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Validation Of Kentucky Wetlands Rapid Assessment Method (ky-Wram) Metrics Using Macroinvertebrate Communities Of Forested Depressional Wetlands

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By

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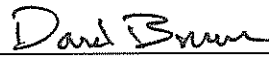

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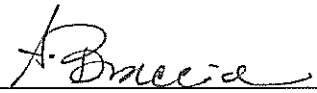

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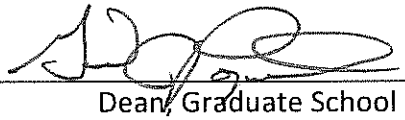

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By

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DEDICATION

This thesis is dedicated to my parents, Brian Huron and Kelly Hay, and my husband, Landon Garrison, for their unending support. My thesis is also dedicated to my aunt, Susan Hay, who has always given me an example of determination, success, compassion, and love.

ACKNOWLEDGMENTS

I would like to thank my co-advisors Dr. Stephen Richter and Dr. David Brown for taking me on as a graduate student and allowing me to be a part in the development and validation of the KY-WRAM. The grant funding they obtained and passed on to graduate student research, including mine, has made all the difference. I thank them for their tireless support and continued input on my academic and personal endeavors. I also thank my committee member Dr. Amy Braccia, whose entomological expertise and frequent affirmations in my project design were absolutely integral to my research and sanity. I extend a special thanks to Michelle Guidugli for providing guidance from the very start of my career as a graduate student. Michelle's oversight of the KY-WRAM project ensured all research equipment, communications, SOPs, and more were aligned and ready, making my and other's research associated with the KY-WRAM development possible. I also thank Jesse Godbold, undergraduate research assistant, for his integral part in the field season, also for his continued positive and enthusiastic outlook during trying times collecting data. I thank Sherrie Lunsford and Jeremee Lewis for their assistance in laboratory analysis and picking of invertebrates. Their meticulous efforts are commendable!

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ABSTRACT

The Kentucky Wetlands Rapid Assessment Method (KY-WRAM) is currently being developed by the collaborative efforts of the Kentucky Division of Water and Eastern Kentucky University as a tool to measure the function and condition of wetlands. To ensure the rapid assessment method properly evaluates wetland condition, the KY-WRAM needs to be validated by comparison to intensive biological data. This project initiated such a comparison using macroinvertebrate communities. Macroinvertebrates play a critical role in wetland ecosystem functioning, thus it is imperative to have an understanding of the macroinvertebrate community responses to degradation of wetlands. Whereas indices of wetland invertebrate communities have been developed for several states including Minnesota, Ohio, California, and Michigan, such research is lacking in Kentucky wetlands. The objectives of this study were to: (1) Determine which habitat and water quality variables macroinvertebrate communities are sensitive to; (2) Recommend macroinvertebrate metrics to be used in a multimetric index for macroinvertebrate biotic integrity for Kentucky wetlands; (3) Assess the correlation of macroinvertebrate communities of forested depressional wetlands in the Upper Cumberland basin to KY-WRAM scores. Nineteen naturally forested, isolated, ridge-top, ephemeral wetlands were selected in the Daniel Boone National Forest for study. Macroinvertebrates were collected conducting 1-meter sweeps with a D-frame dipnet every 5 meters in the emergent vegetation zone around the perimeter of the wetland. Habitat parameters were measured at the same time as macroinvertebrate sampling and water quality samples were taken one week after the completion of habitat and biotic sampling. All wetlands were scored using the Spring 2011 draft of the KY-WRAM and the Ohio Rapid Assessment

Method. Multiple regression analysis comparing macroinvertebrate metrics to habitat variables, water quality, and RAMs were conducted as separate models. Principle Components Analysis was used to evaluate the amount of species variation explained by the RAMs and RAM metrics. A Redundancy Analysis was conducted to model the amount of species variation explained due to habitat and water quality variables. In both multiple regression and multivariate analyses, water quality was not significant and showed few significant relationships to macroinvertebrate community composition. Maximum depth, canopy closure, hummocks and tussocks, and percent vegetative cover were significant in explaining macroinvertebrate community composition in several analyses. These habitat features are also reflected in KY-WRAM metrics that were shown to have significant correlations to the macroinvertebrate community composition, including the KY-WRAM total score and metrics 2, 4, and 6. Some invertebrate metrics are recommended for further research. Because several significant habitat features are also reflective of certain metrics in the KY-WRAM, it is recommended these metrics that reflect important habitat features be considered for adjustment with greater weight so total KY-WRAM score will provide a better reflection of the status of macroinvertebrates. Because this study focused on forested, depressional wetlands, these results should be corroborated by similar macroinvertebrate studies in different wetland types across the varied regions of Kentucky for continued KY-WRAM calibration.

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I. INTRODUCTION

The most important legislation for the protection and management of wetlands are Sections 404 and 104(b)(3) of the Clean Water Act (1972 and 1974). In Kentucky, the Kentucky Division of Water (KDOW) and U.S. Army Corps of Engineers (USACE) regulate impacts to wetlands under Section 404, which requires that wetland mitigation consist of acreage replacement. A goal of the Clean Water Act is to restore and maintain the chemical, physical, and biological integrity of the nation's waters (PL 92-500, Clean Water Act § 101[a]). With the current mitigation system, it is unclear if this goal is being met because it does not take into account the quality or ecological integrity of the wetland being impacted or of the mitigation wetland.

With only 4% of the nation's wetlands assessed (U.S. EPA 2002), it is crucial to develop a tool to rapidly assess the current state of wetlands to save on time and cost, including the level of expertise required to evaluate the health of a wetland. California, Ohio, North Carolina, Minnesota, and others have developed wetland rapid assessment methods (RAMs). Currently, a rapid assessment for wetlands is being developed for Kentucky by the collaborative efforts of the KDOW and Eastern Kentucky University with guidance from a technical work group consisting of various state and federal agencies. The proposed applications of this method include (1) evaluating pre-impact functions and ecological services of wetlands, (2) determining appropriate mitigation, (3) supporting enforcement of illegal impacts, and (4) supporting the development of regulations to protect high-quality wetlands (Barbara Scott, Development of a rapid wetland assessment method for Kentucky, Grant Proposal).

RAMs rely on the premise that certain visible field metrics of biological and physical attributes can be used to indicate the ecological condition of wetlands (Stein et al. 2009). Thus, validation is an important part of developing and calibrating RAMs to ensure that the metrics used are indeed reflective of the wetland's ecological condition. As stated by Fennesy et al. (2007), a three-tiered approach can be used to evaluate the condition of wetlands, and each approach can be used to validate results from other levels. Level one includes remote sensing techniques to examine the wetland from a large-scale, coarse perspective, level two includes the rapid assessments, and level three includes collecting and analyzing intensive data from the wetland. Several studies have conducted level three assessments to compare to results collected from rapid assessments, determine if the rapid assessment is reflective of the ecological condition indicated by the intensive data for wetland sites, and calibrate metrics of the rapid assessment to reflect the increasing compilation of level 3 data conclusions on wetland condition (Mack 2001a, Micacchion 2004, Stapanian et al. 2004, Wardrop et al. 2007, Stein et al. 2009). While it is important to use multiple assemblages for validation, this study will focus on the use of macroinvertebrates to indicate wetland condition and provide insight on how KY-WRAM metrics relate to the invertebrate communities of wetlands.

Macroinvertebrates play a vital role in wetland ecosystems. They are critical in detritus processing and nutrient cycling in wetlands (Batzer et. al 1999, Duffy 1999, Fairchild et al. 1999) and serve as energy for higher trophic levels (Batzer and Wissinger 1996, Batzer et. al 1999, Longcore et al. 2006). Cooper and Anderson (1996) found that increased abundance of macroinvertebrates corresponds with higher brood densities of waterfowl in wetlands, and Magee et al. (1993) suggested that increased diversity of

macroinvertebrates helps waterfowl meet dietary needs. Conversely, some groups of macroinvertebrates negatively impact other populations; for example, some are major predators of amphibian larvae (Baber et al. 2004).

Benthic macroinvertebrates are the most widely used group of organisms to assess the condition of aquatic ecosystems, thus have been used extensively in bioassessments to indicate the health of streams and rivers (Rosenburg and Resh 1993). While indices of biotic integrity (IBI) are well established for these lotic systems, macroinvertebrate IBIs are still being developed for wetlands in several geographic regions including Minnesota (Helgen 2002), Lakes Huron and Michigan fringing wetlands (Burton et al 1999, Urzaski et al 2004) and Italy (Solimini and Bazzanti 2008). Variability in wetland type and ecoregional influences on wetland function prevent the development of a uniform macroinvertebrate IBI for wetlands, thus the US EPA has set forth guidelines to assist states with developing and implementing macroinvertebrate indices of biotic integrity (EPA 2002).

Unfortunately, it is unfeasible to simply adjust established bioassessment methods of streams and rivers to wetland habitats. Wetland macroinvertebrate communities have fundamental differences from lotic systems because of differences in hydrology and water chemistry (Williams 1987, Rader and Richardson 1992, Batzer et al. 2001, Davis et al. 2006). However, Davis et al. (2006) conducted a study in Australia to determine if AUSRIVAS (a predictive modeling technique used for rivers in Australia) could be modified for wetland assessment and monitoring. The AUSRIVAS bioassessment was demonstrated to be adaptable to wetland systems; however, it did not rely on established macroinvertebrate metrics with sensitivity scores (tolerant/intolerant) as is the case with

North American IBI procedures. The AUSRIVAS model includes the use of reference wetlands to establish expected macroinvertebrate communities and compares those communities to degraded wetlands. Thus, specific indices must be developed for each region and wetland type.

Stein et al. (2009) used data from several biotic assemblages to validate the California Rapid Assessment Method (CRAM), including macroinvertebrate data. They found that scores from the California benthic macroinvertebrate IBI for riverine wetlands exhibited a significant positive correlation to CRAM scores. Conversely, Suren et al. (2010) found only weak correlations when they attempted to use macroinvertebrate data in New Zealand to validate two wetland scoring systems, the wetland condition index (WICI) developed by Clarkson et al. (2003) and the index of ecological integrity (IEI) developed by Ausseil et al. (2008).

Other studies have compared macroinvertebrate communities to the physical structure and chemistry of wetlands without the use of IBI scores or wetland condition scores. Cooper et al. (2006) assessed responses of macroinvertebrate communities to chemical-physical variables, land use and cover, and vegetation types in a Lake Michigan drowned river mouth wetland. This large wetland included a gradient of degraded water quality and land use from upland to lowland areas. The authors found that macroinvertebrate community structure correlated most closely with water quality variables, which were influenced by the land use of the surrounding area. Vegetation type seemed to account for the least amount of variation in macroinvertebrate community structure. This was contradictory to the findings of Burton et al. (2002, 2004), who found that vegetation type was the most influential factor for macroinvertebrate communities in

the great Lakes coastal wetlands. Similar to the findings of Cooper et al. (2006), the Ohio EPA, in an effort to develop a wetland invertebrate community index (WICI) for Ohio, found that relative abundance was associated with water and soil characteristics, rather than landscape attributes (Ohio EPA 2004).

Given the discrepancies presented above, it seems valuable to not only consider how macroinvertebrate communities reflect the metrics of RAMs (usually based on physical and habitat attributes), but also water quality and landscape integrity. There are several water chemistry variables, which may or may not be related to KY-WRAM metrics, to consider that could influence macroinvertebrate communities. These include nutrient enrichment, dissolved oxygen, pH, and conductivity.

Nutrient enrichment has been shown to have differing effects on benthic macroinvertebrates communities. Suren et al. (2003) found that in wetlands with poor water quality (low dissolved oxygen and high nutrient loads), aquatic macroinvertebrate communities were often dominated by few taxa, such as oligochaetes and snails, which reduced community diversity. McCormick et al. (2004) found that eutrophication may cause a shift in community composition to include more tolerant taxa. This is similar to the findings of Doughtery (1991), who found that richness and diversity were stable or even increased in the nutrient enriched Pine Barren watershed of New Jersey; however, a closer look into the community composition reveals a shift from macroinvertebrate taxa typically found in the region to a more tolerant composition. Conversely, other studies have shown that nutrient enrichment actually increases macroinvertebrate species richness, diversity, and abundance because the nutrients are more bioavailable (Rader and Richardson 1994) and encourages establishment of macrophytes that macroinvertebrates

use for cover and food (Batzer 2004, Longcore et al. 2006). These studies underscore the need to examine relationships regionally.

Other studies indicate that macroinvertebrate communities respond to nutrient enrichment by means of its impact on dissolved oxygen concentration (Spieles and Mitsch 2000, Campel et al. 2003). Nutrient enrichment allows for greater bacterial respiration, which consumes dissolved oxygen. Many studies have concluded that decreases in macroinvertebrate abundance, diversity, and richness is caused by low dissolved oxygen levels (Nelson et al. 2000, Spieles and Mitsch 2000, 2003) because reduced oxygen levels can negatively affect the growth and survival of aerobic organisms in aquatic systems (Eriksen et al. 1996). Spieles and Mitsch (2000) found that over half the variation in macroinvertebrate community index (ICI) scores was accounted for by variation in dissolved oxygen. Even though dissolved oxygen has been established as an important driver of macroinvertebrate communities (Spieles and Mitch 2000, 2003, Nelson et al. 2000), other studies found only weak relationships between macroinvertebrate communities and water quality measures, including dissolved oxygen and nutrients (Battle and Golladay 2001, Steinman et al. 2003).

Changes in pH can also cause shifts in macroinvertebrate community structure. Sommer and Horwitz (2001) found that the macroinvertebrate community of western Australian wetlands shifted dramatically with the pH shift from ca. 6–8 to ca. 4–5 over a period of 4 years. Longcore et al. (2006) found that the number of invertebrate taxa was higher in wetlands with a pH greater than 5.5 and that low pH wetlands negatively affected acid-intolerant invertebrates (e.g., Ephemeroptera).

Conductivity is known to impact macroinvertebrates by damaging tissues of invertebrates, altering the bioavailability of nutrients and heavy metals, and altering communities at other trophic levels (algae, fish, amphibians, and more), which may cause population limitation for invertebrates (Adamus et al. 2001). A study in Minnesota wetlands found that macroinvertebrate species richness declines were correlated with increasing chloride ions, a product of road salt that increases conductivity within waterbodies (Gernes and Helgen 1999). Spieles and Mitsch (2000) found that conductivity explained 16.4% of the total variation in the invertebrate community scores for the wetland sites they studied.

In addition to the role of water quality in shaping community composition, this research focused on the relationship between KY-WRAM metrics and macroinvertebrate communities. Several variables known to impact macroinvertebrate communities are directly integrated in traditional RAM metrics including forested buffer (upland habitat), hydrology, wetland size, land use, and vegetation. The KY-WRAM metrics included in the April 2011 draft are listed in Table 1.

Table 1. List of Kentucky Wetland Rapid Assessment Method metrics, April 2011 Draft.

Kentucky Wetland Rapid Assessment Method (KY-WRAM) metrics	
1. Wetland size and distribution	
	1a. Wetland size
	1b. Wetland scarcity
2. Upland buffers/ Intensity of surrounding land use	
	2a. Average buffer width around wetland perimeter
	2b. Intensity of surrounding land use within 1,000 feet of the wetland
	2c. Connectivity to other natural areas
3. Hydrology	
	3a. Sources of water
	3b. Connectivity
	3c. Duration of inundation/ saturation
	3d. Alterations to natural hydrologic regime
4. Habitat alteration and habitat structure development	
	4a. Substrate/ soil disturbance
	4b. Habitat alteration
	4c. Habitat structure development
5. Special wetlands	
	5a. High ecological value
	5b. Forested wetland
	5c. Urban/ suburban wetland
	5d. Low-quality wetland
6. Vegetation, interspersion, and habitat features	
	6a. Wetland vegetation components
	6b. Open water component
	6c. Coverage of highly invasive plant species
	6d. Horizontal interspersion
	6e. Habitat features

Source: Kentucky Wetland Rapid Assessment Method, Spring 2011 Draft, Eastern Kentucky University, unpublished.

In order to avoid circularity when evaluating candidate metrics for the recommended MIBI, several landscape integrity variables will be measured to directly indicate the condition of the wetland. These habitat variables include diameter at breast

height of standing live and dead trees, number of trees within the wetland, distance to the nearest paved road, percent canopy closure, percent vegetative cover over the wetland waters, volume of coarse woody debris in the wetland, leaf litter depth and maximum water depth. These variables are intended to objectively measure some of the RAM metrics discussed further below.

Forested buffer, or upland habitat, is a common metric of RAMs to indicate the condition of wetlands. Macroinvertebrates use upland habitats to migrate to other pools and feed (Merrit and Cummins 1996). Few studies have been conducted on the impact of forested buffers on wetland macroinvertebrates, although numerous studies exist for streams (Fuchs et al. 2003, Kiffney et al. 2003, Kreutzweiser et al. 2005, Gomi et al. 2006). These stream studies generally conclude that macroinvertebrate abundance increases with decreasing riparian buffer due to solar influx, which allows for greater macrophyte growth, thus the herbivorous species increased in abundance. The increase in solar influx also elevates water temperatures (Gomi et al. 2006), which has been shown to correlate with increased colonization rates (Nilsson and Svensson 1995). Theriault (2009) studied the effects of forested buffers on Maine vernal pool macroinvertebrates and found that non-predaceous species richness and composition differed significantly between the clear-cut buffer treatments and reference sites. Batzer (2005) found that macroinvertebrate community composition shifted with timber harvest in a South Carolina bottomland hardwood wetland. Related to forested buffer, canopy cover has been shown to impact macroinvertebrates of wetlands. For the same reasons as buffer zones, decreasing overstory canopy cover typically results in increased species richness (e.g., Batzer et al. 2004).

Certain aspects of hydrology are also common metrics for RAMs, which is to be expected since wetland hydrology is one of the three qualifying characteristics of a wetland. Hydroperiod is perhaps the most important wetland characteristic influencing the occurrence and abundance of macroinvertebrates (Batzer and Wissinger 1996). Theriault (2009) found that hydroperiod was the most important factor influencing the composition, richness, diversity and evenness of macroinvertebrates in central Maine vernal pools. Similarly, Euliss and Mushet (2004) found that artificially lengthened hydroperiods in western North Dakota wetlands increased macroinvertebrate richness. Predatory taxa are more common in wetlands with longer hydroperiods (Batzer and Wissinger 1996, Euliss and Mushet 2004, Therialut 2009), which can greatly impact the amphibian and macroinvertebrate communities from a top-down response (Baber et al. 2004). However, Studinski and Grubbs (2007) did not find that species richness increased with hydroperiod in the temporary ponds of Mammoth Cave National Park, KY. Although one metric of the KY-WRAM addresses duration of inundation, this study will not be evaluating the influence of hydroperiod because all wetlands assessed were ephemeral.

Wetland size is often a scoring metric of RAMs, awarding higher scores for larger wetland sizes (Mack 2001b). Batzer et al. (2004) found no relationship between macroinvertebrate species richness and pond surface area, while Studinski and Grubbs (2007) found that richness increased with pond area. Matchik et al. (2010) did not find a relationship between macroinvertebrate species richness or composition to wetland size. The authors further discussed the implications that wetland size should not be used to

extend greater conservation value; small wetlands may be equally valuable for biodiversity.

Surrounding land use is often a characteristic evaluated in RAMs either remotely or in the field. Wetland ecological integrity is degraded by anthropogenic land uses that stress the system (Fennesy et al 2007). Urban development (e.g., highways) impacts macroinvertebrate species composition of wetlands (King et al. 2000). Other than direct habitat alteration, land use can contribute poor water quality from storm water and agricultural runoff, which results in reduced biological integrity and altered macroinvertebrate community composition (Helgen and Gernes 2001). Agricultural land uses can stress the wetland system through herbicides, sedimentation, and habitat homogeneity. Campbell et al. (2009) found that the presence of cattle-degraded water quality and habitat caused a decrease of chironomid taxon richness in Minnesota farm ponds.

Vegetation metrics, such as 'interspersion' and 'percent invasive plants' are measured in some RAMs (Mack 2001b). Vegetation influences the abundance and diversity of macroinvertebrates because it provides cover, dissolved oxygen input through photosynthesis, and food for macroinvertebrates. In fact, macroinvertebrates are important links between the plant community and higher trophic levels (Batzer and Wissinger 1996). Percent vegetative cover is strongly correlated with macroinvertebrate secondary production (Wissinger et al. 2001), and diversity is also greater with increasing plant cover (deSzalay and Resh 2000).

Objectives

The goal of this study was to determine if the macroinvertebrate communities of depressional wetlands of the Upper Cumberland and Kentucky river basins of Kentucky reflect wetland condition as determined by the KY-WRAM. In order to do this, I assessed community composition with diversity, richness, evenness, and abundance measures as well as candidate invertebrate metrics based on percent composition and functional feeding groups. As Ohio EPA (2004) states, " a metric is a characteristic of an organism or an organism group that exhibits a positive or negative association with an environmental factor." It is not the objective of this study to develop a MIBI because a gradient of wetlands ranging from reference to degraded conditions must be known and established and then used to measure the responsiveness of said invertebrate metrics to the condition of the wetland. For this study, wetlands were selected in the Upper Cumberland River Basin without knowledge of their condition. Thus, correlations between the macroinvertebrate community and assessed wetland condition were used to recommend some metrics that seemed to have responsiveness to the narrow disturbance gradient presented by the study sites.

For each wetland, in addition to the intensive measurement of the macroinvertebrate community, I measured water quality, habitat variables, and scored the wetland using the spring 2011 draft KY-WRAM. The specific objectives for this intensive biological survey were to determine how macroinvertebrate communities of sampled wetlands vary according to 1) habitat variables, 2) water quality parameters, and 3) wetland assessment methods including KY-WRAM metrics, Landscape Development Intensity (LDI) scores, and Ohio Rapid Assessment Method (ORAM) metrics.

II. METHODS

Study Sites

All of the known natural forested depressional wetlands within the Daniel Boone National Forest of Jackson, Rockcastle and Laurel counties were investigated for use in this study. While the focus of this research is within the Upper Cumberland River Basin of Kentucky, four of the sampled wetland sites lay north of this boundary in the Kentucky River Basin (Table A1, Appendix; Figure 1). The sampled wetlands are naturally forested, isolated on ridge-tops, and ephemeral. In this region, mixed mesophytic forests prevail, although most of the wetlands were located in upland habitat that is dominated by mixed oaks and hickories (Woods et al. 2002). Twenty-one sites were determined to be suitable for the study. After ground-truthing the wetlands, 19 were used as study sites because two had already dried completely at the time of sampling.

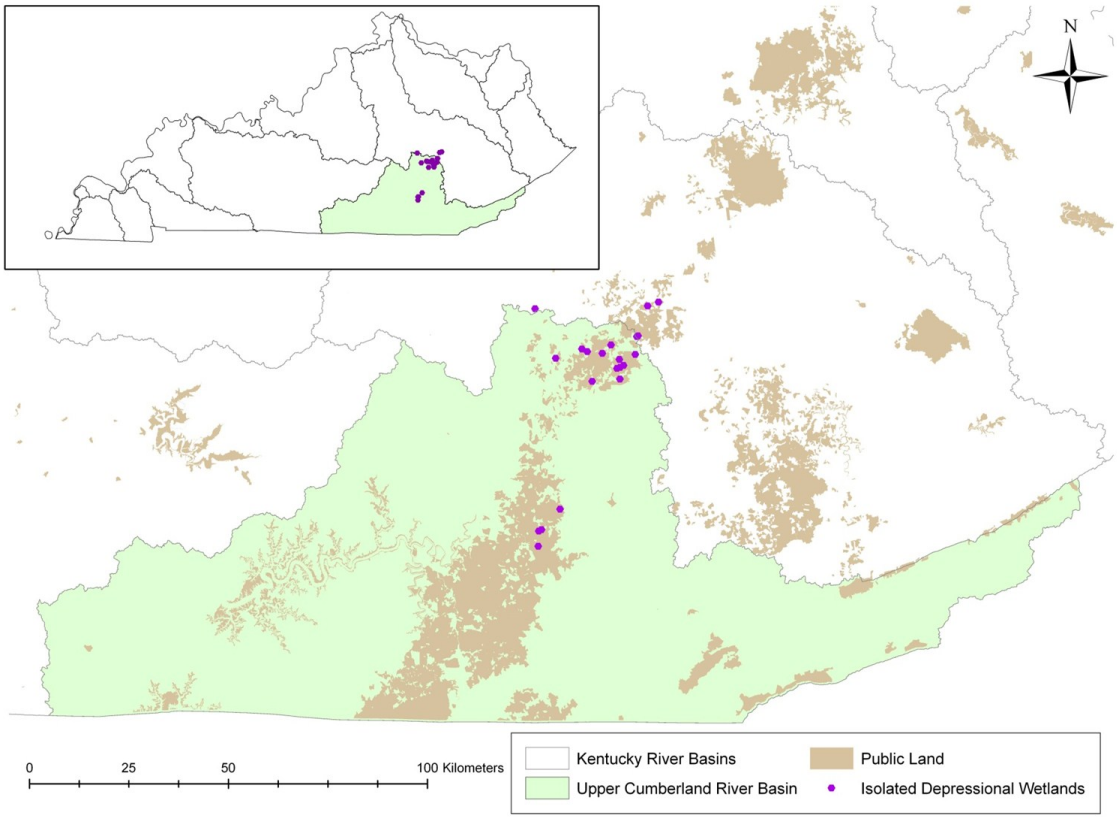

Figure 1. Wetland study sites of the Upper Cumberland River Basin of Kentucky. Four forested depressional wetlands included in the study lie just outside the boundary in the Kentucky River Basin.

Macroinvertebrate Sampling

I used both semi-quantitative and qualitative sampling methods to assess macroinvertebrate communities. Semi-quantitative sampling was conducted using a D-frame dipnet and activity traps. Qualitative, multihabitat sampling was conducted with a D-frame dip net followed by picking through vegetation and debris. In this region of the United States, sampling at anytime from February through June should yield enough macroinvertebrates for research and monitoring purposes because the invertebrates are large enough to be identified, but have not yet emerged from the wetland (Batzer et al. 2000). Aquatic invertebrates tend to emerge and become active in February. By June,

many wetlands are severely reduced in water volume and some dry completely in late summer, making macroinvertebrate collection unfeasible due to migration, maturation to terrestrial stage, retreat to substrate, or mortality. As recommended by Davis et al. (2006) and Batzer et al. (2000), it is important to collect macroinvertebrates in the same time frame (i.e. the same week or month) at sites that will be compared statistically. For this study, macroinvertebrate samples were collected during one sampling period, 11–26 May 2011.

Semi-quantitative Sampling

For the semi-quantitative, standardized dip netting procedure, I conducted 1-meter sweeps every 5 meters (approximated by 10 steps) in undisturbed areas of the wetland's emergent vegetation zone using a D-frame 500 micron mesh dipnet. Batzer et al. (2000) and Davis et al. (2006) recommended choosing a single habitat type that contains the greatest abundance and diversity to reduce cost of sampling and also because detection of wetland impairment may be confounded by type and variety of habitat. In past studies, the greatest abundance of macroinvertebrate communities was found in the emergent zone (Brown and Batzer 2001). Since this study was not designed to compare habitat types within the wetland, sampling from more than one habitat type would jeopardize the significance of comparisons between wetlands. Thus, the emergent vegetation zone (within 5 meters of the shoreline) was selected as the standard habitat to be sampled and compared for each wetland. Also, sampling within the shoreline minimizes disturbance to the wetland. During the 1-meter sweep, I tapped the substrate three times during the sweep to ensure entrapment of benthic macroinvertebrates and pulled the net up with the open mouth of the net facing the water surface to ensure that macroinvertebrates did not

escape. The sweeps were conducted rapidly. Within the dipnet, any large debris was rinsed to remove any aquatic macroinvertebrates clinging to the surface, excess water was drained, and macroinvertebrates, fine materials, and detritus were placed into a wide-mouthed, 1-liter sample jar. Sample jars did not exceed half full, thus additional jars were used as needed. The jars were filled with 95% ethyl alcohol to preserve the specimens for taxonomic identification in the laboratory.

Activity traps are designed to collect actively swimming or nocturnally active invertebrates that may not be represented in the dipnet samples. I constructed activity traps from 2-liter clear plastic bottles, cut at the shoulder. The funnel-shaped bottle top was inverted to fit inside the bottom half of the bottle. Two holes were cauterized through the plastic and positioned so that a plastic coated iron rebar was inserted through the top and bottom of the mouth of the trap. The stick was pushed into the substrate of the wetland so that it holds the funnel of the activity trap horizontally (i.e., parallel to the substrate) under water. Activity traps were placed in the emergent vegetation zone every 10 meters just inside the wetland perimeter and left overnight. To attract invertebrates, glow-sticks were placed in every other trap. The following day, the activity traps were removed, the contents poured through the D-frame dip net (500 micron sieve), and macroinvertebrates placed in a labeled sample jar with 70% ethyl alcohol.

The macroinvertebrates collected from the activity traps were not used in this study. Many amphibians were caught in the traps, which in turn depredated macroinvertebrates and thus biased the sample. The activity traps also tended to have very few individuals so that little information was added to the data collected from dip-net sampling.

Qualitative Sampling

A multihabitat sample was taken at each wetland to obtain as many additional taxa as possible to be coupled with the semi-quantitative samples to calculate the taxa richness of each wetland. To do so, the dipnet was swept in habitats not sampled by semi-quantitative sampling (i.e. open water column, tussocks, root-mats, and other unique habitats). After each sweep, materials were transferred to the sorting pan. Large debris was rinsed thoroughly and discarded. Using field forceps, macroinvertebrates were collected from the sorting pan until one or several individuals of each taxon observed were collected. Leaf packs and other debris were selected and picked of macroinvertebrates to find additional taxa that may otherwise be unrepresented. Multihabitat sampling was conducted for no fewer than 40 minutes. All aquatic macroinvertebrates collected were preserved in labeled 0.5-liter sample jars with 70% ethyl alcohol.

Lab Processing

Dissecting scopes and microscopes were used for sorting and identifying macroinvertebrates in the laboratory. Organisms were identified to the lowest taxonomic level possible. Reference specimens were placed within vials containing the original site information and stored in jars for reference when identifying similar organisms. Lab specimens were stored in 70% ethanol alcohol.

Once in the lab, samples were washed using a 500-micron sieve pan. One tablespoon at a time was transferred to a 12.7 X 17.8 cm sorting pan with water and picked by eye for macroinvertebrates. All macroinvertebrates picked were sorted and stored in 70% ethanol alcohol in a jar labeled with site location and date of collection.

After samples were picked by eye, debris was transferred to another 12.7 X 17.8 cm sorting pan and swirled for homogeneity. A metal grid was placed in the sorting pan to divide the sample into 18 equal cells. Six cells (equaling one-third of the sample) were randomly selected to be picked under the dissecting scope. The cells were chosen by drawing 6 of 18 numbered marbles from a jar.

Water Quality

The week following the macroinvertebrate sampling completion, I and other colleagues collected water quality measures including dissolved oxygen, temperature, salinity, conductivity, and pH using a YSI multi-parameter water meter. Three wetlands had dried down by the first week of sampling and were sampled after rains on a later date. Chlorophyll-a was measured in the surface water using a Turner design Cyclops 7 fluorometer. Surface water was sampled for laboratory analyses of nitrates, nitrites, ammonia, total N, and total P at the Environmental Services Branch of the KDOW, but these results were not used due to a preservation error of the surface water samples.

Habitat Variables

Habitat variables were measured, including the diameter at breast height (DBH) of all standing dead and live trees in or touching the water of the wetland, measuring angular canopy closure, the distance to the nearest paved road, maximum water depth, leaf litter depth, the cubic volume of coarse woody debris, and the area of hummocks and tussocks in the wetland.

DBH was measured using a standard diameter tape. Angular canopy closure was measured with a spherical densiometer from each of the four cardinal directions at the edge of the wetland, and averaged. Leaf litter depth was measured 1 meter from the

shoreline within the wetland at the cardinal directions and averaged. Distance to the nearest paved road was measured as the shortest distance from the wetland edge to a paved road. Maximum pond depth was taken from the deepest part of the pond during macroinvertebrate sampling. Coarse woody debris and percent vegetation within the wetland were measured by establishing two transects that bisect the ponds in the cardinal directions. Thus, the two transects were perpendicular to one another. Coarse woody debris greater than 10 cm in diameter at its narrowest, that intersected a transect was measured for total length, diameter at its narrowest point, and diameter at its widest point. These measurements were used to estimate the cubic volume of the coarse woody debris (Waddell 2002, DeVries 1973). Total area of the wetland was calculated by establishing a set of two transects, one stretching the longest length of the wetland and the other oriented in perpendicular direction. The formula for calculating the area of an ellipse was used to estimate the area of the wetland. The diameter of the vegetation falling on the transect was measured to get percent vegetation using a line-intercept method.

Rapid Assessment Methods

Each wetland was scored by four trained technicians, including myself, using the Spring 2011 draft KY-WRAM and the Ohio Rapid Assessment Method (ORAM). Landscape Development Intensity (LDI) index scores were calculated using ArcGIS within a 1000 m buffer according to the methods of Brown and Vivas (2005) to provide a level 1 assessment for comparison purposes. The LDI score provides an indication of human land-use impacts for a given site. LDI is calculated on a scale of 1–10 with higher scores indicating more intense land-use and alteration by humans.

Analyses

All correlation and regression statistics were done in SPSS v. 19, and ordination analyses were conducted in program R (Version 2.15.1) using Package Vegan (The R Foundation for Statistical Computing 2010). Using SPSS, a univariate correlation matrix was prepared to show the associations of each invertebrate metric to the water quality variables, habitat variables, and WRAM metrics. These data were used to identify important trends and associations to corroborate and compare with the multiple regression analysis, a more powerful statistical tool in evaluating the relationship of all of the environmental variables as a whole to a single invertebrate metric.

I selected 17 invertebrate metrics to use in multiple regression analyses. I calculated the 17 macroinvertebrate community indices using Microsoft Excel and Program PAST (Table 2.). These 17 metrics were selected based on their use in other biological indices and studies that found some significant responsiveness to habitat and water quality variables (Ohio EPA 2004; Gernes and Helgen 2002). These invertebrate metrics are a description of the overall community and each metric was included as a dependent variable in separate multiple regression analyses against independent variables of water quality, habitat variables, as well as rapid assessment total scores and metrics to describe patterns of macroinvertebrate community variation.

Table 2. List of metrics describing the macroinvertebrate communities of forested depressional wetlands of the Upper Cumberland and Kentucky River basins sampled from May 2011.

Macroinvertebrate metric	**Calculations**
1) Abundance per 1 meter dip	Abundance divided by the number of dips. (Dips calculated from perimeter.)
2) Simpson's index of diversity	$D = 1 - \Sigma(p_i)^2$
3) Shannon-wiener diversity index	$H' = \Sigma(p_i)(\log_2 p_i)$
4) Richness	Semi-quantitative + Qualitative taxa
5) Evenness	Calculated using program PAST
6) Percent predators	(Sum of group's abundance / sum of total abundance for each site) *100
7) Percent collector gatherers and filterers	" "
8) Percent shredders and scrapers	" "
9) Percent Odonata	" "
10) Percent Corixidae	" "
11) ETO taxa	The total number of Ephemeroptera, Tricoptera, and Odonoata taxa .
12) Percent Nematoda	" "
13) Percent Oligochaeta	" "
14) Percent Chironomidae	" "
15) Simpson's index of diversity excluding Nematoda	Same as Simpson's calculation, after removing Nematoda data.
16) Richness excuding Nematoda	Same as Richness calculation, after removing Nematoda data.
17) Abundance per 1 meter dip excluding Nematoda	Same as abundance calculation, after removing Nematoda data.

In order to select water quality, habitat, and WRAM metrics to include in each multiple regression model, I first conducted correlation analysis (bivariate correlation matrix) to determine if environmental variables, including water quality and habitat, were intercorrelated. For cases in which two independent environmental variables were correlated, I excluded the one with lower statistical and biological significance and included the other in the multiple regression model (Table 3). For the water quality multiple regression analysis, one wetland site, Sandgap, was excluded because the

wetland had dried down at the time of water sampling. The pH value for the site 'Cliff Palace' appeared to be an outlier and so was not entered into the models.

Table 3. Habitat variables, water quality variables, and wetland assessment measures used in each of the three multiple regression models.

Habitat Variables	**Water Quality Variables**	**Wetland Assessment Measures**
Maximum depth (cm)	Percent DO	Landscape development index
Number of trees	pH	KY-WRAM average score
Average DBH of dead trees	Salinity	ORAM average score
Leaf litter depth (cm)	Chlorophyll-a (μg/L)	Metric 1 score
Tussocks & Hummocks	Air temperature (°C)	Metric 2 score
Average Canopy Closure	Water temperature (°C)	Metric 3 score
Course Woody Debris (m^3)		Metric 4 score
Percent vegetative cover		Metric 6 score
Distance to paved road (m)		
Perimeter (m)		

I used a backward stepwise multiple regression approach andcalculated standardized residuals for each final model. I assessed the assumptions of normality and homogeneity of variance by viewing histograms of the residuals from each analysis. If there was an outlying residual, I removed the wetland study site that was causing the violation of the assumption and ran the multiple regression again.

The 20 most commonly occurring taxa (occurring in 5 or more of the 19 study sites) were used to conduct multivariate ordination analysis (Table 4). Following a Hellinger transformation of the taxa abundance data, a Principle Components Analysis (PCA) was used to illustrate the similarity of taxa in multivariate space. A Hellinger transformation is an accepted transformation for species community data to resolve skewed biplots and the tendency of many species to have low abundance while few have high abundance. I also compared the PCA results of the macroinvertebrate community structure to the KY-WRAM total score and metrics 1–4, 6 and to ORAM using an Envfit

function in Package Vegan. I expected that habitat and water quality variables would directly affect the macroinvertebrate community; therefore, I used a constrained ordination approach to determine the amount of macroinvertebrate community structure that could be explained by habitat and water quality variables together. We used forward, stepwise Redundancy analysis (RDA) to eliminate non-significant environmental variables, and then re-ran the most parsimonious model.

III. RESULTS

A total of 17,849 individual invertebrates consisting of 84 taxa were collected, picked, and identified (Table A2a and Table A2b, Appendix). Twenty taxa occurred in 5 or more wetlands (Table 4).

Table 4. List of the most frequently occurring macroinvertebrate taxa in 19 depressional wetlands from the Upper Cumberland and Kentucky River basins from May 2011.

Order*	**Family***	**Genus**
(P)Annelida	(SC)Oligochaeta	
(P) Amphipoda		
Cladocera	Daphniidae	
Coleoptera	*Egg/ larvae encased	
Coleoptera	Dytiscidae	*Dytiscus* (larvae)
Coleoptera	Dytiscidae	*Hydrotrupes* (adult)
Coleoptera	Hydrophilidae	*Helochares* (larvae)
Copepoda	Cyclopoida	
Diptera	Culicidae	*Aedes*
Diptera	Ceratopogonidae	*Bezzia*
Diptera	Chironomidae	
Diptera	Culicidae	*Culex*
Diptera	Phoridae	
Diptera	Dolichopodidae	
Hemiptera	(SF) Corixini	(immatures)
Hemiptera	Notonectidae	*Notonecta*
Hemiptera	Gerridae	*Trepobates*
Nematoda		
Ostracoda		
Odonata	Aeshnidae	*Remartinia*

Note: Higher taxonomic classification indicated in parenthesis (P = Phylum, SC = Subclass)

A pairwise univariate correlation matrix was prepared to show the general associations of each invertebrate metric to the water quality variables (Table A3, Appendix), habitat variables (Table A4, Appendix), and WRAM metrics (Table A5, Appendix; Table 5). Some of the variables excluded from the multiple regression analysis due to intercorrelation appear in this univariate correlation analysis. There were 9 invertebrate metrics significantly correlated to environmental variables and WRAM metrics at the P = 0.01 level. Total abundance per dip was positively correlated with perimeter, salinity, and conductivity. Richness was positively correlated to landscape development index (LDI) scores. Percent collectors and filterers was negatively correlated to fluorescence (RFUs). Percent shredders and scrapers was positively correlated to number of trees. Percent Corixini was positively correlated to distance to paved road and negatively correlated to pH. Percent Nematoda was negatively correlated to total KY-WRAM score, and metric 6 of the KY-WRAM and positively correlated to fluorescence and chlorophyll-a. The Simpson's diversity index, excluding Nematoda, was negatively correlated to average canopy closure. Richness, excluding Nematoda, was positively correlated to LDI scores. Abundance per dip, excluding Nematoda, was positively correlated to salinity and conductivity.

Table 5. Pairwise univariate correlation matrix of the macroinvertebrate metrics to habitat variables, water quality variables, and wetland assessment metrics. Pearson's correlation (R) and significance values (P) are given. Significant correlations at the 0.05 level are highlighted in grey. Significant correlations at the 0.01 level are highlighted in blue. Abbreviations: Percent PP, PE = Predator-piercers, predator-engulfers; Percent CG, CF = collector-gatherers, collector-filterers; Percent SH, S = Shredders, Scrapers.

		Abundance per dip	Simpson's Diversity	Richness	ETO	Shannon Wiener	Evenness	Percent PP, PE	Percent CG, CF	Percent SH, S	Percent Odonata	Percent Corixini	Percent Nematoda	Percent Ologocheata	Percent Chironomidae	Simpson's Diversity without Nematoda	Richness Without Nematoda	Abundance Per Dip without Nematoda
Max depth (cm)	R	0.018	0.127	0.212	0.388	-0.01	-0.153	-0.088	.488*	-0.08	-0.037	0.05	-.468*	0.083	-0.04	-0.113	0.231	0.092
	P	0.942	0.605	0.383	0.101	0.967	0.531	0.722	0.034	0.746	0.88	0.839	0.043	0.737	0.871	0.646	0.342	0.709
Area (m^2)	R	-.513*	0.168	0.318	0.121	0.166	-0.092	0.051	0.321	0.077	0.06	0.074	-.486*	0.241	0.003	-0.104	0.321	-0.454
	P	0.025	0.492	0.185	0.623	0.498	0.708	0.837	0.181	0.754	0.808	0.763	0.035	0.32	0.99	0.671	0.18	0.051
Perimeter (m)	R	.583**	-0.33	-0.113	-0.161	-0.265	-0.33	-0.269	-0.146	-0.07	-0.268	-0.29	0.378	-0.419	0.303	-0.053	-0.12	.554*
	P	0.009	0.168	0.646	0.51	0.274	0.168	0.266	0.55	0.777	0.268	0.229	0.11	0.074	0.207	0.828	0.626	0.014
Dist. to paved rd (m)	R	0.058	0.154	-0.372	-0.15	-0.065	0.157	0.123	-0.444	0.047	0.315	.583**	0.435	-0.095	-0.21	0.414	-0.363	0.046
	P	0.812	0.53	0.117	0.539	0.791	0.521	0.616	0.057	0.848	0.189	0.009	0.063	0.7	0.388	0.078	0.127	0.852
Avg DBH live trees	R	-0.176	-0.356	-0.18	-0.208	-0.332	0.046	-0.043	0.441	-0.402	-0.18	-0.114	-0.421	.501*	0.163	-.563*	-0.187	-0.168
	P	0.47	0.135	0.46	0.394	0.165	0.851	0.86	0.059	0.088	0.46	0.643	0.073	0.029	0.505	0.012	0.443	0.491
Avg DBH dead trees	R	0.083	-0.363	0.213	-0.114	-0.162	-0.204	-0.216	0.275	0.009	-0.213	-0.318	-0.254	-0.08	0.207	-.473*	0.217	0.079
	P	0.735	0.127	0.38	0.643	0.507	0.402	0.375	0.255	0.972	0.382	0.184	0.293	0.744	0.395	0.041	0.372	0.749
Number of trees	R	-0.183	-0.373	0.243	0.232	0.341	0.004	-0.077	-0.013	.652**	-0.097	-0.178	-0.308	-0.19	0.083	0.061	0.256	-0.336
	P	0.453	0.116	0.316	0.339	0.154	0.988	0.754	0.957	0.002	0.694	0.467	0.2	0.436	0.736	0.805	0.29	0.16
Leaf litter depth(cm)	R	-0.053	0.207	0.266	0.085	0.292	-0.029	0.322	-0.004	0.267	0.351	0.191	-0.29	-0.208	-0.093	0.073	0.29	0.012
	P	0.83	0.395	0.271	0.729	0.225	0.908	0.179	0.989	0.269	0.141	0.435	0.228	0.392	0.705	0.766	0.228	0.961
Tussocks /m^2	R	0.387	-0.306	0.09	-0.319	-0.186	-0.294	-0.208	-0.111	-0.134	-0.176	-0.148	0.3	-0.322	0.193	-0.052	0.08	0.398
	P	0.102	0.203	0.714	0.184	0.447	0.221	0.393	0.651	0.586	0.471	0.546	0.212	0.179	0.428	0.833	0.746	0.091
Hummocks /m^2	R	0.115	-0.397	0.313	-0.221	-0.138	-0.33	-0.175	0.203	0.136	-0.163	-0.157	-0.212	-0.346	0.433	-0.453	0.317	0.128
	P	0.64	0.092	0.191	0.363	0.574	0.167	0.475	0.405	0.579	0.504	0.521	0.384	0.147	0.064	0.052	0.187	0.601
Tussocks+ Hummocks	R	0.158	-0.316	0.348	-0.26	-0.101	-0.356	-0.185	0.154	0.059	-0.182	-0.152	-0.109	-0.371	0.355	-0.307	0.346	0.181
	P	0.518	0.187	0.145	0.283	0.68	0.134	0.448	0.528	0.81	0.456	0.534	0.656	0.118	0.135	0.2	0.147	0.457
Canopy closure	R	-0.187	-.528*	-0.142	-0.036	-.489*	-0.235	-0.35	0.366	0.176	-0.027	0.041	-0.379	0.065	.493*	-.620**	-0.124	-0.14
	P	0.443	0.02	0.561	0.883	0.033	0.334	0.141	0.124	0.47	0.912	0.868	0.109	0.792	0.032	0.005	0.612	0.567
CWD m^3	R	-0.21	0.037	0.354	-0.075	0.09	-0.316	-0.163	0.287	0	-0.168	-0.127	-0.265	-0.032	0.239	-0.108	0.346	-0.184
	P	0.388	0.881	0.137	0.761	0.714	0.187	0.506	0.233	0.999	0.492	0.604	0.272	0.896	0.324	0.66	0.146	0.451
Veg cover (%)	R	0.001	0.272	0.085	0.259	0.32	0.109	0.422	-0.247	0.211	.500*	0.137	0.062	-0.04	-0.287	0.303	0.101	0.019
	P	0.997	0.259	0.731	0.284	0.181	0.658	0.072	0.307	0.385	0.029	0.576	0.801	0.87	0.234	0.207	0.682	0.937
DO (%)	R	-0.011	-0.105	-0.452	-0.299	-0.313	-0.04	-0.291	-0.251	-0.139	-0.222	-0.033	.490*	-0.029	0.18	0.128	-.474*	-0.103
	P	0.964	0.678	0.06	0.228	0.205	0.874	0.242	0.314	0.581	0.376	0.896	0.039	0.909	0.475	0.613	0.047	0.685
pH	R	0.202	-0.272	0.193	0.382	-0.295	-.476*	-.584*	0.3	-0.213	-.528*	-.608**	0.09	-0.152	0.352	-0.217	0.162	0.177
	P	0.421	0.275	0.442	0.118	0.235	0.046	0.011	0.227	0.396	0.024	0.007	0.722	0.547	0.153	0.388	0.52	0.482
Conductivity (µS)	R	.601**	-0.396	-0.101	0.426	-0.425	-0.339	-0.287	0.446	-0.09	-0.194	-0.197	-0.3	-0.289	0.415	-.503*	-0.076	.621**
	P	0.008	0.103	0.69	0.078	0.079	0.169	0.249	0.064	0.723	0.442	0.433	0.227	0.246	0.087	0.033	0.764	0.006
Water temp (°C)	R	0.031	0.227	-0.14	0.021	0.13	0.127	-0.023	-0.238	0.158	0.064	-0.013	0.229	-0.127	-0.093	0.358	-0.132	0.063
	P	0.902	0.365	0.579	0.933	0.606	0.617	0.927	0.341	0.531	0.8	0.96	0.362	0.616	0.713	0.145	0.602	0.805
Salinity (ppt)	R	.601**	-0.412	-0.115	0.433	-0.447	-0.343	-0.291	0.436	-0.092	-0.189	-0.179	-0.279	-0.293	0.426	-.509*	-0.091	.616**
	P	0.008	0.089	0.649	0.073	0.063	0.163	0.241	0.07	0.716	0.454	0.477	0.262	0.238	0.078	0.031	0.719	0.007
fluorometer	R	0.117	0.029	-0.265	-0.069	-0.033	0.162	0.068	-.678**	0.134	0.317	.540*	.765**	-0.239	-0.22	0.466	-0.278	0.099
	P	0.643	0.909	0.287	0.787	0.895	0.522	0.788	0.002	0.597	0.199	0.021	<0.00	0.34	0.379	0.051	0.264	0.695
Chl-a (µg/L)	R	0.355	-0.105	-0.284	-0.16	-0.16	-0.016	-0.125	-.485*	-0.095	0.012	0.111	.713**	-0.232	-0.085	0.331	-0.301	0.348
	P	0.148	0.678	0.254	0.526	0.526	0.951	0.62	0.041	0.709	0.962	0.66	0.001	0.355	0.737	0.18	0.224	0.157
Air temp (°C)	R	0.053	-0.01	0.393	0.215	0.184	-0.073	0.231	0.013	-0.153	0.285	0.049	0.003	-0.067	-0.197	-0.011	0.383	0.05
	P	0.829	0.968	0.096	0.377	0.452	0.766	0.342	0.958	0.532	0.237	0.843	0.99	0.785	0.419	0.963	0.106	0.84
LDI SCORE	R	-0.269	0.029	.669**	0.227	0.301	-0.187	-0.011	0.332	0.15	-0.081	-0.416	-0.455	-0.157	0.098	-0.234	.661**	-0.234
	P	0.266	0.907	0.002	0.35	0.211	0.442	0.966	0.166	0.54	0.742	0.076	0.05	0.522	0.69	0.334	0.002	0.336
KY-WRAM	R	-0.304	-0.2	0.106	0.032	-0.099	-0.156	0.136	0.446	0.047	0.168	0.221	-.641**	0.308	0.204	-.487*	0.119	-0.237
	P	0.206	0.411	0.666	0.897	0.686	0.523	0.58	0.055	0.85	0.492	0.363	0.003	0.2	0.403	0.035	0.627	0.33
ORAM Score	R	-0.304	-0.124	-0.057	0.003	-0.111	-0.071	0.126	0.384	0.026	0.158	0.27	-.557*	0.399	0.135	-0.372	-0.044	-0.24
	P	0.206	0.613	0.816	0.989	0.651	0.773	0.607	0.104	0.915	0.52	0.263	0.013	0.091	0.58	0.117	0.859	0.322
Metric 1	R	-0.368	0.21	0.362	0.109	0.235	-0.086	0.102	0.283	-0.026	-0.006	-0.056	-0.418	0.226	-0.089	-0.041	0.361	-0.321
	P	0.121	0.389	0.128	0.657	0.333	0.726	0.679	0.241	0.916	0.981	0.821	0.075	0.353	0.716	0.866	0.129	0.18
Metric 2	R	-0.269	-0.038	-0.294	0.002	-0.187	0.145	0.106	0.153	-0.12	0.178	.487*	-0.182	.456*	-0.036	-0.131	-0.289	-0.256
	P	0.266	0.876	0.221	0.994	0.443	0.553	0.665	0.531	0.625	0.465	0.035	0.457	0.05	0.884	0.594	0.229	0.291
Metric 3	R	-0.344	-0.246	-0.193	-0.029	-0.217	-0.059	0.069	-0.011	0.052	0.32	0.366	-0.052	0.269	0.131	-0.182	-0.2	-0.314
	P	0.15	0.31	0.43	0.905	0.373	0.812	0.778	0.966	0.832	0.182	0.123	0.833	0.265	0.593	0.455	0.412	0.191
Metric 4	R	-0.23	-0.313	-0.107	-0.065	-0.248	-0.122	-0.002	0.424	0.039	0.062	0.045	-.555*	0.386	0.264	-.535*	-0.091	-0.171
	P	0.344	0.193	0.663	0.793	0.307	0.618	0.994	0.07	0.873	0.8	0.855	0.014	0.103	0.274	0.018	0.711	0.484
Metric 6	R	-0.138	-0.065	0.41	0.08	0.114	-0.226	0.161	.507*	0.111	0.023	0.076	-.747**	0	0.214	-.464*	0.428	-0.083
	P	0.574	0.79	0.081	0.745	0.643	0.352	0.51	0.027	0.652	0.927	0.757	<0.00	0.999	0.378	0.045	0.068	0.734

Multiple Regression Analysis Results

The multiple regression analyses of macroinvertebrate metrics to and 10 habitat variables revealed eleven significant models at the P = 0.05 level (Table 6). For seven invertebrate metrics, the variables retained in the final multiple regression models accounted for greater than 50% of the variance in the invertebrate metric; including: Simpson's diversity index, Shannon Weiner diversity index, taxa richness, percent collector-gatherers and filterers, percent shredders and scrapers, Simpson's diversity index without Nematoda, and richness without Nematoda (Table 6). Among the macroinvertebrate metrics, the model for Simpson's diversity accounted for the most variation. Positive slopes suggest that wetlands with more trees, greater vegetative cover, and deep water tend to have higher Simpson's diversity. Negative slopes for suggest that, after accounting for the other variables, wetlands with lower averages of DBH of dead trees, fewer hummocks and tussocks, and less canopy closure would have a higher Simpson's diversity of macroinvertebrates.

Table 6. Final model of multiple regressions of macroinvertebrate metrics regressed with habitat variables. Statistics for Beta (B), P values (P), R^2, and F values are listed. Non-significant models are denoted by "ns".

	B	P	R^2	F
Abundance		**ns**	**ns**	**ns**
Simpson's Diversity		**0.003**	**0.770**	**6.696**
Number of trees	0.619	0.002	0.757	
Avg DBH dead trees	-0.316	0.072	-0.494	
Tussocks and Hummocks	-0.279	0.094	-0.465	
Canopy Closure	-0.566	0.003	-0.724	
Vegetative Cover	0.298	0.092	0.468	
Maximum Depth	0.294	0.087	0.474	
Shannon Weiner		**0.003**	**0.601**	**7.543**
Number of trees	0.599	0.004	0.662	
Canopy closure	-0.551	0.005	-0.643	
Vegetative Cover	0.398	0.037	0.509	

Table 6. (Continued)

	B	P	R^2	F
Richness		**0.005**	**0.566**	**6.510**
Perimeter	0.672	0.002	0.694	
Tussocks and Hummocks	0.478	0.017	0.570	
Canopy Closure	-0.423	0.035	-0.514	
ETO		**0.067**	**0.287**	**3.220**
Vegetative Cover	0.383	0.099	0.401	
Maximum Depth	0.485	0.041	0.486	
Percent PP, PE		**0.072**	**0.178**	**3.693**
Vegetative Cover	0.422	0.072	0.422	
Percent CG, CF		**0.004**	**0.495**	**7.828**
Distance to paved road	-0.510	0.012	-0.580	
Maximum Depth	0.550	0.007	0.609	
Percent S, SH		**<0.001**	**0.620**	**13.064**
Number of trees	0.800	<0.001	0.776	
Vegetative Cover	0.466	0.011	0.583	
Percent Odonata		**0.024**	**0.371**	**4.722**
Leaf Litter depth	0.348	0.099	0.402	
Vegetative Cover	0.498	0.023	0.532	
Percent Corixini		**0.009**	**0.340**	**8.739**
Distance to paved road	0.583	0.009	0.583	
Percent Nematoda		**0.007**	**0.464**	**6.918**
Distance to paved road	0.498	0.016	0.560	
Maximum Depth	-0.528	0.011	-0.582	
Percent Oligochaeta		**ns**	**ns**	**ns**
Percent Chironomidae		**0.032**	**0.243**	**5.468**
Canopy Closure	0.493	0.032	0.493	
Simpson's diversity, without Nematoda		**<0.001**	**0.723**	**13.034**
Number of trees	0.395	0.017	0.571	
Canopy Closure	-0.748	<0.001	-0.801	
Distance to paved road	0.567	0.001	0.716	
Richness without Nematoda		**0.005**	**0.565**	**6.482**
Perimeter	0.678	0.002	0.697	
Tussocks and Hummocks	0.464	0.018	0.567	
Canopy Closure	-0.406	0.042	-0.498	
Abundance without Nematoda		**ns**	**ns**	**ns**

Nine of the 17 macroinvertebrate community metrics were significantly related to water quality measures (Table 7). Only the models for abundance metrics, ETO taxa

richness, and percent Nematoda explained greater than 50% of the variance. Salinity and chlorophyll-a were significant predictors of abundance. Chlorophyll-a and DO were significant predictors of percent Nematoda, and salinity and pH were significant predictors of ETO taxa. Regarding the ETO taxa metric, pH and salinity had significant positive slopes, indicating that the higher the pH and salinity, the greater number of ETO taxa. Dissolved oxygen and chlorophyll-a had significant positive slopes as predictors of the proportion of Nematoda, indicating that the higher the DO and chlorophyll-a, the higher the percentage of Nemaoda in a wetland. For abundance with Nematoda excluded, positive slopes indicate greater macroinvertebrate abundance should be found in wetlands with higher chlorophyll-a and salinity.

Table 7. Water quality variables retained in the final model of the multiple regression analysis compared to macroinvertebtrate metrics. Multiple regression beta (B), significance (P), R^2, F values are given in bold for the final models. No data for wetland #3, Sandgap was recorded due to early dry-down. pH for wetland #13, Cliff Palace was excluded as an outlier.

	B	P	R^2	F
Abundance per net dip		**0.008**	**0.496**	**6.894**
Salinity (ppt)	0.617	0.006	0.655	
Chlorophyll-a (µg/L)	0.386	0.062	0.477	
Richness		**0.029**	**0.398**	**4.627**
DO (%)	-0.458	0.045	-0.506	
Air temp (°C)	0.391	0.082	0.448	
Percent Collector gatherers and filterers		**0.016**	**0.444**	**5.593**
Salinity (ppt)	0.38	0.078	0.454	
Chlorophyll-a (µg/L)	-0.521	0.021	-0.572	
ETO		**0.008**	**0.501**	**7.021**
pH	0.571	0.009	0.627	
Salinity (ppt)	0.368	0.073	0.46	
Percent Nematoda		**<0.001**	**0.737**	**19.63**
DO (%)	0.483	0.003	0.686	
Chlorophyll-a (µg/L)	0.714	**<0.001**	0.812	
Simpson's Diversity, excluding Nematoda		**0.043**	**0.245**	**4.871**
Salinity (ppt)	-0.495	**0.043**	-0.495	
Richness excluding Nematoda		**0.028**	**0.401**	**4.685**
DO (%)	-0.475	0.039	-0.521	
Chlorophyll-a (µg/L)	0.375	0.093	0.434	
Abundance excluding Nemtatoda		**0.007**	**0.512**	**7.289**
Salinity (ppt)	0.632	0.005	0.669	
Chlorophyll-a (µg/L)	0.380	0.062	0.476	

Wetland assessment and LDI scores were significantly related to macroinvertebrate community metrics in 10 of the 17 models (Table 8). The wetland assessment metric variables explained 50% or more of the variance in the final models for the richness and for percent Nematoda macroinvertebrate metrics. Variables retained in models for Simpson's diversity index, percent Oligochaeta, and percent Corixini account for between 45% and 50% of the variance in the metrics. Of the wetland

assessment metrics, LDI scores, KY-WRAM metrics 2 and 4, and total KY-WRAM score were retained most often in regression models. LDI was important in 6 of the models. Metric 4 was retained in seven of the models. Finally, metric 2 and the total KY-WRAM score were retained in five of the final models. For taxa richness, KY-WRAM had a significant positive slope, indicating wetlands scoring higher on the KY-WRAM would be expected to have greater taxa richness, after controlling for all other assessment metrics in the model. However, after accounting for other variables, negative slopes suggest that wetlands with higher metric 2 and 4 scores would have lower taxa richness.

Table 8. Wetland rapid assessment metrics retained in the final model of the multiple regression analysis compared to macroinvertebtrate metrics. Multiple regression beta (B), significance (P), R^2, F values are given for the final models. Non-significant models are denoted by "ns". * Metric 5 was not included as it has no bearing on the community composition of the wetland.

	B	P	R^2	F
Abundance per 1 meter dip		**0.080**	**0.271**	**2.97**
LDI	-0.504	0.053	-0.463	
Metric 2	-0.504	0.053	-0.463	
Simpson's Diversity		ns	ns	ns
Richness		**0.013**	**0.501**	**5.025**
KYRAM	1.593	0.003	0.672	
Metric 2	-0.719	0.01	-0.604	
Metric 4	-1.179	0.015	-0.58	
Shannon Wiener		ns	ns	ns
Evenness		ns	ns	ns
Percent Predators		ns	ns	ns
Percent Collector gatherers and filterers		**0.037**	**0.338**	**4.081**
LDI score	0.401	0.069	0.438	
Metric 4	0.482	0.032	0.506	
Percent Shredders and scrapers		ns	ns	ns
Percent Odonata		ns	ns	ns
Percent Corixini		**0.018**	**0.478**	**4.573**
LDI score	-0.591	0.009	-0.612	
KY-WRAM	1.291	0.01	0.607	
Metric 4	-1.195	0.016	-0.573	
Percent Nematoda		**<0.001**	**0.746**	**14.713**
LDI score	-0.502	0.002	-0.7	
ORAM	-0.928	0	-0.82	
Metric 3	0.518	0.01	0.603	
Percent Oligochaeta		**0.039**	**0.491**	**3.37**
KY-WRAM	-1.621	0.025	-0.557	
Metric 1	0.617	0.033	0.533	
Metric 2	0.678	0.024	0.559	
Metric 4	1.401	0.021	0.572	
Percent Chironomidae		ns	ns	ns
Simpson's Diversity excluding Nematoda		**0.022**	**0.462**	**4.293**
ORAM	0.802	0.086	0.429	
Metric 4	-0.979	0.021	-0.554	
Metric 6	-0.51	0.063	-0.46	

Table 8. (Continued)

	B	P	R^2	F
Richness excluding Nematoda		**0.013**	**0.500**	**5.004**
KY-WRAM	1.592	0.003	0.671	
Metric 2	-0.725	0.01	-0.607	
Metric 4	-1.109	0.016	-0.572	
Abundance per dip without Nematoda		**0.131**	**0.225**	**2.317**
LDI score	-0.451	0.089	-0.413	
Metric 2	-0.466	0.079	-0.424	

Ordination Results

The first two axes of the PCA explained 38.01% of the variation in the macroinvertebrate communities, with PC1 and PC2 accounting for 19.98% and 18.03% of the variation, respectively (Figure 2). The ordination suggests clustering of some taxonomic groups because of associations with particular wetland sites (Table 9). For example, *Bezzia* and *Dytiscus* are associated along both PC1 and PC2 and show high dissimilarity to *Culex* on PC2. This means that *Bezzia* and *Dytiscus* are more likely to occur together than in association with *Culex.*

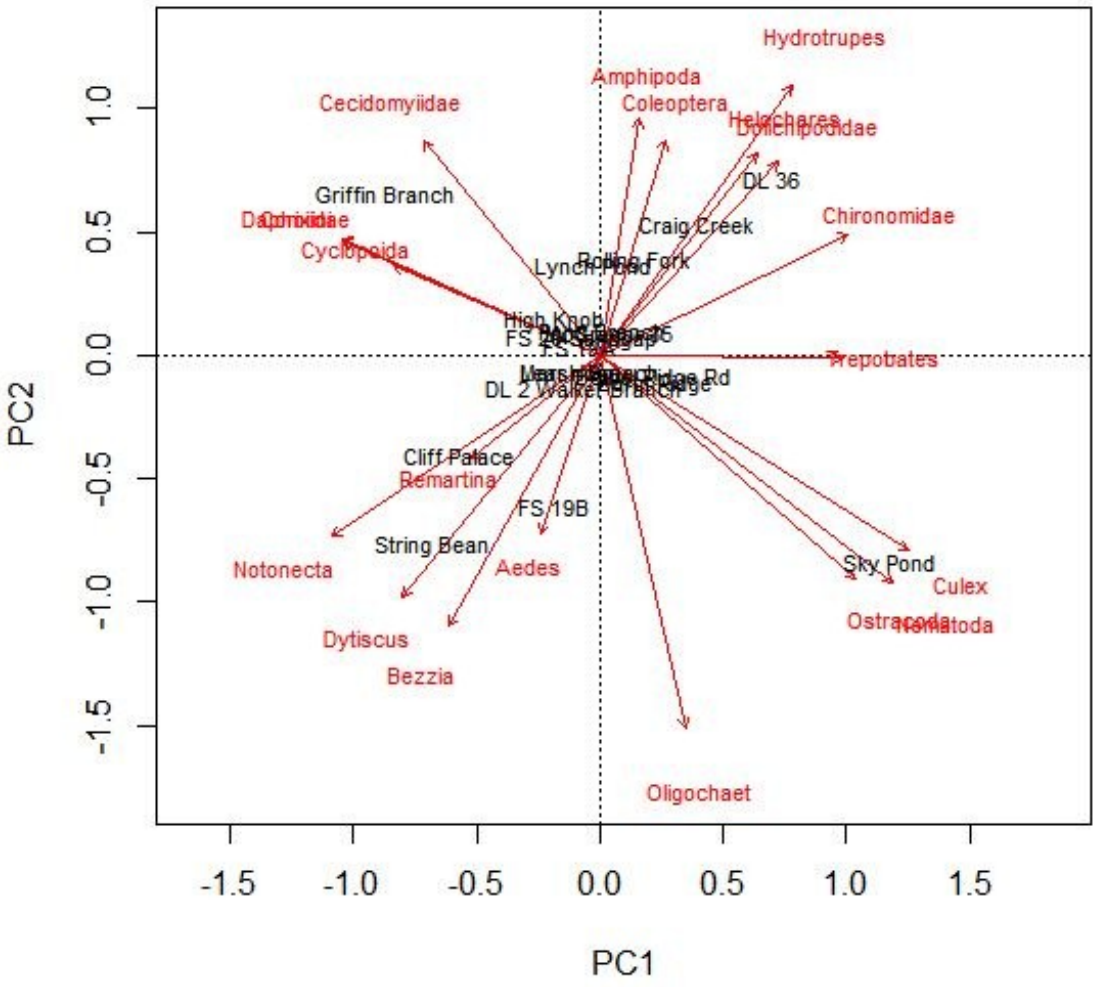

Figure 2. Principal component analysis (PCA) of the macroinvertebrate communities of 19 forested depressional wetlands of the Upper Cumberland Basin of Kentucky. Length of the vectors indicate the correlation of individual taxa with each of the axes of the PCA. Correction: Cecidomyiidae should read Phoridae. Coleoptera refers to the Coleoptera egg cases.

Table 9. Loading scores of each taxon for PC1 and PC2. Taxa included are the most commonly occurring taxa of the 19 wetlands assessed in the Upper Cumberland River Basin of Kentucky, May 2011.

	PC1	PC2
Aedes	-0.12779	-0.361171
Amphipoda	0.08445	0.479752
Bezzia	-0.3227	-0.547992
Cecidomyiidae	-0.37698	0.434675
Chironomidae	0.52769	0.246387
Coleoptera_egg_case	0.13935	0.435734
Corixini	-0.54893	0.236624
Culex	0.6581	-0.393342
Cyclopoida	-0.442	0.181392
Daphniidae	-0.54949	0.233574
Dolichipodidae	0.37891	0.393391
Dytiscus	-0.42125	-0.488273
Helochares	0.33629	0.411272
Hydrotrupes	0.40944	0.546276
Nematoda	0.6257	-0.459875
Notonecta	-0.5706	-0.365037
Oligochaeta	0.18228	-0.754052
Ostracoda	0.5452	-0.453108
Remartina	-0.27258	-0.208976
Trepobates	0.5178	-0.005515

Two of seven wetland assessment variables, metric 2 and metric 6, were significantly correlated with the principal component scores (Figure 3, Table 10). Sites with higher abundance of Cecidomyiidae, Daphniidae, and Corixini had higher KY-WRAM and Metric 6 scores, whereas sites with higher KY-WRAM, ORAM, and metric scores tended to have lower abundances of Oligochaeta, Ostracoda, Nematoda, and Culex.

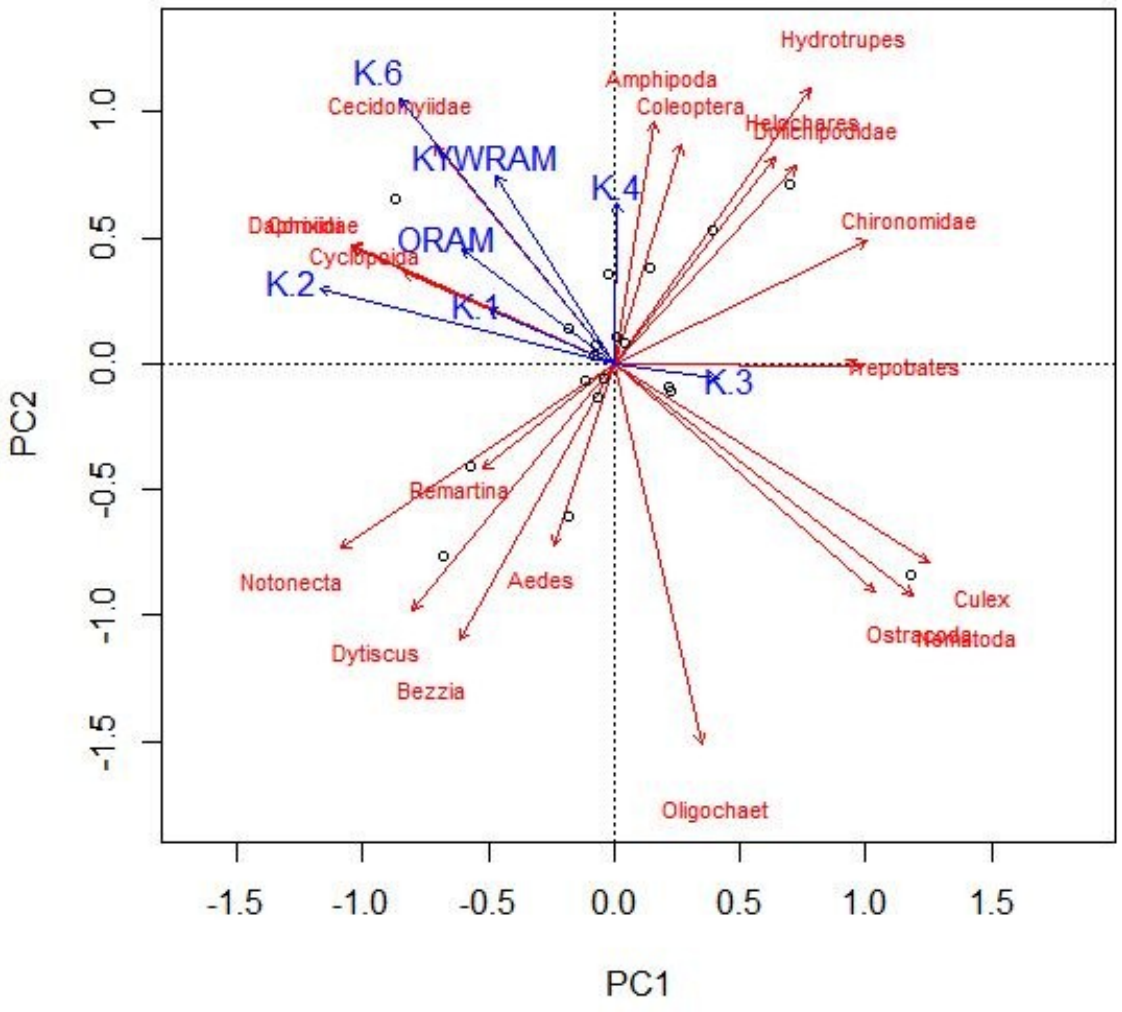

Figure 3. Principal component analysis (PCA) of the macroinvertebrate communities of 19 forested depressional wetlands of the Upper Cumberland Basin of Kentucky. Correlations of KY-WRAM metric scores with PCA axes is shown with blue vectors. Length of the red vectors indicate the strength of correlations of individual taxa with the PCA axes. Correction: Cecidomyiidae is incorrect and should read Phoridae. "Coleoptera" refers to the Coleoptera egg cases.

Table 10. Table of correlations of wetland assessment scores to PC1 and PC2. These coordinates are plotted in Figure 3 to compare with macroinvertebrate species groupings. The R^2 values and significance values (P) are also included.

Vectors	PC1	PC2	R^2	P
KY-WRAM	-0.532261	0.84658	0.1742	0.225
ORAM	-0.079845	0.602061	0.1287	0.359
Metric 1	-0.916728	0.399511	0.0663	0.554
Metric 2	-0.968897	0.247465	0.3264	0.036*
Metric 3	0.991325	-0.131434	0.0387	0.754
Metric 4	0.016714	0.99986	0.0921	0.478
Metric 6	-0.629199	0.777245	0.4127	0.012*

Note: * Statistically significant

The forward, stepwise approach to Redundancy analysis (RDA) yielded a single significant variable and two marginally significant variables: maximum depth (F = 2.640, P = 0.011,), canopy closure (P = 0.069, F = 1.875), and hummocks and tussocks (F = 1.774, P = 0.104). These three variables were included in the final RDA, which explained 25.5% of the variation in the invertebrate communities (F = 2.11, P = 0.007, R^2 = 0.16; Figure 4). When split between the axes, RDA1 and RDA 2 explained 17.1% and 8.4% of the variance, respectively. When inspecting the figure, it appears that Daphniidae tends to associate with deeper wetlands, while Chironomidae is associated with more canopy closure.

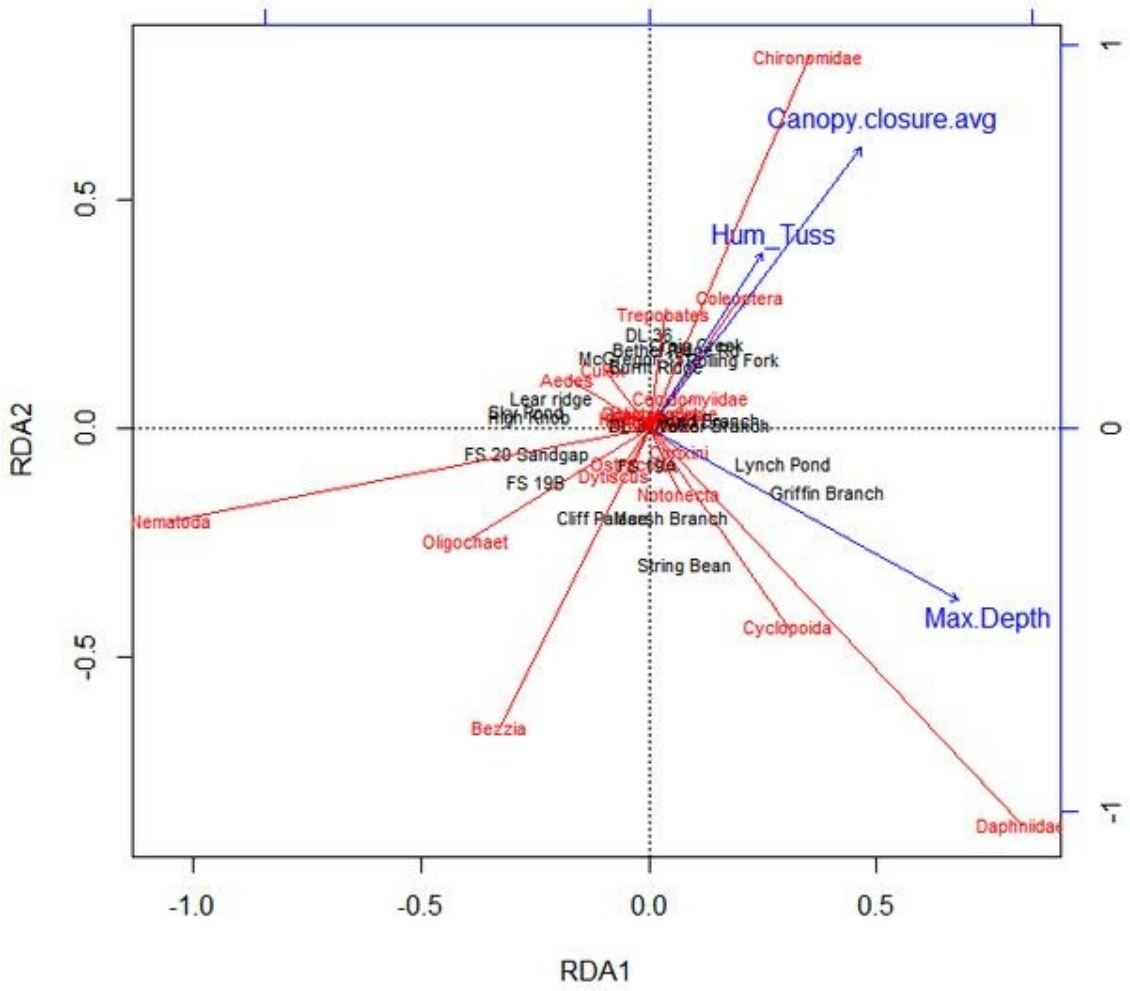

Figure 4: Redundancy Analysis (RDA) of macroinvertebrate communities explained by nine habitat variables and 6 water quality variables, of which only maximum depth was significant and canopy closure and hummocks + tussocks were marginally non-significant and added to the model. Of the variation in species data, 25% can be explained by the three habitat variables. Correction: Cecidomyiidae is incorrect and should read Phoridae. "Coleoptera" refers to Coleoptera egg cases.

IV. DISCUSSION

Macroinvertebrate Community Structure Predicted by Habitat Variables and Water Quality

The RDA and multiple regression were consistent in suggesting hydrology has important effects on invertebrate communities. In the RDA, Daphniidae and Cyclopoida are strongly associated with maximum depth. Batzer (2013) found that microcrustacean richness was strongly associated with hydroperiod. In the multiple regression analyses, Daphniidae and Cyclopoida were captured in the "percent collector-filterers and collector-gatherers" (CF/CG) metric, which produced a significant model showing a positive weight to maximum depth, as is corroborated by the RDA. However, metric 3 of the KY-WRAM, which captures hydrology and presumably maximum depth, was not significant in the percent CG/CF final model. Thus, the variation in water depth at the wetlands surveyed for this project is likely natural variation that has been only minimally impacted by human disturbance.

The other three metrics that included maximum depth in the final model include: Simpson's diversity, ETO taxa, and percent Nematoda, No metrics were correlated to maximum depth in the pairwise univariate correlation matrix (Table 5). However, in several long-term, intensive macroinvertebrate studies, hydrology was the most important factor determining variation in macroinvertebrate communities (Batzer 2013). It is important to note that hydroperiod and invertebrate metrics, like taxa richness, do not always exhibit a linear relationship. In fact, some very strong polynomial patterns have emerged in other studies (Batzer 2013). Comparing Simpson's diversity to maximum depth in this study, a quadratic pattern is discernable. It is possible that maximum depth

and hydroperiod could play a larger role than what has been detected by the linear regression analyses approach of this study.

Canopy closure, an important variable in explaining species variation in the RDA model, is significant in six final models of invertebrate metrics tested in the multiple regression analysis, including Simpson's diversity, Shannon Weiner diversity, taxa richness, percent Chironomidae, and Simpson's diversity and richness excluding Nematoda. In all of these metrics, except for percent Chironomidae, canopy closure had a negative slope. This corroborates the strong positive association of Chironomidae to canopy closure in the RDA ordination. Typically in forested wetlands, a high canopy closure is associated with minimal disturbance (King et al. 2000, Batzer 2004, Cooper et al. 2006, Theriault 2009). Expectedly, Ohio EPA (2002) found that chironomid relative abundance and number of taxa were higher in natural sites. Lunde and Resh (2012) found "% Tanypodinae/Chironomidae" to be a positive sensitive metric in the development of an IBI for California wetlands. This suggests that with additional studies and perhaps greater taxonomic resolution in the Chironomidae, percent chironomids or chironomid taxa richness could be a valuable metric to include in development of MIBI's for forested depressional wetlands in Kentucky. However, the percent chironomids metric was not a sensitive metric to reflect wetland assessment scores in this study. The negative relationship of canopy closure in several models was unexpected. Since angular canopy closure was taken at the wetland's edge, it is possible that it is a reflection of the forest around the wetland, rather than the trees within the wetland. Considering that I sampled all known forested depressional wetlands in the Upper Cumberland River Basin within the Daniel Boone National Forest and that the variation in KY-WRAM and ORAM total

scores is relatively low, this system appears to show a weak gradient of human disturbance simply because they are relatively undisturbed. It is possible that wetlands in this system subjected to heavy disturbance are now completely gone. The wetlands that have been impacted by mowing or road impoundments have a more open canopy closure relative to other wetlands in this study, but are more similar to one another when compared to severely impacted wetlands of other types and in other locations in Kentucky. Therefore, the wetlands with lower canopy closures are only intermediately impacted and, thus, the "Intermediate Disturbance Hypothesis" (Townsend et al. 1997) may be applicable here. Wetlands that are intermediately disturbed (the low canopy closure sites) allow enough light in to increase productivity in the photic zone and open niches for more species to colonize, increasing taxa richness, species diversity, and abundance in those intermediately disturbed sites which, in the case of this system, appeared to be the most disturbed sites.

Hummocks and tussocks was a marginally non-significant variable included in the RDA that helps explain some of the variance in the macroinvertebrate community (Figure 4). Hummocks and tussocks was included in only three final models in the multiple regression analyses. While hummocks and tussocks had positive slopes for the models of richness and richness without Nematoda metrics, it had a negative slope in the model of Simpson's diversity. Since Simpson's diversity takes into account richness and evenness, perhaps the evenness component is responsible for the negative association with hummocks and tussocks, indicating that the presence of hummocks and tussocks positively influences species richness, but not evenness. Regarding the univariate analysis, evenness was not significantly correlated to hummocks and tussocks, but there

was a trend for a negative correlation (Table 5). Richness, though not significant in the univariate model, displayed a positive correlation. This, coupled with the positive slopes of richness to hummocks and tussocks in the multiple regression analyses, supports the notion that evenness is contributing to the negative slope in the multiple regression. Not unexpectedly, since evenness evaluates how equal in number each species is, the more hummocks and tussocks, the less even the species distribution becomes. This heterogeneous habitat provides niches for differing species, causing unevenness in the species distribution. The species closely associated with hummocks and tussocks in the RDA model include Trepobates (Coleoptera), a Coleoptera egg casing, and Chironomids. The hummocks and tussocks provide habitat for such taxa, thereby increasing their relative abundance in the system.

Since the RDA and multiple regressions corroborate some important relationships, the macroinvertebrate metrics appear to be sufficient descriptions of the macroinvertebrate community as a whole. However, percent vegetative cover, which was not significant in the RDA model, was important in explaining variance in invertebrate metrics in six multiple regression models including: Simpson's and Shannon Weiner diversity indices, ETO taxa richness, percent predators, percent shredders and scrapers, and percent Odonata, and showed a positive slope for all of these models. Not surprising, second to hydrology, plant associations within the wetland are important in explaining macroinvertebrate community composition in other studies (Battle and Golladay 2001, Batzer 2013).

No water quality variables were found to be significant in the RDA model. However, several regression models indicated that macroinvertebrate metrics were

sensitive to water quality parameters. Interestingly, abundance was positively correlated to salinity and chlorophyll-a in the final multiple regression model (Table 7). Batzer (2013) also discussed similar findings of increased abundance in polluted or higher-nutrient wetlands. This initial rise is usually followed by a decline if the problem persists or becomes severed. This is further evidence of the "Intermediate Disturbance Hypothesis" in this wetland type, and possibly of a sensitive early warning metric for disturbed wetlands.

In the multiple regression analyses of ETO taxa richness, pH and salinity were strong, positive predictors. The pH ranged from 5.15 to 7.18, excluding Cliff Palace, suggesting that the sensitive ETO taxa reside in wetlands with a more neutral rather than acidic pH. As is the case with this study, lower pH values often result in fewer acid-intolerant species (e.g. ETO taxa) (Rosenburg and Resh 1993, Longcore et al. 2006).

The third metric in which greater than 50% of its variance could be explained by water quality parameters was percent Nematoda. Chlorophyll-a and dissolved oxygen were positively related in the model explaining the variance in percent Nematoda. Considering that percent Nematoda was positively correlated to distance to paved road and negatively correlated to maximum depth in the habitat multiple regression model, it is difficult to discern if percent Nematoda is a positive or negative metric for prediction of wetland condition. Bonger and Ferris (1999) reviewed the use of nematode community structure as a bioindicator in environmental monitoring and found that Nematoda taxa vary in sensitivities to environmental disturbance. Since this study did not identify the Nematoda to a lower taxonomic level, it is difficult to draw conclusions of the relevance of this metric. Communities may shift to more tolerant taxa without a decrease in

abundance of Nematoda in general. Since Nematoda occurred frequently in the study sites, it was important to include them in some analyses; however, the decision was made to exclude Nematoda from some metrics such as Simpson's Diversity, richness, and abundance, since its relevance as an indication of the macroinvertebrate community health is unknown.

Macroinvertebrate Community Structure Shifts with Wetland Assessment Scores

The PCA (Figure 3) shows that microcrustaceans (Copepoda [Cyclopoidia] and Cladocera [Daphniidae]) are closely aligned with KY-WRAM metric scores, especially metrics 2 and 6. This suggests that microcrustaceans may be important biological indicators and useful in future analysis of the biological integrity of wetlands. Indeed, Ohio EPA (2004) used microcrustaceans as a metric for the development of a MIBI for wetlands in Ohio. Microcrustacean abundance (higher metric score) was positively related to ecological condition of wetlands. Ohio EPA (2004) suggested that greater taxonomic resolution may be required for microcrustaceans (Ostracods, Copepods, Cladocerans) to develop more precise metrics. Percent CG/CF (mirocrustaceans are captured in this metric) was positively correlated to LDI and Metric 4 (Habitat Alteration) scores in the multiple regression analyses. High LDI scores indicate greater landscape development intensity; therefore, the more developed the site, the greater the proportion of CG/CF. Conversely, a lower score for metric 4 indicates greater habitat alteration. Therefore, the less altered the habitat, the more CG/CF. These conflicting results may be a result of relatively low LDI scores in this system, since all wetlands are in the Daniel Boone National Forest. This gradient may be too small to show meaningful relationships.

Corixini were also highly correlated with Metric 2 (Buffers) and KY-WRAM scores in the PCA (Figure 3), but did not show a strong correspondence to metric 4 (Habitat Alteration), which is corroborated by the multivariate analyses. Corixini was not expected to have a positive relationship to wetland quality based on other studies that observed higher corixid abundance in degraded wetlands. While exploring the development of a wetland invertebrate community index for Ohio, Ohio EPA (2004) found that corixid abundance was higher at the mitigation sites, when compared to reference sites. Gernes et al. (2002) found a significant, but weak correlation: Corixidae proportion increased with their Human Disturbance Score. Burton et al. (2004) found that Corixidae tended to reside in exposed wetlands. In this study, percent Corixini was not significantly correlated to any habitat variables in the multiple regression analyses; however, when considering the WRAM metrics, Corixini was negatively correlated to LDI scores and metric 4 (habitat alteration and soil disturbance) and positively correlated to the KY-WRAM. In this study, Corixini abundances were actually greater in wetlands rated as higher quality, which is opposite of the studies described above. Again, the "Intermediate Disturbance Hypothesis" may apply here.

Richness was positively correlated to KY-WRAM scores and negatively correlated to Metrics 2 and 4 in the multiple regression analyses. Metrics 2 and 4, in the final model, do not account for as much variance as KY-WRAM scores, after effects of other variables are controlled. The KY-WRAM, which was the most significant relationship in the complete model ($P = 0.003$), shows a strong positive relationship ($R^2 = 0.672$). However, metric 6, which was not statistically significant in the multiple regression model, shows a stronger positive relationship to taxa richness if viewed as a

biplot (Figure 5). Perhaps a type 2 error occurred and metric 6 is biologically significant to the macroinvertebrate community.

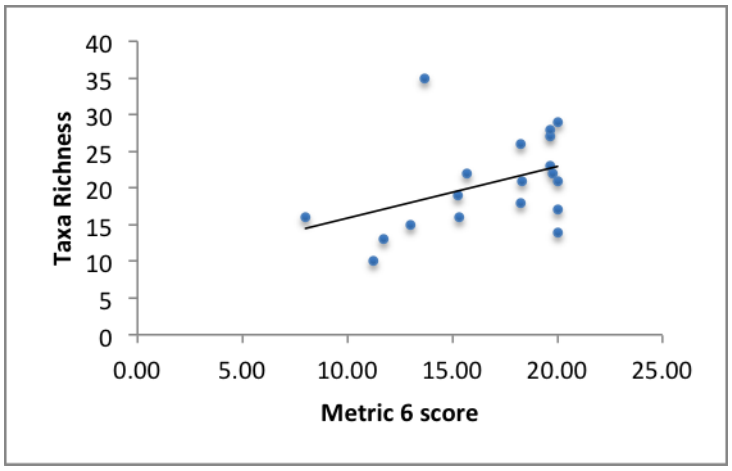

Figure 5. Biplots of Taxa Richness to Metrics 6 scores and also to the KY-WRAM score of the 19 isolated depressional wetlands surveyed in the Upper Cumberland River Basin.

Conclusions and recommendations

It is important to characterize the macroinvertebrate community of wetlands before beginning to understand how anthropogenic changes impact the community. Being isolated, depressional, ridge top wetlands (Table A1, Appendix), most of the sampled wetlands were small, but fairly natural and pristine forested wetlands. The only major human disturbance observed was occasional road impoundment and mowing. Other wetlands of this type that were more severely impacted no longer exist in this region. Therefore, even though every known wetland of this type was surveyed in the Upper Cumberland River Basin, these natural wetlands did not show a wide gradient of wetland conditions with KY-WRAM scoring. Also, the invertebrate community can change during the course of the year and from year to year (Batzer 2013). For example, I returned to one wetland in May 2012 to sample for a different project and found much

greater abundances, some added diversity, and invertebrates in later larval stages. The sampling season of May 2011 had proven to be an unseasonably cold and rainy year, thus many of the macroinvertebrates could have retreated into substrate and could have experienced arrested or slow development. More research may be needed to establish typical communities of this wetland type.

Another limitation in the methods was the water quality sampling having taken place one week following the macroinvertebrate sampling was completed. This, therefore, could not provide a complete picture of the condition of the wetland at the time of macroinvertebrate sampling. This could explain why I found no water quality variables were important in determining species distribution in the RDA and the seemingly spurious relationships that were detected in the multiple regression analyses. Also, the likelihood of type 1 error is great due to the sheer number of variables that were analyzed in this study.

Wetlands vary widely in physical, chemical, and biological characteristics across regions and even across time within the same wetland. Therefore, it is difficult to separate natural from human-induced variation (Rader 2001), and some studies have concluded that the use of macroinvertebrates as indicators of ecological integrity of wetlands may be questionable or require specific indices for region and wetland type (Burton 1999, Davis et al. 2006, Suren et al. 2011). Batzer (2013) also concluded that descriptive approaches to macroinvertebrate studies in wetlands could inevitably produce enigmatic results considering that every wetland has unique attributes and assuming that macroinvertebrates are highly sensitive to environmental conditions.

Whether macroinvertebrates are highly sensitive to specific conditions that change from year to year and wetland to wetland or insensitive and well adapted to the harsh wetland environment, numerous studies have shown that replicating results or attempting to corroborate other work to create generalizations about wetland invertebrates is rarely conclusive and sometimes contradictory (Batzer 2013). In spite of the evidence that generalizations about macroinvertebrate communities might be challenging, several studies have attempted and found some success in creating an index of biotic integrity for wetlands using invertebrates (Helgen and Gernes, 2002; Ohio EPA, 2008; and Lunde and Resh, 2013). However, a gradient of reference to degraded sites sampled over a long period of time is required to develop an MIBI. The development of a final macroinvertebrate index of biotic integrity (MIBI) for depressional wetlands of this region was not the goal of this study, due to limited time, wetlands sites, and the lack of an a priori gradient of wetland conditions with known anthropogenic influences.

However, the findings of this study supply important information to initialize the development of an MIBI for depressional wetlands in this region. Several metrics appear promising for future MIBI development: richness, percent Corixini, percent Chironomidae, percent Oligochaeta, and percent CG/CF (especially considering Daphniidae and Cyclopoida). It is recommended that greater taxonomic resolution be conducted for these groups. Then, the number of taxa and also the sensitivities of those taxa can be used to develop more precise and sensitive metrics. I also recommend for the CG/CF or Daphniidae to become a combined "Microcrustaceans" group to include cladocerans, copepods, and ostracods, as seen in Ohio EPA (2008).

Generally, macroinvertebrate taxa richness decreases as wetland quality declines (Burton et al. 1999, Helgen and Gernes 2001, Urzarski et al 2004). This supports all of my analyses except that richness was negatively correlated with canopy closure and %DO. However, Rader and Richardson (1994) observed an increase in taxa richness in nutrient enriched wetlands. Many nutrient enriched wetland also experience low %DO, although it is uncertain if nutrient enrichment is causing the lower %DO in this study.

Percent Corixini, percent Chironomidae, and percent Oligochaeta all show some significant models as metrics in multiple regression analyses and multivariate ordination. Percent Oligochaeta is a negative predictor for KY-WRAM scores while percent Corixini is a positive predictor (Table 8; Figure 3). Percent Chironomidae did not produce a significant multiple regression model of assessment scores, but was positively correlated with canopy closure in the habitat variable multiple regression model (Table 6) and also strongly associated with canopy closure in the RDA (Figure 4). Chironomidae proportions and/or taxa richness are used as metrics in other MIBI's (Gernes and Helgen, 2002; Ohio EPA, 2008; Lunde and Resh, 2012). Oligocheata proportion or taxa richness has also proven to be a sensitive metric for some wetland types (Ohio EPA, 2008; Lunde and Resh, 2012). Proportion of corixids has been used as a negative metric (Gernes and Helgen, 2002; Ohio EPA, 2008). All of the preliminary analyses agreed with the utility of these metrics with the exception of percent corixids. In previous studies, corixids were an indication of human disturbance or impaired wetlands. However, this study revealed strong relationships of the corixid taxa distribution to sites that received high KY-WRAM scores.

Considering the formation of the "Microcrustacean group", the strong correlations in the PCA and RDA analyses suggest that the cladocerans and copepods are predictors of high wetland quality. Lunde and Resh (2012) did not find any zooplankton metrics were sensitive to wetland quality and questioned the inclusion of zooplankton in sampling and identification since their importance was not demonstrated. However, Ohio EPA (2008) included microcrustaceans as one of 6 metrics in their final recommended MIBI.

Regarding the results of the multiple regression analyses and the multivariate analyses, many macroinvertebrate metrics responded to microhabitat features, notably percent vegetative cover, maximum depth, canopy closure, distance to paved road, and number of trees. Also, the PCA analyses demonstrates that metric 6 (Vegetation, Interspersion, and Habitat Features) is correlated with variation in species distribution, particularly Phoridae, Corixini, Daphniidae, and Cyclopoida. Perhaps, herein lies the Corixidae discrepancy. Murkin et al. (1992) found that microcrustaceans were more abundant in plant stands and corixids were more abundant in open water. Metric 6 includes both of these microhabitat features: 6a) Wetland vegetation components and 6b) Open water components. This would explain why Corixini and Daphniidae are strongly associated with metric 6, though corixids are usually found in lower quality wetlands while daphnids are greater in high scoring wetlands. This could indicate that such sub-metrics may need to be split into different overall metrics and receive differing weights denoting importance to the macroinvertebrate communities expected in reference wetlands. Interestingly, metric 6 explains much of the variance in macroinvertebrate distribution in the PCA, but is only present in one metric multiple regression models (Simpson's diversity, excluding Nematoda) and is negatively weighted in that model.

The PCA also shows that metric 2 is correlated with macroinvertebrate communities. Metric 2 does not address microhabitat features, but the Average Buffer Width Around the Perimeter of the Wetland sub-metric (2a) should reflect the canopy closure over the wetland, which is a marginally non-significant variable in the PCA explaining some of the variance in the macroinvertebrate species distribution. Subsequently, for metrics in which models where canopy closure and metric 2 are significant (Tables 6 and 8), they are both negatively weighted.

Inconsistencies in positive and negative relationships found in the multiple regression analyses (Table 8) could point to a need to adjust the weighting of KY-WRAM metrics such as metric 6 and 2 that reflect habitat features that are important to macroinvertebrates. These weighting adjustments may be needed to better reflect the macroinvertebrate communities of forested depressional wetlands in the KY-WRAM as a whole. Similarly, Suren et al. (2011) notes that the challenge is to discern critical variables that illustrate the status of macroinvertebrates and to use those variables in wetland condition indices so that they are a better reflection of the factors influencing the macroinvertebrate communities. Therefore, it is my recommendation that metrics 2a and metric 6 be considered for adjustment with heavier weighting to better reflect the macroinvertebrate biotic integrity of the wetland in the KY-WRAM as a whole. Corroboration with further studies of additional wetland types will be necessary because, as evident by the multiple regression models, invertebrates respond to influences from many variables, not because they were sensitive to a singular predictor. Therefore, before calibration is conducted to reflect specific metrics and habitat variables, careful studies must be conducted before such generalizations can be made (Batzer 2013).

In addition to macroinvertebrates, intensive research on several different biotic assemblages is needed to establish the validity of the KY-WRAM. Thus, parallel studies have focused on amphibians, birds, and vegetation. As an initial step toward the validation and calibration of the KY-WRAM, this study assessed the macroinvertebrate communities of forested depressional wetlands in the Upper Cumberland Basin. This design (a single wetland type in one river basin) controls for variation among wetland types and region (Rader et al. 2001). Additional studies investigating macroinvertebrate and other biotic assemblages of other wetland types and in other Kentucky regions will be essential to offer a more complete scientific backing to the development and support of KY-WRAM metrics.

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APPENDIX

Table A1. Wetland site identification numbers, site names, location information, and other identification classifications for the 19 wetland sites sampled in the Upper Cumberland River Basin, Kentucky in May, 2011.

Wetland #	Wetland Name	x coordinates	y coordinates	Wetland Subclass	Dominant Vegetation	Wetland Community	River Basin
1	Dale Lynch #1 - Bethel Ridge Road	-84.1185	37.4314	isolated depression	forest	forested ridgetop	Upper Cumberland
2	Dale Lynch #2 - Walker's Branch	-84.103401	37.425098	isolated depression	forest	forested ridgetop	Upper Cumberland
3	Dale Lynch #9 - Sandgap - FS 20 Roadside	-84.060997	37.4207	isolated depression	forest	forested ridgetop	Upper Cumberland
4	Dale Lynch #17 - High Knob	-84.0355	37.439201	isolated depression	emergent	forested ridgetop	Upper Cumberland
5	Dale Lynch #50 - Rolling Fork	-83.967736	37.416675	isolated depression	forest	forested ridgetop	Upper Cumberland
6	Dale Lynch - String Bean	-84.0121	37.361599	isolated depression	shrub	forested ridgetop	Upper Cumberland
7	Dale Lynch - Lear Ridge	-84.090972	37.357694	isolated depression	forest	forested ridgetop	Upper Cumberland
8	Dale Lynch #27 - Sky Pond	-84.012497	37.405998	isolated depression	emergent	forested ridgetop	Upper Cumberland
9	Dale Lynch #25 - 19B Gate	-84.019897	37.385899	isolated depression	emergent	forested ridgetop	Upper Cumberland
10	Dale Lynch #30 - 19A Gate	-84.0112	37.388302	isolated depression	forest	forested ridgetop	Upper Cumberland
11	Dale Lynch #36 - Across from FS road 3380	-84.000999	37.392399	isolated depression	forest	forested ridgetop	Upper Cumberland
12	Burnt Ridge Road - Bighill	37.519419	-84.234458	isolated depression	forest	forested ridgetop	Upper Cumberland
13	Cliff Palace - Keener's Point (DL)	-83.928917	37.526	isolated depression	emergent	forested ridgetop	Upper Cumberland
14	Dale Lynch #51 - Lynch Pond	-83.961861	37.457	isolated depression	forest	forested ridgetop	Upper Cumberland
15	John MacGregor #35 - County Vine - HWY 1209	-83.8983	37.5341	isolated depression	forest	forested ridgetop	Upper Cumberland
16	John MacGregor #3 - Pond Branch	-84.249199	37.021099	isolated depression	forest	forested ridgetop	Upper Cumberland
17	John MacGregor #41 - Craig's Creek	-84.251297	36.986599	isolated depression	forest	forested ridgetop	Upper Cumberland
18	John MacGregor #2 - Marsh Branch	-84.240606	37.024389	isolated depression	emergent	forested ridgetop	Upper Cumberland
19	John MacGregor #1 - Griffin Branch	-84.188202	37.0695	isolated depression	forest	forested ridgetop	Upper Cumberland

Table A2a. Abundance of all macroinvertebrates identified in wetlands 1-10 of the 19 wetlands. Taxa are written as the lowest taxonomic level. Sampling occurred in 19 wetlands of the Upper Cumberland River Basin during May 11–26, 2011. Wetland Site ID # corresponds to site names in Table A1.

		wetland ID #	1	2	3	4	5	6	7	8	9	10
Order	**Family**	**Genus**										
Amphipoda*			0	0	0	0	1	0	0	0	1	0
Arthropoda* (P)	Acari* (SC)	Hydrachnida*	6	0	0	0	3	0	0	0	0	0
Arthropoda* (P)	Arachnida*(SC)		3	1	0	0	2	0	0	0	0	0
Cladocera* (SO)	Daphniidae		0	57	0	0	3	400	0	0	0	9
Cladocera* (SO)	Polyphemidae		139	0	0	0	0	0	0	0	0	0
Coleoptera	Anthicidae larvae		0	0	0	0	1	0	0	0	0	0
Coleoptera	Carabidae		0	0	0	0	0	0	0	0	0	0
Coleoptera	Chrysomelidae larvae		0	0	0	0	0	0	0	0	4	0
Coleoptera	Curculionidae		0	0	0	0	0	1	0	0	0	0
Coleoptera	Dytiscidae	*Acilius* larvae	0	0	0	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	*Agabetes* larvae	0	0	0	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	*Agabus* larvae	0	0	0	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	*Celina* adult	0	0	0	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	*Desmopachria* larvae+adult	0	0	0	0	0	50	0	2	0	0
Coleoptera	Dytiscidae	*Dytiscus* larvae	0	0	0	0	0	17	0	0	2	0
Coleoptera	Dytiscidae	*Hydrodytes* adult	0	0	0	0	0	0	0	0	0	0
Coleoptera	Dytiscidae	*Hydroporus* adult	0	0	0	0	0	0	0	1	0	0
Coleoptera	Dytiscidae	*Hydrotrupe*s adult	0	0	0	0	3	0	0	0	0	1
Coleoptera	Dytiscidae	*Hygrotus* larvae	0	0	0	0	1	0	0	0	0	0
Coleoptera	Dytiscidae	*Laccophilus* adult	0	0	0	1	0	3	0	0	1	0
Coleoptera	Elmidae	*Dubiraphia* adult	0	0	0	0	0	0	0	0	0	0
Coleoptera	Georissidae		0	0	0	0	0	0	1	0	0	0
Coleoptera	Haliplidae	*Peltodytes* larvae	0	0	0	0	0	0	0	2	0	0
Coleoptera	Hydrophilidae	*Helochares* larvae	0	1	0	1	0	0	0	0	0	0
Coleoptera	Hydrophilidae	*Hydrochara* larvae	0	0	0	0	1	0	0	0	0	0
Coleoptera	Hydrophilidae	*Hydrophilus* adult	0	0	0	0	0	0	0	0	1	0
Coleoptera	Hydrophilidae	*Laccobius* adult	0	0	0	0	0	0	0	0	0	0
Coleoptera	Hydrophilidae	*Tropisternus* adult+larvae	0	0	0	1	0	1	0	0	0	0
Coleoptera	Haliplidae adult		0	0	0	1	0	0	0	0	0	0
Coleoptera	Hydraenidae adult		0	0	0	0	0	4	0	0	0	0
Coleoptera	Hydrophilidae	*Enochrus* adult+larvae	0	0	0	0	0	0	0	1	0	0
Coleoptera	Noteridae	*Hydrocanthus* adult	0	0	0	0	0	0	0	0	0	0
Coleoptera egg case			5	0	1	0	46	0	0	0	4	12

Table A2a. (Continued)

		wetland ID #	1	2	3	4	5	6	7	8	9	10
Order	**Family**	**Genus**										
Colepotera	Staphylinidae adult		0	0	0	0	0	0	0	0	0	0
Collembola	Isotomidae		0	0	0	0	0	3	0	0	0	0
Collembola	Poduridae		0	0	0	0	0	0	0	0	0	0
Copepod* (SC)	Harpacticoida* (Order of Copepods)		27	51	0	0	0	0	0	0	0	0
Copepoda* (SC)	Cyclopoida* (Order of Copepods)		6	8	3	0	24	315	3	5	24	0
Decapoda	Cambaridae	*Cambarus*	0	0	0	0	0	0	0	0	0	0
Diptera	Cecidomyiidae		0	0	0	0	0	0	0	0	0	0
Diptera	Ceratopogonidae	*Bezzia* larvae+pupae	3	0	0	1	0	129	0	0	238	0
Diptera	Ceratopogonidae	*Serromyia*	0	0	0	0	0	0	0	0	0	0
Diptera	Chaoboridae	*Chaoborus larvae*+pupae	0	0	0	10	0	13	0	0	1	0
Diptera	Chironomidae (larvae+pupae)		851	411	15	57	103	316	227	95	300	25
Diptera	Culicidae	*Aedes*	9	5	0	0	24	73	6	1	39	0
Diptera	Culicidae	*Anopheles*	0	0	0	0	0	0	0	0	0	0
Diptera	Culicidae	*Culex* larvae+pupae	0	0	0	1	3	1	0	21	4	0
Diptera	Culicidae	*Mansonia*	0	0	0	0	0	0	0	1	8	0
Diptera	Culicidae	*Psorophora*	0	0	0	0	0	0	0	0	0	0
Diptera	Dolichopodidae	Dolichopodidae	4	1	0	0	0	0	0	0	0	0
Diptera	Mycetophilidae		0	0	0	0	0	0	0	0	0	0
Diptera	Phoridae		0	1	5	1	3	1	1	0	0	0
Diptera	Psychodidae	*Psychoda* larvae+pupae	0	0	0	0	0	0	0	2	33	4
Diptera	Stratiomyidae		0	0	0	0	0	0	0	0	327	0
Emphemeroptera	Baetidae		0	0	0	0	0	0	0	0	3	0
Gastropoda* (C)	Planorbidae	*Micromenetus*	0	0	0	0	1	0	0	0	0	0
Gastropoda* (C)	Succinidae		0	0	0	0	0	0	0	0	0	0
Hemiptera	Belostomatidae	*Belostoma*	0	0	0	0	0	6	0	0	0	0
Hemiptera	Corixini* (Tribe)		0	0	0	18	0	3	9	0	0	0
Hemiptera	Gerridae	*Gerris*	0	0	0	0	0	1	0	0	0	0
Hemiptera	Gerridae	*Limnoporous*	0	0	0	0	0	0	0	0	0	0
Hemiptera	Gerridae	*Trepobates*	6	3	1	0	9	3	1	1	0	0
Hemiptera	Mesoveliidae		0	0	0	0	3	0	0	0	0	0
Hemiptera	Nepidae	*Ranatra*	0	0	0	1	0	1	0	0	0	0
Hemiptera	Notonectidae	*Notonecta*	0	11	0	0	0	18	4	0	7	7
Hemiptera	Veliidae	*Microvelia*	0	0	0	0	0	0	0	1	0	0
Hymenoptera			0	0	0	0	0	0	0	0	0	0
Isopoda			0	0	0	0	45	0	0	0	0	0

Table A2a. (Continued)

		wetland ID #	1	2	3	4	5	6	7	8	9	10
Order	**Family**	**Genus**										
Lepidoptera	Crambidae		0	0	0	0	1	0	1	0	0	0
Lepidoptera	Noctuidae		0	0	0	0	1	0	0	0	0	0
Megaloptera	Corydalidae	*Chauloides*	0	0	0	0	0	0	0	0	0	0
Megaloptera	Sialidae	*Sialis*	0	0	0	0	0	0	0	0	0	0
Nematoda* (P)			0	9	48	105	0	106	219	180	253	3
Nematomorpha* (P)			0	0	0	0	0	0	0	0	0	0
Odonata	Aeshnidae	*Remartinia*	2	1	0	0	3	0	0	0	0	0
Odonata	Anispotera	*Dorocordula*	0	0	0	0	0	0	0	0	0	0
Odonata	Lestidae	*Lestes*	0	0	0	11	0	10	0	0	0	0
Odonata	Libellulidae	*Pachydiplax*	0	0	0	0	0	0	0	0	0	0
Odonata	Libellulidae	*Sympetrum*	0	0	0	0	0	0	0	0	0	0
Oligochaeta* (C)			347	279	55	13	11	312	80	26	411	106
Ostracoda* (C)			1	3	0	0	3	8	0	5	6	0
Trichoptera	Branchyceridae A		0	0	0	0	0	0	0	0	0	0
Trichoptera	Lemniphilidae	*Limnephilus* larvae+pupae	105	0	0	0	0	0	0	0	0	0
Trichoptera	Phryganiidae		0	0	0	0	0	0	0	0	0	1

Table A2b. Abundance of all macroinvertebrates identified in wetlands 11-19 of the 19 wetlands, including a tallied total abundance for all 19 wetlands. Taxa are written as the lowest taxonomic level. Sampling occurred in 19 wetlands of the Upper Cumberland River Basin during May 11–26, 2011. Wetland Site ID # corresponds to site names in Table A1.

		wetland ID #	11	12	13	14	15	16	17	18	19	Total
Order	**Family**	**Genus**										
Amphipoda*			1	0	0	3	0	1	3	0	3	**13**
Arthropoda* (P)	Acari* (SC)	Hydrachnida*	6	0	30	6	4	15	1	2	0	**73**
Arthropoda* (P)	Arachnida*(SC)		0	0	0	0	0	1	0	4	0	**11**
Cladocera* (SO)	Daphniidae		9	0	0	390	0	41	1	1	572	**1483**
Cladocera* (SO)	Polyphemidae		0	0	0	0	0	0	0	18	0	**157**
Coleoptera	Anthicidae larvae		0	0	0	0	1	0	0	0	0	**2**
Coleoptera	Carabidae		0	0	0	0	0	0	0	0	1	**1**
Coleoptera	Chrysomelidae_larvae		0	0	0	0	0	0	0	1	0	**5**
Coleoptera	Curculionidae		0	0	0	0	1	0	0	0	0	**2**
Coleoptera	Dytiscidae	*Acilius* larvae	0	0	0	0	0	0	0	0	1	**1**
Coleoptera	Dytiscidae	*Agabetes* larvae	0	0	0	3	0	0	0	0	0	**3**
Coleoptera	Dytiscidae	*Agabus* larvae	9	3	0	0	0	0	0	0	0	**12**
Coleoptera	Dytiscidae	*Celina* adult	0	0	0	0	0	0	0	1	0	**1**
Coleoptera	Dytiscidae	*Desmopachria* larvae+adult	0	0	0	0	0	0	1	6	0	**59**
Coleoptera	Dytiscidae	*Dytiscus* larvae	1	0	5	0	0	0	0	1	0	**26**
Coleoptera	Dytiscidae	*Hydrodytes* adult	0	0	0	0	0	1	0	0	0	**1**
Coleoptera	Dytiscidae	*Hydroporus* adult	0	1	0	0	0	0	0	0	0	**2**
Coleoptera	Dytiscidae	*Hydrotrupe*s adult	3	0	0	0	0	0	1	1	0	**9**
Coleoptera	Dytiscidae	*Hygrotus* larvae	1	0	0	0	0	0	3	9	0	**14**
Coleoptera	Dytiscidae	*Laccophilus* adult	0	0	0	0	0	0	0	0	0	**5**
Coleoptera	Elmidae	*Dubiraphia* adult	0	0	2	0	0	0	0	0	0	**2**
Coleoptera	Georissidae		0	0	0	0	0	0	0	0	0	**1**
Coleoptera	Haliplidae	*Peltodytes* larvae	0	0	0	0	0	0	0	11	0	**13**
Coleoptera	Hydrophilidae	*Helochares* larvae	15	3	0	10	0	0	0	0	0	**30**
Coleoptera	Hydrophilidae	*Hydrochara* larvae	0	0	0	0	0	1	0	0	0	**2**
Coleoptera	Hydrophilidae	*Hydrophilus* adult	0	0	0	0	0	0	0	0	0	**1**
Coleoptera	Hydrophilidae	*Laccobius* adult	0	0	0	3	0	0	0	0	0	**3**
Coleoptera	Hydrophilidae	*Tropisternus* adult+larvae	0	0	0	0	0	0	0	0	0	**2**
Coleoptera	Haliplidae adult		0	0	0	0	0	0	0	0	0	**1**
Coleoptera	Hydraenidae adult		0	0	0	0	0	0	0	0	0	**4**
Coleoptera	Hydrophilidae	*Enochrus* adult+larvae	0	2	0	0	0	0	0	0	0	**3**
Coleoptera	Noteridae	*Hydrocanthus* adult	0	0	0	0	0	0	0	1	0	**1**
Coleoptera egg case			5	0	3	27	23	20	10	5	1	**162**

Table A2b. (Continued)

		wetland ID #	11	12	13	14	15	16	17	18	19	Total
Order	**Family**	**Genus**										
Colepotera	Staphylinidae adult		1	0	0	0	2	0	0	0	0	**3**
Collembola	Isotomidae		0	0	0	0	3	3	34	0	3	**46**
Collembola	Poduridae		0	0	0	0	0	0	3	6	0	**9**
Copepod* (SC)	Harpacticoida* (Order of Copepods)		0	0	0	0	0	0	0	0	0	**78**
Copepoda* (SC)	Cyclopoida* (Order of Copepods)		3	69	9	15	0	190	106	286	446	**1512**
Decapoda	Cambaridae	*Cambarus*	0	0	0	0	0	3	1	0	0	**4**
Diptera	Cecidomyiidae		0	0	0	0	4	1	0	0	0	**5**
Diptera	Ceratopogonidae	*Bezzia* larvae+pupae	0	0	63	0	4	0	1	32	0	**471**
Diptera	Ceratopogonidae	*Serromyia*	0	0	0	0	0	0	0	2	0	**2**
Diptera	Chaoboridae	*Chaoborus larvae*+pupae	0	0	0	0	0	0	0	0	3	**27**
Diptera	Chironomidae (larvae+pupae)		1943	725	13	445	417	594	768	73	695	**8073**
Diptera	Culicidae	*Aedes*	37	0	42	5	1	6	18	1	0	**267**
Diptera	Culicidae	*Anopheles*	0	0	0	0	0	0	3	0	0	**3**
Diptera	Culicidae	*Culex* larvae+pupae	24	0	1	1	0	0	22	0	0	**78**
Diptera	Culicidae	*Mansonia*	0	0	0	6	0	0	0	0	0	**15**
Diptera	Culicidae	*Psorophora*	0	0	0	0	0	1	0	0	0	**1**
Diptera	Dolichopodidae	Dolichopodidae	13	0	0	3	0	3	0	0	0	**24**
Diptera	Mycetophilidae		6	0	0	0	0	0	0	0	0	**6**
Diptera	Phoridae		0	0	0	9	7	8	3	0	16	**55**
Diptera	Psychodidae	*Psychoda* larvae+pupae	75	0	0	0	0	0	0	0	0	**114**
Diptera	Stratiomyidae		0	0	0	0	0	0	0	0	0	**327**
Emphemeroptera	Baetidae		0	0	0	0	0	0	0	0	0	**3**
Gastropoda* (C)	Planorbidae	*Micromenetus*	0	0	0	0	0	0	0	0	0	**1**
Gastropoda* (C)	Succinidae		0	0	0	0	0	0	0	22	0	**22**
Hemiptera	Belostomatidae	*Belostoma*	0	0	0	0	0	0	0	0	0	**6**
Hemiptera	Corixini* (Tribe)		0	0	24	8	0	7	3	0	23	**95**
Hemiptera	Gerridae	*Gerris*	0	0	6	0	0	0	0	0	0	**7**
Hemiptera	Gerridae	*Limnoporous*	0	0	0	2	0	0	0	0	0	**2**
Hemiptera	Gerridae	*Trepobates*	1	11	1	13	8	13	10	0	0	**81**
Hemiptera	Mesoveliidae		0	0	0	0	0	1	0	0	0	**4**
Hemiptera	Nepidae	*Ranatra*	0	0	0	0	0	0	0	0	0	**2**
Hemiptera	Notonectidae	*Notonecta*	0	7	13	13	4	4	0	5	5	**98**
Hemiptera	Veliidae	*Microvelia*	0	0	0	0	0	0	0	0	0	**1**
Hymenoptera			0	0	0	0	0	0	0	6	0	**6**
Isopoda			0	0	0	0	0	23	9	0	0	**77**
Lepidoptera	Crambidae		0	0	0	0	0	0	0	0	0	**2**
Lepidoptera	Noctuidae		0	0	0	0	0	0	0	0	0	**1**

Table A2b. (Continued)

		wetland ID #	11	12	13	14	15	16	17	18	19	Total
Order	**Family**	**Genus**										
Megaloptera	Corydalidae	*Chauloides*	0	0	1	0	0	0	0	4	0	**5**
Megaloptera	Sialidae	*Sialis*	0	1	0	0	0	0	0	0	0	**1**
Nematoda* (P)			301	57	0	3	33	18	35	9	0	**1379**
Nematomorpha* (P)			0	0	0	6	0	0	0	0	6	**12**
Odonata	Aeshnidae	*Remartinia*	0	0	25	0	0	0	0	3	0	**34**
Odonata	Anispotera	*Dorocordula*	0	1	0	0	0	0	0	0	0	**1**
Odonata	Lestidae	*Lestes*	0	0	0	0	0	0	0	20	0	**41**
Odonata	Libellulidae	*Pachydiplax*	0	0	0	0	0	0	0	11	1	**12**
Odonata	Libellulidae	*Sympetrum*	0	0	1	0	0	0	0	0	0	**1**
Oligochaeta* (C)			24	161	115	93	200	236	6	54	0	**2529**
Ostracoda* (C)			0	0	4	6	0	9	8	39	0	**92**
Trichoptera	Branchyceridae A		0	0	0	0	0	1	0	0	1	**2**
Trichoptera	Lemniphilidae	*Limnephilus* larvae+pupae	0	0	0	0	0	0	0	0	0	**105**
Trichoptera	Phryganiidae		0	0	0	0	0	0	0	0	0	**1**

Table A3. Water quality data collected from the 19 depressional wetlands sampled in the Upper Cumberland River Basin, Kentucky. Wetlands in which water quality data could not be taken due to environmental conditions are denoted with "na". Time is recorded on a 24-hour clock timeline.

Wetland ID #	Sample date	Sample time	Depth sample point (cm)	DO (%)	pH	Conduc-tivity (µS)	Water temp (°C)	Salinity (ppt)	chlorophyll-a (µg/L)	Air temp (°C)
1	6/8/11	11:34	11	13	6.48	101.4	22.6	0.05	10.5	55
2	6/6/11	7:45	10	61	6.03	48.4	20.9	0.02	5.7	64
3	6/6/11	na	na	na	na	na	na	na	na	63
4	6/6/11	9:00	12.5	17	5.72	18.9	22.1	0.01	370.048	64
5	6/6/11	11:15	8.1	14	5.7	74.8	22.9	0.03	0.4445	50
6	6/7/11	7:22	15.5	0	5.2	13.1	22.8	0	48.3	64
7	6/23/11	12:20	na	110	5.74	14.2	22.4	0.01	6.76	63
8	6/6/11	10:35	7	24	5.61	11.5	24.4	0	973.44	66
9	6/24/11	9:20	12.9	23	7.18	39.4	20.7	0.02	3.813	70
10	6/7/11	8:15	15	-1	5.8	46.4	20.8	0.02	0.2	68
11	6/24/11	10:40	9.5	38	5.88	170.7	19.7	0.08	3.145	68
12	6/8/11	10:40	9.5	10	6.58	215.9	23.5	0.1	340.992	52
13	6/7/11	9:40	15.5	-2	1.26	27.1	22.2	0.01	11.2	68
14	6/7/11	10:35	16.5	-1	5.63	21.4	21.6	0.01	3.0	48
15	6/7/11	11:28	13.5	9	5.72	30.7	24.5	0.01	43.7	48
16	6/8/11	7:00	38	-2	5.63	13.8	22.1	0	0.3828	81
17	6/8/11	7:40	9.5	0.1	5.97	40.1	21.1	0.02	4.4992	81
18	6/8/11	8:10	29	0	6.88	66.7	22.6	0.03	45.76	86
19	6/8/11	9:00	27.5	-2	5.15	260	22.3	0.12	81.32	68

Table A4. Habitat parameter data for the 19 depressional wetlands sampled in the Upper Cumberland River Basin, Kentucky.

Wetland ID #	Max Depth (cm)	Area (m^2)	Wetl a-nd area (acres)	Perimet-er (m)	Dista-nce to paved road (m)	Avg DBH live trees	Live trees (#)	Avg DBH dead trees	Stand-ing dead trees/ snags (#)	Leaf litter depth (cm)	Tuss-ocks	Hum-mocks	Canop y closure (Avg. %)	CWD (m3)	Vegeta tive cover (%)
1	45	353.43	0.09	110.3	8.0	4.83	3	2.6	4	4.88	2	1	88.30	0.11	48.55
2	50	785.40	0.19	110.0	500.0	20.15	38	0.0	0	5.81	0	0	94.22	1.62	11.90
3	24	270.96	0.07	46.8	10.1	18.83	3	16.0	1	3.38	3	2	81.61	0.00	1.43
4	46	596.90	0.15	101.0	500.0	0.00	0	0.0	0	5.63	0	0	79.07	0.52	9.88
5	29	628.32	0.16	185.0	6.0	6.34	401	23.8	25	8.00	19	17	91.36	1.40	1.36
6	37	490.87	0.12	100.0	0.0	5.63	19	14.1	7	7.38	6	2	49.95	1.49	4.36
7	23	274.89	0.07	88.0	500.0	10.42	14	0.0	0	2.75	0	0	79.79	0.00	18.96
8	22	58.91	0.02	35.0	500.0	0.00	0	0.0	0	7.25	27	0	62.30	0.00	37.78
9	37	367.57	0.09	100.0	1.2	10.04	5	0.0	0	2.00	1	0	45.65	0.17	18.95
10	64	785.40	0.19	140.0	80.0	22.25	19	22.3	2	4.50	0	2	93.89	0.13	6.50
11	27	397.26	0.10	140.0	38.0	19.82	9	47.0	1	6.63	0	6	90.84	1.34	1.07
12	36	197.92	0.05	85.0	25.8	15.97	3	0.0	0	5.88	1	1	92.07	0.44	1.12
13	39	628.32	0.16	97.4	500.0	21.62	6	0.0	0	9.38	1	2	87.66	0.00	40.01
14	66	942.48	0.23	189.6	56.6	19.26	77	7.7	9	9.75	26	12	82.58	0.88	5.06
15	56	1255.07	0.31	150.0	23.9	14.13	95	11.5	9	8.56	2	3	89.73	1.97	0.95
16	66	1099.56	0.27	240.0	15.3	13.71	161	17.4	23	4.13	4	3	87.72	7.66	0.32
17	22	235.62	0.06	75.7	18.6	13.71	31	6.1	4	2.63	109	16	94.41	0.24	20.89
18	46	549.78	0.14	134.0	12.9	3.68	21	12.4	12	7.03	0	0	75.76	0.47	53.30
19	100	628.32	0.16	119.7	500.0	11.94	21	18.0	6	7.15	0	0	92.40	0.29	0.00

Table A5. Assessment scores for each of the 19 wetlands sampled in the Upper Cumberland River Basin, Kentucky. Assessment scores include Landscape Development Intensity (LDI), the total score and individual metric scores from the Kentucky Wetlands Rapid Assessment Method (KY-WRAM), and the total Ohio Rapid Assessment Method (ORAM) score. LDI scores were calculated with GIS. All KY-WRAM, ORAM, and metric scores are averages based on the scoring of four field technicians.

Wetland ID #	LDI	KY-WRAM	ORAM	Metric 1	Metric 2	Metric 3	Metric 4	Metric 5	Metric 6
1	1.0378	64.88	60.38	3.50	10.50	13.63	17.75	6.50	13.00
2	1.0000	75.63	72.63	4.00	11.25	14.88	18.25	9.00	18.25
3	1.4789	46.88	33.63	3.50	9.25	9.63	9.25	4.00	11.25
4	1.0030	61.63	55.75	3.75	11.50	14.63	10.00	6.50	15.25
5	2.1867	58.25	46.25	4.00	7.63	10.00	11.38	5.50	19.75
6	1.6418	55.75	50.00	4.00	9.50	9.63	8.88	5.50	18.25
7	1.0167	52.00	44.50	3.25	10.75	12.25	10.00	4.00	11.75
8	1.0000	44.50	34.13	3.50	7.25	12.63	7.88	5.25	8.00
9	1.4476	49.67	35.83	4.00	9.33	7.83	7.17	5.67	15.67
10	1.4500	80.00	78.00	4.33	12.00	15.00	19.33	9.33	20.00
11	1.4251	64.50	53.33	4.00	8.83	12.00	14.33	5.67	19.67
12	1.7655	60.17	53.17	3.33	9.33	14.83	11.67	5.67	15.33
13	1.0000	78.00	73.33	4.00	11.67	15.00	17.67	9.67	20.00
14	2.1772	71.00	61.50	4.67	9.33	13.67	14.00	9.33	20.00
15	1.7967	66.33	59.83	5.00	10.50	12.17	13.00	7.33	18.33
16	2.0846	66.00	57.33	5.00	10.17	12.83	11.33	7.00	19.67
17	1.6679	70.17	53.50	3.00	10.33	11.17	16.33	9.67	19.67
18	2.2232	55.83	41.83	4.00	8.33	12.33	10.50	7.00	13.67
19	1.0722	62.17	54.83	4.00	11.00	9.00	10.83	7.33	20.00