# BIOASSESSMENT OF FISH AND AQUATIC MACROINVERTEBRATE COMMUNITIES OF LAUREL CREEK IN ROWAN COUNTY, KENTUCKY

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Accepted by the faculty of the College of Science and Technology, Morehead State University, in partial fulfillment of the requirements for the Master of Science degree.

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# BIOASSESSMENT OF FISH AND AQUATIC MACROINVERTEBRATE COMMUNITIES OF LAUREL CREEK IN ROWAN AND ELLIOT COUNTIES, KENTUCKY

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## <u>Abstract</u>

Stream restoration is increasing as a method to repair streams damaged by anthropogenic activities; however, subsequent biological monitoring is still limited. The goal of the Clean Water Act (1972) is the protection and maintenance of the chemical, physical and biological integrity of the Nation's waters which supports the idea of stream restoration. Biological monitoring is critical in assessing the effect of restoration on aquatic biodiversity and to provide baseline data for future comparison. In 2004, the Rowan County Road Department, Kentucky, constructed a road through the valley of Laurel Creek, severely impacting 716 meters of high quality, headwater stream and 259 meters of small tributaries. Stream restoration occurred in Fall 2007 and Fall 2008, with the majority of the restoration activity occurring in Fall 2008. A bioassessment of Laurel Creek using fish, aquatic macroinvertebrates, habitat assessments and water quality was conducted to determine the biological integrity of the stream and to provide baseline data for future monitoring of the watershed. The objective of this study was to compare the fish and aquatic macroinvertebrate communities in Laurel Creek before (June 2008) and after restoration (Spring and Summer 2009). Sampling was conducted above, within, and below the restored area using Kentucky Division of Water standard bioassessment protocols.

The Kentucky Index of Biotic Integrity score decreased from Summer 2008 to Summer 2009 at sites within and below the restored area. Other metrics applied in the KIBI, such as relative abundance of tolerant and insectivorous fishes, also revealed a disturbed fish community. In addition, fish abundance and biomass slightly decreased after restoration at sites within and below the restoration. In contrast, the Macroinvertebrate Biotic Index for all sites in Laurel Creek increased from Summer 2008 to Summer 2009. Relative abundance and diversity of aquatic macroinvertebrates increased at all sampling sites between 2008 and 2009, including the intolerant orders of Ephemeroptera, Plecoptera, and Trichoptera. The functional feeding guild composition more closely resembles a balanced feeding structure.

Decline in the fish community may be a result of the intermittency observed in headwater streams. The improvement seen in aquatic macroinvertebrate community health may be attributed to the length of time between stream restoration activities and bioassessment sampling. Stream restoration appeared to have a slight negative impact on the fish community abundance and biomass, and no effect on the macroinvertebrate communities. This study only addressed the short-term (1-year) effects of restoration; additional monitoring is needed to examine long-term effects of restoration on fish and macroinvertebrate communities. Accepted by:

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#### **Introduction**

## Importance of Stream Restoration

The single most prominent threat to streams and biological communities within them is anthropogenic-related habitat degradation (Giller and Malmqvist 1999, Walters et al. 2009). During the past few decades, stream restoration has become an important focus in restoration ecology, ecological management, and as a scientific discipline (Kondolf and Micheli 1995, Muotka and Laasonen 2002). The goal of natural resource management is to restore ecological integrity to streams; that is, to return streams to a physically, ecologically, and functionally self-sustaining state of resilience and health, able to support a diverse community of organisms (Karr 1987). Aquatic systems possessing ecological integrity are able to better withstand perturbations from natural disturbances and may be able to survive anthropogenic disturbance (Karr et al. 1986). At a minimum, stream restoration is a method employed to retard the loss of biodiversity and re-establish biotic and abiotic heterogeneity of a stream (Giller and Malmqvist 1999, Muotka and Laasonen 2002). A more desirable goal for stream restoration is the rehabilitation or improvement of a degraded aquatic habitat to something resembling a naturally functioning system (Helfman 2007, Spänhoff and Arle 2007). Biological monitoring is a method commonly used to detect, record, and evaluate changes in a biological system from both natural and human induced causes to ensure that incremental improvements in rehabilitation/restoration are met (Helfman 2007).

#### **Biological Monitoring History**

Anthropogenic activities, both past and present, have impacted the quality of water and the biological community within it (Karr 1981). The Clean Water Act of 1972 (specifically amendments 33 U.S.C. §§ 1251-1376) was enacted to protect and maintain water quality and to monitor the waters of the United States. Early traditional measures focused on chemical monitoring (Karr 1981, Cairns and Pratt 1993). Although useful, chemical parameters focus on point-source or discharge pollution, essentially showing only a "snapshot" or a short term effect of anthropogenic disturbance to streams (Carter et al. 2006). Chemical parameters fail to account for the physical and biological damage to the waterways disturbing the aquatic fauna (Karr 1981). Chemical parameters also fail to account for natural disturbance, such as drought or geographic variation of chemicals (Karr 1981).

In contrast, a combined spatial and temporal view of ecosystem health can be obtained by biological monitoring which offers a moving picture of past and present land use (Carter et al. 2006). Biological monitoring (biomonitoring or bioassessment) is a systematic approach used to evaluate changes in the environment using biological organisms with the intent to document the health of the community (Rosenberg and Resh 1996). Aquatic macroinvertebrates, those organisms retained by mesh sizes  $\geq$ 200 to 500 µm (Rosenberg and Resh 1996), and fishes offer a long term perspective of water quality and ecological integrity which reflect watershed conditions and use (Karr 1981, Karr et al. 1986, Barbour et al. 1999, KDOW 2008). Biological communities integrate the effects of different disturbances of the watershed, thus are

continuous monitors of fluctuating abiotic and biotic factors; whereas chemical measurements may not detect a disturbance that occurred between sampling periods (KDOW 2008).

#### Importance of Fishes in Biological Monitoring

Fishes are valuable tools with many advantages for biological monitoring. Fishes are used in biological monitoring because they are good indicators of longterm effects and broad habitat conditions, and generally include species that represent a variety of tropic levels (Karr et al. 1986, Barbour et al. 1999, KDOW 2008). Fish are relatively easy to identify and specimens may be released unharmed after identification. Natural history and sensitivity to disturbances are well documented for fishes (Karr et al. 1986, Etnier and Starnes 2001). Furthermore, the absence of certain species can be indicative of what is occurring in a stream's watershed. For example, an intolerant, simple lithophilic species, such as *Clinostomus funduloides* (rosyside dace), would be predicted to have reduced numbers in a stream with high sedimentation or silt. Silt fills interstitial spaces between gravel and cobble, destroying habitats that many aquatic organisms require, and disrupting predation refuges and feeding guilds, potentially causing the loss of sensitive species. (Giller and Malmqvist 1999). Scott (2006) examined 36 streams undergoing deforestation in the southeastern Appalachian Mountains, and concluded that loss of biotic integrity and increased homogenization in fishes followed habitat modification. Many studies have used fish community structure to assess the biological integrity of streams because they are continuously affected by anthropogenic perturbations and are

sensitive to early stages of restoration. Fish responsiveness to stream restoration can be measured with biotic indices and is commonly conducted (Kinsolving and Bain 1993, Paller et al. 2000, Miltner et al. 2004, Price and Birge 2005, Morgan and Cushman 2005, Zhu and Chang 2008, Walters et al. 2009).

Biological monitoring of fishes is also of value to the general public (Karr 1981). If the general public can make a connection to the overall fish community health, then they may be more likely to recognize the importance of protecting and maintaining water quality for both fish and themselves.

### Importance of Aquatic Macroinvertebrates in Biological Monitoring

Aquatic macroinvertebrates are also useful tools for biological monitoring of streams and other aquatic systems. Since the early 1900s, macroinvertebrates have been used to assess changes in habitat and water quality (Carpenter 1924, Cairns and Pratt 1993). Due to the complex life cycle of macroinvertebrates, long term studies can be employed to monitor the improvement of stream restoration (Barbour et al. 1999, KDOW 2008). Aquatic macroinvertebrates are diagnostic in measuring the health and quality of a stream or river because they are ubiquitous and affected by all types of anthropogenic perturbations in aquatic systems (Barbour et al. 1999, Carter et al. 2006, KDOW 2008). The large number of aquatic macroinvertebrate species provides a variety of responses to perturbations. The sedentary lifestyle of many macroinvertebrate species allows for a spatial analysis of disturbance effects (Barbour et al. 1999, Carter et al. 2006).

Aquatic macroinvertebrates represent a fundamental part of an aquatic lotic system by providing energy and nutrients to higher trophic levels (Vannote et al. 1980, Wallace and Webster 1996). Thus, an understanding of anthropogenic influences on their spatial distribution and abundance is critical for a comprehensive bioassessment before and after stream restoration. Likewise, they integrate the effects of short-term environmental stressors in the aquatic environment and may be used to assess site specific impacts (i.e., stressed versus unstressed areas). Benthic aquatic macroinvertebrate communities constitute a range of trophic levels, and community responses to many types of pollution have been established (Cairns and Pratt 1993, Barbour et al. 1999, Carter et al. 2006). A decrease or absence of species intolerant to habitat degradation, such as ephemeropterans (mayflies), plecopterans (stoneflies), and trichopterans (caddisflies), may reflect disturbances in an aquatic system. Many studies have focused on the use of aquatic macroinvertebrate communities for biological monitoring to assess ecosystem disturbance and environmental conditions (Wallace 1990, Richardson and Kiffney 2000, Muotka and Laasonen 2002, Pond 2000, Korsu 2004, Bae et al. 2005, Churchel and Batzer 2006, Walther and Whiles 2008).

Another aspect of biomonitoring, which is recommended in Barbour et al. (1999), is the use of trophic measures to evaluate the balance of feeding strategies in benthic assemblages. The functional feeding guild (FFG) proportions have been used to detect the severity of disturbance in streams by evaluating the balance of feeding strategies. Feeding guilds are defined by how organic matter is acquired. One group

of the FFG are shredders, and they feed on coarse particulate organic matter (CPOM; >1mm; Cummins and Klug 1979). Decomposition or mechanical breakdown of CPOM releases fine particulate organic matter (FPOM,  $50\mu$ m-1mm) and ultra fine particulate organic matter (UPOM, 0.5-  $50 \mu$ m). Collectors comprise another important group within the FFG, and they feed primarily on FPOM and UPOM by gathering or filtering the organic matter. They can be further split into two groups: collector-filterers and collector-gatherers (Vannote et al. 1980). A third group within a stream's FFG are scrapers (grazers), and they graze or scrape organic matter (algae) from the substrate and primarily feed on periphyton. A final component of the FFG consists of predators that actively seek out and capture prey (Cummins and Klug 1979).

The characteristics of a particular stream reach directly influence the specific proportion of each member of the macroinvertebrate FFG that comprise the community. The River Continuum Concept (RCC), which was developed by Vannote et al. (1980), is a paradigm that explains the FFG composition in relation to stream order and both allochthonous and autochthonous input. The composition of the community shifts according to the lotic gradient from the headwater to the mouth. According to Vannote et al. (1980) headwater streams (orders 1-3) are strongly influenced by allochthonous organic matter from surrounding riparian vegetation, thus autochthonous production is decreased by riparian shading. Shredders and collectors are proposed to be the dominant macroinvertebrates in headwater streams because of the allochthonous input. Middle reaches of streams (orders 4-6) are

dominated by collectors which acquire nutrients from FPOM input transported from upstream. Because middle reaches of streams are not limited by light, scrapers also dominate due to increased autochthonous input from periphyton. Higher order streams (>6) receive little shading and rely on large amounts of FPOM and UPOM transported from upstream, creating a community where collectors dominate.

Changes from the expected macroinvertebrate functional feeding guild proportions within a given reach may suggest that disturbance has occurred, creating a shift in the macroinvertebrate community. Anthropogenic activity such as clearing of riparian vegetation, increased channelization, and sediment inputs can lead to increased stress on a lotic system. Natural fluctuations, such as intermittent stream flow, can also induce stressed functional feeding guilds (Pond et al. 2003).

## Importance of Habitat Assessments

Habitat assessments are conducted, in addition to chemical and biological monitoring efforts, in order to measure, record, and evaluate habitat parameters (KDOW 2008). Stream ecosystems are strongly influenced by the condition of the riparian zone which can impact stream substrate, water temperature, water chemistry, hydrology, and energy flow of a lotic system (Harding et al. 1998). Along with chemical and biological monitoring, habitat assessments provide an integrated picture of factors impacting an aquatic ecosystem, and they help to show a comprehensive view of an aquatic ecosystem.

Biological communities within an aquatic ecosystem are affected by both the quality and quantity of available physical habitat (Barbour et al. 1999). All physical

habitat parameters, including the catchment area, can potentially influence the health of the aquatic ecosystem (Harding et al. 1998, Barbour et al. 1999). Riparian zones function as a buffer by intercepting sediments from upland sources, reducing stream bank erosion, processing nutrients, and controlling the range and elevation of temperature (Helfman 2007). Research has focused on relating watershed use to instream physical and biological components. For example, Roy et al. (2003) and Cuffney et al. (2005) concluded that a decrease in forest cover reduced aquatic macroinvertebrate richness, and increased the abundance of tolerant organisms. Also, Miltner et al. (2004) found that urban streams with good Index of Biotic Integrity (IBI) scores were maintained because those sites either had intact riparian zones and undeveloped floodplains, or were supported by large amounts of groundwater. Importance of Water Chemistry

In addition to biological monitoring and habitat assessments, water chemistry measurements can detect patterns of chemical variation influencing the aquatic community, which can provide insight about the ability of the stream to support a healthy aquatic community. Common water chemistry parameters that are measured include temperature, dissolved oxygen, conductivity, pH, total suspended solids, and turbidity. Total suspended solids (TSS) is a measure of sediment loading, which describes the mass of sediment suspended in water (mg/L). Another measure of sediment loading is turbidity, which measures the degree of light penetration as a function of suspended material in a unit of water (Helfman 2007). These common water quality measurements help detect the effects of disturbance to a stream.

Temperature is one variable that plays in important role in biological and chemical processes. Many aquatic organisms can only tolerate a specific temperature range. If water temperatures are outside of an organism's optimal range for a prolonged period, the organism can become stressed and/or die (Dohner et al. 1997). Temperature is closely correlated to the oxygen content of water; that is, oxygen levels decrease with increased water temperatures (Brower et al. 1998). Likewise, metabolic rates of aquatic organisms, photosynthesis by aquatic mosses and plants, and sensitivity of organisms to toxicants are influenced by water temperature (Dohner et al. 1997, Newman and Unger 2003). Anthropogenic factors that can influence water temperature include removal of stream riparian zones (which can increase water temperatures), increased storm water runoff, and dam-created impoundments (Dohner et al. 1997).

Aquatic organisms require oxygen for cellular processes (Brower et al. 1998). Increased runoff from farmland and impervious surface can decreased the amount of available oxygen in a stream ecosystem. Oxygen in water is measured as dissolved oxygen (mg/L or % saturation), and if more oxygen is consumed than produced by the aquatic ecosystem, dissolved oxygen levels decline and sensitive organisms (e.g., trout, stoneflies) may become stressed or die (Dohner et al. 1997).

Conductivity (µS/cm) is a measure of the water's ability to conduct an electrical current, and is affected by inorganic (anions and cations) and organic dissolved solids (Dohner et al. 1997). Polluted waters generally have a higher and a less stable conductivity than non-polluted waters, therefore conductivity can be used

as a measure of pollution. Sensitive species will decline with increased conductivity (KDOW 2008).

The hydrogen ion concentration, pH, also plays a major role in many chemical and biological processes in the water. pH outside of the average range (6-8.5), can reduce the diversity of stream organisms because it stresses their physiology. Low pH can dissociate toxic elements, leading to conditions that can stress the aquatic community (Newman and Unger 2003). Measuring the ability of a stream to neutralize acidic conditions is important. Alkalinity is a measurement of the alkaline compounds in the water such as bicarbonates, carbonates, and hydroxides which act as a buffering system (APHA 1998, Wetzel 2001). Alkalinity is influenced by atmospheric deposition (acid rain), surrounding rock, runoff, and wastewater discharges which can alter the pH of a stream.

Ultimately, a biological assessment supported by habitat and water chemistry measurements provides a comprehensive view of stream health and integrity. A knowledge and understanding of watershed use and aquatic relationships is essential to properly understand the structure and function of a stream ecosystem. Disturbance to a lotic system can alter the water chemistry and habitat in turn influencing the biological communities.

# Laurel Creek Background Information

Laurel Creek is a second order stream in Rowan County, Kentucky, and part of the Little Sandy River drainage (Fig. 1). Laurel Creek watershed, which drains 61.38 km<sup>2</sup>, lies within the unglaciated Western Allegheny Plateau ecoregion of

Kentucky (KDOW 2008), and has ecologically sensitive species such as *Clinostomus funduloides* (rosyside dace) and *Cottus bairdii* (mottled sculpin). Laurel Creek is a high gradient headwater stream with horizontally bedded Pennsylvanian sedimentary rock containing sandstone, siltstone, shale, and coal (KDOW 2008). The headwaters of Laurel Creek experience periods of intermittency, or low flow, during summer and fall months. This intermittency can create dry stretches interspersed by isolated pools.

Historically Laurel Creek has been minimally impacted by anthropogenic factors and recognized by the Kentucky Division of Water as a Special Use Water. Special Use Waters are worthy of extra protection because they are thought to have exceptional water quality and are able to support indigenous life (KDOW 2009). According to Kentucky Administrative Regulations (401 KAR 10:026) Laurel Creek is listed as a cold water aquatic habitat, an outstanding state resource, a primary contact recreation, and a secondary contact recreation from Stegal-Cold Springs Road Bridge in Elliott County to its headwaters in Rowan County.

In 2004 the Rowan County Road Department illegally constructed a road through the valley of Laurel Creek. Necessary permits required to perform construction in Laurel Creek were not obtained. Following the road construction in Laurel Creek, 716 meters of the Laurel Creek mainstem and 259 meters of small tributaries to Laurel Creek were severely impacted. Laurel Creek experienced excessive sediment deposition, extreme channel alteration, substantial turbidity in riffles and runs, and extensive riparian zone de-vegetation (personal communication with KDOW biologists 8 October 2009). In addition, the road increased public access

to the area which caused further degradation to in-stream habitat from all terrain vehicle activity (ATV). The unpermitted activity and substantial environmental damage triggered action by the Kentucky Division of Water and the United States Environmental Protection Agency, Region 4. To avoid paying a considerable fine, Rowan County elected to restore the creek. A restoration plan was developed by CPD Engineers, and restoration occurred in Fall 2007 and Fall 2008. A minimal amount of restoration (128 meters) occurred in Fall 2007, and a majority of the restoration activity (847 meters) occurred in Fall 2008. The restoration goal was to enhance the creek through proper reshaping of impacted sections, installing grade control structures, re-vegetating and stabilizing disturbed sections of the riparian zone, controlling invasive plant species, and removing culverts in tributaries to restore natural drainage. Biological and chemical monitoring of Laurel Creek was funded by Rowan County.

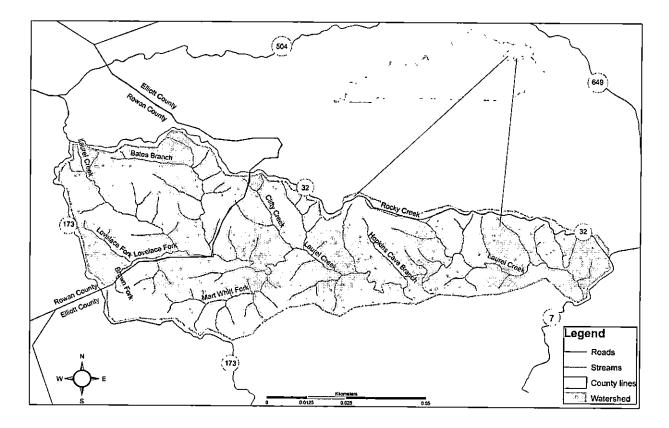


Figure 1. Laurel Creek watershed in Rowan and Elliot Counties, Kentucky. (KDGI 2009)

#### Study Objective

Pre- and post-restoration biological assessments of Laurel Creek using fishes, aquatic macroinvertebrates, water quality, and habitat assessments were conducted to determine the biological integrity of the stream and to provide baseline data for future monitoring of the watershed. The main objective of this study was to compare the biological communities of fishes and aquatic macroinvertebrates in Laurel Creek before and after restoration. Data from the bioassessment were used to determine if the restoration affected the biological communities. If restoration has affected the biological communities, change in those communities would be expected in sites within and possibly below the restoration, while no change in fish and aquatic macroinvertebrate communities would be expected in sites above the restoration. Stream restoration would be expected to disturb the fish and aquatic macroinvertebrates communities within and below the restoration by causing a decline in diversity and abundance, especially in those groups that are sensitive to disturbance. If a system-wide change (all sites change in a similar fashion) or no change in the communities is detected, this suggests that restoration has not affected the biological community.

#### Methods and Materials

#### Study Area

Eight sites in the Laurel Creek watershed were surveyed for fishes in Summer 2008 (17, 18, and 23 June 2008), Spring 2009 (17 and 19 March, and 5 April 2009), and Summer 2009 (1 and 2 July 2009); these same sites were surveyed for aquatic macroinvertebrates in Summer 2008 (21, 23, 24, and 30 June 2008) and Summer 2009 (14, 15, 16, and 18 June 2009) (Fig. 2). In general, collection methods and data analysis followed guidelines in Methods for Assessing Biological Integrity of Surface Waters in Kentucky (KDOW 2008), Development and Application of the Kentucky Index of Biotic Integrity (KIBI) (Compton et al. 2003), and The Kentucky Macroinvertebrate Bioassessment Index (Pond et al. 2003). Two sites were selected above the restored area (Above 1 (A1) and Above 2 (A2)), along with three sites within the restored area (Damaged 1 (D1), Damaged 2 (D2), and Damaged 3 (D3)), and three sites below the restored area (Below 1 (B1), Below 2 (B2), and Below 3 (B3)) for biological monitoring (Fig. 2, Table A1). All reach lengths were 100 meters and incorporated multiple habitats such as a riffle, a run, and a pool. Fifty-five meters of the most upstream reach on Laurel Creek were sampled in June 2008 and March and April 2009, and 100 m were sampled in July 2009. All tables with locality information are provided in Appendix A.

## Fish Sampling and Analysis

Fishes were sampled in Summer 2008 (17, 18, 23 June 2008), Spring 2009 (17 and 19 March 2009, and 5 April 2009), and Summer 2009 (1 and 2 July 2009). In

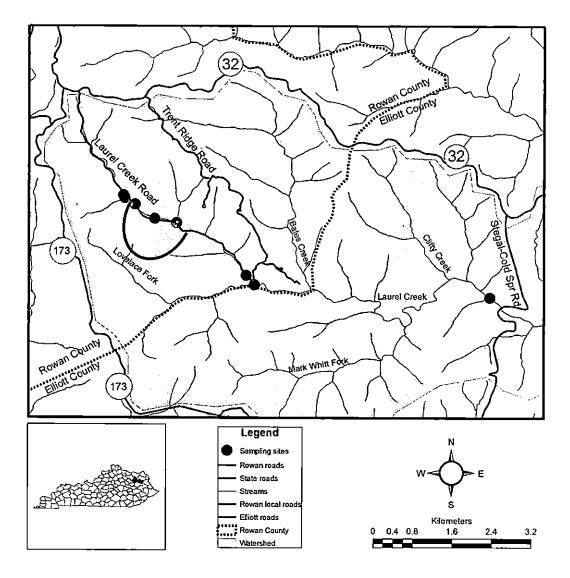


Figure 2. Map displaying sample sites in Laurel Creek, Rowan/ Elliot Counties, KY, indicated by dots. The restored area of the stream is within the curved bracket (KDGI 2009).

general, collection methods and data analysis followed guidelines in KDOW (2008) and Compton et al. (2003).

Fishes were sampled using a Smith-Root, Inc. LR-24 backpack electrofisher and a  $1.6 \times 4$  m seine, as recommended by KDOW (2008). Complex habitats, such as boulders, riffles, and undercut banks, were sampled with a backpack electrofisher; gravel riffles, root masses, and pools were sampled with a seine. Sampling was conducted by two to three personnel. The electrofisher operator and "main netter" always were experienced personnel. Shocking was conducted by moving upstream in a side-to-side/bank-to-bank sweeping technique with one pass of the reach. Seining was also accomplished with two people pulling/holding the seine, and the other crew member kicking into the seine. If only two people were available one person held the seine and another person kicked into the seine. All sites were sampled by electofishing and the lower most site (B3) was supplemented with the seine to ensure that the wide shallow pools were sampled effectively. For each reach, fishes collected were temporarily retained in a five gallon bucket until the entire reach had been sampled, and then identified, counted, weighed, and released unharmed, or if small size prevented identification, preserved in 10% formalin and returned to the laboratory for identification. All fishes were identified to species level. Vouchered specimens were fixed in the field with 10% formalin, identified in the laboratory, and then permanently preserved in 70% ethanol in the Morehead State University Fishes Collection.

Fish community health was evaluated, in part, using biomass, abundance, and Shannon Diversity Index. The Shannon Diversity Index (H') was calculated to describe species diversity at each site. The Shannon Diversity Index is a measure incorporating both taxa richness and taxa abundance, and is maximized by having high richness and high abundance values across all taxa. Richness is a measure of the total number of taxa recorded at each sample site, and generally decreases with decreasing water quality and stream health. Using the H' values, evenness (J') was calculated to estimate how evenly the species were distributed at each site. This metric is maximized when the abundance of all taxa in a sample are equal. Shannon Diversity Index and evenness were calculated as follows (Lugwig and Reynolds 1988, Brower et al. 1998):

 $H'=-\sum(p_i \log p_i)$ J'=H'/In(S)

Fish community health was evaluated with the *Kentucky Index of Biotic Integrity (KIBI)* (Compton et al. 2003). The Index of Biotic Integrity (Karr 1981) originally was used to assess fish community and structure in warm-water Midwestern streams. The KIBI was developed specifically for Kentucky's streams and aquatic fauna, and followed a framework of Karr (1981) and Karr et al. (1986). The KIBI incorporates stream size in the analysis, and includes seven metrics that measure fish community attributes which show responsiveness to anthropogenic disturbances. The metrics include NAT, DMS richness, INT richness, SL richness, %Insct, %Tol, and %FHW,

and these values are incorporated into an overall KIBI score used to rate fish community health.

1. Native species richness (NAT) is a count of the number of native species present. Native fish species were determined using the ecological designation from Appendix A of Compton et al. (2003). Native species are expected to decline with disturbance (KDOW 2008). This metric is used only in wadeable streams.

2. Darter, madtom, sculpin richness (DMS) is a count of the number of intolerant species included in the tribe Etheostomatini (darters), the genus *Noturus* (madtoms), and the genus *Cottus* (sculpins). These orders are sensitive to pollution and disturbance and are expected to decline with impairment (KDOW 2008).

3. Intolerant species richness (INT) is a count of the number of intolerant species collected from a sample. Intolerant fish species were determined using the ecological designation from Appendix A of Compton et al. (2003). Intolerant species are expected to decline with impairment (KDOW 2008).

4. Simple lithophilic spawning species richness (SL) is a count of the total number of simple lithophilic spawning species. Simple lithophile fish species were determined using the ecological designation from Appendix A of Compton et al. (2003). Simple lithophile species require clean gravel to spawn on and do not build nests. This metric will decline with increasing sedimentation and habitat instability.

5. Relative abundance of insectivorous individuals (%INSCT) is a count of the total number of insectivorous individuals in a sample, excluding tolerant individuals. Insectivorous fishes were determined using the ecological designation

from Appendix A of Compton et al. (2003). To determine the %INSCT for each reach, abundance of insectivorous fish species (tolerant insectivore species are excluded) are summed, and then divided by the total number of individuals in that sample and multiplied by 100. Because disturbances, particularly siltation, affect aquatic insects by filling in interstitial spaces, degraded sites have fewer insectivorous fish species.

6. Relative abundance of tolerant individuals (%TOL) is a count of the total number of tolerant individuals from a site, divided by the total number of individuals from the sample. Tolerant fish species were determined using the ecological designation from Appendix A of Compton et al. (2003). Because tolerant individuals are not considered susceptible to disturbance, the relative abundance of tolerant individuals will increase with decreasing water quality, habitat diversity, and/or habitat stability.

7. Relative abundance of facultative headwater individuals (%FHW) is a count of the number of individuals of facultative headwater species, divided by the total number of individuals from the sample. A watershed of less than 15.4 km<sup>2</sup> is considered a headwater stream. Facultative headwater fish species were determined using the ecological designation from Appendix A of Compton et al. (2003). Facultative headwater species are typically less common in pristine headwater streams, but tend to increase in abundance in impaired streams. More facultative headwater individuals suggest recent or ongoing disturbance, however, this may increase from natural disturbance such as flood scouring or drought.

## Aquatic Macroinvertebrate Sampling and Analysis

The same selected sites and reaches for fish sampling were surveyed for macroinvertebrates in Summer 2008 (21, 23, 24, and 30 June 2008) and again in Summer 2009 (14, 15, 16, 18 June 2009). Aquatic macroinvertebrate communities were sampled using both a semi-quantitative riffle and a multiple habitat qualitative method as outlined in KDOW (2008). In a semi-quantitative riffle collection, nine Dframe dipnet (0.11m<sup>2</sup>) sweeps are stratified within the deepest portion (thalweg) of the cobble-boulder-riffle habitat to make a 1m<sup>2</sup> sample. Working upstream in the measured reach, the substrate above the D-frame dipnet was disturbed to dislodge any macroinvertebrates living within or on the rocks. This method was used in the riffles of each reach, and triplicate samples were collected. Riffle habitat is targeted due to the high species richness and abundance, and to ensure flow and substrate stability within a high gradient headwater stream (KDOW 2008). A dipnet is 35 cm wide, and a sample is made the width of the net to about 35 cm above the net. These nine samples were combined in a wash bucket to collect a 1m<sup>2</sup> semi-quantitative sample. The combined sample was partly processed in the field using a 500 µm sieve (US #35) to remove any large gravel, cobble, leaves, or pieces of woody debris, which were separately inspected and rinsed off for any invertebrates, then discarded.

In a multiple habitat qualitative sample, collections from diverse habitats are targeted, such as leaf packs, boulders, woody debris, aquatic mosses, and submerged roots. When any of the multiple habitat types were not available, such as submerged roots or woody debris, more time was invested in other habitat areas (e.g., leaf packs and small boulders). A one hour effort per sample was divided between two people into a 30 minute segment per person. The time period allowed for equal effort among sites and enabled comparison between sites.

Samples were preserved in the field using 50% ethanol, and taken to the laboratory for sorting and identification. In the laboratory, the semi-quantitative riffle sample was processed separately from the qualitative multiple habitat sample. The semi-quantitative riffle sample was meticulously sorted with the aid of a dissecting microscope and fine forceps to search for all macroinvertebrates in the sample. All organisms were identified to the lowest possible taxonomic level, usually genus. Early instar individuals were left at higher taxonomic levels unless it could be determined with a high probability that they belonged to a lower taxonomic ranking. If, for example, some early instar individuals could be diagnosed to be one of two taxa, but only one of those taxa was present within the sample, then the early instar individuals would be added to the total for the taxon that was previously recorded from the site. Vouchered specimens were placed in the Morehead State University aquatic invertebrate collection. The same sorting and preservation procedure was employed for the qualitative multiple habitat sample.

Chironomids were mounted on microscope slides with CMC media for identification following the methods described in the *Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina* (Epler 2001). Identification was conducted with the use of a compound microscope using 40x magnification and oil emersion. Chironomids were identified to genus. Early instar

individuals were left at higher taxonomic levels unless it could be determined with a high probability that they belonged to a lower taxonomic ranking. Questionable chironomid identifications were verified by Mark Vogel (Kentucky Division of Water).

Macroinvertebrate identification primarily followed *An Introduction to the Aquatic Insects of North America* by Merritt and Cummins (1996), Merritt et al. (2008), the *Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina* by Epler (2001), and *The Crayfishes of Kentucky* by Taylor and Schuester (2004). Aquatic macroinvertebrate community health was evaluated using the *Kentucky Macroinvertebrate Bioassessment Index* (MBI) (Pond et al. 2003) and *Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (KDOW 2008), Shannon Diversity Index, and Functional Feeding Guild composition. Shannon Diversity Index (H') and evenness (J') were calculated to describe species diversity and evenness at each site using the semi-quantitative sample. Aquatic macroinvertebrate community health was evaluated using the MBI which includes seven metrics that show responsiveness to anthropogenic disturbance.

1. Taxa richness (TR) is a measure of the total number of distinct genera present in the composited sample (both semi-quantitative and qualitative multiple habitat sample combined). For taxa not identifiable to genus, the family taxon was counted only if no genera were identified and counted. To obtain a MBI score at each site, the TR values were divided by 63 and multiplied by 100 (Table A2). Taxa richness generally decreases with decreasing water quality and stream health.

2. Ephemeroptera, Plecoptera, Trichoptera richness (EPT) is a measure of the total number of distinct genera (using a composite of the semi-quantitative riffle and qualitative multiple habitat sample) within the pollution sensitive orders of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). For the EPT value of each site the number of distinct genera were counted. For taxa not identifiable to genus, the family taxon was counted only if no genera were identified and counted. To obtain a MBI score at each site, the EPT value was divided by 33 and multiplied by 100 (Table A2). The metric generally increases with increasing water quality, habitat diversity, and stability (KDOW 2008).

3. The modified Hilsenhoff Biotic Index (mHBI) was modified from the Hilsenhoff Biotic (HBI) Index, and originally only included benthic arthropod communities from Wisconsin. The HBI was used to evaluate organic stream pollution based on tolerance values for benthic arthropod communities. The HBI has been regionally modified for southeastern United States streams, and tolerance values have been developed from North Carolina Division of Environmental Management and KDOW data (KDOW 2008). For the mHBI value for each site, tolerance values (TV) from Appendix D-1 of KDOW (2008) were assigned to each taxon. If a tolerance value for a taxon was not available, the family tolerance value was used. For the genera *Hydrobiomorpha, Paratrichocladius, Reomyia, Serromyia, Maccerffertium, Nixe*, and *Stylogomphus* the family tolerance values were used. The number of each taxon (up to 25 individuals) was multiplied by their tolerance value to yield a score for each genus, and then were summed. The summed value was then divided by the

total number of individuals for each site (up to 25 individuals per taxon). To obtain a MBI score, the mHBI value for each site was subtracted from 10, then divided by 7.82 and multiplied by 100 (Table A2). An increasing mHBI value indicates decreasing water quality. Data used to calculate this metric are taken only from the semi-quantitative riffle sample.

4. Modified EPT (m%EPT) Richness is a measure of the abundance of the generally pollution sensitive insect orders Ephemeroptera, Plecoptera, and Trichoptera recorded from each semi-quantitative sample. Species of Trichoptera genus *Cheumatopsyche* are excluded from this calculation because they have been documented as being a pollution tolerant. For the m%EPT value of each site, the abundance of these three taxa (excluding *Cheumatopsyche*) are summed and divided by the total number of individuals collected for each site. To obtain a MBI score, the m%EPT value was divided by 86.9 then multiplied by 100 (Table A2). The higher the m%EPT value indicates increasing water quality and/or habitat conditions (KDOW 2008). Data used to calculate this metric are taken only from the semi-quantitative riffle sample.

5. Percent Ephemeroptera (%Ephem) is a measure of the abundance of mayflies. Mayflies are generally considered susceptible to impacts of heavy metals and high conductivity associated with mining and oil well alterations (KDOW 2008). For the %Ephem value of each site, the relative abundance of mayflies was summed, and divided by the total number of individuals in each sample. To obtain a MBI score for each site, the %Ephem was divided by 66.5 then multiplied by 100 (Table 2).

With increased pollution from the above sources, the percent of Ephemeroptera will decrease (KDOW 2008). Mayflies harbored in headwater streams have been documented as more sensitive than those in wadeable streams, therefore this metric is only used in headwater streams. Data used to calculate this metric are taken only from the semi-quantitative riffle sample.

6. Percent Chironomidae+Oligochaeta (%Chir+%Olig) is a measure of the relative abundance of chironomids (midges) and oligochaetes (segmented worms) in each sample. These organisms are generally considered pollution tolerant (KDOW 2008). For the %Chir +%Olig value of each site, the abundance of Chironomidae and Oligocheata individuals were summed, and then divided by the total number of individuals in each sample. To obtain a MBI score for each site, the %Chir+%Olig value was subtracted from 100, divided by 99.32, and multiplied by 100 (Table A2). Increasing abundance in Chironomide and Oligochaeta generally indicates decreasing water quality from a variety of sources including municipal waste, agriculture, and coal mining (KDOW 2008). This index value generally will increase with decreasing habitat diversity and/or stability. Data used to calculate this metric are taken only from the semi-quantitative riffle sample.

7. Percent Primary Clingers (%Clingers) is a measure of the abundance of those organisms that require hard, silt-free substrate to "cling" to (KDOW 2008). For the %Clinger value of each site, the abundance of each clinger taxon (marked by an "X" in the habitat column of Appendix D-1 of KDOW (2008)) was summed, and then divided by the total number of individuals of each site. To obtain a MBI score, the

%Clinger value was divided by 75.5 and then multiplied by 100 (Table A2). The increasing abundance of clingers indicates higher quality habitat and substrate stability. Data used to calculate this metric are taken only from the semi-quantitative riffle sample.

8. The macroinvertebrate biotic index (MBI) is a composite average of the seven bioassessment metrics that have been standardized to an approximated "best" value found in the KDOW statewide database. In order to rate a given site, the MBI value for the site is compared to a narrative description determined by the KDOW (Pond et al. 2003). The KDOW have determined reference sites throughout the state to establish baseline data for macroinvertebrate assessments. For headwater streams in the mountain regions of Kentucky, the MBI values range from 0-23 (very poor), 24-47 (poor), 48-71 (fair), 72-82 (good), and above 82 is rated as excellent.

Functional feeding group composition was included to evaluate trophic relationships. Functional feeding groups were analyzed from the semi-quantitative samples because they have a more standard collecting procedure in comparison to the qualitative sampling. Functional feeding group classifications were obtained by using Appendix D-1 of KDOW (2008) and Merritt et al. (2008). The macroinvertebrates collected for each of the eight sites in Summer 2008 and Summer 2009 were divided into four different functional feeding groups (FFGs). These included predators, scrapers, shredders, and collectors. The collectors were further subdivided into collector-gathers and filter feeders. Relative abundance for each functional feeding group was calculated. If a trophic relationship was not available for a taxon in

Appendix D-1 of KDOW (2008), the trophic relationship was obtained from Merritt et al. (2008). A functional feeding group classification for *Reomyia* sp. was not available.

Inferential statistics were not used to evaluate the biological community, water chemistry, or habitat data. The design of this study would have statistical limitations (i.e., pseudoreplication) that limit inferences that can be made (Eberhardt and Thomas 1991, Richardson and Kiffney 2000). Pseudoreplication is a source of error consisting of assigning an exaggerated estimate of statistical significance by treating a data set as independent observations when in fact the observations are interdependent (Hurlbert 1984). The design of the study can demonstrate differences between sample sites within Laurel Creek by this use of widely accepted multimetric biotic indices and community measures for aquatic ecosystems (Washington 1984). Multimetric biotic indices are justifiable because they integrate multiple attributes of a biological system that respond to a variety of disturbances which generally affect the aquatic community (Gerristen 1995, Karr 1999, Kilgour et al. 2004).

## Habitat Assessment

In addition to macroinvertebrate and fish community health, habitat was evaluated with high gradient habitat data sheets from KDOW (2008) to evaluate the quality of in-stream and riparian habitat and to determine if any parameters were affecting the biological community (e.g., increased sedimentation). Only the reaches within the restored area in which macroinvertebrate and fishes were sampled were included in the habitat evaluation for a pre- and post- analysis. Sites D1, D2, and D3

were evaluated on 13 August 2009. The reaches above and below the impacted area were evaluated in Summer 2009 (4 July 2009) post-restoration.

The availability of quality habitat directly influences the integrity of the stream (KDOW 2008). It is important to evaluate the condition of the habitat to determine what is happening within the stream itself. Habitat assessments also provide documentation of current habitat condition for future references. Habitat assessments are subjective; therefore, to maintain consistency in evaluations, the same person must fill out the habitat data forms each sampling period. Ten habitat parameters were measured: epifaunal substrate/available cover, embeddedness, velocity/depth regime, sediment deposition, channel flow status, channel alteration, frequency of riffles (or bends), bank stability (right and left bank), vegetative protection (right and left bank), and riparian vegetative zone width (right and left bank).

1. Epifaunal substrate/available cover is a parameter that measures the quantity and variety of diverse in-stream structures such as cobble, fallen trees, undercut banks, root mass, etc., which offer refuge, feeding opportunities, and nursery sites to aquatic organisms. The assessment is conducted for the entire 100 m reach.

2. Embeddedness is a measurement of the degree of silt, sand, or mud that cover or surround the rocks. Increased embeddedness decreases the available habitat providing shelter, spawning sites, and incubation sites for fishes and aquatic macroinvertebrates. The upstream and middle section of riffle habitat are assessed.

3. Velocity/depth regime is a measurement evaluating the four velocity regimes present in a high-gradient stream. The four velocity regimes are slow-deep, slow-shallow, fast-deep, and fast-shallow. This parameter is used to determine a stream's ability to provide and maintain a stable aquatic environment.

4. Sediment deposition is a measurement of the amount of sediment that has accumulated in the bottom of pools and stream bottom. The formation of island, point bars or shoals is a direct effect of sediment deposition, and often results in filling in of runs and pools (KDOW 2008). An unstable and frequently changing environment will have increased sediment deposition, which may render the habitat unsuitable for many organisms.

5. Channel flow status is a measurement of the wetted width (water that reaches the base of both lower banks) of 100 m reach. The measurement varies seasonally.

6. Channel alteration is a measurement of the degree of channelization (straightening of the stream), amount of bank stabilization structures (rip-rap), dams or bridges present that obstruct flow, and dredging detected within the last 20 years.

7. Frequency of riffles is a measurement of the heterogeneity of a stream. To obtain the occurrence of riffles, the ratio of distance between each riffle was divided by the width of the stream in each reach.

8. Bank stability is a parameter which evaluates the amount of erosion, or the potential for erosion, for each stream bank. The right and the left bank (determined by facing downstream) are scored separately on a 0 to 10 scale.

9. Vegetative protection is a parameter which measures the immediate riparian zone by evaluating the degree of cover. Riparian zones function as a buffer in that they intercept sediments from upland sources, reduce stream bank erosion, provide allochthonous organic matter to organisms in the stream, and control the range and elevation in temperatures (Helfman 2007). The right and the left bank (determined by facing downstream) are scored separately on a 0 to 10 scale, and native vegetation scores higher than invasive vegetation.

10. Riparian vegetative zone width is a parameter evaluating the width of vegetative cover from the stream bank through the riparian zone. The age of the trees is incorporated into the score, and a vegetative zone with older trees scores higher than that of a vegetative zone with younger trees. The right and the left bank (determined by facing downstream) are scored separately on a 0 to 10 scale.

Each individual parameter is ranked by a score of up to 20 possible points; parameters with a right and a left bank receive a score for each side (up to ten points per side). The total points allotted for each parameter are combined for an overall habitat score (maximum 200 points). For a stream in the Western Allegheny Plateau bioregion of Kentucky, a score of  $\geq$  160 is considered fully supporting, 117-159 is partially supporting, and  $\leq$  116 is non-supporting of aquatic life (KDOW 2008).

# Water Chemistry

Water chemistry parameters were collected and measured concurrently with fishes at the same locations in Laurel Creek during Summer 2008 (17, 18, and 23 of

June 2008), Spring 2009 (17 and 19 March and 5 April 2009), Summer 2009 (1 and 2 July 2009), and with aquatic macroinvertebrates in Summer 2009 (14, 15, 16, and 18 June 2009). Water quality measures were collected to ensure that the basic water chemistry at each site in Laurel Creek was not a limiting factor for the biological communities. Measures of water quality followed Standard Methods for the Examination of Water and Wastewater (APHA 1998) and equipment manufacturer's methods. Parameters measured included: temperature (°C), conductivity (µS/cm), dissolved oxygen (% saturation and mg/L), and pH (standard units). A portable YSI 556 multiparameter system (multiprobe system) was calibrated accordingly to the manufactures's manual. Metrepak pH-pHydrion buffers certified at  $4.00 \pm 0.02$  and  $10.00 \pm 0.02$  certified at 25°C and Fisher Buffer Solution pH 7.00 certified pH of 6.99-7.01 at 25°C were employed for pH standards. Traceable® Conductivity standard certified reference material (99.5  $\mu$ S/cm) were employed for conductivity standards. The YSI probe was placed in the stream at the downstream location of each reach prior to sampling the biological community and measurements were recorded. Field verification, with the use of pH and conductivity standards, was obtained when potentially aberrant readings were observed.

Samples for total suspend solids (TSS) and turbidity (nephelometric turbidity units, NTU) were collected during sampling in Spring 2009 (17 and 19 March and 5 April 2009), and Summer 2009 (1 and 2 July 2009). Total suspended solids were measured following methods outlined in Wyckoff (1964) and *Standard Methods for the Examination of Water and Wastewater* (APHA 1998) (Method 2540 D, total

suspended solids dried at 103-105 °C). Filtered and unfiltered water samples were stored in acid washed polyethylene bottles at 4°C until analyzed. Total suspended solids were determined by suction filtering water through a pre-combusted 0.45 µm pore-size glass-fiber filter (Wyckoff 1964). Turbidity was measured generally following guidelines in Standard Methods for the Examination of Water and Wastewater (APHA 1998) (2130 B, Nephelometric Method) with the use of a HACH Company (Model 2100) portable turbidimeter which measures turbidity of water from 0.1 to 1000 NTUs. The meter was standardized using purchased HACH solid standards 24 hours before sampling. Triplicate representative water samples were collected in manufacturer's glass sample cells (sample cells hold approximately 15 ml) from each designated reach. Sample cells were wiped clean with a lint free cloth, a thin film of silicone oil was applied, and the sample cell was wiped again to ensure an even film of silicone. The application of silicone is used to mask scratches or minor imperfections that may contribute to turbidity or stray light. The sample cell was then placed into the turbidity meter cell compartment and the turbidity, in NTU, was measured and recorded.

Alkalinity was measured due to its significance in aquatic systems. Alkalinity is a measure of the capacity of water to neutralize acids (APHA 1998, Wetzel 2001). Samples for alkalinity were collected and measured in Summer 2009 (1 and 2 June 2009). Alkalinity was measured following methods outlined in *Standard Methods for the Examination of Water and Wastewater* (APHA 1998) (Method 2320 B, titration method). Unfiltered water samples were stored in acid washed polyethylene bottles at

 $4^{\circ}$ C until analyzed. Alkalinity was determined by titrating 0.02 N sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) with a self-zeroing buret into 100 milliliters of sample water mixed with bromcresol green-methyl red indicator until a color change was observed. A pH probe was used to measure the pH (standard units) after titration.

#### <u>Results</u>

#### Fish Community Analysis

From the collection of 3,395 individuals in Laurel Creek, a total of 14 species from six families were identified from Summer 2008 to Summer 2009. All assessments of fish communities are provided in Appendix B (Tables B1-B11). All sites in Laurel Creek were dominated by minnows and sculpins, especially rosyside dace (*Clinostomous funduloides*), blacknose dace (*Rhinichthys atratulus*), creek chubs (*Semotilus atromaculatus*), and mottled sculpins (*Cottus bairdii*). *Clinostomus funduloides* was the dominant species collected in Summer 2008 and Spring 2009. *Semotilous atromaculatus* was the dominant species collected in Summer 2009.

Overall there was little change observed in Darter, Madtom, and Sculpin richness (DMS), intolerant species richness (INT), and simple lithophile spawning species (SL) among years. DMS richness ranged from 2-4 species between Summer 2008 and Summer 2009. The only change in DMS richness observed from Summer 2008 to Summer 2009 was encountered at sites D2 and B1; D2 increased by one species and B1 decreased by two species. *Etheostoma flabellare*, the fantail darter, was collected in Summer 2009 at site D2 and it had not been collected there previously. Site B1 declined by two DMS species; *Cottus bairdii* was the only DMS species collected in Summer 2009. Intolerant species richness ranged from 1-4 species from Summer 2008 to Summer 2009. The only change observed from Summer 2008 to Summer 2009 was an increase at site B3 by one intolerant species. *Oncorhynchus mykiss* was collected at site B3 in Summer 2009 and it was not

previously collected at this site. SL richness ranged from 1-6 between Summer 2008 and Summer 2009. The only change observed from Summer 2008 to Summer 2009 was a decrease of simple lithophile richness by one species at sites A1 and B1. *Catostomus commersonii* was not collected from site A1 in Summer 2009 and *Hypentilium nigricans* was not collected from site B1 in Summer 2009.

## System-Wide Changes Observed Among Sites

There was an observable, system-wide trend, in the relative abundance of insectivorous (%Insct) and tolerant (%Tol) fish species. From Summer 2008 to Summer 2009 a decrease was observed in relative abundance of insectivorous fishes present at all sites excluding the most downstream site (B3; Fig. 3). The two most abundant intolerant fishes were *Cottus bairdii* and *Clinostomus funduloides*. Spring 2009 %Insct values showed an increase at sites A1, D1, D2, D3, and B3 however, the values decreased in Summer 2009 below the values collected in Summer 2008 (Fig. 3). Summer 2008 %Insct values ranged from 42.9-67.4 with the highest relative abundance of insectivorous fishes collected at site B1, and the lowest collected at site A1 (Fig. 3). Spring 2009 %Insct values ranged from 45.3-87.0, with the highest %Insct collected at site B3, and the lowest collected at site B2 (Fig. 3). Summer 2009 %Insct values ranged from 20.0-65.9 with the highest %Insct collected at site B3, and lowest at site A1 (Fig. 3).

A system-wide trend was also observed in relative abundance of tolerant fishes. The two most abundant tolerant fishes were *Rhinichthys atratulus* and *Semotilius atromaculatus*. Relative abundance of tolerant fishes (%Tol) increased at

all sites from Summer 2008 to Summer 2009, excluding the most downstream site (B3; Fig. 4). Spring 2009 %Tol values showed a decrease at sites A1, D1, D2, D3, and B3; however, the values increased in Summer 2009 to values higher than collected in Summer 2008. Summer 2008 %Tol values ranged from 30.4-54.8, Spring 2009 values ranged from 9.42-50.0, and Summer 2009 values ranged from 26.6-77.7. The lowest %Tol values observed in Summer 2008 was at site B1, and site B3 for Spring and Summer 2009. The highest %Tol values from Summer 2008 to Summer 2009 were observed at site A1 (Fig. 4).

Facultative headwater species collected in Laurel Creek were *Campostoma anomalum*, *Rhinichthys atratulus*, *Catostomus commersonii*, and *Etheostoma flabellare*. Relative abundance of facultative headwater species was variable between Summer 2008 and Summer 2009 in comparison to other metrics. Relative abundance of facultative headwater species increased from Summer 2008 to Summer 2009 at all sites excluding D3 and B2 (Fig. 5). Summer 2008 %FHW values ranged from 16.7-51.2, Spring 2009 values ranged from 16.5-58.3, and Summer 2009 values ranged from 21.6-56.0. The lowest %FHW values observed in Summer 2008 and Spring 2009 were in site B1, and in site D3 for Summer 2009. The highest %FHW values from Summer 2008 to Summer 2009 were observed at site A1 (Fig 5).

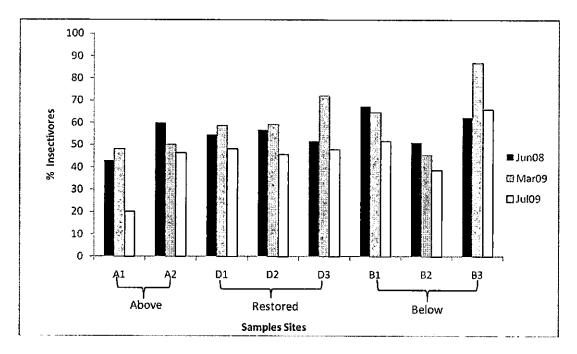


Figure 3. Comparison of relative abundance of insectivorous fish species collected in Laurel Creek from Summer 2008 to Summer 2009.

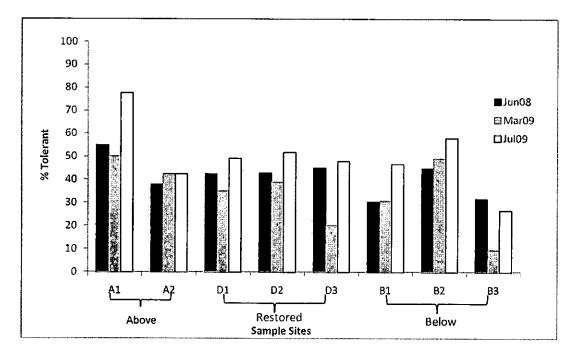


Figure 4. Comparison of relative abundance of tolerant fish species collected in Laurel Creek from Summer 2008 to Summer 2009.

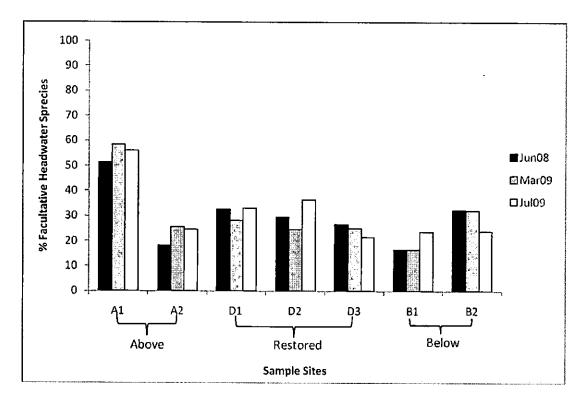


Figure 5. Comparison of relative abundance of facultative headwater fish species collected in Laurel Creek from Summer 2008 to Summer 2009.

# Differential Changes Observed Among Sites

There was an observable change in sites within and below the restored area in KIBI scores, fish biomass, and fish abundance. This same trend was not observed in sites above the restoration. KIBI scores for Summer 2008 ranged from 56-67 and were rated as fair to excellent (Fig. 6; Table B1). KIBI scores for Spring 2009 ranged from 35-77 and were rated as poor to excellent (Fig. 6, Table B2). KIBI scores in Summer 2009 ranged from 55-72 and were rated as good to excellent (Fig. 6; Table B3). KIBI Scores slightly decreased from Summer 2008 to Summer 2009 except for sites A1 and B3. In general, KIBI scores were the lowest in Spring 2009, especially at sites A1, D1, D3, B1, and B2.

An increase in abundance of individuals collected was observed in sites above the restored area, compared to a pronounced decrease in sites within and below the restored area (Fig. 7). Abundance for Summer 2008 ranged from 82-358 individuals, 48-160 individuals in Spring 2009, and 66-173 in Summer 2009. Site A1 had the lowest abundance in Summer 2008 and Spring 2009, but the abundance still increased from Summer 2008 to Summer 2009. Site D2 had the lowest abundance in Summer 2009. The highest abundance of individuals for Summer 2008 was collected at site B1, site D1 in Spring 2009, and site B3 in Summer 2009 (Table B7). The largest decline observed in abundance from Summer 2008 to Summer 2009 was at site B3 with a decrease of 185 individuals. Sites D3 and D2 also had a substantial decline

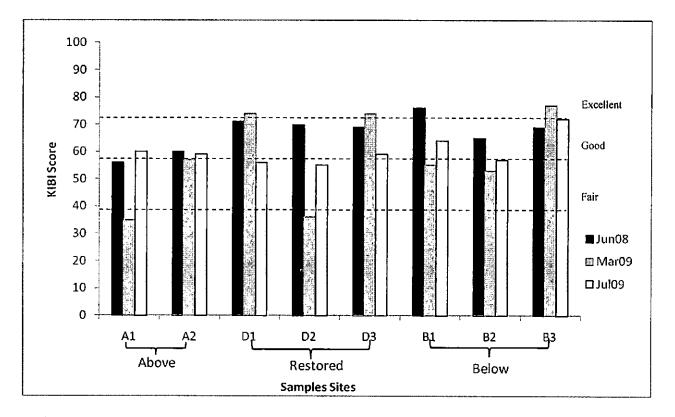


Figure 6. Comparison of Kentucky Index of Biotic Integrity scores in Laurel Creek from Summer 2008 to Summer 2009. Categorical classifications are depicted by the dotted line with the associated narrative classification. Any classification below fair was within the poor or very poor classification.

in abundance; site D3 decreased by 133 individuals and site D2 decreased by 86 individuals.

Total biomass (grams) exhibited a similar change from Summer 2008 to Summer 2009. An increase in total biomass collected was observed in sites above the restored area, compared to a decrease in sites within and below the restored area (Fig. 8). Total biomass for Summer 2008 ranged from 257.3-991.8 grams, 124.0-492.8 grams in Spring 2009, and 214.7-695.5 grams in Summer 2009. Site A1 had the lowest total biomass in Summer 2008 and Spring 2009, and site D2 had the lowest in Summer 2009. The highest biomass for Summer 2008 was encountered at site D3, site D1 for Spring 2009, and site B3 for Summer 2009 (Table B8).

Shannon Diversity Index (H') values are provided in Tables B9-B11 (Appendix B). The highest H' of 2.07 occurred at site B3 in Summer 2008 (Fig. 9, Table B9). The highest H' of 1.80 occurred at sites D1 and B2 in Spring 2009. The highest H' of 2.09 occurred at site B3 in Summer 2009. The lowest H' of 1.60 occurred at site D1 in Summer 2008. The lowest H' of 1.43 occurred at site A1 and B1 in Spring 2009. The lowest H' of 1.32 occurred at site A1 in Summer 2009. Sites A1, D3, B1, B2, and B3 showed a decrease in Shannon Diversity Index from Summer 2008 to Summer 2009, and sites A2, D1, D2, and B3 increased from Summer 2008 to Summer 2009 (Fig. 9). Overall, Shannon Diversity Indices did not vary much among years.

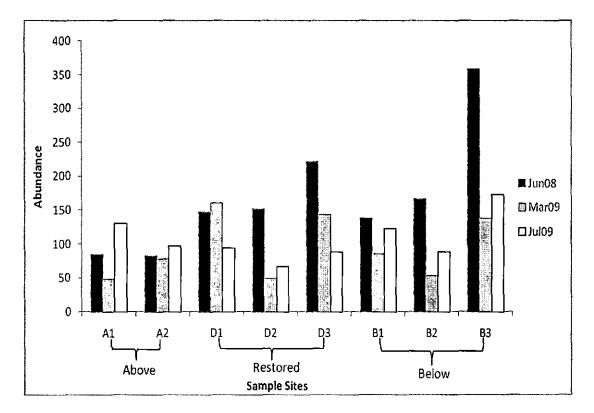


Figure 7. Comparison of abundance of fishes collected in Laurel Creek from Summer 2008 to Summer 2009.

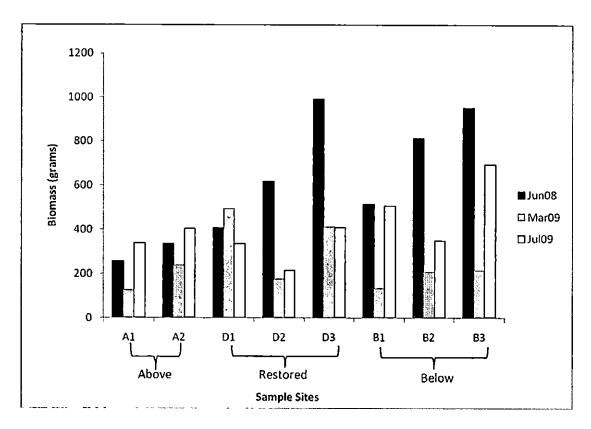


Figure 8. Comparison of total biomass (grams) of fishes collected in Laurel Creek from Summer 2008 to Summer 2009.

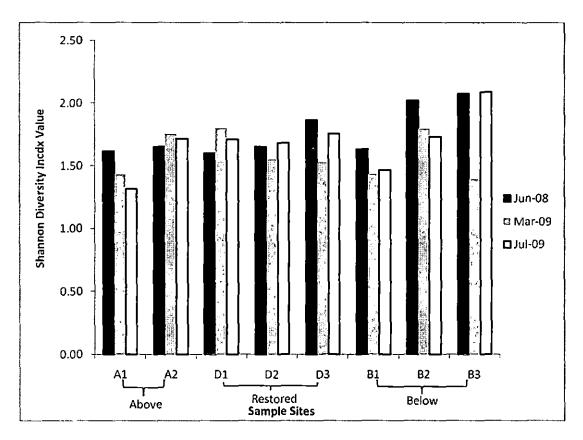


Figure 9. Comparison of Shannon Diversity Index for fish communities in Laurel Creek from Summer 2008 to Summer 2009.

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#### Aquatic Macroinvertebrate Community Analysis

A total of 5,051 macroinvertebrate individuals were collected from Laurel Creek. Seventy-one taxa were identified from Summer 2008 and 105 taxa from Summer 2009; this includes 11 orders and 46 families from both the semi-quantitative and qualitative samples. All assessments of macroinvertebrate communities are provided in Appendix C (Tables C1-C13). Diptera were the most diverse with 25 genera identified in Summer 2008 and 45 genera in Summer 2009. The most frequently encountered taxon recorded from Laurel Creek in Summer 2008 was *Cheumaiopsyche* (Trichoptera, Hydropsychidae), and the most frequently encountered taxon in Summer 2009 was *Allocapnia* (Plecoptera, Capniidae). Taxa collected in Laurel Creek in Summer 2008 and not in Summer 2009 are provided in Table C12, and taxa collected in Summer 2009 and not collected in Summer 2008 are provided in Table C13.

Comparison of site trends in generic taxa richness (TR), EPT richness (EPT), mHBI, relative abundance of EPT (%EPT), relative abundance of mayflies (%Ephem), relative abundance of chironomid midges and oligocheate worms (%Chir + %Olig), relative abundance of clinger organisms (%Clingers), macroinvertebrate biotic index (MBI), Shannon Diversity Index (H'), Shannon Evenness Index ( J'), and functional feeding guild composition of Summer 20008 and Summer 2009 are shown in Tables C5-C11.

Generic taxa richness (TR) increased from Summer 2008 to Summer 2009 at all sites within Laurel Creek (Fig. 10). TR values ranged from 26-39 in Summer 2008

and from 41-57 in Summer 2009. The greatest increase in TR was observed at sites A1 (19 taxa), D1 (25 taxa), B2 (20 taxa), and B3 (20 taxa).

EPT richness increased from Summer 2008 to Summer 2009 in all sites except site A2 (Fig. 11). No change was detected in site A2 from Summer 2008 to Summer 2009. EPT richness ranged from 9-18 in Summer 2008, and from 12-26 in Summer 2009. The greatest increase in EPT richness was observed at sites D1 (12 taxa), D3 (8 taxa), and B3 (8 taxa).

The mHBI decreased from Summer 2008 to Summer 2009, except at sites B2 and B3 (Fig. 12). The mHBI ranged from 3.24-5.54 for Summer 2008 and from 2.11-3.74 in Summer 2009 (Tables C5 and C6). The greatest decrease in mHBI was observed at sites A1 (1.86 decrease) and D3 (1.28 decrease).

Modified EPT richness (m%EPT) showed improvement from Summer 2008 to Summer 2009 (Fig. 13). m%EPT ranged from 21.02-39.27 for Summer 2008, from 41.77-72.68 for Summer 2009. *Allocapnia* were the most abundant EPT macroinvertebrate encountered in Summer 2009, and the relative abundance of this genus increased at all sites from Summer 2008 to Summer 2009. The greatest increases in relative abundance of *Allocapnia* were encountered at site D2, with an increase in relative abundance of 11.48% in Summer 2008 to 50.13% in Summer 2009. *Cheumatopsyche* decreased in relative abundance in all sites from Summer 2008 to Summer 2009. The largest decreases were observed at sites D3, B2, and B3. In addition, several genera of mayflies, stoneflies, and caddisflies were encountered

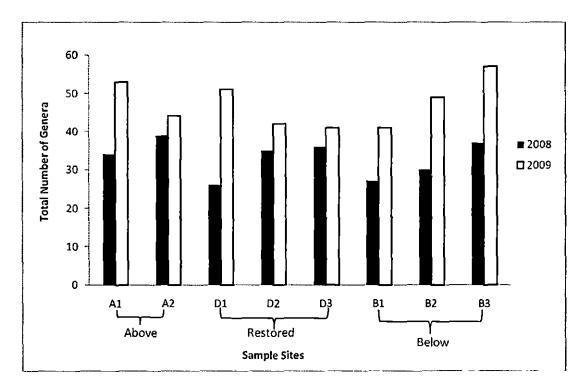


Figure 10. Comparison of taxa richness for macroinvertebrate communities among sample sites in Laurel Creek in 2008 and 2009.

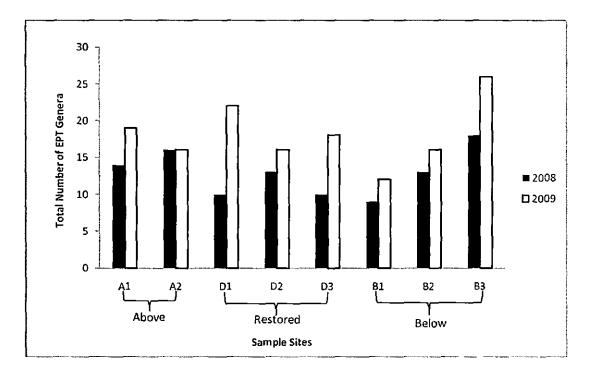


Figure 11. Comparison of EPT richness for macroinvertebrate communities among sample sites in Laurel Creek in 2008 and 2009.

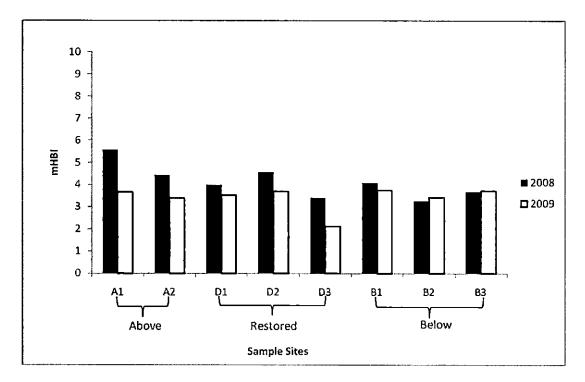


Figure 12. Comparison of macroinvertebrate mHBI among sample sites in Laurel Creek in 2008 and 2009.

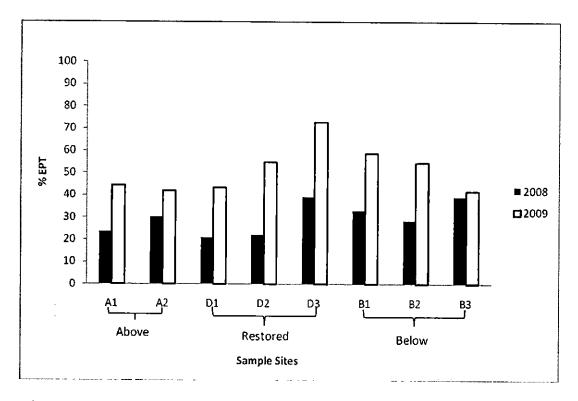


Figure 13. Comparison of modified %EPT for macroinvertebrate communities among sample sites in Laurel Creek in 2008 and 2009.

in Summer 2009 and not in Summer 2008 (Tables C12 and C 13).

Percent Ephemeoptera (%Ephem) showed an overall improvement from Summer 2008 to Summer 2009, except at sites D3 and B3 (Fig. 14). %Ephem ranged from 3.22-11.96 for Summer 2008, and from 11.67-22.09 for Summer 2009. Site D3 decreased in %Ephem by 0.29 and site B3 decreased by 3.52. The greatest increase in %Ephem from Summer 2008 and Summer 2009 was observed at site A1 (12.70%).The most abundant mayfly collected in Summer 2008 was *Isonychia* (Ephemeroptera, Isonychidae). The most frequently encountered mayfly collected in Summer 2009 at sites A1, A2, D1, D3, B1, and B3 was *Acentrella* (Ephemeroptera, Baetidae), and the most frequently encountered mayfly at site D2 was *Isonychia*. *Acentrella* and *Isonychia* were the most frequently encountered mayflies collected at site B2 in Summer 2009 with equal relative abundance.

Relative abundance of chironomids (midges) and oligochaete (segmented worms; %Chir + %Olig) increased in all sites from Summer 2008 to Summer 2009, except at sites B1 and B2 (Fig. 15). %Chir + %Olig ranged from 0-24.29 for Summer 2008, and in from 5.84-27.64 for Summer 2009. The greatest increase in %Chir + %Olig was observed at site A1(13.93%) and D2 (16.64); Fig. 15). The most abundant chironomids collected in Summer 2008 were either in the *Thienemannimyia* group (Diptera, Chironomidae) or *Polypedilum* (Diptera, Chironomidae). The most abundant chironomids collected in Summer 2009 were the relatively intolerant genus *Parametriocnemus* (Diptera, Chironomidae).

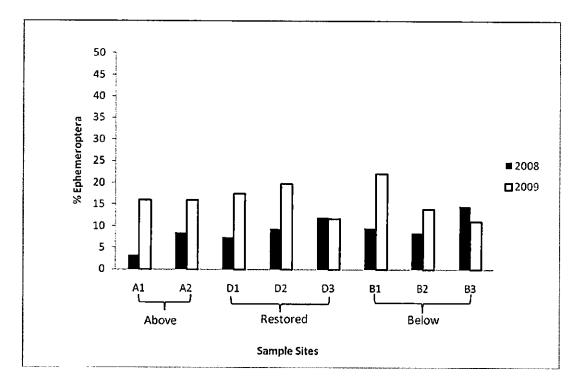


Figure 14. Comparison of % Ephem for macroinvertebrate communities among sample sites in Laurel Creek in 2008 and 2009.

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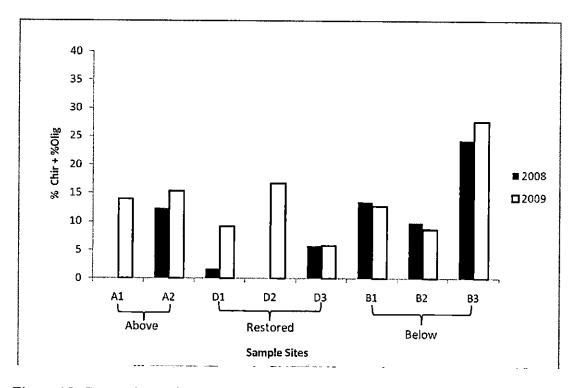


Figure 15. Comparison of %Chir + %Olig for macroinvertebrate communities among sample sites in Laurel Creek in 2008 and 2009.

Relative abundance of clinger organisms (% Clingers; macroinvertebrates adapted to attach to hard, silt-free substrates) decreased at all sites from Summer 2008 to Summer 2009 (Fig. 16). %Clingers ranged from 42.31-73.30 for Summer 2008, and from 28.49-56.66 for Summer 2009. The greatest decrease in %Clingers was observed at sites D3 (31.27), B2 (30.19), B1 (28.25), and A1(26.36) (Fig. 16). *Cheumatopsyche* were the most frequently encountered clinger organism at sites A1, A2, D1, D2, D3, B2, and B3 in Summer 2008, while *Optioservus* larvae (Coleoptera, Elmidae) were the most frequently encountered clinger organism at site B1. *Perlesta* (Plecoptera, Perlidae) were the most frequently encountered clinger organisms at sites D2, D3, B1, and B3 in Summer 2009. *Nigronia* (Megaloptera, Corydalidae) were the most frequently encountered clinger organisms at sites

The overall MBI scores improved from Summer 2008 to Summer 2009 (Fig. 17). MBI scores ranged from 51.39-66.66 in Summer 2008, and ranged from 58.49-67.55 in Summer 2009 (Tables C5 and C6). The greatest improvement in MBI scores was observed in site D1, which increased by 13.10.

From Summer 2008 to Summer 2009 the abundance of aquatic macroinvertebrates increased at all sites, except B1 and B3, with site D3 having the highest abundance of aquatic macroinvertebrates sampled. Site A2 had the highest Shannon Diversity Index of 2.95 in Summer 2008. In Summer 2009, A1 had the highest Shannon Diversity Index of 3.18 (Tables 1 and 2). Site A1 had the lowest Shannon Diversity Index of 2.40 for Summer 2008, and Site D3 had the lowest

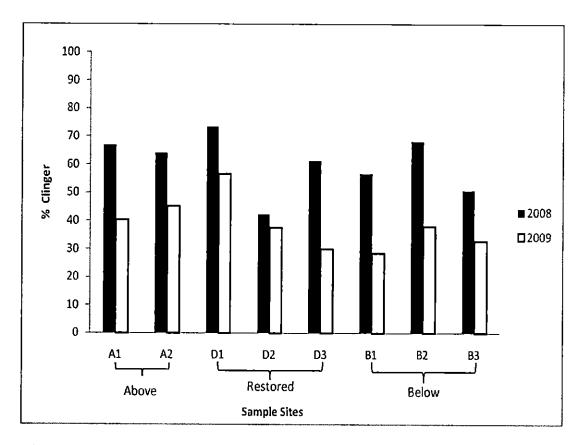


Figure 16. Comparison of %Clinger for macroinvertebrate communities among sample sites in Laurel Creek in 2008 and 2009.

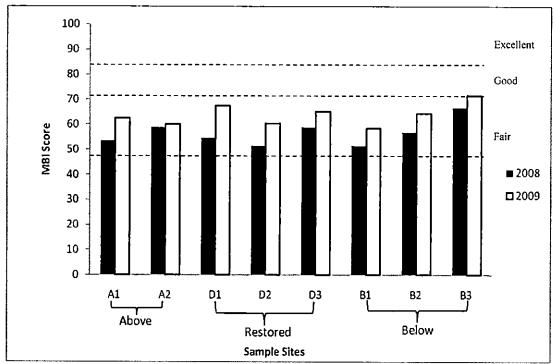


Figure 17. Comparison of MBI scores among sample sites in Laurel Creek in 2008 and 2009. Categorical classifications are depicted by the dotted line with the associated narrative classification listed. Any classification below fair was within the poor or very poor classification.

Sample Site	Abundance	# of Genera	Shannon Diversity Index (H')	Shannon Evenness Index (J')
A1	93	19	2.40	0.82
A2	178	25	2.95	0.92
D1	176	22	2.42	0.78
D2	182	28	2.78	0.83
D3	209	28	2.42	0.73
B1	178	19	2.42	0.82
B2	223	21	2.38	0.78
B3	247	20	2.46	0.82

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 Table 1. Shannon Diversity Index, abundance, and generic richness for macroinvertebrate communities

 of eight sites within Laurel Creek in Summer 2008

Sample		# of	Shannon Diversity Index	Shannon Evenness Index
Site	Abundance	Genera	(H')	(J')
Al	201	48	3.18	0.82
A2	339	38	3.03	0.83
D1	293	43	2.91	0.77
D2	203	38	2.94	0.81
D3	377	35	2.21	0.62
B1	172	33	2.76	0.79
B2	266	44	2.81	0.74
<u>B3</u>	199	42	3.06	0.82

 Table 2. Shannon Diversity Index, abundance, and generic richness for macroinvertebrate communities

 of eight sites within Laurel Creek in Summer 2009.

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Shannon Diversity Index of 2.21 for Summer 2009 (Tables 1 and 2). Overall, Shannon Diversity Index increased in all sites from Summer 2008 to Summer 2009 except site D3.

## Functional Feeding Guild Composition

Changes were observed in the composition of functional feeding group composition from Summer 2008 to Summer 2009. Generally, from Summer 2008 to Summer 2009 collectors (filterers and gatherers) and shredders increased while predators decreased in many of the sites. Scrapers were not frequently encountered and lower in abundance compared to the other feeding groups. All values for the functional feeding guild composition are provided in Tables C9-C11.

At sites above the restored area (A1 and A2) predators were the most abundant feeding group in Summer 2008, ranging from 30.34-53.76% (Fig. 18). Collectors (filterers and gatherers) were the second most abundant feeding group, ranging from 31.46-40.86%. Scrapers were not a large component of the feeding guilds, ranging from 4.30-13.48%, and shredder abundance ranged from 1.08 -23.60%. The prevalent trends observed in Summer 2009 were a decrease in predators at site A1 and an increase in shredder abundance at sites A1 and A2. Shredder abundance ranged from 23.89-24.87% in Summer 2009. Collectors (filterers and gatherers) were the most abundant feeding group in Summer 2009 with values ranging from 37.31-43.37% (Fig. 18).

Within the restored area (D1, D2, and D3) predators were the most abundant group at sites D1 and D2, with values ranging from 45.60-50.00% in Summer 2008

(Fig. 19). Collectors (filterers) were the most abundant group at site D3 in Summer 2008 with a relative abundance of 56.46%. Predators decreased in abundance at sites D1 and D2 in Summer 2009 to 25.94-36.95%, but increased at site D3 to 24.40% relative abundance. Collectors (filterers and gatherers) increased in abundance at site D1 in Summer 2009 to 51.20% relative abundance and decreased at sites D2 and D3 to 34.97% and 15.91% (Fig. 19). Shredder abundance increased at all sites from a relative abundance of 3.41-13.40% in Summer 2008 to 16.38-51.19% in Summer 2009 (Fig. 19). Scrapers were not a large component of the feeding guild in Summer 2008 or Summer 2009; their values ranged from 7.14-13.07% in 2008 and 4.93-8.49% in 2009.

At sites below the restored area (B1, B2, and B3) shredders were the most abundant group at site B1 (26.40%) and collectors (filterers and gatherers) were the most abundant group at sites B2 and B3 with relative abundance of 39.91% and 45.75% respectively (Fig. 20). Shredders increased in abundance at all sites from Summer 2008 to Summer 2009 with relative abundance values ranging from 28.14-33.09%. Collectors increased at site B1 from Summer 2008 to Summer 2009 and decreased in abundance at sites B2 and B3 (Fig. 20). The relative abundance of collectors ranged from 27.82-39.70% in Summer 2009. Predators relative abundance decreased at each site below the restored area from Summer 2008 to Summer 2009 (Fig. 20). Relative abundance of predators ranged from 25.84-39.01% in Summer 2008 and from 23.84-29.32% in Summer 2009. Scrapers were not a large component of the feeding guilds in Summer 2008 or in Summer 2009. Relative abundance of

scraper relative abundance vales ranged from 5.67-20.79% in Summer 2008 and 8.04-9.77% in Summer 2009.

The functional feeding guild composition of Laurel Creek in Summer 2009 is closer to the hypothetical community structure provided by Vannote et al. (1980) in comparison to the Summer 2008 composition. Functional feeding guild composition for sites A2, D3, and B2 in Summer 2008, and A1 in Summer 2009 do not equal 100%, because a functional feeding guild classification was not available for the *Reomyia* (Diptera, Chironomidae) found at those sites. Relative abundance for *Reomyia* in Summer 2008 was 1.12% at site A2, 0.48% at site D3, and 0.45% at site B2. Relative abundance for *Reomyia* in Summer 2009 was 0.50% at site A1.

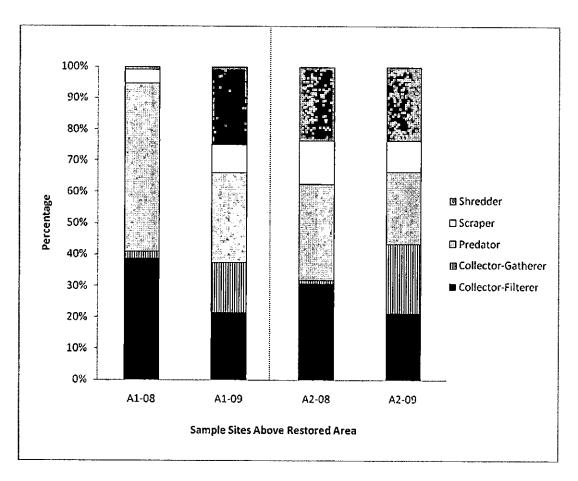


Figure 18. Comparison of macroinvertebrate functional feeding group assemblages in in sites above the restored area in Laurel Creek in Summer 2008 and Summer 2009.

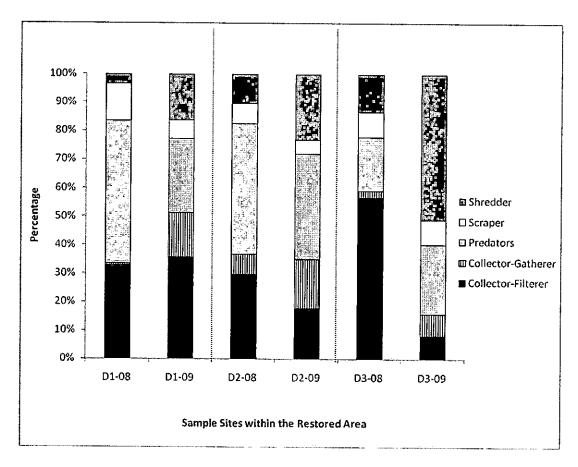


Figure 19. Comparison of macroinvertebrate functional feeding group assemblages in sites within the restored area in Laurel Creek in Summer 2008 and Summer 2009.

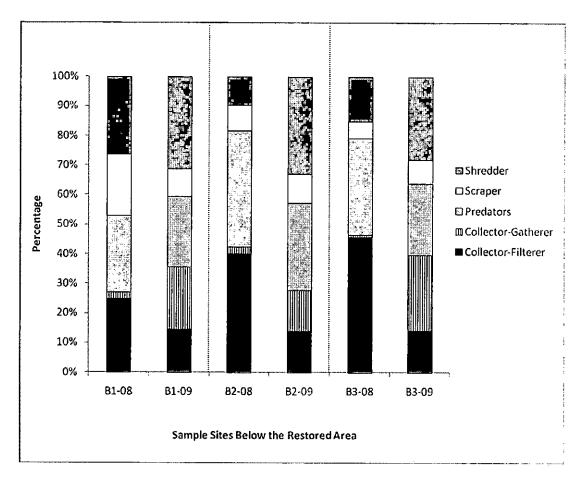


Figure 20. Comparison of macroinvertebrate functional feeding group assemblages in sites below the restored area in Laurel Creek in Summer 2008 and Summer 2009.

#### Habitat Assessment

Pre-restoration and post- restoration habitat assessment scores for Laurel Creek in Summer 2008 and Summer 2009 are presented in Tables 3 and 4. Sites within the restored area (D1, D2, and D3) were evaluated in Summer 2008, and all sites were evaluated in Summer 2009. In Summer 2008 overall habitat scores were all rated as partially supporting aquatic life (Fig. 21). In Summer 2009, sites A2 and D3 were rated as fully supporting aquatic life, and all other sites were rated as partially supporting aquatic life in Laurel Creek (Fig. 21).

Evaluations of habitat assessments conducted in Summer 2008 revealed the lowest scores in the following parameters: velocity/depth regime, channel flow status, bank stability, vegetative protection zone, and riparian vegetative zone width. For velocity/depth regime, site D1 scored in the poor category and sites D2 and D3 scored in the low end of the marginal category. For channel flow status, sites D2 and D3 scored in the low end of the marginal category. Water filled only 25-75% of the available channel. For bank stability, the right bank of D1 scored in the low end of the marginal category. Thirty to sixty percent of the stream bank had potential for future erosion. For vegetative protection zone, site D2 scored in the poor category. Kudzu (*Pueraria lobata*), a non-native invasive species of vine, was the dominant vegetation on the right bank of site D2. For riparian vegetative zone width, site D1 scored in the high end marginal category; the riparian habitat was only 6 to 12 meters wide.

Evaluations of habitat assessments conducted in Summer 2009 revealed the lowest scores in the following parameters: epifaunal substrate/available cover,

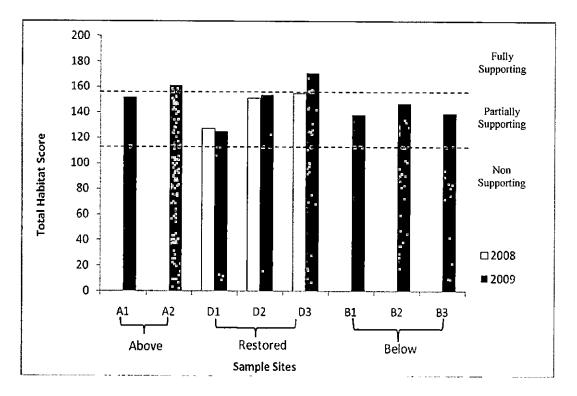


Figure 21. Comparison of overall habitat scores for sites in Laurel Creek in Summer 2008 and Summer 2009.

embeddedness, bank stability, vegetative protection zone, and riparian vegetative zone width. For epifaunal substrate/available cover site D1 scored in the marginal category. Habitat availability was less than desirable for colonization. For embeddedness, site B3 score in the high in marginal category. Gravel and cobble particles were 50-75% surrounded by fine sediment. For bank stability, site D1 scored in the high end of the marginal category. Thirty to sixty percent of the stream bank had potential for future erosion. For vegetative protection zone, sites D2 and B1 scored in the poor category. Kudzu (*Pueraria lobata*), a non-native invasive species of vine, was the dominant vegetation on the right bank of site D2. For riparian vegetative zone width, site D2 and B2 scored in the marginal category. The riparian habitat was only 6 to 12 meters wide at those sites.

Epifaunal Substrate available cover			D3
	12	15	16
Embeddedness	8	18	15
Velocity/Depth Regime	5	8	8
Sediment Deposition	17	20	18
Channel Flow Status	17	7	7
Channel Alteration	16	20	20
Frequency of Riffles (or bends)	10	18	17
Bank Stability- Left Bank	6	8	9
Bank Stability-Right Bank	4	9	8
Vegetative Protection- Left Bank	9	9	10
Vegetative Protection- Right Bank	8	3	9
Riparian Vegetative Zone Width-Left bank	5	10	9
Riparian Vegetative Zone Width-Right bank	10	6	9
Total Score	127	151	155
Narrative Classification <sup>1</sup>	PS	PS	PS

Table 3. Habitat assessment scores for sites within the restored area, pre-restoration, Summer 2008.

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Parameter	A1	A2	D1	D2	D3	B1	B2	B3
Epifaunal Substrate available cover	13	18	10	15	18	13	16	8
Embeddedness	16	15	13	16	15	11	15	10
Velocity/Depth Regime	10	14	12	13	15	13	13	13
Sediment Deposition	18	17	15	18	18	16	17	12
Channel Flow Status	14	15	15	15	15	12	15	15
Channel Alteration	16	18	12	18	19	15	16	16
Frequency of Riffles (or bends)	13	16	11	16	16	15	16	13
Bank Stability- Left Bank	9	10	5	8	9	6	8	8
Bank Stability-Right Bank	9	6	8	6	9	8	8	8
Vegetative Protection- Left Bank	10	10	6	9	10	6	8	8
Vegetative Protection- Right Bank	8	6	7	5	9	8	5	8
Riparian Vegetative Zone Width-Left Bank	10	10	3	10	10	5	6	10
Riparian Vegetative Zone Width-Right Bank	6	6	8	5	8	10	4	10
Total Score	152	161	125	154	171	138	147	139
Narrative Classification <sup>1</sup>	$\mathbf{PS}$	FS	PS	$\mathbf{PS}$	FS	PS	PS	PS

Table 4. Habitat assessment scores for all sites in Laurel Creek, post-restoration, in Summer 2009.

1. PS= partially supporting and FS= fully supporting

#### Water Chemistry

Results of water quality measurements from Laurel Creek are presented in Tables 5-6. Notable differences were not found between sample sites or between years. The water quality data was what one might expect from a stream in the Little Sandy River Drainage of the Western Allegheny Plateau as compared to KDOW water quality data. All parameters measured were similar for all eight sampling sites between years, except for a slight increase in conductivity and decrease in dissolved oxygen (% saturation and mg/L) in Summer 2009. The levels of conductivity and dissolved oxygen were not outside normal conditions to support aquatic life. Furthermore, a comparison was made with KDOW water quality background levels for reference reach headwater streams in the Western Allegheny Plateau region of eastern Kentucky. The levels collected were within the expected range with the exception of a high total suspended solids and turbidity reading at site B2 in Spring 2009 and a low pH reading at site A2 in Summer 2009.

A high measurement of total suspended solids and turbidity were collected and measured at site B2. This measurement was high due to logging upstream in Lovelace Fork that caused substantial turbidity and sediment loading to the creek in Spring 2009. At site B2 on 18 June 2008 a high pH reading of 10.14 and at A2 on 14 June 2009 a low pH reading of 2.62 were measured and recorded (Table 5). These measurements are thought to be due to faulty probe because the aquatic life was still flourishing at the site. In addition, water samples were taken back to the lab and

measured with a hand held pH meter; values were within the expect range of a pH of 6-9.

Site	Date	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (% Saturation)	Dissolved Oxygen (mg/L)	pH (standard Unit)
Al	17-Jun-08	15.85	227	129.5	12.81	6.07
A2	17-Jun-08	17.32	216	111.5	10.62	6.18
Dl	17-Jun-08	21.17	223	131.1	11.64	6.43
D2	17-Jun-08	20.36	207	118.7	10.70	6.07
D3	23-Jun-08	15.16	201	107.5	12.50	7.20
BI	18-Jun-08	14.21	250	111.4	11.39	6.42
B2	18-Jun-08	14.99	273	110.2	11.05	10.14
B3	23-Jun-08	15.65	201	101.5	10.56	7.01

Table 5. Water quality data from Laurel Creek in Summer 2008.

Site	Date	Temperature (°C)	Conductivity (µS/cm)	Dissolved Oxygen (% Saturation)	Dissolved Oxygen (mg/L)	pH (standard Unit)	Turbidity (average NTU)	TSS (mg/L)	Alkalinity as CaCO <sub>3</sub> (mg/L)
A1	17-Mar-09	12.20	120	108.8	11.66	7.11	4.37	4.8	
A2	17-Mar-09	11.17	121	106.8	11.60	7.23	4.26	3.6	-
D١	17-Mar-09	6.00	122	107.5	13.36	7.00	5.48	2.0	-
D2	17-Mar-09	7.29	114	109.4	13.20	7.29	9.82	1.6	_
D3	17-Mar-09	8.35	104	104.7	12.25	7.10	3.06	2.4	-
B1	19-Mar-09	8.63	123	101.0	11.78	6.80	2.95	2.0	_
B2	5-Apr-09	6.73	88	106.7	12.90	5.11	7.98	11.2	-
B3	19-Mar-09	8.51	99	111.7	13.06	7.03	17.70	8.0	-
A1	14-Jun-09	17.07	119	95.5	9.05	5.97	_		_
A2	14-Jun-09	18.10	124	76.7	7.22	2.62	_	-	-
DI	16-Jun-09	16.79	118	102.2	9.92	6.74	_	-	-
D2	16-Jun-09	16.88	107	100.2	9.72	5.56	_	-	-
D3	16-Jun-09	16.37	103	97.0	9.46	6.94	_	-	-
B1	16-Jun-09	16.67	112	88.8	8.65	6.03	-	-	_
B2	17-Jun-09	16.50	105	97.1	9.48	6.53	_	-	-
B3	18-Jun-09	15.84	107	97.7	9.63	6.61		-	-
Al	1-Jul-09	17.89	157	86.8	8.23	9.04	4.28	0.4	42
A2	1-Jul-09	18.08	163	83.3	7.87	8.2	4.09	0.8	40
D1	1-Jul-09	18.08	160	83.0	7.84	8.41	4.02	0.8	43
D2	1-Jul-09	17.23	155	88.9	8.53	7.54	4.27	0.8	46
D3	1-Jul-09	16.80	152	87.3	8.46	7.07	3.01	4.0	50
BI	2-Jul-09	16.34	164	80.5	7.88	8.30	3.67	0.4	61
B2	2-Jul-09	16.82	133	85.6	8.29	8.35	4.04	0.4	45
B3	<u>2-J</u> ul-09	16.54	140	83.2	8.11	7.28	4.03	0.8	51

Table 6. Water quality data from Laurel Creek in Spring and Summer 2009.

The (-) indicates parameter was not collected during the sample period.

### **Discussion**

#### Fish Community Health

Overall, the diversity of fishes, biomass, abundance, Shannon Diversity Index, and KIBI scores suggest fish communities are in fair to good condition. However, the fish community structure reflects a community that has been disturbed. The observed slight decline in abundance of intolerant, tolerant, and facultative headwater species, across all sites, may indicate a more transient response in the fish community to unusually low flows in Summer and Fall 2008. However, the decline in KIBI scores, abundance, and biomass of fishes only within and below the restored area may suggest that restoration has had a slight negative impact on the fish community.

Summer 2009 KIBI scores for Laurel Creek at all sites, excluding B3, were in the fair and good classification, and this was a decline from Summer 2008. Summer 2008 KIBI scores ranged from fair to excellent, with most sites described as good or excellent. Communities rated as "fair" and "good" are defined by fewer species, loss of intolerant species, and a stressed trophic structure (Karr et al. 1986). Site B3 was classified as excellent in Summer 2009, however, this is a wadeable (higher order) site whereas the other sites are headwater (lower order) sites. Site B3 is approximately 9.66 km downstream from the headwater sites and is affected by a variety of factors such as increased watershed size, additional tributaries, and heavy all terrain vehicle activity. Because site B3 is relatively insensitive to change in upper Laurel Creek (i.e. restoration), it is not directly comparable to the seven other headwater sites sampled in Laurel Creek.

## System-wide changes observed among sites

System-wide changes were those that were similar across all sites, and might suggest a source of disturbance to the fish communities other than the restoration. From Summer 2008 to Summer 2009 there was a slight system-wide decrease in abundance of intolerant fishes, such as *Clinostomus funduloides* and *Cottus bairdii*, and a system-wide increase in abundance of tolerant. In addition, a system-wide increase was observed in facultative headwater species at sites A1, A2, D1, D2, and B1. In general, facultative headwater species are generalist species that are not primarily associated with headwater streams, and natural or anthropogenic disturbance can cause an increase in these species (KDOW 2008). Because these changes occurred at all sites, factors other than restoration activities might have affected the fish communities.

Headwater streams undergo periods of intermittent flow during summer or fall months which could impact recruitment and overall densities from year to year. During periods of low flow, fishes are particularly vulnerable and re-colonization depends on distance an organism must travel, the available resources in the new section, and the pool of available colonists (Peterson and Bayley 1993, Moerke and Lamberti 2003). Generalist fish species may provide a base of fish species that are variable in composition, but remain relatively constant, thus providing a base for colonization (Kinsolving and Bain 1993). Generalist species are also better able to cope with environmental change compared to intolerant species (Poff and Ward 1990).

Unusually low flow in late Summer and Fall of 2008 may have affected the composition of fish species. In August 2008, the headwaters of Laurel Creek experienced sections of de-watering creating in-stream barriers, and the unusually low water levels continued throughout Fall 2008. This may help to explain why a system-wide increase in relative abundance of tolerant and facultative headwater species and a decrease in intolerant fishes were observed. Tolerant (generalist) fish species may have been able to colonize the sites above, within, and below the disturbed area following the months of low water levels and post-restoration disturbance. To fully address this observation, follow up bioassessments should be conducted to further assess the fish community.

## Differential changes observed among sites

Differential changes are those measures which showed a decline in fish community health within and below the restoration, but did not show the same decline in sites above the restoration. This may indicate restoration as the disturbance. A decline in abundance, biomass, and overall KIBI scores were observed in sites within and below the restoration, but the same decline was not observed in sites above the restoration. Similar studies found that areas disturbed by anthropogenic sources were not devoid of fish, but disturbed sections had lower density and fewer fish species compared to less disturbed sections (Kinsolving and Bain 1993, Paller et al. 2000, Moerke and Lamberti 2003, Miltner 2004, Helfman 2007). Other studies reported an increase in abundance and biomass following in-stream restoration.

However, the species that increased in abundance were categorized as tolerant species (Shields et al. 2003, Hrodey and Sutton 2008).

The timing of the major restoration activity in Laurel Creek also coincided with a period of unusually low flow in 2008. During periods of low flow, pools within streams offer a place of refuge for the fish community. Fishes may become concentrated in the remaining isolated pools and conditions within these pools may be less than optimal. Therefore, effects from restoration activities, such as major moving of earth, installing cross vane weirs with large machinery in the creek, and disturbance to the riparian zone from removing non-native invasive plant species, may have created further disturbance to the fish community during this vulnerable period.

Fish are important to use in bioassessments of restoration as they are present in a stream continuously and they respond to transient effects which may not be detected by other measurements such as water quality or habitat assessments. Periods of intermittency experienced in Laurel Creek may help to explain the increase in relative abundance of tolerant species, decrease in relative abundance of intolerant species, and increase in relative abundance of facultative headwater species. Other fish community measures, such as the decline in abundance, biomass, and KIBI scores suggest restoration has had some effect on the fish community. The magnitude of these declines was not high, suggesting that negative effects on the fish community were only minimal. To fully address these observations, continued bioassessments

should be conducted in Laurel Creek to determine if these effects persist for a longer period of time.

#### Aquatic Macroinvertebrate Community Health

Overall, a positive system-wide response was observed in aquatic macroinvertebrates between Summer 2008 and 2009. Many of the biotic metrics within the MBI increased, the Shannon Diversity Index improved, and functional feeding guild composition more closely resembled the hypothetical community structure in Summer 2009 compared to Summer 2008. Restoration does not seem to have impacted the aquatic macroinvertebrate community.

Much research has focused on using macroinvertebrate community structure for biomonitoring lotic systems after disturbance (Hynes 1970, Wallace 1990, Richardson and Kiffney 2000, Muotka and Lassonen 2002, Pond 2000, Korsu 2004, Bae et al. 2005, Churchel and Batzer 2006, Walther and Whiles 2008). Several studies concluded that aquatic macroinvertebrate communities recovered quickly after disturbance from colonization, but the macroinvertebrate species composition changed following restoration (Wallace 1990, Pond 2000, Korsu 2004). The results of this study indicate improvement in the health of the aquatic macroinvertebrate communities from Summer 2008 to Summer 2009; however some MBI metrics and the overall MBI scores were somewhat lower than what is expected from an undisturbed headwater stream in the Little Sandy River drainage (Pond et al. 2003). The improvement seen in aquatic macroinvertebrate community health may be attributed to the length of time between stream restoration activities and bioassessment sampling. During that seven month period, aquatic macroinvertebrates could have re-colonized the stream (Molles 1985, Wallace 1990, Korsu 2004). The recovery seen in the aquatic macroinvertebrate community is probably due in part to the mobility of the adults and rapid life cycles of some aquatic macroinvertebrates (e.g., dipterans; Hynes 1970, Davies 1976, Pinder 1986, Wallace 1990, Merritt and Cummins 1996, Merritt et al. 2008).

While there was an improvement seen in the overall macroinvertebrate community health, values are lower for all sampled sections of Laurel Creek when compared to reference reach data for mountain headwater streams of Kentucky (Pond et al. 2003). Taxa richness, EPT richness, m%EPT, %Ephem, %Chir + %Olig values, and MBI scores recorded for Laurel Creek increased from Summer 2008 to Summer 2009, but these metrics were not within the expected ranged determined by Pond et al. (2003). These results may be attributed to when the bioassessment sampling was conducted. Bioassessment activity was outside of the suggested sampling period for a headwater stream in the Little Sandy River drainage (Pond et al. 2003). Spring (i.e., February to May) is the most appropriate time to sample a mountainous headwater stream in Kentucky (Pond et al. 2003, KDOW 2008); the lower scores may be a result of sampling for aquatic macroinvertebrates in June of 2008 and June 2009. Sampling in summer months may fail to record temperature and oxygen sensitive species, as

well as species that have emerged from the stream in early Spring (e.g., plecopterans and ephemeropterans; Hynes 1970, Pond et al. 2003).

One metric of the MBI, %clingers, showed a large decline in relative abundance of clinger organisms in Laurel Creek between Summer 2008 and Summer 2009. However, the un-standardized data for the relative abundance of clingers at the sites above and two sites within the restored area actually showed an increase in the relative abundance of clingers. The un-standardized data for one site within and three sites below the restored area showed a decrease in relative abundance of clingers. The overall abundance of macroinvertebrates at each site is numerically masking the abundance of clingers at those sites. Clingers are good indicators of sedimentation in a lotic system since they require hard silt-free substrates to cling to (KDOW 2008). Restoration activities might have increased sedimentation downstream, but prerestoration data are unavailable for comparison.

## Functional Feeding Guild Composition

Although many aquatic macroinvertebrates are considered omnivorous feeders, they can still be classified into feeding groups based on their resource preference (Vannote et al. 1980, Pond 2000). Relative abundance of functional feeding group composition provides useful information on the overall trophic organization and food resource dynamics of streams. The abundance of various functional feeding groups shifted from Summer 2008 to Summer 2009. The functional feeding guild present in Laurel Creek in Summer 2009 resembled a pattern

close to the River Continuum Concept (Vannote et al. 1980). Scrapers were not a large component of the feeding guilds in Summer 2008 or Summer 2009. Scrapers are not as prevalent in forested headwaters streams because they graze on benthic algal communities which are limited by light (Vannote et al. 1980, Muotaka and Laasonen 2002, Pond 2000). Predator abundance decreased at most sites from Summer 2008 to Summer 2009, however, the decrease was not substantial. Predators are expected to make up <15% of the functional feeding guild (Vannote et al. 1980). This study found a somewhat higher predator value (23-36%). Collectors and shredders dominated the functional feeding community in Summer 2009, comprising more than 58% of the community at each site. The relative abundance of collectors increased at some sites and decreased at other sites from 2008 to 2009, independent of sample site. Shredders increased at all sites from Summer 2008 to Summer 2009. However, shredder abundance is still somewhat lower than the hypothetical value (35%) expected in a functional feeding guild of an undisturbed headwater stream (Vannote et al. 1980). Two explanations are possible for these findings: shredders could have been underestimated because non-riffle habitats were not included in the functional feeding guild calculations (which are based only on the semi-quantitative samples), and lack of sufficient riparian vegetation may be a limiting factor to the shredder community.

Shredders should have comprised a larger proportion of the functional feeding guild than was observed in Laurel Creek in both Summer 2008 and 2009 in comparison to Vannote et al. (1980). However, since quantitative sampling was

conducted in riffles and functional feeding guild composition was calculated from those samples, the shredder composition may have been underestimated. Shredders, such as *Eurylophella* (Ephemerellidae), *Pycnopsyche* (Limnephilidae), and *Lepidostoma* (Lepidostomatidae) were found in other habitats within each sample site that were not included in the calculations (Muotka and Laasonen 2002, Pond 2000).

In addition, shredder composition could have been limited by allochthonous input from the riparian vegetative zone. Riparian vegetative zone width was degraded at sites where the road was adjacent to the creek or other anthropogenic disturbance was present. Thus, riparian vegetative zone width was most likely limited by the existence of the road, which was barricaded to vehicle traffic prior to restoration in Fall 2008, but never fully removed. Therefore, as time progresses and the riparian zone further recovers, one might expect to see increased shredder abundance in years to come.

Overall, a positive system-wide change was observed in aquatic macroinvertebrates between Summer 2008 and 2009. Many of the measures of aquatic macroinvertebrate community health indicated improvement between years and probably suggest that restoration has not impacted the macroinvertebrate communities.

# **Conclusion**

The results from this study indicate that restoration may have had a minimal negative impact on the fish community as supported by decreased KIBI scores,

abundance, and biomass within and below the restored area of Laurel Creek, but it does not appear to have impacted the aquatic macroinvertebrate community. Fish populations were heavily impacted during the unusually low flow events in late Summer and Fall 2008, and would not have had time to recover by Summer 2009 sampling. However, it is not surprising that the macroinvertebrate communities showed an improvement between years considering many aquatic macroinvertebrates possess short life cycles and the ability to rapidly re-colonize a disturbed area (Molles 1985, Wallace 1990, Merritt et al. 2008, Korsu 2004). The results from this study do not suggest ecologically significant impacts since the trends observed in the fish community were minor and the macroinvertebrate community seemed to improve. Continued biological monitoring is needed to examine long-term effects of restoration in Laurel Creek to the biological communities.

# Future Direction

Stream restoration projects are becoming increasingly common, but biological assessments following restoration are still lacking (Alexander and Allan 2006, Walther and Whiles 2008). Ecological restoration will continue to play an important role in natural resource management to restore function to streams and rivers. Preand post-restoration data are needed to document changes in the abiotic and biotic components of an ecosystem to determine if restoration has been successful (Kondolf and Micheli 1995, Helfman 2007, Walther and Whiles 2008).

The results of this study lend insight into the short-term effects (one year) of restoration on the biological community of Laurel Creek. It is very likely that this study was not long enough to document full recovery of the biological community (Detenbeck et al. 1992). Additional monitoring is needed to examine long-term effects of restoration on fish and macroinvertebrate communities, which will allow for a better understanding of these restoration efforts.

In addition, there were limitations inherent in the design of this study. Future research should focus on increasing the number of sample sites within the stream being restored as well as sampling ecologically and geologically equivalent streams for replication. Sampling should be conducted at the appropriate assessment season for each biological community (in the Spring for aquatic macroinvertebrates and in the Summer or Fall for fishes) to obtain accurate abundance and diversity measures. Bioassessment monitoring of stream restoration projects is valuable for determining the ecological integrity of a stream, and documenting the health and resilience of the stream community (Karr et al. 1986).

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Appendix A Sample Site Locations and Formula Table

Site	Latitude	Longitude	County	Watershed
Above 1 (A1)	N38.15285°	W083.25982°	Rowan	2.31 km <sup>2</sup>
Above 2 (A2)	N38.1537°	W083.26102°	Rowan	2.12 km <sup>2</sup>
Damaged 1 (D1)	N38.15060°	W083.25906°	Rowan	2.46 km <sup>2</sup>
Damaged 2 (D2)	N38.14640°	W083.25529°	Rowan	3.73 km
Damaged 3 (D3)	N38.14331°	W083.24782°	Rowan	5.54 km <sup>2</sup>
Below 1 (B1)	N38.13491°	W083.23613°	Rowan	7.54 km <sup>2</sup>
Below 2 (B2)	N38.13277°	W083.23380°	Rowan/Elliott	13.49 km <sup>2</sup>
Below 3 (B3)	N38.13164°	W083.19317°	Elliott	37.16 km <sup>2</sup>

Table A1. List of the eight sites sampled in Laurel Creek watershed, Rowan and Elliott Counties, Kentucky.

Metric	95%ile or 5%ile	Formula for calculation MBI subcomponents
TR	63	TR(100)/63
EPT	33	EPT(100)/33
m%EPT	86.9	m%EPT(100)/86.9
%Ephem	66.5	%Ephem(100)/66.5
%Chir + %Olig	0.68	((100-%chir+%Olig)/(100-0.68))100
%Clingers	75.5	%Clingers(100)/75.5
%mHBl	2.18	((10-mHBI)/(10-2.18))100

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 Table A2. Statewide 95%ile or 5%ile values used by KDOW for each metric for headwater streams

 (Pond et al. 2003).

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Appendix B Fish Community Data

Fish family or species	Above 1	Above 2	Damaged 1	Damaged 2	Damaged 3	Below 1	Below 2	Below 3
Petromyzontidae	-				Ç			
Lampetra aepyptera		1	2		2	2 ·	1	5
(least brook lamprey)								
Cyprinidae								
Campostoma anomalum (central stoneroller)	2	1	3	1	6	1	7	7
Clinostomus funduloides (rosyside dace)	17	34	54	51	61	34	34	102
Notropis buccatus (silverjaw minnow)								10
Notropis photogenis (silver shinner)								24
Pimephales notatus (bluntnose minnow)								6
(blacknose dace)	32	13	39	33	39	16	32	41
Semotilus atromaculatus (creek chub)	13	9	21	25	37	24	34	62
Catostomidae								
Catostomus commersonii (white sucker)	1	9	2	7	23	2	8	4
<i>Hypentilium nigricans</i> (nothern hog sucker)						I		6
Salmonidae				•				
Salmo trutta							1	
(brown trout)								

Table B1. Fishes collected in Laurel Creek, Rowan County, KY in June 2008.

Fish family or species	Above 1	Above 2	Damaged 1	Damaged 2	Damaged 3	Below 1	Below 2	Below
Cottidae		-						
Cottus bairdii	10	13	20	24	36	51	21	36
(mottled sculpin)								
Percidae								
Etheostoma caeruleum							11	4
(rainbow darter)								
Etheostoma flabellare		1			3	I	2	
(fantail darter)								
Etheostoma nigrum	9	1	6	11	14	6	15	51
(johnny darter)								
Total individuals	84	82	147	152	221	138	166	358
Total Species	7	9	8	7	9	10	11	13
Biomass (grams)	257.3	336.4	407.9	616.8	991.8	516.2	813.9	953.5
KIBI score (headwater)	56	60	71	70	69	76	65	69
narrative classification	Fair	Good	Excellent	Good	Good	Excellent	Good	Good

Table B1. (Continued) Fishes collected in Laurel Creek, Rowan County, KY in June 2008.

Fish family or species	Above 1	Above 2	Damaged 1	Damaged 2	Damaged 3	Below 1	Below 2	Below 3
Petromyzontidae								
Lampetra aepyptera		3	4		6		1	1
(least brook lamprey)								
Cyprinidae								
Campostoma anomalum	1	3	6	1	5	3	2	3
(central stoneroller)								
Clinostomus funduloides		21	60	18	69	14	14	14
(rosyside dace)								
Notropis buccatus						1		1
(silverjaw minnow)								
Notropis photogenis (sliver minnow)								88
Rhinichthys atratulus	16	16	26	10	24	9	11	7
(blacknose dace)								
Semotilus atromaculatus	8	14	24	8	4	17	14	5
(creek chub)								
Catostomidae								
Catostomus commersonii		3	6	1	1		1	I
(white sucker)								
Hypentilium nigricans								1
(northern hog sucker)								
Cottidae								
Cottus bairdii	12	17	19	10	26	40	5	8
(mottled sculpin)								

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Table B2. Fishes collected in Laurel Creek, Rowan County, KY in March and April 2009.

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Fish family or species	Above 1	Above 2	Damaged 1	Damaged 2	Damaged 3	Below 1	Below 2	Below 3
Percidae								
Etheostoma caeruleum								2
(rainbow darter)								
Etheostoma flabellare			2		1		1	
(fantail darter)								
Etheostoma nigrum	11	t	13	1	7	1	4	7
(johnny darter)								
Total individuals	48	78	160	49	143	85	53	138
Total species	5	8	9	7	9	7	9	12
Biomass (grams)	124.0	239.1	492.8	174.6	411.9	132.7	206.9	215.1
KIBI score (headwater)	35	57	74	36	74	55	53	77
narrative classification	Poor	Fair	Excellent	Poor	Excellent	Fair	Fair	Excellent

Table B2. (Continued) Fishes collected in Laurel Creek, Rowan County, KY in March and April 2009.

Fish family or species	Above 1	Above 2	Damaged 1	Damaged 2	Damaged 3	Below 1	Below 2	Below 3
Petromyzontidae								
Lampetra aepyptera		7	3	1	3		1	4
(least brook lamprey)								
Cyprinidae								
Campostoma anomalum	3	4		1	1	2	2	6
(central stoneroller)								
Clinostomus funduloides	13	31	21	20	17	24	11	31
(rosyside dace)								
Notropis buccatus								3
(silverjaw minnor)								
Notropis photogenis								38
(silverjaw minnow)								
Pimephales notatus								1
(bluntnose minnow)								
Rhinichthys atratulus	65	17	22	19	11	26	15	11
(blacknose dace)								
Semotilus atromaculatus	36	22	22	12	26	30	33	30
(creek chub)								
Catostomidae								
Catostomus commersonii		2	2	3	5	1	3	4
(white sucker)								
Hypentilium nigricans								2
(northern hog sucker)								
Salmonidae								
Oncorhynchus mykiss								1
(rainbow trout)								

Table B3. Fishes collected in Laurel Creek, Rowan County, KY in July 2009.

Fish family or species	Above 1	Above 2	Damaged 1	Damaged 2	Damaged 3	Below 1	Below 2	Below 3
Cottidae								
Cottus bairdii	8	12	17	8	22	39	18	33
(mottled sculpin)								
Percidae								
Etheostoma caeruleum							2	2
(rainbow darter)								
Etheostoma flabellare		1		1	1		2	
(fantail darter)								
Etheostoma nigrum	5	1	7	1	2		1	7
(johnny darter)								
Total individuals	130	97	94	66	88	122	88	173
Total species	6	9	7	9	9	6	10	14
Biomass (grams)	339.0	405.2	336.4	214.7	410.7	507.5	349.9	695.5
KIBI score (headwater)	60	59	56	55	59	64	57	72
narrative classification	Good	Good	Fair	Fair	Good	Good	Good	Excellent

Table B3. (Continued) Fishes collected in Laurel Creek, Rowan County, KY in July 2009.

Metric	Date	A 1	A 2	D1	D 2	D 3	B 1	B 2	B3
DMS	Jun08	2	3	2	2	3	3	4	3
INT	Jun08	2	2	2	2	2	2	2	3
SL	Jun08	3	3	3	3	3	4	4	6
%INSCT	Jun08	42.9	59.8	54.4	56.6	51.6	67.4	51.0	62.3
%TOL	Jun08	54.8	37.8	42.3	42.8	44.8	30.4	44.6	31.6
%FWH	Jun08	51.2	18.3	32.7	29.6	26.7	16.7	32.5	NA

Table B4. Values for core Kentucky Index of Biotic Integrity Scores- June 2008

Metric	Date	Above 1	Above 2	Damage 1	Damage 2	Damage 3	Below 1	Below 2	Below 3
DMS	Mar 09	2	2	3	2	3	2	3	3
INT	Mar 09	1	2	2	2	2	2	2	3
SL	Mar 09	1	3	3	3	3	2	3	6.0
%INSCT	Mar 09	47.9	50	58.8	59.2	72.0	64.7	45.3	87.0
%TOL	Mar 09	50.0	42.3	35.0	38.8	20.3	30.6	49.1	9.42
%FWH	<u>Mar</u> 09	58.3	25.6	28.1	24.5	25.2	16.5	32.1	NA

Table B5. Values for core Kentucky Index of Biotic Integrity Scores- March 2009.

Metric	Date	Above 1	Above 2	Damage 1	Damage 2	Damage 3	Below 1	Below 2	Below 3
DMS	Jul09	2	3	2	3	3	1	4	3
INT	Jul09	2	2	2	2	2	2	2	4
SL	Jul09	2	3	3	3	3	3	4	6
%INSCT	Jul09	20.0	46.4	47.9	45.5	47.7	51.6	38.6	65.9
%TOL	Jul09	77.7	42.3	48.9	51.5	47.7	46.7	57.8	26.6
%FWH	Jul09	56.0	24.7	33.0	36.4	21.6	23.7	23.9	NA

Table B6. Values for core Kentucky Index of Biotic Integrity Scores- July 2009

10 2009	•		
Sites	June 2008	March 2009	July 2009
A1	84	48	130
A2	82	78	97
D1	147	160	94
D2	152	49	66
D3	221	143	88
B1	138	85	122
B2	166	53	88
B3	358	138	173

Table B7. Total abundance of fishes collected in Laurel Creek from 2008 to 2009.

Table B8. Total biomass (grams) of fishes collected in Laurel Creek from 2008 to 2009.

10200			
Sites	June 2008	March 2009	July 2009
A1	257.3	124.0	339.0
A2	336.4	239.1	405.2
D1	407.9	492.8	336.4
D2	616.8	174.6	214.7
D3	991.8	411.9	410.7
B1	516.2	132.7	507.5
B2	813.9	206.9	349.9
B3	953.5	215.1	695.5

			Shannon's	
Sample		# of	Diversity	Shannon's Evenness Index
Site	Abundance	Species	Index (H')	(J')
A1	84	7	1.61	0.83
A2	82	9	1.65	0.75
D1	147	8	1.60	0.77
D2	152	7	1.65	0.85
D3	221	9	1.87	0.85
B1	138	10	1.63	0.71
B2	166	11	2.02	0.84
B3	358	13	2.07	0.81

Table B9. Shannon Diversity Index values for fish communities in Laurel Creek inSummer 2008.

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			Shannon	
Sample		# of	Diversity	Shannon Evenness Index
Site	Abundance	Species	Index (H')	(J')
A1	48	5	1.43	0.89
A2	78	8	1.75	0.84
D1	160	9	1.80	0.82
D2	49	7	1.55	0.80
D3	143	9	1.53	0.70
B1	85	7	1.43	0.74
B2	53	9	1.80	0.82
B3	138	12	1.39	0.56

Table B10. Shannon Diversity Index values for fish communities in Laurel Creek in Spring 2009.

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Comple			Shannon	
Sample Site		# of	Diversity	Shannon Evenness Index
Sile	Abundance	Species	Index (H')	(J')
A1	130	6	1.32	0.73
A2	97	9	1.71	0.78
D1	94	7	1.71	0.88
D2	66	9	1.68	0.76
D3	88	9	1.75	0.80
B1	122	6	1.47	0.82
B2	88	10	1.73	0.75
B3	173	14	2.09	0.84

 Table B11. Shannon Diversity Index values for fish communities in Laurel Creek in

 Summer 2009.

Appendix C. Aquatic Macroinvertebrate Community Data

		TV'	$\overline{CL}^2$	A1	A2	D1	D2	D3	BI	B2	<b>B</b> 3
Coleoptera											
Dryopidae	Helichus	4.6	х	2	9	6	6	12	6	7	
Elmidae	Dubiraphia	6.4									
Elmidae	Optioservus	2.36	х			4	2	2	30	8	3
Elmidae	Oulimnius	1.78	х					1	I		
Elmidae	Stenelmis	5.1	x		10			1		1	
Hydrophilidae	Tropisternus	9.7	x						1		
Psephenidae	Ectopria	4.61	x			4		1		1	
Psephenidae	Psephenus herricki	2.35	x		Ι	5	4				
Ptilodactylidae	Anchytarsus bicolor	3.65	x		19	1					
Diptera											
Ceratopogonidae	Atrichopogon	6.49					1				
Ceratopogonidae	Bezzia	6.9							3		
Chironomidae	Chironomus	9.63									
Chironomidae	Cricotopus	7					I		1		
Chironomidae	Helopelopia	6.2			2	I	I	3		1	5
Chironomidae	Microtendipes	5.5									
Chironomidae	Parametriocnemus	3.65			2		9	2	3	7	1
Chironomidae	Polypedilum	6.8	х		8			3	11	2	10
Chironomidae	Reomyia	7			2			1		1	
Chironomidae	Rheocricotopus	7.3						1			
Chironomidae	Tanytarsus	6.7	x				2				
Chironomidae	Thienemannimyia gp	5.9			8	2	б	2	9	11	44
Chironomidae	Żavrelimyia	5.3					1				
Simulidae	Prosimulium	4.01	х	1							
Simulidae	Simulium	4.4	x								9
Tabanidae	Tabanus/Whitneyomyia	9.22								1	

Table C1. Taxa collected in the semi-quantitative samples in Laurel Creek, Summer 2008.

		$TV^1$		A1	A2	D1	D2	D3	B1	B2	B3
Tipulidae	Antocha	4.25	х	2							
Tipulidae	Crytolabis	4.9							1		
Tipulidae	Dicranota	0		5	5	1	4	2		6	5
Tipulidae	Hexatoma	4.31		1	2	3	20	12	8	6	
Tipulidae	Pseudolimnophila	7.22							2	1	
Tipulidae	Tipula	7.3			2	2	2	1			1
Ephemeroptera											
Baetidae	Unid Baetid	5		2			1			5	
Caenidae	Caenis	7.41					1	1			
Ephemerellidae	Eurylophella	4.34	x				1	1			
Ephemeridae	Ephemera	1.1					1				
Heptageniidae	Stenacron	4	x			1					
Heptageniidae	Stenonema	4.2	x	1	2	4		1		2	11
Isonychiidae	Isonychia	3.45			13	8	13	22	17	12	24
Leptophlebiidae	Leptophlebia	6.23									1
Hemiptera											
Veliidae	Microvelia	9		12	4	5	7	4	1		
Veliidae	Rhagovelia	9		8	5	3	2	1	-		10
Megaloptera	0				-	-	-	•			
Corydalidae	Nigronia	5.3	х	16	5	50	12	6	18	42	5
Odonata	0				-			Ŭ	10	12	5
Aeshnidae	Boyeria	6		1	1	3	8	4			
Cordulegastridae	Cordulegaster	5.73				-	·	1			
Gomphidae	Gomphus	5.8		1	5	16	17	2			
Plecoptera	•				-			-			
Capniidae	Allocapnia	2.52		1	13	3	16	24	34	20	27
Perlidae	Acroneuria	1.4	х	-	1	÷		~ 1	2-1	20	41
Perlidae	Perlesta	4.7	x	4	16	4	2	2	5	12	10

Table C1. (Continued) Taxa collected in the semi-guantitative samples in Laurel Creek, Summer 2008.

		TV <sup>1</sup>	$CL^2$	<u>A1</u>	A2	D1	D2	D3	Bİ	B2	B3
Trichoptera											
Goeridae	Goera	0.13			2		1				
Hydropsychidae	Ceratopsyche	1.4	x	3			1				
Hydropsychidae	Cheumatopsyche	6.22	x	22	20	36	37	65	24	64	57
Hydropsychidae	Hydropsyche	4	x	8							12
Philopotamidae	Chimarra	2.76	х		8	13	3	29	3	12	8
Philopotamidae	Dolophilodes	0.81	x	2	13	1		2		1	1
Polycentropodidae	Cyrnellus	7.34	x								2
Rhyacophilidae	Rhyacophila	0.8	x								1
Uenoidae	Neophylax	2.2	x	1							

Table C1. (Continued) Taxa collected in the semi-quantitative samples in Laurel Creek, Summer 2008.

		A1	A2	Dl	D2	D3	B1	B2	B3
Coleoptera					•	_			
Dryopidae	Helichus								29
Elmidae	Dubiraphia	I							5
Psephenidae	Ectopria				1				
Decapoda									
Cambaridae	Cambarus					1			
Cambaridae	Cambarus bartonii								1
Cambaridae	Cambarus robustus								1
Cambaridae	Orconectes				1				
Diptera									
Chironomidae	Chironomus	1	1				1		
Chironomidae	Helopelopia	1							
Chironomidae	Microtendipes	7	4			5	2		1
Chironomidae	Reomyia	3							
Chironomidae	Stictochironomus	10	30			1			2
Chironomidae	<i>Thienemannimyia</i> gp.								
Chironomidae	Xylotopus		1						
Culicidae	Unid Culicidae	2						1	
Dixidae	Dixella	1							
Tipulidae	Tipula						3	1	

Table C2. Taxa collected in the qualitative samples, but not in the semi-quantitative samples, Summer 2008.

		A1	A2	D1	D2	D3	B1	B2	B3
Ephemeroptera									
Baetidae	Unid Baetid		2						
Baetiscidae	Baetisca								1
Caenidae	Caenis								2
Ephemerellidae	Eurylophella	5	2				1		6
Ephemeridae	Ephemera								3
Heptageniidae	Stenacron	10	4		3		9	3	
Heptageniidae	Stenonema				5		1		
Isonychiidae	Isonychia	2							
Leptophlebiidae	Leptophlebia	1	1						
Hemiptera									
Gerridae	Aquarius	1	2	3	2	1		7	2
Gerridae	Gerris		1	2		I			1
Megaloptera									-
Sialidae	Sialis	2							
Odonata									
Aeshnidae	Boyeria						1		3
Calopterygidae	Calopteryx							2	- 1
Cordulegastridae	Cordulegaster		2					_	
Plecoptera	Ų								
Nemouridae	Amphinemura							2	
Trichoptera	•								
Glossosomatidae	Glossosoma								1
Limnephilidae	Pycnopsyche	14	13	27	17	26		2	20
Philopotamidae	Wormaldia					I		-	
Polycentropodidae	Cyrnellus		1			-			
Rhyacophilidae	Rhyacophila							2	
Uenoidae	Neophylax		3	1	22	21	21	2	22

Table C2. (Continued). Taxa collected in the qualitative samples, but no in the semi-quantitative samples, Summer 2008.

		$TV^1$	CL <sup>2</sup>	A1	A2	D1	D2	_D3	B1	B2	B3
Coleoptera											
Dryopidae	Helichus	4.6	х	2	2	2	1	2	4	3	
Dytiscidae	Hydroporous	8.62		2	1					2	
Elmidae	<b>Optioservus</b> larvae	2.36	x	1	20	4		7	8	14	8
Elmidae	Oulimnius	1.78	х					1			
Elmidae	Stenelmis	5.1	х		1				1	1	
Hydrophilidae	Helochares	8.3							1		
Hydrophilidae	Hydrobiomorpha	9					1				
Psephenidae	Ectopria	4.16	х	4	2	3	1	4			
Psephenidae	Psephenus herricki	2.35	х	1	1	4			I	1	
Ptilodactylidae	Anchytarsus bicolor	3.64	x	3	9	t					
Decapoda											
Cambaridae	Cambarus	4.9								1	
Cambaridae	Cambarus bartonii	4.59					1				
Cambaridae	Orconectes cristavarius	5.47		1		1		1		1	1
Diptera											
Athericidae	Atherix	2.1									1
Ceratopogonidae	Bezzia/Palpomyia	6.9		1			1		3	3	3
Ceratopogonidae	Serromyia	7								1	
Chironomidae	Cladotanytarsus	4.09									1
Chironomidae	Chironomus	9.63				1					
Chironomidae	Corynoneura	6.01			t					1	
Chironomidae	Cricotopus	7			2	1		1	1	1	6
Chironomidae	Demicrytochironomus	2.12									1
Chironomidae	Dicrotendipes	8.1			2						
Chironomidae	Diplocladius	7								1	
Chironomidae	Helopelopia	6.2			2	2		1			
Chironomidae	Krenosmittia	0		1							

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Table C3. Taxa collected in the semi-quantitative samples in Laurel Creek, Summer 2009

1. Tolerance values (KDOW 2008) 2. Clinger organisms denoted by "x." (KDOW 2008)

		$TV^1$	$CL^2$	Al	A2	DI	D2	D3	BI	B2	B3
Chironomidae	Microspectra	1.52		1		1	2	1		_	1
Chironomidae	Microtendipes	5.5		2	1	3	1			1	
Chironomidae	Natarsia	9.95						1			
Chironomidae	Orthocladius	7.3		1							1
Chironomidae	<b>Paracricotopus</b>	4.7		1	1	1					
Chironomidae	Parakiefferiella	5.4		1							
Chironomidae	Parametriocnemus	3.65		8	13	6	9	4	2	2	17
Chironomidae	Paratendipes	5.11								1	
Chironomidae	Paratrichocladius	7			5	1		2	2	4	1
Chironomidae	Phaenospectra	6.5	х						1		
Chironomidae	Polypedilum	6.8	х	2	4	2	5	3	3	3	6
Chironomidae	Potthastia	6.4							1		
Chironomidae	Pseudorthocladius	1.51					1		1	1	11
Chironomidae	Reomyia	7		I							
Chironomidae	Rheotanytarsus	6.4	х	1							
Chironomidae	Stictochironomus	6.52		1							
Chironomidae	Subletta	7					1				
Chironomidae	Tanytarsus	6.7	x	1		1	1	1	2	1	2
Chironomidae	Thienemannimyia gp.	5.9		6	8	6	7	6	5	1	5
Chironomidae	Tvetenia	3.6			11	2	6	2	2	2	1
Chironomidae	Zavrelimyia	5.3		1	2		1				
Dixidae	Dixa	2.55				I					
Empididae	Chelifera	8.1								1	1
Simuliidae	Prosimulium	4.01	x	4	15	9	4	4	1	4	9
Simuliidae	Simulium	4.4	x	10	23	26	11	4	8	8	9

Table C3. (Continued) Taxa collected in the semi-quantitative samples in Laurel Creek, Summer 2009.

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		τv'	CL <sup>2</sup>	_ A1	A2	DI	D2	D3	B1	B2	B3
Tabanidae	Tabanus/Whitneyomyia	9.2									1
Tipulidae	Antocha	4.25	x	2							
Tipulidae	Dicranota	0		12	18	5	9	13	2	4	5
Tipulidae	Hexatoma	4.31		1	4	2	3	4	1	5	1
Tipulidae	Pseudolimnophila	7.22		1			2	5	9	6	2
Tipulidae	Tipula	7.3					1				
Tipulidae	Unid Tipulid	7.33			2					3	
Ephemeroptera											
Baetidae	Acentrella	3.6		15	35	30	15	15	25	15	13
Caenidae	Caenis	7.41								ľ	
Ephemerellidae	Drunella	0.7	x		5	1	1	3		1	
Ephemerellidae	Eurylophella	4.34	х	1		2	1	5			1
Ephemerellidae	Timpanoga	2	х							1	
Ephemeridae	Ephemera	1.1				1					
Heptageniidae	Maccaffertium	3									4
Heptageniidae	Stenacron	4									1
Heptageniidae	Stenonema	4.1	x	6	2	3	3	6		4	1
Isonychiidae	Isonychia	3.45		7	5	14	16	7	13	15	1
Letophlebiidae	Habrophlebia	0.5		1							
Letophlebiidae	Habrophlebiodes	2.3						8			
Letophlebiidae	Paraleptophlebia	0.94	x	2	7						
Leptophlebiidae	Unid Leptophlebid	3.3					4				1
Hemiptera											
Veliidae	Microvelia	9				1					
Megaloptera											
Corydalidae	Nigronia	5.3	x	16	13	26	15	23	7	28	2
Sialidae	Sialis	7.17					1			1	

Table C3. (Continued) Taxa collected in the semi-quantitative samples in Laurel Creek, Summer 2009.

		TV	CL <sup>2</sup>	Al	A2	D1	_ D2	D3	B1	B2	B3
Odonata											
Aeshnidae	Boyeria	6		1	2			1	I		
Cordulegastridae	Cordulegaster	5.73					1				
Gomphidae	Stylogomphus	6		8	8	4	5	3		3	13
Plecoptera											
Capniidae	Allocapnia	2.52		40	61	39	37	189	43	81	41
Nemouridae	Amphinemura	3.33		4	I	3	2		7	2	2
Peltoperidae	Unid. Peltoperlid	2	x		I	1	2				
Perlidae	Acroneuria	1.4	x			1					
Perlidae	Eccoptura xanthenes	3.74	x	1		3		1			
Perlidae	Perlesta	4.7	x	4	20	23	28	33	11	22	13
Perlodidae	Isoperla	1.8	x	1							
Trichoptera	-										
Glossosomatidae	Agapetus	0	x								1
Goeridae	Goera	0.13		1		1			1		
Hydropsychidae	Ceratopsyche	1.4	х	2							
Hydropsychidae	Cheumatopsyche	6.22	x	14	24	50	1	9	I	8	5
Hydropsychidae	Hydropsyche	4	x			1					
Lepidostomatidae	Lepidostoma	0.9				1					
Limnephilidae	Pycnopsyche	2.52		1	1					1	1
Philopotamidae	Chimarra	2.76	х								1
Philopotamidae	Dolophilodes	0.81	х	2	2	2	1	4			
Philopotamidae	Wormaldia	0.65	x	x	2			2			1
Rhyacophilidae	Rhyacophila	0.8	х			1	1	1	1	1	I
Uenoidae	Neophylax	2.2	x	1						I	
Amphipoda	Unid. Amphipod	7.97							1		
Annelida	Unid. Oligocheata	8.2							2	4	2

Table C3. (Continued) Taxa collected in the semi-quantitative samples in Laurel Creek, Summer 2009.

		Al	A2	D1	D2	D3	B1	B2	B3
Coleoptera					_				
Dryopidae	Helichus								21
Psephenidae	Psephenus herricki				1				
Diptera									
Chironomidae	Brilla							I	
Chironomidae	Microspectra		1						
Chironomidae	Paratanytarsus			1					
Chironomidae	Xylotopus			1					
Tipulidae	Tipula	1	2				1	7	6
Decapoda									
Cambaridae	Camabrus		1						
Cambaridae	Orconectes cristavarius						1		
Ephemeroptera									
Baetiscidae	Baetisca								1
Caenidae	Caenis								2
Ephemerellidae	Eurylophella							5	
Ephemeridae	Ephemera								1
Heptageniidae	Stenacron	2	2	4	1	3	10		
Leptophlebiidae	Unid <i>Leptophlebid</i>			1			1		
Hemiptera									
Gerridae	Aquarius					1			
Gerridae	Gerris	1					1	2	
Veliidae	Microvelia								9

Table C4. Taxa collected in the qualitative samples, but not in the semi-quantitative samples In Laurel Creek, Summer 2009.

		A1	A2	D1	D2	D3	B1	B2	B3
Odonata									
Aeshnidae	Boyeria			2					2
Cordulegastridae	Cordulegaster	1							
Plecoptera									
Perlidae	Acroneuria								2
Perlidae	Eccoptura xanthenes								1
Perlodidiae	Isoperla			3			1	2	1
Taeniopteryx	Taeniopteryx					1			
Trichoptera									
Glossosomatidae	Agapetus					1			
Glossosomatidae	Glossosoma								1
Hydropsychidae	Diplectrona								1
Lepidostomatidae	Lepidostoma	1	1				1		8
Limnephilidae	Pycnopsyche			7	6	16	8		
Philopotamidae	Chimarra		1	3					
Uenoidae	Neophylax				1	14	20		7

 Table C4. (Continued) Taxa collected in the qualitative samples, but not in the semi-quantitative

 Laurel Creek, Summer 2009.

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Metric	A1	A2	D1	D2	D3	B1	B2	B3
TR	34	39	26	35	36	27	30	37
EPT	14	16	10	13	10	9	13	18
mHBI	5.54	4.41	3.96	4.54	3.39	4.08	3.24	3.66
m%EPT	23.66	30.20	21.02	21.98	39.23	33.15	28.70	39.27
%EPHEM	3.22	8.43	7.39	9.34	11.96	9.55	8.52	14.57
%CHIR+OLIG	0	12.36	1.70	0.11	5.74	13.48	9.87	24.29
%Clinger	66.66	64.04	73.30	42.31	61.24	56.74	68.16	50.61

Table C5. Values for core macroinvertebrate metrics, Summer 2008.

Table C6. Standardized values for MBI Scores 2008 (values from Table C5 inputted into formulae in Table A2).

	A1	A2	D1	D2	D3	B1	B2	B3
TR	53.96	61.9	41.27	55.55	57.14	42.86	47.62	50
EPT	42.42	48.48	30.3	39.39	30.3	27.27	39.39	60
mHBI	57.03	71.48	77.24	68.82	84.53	75.7	86.45	92.02
m%EPT	27.22	43.96	24.19	25.29	45.14	38.14	33.03	53.07
%EPHEM	4.84	12.68	11.11	14.05	17.99	14.36	9.8	NA
%CHIR+%OLIG	100.68	88.24	99.97	100.57	94.91	86.59	90.75	76.47
%Clinger	88.29	84.82	97.08	56.04	81.11	75.15	90.28	68.39
MBI score	53.49	<u>58.</u> 79	54.45	51.39	58.73	51.44	56.76	66.66

Metric	A1	A2	D1	D2	D3	B1	B2	B3
TR	53	45	51	42	41	41	50	57
EPT	19	16	22	16	18	12	16	26
mHBI	3.68	3.39	3.53	3.70	2.11	3.74	3.41	3.72
m%EPT	44.28	41.89	43.34	54.68	72.68	58.72	54.51	41.71
%EPHEM	15.92	15.93	17.41	19.70	11.67	22.09	13.91	11.05
%CHIR+%OLIG	13.93	15.34	9.22	16.75	5.84	12.79	8.65	27.64
%Clinger	40.30	45.13	56.66	37.44	29.97	28.49	37.97	32.66

Table C7. Values for core macroinvertebrate metrics, Summer 2009.

Table C8. Standardized values for MBI Scores 2009 (values from Table C6 Inputted into formulae in Table A2).

	A1	A2	D1	D2	D3	B1	B2	B3
TR	84.13	69.84	80.95	66.66	65.08	66.66	77.78	77.03
EPT	57.57	48.48	66.66	48.48	54.54	36.36	48.48	86.66
mHBI	80.81	84.5	82.74	80.56	100.9	80.05	84.27	91.15
%EPT	50.95	48.2	49.89	62.92	83.64	67.57	62.73	56.36
m%EPHEM	23.94	23.95	26.17	29.63	17.55	33.22	20.92	NA
%CHIR+%OLIG	86.67	85.24	91.4	83.82	94.8	87.81	91.97	73.09
%Clinger	53.38	59.78	75.04	49.59	39.69	37.73	50.29	44.14
MBI score	62.49	60.00	67.55	60.24	65.17	58.49	62.35	71.41

Table C9. Metric values and MBI scores for mountain headwater and wadeable sites in the Little Sandy River basin. (Pond et al. 2003).

Metric	Headwater	Wadeable
TR	41-53	52-69
EPT	17-25	27-33
mHBI	2.95-3.71	3.55-4.32
M%EPT	63.8-77.4	47.7-75.0
%Ephem	14.7-31.5	$NA^1$
%Chir + %Olig	2.3-7.2	5.3-17.6
%Clinger	31.7-43.9	47.4-75.5
MBI Scores	67.0-75.8	77.5-94.8

1.% Ephem used only with headwater stream assessments.

Table C10. Comparison of functional feeding group composition of sites above the restoration from Summer 2008 to Summer 2009.

%FFG	A1-08	<u>A</u> 1-09	A2-08	A2-09
Collector/Filterer	38.71	21.39	30.34	21.24
Collector/Gatherer	2.15	15.92	1.12	22.13
Predator	53.76	28.36	30.34	23.01
Scraper	4.30	8.96	13.48	9.73
Shredder	1.08	24.87	23.60	23.89

Table C11. Comparison of functional feeding group composition of sites within the restored area from Summer 2008 to Summer 2009.

%FFG	D1-08	D1-09	D2-08	D2-09	D3-08	D3-09
Collector/Filterer	32.95	35.84	29.67	17.73	56.46	8.22
Collector/Gatherer	0.57	15.36	7.14	17.24	2.39	7.69
Predators	50.0	25.94	45.60	36.95	18.66	24.4
Scraper	13.07	6.48	7.14	4.93	8.61	8.49
Shredder	3.41	16.38	10.45	23.15	13.4	51.19

Table C12. Comparison of functional feeding group composition of sites below the restoration from Summer 2008 to Summer 2009.

<u>%F</u> FG	B1-08	B1-09	B2-08	B2-09	B3-08	B3-09
Collector/Filterer	24.72	14.53	39.91	13.91	45.75	14.07
Collector/Gatherer	2.25	20.93	2.24	13.91	0.81	25.63
Predators	25.84	23.84	39.01	29.32	32.39	24.12
Scraper	20.79	9.30	8.52	9.77	5.67	8.04
Shredder	26.40	31.40	9.87	33.09	15.38	28.14

Table C13. Aquatic macroinvertebrate taxa collected Laurel Creek in Summer 2008 and not collected in Summer 2009.

Order	Family	Genus
Diptera	Ceratopogonidae	Atrichopogon
Diptera	Chironomidae	Rheocricotopus
Diptera	Culicidae	Unid Culicidae
Diptera	Dixidae	Dixella
Odonata	Calopterygidae	Calopteryx
Odonata	Gomphidae	Gomphus

Order	Family	Genus
Coleoptera	Dytiscidae	Hydroporous
Coleoptera	Hydrophilidae	Helochares
Coleoptera	Hydrophilidae	Hydrobiomorpha
Diptera	Athericidae	Atherix
Diptera	Ceratopogonidae	Serromyia
Diptera	Chironomidae	Brilla
Diptera	Chironomidae	Cladotanytarsus
Diptera	Chironomidae	Corynoneura
Diptera	Chironomidae	Demicrytochironomus
Diptera	Chironomidae	Dicrotendipes
Diptera	Chironomidae	Diplocladius
Diptera	Chironomidae	Krenosmittia
Diptera	Chironomidae	Microspectra
Diptera	Chironomidae	Natarsia
Diptera	Chironomidae	Orthocladius
Diptera	Chironomidae	Paracricotopus
Diptera	Chironomidae	Parakiefferiella
Diptera	Chironomidae	Paratanytarsus
Diptera	Chironomidae	Paratendipes
Diptera	Chironomidae	Paratrichocladius
Diptera	Chironomidae	Phaenospectra
Diptera	Chironomidae	Potthastia
Diptera	Chironomidae	Pseudorthocladius
Diptera	Chironomidae	Rheotanytarsus
Diptera	Chironomidae	Subletta
Diptera	Chironomidae	Tvetenia
Diptera	Dixidae	Dixa
Diptera	Empididae	Chelifera
Ephemeroptera	Baetidae	Acentrella
Ephemeroptera	Ephemerellidae	Drunella
Ephemeroptera	Ephemerellidae	Timpanoga
Ephemeroptera	Heptageniidae	Maccaffertium
Ephemeroptera	Letophlebiidae	Habrophlebia
Ephemeroptera	Letophlebiidae	Habrophlebiodes
Ephemeroptera	Letophlebiidae	Paraleptophlebia

Table C14. Aquatic macroinvertebrate taxa collected in Laurel Creek in Summer 2009 and not collected in Summer 2008.

Table C14. (Continued) Aquatic macroinvertebrate taxa collected in Laurel Creek in Summer 2009 and not collected in Summer 2008.

Order	Family	Genus
Odonata	Gomphidae	Stylogomphus
Plecoptera	Peltoperidae	Unid. Peltoperlid
Plecoptera	Perlidae	Eccoptura xanthenes
Plecoptera	Perlodidae	Isoperla
Plecoptera	Taeniopteryx	Taeniopteryx
Trichoptera	Glossosomatidae	Agapetus
Trichoptera	Hydropsychidae	Diplectrona
Trichoptera	Lepidostomatidae	Lepidostoma