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ASSESSMENT OF THE MACROINVERTEBRATE ASSEMBLAGES FROM THE MESOHABITATS OF A HEADWATER STREAM-WETLAND HYDROLOGIC RESTORATION IN EASTERN KENTUCKY

By

Rebecca J. Roberts

Thesis Approved:

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Bachelor of Science
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2013

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
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DEDICATION

This thesis is dedicated to my mother and sister,

Gail and Cassie Roberts.

“You’re entirely bonkers. But I’ll tell you a secret,

all of the best people are.”

– Lewis Carroll

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ABSTRACT

The bulk of streams in the U.S. have been negatively impacted by anthropogenic disturbances and the streams of Kentucky are no exception. In recent decades stream restoration has become a common practice in order to improve habitat degradation resulting from land use practices such as channelization. Despite the large amount of effort and funding stream restoration projects represent, only a small portion have undergone post-restoration assessments of the ecological response in the restored streams. Slabcamp Creek, a headwater stream located in the Licking River basin in eastern Kentucky, underwent a stream-wetland hydrologic restoration in 2010 in order to improve hydrologic functioning and degraded habitat that resulted from channelization. The goal of this study was to quantify macroinvertebrate assemblages from Slabcamp Creek and compare the assemblages to a site representing Kentucky Division of Water's headwater reference conditions and a pre-restoration condition control site. Specific objectives included: 1) compare macroinvertebrate assemblage structure and function across study sites, 2) determine if mesohabitats (pools and riffles) support unique macroinvertebrate assemblages within and between study sites, 3) determine if macroinvertebrate assemblages varied at the study sites seasonally between high base flow (winter) and low base flow (summer), 4) explore relationships between the macroinvertebrate assemblages and microhabitat variables at the study sites, and 5) determine how accounting for the availability of mesohabitats at the reach scale (habitat weighting the data) compares to patch scale analyses for these objectives. Overall, findings indicated restored Slabcamp Creek was more similar to the reference condition site than the pre-restoration condition control site. It appeared that habitat-specific sampling may play an important role in assessing hydrologic restoration, since invertebrate densities, biomass and assemblage structure and function from riffles were fairly similar across sites while stark differences were detected in pools. This could be a result of the restoration improving hydrologic functioning and thus the underlying fluvial geomorphological processes that create pools which are disrupted by channelization. Subsequently, improved hydrologic function may have led to increased habitat complexity, substrate stability, and organic matter retention. Post restoration monitoring should continue at these study sites to see if these results vary or persist over time.

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CHAPTER I

INTRODUCTION

1.1 History and consequences of channel alteration

Prior to European settlement, Kentucky's freshwater ecosystems likely looked and functioned very differently than their modern counterparts. Many of the streams Daniel Boone would have laid eyes upon drained old uncut forests; a large portion of Kentucky's streams were likely heavily influenced by natural discontinuities such as dams built by native beaver (*Castor canadensis*). Natural channel discontinuities create intricate connections with wetland habitat in streams' floodplains (Burchsted et al. 2010, Parola and Bigbiehauser 2011). Settlement of the region brought a legacy of anthropogenic disturbances that altered the natural structure and hydrologic function of many stream and river channels. The intensity and frequency of land use disturbance has continued to increase; many streams and rivers have been channelized as a result of historical and current human activities in order to control floods, increase space for agriculture, and build roads.

As a result of natural fluvial geomorphological processes, streams in eastern Kentucky exhibit channels characterized by features called pools and riffles. Under normal flow conditions, shallow, high velocity erosional areas (riffles) alternate with deeper, slower velocity depositional areas (pools) within stream channels. Riffles tend to be characterized by larger, coarse substrates such as cobble and gravel while pools are characterized by finer substrates such as sand, clay, and silt (Allan and Castillo 2007).

Pools and riffles are mesohabitats, i.e. “medium-scale habitats which arise through the interactions of hydrological and geomorphological forces” (Tickner et al. 2000). Aspects of macroinvertebrate and fish assemblages such as abundance, biomass, diversity, and assemblage structure have been shown to vary across stream mesohabitats because of substrate composition, food availability, water depth and current velocity (Beisel et al. 1998, Beisel et al. 2000, Jackson et al. 2001, Jowett 2003, Merritt and Cummins 2008, Schwartz and Herricks 2008).

Channelization is the practice of converting a complex meandering stream into a simple straight channel, which changes the natural flow regime. In Kentucky, many streams have been straightened and moved to valley sides in order to make more land available for growing crops (Parola and Biebighauser 2011). Despite perceived advantages, such as creating room for agriculture and flood control, there is a preponderance of evidence that suggests many streams and rivers have lost their hydrologic function as a result of widespread alteration of channel structure and flow regimes, culminating into one of the most severe problems facing streams today (Poff et al. 1997, Bunn and Arthington 2002, Elosegi et al. 2010, Bernhardt and Palmer 2011). The most recent Kentucky Water Quality Assessment Report indicates 66.8% of evaluated miles of streams are impaired under the Clean Water Act (1972) (EPA 2012).

The ecological consequences of anthropogenic channel alteration are numerous, and result in major stressors to biota such as disconnection from the floodplain, drying, erosional down cutting that removes substrate and deepens the channel, unstable beds, and noncomplex habitat; overall it diminishes the ecosystem’s structure and ability to function. Loss of functioning results in a net loss of ecosystem services (e.g., biogeochemical cycling, production, water quality regulation, food, and recreation amongst others) (Thorp et al. 2010) and in many instances may facilitate the establishment of harmful invasive species; all of which result in the decline of biodiversity (Bunn and Arthington 2002). Channelization influences the natural fluvial geomorphological processes that create pool and riffle mesohabitats and results in a more homogenous channel (Negishi et al. 2002). For instance, channelization can lead to an increase in velocity and discharge that increases the stream's sediment transport capacity and thus sediment storage and composition (Montgomery and Buffington 1997). Increased sediment transport capacity essentially leads to a decline of the finer substrates (both sediments and organic matter) which are characteristic of pool mesohabitat. Substrate (sediment) composition and stability is very important to biota and different taxa exhibit different substrate preferences (Vannote et al. 1980, Beisel et al. 1998, Beisel et al. 2000, Jowett 2003, Merritt and Cummins 2008, Thorp and Covich 2009). A loss of fines may have implications on the assemblage structure of channelized streams. Substrate serves as refuge, foraging ground, and a place for reproduction and development (Allan and Castillo 2007, Merritt and Cummins 2008, Thorp and Covich 2009). Drying can be a major stressor to biota when channelization disconnects a stream from its floodplain and groundwater source, particularly in streams with non-complex habitat. During drought events, naturally functioning pools often retain water much longer than riffles; although little research has been done, some evidence suggests pools can function as a refuge from drying for macroinvertebrate taxa (Miller and Golladay 1996, Boulton 2003).

1.2 Stream Restoration and post restoration monitoring

In order to address the negative consequences of anthropogenic disturbance, stream restoration has become an increasingly common and important practice in recent decades (Bernhardt et al. 2005, Lake et al. 2007, Bernhardt and Palmer 2011). Stream restoration may occur for mitigation purposes, to enhance habitat for threatened and endangered species, or simply to return a stream to its former condition that better supported biodiversity and provided ecosystem services. The singular most important piece of legislation regarding water quality in the United States names restoration as a goal. The Clean Water Act (CWA), the 1972 amendments to the Federal Water Pollution Control Act, states its main objective is the restoration and maintenance of the chemical, physical, and biological integrity of the Nation's waters (Clean Water Act of 1972).

Stream restoration practices are diverse, but the most common practices to date involve channel reconfiguration, bank stabilization, introducing various structural features such as boulders and woody debris to increase habitat heterogeneity, and planting trees in riparian zones (Lave 2009, Tullos et al. 2009, Bernhardt and Palmer 2011). In the United States alone these restoration practices represent a substantial expenditure of resources, surpassing 1 billion dollars each year (Bernhardt et al. 2005).

Interestingly enough, despite the large amount of effort and funding represented by stream restorations, only a small portion of projects have undergone post-restoration assessments of the ecological response (Sudduth et al. 2007, Tullos et al. 2009, Bernhardt and Palmer 2011), perhaps due to the additional cost represented by continued monitoring. According to surveyed project managers, common tools for assessing the success of a restoration include the appearance of the restored site and public opinion on the project (Bernhardt et al. 2005). In Kentucky, stream restorations have been sparsely evaluated for ecological success (Jack et al. 2003, Suddeth et al. 2007). In an instance where post-project assessment was attempted in Kentucky, Price and Birge (2005) found degraded habitat and no difference in fish species assemblages in two restored reaches relative to control reaches. Given the rise in the number of restoration projects throughout the years, as well as the importance of their success in improving the ecological integrity of streams and rivers, it has become increasingly important to evaluate ecological responses in order to ensure limited resources are maximally utilized and efforts are not in vain.

There is no consensus in the scientific community as to what characteristics a “successful” restoration might exhibit (Palmer et al. 2005). A variety of indicators such as aesthetics, stakeholder satisfaction, economic cost or benefit, and biological indicators have been used to judge restoration success. Palmer et al. (2005) argues for measures of ecological success since the very definition of restoration implicates environmental improvement as a goal. Ecological success can be evaluated by summarizing the structure of aquatic communities or measuring ecosystem functions (e.g., secondary production, decomposition, nutrient retention). Biological indicators are organisms which are commonly used to evaluate the quality or health of an aquatic environment, making them ideal measures of the ecological success of stream restoration. The composition of macroinvertebrates and fish communities are frequently used to judge the biological integrity of streams and rivers (Hughes 1995, Carter and Resh 2001, Merritt and Cummins 2008). Benthic macroinvertebrates are especially useful in biomonitoring efforts since they: (1) are diverse and abundant with many taxa varying in their tolerance to environmental stressors, (2) occur in a variety of microhabitats, and (3) are relatively sedentary (Merritt and Cummins 2008). In addition, macroinvertebrates are important in food webs, and they influence ecosystem functions (Wallace and Webster 1996). Specific aspects of stream restoration practices that influence the distribution and abundance of macroinvertebrates in restored channels might be revealed if measures of water quality (pH, conductivity), physical habitat (flow, composition of inorganic substrate), riparian condition (canopy cover, vegetation assessment), and benthic food resources (benthic organic matter, periphyton) are collected concurrently with benthic macroinvertebrates.

Studies using macroinvertebrate communities to judge restoration success indicate current restoration practices (i.e., channel reconfiguration and increasing structural habitat heterogeneity), have had limited success at eliciting a positive ecological response (Miller et al. 2009, Tullos et al. 2009, Palmer et al. 2010). Palmer et al. (2010) found only two out of 78 studies reported an increase in macroinvertebrate taxa richness following restoration. Another study from restored streams in North Carolina found restoration practices negatively influenced food availability and habitat, leading to benthic macroinvertebrate communities dominated by tolerant taxa and species with life histories adapted for frequent disturbance (Tullos et al. 2009). Miller et al. (2009) performed a meta-analysis of 24 published restoration studies and found a significant increase in macroinvertebrate richness but not density. Density could be a very important measure when determining the ecological success of a restoration because it speaks to trophic level dynamics and the ability of an ecosystem to support higher organisms (Lake et al. 2007).

1.3 Recommendations for improved restoration practices and post restoration monitoring

1.3.1 Restoration practices

The revelation that common restoration practices have had limited success calls for a reevaluation of restoration and post restoration monitoring methodology. As a result of the above studies and others, many ecologists have pointed out shortcomings of current restoration practices and have made recommendations for improving future stream restorations (Bernhardt et al. 2005, Lake et al. 2007, Miller et al. 2009, Tullos et al. 2009, Burchsted et al. 2010, Palmer et al. 2010, Bernhardt and Palmer 2011). Restorations should occur within the context of the disturbances present in individual streams and the goals to be achieved (Palmer et al. 2005, Palmer et al. 2010, Bernhardt and Palmer 2011). Palmer et al. (2010) points out that despite the great diversity of reasons for stream restoration (channelization, agriculture, urbanization, etc.), the majority are addressed with the same practices, i.e. practices that focus on channel reconfiguration and introduce structural features such as boulders and woody debris to increase habitat heterogeneity. Given the lack of biological improvement following these types of restoration practices (Miller et al. 2009, Tullos et al. 2009, Palmer et al. 2010), it is likely there are other more important factors that need to be addressed depending on the individual projects.

One important consideration for restorations is the regional species pool. If the area (watershed) surrounding a restoration is ecologically impaired it is unlikely a source of colonists will be available to recolonize restored sites (Lake et al. 2007, Sundermann et al. 2011). Additionally, Lake et al. (2007) argue that the widespread failure to apply ecological theory to restoration practices is responsible for many failures. Many restorations are implemented without the input of ecologists or biologists and incorrectly focus on improving structural components of habitat while overlooking function (ecosystem processes) and life history aspects of the biota, with the assumption that if the basic habitat structure is present biota will recover (Hilderbrand et al. 2005, Lake et al. 2007). Another consideration that is frequently overlooked is that stream ecosystems are intricately connected with surrounding terrestrial ecosystems in terms of trophic level dynamics. Allochthonous organic matter inputs from terrestrial ecosystems are an important source of energy in the form of food for stream biota, the presence or lack thereof can have profound implications on trophic structure. However, organic matter inputs and retention within streams are rarely the focus of common restoration practices (Lake et al. 2007) and should be an area of greater concern in the future. Finally, future projects should emphasize restoring natural flow regimes and hydrologic functioning (Palmer et al. 2010, Bernhardt and Palmer 2011), which could result in more stable and complex habitat rather than simply manipulating structural habitat features.

Restoring hydrology results in streams that are properly connected to their floodplain, which prevents channel erosion and allows wetlands to develop adjacent to channels (Parola and Biebighauser 2011). The presence of the additional wetland habitat in the floodplain creates food and nursery advantages for aquatic organisms and subsequently may increase biodiversity (Bunn and Arthington 2002). Lateral connections to the floodplain and surrounding stream network also improves the dispersal and recruitment of aquatic organisms (Bunn and Arthington 2002, Lake et al. 2007). Perhaps most importantly, reconnecting streams with their groundwater sources enables channels to stay wet longer, which is beneficial to aquatic organisms that would otherwise perish during drying events (Parola and Biebighauser 2011). Stream restoration goals and practices must vary according to project location, but in order to increase restoration success, it is important to recognize the historical condition of watersheds prior to the implementation of restoration efforts (Foster et al. 2003). For instance, it is often overlooked that before European settlement, many Kentucky streams likely drained forested watersheds, and hydrology and habitat were heavily influenced by native beaver (*Castor canadensis*) (Naiman et al. 1988, Parola and Bigbiehauser 2011). Ignoring the influence beaver modifications once had on stream hydrology is to ignore the baseline conditions and render a return to pre-disturbance conditions impossible in areas where they once thrived (McDowell and Naiman 1986, Burchsted et al. 2010).

1.3.2 Post restoration monitoring

There is a general consensus that the first step towards more successful restoration practices is to increase both short and long term post-restoration monitoring, preferably with a more standardized approach for evaluating success (Jack et al. 2003, Bernhardt et al. 2005, Palmer et al. 2005, Bernhardt et al. 2007, Miller et al. 2010). In addition to emphasizing ecological indicators as a measure of success in future monitoring projects

(Palmer et al. 2005, Lake et al. 2007, Pander and Geist 2013), sampling design and methodology need to be carefully considered in the context of the goals of the restoration.

In terms of using macroinvertebrates as indicators, it is widely thought that targeting riffle habitat produces “the most bang for your buck” (Carter and Resh 2001, Beauger and Lair 2007), and this is likely true for *general* bioassessment purposes, such as in the case of rapid biomonitoring protocol used for water quality assessments and determining use attainment under the Clean Water Act. In addition to targeting one specific habitat, many bioassessment protocols choose sampling location based on “expert opinion”. Investigators visually inspect the sample reach and collect macroinvertebrates in what they believe is the best available riffle habitat (patch scale) and then extrapolate their findings to the reach scale (Carter and Resh 2001) without considering the amount of available mesohabitat within a given reach. Habitat changes both at the patch and reach scale and macroinvertebrate taxa exhibit a diversity of habitat preferences. Limiting a study to a single habitat likely does not produce results reflective of the macroinvertebrate assemblage structure as a whole (Grubaugh et al. 1996). If the goals of a restoration project are to restore hydrologic functioning and subsequently improve habitat complexity, it is likely that targeting riffle habitat during post restoration monitoring is an insufficient method since improved and more abundant mesohabitats may be available. Habitat availability should be accounted for at the reach scale so that investigators can adequately detect changes in macroinvertebrate assemblage structure at the scale of the restoration.

When possible, it may be beneficial to incorporate the concept of reference condition into post restoration monitoring study designs. Reference condition streams are those that are the least altered or disturbed by human activities, they are thought of as representing the most "natural" state of streams observable today and could serve as a benchmark for restoration goals (Hughes 1995, Stoddard et al. 2006). In Kentucky, reference reaches are used by government agencies to represent the best-attainable condition for streams. Reference reaches have "minimal human impact" and exemplify the "biological potential" of streams from the same region (Pond et al. 2003). Streams representing regional reference conditions were used to develop the Kentucky Macroinvertebrate Biotic Index (MBI), which is a tool widely used to judge the use attainment of streams throughout Kentucky. Since reference reaches are the standard to which streams are held in Kentucky (and often elsewhere), it could be worthwhile to see how the restored streams compare to this "ideal" condition. Not only *where* samples are collected, but *how* samples are collected should be a careful consideration as well. Quantitative sampling methods allow for the calculation of macroinvertebrate densities and biomass, which allows investigators to address questions about secondary production and trophic level dynamics (Benke 1984).

1.4 A hydrologic restoration in eastern Kentucky: Slabcamp Creek

Stream restoration practices used by the Stream Institute (University of Louisville) go beyond common restoration practices and focus on restoring hydrology. In addition to restoring hydrologic function, the Stream Institute attempts to address hydrologic dynamics once present in Kentucky streams due to beaver influence when feasible and reintroduce stream-wetland complexes along channels. One of the Stream Institute's restoration sites, Slabcamp Creek, was the focus of this study.

The Slabcamp Creek restoration was conducted by the Stream Institute on a 3.6 km first-order section of the stream (see methods section for details on site location and description), which was historically damaged by channelization associated with agricultural practices. Prior to the restoration, the channel was surrounded by second growth forest, but the stream was disconnected from its floodplain and aquifer. As a result, the channel was incised, had unstable substrates and dried during late summer (Biebighauser 2006).

The USDA Forest Service decided in 2006 Slabcamp Creek would be restored to pre-settlement conditions for the purpose of improving habitat for wildlife, improving water quality, preventing erosion, and reinstating a more natural flooding pattern (Biebighauser 2006). The restoration practices involved moving the channel from the side of the valley back to the center, removing built up sediment to reinstate long-buried natural gravel riffles from the pre-settlement condition stream, planting native vegetation, introduction of woody debris and channel discontinuities, which resulted in wetland habitat in the floodplain (Parola and Biebighauser 2011). The Slabcamp Creek restoration was completed in late 2010.

1.5 Study Objectives

The goal of this study was to quantify macroinvertebrate assemblages from Slabcamp Creek and compare the assemblage to a site representing Kentucky Division of

Water's (KDOW) headwater reference conditions (Bucket Branch) and a pre-restoration condition control site (White Pine Branch). Specific objectives include:

1. Compare macroinvertebrate assemblage structure and function at Slabcamp Creek to sites that represent the reference condition and the pre-restoration condition (a control site).
2. Determine if mesohabitats (pools and riffles) support unique macroinvertebrate assemblages within and between the study sites.
3. Determine if macroinvertebrate assemblages vary at the study sites seasonally between high base flow (winter) and low base flow (summer).
4. Explore relationships between the macroinvertebrate assemblages and microhabitat variables at the study sites.
5. Determine how accounting for the availability of mesohabitats (pools and riffles) at the reach scale (habitat weighting the data) compares to patch scale analyses for the above objectives.

Findings may provide insight into the effectiveness of the stream restoration practices used by the Stream Institute at the University of Louisville and could provide guidance for future post-restoration monitoring efforts.

CHAPTER II

METHODS

2.1 General study design and study sites

With assistance from the United States Forest Service and the Stream Institute, various criteria were established and implemented to select a control site that would represent conditions at Slabcamp Creek prior to the restoration:

1) Located within the North Fork of the Licking River watershed (HUC 10). Streams within the same watershed can be expected to be under the influence of similar external inputs more so than streams in different watersheds. This criteria aids in controlling for extra variation due to differences in environmental and anthropogenic influences on the streams.
2) Located in the same bioregion. The concept of bioregions has been utilized to control for natural variation in biological assemblages that occurs between geographic regions with different physical characteristics (Pond et al. 2003).
3) Drain approximately the same amount of land within its own watershed. Streams of a similar size are more comparable than those which are not in terms of discharge, physical characteristics and macroinvertebrate fauna.
4) Defined by similar geologic features. Comparing sites with similar geology controls for differences which would be inherent in streams influenced by different physical characteristics.

5) Historically subjected to the same anthropogenic disturbances. Historical disturbance was determined by local literature review and ground-truthing via visual observation of stream characteristics such as channelization and bank stability.

The reference condition stream was selected using the first four criteria listed above for the control reach, as well as reference condition criteria defined by KDOW. Various physical criteria applied by KDOW to select reference condition streams include a minimal amount of suspended solids present in the stream, a conductivity level not above what is normal for the ecoregion, absence of garbage at the site (or at least a minimal amount), and no recent disturbance due to a change in land-use. Other criteria utilized by KDOW for determining reference condition are scored using a Rapid Bioassessment Protocol (RBP) habitat assessment, for example: riparian zone condition, bank stability, sedimentation, and habitat availability. These parameters are rated numerically and depending on which percentile the score falls into they are assigned a number of "habitat stress points" (ex. a parameter scoring in the 50^{th} to 75^{th} percentile will receive 1 habitat stress point). The total number of habitat stress points for the RBP is then calculated and the site is assigned a "habitat stress code". A reference condition stream should have a habitat stress code 1, which has 0 – 4 habitat stress points (Pond et al. 2003).

The restored (Slabcamp Creek), pre-restoration condition control (White Pine Branch), and reference condition (Bucket Branch) reaches for this study are located in southern Rowan and northern Morgan counties, Kentucky in the Daniel Boone National Forest (Fig. 1 and 2).[1] The three study reaches are all first order tributaries within the

[1]All figures and tables are presented in the appendices

North Fork Licking River watershed. The study reaches have similar drainage areas, geologic features (Western Allegheny Plateau ecoregion), and biology (Mountain bioregion) (Table 1). All sites have similar land use and are mostly forested (Table 1, Fig. 3).

2.2 Sampling design

Study sites consisted of 150-m reaches in each stream. Each site was sampled twice: once during high seasonal base flow in late winter 2014 and once at low seasonal base flow during late summer 2014. During the winter sampling event, benthic samples were collected randomly from five riffles and five pools for each site. During the summer, riffles did not have adequate flow for sampling in all three streams so they were omitted, but five pool replicates were collected from each site. This sampling design amounted to five riffle and five pool replicates from each stream during the winter and five pool replicates from each stream during the summer, totaling 45 benthic samples for the entire study.

At each sample location, water depth (cm) was measured with a ruler and substrate composition was visually estimated before benthic samples were collected. Inorganic substrate was estimated as: % cobble, % gravel, % pebble and % fine. A quantitative bottom area sampler (modified Hess, 250 µm, 0.086 m^2) was used to collect macroinvertebrates and benthic organic matter. The Hess sampler was modified to include a bottomless bucket which aids in the collection of fine benthic organic matter (FPOM). The sampler was placed in the thalweg of the stream and the benthic material in the bottom was agitated. If the sample was collected from a riffle where it was difficult to maintain a seal between the sampler and the stream bed, a towel was used to prevent fine benthic material from escaping from the bottom of the sampler. Once the material in the Hess was agitated, a 200-mL subsample of water was collected for fine benthic organic matter (FPOM, less than 1 mm) analysis. These samples were transported in ice and frozen until analysis. Additionally, water depth inside the sampler was measured at four equidistant points to estimate total volume. Following FPOM collection, the bottomless bucket was then removed from the Hess and remaining benthic material, macroinvertebrates and course benthic organic material (CPOM, organic matter greater than 1 mm), were captured in the collecting net as water flowed through the sampler. Each sample collected was rinsed into individual plastic bags and preserved with 95% ethanol. Following benthic sampling, wetted area covered by predominant channel habitat (riffles, run, pools and bedrock) units was estimated at each site by measuring the total length and average width of each habitat type. Estimating wetted area of these habitat units enabled habitat weighted estimates of macroinvertebrate abundance (TNI/100 m^2) and biomass (mg AFDM/100 m^2).

2.3 Laboratory methods

In the laboratory, benthic samples were rinsed through two stacked sieves (1 mm and 250 μm) and material retained on the 250 μm was subsampled with a sample splitter. Macroinvertebrates from both sieves were sorted using a dissecting microscope and identified to the lowest practical taxonomic level, typically genus, and counted. Early instar specimens and Chironomidae (non-biting midges) were left at the family level.

Individuals were measured to the nearest 0.5 mm using grid paper behind a clear watch glass, and estimates of standing stock biomass (mg AFDM/m^2) were calculated from previously published methods (Benke et al. 1999). CPOM captured in the benthic samples was dried to a constant weight at 60º C then dry mass was weighed. Fine benthic organic matter (FPOM) was filtered onto pre-ignited filter paper and again dry mass was weighed. Both CPOM and FPOM were ignited at 500º C and reweighed for ash free dry mass (AFDM).

2.4 Data analyses

2.4.1 Macroinvertebrate density and biomass between mesohabitats and seasons

Since a quantitative bottom area sampler was used, macroinvertebrate density and standing stock biomass were reported per m^2 of stream bottom area. In order to explore the importance of mesohabitat (pool and riffle) availability at the reach scale, analyses were run on both patch scale (per m^2) and habitat weighted (habitat weighted results will be referred to as "reach scale", per 100 m^2) data. Habitat weighting was achieved by using estimates of wetted channel units to calculate proportions of available pool and riffle habitat in each reach. Habitat-specific (i.e. riffle, pool), patch-scale (m^2) density and biomass values were then multiplied by the proportion of available habitat, and finally multiplied by 100 so habitat weighted values could be expressed per 100 m^2 of stream reach (Negishi et al. 2002, Grubaugh et al. 1996).

Eight separate two-way repeated measures Analysis of Variance (ANOVA) were conducted using Minitab Statistical Software (Minitab Inc., PA, USA, 2014) to compare macroinvertebrate density (TNI/100 m^2) and biomass (mg AFDM/100 m^2) at both patch and reach scales between the study sites. Habitat and season were not included as factors within a single ANOVA as a result of channel drying during the summer collection. The first set of four two-way repeated measures ANOVAs analyzed density and biomass at the patch and reach scales between mesohabitats among study sites during the winter season: site, habitat, and the interaction between site and habitat were included as factors. The second set of four two-way repeated measures ANOVAs analyzed density and biomass at the patch and reach scales between seasons among study sites: stream (site), season, and the interaction between stream and season were included as factors. Prior to analyses, assumptions were tested using the Ryan-Joiner test for normality (similar to Shapiro-Wilk), Levene's test for homogeneity of variance, and by visual interpretation of probability plots and histograms. Subsequently, in order to satisfy the assumptions of ANOVA, the macroinvertebrate data were $\log_{10}(X+1)$ transformed. The $\log_{10}(X+1)$ transformation is commonly used in macroinvertebrate analyses due to the clumped nature of their distributions (Tiemann et al. 2004, Zar 2007, O'Conner 2016). Additionally, uncommonly large individuals (>1.5 mg AFDM) such as Cambaridae, *Tipula*, and *Pycnospyche* were removed from the biomass dataset for all sites. Once the data were transformed and large individuals were removed, the assumptions were re-tested and found to be satisfied. Tukey's tests were performed for pairwise comparisons when ANOVA results were significant. A p-value ≤ 0.05 was considered significant for ANOVAs and pairwise comparisons.

2.4.2 Exploration of relationships between macroinvertebrate density and biomass and microhabitat variables

Pearson correlations were conducted for patch- and reach-scale data using Minitab to determine if relationships exist between macroinvertebrate density and biomass, organic matter ("food" for many macroinvertebrates), and habitat variables. Macroinvertebrate, organic matter, and depth data were transformed using the log(x+1) transformation, and the arcsine square root transformation was used on percent substrate composition data in order to meet the normality assumption of Pearson's test (Zar 2007).

2.4.3 Macroinvertebrate assemblage structure between mesohabitats and seasons

Macroinvertebrate assemblage structure was analyzed by conducting two non-metric multidimensional scaling (NMDS) of density data of each taxon (data were grouped by site, habitat, and season), one for patch-scale data and one for habitat-weighted reach-scale data, using PRIMER version 6 software (PRIMER-E Ltd., Plymouth, UK). NMDS is a robust and commonly used multivariate ordination method that is ideal for ecological data. NMDS makes few assumptions about the form of the data or the relationships among samples, and it has a greater ability to represent relationships in fewer dimensions relative to other ordination methods. The measure of fit associated with NMDS is known as the stress value. The stress value is a measure of how well the ordination summarizes distances between samples and ordinations with a stress value of <0.20 can be considered useful (Clarke and Warwick 2001). Prior to analysis, rare taxa, i.e., those that made up <0.5% of the total abundance, were removed (see

Appendix). The NMDS parameters for both tests were: Bray-Curtis similarity as the distance measure, number of restarts = 250, and minimum stress = 0.01. Following the NMDS, analyses of similarity (ANOSIM) were performed to test for differences in assemblage composition between groups (Bray-Curtis similarity as the distance measure, maximum permutations = 999). ANOSIM is a non-parametric permutation test with a test statistic, R, that typically ranges from 0 to 1 where an R value of 0 indicates a true null hypothesis (no difference between groups) where 1 indicates dissimilarity between groups. It is possible to obtain an r value of less than 0, which indicates differences within groups are greater than between groups (Clarke and Warwick 2001). ANOSIM R values were considered significant if $p < 0.01$.

NMDS was also used to analyze macroinvertebrate functional feeding group (FFG) data (based on total abundance) at the patch and reach scales. The NMDS parameters for both tests were: Bray-Curtis similarity as the distance measure, number of restarts = 250, and minimum stress = 0.01. Following NMDS, ANOSIMs were performed to test for differences in FFG composition between groups (Bray-Curtis similarity as the distance measure, maximum permutations = 999).

2.4.4 Macroinvertebrate assemblage structure based on common water quality metrics

Metrics commonly used for water quality assessment purposes were calculated to summarize various aspects of the assemblages: taxa richness, EPT taxa richness, Hilsenhoff’s Biotic Index, and % top five dominant taxa. Jaccard’s index (Krebs 1999) was calculated to examine similarity between sites.

CHATPER III

RESULTS

During winter 2014 Slabcamp Creek and White Pine Branch had similar wetted channel areas (approximately 390 m^2 each). As a result of large pools and a wider channel, Bucket Branch had approximately 160 m^2 more wetted area than Slabcamp Creek and White Pine Branch (Table 2). During low base flow (summer 2014), wetted area decreased as a result of drying at all sites. Slabcamp Creek and Bucket Branch lost 24% and 20% wetted area, respectively, while White Pine Branch lost 68%. Riffle wetted area decreased dramatically at all sites and dried entirely at both Bucket Branch and White Pine Branch, while pool wetted area was more apt to be retained (Fig. 4).

A total of 14,301 macroinvertebrates were collected for the entire study (Appendix). See Tables 3 – 4 for mean (±1 SE) macroinvertebrate density and biomass reported at the patch and reach scales. For all habitats and seasons combined at the sample scale, Slabcamp Creek supported greater macroinvertebrate density and biomass (7291 TNI, 231 mg AFDM) than either Bucket Branch (4823 TNI, 170 mg AFDM) or White Pine Branch (2187 TNI, 145 mg AFDM).

3.1 Macroinvertebrate density and biomass between mesohabitats

Repeated measures two-way ANOVAs on density at the patch and reach scales between mesohabitats among study sites returned some significant results. Density results indicated significant differences at the patch scale among sites and between habitats but not the site x habitat interaction (Table 5 A, Fig. 5 A). Macroinvertebrate densities were greater from pools ($p = 0.003$), and Tukey's pairwise comparisons showed Slabcamp Creek had significantly greater densities than White Pine Branch but not Bucket Branch (SCxWP $p = 0.006$, SCxBB $p = 0.299$, BBxWP $p = 0.158$). At the reach scale, ANOVAs indicated significant differences in density for site, habitat, and the site x habitat interaction (Table 6 A, Fig. 5 C). Macroinvertebrate densities were again greater from pools ($p = 0.015$). Tukey's showed Slabcamp Creek and Bucket Branch were significantly different from White Pine Branch but not from one another (SCxWP $p < 0.001$, BBxWP $p = 0.002$, SCxBB $p = 0.612$). The significant interaction term indicates differences in densities between habitats were site dependent. Riffles supported similar densities between sites at both the patch and reach scales. However, macroinvertebrate densities from pools at Slabcamp Creek and Bucket Branch were several times greater than densities from White Pine Branch, and this was especially pronounced from habitat-weighted data, which accounts for mesohabitat availability at the reach scale (Fig. 5 A and C). Additionally, within sites both Slabcamp Creek and Bucket Branch supported densities three to four times greater in pools than in riffles, but White Pine Branch pools supported less than half the density of its riffles.

Results of repeated measures two-way ANOVAs on biomass at the patch and reach scales between mesohabitats among study sites were not as significant as density results. At the patch scale, biomass results indicated significant differences between habitats but not sites or the site x habitat interaction (Table 5, Fig. 5 B). At the reach scale, no significant differences were found, although p-values were approaching significance (Table 6, Fig. 5 D). At the patch scale, biomass was significantly higher in pools ($p = 0.015$). Although habitat-weighted results were not significant, reach-scale biomass was higher in pools than riffles at both Slabcamp and Bucket Branch but not at White Pine Branch. White Pine Branch riffles supported more than twice the biomass of its pools (Fig. 5 D).

3.2 Macroinvertebrate density and biomass between seasons

Repeated measures two-way ANOVAs on density at the patch and reach scales between seasons among study sites indicated no significant differences between high base flow (winter) and low base flow (summer). Both the patch and reach scale density models returned significant results for the site factor but not for season or the site x season interaction (Tables 7 and 8, Fig. 6 A and C). Slabcamp Creek and Bucket Branch supported greater densities than White Pine Branch at both the patch (SCxWP $p < 0.001$, BBxWP $p < 0.001$, SCxBB $p = 0.522$) and reach scales (SCxWP $p < 0.001$, BBxWP $p < 0.001$, SCxBB $p = 0.906$) but were not significantly different from each other.

Repeated measures two-way ANOVAs on biomass at the patch and reach scale between seasons among study sites also indicated no significant differences between high base flow (winter) and low base flower (summer). The patch scale model returned no significant results, while the reach scale model results indicated site was significant but season and the site x season interaction were not (Tables 7 and 8, Fig. 6 B and D). At the reach scale, Slabcamp Creek and Bucket Branch supported significantly greater densities than White Pine Branch, but were not different from one another (SCxWP $p = 0.001$, BBxWP $p < 0.001$, SCxBB $p = 0.885$).

3.3 Exploration of relationships between macroinvertebrate density and biomass and microhabitat variables

Pearson correlation results showed that some relationships existed between microhabitat variables and the macroinvertebrate assemblages across the study sites (Table 9). Macroinvertebrate density and biomass were positively correlated at all sites: more so at Slabcamp Creek (patch r = 0.81, reach r = 0.89) and Bucket Branch (patch r = 0.78, reach r = 0.83) than White Pine Branch (patch r = 0.67, reach r = 0.60). Organic matter, CPOM and FPOM, were also positively correlated with the macroinvertebrate assemblages at all sites. In general, stronger relationships existed between the assemblages with FPOM than CPOM. Positive correlations between the macroinvertebrate assemblages and depth existed at both Slabcamp Creek (reach density r = 0.65, reach biomass r = 0.73) and Bucket Branch (patch density r = 0.57, reach density r = 0.59), but White Pine Branch macroinvertebrate density was negatively correlated with depth (reach density r = -0.58). At Slabcamp Creek (reach density r = 0.65, reach biomass r = 0.56) and Bucket Branch (patch density r = 0.69, reach density r = 0.77) relationships existed between the macroinvertebrate assemblages and fine substrates, whereas at White Pine Branch the macroinvertebrate biomass was correlated with pebble substrates (patch biomass r = 0.60, reach biomass r = 0.53). In general, habitat weighting to the reach scale improved correlations between the macroinvertebrate assemblages and microhabitat variables at Slabcamp Creek and Bucket Branch, but this did not appear to be the case at White Pine Branch. For instance, the relationship between density and biomass was improved by habitat weighting at Slabcamp Creek (patch r =

0.81, reach r = 0.89) and Bucket Branch (patch r = 0.78, reach r = 0.83) but resulted in a weaker correlation at White Pine Branch (patch r = 0.67, reach r = 0.60). Similar results were seen with density and depth: Slabcamp Creek (patch r = 0.44, reach r = 0.65), Bucket Branch (patch r = 0.57, reach r = 0.59), White Pine Branch (patch r = -0.12, reach r = -0.58).

3.4 Macroinvertebrate assemblage structure

A total of 92 taxa were collected from the three study sites during winter and summer 2014 (Appendix). Thirty-six taxa were considered rare (those that made up < 0.5% of the total abundance), and 20 of the rare taxa were unique to one site (Table 10). The dominant taxa from riffles and pools varied among study sites (Table 11). In general, burrowing taxa (Oligochaeta, and *Ephemera*, and usually Chironomidae) were numerically dominant in the pools at Slabcamp Creek and Bucket Branch during winter (76% and 77% respectively), but these taxa comprised only 38% of the pool assemblage at White Pine Branch. This pattern was also apparent during summer, although to a lesser extent, when taxa exhibiting both burrowing and collecting traits comprised 45 – 53% of the pool assemblage at Slabcamp Creek and Bucket Branch, but only 26% in pools of White Pine Branch. White Pine Branch pools had greater dominance of clinging taxa (22% in winter, 9% in summer) such as *Eurylophella*, *Cinygmula*, *Haploperla*, and *Psephenus* than either Slabcamp Creek (0% in both winter and summer) or Bucket Branch (2% in winter, 0% in summer). In riffles, burrowers were again dominant at all sites; 34% at Slabcamp Creek, 31% at Bucket Branch, and 30% at White Pine Branch. Clingers had a more dominant presence at Slabcamp Creek (*Allocapnia* - 32%) and

White Pine Branch (*Cinygmula* and *Prosimulium* - 23%) than Bucket Branch (*Neophylax* and *Eurylophella* - 7%).

3.4.1 Macroinvertebrate assemblage structure based on taxa densities

The NMDS of patch scale taxa density produced a final stress value of 0.14 (Fig. 7). Slabcamp Creek riffles grouped together relatively well in ordination space compared to Bucket Branch and White Pine Branch riffles, which are more spread out, White Pine Branch more so than Bucket Branch. Slabcamp Creek and Bucket Branch winter and pools grouped together relatively closely and were separate from riffles in ordination space, whereas White Pine Branch winter pools were separate from the winter pools of the other sites and in closer proximity with riffles. This pattern was also apparent in the summer but to a lesser extent, White Pine Branch summer pools were separate from the summer pools of the other sites but were further from riffles than White Pine Branch's winter pools were. Eight taxa explaining variation were identified by the analysis and are shown as vectors on the plot: *Caenis* (-0.48, NMDS axis 1), Ceratopogonidae (-0.47, NMDS axis 1), Chironomidae (-0.70 NMDS axis 1), Copepoda (-0.40, NMDS axis 1), *Neophylax* (-0.48, NMDS axis 2), Oligochaeta, *Paraleptophlebia* (0.61, NMDS axis 2), and *Psephenus* (0.52, NMDS axis 2). The taxa that really appear to be driving the placement of Slabcamp Creek and Bucket Branch pools in ordination space are often associated with depositional (i.e. pool) habitats (especially *Caenis* and Oligochaeta) and exhibit burrowing and sprawling habits (Poff et al. 2006). *Psephenus* shows up as a vector influencing the placement of White Pine Branch's pools, *Psephenus* is a clinger associated with erosional (i.e. riffle) habitats (Poff et al. 2006).

The NMDS of habitat-weighted reach-scale taxa density produced a final stress value of 0.12 (Fig. 8). Compared to the replicates of other groups, Slabcamp Creek and Bucket Branch winter and summer pools' proximity to one another in ordination space remained relatively stable between the patch and reach scale ordinations. Slabcamp Creek and Bucket Branch winter and summer pool samples grouped together and were separate from riffles at both scales. Habitat weighting to the reach scale improved the grouping of Slabcamp Creek and Bucket Branch pools relative to the patch scale. White Pine Branch winter and summer pools noticeably shifted between the patch- and reach-scale ordinations, at the reach scale they became even further separated from the pools of other sites (more so in the winter than the summer) and in closer proximity to White Pine Branch's riffles than the other sites' pools were to their own respective riffles. Five taxa explaining variation were identified by the analysis and are shown as vectors on the plot: *Caenis* (-0.49, NMDS axis 1), Ceratopogonidae (-0.56, NMDS axis 1), Chironomidae (-0.75, NMDS axis 1), Copepoda (-0.44, NMDS axis 1), Oligochaeta (-0.62), and *Paraleptophlebia* (-0.50, NMDS axis 2). Taxa associated with depositional habitats (especially Caenis and Oligochaeta) again appeared to be driving the placement of Slabcamp Creek and Bucket Branch winter and summer pools. Copepoda influenced the placement of summer pools for all sites and *Paraleptophlebia*'s top dominance (Table 11) in White Pine Branch's summer pools appears to have pulled it away from the other sites in ordination space.

ANOSIM results for macroinvertebrate taxa density indicated some significant differences between sites, habitats, and seasons at both the patch and reach scales (Table 12). Winter pools and riffles within sites were significantly different from one another in both Slabcamp Creek (patch R = 0.61, reach R = 0.74) and Bucket Branch (patch R = 0.85, reach R = 0.89) at the patch and reach scales, but the comparisons between White Pine Branch's winter pools and riffles (patch R = 0.23, reach R = 0.36) returned low R values indicating high similarity between the groups. Slabcamp Creek's winter pools had high similarity to Bucket Branch's winter pools (patch R = 0.30, reach R = 0.29) and were significantly different from White Pine Branch's at the reach scale (patch R = 0.58, reach R = 0.93). This trend was also true for summer pools, Slabcamp Creek was very similar to Bucket Branch (patch R = 0.10, reach R = 0.09), but significantly different from White Pine Branch (patch R = 0.92, reach R = 0.98). Seasonally, within sites, Slabcamp Creek's winter pools were not significantly different from its summer pools (patch R = 0.46, reach R = 0.44) and neither were Bucket Branch's (patch R = 0.47, reach R = 0.38), but White Pine Branch's winter and summer pools were significantly different (patch R = 0.58, reach R = 0.77).

3.4.2 Macroinvertebrate assemblage structure based on functional feeding groups

The NMDS based on density of functional feeding groups (FFG) at the patch scale produced an ordination with a final stress value of 0.07 (Fig. 9), The patch-scale FFG NMDS showed trends that were similar to NMDS ordinations based on macroinvertebrate taxa densities; Slabcamp Creek and Bucket Branch winter and summer pools grouped together well and were separate from riffles in ordination space, while White Pine Branch's pools were separate from pools of the other sites and in closer proximity to its riffles. All five functional feeding groups explained variation on the plot and were correlated with NMDS axes: collector-gatherers (0.82, NMDS axis 1), collector-filterers (-0.58, NMDS axis), scrapers (-0.52, NMDS axis 2), predators (-0.62, NMDS axis 2), and shredders (-0.70, NMDS axis 2). Collector-gatherers, macroinvertebrates that feed on fine organic matter (Merritt and Cummins 2008), were especially important in driving the placement of Slabcamp Creek and Bucket Branch pools, and to a lesser degree some but not all of White Pine Branch's pools. The other four FFGs had more influence on the riffles of all sites and White Pine Branch's pools. Riffles across all sites were spread out in ordination space and intermingled with one another.

The NMDS of reach-scale functional feeding group density data produced an ordination with a final stress value of 0.06 (Fig. 10). The FFG ordinations showed Slabcamp Creek and Bucket Branch winter and summer pools' proximity to one another in ordination space remained relatively stable between the patch and reach scales. Slabcamp Creek and Bucket Branch winter and summer pool samples grouped together and were separate from riffles at both scales. This trend was improved by habitat weighting to the reach scale. White Pine Branch winter and summer pools noticeably shifted between the patch and reach scale ordinations. At the reach scale White Pine Branch's pools became even further separated from the pools of the other sites (more so in the winter than the summer) and remained in close proximity to the riffles from all sites. The collector-gatherer influence on and the placement of Slabcamp Creek and

Bucket Branch's pools was stronger in the reach-scale FFG ordination than the patch-scale FFG ordination (-0.85 NMDS axis 1) while the other four FFGs were associated with riffles across sites and White Pine Branch's pools: collector-filterers (0.56, NMDS axis 2), scrapers (-0.35 NMDS axis 1), predators (-0.53, NMDS axis 1), and shredders (0.74, NMDS axis 2).

ANOSIM results for within site comparisons (Table 13) based on FFG densities indicated that riffles and pools from Bucket Branch supported significantly different assemblages (patch R = 0.76, reach R = 0.83). Slabcamp Creek pools were not significantly different from its riffles (patch R = 0.45, reach R = 0.53), but they were not as similar as White Pine Branch's pools were to its riffles (patch R = 0.14, reach R = 0.37). Comparisons among sites indicated that Slabcamp Creek riffles are similar to both Bucket Branch riffles (patch R = 0.03, reach R = 0.25) and White Pine Branch riffles (patch R = 0.24, reach R = 0.29). The assemblage from the winter pools of Slabcamp Creek were very similar to the pools of Bucket Branch (patch R = 0.11, reach R = 0.10) and different from the pools of White Pine Branch (patch R = 0.52, reach R = 0.88). This pattern was also evident from summer pools. Slabcamp Creek summer pools were similar to Bucket Branch (patch R = 0.24, reach R = -0.13) and significantly different from White Pine Branch (patch R = 0.75, reach R = 0.96).

Visual interpretation of percent FFG composition bar charts (Fig. 11) and percent top dominant taxa (Table 11) revealed that collector-gatherers (Chironomidae, Oligochaeta, *Caenis*, *Ephemera*, *Habrophlebia*, and *Eurylophella*) were the numerically-dominant FFG from pools of Slabcamp Creek and Bucket Branch, and they comprised 82% and 84% respectively during winter. White Pine Branch winter pools were 43%

dominated by the collector-gatherers Chironomidae, Oligochaeta, and *Paraleptophlebia*. Collector-gatherer dominance was high and similar across all sites in summer pools: 82% at Slabcamp Creek, 75% at Bucket Branch, and 76% at White Pine Branch. Scrapers were not dominant in the pools of Slabcamp Creek or Bucket Branch pools, but the scraping macroinvertebrates *Cinygmula* and *Psephenus* dominated 14% and 6% of White Pine Branch's winter and summer pools, respectively. The riffles of Slabcamp Creek were numerically dominated by the shredding stoneflies *Allocapnia* and *Prostoia* (35%) and collector-gatherers (Oligochaeta and Chironomidae – 34%). Bucket Branch's riffles were numerically dominated by collector-gatherers (Chironomidae, *Paraleptophlebia*, *Eurylophella* – 52%) and the shredding stonefly *Leuctra* (15%). Riffles at White Pine Branch were numerically dominated by collector-gatherers (Chironomidae, Oligochaeta, *Paraleptophlebia* – 34%) and the scraping mayfly *Cinygmula* (19%), scrapers were not represented by dominant taxa in either Slabcamp Creek or Bucket Branch.

3.4.3 Macroinvertebrate assemblage structure based on common water quality metrics

Total taxa richness was very similar among sites, and Bucket Branch and White Pine Branch supported a few more EPT taxa than Slabcamp Creek (Table 14). Modified Hilsenhoff's Biotic Index results were also similar among sites. Percent top 5 dominant taxa was higher at Slabcamp Creek and Bucket Branch than White Pine (Table 14). Jaccard's similarity index showed that Slabcamp Creek and Bucket Branch were more similar to one another in terms of macroinvertebrate taxa composition than either was to White Pine Branch (Table 15).

CHAPTER IV

DISCUSSION

Overall, findings from my study indicated that restored Slabcamp Creek was more similar to the reference condition site (Bucket Branch) than the pre-restoration control condition site (White Pine Branch) in terms of macroinvertebrate density, standing stock biomass, and assemblage structure and function. It appears that habitat availability, both at the patch and reach scales, could play an important role in assessing stream restorations. If this study had not accounted for pool habitat and had instead focused on riffles alone, which is a common monitoring practice (Carter and Resh 2001, Beauger and Lair 2007), differences between sites would not have been detected because, in general, analyses returned similar results for riffles among sites. Analyses showed that seasonal influences had less influence on the macroinvertebrate assemblages than habitat.

4.1 Pool and riffle mesohabitats

In general, results indicated that within Slabcamp Creek and Bucket Branch pool and riffle mesohabitats supported macroinvertebrate assemblages distinct from one another, but this did not appear to be the case at White Pine Branch. Significant or nearly significant interaction terms in two reach-scale repeated measures two-way ANOVA models suggests that differences in macroinvertebrate densities and biomass between pool and riffle mesohabitats were site dependent. Notably, the patch-scale models did not return significant results for the site x habitat interaction. This supports the notion that targeting mesohabitats that are preferred by macroinvertebrates at the patch scale, but not accounting for the availability of those mesohabitats at the reach scale, may conceal differences in macroinvertebrate density and biomass between study sites. This may be of particular importance in the case of post-restoration monitoring because restoration generally occurs at the reach scale. This study detected greater differences in macroinvertebrate density and biomass among sites at the reach scale than at the patch scale. Macroinvertebrates are an important food source for many organisms. Biomass is a surrogate measure of secondary production, i.e. macroinvertebrate biomass provides energy to food webs both within the stream and the surrounding terrestrial environment (Benke et al. 1984, Huryn and Wallace 2000, Stagliano and Whiles 2002).

In addition to greater densities and biomass, the structure of the macroinvertebrate assemblage differed between mesohabitats of the reference and restored sites, but not the control site. Pools and riffles of White Pine Branch had similar macroinvertebrate assemblage structure, while the pools and riffles of Slabcamp Creek and Bucket Branch had distinct assemblage structure. Taxa characteristic of Slabcamp Creek and Bucket Branch pools were macroinvertebrates that are often associated with depositional (i.e. pool) habitats. Burrowing or sprawling taxa such as Chironomidae, Oligochaeta, *Caenis*, and *Ephemera* that are typically associated with pools had greater numerical dominance in Slabcamp Creek and Bucket Branch than in White Pine Branch pools. Additionally, *Psephenus'* notable presence in White Pine Branch's pools was unusual because it is a clinger associated with erosional (i.e. riffle) habitats (Poff et al. 2006).

The differences within and between study sites' mesohabitats were likely due to the underlying fluvial geomorphological processes at work. Channelization alters processes that create and maintain riffle-pool sequences in streams, and it often leads to increased sediment transport capacity and scouring discharge during high flow events which impacts a stream's ability to retain fine substrates (Montgomery and Buffington 1997). These disruptive processes could be occurring at the pre-restoration condition control, White Pine Branch.

Fine substrates, i.e. organic matter and inorganic sediments, are characteristic of pools (Allan and Castillo 2007), which are the preferred habitat of some macroinvertebrate taxa. The inability of a stream to retain fine sediment in pools may negatively impact macroinvertebrate taxa as well. There is evidence to suggest finer sediments have a greater "detritus storage capacity", that is they hold organic matter ("food" for some taxa) better than coarser substrates, which could explain why some macroinvertebrates show preference for finer substrates (Rabeni and Minshall 1977, Parker 1989). At Slabcamp Creek and Bucket Branch, aspects of the macroinvertebrate assemblages were positively correlated with fine substrates and depth (Table 9). At White Pine Branch aspects of the assemblage were positively correlated with coarser pebble substrates and negatively correlated with depth, this could indicate that areas that would normally serve as depositional (pool) habitat where macroinvertebrate density and biomass would be concentrated were impaired at White Pine Branch. While processing macroinvertebrate samples I observed that early instar juvenile specimens were abundant in Slabcamp Creek and Bucket Branch pools but scarce in White Pine Branch. It is possible that juvenile macroinvertebrates within pools influenced the density-biomass and density-depth correlations at the study sites. Fewer juveniles in the pools of White Pine Branch could indicate mortality due to channel drying or export during high flow events. In sum, the results of this study suggest that habitat at White Pine Branch is homogenous; from the macroinvertebrate perspective, pool and riffle mesohabitats at White Pine Branch appeared to be functionally similar.

It is well known that stream ecosystems are intricately connected with their surrounding terrestrial ecosystems, but functional aspects of this relationship are often difficult to measure (Lake et al. 2007). One simplified way in which benthic ecologists address trophic level dynamics is by categorizing taxa according to functional feeding groups (FFGs – shredders, predators, scrapers, collector-filterers, and collector-gatherers) describing morpho-behavioral mechanisms of food acquisition that reflect macroinvertebrates' adaptations to their environment (Townsend and Hildrew 1994, Cummins 2002, Merritt and Cummins 2008). Research conducted on functional feeding groups has indicated relationships exist between coarse particulate organic matter (CPOM) and shredders, fine particulate organic matter (FPOM) and collectors, and primary production and scrapers (Merritt and Cummins 2008). Headwater streams of the temperate deciduous region, such as the study sites, are typically allochthonous systems meaning they receive a large amount of energy from the surrounding terrestrial ecosystems in the form of leaf litter (Vannote et al. 1980, Webster and Wallace 1996, Richardson and Danehy 2007). These terrestrial energy inputs are important for food webs in forested headwater streams because primary production by photosynthesizing organisms, such as algae, is limited by canopy shading (Hill et al. 1995, Richardson and Danehy 2007). When CPOM enters streams from the surrounding terrestrial environment, shredders process it into finer material (FPOM) as they feed and this has implications on stream detrital processes as well as collector-gatherers and collector-filterers that feed on FPOM (Cummins 2002, Merritt and Cummins 2008).

During the restoration at Slabcamp Creek trees were removed so that engineers could reconnect the stream with its groundwater source (Parola and Biebighauser 2011), and thus it might be expected that shredding macroinvertebrates that feed on CPOM would be scarce at Slabcamp Creek relative to Bucket Branch and White Pine Branch. However, shredders represented a greater proportion of the abundance at Slabcamp Creek than either Bucket Branch or White Pine Branch. The shredding stoneflies, *Allocapnia* and *Prostoia*, were dominant (35%) in Slabcamp Creek riffles. At Bucket Branch the shredding stonefly, Leuctra, was 15% dominant in riffles. There were no dominant shredding taxa in White Pine Branch riffles. A scraping mayfly taxon, *Cinygmula,* was the numerically dominant taxon (19%) in White Pine Branch riffles while there were no dominant scraping taxa in either Slabcamp Creek or Bucket Branch riffles. Scrapers graze on periphyton, algae, and microbiota attached to substrates (Wallace and Webster 1996). Scraper dominance at the pre-restoration condition control, White Pine Branch, suggests that organic material inputs from the surrounding forest may not be retained well and the system has become more autochthonous, i.e. the food web may be more heavily fueled by energy from primary production than what might be considered typical of a forested headwater stream. Stone and Wallace (1998) found that a mountain stream disturbed by clear cutting experienced a shift from an allochthonous to an autochthonous based system and an increase in scraper secondary production. Shredder dominance in Slabcamp Creek riffles could be a product of improved organic material retention at Slabcamp due to the hydrologic restoration.

The composition of FFGs in pools at Slabcamp Creek were more similar to Bucket Branch than White Pine Branch. Collector-gatherers, macroinvertebrates that feed on FPOM, made up a greater proportion of the total abundance in Slabcamp Creek and Bucket Branch pools than White Pine Branch pools, particularly in the winter, this could be a result of the lack of shredders at White Pine Branch. Scraping taxa were not numerically dominant in Slabcamp Creek or Bucket Branch pools, but scrapers were numerically dominant in White Pine Branch pools during the winter (*Cinygmula*) and the summer (*Psephenus*). The dominant presence of scrapers in White Pine Branch riffles and pools again demonstrates that habitat is likely functionally homogenous at the control site. The higher dominance of collector-gatherers in Slabcamp Creek relative to White Pine Branch is further evidence of improved organic material retention at the restored site.

4.2 Season

Based on the results of statistical analyses of this study, seasonal influences had a lesser impact on macroinvertebrate assemblages from study sites than habitat does. During winter Slabcamp Creek and White Pine Branch had similar wetted channel areas, approximately 390 m^2 each, and Bucket Branch had approximately 160 m^2 more wetted area as a result of large pools and a wider channel. During summer, wetted area decreased as a result of drying at all sites. Slabcamp Creek and Bucket Branch lost 24% and 20% wetted area, respectively, while White Pine Branch lost 68%. Riffle wetted area decreased dramatically at all sites (78% loss at Slabcamp Creek and 100% loss at Bucket

Branch and White Pine Branch), while pool wetted area was retained at all sites. Due to drying, riffle samples could not be part of the statistical analyses. Although sampling and analysis methods did not allow me to express this quantitatively, Slabcamp Creek retained water in riffles during the summer and thus provided habitat for aquatic macroinvertebrates while Bucket Branch and White Pine Branch riffles did not. The habitat weighting method used in this study used proportions of available pool and riffle mesohabitats at each study site, rather than absolute area, which may have masked seasonal differences within and between sites. If habitat weighting had been done using absolute area, the extreme loss of wetted area at White Pine Branch during the summer relative to the other study sites may have had more noticeable impacts on macroinvertebrate density and biomass analyses results.

The differences seen between seasons in the NMDS ordinations could be attributable to natural seasonal variation in macroinvertebrate assemblage structure (Beche et al. 2006, Sporka et al. 2006) rather than a seasonally driven disturbance acting as a stressor on the pools of any of the sites. Beche et al. (2006) found although taxonomic composition and abundance varies significantly across seasons, trait composition (including functional feeding group composition) is relatively stable. This could explain why winter and summer pools grouped together more closely in the FFG ordination than they do in the total abundance ordination. Although the taxa themselves change seasonally, the proportions of feeding habits exhibited by taxa were relatively the same.

In summary, considering the extent of wetted area loss at the pre-restoration condition control site relative to the restored and reference sites, it seems likely that seasonal drying negatively influenced the macroinvertebrate assemblage at White Pine Branch. However, the results of this study indicate that homogenous habitat was the greater stressor. Drying was not nearly as extensive at Slabcamp Creek, likely as a result of the hydrologic restoration that reconnected the channel with its groundwater source (Parola and Biebighauser 2011).

4.3 Habitat weighting – implications for post restoration monitoring

In general, it appears that habitat weighting the data better enabled analyses to detect differences among study sites. Results indicated that at the patch scale mesohabitats are similar among study sites but at the reach scale mesohabitats are not equally available, which influences macroinvertebrate density and biomass. It appears that patch scale analyses of targeted habitat mask differences between study sites. Stream restorations typically occur at the reach scale, if improving habitat for biota is a restoration goal then monitoring should account for habitat availability at the scale of the restoration. This is particularly important in terms of trophic dynamics and ecosystem functioning.

4.4 Macroinvertebrate assemblage structure based on common water quality metrics

Commonly used water quality metrics such as total taxa richness, EPT taxa richness, modified Hilsenoff's Biotic Index (mHBI), and percent top 5 dominant taxa did not detect differences between study sites as clearly or conclusively as the more detailed analyses of this study. The results of these metrics could be considered inconclusive or even contradictory with the more detailed macroinvertebrate density, biomass, and assemblage structure/function analyses. For instance, White Pine Branch had the lowest scores for mHBI and percent 5 dominant taxa, which might seem to indicate the macroinvertebrate assemblage is "healthier" at the control site than the restored or reference condition sites. Jaccard's similarity index simply indicated that the macroinvertebrate assemblage at Slabcamp Creek is more similar to Bucket Branch than White Pine Branch. These metric results, and results of other analyses that did not detect strong differences in riffle mesohabitats among sites, suggest that current rapid sampling methods (many protocols target riffle habitat) and metrics commonly employed for general water quality assessment purposes may not be appropriate for assessing hydrologic restorations.

Rapid sampling methods are used for a variety of reasons. Less man power is required and sampling takes less time, so resource expenditure is reduced and more sites can be visited. Additionally, results are more easily summarized and interpreted by politicians and the general public. However, there is a tradeoff between these perceived advantages and the quality of the data that is obtained (Hannaford and Resh 1995). There are few comparable studies on macroinvertebrates, restoration, and the effectiveness of rapid sampling methods. One study on the Environmental Protection Agency's Rapid Bioassessment Protocol showed that rapid sampling method is also likely not appropriate for assessing restorations (Hannaford and Resh 1995). A variety of studies have shown rapid methods in general have limitations compared to quantitative sampling (Dolph et al. 2015, Verdonschot et al. 2015, Everall et al. 2017).

4.5 Conclusion

Due to the design of this study, the conclusions that may be drawn about hydrologic restoration are limited. In the case of Slabcamp Creek, the hydrologic restoration appears to have shifted macroinvertebrates (in terms of density, biomass, and assemblage structure) away from the pre-restoration control condition (White Pine Branch) towards Kentucky's headwater reference condition (Bucket Branch). This could be a result of the restoration improving hydrologic functioning and thus habitat complexity, substrate stability, and organic matter retention. Post-restoration monitoring should continue at these study sites to see if these results vary or persist over time. If future studies were to replicate at the stream level (multiple restoration, control, and reference condition treatments) it is possible that more overarching conclusions about the success of hydrologic restoration could be made.

The results of this study imply that habitat may be critical for evaluating restoration success. Completely random sampling within study reaches would likely eliminate the bias created by targeting habitats preferred by macroinvertebrates at the patch scale, but if improving habitat for biota is a restoration goal it may be desirable to target specific habitats. Future studies that target habitats should account for both habitat type and availability at the reach scales. Habitat weighting appears to better enable analyses to detect differences between study sites. When possible, comparisons to the regional reference condition could be beneficial. Regions similar to eastern Kentucky with streams dominated by riffle-pool morphology that have been subjected to channelization may benefit from a focus on pool habitats. It is possible macroinvertebrates in pools are more susceptible to the damage caused by channelization that restoration seeks to improve. Measuring habitat at any scale can be a laborious and time-consuming process, however the end likely justifies the means. I recommend future restoration studies invest in more intensive, quantitative habitat measures than those employed by my study, such as pebble counts, which would result in more accurate measures of substrate composition than visual estimates of percent composition. Measures of ecosystem function at restored, pre-restoration condition controls and reference condition sites could provide valuable information as well (Lake et al. 2007). I recommend future investigators consider incorporating measures of ecosystem processes, such as decomposition or organic matter and nutrients retention into their studies. Results from this study and future studies could help guide future post restoration monitoring efforts towards a more effective and standardized approach.

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APPENDICES

APPENDIX A:

Taxa List

Taxa List. Macroinvertebrate abundance from samples collected from pool and riffle mesohabitats during winter and summer 2014 at restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Values are the total number of individuals from five Hess samples. Superscripts [R] indicate rare taxa in the collection where total abundance <0.05%, number superscripts indicate taxa that required a length-mass substitution for biomass estimates, [*] indicates taxa that were omitted from functional feeding group analyses and metric calculations due to a lack of species traits information.

					Slabcamp Creek			Bucket Branch			White Pine Branch		
Phylum	Class	Order	Family	Genus (or Final ID)	Winter Pools	Winter Riffles	Summer Pools	Winter Pools	Winter Riffles	Summer Pools	Winter Pools	Winter Riffles	Summer Pools
Annelida	Oligochaeta			Oligochaeta	588	262	439	216	10	180	57	94	10
Arthropoda	Arachnida	Trombidiformes		Hydracarina [*,R]	0	0	0	0	2	0	0	0	0
	Insecta	Ephemeroptera	Ameletidae	*Ameletus* [R]	2	7	0	0	0	11	12	0	0
			Baetidae	Baetidae [1]	4	2	0	0	3	1	0	0	0
				Acentrella	0	1	0	0	0	0	1	5	0
				Acerpenna [1]	9	4	63	0	0	1	0	0	1
				Baetis	1	0	0	0	0	0	1	10	3
				Diphetor [1]	5	7	32	10	13	16	10	9	8
				Heterocleon [R]	0	1	0	0	0	0	0	3	0
			Caenidae	*Caenis*	179	5	110	0	0	0	0	0	0
			Ephemeridae	*Ephemera*	95	9	313	20	1	27	8	0	17
			Ephemerellidae	*Ephemerella*	0	1	0	0	14	0	0	1	0
				Eurylophella	10	3	0	57	22	47	20	5	24
				Serratella [R]	0	0	0	6	0	0	0	0	0
			Heptageniidae	*Cinygmula*	1	8	0	0	4	0	111	116	0
				Epeorus	1	13	0	0	2	0	6	21	0
				Leucrocuta [R]	0	0	0	6	0	0	0	0	0
				Maccaffertium [2]	18	17	7	7	5	32	10	2	6
				Stenacron	32	0	78	21	0	46	0	0	0
				Stenonema	19	1	19	4	2	0	1	0	4
			Leptophlebiidae	*Habrophlebia*	0	0	0	108	0	0	0	0	0
				Paraleptophlebia [3]	4	4	128	11	117	208	43	25	216
		Odonata	Aeshnidae	*Boyeria* [R]	0	0	1	0	0	1	0	0	0
			Coenagrionidae	*Argia*	5	1	11	0	0	0	0	0	0
			Cordulegaster	*Cordulegaster* [R]	1	0	1	0	0	1	2	0	0
			Gomphidae	*Lanthus*	0	2	0	10	3	0	4	0	0
				Stylogomphus	0	0	3	1	0	19	0	0	15
		Plecoptera	Capniidae	Capniidae [4]	20	0	8	5	19	12	0	21	0
				Allocapnia	6	469	14	0	5	7	4	15	0
				Paracapnia	0	0	0	4	5	0	5	0	0
			Chloroperlidae	Chloroperlidae [5]	1	16	0	0	2	40	4	37	14
				Haploperla [5]	0	0	0	21	17	0	67	6	0
				Sweltsa [5]	8	13	0	1	0	0	22	5	0
			Leuctridae	*Leuctra*	19	13	0	56	95	13	12	8	13
			Nemouridae	Nemouridae [6]	0	0	0	0	0	4	0	0	4
				Amphinemura	5	15	0	1	2	0	2	5	0
				Prostoia	3	48	0	0	0	0	1	2	0
			Perlidae	*Acroneuria* [R]	0	1	0	1	4	0	0	0	0
				Eccoptura [R]	0	0	0	0	1	0	1	0	1
				Perlesta [R]	0	0	0	1	0	0	0	0	0
			Perlodidae	*Isoperla* [R]	0	0	0	0	1	0	0	0	0
		Megaloptera	Corydalidae	*Nigronia* [R]	0	0	0	0	1	0	0	0	0
			Sialidae	*Sialis* [R]	2	0	2	0	0	0	0	0	0

Taxa list (continued)

Phylum	Class	Order	Family	Genus (or Final ID)	Slabcamp Creek Winter Pools	Slabcamp Creek Winter Riffles	Slabcamp Creek Summer Pools	Bucket Branch Winter Pools	Bucket Branch Winter Riffles	Bucket Branch Summer Pools	White Pine Branch Winter Pools	White Pine Branch Winter Riffles	White Pine Branch Summer Pools
		Trichoptera	Calamoceratidae	*Anisocentropus* [R]	0	0	0	0	0	0	2	0	1
			Glossosomatidae	*Glossosoma* [R]	0	0	0	0	1	0	0	1	0
			Hydropsychidae	*Cheumatopsyche* [R]	0	0	0	0	0	0	5	0	0
				Diplectrona [7]	0	0	0	0	0	0	4	6	0
				Hydropsyche [R]	1	0	0	0	0	0	1	0	0
			Hydroptilidae	*Hydroptila*	7	0	0	4	0	17	0	0	0
			Lepidostomatidae	*Lepidostoma* [R]	0	0	0	2	0	0	0	0	0
			Limnephilidae	*Pycnopsyche*	0	0	0	8	0	1	0	0	0
			Philopotamidae	*Chimarra* [R]	1	1	0	0	1	0	0	0	0
				Dolophilodes [R, 8]	0	0	0	1	0	0	0	1	0
				Wormaldia [R]	0	0	0	0	0	0	1	0	0
			Polycentropodidae	*Nyctiophylax* [R]	0	0	0	1	0	0	0	0	0
			Psychomyiidae	*Lype* [R]	0	0	0	0	0	0	1	0	0
			Rhyacophilidae	*Rhyacophila*	1	0	0	1	14	0	1	2	0
			Uenoidae	*Neophylax* [9]	16	39	0	11	27	0	10	8	0
		Coleoptera	Elmidae	*Dubiraphia*	12	0	18	0	0	0	0	0	0
				Optioservus	1	0	0	2	1	0	3	2	0
				Stenelmis	2	1	0	0	0	20	0	0	14
			Dryopidae	*Helichus*	0	16	0	0	0	0	2	0	0
			Psephenidae	*Ectopria* [R]	0	0	0	1	0	0	0	0	0
				Psephenus	1	2	4	9	2	26	1	0	44
		Diptera		Diptera (Unknown #1) [*,R]	0	0	0	1	0	0	0	0	0
				Diptera (Unknown #2) [*,R]	0	0	0	5	0	0	0	0	0
			Cecidomyiidae	Cecidomyiidae [*,R]	0	0	0	0	0	1	0	0	0
			Ceratopogonidae	Ceratopogonidae	152	55	55	50	12	47	29	22	7
				Atrichopogon [R]	0	0	0	0	0	0	1	0	1
			Chironomidae	*Dasyhelea*	8	1	0	6	2	0	1	0	0
			Dixidae	Chironomidae	1672	231	720	1564	205	646	250	83	207
			Empididae	*Dixa* [10]	0	0	0	0	0	27	0	0	0
				Hemerodromia	4	8	5	5	2	0	3	0	0
				Metachela	2	0	4	11	0	2	4	3	0
			Psychodidae	*Pericoma* [R]	0	0	2	0	0	0	0	0	0
			Sciaridae	Sciaridae [R]	1	0	0	0	2	0	0	0	0
			Simuliidae	*Prosimulium*	2	47	0	0	8	0	2	24	0
				Simulium	2	23	0	0	0	0	1	14	0
			Tabanidae	*Chrysops* [R]	0	0	0	0	0	0	0	0	4
			Tipulidae	Tipulidae (Unknown #1) [R,11]	0	0	0	0	1	0	0	0	0
				Antocha [R]	0	1	0	0	0	0	0	0	0
				Hexatoma	0	1	0	0	1	13	3	2	5
				Molophilus [R]	0	0	0	2	1	0	0	0	0
				Pseudolimnophila [R]	0	0	0	2	0	0	1	0	0
				Tipula [R]	0	0	0	2	0	0	1	0	0
	Malacostraca	Amphipoda	Crangonyctidae	*Crangonyx*	4	1	0	0	0	0	30	11	3
		Decapoda	Cambaridae	Cambaridae [*]	5	0	2	0	0	0	1	0	3
		Isopoda	Asellidae	*Lirceus*	0	0	1	0	0	0	18	12	2
	Maxillopoda			*Copepoda* [*]	16	0	650	6	0	348	0	0	157
	Ostracoda			Ostrocoda [*]	66	2	12	0	1	4	1	1	4
Mollusca	Bivalvia	Veneroida	Sphaeriidae	Sphaeriidae [*]	11	3	14	12	0	17	3	1	0
	Gastropoda			Gastropoda [*]	0	3	0	5	1	0	1	0	0
			Planorbidae	*Ferrissia* [*]	3	1	15	1	0	0	0	0	0
Nematomorpha				Nematomorpha [*]	24	22	2	20	0	0	1	0	0

Regression equation substitutions: [1] *Baetis*, [2] *Stenonema*, [3] *Paraleptophlebia*, [4] *Allocapnia*, [5] *Sweltsa*, [6] *Amphinemura*, [7] *Hydropsyche*, [8] *Chimarra*, [9] *Limnephilus*, [10] Chironomidae, [11] *Tipula*

APPENDIX B:

Tables

Table 1. Summary information for the study reaches: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky.

Site Name	Study Designation	Bioregion*	Basin	Drainage Area (mi^2)	%Forested**	Geology*	Dominant Land Use*
Slabcamp Creek	Restoration	Mountain	Licking	0.88	79.5	Western Allegheny Plateau ecoregion geology: "Horizontally-bedded, Pennsylvanian sedimentary rock containing sandstone, siltstone, shales, and coal. Some areas have eroded down to limestone and may have localized karst development.	"Silviculture, mining, oil and gas drilling, moderate agriculture, residential."
Bucket Branch	Reference Condition	Mountain	Licking	0.70	86.1	"	"
White Pine Branch	Pre-restoration Condition Control	Mountain	Licking	0.92	99.8	"	"

**Source*: Pond, G. J., Call, S.M., Brumley, J.F., and Compton, M.C. (2003). The Kentucky macroinvertebrate bioassessment index. Kentucky Department for Environmental Protection Division of Water, Water Quality Branch.
**Calculated using Landsat 8 imagery and ERDAS imagine remote sensing software

Table 2. Reach scale physical habitat measurements from winter and summer 2014 at restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Values are means (± 1 SE).

	Winter 2014			Summer 2014		
	SC	BB	WP	SC	BB	WP
Channel width (m)	2.26 ± 0.18	3.37 ± 0.20	2.49 ± 0.13	1.60 ± 0.12	2.73 ± 0.21	1.37 ± 0.19
Total wetted area (m^2)	388.85	549.83	392.97	296.02	438.59	164.52
Total pool area (m^2)	211.34	304.57	45.59	161.44	321.23	51.59
Total riffle area (m^2)	112.13	230.30	202.60	24.29	0.00	0.00
Pool depth (m)	0.19 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.14 ± 0.003	0.12 ± 0.01	0.09 ± 0.009
Riffle depth (m)	0.07 ± 0.003	0.05 ± 0.003	0.07 ± 0.005	0.02 ± 0.003	0.00	0.00
Pool volume (m^3)	40.15	48.73	6.38	22.60	38.55	4.64
Riffle volume (m^3)	7.85	11.52	14.18	0.49	0.00	0.00

Table 3. Mean (±1 SE) macroinvertebrate density at the patch and reach scales for the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Sample sizes for each of the nine groups are even (N = 5). TNI = total number of individuals.

	Slabcamp Creek		Bucket Branch		White Pine Branch	
	Patch Scale (TNI/m^2)	Reach Scale (TNI/$100m^2$)	Patch Scale (TNI/m^2)	Reach Scale (TNI/$100m^2$)	Patch Scale (TNI/m^2)	Reach Scale (TNI/$100m^2$)
Winter						
Pools	7,259 ± 2,199	392,013 ± 118,736	5,380 ± 1,002	295,886 ± 55,085	1,863 ± 136	22,361 ± 1,631
Riffles	3,397 ± 720	98,516 ± 20,879	1,531 ± 583	64,318 ± 24,489	1,407 ± 511	73,189 ± 26,586
Summer						
Pools	6,390 ± 558	351,436 ± 30,708	4,365 ± 698	318,648 ± 50,978	1,842 ± 72	57,113 ± 12,666

Table 4. Mean (±1SE) macroinvertebrate biomass* at the patch and reach scales for the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Sample sizes for each of the nine groups are even (N = 5). AFDM = ash free dry mass.

	Slabcamp Creek		Bucket Branch		White Pine Branch	
	Patch Scale (mgAFDM/m^2)	Reach Scale (mgAFDM/$100m^2$)	Patch Scale (mgAFDM/m^2)	Reach Scale (mgAFDM/$100m^2$)	Patch Scale (mgAFDM/m^2)	Reach Scale (mgAFDM/$100m^2$)
Winter						
Pools	274 ± 72	14,789 ± 3884	144 ± 37	7,941 ± 2054	161 ± 36	1,937 ± 428
Riffles	122 ± 23	3,528 ± 661	85 ± 40	3,568 ± 1697	107 ± 44	5,551 ± 2265
Summer						
Pools	145 ± 13	7,955 ± 689	167 ± 58	12,227 ± 4240	72 ± 26	2,224 ± 801

*Values are for adjusted biomass; rare large individuals (>1.5 mg AFDM) were removed.

Table 5. Two-way Repeated Measures Analyses of Variance results for patch scale macroinvertebrate density and biomass between mesohabitats (pools and riffles) and among sites (restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch) located in eastern Kentucky.

Variable	SS	df	MS	F	P	R-sq
Density						50.76%
Site	1.31	2	0.65	5.92	0.008*	
Habitat	1.21	1	1.21	10.96	0.003*	
Site x Habitat	0.22	2	0.11	0.97	0.392	
Biomass						29.40%
Site	0.45	2	0.23	1.56	0.231	
Habitat	0.98	1	0.98	6.80	0.015*	
Site x Habitat	0.01	2	0.01	0.04	0.960	

* indicates significance at p ≤0.05

Table 6. Two-way Repeated Measures Analyses of Variance results for habitat-weighted (reach scale) for macroinvertebrate density and biomass between mesohabitats (pools and riffles) and among study sites (restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch) located in eastern Kentucky.

Variable	SS	df	MS	F	P	R-sq
Density						66.01%
Site	2.83	2	1.41	12.77	0.000*	
Habitat	0.76	1	0.76	6.89	0.015*	
Site x Habitat	1.56	2	0.78	7.09	0.004*	
Biomass						41.14%
Site	0.97	2	0.49	3.18	0.059	
Habitat	0.62	1	0.62	4.06	0.055	
Site x Habitat	0.96	2	0.48	3.17	0.060	

* indicates significance at p ≤0.05

Table 7. Two-Way Repeated Measures Analyses of Variance results for patch scale macroinvertebrate density and biomass between seasons (winter and summer 2014) and among sites (restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch) located in eastern Kentucky.

Variable	SS	df	MS	F	P	R-sq
Density						58.11%
Site	1.52	2	0.76	16.28	0.000*	
Season	0.00	1	0.00	0.08	0.782	
Site x Season	0.03	2	0.02	0.32	0.728	
Biomass						35.08%
Site	0.65	2	0.32	2.81	0.080	
Season	0.45	1	0.45	3.89	0.060	
Site x Season	0.40	2	0.20	1.73	0.200	

* indicates significance at $p \leq 0.05$

Table 8. Two-way Repeated Measures Analyses of Variance results for reach scale (habitat weighted) macroinvertebrate density and biomass between seasons (winter and summer 2014) and among study sites (restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch) located in eastern Kentucky.

Variable	SS	df	MS	F	P	R-sq
Density						85.14%
Site	6.06	2	3.03	64.84	0.000*	
Season	0.19	1	0.19	4.06	0.055	
Site x Season	0.17	2	0.09	1.86	0.177	
Biomass						59.17%
Site	4.11	2	2.05	16.57	0.000*	
Season	0.04	1	0.04	0.31	0.584	
Site x Season	0.17	2	0.08	0.67	0.523	

* indicates significance at $p \leq 0.05$

Table 9. Pearson correlations for macroinvertebrate, organic matter, and habitat data at the patch and reach scales for the study sites: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. CPOM = coarse benthic organic matter, FPOM = fine benthic organic matter.

		Density		Biomass	
		Patch (TNI/m^2)	Reach ($TNI/100m^2$)	Patch ($mgAFDM/m^2$)	Reach ($mgAFDM/100m^2$)
Slabcamp Creek	Density	—	—	—	—
	Biomass	0.81**	0.89**	—	—
	CPOM	0.52*	0.66**	0.33	0.53*
	FPOM	0.43	0.67**	0.46	0.71**
	Depth	0.44	0.65**	0.5	0.73**
	%Bedrock	N/A	N/A	N/A	N/A
	%Cobble	-0.06	-0.12	-0.03	-0.1
	%Pebble	-0.25	-0.4	-0.15	-0.36
	%Fines	0.47	0.65**	0.31	0.56*
Bucket Branch	Density	—	—	—	—
	Biomass	0.78**	0.83**	—	—
	CPOM	0.55*	0.45	0.41	0.45
	FPOM	0.62*	0.74**	0.19	0.4
	Depth	0.57*	0.59*	0.29	0.37
	%Bedrock	N/A	N/A	N/A	N/A
	%Cobble	-0.24	-0.31	0.11	-0.02
	%Pebble	-0.33	-0.37	-0.17	-0.25
	%Fines	0.69**	0.77**	0.31	0.46
White Pine Branch	Density	—	—	—	—
	Biomass	0.67**	0.60*	—	—
	CPOM	0.53*	0.61*	0.51	0.5
	FPOM	0.59*	0.57*	0.74**	0.71**
	Depth	-0.12	-0.58*	0.1	-0.22
	%Bedrock	-0.14	-0.09	-0.27	-0.24
	%Cobble	-0.3	0.02	-0.31	-0.14
	%Pebble	0.33	0.15	0.60*	0.53*
	%Fines	0.21	-0.16	0.03	-0.21

*indicates significance at $p \leq 0.05$

**indicates significance at $p \leq 0.001$

Table 10. Macroinvertebrate taxa that were unique to one of the three study sites: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky.

	Slabcamp Creek	Bucket Branch	White Pine Branch
Winter Pools	N/A	Diptera (unknown #1) Diptera (unknown #2) *Ectopria* *Lepidostoma* *Leucrocuta* *Nyctiophylax* *Perlesta* *Serratella*	*Cheumatopsyche* *Helichus* *Lype* *Wormaldia*
Winter Riffles	*Antocha*	Hydracarina *Isoperla* *Nigronia*	N/A
Summer Pools	*Dubiraphia* *Pericoma*	Cecidomyiidae	*Chrysops*

Table 11. Top five dominant macroinvertebrate taxa in pool and riffle mesohabitats for the study sites in winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Numbers are percentages calculated from total abundance. Sample sizes for each of the nine groups are even (N = 5).

	Slabcamp Creek		Bucket Branch		White Pine Branch	
Winter 2014						
Pools	Chironomidae	54	Chironomidae	68	Chironomidae	31
	Oligochaeta	19	Oligochaeta	9	*Cinygmula*	14
	Caenis	6	*Habrophlebia*	5	*Haploperla*	8
	Ceratopogonidae	5	*Eurylophella*	2	Oligochaeta	7
	Ephemera	3	*Leuctra*	2	*Paraleptophlebia*	5
Riffles	*Allocapnia*	32	Chironomidae	31	*Cinygmula*	19
	Oligochaeta	18	*Paraleptophlebia*	18	Oligochaeta	16
	Chironomidae	16	*Leuctra*	15	Chironomidae	14
	Ceratopogonidae	4	*Neophylax*	4	*Paraleptophlebia*	4
	Shipsa/Prostoia	3	*Eurylophella*	3	*Prosimulium*	4
Summer 2014						
Pools	Chironomidae	26	Chironomidae	35	*Paraleptophlebia*	27
	Copepoda	24	Copepoda	19	Chironomidae	26
	Oligochaeta	16	*Paraleptophlebia*	11	Copepoda	20
	Ephemera	11	Oligochaeta	10	*Psephenus*	6
	Paraleptophlebia	5	Ceratopogonidae	3	*Eurylophella*	3

Table 12. R values from Analysis of Similarity (ANOSIM) tests (conducted at the patch and reach scales) based on density of macroinvertebrates from pool and riffle mesohabitats of the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. SC = Slabcamp Creek, BB = Bucket Branch, WP = White Pine Branch, W = winter, S = summer, P = pools, R = riffles.

	SC_W_P		SC_S_P		BB_W_P		BB_S_P		WP_W_P		WP_S_P		SC_W_R		BB_W_R		WP_W_R	
	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach
SC_W_P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SC_S_P	0.46	0.44	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BB_W_P	0.30	0.29	0.93*	0.93*	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BB_S_P	0.34	0.28	0.10	0.09	0.47	0.38	—	—	—	—	—	—	—	—	—	—	—	—
WP_W_P	0.58	0.93*	1.00*	1.00*	0.98*	1.00*	0.68*	1.00*	—	—	—	—	—	—	—	—	—	—
WP_S_P	0.73*	0.86*	0.92*	0.98*	1.00*	1.00*	0.41*	0.84*	0.58*	0.77*	—	—	—	—	—	—	—	—
SC_W_R	0.61*	0.74*	0.92*	0.99*	0.96*	1.00*	0.79*	0.98*	0.75*	0.96*	0.96*	0.95*	—	—	—	—	—	—
BB_W_R	0.64	0.68	0.88*	0.94*	0.85*	0.89*	0.62*	0.80*	0.34	0.43*	0.48*	0.46*	0.73*	0.67*	—	—	—	—
WP_W_R	0.66	0.66	0.84*	0.87*	0.85*	0.86*	0.69*	0.81*	0.23	0.36	0.62*	0.60*	0.51*	0.47*	0.32	0.28	—	—

*indicates a significant R value at $p \leq 0.01$

Table 13. R values from Analysis of Similarity (ANOSIM) tests (conducted at the patch and reach scales) based on the density of macroinvertebrate functional feeding groups from pool and riffle mesohabitats of the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. SC = Slabcamp Creek, BB = Bucket Branch, WP = White Pine Branch, W = winter, S = summer, P = pools, R = riffles.

	SC_W_P		SC_S_P		BB_W_P		BB_S_P		WP_W_P		WP_S_P		SC_W_R		BB_W_R		WP_W_R	
	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach	Patch	Reach
SC_W_P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SC_S_P	0.16	0.14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BB_W_P	0.11	0.10	-0.14	-0.14	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BB_S_P	0.28	0.23	0.24	-0.13	0.32	-0.06	—	—	—	—	—	—	—	—	—	—	—	—
WP_W_P	0.52	0.88*	0.99*	1.00*	1.00*	1.00*	0.87*	1.00*	—	—	—	—	—	—	—	—	—	—
WP_S_P	0.46	0.58	0.75*	0.96*	0.77*	0.96*	0.47	0.96*	0.04	0.43	—	—	—	—	—	—	—	—
SC_W_R	0.45	0.53	0.82*	0.99*	0.85*	0.98*	0.55*	0.96*	0.32	0.89*	0.34	0.32	—	—	—	—	—	—
BB_W_R	0.43	0.54	0.75*	0.83*	0.76*	0.83*	0.60	0.82*	0.29	0.24	0.12	0.03	0.25	0.10	—	—	—	—
WP_W_R	0.44	0.46	0.65*	0.66*	0.64*	0.66*	0.57*	0.65*	0.14	0.37	0.25	0.24	0.29	0.18	0.12	0.08	—	—

*indicates a significant R value at p≤0.01

Table 14. Macroinvertebrate metrics commonly used in water quality assessment for the three study sites: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky.

	Slabcamp Creek	Bucket Branch	White Pine Branch
Total Taxa Richness	55	56	55
EPT Taxa Richness	27	34	32
Hilsenhoff's Biotic Index	5.79	5.48	4.41
% Top 5 Dominant Taxa	66.66	70.70	53.05

Table 15. Jaccard's similarity index for the three study sites: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky.

	Slabcamp Creek	Bucket Branch	White Pine Branch
Slabcamp Creek	—	—	—
Bucket Branch	0.55	—	—
White Pine Branch	0.34	0.34	—

APPENDIX C:

Figures

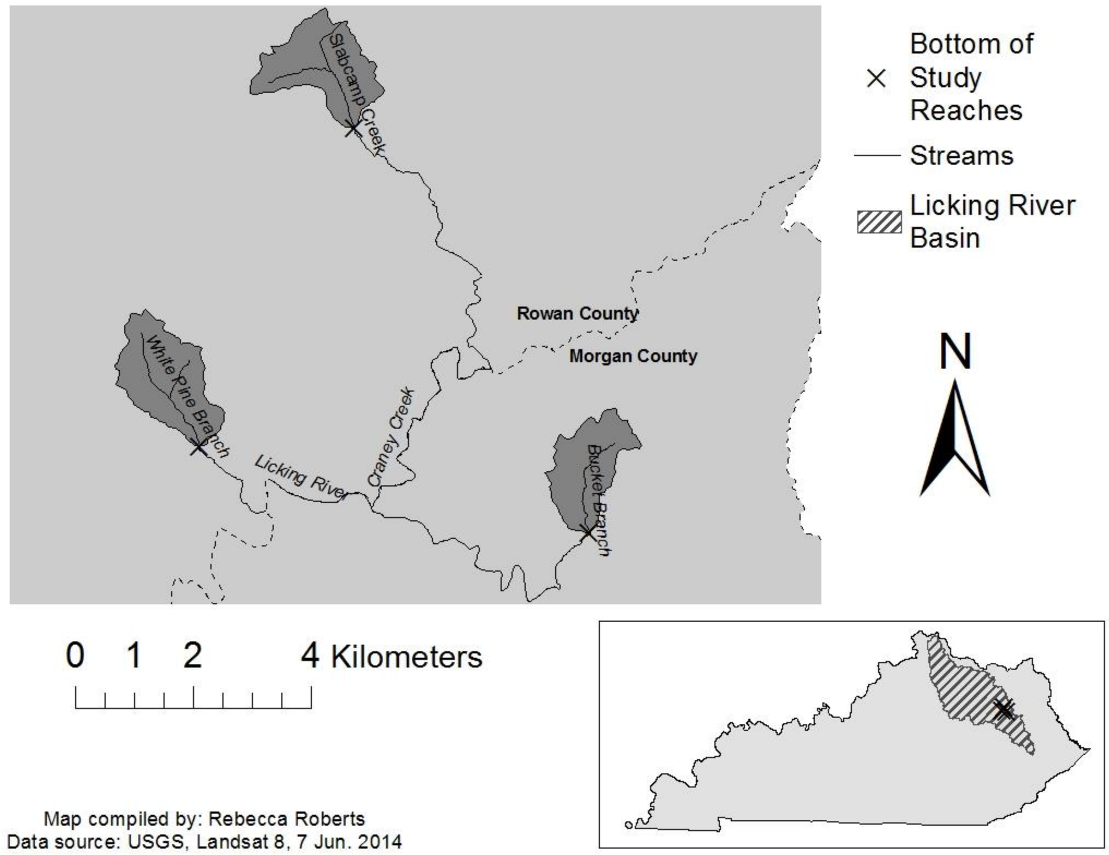

Figure 1. Approximate location of study reaches within their watersheds: restored Slabcamp Creek (38.12282, -83.3527), reference condition Bucket Branch (38.05474, -83.3162), and pre-restoration condition control White Pine Branch (38.07482, -83.3845) located in eastern Kentucky.

Figure 2. Study site photos. From left to right images are: restored Slabcamp Creek in summer 2014 (38.12282, -83.3527), reference condition Bucket Branch in spring 2015 (38.05474, -83.3162), and pre-restoration condition control White Pine Branch in summer 2014 (38.07482, -83.3845) located in eastern Kentucky.

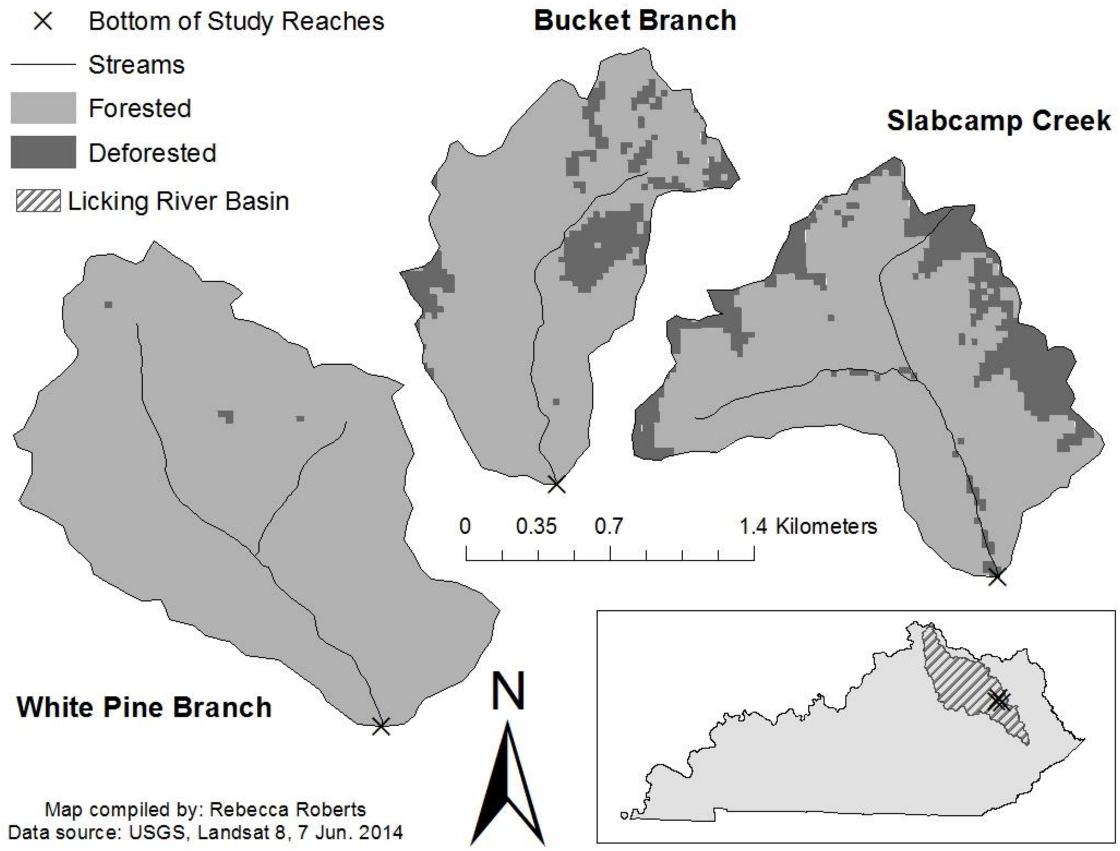

Figure 3. Land use within study reaches' watersheds: restored Slabcamp Creek (38.12282, -83.3527), reference condition Bucket Branch (38.05474, -83.3162), and pre-restoration condition control White Pine Branch (38.07482, -83.3845) located in eastern Kentucky. Compiled using Landsat 8 imagery and ERDAS imagine remote sensing software.

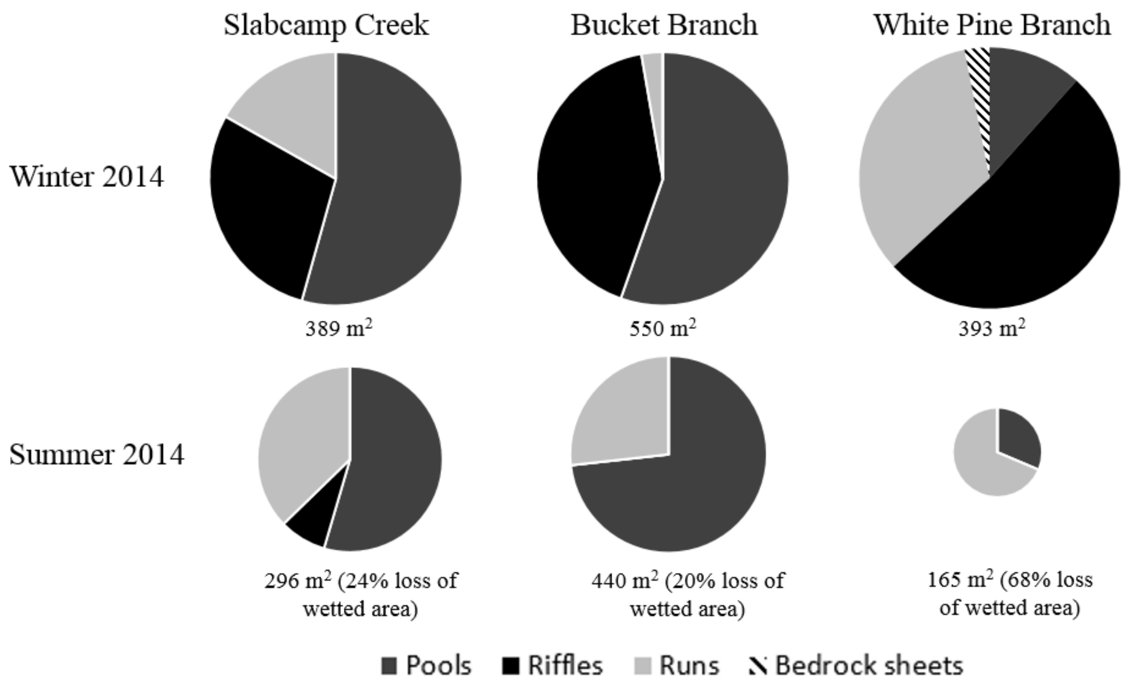

Figure 4. Habitat composition at the reach scale (150m) during 2014 sampling events for restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Pie chart size decrease within sites from winter to summer is proportional to the amount of wetted area lost as a result of drying.

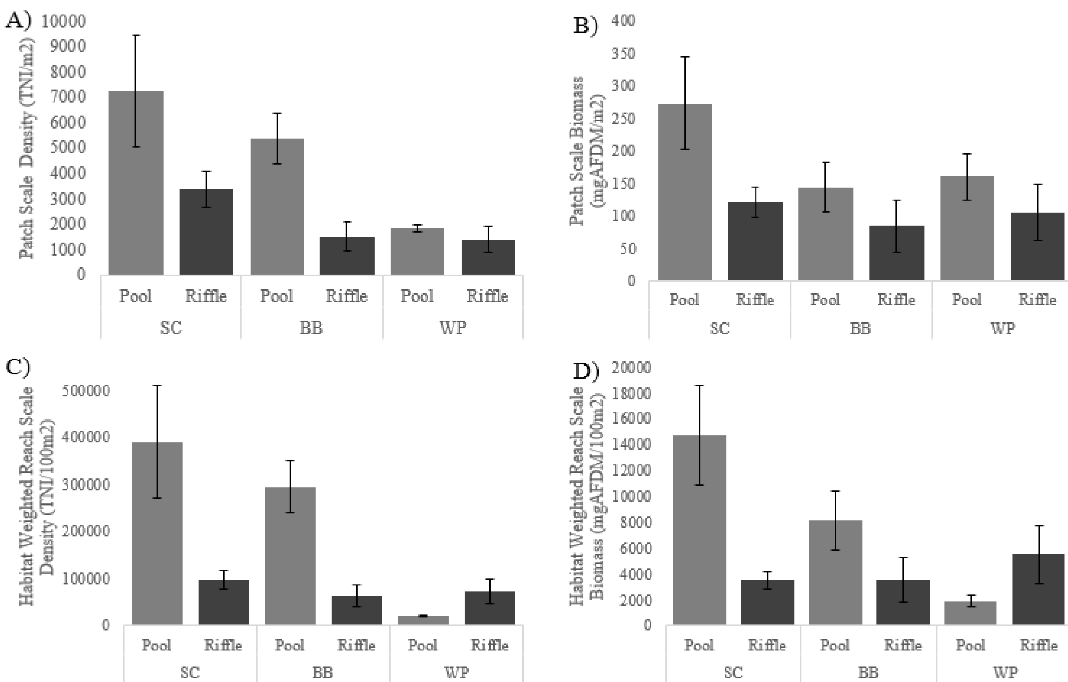

Figure 5. Mean (±1SE) macroinvertebrate density (TNI/100m^2) and biomass (mg AFDM/100m^2) in riffle and pool mesohabitats at the patch and reach scales for the study sites during winter 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky.

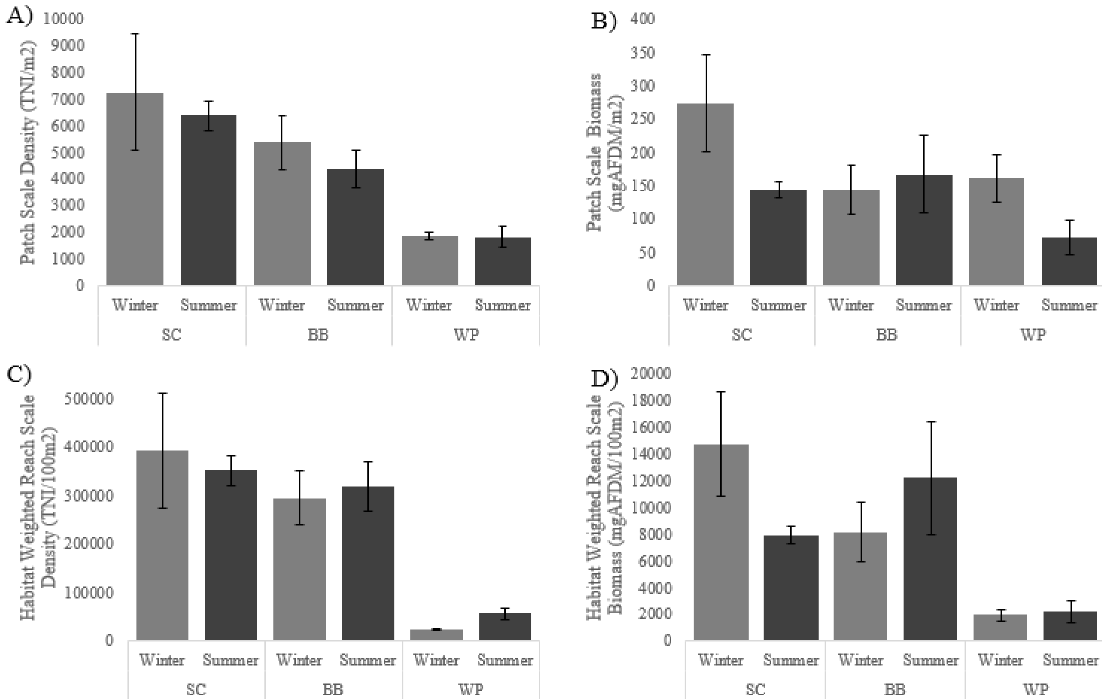

Figure 6. Mean (±1SE) macroinvertebrate density (TNI/100m2) and biomass (mg AFDM/100m2) in pool mesohabitat at the patch and reach scales for the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition White Pine Branch located in eastern Kentucky.

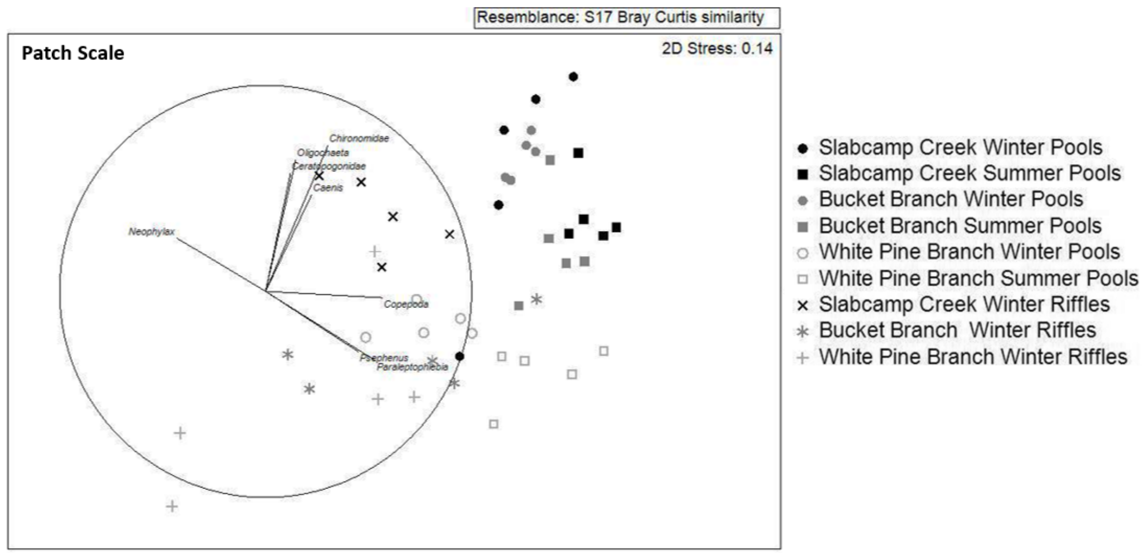

Figure 7. Nonmetric multidimensional scaling of macroinvertebrate taxa density data from riffle and pool mesohabitats at the patch scale for the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Macroinvertebrate taxa explaining variation are shown as vectors on the plot.

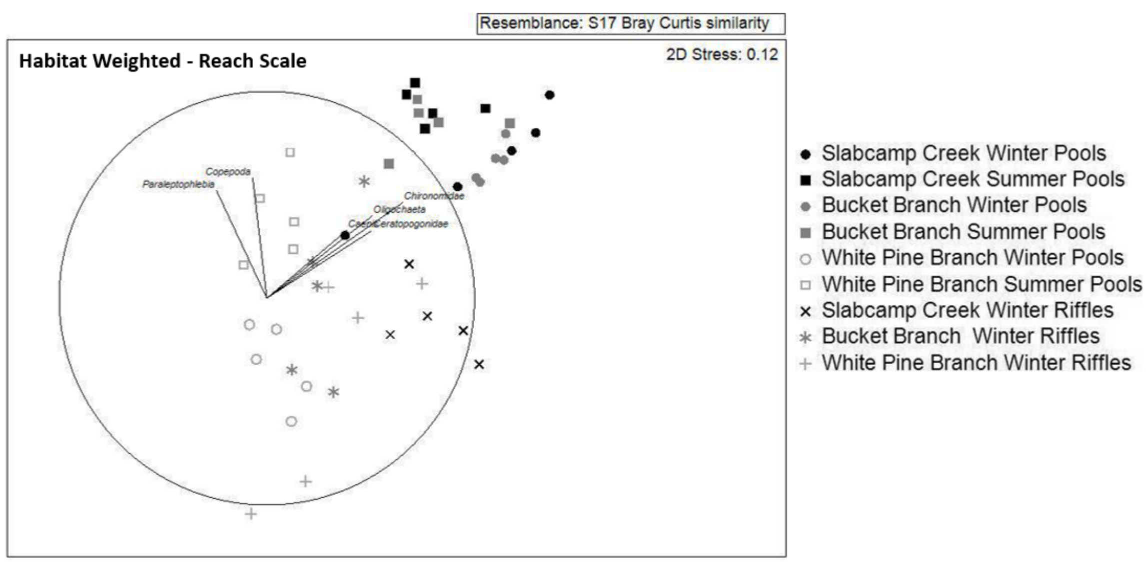

Figure 8. Nonmetric multidimensional scaling of macroinvertebrate taxa density data from pool and riffle mesohabitats at the reach scale for the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Macroinvertebrate taxa explaining variation are shown as vectors on the plot.

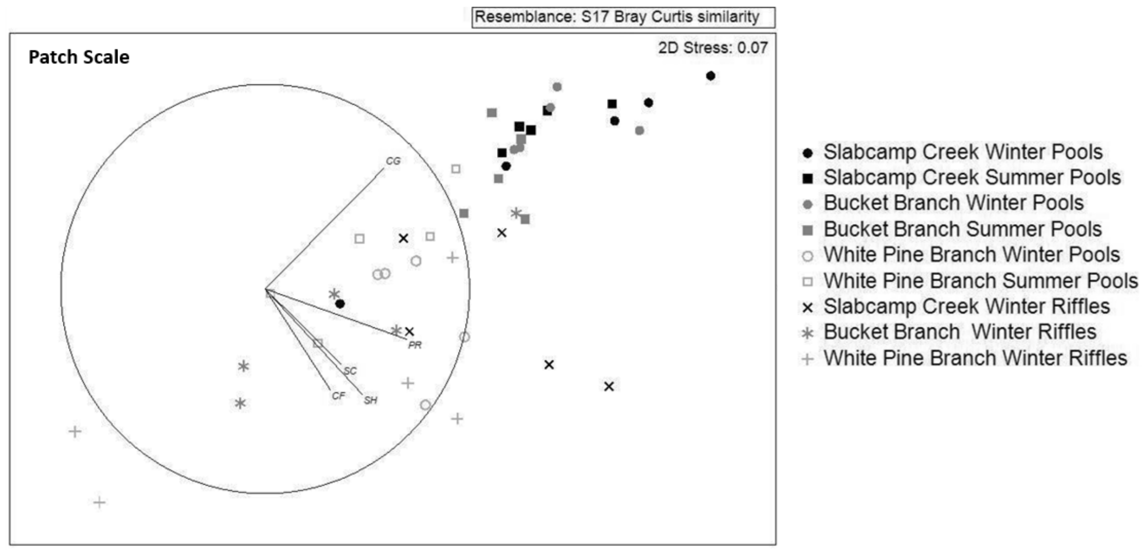

Figure 9. Nonmetric multidimensional scaling of macroinvertebrate functional feeding group density data from pool and riffle mesohabitats at the patch scale for the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Functional feeding groups explaining variation are shown as vectors on the plot. CG = collector-gatherers, CF = collector-filterers, SC = scrapers, PR = predators, SH = shredders.

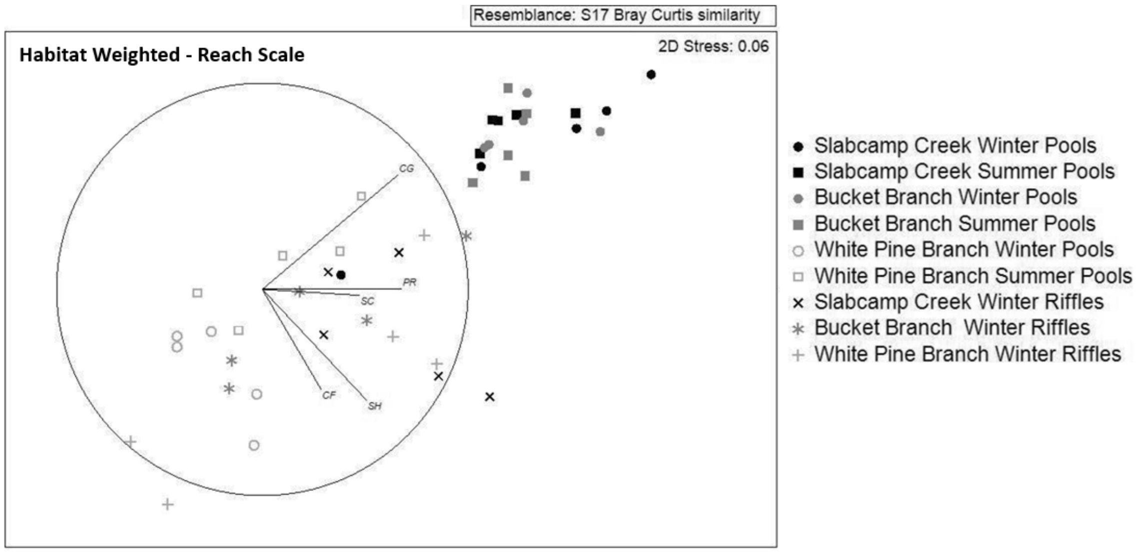

Figure 10. Nonmetric multidimensional scaling of macroinvertebrate functional feeding group density data from pool and riffle mesohabitats at the reach scale for the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky. Functional feeding groups explaining variation are shown as vectors on the plot. CG = collector-gatherers, CF = collector-filterers, SC = scrapers, PR = predators, SH = shredders.

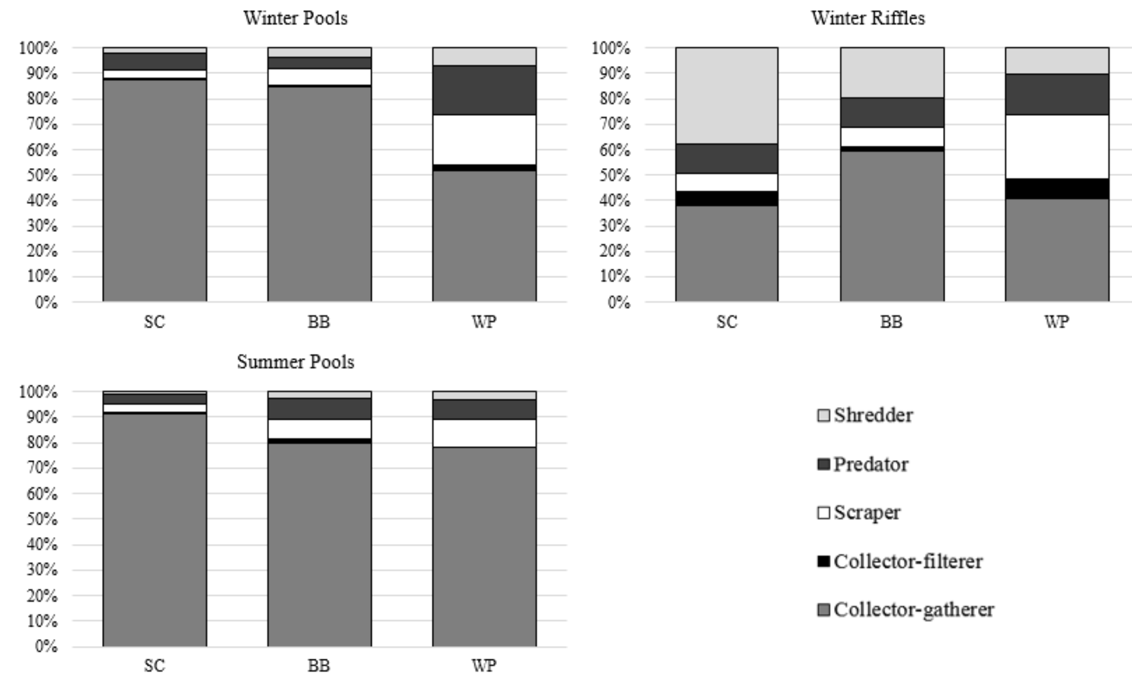

Figure 11. Macroinvertebrate assemblage composition based on the abundance of functional feeding groups from pool and riffle mesohabitats of the study sites during winter and summer 2014: restored Slabcamp Creek, reference condition Bucket Branch, and pre-restoration condition control White Pine Branch located in eastern Kentucky.