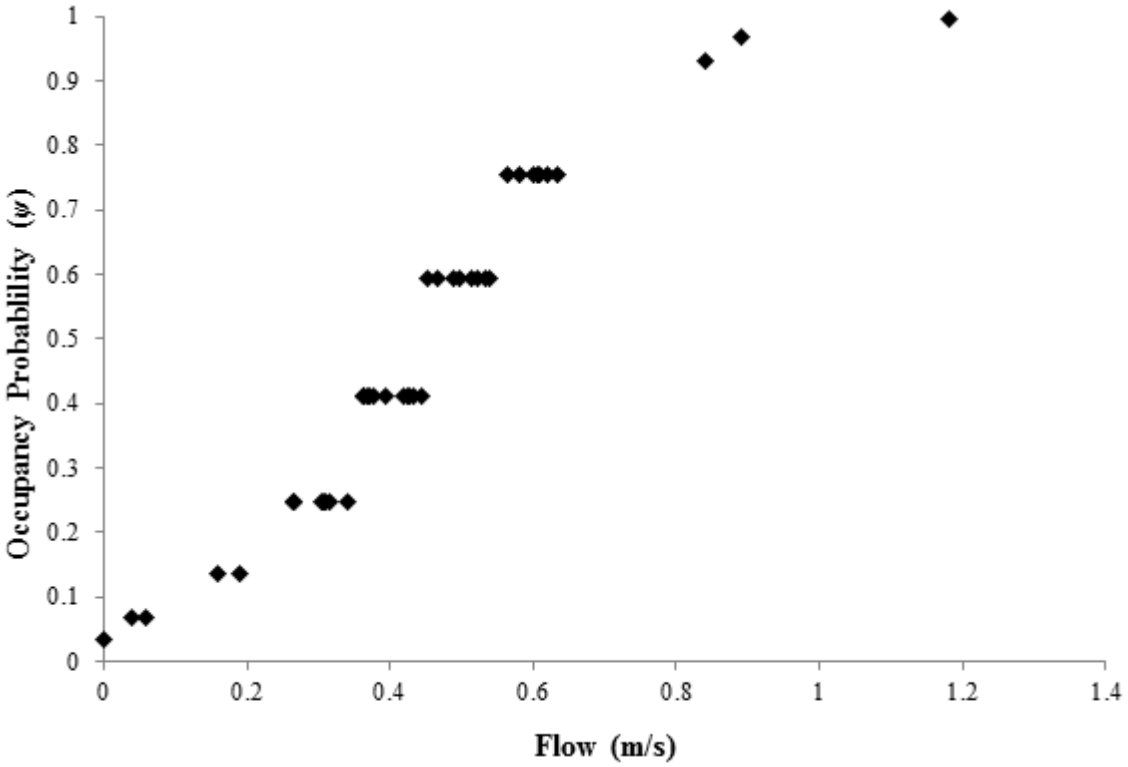

Figure 2.5: Relationship between probability of site occupancy by *B. simmonsi* and flow. Taken from the highest ranked model by AIC (Table 2.7). Flow is measured in meters per second.

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CHAPTER 3: TROPHIC ECOLOGY OF *BARBICAMBARUS* SPECIES

INTRODUCTION

All life requires some transfer of energy or nutrients in order to survive. Where nutrients are acquired from varies for every different species. Generalist species can utilize a broad range of energy sources, while some other species have narrower dietary niches. In either situation, diet is a fundamental part of species ecology, when it comes to conservation. By investigating diet, key resources can be identified that are important for species fitness and population densities (Coogan et al. 2014). Once identified, knowledge of diet can be implemented to develop management and conservation plans based around prey species. For example, Hayward et al. 2006 identified Thomson's gazelle as the most important prey item for cheetahs on the Serengeti. As a result, managing for increased abundance of gazelle may help lead to a corresponding increase in cheetah (Hayward et al. 2006).

Trophic position studies are a good method to study the diet of an organism. Trophic position studies are effective because they can look at the food web of a site, determine functional roles of organisms, and trace the flow of energy and nutrients throughout a system (Post 2002, Vander Zanden and Rasmussen 1996). Assessing trophic position and food web relationships in aquatic food webs is effectively done by means of stable isotope analysis. Stable isotope analysis reflects the isotopic ratios of all food sources consumed and gives a long-term indication of diet and functional role compared to the small time frame represented by other methods such as gut content analysis (Stenroth et al. 2006; Taylor and Soucek 2010). Stable isotope analysis is also effective at measuring levels of omnivory (Vander Zanden and Rasmussen 1996) making it an excellent tool to study trophic positon of crayfishes.

The diversity of aquatic species in North American streams is matched by the many different functional roles they play. Crayfish are well documented as opportunistic omnivores and utilize a wide variety of food sources (Momot 1995; Alcorlo 2004). They are capable of fulfilling multiple levels of the trophic scale including primary consumer, scavenger, and higher-level predator (Momot 1995; Parkyn et al. 2001; Taylor and Soucek 2010; Thomas and Taylor 2013; Vollmer 2014). Because crayfish can occupy multiple levels within an aquatic ecosystem and aspects of their behavior, they can significantly alter their habitat in various ways, including the transfer of energy between trophic levels, vegetation reduction, substrate disturbance, and direct competition with each other (Momot 1995; Geiger et al. 2005; Westhoff and Rabeni 2013; Jackson et al. 2014). The large effects crayfish have on their ecosystem have led many to declare them to be ecosystem engineers of aquatic systems (Statzner et al 2000; Statzner et al. 2003; Creed Jr. and Reed 2004; Reynolds et al. 2013). To properly understand and manage aquatic ecosystems, it is important to determine the functional roles of individual crayfish species present. Different functional roles could indicate a variation of diet, and understanding preferred prey is an important step in managing a species (Lyngdoh et al. 2014). While diet and trophic position of crayfish is largely generalized in the literature, there is a lack of sufficient species-specific data regarding these life history aspects (Moore et al. 2013).

In my study, I hoped to quantify diet and trophic positon of both species in the genus *Barbicambarus*, *B. simmonsi* and *B. cornutus*, two species for which basic ecological information is lacking. The objectives of my study were to: 1) determine trophic position using stable isotope analysis; 2) determine diet from both stable isotope analysis and gut content analysis; and 3) to perform a small side project investigating the unique setae on *Barbicambarus* antennae, which potentially aid in feeding.

METHODS

Study Area

My study was conducted within the native ranges of both species of *Barbicambarus*. For *B. simmonsi*, I sampled populations from Shoal Creek at the Busby road bridge, Lawrence County, Tennessee and Shoal Creek at the AL County Road 8 bridge, Lauderdale County, Alabama. For *B. cornutus* I sampled populations from Trammel Fork at the Pope road bridge, Allen County, Kentucky and from the Nolin River at Taylor Bend Park, Hardin County, Kentucky (Green River drainage). Site localities for *B. simmonsi* were taken from Taylor and Schuster (2010) and previous field work by the author. Historic localities for *B. cornutus* were obtained from the Illinois Natural History Survey Crustacean Collection and Eastern Kentucky University Crustacean Collection records.

Sampling Methods

Crayfish were collected from the sites by performing kick sets using a 1.5x3 m mesh minnow seine. Kick sets consist of disturbing the substrate in 1 m^2 of the streambed while the seine is held immediately downstream to catch any disturbed organisms. The streams were sampled until at least seven crayfish were captured from each site.

For large *B. simmonsi* , I used a non-lethal approach to collect muscle tissue for stable isotope analysis by removing 1-2 legs, then re-releasing the crayfish at Shoal Creek. Preliminary analysis showed the substitution of legs for tail tissue did not affect the isotopic signature. I used a non-lethal approach in order to reduce any damage done to the population at the site, as I did not have knowledge of population sizes. Specimens too small or accidentally killed during sampling were used as whole body samples. At my two sites for *B. cornutus*, all specimens were collected as whole specimens. Whole body specimens were frozen for later analysis.

I also collected representative species of other feeding groups for stable isotope analysis at each site including fine particulate organic matter (FPOM), algae, macrophytes, leaf litter, snails, predatory insects, predatory fish, and other sympatric crayfish species. I collected fine particulate organic matter by disturbing the substrate and scooping up water into a Whirlpak while sediment was suspended in the water column. I collected plants by hand and all insects, sympatric crayfish, and fish were collected with a 1.5x3meter mesh minnow seine. All samples were frozen for later analysis.

Stable Isotope Analysis

All samples were prepared for stable isotope analysis in a laboratory on the University of Illinois Urbana-Champaign campus. Samples were removed from the freezer and allowed to thaw. Plant specimens were cleansed of any dirt or contamination and placed in an aluminum weighing dish. Insect specimens were placed into the dishes as either whole specimens or just their legs depending on the species and the number of individuals needed. In some cases, 3-4 insects were combined to obtain enough tissue to make a proper sample. Snails had their operculum removed, were removed from their shells, and had their gut removed. Tissue from most crayfish samples was attained by removing a muscle plug from the tail tissue or legs. For fish specimens, scales were scraped away and a muscle plug was removed from the caudal region of the fish. All tissues were placed in the aluminum weighing dishes then dried in an incubator set at 45 °C for 48 hrs. to dry. After drying, plant tissues and FPOM samples were placed in a desiccator with hydrochloric acid for an additional 6 hrs. to remove inorganic carbonates. All specimens were ground into a fine powder with a mortar and pestle and weighed on a scale to either 2.00mg for animal tissue or 3.00mg for plant tissue as requested by the facility performing the analysis. The powder from each sample was placed into a small tin

capsule, closed, and sent to Southern Illinois University Carbondale's Mass Spectrometry Facility for analysis.

The samples were analyzed using continuous flow EA-IRMS for $\delta^{15}N$ and $\delta^{13}C$. I used the results to calculate a trophic position (TP) using the equation:

$$TP_{consumer} = TP_{base} + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta_N$$

where algae was used as the base and $TP_{base} = 1$. The $\Delta_N$was set at 3.40 based on an established average fractionation rate (Petersen and Fry 1987, Vander Zanden and Rasmussen 2001, Post 2002, Nilsson et al 2011).

The data for each site was checked for normality in JMP by analyzing the distribution for continuous fit and then looking at goodness-of-fit. Normal data was analyzed for homogeneity of variance by analyzing the fit of species by trophic position and checking for unequal variances. Trophic positions of all crayfishes from each site were plotted using linear regression analysis in JMP (Ver. 9.0.0). Non-parametric data was analyzed using a Wilcoxon Signed Rank test.

Gut Content Analysis

Gut content analysis was conducted on specimens collected for the stable isotope portion of my study. Because I saved a low number of whole *B. simmonsi* specimens, I supplemented those with 6 individuals from a collection of *B. simmonsi* made at the Shoal Creek at AL County Road 8 crossing site on 9 November 2010 that were collected in the Illinois Natural History Survey Crustacean Collection. Crayfish were dissected by cutting the connective tissue between the carapace and the abdomen and carefully lifting the carapace up. The stomachs were carefully removed to avoid rupture as best as possible. Upon removal, the stomachs were opened up and the contents were spread across an 8 by 8 grid of 1 cm squares on a petri dish. The gut contents were viewed under a stereo dissecting microscope and a percent estimate to the nearest 5% was made for each kind of food type present in each grid cell. Contents were categorized into one of

nine categories: fish, crayfish, macroinvertebrates, leaf litter, macrophytes, algae, wood, sediment, and unidentified organic material. Percent values of gut contents were averaged together for all crayfish at a site and the means were plotted to show the average percentages of each food type at all four sites.

Antennal Structure

I compared the antennae of *Barbicambarus* crayfish to other North American species by examining samples using the Environmental Scanning Electron Microscope (ESEM) housed at the University of Illinois' Microscopy Suite. I compared the antennae of five different crayfish species, *B. cornutus, Orconectes virilis, Procambarus lephotus, Cambarus girardianus and Cambarus graysoni. Barbicambarus* antennae samples used were taken from frozen crayfish collected for stable isotope analysis. I clipped the antennae into small pieces and dried them under a fume hood for 2 days. I mounted the dry antennae onto ESEM mounts and sputter coated them with gold/palladium. Each crayfish had at least one cross section of an antenna and one lengthwise antenna on the mount. I viewed the mounts with the microscope, and images were taken of the antennae and setae at 5 µm, 50 µm, and 500 µm. Images taken at 500 µm were taken as both top down cross sections and as lateral views of the antennae. For *B. simmonsi* and *O. virilis* lateral images were taken at a scale of 1 mm and 200 µm respectively, as these magnifications provided the best lateral view of the antennae. Lateral images for *B. simmonsi* and *O. virilis* were taken at 1 mm and 200 µm respectively, instead of 500 µm, as these magnifications provided a better lateral image for comparison.

RESULTS

I collected a total of 21 *B. simmonsi* and 23 *B. cornutus* to be analyzed with stable isotope analysis. I collected 14 individuals from Shoal Creek at Busby Road and seven from the Shoal

Creek site. From Kentucky, I collected 10 individuals from the Nolin River and 13 individuals from Trammel Fork.

Stable Isotope Analysis

Trophic position for *B. simmonsi* ranged from 1.80-2.75 and 1.77-2.49 at the Busby Road site and the AL Co. Road 8 site respectively (**Table 3.1**). Trophic position for *B. cornutus* ranged from 3.35-4.08 and 2.52-3.17 at the Trammel Fork site and the Nolin River site respectively (**Table 3.2**). This placed *Barbicambarus* crayfish at a higher trophic position compared to other sympatric occurring species of crayfish within sites and at a lower trophic position than predatory fishes with the exception of Nolin River in Kentucky (**Tables 3.3-3.6**). Site specific, general food web positions of community members plotting $\delta^{13}C$ vs $\delta^{15}N$ are presented in **Figure 3.1A-3.1D**. I observed similar structure between all four site where the levels of $\delta^{15}N$ for *Barbicambarus* is higher than all other plant and invertebrate organisms present. At all sites, except for the Nolin River site in Kentucky, predatory fish had higher levels of $\delta^{15}N$. My results show *Barbicambarus* occupying a trophic position below predatory fish and above plants and invertebrates, with the exception of the Nolin River site where they occupy the highest trophic position.

At three sites, I observed no significant relationship between trophic position and body size for all crayfish at the site with the exception of *Barbicambarus* crayfish. At Nolin Creek in Kentucky, *C. graysoni* (p=0.2477), *O. barrenensis* (p=0.2491), and *O. rusticus* (p=0.5711) all showed no relationship between carapace length and trophic position, while *Barbicambarus cornutus* (p=0.0316) showed a positive relationship between the two variables (**Fig. 3.2A**). At Trammel Fork in Kentucky, *O. compressus* (p=0.9361) and *O. putnami* (p=0.7035) both showed no relationship between the two variables while *B, cornutus* (p=<0.0001) again showed a

positive relationship between trophic position and carapace length (**Fig. 3.2B**). At Shoal Creek at County road 8 crossing in Alabama, neither *O. erichsonianus* (p=0.3885) or *O. forceps* (p=0.0494) showed relationships between the two variables while *B. simmonsi* (p=0.0010) showed a strong positive relationship between the two (**Fig. 3.2C**). Lastly at Shoal Creek at Busby road Tennessee *C. girardianus* (p=0.5893)) did not show a significant relationship between trophic position and carapace length, while *B. simmonsi* (p=<0.0001) showed a significant positive relationship (**Fig. 3.2D**).

Gut Content Analysis

Gut content analysis of 11 *B. simmonsi* and 22 *B. cornutus* revealed both species had large amounts of plant material and unidentified organic matter, moderate amounts of algae and leaf litter, and small amounts of wood, sediment, and macroinvertebrates in their guts. One individual from Kentucky was found to contain a fish scale. Average percentages of gut contents did not seem to vary by large amounts between species or between sites (**Fig. 3.3A-3.3D**).

Antennal Analysis

My antennal analysis showed setae structure varied considerably across crayfish species. Variation in structure is solely based on the photos included and is purely observational. I cannot draw any major conclusions from the images, but I can show a physical difference between the antennal setae of different crayfish genera. The images can be seen in **Figure 3.4** and **Figure 3.5**. **Figure 3.4** shows that *B. cornutus* has longer and larger setae, in higher densities than other crayfish species. Figure 3.5 shows close ups of the surface of the setae, which are covered in small spines varying in size and shape across species.

DISCUSSION

Stable Isotope Analysis

My stable isotope analysis shows *Barbicambarus* species are feeding at higher trophic levels than other sympatric crayfish and may occupy a higher trophic level as an omnivore or a predator. Thus, indicating *B. simmonsi* are acquiring energy from feeding on primary or secondary consumers. Results of comparing carapace length and trophic position show *Barbicambarus* crayfish increase in trophic position as they grow larger while other sympatric species do not, indicating interspecies niche partitioning. It is also possible *Barbicambarus* species are occurring at higher trophic positions because they are larger. Vollmer and Gall (2014) showed larger crayfish more easily captured salamander larvae. The large size of *Barbicambarus* crayfish could afford them the ability to capture larger or higher trophic level prey items. Thus, resulting in the more enriched level of nitrogen observed. In addition, studies have shown that crayfish rely on animal protein to grow (Momot 1995; Parkyn et al. 2001; Hollows et al. 2002). Therefore, it is possible *Barbicambarus* crayfish are achieving larger sizes by assimilating larger amounts of animal protein, further supporting the idea that *Barbicambarus* crayfish are acting as predators.

Gut Content Analysis

The identifiable gut contents of the crayfish I investigated consisted largely of plant, algae, and leaf litter, with smaller amounts of animal matter. These types of findings in crayfish gut contents are not uncommon and have been reported in various studies (Whitledge and Rabeni 1997; Whitemore and Huryn 1999; Helms and Creed 2005). My results indicate *Barbicambarus* are performing the function of a primary consumer. However, gut contents only represent what has been ingested over a short period of time and thus may not be indicative of the long-term diet

of a species. The vast majority of the gut contents across species and sites were unidentifiable organic material. Crayfish have been shown to eat high amounts of animal protein (Momot 1995; Parkyn et al. 2001; Stenroth et al. 2006; Thomas and Taylor 2013). As such, it is possible some of the unidentifiable material is actually derived from animal tissue. Animal tissue is much softer and digests at faster rates than plant tissue, which could explain why it has been turned into an unidentifiable pulp. Crayfish also have grinding mandibles and a second set of gastric teeth, which could easily grind up soft animal tissue. In my study, there were instances where hard pieces of animal matter such as pieces of snail shell, crayfish carapace, and fish scales were found, but not soft tissue was able to be identified which again, possibly accounts for the large amount of unidentified material within the gut contents. A stable isotope analysis on the unidentifiable organic matter could provide additional insight on the source of the matter and the diets of crayfish in general.

Conclusions

My stable isotope and gut content data yield differing results. Conflicting data between the two types of analysis has been documented before in other crayfish species (Parkyn et al. 2001; Hollows et al. 2002; Evans-White et al. 2001). The difference in results could be a result of the two types of data providing information of different scales. Stable isotope analysis serves as a measure of long-term energy assimilation whereas gut contents represent short-term recent ingestion. Other reasons accounting for the lack of comparable results between gut contents and stable isotope analysis is the sample size being too small to draw comparable conclusions from. I collected a small number of individuals for gut content analysis because I did not wish to affect the local populations and the number of *B. simmonsi* encountered was low. The small sample size could account for the lack of many individuals containing signs of animal protein. In

addition, I only sampled on one occasion. Perhaps there is seasonality to the diet of *Barbicambarus* crayfish, and another gut content analysis spread over different seasons could yield more accurate results.

My study indicates all crayfish within a site are capable of functioning at different trophic levels, which is indicative of resources partitioning and reduced interspecific interaction. Jackson et al. (2014) observed an indicated shift in diet between crayfish species, where the introduction of one species drove up the nitrogen levels of the other. Other studies indicate similar phenomenon among stream dwelling fish (Naman et al. 2014). Understanding differences in diet will have implications in conservation, as conservation of key prey species is crucial in the survival of any organism (Lyngdoh et al. 2014). Thus, knowing trophic position and dietary habits of *B.simmonsi* will aid management efforts for its continued persistence.

Antennal Analysis

My antennal analysis was purely an observational study, and as such, I cannot draw any major inferences from it. However, my SEM imagery shows definite structural difference between the setae of different crayfish genera. *Barbicambarus cornutus* had antennae covered in long dense setae where as other crayfish had shorter and far less dense setae. When the setae were magnified even more I could see *B. cornutus* setae were covered in small needle like projections, where as other species such as *O. virilis* had small stunted projections, and others such as *C. girardianus* appeared to have smooth setae without small projections on them at all. The differences in structure could indicate that antennal function is unique among genera. Antennae are known to function as chemoreceptive organs as well as being involved with tactile sensation (Holdich and Reeve 1988). Other studies have shown setae on the second antennae are predominantly mechanosensory and respond to vibrations in the water (Tautz et al. 1981;

Sandeman 1989). Perhaps, different genera have varying levels of mechanosensory sensitivity determined by the shape and structure of their antennal setae. To draw more conclusions, a lab study would need to be done looking at sensory ability of different genera.

TABLES AND FIGURES

Table 3.1: Trophic Positions (TP) of all *B. simmonsi* collected ranked from lowest to highest.

Species	Site	TP
B. simmonsi	Shoal Creek @AL Co. road 8	1.77
B. simmonsi	Shoal Creek @Busby Rd	1.80
B. simmonsi	Shoal Creek @Busby Rd	1.82
B. simmonsi	Shoal Creek @Busby Rd	2.04
B. simmonsi	Shoal Creek @Busby Rd	2.05
B. simmonsi	Shoal Creek @Busby Rd	2.06
B. simmonsi	Shoal Creek @Busby Rd	2.10
B. simmonsi	Shoal Creek @AL Co. road 8	2.11
B. simmonsi	Shoal Creek @Busby Rd	2.24
B. simmonsi	Shoal Creek @AL Co. road 8	2.25
B. simmonsi	Shoal Creek @Busby Rd	2.30
B. simmonsi	Shoal Creek @Busby Rd	2.33
B. simmonsi	Shoal Creek @AL Co. road 8	2.41
B. simmonsi	Shoal Creek @AL Co. road 8	2.44
B. simmonsi	Shoal Creek @Busby Rd	2.46
B. simmonsi	Shoal Creek @AL Co. road 8	2.47
B. simmonsi	Shoal Creek @AL Co. road 8	2.50
B. simmonsi	Shoal Creek @Busby Rd	2.52
B. simmonsi	Shoal Creek @Busby Rd	2.62
B. simmonsi	Shoal Creek @Busby Rd	2.75
B. simmonsi	Shoal Creek @Busby Rd	2.75

Table 3.2: Trophic Positions (TP) of all *B. cornutus* collected ranked from lowest to highest.

Species	Site	TP
B. cornutus	Nolin River @Taylor Bend Park	2.52
B. cornutus	Nolin River @Taylor Bend Park	2.64
B. cornutus	Nolin River @Taylor Bend Park	2.71
B. cornutus	Nolin River @Taylor Bend Park	2.78
B. cornutus	Nolin River @Taylor Bend Park	2.84
B. cornutus	Nolin River @Taylor Bend Park	2.86
B. cornutus	Nolin River @Taylor Bend Park	2.87
B. cornutus	Nolin River @Taylor Bend Park	2.89
B. cornutus	Nolin River @Taylor Bend Park	2.91
B. cornutus	Nolin River @Taylor Bend Park	3.17
B. cornutus	Trammel Fk @Pope road	3.36
B. cornutus	Trammel Fk @Pope road	3.39
B. cornutus	Trammel Fk @Pope road	3.47
B. cornutus	Trammel Fk @Pope road	3.51
B. cornutus	Trammel Fk @Pope road	3.57
B. cornutus	Trammel Fk @Pope road	3.58
B. cornutus	Trammel Fk @Pope road	3.76
B. cornutus	Trammel Fk @Pope road	3.82
B. cornutus	Trammel Fk @Pope road	3.83
B. cornutus	Trammel Fk @Pope road	3.87
B. cornutus	Trammel Fk @Pope road	3.92
B. cornutus	Trammel Fk @Pope road	3.97
B. cornutus	Trammel Fk @Pope road	4.08

Table 3.3: Trophic positions of all sample groups collected at Shoal Creek at Busby road, Lawrence County, TN

Site	Sample	TP
Shoal Creek @Busby Rd	Leaf	-0.19
Shoal Creek @Busby Rd	FPOM	0.58
Shoal Creek @Busby Rd	Algae	1.00
Shoal Creek @Busby Rd	*Odonata*	1.69
Shoal Creek @Busby Rd	Hellgrammite (*Megaloptera*)	1.72
Shoal Creek @Busby Rd	Snails (*Gastropoda*)	1.78
Shoal Creek @Busby Rd	Crayfish (*C. girardianus*)	2.00
Shoal Creek @Busby Rd	*B. simmonsi*	2.28
Shoal Creek @Busby Rd	Darter (*Etheostoma*)	2.77
Shoal Creek @Busby Rd	Rock Bass (*Ambloplites*)	3.11

Table 3.4: Trophic positions of all sample groups collected at Shoal Creek at AL County road 8, Lauderdale County, AL

Site	Sample	TP
Shoal Creek @AL Co. road 8	Leaf	-0.95
Shoal Creek @AL Co. road 8	FPOM	0.30
Shoal Creek @AL Co. road 8	Algae	1.00
Shoal Creek @AL Co. road 8	*Ephemeroptera*	1.14
Shoal Creek @AL Co. road 8	*Odonata*	1.23
Shoal Creek @AL Co. road 8	Hellgrammite (*Megaloptera*)	1.50
Shoal Creek @AL Co. road 8	Crayfish (*O. erichsonianus*)	1.67
Shoal Creek @AL Co. road 8	Snail (*Gastropoda*)	1.67
Shoal Creek @AL Co. road 8	Crayfish (*O. forceps*)	1.71
Shoal Creek @AL Co. road 8	Crayfish (*C. girardianus*)	1.87
Shoal Creek @AL Co. road 8	*B. simmonsi*	2.28
Shoal Creek @AL Co. road 8	Sunfish (*Lepomis*)	2.40
Shoal Creek @AL Co. road 8	Darter (*Etheostoma*)	2.54

Table 3.5: Trophic positions of all sample groups collected at Trammel Fork at Pope road, Allen County, KY

Site	Sample	TP
Trammel Fk @Pope road	Algae	1.00
Trammel Fk @Pope road	FPOM	1.55
Trammel Fk @Pope road	Leaf	1.03
Trammel Fk @Pope road	Cranefly (*Diptera*)	1.76
Trammel Fk @Pope road	Crayfish (*O. putnami*)	2.99
Trammel Fk @Pope road	*Odonata*	3.35
Trammel Fk @Pope road	Crayfish (*O. compressus*)	3.46
Trammel Fk @Pope road	Snail (*Gastropoda*)	3.47
Trammel Fk @Pope road	*B. cornutus*	3.70
Trammel Fk @Pope road	Darter (*Etheostoma*)	4.05
Trammel Fk @Pope road	Bass (*Micropterus*)	4.59

Table 3.6: Trophic positions of all sample groups collected at Nolin River at Taylor Bend Park, Hardin County, KY.

Site	Sample	TP
Nolin River @Taylor Bend Park	Leaf	-0.03
Nolin River @Taylor Bend Park	FPOM	0.19
Nolin River @Taylor Bend Park	Algae	1.00
Nolin River @Taylor Bend Park	Damselfly (*Odonata*)	1.85
Nolin River @Taylor Bend Park	Crayfish (*O. rusticus*)	1.95
Nolin River @Taylor Bend Park	Crayfish (*C. graysoni*)	1.97
Nolin River @Taylor Bend Park	Hellgrammite (*Megaloptera*)	2.03
Nolin River @Taylor Bend Park	Crayfish (*O. barrenensis*)	2.32
Nolin River @Taylor Bend Park	Snail (*Gastropoda*)	2.48
Nolin River @Taylor Bend Park	Sunfish (*Lepomis*)	2.51
Nolin River @Taylor Bend Park	Sculpin (*Cottus*)	2.66
Nolin River @Taylor Bend Park	*B. cornutus*	2.82

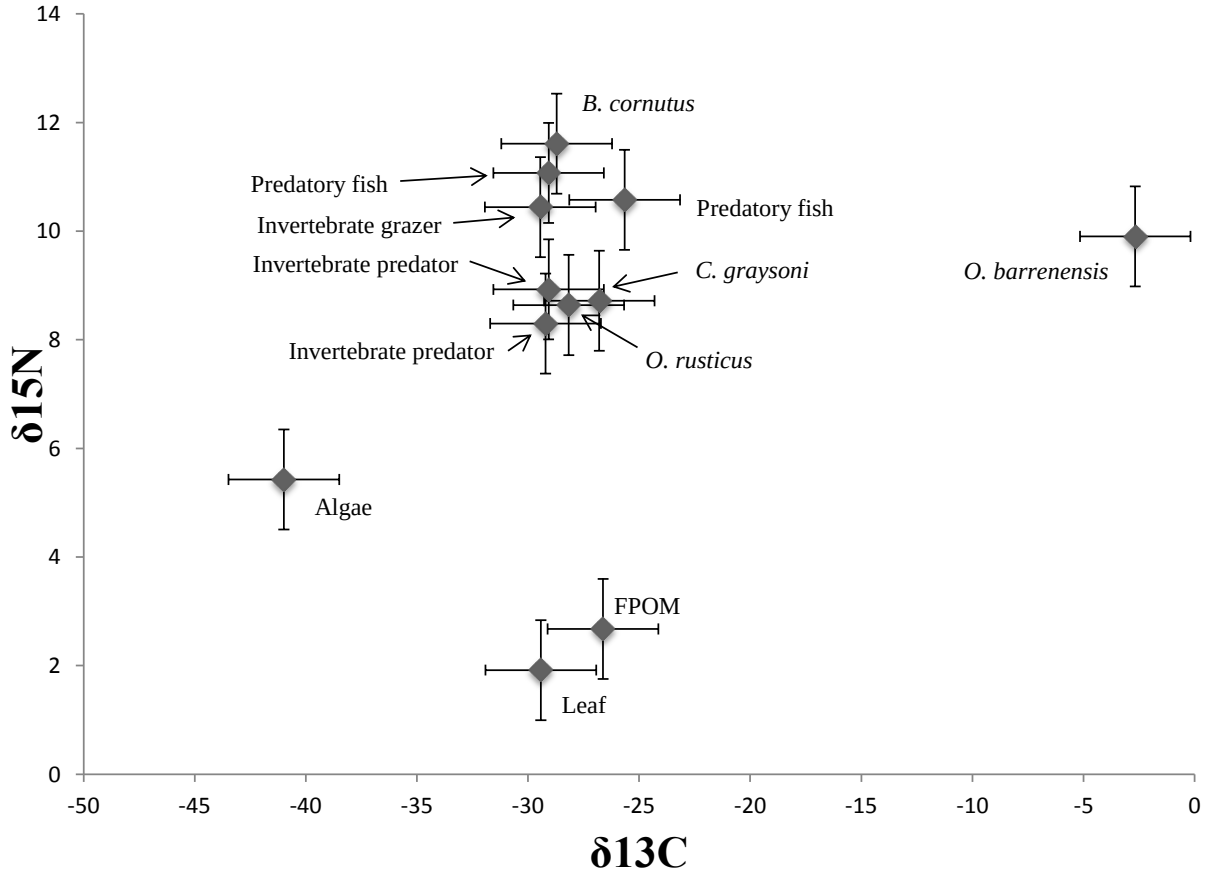

Figure 3.1A: $\delta^{13}C$ vs $\delta^{15}N$ plots for all organisms collected within the Nolin River site in Kentucky. Trophic position can be interpreted from the levels of $\delta^{15}N$. (*B. cornutus* n=10; *Cambarus graysoni* n=4; *O. barrenensis* n=8; *O. rusticus* n=8)

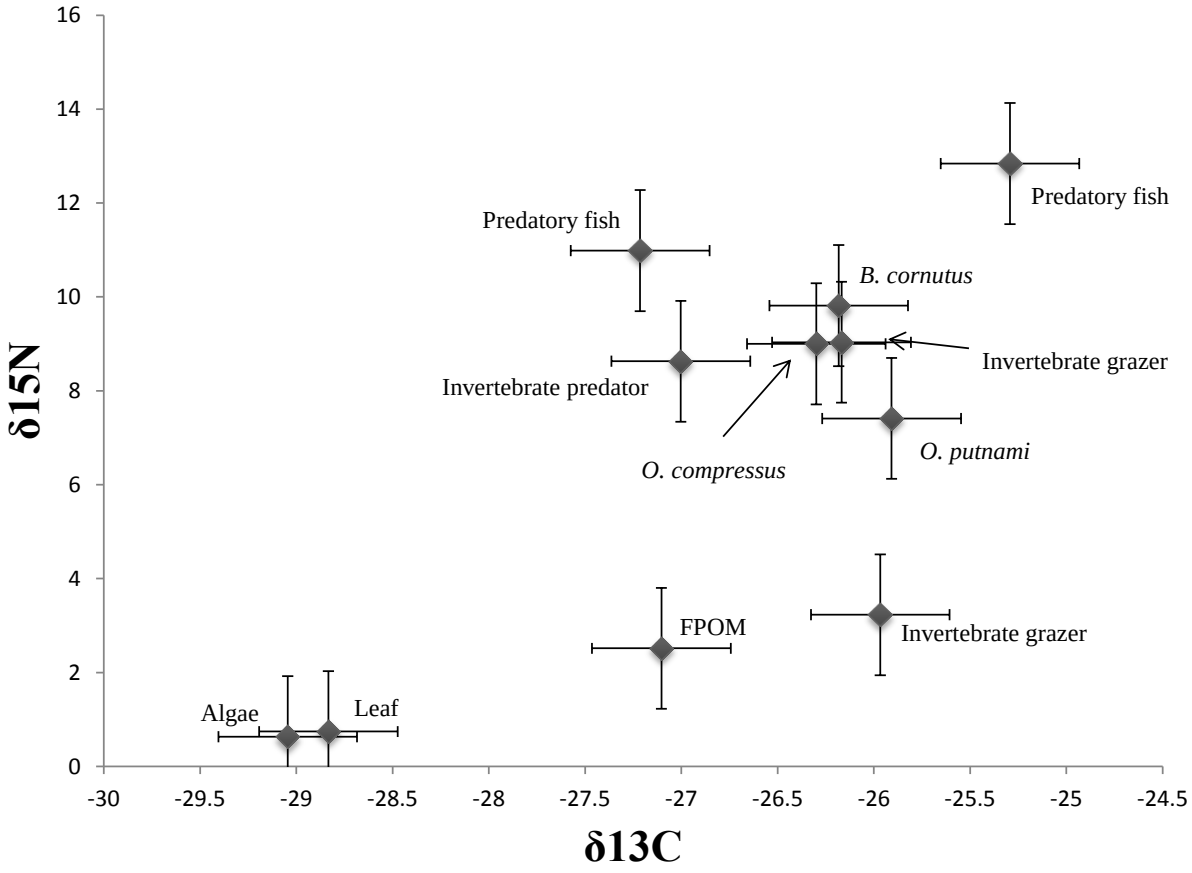

Figure 3.1B: $\delta^{13}C$ vs $\delta^{15}N$ plots for all organisms collected within the Trammel Fork site in Kentucky. Trophic position can be interpreted from the levels of $\delta^{15}N$. (*B. cornutus* n=13; *O. compressus* n=8; *O. putnami* n=7)

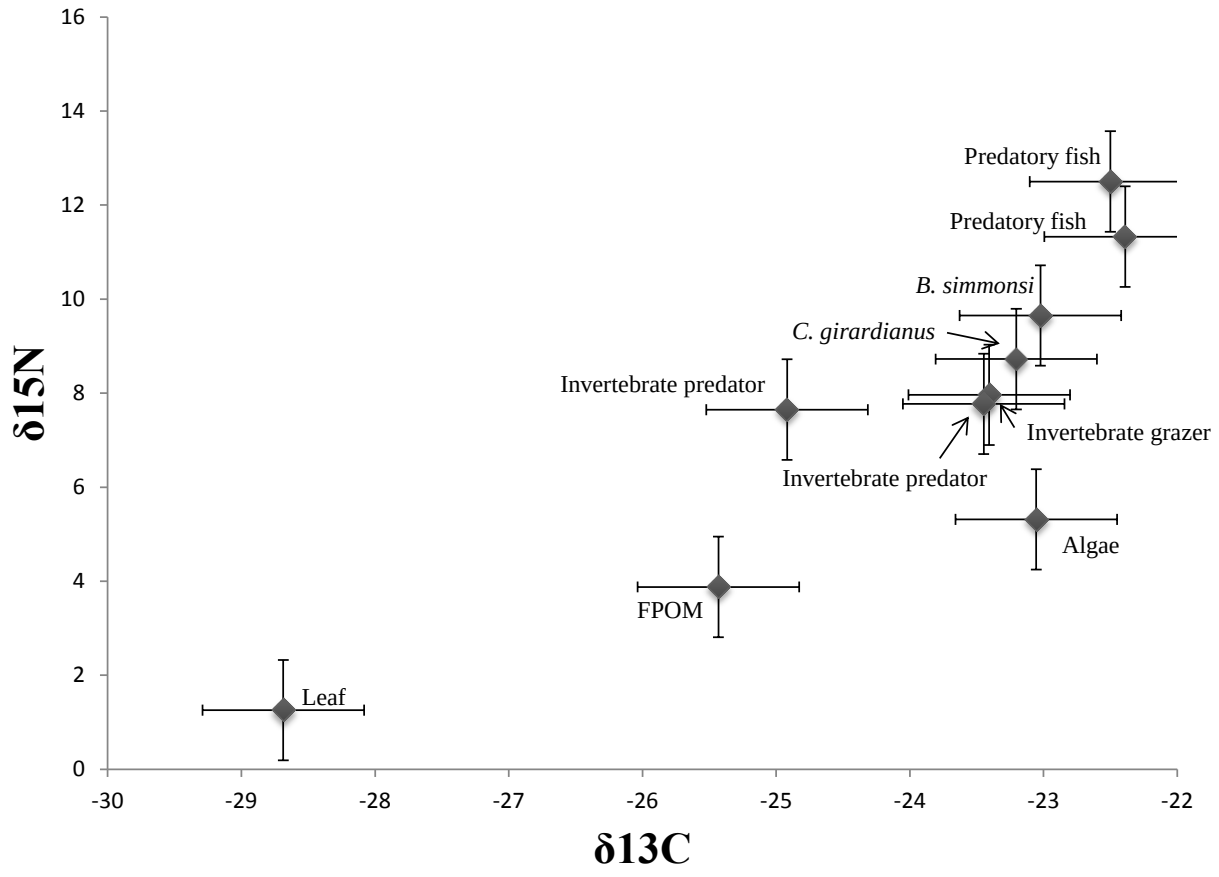

Figure 3.1C: $\delta^{13}C$ vs $\delta^{15}N$ plots for all organisms collected within the Shoal Creek at Busby road site in Tennessee. Trophic position can be interpreted from the levels of $\delta^{15}N$. (*B. simmonsi* n=14; *C. girardianus* n=8)

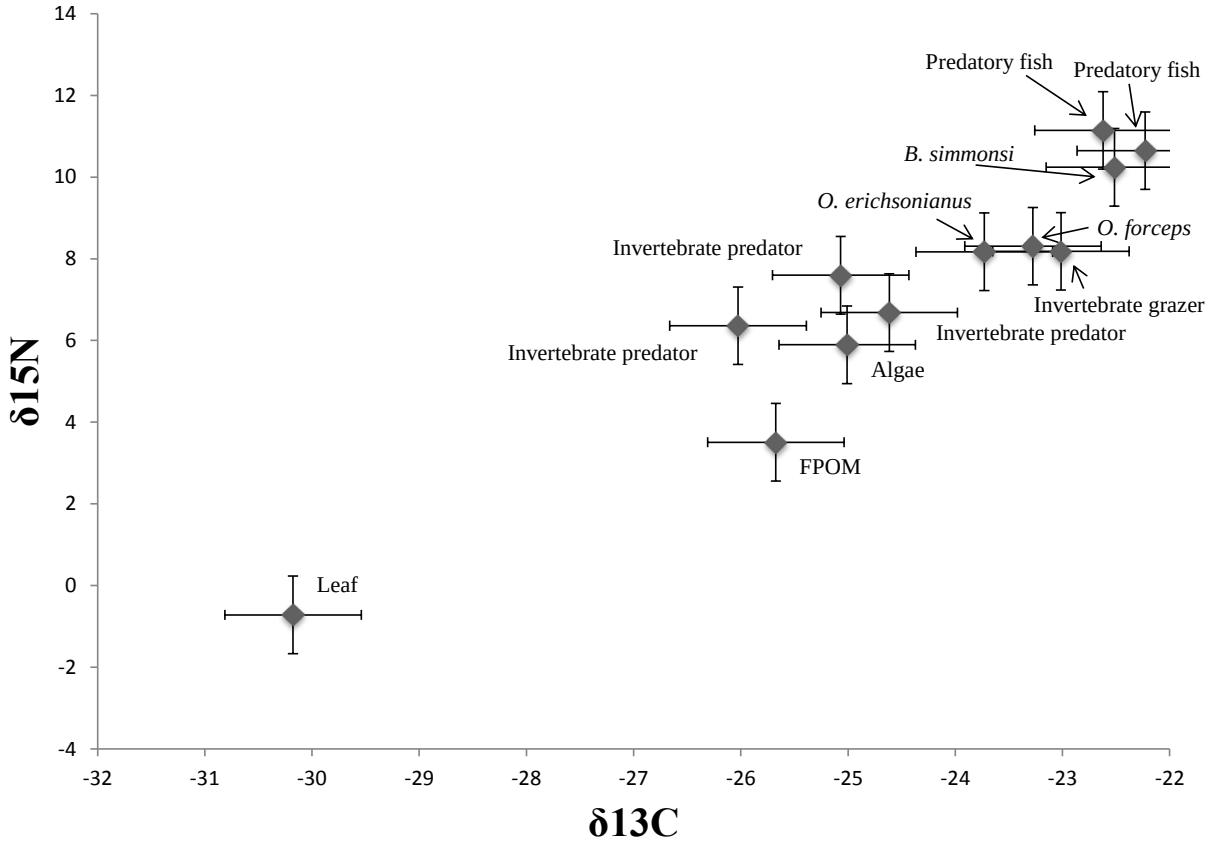

Figure 3.1D: $\delta^{13}C$ vs $\delta^{15}N$ plots for all organisms collected within the Shoal Creek at AL county road 8 in Alabama. Trophic positions can be interpreted from the levels of $\delta^{15}N$. (*B. simmonsi* n=7; *O. erichsonianus* n=5; *O. forceps* n=5)

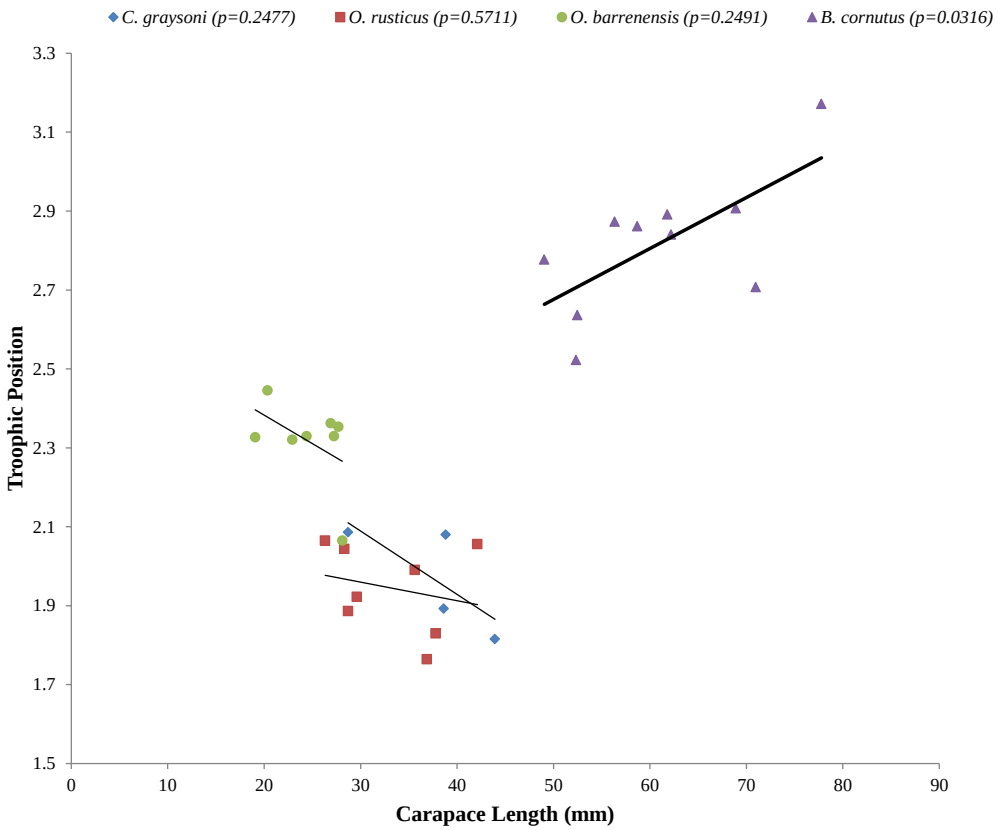

Figure 3.2A: Linear regression of trophic position and body size for *B. cornutus* (p=0.0316), *C. graysoni* (p=0.2477), *O. barrenensis* (p=0.2491), and *O. rusticus* (p=0.5711) collected at the Nolin Creek site, Kentucky.

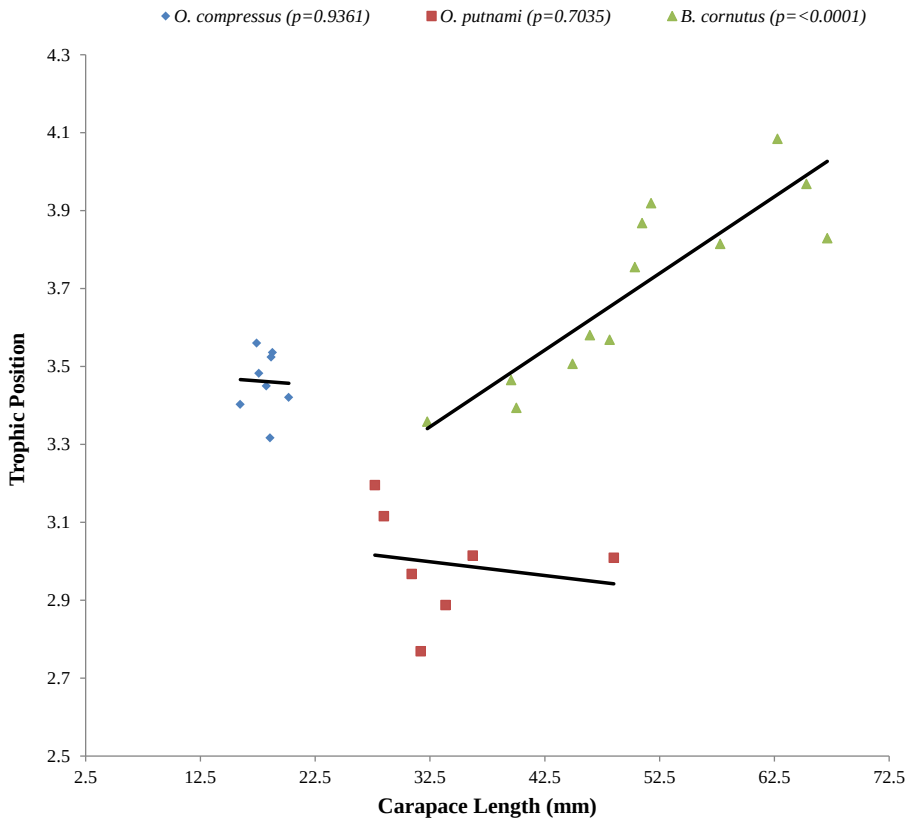

Figure 3.2B: Linear regression of trophic position and body size for *B. cornutus* (p=<0.0001), *O. compressus* (p=0.9361), and *O. putnami* (p=0.7035) collected at the Trammel Fork site, Kentucky.

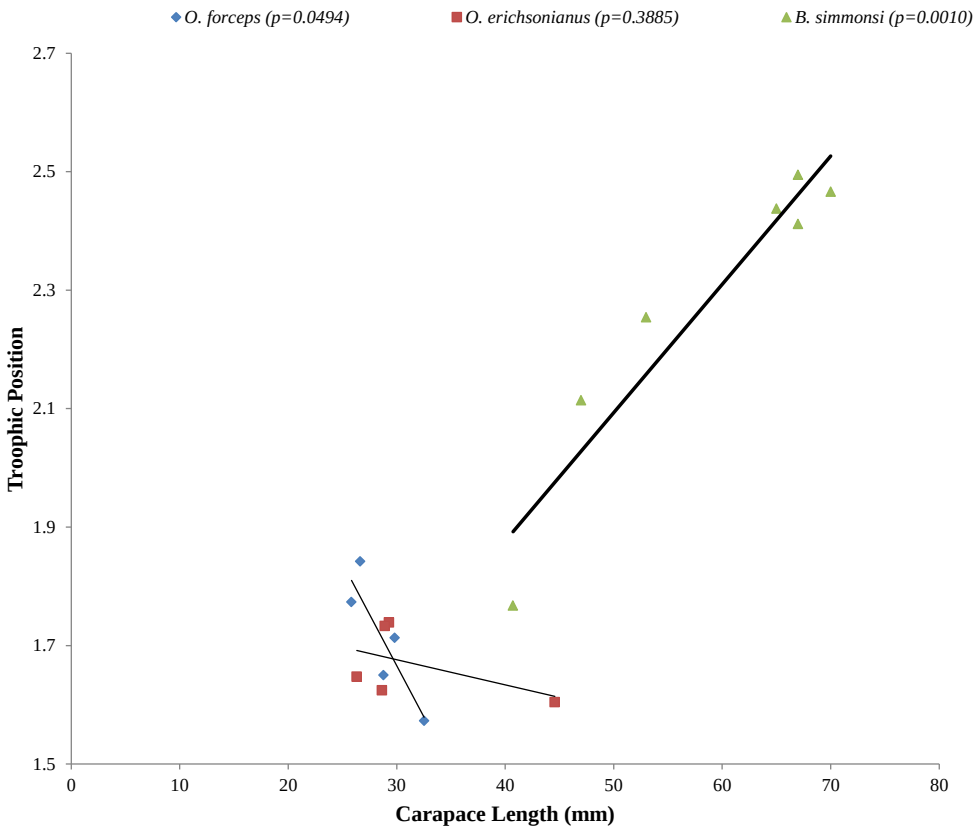

Figure 3.2C: Linear regression of trophic position and body size for *B. simmonsi* (p=0.0010), *O erichsonianus* (p=0.3885), and *O. forceps* (p=0.0494) collected at the Shoal Creek at AL County road 8 crossing, Alabama.

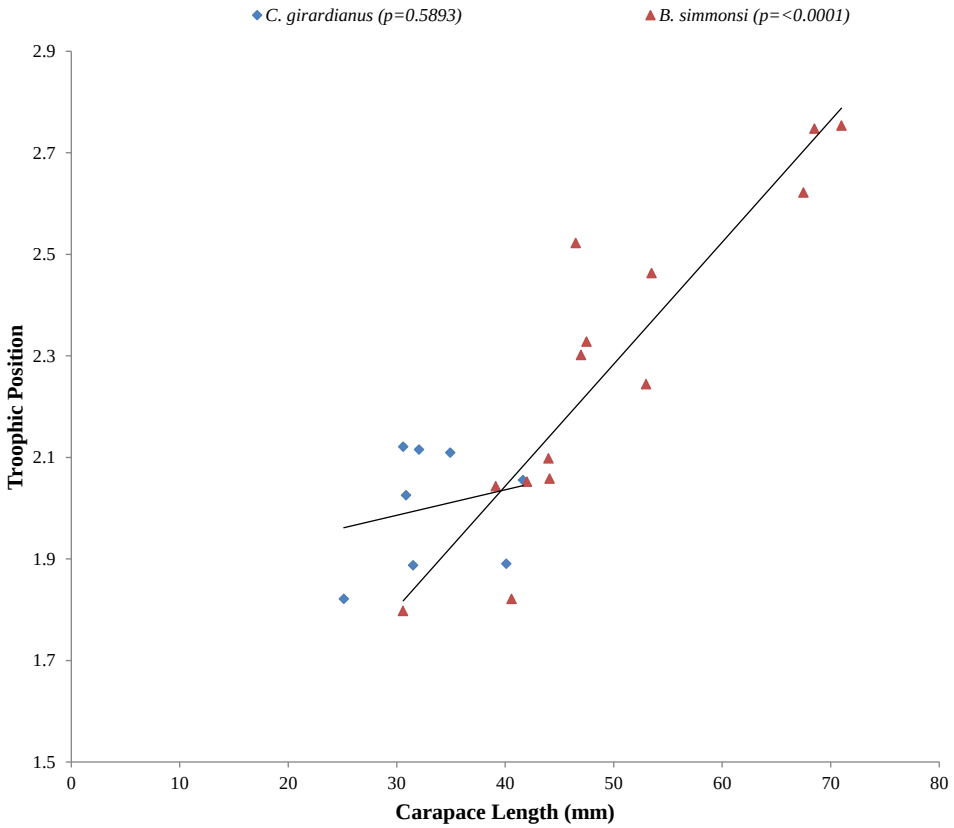

Figure 3.2D: Linear regression of trophic position and body size for *B. simmonsi* (p=<0.0001) and *C. girardianus* (p=0.5893) collected at the Shoal Creek at Busby road crossing, Tennessee.

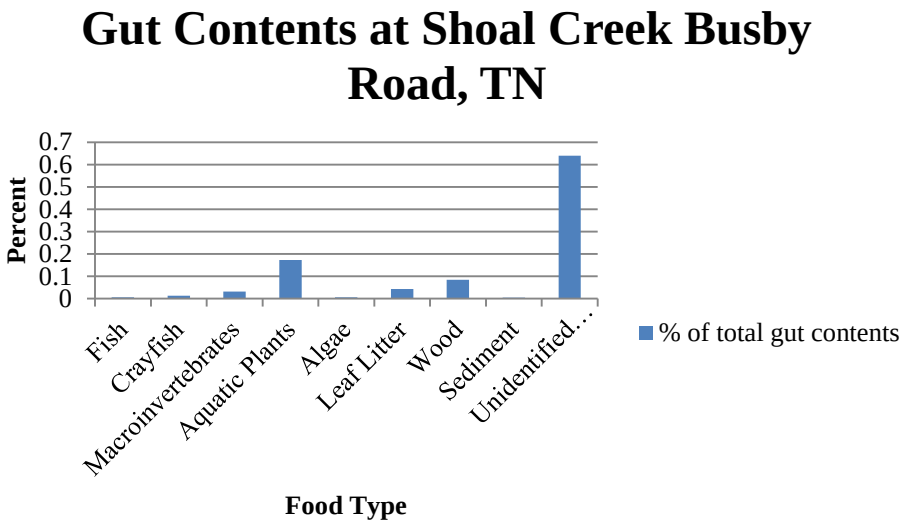

Figure 3.3A: Percent of total gut contents for N=5 *B. simmonsi* at Shoal Creek at Busby Road, Lawrence County, TN

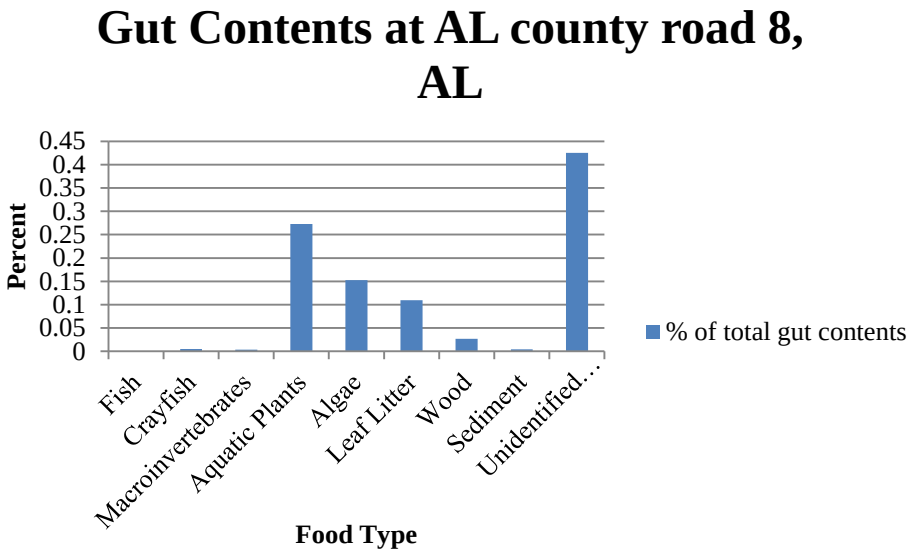

Figure 3.3B: Percent of total gut contents for N=7 *B. simmonsi* at Shoal Creek at AL county road 8, Lauderdale County, AL

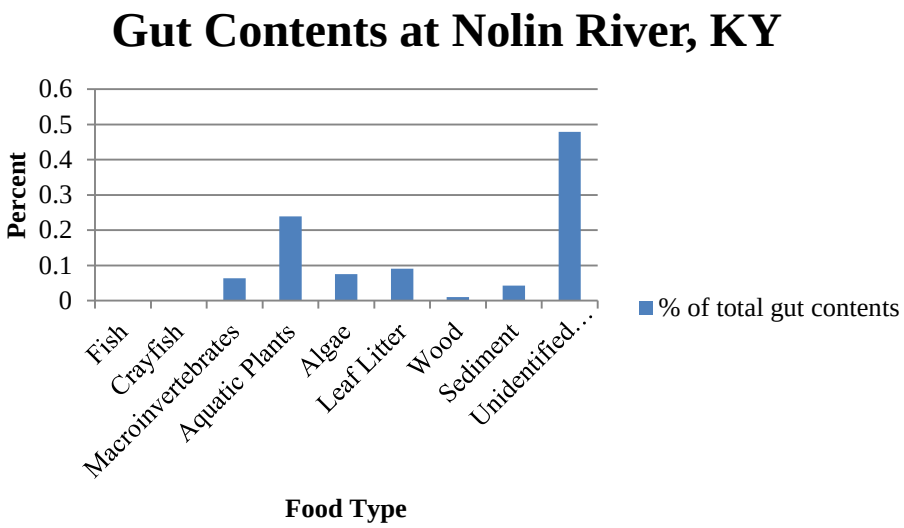

Figure 3.3C: Percent of total gut contents for N=10 *B. cornutus* at Nolin River at Taylor Bend Park, Allen County, KY

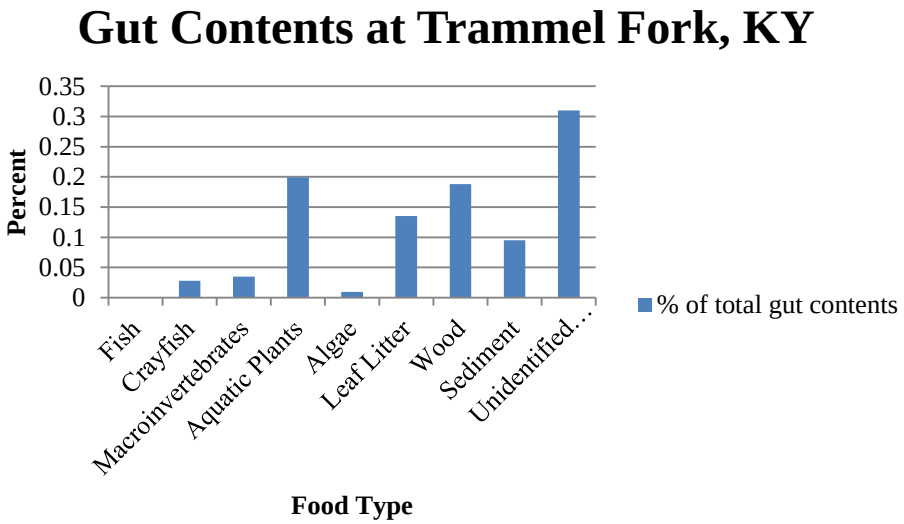

Figure 3.3D: Percent of total gut contents for N=13 *B. cornutus* at Trammel Fork at Pope Road, Hardin County, KY

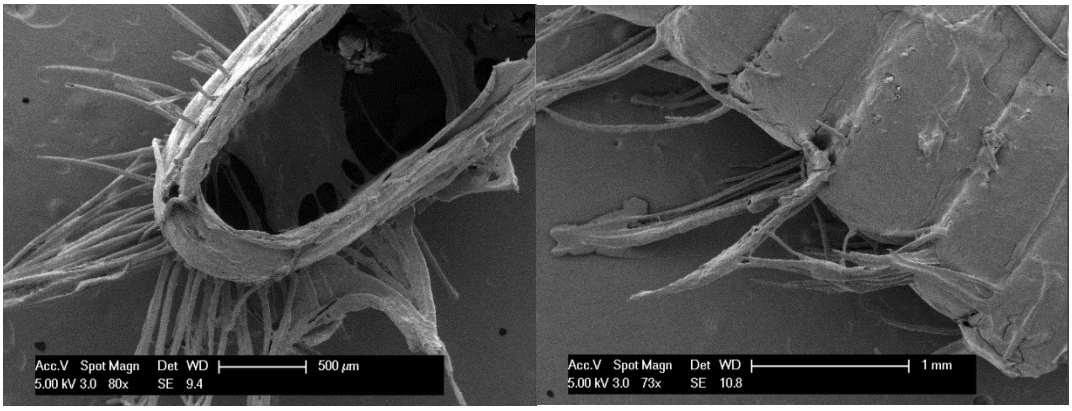

Figure 3.4A: ESEM images of *B. cornutus* antennae taken at 500 µm. The left image is a top down view, while the image on the right is a side view.

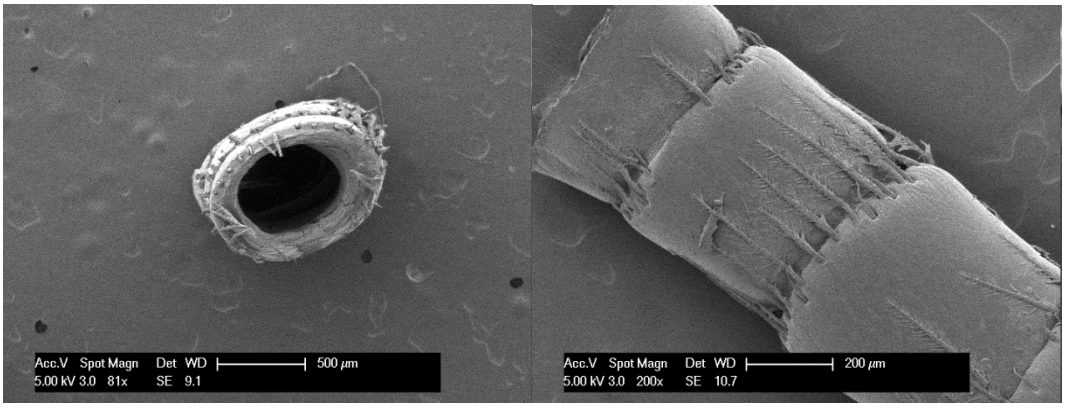

Figure 3.4B: ESEM images of *O. virilis* antennae taken at 500 µm. The left image is a top down view, while the image on the right is a side view.

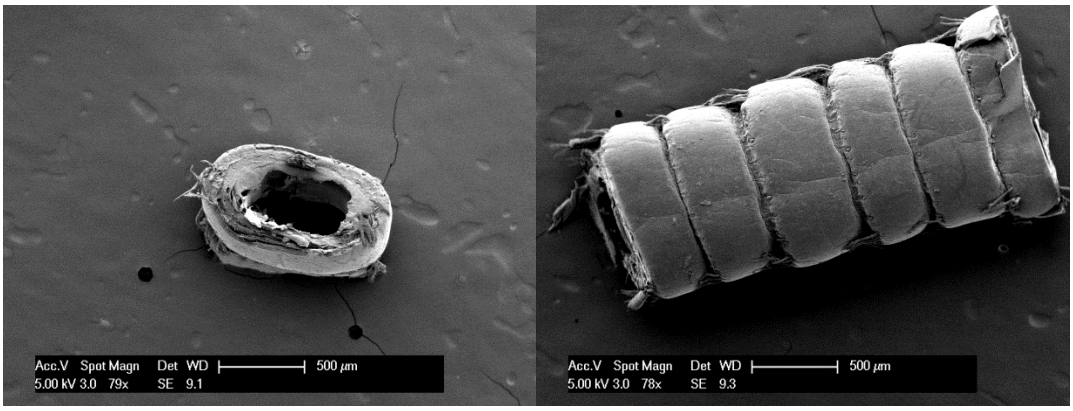

Figure 3.4C: ESEM images of *C. girardianus* antennae taken at 500 µm. The left image is a top down view, while the image on the right is a side view.

Figure 3.4 cont.

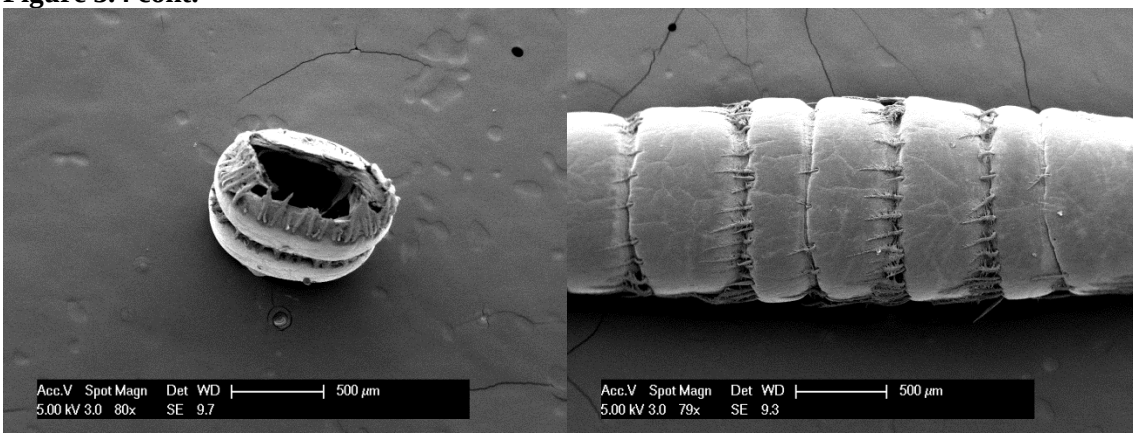

Figure 3.4D: ESEM images of *C. graysoni* antennae taken at 500 µm. The left image is a top down view, while the image on the right is a side view.

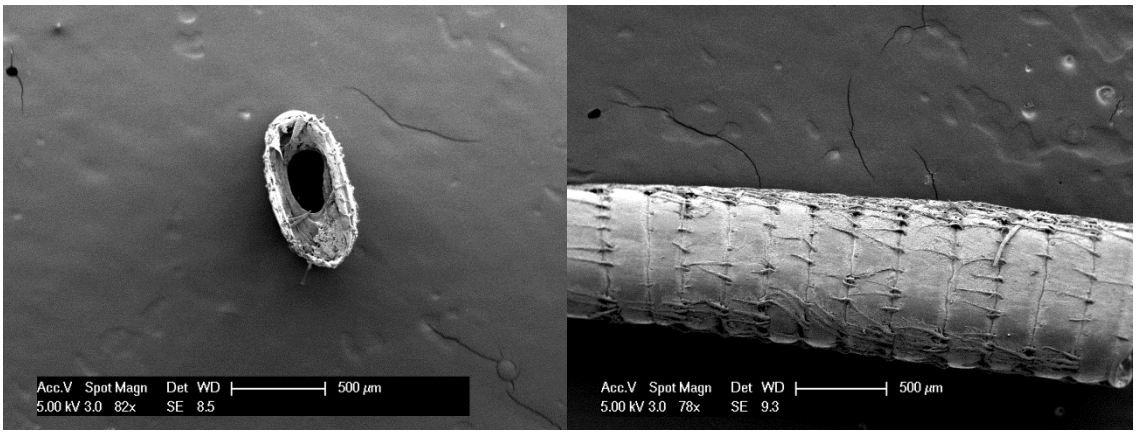

Figure 3.4E: ESEM images of *P. lephotis* antennae taken at 500 µm. The left image is a top down view, while the image on the right is a side view.

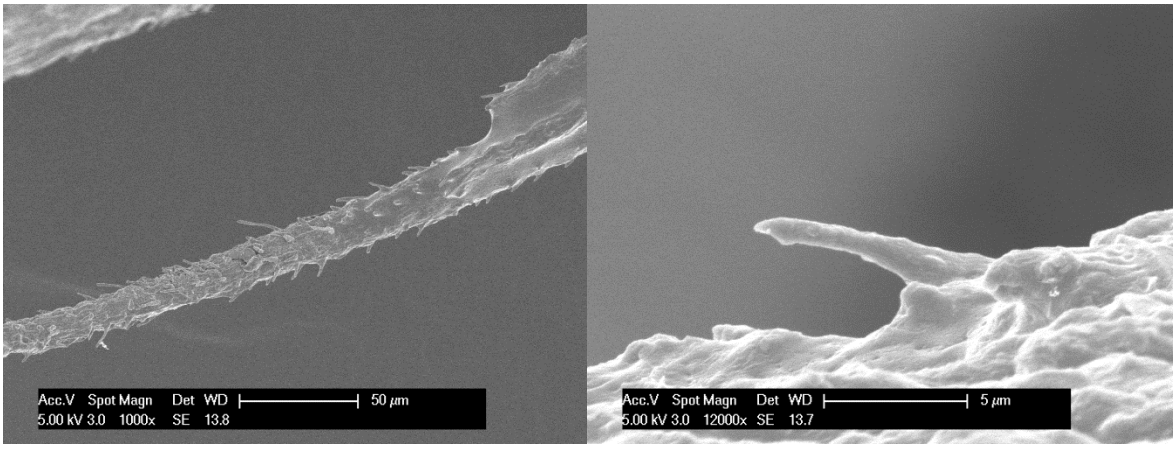

Figure 3.5A: The antennal setae of *B. cornutus*\ at a scale of 50 µm (left) and 5 µm (right)

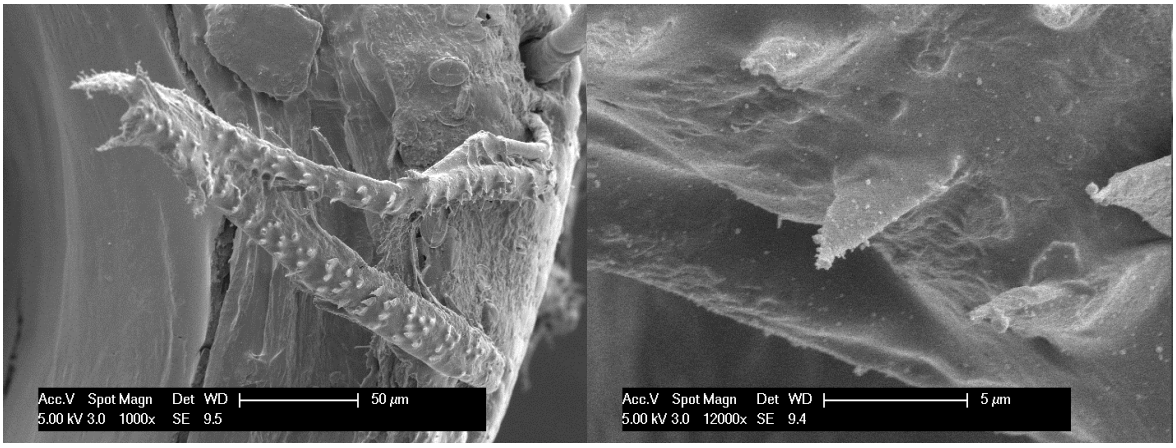

Figure 3.5B: The antennal setae of *O. virilis* at a scale of 50 µm (left) and 5 µm (right)

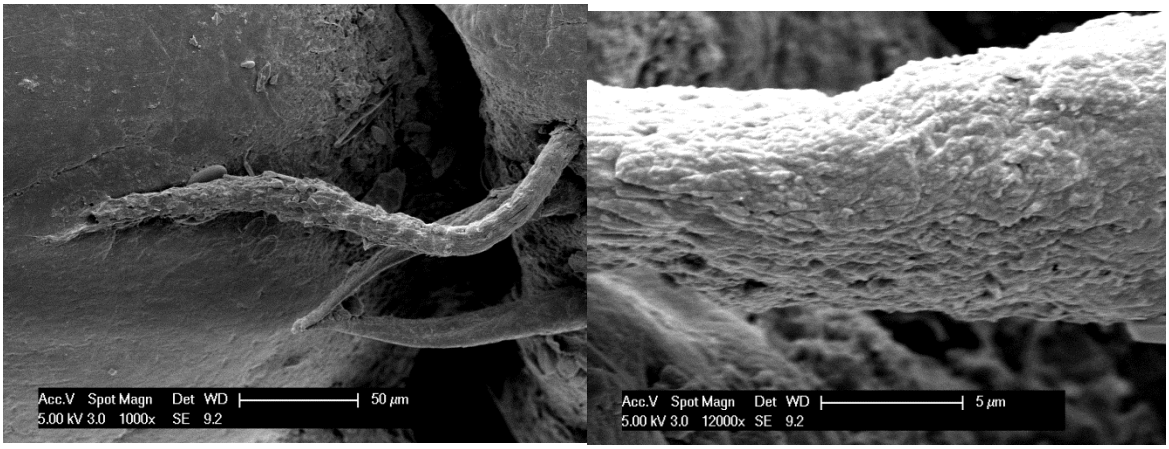

Figure 3.5C: The antennal setae of *C. girardianus* at a scale of 50 µm (left0 and 5 µm (right)

Figure 3.5 cont.

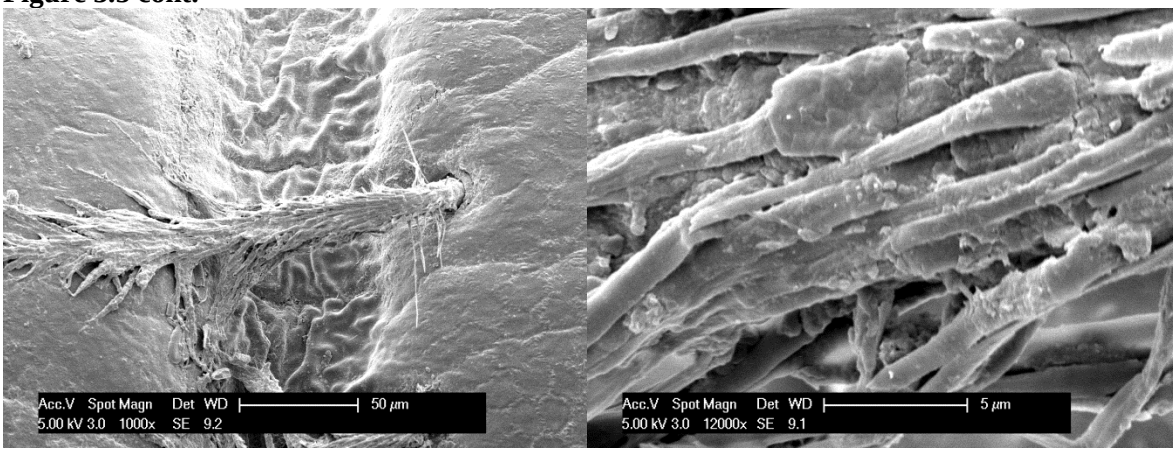

Figure 3.5D: The antennal setae of *C. graysoni* at a scale of 50 µm (left) and 5 µm (right)

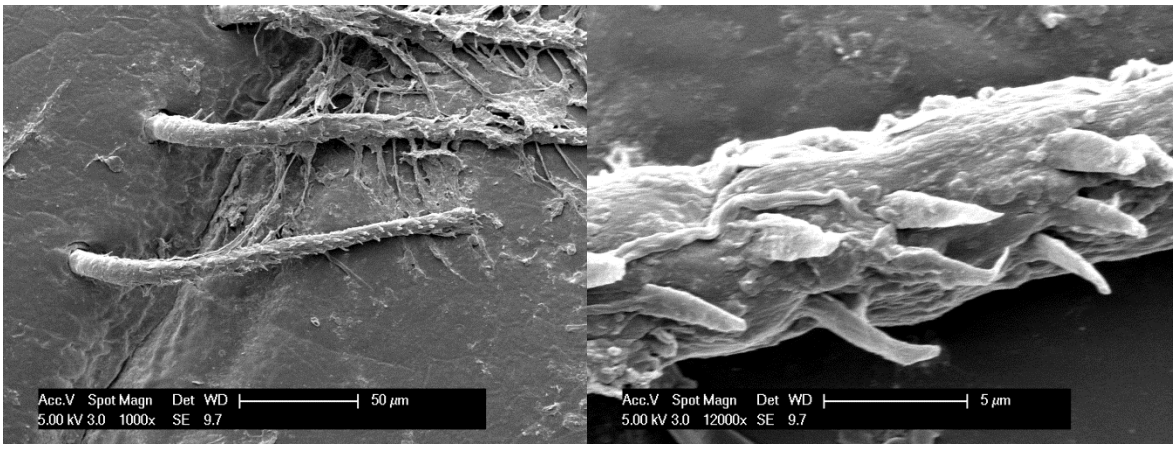

Figure 3.5E: The antennal setae of *P. lephotis* at a scale of 50 µm (left) and 5 µm (right)

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CHAPTER 4: SUMMARY

The southeast United States is a known hotspot in North America for aquatic biodiversity. However, many of the aquatic species within this assemblage of rich diversity are endemic to certain drainages and water bodies. Small distributions make these endemic groups more vulnerable to negative affects by any kind of natural or man-made disaster. It is important to do as much as possible to understand and maintain the high level of biodiversity in the southeast United States. One major aquatic group, crayfish, has a large knowledge gap in basic natural history for many species. Natural history knowledge is essential if efforts ever need to be made to attempt to manage for an existing species.

My study of the distribution and habitat use of the Tennessee Bottlebrush Crayfish, yielded valuable information expanding the known distribution from only 3 sites to 14 and determined habitat use and site covariates that may be indicative of site occupancy. My results provide information that was previously unknown or only speculated at, and can be beneficial in the event that a management plan is needed for *B. simmonsi*.

I also performed a stable isotope analysis on *B. simmonsi* and *B. cornutus*. I yielded similar results between the two species indicating *Barbicambarus* crayfish occupy a higher trophic level than other crayfish within their sites, indicating the possibility of some level of niche separation and differentiation in ecosystem roles. I also observed the larger body size of *Barbicambarus* crayfish is likely responsible for their elevated trophic position, as they show a positive correlation between body size and trophic positon, unlike other crayfish species. This information is important because it suggests *Barbicambarus* crayfish perform a different functional role within the stream, and could put a greater emphasis on why we should ensure they maintain stable populations.

Appendix A: Occupancy Modeling Supplementary Material

Table A.1 Ranking of occupancy models for *Barbicambarus simmonsi* in Shoal Creek based on Akaike's Information Criterion (AIC). Detection covariates include depth and substrate. Occupancy covariates included site width, flow, substrate, depth, type of site, and the presence-absence of other crayfish species (OS). ΔAIC = AIC for a given model minus AIC for the best model. *K* = number of model parameters, w_i = Akaike weights, LL is the log-likelihood, and Likelihood is the model likelihood.

Model	ΔAIC	W_i	K	-2*LL	Likelihood
ψ(flow),p(depth,substrate)	0	0.1444	5	98.37	1
ψ(flow,OS),p(depth,substrate)	1.18	0.08	6	97.55	0.5543
ψ(flow,depth),p(depth,substrate)	1.99	0.0534	6	98.36	0.3697
ψ(flow,substrate),p(depth,substrate)	1.99	0.0534	6	98.36	0.3697
ψ(width,flow),p(depth,substrate)	2	0.0531	6	98.37	0.3679
ψ(site,flow),p(depth,substrate)	2	0.0531	6	98.37	0.3679
ψ(.),p(depth,substrate)	2.36	0.0444	4	102.73	0.3073
ψ(site,flow,OS),p(depth,substrate)	2.79	0.0358	7	97.16	0.2478
ψ(flow,depth,OS),p(depth,substrate)	2.94	0.0332	7	97.31	0.2299
ψ(flow,substrate,OS),p(depth,substrate)	2.94	0.0332	7	97.31	0.2299
ψ(width,flow,OS),p(depth,substrate)	3.17	0.0296	7	97.54	0.2049
ψ(OS),p(depth,substrate)	3.64	0.0234	5	102.01	0.162
ψ(width,flow,substrate),p(depth,substrate)	3.98	0.0197	7	98.35	0.1367
ψ(site,flow,depth),p(depth,substrate)	3.98	0.0197	7	98.35	0.1367
ψ(site,flow,substrate),p(depth,substrate)	3.98	0.0197	7	98.35	0.1367
ψ(width,flow,depth),p(depth,substrate)	3.98	0.0197	7	98.35	0.1367
ψ(width,site,flow),p(depth,substrate)	3.99	0.0196	7	98.36	0.136
ψ(flow,depth,substrate),p(depth,substrate)	3.99	0.0196	7	98.36	0.136
ψ(width),p(depth,substrate)	4.14	0.0182	5	102.51	0.1262
ψ(depth),p(depth,substrate)	4.23	0.0174	5	102.6	0.1206
ψ(substrate),p(depth,substrate)	4.23	0.0174	5	102.6	0.1206
ψ(site),p(depth,substrate)	4.34	0.0165	5	102.71	0.1142
ψ(site,flow,depth,OS),p(depth,substrate)	4.72	0.0136	8	97.09	0.0944
ψ(width,flow,depth,OS),p(depth,substrate)	4.93	0.0123	8	97.3	0.085
ψ(site,OS),p(depth,substrate)	5.02	0.0117	6	101.39	0.0813
ψ(width,OS),p(depth,substrate)	5.24	0.0105	6	101.61	0.0728
ψ(substrate,OS),p(depth,substrate)	5.63	0.0086	6	102	0.0599
ψ(depth,OS),p(depth,substrate)	5.63	0.0086	6	102	0.0599
ψ(site,flow,depth,substrate),p(depth,substrate)	5.98	0.0073	8	98.35	0.0503
ψ(width,site,flow,depth),p(depth,substrate)	5.98	0.0073	8	98.35	0.0503
ψ(width,site,flow,substrate),p(depth,substrate)	5.98	0.0073	8	98.35	0.0503
ψ(width,flow,depth,substrate),p(depth,substrate)	5.98	0.0073	8	98.35	0.0503
ψ(width,depth),p(depth,substrate)	6	0.0072	6	102.37	0.0498
ψ(width,substrate),p(depth,substrate)	6	0.0072	6	102.37	0.0498

Table A.1 cont'd

Model	ΔAIC	W_i	K	-2*LL	Likelihood
ψ(site,substrate),p(depth,substrate)	6.03	0.0071	6	102.4	0.049
ψ(site,depth),p(depth,substrate)	6.03	0.0071	6	102.4	0.049
ψ(width,site),p(depth,substrate)	6.12	0.0068	6	102.49	0.0469
ψ(depth,substrate),p(depth,substrate)	6.23	0.0064	6	102.6	0.0444
ψ(site,depth,OS),p(depth,substrate)	6.77	0.0049	7	101.14	0.0339
ψ(site,substrate,OS),p(depth,substrate)	6.77	0.0049	7	101.14	0.0339
ψ(width,site,OS),p(depth,substrate)	6.93	0.0045	7	101.3	0.0313
ψ(width,depth,OS),p(depth,substrate)	7.24	0.0039	7	101.61	0.0268
ψ(width,substrate,OS),p(depth,substrate)	7.24	0.0039	7	101.61	0.0268
ψ(depth,substrate,OS),p(depth,substrate)	7.63	0.0032	7	102	0.022
ψ(width,site,substrate),p(depth,substrate)	7.96	0.0027	7	102.33	0.0187
ψ(width,site,depth),p(depth,substrate)	7.96	0.0027	7	102.33	0.0187
ψ(width,depth,substrate),p(depth,substrate)	8	0.0026	7	102.37	0.0183
ψ(site,depth,substrate),p(depth,substrate)	8.03	0.0026	7	102.4	0.018
ψ(site,depth,substrate,OS),p(depth,substrate)	8.77	0.0018	8	101.14	0.0125
ψ(width,depth,substrate,OS),p(depth,substrate)	9.24	0.0014	8	101.61	0.0099

Table A.2: Matrix of Pearson correlation coefficients (*r*) for occupancy covariates. Covariates include stream width, flow, depth, substrate, presence/absence of other crayfish species (OS), and site type. Values marked with an asterisk indicates a significant correlation (*P* <0.05)

	Width	**Flow**	**Depth**	**Substrate**	**OS**	**Site Type**
Width	1	-0.274	0.340*	-0.094	0.044	0.193
Flow	.	1	-0.245	0.200	-0.062	-0.055
Depth	.	.	1	0.194	-0.409*	0.813*
Substrate	.	.	.	1	-0.178	0.204
OS	.	.	.	.	1	-0.511*
Site Type	.	.	.	.	.	1

Table A.3: Table of Spearman correlation coefficients (ρ) for occupancy covariates. Covariates include stream width, flow, depth, substrate, presence/absence of other crayfish species (OS), and site type. Values marked with an asterisk indicates a significant correlation (*P* <0.05)

Variable	by Variable	Spearman ρ	Prob>|ρ|
Flow	Width	-0.3463	0.0229*
Depth	Width	0.1088	0.4875
Depth	Flow	-0.0305	0.8459
Substrate	Width	-0.1711	0.2727
Substrate	Flow	0.1496	0.3383
Substrate	Depth	0.2592	0.0932
OS	Width	0.0594	0.705
OS	Flow	-0.083	0.5968
OS	Depth	-0.3672	0.0154*
OS	Substrate	-0.0935	0.5507
Site Type	Width	0.1976	0.2097
Site Type	Flow	-0.1735	0.2718
Site Type	Depth	0.6689	<.0001*
Site Type	Substrate	0.0654	0.6807
Site Type	OS	-0.5447	0.0002*

Appendix B: Crayfish Sampling Supplementary Material

Table B: Habitat covariates at each kick set location where *B. simmonsi* was not collected, at sampling reaches where *B. simmonsi* was present. Substrate measurements were recorded at each point of a substrate cross giving 5 readings. B=Boulder, C=Cobble, P=Pebble, G=Gravel, S=Sand, Si=Silt, and Bed=Bedrock. Lat/longs are unique to the site not the kick set.

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Shoal Creek	35.13384, -87.44829	GCPPP	0.14	0.2	Riffle/Run
Shoal Creek	"	CPGGG	0.16	0.9	Riffle/Run
Shoal Creek	"	PPGGC	0.505	0.8	Riffle/Run
Shoal Creek	"	PPPCC	0.5	0.7	Riffle/Run
Shoal Creek	"	PPGGC	0.2	1.1	Riffle/Run
Shoal Creek	"	CCCPG	0.26	0.9	Riffle/Run
Shoal Creek	"	CCPPG	0.32	1	Riffle/Run
Shoal Creek	"	CPPGG	0.24	1	Riffle/Run
Shoal Creek	"	PGGGG	0.26	0.5	Riffle/Run
Shoal Creek	"	PGGGG	0.185	0.7	Riffle/Run
Shoal Creek	"	CCPGG	0.25	1	Riffle/Run
Shoal Creek	"	CCPPP	0.39	0.7	Riffle/Run
Shoal Creek	"	CCPPG	0.425	0.4	Riffle/Run
Shoal Creek	"	CPPPG	0.475	0.7	Riffle/Run
Shoal Creek	"	CGGGG	0.08	0	Riffle/Run
Shoal Creek	"	CCGGG	0.08	0	Riffle/Run
Shoal Creek	"	CPPGG	0.635	0.8	Riffle/Run
Shoal Creek	"	CPGGS	0.08	0	Riffle/Run
Shoal Creek	"	BBPPG	0.08	0	Riffle/Run
Shoal Creek	"	CCPPG	0.37	1.5	Riffle/Run
Shoal Creek	"	PPSSS	0.08	0.2	Riffle/Run
Shoal Creek	"	PPPGS	0.08	0.2	Riffle/Run
Shoal Creek	"	CPPPP	0.255	1.4	Riffle/Run
Shoal Creek	"	GGGGBed	0.16	0.7	Riffle/Run
Shoal Creek	"	GGGGS	0.23	0.7	Riffle/Run
Shoal Creek	"	CCCPP	0.61	0.6	Riffle/Run
Shoal Creek	"	PSSSS	0.2	0.2	Riffle/Run
Shoal Creek	"	GGGGG	0.16	0.3	Riffle/Run
Shoal Creek	"	GGGGG	0.23	0.7	Riffle/Run
Shoal Creek	"	BBCCC	0.21	0.1	Riffle/Run
Shoal Creek	35.1066, -87.50932	BPPGG	0.365	0.7	Riffle/Run
Shoal Creek	"	Bedx5	0.525	1	Riffle/Run
Shoal Creek	"	BBGGG	0.01	0.1	Riffle/Run
Shoal Creek	"	SSBedx3	0.57	0.1	Riffle/Run

Table B cont'd

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Shoal Creek	"	CCPPP	0.255	0	Riffle/Run
Shoal Creek	"	CPPPG	0.2	0	Riffle/Run
Shoal Creek	"	BCBedx3	0.59	1.1	Riffle/Run
Shoal Creek	"	PGGGG	0.59	0.2	Riffle/Run
Shoal Creek	"	SiSiSiSiBed	0.62	0.2	Riffle/Run
Shoal Creek	"	CPPPP	0.13	0.1	Riffle/Run
Shoal Creek	"	PGGGG	0.32	0	Riffle/Run
Shoal Creek	"	PPGGG	0.335	0	Riffle/Run
Shoal Creek	"	PPPPP	0.82	1	Riffle/Run
Shoal Creek	"	PPPPP	0.125	0	Riffle/Run
Shoal Creek	"	PGGGG	0.255	0.4	Riffle/Run
Shoal Creek	"	CPPPP	0.175	1.5	Riffle/Run
Shoal Creek	"	PPGGG	0.08	0.5	Riffle/Run
Shoal Creek	"	CCPPP	0.08	0.7	Riffle/Run
Shoal Creek	"	CCPPP	0.195	1	Riffle/Run
Shoal Creek	"	PPPGG	0.32	0.9	Riffle/Run
Shoal Creek	"	BBBBG	0.25	0.9	Riffle/Run
Shoal Creek	"	CCPPP	0.45	0.9	Riffle/Run
Shoal Creek	"	PPPPP	0.08	0	Riffle/Run
Shoal Creek	"	PPPPP	0.08	0	Riffle/Run
Shoal Creek	"	PPPGG	0.555	0.6	Riffle/Run
Shoal Creek	"	GGGGG	0.35	0	Riffle/Run
Shoal Creek	"	GGSSS	0.495	0	Riffle/Run
Shoal Creek	"	CPGGG	0.62	0.7	Riffle/Run
Shoal Creek	"	PPGGG	0.08	0.1	Riffle/Run
Shoal Creek	35.10046, -87.52119	CPPGG	0.125	0.3	Riffle/Run
Shoal Creek	"	GGGBP	0.43	1.3	Riffle/Run
Shoal Creek	"	BBBPG	0.32	0.9	Riffle/Run
Shoal Creek	"	BBCCP	0.3	0.1	Riffle/Run
Shoal Creek	"	CCCCC	0.31	0.4	Riffle/Run
Shoal Creek	"	CCPGG	0.135	0.5	Riffle/Run
Shoal Creek	"	PPGSS	0.195	0.4	Riffle/Run
Shoal Creek	"	PPPPG	0.21	0.7	Riffle/Run
Shoal Creek	"	PPPPC	0.8	0.2	Riffle/Run
Shoal Creek	"	PPPPG	0.8	0.4	Riffle/Run
Shoal Creek	"	CPGGG	0.155	0.7	Riffle/Run
Shoal Creek	"	BBGGG	0.535	1.2	Riffle/Run
Shoal Creek	"	CCPGG	0.17	0.5	Riffle/Run

Table B cont'd

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Shoal Creek	"	GGGGW	0.19	0.4	Riffle/Run
Shoal Creek	"	Bedx5	0.79	0.6	Riffle/Run
Shoal Creek	"	PPGGG	0.36	0.4	Riffle/Run
Shoal Creek	"	PPGGG	0.395	0.5	Riffle/Run
Shoal Creek	"	BGGGBed	0.69	0.6	Riffle/Run
Shoal Creek	"	Bedx5	0.87	0.8	Riffle/Run
Shoal Creek	"	Bedx5	0.87	0.7	Riffle/Run
Shoal Creek	"	BGGGG	0.88	0.9	Riffle/Run
Shoal Creek	"	GGGGG	0.175	0.2	Riffle/Run
Shoal Creek	"	PPGGG	0.2	0.2	Riffle/Run
Shoal Creek	"	CPPPG	0.41	0.3	Riffle/Run
Shoal Creek	"	GGGGG	0.51	0.5	Riffle/Run
Shoal Creek	"	GGGGG	0.45	0.5	Riffle/Run
Shoal Creek	"	GGGGG	0.54	1.4	Riffle/Run
Shoal Creek	"	PPGGG	0.375	0.9	Riffle/Run
Shoal Creek	"	BBGGG	0.33	0.9	Riffle/Run
Shoal Creek	"	BGGGG	0.59	0.9	Riffle/Run
Shoal Creek	35.08008, -87.54720	GGGGG	0.295	0.1	Riffle/Run
Shoal Creek	"	CCCCC	0.44	0.4	Riffle/Run
Shoal Creek	"	Bedx5	0.56	1	Riffle/Run
Shoal Creek	"	Bedx5	0.54	1	Riffle/Run
Shoal Creek	"	Bedx5	0.95	0.5	Riffle/Run
Shoal Creek	"	GGGGG	0.555	0.1	Riffle/Run
Shoal Creek	"	PPGSS	0.635	0.3	Riffle/Run
Shoal Creek	"	Bedx5	0.885	0.9	Riffle/Run
Shoal Creek	"	BBBedx3	0.38	0.5	Riffle/Run
Shoal Creek	"	Bedx5	0.45	0.7	Riffle/Run
Shoal Creek	"	PPPPG	0.65	0.1	Riffle/Run
Shoal Creek	"	GGGGG	0.395	0	Riffle/Run
Shoal Creek	"	CCPPP	0.38	0	Riffle/Run
Shoal Creek	"	GGGGG	0.68	0.9	Riffle/Run
Shoal Creek	"	BGBedx3	0.45	0.9	Riffle/Run
Shoal Creek	"	BPPPP	0.6	1.2	Riffle/Run
Shoal Creek	"	SiSiSiSiSi	0.205	0.5	Riffle/Run
Shoal Creek	"	PPPPP	0.235	1.2	Riffle/Run
Shoal Creek	"	CGGGG	0.42	1.1	Riffle/Run
Shoal Creek	"	BBCCG	0.44	1.3	Riffle/Run
Shoal Creek	"	BBBedx3	0.56	1.1	Riffle/Run

Table B cont'd

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Shoal Creek	"	BBBBP	0.34	0.4	Riffle/Run
Shoal Creek	"	GGGGG	0.08	0.3	Riffle/Run
Shoal Creek	"	GGGGG	0.12	0.3	Riffle/Run
Shoal Creek	"	BBBGG	0.365	0.7	Riffle/Run
Shoal Creek	"	BPBedx3	0.36	0.5	Riffle/Run
Shoal Creek	"	BBBedx3	0.345	0.8	Riffle/Run
Shoal Creek	"	BBBGG	0.35	0.8	Riffle/Run
Shoal Creek	"	GGGGG	0.16	0.1	Riffle/Run
Shoal Creek	35.05922, -87.56850	PPGGG	0.29	0	Riffle/Run
Shoal Creek	"	PPPPP	0.47	0.3	Riffle/Run
Shoal Creek	"	CSSSS	0.545	0.6	Riffle/Run
Shoal Creek	"	CPGSS	0.375	0.6	Riffle/Run
Shoal Creek	"	BBBGG	0.235	0.4	Riffle/Run
Shoal Creek	"	PPPGG	0.14	0.1	Riffle/Run
Shoal Creek	"	PPGGG	0.16	0.1	Riffle/Run
Shoal Creek	"	BBBPG	0.465	0.5	Riffle/Run
Shoal Creek	"	CGGGG	0.205	0.4	Riffle/Run
Shoal Creek	"	BCGGG	0.27	0.2	Riffle/Run
Shoal Creek	"	GGGGG	0.45	0	Riffle/Run
Shoal Creek	"	BBBBB	0.7	0	Riffle/Run
Shoal Creek	35.05085, -87.56524	BBBGG	0.27	0.2	Riffle/Run
Shoal Creek	"	Bedx5	0.525	0.4	Riffle/Run
Shoal Creek	"	CCPGG	0.41	0.3	Riffle/Run
Shoal Creek	"	CPPPG	0.405	0.3	Riffle/Run
Shoal Creek	"	Bedx5	0.53	0.8	Riffle/Run
Shoal Creek	"	Bedx5	0.175	0.2	Riffle/Run
Shoal Creek	"	Bedx5	0.21	0.1	Riffle/Run
Shoal Creek	"	BBCGBed	0.43	0.6	Riffle/Run
Shoal Creek	"	BCGGG	0.38	0.5	Riffle/Run
Shoal Creek	"	GGGGG	0.33	0.5	Riffle/Run
Shoal Creek	"	Bedx5	0.535	0.4	Riffle/Run
Shoal Creek	"	BBedx4	0.12	0.1	Riffle/Run
Shoal Creek	"	Bedx5	0.155	0.1	Riffle/Run
Shoal Creek	"	BBedx4	0.435	0.4	Riffle/Run
Shoal Creek	"	GGGBedx2	0.47	0.3	Riffle/Run
Shoal Creek	"	GGGGBed	0.48	0.3	Riffle/Run
Shoal Creek	"	Bedx5	0.46	0.4	Riffle/Run

Table B cont'd

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Shoal Creek	"	BBedx4	0.085	0.2	Riffle/Run
Shoal Creek	"	Bedx5	0.07	0.2	Riffle/Run
Shoal Creek	"	GGGBedx2	0.37	0.4	Riffle/Run
Shoal Creek	"	PPPPP	0.19	0.1	Riffle/Run
Shoal Creek	"	PPGGG	0.2	0.1	Riffle/Run
Shoal Creek	"	GGGGG	0.545	0.3	Riffle/Run
Shoal Creek	"	Bedx5	0.245	0	Riffle/Run
Shoal Creek	"	Bedx5	0.2	0	Riffle/Run
Shoal Creek	"	Bedx5	0.68	0.3	Riffle/Run
Shoal Creek	"	GGGGG	0.34	0	Riffle/Run
Shoal Creek	"	PGGGG	0.4	0	Riffle/Run
Shoal Creek	"	SBedx4	0.64	0.5	Riffle/Run
Shoal Creek	35.03847, -87.56725	GGGGG	0.26	0.4	Riffle/Run
Shoal Creek	"	Bedx5	0.43	0.4	Riffle/Run
Shoal Creek	"	GGGGG	0.3	0.2	Riffle/Run
Shoal Creek	"	GGSSS	0.26	0.2	Riffle/Run
Shoal Creek	"	Bedx5	0.345	0.5	Riffle/Run
Shoal Creek	"	GSiSiSiSi	0.295	0.8	Riffle/Run
Shoal Creek	"	GGGGG	0.29	0.8	Riffle/Run
Shoal Creek	"	PGBedx3	0.435	0.5	Riffle/Run
Shoal Creek	"	Bedx5	0.455	0.6	Riffle/Run
Shoal Creek	"	Bedx5	0.515	0.6	Riffle/Run
Shoal Creek	"	GBedx4	0.535	0.2	Riffle/Run
Shoal Creek	"	GGGGG	0.57	0.2	Riffle/Run
Shoal Creek	"	Bedx5	0.59	0.9	Riffle/Run
Shoal Creek	"	BBCCP	0.22	0.1	Riffle/Run
Shoal Creek	"	BCPPG	0.1	0.1	Riffle/Run
Shoal Creek	"	Bedx5	0.56	0.5	Riffle/Run
Shoal Creek	"	SSSSS	0.2	0	Riffle/Run
Shoal Creek	"	SSSSS	0.225	0	Riffle/Run
Shoal Creek	"	SSSSBed	0.67	0.7	Riffle/Run
Shoal Creek	"	PGGGS	0.365	0	Riffle/Run
Shoal Creek	"	PPGSS	0.39	0	Riffle/Run
Shoal Creek	"	GGBedx3	0.635	0.6	Riffle/Run
Shoal Creek	"	CPPGG	0.255	0.3	Riffle/Run
Shoal Creek	"	PPPPP	0.32	0.3	Riffle/Run
Shoal Creek	"	GGGGG	0.67	0.4	Riffle/Run
Shoal Creek	"	Bedx5	0.53	0.1	Riffle/Run

Table B cont'd

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Shoal Creek	"	Bedx5	0.53	0.1	Riffle/Run
Shoal Creek	"	GBedx4	0.545	0.5	Riffle/Run
Shoal Creek	"	PPPPP	0.39	0.2	Riffle/Run
Shoal Creek	35.03237, -87.57729	PPPPG	0.11	0.1	Riffle/Run
Shoal Creek	"	CPPPP	0.33	0.7	Riffle/Run
Shoal Creek	"	PPGGG	0.265	0.4	Riffle/Run
Shoal Creek	"	GGGGG	0.32	0.1	Riffle/Run
Shoal Creek	"	PPPPP	0.4	0.8	Riffle/Run
Shoal Creek	"	CPPGG	0.27	0.6	Riffle/Run
Shoal Creek	"	BPPPG	0.175	0.5	Riffle/Run
Shoal Creek	"	PPPGG	0.31	0.7	Riffle/Run
Shoal Creek	"	PPPPP	0.195	0.7	Riffle/Run
Shoal Creek	"	PPPPP	0.245	0.6	Riffle/Run
Shoal Creek	"	PPPGG	0.385	0.5	Riffle/Run
Shoal Creek	"	PPPPP	0.085	0.1	Riffle/Run
Shoal Creek	"	PPPPG	0.16	0.1	Riffle/Run
Shoal Creek	"	GGGGG	0.435	0.3	Riffle/Run
Shoal Creek	"	PPGGG	0.28	0.1	Riffle/Run
Shoal Creek	35.00348, -87.57726	CCPPP	0.26	0.7	Riffle/Run
Shoal Creek	"	GGGGG	0.43	0.8	Riffle/Run
Shoal Creek	"	PGGGBed	0.3	0.7	Riffle/Run
Shoal Creek	"	PPPPG	0.26	0.5	Riffle/Run
Shoal Creek	"	GGGGG	0.345	0.9	Riffle/Run
Shoal Creek	"	PPGGG	0.295	0.3	Riffle/Run
Shoal Creek	"	PPGGG	0.29	0.2	Riffle/Run
Shoal Creek	"	PPPPP	0.435	1.1	Riffle/Run
Shoal Creek	"	CCGGG	0.45	0.5	Riffle/Run
Shoal Creek	"	BBBGG	0.455	0.5	Riffle/Run
Shoal Creek	"	PPPPP	0.515	1.3	Riffle/Run
Shoal Creek	"	CPPPP	0.535	0.1	Riffle/Run
Shoal Creek	"	PGGGG	0.57	0.1	Riffle/Run
Shoal Creek	"	GGGGG	0.59	0.8	Riffle/Run
Shoal Creek	"	PPPPP	0.22	0.7	Riffle/Run
Shoal Creek	"	PPPPP	0.1	0.7	Riffle/Run
Shoal Creek	"	CPPPP	0.56	0.9	Riffle/Run
Shoal Creek	"	CPPPG	0.2	0.4	Riffle/Run
Shoal Creek	"	CCPPG	0.225	0.3	Riffle/Run
Shoal Creek	"	PPPPG	0.67	0.5	Riffle/Run

Table B cont'd

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Shoal Creek	"	CPPPP	0.365	0.3	Riffle/Run
Shoal Creek	"	PPPGS	0.39	0.3	Riffle/Run
Shoal Creek	"	PGGGG	0.635	0.4	Riffle/Run
Shoal Creek	"	BGGGG	0.255	0.2	Riffle/Run
Shoal Creek	"	PPGGG	0.32	0.2	Riffle/Run
Shoal Creek	"	PPPPP	0.53	0.3	Riffle/Run
Shoal Creek	"	PPPGG	0.53	0.3	Riffle/Run
Shoal Creek	"	GGGGG	0.545	0.4	Riffle/Run
Shoal Creek	"	CGGGG	0.39	0.1	Riffle/Run
Shoal Creek	35.04272, -87.56043	Bedx5	1.9	0.6	Pool
Shoal Creek	"	Bedx5	1.7	0.5	Pool
Shoal Creek	35.01212, -87.57323	BBBBB	1.25	1	Pool
Shoal Creek	"	CGGGG	1.3	1.2	Pool
Shoal Creek	"	BBBGP	1.38	1.1	Pool
Shoal Creek	"	Bedx5	1	1.4	Pool
Shoal Creek	35.1203, -87.5089	PPPGG	0.165	0.4	Riffle/Run
Shoal Creek	"	CCPGG	0.61	1.2	Riffle/Run
Shoal Creek	"	CCGGG	0.05	0.4	Riffle/Run
Shoal Creek	"	CPGGG	0.18	0	Riffle/Run
Shoal Creek	"	PPPGS	0.495	0.7	Riffle/Run
Shoal Creek	"	PPPPG	0.125	0.5	Riffle/Run
Shoal Creek	"	PPGGG	0.19	0.4	Riffle/Run
Shoal Creek	"	GGGGG	0.675	0.5	Riffle/Run
Shoal Creek	"	Bedx5	0.77	0	Riffle/Run
Shoal Creek	"	SiSiSiSiSi	0.72	0	Riffle/Run
Shoal Creek	"	CPPPG	0.275	0.6	Riffle/Run
Shoal Creek	"	GGGGG	0.27	0.3	Riffle/Run
Shoal Creek	"	PGGGG	0.335	0.2	Riffle/Run
Shoal Creek	"	PPPGG	0.34	0.6	Riffle/Run
Shoal Creek	"	SSSSS	0.32	0	Riffle/Run
Shoal Creek	"	SSSSS	0.245	0.1	Riffle/Run
Shoal Creek	"	PPGGG	0.385	0.4	Riffle/Run
Shoal Creek	"	GGGSiSi	0.36	0.1	Riffle/Run
Shoal Creek	"	GGGGSi	0.365	0.1	Riffle/Run
Shoal Creek	"	CPPGG	0.435	0.5	Riffle/Run
Shoal Creek	"	SiSiSiSiSi	0.321	0.1	Riffle/Run
Shoal Creek	"	SiSiSiSiSi	0.262	0.2	Riffle/Run
Shoal Creek	"	PPPPP	0.415	0.5	Riffle/Run

Table B cont'd

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Shoal Creek	"	BBPPP	0.205	0.3	Riffle/Run
Shoal Creek	"	CGGGG	0.175	0.2	Riffle/Run
Shoal Creek	"	PPGGG	0.395	0.4	Riffle/Run
Shoal Creek	"	PGGSS	0.525	0.1	Riffle/Run
Shoal Creek	"	GGGSS	0.45	0.1	Riffle/Run
Shoal Creek	"	GGGGS	0.4	0.4	Riffle/Run
Shoal Creek	"	BBBGG	0.305	0.3	Riffle/Run
Shoal Creek	34.95339, -87.59387	CCCPP	0.28	0.5	Riffle/Run
Shoal Creek	"	Bedx5	0.18	0.6	Riffle/Run
Shoal Creek	"	Bedx5	0.26	0.2	Riffle/Run
Shoal Creek	"	Bedx5	0.095	0.2	Riffle/Run
Shoal Creek	"	CGGGBed	0.33	0.6	Riffle/Run
Shoal Creek	"	PPPGG	0.42	0.8	Riffle/Run
Shoal Creek	"	CCPGG	0.335	0.8	Riffle/Run
Shoal Creek	"	GGBedx3	0.405	0.6	Riffle/Run
Shoal Creek	"	CCBedx3	0.29	0.1	Riffle/Run
Shoal Creek	"	Bedx5	0.25	0.1	Riffle/Run
Shoal Creek	"	BBGGP	0.44	0.4	Riffle/Run
Shoal Creek	"	BBCGG	0.275	0.4	Riffle/Run
Shoal Creek	"	BCGGG	0.27	0.4	Riffle/Run
Shoal Creek	"	CGGGG	0.36	0.3	Riffle/Run
Shoal Creek	"	Bedx5	0.1	0.1	Riffle/Run
Shoal Creek	"	GGGGBed	0.1	0.1	Riffle/Run
Shoal Creek	"	BBGGG	0.345	0.4	Riffle/Run
Shoal Creek	"	PGSSS	0.13	0.1	Riffle/Run
Shoal Creek	"	PGSBedx2	0.19	0.1	Riffle/Run
Shoal Creek	"	CCGGBed	0.24	0.5	Riffle/Run
Shoal Creek	"	Bedx5	0.14	0.1	Riffle/Run
Shoal Creek	"	Bedx5	0.11	0.1	Riffle/Run
Shoal Creek	"	BBedx4	0.3	0.5	Riffle/Run
Shoal Creek	"	Bedx5	0.205	0.1	Riffle/Run
Shoal Creek	"	CCBedx3	0.135	0.1	Riffle/Run
Shoal Creek	"	BBBedx3	0.37	0.5	Riffle/Run
Shoal Creek	"	BBGGP	0.25	0.1	Riffle/Run
Shoal Creek	"	Bedx5	0.145	0.1	Riffle/Run
Shoal Creek	"	Bedx5	0.645	0.6	Riffle/Run
Shoal Creek	"	BGGGS	0.38	0	Riffle/Run
Factory Creek	35.10119, -87.53975	PPPGG	0.15	0.3	Riffle/Run

Table B cont'd

Site	Lat/Long	Substrate	Depth (m)	Flow (m/s)	Site Type
Factory Creek	"	BBGBedx2	0.15	0.6	Riffle/Run
Factory Creek	"	SSSSS	0.26	0.6	Riffle/Run
Factory Creek	"	CCPSBed	0.45	0.8	Riffle/Run
Factory Creek	"	CPPPP	0.16	0.1	Riffle/Run
Factory Creek	"	PPPPP	0.18	0.1	Riffle/Run
Factory Creek	"	PPPGC	0.65	0.75	Riffle/Run
Factory Creek	"	SSSGG	0.27	0.4	Riffle/Run
Factory Creek	"	SSSSS	0.25	0.4	Riffle/Run
Factory Creek	"	BBBGG	0.26	0.6	Riffle/Run
Factory Creek	"	GGGPP	0.1	0.3	Riffle/Run
Factory Creek	"	GGPPP	0.12	0.3	Riffle/Run
Factory Creek	"	PPPPP	0.56	0.5	Riffle/Run
Factory Creek	"	SSSPC	0.21	0	Riffle/Run
Factory Creek	"	PPGGG	0.08	0.2	Riffle/Run
Factory Creek	"	BBBGG	0.46	0.6	Riffle/Run
Factory Creek	"	CGPPP	0.4	0.1	Riffle/Run
Factory Creek	"	PPPPG	0.3	0.1	Riffle/Run
Factory Creek	"	Bedx5	0.06	0.1	Riffle/Run
Factory Creek	"	Bedx5	0.05	0.1	Riffle/Run
Factory Creek	"	SSBedx3	0.53	0.5	Riffle/Run
Factory Creek	"	GGGGG	0.37	0.5	Riffle/Run
Factory Creek	"	GGGGS	0.34	0.5	Riffle/Run
Factory Creek	"	CCBedx3	0.46	0.6	Riffle/Run
Factory Creek	"	Bedx5	0.11	0	Riffle/Run
Factory Creek	"	Bedx5	0.12	0	Riffle/Run
Factory Creek	"	GGGPBed	0.51	0.5	Riffle/Run
Factory Creek	"	SSSSS	0.1	0	Riffle/Run