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A Post-Restoration Assessment of the Fishes and Macroinvertebrates of Mill Branch, Knox County, Kentucky, with Emphasis on the Blackside Dace (Chrosomus cumberlandensis)

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By

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DEDICATION

This thesis is dedicated to my wife, Christie, for her love and encouragement throughout my college career. I am truly blessed to have her in my life.

I would also like to dedicate this thesis to my daughters, Rebekah and Meredith, for their patience and understanding. It has brought me great joy watching them both grow and develop over these past years. They are both truly amazing.

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ABSTRACT

The first stream habitat restoration project for the federally threatened blackside dace, *Chrosomus cumberlandensis* (Starnes and Starnes), was initiated in fall 2005 on Mill Branch, a small headwater stream located in the Upper Cumberland River basin, Knox County, Kentucky. The lower 700 meters of Mill Branch were restored through construction of a new, stable channel with specially designed habitat features for blackside dace. In August 2009, the newly restored channel was connected to the mainstem and the old channel was eliminated. A post-restoration survey was conducted on the fishes (with emphasis on the blackside dace) and benthic macroinvertebrate communities, between August 2009 and June 2010, to determine if the restoration effort resulted in biological improvement. It appeared the new channel was gradually becoming more stable in terms of water quality and habitat. The overall fish community showed no improvement for most sections in the new channel. However, the blackside dace population has increased considerably from the first sampling event in 2006 prior to construction. Blackside dace have begun to utilize areas below the new culvert. Two individuals were found over 300 meters downstream of the new culvert. Baseline data for the macroinvertebrate community showed fair water quality conditions within the new channel for all sites sampled in March 2010. Long-term monitoring of Mill Branch should be continued to assess the long-term success of the stream restoration, to evaluate the overall stream ecosystem structure and function of Mill Branch, and to assist in our efforts to successfully enhance other degraded streams.

KEY WORDS: *Chrosomus cumberlandensis*, blackside dace, stream restoration, Upper Cumberland River, fishes of Kentucky, macroinvertebrates

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CHAPTER 1

INTRODUCTION

The southeastern United States supports the richest diversity of freshwater fishes and highest number of endemic fishes of any region in North America north of Mexico (Burr and Mayden 1992, Warren et al. 1997, Warren et al. 2000). Regrettably, a high proportion of the native fish fauna within this region are also threatened with extinction and fish populations continue to decline due to habitat degradation and habitat fragmentation (Warren and Burr 1994, Angermeier 1995, Warren et al. 2000). Alterations of physical habitat such as channelization, impoundments, sedimentation, and flow modification represent the greatest threats to fishes and are responsible for the majority of observed population declines within the southeastern United States (Walsh et al. 1995, Etnier 1997, Burkhead et al. 1997). Many fishes within this region are endemics (Burr and Mayden 1992, Warren and Burr 1994) that are susceptible to extirpation from habitat degradation (Burkhead et al. 1997). For example, the Cumberland River drainage of Kentucky and Tennessee supports 10 endemic fishes including the federally threatened blackside dace, *Chrosomus cumberlandensis* (Starnes and Starnes 1978, Warren and Burr 1994).

The blackside dace is a small minnow restricted to small tributaries in the upper Cumberland drainage in southeastern Kentucky and northeastern Tennessee (Starnes and Starnes 1978, Starnes and Etnier 1986, Biggins 1987). This species is one of seven North American species belonging to the genus *Chrosomus* (Strange and Mayden 2009, Starnes and Jenkins 1988, Skelton 2001): finescale dace, *C. neogaeus* (Cope); southern redbelly dace, *C. erythrogaster* (Rafinesque); northern redbelly dace, *C. eos* (Cope); laurel dace, *C. saylori* (Skelton); mountain redbelly dace, *C. oreas* (Cope); and Tennessee dace, *C. tennesseensis* (Starnes and Jenkins). Based on a color description, the blackside dace was probably first observed by D.S. Jordan and J. Swain in 1883 in Whitley County, Kentucky (Starnes and Starnes 1978). They considered it to be a variation of *C. erythrogaster*. However, their specimens may have been lost in a fire at Indiana University in 1883. It was later collected by N.H. Crisp and B.A. Branson, Eastern Kentucky University, in Whitley County, Kentucky, but was also regarded as a variation of *C. erythrogaster* (Starnes and Starnes 1978). The holotype specimen was collected from Bell County, Kentucky by J. Lowe and G.W. Wolfe, University of Tennessee. This specimen was recognized as a new species by D.A. Etnier (University of Tennessee), W.C. Starnes, and L.C. Starnes; and was scientifically described in 1978 by W.C. Starnes and L.B. Starnes (Starnes and Starnes 1978).

Adult blackside dace typically reach a maximum total length (TL) of 85 mm and have a life span of approximately 3 to 4 years (Starnes and Starnes 1978, Etnier and Starnes 1993). The species can be distinguished from other members of *Chrosomus* by a wide, single black lateral stripe, or two black lateral stripes that converge at the caudal peduncle. Furthermore, this species displays an olivaceous or green-gold dorsolateral background with numerous black speckles, a pale belly (except during spawning season), and bright yellow fins. During the spawning season, in males, the belly, nape, ventral portion of the head, base of the dorsal fins, and lips exhibit a brilliant scarlet color. Also, the lateral stripe becomes a more intense black, and the fins turn to a brighter yellow (Starnes and Starnes 1978, Etnier and Starnes 1993).

Upon its description in 1978, the blackside dace was assigned to the genus *Phoxinus*; however, Strange and Mayden (2009) revised the taxonomy for all seven North American *Phoxinus* species, placing them in the genus *Chrosomus* based on phylogenetic relationships. With the exception of *C. neogaeus*, they recognized three North American clades separated into paired sister species: *C. erythrogaster* and *C.eos*, *C. cumberlandensis* and *C. saylori*, and *C. oreas* and *C. tennesseensis*. In the Upper Cumberland River system, *C. cumberlandensis* and *C. erythrogaster* are sympatric. However, most phylogeographic patterns among *Chrosomus* are allopatric, but vicariant events that separated the ancestral species happened in unknown drainages (Strange and Mayden, 2009). Both *Chrosomus cumberlandensis* and *C. saylori* have restricted distributions, with *C. saylori* being restricted to just six streams in the upper Tennessee River (Skelton, 2001) and *C. cumberlandensis* being restricted to the upper Cumberland River system (O'Bara, 1990; Starnes and Starnes, 1981). Strange and Mayden (2009) argued that a widespread extinction of the shared ancestral species of *C. cumberlandensis* and *C. saylori* is the most parsimonious explanation for the geographic separation of these two species. Starnes and Jenkins (1988) suggested that the ancestor of *C. oreas*, *C. tenneseensis*, and *C. cumberlandensis*, which was more similar to *C. oreas*, was once widespread in the upper Teays River drainage. Starnes and Starnes (1978) surmised that *C. cumberlandensis* dispersed from this ancestral stock into the upper Cumberland River drainage by means of stream capture with tributaries connecting the upper Teays River. The *C. oreas* form may have dispersed up into the Holston River (upper Tennessee River) giving rise to *C. saylori* (Starnes and Jenkins, 1988). *Chrosomus tennesseensis* was likely derived from the re-invasion of the ancestral stock into the Tennessee River system. Strange and Burr (1995) proposed that *C. cumberlandensis* originated in tributaries above Cumberland Falls, more specifically the Stinking Creek, Clear Creek, and Youngs Creek systems (Starnes and Starnes 1978, Starnes and Starnes 1981, O'Bara 1990, Strange and Burr 1995). Moreover, Strange and Burr (1995) suggested that Stinking Creek to Youngs Creek systems makeup one of three metapopulations based on mtDNA haplotypes. The other two metapopulations occur in the upper Poor Fork through Straight Creek stream systems and around Marsh Creek and Jellico Creek. Populations below Cumberland Falls may constitute a fourth metapopulation. However, the technique used for determining these relationships compares mtDNA cut at specific places to look at the same fragment sizes. This technique can only reveal similarities, not phylogenetic relationships.

In 1987, the blackside dace was listed as threatened by the U.S. Fish and Wildlife Service (USFWS) (USFWS 1987, USFWS 1988). At that time, an estimated 151 stream systems supported habitat sufficient for blackside dace; however, only 30 small headwater streams (27 total stream km) were known to be occupied (O'Bara 1990). Currently, blackside dace are found in 205 locations across 113 different streams in Kentucky (KSNPC 2010). Some of those streams are tributaries to the receiving streams which also support blackside dace. These numbers reflect all known historic and current records. Many of these streams harbor small or remnant populations (i.e. less than 10 individuals) (Black and Mattingly 2007). Current estimates suggest that *C. cumberlandensis* has been extirpated from at least 60-70% of its historic range (Starnes 1981).

Typical habitat requirements for blackside dace include small streams with clear, cool water (≤ 23°C), moderate stream flow with relatively clean rocky substrates, and a high amount of periphytic production (Starnes and Starnes 1978, Starnes and Starnes 1981, O'Bara 1990, Etnier and Starnes 1993, Jones 2005). Blackside dace are also relatively secretive and prefer to inhabit shaded pools with a depth between 0.3m and 1.0m and a sufficient amount of cover (e.g. undercut banks and brush piles). Preferred substrates include a mixture of gravel, cobble, and boulder with some areas of bedrock and sand. Streams that have a low overall gradient between one and six percent (Jones 2005), and a riffle/pool-area ratio near or below 60:40 (Starnes and Starnes 1981), harbor more populations. This is indicative of blackside dace streams located in the Cumberland Plateau closer to Cumberland Falls (Starnes and Starnes 1981, O'Bara 1990). However, Cumberland River headwaters located in the Cumberland Mountains region support fewer blackside dace populations due to higher gradient streams and riffle/pool ratios greater than 60:40 (Starnes and Starnes 1981). Predictive models also propose that blackside dace are more persistent in streams with summer temperatures between 14.6°C and 18.5°C and stream conductivities lower than 240 µS (Jones 2005).

Preferred food items for blackside dace are diatoms, algae, organic detritus, root hairs, and benthic macroinvertebrates (Starnes and Starnes 1981). Blackside dace are also known to ingest large amounts of sand. It is unclear if sand is ingested accidentally or intentionally. Foraging patterns noted by Starnes (1981) consisted of grazing on the surface of submerged objects in addition to repeatedly drifting downstream in small groups in search of food.

Blackside dace follow the most primitive spawning mode, known as broadcast spawning, in which females and males release eggs and sperm over selected substrates and then abandon them (Johnston 1999). The spawning season generally occurs between April and July (Etnier and Starnes 2001), where blackside dace can be observed in aggregates composed of individuals 12 months or older, spawning over clean gravel substrates (Starnes and Starnes 1981). In streams with increased silt loads, blackside dace have been observed spawning over clean gravel nests constructed by creek chubs, *Semotilus atromaculatus* (Mitchill) (Cicerello and Laudermilk 1996) and central stonerollers, *Campostoma anomalum* (Rafinesque) (Starnes and Starnes 1981). This type of strategy offers some protection against predators (Starnes and Starnes 1981). Hybridization of *C. cumberlandensis* and *S. atromaculatus* lacking gonads has been documented (Eisenhour and Piller 1997). Breeding behaviors and habitat alterations may be the cause for *C. cumberlandensis X S. atromaculatus* hybrid individuals.

The primary causes of blackside dace population declines are anthropogenic habitat perturbations associated with coal mining, road construction, agriculture, and silviculture, gas/oil well exploration, and inadequate sewage treatment (Starnes and Starnes 1978, Starnes and Starnes 1981, Wood and Armitage 1997, Williams et al. 1989, O'Bara 1990, USFWS 1988). These potential perturbations can result in increased isolation due to the reduction and fragmentation of the geographical range (Butler 2002). The entire blackside dace range is rich in coal and timber reserves, and when exploited, often impacts blackside dace streams by causing negative changes in water quality, increased sediment load, acidification, and elevated stream conductivity (Starnes and Starnes 1981, Brake et al. 2001). Coal mining activities, poor logging practices, and road construction often result in the removal of riparian vegetation which can cause increased sedimentation through bank erosion, a loss of submerged root systems that provide necessary habitat for blackside dace and various macroinvertebrates, and increased water temperatures due to increased solar radiation (O'Bara 1990, Wood and Armitage 1997, Johnson and Jones 2000, Sutherland et al. 2002). Untreated sewage causes excessive nutrient inputs resulting in algal blooms that lower dissolved oxygen concentrations (Mattingly et al. 2005). Drought conditions may also contribute to the decline of blackside dace populations (O'Bara 1990), especially because the species is already fragmented.

Surface coal mining activities represent the most imminent and substantial source of threats to blackside dace populations (USFWS 2010). Surface coal mining (e.g. mountaintop removal) processes include clearing forests, stripping topsoil, and removing rocks to access layers of coal (Palmer et al. 2010, Pond et al. 2008). Most of the excess rock (i.e. mining "spoil) is placed in adjacent valleys next to the surface mine, resulting in valley (hollow) fills. These valley fills permanently bury headwater streams located next to the surface mine. The primary cause of water quality degradation is contaminated groundwater inputs that contain elevated levels of sulfate (SO_4), calcium, magnesium, and bicarbonate ions (USFWS 2010, Palmer et al. 2010). These inputs originate from valley (hollow) fills or as groundwater seeps (springs) along reclaimed slopes (USFWS 2010), and are responsible for increases in pH, conductivity, and total dissolved solids in streams located below valley fills (Palmer et al. 2010). In some cases, surface coal mining activities can permanently alter water quality and degrade physical habitat (USFWS 2010, Palmer et al. 2010). Other coal mining activities can create acid mine drainage (AMD) resulting from oxidation of sulfide ions exposed to the atmosphere through erosion of coal mine spoils (Detar 2004, Mattingly et al. 2005). Streams with AMD are usually detrimental to fish populations due to low pH, increased conductivity, and high metal and sulfate concentrations.

Aquatic ecosystems all over the world are being impacted by human activity at a greater rate than any other time in history, and at a much faster rate than they are being restored (Baron and Poff 2004, NRC 1992). Because fish communities are sensitive to a range of biological, physical, and chemical perturbations, they are often used to detect the degree to which a stream is impaired (Karr 1981). Anthropogenic activities, such as stream channelization, influence biological interactions of fish communities on the local scale by altering physical features such as water depth, current velocity, substrate particle size, cover, and temperature (Bayless and Smith 1964, Jones 1975, Rabeni and Jacobson 1993). By eliminating the natural pool-riffle sequence, stream channelization may also affect the distribution of fishes, both juveniles and adults (Bayless and Smith 1964, Jones 1975).

Stream restoration focuses on reversing stream impairment by increasing habitat diversity for the benefit of various or specific organisms (Gore et al. 1995). Restoration is defined by the National Research Council (1992) as "*The return of an ecosystem to a close approximation of its condition before disturbance. In restoration, ecological damage to the resource is repaired. Both the structure and the functions of the ecosystem are recreated. Merely recreating the form without the functions, or the functions in an artificial configuration bearing little resemblance to a natural resource, does not constitute restoration. The goal is to emulate a natural, functioning, self-regulating* *system that is integrated with the ecological landscape in which it occurs."* Reasons for restoring a stream may vary, but most stream restoration projects focus on problems associated with water quality, riparian vegetation, sediment transport, bed and bank stability, channel stability, natural stream flow, instream habitat, fish assemblages, aesthetics, and recreation (Bernhardt 2005, Bernhardt et al. 2005, Palmer et al. 2005). However, few investigators have monitored or assessed the long-term effects that stream restoration projects have within aquatic ecosystems (Bernhardt et al. 2005, Palmer et al. 2005). Long-term post restoration monitoring efforts can provide opportunities to develop our current understanding of stream ecosystem structure and function; therefore, aiding in our abilities to repair degraded ecosystems.

In fall 2005, the USFWS (Kentucky Ecological Services Field Office), Kentucky Department of Fish and Wildlife Resources (KDFWR), and the Natural Resources Conservation Service (NRCS), along with the cooperation of several private landowners, decided to implement the first habitat restoration project for the blackside dace by restoring the lower 700 meters (m) of Mill Branch (KDFWR 2007). This was the first large-scale habitat restoration project for a federally listed fish species in Kentucky (Michael Floyd, personal communication, USFWS, 2010). Natural channel design techniques were used in the restoration, and baseline biological information was collected before and during the restoration. Construction was initiated in fall of 2007 and completed in August 2009 with the connection of the new channel with the mainstem. During this time, the old channel was retained and the restored channel below the new culvert was restricted by a flow pipe and berm to allow for riparian vegetation growth.

The stream reconstruction process focused on creating a forested riparian buffer and adjacent floodplain wetlands, developing riffles and pools, and restoring a stable, sinuous channel (KDFWR 2007). Particular attention was given to in-stream habitat designed specifically for blackside dace and placed throughout the restored channel. The process also included the replacement of a perched culvert with a concrete stream simulation culvert, allowing for fish passage. A three percent channel gradient was created upstream of the culvert; while areas below the culvert were designed with less than a one percent slope (Art Parola, personal communication, University of Louisville Stream Institute, 2010). Prior to the restoration of Mill Branch, lack of continuous flow throughout certain parts of the year restricted fish movement (Michael Floyd, personal communication, USFWS, 2010). The restored channel was designed so the stream bed would be located below the water table to ensure perennial flow that would allow fish to utilize the entire stream. Biodegradable fiber mattresses held in place by wooden stakes were placed along the streambank to minimize sediment loading and help reduce erosion. Riparian vegetation was also planted along the streambank to stabilize the channel and provide shade, food, and habitat as well as reduce runoff and erosion. Finally, adjacent floodplain wetlands were established along the new stream corridor to buffer and treat precipitation.

Annual fish surveys of Mill Branch were conducted annually beginning in 2006 (Michael Floyd, personal communication, USFWS, 2010) and extending into 2009 prior to reconnection of the new channel with upstream reaches of Mill Branch. Surveys were completed by personnel from several federal and state agencies, academic institutions, and other volunteers. During most years, Mill Branch was electrofished in its entirety, with sampling teams allocated to four separate survey reaches. Efforts were focused on obtaining blackside dace abundance and age class structure (Figures 1-4[1]). Four age classes of blackside dace were documented across sampling years, with highest recruitment between 2006 (76 individuals) and 2007 (525 individuals) including 450 age-0 fishes (Michael Floyd, USFWS, personal communication, 2009). Over 400 individuals were collected in 2008 and 2009.

In March 2009, 43 blackside dace were found in the restored channel of Mill Branch above the large culvert. However, the restored channel below the culvert was restricted by a flow pipe and berm, thus, limiting its connection to the main channel to allow for riparian vegetation growth. Even though connectivity was limited in this reach, annual fish sampling in 2008 and 2009 yielded 11 and 15 fish species, respectively. No blackside dace were observed in this reach.

In addition to blackside dace, all other fishes were identified and enumerated. A total of 7,123 individuals, including 26 species and 17 families were identified from Mill Branch during the four-year sampling period (Tables 1-3). Only one Cumberland arrow darter, *Etheostoma s. sagitta* (Jordan and Swain) individual was collected during this time. This individual was collected in the old channel during the 2007 sampling event within 600m from the confluence with Stinking Creek. The Cumberland arrow darter is known to co-occur with blackside dace (Starnes and Starnes 1978, O'Bara 1990), is listed by KDFWR as a Species of Greatest Conservation Need (SGCN), and is being evaluated currently by the USFWS for possible federal listing.

Aquatic organisms have been widely used in monitoring water quality since the early 1900s (KDOW 2002), especially since chemical and physical criteria are ineffective for

[1] Tables are located in Appendix A and Figures are located in the Appendix B.

measuring biotic integrity (Karr and Dudley 1981). For example, chemical monitoring fails to detect many human induced perturbations such as flow alterations and habitat degradation (Karr, 1981). Karr and Dudley (1981) defined the concept of biological integrity as "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region." Benthic macroinvertebrates (Merritt et al. 2008) and fish assemblages (Karr 1981) are important indicators for detecting changes in habitat and water quality. Fishes are used to detect environmental changes to water quality by examining community attributes (e.g. species richness and relative abundances) and comparing them with regional reference sites (Karr 1981). However, benthic macroinvertebrates are the most frequently used group of organisms for assessing water quality because they are ubiquitous, the many species within this group demonstrate a range of responses to environmental stressors, they are useful in determining the spatial extent of changes to the aquatic environment because of their sedimentary lifestyle, and temporal changes can be observed more efficiently by examining abundance and age class structure (Merritt 2008).

Benthic macroinvertebrates are used throughout the world today as environmental indicators of biological integrity for water quality assessments (KDOW 2002). In Kentucky, benthic macroinvertebrate communities are used by KDOW for assessing and listing Exceptional Water designations to streams and rivers throughout the state for the purpose of preparing integrated reports to Congress in accordance with Sections 305(b) and 303(d) of the Federal Clean Water Act. Benthic macroinvertebrates are defined by KDOW (2002) as organisms large enough to be seen by the unaided eye, can be retained by a U.S. Standard No. 30 sieve (28 mesh/inch, 600 μm openings) and live at least part of their life cycle within or upon available substrates of a waterbody.

Karr (1981) first developed the Index of Biotic Integrity (IBI), a multimetric system for assessing the biotic integrity of rivers and streams using fish communities. He argued that the biotic integrity of a local water resource could be rapidly assessed by monitoring fish communities. The evaluation of fish community structure has been an important biological component in monitoring streams, and provides reliable assessments for preparing reports in accordance with Section 305(b) of the Clean Water Act (KDOW 2002). The most accurate means of evaluating fish community structure is to perform a fish Index of Biotic Integrity (IBI) for each sampling event. Karr's (1981) original IBI has been modified many times, both for fish and macroinvertebrates, to ensure accuracy and precision within a particular region and/or water body type (KDOW 2002). Currently, Kentucky uses a modified IBI version called the Kentucky Index of Biotic Integrity (KIBI) (DOW 2002).

The purpose of this project was to conduct post-restoration monitoring and assessment of the fish (with emphasis on the federally threatened blackside dace, *C. cumberlandensis*) and benthic macroinvertebrate communities of Mill Branch with the goal of determining whether the stream restoration effort resulted in biological improvement. The study had the following objectives: 1) determine the distribution and abundance of blackside dace in the restored channel of Mill Branch just prior to and post connection of the newly restored channel to the mainstem; 2) relate blackside dace abundance to stream habitat and physicochemical parameters measured throughout the lower three reaches of Mill Branch post-restoration; and 3) assess the biotic integrity of

Mill Branch, with emphasis on the newly restored channel, using fish and macroinvertebrates as bioindicators. Data will provide baseline information for future comparison through long-term monitoring to determine the overall success of the restoration project.

CHAPTER 2

STUDY AREA

Mill Branch is a small headwater tributary to Stinking Creek located within the Central Appalachians (Level IV) and Dissected Appalachian Plateau (Level III) ecoregions (Woods et al. 2002) in Knox County, Kentucky, in the upper Cumberland River basin (Figure 5). This ecoregion is mostly forested with narrow ridges, deep coves, and narrow valleys (Woods et al. 2002). Most streams within this ecoregion are cool, with high gradients, numerous riffles, and large substrate, such as cobble and boulder. Forest composition is highly variable but mostly deciduous. The geology is characterized by Pennsylvanian shale, siltstone, sandstone, and coal. Many streams within the Dissected Appalachian Plateau have decreased biological productivity and impaired water quality due to surface and underground coal mining, logging, and gas and oil extraction activities (Woods et al. 2002).

The drainage area of Mill Branch encompasses approximately six square kilometers (km). The surrounding area upstream of the restored channel is characterized by having a dense deciduous forest dominated by several oak species (*Quercus* spp.), American beech (*Fagus grandifolia*), boxelder (*Acer negundo*), and American sycamore (*Platanus occidentalis*). The lower section (restored channel) is surrounded by mostly pasture land, with very little forest. There are three residential homes located approximately 100m from Mill Branch in the middle and upper reaches, and one near the restored channel less than 300m away.

Historical impacts from agricultural and residential land uses, along with poor logging practices and surface coal mining activities have altered the once natural physical and chemical composition of the stream through stream channelization, increased sedimentation, removal of riparian zones, and elevated conductivity (KDFWR 2007). Prior to the restoration, other threats included a lack of perennial flow and a perched culvert (on Walker Road) located approximately 500m from the confluence with Stinking Creek. The perched culvert inhibited upstream and downstream movements of fishes, including blackside dace.

CHAPTER 3

MATERIALS AND METHODS

Four different fish sampling events were included in the post-restoration monitoring and assessment. One sampling event was completed for Mill Branch a few days prior to the connection of the restored channel with the mainstem (August 2009), and three sampling events were completed after the connection of the restored channel with the mainstem. Thus, sampling events included the following: (1) August 18-22, 2009; (2) October 9 – November 4, 2009; (3) March 27 – April 15, 2010; and (4) June 3, 2010. From this point forward the post-restoration sampling events will be referred to as August 2009, October 2009, March 2010, and June 2010.

The lower 2,000m of Mill Branch were divided into three reaches (Figure 6). Reach 1 began at the confluence with Stinking Creek and extended 100m upstream of the new culvert. Reach 2 began at the upstream limit of Reach 1 within the restored channel, and extended upstream to approximately 100m within the non-restored channel. Reach 3 began at the third culvert and extended upstream to a head-cut, beside an old chicken coop. A wire fence was placed across the stream that prohibited further sampling.

These reaches were further divided into 200m sections to gain more insight into the overall biological structure and dynamics of the stream. Reaches 1 and 2 each included three 200m sections, each totaling 600m, and reach 3 included four 200m sections, totaling 800m. Reach 1: Section 1 began at the confluence with Stinking Creek. The new culvert was located within Reach 1: Section 3, 100m upstream from where this reach was established and 100m downstream from where Reach 2: Section 1 began. Reach 1:

Section 3 was divided into two 100m sections. Reach 1: Section 3 downstream the new culvert (DC), and Reach 1: Section 3 upstream of the new culvert (UC). Global positioning system (GPS) coordinates were recorded at the most downstream point of each sampling section with a Garmin© Colorado 400t (Table 4).

August 2009 fish sampling was performed only on the restored channel of Mill Branch because reaches and sections had not been defined at that time. The October 2009 sampling event covered 2,000m of Mill Branch, including the restored channel. March 2010 sampling included the lower 1,800m of Mill Branch. Reach 3: Section 4 was not sampled during this time because blackside dace were not found to inhabit the last 100m of reach 3: section 3. Approximately 25m of section 1 was not thoroughly sampled due to a large deep pool created by the construction of a beaver dam. During the June 2010 sampling event, approximately 90 percent of Reach 1: Section 1 was relatively impossible to sample due to hazardous sampling conditions created by the construction of beaver dams. The remainder of Reach 1, and approximately 50m of Reach 1: Section 2 were thoroughly sampled. No sampling occurred past this point because of the possible increase in blackside dace mortality due to increased summer temperatures.

Fish Sampling

Fish sampling was conducted via single-pass backpack electrofishing using a Smith-Root LR-24 electroshocker. Sampling began at the most downstream section, and was conducted by moving upstream in a sweeping pattern, covering all possible habitats within each 200m section of stream. Sampling time (seconds) was recorded at the end of each section. The voltage was adjusted on the electroshocker to minimize injury to fish, especially blackside dace. A team of three individuals (rarely four) sampled on all occasions. One individual operated the electroshocker and a dipnet, another individual used a dipnet to capture fish, and a third individual carried a five gallon bucket for the purpose of either holding captured fish, or allowing blackside dace to recover before releasing them back into the stream. The third individual also recorded data.

All fish were identified, enumerated, and released in the field, except for voucher specimens. No blackside dace were retained. All blackside dace were measured for total length (mm) and released. Voucher specimens were fixed in 10% formalin for approximately one month, rinsed in water for 24 to 48 hours, and preserved in 70% ethanol. All voucher specimens were deposited in the Branson Museum of Zoology fish collection at Eastern Kentucky University. All redbreast sunfish, *Lepomis auritus* (Linnaeus) were either discarded or deposited into the Branson Museum along with the other voucher specimens. Redbreast sunfish are non-native fish stocked by state agencies in the past, and are considered a potential predator of blackside dace (USFW 2010). Recruitment, age structure, and abundance of blackside dace were examined by constructing a total length (mm) frequency histogram (Figures 7-10) for each sampling event. These histograms were then compared to previously obtained data on blackside dace abundance and age structure (Figures 1-4).

Macroinvertebrate Sampling

Macroinvertebrate communities were sampled during August 2009, and again in March 2010, using quantitative and qualitative methods outlined by KDOW (2002). Three sites were randomly selected in the restored channel of Mill Branch for the purpose of computing a water quality rating. Site 1 was located within the first 100 meters from the mouth of Stinking Creek. Site 2 was located within 100 meters downstream of Mill Branch Rd. Site 3 was located within 100 meters upstream from Mill Branch Rd (Figure 11). Global positioning system (GPS) coordinates were recorded at the most downstream point of each site with a Garmin© Colorado 400t (Table 5). All macroinvertebrate samples were kept separate from one another, including quantitative and qualitative samples. Macroinvertebrates were identified to the lowest taxonomic level possible using Merritt et. al (2008) taxonomic keys. All macroinvertebrates were deposited into the Branson Museum of Zoology at Eastern Kentucky University.

At each site, a total of four quantitative riffle samples were collected from the thalweg of different sections of one to two riffles using a surber sampler with a mesh size less than 500 µm. Surber samplers are recommended when riffles are 20cm or less (KDOW 2002). All samples were processed on the stream bank because very little sediment was collected in the riffles; therefore, macroinvertebrates that were collected were easily and thoroughly picked. All sample contents at each site (ie. leafpacks, soil, rocks, etc.) were washed into a large white plastic tray and processed on the stream bank. The surber sampler was then inspected for any remaining macroinvertebrates. All macroinvertebrates were placed into a labeled container filled with 70 percent ethanol.

A qualitative sample was collected for each of the three sites. This sampling effort consisted of using the Sweep Sampling Method (KDOW 2002). The sweep sampling method was done using an 800 x 900 µm mesh triangular D-frame dipnet. This method focused on various habitats within several runs and pools within the reach such as undercut banks, rootwads, leafpacks, marginal vegetation, and bedrock. A total of three replicate samples were collected from each habitat. Qualitative samples were processed on the stream bank. Processing each qualitative sample consisted of all contents being washed over a large plastic tray, and all macroinvertebrates picked and placed into a label jar containing 70 percent ethanol.

Physicochemical Conditions

Water quality data (i.e. dissolved oxygen (mg/l), conductivity (µS), temperature (°C), and pH (Standard Units)) and physical measurements (e.g. flow (m/s), depth (m), and wetted width (m)) were collected at each sample section. Water quality data were collected at the downstream point of each section at the mid-channel, approximately 5 cm below the surface. Dissolved oxygen (mg/l) readings were taken with a YSI 55 meter (Yellow Springs Instruments, Yellow Springs, OH), and conductivity (µS), temperature (°C), and pH readings were taken with a YSI 30 meter (Yellow Springs Instruments, Yellow Springs, OH). Physical measurements were taken along three randomly selected transects (i.e. a riffle, a run, and a pool) within each section. Wetted width (m) was determined with a forester's measuring tape, and three measurements of flow (m/s) and depth (m) were taken at subsections along each transect (i.e. left bank, middle, and right bank) with a Marsh McBirney Flo-Mate 2000 (Hach Co., Loveland, Co) and incremented top-set wading rod. All depths were less than 1m and thus, all flow measurements were taken at 60% of each depth measured. Discharge was calculated for each transect of data using the following equation:

$$Q = \sum (a\ v)$$

were Q is Discharge, a is the product of width (m) and depth (m) for that transect, and v is the velocity (m/s) of the current in a subsection (Gore 2006). Total discharge for each transect was calculated by summing the three subsections for that transect. Average discharge was calculated by summing the total discharge of the three transects (i.e. riffle, run, pool) and dividing by three.

Kentucky Division of Water's (KDOW) visually-based habitat assessment for high gradient streams was used for each 200m section of stream to evaluate ten habitat parameters (KDOW 2002). The ten parameters rated instream habitat, bank stability, channel morphology, and riparian vegetation for each section sampled. These parameters were ranked on a numerical scale of 0 (lowest) to 20 (highest). The numbers were then added together to produce a final score for each section. The final score was used to measure relative habitat quality. Scores of 165 and above were fully supporting of the aquatic life use designation, scores of 164-156 were supporting, but threatened of the aquatic life use designation, scores of 155-145 indicated partial support for the aquatic life use designation, and scores of 144 and below indicated non-support of the aquatic life use designation (KDOW 2002).

Assessment of Biological Integrity

The Kentucky Index of Biotic Integrity (KIBI) (KDOW 2009) was calculated for each section sampled. The purpose of the KIBI was to examine the fish community structure in order to assess stream health within all sections of Mill Branch sampled. The KIBI metrics included Native Species Richness (NAT), Darter, Madtom, Sculpin Richness (DMS), Intolerant Species Richness (INT), Simple Lithophic Spawning Species Richness (SL), Percent of Insectivores, excluding tolerant individuals (%INSCT), Percent of Tolerant Individuals (%TOL), and Percent of Facultative Headwater Individuals (%FHW) (KDOW 2002). The values were calculated, and each 200m section of stream sampled was ranked as very poor, poor, fair, good, or excellent.

All macroinvertebrates, both Quantitative and qualitative samples, were identified to the lowest possible taxonomic level (mostly genus level) using taxonomic keys from Merritt et. al (2008), and a macroinvertebrate bioassessment index (MBI) score (KDOW 2002) was calculated for each of the three sites for both sampling seasons. Kentucky core metrics included Taxa Richness (G-TR), EPT Index (G-EPT), Modified HBI Index (mHBI), Modified percent EPT Abundance (m%EPT), Percent Ephemeroptera (%Emph), Percent chironomids + oligochaetes (%C+O), and Percent primary clingers (%ClngP).

Statistical Analysis

Two stepwise regressions were performed, using SAS software (SAS Institute 2008), on data collected in October 2009 and March 2010. A model was determined to identify the best predictors of the dependent variable (i.e. blackside dace abundance) among independent variables (including abundance of all fish species collected in all reaches and sections of Mill Branch and physicochemical parameters, such as dissolved oxygen (mg/l), conductivity (μS), temperature (°C), pH, and average discharge (m^3/s)).

CHAPTER 4

RESULTS

Fishes

Surveys conducted in the restored channel during the post-restoration survey (August 2009 through June 2010) yielded a total of 7,865 fishes, representing 26 species and 17 families (Tables 6-9). Greater species richness was recorded downstream of Walker Road (new culvert) for all sampling events, totaling 25 species (Tables 6-7). No Cumberland arrow darters were collected during the post restoration survey. However, one greenside darter, *Etheostoma blennioides* (Rafinesque) individual was observed in Mill Branch for the first time in October 2009. This individual was captured in the upper, non-restored channel of Mill Branch approximately 800m upstream of the mouth (Table 8).

During the post-restoration survey, the dominant species in the lower restored channel, downstream of the new culvert was bluntnose minnow, *Pimephales notatus* (Rafinesque) (Tables 6-8). The creek chub, *Semotilus atromaculatus* (Mitchill) was the most abundant species in the upper reaches during the October 2009 and March 2010 sampling events (Tables 8-9). Furthermore, the creek chub was the only fish species that was captured in all 10 sections of Mill Branch during all three sampling events (i.e. October 2009, March 2010, and June 2010). The blackside dace was the dominant species in the restored channel upstream of the new culvert.

Four age classes of blackside dace have been documented over the entire post-restoration sampling period at Mill Branch, with high recruitment between March 2009 and March 2010 (Figures 4 and 9). Age classes were 0-1 years, 1-2 years, 2-3 years, and

3+ years. There was a total of 134 individuals captured in August 2009 (restored section only), 564 individuals captured in October 2009 (210 individual were captured in the restored section), 239 individuals captured in March 2010 (71 individuals were captured in the restored section), and 58 individuals captured in June 2010 (restored section only) (Figures 7-10). Blackside dace inhabited a total of approximately 1,600m of Mill Branch from August 2009 to June 2010, including the new channel (Tables 6-9).

In the restored section, capture rates for all fishes, including blackside dace, were highest in Reach 1: Section 3 upstream of the new culvert for the October 2009 and March 2010 surveys (Tables 10-11). However, during October 2009 and March 2010, blackside abundance was much higher in the lower and middle non-restored sections of Mill Branch (Tables 8-9). Capture rates for blackside dace in the restored channel increased below the new culvert. Blackside dace were observed using several different habitats below the culvert in March and June 2010, including riffle areas, undercut banks, and the underside of log structures. Blackside dace were collected in fewer habitats within the restored channel during October 2009. They were mostly collected in aggregates in undercut banks and in pools under rootwads and log structures. However, during the March 2010 sampling event (spawning season), blackside dace were scattered. These fish were collected in riffles, under logs, in snags, and in open pools throughout the restored and non-restored channels of Mill Branch. Age-0 individuals were captured during the June 2010 sampling of the restored channel. Also, blackside dace were observed in March 2010 spawning over creek chub nests upstream in the non-restored channels, from Reach 2: Section 2 through Reach 3: Section 2. These sections combined had the highest capture rate of blackside dace.

Macroinvertebrates

Quantitative and qualitative sampling of macroinvertebrates in August 2009 and March 2010 produced a total of 30 and 41 taxa, respectively (Table 12). The three dominant taxonomic groups from August 2009 included mayflies (Order Ephemeroptera), dragonflies and damselflies (Order Odonata), and true flies (Order Diptera). Only one species in the Order Plectoptera (*Eccoptera* sp.) and one species in the Order Trichoptera (*Cheumatopsyche* sp.) was collected in August 2009, each at sites 2 and 3 (Table 12). The three dominant taxonomic groups from March 2010 were mayflies (Order Ephemeroptera), true flies (Order Diptera), and caddisflies (Order Trichoptera). Quantitative data from August 2009 showed a species richness for, sites 1, 2, and 3 of 1, 7, and 7, respectively. For March 2010, species richness increased for sites 1, 2 and 3 to 2, 8, and 10, respectively. The total number of individuals (TNI) collected in riffle areas for August 2009 and March 2010 were 28 and 42, respectively.

Physicochemical Conditions

Mean conductivity recorded in the restored channel decreased from August 2009 (158.27 ±27) to October 2009 (143.00 ±10), and from March 2010 (75.90 ±10) (Table 13). However, there was an increase in mean conductivity from March 2010 (75.90 ±10) to June 2010 (144.53 ±5) (Table 13). Mean temperatures during the summer months (e.g. August 2009 and June 2010) were 21.07 ±1 ^{o}C and 23.10 ±1 ^{o}C, respectively (Table 14). Mean pH ranged from 7.02 ±0.7 (March 2010) to 7.81 ±0.8 (October 2009) (Table 14).

Mean dissolved oxygen ranged between 8.31 ±0.2 (June 2010) and 11.15 ±2 (March 2010) (Table 14).

Habitat scores for all 200m sampling sections scored below 144 (Table 15). The upper non-restored reaches of Mill Branch scored consistently higher overall than the restored channel for October 2009 and March 2010. However, scores increased in the restored channel from October 2009 to March 2010, except for Reach 1: Section 1. Habitat scores for Reach 1: Section 1 for October 2009 and March 2010 was 98 and 84, respectively.

Assessment of Biological Integrity

Results from the KIBI scores varied from fair to excellent throughout all reaches sampled for all sampling events during the post-restoration survey (Table 15). Scores decreased for Reach 1: Section 1 and Reach 1: Section 3 (both above and below the new culvert) between October 2009 and March 2010 (Table 15). The opposite occurred for Reach 1: Section 2 between October 2009 and March 2010. Furthermore, the percentage of tolerant individuals (%Tol) increased in Reach 1: Section 1 and Reach 1 Section 3 (downstream of the new culvert) between October 2009 and March 2010, while the percentage of tolerant individuals (%Tol) decreased in Reach 1: Section 3 (upstream of the new culvert). The total number of individuals (TNI) collected in Reach 1: Section 3 (upstream of the new culvert) decreased from October 2009 to March 2010. Reach 2: Section 1 had a KIBI score of 77 (Excellent) for March 2011, an increase from October

2009 (61-Good). Total number of individuals collected was greater than 100 for all sampling events, except for Reach 1: Section 1 in June 2010.

Macroinvertebrate Biotic Index scores in the restored channel for the August 2009 sampling period produced water quality ratings of Poor at site 1, Poor at site 2, and Very Poor at site 3 (Table 16). Water quality ratings for the March 2010 sampling period were Fair at all three sites (Table 16). EPT values were higher for March 2010 than August 2009 (Table 16). Percentage of EPT taxa for August 2009 for sites 1, 2, and 3 were 11.4%, 35.5%, and 5.0%, respectively. Percentage of EPT taxa for March 2010 for sites 1, 2, and 3 were 60.9%, 71.43%, and 74.7%, respectively. Percent Chironomids & Oligochaetes for August 2009 were 37.1%, 9.7%, and 71.7% for sites, 1, 2, and 3, respectively (Table 16). Percent Chironomids & Oligochaetes for March were 18.8%, 12.1%, and 6.7% for sites, 1, 2, and 3, respectively. The number of EPT taxa was also greater for all three sites in March 2010 than August 2009.

Statistical Analysis

October 2009 stepwise regression results showed that creek chub abundance contributed to 50% and conductivity to 26% of the variation in blackside dace abundance with $R^2 = 0.76$. Results from March 2010 showed that water temperature, bluntnose minnow abundance, and conductivity contributed to 69%, 13% and 10%, respectively, of the variation in blackside dace abundance with $R^2 = 0.92$.

CHAPTER 5

DISCUSSION

The stream restoration effort resulted in biological improvement within the fish community. Improvement within the macroinvertebrate community cannot be demonstrated because this was the first post-restoration data collected within the new channel. Furthermore, data from the macroinvertebrate samples were not compared because sampling in August 2009 was performed outside the sample index period. Macroinvertebrate samples prior to restoration were deposited into the Branson Museum at Eastern Kentucky University, but have not been assessed to date. Post-restoration survey data showed an increase in abundance of blackside dace in the restored channel of Mill Branch. The overall morphology of the restored channel changed considerably from August 2009 through June 2010. The stream bed had become more firm with less impact from sediment deposition. Pools, both upstream and downstream of the passable culvert, had begun to fill in due to the accumulation of upstream sediment creating more suitable habitat for blackside dace. Sedimentation had decreased in the riffles and shallow runs. Banks also had more undercut sections with exposable rootwads than observed in October 2009.

Capture rates recorded for fishes in the restored channel were considered to serve as baseline information for future studies at Mill Branch. A comparison of pre- and post-restoration data revealed a considerable increase in abundance of sunfishes in the restored channel, especially *L. auritus*. Increased capture rates and increased *L. auritus* abundance in the restored channel, below the new culvert, were likely due to several factors, including proximity of the restored channel to the Stinking Creek confluence and an established beaver dam near the Stinking Creek confluence. Also, this section of the channel was deeper and contained more suitable habitat for a number of species that were not collected above the culvert, including rock bass, *Ambloplites rupestris* (Rafinesque), yellow bullhead, *Ameiurus natalis* (Lesueur), brown bullhead, *Ameiurus nebulosus* (Lesueur), northern hogsucker, *Hypentelium nigricans* (Lesueur), warmouth, *Lepomis gulosus* (Cuvier), bluegill, *Lepomis macrochirus* (Rafinesque), longear sunfish, *Lepomis megalotis* (Rafinesque), scarlet shiner, *Lythrurus fasciolaris* (Gilbert), largemouth bass, *Micropterus salmoides* (Lacepède), and golden shiner, *Notemigonus crysoleucas* (Mitchill).

The presence of four age classes and high recruitment of blackside dace was evident throughout the study (e.g. pre- and post restoration), with the exception of the 2006 sampling event. This was likely due to improvement of water quality, habitat conditions, and perennial flow. Histograms for August 2009 through June 2010 showed unbalanced age distributions of blackside dace, with low numbers of juveniles. The low number of juveniles was expected due to the cautionary use of lower output voltage settings on the backpack electroshocker, which tends to produce fewer juveniles due to the smaller size (surface area) of younger individuals. Age-0 individuals captured during the June 2010 sampling of the restored channel, upstream of the new culvert, indicated that reproduction was occurring within the new channel. Blackside dace was also utilizing restored instream habitats such as rootwads, and intentionally placed log structures, especially in areas upstream of the new culvert.

The River Continuum Concept (Vannote et al. 1980) proposes that biotic communities downstream take advantage of the processing inefficiencies of upstream biotic communities. Macroinvertebrate communities in small order streams are comprised mostly of collectors and shredders because of allochthonous inputs such as leaves and woody debris (CPOM) via riparian vegetation. Ideally, riffle habitat is the predominate habitat for macroinvertebrates in headwater steams; and therefore, offer the highest potential diversity and abundance (KDOW 2002); therefore, macroinvertebrate abundance and species richness may have been low within riffles areas of the new channel. The cause of this may be due to how the riffles were constructed. These riffle areas were constructed using large cobble and small boulders that were dumped into piles, leaving very little, or no interstitial space for species that are typically found within this type of habitat, including members belonging to Ephemeroptera, Plecoptera, Trichoptera, and Diptera. However, the new channel is expected to change over time, and move to a more natural state.

The sampling index period for macroinvertebrates in headwater streams is between mid-February and early June (KDOW 2002). These sampling protocols were designed and calibrated for seasonality. Only one sampling event, March 2010, was within the sampling index period; and therefore, was used only to assess the biotic integrity of the new channel for that particular time. The August 2009 macroinvertebrate data was collected for the purpose of investigating the macroinvertebrate community at the time of the connection of the new channel to the mainstem. Sometimes sampling outside of sampling index periods is necessary in order to assess immediate impacts (in this case, restoration) by following USFWS guidelines or KDOW Standards and Specifications Section for monitoring and assessing streams with federally listed species (KDOW 2002).

Large boulders were placed underground at the most upstream section of the restored channel to divert runoff and groundwater seeps from a reclaimed mine into an adjacent floodplain wetland. This is one reason for the decrease in conductivity since August 2008. The highest conductivity measure for Mill Branch, 271.1 µS/cm, was recorded in the non-restored channel at an illegal dump site during that time. Perennial flow may have also contributed to the decline in conductivity within the restored channel, diluting groundwater seeps and runoff. The decrease in conductivity may have also influenced blackside dace inhabitance in the new channel, especially since predictive models have indicated the optimal conductivity value for healthy populations of blackside dace is less than 240 µS/cm (Jones 2005, Black and Mattingly 2007). Dissolved Oxygen (mg/L) and pH fell within the normal range to support aquatic life for all reaches, including those in the new channel for all sampling events. Most aerobic organisms need dissolved oxygen levels to be at least 5mg/L. Dissolved Oxygen is typically at or near saturation in most small order streams. Dissolved Oxygen and carbon dioxide are usually inversely related due to photosynthesis and respiration. Carbon dioxide acts as an acid in freshwater and can alter pH by forming carbonic acid. pH is highly dependent on vegetation and geology. Limestone, like the cobble and boulders used to create the riffles within the new channel, contains high concentrations of bicarbonate ions, resulting in alkaline waters. Most streams have a pH between 6.5 and 8.5. Temperature and dissolved oxygen are also inversely related. The slightly high temperatures recorded in the channel during August

2009 and June 2010 was expected, especially since the riparian vegetation had not had sufficient time to provide shade to this section of stream.

Rapid Habitat Assessment scores indicated that both the restored and non-restored channels of Mill Branch are not supporting the aquatic life use designation. Reach 1: Section 1 scored lower in March 2010 than it did in August 2009 primarily because of the large deep pools created by beaver dams. Reach 1: Section 2 scored lower in August 2009 because of sediment deposition (embeddedness) issues, especially below the new culvert. Embeddedness is a term used to describe the degree in which rocks or snags on the stream bottom are covered or sunken into the silt, sand, mud or biofilms (KDOW 2002). Embeddedness decreases the amount of available surface area for macroinvertebrates and fish for shelter, egg incubation, or to spawn. Scores for Reach 1: Section 3 and Reach 2: Section 1 indicated an improvement in habitat from October 2009 to March 2010. Approximately 100m of Reach 2: Section 1 is located in the restored channel. The other 100m is in the non-restored channel and is channelized. The channelized section holds less than half the normal water flow during the low flow months. This was observed in August 2009 and again in June 2010.

KIBI scores were consistent in the non-restored section of Mill Branch, with exceptions of Reach 2: Sections 1 and 2. One hundred meters of Reach 1: Section 2 is located in the non-restored section. This section of Reach 2: Section 1 is channelized with very little in-stream habitat, especially during low flow months. As mention previously, the lower portion of Reach 1: Section 1 could not sampled during March 2010 because of a large pool created by a beaver dam. This could have contributed to the lower KIBI score from October 2009 to March 2010. The decreased KIBI score for Reach 1: Section

2 could be due to an increase in percentage of insectivores and a decrease in percentage of tolerant individuals from October 2009 to March 2010. The decreased KIBI score for Reach 1: Section 3 (upstream of the new culvert) from October 2009 to March 2010 may partly be contributed to a decrease in total number of individuals (TNI) and percentage of facultative headwater individuals. Blackside dace abundance decreased in this section of stream from October 2009 to March 2010. Furthermore, the moderate increase in the percentage of insectivores and tolerant individuals could have also contributed to the decrease score for this section of Mill Branch.

One major difference in MBI scores from August 2009 to March 2010 is due to % EPT abundance. March 2010, produced more EPT taxa than August 2009. Most taxa within these orders are generally pollution sensitive, and their increased abundance is indicative of increasing water quality and/or habitat conditions (KDOW 2002). Diversity and abundance is expected to be higher within the sampling index period (mid-February to early-June). Furthermore, the decrease in chironomids and oligochaetes for sites 1 and 3 may have contributed to an increased MBI score for March 2010. The decrease in abundance of chironomids and oligochaetes is associated with increasing water quality (KDOW 2002). August 2009 and March 2010 data only serve as baseline information for future surveys or monitoring efforts at Mill Branch. August 2009 and March 2010 data were not intended to be compared to one another because the difference in community structure was likely due to differences in insect life cycles.

As the riparian vegetation matures and the riffles, runs, and pools become less embedded, Mill Branch will provide more cover and food, creating suitable habitats for EPT taxa, including shredders and collectors. This in turn will provide a more stable food source for other predator insects and fish, including blackside dace. Thus, macroinvertebrate and fish communities are expected to improve as riparian vegetation matures along the stream corridor and in-stream conditions improve (e.g. reduced sediment deposition),

The October 2009 stepwise regression results indicated that creek chub abundance contributed 50% of the variation in blackside dace abundance. This was likely due to the high abundance of both species in shallow pool areas throughout sections where they were collected. Both blackside dace and creek chub abundance was higher in shallow pool areas than other areas during that time. It is possible that both species sought refuge from the elevated temperatures. During March 2010 sampling, blackside dace individuals were scattered throughout different habitat types (i.e. pool areas, run areas, and riffle areas). Blackside dace and creek chub abundance, as well as temperatures were slightly higher in the non-restored channel at that time. This could explain the positive correlation between temperature and blackside dace abundance for the March 2010 stepwise regression model.

The overall blackside dace population seems to be utilizing more habitat than previously observed in March 2009. March 2009 was the first time blackside dace were observed in the new channel. In June 2010, blackside dace were observed in sections of Mill Branch over 300m downstream from where the newly constructed passable culvert is located. This observation is an indication that the stream was gradually changing in a positive direction that will hopefully benefit the blackside dace population. However, the beaver population had moved upstream from March 2010 to June 2010, as more dams were being constructed. This could be detrimental to the blackside dace population if they persist and create additional impounded reaches, thereby creating favorable conditions for predatory fishes. As of June 2011, the beaver had been trapped and relocated, and the lower reaches of Mill Branch were free-flowing (Michael Floyd, USFWS, 2010).

The Cumberland arrow darter could have been extirpated from Mill Branch, especially since only one individual was collected from 2006-2010. There is an obvious association between the Cumberland arrow darter and blackside dace in other tributaries within the Cumberland River basin (Starnes and Starnes 1978, O'Bara 1990). The new channel design, including instream habitat and flow modifications could allow for the re-introduction of Cumberland arrow darters into Mill Branch, primarily because it is known to co-occur with black dace throughout most of its range; and therefore, is susceptible to the same threats.

Rapid human population growth within the Cumberland River basin and the associated increase of natural resource extraction activities are the greatest threats to blackside dace and other fishes that inhabit this drainage system. For Mill Branch, the most immediate threat was an increasing beaver population. Thus, it is recommended that the lower portion of Mill Branch be monitored frequently for beavers. It is also recommended that long-term monitoring be done on both the fish and macroinvertebrate communities of Mill Branch. Long-term monitoring can greatly improve our understanding of blackside dace, and more importantly, the overall physical and chemical dynamics of stream structure and function. Restoration requires knowledge of the complex interactions within stream ecosystems. Furthermore, additional information is needed on how these ecosystems react to the physical and chemical perturbations that act within their watersheds. This knowledge will greatly determine the success of future restoration projects.

LIST OF REFERENCES

1. Angermeier, P. L. 1995. Ecological attributes of extinction-prone species: loss of freshwater fishes of Virginia. Conservation Biology 9:143-158.
2. Baron, J.S., N.L. Poff. 2004. Sustaining Healthy Freshwater Ecosystems. Universities Council on Water Resources: *Water Resources Update*. 127:52-58.
3. Bayless, J., and W. B. Smith. 1964. The effects of channelization upon the fish populations of lotic waters in eastern North Carolina. Proceedings of the North Carolina Wildlife Resources Committee, Raleigh.
4. Black, T. and H. T. Mattingly. 2007. Conservation status of the threatened blackside dace in selected Cumberland River streams. Unpublished final project report submitted to Tennessee Ecological Services Field Office and Kentucky Ecological Services Field Office, U. S. Fish and Wildlife Service. Cookeville, Tennessee and Frankfort, Kentucky. 51 pp.
5. Burkhead, N. M., S. J. Walsh, B. J. Freeman, and J. D. Williams. 1997. Status and restoration of the Etowah River, an imperiled southern Appalachian ecosystem. Pages 375-444, *In* G. W. Benz and D. E. Collins, eds. Aquatic fauna in peril: the Southeastern perspective. Special Publ. 1, Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, GA.
6. Bernhardt, E. S. 2005. National River Restoration Science Synthesis (NRRSS) statistics page for all node areas. U.S. Geological Survey. Available: nrrss.nbii.gov. (December 2005).
7. Bernhardt, E. S., M. A. Palmer, J. D. Allan, G. Alexander, K. Barnas, S. Brooks, J. W. Carr, S. Clayton, C. N. Dahm, J. F. Follstad-Shah, D. L. Galat, S. G. Gloss, P. Goodwin, D. D. Hart, B. Hassett, R. Jenkinson, S. Katz, G. M. Kondolf, P. S. Lake, R. Lave, J. L. Meyer, T. K. O'Donnell, L. Pagano, B. Powell, and E. Sudduth. 2005. Synthesizing U.S. river restoration efforts. Science 308:636–637.

8. Brake, S.S., K.A. Connors, and S.B. Romberger. 2001. A river runs through it: impact of acid mine drainage on the geochemistry of West Little Sugar pre- and post-reclamation at the Green Valley coal mine, Indiana, USA. Environmental Geology 40:1471-1481.
9. Burr, B. M., and R. L. Mayden. 1992. Phylogenetics and North American freshwater fishes. Pages 18-75, *In* R. L. Mayden, ed. Systematics, historical ecology, and North American freshwater fishes. Stanford University Press, Stanford, CA.
10. Butler, R.S. 2002. Imperiled fishes of the lower Tennessee Cumberland ecosystem, with emphasis on the non-federally listed fauna. U.S. Fish and Wildlife Service, Asheville, North Carolina. 38 pp.
11. Cicerello, R.R., and E.L. Laudermilk. 1996. Nesting association of the cyprinid fishes *Phoxinus cumberlandensis* and *Semotilus atromaculatus* (Cyprinidae). Transactions of the Kentucky Academy of Science 57:47.
12. Detar, J.E. 2004. Population densities and movement patterns of the threatened blackside dace, *Phoxinus cumberlandensis*. M.S. Thesis, Tennessee Technological University, Cookeville, Tennessee. 75 pp.
13. Eisenhour, D.J. and K.R. Piller. 1997. Two intergeneric hybrids involving *Semotilus atromaculatus* and the genus *Phoxinus* with analysis of additional *Semotilus atromaculatus*-*Phoxinus* hybrids. Copeia 1997:204-209.
14. Etnier, D.A. 1997. Jeopardized southeastern freshwater fishes: a search for causes. Pages 88-104, *In* G.W. Benz and D.E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Special Publication 1, Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, Georgia.
15. Etnier, D.A. and W.C. Starnes. 1993. The Fishes of Tennessee. The University of Tennessee Press, Knoxville, Tennessee. 689 pp.
16. Floyd, M. 2009. Personal communication. Fish and Wildlife Biologist. Kentucky Ecological Services Field Office, U. S. Fish and Wildlife Service. Frankfort, Kentucky.

17. Floyd, M. 2010. Personal communication. Fish and Wildlife Biologist. Kentucky Ecological Services Field Office, U. S. Fish and Wildlife Service. Frankfort, Kentucky.
18. Gore, J. A., F. L. Bryant, and D. J. Crawford. 1995. River and stream restoration. Pages 245–275, *In* J. Cairns, editor. Rehabilitating damaged ecosystems. Lewis Publishers, Boca Raton, Florida.
19. Gore, J. A. 2006. Discharge measurments and streamflow analysis. Pages 51-78 In R. F. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology, 2nd edition. Academic Press. Burlington, MA
20. Johnson, S.L. and J.A. Jones. 2000. Stream temperature responses to forest harvest and debris flows in western Cascades, Oregon. Canadian Journal of Fisheries and Aquatic Science 57:30-39.
21. Johnston, C.E. 1999. The Relationship of Spawning Mode to Conservation of North American Minnows (Cyprinidae). Environmental Biology of Fishes. 55:21-30.
22. Jones, B.K. 2005. Predictive habitat models for conservation for the threatened blackside dace (*Phoxinus cumberlandensis*). M.S. Thesis, Tennessee Technological University, Cookeville, Tennessee. 75 pp.
23. Jones, N. A. 1975. A preliminary study of fish segregation in salmon spawning streams. Journal of Fish Biology 7:95–104. Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21–27.
24. Karr, J. 1981. Assessment of Biotic Integrity Using Fish Communities. Fisheries. 6:21-27.
25. Karr, J.R., and D.R. Dudley. 1981. Ecological perspective on water quality goals. Environmental Management 5:55-68.
26. Kentucky Department of Fish and Wildlife Resources (KDFWR). 2007. Knox County Mill Branch stream restoration project is underway. Kentucky Fish and Wildlife Commissioner's Newsletter. 2(11):2. Frankfort, Kentucky.
27. Kentucky Division of Water (KDOW). 2002. Methods for assessing biological integrity of surface waters. Kentucky Department for Environmental Protection, Ecological Support Section. Frankfort, Kentucky. 182 pp.

28. Kentucky State Nature Preserves Commission (KSNPC). 2010. Kentucky State Nature Preserves Commission Biotics database. Frankfort, Kentucky.
29. Mattingly, H.T., and Contributors. 2005. Factors affecting the distribution and recovery of the threatened blackside dace in Kentucky and Tennessee. Final project report, U.S. Fish and Wildlife Service, Cookeville Field Office, Cookeville, TN. 126 pp.
30. Merritt, R.W., K.W. Cummins, M.B. Berg. 2008. An Introduction to the Aquatic Insects of North America. Kendall Hunt.
31. National Research Council (NRC). 1992. Restoration of aquatic ecosystems. National Academy Press, Washington, D.C.
32. Natural Resources Conservation Service Newsletter, 2008. http://www.ky.nrcs.usda.gov/news/BlacksideDACE.html, accessed April 16, 2009.
33. Palmer, M. A., E. S. Bernhardt, J. D. Allan, P. S. Lake G. Alexander, S. Brooks, J. W. Carr, S. Clayton, C. N. Dahm, J. F. Follstad-Shah, D. L. Galat, S. Gloss, P. Goodwin, D. D. Hart, B. Hassett, R. Jenkinson, G. M. Kondolf, R. Lave, J. L. Meyer, T. K. O'Donnell, L. Pagano, and E. Sudduth. 2005. Standards for ecologically successful river restoration. Journal of Applied Ecology 42:208–217.
34. Palmer, M.A., E.S. Bernhardt, W.H. Schlesinger, K.N. Eshleman, E. Foufoula-Georgiou, M.S. Hendryx, A.D. Lemly, G.E. Likens, O.L. Loucks, M.E. Power, P.S. White, and P.R. Wilcock. Mountaintop Mining Consequences. 2010. Science. 327: 148-149.
35. Parola, A. 2010. Personal Communication. University of Louisville Stream Institute: Department of Civil and Environmental Engineering. Louisville, Kentucky.
36. Pond, G.J., M.E. Passmore, F.A. Borsuk, L. Reynolds, and C.J. Roses. 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family-and genus-level macroinvertebrate bioassessment tools. Journal of North American Benthological Society. 27: 717-737.
37. O'Bara, C.J. 1990. Distribution and ecology of the blackside dace *Phoxinus cumberlandensis* (Osteichthyes: Cyprinidae). Brimleyana. 16:9-15.

38. Rabeni, C. F., and R. B. Jacobson. 1993. The importance of fluvial hydraulics to fish-habitat restoration in low-gradient alluvial streams. Freshwater Biology 29:211–220.

39. SAS Institute Inc., SAS 9.2 Help and Documentation, Cary, NC: SAS Institute Inc., 2002-2008.

40. Skelton, C.E. 2001. New Dace of the Genus *Phoxinus* (Cyprinidae: Cypriniformes) from the Tennessee River Drainage, Tennessee. Copeia. 2001: 118-128.

41. Starnes, L.B. and W.C. Starnes. 1978. A new cyprinid fish of the genus *Phoxinus* from the Tennessee River drainage with comments on relationships and biogeography. Proceedings of the Biological Society of Washington 101:517-529.

42. Starnes, L.B. and W.C. Starnes. 1981. Biology of the blackside dace *Phoxinus cumberlandensis*. American Midland Naturalist 106:360-372.

43. Starnes, W.C. 1981. Listing package for the blackside dace, *Phoxinus cumberlandensis*. U.S. Fish and Wildlife Service, Asheville, North Carolina.

44. Starnes, W.C., and D.A. Etnier. 1986. Drainage evolution and fish biogeography of the Tennessee and Cumberland rivers drainage realm. Pages 325-361, *In* C.H. Hocutt and E.O. Wiley, editors. The Zoogeography of North American Freshwater Fishes. John Wiley, New York, New York.

45. Strange, R.M., and B.M. Burr. 1995. Genetic Variability and Metapopulation Dynamics in Federally Threatened Blackside Dace, *Phoxinus cumberlandensis* (Pisces: Cyprinidae). Final Report to Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky.

46. Strange, R.M., and R.L. Mayden. 2009. Phylogenetic Relationships and a Revised Taxonomy for North American Cyprinids Currently Assigned to *Phoxinus* (Actinopterygii: Cyprinidae). Copeia 3:494-501.

47. Sutherland, A.B., J.L. Meyer, and E.P. Gardiner. 2002. Effects of land cover on sediment regime and fish assemblage structure in four southern Appalachian streams. Freshwater Biology 47:1791-1805.

48. USFWS. 1987. Determination of threatened species status for the blackside dace. Federal Register 52(113):22580-22585.

49. USFWS. 1988. Recovery plan for the blackside dace *Phoxinus cumberlandensis*. U.S. Fish and Wildlife Service, Atlanta, Georgia. 23 pp.
50. USFWS. 2010. Blackside Dace *Phoxinus cumberlandensis* (=*Chrosomus cumberlandensis*) Draft 5-Year Review: Summary and Evaluation. Kentucky Ecological Services Field Office, U. S. Fish and Wildlife Service. Frankfort, Kentucky.
51. Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, C.E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences. 37:130-137.
52. Walsh, M.J., N.M. Burkhead, and J.D. Williams. 1995. Southeastern freshwater fishes. Pages 144-147 *in* E.T. Laroe, G.S. Farris, C.E. Puckett, P.D. Doran, and M.J. Mac, editors. Our living resources: a report to the nation on the distribution, abundance, and health of U.S. plants, animals, and ecosystems. U.S. Department of the Interior, Washington, DC.
53. Warren, M.L., and B.M. Burr. 1994. Status of freshwater fishes of the United States: overview of an imperiled fauna. Fisheries 19:6-18.
54. Warren. M.L., P.L. Angermeier, B.M. Burr, and W.R. Haag. 1997. Decline of a diverse fish fauna: patterns of imperilment and protection in the southeastern United States. Pages 105-164, *In* G.W. Benz and D.E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Special Publication 1, Southeastern Aquatic Research Institute, Lenz Design and Communications, Decatur, Georgia.
55. Warren, M.L. Jr., B.M. Burr, S.J. Walsh, H.L. Bart Jr., R.C. Cashner, D.A. Etnier, B.J. Freeman, B.R. Kuhajda, R.L. Mayden, H.W. Robison, S.T. Ross, W.C. Starnes. (2000). Diversity, Distribution, and Conservation Status of the Native Freshwater Fishes of the Southern United States. Fisheries. 25:7-31.
56. Williams, J.E., J.E. Johnson, D.A. Hendrickson, S. Contreras-Balderas, J.D. Williams, M. Navarro-Mendoza, D.E. McAllister, and J.E. Deacon. 1989. Fishes of North America endangered, threatened, or of special concern: 1989. Fisheries. 14:2-20.

57. Wood, P.J. and P.D. Armitage. 1997. Biological effects of fine sediment in the lotic environment. Environmental Management 21:203-217.

58. Woods, A.J., J.M. Omernik, W.H. Martin, G.J. Pond, W.M. Andrews, S.M. Call, J.A. Comstock, D.D. Taylor. 2002. Ecoregions of Kentucky (color poster with map, descriptive text, summary tables, and photographs): Reston, VA., U.S. Geological Survey (map scale 1:100,000).

APPENDIX A: TABLES

Table 1. Representative fish identified and enumerated by Michael Floyd, USFWS within the Reach 1 of Mill Branch, Knox Co., KY. Data collected between 2006-2009 (pre- and post-restoration) but prior to eliminating old channel. (Note: New refers to restored channel and old refers to old channel. New-above and New-below refers to upstream and downstream of newly installed culvert).

Species	2006	2007	Reach 1 Mar-08		Nov-08	2009	
			Old	New	New	New-above	New-below
Lampetra aepyptera	0	88	0	0	0	0	0
Campostoma anomalum	12	140		10	85	1	195
Cyprinella spiloptera	24	7	6	27	0	0	3
Luxilus chrysocephalus	80	62	5	39	0	0	28
Lythrurus fasciolaris	0	0	2	0	0	0	0
Notropis buccatus	0	2	0	1	0	0	1
Notropis rubellus	0	0	0	16	0	0	0
Pimnephales notatus	22	105	25	48	240	1	134
Chrosomus cumberlandensis	6	75	0	0	0	43	0
Chrosomus erythrogaster	0	11	0	0	0	2	1
Semotilus atromaculatus	123	478	1	2	7	76	20
Rhinichthys obtusus	1	2	0	0	0	0	0
Catastomus commersoni	2	13	0	0	169	77	44
Hypentelium nigricans	0	0	1	0	0	0	0
Ameiurus natalis	0	0	0	0	4	0	0
Lepomis auritus	14	42	4	9	95	0	37
Lepomis cyanellus	4	0	0	2	0	0	3
Lepomis gulosus	0	0	0	0	2	0	3
Lepomis macrochirus	3	0	0	0	26	0	21
Lepomis megalotis	0	0	0	0	3	0	0
Ambloplites rupestris	0	0	0	0	7	0	0
Micropterus salmoides	0	0	0	0	0	0	0
Micropterus punctulatus	0	0	0	0	26	0	13
Etheostoma caeruleum	2	38	1	19	28	6	107
Etheostoma kennicotti	7	56	5	4	5	25	21
Ethostoma sagitta sagitta	0	1	0	0	0	0	0

Table 2. Representative fish identified and enumerated by Michael Floyd, USFWS within Reach 2 of Mill Branch, Knox Co., KY. Data collected between 2006-2009 (pre- and post-restoration) but prior to eliminating old channel.

	Reach 2			
Species	**2006**	**2007**	**2008**	**2009**
Campostoma anomalum	5	1	0	2
Luxilus chrysocephalus	0	0	15	0
Pimnephales notatus	0	0	1	0
Chrosomus cumberlandensis	63	386	406	316
Chrosomus erythrogaster	1	13	28	20
Semotilus atromaculatus	118	726	209	429
Rhinichthys obtusus	0	0	0	0
Catastomus commersoni	16	23	9	19
Lepomis auritus	1	0	0	0
Lepomis cyanellus	1	0	0	0
Micropterus salmoides	1	0	0	0
Etheostoma caeruleum	0	0	0	1
Etheostoma kennicotti	18	50	1	18

Table 3. Representative fish identified and enumerated by Michael Floyd, USFWS within the upper non-restored reaches of Mill Branch, Knox Co., KY. Data collected between 2006-2009 (pre- and post-restoration) but prior to eliminating old channel.

	Reach 3				Reach 4			
Species	**2006**	**2007**	**2008**	**2009**	**2006**	**2007**	**2008**	**2009**
Pimephales notatus	0	0	0	7	0	0	0	0
Chrosomus cumberlandensis	7	64	0	44	0	0	0	0
Chrosomus erythrogaster	1	0	0	9	0	0	0	0
Semotilus atromaculatus	112	257	7	439	49	19	0	1
Catastomus commersoni	0	5	0	0	0	0	0	0

Table 4. Fish Sampling Sites in August 2009 through June 2010 in Mill Branch, Knox Co., KY, indicating a total of 3 reaches, each 600 meters in length, Each 600 meter reach has 3 sections within, measuring 200 meters each. Reach 1 begins at the confluence of Mill Branch and Stinking Creek.

Reach : Section	Latitude	Longitude
Reach 1: Section 1	N 36.8733	W -83.7323
Reach 1: Section 2	N 36.8722	W -83.7309
Reach 1: Section 3	N 36.8706	W -83.7305
Reach 2: Section 1	N 36.8692	W -83.7295
Reach 2: Section 2	N 36.8676	W -83.7289
Reach 2: Section 3	N 36.8663	W -83.7276
Reach 3: Section 1	N 36.8653	W -83.7259
Reach 3: Section 2	N 36.8651	W -83.7237
Reach 3: Section 3	N 36.8647	W -83.7218
Reach 3: Section 4	N 36.8648	W -83.7201

Table 5. Macroinvertebrate Sampling Sites in August 2009 and March-April 2010 in Mill Branch, Knox Co., KY. (Note: All macroinvertebrate collection sites are located within the restored section. Site 1 is located within the first 100 meters from the mouth of Stinking Creek. Site 2 is located within 100 meters downstream of Mill Branch Rd. Site 3 is located within 100 meters upstream from Mill Branch Rd.)

Site #	Latitude	Longitude
1	N 36.872625	W 83.731753
2	N 36.870522	W 83.730467
3	N 36.869783	W 83.729989

Table 6. Representative fish within the restored section of Mill Branch, Knox Co., KY. Data collected August 18-22, 2009 after connection of new channel to main stem.

Species	Below Culvert	Above Culvert
Ambloplites rupestris	7	0
Ameiurus nebulosus	1	0
Campostoma anomalum	68	47
Catastomus commersoni	141	45
Chrosomus cumberlandensis	2	132
Etheostoma caeruleum	8	4
Etheostoma kennicotti	9	9
Hypentelium nigricans	1	0
Lepomis auritus	178	30
Lepomis cyanellus	2	1
Lepomis gulosus	2	0
Lepomis macrochirus	33	3
Lepomis megalotis	1	6
Luxilus chrysocephalus	13	0
Micropterus punctulatus	3	0
Micropterus salmoides	7	0
Notemigonus crysoleucas	11	0
Notropis buccatus	5	0
Pimephales notatus	402	53
Semotilus atromaculatus	114	96

Table 7. Fish species abundance for reach 1 (restored channel). Data collected from October 2009 through June 2010 located within Mill Branch, Knox Co., KY, after connection of restored channel to the main stem.

Species	Reach 1: Section 1			Reach 1: Section 2			Reach 1: Section 3 (Below Culvert)		Reach 1: Section 3 (Above Culvert)		Reach 1: Section 3 (ALL)
	Oct 2009	Mar 2010	Jun 2010	Oct 2009	Mar 2010	Jun 2010	Oct 2009	Mar 2010	Oct 2009	Mar 2010	Jun 2010
Ambloplites rupestris	0	0	0	0	0	1	1	0	0	0	0
Ameiurus natalis	7	2	0	0	0	0	0	0	0	0	0
Ameiurus nebulosus	0	1	0	0	0	0	0	0	0	0	0
Campostoma anomalum	28	14	1	53	37	0	13	17	9	0	19
Catastomus commersoni	37	73	3	35	29	38	20	44	44	9	50
Chrosomus cumberlandensis	0	0	2	0	0	1	4	13	124	32	48
Chrosomus erythrogaster	0	0	0	0	0	0	0	0	0	1	0
Cyprinella spiloptera	15	29	0	8	16	0	9	13	2	5	0
Etheostoma caeruleum	2	10	0	12	16	12	5	1	3	8	9
Etheostoma kennicotti	22	5	11	60	24	0	31	38	34	0	62
Hypentelium nigricans	2	0	0	1	1	0	0	0	0	0	0
Lampetra aepyptera	0	1	0	0	2	0	0	0	0	2	0
Lepomis auritus	77	76	25	33	31	35	15	49	14	5	47
Lepomis cyanellus	3	1	4	4	4	14	1	4	0	0	2
Lepomis gulosus	3	3	0	5	1	0	0	0	0	0	0

Table 7. (Continued)

Species	Reach 1: Section 1			Reach 1: Section 2			Reach 1: Section 3 (Below Culvert)		Reach 1: Section 3 (Above Culvert)		Reach 1: Section 3 (ALL)
	Oct 2009	Mar 2010	Jun 2010	Oct 2009	Mar 2010	Jun 2010	Oct 2009	Mar 2010	Oct 2009	Mar 2010	Jun 2010
Lepomis macrochirus	20	15	0	10	4	0	2	3	0	0	1
Lepomis megalotis	22	3	0	1	0	0	2	0	0	0	0
Luxilus chrysocephalus	10	79	0	6	23	0	0	1	0	4	0
Lythrurus fasciolaris	1	0	0	1	0	0	0	0	0	0	0
Micropterus punctulatus	5	0	0	0	0	0	0	0	1	0	0
Micropterus salmoides	4	1	0	0	0	0	0	1	0	0	0
Notemigonus crysoleucas	2	6	0	0	0	0	0	0	0	0	0
Notropis buccatus	1	5	0	0	2	0	0	0	0	0	0
Pimephales notatus	154	324	6	186	29	68	63	26	45	7	40
Semotilus atromaculatus	26	65	13	55	35	53	58	32	102	78	132
L. cyanellus/auritus hybrid	0	0	0	0	0	1	0	0	0	0	0

Table 8. Fish species abundance for reach 2 data collected from August 2009 through June 2010 located within Mill Branch, Knox Co., KY. (Note: only 50 meters of Reach 2: Section 1 was sampled due to extreme temperatures that could have caused high mortality in blackside dace.) No blackside dace were found in the restored section of Reach 2.

Species	Reach 2: Section 1			Reach 2: Section 2		Reach 2: Section 3	
	Oct 2009	Mar 2010	Jun 2010	Oct 2009	Mar 2010	Oct 2009	Mar 2010
Campostoma anomalum	9	0	0	35	15	1	1
Catastomus commersoni	18	3	0	11	1	3	10
Chrosomus cumberlandensis	82	74	8	191	30	90	52
Chrosomus erythrogaster	0	3	0	2	2	7	1
Cyprinella spiloptera	1	0	0	2	0	0	0
Etheostoma blennioides	0	0	0	1	0	0	0
Etheostoma caeruleum	3	4	0	13	3	12	1
Etheostoma kennicotti	26	9	21	52	33	6	10
Lampetra aepyptera	0	17	0	0	7	0	2
Lepomis auritus	10	3	0	9	3	8	2
Lepomis cyanellus	0	0	0	1	0	0	0
Notropis buccatus	1	0	0	0	0	0	0
Pimephales notatus	11	1	0	13	0	0	0
Semotilus atromaculatus	201	97	37	230	84	194	143

Table 9. Fish species abundance for reach 3. Data collected from August 2009 through June 2010 within Mill Branch, Knox Co., KY.)

Species	Reach 3: Section 1		Reach 3: Section 2		Reach 3: Section 3		Reach 3: Section 4
	Oct-09	Mar-10	Oct-09	Mar-10	Oct-09	Mar-10	Oct-09
Campostoma anomalum	0	7	3	0	0	0	0
Catastomus commersoni	2	9	0	2	0	0	0
Chrosomus cumberlandensis	49	15	9	23	9	17	8
Chrosomus erythrogaster	1	1	0	3	2	2	9
Etheostoma caeruleum	10	0	0	0	0	0	0
Etheostoma kennicotti	16	10	6	0	2	0	0
Lampetra aepyptera	0	1	0	1	0	0	0
Lepomis auritus	9	2	0	0	0	0	0
Semotilus atromaculatus	134	38	232	149	225	76	290

Table 10. Capture rates (fish/minute) for all fish species. Note: (-) indicates the section was not sampled. R1: S3 was divided into two 100 meter sections, indicated as BC (below culvert) and AC (above culvert). For June 2010, R1: S3 was combined into 200 meters.

Reach (R): Section (S)	October 2009	March 2010	June 2010
R1: S1	9.88	7.55	6.50
R1: S2	11.37	3.86	3.46
R1: S3 (BC)	14.88	13.96	6.10
R1: S3 (AC)	17.30	5.94	
R2: S1	9.18	5.77	-
R2: S2	13.55	4.76	-
R2: S3	8.83	6.77	-
R3: S1	6.58	2.84	-
R3: S2	5.95	6.90	-
R3: S3	5.19	4.87	-
R3: S4	7.76	-	-

Table 11. Blackside dace capture rates in the new channel. (Note: BC = below new culvert, AC = above new culvert. Reach 1: Section 3 was divided into 100 meter reaches, 100 meters BC and 100 meters AC. R = Reach : S = Section.)

<table>
<tr><th colspan="5">Capture Rates (blackside Dace/minute)</th></tr>
<tr><td>Sampling Date</td><td>R1 : S2</td><td>R1 : S3 (BC)</td><td>R1 : S3 (AC)</td><td>R2 : S1</td></tr>
<tr><td>Oct/Nov 2009</td><td>0.00</td><td>0.27</td><td>5.68</td><td>0.00</td></tr>
<tr><td>Mar/Apr 2010</td><td>0.00</td><td>0.75</td><td>1.26</td><td>2.02</td></tr>
<tr><td>June 2010</td><td>0.02</td><td colspan="2">0.71</td><td>N/A</td></tr>
</table>

Table 12. Quantitative and qualitative Macroinvertebrate data collected from Mill Branch, Knox County, Kentucky, August 2009 and March 2010. (Note: Asterisks represent qualitative data, and numbers represent quantitative data).

			August 2009			March-2010		
Order	**Family**	**(subfamily) Genus species**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
Odonata	Aeschnidae	*Boyeria* sp.	*	*				
Odonata	Calopterygidae	*Calopteryx* sp.	*	*		*	*	
Odonata	Coenagrionidae	*Chromagrion* sp.				*		
Odonata	Cordulagastridae	*Cordulagaster* sp.	*					
Odonata	Corduliidae	*Helocordulia* sp.				*		
Odonata	Corduliidae	*Sematochlora* sp.			*			
Odonata	Gomphidae	*Arigomphus* sp.	*					
Odonata	Gomphidae	*Gomphus* sp.				*		
Odonata	Gomphidae	*Hagenius sp*	1*					
Odonata	Gomphidae	*Stylogomphus* sp.	*					
Odonata	Macromiidae	*Didymops* sp.				*		
Ephemeroptera	Baetidae	*Baetis* sp.	*		1	*		*
Ephemeroptera	Baetidae	*Centroptilum* sp.	*					
Ephemeroptera	Baetidae	*Paracloeodes* sp.				*		
Ephemeroptera	Caenidae	*Caenis* sp.	*	*				
Ephemeroptera	Ephemeridae	*Ephemera* sp.	*				*	
Ephemeroptera	Ephemeridae	*Hexagenia* sp.				*		*
Ephemeroptera	Ephemerellidae	*Ephemerella* sp.						*
Ephemeroptera	Ephemerellidae	*Eurylophella* sp.					1	1*
Ephemeroptera	Ephemerellidae	*Serratella* sp.					*	

Table 12. (Continued)

Order	Family	(subfamily) Genus species	August 2009			March-2010		
			Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Ephemeroptera	Heptageniidae	*Epeorus* sp.						*
Ephemeroptera	Heptageniidae	*Maccaffertium* sp.		*			*	1*
Ephemeroptera	Heptageniidae	*Stenacron* sp.		*				
Ephemeroptera	Heptageniidae	*Stenonema femoratum*				*		
Ephemeroptera	Isonychiidae	*Isonychia* sp.		3				
Ephemeroptera	Leptophlebiidae	*Leptophlebia* sp.				*	*	
Plecoptera	Perlidae	*Eccoptera* sp.		2	1			
Plecoptera	Perlodidae	*Cleoperla clio*				1*		
Plecoptera	Perlodidae	*Cultus* sp.				4*	3	1
Plecoptera	Perlodidae	*Isoperla* sp.				*	3*	4*
Trichoptera	Glossosomatidae	*Glossosoma* sp.					3	
Trichoptera	Hydropsychidae	*Ceratopsyche* sp.						1
Trichoptera	Hydropsychidae	*Cheumatopsyche* sp.		3	1	*	1	
Trichoptera	Limnephilidae	*Pycnopsyche* sp.					*	*
Trichoptera	Polycentropodidae	*Polycentropus* sp.				*		
Trichoptera	Rhyacophilidae	*Rhyacophila* sp.						1*
Trichoptera	Uenoidae	*Neophylax* sp.					5*	
Megaloptera	Corydalidae	*Corydalus* sp.		1			1	
Megaloptera	Corydalidae	*Nigronia* sp.		1				
Megaloptera	Sialidae	*Sialis* sp.				*	*	
Coleoptera	Dryopidae	*Helicus* sp.	*	6	3*			
Coleoptera	Elmidae	*Optioservus* sp.						1

Table 12. (Continued)

Order	Family	(subfamily) Genus species	August 2009			March-2010		
			Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Coleoptera	Hydrophilidae	*Hydrophilus* sp.	*					
Diptera	Ceratopogonidae	*Culicoides* sp.						*
Diptera	Chironomidae	Chironominae	*					
Diptera	Chironomidae	Orthocladinae			*			
Diptera	Chironomidae	Tanypodinae	*		*			
Diptera	Chironomidae	unidentified subfamily		*	1*	*	*	*
Diptera	Sciomyzidae	unid. Genus species					*	
Diptera	Simulidae	*Prosimulium* sp.					*	
Diptera	Simulidae	*Simulium* sp.						*
Diptera	Tipulidae	*Limnophila* sp.		*	1			*
Diptera	Tipulidae	*Hexatoma* sp.						3
Diptera	Tipulidae	*Tipula* sp.			*	*	5*	1*
Isopoda	Unidentified Family	Unidentified genus						*
Decapoda	Cambaridae	*Crayfish* sp.	*		2*		*	
Decapoda	Cambaridae	*Orconectes* sp.	*	1*				
Decapoda	Cambaridae	*Cambarus buntingi*	*	*				
Decapoda	Cambaridae	*Cambarus* sp.	*	*	*			*
Unid. Oligochaete	Unid. Oligochaete		*	*	*	*		1

Table 13. Mean conductivity, temperature, dissolved oxygen, and pH for the restored channel of Mill Branch, Knox County, Kentucky. (Note: August 2008 (N = 6; ± = Standard Deviation); August 2009 (N = 3; ± = Standard Deviation); October 2009 (N = 4; ± = Standard Deviation); March 2010 (N = 4; ± = Standard Deviation); June 2010 (N = 3; ± = Standard Deviation).

Date	Conductivity (µS)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH
August 2008	235.12 ±22	22.18 ±2	-	-
August 2009	158.27 ±27	21.07 ±1	-	7.61 ±0.3
October 2009	143.00 ±10	16.39 ±1	10.69 ±3	7.81 ±0.8
March 2010	75.90 ±10	12.73 ±4	11.15 ±2	7.02 ±0.7
June 2010	144.53 ±5	23.10 ±1	8.31 ±0.2	7.45 ±0.1

Table 14. Rapid Habitat Assessment scores for Mill Branch, Knox Co., KY, for all reaches. Sampled October 2009 and March 2010.

Reach: Section	Date	Rapid Habitat Assessment Score
Reach 1: Section 1	10/9/09	99
	3/27/10	84
Reach 1: Section 2	10/9/09	113
	3/22/10	123
Reach 1: Section 3	10/12/09	124
	4/1/10	127
Reach 2: Section 1	10/12/09	109
	4/6/10	114
Reach 2: Section 2	10/13/09	125
	4/6/10	125
Reach 2: Section 3	10/27/09	129
	4/15/10	127
Reach 3: Section 1	10/27/09	129
	4/15/10	133
Reach 3: Section 2	11/4/09	132
	4/15/10	132
Reach 3: Section 3	11/4/09	123
	4/15/10	129

Table 15. Fish metric values for Kentucky Index of Biotic Integrity (KIBI) for each 200 meter section sampled October 2009 through June 2010 from Mill Branch, Knox County, Kentucky.

Reach: Section	Sample Date	Catchment Area (CA) (mi^2)	TNI	NAT	DMS	INT	SL	%INSCT	%TOL	%FHW	KIBI	SCORE
Reach 1: Section 1	10/9/09	2.32	441	19	2	0	5	36.73	65.99	88.21	61	G
	3/27/10	2.32	712	17	2	2	4	31.18	90.87	87.08	47	F
	6/4/10	2.32	63	6	1	0	2	63.49	80.95	79.37	58	F
Reach 1: Section 2	10/9/09	2.26	470	14	2	0	5	30.00	70.00	72.77	57	F
	3/22/10	2.26	254	14	2	0	5	47.24	61.02	69.29	66	G
	6/4/10	2.26	222	8	1	1	3	58.11	93.69	70.27	66	G
Reach 1: Section 3 (Below Culvert)	10/12/09	2.22	224	12	2	1	4	24.55	70.98	56.25	68	G
	4/1/10	2.22	242	12	2	1	5	39.67	47.52	47.11	54	F
Reach 1: Section 3 (Above Culvert)	10/12/09	2.19	378	9	2	1	4	14.02	54.23	30.42	63	G
	4/1/10	2.19	151	9	1	2	4	21.85	68.21	19.87	49	F
Reach 2: Section 1	10/12/09	2.06	362	9	2	1	4	11.05	66.30	13.54	61	G
	4/1/10	2.06	211	8	2	1	5	7.58	49.29	3.32	77	E
Reach 2: Section 2	10/12/09	1.91	560	11	3	2	6	13.93	47.14	12.86	67	G
	4/6/10	1.91	178	8	2	2	5	21.91	49.44	10.67	53	F
Reach 2: Section 3	10/27/09	1.78	321	8	2	2	5	8.10	63.86	3.74	63	G
	4/6/10	1.78	222	8	2	2	5	5.86	69.82	5.86	61	G
Reach 3: Section 1	10/27/09	1.63	221	7	2	2	5	15.84	65.61	4.98	54	F
	4/15/10	1.63	83	7	1	2	4	14.46	59.04	21.69	50	F
Reach 3: Section 2	11/4/09	1.46	250	4	1	1	2	2.40	92.80	1.20	58	F
	4/15/10	1.46	178	5	0	2	3	0.00	84.83	1.12	55	F
Reach 3: Section 3	11/6/09	1.32	238	4	1	2	3	0.84	94.54	0.00	58	F
	4/15/10	1.32	95	3	0	2	2	0.00	80.00	0.00	58	F
Reach 3: Section 4	11/5/09	1.13	307	3	0	2	2	0.00	94.46	0.00	49	F

Table 16. Kentucky core macroinvertebrate metrics, MBI score, and water quality rating for Mill Branch, Knox County, Kentucky, during August 2009 and March 2010. Note: The ratings refer to water quality (VP = very poor, P = poor, F = fair).

Sample Date	Sample Site	G-TR	G-EPT	mHBI	m%EPT	%Ephem	%C+O	%ClngP	TotInd	MBI	Rating
8/18/2009	1	19	4	6.25	11.43	11.43	37.14	2.86	35	26.81	P
8/18/2009	2	17	6	5.28	35.48	19.35	9.68	48.39	31	45.62	P
8/18/2009	3	13	3	6.49	5.00	1.67	71.67	10.00	60	17.54	VP
3/27/2010	1	20	11	5.96	60.87	40.58	18.84	36.23	69	53.22	F
4/6/2010	2	19	11	4.71	71.43	50.55	12.09	63.74	91	65.86	F
4/6/2010	3	21	11	4.33	74.67	54.67	6.67	40.00	75	64.89	F

Table 17. Combined discharge (riffle, run, pool) of each 200 meter section, indicating date and corresponding reach and section.

Reach: Section	Date	Discharge (m^3/s)
Reach 1: Section 1	10/9/09	0.090 ±0.132
	3/27/10	0.200 ±0.117
Reach 1: Section 2	10/9/09	0.051 ±0.20
	3/22/10	0.079 ±0.041
	6/4/10	0.161 ±0.062
Reach 1: Section 3	10/12/09	0.096 ±0.054
	4/1/10	0.236 ±0.047
	6/4/10	0.312 ±0.005
Reach 2: Section 1	10/12/09	0.014 ±0.005
	4/6/10	0.178 ±0.016
	6/4/10	0.098 ±0.105
Reach 2: Section 2	10/13/09	0.045 ±0.041
	4/6/10	0.066 ±0.040
Reach 2: Section 3	10/27/09	0.026 ±0.021
	4/15/10	0.159 ±0.028
Reach 3: Section 1	10/27/09	0.015 ±0.009
	4/15/10	0.056 ±0.015
Reach 3: Section 2	11/4/09	0.069 ±0.079
	4/15/10	0.071 ±0.022
Reach 3: Section 3	11/4/09	0.055 ±0.030
	4/15/10	0.089 ±0.033

APPENDIX B: FIGURES

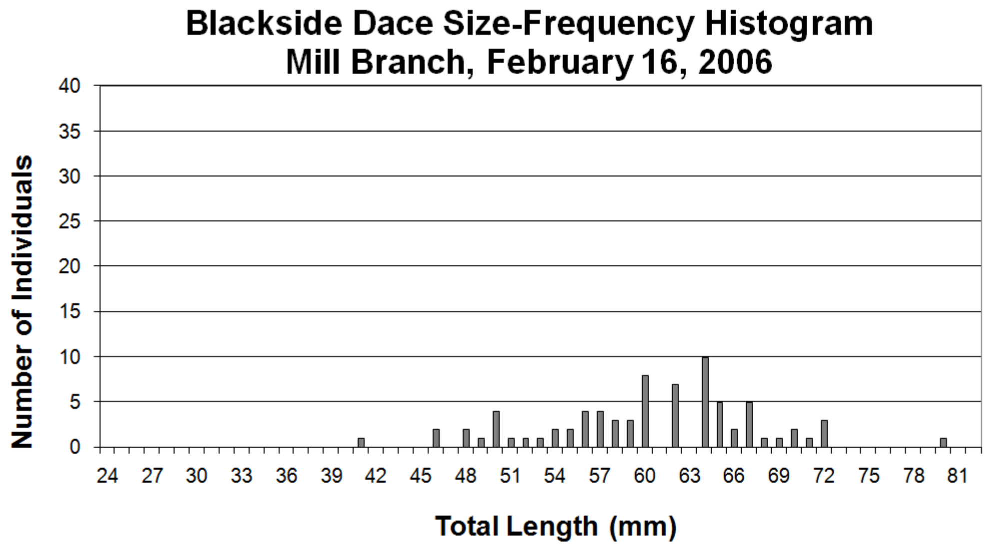

Figure 1. Blackside dace (*Chrosomus cumberlandensis*) length-frequency histogram, Mill Branch, February 16, 2006 (Figure courtesy of Mike Floyd, USFWS). A total of 76 individuals were collected.

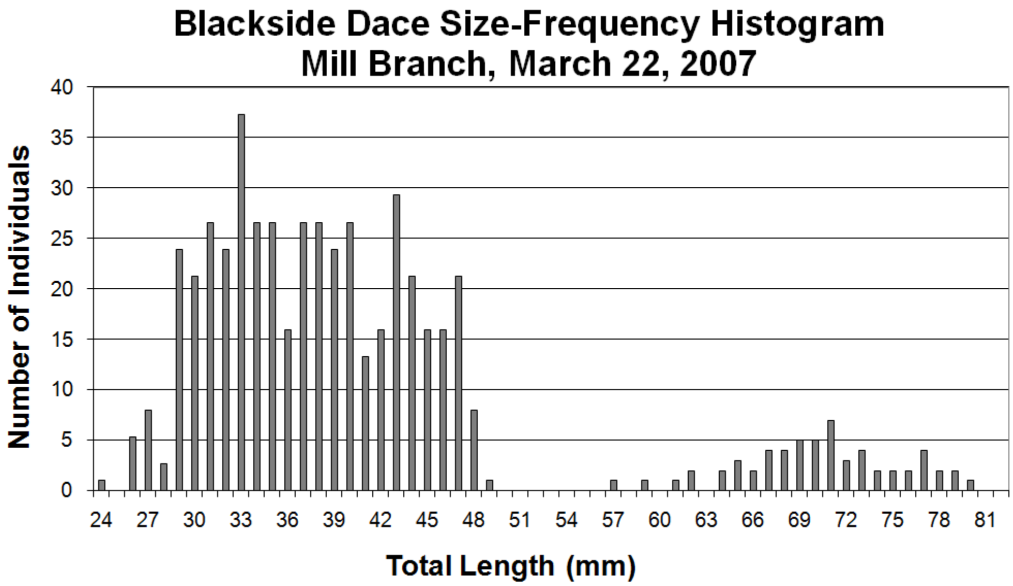

Figure 2. Blackside dace (*Chrosomus cumberlandensis*) length-frequency histogram, Mill Branch, March 22, 2007 (Figure courtesy of Mike Floyd, USFWS). A total of 525 individuals were collected.

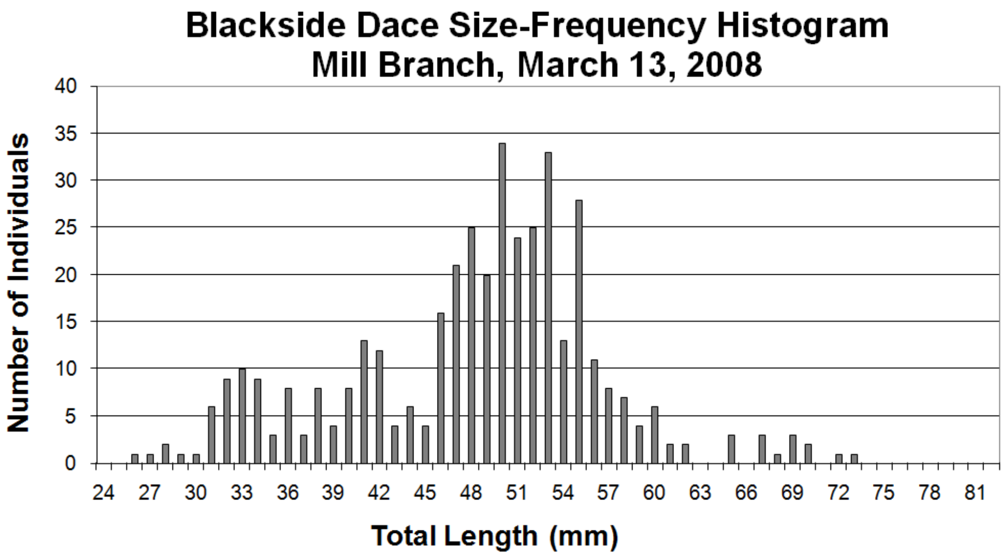

Figure 3. Blackside dace (*Chrosomus cumberlandensis*) length-frequency histogram, Mill Branch, March 13, 2008 (Figure courtesy of Mike Floyd, USFWS). A total of 406 individuals were collected.

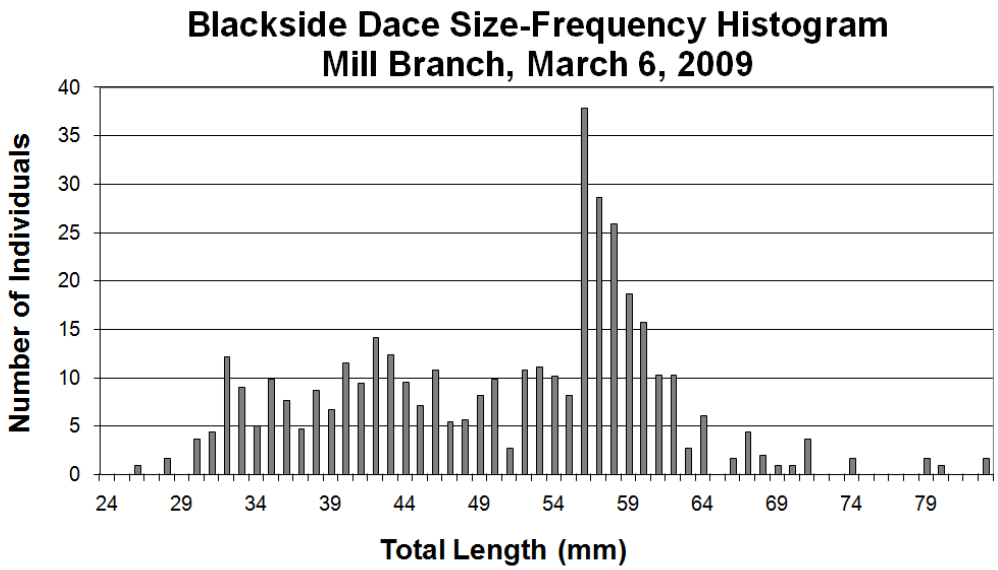

Figure 4. Blackside dace (*Chrosomus cumberlandensis*) length-frequency histogram, Mill Branch, March 6, 2009 (Figure courtesy of Mike Floyd, USFWS). A total of 397 individuals were collected.

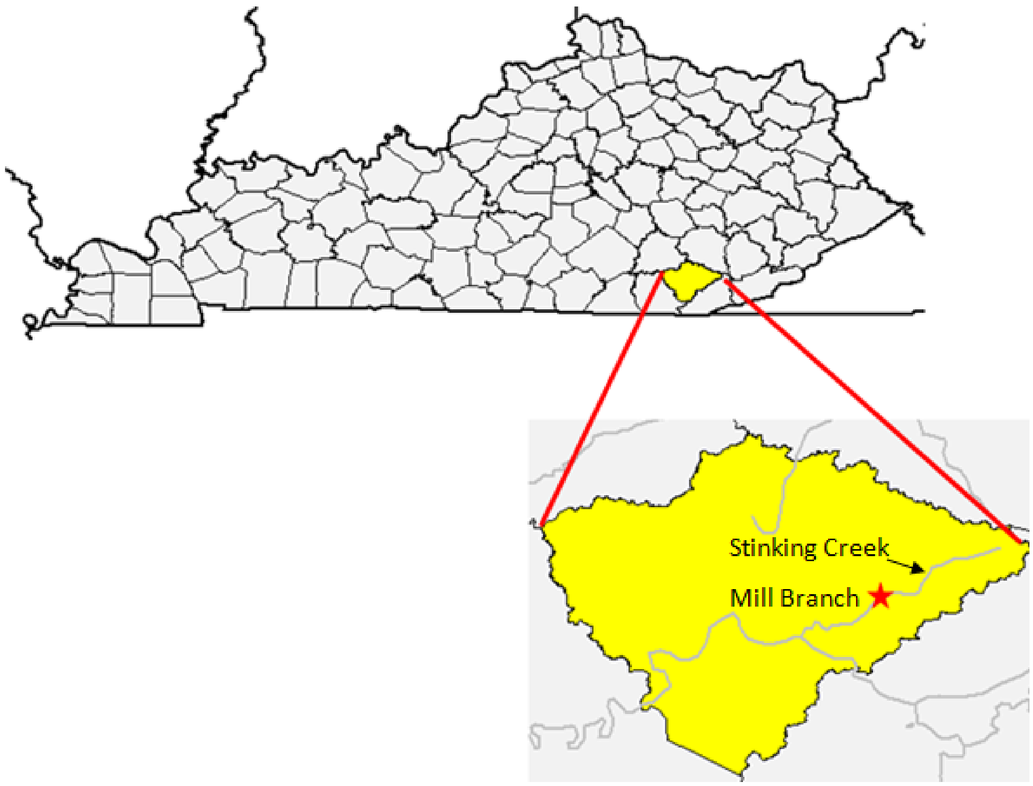

Figure 5. Map of Mill Branch, Knox County, Kentucky Note: Mill Branch is a small headwater tributary to Stinking Creek. Stinking Creek is indicated by the black arrow and Mill Branch is indicated by the red star.

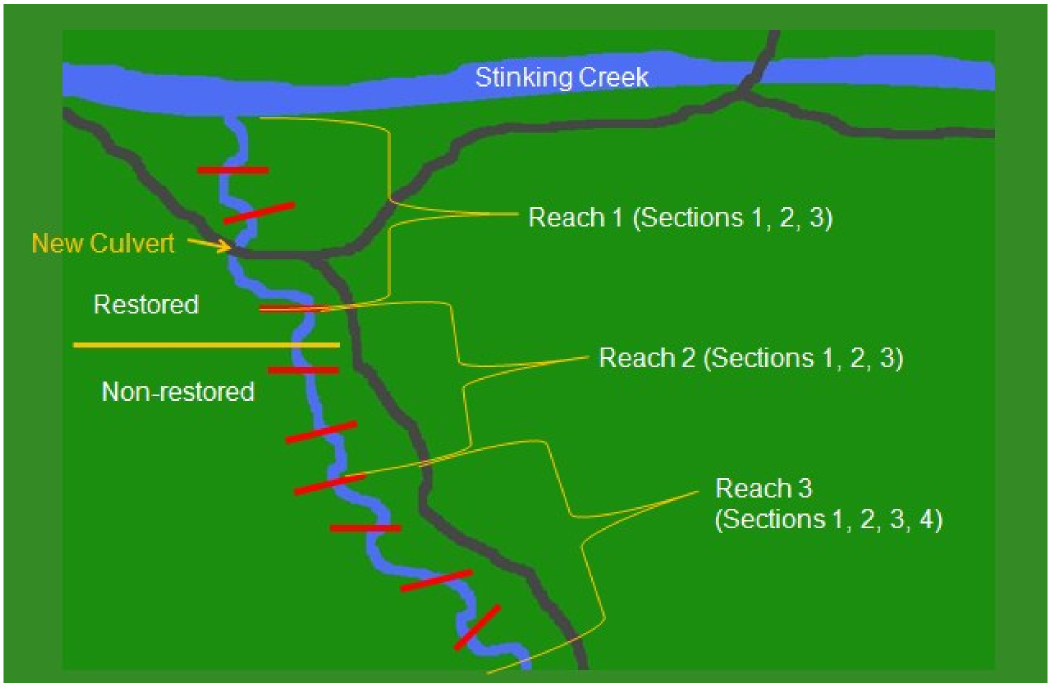

Figure 6. Defined reaches (indicated by yellow braces) and sections (indicated by red lines) of Mill Branch, Knox County, Kentucky, post-restoration and following connection of the new channel to the mainstem.

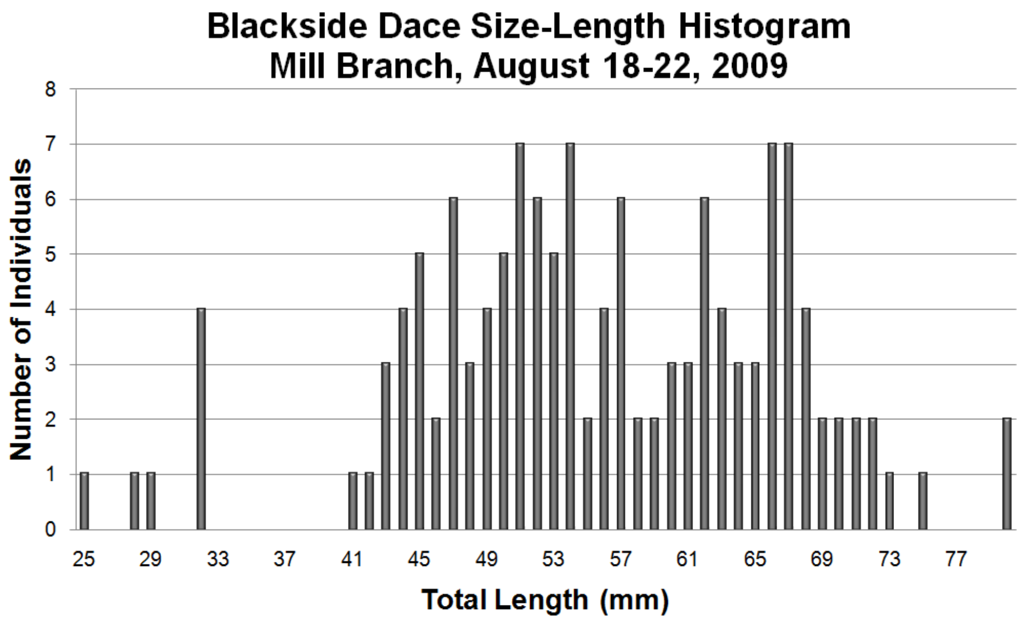

Figure 7. Blackside dace (*Chrosomus cumberlandensis*) length-frequency histogram for August 2009. Note: only includes restored channel. A total of 134 individuals were collected.

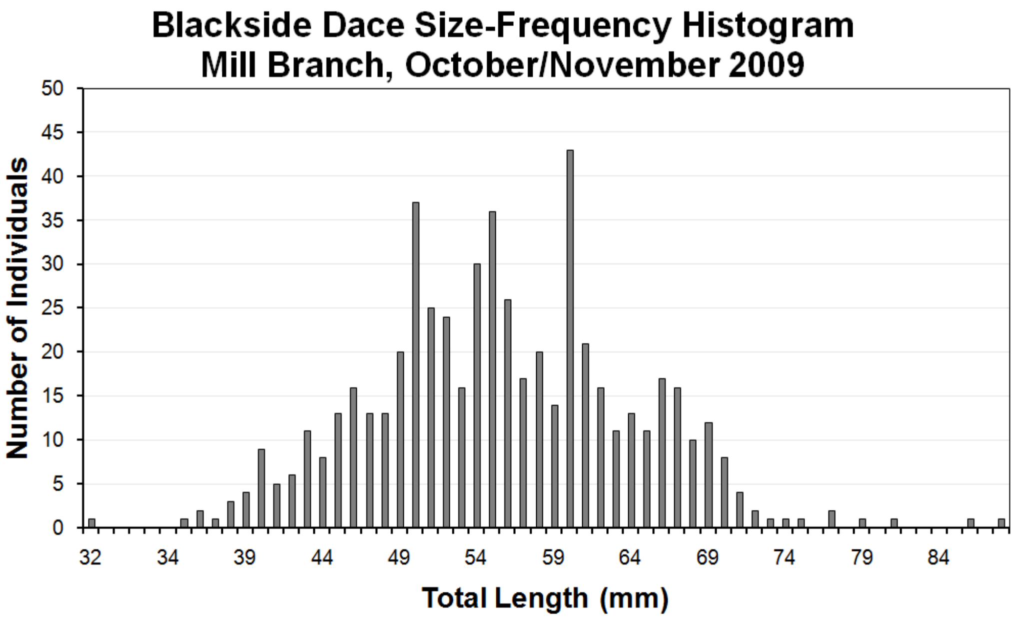

Figure 8. Blackside dace (*Chrosomus cumberlandensis*) length-frequency histogram for October/November 2009. A total of 564 individuals were collected.

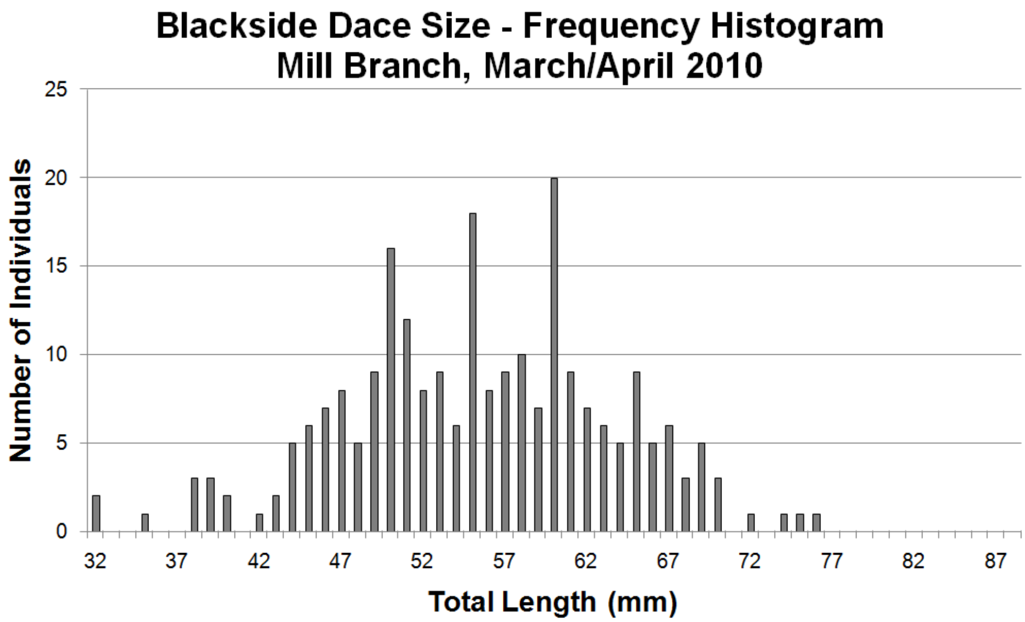

Figure 9. Blackside dace (*Chrosomus cumberlandensis*) length-frequency histogram for March/April 2010. A total of 239 of the 254 blackside dace observed are included, however 15 blackside dace were observed with several creek chubs (*Semotilis atromaculatus*) located near spawning beds; and therefore, were not captured and measured.

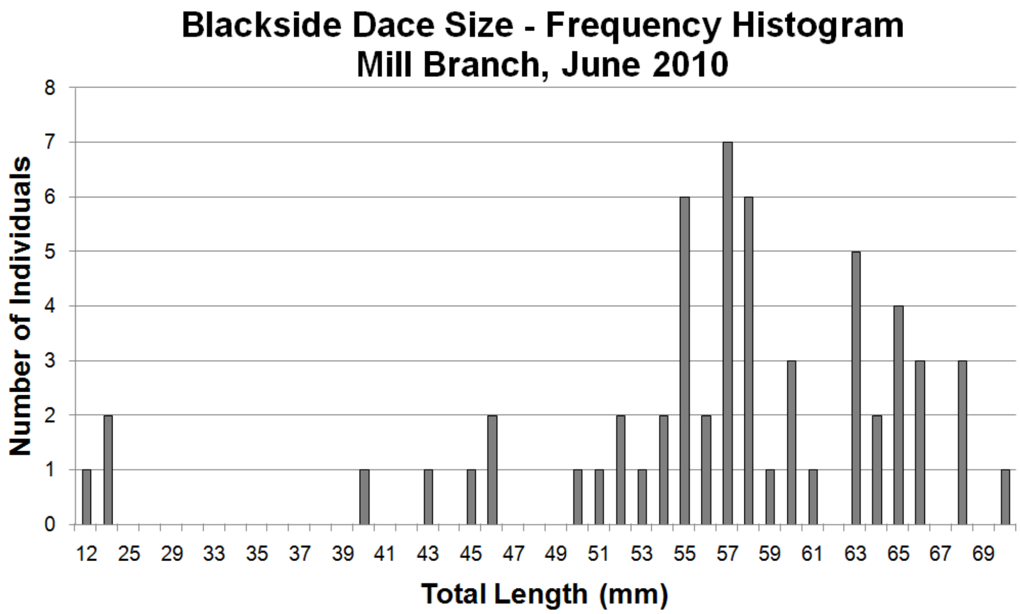

Figure 10. Blackside dace (*Chrosomus cumberlandensis*) length-frequency histogram for June 2010. A total of 58 blackside dace were collected.

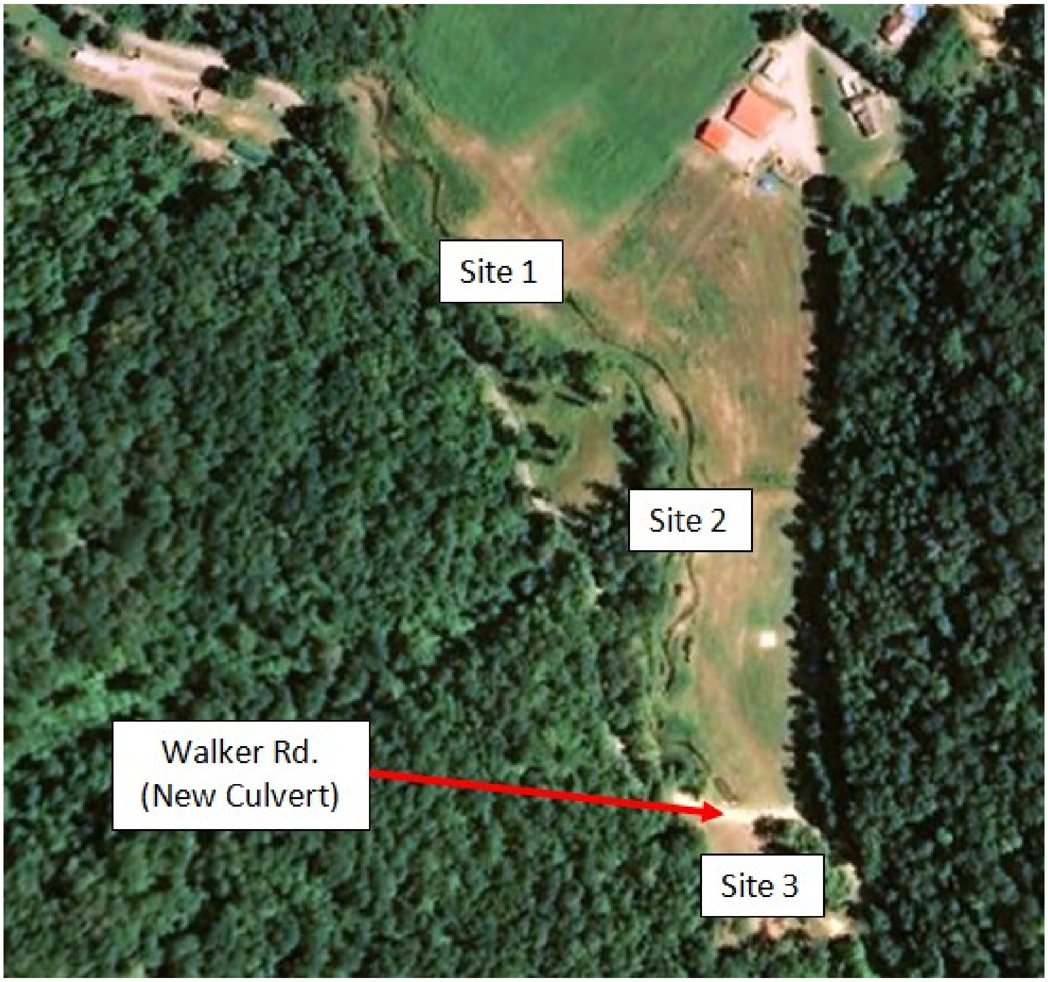

Figure 11. Sampling sites for macroinvertebrate sampling during August 2009 and March 2010 in the new channel of Mill Branch, Knox County, Kentucky post-restoration survey.

VITA

Dwayne Kevin Merrill was born on March 21, 1973 in Brevard, North Carolina. He entered the workforce after graduating from Brevard High School in 1991. He received an Associate of Science degree from Madisonville Community College in May, 2005. The following August he entered Eastern Kentucky University and in May, 2009 received the degree of Bachelor of Science in Biology Teaching (8-12) with a minor in Chemistry Teaching. He reentered Eastern Kentucky University in August, 2009 and is a candidate for the Master of Science degree.