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# Benthic Macroinvertebrate Community Responses to a Headwater Valley Restoration at Slabcamp Creek, Rowan Co., Kentucky

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BENTHIC MACROINVERTEBRATE COMMUNITY RESPONSES TO A HEADWATER  
VALLEY RESTORATION AT SLABCAMP CREEK, ROWAN CO., KENTUCKY


By


Nicholas P. Revetta

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Chair, Advisory Committee

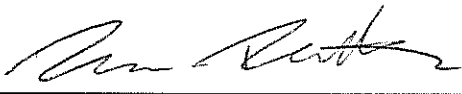
  
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BENTHIC MACROINVERTEBRATE COMMUNITY RESPONSES TO A  
HEADWATER VALLEY RESTORATION AT SLABCAMP CREEK, ROWAN CO.,  
KENTUCKY

By

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Bachelor of Science

Eastern Kentucky University

Richmond, Kentucky

2014

Submitted to the Faculty of the Graduate School of  
Eastern Kentucky University  
in partial fulfillment of the requirements  
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## DEDICATION

This thesis is dedicated to my family  
Randy, Janice, and Mitch Revetta  
for their constant support.

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## ABSTRACT

The practice of stream restoration is well underway in the U.S., but there are few quantitative post-restoration studies of macroinvertebrate communities in restored streams in Kentucky. Slabcamp Creek, a first order tributary within the Licking River Basin, was recently restored to improve hydrology and degraded habitat caused by historical land use. The primary goal of my study was to begin baseline studies of the macroinvertebrate community in the restored section of Slabcamp Creek and to compare those findings to White Pine Branch (a pre-restoration control site) during the first post-restoration year. Specific objectives of my study were to: 1) report seasonal estimates of macroinvertebrate abundance and biomass from riffles, 2) describe the macroinvertebrate community structure from riffles, and 3) measure channel habitat at the study sites. Results from habitat measures indicated that, during low base flow, both channels lost wetted habitat, but the difference in wetted habitat lost between spring and summer was greater at White Pine Branch than Slabcamp Creek. Relative to White Pine Branch, Slabcamp Creek had more large woody debris, less canopy cover, and greater amounts of fine sediments and no bedrock. Macroinvertebrates were collected from five riffles in each stream with a bottom area sampler during fall 2011, winter 2012, and spring 2012. Repeated measures ANOVA indicated greater macroinvertebrate abundance and biomass at Slabcamp Creek, but no difference in taxa richness was detected between streams. Community metrics based on absolute abundance revealed greater abundance of EPT taxa, scrapers/grazer, clingers, slow seasonal developers, taxa with a large body size at maturity, and low rheophilic taxa at Slabcamp Creek. Finally, patterns from multivariate ordinations showed more seasonal variation in macroinvertebrate community composition at White Pine Branch. Overall findings from this study suggest that differences in macroinvertebrate communities between streams during the first post-restoration year likely resulted from improved hydrology, channel bed stability, and benthic food resources associated with the restoration practices at Slabcamp Creek.



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## CHAPTER I

### **Introduction:**

#### *Historical land use and channel alteration*

Many stream and river channels of the United States, including those of the Appalachian physiographic region, have been modified as a result of historical and current land use practices (Brookes 1988, Yarnell 1998). By the time national forests were established in the early 20<sup>th</sup> century, nearly 70% of Appalachian forests were cutover for lumber (Yarnell 1998). Historical timber harvest, lumbering, and farming practices introduced sediments from hillsides to valley bottoms and altered the structure of stream channels, and the effects remain to this day. In 2008, it was estimated that nearly 99% of perennial streams in the Appalachian Highland physiographic region showed some sign of modification as a result of historic land use practices (Mastin 2008).

Land use practices that relocate, straighten, widen, or deepen natural channels (i.e., channelization) disrupt natural flow regimes and ultimately degrade the physical and hydrologic integrity of streams and rivers (Brookes 1988, Bunn and Arthington 2002, Shankman and Smith 2004, Asmus et al. 2009). Altered channels can become disconnected from their natural floodplains and aquifers, and thus leads to intermittent flow patterns (Asmus et al. 2009, Shields et al. 1994). Channels with less connection to floodplains are also unable to dissipate the force of flows, so the frequency and magnitude of erosive, scouring events increase (Schumm et al. 1984, Poff et al. 1997,

Shields et al. 2010). These altered flow patterns homogenize the physical habitat of streams and cause incised channels with unstable banks and bed substrates (Waters 1995, Asmus 2009, Kroes and Hupp 2010). Furthermore, since channels with reduced complexity are less retentive, downstream reaches can receive elevated sediments and nutrient loads (Shields et al. 1994, Noe and Hupp 2005, Milner and Gilvear 2012).

General findings from field studies indicate that the aquatic biota from channelized reaches are less diverse and abundant than the biota from unaltered reaches (Maul et al. 2004, Smiley and Dibble 2005, Lau et al. 2006, Engman et al. 2012). Moyle (1976) reported that, in addition to differences in the species composition, a channelized reach of a stream in California supported less than one-third of the invertebrate and fish biomass than the unchannelized reach. Negishi et al. (2002) found that effects of channelization on macroinvertebrates densities were especially pronounced following spates, which they attributed to less refugia and habitat heterogeneity in a channelized reach. Rohasliney and Jackson (2008) attributed less invertebrate richness and abundance in a channelized reach to powerful flushing flows, sediment transport, and lack of stable attachment as a result of the long term, persistent, negative impacts of channelization in Mississippi streams. In addition, a study performed by Paetzold et al. (2008) from channelized streams in Europe provides some evidence that riparian arthropods were negatively correlated with flood frequency and substrate embeddedness, which suggests that channelization has negative impacts on riparian as well as aquatic communities.

The best attainable stream condition today is an altered condition since there are truly no anthropogenically undisturbed streams (Foster et al. 2003). For monitoring and assessment purposes in Kentucky, the best attainable stream condition (the reference condition) is represented by streams with well developed forested riparian zones, relatively stable banks, low conductivity and fine sediments (less than 25%), water that is free from suspended solids, algal mats, and solid waste,  $\geq 70\%$  mix of stable habitat for aquatic biota, and land use conditions that are unchanged compared to recent maps (Pond et al. 2003). However, many streams that represent the state's reference condition are likely still adjusting to the effects of historic land use practices. Slabcamp Creek and White Pine Branch are tributaries in eastern Kentucky that have many attributes of the headwater reference condition. Today the streams drain watersheds of second growth forests that are owned by the USDA Forest Service and Slabcamp Creek is classified as an "exceptional" and "outstanding state resource water" in Kentucky Division of Water's Antidegradation Policy (401 KAR 10:030). However, many years ago trees were removed from the watershed and sections of the streams were moved from the center of their valleys to the base of the mountain, in order to support farming, and as a result, the streams lost their hydrologic functions. Sections of the channels eroded to bedrock and developed unstable habitat for macroinvertebrates and fish. The streams maintained a low water table, which resulted in an intermittent flow pattern and channel drying during late summer (Biebighauser 2006). In 2006, approximately one mile of Slabcamp Creek's headwater valley and its tributaries were restored in order to improve the hydrologic functions and degraded habitat caused by past land use (USDA Forest Service 2006).

### *Stream restoration*

According to the United States Environmental Protection Agency USEPA (2000), restoration is the return of a degraded ecosystem to a close approximation of its natural potential, and attempting to restore stream ecosystems has become an increasingly common practice (Bernhardt et al. 2005, Bernhardt and Palmer 2011). The number of restoration projects has risen dramatically over the last several decades, and as of July 2004 there were 37,099 stream restorations recorded within the United States with at least 14-15 billion dollars spent on these projects between 1990 and 2004 (Bernhardt et al. 2005). In Kentucky alone, as of 2013, there were 59 ongoing restoration projects (Kentucky Department of Fish and Wildlife Resources 2014).

Despite the increase in stream restoration projects, only a small proportion of restored streams have been assessed for ecological improvement). Bernhardt et al. (2005) reported only 10% of 37,099 restoration projects completed in the US have been assessed or monitored. Alexander and Allan (2006) reported similar findings and found only 11% of projects in the upper Midwest had been monitored. These findings are synonymous for Kentucky as well, where there has been very little post-restoration monitoring of the ecological success of stream restoration projects (Jack et al. 2003).

Although restoration success can be judged in a variety of ways, using ecological responses as a measure of success is in accordance with the USEPA's broader definition. There are many possible ecological responses to stream restoration, and there is currently debate as to what the appropriate indicator for ecological success for restoration projects should be (Bernhardt and Palmer 2011). For example, there is currently a disagreement

as to whether we should be using structural ecosystem attributes (e.g. richness, diversity) or measures of ecosystem function (e.g., production, nutrient uptake, decomposition) to measure success (Ryder et al. 2005). Despite this argument, macroinvertebrate community structure has often been used as a biological indicator of success in many post-restoration studies (Palmer et al. 2010). Although benthic macroinvertebrates assemblages are only one component of aquatic communities, they perform important functional roles in stream ecosystems. Macroinvertebrates play a significant role at intermediate levels of food webs and they influence important ecosystem processes (e.g. decomposition) (Wallace and Webster 1996, Wallace et al. 1996). Furthermore, metrics that summarize the structure of macroinvertebrate communities (e.g., EPT index, % clingers) are widely used to assess the biological integrity of freshwater ecosystems (Cairns and Pratt 1993, Rosenberg and Resh 1993, Bonada et al. 2006).

Despite the lack of ecological studies from restored streams, a general pattern has emerged from studies that have used macroinvertebrate community structure responses to judge the ecological success of stream restoration projects. Although positive macroinvertebrate responses, such as rapid colonization and greater abundance and biomass of sensitive (EPT) taxa have been reported from restored streams (Pederson et al. 2007, Walther and Whiles 2008, Heinrich et al. 2014), findings from most studies indicate that current restoration practices rarely yield positive or significant results in terms of macroinvertebrate richness, diversity, and densities (Miller et al. 2010, Palmer et al. 2010, Louhi et al. 2011). For example, Palmer et al. (2010) found only two out of 78 studies showed an increase in macroinvertebrate diversity (as measured by species richness) following restoration. In response to these findings, ecologists have identified



shortcomings in common restoration practices and have made recommendations for improvement. The recommendations that appear most frequently in the literature include, but are not limited to:

1. More post-restoration ecological studies that occur over longer time frames are needed (Bernhardt et al. 2005, Palmer 2009, Miller et al. 2010).
2. Restorations should take place where the surrounding area (i.e. watershed) contains viable macroinvertebrate colonizers to inhabit the newly restored stream (Palmer et al. 2010, Sundermann et al. 2011).
3. Restoration practices should place more emphasis on restoring natural flow regimes, hydrologic, and geomorphic processes as opposed to simply focusing on channel reconstruction (Palmer et al. 2010, Bernhardt and Palmer 2011).

The majority of current restoration projects attempt to restore degraded streams by using natural channel design practices that involve reconfiguring channels and introducing structures to enhance habitat diversity (Shields et al. 2003, Lave 2009, Tullos et al. 2009, Rosenfeld et al. 2011, Bernhardt and Palmer 2011), but the restoration of Slabcamp Creek involved practices that went beyond channel reconfiguration and restored hydrologic functions. The restoration practices used to restore Slabcamp Creek first involved removing trees and post-settlement alluvium from the center of the valley in order to expose the bed substrates of the original channel. Then wood was introduced within the channel to control gradients, and the floodplain and new stream channel were formed by scour and natural deposition processes around the woody debris. Small tributaries were also restored to reduce upstream supply of sediment and native

vegetation was planted in the floodplain (Parola and Biebighauser 2011). The restoration was completed in October 2011 and since then, continuous flow monitoring records, as well as personal observations indicate a restored annual flow pattern with riffles and pools remaining connected through the late summer and early fall (Fig. 1)<sup>1</sup>.

The primary goal of my study was to begin baseline studies of the macroinvertebrate community in the restored section of Slabcamp Creek and to compare those findings to White Pine Branch (a pre-restoration control site) during the first post-restoration year. Specific objectives of my study were to: 1) report seasonal estimates of macroinvertebrate abundance and biomass from riffles, 2) describe the macroinvertebrate community structure from riffles, and 3) measure channel habitat at the study sites. Due to the restored flow regime and increased habitat complexity and stability, I expected greater densities of clingers, burrowers, and slow seasonal developers in Slabcamp Creek relative to White Pine Branch throughout all seasons. I also expected differences in macroinvertebrate trophic groups between the streams as a result of the tree removal during restoration construction at Slabcamp Creek. More specifically, I expected greater densities of scraper/grazers and reduced densities of shredders in Slabcamp Creek relative to White Pine Branch.

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<sup>1</sup> All figures and tables are listed in the appendix

## CHAPTER II

### **Methods:**

#### *Study design*

I was unable to use a highly desirable Before-After-Control-Impact design for this study (Stewart-Oaten et al. 1986, Osenberg et al. 2006) since there was not an opportunity to sample Slabcamp Creek prior to the restoration. Furthermore, adequate upstream control reaches were unavailable for sampling since the restoration extended to Slabcamp Creek's upstream tributaries. Therefore, I selected a stream in a similar setting (i.e., same geology, bioregion and watershed size) that showed evidence of similar historical disturbance (as indicated by presence of current and historic farm fields, channel movement and straightening to support agriculture in the valley, head-cuts causing erosion, vertical eroding stream banks, channel bed dominated by bedrock and gravel, and an intermittent annual flow pattern) to serve as a comparison site for my studies in Slabcamp Creek. Following GIS mapping, ground-truthing and conversations with USDA Forest Service personnel, I selected White Pine Branch to serve as the pre-restoration control site for this study.

### *Study area and study sites*

Slabcamp Creek and White Pine Branch are first order tributaries within the North Fork Licking River watershed. The study area is located within the Western Allegheny Plateau ecoregion and Mountain bioregion of Kentucky (Omernik 1987, Pond et al. 2003), which is characterized by horizontally-bedded sedimentary rock containing sandstone, siltstone, shale, and coal, and some areas that have eroded to limestone and possibly contain a landscape of karst. The potential natural vegetation of the ecoregion contains mixed mesophytic forests, though mixed oak forests are common in drier sites (Omernik 1987). The study sites drained similar watershed sizes (Slabcamp Creek drainage: 229.457 ha and White Pine Branch drainage: 239.169 ha) and were within the boundaries of the Daniel Boone National Forest in Rowan Co. Kentucky (Fig. 2).

### *Physical habitat estimates*

Reach-scale physical habitat was measured twice throughout the study to estimate the amount of wetted channel habitat at the study sites during high (spring 2012) and low (summer 2012) base flow conditions. Physical habitat was measured every 3 mean stream widths (i.e. every 9 m at Slabcamp Creek and every 12 m at White Pine Branch) for a total of 13 transects per stream. At each transect wetted channel width (m) was measured and the habitat unit (i.e., riffle, run, pool) was determined. In addition, flow (m/s), depth (m), and the percent inorganic substrate (% cobble, % gravel, % pebble, % fine, and % bedrock) were visually estimated from the thalweg at each transect. During

spring 2012, channel canopy closure and the frequency of large wood were estimated in each stream. Canopy closure was determined with a concave spherical densitometer held at breast height from the center of the channel at each transect. Large wood frequency was determined by identifying wood with a diameter  $\geq 5$  cm in an area one meter up and downstream of each transect. Finally, the total length of each habitats unit was determined from direct longitudinal measures along the reach during benthic sampling events.

#### *Benthic sampling design*

Benthic macroinvertebrates were collected from five riffles at each site during winter 2011, fall 2012, and spring 2012. Samples were collected from the thalweg with a Hess bottom area sampler (dia 0.33 m, 243  $\mu\text{m}$  mesh) that was inserted approximately 10 cm into the stream bed. This design resulted in five replicates from riffles in each stream and amounted to a total of 30 benthic samples for the entire study. Benthic samples were rinsed into separate plastic bags, preserved with 95% ethanol, and transported to the laboratory for analysis. Benthic samples from pools were also collected using the same methods that were used for riffle samples, but benthic samples from pools were not analyzed for this study.

### *Laboratory methods*

To facilitate sample processing, benthic samples were rinsed through two stacked sieves (1 mm and 250  $\mu$ m). Samples from both fractions were sorted to their entirety under a dissecting microscope. Most individuals were identified to genus using keys in Merritt et al. (2008) except for Chironomidae, Sphaeriidae, and Cambaridae which were identified to family, and Oligochaeta and Gastropoda were identified to class using keys in Thorp and Covich (2009). All individuals were enumerated and measured to the nearest 0.5 mm in order to estimate standing stock from published length-mass regressions (Benke et al. 1999). For any taxa where length-mass regressions were not available, an equation for an alternative taxa with a similar body form was used (Table 1).

### *Data analysis*

Total macroinvertebrate density and biomass for each sample was reported per square meter of stream bottom. Standing stock biomass (mg ash free dry mass (AFDM) per square meter of stream bottom) was calculated as the sum of each length class for all taxa present. Three separate repeated measures analyses of variance (ANOVA) were performed using SAS v. 9.3 (SAS Inc., Cary, NC, USA) in order to compare total macroinvertebrate density, biomass, and taxa richness between streams. Stream (site), season, and the interaction between stream and season were included as factors in the

repeated measures ANOVA. Due to the clumped distribution of the macroinvertebrates, data were  $\log_{10}(X+1)$  transformed prior to analysis to improve normality (Zar 2007).

The structure of the macroinvertebrate communities were summarized by summing the abundance of each taxon from all benthic samplings for each sampling period. Any taxon that constituted  $< 0.5\%$  of the total abundance was considered a rare taxon in the collection (Table 1). Jaccard's similarity index (Krebs 1999) was calculated to examine similarity based on presence and absence of taxa. This was calculated by dividing the total number of taxa present at both sites by: that value, the number of individuals unique to Slabcamp Creek, and the number of individuals unique to White Pine Branch. This index was interpreted on a scale of 0-1, and the communities were considered more diverse if values were closer to 0. Nonmetric Multidimensional Scaling (NMS) based on Sorensen distance, a multivariate ordination used to graphically represent community relationships, was performed to explore the similarity of the macroinvertebrate community structure over time within ordination space and was run on the abundance data of taxa that constituted  $\geq 0.5\%$  of the total abundance; rare taxa in the collection were removed prior to analysis. NMS analysis was conducted using PC-ORD Version 6.0 (McCune and Mefford 2011). Random starting coordinates were used, and the analysis parameters were as follows: runs with real data = 250, stability criterion  $< 0.0000001$ , maximum iterations = 250, and step length = 0.20. Following the NMS, benthic samples were grouped by season and then multi-response permutation procedures (MRPP) were performed to test for similarity in community structure among groups. MRPP is a nonparametric procedure that tests for differences in community structure between groups and the analysis provides a measure of effect size (an A-value). If A-

values are equal to one, then all samples within groups are identical. A-values from ecological studies typically range from 0.1 to 0.3, and higher A-values indicate greater differences in community structure between groups (McCune et al. 2002).

Finally, the community structure was summarized with metrics that describe various attributes of macroinvertebrate communities. For the metric analysis I first selected 27 candidate metrics that described various attributes of the community. Macroinvertebrate abundance data from all seasons in each stream was then combined and traits were assigned to each taxon using information reported by Poff et al. (2006) and Kentucky Division of Water's (KDOW) master taxa list (updated fall 2013). Candidate metrics were placed into one of four categories (i.e., trophic habitat, habits and habitat preferences, life history, and tolerance) (Table 2). To reduce redundancy in the metric analysis, Pearson's correlation was performed using SAS on metrics within each of these categories. If metrics within a category were correlated ( $r \geq 0.7$ ), then redundant metrics that are not commonly reported in the literature were removed and not included in further analyses.

Graphical analyses of box and whisker plots were then performed on the final set of metrics for both absolute and relative abundance data in order to visually interpret the data and make inferences about the biological significance of the metrics. Since absolute abundance data were skewed, I performed  $\log_{10}(X+1)$  transformations to improve interpretation of the box and whisker plots. Results from the box and whisker plots were then interpreted on a scale of 0 – 3 following methods described by Pond et al. (2003). If the interquartile ranges did not overlap between the groups, then metrics received a score



of 3 and were considered to have excellent discriminatory power between streams. If there was some degree of overlap of the interquartile ranges of the groups, but not of the medians, the metrics were considered to have good discriminatory power and were assigned a score of 2. If the median of one box overlapped with the interquartile range of the other, the metric showed fair discriminatory power and were assigned a 1. Finally, if both the medians overlapped the interquartile ranges of the groups, then metrics were interpreted to have poor discriminatory power and received a score of 0.

As a complement to the box and whisker plot analysis, standardized effect sizes, Cohen's  $d$  ( $\pm$  95% confidence intervals), were calculated for each absolute and relative abundance test metric using Microsoft Excel 2007. Effect size calculations complement inferential statistics by measuring the strength of the difference between groups, allowing for a better understanding of the magnitude and direction of an effect (Nakagawa 2004, Nakagawa and Cuthill 2007, McCabe et al. 2012). Effect size (Cohen's  $d$ ) values were calculated by dividing the mean difference between two groups by the pooled standard deviation. Effect size values were interpreted based off of Hill et al. (2008) where, on a scale of 0 – 1, 0 – 0.33 was a small effect, 0.34 - 0.66 was a medium effect, and 0.67 - 1 was a large effect.

## CHAPTER III

### **Results:**

#### *Reach scale habitat*

As a result of the wider channel at White Pine Branch, Slabcamp Creek had less wetted channel habitat during spring (Table 3). During low base flow, both channels lost wetted habitat, but the difference in wetted habitat lost between spring and summer was greater at White Pine Branch – wetted habitat decreased by 96% at White Pine Branch and by only a 33% at Slabcamp Creek. The composition of channel bed substrates remained fairly consistent between spring and summer within each stream, but relative to White Pine Branch, the bed of Slabcamp Creek consisted of greater amounts of fine sediment and no bedrock. Finally, Slabcamp Creek had more large wood and less canopy cover than White Pine Branch (Table 3).

#### *Macroinvertebrate abundance and biomass*

A total of 4,070 individuals were collected for the entire study. When benthic samples from all seasons were combined, riffles from Slabcamp Creek supported greater mean annual macroinvertebrate densities (mean = 2145 ind/m<sup>2</sup>; SE = 591; *n* = 15) than the riffles of White Pine Branch (mean = 1029 ind/m<sup>2</sup>; SE = 366; *n* = 15), showed fair “1” discrimination according to the box and whisker plots (Fig. 3), and had a strong

effect (Cohen's  $d = 0.59$ , CI = -0.16- 1.3). Likewise, annual mean standing stock biomass estimates from riffles was greater in Slabcamp Creek (mean = 199 mg AFDM/m<sup>2</sup>; SE = 46;  $n = 15$ ) than White Pine Branch (mean = 36 mg AFDM/m<sup>2</sup>; SE = 16;  $n = 15$ ), showed excellent "3" discrimination between streams in the box and whisker plot analysis (Fig. 3), and had a strong effect (Cohen's  $d = 1.22$ , CI = 0.41- 1.96).

Total macroinvertebrate abundance was fairly similar between the streams in fall, but during winter and spring macroinvertebrates were 3-7 times more abundant in Slabcamp Creek (Table 4). Total macroinvertebrate biomass from riffles of Slabcamp Creek was at least 6 times the amount from riffles of White Pine Branch during every season (Table 4). Repeated measures ANOVA indicated significant differences in absolute abundance and biomass between streams, but there was no significant difference in these responses over time (Table 5, Fig. 4).

#### *Macroinvertebrate community structure*

A total of 59 taxa were collected from the two study sites and 15 taxa were considered rare in the collection (Table 1). For all seasons combined, Slabcamp Creek had greater taxa richness (52 total taxa) than White Pine Branch (45 total taxa). Eleven rare taxa were collected from riffles of Slabcamp Creek, while only five rare taxa were collected from riffles of White Pine Branch. Results from Jaccard's similarity between the streams, in each season, ranged from 0.17-0.65 (Table 6) indicating that the communities were the most similar in the fall (0.65), but appeared very different in other

seasons. Jaccard's similarity values from Slabcamp Creek remained fairly similar (0.44-0.51), but White Pine Branch had a greater range from 0.28-0.63.

Taxa richness between the sites was similar in the fall and spring, but 11 more taxa were collected from riffles in Slabcamp Creek during winter (Table 4). Repeated measures ANOVA indicated no significant differences in taxa richness between sites or over time (Table 5, Fig. 5).

When macroinvertebrate abundance data from all seasons were combined, the top five dominant taxa in Slabcamp included Chironomidae (38%), Capniidae (11%), *Maccaffertium* (10%), *Chimarra* (7%), and *Acerpenna* (6%). In White Pine Branch, the top five dominant included: Chironomidae (44%), Capniidae (10%), *Acerpenna* (8%), Leptophlebiidae (5%), and *Epeorus* (4%). When dominant taxa were examined by season, Chironomidae dominated abundance in every season in both streams (Table 7). In Slabcamp Creek, *Cheumatopsyche* contributed 5-7% to total community abundance in fall and spring, while *Amphinemura* contributed 3% in winter. In White Pine Branch, five additional taxa (*Haploperla*, Leuctridae, *Lirceus*, *Cinygmula*, *Amphinemura*, and *Baetis*) contributed to seasonal macroinvertebrate abundance, but the dominance of each of taxon changed across seasons (Table 7). Several taxa were not dominant or rare in the collection but were unique to each stream. *Ephemera*, *Stenelmis*, *Corydalus*, and *Sialis* were collected only from riffles in Slabcamp Creek while *Crangonyx*, *Diphetera*, *Eurylophella*, and *Lepidostoma* were collected only from riffles in White Pine Branch (Table 1)

NMS ordination produced a three-dimensional solution with a final stress value of 10.98 (instability < 0.00001). NMS axes 1, 2, and 3 explained 59.5%, 13%, and 2.6% of the variation in the community data (Fig. 6). Taxa with the strong positive correlations with axis 1 included Chironomidae ( $r = 0.79$ ), Oligochaeta ( $r = 0.76$ ), *Acerpenna* ( $r = 0.72$ ), *Maccaffertium* ( $r = 0.71$ ), *Cheumatopsyche* ( $r = 0.69$ ), and *Chimarra* ( $r = 0.55$ ). *Eurylophella* ( $r = -0.45$ ) and *Lirceus* ( $r = -0.41$ ) had negative correlations with axis 1. Taxa with strong positive correlations with axis 2 included Leuctridae ( $r = 0.60$ ), Oligochaeta ( $r = 0.58$ ), *Haploperla* ( $r = 0.54$ ), and *Cinygmula* ( $r = 0.51$ ). No taxa had a strong negative correlation with axis 2.

MRPP results indicated no significant differences in community structure among seasons within Slabcamp Creek, while differences among seasons were detected within in White Pine Branch (Table 8). Significant differences in community structure were detected between the streams throughout all seasons, and the degree of community change (as indicated by A-values) was greater than seasonal changes within each stream (Table 8). The strongest difference in community structure between streams occurred during the fall and winter seasons (Table 8).

#### *Macroinvertebrate community metrics*

After correlations between metrics were examined, nine metrics were retained for further analysis (Table 9). Seven out of the nine community metrics based on absolute abundance discriminated between the streams. Scrapers/grazers and taxa associated with

depositional habitats (i.e., low rheophily) received an “excellent” metric rating according to graphic interpretation (Fig. 7). The abundance of EPT taxa, clingers, taxa with slow seasonal development, and taxa with a large body size at maturity showed good discrimination between the streams (Fig. 7). Box and whisker plots revealed no differences in the abundance of shredders or semivoltine taxa between streams (Fig. 7). No metrics based on relative abundance received an ‘excellent’ rating (Table 9, Fig. 8). Two metrics based on relative abundance (% scrapers and % low rheophily) received a metric score of 2, suggesting good separation between sites (Table 9, Fig. 8). Results from effect size analysis generally supported the results from the box-and whisker plot analysis, and metrics that had the highest discriminatory power also had the strongest effect sizes (Table 10). Exceptions included semivoltine abundance and % EPT abundance which had large effects, but scored a “0” on the box and whisker plots.

## CHAPTER IV

### **Discussion:**

Overall findings from my study indicate that, relative to the non-restored site, Slabcamp Creek had a different community structure and greater total macroinvertebrate abundance, biomass, and sensitive EPT taxa. Greater macroinvertebrate abundance and biomass implies greater abundance of emerging aquatic insects and has implications for higher trophic levels. Several studies have shown that emerging aquatic insects subsidize riparian food webs (Nakano and Murakami 2001, Sabo and Power 2002, Balinger and Lake 2006). Heinrich et al. (2014) found that greater emergence of larger-bodied insect taxa from stabilized riffles of the Illinois River resulted in a positive numerical response by riparian birds. Quantitative benthic studies should continue at Slabcamp Creek and White Pine Branch in order to determine if the observed differences in community structure, particularly for density and biomass of macroinvertebrates from the first year following restoration will persist over time. Furthermore, future studies should relate aquatic insect emergence to the abundance and richness of wildlife species that known to feed on them, such as amphibians, birds, and bats.

The mean annual macroinvertebrate density and biomass estimates from my study, especially those estimates from White Pine Branch, are low relative to values reported from other headwater streams in the Appalachian region (Angradi 1996, 1997, 1999, Whiles and Wallace 1995), but are within the range of studies from other regions

of the US (e.g. Smock et al. 1992, Entekin et al. 2007). However, comparing my estimates to those from other studies should be done with caution since density and biomass estimates are highly influenced by study and sampling designs, including specific field and lab methods. It is likely that as a result of the restoration, the differences in total macroinvertebrate density and biomass between streams in this study may have resulted from a combination of factors including: increased bed stability, more complex habitat and refugia, a perennial flow pattern, and different food resources for macroinvertebrates.

Improved bed stability and the presence of refugia likely reduced macroinvertebrate export during flooding events at Slabcamp Creek. Streams are naturally dynamic systems that can be frequently disturbed from flow-generated bed movement, and macroinvertebrate densities and diversity have been shown to decrease when bed substrates become dislodged and mobilized as a result of erosive flooding events (Cobb et al. 1992, Miller and Golladay 1996, Bond and Downes 2003, Schwendel et al. 2011). Some macroinvertebrate taxa use cues from rainfall and flow to take shelter and avoid floods or droughts (Lytle and White 2007, Lytle et al. 2008). Hyporheic zones and large woody debris have been shown to serve as refugia and contribute to faster rates of community recolonization following spates (Poole and Stewart 1976, Sedell et al. 1990, Borchardt 1993, Gjerlov et al. 2003, Stubbington 2012). Large wood and new connections to the hyporheic zone and floodplain likely allowed the macroinvertebrate community to reach pre-spate densities at faster rates than at White Pine Branch.



The restoration practices also resulted in bed substrates composed of greater proportions of fine sediments (particle size < 2 mm) and this resulted in greater abundance of taxa that are frequently associated with soft-bottomed depositional habitats (i.e., low rheophily) in riffles of Slabcamp Creek. Excessive fine sediments in streams are viewed as negative, and headwater and wadeable streams with greater amounts of fine sediments relative to reference conditions receive lower ratings in the embeddedness category of visual rapid habitat assessments (Barbour et al. 1999, Pond et al. 2003). Excessive sediments may increase drift rates, alter respiration and feeding habits, and decrease the richness, densities and biomass of macroinvertebrates communities, especially for EPT taxa (Rosenberg and Wiens 1978, Waters 1995, Wood and Armitage 1997, Shaw and Richardson 2001). Macroinvertebrate communities from streambeds and patches with excessive deposited sediments can be dominated by burrowing chironomid midges and oligochaetes with fewer taxa that cling to stable substrates (Rosenberg and Wiens 1978, Gray and Ward 1982, Zweig and Rabeni 2001, Rabeni et al. 2005). In addition, studies have shown that increased deposition of fine sediment can reduce EPT taxa richness and the abundance of clingers and sprawlers (Kaller and Hartman 2004, Rabeni et al. 2005) In my study however, the abundance of clingers and sensitive taxa (EPT) was greater in Slabcamp Creek which suggests that the 28% additional fine sediments in riffles (relative to White Pine Branch) was not excessive enough to exclude these taxa. In fact, my findings indicate that, in addition to clinger taxa, the riffles of Slabcamp Creek also supported a variety of taxa that burrow or sprawl on soft substrates (e.g., *Ephemera*, *Caenis*, *Baetis*, *Oligochaeta*), and the presence of these taxa contributed

to the overall greater macroinvertebrate abundance and taxa richness in the riffles of Slabcamp Creek.

While sorting pool samples that were not included in this study, I observed that *Ephemera* was more abundant in pools than in riffles, and was much more abundant in pools from Slabcamp Creek than in pools from White Pine Branch. Greater abundance of *Ephemera* in pools and riffles at Slabcamp Creek likely reflects the improved bed stability and flow patterns at Slabcamp Creek. Although this taxon was not extremely abundant in riffles, *Ephemera* contributed to several metrics (i.e. low rheophily, slow seasonal developer, and EPT abundance) that discriminated between the streams. *Ephemera* is the largest genus of Ephemeridae (burrowing mayflies) worldwide and seven species are recognized in North America (McCafferty 1975). I was unable to identify *Ephemera* beyond genus in my study since last instar nymphs or adults are required for species determinations, but according to McCafferty et al. (2010), *Ephemera* in Slabcamp Creek could be *E. blanda*, *E. guttulata*, *E. simulans*, or *E. varia*. Regardless of the specific species collected from Slabcamp Creek, species within *Ephemera* have similar life history characteristics that would be favored by an annual flow pattern, increased fine sediments, and bed stability. *Ephemera* nymphs are burrowers and they require deposits of silt, sand, and fine sediment to construct their burrows (McCafferty 1975, Poff et al. 2006). Burrowing mayflies are also larger and longer-lived relative to other mayflies and they are generally semivoltine with slow-seasonal development (McCafferty 1975, Poff et al. 2006). Britt (1962) found that *E. simulans* required one year for development and that the eggs hatched in July and nymphs emerged the following June in Lake Erie. *Ephemera* nymphs are collector-gatherers that feed on

diatoms, algae, detritus, and are important food resources for fish and birds (Britt 1962). Further, when assessing the biological integrity of streams in Kentucky, *Ephemera* is considered a sensitive taxon that has a pollution tolerance value of 2.2 out of 10, where 10 is very pollution tolerant (KDOW 2002). Considering these life history characteristics, absence of *Ephemera* from riffles in White Pine Branch, may have resulted from unsuitable substrate conditions and loss of wetted riffle habitat during summer/early fall. Future studies should consider benthic samples from pools and *Ephemera* abundance as a potential indicator of bed stability and annual flow patterns in headwater mountain streams of Eastern Kentucky.

Additionally, throughout the course of my study I detected greater abundance of taxa with slow-seasonal development (i.e. taxa that take longer to reach maturity) in riffles of Slabcamp Creek than in White Pine Branch. This also provides some evidence of continuous flow during summer and early fall. During the summer sampling period, flow in riffles at Slabcamp Creek was too low for benthic collection, but I observed that riffles and pools maintained flow connections, and this observation has been confirmed from continuous flow monitoring equipment operated by the University of Louisville (Art Parola, personal communication). The majority of taxa (33 total taxa) that I collected from Slabcamp Creek and White Pine Branch have slow-seasonal development. Of these slow-seasonal taxa, *Chimarra* and *Cheumatopsyche* numerically dominated this metric and they were far more abundant in the riffles of Slabcamp Creek and contributed to overall greater macroinvertebrate abundance within the stream relative to White Pine Branch.

Persistence of the macroinvertebrate community structure across seasons at Slabcamp Creek, as indicated by the richness, abundance, NMS, and MRPP results, may also be explained by the improved bed stability and continuous flow pattern created from the restoration practices. It is known that invertebrate community compositions shift in streams along longitudinal and seasonal gradients (Hynes 1970). Seasonal variation of stream invertebrates is a result of various life history strategies (e.g. voltinism) and adaptations to environmental variables (e.g. drought) (Butler 1984, Sweeney et al. 1986, Delucchi and Peckarsky 1989, Murphy and Giller 2000). Seasonal changes in macroinvertebrate community structure are also often a result of natural disturbances such as floods, extreme temperatures, and drought (Townsend et al. 1987, Matthaei et al. 1997, Bradt et al. 1999). However, the degree of community change over time (both seasonally and annually) can depend on the stream setting. Greater seasonal variation has been documented from streams with unpredictable disturbance and there is evidence that consistent habitat conditions promote the persistence (similarity in the composition of assemblages of invertebrate communities) of aquatic biota over time (Weatherly and Omerod 1990). Several studies have shown that the community persistence over years is greatest when environmental conditions remain consistent (Robinson et al. 2000, Scarsbrook 2002). For example, Scarsbrook (2002) showed that over 9 years, community persistence was greater when flow conditions remained constant. Maul et al. (2004) reported that reference sites had more similar macroinvertebrate communities between years than impaired sites. Likewise, data from a 6 year study provided by Robinson et al. (2000) reported annual persistence in macroinvertebrate community structure from pristine streams in Idaho. My findings only indicate greater community

persistence among seasons during the first year following restoration. Continuous long-term monitoring at Slabcamp Creek will be necessary to determine if this trend persists over a longer time period.

Although the restoration at Slabcamp Creek improved hydrology and habitat, canopy removal during restoration could have altered food resources and contributed to differences in macroinvertebrate community abundance and structure between streams. Field studies have shown that canopy removal from riparian zones, usually from timber harvest operations, can increase macroinvertebrate abundance and biomass presumably as a result of primary production stimulated by increased sunlight (Hawkins et al. 1982, Behmer and Hawkins 1986, Quinn et al. 1997). Greater abundance of scraper/grazer taxa in my study suggests periphyton food resources were more abundant on riffle substrates in Slabcamp Creek during the first year following restoration. Although I did not measure food resources for macroinvertebrates in this study, several studies have shown that scraper abundance can increase in reaches or habitat patches where periphyton food resources are abundant (Wallace and Gurtz 1986, Richards and Minshall 1988, Dudgeon and Chan 1992). Slabcamp Creek contained 55% less canopy closure at breast height compared to White Pine Branch and although the channel appeared to have some shade from floodplain vegetation, tree canopy removal during construction of the new channel could have resulted in increased primary production that in turn contributed to greater macroinvertebrate abundance. However, periphyton is more abundant on stable substrates (Robinson and Minshall 1986, Biggs 1995), so scraper taxa in the riffles of Slabcamp Creek may have been favored by a combination of primary production and substrate stability. The elevated abundance of scrapers/grazers in the riffles of Slabcamp

Creek may be a short term response to the restoration since the abundance of macroinvertebrate trophic groups can reflect food resources in streams. Stone and Wallace (1998) reported a change in the dominant functional feeding groups from scrapers/grazers to shredders as a result of the re-growth of the forest surrounding their study stream after logging. Native trees were planted in the floodplain shortly after construction was complete, and over time these trees should provide shade and litter inputs that might reduce periphyton resources and scraper abundance. I expected that canopy removal would decrease riparian litter inputs into the channel at Slabcamp Creek, which would result in fewer taxa that comminute large pieces of dead plant matter (i.e. shredders) (Wallace and Webster 1996). Dominant shredders in my study included small Capniidae, Leuctridae, and *Allocapnia*, but I did not detect a difference in their abundance between streams. This finding suggests that coarse organic inputs, likely from newly planted floodplain vegetation or deciduous trees in the valley at Slabcamp Creek, provided sufficient food resources for shredders during the first year following restoration.

The macroinvertebrate community responses that I detected between the streams was likely a result of the practices used to restore Slabcamp Creek. These practices caused multiple changes to the physical habitat within Slabcamp Creek as well as to the potential food resources utilized by the macroinvertebrates that inhabit the channel. However, my study was not designed to determine the relative influence of each of these factors on the macroinvertebrate communities. In order to determine the mechanisms driving the community differences that I detected between streams, I recommend future studies that incorporate simultaneous measures of benthic food resources, especially

periphyton, as well as spate-driven sampling designs. Additionally, it would be worthwhile to determine if the responses I detected in my study can be detected with the Kentucky Macroinvertebrate Bioassessment Index (KMBI).

The KMBI is a biological monitoring tool that was developed to compare the biological integrity of streams to the regional reference conditions (Pond et al. 2003). KMBI methodology is a valid and useful rapid assessment tool that allows KDOW personnel to assess headwater and wadeable streams throughout Kentucky. KMBI methodology involves semi-quantitative collections from riffles that are combined in the field. The riffle sample is processed in the lab and following macroinvertebrate identification, a suite of seven metrics (five based on relative abundance) are calculated to determine the final stream score. Since the KMBI is the only available tool for assessing the biological status of streams, resource managers rely on it for assessing biological responses following stream restorations in Kentucky. Comparisons between metrics based on absolute and relative abundance data from my study indicate that most of the positive responses that I documented would not have been detected if I had relied solely on metrics based on relative abundances. Future studies that incorporate the recommendations that I have mentioned throughout this discussion should be expanded to other restored streams in order to gain a more comprehensive understanding of the biological responses to practices that restore the hydrology of channelized streams. Knowledge gained from these studies could be used to develop a rapid tool that is specifically designed for assessing biological responses to restoration projects.

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## APPENDIX

Table 1. Taxa List. Macroinvertebrate abundance from seasonal samples (fall 2011, winter 2012, spring 2012) at Slabcamp Creek and White Pine Branch. Values are the total individuals from five Hess samples in each season. Superscripts <sup>R</sup> indicates rare taxa in the collection where total abundance < 0.05%, numbers indicate taxa that required a length-mass substitution for biomass estimates, \* indicates taxa that were omitted from metric calculations due to lack of species traits information.

Class	Order	Family	Genus	Slabcamp Creek			White Pine Branch				
				Fall	Winter	Spring	Fall	Winter	Spring		
Malacostraca	Amphipoda	Crangonyctidae	<i>Crangonyx</i> <sup>*</sup>			1		3	2	5	
	Decapoda	Cambaridae <sup>*,R</sup>				4		3	14	1	
	Isopoda	Asellidae	<i>Lirceus</i>	37	21	32		31	2	3	
Oligochaeta <sup>*</sup>					1						
Gastropoda <sup>*,R</sup>						4					
Bivalvia	Veneroida	Sphaeriidae						2			
Insecta	Ephemeroptera	Ameletidae	<i>Ameletus</i> <sup>R</sup>		1						
		Baetidae	<i>Acerpenna</i> <sup>1</sup>	68	53	50		7		104	
			<i>Baetis</i>				11		2	1	24
			<i>Diphetero</i> <sup>1</sup>								
		Caenidae	<i>Caenis</i>		18	24	1		1		1
		Ephemeridae	<i>Ephemerella</i>		8	8					
		Ephemerellidae	<i>Eurylophella</i>						1	2	
		Heptageniidae	<i>Maccaffertium</i> <sup>2</sup>		75	53	149		6		6
			<i>Nixe</i>			5				3	
			<i>Stenonema</i> <sup>R</sup>		1	1					
			<i>Cinygmula</i>		3	4	2		6	13	1
			<i>Epeorus</i>		15	6	8		52		1
			<i>Isonychia</i> <sup>R</sup>		1						
	<i>Paraleptophlebia</i>		4		1		1	1	3		
	Unknown <sup>3</sup>		6	12	5		18	1	45		
Odonata	Gomphidae		1								
Megaloptera	Corydalidae		<i>Lanthus</i> <sup>R</sup>								
			<i>Corydalus</i>	6							
			<i>Nigronia</i> <sup>R</sup>						1		
	Sialidae					1			3		

Table 1. (continued)

Class	Order	Family	Genus	Slabcamp Creek			White Pine Branch			
				Fall	Winter	Spring	Fall	Winter	Spring	
Plecoptera		Capniidae	<i>Allocapnia</i>	63			24			
			Unknown <sup>4</sup>	22	205	79	89	25	19	
			<i>Haploperla</i> <sup>5</sup>	3	4	6	39	4	5	
		Leuctridae	<i>Sweltsa</i> <sup>5,R</sup>	4	1				1	
			<i>Leuctra</i>	4	25	35	34	4	1	
		Nemouridae	<i>Amphinemura</i>		29				6	
			<i>Soyedina</i>	2			17			
			Unknown <sup>6</sup>	33		1	6		1	
		Perlidae	<i>Acroneuria</i> <sup>R</sup>			2				
			<i>Eccoptura</i> <sup>R</sup>	1						
			<i>Perlesta</i> <sup>R</sup>						1	
			Unknown <sup>7,R</sup>		1				1	
			<i>Isoperla</i> <sup>R</sup>		1					
Trichoptera		Taeniopterygidae <sup>R</sup>		1						
			<i>Cheumatopsyche</i>	42	10	78			11	
		Hydropsychidae	<i>Diplectrona</i> <sup>9</sup>	4	1	1	4		4	
			<i>Hydropsyche</i>	15		3			1	
		Lepidostomatidae	<i>Lepidostoma</i>				2		5	
			<i>Chimarra</i>	38	9	152			2	
		Philopotamidae	<i>Dolophilodes</i> <sup>10</sup>				1			
			<i>Rhyacophila</i>			5	6		7	
			<i>Neophylax</i> <sup>11</sup>	2	4		2		4	
		Coleoptera		Uenoidea	2	3	2	2		2
				Elmidae	1	1	2			1
				Psephenidae	4		1			
				<i>Stenelmis</i>	9	4	2	4	1	3
Diptera		Ceratopogonidae								

Table 1. (continued)

Class	Order	Family	Genus	Slabcamp Creek			White Pine Branch		
				Fall	Winter	Spring	Fall	Winter	Spring
		Chironomidae		265	374	417	413	45	117
		Dixidae	<i>Dixa</i> <sup>*12</sup>		1	2			4
		Empididae	<i>Hemerodromia</i>	3	3		2		3
			<i>Metachela</i>	12			2		13
		Simuliidae	<i>Prosimulium</i>	17	14	2	9		1
			<i>Simulium</i> <sup>R</sup>					1	
		Tipulidae	<i>Hexatoma</i> <sup>*</sup>	1			1		1
			Unknown <sup>13</sup>			2			1

Regression equation substitutions: <sup>1</sup>*Baetis*, <sup>2</sup>*Stenonema*, <sup>3</sup>*Paraleptophlebia*, <sup>4</sup>*Allocapnia*, <sup>5</sup>*Sweltsa*, <sup>6</sup>*Amphinemura*, <sup>7</sup>*Eccopectura*, <sup>8</sup>*Linata*, <sup>9</sup>*Hydropsyche sparna*, <sup>10</sup>*Chimarra*, <sup>11</sup>*Limnephilus*, <sup>12</sup>Chironomidae, <sup>13</sup>*Tipula*

Table 2. Candidate Metrics. List of candidate metrics and their expected response to the restoration at Slabcamp Creek. Superscript <sup>KY</sup> indicates a core metric in the Kentucky MBI

Community metrics	Expected Response
<b>Trophic group</b>	
Collector-gatherer	Variable
Collector-filterer	Variable
Scraper/grazer	Increase
Predator	Variable
Shredder	Decrease
<b>Habits and habitat associations</b>	
Burrower	Increase
Sprawler	Variable
Swimmer	Variable
Clinger <sup>KY</sup>	Increase
# Clinger Taxa	Increase
Low rheophily (depositional)	Variable
High rheophily (erosional)	Variable
Cold stenothermal	Decrease
<b>Life history</b>	
Fast-seasonal development	Variable
Slow-seasonal development	Increase
Non-seasonal development	Increase
Semivoltine	Increase
Univoltine	Increase
Multivoltine	Variable
Large body size at maturity	Increase
Desiccation resistance	Decrease
<b>Tolerance</b>	
EPT taxa <sup>KY</sup>	
EPT <sup>KY</sup>	Increase
Ephemeroptera <sup>KY</sup>	Increase
Plecoptera	Increase
Trichoptera	Increase
Top 5 dominant	Variable

Table 3. Physical Habitat Measurements. Reach-scale physical habitat from spring 2012 (high base flow) and summer 2012 (low base flow) at Slabcamp Creek and White Pine Branch. Means ( $\pm 1$  S.E.) were determined from equally spaced transects ( $n = 13$ ) at each study site. Dashes indicate where a parameter was not measured.

	Spring 2012		Summer 2012	
	Slabcamp Creek	White Pine Branch	Slabcamp Creek	White Pine Branch
Channel width (m)	3.3 $\pm$ 0.3	3.8 $\pm$ 0.3	2.4 $\pm$ 0.2	4.5 $\pm$ 0.2
Wetted channel area (m <sup>2</sup> )	495	570	330	24
Depth (m)	0.17 $\pm$ 0.02	0.1 $\pm$ 0.01	0.1 $\pm$ 0.04	0 $\pm$ 0
Flow (m/s)	0.08 $\pm$ 0.02	0.11 $\pm$ 0.02	0 $\pm$ 0	0 $\pm$ 0
Canopy Closure (%)	38 $\pm$ 7	93 $\pm$ 1	—	—
Wood frequency (%)	100	23	—	—
Substrate				
% Bedrock	0 $\pm$ 0	31 $\pm$ 13	0 $\pm$ 0	36 $\pm$ 13
% Cobble	36 $\pm$ 9	35 $\pm$ 9	21 $\pm$ 7	17 $\pm$ 5
% Gravel	28 $\pm$ 7	25 $\pm$ 6	45 $\pm$ 4	38 $\pm$ 11
% Fine	35 $\pm$ 9	10 $\pm$ 3	34 $\pm$ 4	9 $\pm$ 4



Table 4. Total Macroinvertebrate Abundance, Biomass, and Richness. Data are from riffles of Slabcamp Creek and White Pine Branch. Values are totals from five Hess samples.

	Slabcamp Creek			White Pine Branch		
	Fall 2011	Winter 2012	Spring 2012	Fall 2011	Winter 2012	Spring 2012
Total number of individuals	788	878	1085	791	127	401
Total biomass (mg AFDM)	115	62	78	33	6	7
Taxa richness	36	29	33	32	18	33

Table 5. Results from Repeated Measures ANOVA. Three separate ANOVAs were run between Slabcamp Creek and White Pine Branch throughout three sampling seasons for total abundance, biomass, and richness. \* indicates  $p \leq 0.01$ , \*\* indicates  $p \leq 0.001$ .

	Site <i>F</i> <sub>1,8</sub>	Time <i>F</i> <sub>1,8</sub>	Site x Time <i>F</i> <sub>1,8</sub>
Total Abundance	14.29*	1.55	1.4
Total Biomass	42.70**	3.34	0.19
Richness	4.31	4.82	3.09

Table 6. Jaccard's Similarity Index. Values are reported seasonally between Slabcamp Creek and White Pine Branch.

	SC fall	SC winter	SC spring	WP fall	WP winter	WP spring
SC fall	—					
SC Winter	0.51	—				
SC spring	0.50	0.44	—			
WP fall	0.51	0.39	0.48	—		
WP winter	0.17	0.31	0.24	0.32	—	
WP spring	0.57	0.55	0.65	0.63	0.28	—

Table 7. Top 5 Dominant Taxa. Values are from the riffles of Slabcamp Creek and White Pine Branch. Numbers are percents and were determined from the total abundance from five Hess samples in each season.

	Slabcamp Creek		White Pine Branch	
Fall 2011	Chironomidae	34	Chironomidae	51
	<i>Maccaffertium</i>	10	Capniidae	11
	<i>Acerpenna</i>	9	<i>Epeorus</i>	6
	<i>Allocapnia</i>	8	<i>Haploperla</i>	5
	<i>Cheumatopsyche</i>	5	Leuctridae	4
Winter 2012	Chironomidae	43	Chironomidae	35
	Capniidae	23	Capniidae	20
	<i>Acerpenna</i>	6	<i>Lirceus</i>	11
	<i>Maccaffertium</i>	6	<i>Cinygmula</i>	10
	<i>Amphinemura</i>	3	<i>Amphinemura</i>	5
Spring 2012	Chironomidae	38	Chironomidae	29
	<i>Chimarra</i>	14	<i>Acerpenna</i>	26
	<i>Maccaffertium</i>	14	Leptophlebiidae	11
	Capniidae	7	<i>Baetis</i>	6
	<i>Cheumatopsyche</i>	7	Capniidae	5

Table 8. A-values from MRPP Results. \* indicates  $p \leq 0.01$ , \*\* indicates  $p \leq 0.001$ .

	SC fall	SC winter	SC spring	WP fall	WP winter	WP spring
SC fall	—					
SC winter	0.10	—				
SC spring	0.04	0.08	—			
WP fall	0.14*	0.17*	0.11	—		
WP winter	0.30**	0.30*	0.24*	0.20*	—	
WP spring	0.12*	0.16*	0.10*	0.12	0.19*	—

Table 9. Box and Whisker Plot Results from Community Metrics. Numerical scoring of the box and whisker plot results for community metrics based on absolute and relative abundance of macroinvertebrates from riffles. Numbers are metric scores based on discriminatory power (0 = none, 1 = poor, 2 = good, 3 = excellent) according to visual interpretation. Superscript <sup>KY</sup> indicates a core metric in the KMBI.

Community metrics	Absolute abundance	Relative abundance
<b>Trophic group</b>		
Scraper/grazer	3	2
Shredder	0	0
<b>Habits and habitat associations</b>		
Clinger <sup>KY</sup>	2	1
Low rheophily	3	2
<b>Life history</b>		
Slow-seasonal development	2	0
Semivoltine	0	0
Large body size at maturity	2	0
Desiccation resistance	1	0
<b>Tolerance</b>		
EPT <sup>KY</sup>	2	0

Figure 10. Standardized Effect Sizes (Cohen's  $d$ ) and 95% Confidence Intervals (CI). Values are from macroinvertebrate community metrics from the riffles of Slabcamp Creek and White Pine Branch. Superscript <sup>KY</sup> indicates a core metric in the KMBI.

Trophic group	Absolute abundance			Relative abundance		
	Cohen's $d$	Lower CI	Upper CI	Cohen's $d$	Lower CI	Upper CI
Scraper/grazer	1.15	0.35	1.88	0.89	0.11	1.61
Shredder	0.53	-0.21	1.24	-0.08	-0.79	0.64
Habits and habitat associations						
Clinger <sup>KY</sup>	0.72	-0.04	1.44	0.49	-0.25	1.21
Low rheophily	1.14	0.34	1.88	0.95	0.17	0.68
Life history						
Slow-seasonal development	0.65	-0.10	1.37	0.63	-0.12	1.34
Semivoltine taxa	0.89	0.12	1.62	0.82	0.05	1.54
Large size at maturity	0.56	-0.18	1.28	-0.27	-0.98	0.45
Desiccation resistance	0.60	-0.15	1.31	-0.26	-0.97	0.46
Tolerance						
EPT <sup>KY</sup>	0.66	-0.10	1.37	0.82	0.05	1.54



Figure 1. Study Site Photos. Images are of Slabcamp Creek (left) and White Pine Branch (right) and were taken in March 2014.



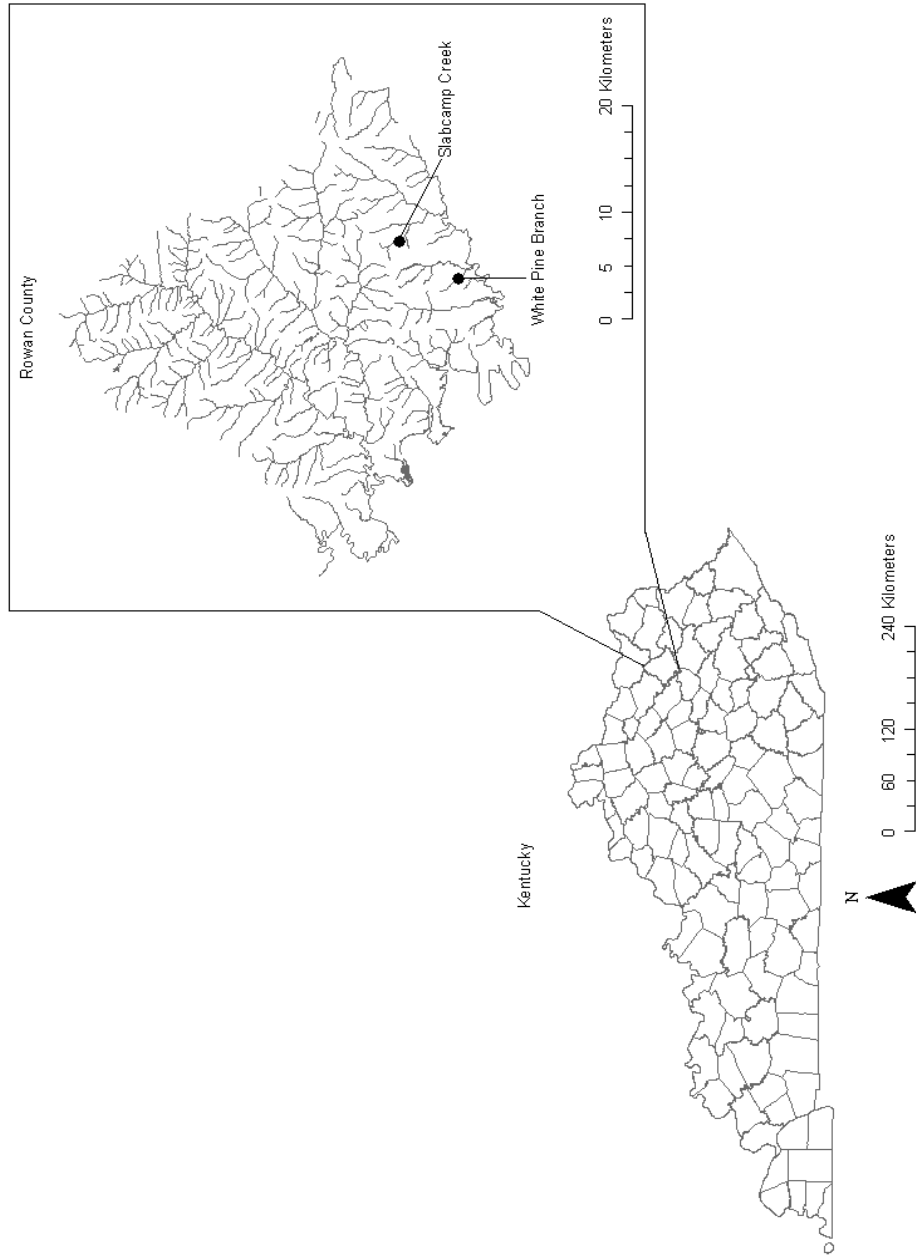


Figure 2. Map of Study Sites. Slabcamp Creek and White Pine Branch are pictured above in Rowan Co., Kentucky.

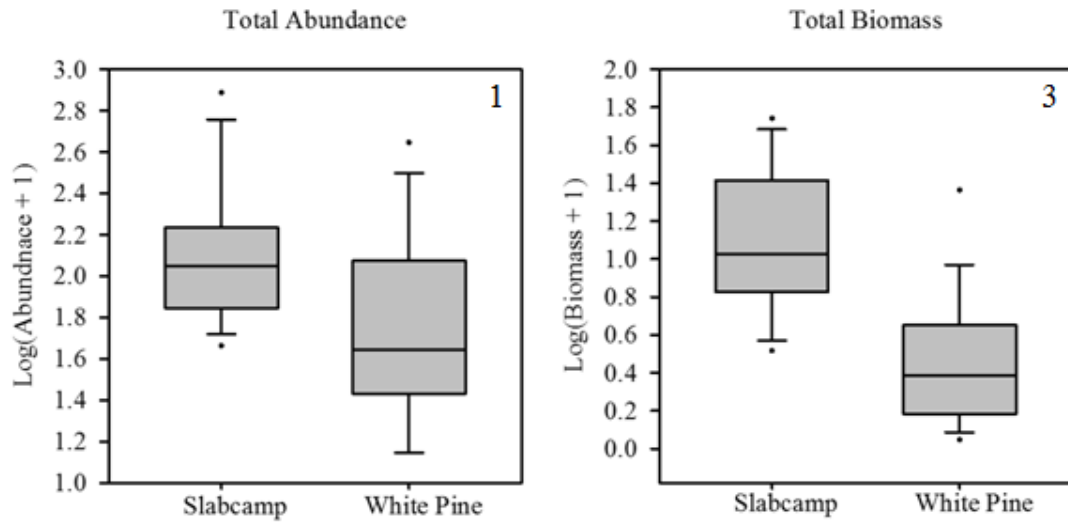


Figure 3. Box Plots of Total Abundance and Total Biomass. Results are total macroinvertebrate abundance and biomass from riffles in Slabcamp Creek ( $n=15$ ) and White Pine Branch ( $n=15$ ), with seasonal samples combined and  $\log_{10}(X+1)$  transformed data. Numbers in the top right corners are the score that plot received on a scale of 0-3 from the visual interpretation.

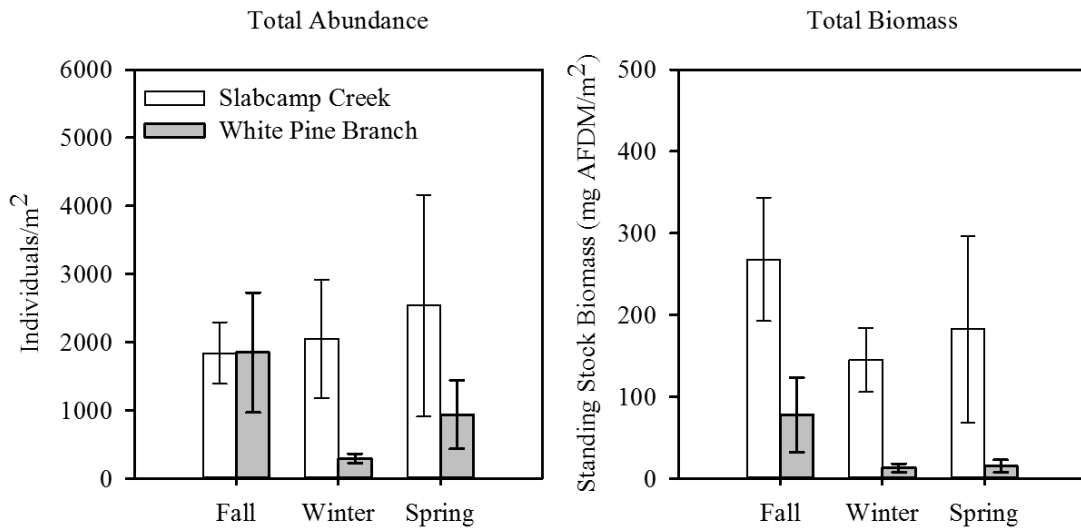


Figure 4. Mean ( $\pm 1$  SE) Macroinvertebrate Abundance and Biomass Across Seasons. Data are taken from riffle habitats from Slabcamp Creek ( $n=5$ ) and White Pine Branch ( $n=5$ ) per season.

## Richness

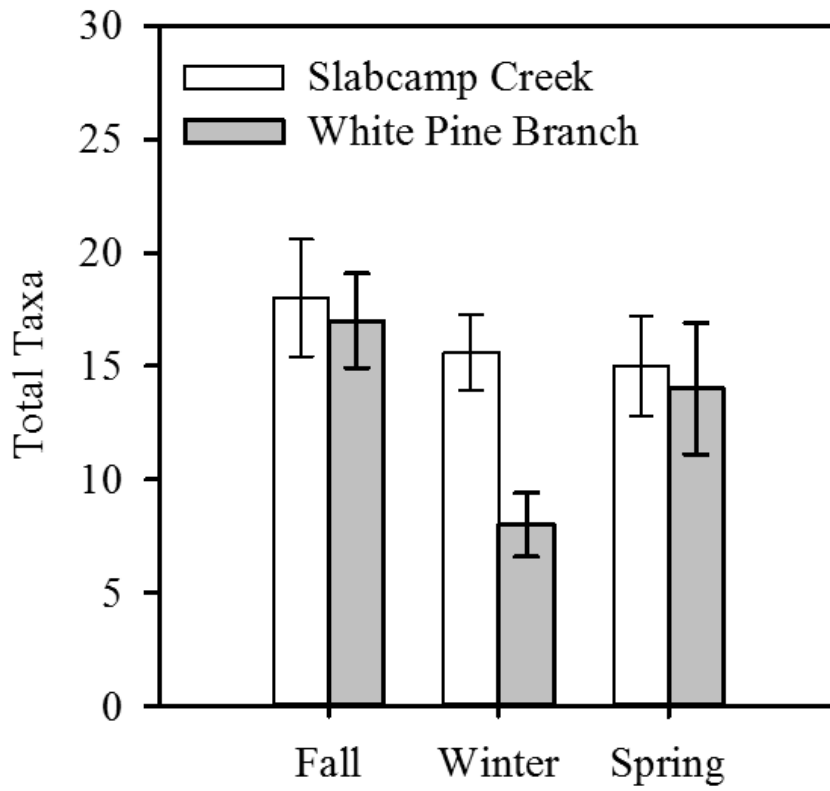


Figure 5. Mean ( $\pm 1$  SE) Macroinvertebrate Taxa Richness Across Seasons. Values are the total number of taxa present from riffles in Slabcamp Creek ( $n=5$ ) and White Pine Branch ( $n=5$ ) per season.

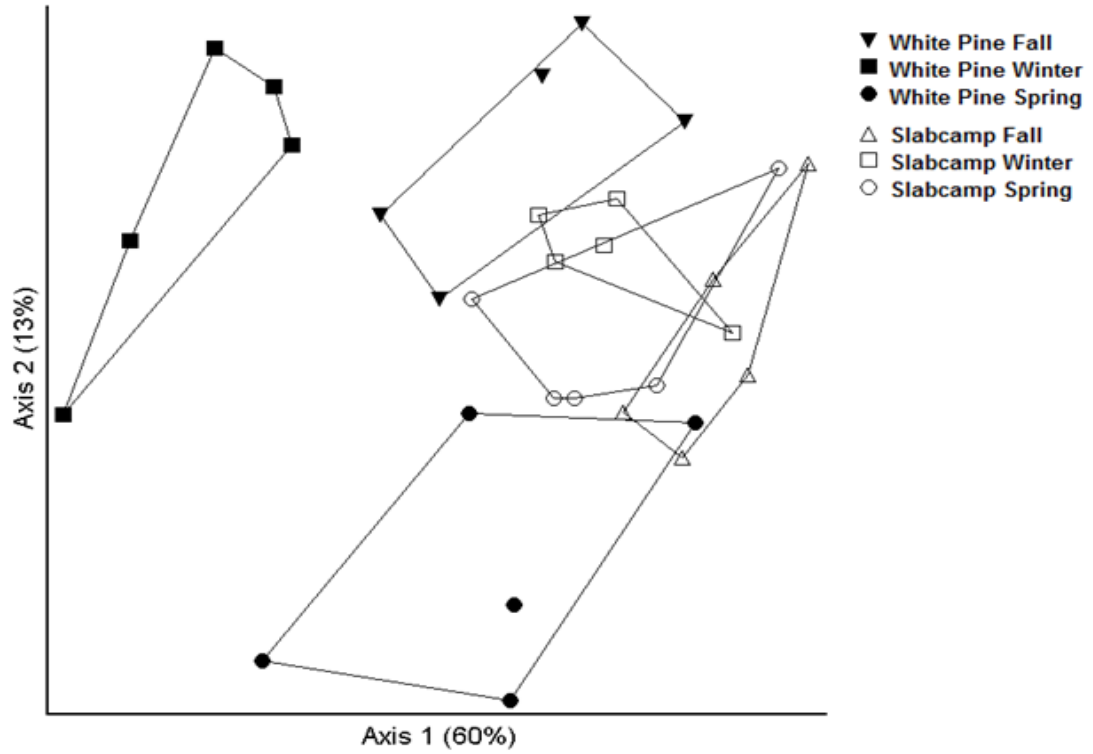


Figure 6. Nonmetric Multidimensional Scaling. Symbols represent macroinvertebrate abundance from benthic samples. Lines connected to symbols indicate the ordination space the benthic samples occupied within each stream by season.

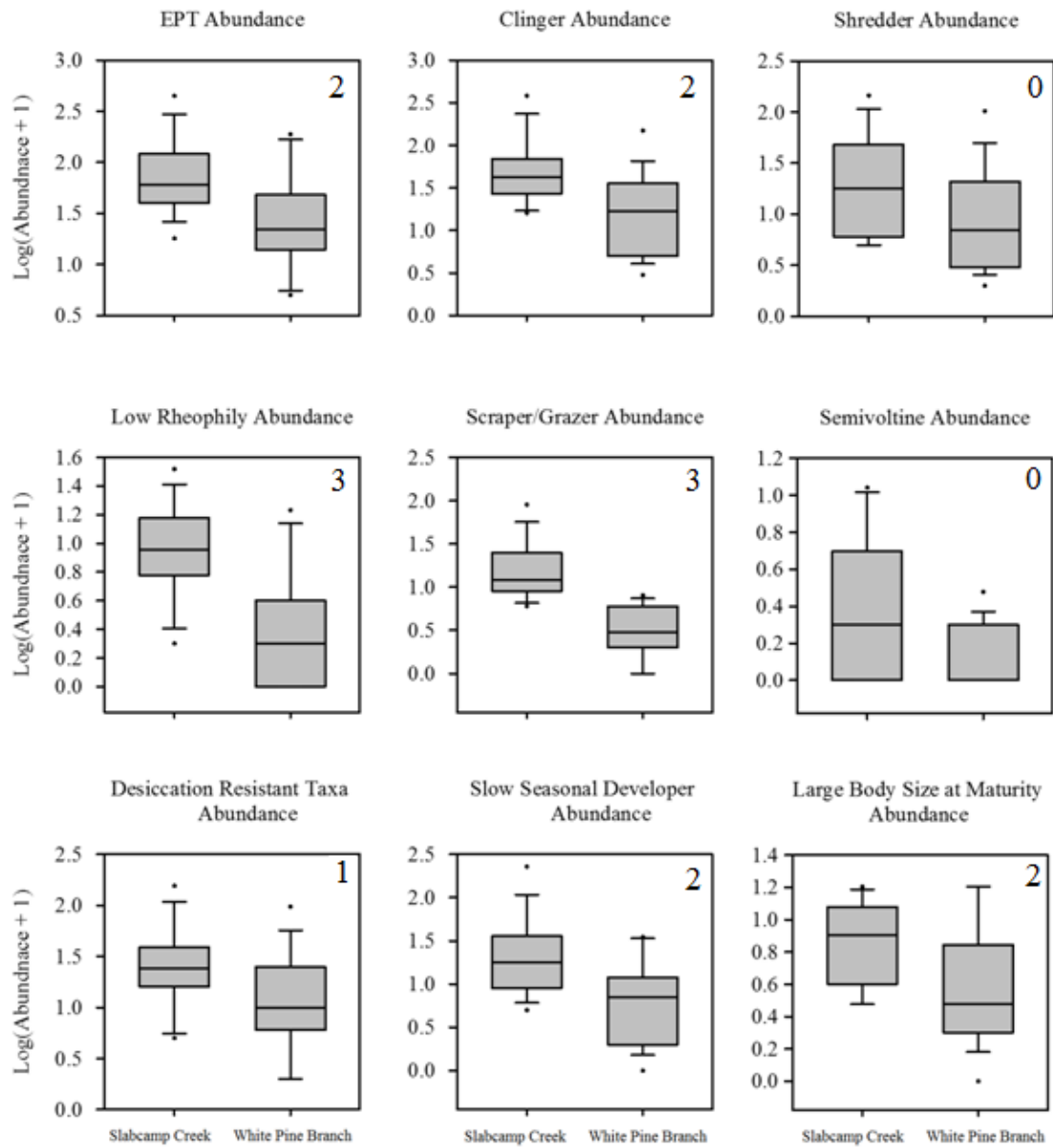


Figure 7. Box Plots of Absolute Abundance Metrics. Value are from macroinvertebrate community metrics based on absolute abundance from riffles in Slabcamp Creek ( $n=15$ ) and White Pine Branch ( $n=15$ ), with seasonal samples combined and  $\log_{10}(X+1)$  transformed data. Numbers in the top right corners are the score that plot received on a scale of 0-3 from the visual interpretation.

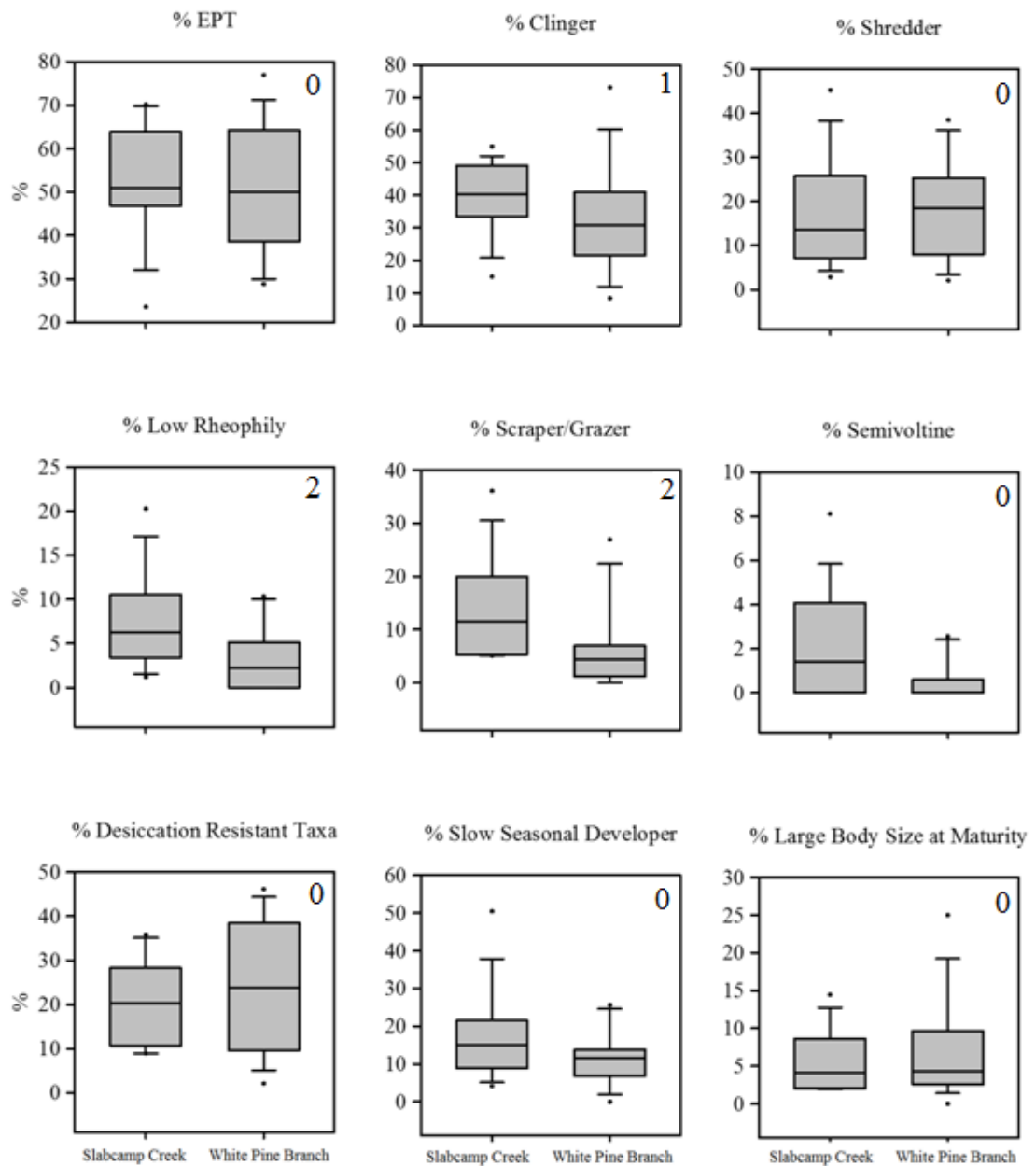


Figure 8. Box Plots of Relative Abundance Metrics. Values are from macroinvertebrate community metrics based on relative abundance from riffles in Slabcamp Creek ( $n=15$ ) and White Pine Branch ( $n=15$ ). Numbers in the top right corners are the score that plot received on a scale of 0-3 from the visual interpretation.