ASSESSING THE BIOLOGICAL INTEGRITY AND FISH ASSEMBLAGES OF TWO WATERSHEDS LOCATED WITHIN A KARST AGRICULTURAL PLAIN, LOGAN COUNTY, KENTUCKY

DERECK L. EISON

ASSESSING THE BIOLOGICAL INTEGRITY AND FISH ASSEMBLAGES OF TWO WATERSHEDS LOCATED WITHIN A KARST AGRICULTURAL PLAIN, LOGAN COUNTY, KENTUCKY

A Thesis

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Austin Peay State University

Dereck L. Eison

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DEDICATION

This thesis is dedicated to my wife, Casey. Without her endearing love and support, my accomplishments throughout my graduate career would not have been possible.

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ABSTRACT

Pleasant Grove Creek is located within the Western Pennyroyal Karst Plain Ecoregion north of Tennessee. The watershed is the focus of several surveys by the Kentucky Division of Water, Environmental Protection Agency, and Austin Peay State University. Pleasant Grove Creek has been identified as an impaired water body on the Environmental Protection Agency's 303(d) list since 2002. The majority of this subwatershed of the Red River is privately owned and extensively farmed. Primary concerns about this watershed include the impacts of improper land use practices and the presence of faulty septic systems. Because of the close interactions between ground and surface waters in a karst system, the number of sinkholes located adjacent to the creek has also become a concern. Objectives of this study were to assess the environmental health of Pleasant Grove Creek utilizing the Kentucky Index of Biotic Integrity (IBI) and comparing historical data with data collected from 2007 to 2009. All protocols recommended by the Kentucky Division of Water were employed except for electroshocking. The Pennyroyal Ichthyoregion scoring classification was used to assess IBI scores. Fish assemblages were compared to habitat changes and an adjacent watershed, Whippoorwill Creek. Whippoorwill Creek is a creek identified by Kentucky as an exceptional water resource. Dissolved oxygen (DO) samples collected from these streams suggest that DO concentrations experience extreme diurnal fluctuations. IBI scores indicate that the biological integrity of Pleasant Grove Creek has improved; however, this improvement was not significant. A correlation between the riparian zone area adjacent to sample reaches

and the number of fish collected at each reach was shown to exist as well. From assessing the DO levels, IBI scores, and the number and species composition of fish collected during sampling events, it was concluded that both Pleasant Grove and Whippoorwill Creeks are experiencing some degree of degradation. Furthermore, Pleasant Grove Creek continues to be an impaired stream, and the best management practices implemented in the mid-1990's have had little impact on the biotic integrity of this stream.

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INTRODUCTION

A History of Water Pollution Control in the United States

During the 19th and early 20th centuries, the urban population of the United States grew from approximately 300,000 in 1800 to 54 million in 1920 (Andreen, 2003a). Population growth was attributed to a rush of immigration from Europe and an increase in migration from rural areas to meet the demands of industrialism. During this time, urban areas had to meet the complexities of a growing population, such as the outbreak of diseases that had arisen due to inadequate waste disposal systems. Despite the influx of immigrants, cities did not take into account the importance of a supply of clean water until epidemics of yellow fever, cholera, and typhoid fever became associated with filth and contaminated water. These events prompted city officials in the Northeastern United States to construct sewage systems in an attempt to rid industrialized cities of contaminated water. Unfortunately, these systems were designed to transport waste from the city to local streams, rivers, and ponds (Andreen, 2003a).

As early as 1869, scientists had warned cities that sewage deposited into aquatic systems were "choking our rivers with foul deposits" (Andreen, 2003a). Although sewer systems were improving local sanitary conditions, stream pollution increased, thus transporting water quality issues to downstream cities. Consequently, Congress created the National Board of Health in 1879 and passed the Rivers and Harbors Act of 1899, also known as the Refuse Act, to combat waterborne diseases and protect the Nation's waterways (Andreen, 2003b). Section 13 of the Refuse Act has commonly been referred to as the first article of federal legislation that addressed water pollution within the United States (Karr, 1991). This section of the Refuse Act prohibited the discharge of foreign matter into any navigable water unless a permit was obtained by the Army Corps of Engineers (Andreen, 2003b).

Although water quality awareness in the United States was increasing in the late 1800's, streams were still being severely polluted. By 1900, approximately 40% of pollutant loading to American rivers was industrial in origin. In addition, the increased production demands of War Worlds I and II caused industrial discharges to increase, which led to severe fish kills and the destruction of aquatic habitats (Andreen, 2003a). To alleviate the issues surrounding industrial point source pollution, Congress introduced several acts of legislation between 1924 and 1970 (Table 1). These efforts were often flawed, however, because of narrowly defined problems which resulted in solutions that were too narrow in scope to remedy the problem (Andreen, 2003a).

Possibly the most influential act of legislation related to water quality is the Clean Water Act (CWA) of 1972. Although this act contained lofty goals, such as the strict deadline to end all pollution by 1985, the objective of restoring and maintaining the physical, chemical, and biological integrity of the nation's waters was viewed as a revolutionary statement. Besides becoming synonymously interpreted as the ultimate goal of water quality management, it was also the first time that biological integrity was included as an integral factor related to aquatic resource management (Karr, 1991). Biological integrity has been defined by Karr and Dudely (1981) as "the ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region." In addition, this act was unique from previous water quality legislation because it applied to all point source discharges, whether existing or new, municipal or industrial (Andreen, 2003a).

The 1972 CWA was effective in combating point source effluents associated with industrial and municipal operations; however, nonpoint source pollution quickly replaced point sources in the years following as the major contributor to poor water quality in the United States. Agricultural activities became a concern as a significant source of nonpoint source pollutants, and the only issue associated with agriculture addressed in the 1972 CWA was the practice of confined animal feeding operations (CAFOs). Therefore, Congress revised the CWA in 1987 to address nonpoint sources, toxic pollutants, and storm water discharge (Murchison, 2005).

Presently, the foremost questions revolving around the CWA is what progress is being made to achieve its goals. Section 319 of the 1987 CWA revision required states to develop and implement programs to control nonpoint source pollutants. Programs that address agricultural land use practices, such as soil conservation and water quality protection, have allowed landowners to voluntarily adopt new practices set forth by policy makers (Bosch, 1995). Also, management decisions by individual landowners are more difficult to regulate than those of industrial sources. In addition to a slow response by landowners during the last three decades to adopt water quality initiatives, state governments cite lack of funding as their primary concern when implementing management programs (Copeland, 2006).

Table 1. Water quality legislation passed by the United States Congress between 1924 and 1970.

Legislative Act	*Objectives*
The Oil Pollution Act of 1924	Banned the discharge of oil to coastal navigable waters from vessels; gave the Army Corps of Engineers the power to regulate the loading and unloading of oil.
The Federal Water Pollution Control Act of 1948	Primary responsibility for water pollution was given to states; authorized the Surgeon General to give notice to states and to recommend remedial measures whenever water pollution jeopardized public health.
The Federal Water Pollution Control Act Amendments of 1956	Provided more grant money for municipal sewage plant construction; eliminated the power of a polluting state to veto any federal court action.
The Federal Water Pollution Control Act Amendments of 1961	Increased grant funding; enhanced federal employment; transferred administrative responsibilities from the Surgeon General to the Secretary of Health, Education, and Welfare.
The Water Quality Act of 1965	Created the Federal Water Pollution Control Administration (FWPCA) to administer the federal program; expanded the grant program; required states to adopt water quality standards subject to approval by the federal government.
The Clean Water Restoration Act of 1966	Extended the Oil Pollution Act's jurisdiction to inland waters; administrative duties of the 1924 Act shifted from the Corp of Engineers to the FWPCA.
The Water Quality Improvement Act of 1970	Prohibited all facilities from discharging oil into the navigable waters of the U.S.; created new compliance mandates for entities applying for permits to discharge into navigable waters.

Nonpoint Source Pollutants Associated with Agricultural Practices

Unfortunately, the biological integrity of aquatic ecosystems is commonly and continually degraded because of human practices (Karr and Dudely, 1981; Karr, 1981; Miller et al., 1988; Harding et al., 1998; Jones III et al., 1999). Karr (1985) suggests that some human activities that have had the greatest impact on aquatic fauna include: "agriculture, which results in a lowering of the water table and nutrient enrichment; navigational locks and channels in larger rivers; impoundments, levees, and milldams; discharge of oxygen demanding wastes and toxic chemicals, overconsumption of water; and introduction of exotic species." When naturally vegetated landscapes are converted for agricultural uses, physical and biological relationships with adjacent streams are impacted (Roth et al., 1996). These impacts include the degradation of habitat and a decline in stream biota diversity (Karr and Schlosser, 1978; Schlosser 1991). The U.S. Environmental Protection Agency (EPA) has acknowledged that nonpoint source pollution from agricultural activities is a high priority water quality problem (United States Environmental Protection Agency, 1989), and agriculture has been listed as a source of pollution for 48% of the impaired river miles in the United States (United States Environmental Protection Agency, 2003).

Causes of nonpoint source pollutants in agricultural land use watersheds include increased levels of nutrients and pesticides from surface runoff, bacteria from animal wastes, and sedimentation due to soil erosion (Berryhill, 1989). Two nutrients that have been repeatedly identified as having adverse effects on surface waters are nitrogen and phosphorous (Carpenter et al., 1998). Human health implications and eutrophication of tainted waters are two common impacts associated with these nutrients. Major sources of nutrient contamination have been linked to commercial fertilizers or manure applied to fields for crop production, manure storage facilities, concentrated feedlots, dairy parlors, poultry and hog houses, and over used pastures (Johnson et al., 1982a; United States Environmental Protection Agency, 2003). Additionally, McCollum (2004) concluded that pasture use by livestock and row crop cultivation contribute most to stream degradation within agricultural land use watersheds.

As a result of farmers increasing soil conservation practices by no-till and reduced-till cultivation within watersheds, increased pesticides have become another source of aquatic pollution. Pesticides are relied upon to kill cover crop plants or to control weeds, insects, and disease producing organisms (Berryhill, 1989). The primary routes that pesticides enter aquatic systems are by direct application to surface waters, surface runoff, aerial drift through volatilization, and subsequent atmospheric deposition. Also, pesticides enter waterways through uptake by biota and subsequent movement through the food web. Adverse impacts resulting from improper pesticide use includes effecting surface waters downstream, infiltration of pesticides in municipal water supplies, and ultimately fish kills (Maas et al., 1984; Berryhill, 1989).

Erosion usually results from bad tillage practices and overly grazed pastures by livestock without rotation to alternate pastures (Berryhill, 1989; Hall et al. 1999). Johnson et al. (1982b) reported that farmland contributes over 6.4 billion tons of topsoil to surface waters each year. Consequently, sedimentation is a transport system for all sources of pollutants listed above, and an increase of suspended solids can significantly impact aquatic systems (Berryhill, 1989). Suspended solids reduce sunlight availability to primary producers (algae), interfere with the filtering capacity of filter feeders, disrupt the respiration of fishes, and cover spawning habitats of fish. Ultimately, the productivity of an aquatic system diminishes due to reduced plant, macroinvertebrate, and fish populations (United States Environmental Protection Agency, 2003).

The degradation of surface waters in agricultural watersheds due to bacteria and sedimentation is commonly associated with animal waste from livestock production. Additional sources of bacteria and microorganisms entering aquatic systems include faulty septic systems, dead animals, and biologic waste discarded into streams by humans. Runoff laden with manure and wastewater adds excess nutrients and organic materials. Consequently, an increase in nutrient levels creates excessive plant and algae growth. When these organisms decompose, an anaerobic environment is produced; thus, creating conditions favorable for fish kills and the release of greenhouse gases. Eventually, the addition of nonpoint source pollutants to surface waters creates an aquatic environment unsuitable for drinking, fishing, and other recreational uses (United States Environmental Protection Agency, 2003).

Riparian Zone Removal in Agricultural Watersheds

Riparian zones play a significant role when managing the water quality impacts of nonpoint source pollution (Duehr et al., 2006). An example of a riparian ecosystem and its relationship with surrounding wetlands and uplands can be seen in Figure 1. These areas help in reducing nonpoint source pollution by intercepting surface runoff laden with pollutants such as those discussed above. Generally, the velocity of the surface runoff is slowed as it passes through these buffer areas; thereby increasing the rate of infiltration of pollutants. The description or definition of a riparian zone can vary. For example, the United States Environmental Protection Agency (2005) defines riparian areas as "vegetated ecosystems along a water body through which energy, materials, and water pass." In addition, Naiman and Decamps (1997) described a riparian zone as an interface which acts like a semi-permeable membrane between an aquatic system and the adjacent land. Regardless of its description, these areas improve the quality of an aquatic system by providing a buffer which aides in reducing the affects of human activities (United States Environmental Protection Agency, 2005).

The riparian zone also plays a vital role in the maintenance of the natural processes that occur in aquatic systems. Woody debris from these zones provides cover for organisms and influences channel morphology. Energy subsidies are provided through the accumulation of leaf litter and other organic detritus.

In addition to regulating sediment transportation, vegetation along stream banks also provides shade necessary for natural temperature regimes (Roth et al., 1996). In-stream processing of both nonpoint source and point source pollutants are also enhanced in the presence of riparian buffers; thus, reducing the affects of these pollutants on downstream rivers and estuaries (Sweeney et al., 2004). Riparian zones in agricultural watersheds are frequently cut to stream edges in order to maximize land for cultivation of crops and livestock production (Kalff, 2002). Alteration of these areas can inhibit their ability to treat the adverse affects of pollutants (United

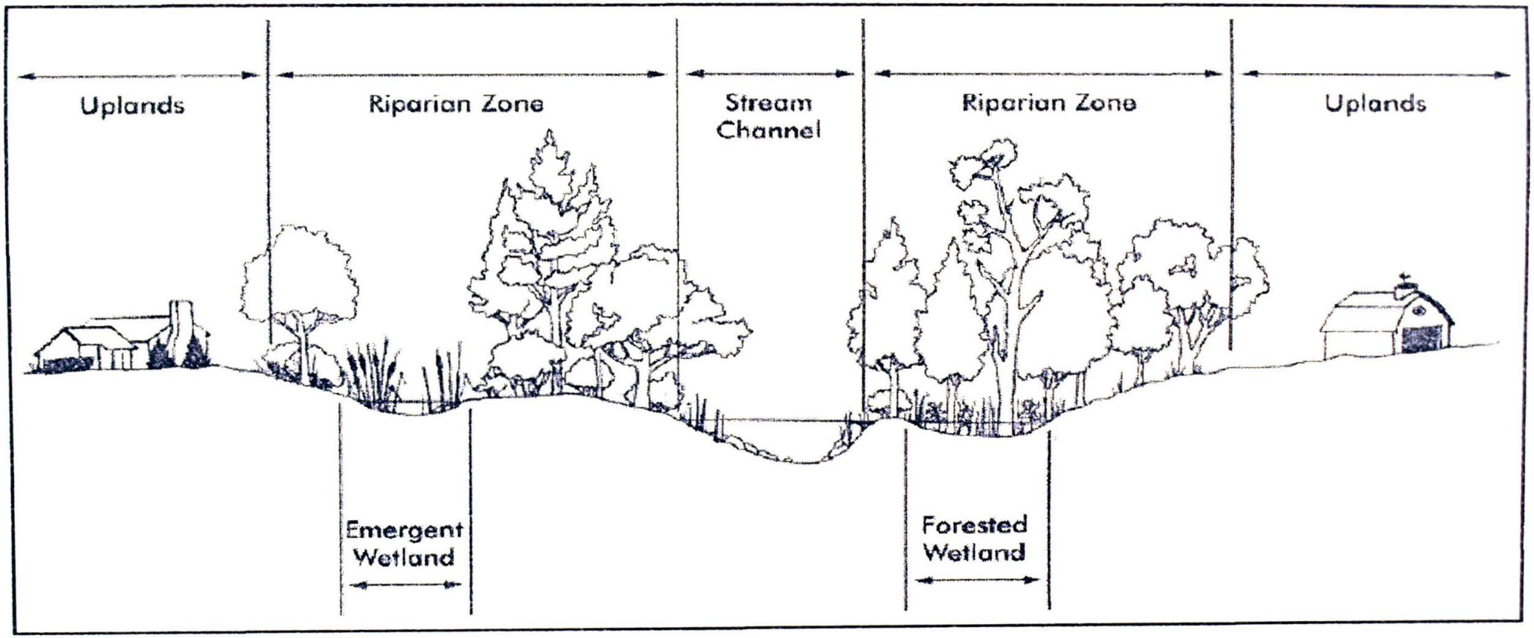

Figure 1. Spatial relationship between riparian ecosystems, wetlands, uplands, and a stream channel (Modified from USEPA, 2005).

States Environmental Protection Agency, 2005). The deforestation of riparian habitat along channels within agricultural watersheds contributes to more rapid sediment inflows to waterways (Berryhill, 1989; Currens, 2002; Duehr et al., 2006; Harding et al. 1998; and Jones III et al. 1999). Increases in fertilizers and biocides in streams and the alteration of light and thermal regimes occurs as a result of canopy loss as well (Jones III et al., 1999).

Species diversity and assemblage composition of aquatic fauna can be negatively impacted as well by landscape alteration and riparian vegetation removal, especially in severely impacted agricultural watersheds. When streamside vegetation is removed, a shift from allochthonous to autochthonous production can occur. The absence of woody debris alters current velocity, depth, and substrate type which results in wide shallow streams with little habitat heterogeneity (Roth et al., 1996) The increased amount of silt along the stream bottom reduces the availability of clean substrate for spawning habitat required by many fish species (Compton et al., 2003). Miller et al. (1989) stated that habitat alteration is a contributing cause in the decline of 73% of fish species extinctions in North America. Roth et al. (1996) found that stream biotic integrity and habitat quality were negatively correlated with the extent of agriculture in a given area. Often the riparian zone area has been shown to correlate with fish density in areas where riparian buffers have been altered as well (Jones III et al., 1999).

The Impact of Agriculture within Karst Watersheds

Karst terrain is formed by the dissolution of soluble rock, such as limestone and dolomite, and is usually accompanied by aquifers that are capable of providing large supplies of water. Characteristics of this terrain include sinkhole plains, underground caves and streams, and springs (United States Geological Survey, 2007). The solvents usually associated with this dissolution of carbonate rock are carbonic acid and humic acid from soil detritus. Consequently, because karst aquifers contain larger conduits that allow for a more turbulent flow, they are significantly different from that of porous media aquifers. Velocity of ground water flow in karst aquifers can approach that of surface streams; but, the movement of ground water through porous media aquifers is significantly slower, usually only a few meters per year (Crawford, 1988).

The same characteristics of groundwater within karst terrain that make it unique also allow it to be vulnerable to degradation by nonpoint source pollution, which can cause irreparable damage in these watersheds. Self treatment of water borne contaminants is often ineffective as well. Ulrich (2002) reported several reasons why self treatment is difficult in a karst system:

- There is little opportunity for percolating water to purify itself due to the absence of microorganism colonies responsible for absorption and ion exchange
- The evaporative processes that help in the removal of volatile substances is reduced due to the rapid infiltration of surface waters
- The presence of sinkholes and sinking streams assists in the transmission of particulate contaminants into karst systems
- Efficiency of filtration is reduced due to the patchy, shallow soils that are characteristic of karst

- High flow velocities and short flow through times allow pathogens and pollutants that would normally die off or be degraded to emerge at karst springs, contaminating surface waters

Human activities in karst watersheds can exacerbate the issues listed above. For example, agricultural practices can lead to increased surface runoff and erosion deposited into karst systems. Thus, degrading soils within these areas and depositing large amounts of sediments into caves (Ulrich, 2002). Other activities that degrade water quality and alter habitats within streams located in a karst system include excess fertilizer application to row crops, improper timing of chemical application, poor manure storage management, and failure to restrict livestock access to sinking streams, cave entrances, and sinkholes (Berryhill, 1989; Ryan and Meiman, 1996; Ulrich, 2002).

The Index of Biotic Integrity

In order to assess the biotic integrity of surface waters and to provide some criteria on which stream degradation could be measured, Karr (1981) developed the Index of Biotic Integrity (IBI) using fish community structure of warm water Midwestern United States streams. Karr's IBI utilized 12 equally weighted metrics that were grouped into the categories of Species Richness and Composition, Trophic Composition, and Fish Abundance and Condition (Table 2). These three classes evaluated the extent of human impacts on the energy base and trophic dynamics of fish assemblages of Midwestern streams (Karr, 1990). Values, or scores, were assigned to each metric and were based on whether or not the obtained score strongly approximated the expected value (5), somewhat approximated the expected value (3),

Table 2. IBI metrics and categories used to assess human impacts on the biotic integrity of Midwestern United States streams (Karr 1981).

Category	Metric	Scoring Criteria 5	3	1
Species Richness and Composition	1. Total number of fish	Expectations for metrics 1-5 vary with stream size and region.		
	2. Number and identity of darter species			
	3. Number and identity of sunfish species			
	4. Number and identity of sucker species			
	5. Number and identity of intolerant species			
	6. Proportion of individuals as green sunfish	< 5%	5-20%	> 20%
Trophic Composition	7. Proportion of individuals as omnivores	< 20%	20-45%	> 45%
	8. Proportion of individuals as insectivorous cyprinids	> 45	45-20%	< 20
	9. Proportions of individuals as piscivores	> 5	5-1%	< 1%
Fish abundance and condition	10. Number of individuals in sample	Expectations for metric 10 vary with stream size and other factors.		
	11. Proportion of individuals as hybrids	0%	> 0-1%	> 1%
	12. Proportion of individuals with disease, fin damage, and skeletal anomalies	0-2%	> 2-5%	> 5%

or did not approximate the expected value (1). The total IBI score, ranging from 12-60, was achieved by summing the individual metric scores. Five classifications, Excellent, Good, Fair, Poor, Very Poor, were then assigned to describe the quality of the fish community at each site (Compton et al., 2003). This form of biological assessment has proven to be very useful and popular among resource managers, and as of 1995, 45 states within the U.S. had implemented some variation of the IBI (United States Environmental Protection Agency, 2007). In addition, Fausch et al. (1984) and Angermeier et al. (2000) concluded that the development of criteria for an IBI must be *region specific* to account for discrepancies between ecoregions, or areas with similar ecosystems that possess the same type, abundance, and quality of environmental resources.

In addition of the IBI becoming a viable alternative to physiochemical monitoring programs, there are several advantages of using an index that employs fish to assess the environmental health of water systems:

- Fish are relatively long lived and motile
- Fish assemblage structure is composed of a range of species representing several trophic levels
- Fish are relatively easy to collect and identify
- Because fish can be argued as being the top of the aquatic food web and are consumed by humans, it is important their habitat be assessed regularly
- The life history of most fish species is well documented
- Fish have varying tolerance levels to pollution

(Karr, 1981; Simon, 1991; Barbour et al., 1999).

Other advantages of using an IBI include its flexibility to be used to determine local conditions or be used to evaluate entire basins by sampling different sites throughout the watershed, and this multi-parameter index allows for water quality trends to be compared when multiple samples are taken over a period of time (Karr, 1990).

Purpose of Study

For this study, a region-specific IBI was employed to assess the impacts of agriculture in two karst watersheds, Pleasant Grove Creek and Whippoorwill Creek, in Logan County, Kentucky. The Pleasant Grove Creek watershed has been the focus of several water quality investigations and a continued focus for pollutant reduction actions (Sampson, 1995; McMurray, 1999; Currens, 2002). The percentage of land used for agricultural practices in lower Pleasant Grove Creek is 86% (Ridenour, 2006) and in 1994, the Kentucky Division of Water began monitoring Pleasant Grove Creek prior to the implementation of best management practices (BMP) within the watershed as part of the U.S. Department of Agriculture Water Quality Incentive Program. Based on pre-BMP fish and macroinvertebrate surveys, professional water quality staff concluded that Pleasant Grove Creek was degraded (Sampson, 1995). Sampson (1995) suggested that post-BMP data should be collected to "assist in evaluating BMP effectiveness." In 1998, the Kentucky Division of Water revisited Pleasant Grove Creek to assess the impact of BMPs implemented within the watershed and found that water quality within the stream was still degraded and did not meet state water quality standards (Table 3). Data suggested that the assessment was still too early for the BMPs to have any "demonstrable" effects on water

Table 3. Pollutants present in Pleasant Grove Creek (Kentucky Division of Water, 2008a).

Stream Name	County	River Miles	Pollutant
Pleasant Grove Creek into the Red River	Logan	0.0 to 2.2	Fecal Coliform
Pleasant Grove Creek into the Red River	Logan	0.0 to 2.2	Nutrient/Eutrophication Biological Indicators
Pleasant Grove Creek into the Red River	Logan	0.0 to 2.2	Organic Enrichment (Sewage) Biological Indicators

quality (McMurray, 1999). In 2002, this watershed was identified and listed as an impaired water body on the EPA's 303(d) list of impaired streams (Kentucky Division of Water, 2003). Impaired uses of the Pleasant Grove Creek watershed include partially supporting warm water aquatic habitat and non-supporting of primary contact for recreational purposes. Suspected sources of pollutants mentioned in Table 3 include agricultural practices, livestock grazing in riparian or shoreline zones, managed pasture grazing, and on-site treatment systems (Kentucky Division of Water, 2008a).

The primary objectives of this study were to investigate fish assemblages within the Pleasant Grove Creek watershed. The study aims to determine if water quality has improved since BMPs were implemented over a decade ago, and to provide baseline data for fish diversity and the species of fish inhabiting these watersheds for both the current study and future studies. Secondary objectives of this study included the investigation of the varying riparian zone width and area along the Pleasant Grove Creek and Whippoorwill Creek channels and its relationship to fish abundance. This information will be compared to the limited historical IBI scores calculated from fish surveys before and after BMPs were implemented with IBI scores calculated from fish surveys of the Pleasant Grove Creek and Whippoorwill Creek watersheds.

CHAPTER II

METHODS AND MATERIALS

Study Area Description

The Red River watershed (Figure 2), designated by the United States Geological Survey as HUC 05130206, is located along South-central Kentucky and northern Middle Tennessee. The Red River, a tributary of the Cumberland River, supplies ground and surface water for livestock watering, serves as a source for irrigation, and is a source for domestic and municipal drinking water for 10 counties located along the Kentucky-Tennessee state border. From its headwaters to its confluence with the Cumberland River in Clarksville, Tennessee, the Red River watershed encompasses and area of 3740 square kilometers (Tennessee Department of Environment and Conservation, 2007). Although this watershed has been identified as a significant catfish, bluegill, bass, and crappie fishery by the Tennessee Wildlife Resources Agency, there are water quality problems in urban and agricultural areas throughout the watershed. Examples of water quality challenges associated with farming practices include: cattle having access to streams, filling of sinkholes with debris, and allowing pesticide and nutrient laden surface runoff from row crops to enter into the groundwater by way of poorly buffered sinkholes (Red River Watershed Association, 2008).

The focus areas for this project were two subwatersheds of the Red River, Pleasant Grove and Whippoorwill creeks. These creeks are located in the Western Pennyroyal Karst Plain Ecoregion (ecoregion level IV, 71e; Woods et al., 2002)

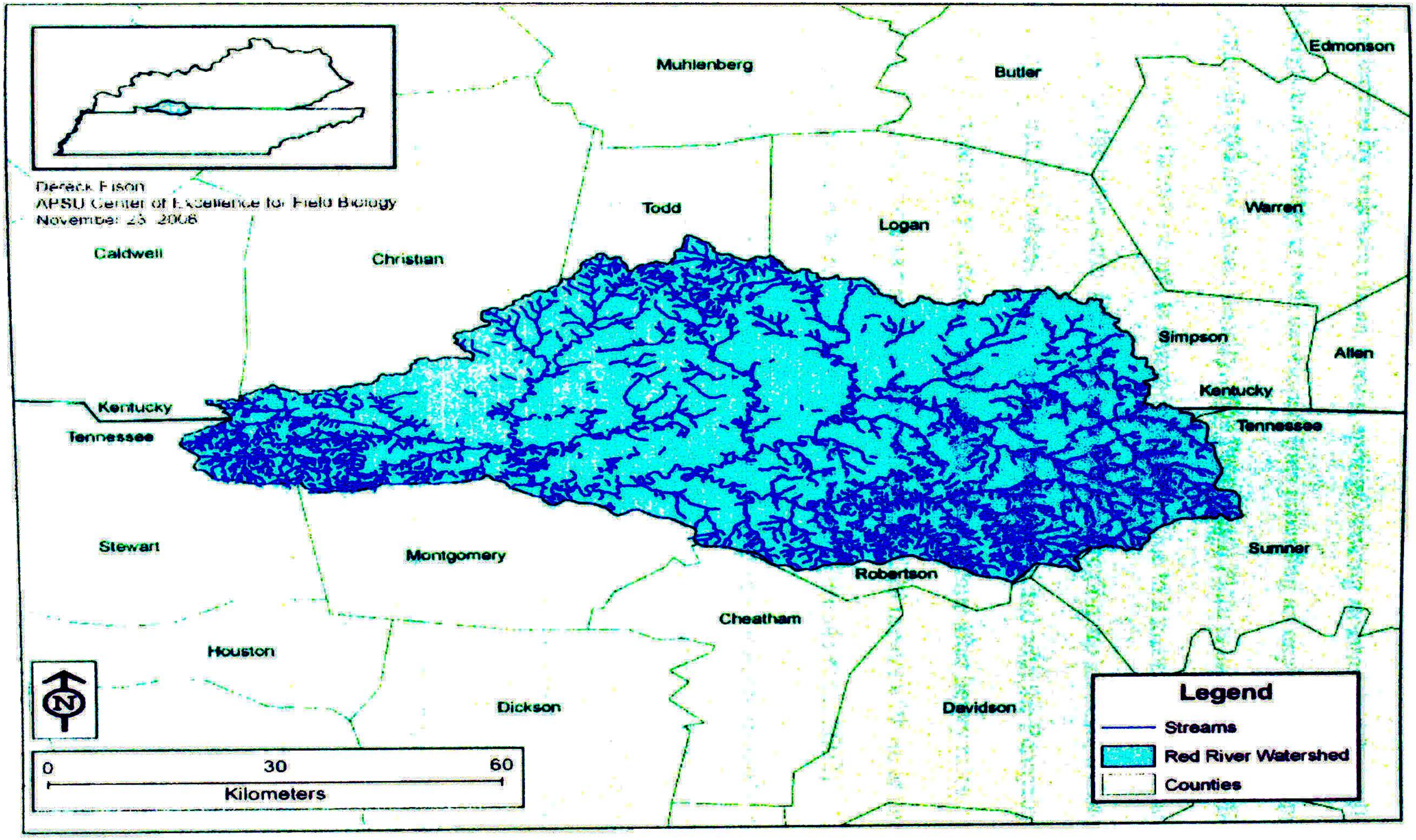

Figure 2. Map of the Red River watershed.

north of the Tennessee state line in Logan and Todd counties, Kentucky (Figure 3). This ecoregion, commonly referred to as the Pennyroyal or Pennyrile Plain, is characterized by sinkhole plains, ponds, springs, sinking streams, and dry valleys. Underground drainage is well developed, thus providing an explanation for quick drying soils and low stream density. The Pennyroyal Plain is underlain by Middle Mississippian limestone and is commonly farmed due to a mantle of thin loess soil that overlies the limestone. Chief exports of the ecoregion are tobacco, livestock, corn, soybeans, and wheat. Water quality issues associated with the Pennyroyal Plain include the washing of sediment and nitrogen into streams after heavy rains. The range in elevation for this region is between 152 and 229 meters with an annual mean precipitation range between 114 and 135 centimeters (Woods et al., 2002).

The Pleasant Grove Creek subwatershed (Figure 4) is approximately 88 square kilometers. This aquatic system is identified as an interrupted stream that resurfaces from a karst aquifer. Pleasant Grove Creek has no significant surface tributaries and resurfaces from the ground approximately 3.5 kilometers above its confluence with the Red River.

The Whippoorwill Creek subwatershed (Figure 5) is approximately 298 square kilometers. This watershed is located adjacent to the Pleasant Grove Creek watershed. Whippoorwill Creek has been recognized by the Commonwealth of Kentucky as an exceptional state water resource as well as a reference reach stream (Kentucky Division of Water, 2008c). Due to its location adjacent to Pleasant Grove Creek and its listing as a reference reach stream, Whippoorwill Creek was used as a paired analysis stream for this project.

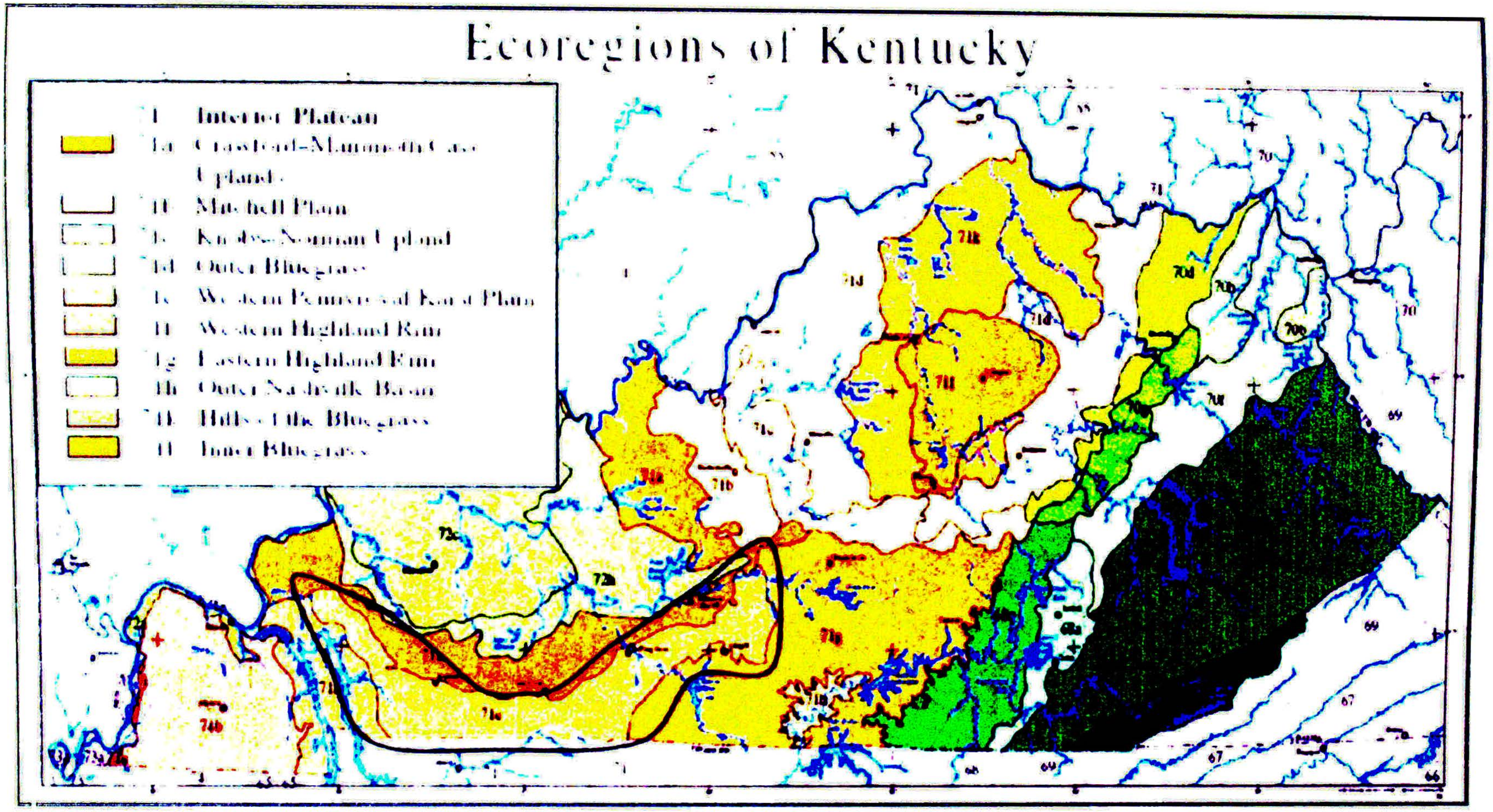

Figure 3. Level IV Ecoregions of Kentucky (Woods et al., 2002). The Western Pennyroyal Karst Plain (71e) is circled in black. Pleasant Grove Creek and Whippoorwill Creek are located within this level IV ecoregion.

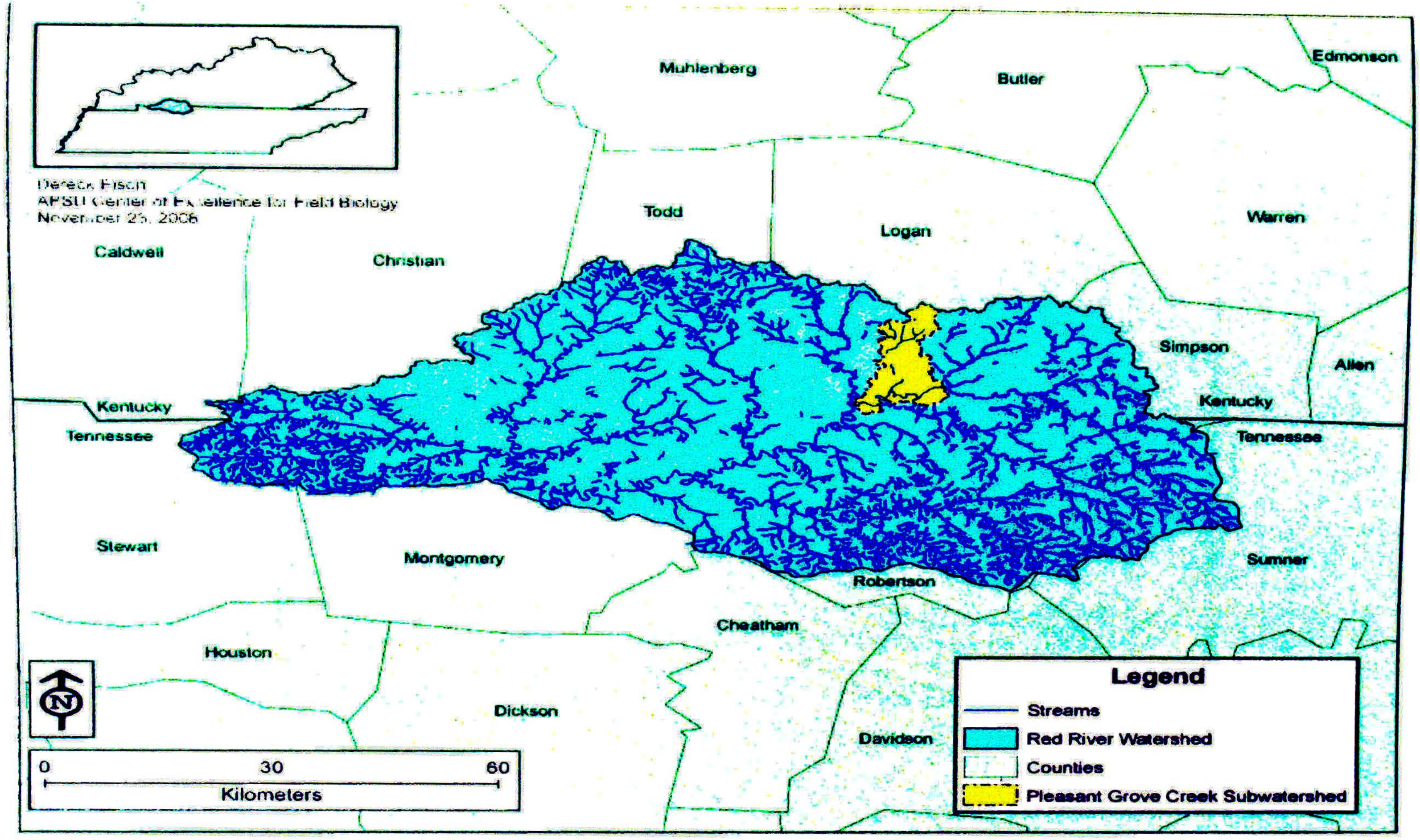

Figure 4. Map of the Pleasant Grove Creek subwatershed of the Red River watershed, Kentucky and Tennessee.

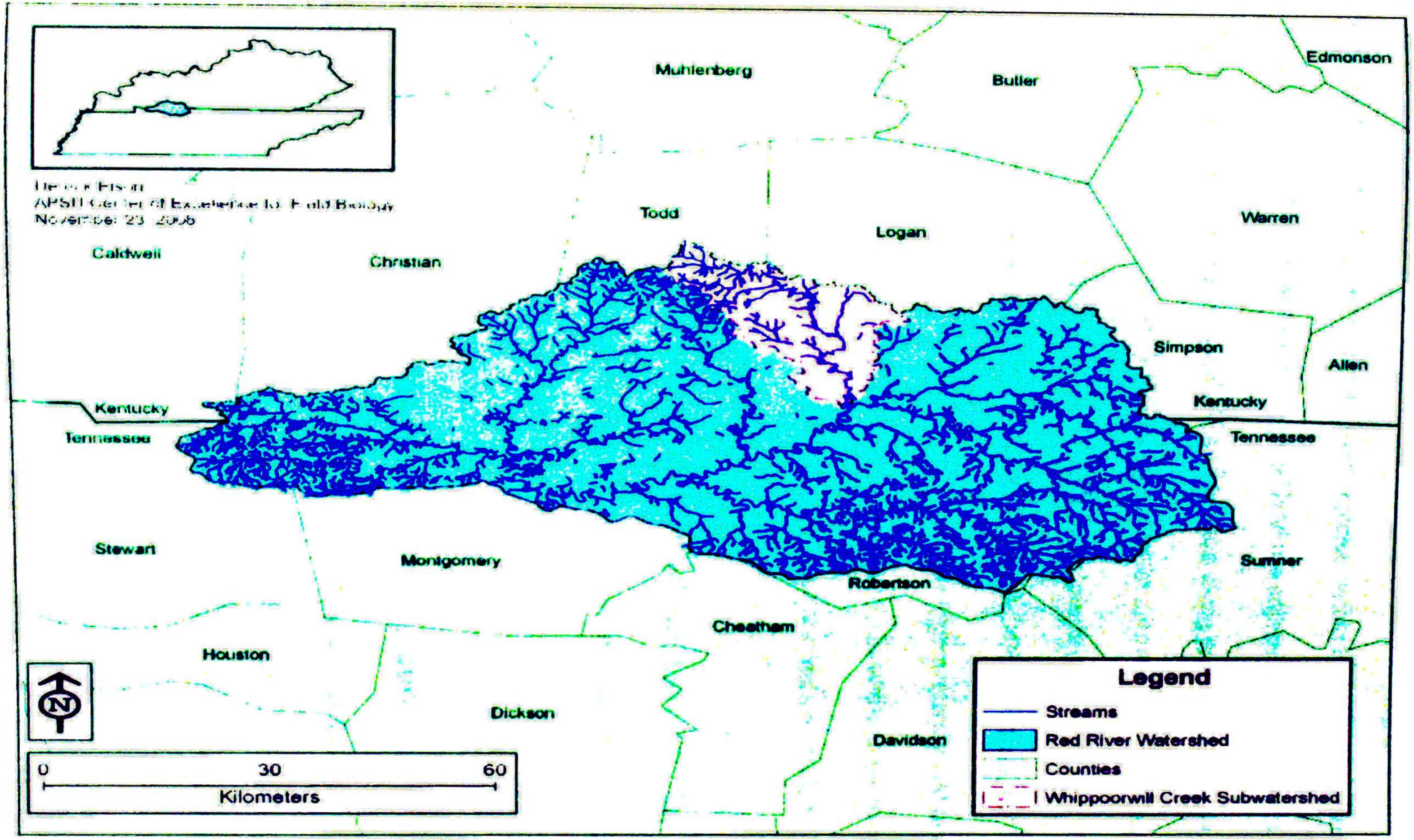

Figure 5. Map of the Whippoorwill Creek subwatershed of the Red River watershed, Kentucky and Tennessee.

Sample Reaches

A total of six stream sampling reaches were chosen using ArcGIS 9.2 software (Figures 6 and 7). Three reaches were selected within both Pleasant Grove and Whippoorwill creeks (Appendix I). Type of land use adjacent to the stream was the major criteria for choosing sampling sites. Land use along reaches chosen for the study was either used for the production of livestock or for cultivation of row crops.

Water quality sampling parameters of each reach sampled followed parameters set forth by the Kentucky Division of Water's (KDOW) protocol for sampling surface water (2008b). Each sampling reach was approximately 100 meters in length and consisted of at least two riffles, runs, and pools. In addition, sample reaches were located at least 50 meters away from any road obstruction (bridge, culvert, etc.) that could possibly bias results.

Visual habitat assessments were performed at each sampling reach. The Habitat Assessment Field Data Sheet for High Gradient Streams (Kentucky Division of Water, 2008b) was used to perform these assessments. Ten parameters (Table 4) were used to assess in-stream habitat, channel morphology, bank stability, and riparian vegetation of each sampling reach. A numerical score of 0 (lowest) to 20 (highest) was used for each parameter. Parameter scores were divided into the following conditions: Optimal (20-16), Suboptimal (15-11), Marginal (10-6), and Poor (0-5). Parameter scores were then totaled to obtain an overall habitat assessment score.

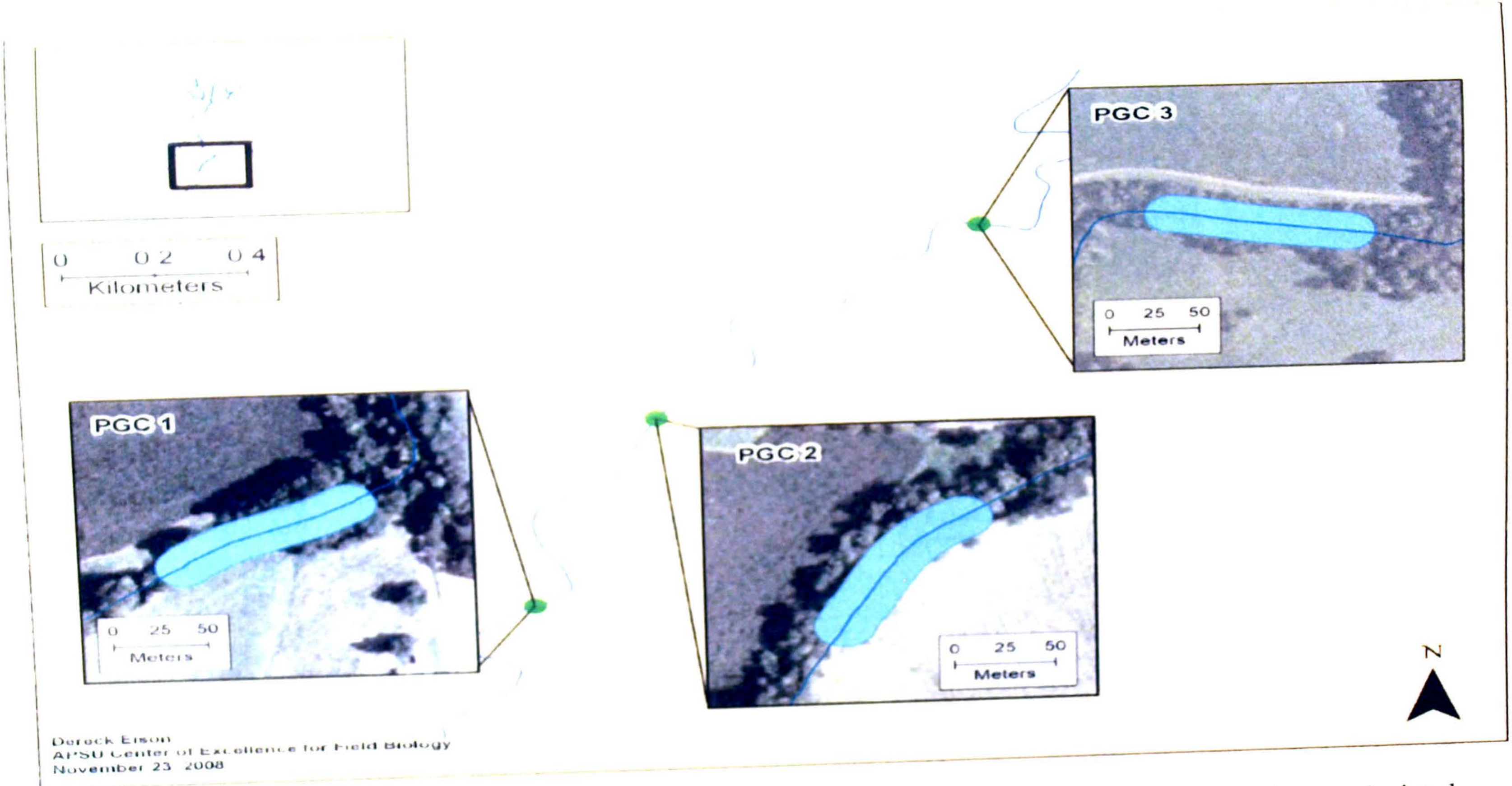

Figure 6. Stream sampling reach locations within Pleasant Grove Creek, Logan County, Kentucky. Reaches are depicted within inset maps of sample locations. Each 100 meter sampling reach is highlighted in blue.

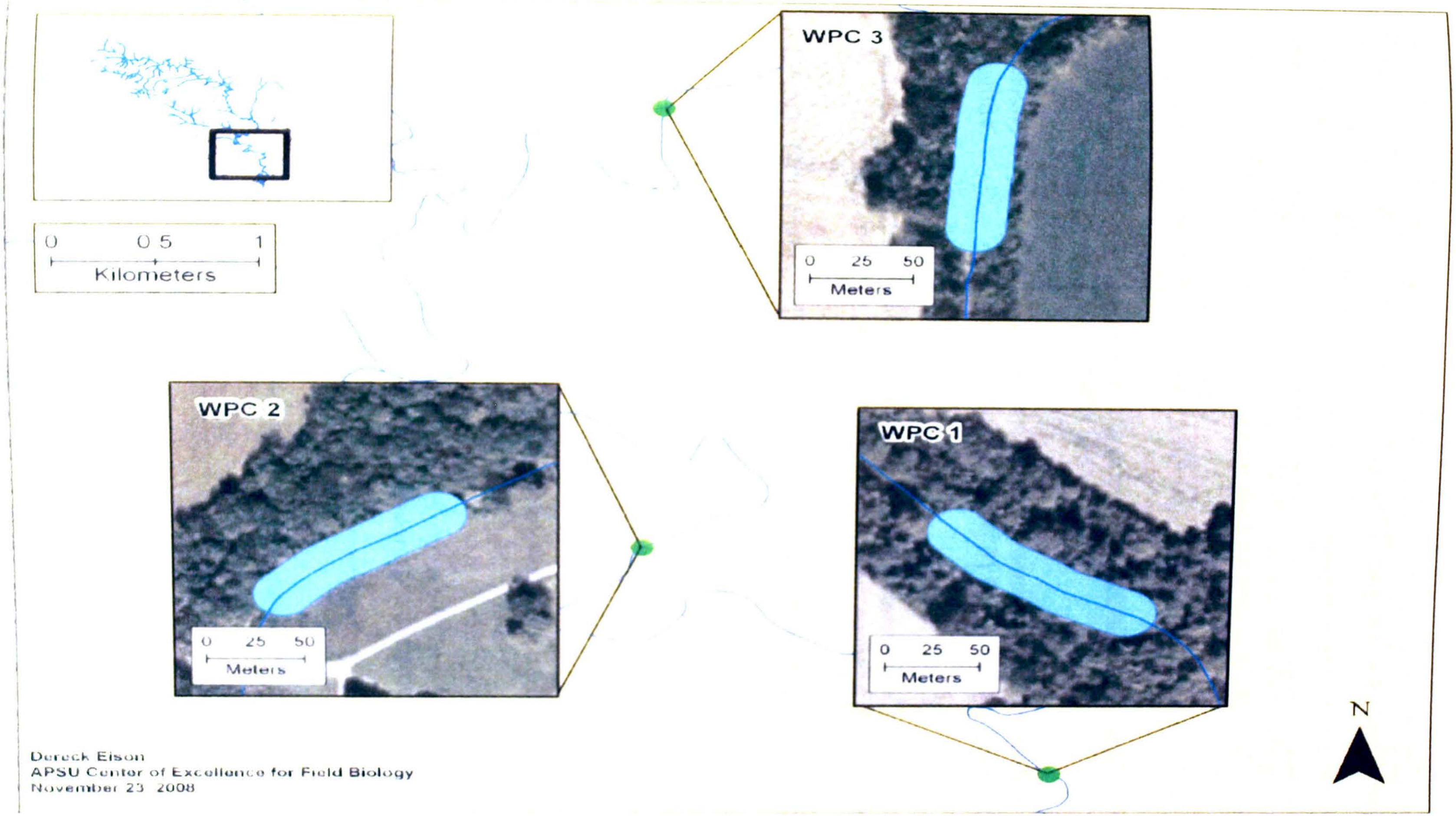

Figure 7. Stream sampling reach locations within Whippoorwill Creek, Logan County, Kentucky. Reaches are depicted within inset maps of sample locations. Each 100 meter sampling reach is highlighted in blue.

Table 4. Parameters used to assess habitats of high-gradient wadeable streams in the Interior Plateau Ecoregion of Kentucky.

Parameter	*Description*
Epifaunal Substrate/ Available Cover	Measures the relative quantity and the variety of structures providing refuge, feeding opportunities, and sites for spawning
Embeddedness	Measures the extent to which silt, sand, mud, or biofilms cover rocks and snags
Velocity/Depth Regime	The following patterns occur in the best streams: 1) slow-deep, 2) slow-shallow, 3) fast-deep, 4) fast-shallow
Sediment Deposition	Measures the amount of sediment that has accumulated in pools and changes that have occurred due to deposition
Channel Flow Status	The degree to which the channel is filled with water
Channel Alteration	Measures the large-scale changes in the shape of the stream channel
Frequency of Riffles	Measures the sequence of riffles and thus the heterogeneity occurring in a stream
Bank Stability	Measures the extent to which stream banks are eroded or the bank's potential to erode
Riparian Vegetative Zone Width	Measures the width of vegetation of each bank. Width is measured from the edge of the streambank through the riparian zone

Data Collection

Abiotic data was collected using a YSI 600QS multi-parameter sampling unit. The following are the physical stream characteristic recorded: dissolved oxygen (mg/L and % saturation), pH, temperature (°C), specific conductance (mS/cm), and total dissolved solids (mg/L). Measurements were recorded from each reach during sampling events.

Biotic data collected was solely related to fish species inhabiting each stream sampling reach. All sampling protocols for sampling fish set forth by KDOW were followed, with the exception of electroshocking (Compton et al., 2003). Fish were collected using a seine (3.4 x 1.8 m with 0.3 cm mesh). Sampling was performed for one hour at each sample reach to maintain consistency. One upstream pass was performed, making sure that all habitats (riffles, runs, and pools) were sufficiently sampled by seining techniques.

In order to maximize survival of collected specimens, fish were identified on site and released. Sampson (1995) and McMurray (1999) reported extremely low numbers of fish being collected in Pleasant Grove Creek, so survival of individuals was an issue during repeated sampling events. Fish were identified using Etnier and Starnes (1993) and Page and Burr (1991) taxonomic texts.

For purposes of identification and vouchering, a photo-box (20.3 x 20.3 x 5.7 cm; Figure 8) was constructed using 0.6 centimeter Plexiglas and silicone. The photo-box was used as a reservoir to hold fish as they were being photographed. A photograph was taken of each species collected using a Nikon D60 DSLR camera with an 18-55 millimeter vibration reduction lens. Total length of fish was recorded

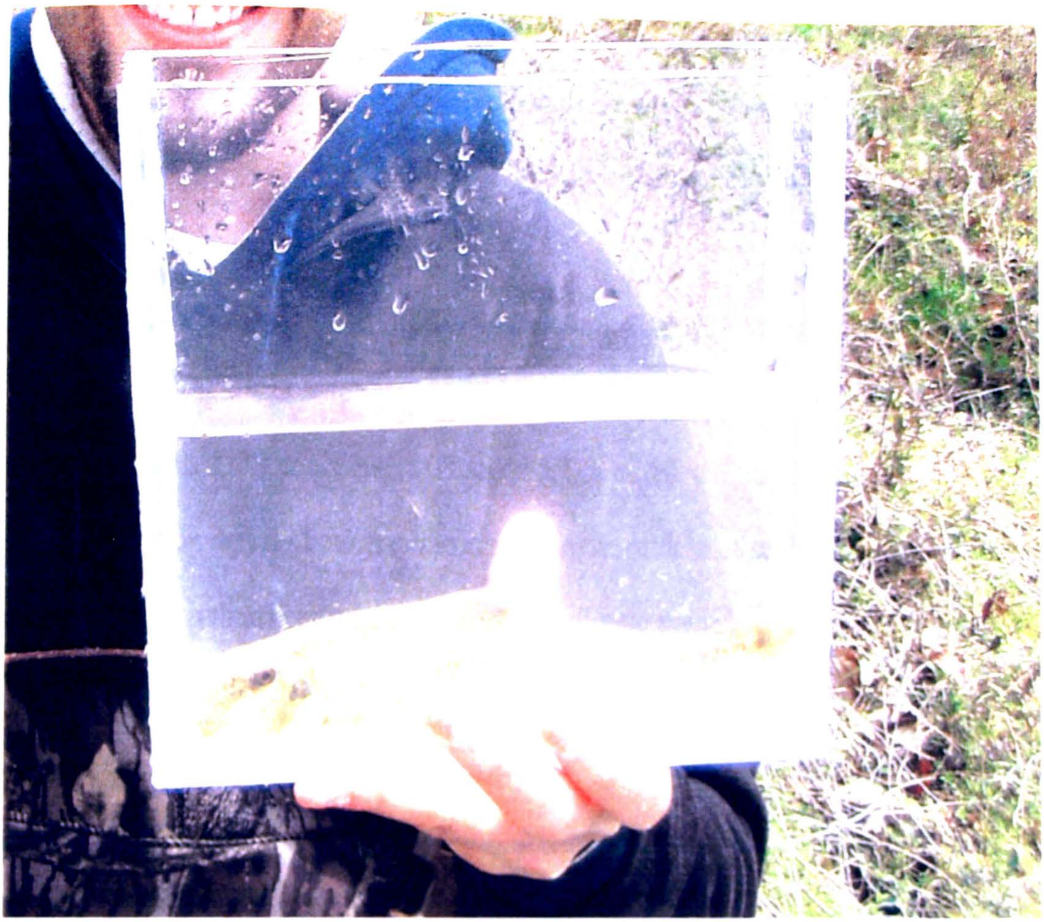

Figure 8. Photo-box used to hold fish specimens during the field identification and photography.

to the nearest millimeter, and fish were then released. To confirm the identification of fishes, digital photographs were further analyzed using Nikon and Microsoft Photoshop computer software in the lab. Voucher photos were saved and catalogued by collection date and sample reach.

Using ArcGIS 9.2 to Determine Riparian Zone Area and Width

ArcGIS 9.2 was employed to digitize the riparian zone adjacent to each sampling reach. GPS coordinates (Appendix I) were plotted in ArcMap on a two meter resolution aerial photograph to represent the middle of each sampling reach. A 50 m buffer was then created around each coordinate point to find the upstream and downstream boundary of each 100 m sampling reach (Figure 9). The adjacent riparian corridor of each sampling reach was digitized into a polygon and the area of each polygon was calculated using the measuring tool from the ArcMap toolbar (Figure 10).

Due to riparian zone polygons being irregularly shaped, mean width of each polygon was calculated. The ArcMap measuring tool was employed to perform these calculations. Concentric buffers rings were created around each mid-reach coordinate to create a guide by which riparian zone width could be measured. At each reach, ten measurements were taken at random along buffer ring tangents and then averaged to obtain a mean width (Figure 11).

Assessment of Biotic Integrity

The Kentucky Index of Biotic Integrity (KIBI; Compton et al., 2003) was used to assess the biotic integrity of both Pleasant Grove and Whippoorwill creeks. The KIBI uses seven metrics (Table 5) to assess the quality of headwater and wadeable

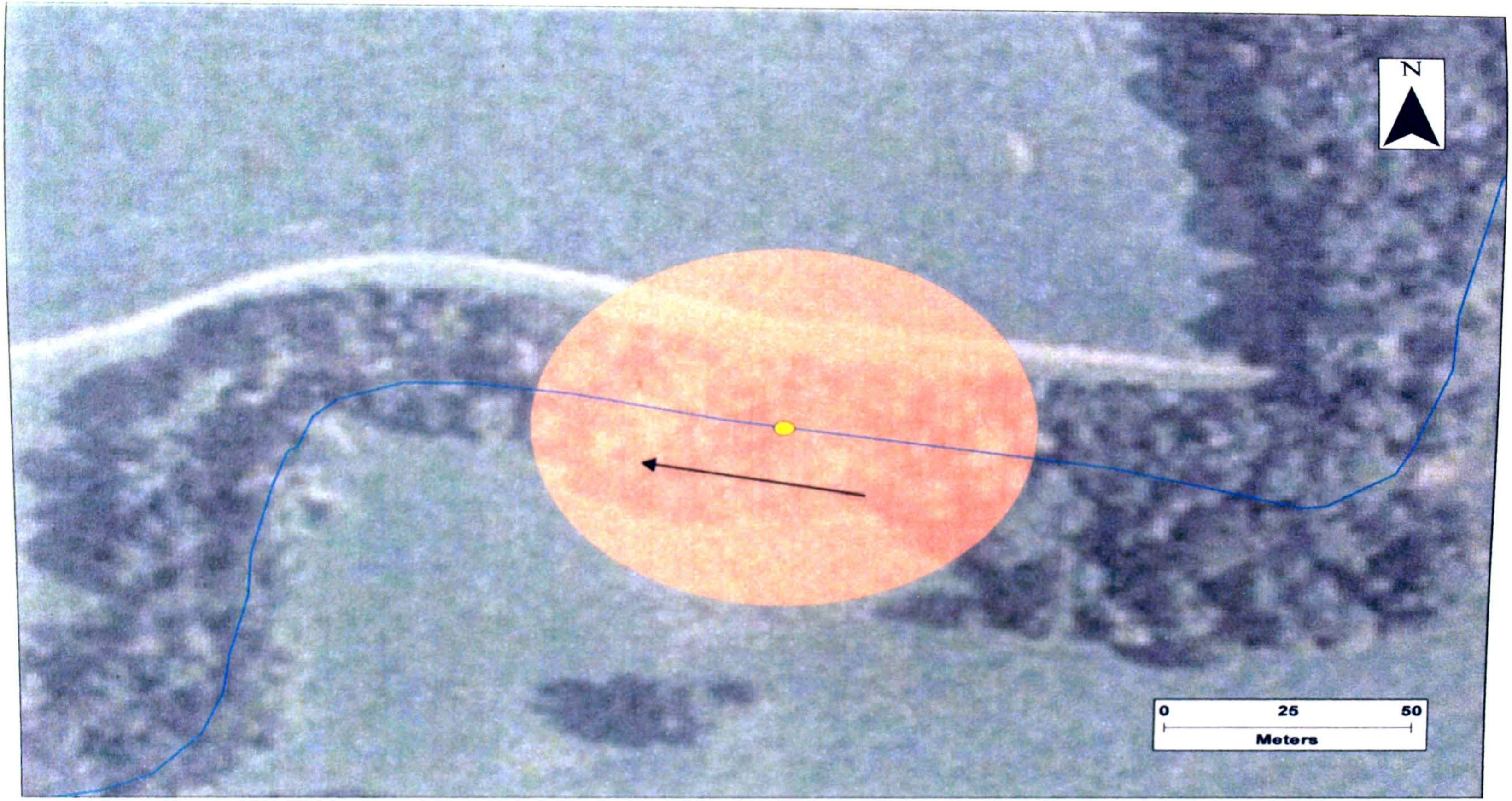

Figure 9. Fifty meter buffer created around the mid-reach coordinate at PGC 3. The mid-reach coordinate is represented by the yellow circle. The buffer is represented by the transparent orange circle. The arrow is pointing downstream.

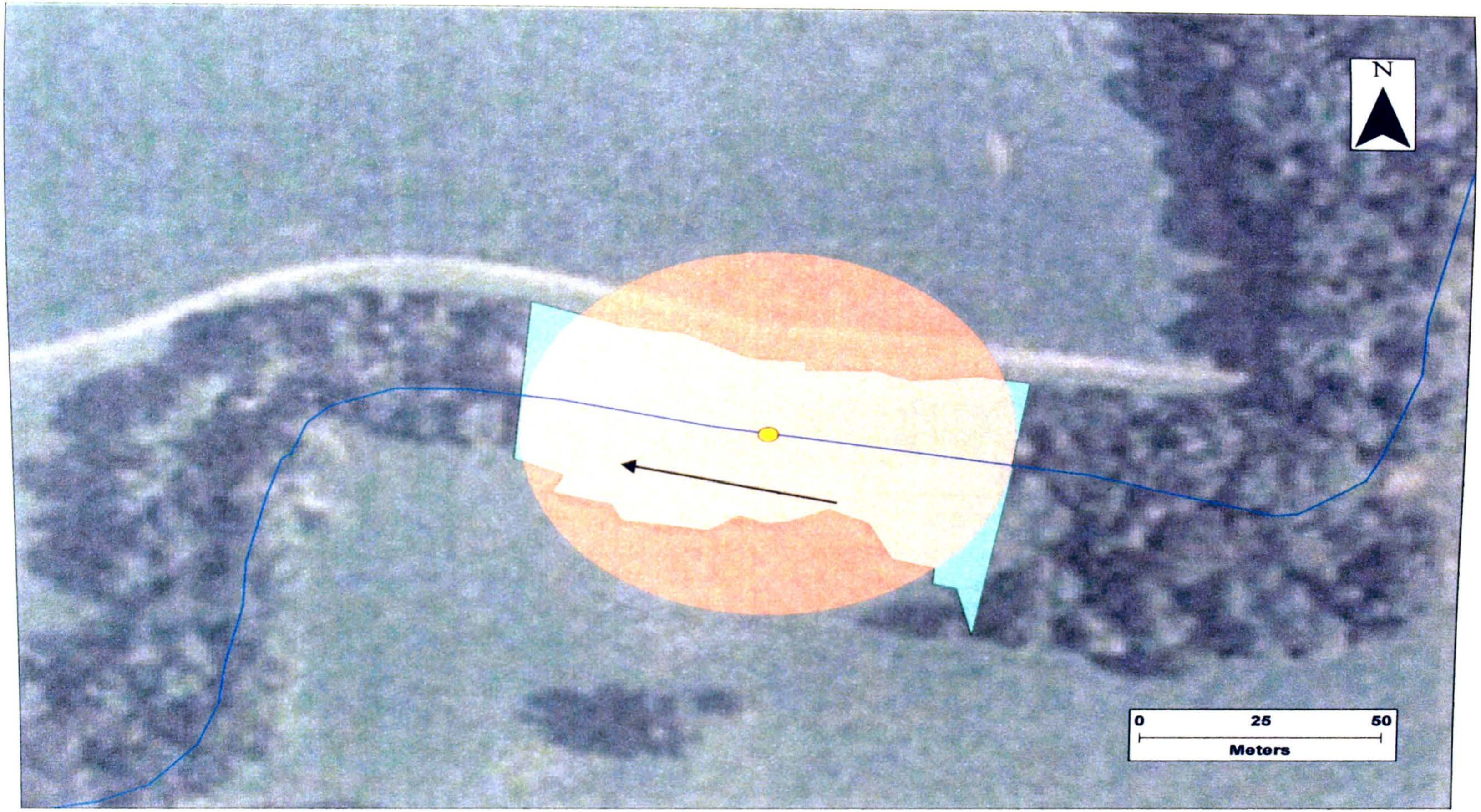

Figure 10. Digitized riparian zone located along the PGC 3 sampling reach. The mid-reach coordinate is represented by the yellow circle. The 50 m buffer is represented by the transparent orange circle. The polygon represents the riparian zone area along the sample reach. The arrow is pointing downstream.

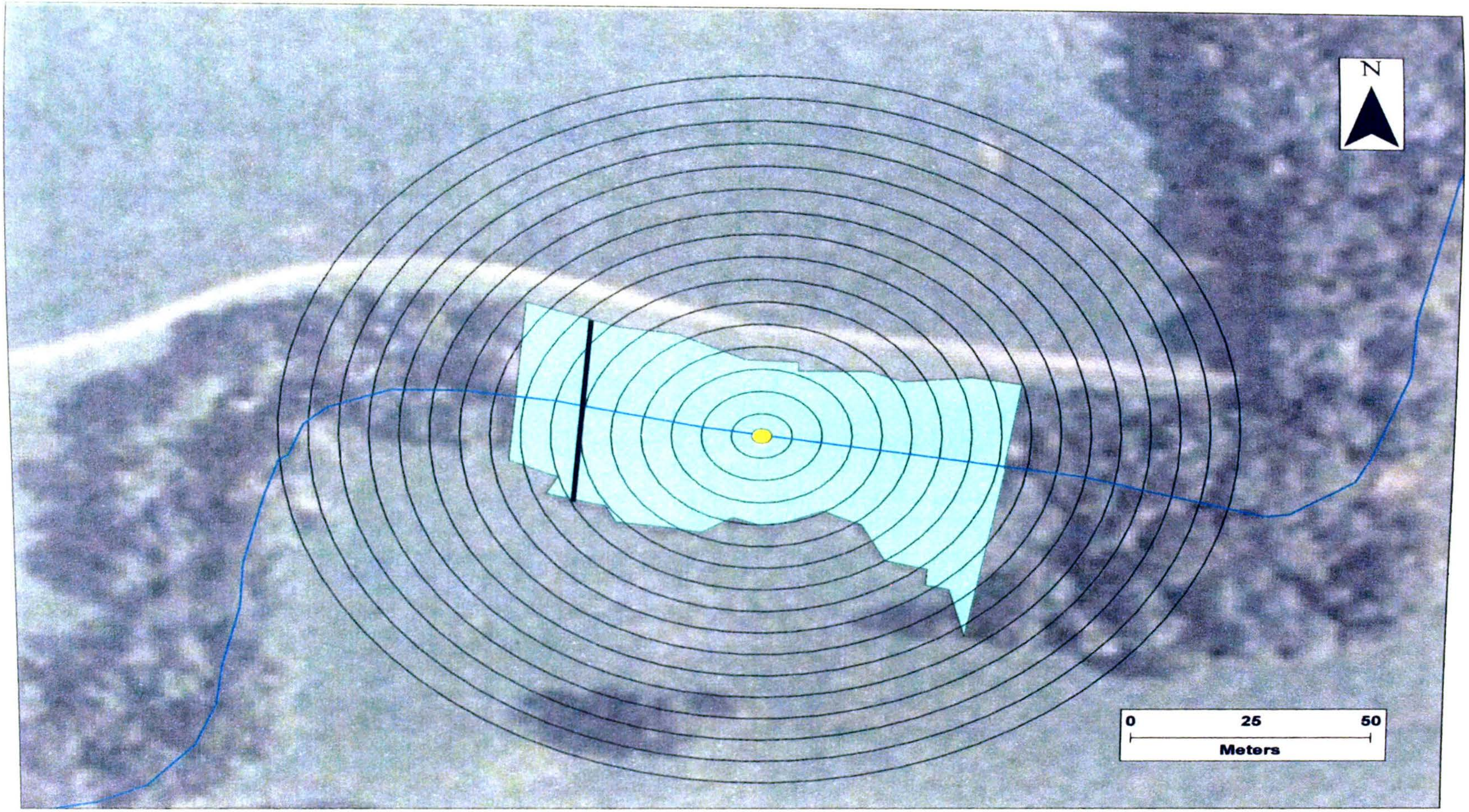

Figure 11. Concentric buffer rings used to measure riparian zone width. The bolded line represents a tangent line used to measure riparian zone width. The mid-reach coordinate is represented by the yellow circle. The polygon represents the riparian zone area along the sample reach

Table 5. Metrics used to calculate the Kentucky Index of Biotic Integrity (Compton et al. 2003).

Metrics	*Abbreviation*	*Response*
Total Number of Native Species*	NAT	Decrease
Total Number of Darters, Madtoms, and Sculpins Species	DMS	Decrease
Total Number of Intolerant Species	INT	Decrease
Total Number of Simple Lithophilic Spawning Species	SL	Decrease
Relative Abundance of Insectivorous Individuals (excluding Tolerant Individuals)	%INSCT	Decrease
Relative Abundance of Tolerant Individuals	%TOL	Increase
Relative Abundance of Facultative Headwater Individuals**	%FHW	Increase

*Used when sampling wadeable streams (drainage area > 12 $mi.^2$)

**Used when sampling headwater streams (drainage area < 12 $mi.^2$)

streams. Metrics are calculated on a 0 to 100 point scale and then averaged to obtain an overall KIBI score. Rules and instructions of how the KIBI is calculated can be seen in Figure 12. Kentucky developed six regions *a posteriori* to compensate for the physical influences of ecoregions and river basins on KIBI scores (Figure 13). KIBI scores of each ichthyoregion are based on a 0-100 point scale, but each ichthyoregion has a different scoring range for classifications (Very Poor, Poor, Fair, Good, and Excellent). For example, a score of 53 is classified as Excellent in the Bluegrass Ichthyoregion, but a score of 53 is classified as Good in the Pennyroyal Ichthyoregion.

The scoring criteria used are consistent with that of the criteria used to classify streams within the Pennyroyal Ichthyoregion of Kentucky (Figure 13). All streams within this ichthyoregion use the Pennyroyal scoring criteria except for the Green River watershed (Compton et al., 2003). Scores of the KIBI are classified in the Pennyroyal Ichthyoregion as follows: Excellent (≥ 67), Good (53-66), Fair (35-52), Poor (17-34), and Very Poor (0-16).

Statistical Analysis

ANOVA was used to test for statistical differences among sample reaches for mean dissolved oxygen (mg/L and % saturation), pH, temperature (°C), specific conductance (mS/cm), and total dissolved solids (mg/L), KIBI scores, and fish species richness. This parametric test was used because these data sets were determined as having normal distributions by the Bartlett's test of homogeneity of variances. The non-parametric Kruskall-Wallis test was used to determine if a

KENTUCKY INDEX OF BIOTIC INTEGRITY (KIBI)

KIBI Calculation Process

1) Convert Catchment Area (sq. miles) to Log 10. This value will represent 'x' in the Reference Regression Equation (RRE).
2) Inverse % INSCT and % TOL metrics (100 minus metric's actual/raw value).
3) Solve for the Expected Value of a particular metric using the Log 10 of a site's catchment area as 'x' in the RRE (given) of the respected metric.
4) Subtract Actual Value (raw data) from the Expected Value (Step 3) to obtain a Residual Value. This number will be positive or negative based on site quality.
5) To normalize Residual Value data for all catchment areas a Catchment Area Constant (CAC) (given) is used for each metric. The CAC is added to the Residual Value (step 4) to obtain the metric value.
6) The metric value is divided by 95th percentile (given) of the Reference Set and multiplied by 100.
7) The average score of the eight metrics is the Final KIBI score on a 0–100 point scale.

Rules

1) Metric Values (step 7) with values >100 score as 100.0.
2) Metric Values (step 7) with negative values score as 0.0.
3) Total Number of Individuals (TNI) ≤ 50 score % Metrics as 0.0.
4) TNI 51-99 score % Metrics as 50.0, unless value is already under 50.0.
5) TNI= 0, then KIBI = 0

KIBI Metrics	Reference Regression Equations	CAC	95th %
NAT*	$y = 10.123x + 4.4279$	20.49	28.2
DMS	$y = 2.967x + 1.5037$	6.21	9.3
INT	$y = 2.6679x - 0.1395$	4.09	7.7
SL	$y = 4.4162x + 0.9526$	7.96	12.5
%INSCT	$y = -10.326x^2 + 44.989x + 17.575$	58.88	87.8
%TOL	$y = -5.4568x^2 + 31.379x + 41.6$	77.65	101.5
%FHW*	$y = 8.9128x^2 - 59.151x + 98.557$	27.14	61.4

* note NAT for wadeable streams and %FHW for headwater streams

Figure 12. Instructions and rules for calculating the Kentucky Index of Biotic Integrity (Compton et al., 2003).

Interior Plateau, Pennyroyal (PR)	
CLASSIFICATION	**SCORE**
EXCELLENT	≥ 67
GOOD	53-66
FAIR	35-52
POOR	17-34
VERY POOR	0-16

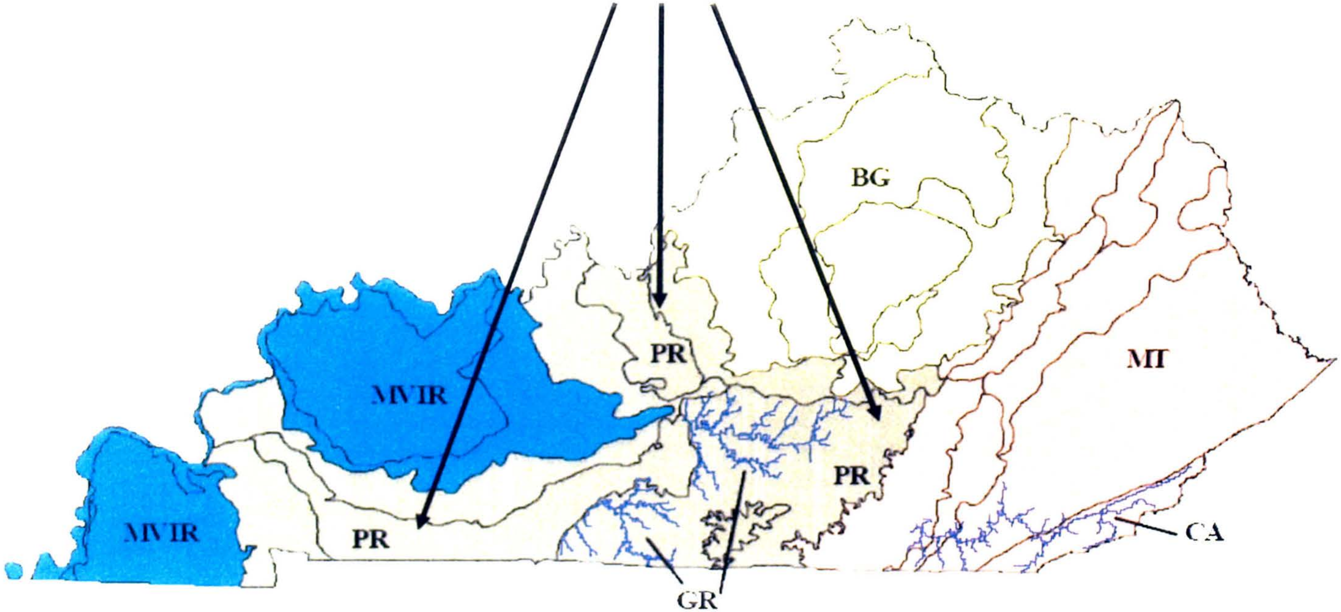

Figure 13. The Pennyroyal Ichtyoregion IBI scoring classification and the Kentucky IBI Ichthyoregions (Compton et al. 2003). BG= Bluegrass, CA= Cumberland above the Falls, GR= Green River, MT= Mountain, MVIR= Mississippi Valley-Interior River, PR= Pennyroyal. Note GR and CA ichthyoregions are river basins within larger ichthyoregions.

rank correlation test was employed to determine if a significant correlation existed among sample reach riparian zone area, sample reach riparian zone width, and sample reach habitat assessment score versus the number of fish collected per sampling event. All calculations were performed using the MYSTAT 12 statistical software package and Microsoft Excel 2007.

CHAPTER III

RESULTS

Abiotic Data

An ANOVA was used to test for statistical differences among sample reaches for mean dissolved oxygen (mg/L and % saturation), pH, temperature (°C), specific conductance (mS/cm), and total dissolved solids (mg/L). A significant difference was found in the abiotic data among mean dissolved oxygen as mg/L (Table 6) and as percent saturation (Table 7). A Tukey-Kramer statistic for unequal sample sizes was performed *a posteriori* to determine which stream reaches exhibited significantly different dissolved oxygen levels as mg/L and as percent saturation. The Tukey-Kramer statistic revealed that a significant difference existed for dissolved oxygen as mg/L ($Q_{0.05\,[6,27]} = 4.13$, AV = 3.85, MSD = 2.72) and as percent saturation ($Q_{0.05\,[6,27]} = 4.13$, AV = 36.4, MSD = 34.2) between PGC 3 and WPC 3.

Biotic Data

Nine fish families, 31 species, and 4,667 individuals were collected at the six sampling reaches (Table 8). The fish family collected most often was Cyprinidae with 11 species collected. The species collected most often was of the cyprinid *Lythrurus fasciolaris*. A total of 3,556, 76% of all fish collected, were individuals of this species. When comparing the number of fish collected per sample reach, WPC 2 had the greatest mean number of individuals collected ($\bar{x} = 275 \pm 73$ SE). Sample reach PGC 3 had the lowest mean number of fish collected, ($\bar{x} = 46 \pm 15$ SE; Figure 14). The Kruskal-Wallis test used to compare the mean number of fish collected

Table 6. Results for an ANOVA comparing mean dissolved oxygen (mg/L) levels collected from sample reaches during 2007-2009 sampling events within Pleasant Grove and Whippoorwill creeks ($\alpha = 0.05$).

	Sum of Squares	*df*	Mean Squares	F-ratio	P-value
Among groups	43.545	5	8.709	3.308	0.019
Within groups	71.081	27	2.633		
Total	114.626	32			

Table 7. Results for an ANOVA comparing mean dissolved oxygen (% saturation) levels collected from sample reaches during 2007-2009 sampling events within Pleasant Grove and Whippoorwill creeks ($\alpha = 0.05$).

	Sum of Squares	*df*	Mean Squares	F-ratio	P-value
Among groups	4517.885	5	903.577	2.840	0.035
Within groups	8591.093	27	318.189		
Total	13108.978	32			

Table 8. Total number of fish species collected per sample reach from 2007-2009 sampling events within Pleasant Grove (PGC) and Whippoorwill creeks (WPC).

Fish	Site					
	PGC 1[a]	PGC 2[b]	PGC 3[a]	WPC 1[b]	WPC 2[a]	WPC 3[b]
Petromyzontidae						
Lampetra aepyptera	1	1	3	**	**	**
Cyprinidae						
Campostoma anomalum	3	1	27	3	2	7
Cyprinella galactura	**	**	**	1	9	1
Hybopsis amblops	1	**	**	1	3	6
Luxilus chrysocephalus	5	14	15	20	6	17
Lythrurus fasciolaris	529	52	144	582	1706	543
Nocomis effusus	1	1	1	12	24	16
Nocomis micropogon	**	**	**	**	**	1
Notropis boops	2	**	**	**	**	**
Phoxinus erythrogaster	20	31	16	**	1	**
Pimephales notatus	7	**	3	1	13	27
Semotilus atromaculatus	4	**	2	**	6	5
Catostomidae						
Hypentelium nigricans	**	1	1	6	1	1
Esocidae						
Esox americanus vermiculatus	1	2	**	**	**	**
Fundulidae						
Fundulus olivaceus	**	**	**	1	2	1

Table 8 Continued.

Fish	Site					
	PGC 1[a]	PGC 2[b]	PGC 3[a]	WPC 1[b]	WPC 2[a]	WPC 3[b]
Poeciliidae						
Gambusia affinis	**	**	7	**	**	**
Centrachidae						
Ambloplites rupestris	**	**	**	3	1	1
Lepomis cyanellus	**	**	**	**	1	**
Lepomis macrochirus	**	**	**	**	1	**
Micropterus dolomieu	**	**	**	**	1	1
Micropterus punctulatus	**	**	**	**	**	1
Percidae						
Etheostoma blennioides	**	**	1	3	1	6
Etheostoma flabellare	2	**	**	1	**	**
Etheostoma flavum	19	8	10	12	23	10
Etheostoma rafinesquei	6	**	**	**	4	1
Etheostoma rufilineatum	**	**	**	14	15	1
Etheostoma simoterum	33	18	**	44	82	94
Etheostoma spectabile	**	**	**	1	11	**
Etheostoma squamiceps	16	97	50	**	4	**
Percina maculata	1	**	**	**	**	**
TOTAL	*683*	*281*	*318*	*707*	*1922*	*741*

[a] Seven sample events were performed from 2007-2009.
[b] Four sample events were performed from 2008-2009.
** Species was not collected.

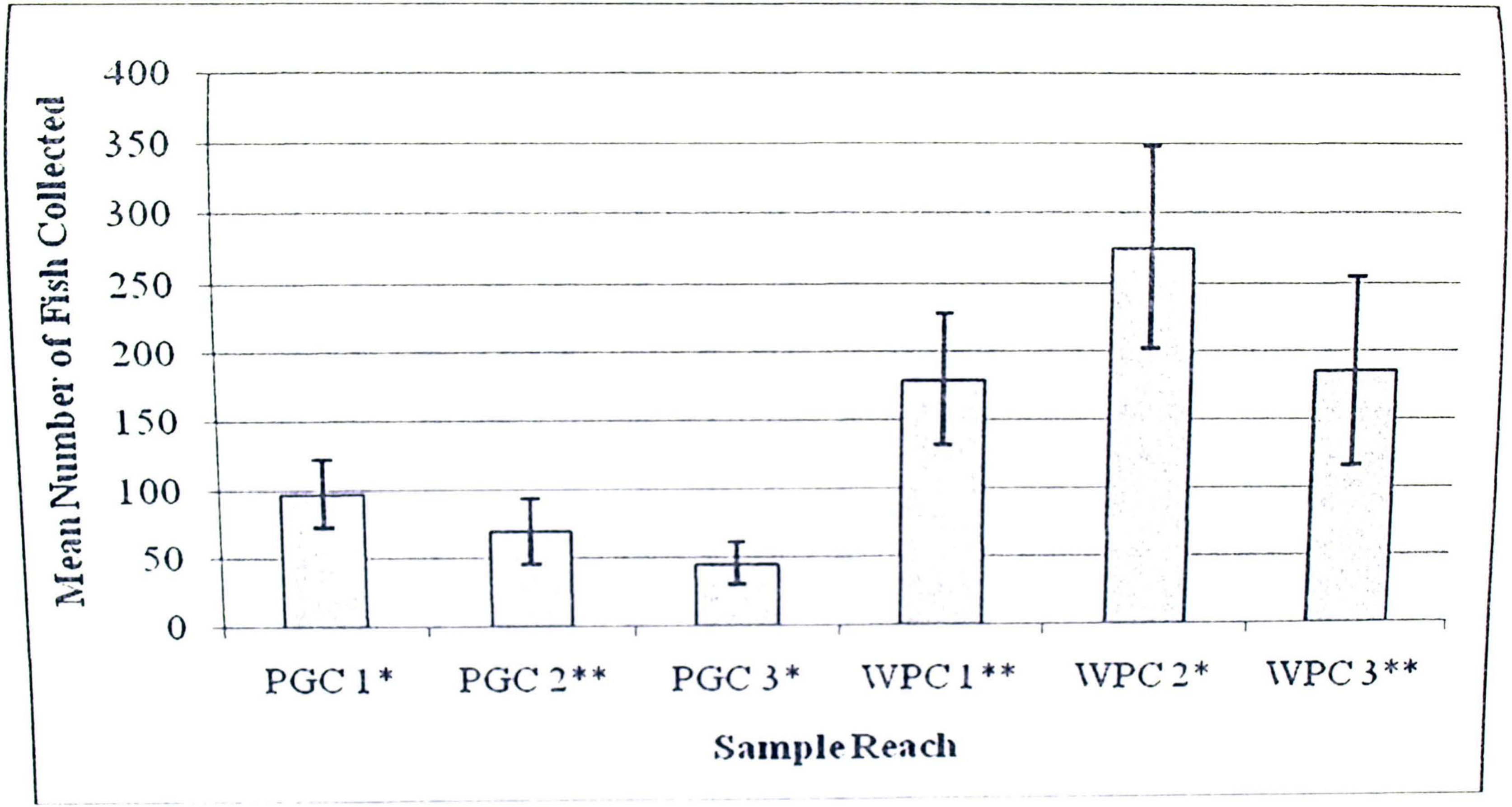

Figure 14. Number of fish collected per sampling reach within Pleasant Grove and Whippoorwill creeks during 2007-2009 sampling events. Bars represent mean ± one standard error. *Sample size = 7; **Sample size = 4.

per sample reach resulted in a significant difference existing between sample reaches ($X^2_{0.05\,[5]} = 15.76$). *A posteriori* analysis of the mean number of fish collected per sample reach was performed using Dunn's nonparametric multiple comparison statistic for unequal sample sizes (Dunn, 1964). Zar (2009) proposed that this test is a more stringent and conservative test for unequal sample sizes than the nonparametric Tukey-type multiple comparisons statistic. Dunn's statistic revealed that a significant difference existed between the mean number of fish collected at sample reaches PGC 3 and WPC 2 ($Q_{0.05\,[6]} = 2.936$, AV = 3.60, MSD = 2.94). When comparing mean number of species collected per site, PGC 3 had the lowest mean species richness ($\bar{x} = 6 \pm 1$ SE) and WPC 3 had the greatest mean species richness ($\bar{x} = 10 \pm 2$ SE; Figure 15). An ANOVA test for mean species richness per sample reach resulted in no significant difference existing between sites (Table 9).

A Spearman's rank correlation test revealed a significant correlation between the number of fish collected per sampling event and sampling reach riparian zone area (Figure 16), sample reach mean riparian zone width (Figure 17), and sample reach habitat assessment scores (Figure 18). Habitat assessment scores are presented in Table 10. Habitat assessment scores of sample reaches within Pleasant Grove Creek were lower than scores calculated from sample reaches within Whippoorwill Creek. Whippoorwill Creek habitat assessment scores reflected habitats that fully support aquatic life. Conversely, Pleasant Grove Creek habitat assessment scores reflected habitats that partially supported aquatic life or habitats that supported aquatic life but were degrading.

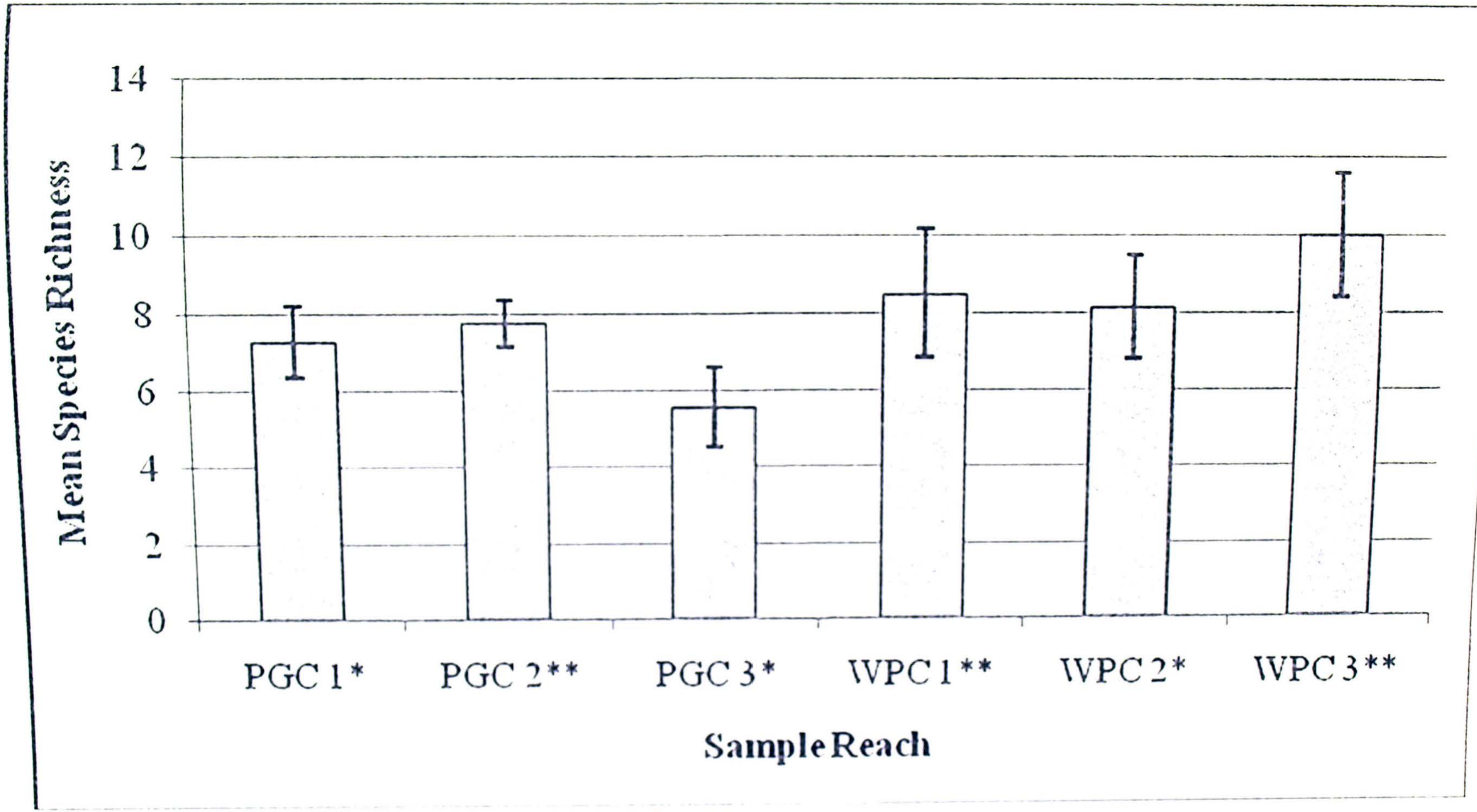

Figure 15. Fish species richness collected per sampling reach within Pleasant Grove and Whippoorwill creeks during 2007-2009 sampling events. Bars represent mean ± one standard error. *Sample size = 7; **Sample size = 4.

Table 9. Results for an ANOVA comparing mean fish species richness between sample reaches within Pleasant Grove and Whippoorwill creeks. Sampling events transpired during 2007-2009 ($\alpha = 0.05$).

	Sum of Squares	*df*	Mean Squares	F-ratio	P-value
Among groups	57.886	5	11.577	1.385	0.261
Within groups	225.750	27	8.361		
Total	283.636	32			

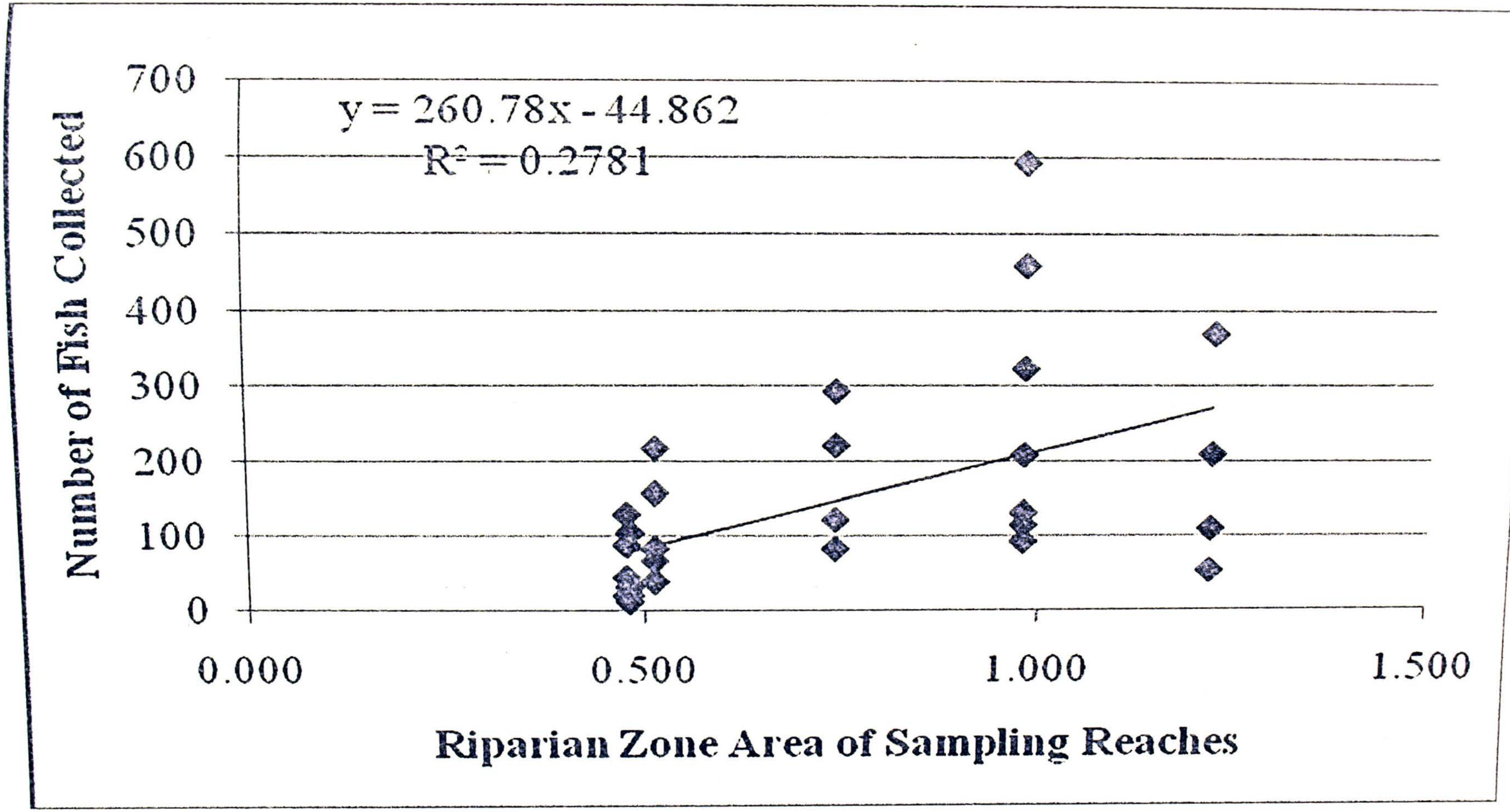

Figure 16. Results of the Spearman's rank correlation test used to determine significant correlation between total number of fish collected per sampling reach and adjacent riparian zone area (hectares) of sampling reaches within Pleasant Grove and Whippoorwill creeks ($n = 33$, $r = 0.528$).

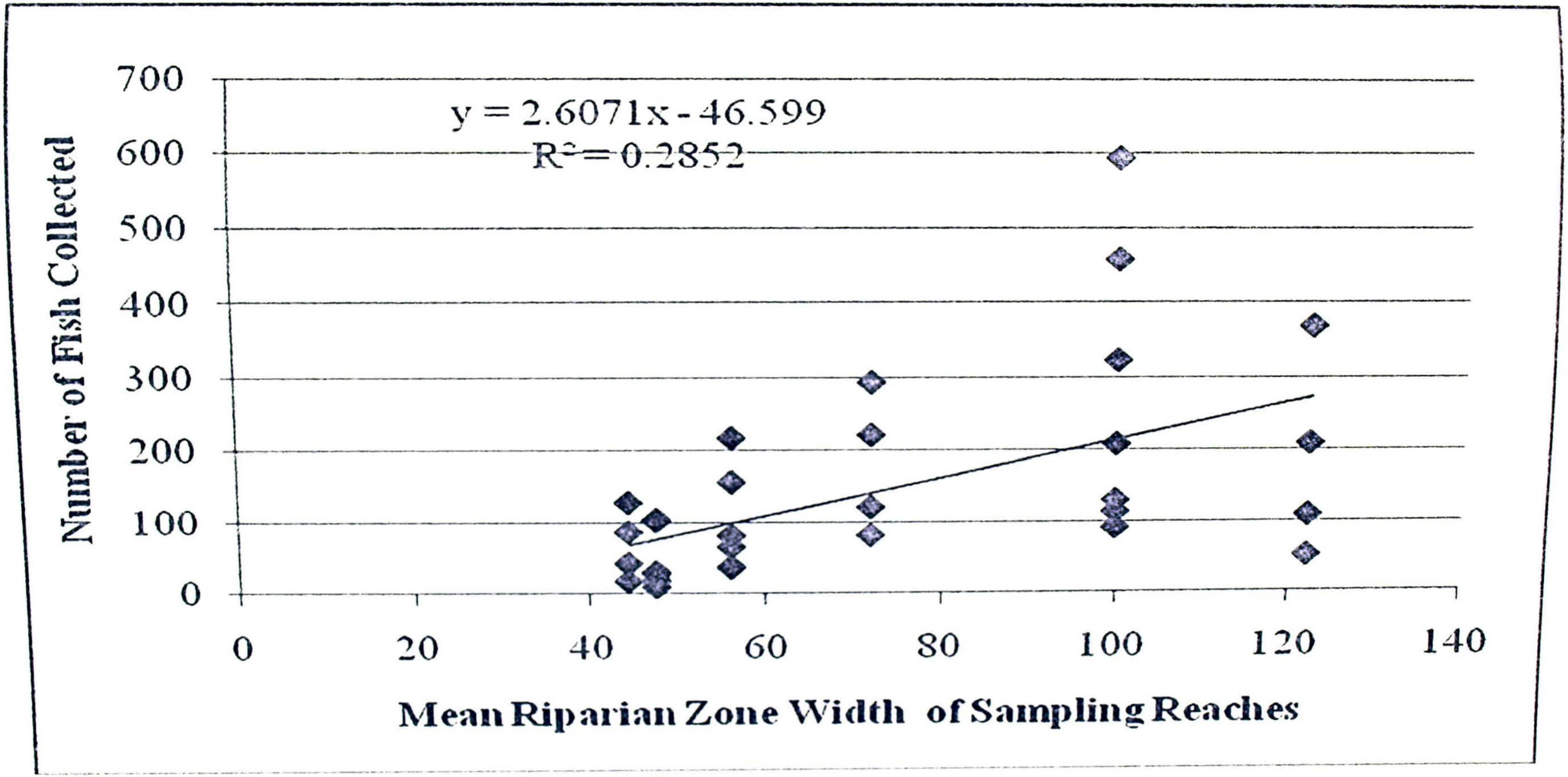

Figure 17. Results of the Spearman's rank correlation test used to determine if a significant correlation existed between total number of fish collected per sampling reach and mean riparian zone width (meters) of sampling reaches within Pleasant Grove and Whippoorwill creeks (n = 33, r = 0.534).

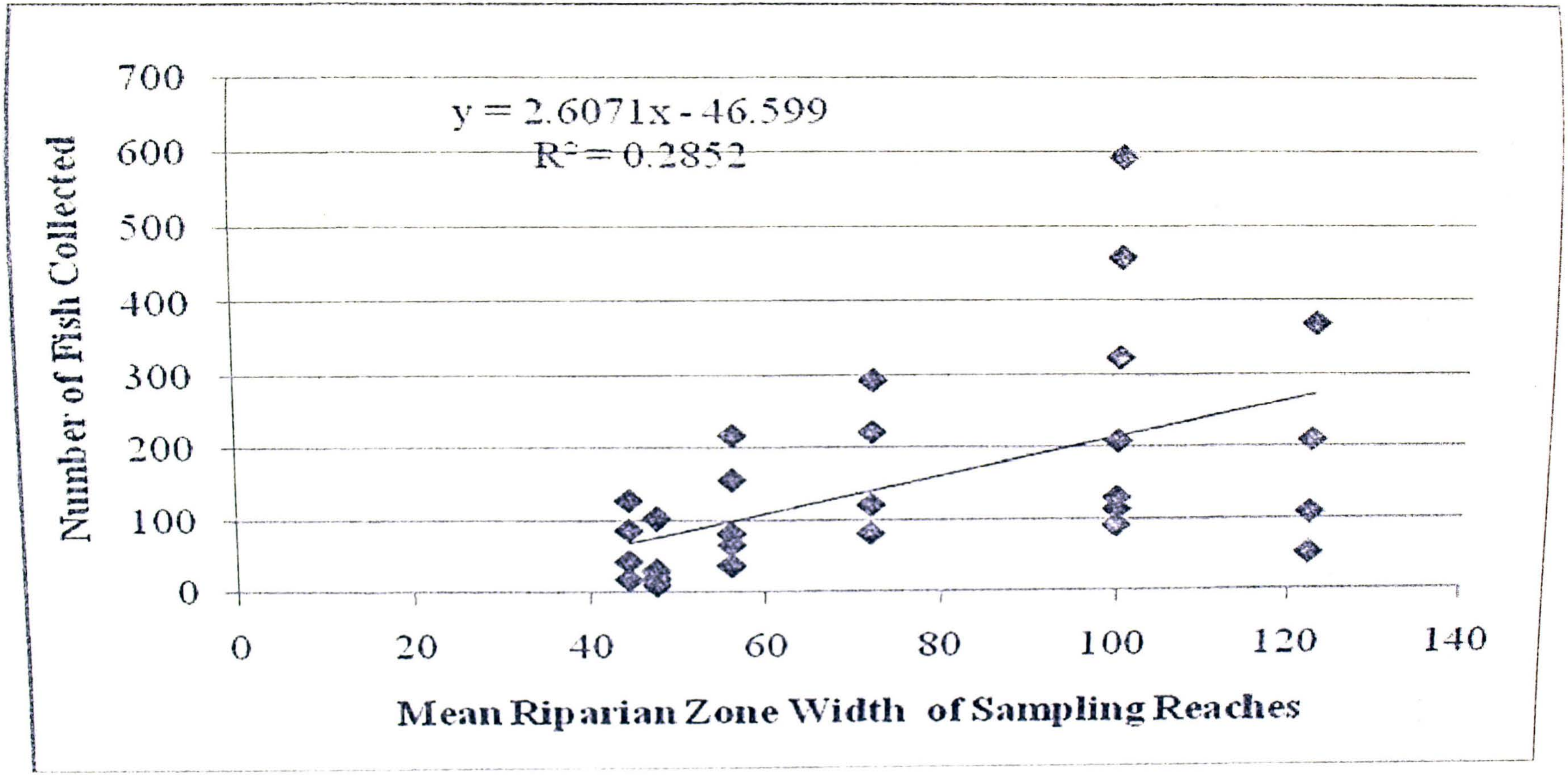

Figure 17. Results of the Spearman's rank correlation test used to determine if a significant correlation existed between total number of fish collected per sampling reach and mean riparian zone width (meters) of sampling reaches within Pleasant Grove and Whippoorwill creeks (n = 33, r = 0.534).

Table 10. Habitat assessment scores and criteria of sampling reaches in Pleasant Grove and Whippoorwill creeks. Criteria are for high-gradient streams located within the Interior Plateau ecoregion of Kentucky.

Site	Score	Criteria Range
PGC 1	129	Partially Supporting
PGC 2	133	Supporting, But Threatened
PGC 3	141	Supporting, But Threatened
WPC 1	161	Fully Supporting
WPC 2	164	Fully Supporting
WPC 3	162	Fully Supporting

Biotic Integrity

When comparing mean Kentucky Index of Biotic Integrity (KIBI) scores between sampling reaches, PGC 3 had the lowest mean KIBI score ($\bar{x}$ = 24 ± 10 SE). WPC 2 had the greatest mean KIBI score, ($\bar{x}$= 44 ± 4 SE). The mean KIBI score and biotic integrity classification of each sample reach are presented in Table 11. An ANOVA comparing the mean KIBI scores between sampling reaches resulted in no significant difference between these means (Table 12). When comparing fish IBI scores calculated from Kentucky Department of Water (KDOW) samples collected in 1994 and 1998 with mean fish KIBI scores collected from 2007 to 2009, Pleasant Grove Creek continues to have a biotic integrity designated as Poor (Figure 19). The KIBI score for Pleasant Grove Creek is nearly in the Fair range and higher than the IBI scores from the KDOW studies.

Table 11. Mean KIBI score and biotic integrity classification of each sample reach calculated from fish samples collected in 2007-2009.

Site	*n*	Mean KIBI Score ± 1SE	Biotic Integrity Classification
PGC 1	7	41 ± 7	Fair
PGC 2	4	37 ± 9	Fair
PGC 3	7	24 ± 10	Poor
WPC 1	4	42 ± 7	Fair
WPC 2	7	44 ± 4	Fair
WPC 3	4	44 ± 7	Fair

Table 12. An ANOVA comparing mean KIBI scores between sample reaches of Pleasant Grove and Whippoorwill creeks. KIBI scores were calculated from sampling events that transpired during 2007-2009 ($\alpha = 0.05$).

	Sum of Squares	*df*	Mean Squares	F-ratio	P-value
Among groups	1891.242	5	378.248	1.167	0.351
Within groups	8753.000	27	324.185		
Total	10644.242	32			

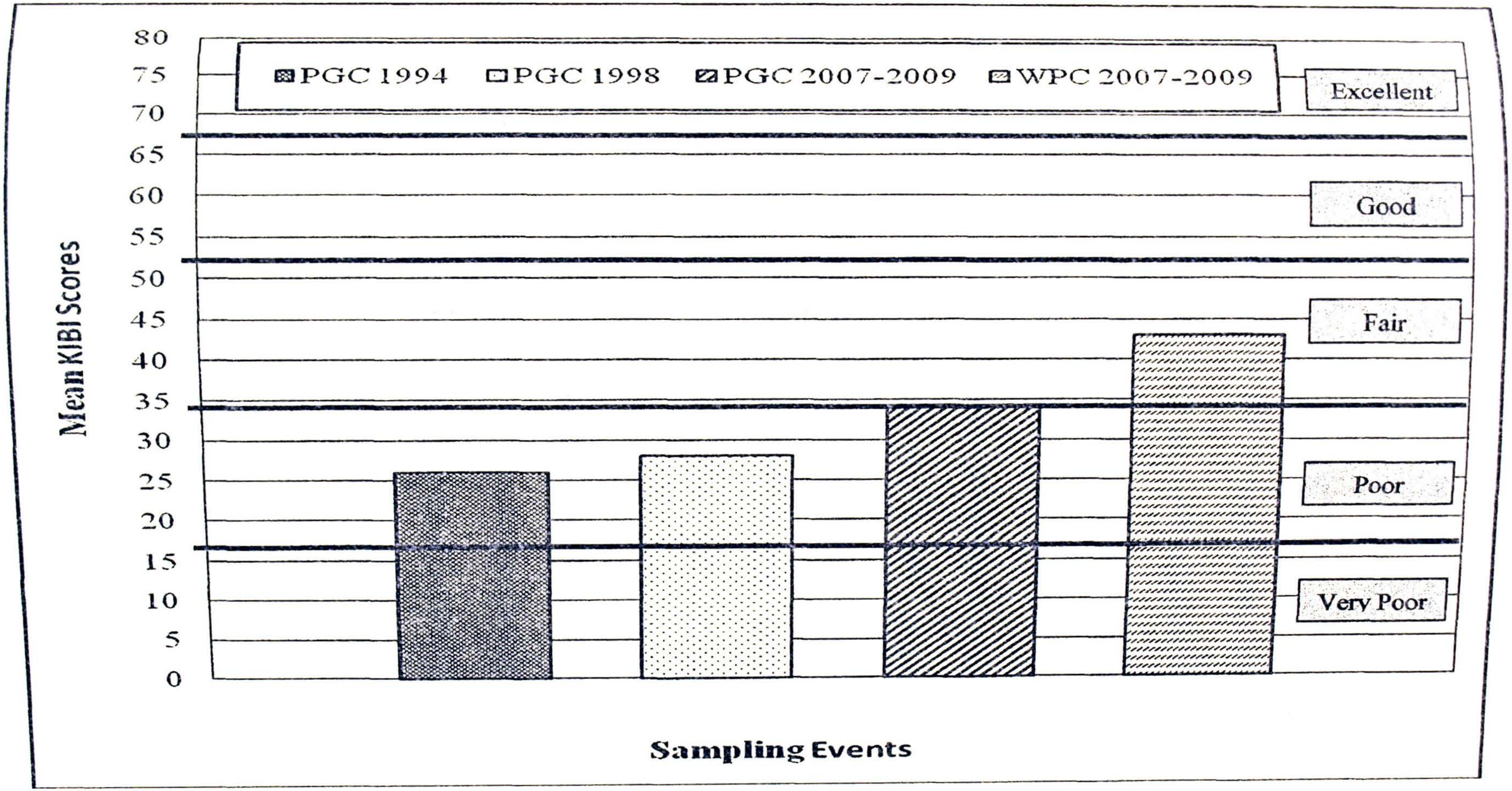

Figure 19. Comparison of fish IBI scores calculated from Kentucky Department of Water sample events in 1994 and 1998 and mean fish Kentucky Index of Biotic Integrity (KIBI) scores calculated from 2007-2009 sampling events. Boxes on the right represent biotic integrity classifications and bolded lines represent KIBI scoring ranges for the Pennyroyal Ichthyoregion.

CHAPTER IV

DISCUSSION

Fish Abundance and Dissolved Oxygen

When assessing the potential ecological functions within an aquatic system, dissolved oxygen (DO) has been argued to be one of the most significant measurements of water quality (Slack, 1971). Concentrations of dissolved oxygen have been shown to reflect the balance between the amount of oxygen available and the amount of oxygen being consumed by metabolic processes within an aquatic community (Kalff, 2002). Mulholland et al. (2005) suggested that DO concentration can be a useful indicator of stream metabolism and that creating profiles of DO concentration can provide valuable information needed to make determinations about the metabolism of a given stream.

Dissolved oxygen levels within streams have been shown to have diurnal fluctuations (Lebkuecher et al., 1998). Small streams in particular can have pronounced changes in DO over a 24 h period, and community respiration rates in most streams are only partially offset by the photosynthetic activities of primary producers during the day. Extreme diurnal oscillations in DO levels can indicate an increase in primary production by way of nutrient loading (Kalff, 2002). Due to the often excessive amounts of nitrogen and phosphorus added to crops and soil in agricultural watersheds, nutrient loading is a common occurrence in streams associated with this type of land use. Nitrogen and phosphorus are the primary nutrients that limit algae and macrophyte growth in lotic environments. When concentrations of these nutrients are elevated in streams, plant productivity can increase substantially (United States Department of Agriculture, 1983). An increase in primary production within a stream will create supersaturated DO levels during the day, but DO levels will plummet at night due to an increase in overall community respiration. Such was the case of Walker Branch, a stream in Roane County, Tennessee. Marzolf et al. (1994) demonstrated that gross primary production within Walker Branch was only occurring during the midday hours, and that Walker Branch exhibited an overall negative net rate of oxygen production over a 24 hour period. Scenarios such as this create unfavorable environments for higher order aquatic trophic levels, such as fish (Williamson et al., 1998).

Low DO levels are the primary concern when assessing a stream's ability to support aquatic life (Slack, 1971). Changes in DO, or abnormal levels, have been observed to have adverse effects on aquatic organisms (Ostrand, 2000, Helms, 2008). Stream reaches that exhibit abnormal DO concentrations essentially act as a physical barrier to fish species that are intolerant of such environmental conditions. Fish exposed to abnormal DO concentrations will be restricted in their local habitat range, and changes in DO have been demonstrated to disrupt fish behavior. Poon et al. (2002) stated that when faced with low DO levels, fish reduce food intake and restrict movement to expend less energy while preserving oxygen. This phenomenon was demonstrated by Hubbs et al. (1967) when their results showed that killifish (*Crenichthys baileyi* and *Crenichthys nevadae*) inhabiting warm water spring reaches in eastern Nevada exhibited reduced activity in areas with low DO levels (1.5 ppm), while nearby populations (sampling stations were 50 m apart) were active in localities with normal concentrations of dissolved oxygen. A reduction in fertilization success can result as well due to decreased dissolved oxygen. Zhou et al. (2001) demonstrated that individuals of *Cyprinus carpio*, the common carp, exposed to hypoxic conditions (1 mg/L DO) displayed significantly retarded gonad development and a reduction in fertilization success, larval hatching, and larval survival success.

Alternatively, other studies have shown that dissolved oxygen tolerance can be species specific. Hill et al. (1978) reported that western mosquitofish (*Gambusia affinis*) and Red River pupfish (*Cyprinodon rubrofluviatilis*) were observed to tolerate extremely high levels of DO (20.0 ppm) in laboratory conditions. The authors went on to suggest that the high tolerance to such supersaturated DO concentrations explains their observations of *G. affinis* and *C. rubrofluviatilis* inhabiting stream reaches with large algal beds present along the substrate of western Oklahoma streams.

The scenarios discussed above are all possible explanations as to why fish abundance and IBI scores were so low at stream reaches in Pleasant Grove Creek, especially at PGC 2 (mean KIBI score = 37; Biotic Integrity Classification = Fair) and PGC 3 (mean KIBI score = 24; Biotic Integrity Classification = Poor). These scores were the lowest mean KIBI scores calculated for this study (Table 11). Dissolved oxygen levels recorded over the course of this study suggest that stream metabolism within Pleasant Grove Creek is being heavily influenced by increased levels of primary production (Lebkuecher, J.G., personal communication). Mean dissolved oxygen levels calculated from samples taken at PGC 2 (106.8%) and PGC 3 (108.5%) indicate that supersaturated DO concentrations frequently occur within this stream. Dissolved oxygen samples from these sites were taken during midday. Due to an increase in the availability of solar energy during this time period, higher rates of primary production occur (Kalff, 2002). Samples collected during midday and afternoon periods within WPC 2 exceeded saturation levels of 100 percent as well (102.4%, 109.3%, and 107.7%), suggesting that this creek may also be experiencing exaggerated diurnal DO fluctuations. One instance of low DO being recorded (4.23 mg/L) at WPC 3 during the morning hours (8:15am) further indicates that levels fall below limits that are required for fish to maintain normal metabolic processes. In order maintain the quality of life required by intolerant aquatic organisms, the Kentucky Division of Water (2009) states that instantaneous dissolved oxygen samples should not fall below 5.00 mg/L. Furthermore, the time at which samples were taken at WPC 3 ($\bar{x}$ = 8:50 am) and the evidence of supersaturated DO concentrations at PGC3 (Appendix III, Table B) provide an explanation for the significant difference that existed between mean dissolved oxygen as mg/L ($Q_{0.05\,[6,27]}$ = 4.13, AV = 3.85, MSD = 2.72) and as percent saturation ($Q_{0.05\,[6,27]}$ = 4.13, AV = 36.4, MSD = 34.2) between PGC 3 and WPC 3.

Data collected from other studies conducted simultaneously with this project support the hypothesis regarding increased primary productivity is a reflection of increased nutrient loading within Pleasant Grove Creek. Lebkuecher (2008; unpublished report) demonstrated that a very large standing crop of benthic photoautotrophs is present within Pleasant Grove Creek. Autotrophic Index values calculated from periphyton samples taken from PGC 2 and WPC 2 suggest that these reaches exhibited poor water quality (Lebkuecher, 2008, unpublished report). Eaton et al., (2005) state that the Autotrophic Index (AI) allows one to determine the trophic nature of the periphyton community. Values of the AI can range from 50 to 200. Values at the upper end of this range denote heterotrophic associations, which indicate poor water quality. Lebkeucher (2008; unpublished report) reported that AI values from PGC 2 (Range =135 to 236) and values from WPC 2 (Range =140 to 261) indicate that both of these stream reaches exhibit poor water quality.

The existence of poor water quality has ultimately caused a reduction in the KIBI scores of sampled stream reaches within Pleasant Grove Creek. Due to environmental perturbations, such as low concentrations of DO, the relative abundance of intolerant fish species will decrease in samples taken from stream reaches exhibiting poor water quality (Compton et al. 2003). Mean DO levels recorded within Pleasant Grove Creek provide an explanation as to why KIBI scores were low, especially in PGC 3 (mean IBI = 24). The IBI scores, such as those observed at PGC 3 (Appendix II), strongly indicate that this reach is experiencing diminished fish abundance as a direct result of insufficient environmental conditions. When compared to all other stream reaches within Pleasant Grove and Whippoorwill Creeks, mean KIBI scores for the Intolerant Species Richness metric (INT; Appendix II) was lowest at PGC 3 ($\bar{x}$ INT score = 29.70). Mean KIBI scores for the INT metric score was highest at WPC 2 ($\bar{x}$ INT score = 51.06). Mean INT metric scores for PGC 3 and WPC 2 reflect the degree of habitat quality available for fish within these stream reaches. The threatened habitat quality (Habitat Assessment Score = 141; Table 10) reflected in the mean INT metric score of PGC 3 (29.70) and the fully supporting habitat quality (Habitat Assessment Score = 164; Table 10) indicated by the mean INT metric scores of WPC 2 (51.06) may provide an explanation for why the mean number of fish collected was significantly greater at WPC 2.

The Kentucky Index of Biotic Integrity and Using Fish as Indicators of Water Quality

The total number of fish collected and the various fish species collected during this study may be able to provide reasonable explanations for the low IBI scores calculated from Pleasant Grove Creek samples. The pronounced difference in the total number of fish collected within Pleasant Grove and Whippoorwill Creeks could be a product of the difference in watershed size (Wang et al., 2003; Smith and Kraft, 2005). Our data suggest that other environmental factors are adding to the low number of fish observed to be in Pleasant Grove Creek (Figure 14). The Relative Abundance of Tolerant Individuals (%TOL) metric scores calculated from data collected at stream reaches within Pleasant Grove Creek demonstrate that this stream is experiencing some degree of degradation (Appendix II). Compton et al. (2003) states that the %TOL metric is the only metric positively correlated with increasing stream impairment.

The significant difference that exists between the mean number of fish collected at PGC 3 and WPC 2 is reflected in the mean %TOL metric scores of these stream reaches. The mean %TOL score of PGC 2 (27.84) is the lowest mean %TOL score calculated for this study. The highest mean %TOL metric score (82.38) was calculated from fish samples collected at WPC 2. An inverse relationship exists between %TOL metric scores and the relative abundance of tolerant individuals collected from a stream reach (Compton et al., 2003). As stated in the previous paragraph, the %TOL metric score ultimately reflects the habitat quality of a stream reach. The mean %TOL metric scores calculated from sampling events at PGC 3 and WPC 2 further suggest that PGC 3 exhibits degraded habitat quality due to land use practices within the Pleasant Grove Creek watershed.

Fish species collected within Pleasant Grove Creek that have been described by Compton et al. (2003) to be tolerant individuals include: the striped shiner (*Luxilus chrysocephalus*),the bluntnose minnow (*Pimephales notatus*),the creek chub (*Semotilis atromaculatus*), and the western mosquitofish (*Gambusia affinis*). These species are common inhabitants of small streams such as Pleasant Grove Creek (Etnier and Starnes, 1993). Previous studies have demonstrated that these fish species were able to survive degraded environmental conditions such as decreased levels of dissolved oxygen (*S. atromaculatus* and *G. affinis;* Starrett, 1950; Schweizer and Matlock, 2005) and siltation (*L. chrysocephalus, P. notatus,* and *S. atromaculatus*; Schweizer and Matlock 2005; Duehr et al., 2006; and Branson et al., 1974).

Perhaps one observation that suggests why these fish species to survive in harsh conditions when other species cannot is their ability to exploit multiple food resources, in particular, prey taken at the water's surface (Kraatz, 1928; Barkinol, 1941; and Lotrich, 1973). Studies have demonstrated that the diet of *Luxilus chrysocephalus* is composed primarily of terrestrial invertebrates (coleopterans and dipterans), filamentous and unicellular algae, and ephemeropteran nymphs (Lotrich, 1973; Etnier and Starnes, 1993). *Pimephales notatus* usually forages on detritus and various midge larvae, but is also known to supplement its diet with surface dwelling insects and plankton (Kraatz, 1928; Moyle, 1973; Boschung et al. 2003). The diet of

S. atromaculatus is mainly composed of small fish, crayfish, and other large invertebrates (Etnier and Starnes, 1993); however, use of terrestrial insects as food resources occurs as well (Lotrich, 1973). Barkinol (1941) showed that *G. affinis* fed primarily on terrestrial insects, aquatic larvae, microcrustaceans, small snails, and larval fishes, including their own young.

The abundance of the scarlet shiner (*Lythrurus fasciolaris*) individuals at all stream reaches within Pleasant Grove and Whippoorwill creeks (Table 8) may be explained by using its diet composition, but its preference in habitat could also give some insight as to why this species was so numerous within samples from both streams. This species is an extremely common inhabitant of small to medium streams. Adults of this species primarily feed in mid-water areas within the water column and from the water's surface (Etnier and Starnes, 1993). Studies have demonstrated that terrestrial insects and algae comprise most of its diet (Surat et al., 1982). Conversely, this species has been reported to decrease with increasing levels of siltation (Lotrich, 1973). The relative abundance of this species did decrease as samples were taken further upstream in Pleasant Grove Creek; yet, the relative abundance of *L. fasciolaris* between sample reaches within Whippoorwill Creek showed little variation (Table 8). This observation, including the presence of the tolerant individuals discussed previously, suggest that Pleasant Grove Creek is experiencing some degree of increased sedimentation.

Additional data providing evidence that Pleasant Grove Creek is suffering from affects of adjacent land uses are the IBI scores calculated for the following metrics: Darter, Madtom, and Sculpin Species Richness (DMS) and Simple

Lithophilic Spawning Species Richness (SL). Mean scores of these metrics suggest that Pleasant Grove Creek exhibits poor water quality (Appendix II). The environmental factor that has an influence on these metrics is increased or persistent sedimentation (Ohio Environmental Protection Agency, 1987; Compton et al., 2003; Corrao and Roberts, 2003). Mean scores for DMS and SL metrics, along with mean scores for the Abundance of Native Species (NAT), indicate that all reaches sampled within Pleasant Grove and Whippoorwill creeks exhibited poor water quality (Appendix II). These results support our hypothesis that the land use practices within these watersheds are diminishing the environmental condition of surface waters flowing through these karst regions.

Such low IBI scores were not expected for Whippoorwill Creek stream reaches. This study suggests that the water quality within Whippoorwill Creek, a Kentucky ecoregion reference stream, is also influenced by the agricultural activities within its watershed. Although fish abundance was greater within stream reaches of Whippoorwill Creek (Figure 14), the mean KIBI score (43) for this creek was only Fair for streams within the Pennyroyal Ichthyoregion (Figure 13).

As reflected by its low IBI scores, PGC 3 is perhaps the most impacted stream reach sampled throughout this study (Appendix II). Fish species within this reach belonging to the INT and SL metrics were extremely rare. The Tennessee snubnose darter (*Etheostoma simoterum*) was a common species that was collected at all sampled reaches of Pleasant Grove and Whippoorwill creeks except for the sample reach PGC 3. This percid species is known to be intolerant of habitat degradation and is listed as a simple lithophilic spawner (Compton et al., 2003). Due to their strict habitat requirements and the loss of the gas bladder (Etnier and Starnes 1993), darter species that are simple lithophilic spawners are especially vulnerable to environmental change (Page, 1983). The absence of this species and the low abundance of fish collected at PGC 3 suggest that this portion of Pleasant Grove Creek is severely impacted.

Not all fish species that are known to inhabit benthic environments seemed to decrease so rapidly in relative abundance within Pleasant Grove Creek as did the previous example. The high relative abundance of the banded sculpin (*Cottus carolinae*) and the spottail darter (*Etheostoma squamiceps*) collected at stream reaches within Pleasant Grove Creek could be attributed to their reproductive behavior. Compton et al. (2003) does not recognize these benthic species as simple lithophilic spawners, meaning that they do not broadcast their eggs over the stream substrate. Parental care is not involved in simple lithophilic spawning (Shaner, 1999); as a result, eggs deposited by fish belonging to this reproductive guild are more vulnerable to environmental changes and to predation. These impacts will reduce the abundance of these individuals in a given stream reach. Benthic individuals, such as *C. carolinae* males and *E. squamicpes* males, are known to guard their nest sites; thus, increasing the probability that offspring of these species will survive. Furthermore, *E. squamiceps* belongs to the subgenus *Catonotus*. Male individuals within this subgenus have been reported to keep nests clean by sweeping away silt or debris with their dorsal fins or removing foreign material from the nest with their mouth (Etnier and Starnes, 1993). Poly and Wilson (1998) suggested that because of the reproductive strategies described above, members of *Catonotus* were able to curtail the impacts of increased sedimentation in the Cache River basin in southern Illinois. Thus, the relatively high abundance of *C. carolinae* and *E. squamiceps* in PGC 2 and PGC 3 could be a result of little competition for habitat and food resources due to the decrease of more intolerant benthic species (Table 8).

The species composition of fish samples collected within Pleasant Grove and Whippoorwill creeks and the IBI scores calculated during this study suggest that both streams are experiencing some degree of degradation because of the land use practices within these watersheds. When IBI scores are compared, data suggests that the degree of impairment is greater within Pleasant Grove Creek (Figure 19). An increase in the relative abundance of tolerant individuals within PGC 3 coupled with a decrease in the relative abundance of intolerant and simple lithophilic species within PGC 3 suggests that this region and stream reach of Pleasant Grove Creek is experiencing greater water quality impacts from the land use activities within this watershed.

The Correlation Between Riparian Zone Width, Riparian Zone Area, and Abundance of Fish

The intention of this portion of my study was to determine if fish density correlating with adjacent riparian zone area is true in streams flowing through karst environments. My study demonstrated that correlation coefficients used to evaluate adjacent riparian zone widths and areas showed little variation (Figures 16 and 17). Results suggest that riparian zone area could be considered a viable alternative to using riparian zone width when assessing stream corridors within a karst agricultural watershed. Because altered stream corridors used during this study do not have uniform riparian zone widths (Figures 6 and 7), riparian zone area should be taken into account when performing habitat assessments in similar streams with variable riparian buffer habitats. Consequently, due to the small scope of this study in regards to the number of streams assessed and because of the small sample size for stream reaches per stream, the data suggests that more research be done in karst regions to further examine this hypothesis.

Although my results demonstrated that riparian zone area did correlate with fish density within Pleasant Grove and Whippoorwill creeks (r =0.528), this correlation does not suggest that the overall biological integrity in these streams is adequate (Figure 19). The Kentucky Division of Water (2008b) recommends that a given stream reach have a riparian zone buffer of at least an 18 m width along each stream bank. This equates to 36 m of total riparian buffer width along a stream reach. A 36 x100 m riparian zone equates to 0.36 hectares. Stream reaches used during this study had riparian zone areas exceeding 0.36 hectares (Appendix IV); although, mean KIBI scores of all stream reaches within Pleasant Grove Creek suggest that the biotic integrity within this stream is marginal (Table 11). In some instances, such as PGC 3, poor biotic integrity is exhibited. Furthermore, WPC 2 has a riparian zone area three fold greater (1.22 hectares) than the suggested optimal riparian zone area of 0.36 hectares for a 100 m stretch; yet, its KIBI score is categorized as Fair (Table 11). My data supports other studies that have suggested that riparian zone characteristics do have an indirect connection to fish communities (Roth et al. 1996; Jones III et al., 1999; Stauffer et al., 2000; Wang et al., 2003); but, it should be noted that riparian zone area alone is not a relevant indicator of water quality within

Pleasant Grove and Whippoorwill creeks.

Conclusions

When comparing IBI scores calculated from Sampson (1995) and McMurray (1999) with KIBI scores calculated from sampling events that occurred during this study, the biotic integrity of Pleasant Grove Creek has improved (Figure 19). Nonetheless, the species composition of fish samples and other abiotic data collected from Pleasant Grove Creek indicate that areas of this stream continue to be impacted by environmental perturbations, such as extreme fluctuations in dissolved oxygen and increased sedimentation. The IBI scores calculated from fish samples collected from Whippoorwill Creek suggest that this stream is experiencing some degree of degradation as well (Table 11).

Although an index of biotic integrity cannot pinpoint the exact perturbations that are occurring within an impacted stream, it can provide a measureable account of the overall degradation taking place. Streams such as Pleasant Grove and Whippoorwill creeks seem to be heavily influenced by the land use practices occurring within their karst watersheds. The level of agricultural activity occurring within these watersheds along with the extensive karst topography through which these streams flow is creating an adverse environment for fish assemblages within Pleasant Grove and Whippoorwill creeks.

The BMPs established during the mid-1990s have had little impact, if any, on the biotic integrity of Pleasant Grove Creek. Although this study demonstrated that riparian buffers correlate with fish assemblages within Pleasant Grove and Whippoorwill creeks, we do not believe the management of riparian buffers alone will improve the water quality of these two streams. The importance of riparian buffers along stream corridors has been a well researched subject (Naiman et al., 2005). Additionally, Lowrance et al. (1995) documented that the presence of woody and herbaceous plants within a 20 m wide (Area = 0.040 hectares for 20 x 100 m stream reach) riparian zone can substantially reduce the amount of sediment (89.8%), nitrogen (74.3%), and phosphorus (70.0%) that would have otherwise entered into surface waters in the Chesapeake Bay Catchment. The influence of herbaceous or woody plants on runoff laden with sediment and nutrients was less when just one of these types of vegetative buffer was present (Lowrance et al., 1995). The value of riparian canopy cover in regards to the addition of energy subsidies and the regulation of temperature regimes has also been well researched (Naiman et al., 2005). The riparian zone areas of stream reaches used to assess the riparian habitat along Pleasant Grove and Whippoorwill creeks were variable (Area Range = 0.48 to 1.22 hectares) and exhibited evidence of nutrient loading and increased amounts of sediment deposition. Consequently, the areas and widths of riparian zones associated with stream reaches used in our study exhibit similar physical parameters to those in the Chesapeake Bay study. Therefore, the presence of an extensive ground and surface water interchange in this karst region (Currens, 2002) must strongly influence the overall water quality of Pleasant Grove and Whippoorwill creeks. In order to conserve the biotic integrity of Pleasant Grove and Whippoorwill creeks, proper land use conservation practices should be a priority in this region. In addition to a continued effort to implement riparian buffer management strategies, other land conservation practices should be utilized in karst regions where agriculture is the dominate land use.

LITERATURE CITED

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Andreen, W.L. 2003a. The evolution of water pollution control in the United States-state, local, and federal efforts, 1789-1972: part I. Stanford Environmental Law Journal 22:145-200.

Andreen, W.L. 2003b. The evolution of water pollution control in the United States-state, local, and federal efforts, 1789-1972: part II. Stanford Environmental Law Journal 22:215-294.

Angermeier, P.L., R.A Smogor, and J.R. Stauffer. 2000. Regional frameworks and candidate metrics for assessing biotic integrity in the Mid-Atlantic Highland streams. Transactions of the American Fisheries Society 129:962-981.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water,Washington, D.C.

Barkinol, PG. 1941. Food habits of *Gambusia affinis* from Reelfoot Lake, Tennessee, with special reference to malarial control. Report from Reelfoot Biological Station. Journal of Tennessee Academy of Science 5:5-13.

Barrass, A. N., M.J. Wilson, G.S. Ridenour, J.L. Lowers, and G. Seavey. 2008. Watershed management plan lower Pleasant Grove creek, Logan County, Kentucky. Center for Field Biology. Austin Peay State University. Clarksville, Tennessee.

Berryhill Jr., W.S. 1989. The impact of agricultural practices on water quality in karst regions. Proceedings of the Third Multidisciplinary Conference on Sinkholes and the Engineering Environmental Impacts of Karst 1:159-164.

Bosch, D.J., Z.L. Cook, and K.O. Fuglie. 1995. Voluntary versus mandatory agricultural policies to protect water quality: adoption of nitrogen testing in Nebraska. Review of Agricultural Economics 17:13-24.

Boschung, Jr., H.T. and R.L. Mayden. 2003. Fishes of Alabama. Smithsonian Institution. Washington, D.C.

Branson, B.A. and D.L. Batch. 1974. Additional observations on the effects of strip mining on small-stream fishes in east-central Kentucky. Transactions of the Kentucky Academy of Science 35:81-82.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H.Smith. 1998. Nonpoint pollution of surface waters with nitrogen and phosphorous. Ecological Applications 8:559-568.

Compton, M.C., G.J. Pond, and J.F. Brumley. 2003. Development and Application of the Kentucky Index of Biotic Integrity (KIBI). Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Copeland, C. 2006. CRS report RL33466, water quality: implementing the clean water act. Congressional Research Service, Washington, D.C.

Corrao, J. and J. Roberts. 2003. Use of an index of biotic integrity to assess the condition of Stroubles Creek. Stream Concerns Committee. Virginia Tech Chapter of the American Fisheries Society, pp 1-7.

Crawford, N.C. 1988. Karst hydrologic problems of South Central Kentucky: ground water contamination, sinkhole flooding, and sinkhole collapse. Field Trip Guide Book, Second Conference on Environmental Problems in Karst Terranes and Their Solutions, November 16-18, 1988, Nashville, Tennessee.

Currens, J.C. 2002. Changes in groundwater quality in a conduit-flow-dominated karst aquifer, following BMP implementation. Environmental Geology 42:525-531.

Duehr, J.P., J.P. Siepker, C.L. Pierce, and T.M. Isenhart. 2006. Relation of riparian buffer strips to in-stream habitat, macroinvertebrates and fish in a small Iowa stream. Journal of the Iowa Academy of Science 113:49-55.

Dunn, O.J. 1946. Multiple comparisons using rank sums. Technometrics 6:241-252.

Eaton, A.D., L.S. Clesceri, E.W. Rice, and A.E. Greenberg (Eds.). 2005. *Standard Methods for the Examination of Water and Wastewater, 21st Edition.* American Public Health Association. Washington, D.C.

Etnier, D.A., and W.C. Starnes. 1993. *The Fishes of Tennessee.* The University of Tennessee Press. Knoxville, Tennessee.

Fausch, K.D., J.R. Karr, and P.R. Yant. 1984. Regional application of an index of biotic integrity based on stream fish communities. Transactions of the American Fisheries Society 113:39-55.

Hall, R.I., P.R. Leavitt, R. Quinlan, A.S. Dixit, and J.P. Smol. 1999. Effects of agriculture, urbanization, and climate on water quality in the Northern Great Plains. Limnology and Oceanography 44:739-756.

Harding, J.S., E.F. Benfield, P.V. Bolstad, G.S. Helfman, and E.B.D. Jones III. 1998. Stream Biodiversity: the ghost of land use past. Proceedings from the National Academy of Sciences of the United States of America 95:14843-14847.

Helms, B.S. 2008. Response of aquatic biota to changing land use pattern in streams of west Georgia, USA. Ph.D. Thesis, Auburn University, Auburn, Alabama.

Hill, L.G., W.J. Matthews, and G.D. Schnell. 1978. Locomotor reactions of two cyprinodontid fishes to differences in dissolved oxygen concentrations. The Southwestern Naturalist 23:397-400.

Hubbs, C., R.C. Baird, and J.W. Gerald. 1967. Effects of dissolved oxygen concentration and light intensity on activity cycles of fishes inhabiting warm springs. American Midland Naturalist 77:104-115.

Johnson, D.D., J.M. Kreglow, S.A. Dressing, R.P. Maas, F.A. Koeler, F.J. Humenik, W.K. Snyder, and L. Christensen. 1982a. Best management practices for agricultural nonpoint source control. II. commercial fertilizer. North Carolina Agriculture Extension Service, Biological and Agricultural Engineering Department, North Carolina State University, Raleigh, North Carolina.

Johnson, D.D., J.M. Kreglow, S.A. Dressing, R.P. Maas, F.A. Koeler, F.J. Humenik, W.K. Snyder, and L. Christensen. 1982b. Best management practices for agricultural nonpoint source control. III. sediment. North Carolina Agriculture Extension Service, Biological and Agricultural Engineering Department, North Carolina State University, Raleigh, North Carolina.

Jones III, E.B.D., G.S. Helfman, J.O. Harper, and P.V. Bolstad. 1999. Effects of riparian forest removal on fish assemblages in southern Appalachian streams. Conservation Biology 13:1454-1465.

Lotrich, V.A. 1973. Growth, production, and community composition of fishes inhabiting a first-, second-, and third-order stream of eastern Kentucky. Ecological Monographs 43:377-397.

Kalff, J. 2002. *Limnology*. Prentice-Hall, Inc. Upper Saddle River, New Jersey.

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Karr, J.R. 1990. Biological integrity and the goal of environmental legislation: lessons for conservation biology. Conservation Biology 4:244-250.

Karr, J.R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1:66-84.

Karr, J.R. and D. R. Dudely. 1981. Ecological perspective on water quality goals. Environmental Management 5:55-68.

Karr, J.R., L.A. Toth, D.R. Dudely. 1985. Fish communities of midwestern rivers: a history of degradation. Bioscience 35:90-95.

Kentucky Division of Water. 2003. 2002 303(d) list of waters for Kentucky. Frankfort, Kentucky.

Kentucky Division of Water. 2008a. Integrated report to Congress on the condition of water resources in Kentucky volume II. 303(d) list of surface waters. Frankfort, Kentucky.

Kentucky Division of Water. 2008b. Methods for assessing biological integrity of surface waters in Kentucky. Frankfort, Kentucky.

Kentucky Division of Water. 2008c. Special use waters. http://nrepcapps.ky.gov/special_waters/specialwaters.htm. Accessed 25 July 2008.

Kentucky Division of Water. 2009. Kentucky Administrative Regulations Title 401: Chapter 10, Section 31: Water quality standards. http://www.lrc.ky.gov/kar/401/010/031.htm. Accessed 3 July 2009.

Kraatz, W.C. 1928. Study of the food of the blunt-nosed minnow, *Pimephales notatus*. The Ohio Journal of Science 28:86-98.

Lebkuecher, J.G., T.D. Neville, K.L. Wallace, and L.F. Barber. 1998. Primary production in sandy-bottom streams of the West Sandy Creek watershed of Tennessee. Castanea 63:130-137.

Lebkuecher, J.G. 2008. Measurements of primary production in Pleasant Grove and Whippoorwill Creeks. Unpublished manuscript, Austin Peay State University, Clarksville, Tennessee.

Lowrance, R., L.S. Altier, J.D. Newbold, R.R. Schnabel, P.M. Groffman, J.M. Denver, D.L. Correll, J.W. Gilliam, J.L. Robinson, R.B. Brinsfield, K.W. Staver, W. Lucas, and A.H. Todd. Water quality functions of riparian forest buffer systems in the Chesapeake Bay watershed. United States Chesapeake Bay Program. Annapolis, Maryland.

Marzolf, E.R., P.J. Mulholland, and A.D. Steinman 1994. Improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Canadian Journal of Fisheries and Aquatic Sciences 51:1591-1599.

Maas, R.P., S.A. Dressing, J. Spooner, M.D. Smolen, and F.J. Humenik. 1984. Best management practices for agricultural nonpoint source control. IV. pesticides. North Carolina Agriculture Extension Service, Biological and Agricultural Engineering Department, North Carolina State University, Raleigh, North Carolina.

McCollum, D.S. 2004. Landscape and local influences on the biotic integrity of fish communities in Ohio headwater streams. Ph.D. Thesis, Miami University, Miami, Ohio.

McMurray, S.E. 1999. Post-BMP biological survey of Pleasant Grove Spring, Logan County, Kentucky. Kentucky Division of Water, Water Quality Branch, Nonpoint Source Section, Frankfort, Kentucky.

Miller, D.L., P.M. Leonard, R. M. Hughes, J.R. Karr, P.B. Moyle, L.H. Shrader, B.A. ThompsonR.A. Daniels, K.D. Fausch, G.A. Fitshugh, J.R. Gammon, D.B. Haliwell, P.L. Angermeier, and D.J. Orth. 1988. Regional applications of an index of biotic integrity for use in water resource management. Fisheries 13:12-20.

Miller, R.R., J.D. Williams, and J.E. Williams. 1989. Extinctions of North American fishes during the past century. Fisheries 14:22-36.

Moyle, P.B. 1973. Ecological segregation among three species of minnows (Cyprinidae) in a Minnesota lake. Transactions of American Fisheries Society (1900) 102:794-805.

Murchison, K.M. 2005. Learning from more than five-and-a-half decades of federal water pollution control legislation: twenty lessons for the future. Boston College Environmental Affairs Law Review 32:527-598.

Mulholland, P.J., J.N. Houser, and K.O. Maloney. 2005. Stream diurnal dissolved oxygen profiles as indicators of in-stream metabolism and disturbance effects: Fort Benning as a case study. Ecological Indicators 5:243-252.

Naiman, R.J., and H. Decamps. 1997. The ecology of interfaces: riparian zones. Annual Review of Ecology and Systematics 28:621-658.

Naiman, R.J., H. Decamps, and M.E. McClain. 2005. *Riparia: Ecology, Conservation, and Management of Streamside Communities*. Elsevier Academic Press. Burlington, Maine.

Ohio Environmental Protection Agency. 1987. Biological criteria for the protection of aquatic life: Volume II: Biological field assessment of Ohio Surface waters. Volume III: Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Division of Water Quality Monitoring and Assessment, Columbus, Ohio.

Ostrand, K.G. 2000. Abiotic determinants of fish assemblage structure in the upper Brazos River, Texas. Ph.D. Thesis, Texas Tech University.

Page, L.M. and B.M. Burr. 1991. *The Peterson Field Guide Series: A Field Guide to Freshwater Fishes*. Houghton Mifflin Company. New York, New York.

Pollock, M.S., L.M.J. Clark, and M.G. Dube. 2007. The effects of hypoxia on fishes: from ecological relevance to physiological effects. Environmental Reviews 15:1-14.

Poly W.J. and A.K. Wilson. 1998. The Fringed Darter, *Etheostoma crossopterum*, in the Cache River basin of southern Illinois (Percidae: Subgenus *Catonotus*). Ohio Journal of Science 98:6-9.

Poon, W.L., C.Y. Hung, and D.J. Randall. 2002. The effects of aquatic hypoxia on fish. In: Fish physiology, toxicology, and water quality: Proceedings of the sixth international symposium, La Paz B.C.S. Mexico, January 22-26, 2001. United States Environmental Protection Agency. EPA 600-R-02-097. Washington D.C.

Red River Watershed Association. 2008. Watershed information. http://www.redriverwatershed.org/Red%20River%20Info.htm. Accessed 22 July 2008.

Ridenour, G. S., 2006. Nutrient loading in the Red River watershed. TN Department of Environment and Conservation. Report 2006.

Roth, N.E., J.D. Allen, and D.L. Erickson. 1996. Landscape influences on stream biotic integrity assessed at multiple spatial scales. Landscape Ecology 11:141-156.

Ryan, M. and J. Meiman. 1996. An examination of short-term variations in water quality at a karst spring in Kentucky. Ground Water 34:23-30.

Sampson, B. 1995. Biological survey of Pleasant Grove Spring, Logan County, Kentucky. Kentucky Division of Water, Water Quality Branch, Nonpoint Source Section. Frankfort, Kentucky.

Schweizer, P.E. and G.R. Matlock. 2005. Annual variation in fish assemblages of watersheds with stable and changing land use. American Midland Naturalist 153: 293-308.

Shaner, B.L. 1999. Development of a standardized index of biotic integrity for the Piedmont region of Georgia. Proceedings of the 1999 Georgia Water Resources Conference, March 30-31, 1999. The University of Georgia, Athens, Georgia.

Simon, T.P. 1991. Development of index of biotic integrity expectations for the ecoregions of Indiana. I. central corn belt plain. EPA-905/9-91/025. Environmental Protection Agency, Environmental Science Division, Monitoring and Quality Assurance Branch, Ambient Monitoring Section, Chicago, Illinois.

Slack, K.V. 1971. Average dissolved oxygen: Measurement and water quality significance. Journal (Water Pollution Control Federation) 43:433-446.

Smith, T.A. and C.E. Kraft. 2005. Stream fish assemblages in relation to landscape position and local habitat variables. Transactions of the American Fisheries Society 134:430-440.

Starrett, W.C. 1950. Distribution of the fishes of Boone County, Iowa, with special reference to the minnows and darters. American Midland Naturalist 43:112-127.

Stauffer, J.C., R. M. Goldstein, and R. M. Newman. 2000. Relationship of wooded riparian zones and runoff potential to fish community composition in agricultural streams. Canadian Journal of Fisheries and Aquatic Sciences 57:307-316.

Surat, E.M., W.J. Matthews, and J.R. Bek. 1982. Comparative ecology of *Notropis albeolus*, *N. ardens*, and *N. cerasinus* (Cyprinidae) in the upper Roanoke River drainage. American Midland Naturalist 107:13-24.

Sweeney, B.W., T.L. Bott, J.K. Jackson, L.A. Kaplan, J.D. Newbold, L.J. Standley, W.C. Hession, and R.J. Horwitz. 2004. Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proceedings of the National Academy of Sciences of the United States of America 101:14132 - 14137.

Tennessee Department of Environment and Conservation. 2007. Red River watershed (05130206) of the Cumberland River basin watershed: water quality management plan. Nashville, Tennessee.

United State Department of Agriculture. 1983. Water quality field guide. Soil Conservation Service. SCS-TP-160. Washington, D.C.

United States Environmental Protection Agency. 1989. Nonpoint sources: agenda for the future. EPA 841-S-89-006. Washington, D.C.

United States Environmental Protection Agency. 2002. Fish physiology, toxicology, and water quality: Proceedings of the sixth international symposium, La Paz B.C.S. Mexico, January 22-26, 2001. EPA 600-R-02-097. Washington D.C.

United States Environmental Protection Agency. 2003. National management measures for the control of nonpoint source pollution from agriculture. EPA 841-B-03-004. Washington, D.C.

United States Environmental Protection Agency. 2005. National management measures to protect and restore wetlands and restore riparian areas for the abatement of nonpoint source pollution. EPA 841-B-05-003. Washington, D.C.

United States Environmental Protection Agency. 2007. An introduction to the index of biotic integrity. http://www.epa.gov/bioindicators/html/ibi-hist.html. Accessed 5 January 2008.

United States Geological Survey. 2007. United States Geological Survey ground water information. http://water.usgs.gov/ogw/karst/. Accessed 5 January 2008.

Wang, L., J. Lyons, P. Rasmussen, P. Seelbach, T. Simon, M. Wiley, P. Kanehl, E. Baker, S. Niemela, and P.M. Stewart. 2003. Watershed, reach, and riparian influences on stream fish assemblages in the Northern Lakes and Forest ecoregion, U.S.A. Canadian Journal of Fisheries and Aquatic Sciences 60:491-505.

Williamson, A.K., M.D. Munn, S.J. Ryker, R.J. Wagner, J.C. Ebbert, and A.M. Vanderpool. 1998. Water quality in the Central Columbia Plateau, Washington and Idaho, 1992-1995. United States Geological Survey Circular 1144. Denver, Colorado.

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

Zar, J.H. 2009. *Biostatistical Analysis*, 5th Edition. Pearson Prentice Hall. Upper Saddle River, New Jersey.

Zhou, B.S. 2001. Effects of hypoxia on metabolism, bioenergetics, growth, and reproduction of the common carp (*Cyprinus carpio* L.). Ph.D. Thesis, City University of Hong Kong, Hong Kong.

APPENDICES

Appendix I. Latitude/Longitude coordinates of sample reaches within the Pleasant Grove and Whippoorwill subwatersheds.

Site	Latitude	Longitude
PGC1	36.696839	-86.918533
PGC2	36.702300	-85.915525
PGC3	36.707833	-86.907656
WPC1	36.732636	-86.961181
WPC2	36.729611	-86.984228
WPC3	36.755739	-86.983619

Appendix II. KIBI metric scores calculated from 2007-2009 sampling events within Pleasant Grove (PGC) and Whippoorwill creeks (WPC).

		KIBI Metric Scores					
Site	**Date**	NAT	DMS	INT	SL	%INSCT	%TOL
	Oct-07	12.62	23.25	27.84	17.95	50.00	50.00
	Nov-07	26.80	55.51	53.81	25.95	50.00	50.00
	Mar-08	19.71	12.50	27.84	25.95	0.00	0.00
PGC1	May-08	33.90	55.51	53.81	25.95	0.00	0.00
	Sep-08	30.35	44.76	53.81	33.95	100.00	99.30
	Oct-08	33.90	44.76	66.80	41.95	100.00	96.15
	May-09	37.44	55.51	66.80	41.95	50.00	50.00
Mean		**27.82**	**41.68**	**50.10**	**30.52**	**50.00**	**49.35**
	May-08	30.35	44.76	40.83	33.95	0.00	0.00
	Sep-08	30.35	44.76	66.80	41.95	80.89	97.04
PGC 2	Oct-08	33.90	44.76	53.81	41.95	50.00	50.00
	May-09	23.26	34.00	27.84	17.95	0.00	0.00
Mean		**29.46**	**42.07**	**47.32**	**33.95**	**32.72**	**36.76**
	Oct-07	12.62	12.50	14.85	1.95	0.00	0.00
	Nov-07	16.17	12.50	14.85	9.95	0.00	0.00
	Mar-08	16.17	12.50	14.85	9.95	0.00	0.00
PGC 3	May-08	16.17	12.50	14.85	1.95	0.00	0.00
	Sep-08	33.90	44.76	53.81	33.95	91.37	97.43
	Oct-08	37.44	55.51	66.80	49.95	78.62	97.43
	May-09	19.71	23.25	27.84	9.95	0.00	0.00
Mean		**21.74**	**24.79**	**29.70**	**16.81**	**24.28**	**27.84**

Appendix II continued.

Site	Date	KIBI Metric Scores NAT	DMS	INT	SL	%INSCT	%TOL
	May-08	4.26	27.87	22.49	23.26	50.00	50.00
	Sep-08	14.90	6.37	22.49	15.26	100.00	91.15
WPC 1	Oct-08	29.08	49.38	61.45	31.26	100.00	91.82
	May-09	4.26	6.37	22.49	7.26	93.10	83.47
Mean		**13.13**	**22.50**	**32.23**	**19.26**	**85.78**	**79.11**
	Oct-07	0.00	0.00	0.00	0.00	100.00	93.16
	Nov-07	0.72	6.37	9.50	0.00	100.00	92.69
	Mar-08	14.90	38.63	61.45	23.26	100.00	90.59
WPC 2	May-08	11.35	27.87	35.48	15.26	96.60	85.64
	Sep-08	18.45	27.87	48.46	23.26	100.00	92.83
	Oct-08	25.54	49.38	61.45	15.26	100.00	92.86
	May-09	21.99	17.12	48.46	15.26	50.00	50.00
Mean		**18.45**	**32.17**	**51.06**	**18.46**	**89.32**	**82.38**
	May-08	4.26	6.37	22.49	15.26	50.00	50.00
	Sep-08	25.54	17.12	35.48	23.26	100.00	91.78
WPC 3	Oct-08	29.08	49.38	61.45	31.26	93.79	83.70
	May-09	14.90	17.12	35.48	23.26	84.56	77.00
Mean		**18.45**	**22.50**	**38.72**	**23.26**	**82.09**	**75.62**

Appendix III. Abiotic data collected from 2007-2009 sampling events within Pleasant Grove and Whippoorwill creeks.

Table A. Dissolved oxygen (mg/L) samples collected from stream reaches of Pleasant Grove (PGC) and Whippoorwill creeks (WPC) during 2007-2009.

	Site					
Date	PGC 1	PGC 2	PGC 3	WPC 1	WPC 2	WPC 3
Oct-07	6.76	**	11.62	**	8.16	**
Nov-07	9.77	**	11.21	**	11.24	**
Mar-08	9.52	**	9.57	**	11.64	**
May-08	8.93	11.57	8.83	9.43	9.03	8.99
Sep-08	8.72	9.22	13.78	7.98	9.74	6.96
Oct-08	10.22	10.30	12.04	8.55	5.21	4.23
May-09	9.03	9.16	9.52	8.39	8.88	8.18

**No sampling event occurred.

Table B. Dissolved oxygen (%Saturation) samples collected from stream reaches of Pleasant Grove (PGC) and Whippoorwill creeks (WPC) during 2007-2009.

	Site					
Date	PGC 1	PGC 2	PGC 3	WPC 1	WPC 2	WPC 3
Oct-07	68.0	**	116.8	**	87.4	**
Nov-07	88.9	**	108.6	**	102.4	**
Mar-08	90.7	**	91.7	**	109.3	**
May-08	88.6	142.5	87.9	96.6	90.7	90.5
Sep-08	86.4	94.4	142.7	86.1	107.7	74.5
Oct-08	99.8	98.4	115.3	81.2	50.5	38.3
May-09	90.6	92.0	96.8	87.2	94.1	85.3

**No sampling event occurred.

Appendix III continued.

Table C. pH samples collected from stream reaches of Pleasant Grove (PGC) and Whippoorwill creeks (WPC) during 2007-2009.

	Site					
Date	PGC 1	PGC 2	PGC 3	WPC 1	WPC 2	WPC 3
Oct-07	7.16	**	8.11	**	7.80	**
Nov-07	7.72	**	7.94	**	8.04	**
Mar-08	6.63	**	7.20	**	7.68	**
May-08	7.19	7.92	7.17	7.45	7.54	7.07
Sep-08	7.39	7.52	7.42	7.30	7.51	7.43
Oct-08	7.96	7.87	8.01	7.62	7.94	7.55
May-09	7.61	7.55	7.53	7.61	7.67	7.58

**No sampling event occurred.

Table D. Specific conductance (mS/cm) samples collected from stream reaches of Pleasant Grove (PGC) and Whippoorwill creeks (WPC) during 2007-2009.

	Site					
Date	PGC 1	PGC 2	PGC 3	WPC 1	WPC 2	WPC 3
Oct-07	**	**	**	**	**	**
Nov-07	**	**	**	**	**	**
Mar-08	0.376	**	0.373	**	0.359	**
May-08	0.379	0.422	0.376	0.352	0.360	0.341
Sep-08	0.486	0.474	0.468	0.470	0.462	0.406
Oct-08	0.496	0.486	0.472	0.482	0.465	0.455
May-09	0.415	0.412	0.409	0.419	0.406	0.403

**No sampling event occurred.

Appendix III continued.

Table E. Total dissolved solids (g/L) samples collected from stream reaches of Pleasant Grove (PGC) and Whippoorwill creeks (WPC) during 2007-2009.

	Site					
Date	PGC 1	PGC 2	PGC 3	WPC 1	WPC 2	WPC 3
Oct-07	**	**	**	**	**	**
Nov-07	**	**	**	**	**	**
Mar-08	0.244	**	0.242	**	0.234	**
May-08	0.247	0.275	0.245	0.236	0.234	0.256
Sep-08	0.298	0.308	0.302	0.306	0.301	0.264
Oct-08	0.322	0.316	0.306	0.313	0.302	0.296
May-09	0.270	0.268	0.265	0.272	0.264	0.262

**No sampling event occurred.

Table F. Water temperature (°C) samples collected from stream reaches of Pleasant Grove (PGC) and Whippoorwill creeks (WPC) during 2007-2009.

	Site					
Date	PGC 1	PGC 2	PGC 3	WPC 1	WPC 2	WPC 3
Oct-07	14.69	**	15.67	**	18.63	**
Nov-07	11.07	**	13.93	**	11.15	**
Mar-08	13.06	**	13.38	**	12.54	**
May-08	15.07	18.83	15.14	16.53	15.58	15.68
Sep-08	16.38	16.29	17.06	18.90	20.37	18.54
Oct-08	14.23	13.29	13.34	12.74	13.97	12.94
May-09	15.47	15.54	15.94	17.19	12.15	17.31

**No sampling event occurred.

Appendix IV. Riparian zone areas and mean widths of 100 meter stream reaches used to collect abiotic and biotic data during 2007-2009 sampling events within Pleasant Grove (PGC) and Whippoorwill creeks (WPC).

Site	Mean Width (meters)[a]	Area (hectares)
PGC 1	56.2	0.514
PGC 2	44.4	0.478
PGC 3	47.6	0.482
WPC 1	122.2	1.218
WPC 2	100.0	0.979
WPC 3	72.0	0.741

[a] Riparian width of the left and right banks along each stream reach were added together to obtain an overall width before calculating the mean.

VITA

Dereck Lynn Eison was born in Jackson, Tennessee on October 24, 1981, to Debbie and Jeff Eison of Friendship, Tennessee. He attended Crockett County High School and attained his high school degree in 2000. He enrolled in the Bachelor of Science in Biology degree program at the University of Tennessee-Martin in August 2000. After he finally decided upon a career path, he was conferred a degree in biology with a concentration in organismal biology in May 2006.

Dereck was married to his patient and loving wife on August 4, 2007. During that same month, he and his wife moved to Clarksville, Tennessee, where he enrolled into the Master of Science in Biology program at Austin Peay State University. While at Austin Peay, he worked under the tutelage of Dr. Andrew N. Barrass, who is a professor in the Department of Biological Sciences and also a principal investigator for the Center of Excellence for Field Biology.

Upon graduation in 2009, Dereck plans to pursue a career in the Southeastern United States working in the field of stream ecology with an emphasis on nonpoint source and point source pollution assessment.