January 2016

Wetland Condition Matters: Amphibian Richness and Abundance Change Across Wetland Condition Gradient

Kari Dupler
Eastern Kentucky University

Wetland Condition Matters:

Amphibian Richness and Abundance Change Across Wetland Condition Gradient

By

Kari Dupler

Thesis Approved:

Dr. Stephen Richter, Chair, Advisory Committee

Dr. David Brown, Member, Advisory Committee

Dr. Amy Braccia, Member, Advisory Committee

Dean, Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master of Science degree at Eastern Kentucky University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of the source is made. Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in [his/her] absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature Kari Doyle

Date 6 April 2016

Wetland Condition Matters:

Amphibian richness and abundance change across wetland condition gradient

By

Kari Dupler

Bachelor of Science

Texas Tech University

Lubbock, Texas

2006

Submitted to the Faculty of the Graduate School of

Eastern Kentucky University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

May, 2016

DEDICATION

This thesis is dedicated to my parents for their unwavering support,
and to Buddy for always being my best friend.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Stephen Richter, for allowing me to join the Richter lab to work on the KY-WRAM project. This project has been such a pleasant and amazing experience, and I am so thankful to Stephen for his open door and advice throughout the whole process. I am grateful to my committee member, Dr. David Brown, for providing realistic advice and statistical support, and his help on other projects along the way. Collectively, thank you to Stephen and David for allowing me to be a part of the KY-WRAM team and providing funding and support for my research project. Thank you to my committee member Dr. Amy Braccia for introducing me to the world of aquatic insects and for being a great mentor. Thank you to Michelle for inspiring me to be a better scientist. Field work would not have been the same without you there. Thank you to Steve for your love and support during these last two years, the move was hard, but in the end was worth it. I would like to thank my family for all their hard work and sacrifices that have helped get me to this point, and their ever-present love and support. Last, thank you to my friends who have been nothing but supportive of my crazy idea to move across the country to pursue this degree.

ABSTRACT

In the past century, Kentucky has lost more than 80% of its wetlands, and because state-wide monitoring was historically minimal, this number is likely underestimated. The Kentucky Division of Water, with Eastern Kentucky University and a technical working group, developed a rapid wetland assessment method (i.e. KY-WRAM) to assess wetland quality and aid in establishing mitigation levels and long-term monitoring. Validation of the KY-WRAM's ability to reflect wetland condition requires comparison to intensive biotic assessments of amphibian, plant, and bird communities. Wetland and amphibian surveys for the 2014 and 2015 seasons were conducted at 42 riverine wetlands in the Kentucky and Salt river basins in Kentucky. Wetlands were chosen from across a gradient of low-, medium-, and high-category scores to compare amphibian communities across a range of wetland condition. Seven were in the low category, twenty-four in medium, and eleven in the high category. Wetlands were surveyed for amphibians via dipnetting and minnow-trapping. Species richness and abundance were tested with AIC modeling using nutrients, dissolved oxygen, landscape disturbance, presence of predatory fish, the pesticide atrazine, and KY-WRAM scores as model parameters. Results indicated KY-WRAM score explained the majority in species richness, and was an important predictor of abundance for seven species of amphibians. Additionally, species richness was significantly and positively related to KY-WRAM score ($p<0.001$, $R^2 = 0.62$), and was greater among medium and high category sites than low ones. Species present at low quality sites tended to be present at all sites, and species that are sensitive to disturbance were generally only found at higher-scoring sites. KY-WRAM scores reflect a gradient of wetland condition, and anthropogenic impacts within wetland habitats and surrounding uplands are reflected in these scores. Overall, these results indicate that the KY-WRAM is a good predictor of wetland condition, and strongly relates to amphibian communities.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

1. INTRODUCTION

Wetland area has declined significantly across the United States in the last century, and Kentucky has lost more than 80% of its wetland area (Dahl, 1990). Historically, wetland monitoring within the state has been minimal, so it is likely the area of impact is underestimated. Many wetlands that remain have been impacted by human activities, such as agriculture and urbanization, and as such, both function and condition are impaired. Wetlands perform a variety of ecosystem functions, including flood control and nutrient cycling, and also serve as habitats for a diversity of organisms, including amphibians. Under the auspices of the Clean Water Act of 1974, the United States Environmental Protection Agency (USEPA) defined objectives to maintain biological integrity of wetlands (33 U.S.C. §1251(a)) and established a policy in 1989 of "no net loss" of wetlands, which attempts to balance and mitigate wetland loss by replacement with equal area. Karr and Dudley (1981) defined biological integrity as the ability of an ecosystem to support a community of organisms that has both species composition and diversity comparable to that of the natural habitat of the region. Although the USEPA established policies for protection and restoration of degraded wetlands and has provided guidance for creation of assessment methods, no national protocol is in place for rapid monitoring or assessing wetland condition. Instead, states (e.g., Ohio and California) have taken the initiative to implement their own monitoring in the form of state or region-specific wetland rapid assessment methods (RAMs) (Mack, 2001; Collins et al., 2008). Recently, the Kentucky Division of Water (KDOW) and Eastern Kentucky University, in conjunction with a technical working group composed of state and federal agencies, developed a Kentucky-specific wetland rapid assessment method (KY-WRAM) to evaluate the condition of wetlands throughout the state.

Wetland rapid assessment methods quantify ecological attributes within and surrounding the wetland using metrics to assess characteristics, such as hydrological impacts, substrate disturbances, vegetation cover, wetland type, and surrounding land use. Suitability of habitats can influence abundance and diversity of wetland faunal assemblages, and can be used to make estimates about overall wetland habitat condition (Semlitsch, 2000; Stapanian et al., 2004; Micacchion et al., 2015). Assessment metrics are measures designed to represent specific attributes of ecosystem function and health (Karr and Chu, 1997). To be both effective and rapid, RAMs must combine field observations of habitat parameters into a single score which can then be used to compare wetlands to a reference condition (Fennessy et al., 2007). Following the Karr and Dudley (1981) definition of biological integrity, Fennessy et al. (2007) defined condition as the ability of a wetland to maintain ecological functions and biotic communities similar to one which humans have not impacted (i.e. reference). Because RAM metrics are generally qualitative, and take the place of direct measures of biological and chemical components, they must be validated using data from intensive biological assessments before being employed (Fennessy et al., 2007). Validation of a RAM means achieving repeated significant positive correlation with independent intensive biological assessments (Stein et al., 2009).

The KY-WRAM is similar to those of other states, and uses various features to evaluate levels of disturbance and the overall condition of each wetland (Appendix 1). The scoring methodology is designed to reflect habitat condition within wetlands and the surrounding landscape at multiple scales. For the KY-WRAM, metric 1 assigns points for wetland size and distribution in the landscape. Larger wetlands and those that are isolated within a 2-mile radius receive more points than smaller wetlands or those in large wetland complexes. Metric 2 quantifies and weights intensity of surrounding land use within 150 and 1,000 feet of the wetland edge. Previous studies have determined that wetland buffers are important areas that serve as both habitat and corridors for movement of many organisms, including amphibians (Semlitsch and Bodie, 2003; Browne et al., 2009). Metric 3 assesses various aspects of hydrology, and provides more points to wetlands which have characteristics that are associated with groundwater recharge, filtering and discharge control, and have minimal impacts. Sites with anthropogenic impacts to within-wetland habitats such as draining, grazing, or mowing all receive lower points for metric 4. Likewise, wetlands that are in fair to poor shape receive fewer points than those that reflect reference condition. Metric 5 reflects the state-specific nature of the KY-WRAM and awards points to wetlands that are rare or considered of high-ecological value within the state. Metric 6 is designed to allow points for vegetation, interspersion of different habitat types within the wetland, and microhabitat features (e.g. snags, tussocks, hummocks) which provide habitat and breeding locations for organisms such as amphibians, ducks, and bats.

Intensive bioassessments are useful tools in both evaluation and calibration of metrics for rapid assessment of wetland condition. These assessments rely on the idea that flora and fauna will reflect the condition of their environment along a gradient, and reflect impacts from anthropogenic activities (USEPA, 2002; Karr and Chu, 1997). The California Rapid Assessment Method (CRAM) was validated using data from surveys of birds, macroinvertebrates and plants (Stein et al., 2009). The Ohio Rapid Assessment Method (ORAM) has been used within that state for many years, and was validated and cross-checked using intensive biological assessments of amphibians (Micacchion, 2004), wetland vegetation (Mack and Kentula, 2010), and landscape disturbance (Mack, 2006). Additional work within this state has shown that individual metrics of the ORAM and overall scores also serve as predictors of avian richness (Stapanian et al., 2004). Results from these studies generally indicate positive relationships between the bioassessments and metrics designed to assess wetland condition. They also indicate that testing metrics across multiple taxa provides better resolution in determining metrics that serve as habitat indicators for a variety of organisms.

Pond-breeding amphibians are good indicators of wetland ecosystem health because they make up a significant portion of wetland faunal biomass (Gibbons et al., 2006) and exhibit species-specific sensitivities to habitat change (Welsh & Ollivier, 1998; Dixon et al., 2011; Amburgey et al., 2012). Declines in amphibian populations have been well documented and attributed to a variety of causes including habitat alteration, diseases, climate change, and the additive stress of multiple factors (Lehtinen et al., 1999; Stuart, 2004; Beebee & Griffiths, 2005; Kiesecker, 2011). Due to their biphasic life cycles, most amphibians are sensitive to changes in both inundated areas and surrounding terrestrial habitats (Salice et al., 2011; Hamer and Parris, 2011). The quality of wetland habitats (e.g., amount of sedimentation, duration and timing of the hydroperiod, and contaminant burden) can influence the likelihood of successful oviposition and larval development in amphibians (Semlitsch, 2000; Babbitt et al., 2003; Brühl et al., 2013). Additionally, adults of many species spend non-breeding periods in the terrestrial environment adjacent to wetlands, so conservation of surrounding upland habitats is just as important as the conservation of wetlands (Semlitsch & Bodie, 1998; Babbitt et al., 2009; Dodd & Cade, 2008; Lehtinen et al., 1999).

The objective of my study was to assess the amphibian community composition of riverine wetlands in the Kentucky and Salt river basins, using measures of abundance and species richness to validate the KY-WRAM across a gradient of condition, from low to high. Because amphibians use both upland and wetland habitats, and the KY-WRAM scores reflect condition of both, I anticipated positive relationships between amphibian species richness and KY-WRAM scores.

2. METHODS

Site Selection

Potential sites were identified throughout the Kentucky and Salt river basins using the National Wetlands Inventory (NWI) database and confirmation from EKU and KDOW staff that the wetlands potentially support amphibian communities (i.e., appear to have sufficient hydrology). Sites were randomly chosen from a list of candidate sites, targeting sites within each category (low, medium, high). The narrative ratings (e.g. low, medium, high) for categories were developed by dividing the points into three ranges, based on the lowest score possible (11 points) (Karr and Chu, 1997). These three groups were delineated by diving the possible score range (11-100) evenly into three groups, and were not based on statistical analysis of the distribution of KY-WRAM scores. Low category sites were those under 41, medium category sites are those 42–72, and high 73–100, as scored by the KY-WRAM. These ratings were designed to reflect a range of sites along a condition gradient. Within each basin, I selected 5 low category sites, 11 medium, and 6 high quality sites to reflect a condition gradient, as scored by the KY-WRAM. Prior to sampling, sites were ground-truthed to confirm wetlands contained water and were located in the floodplain of rivers or streams (HGM riverine classification). In total, 44 wetlands were chosen, 22 from the Kentucky basin and 22 from the Salt (Figure 1). Two sites from the Kentucky basin were dropped mid-season (2014) due to revocation of landowner permission.

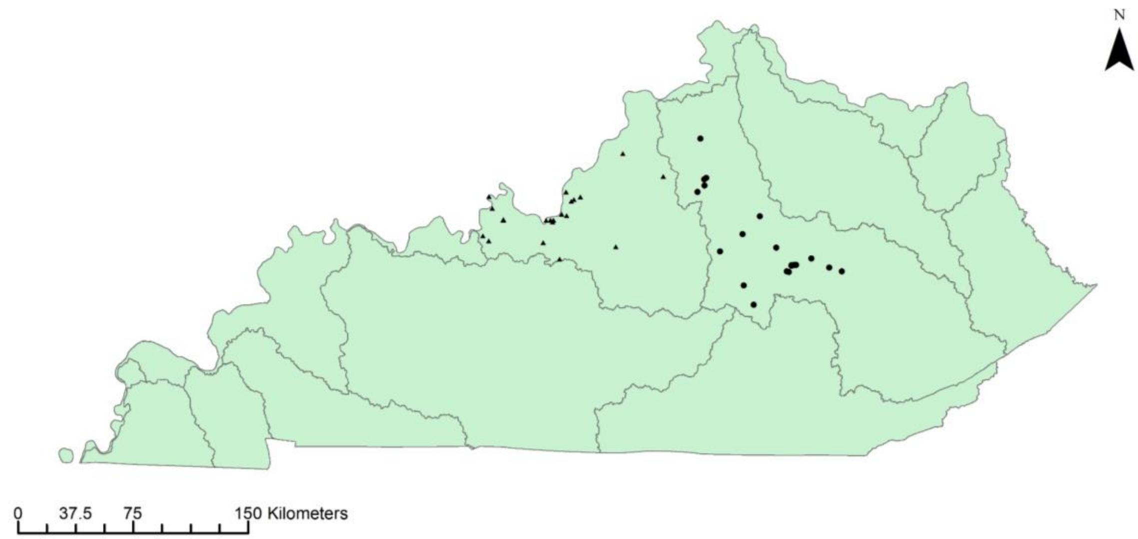


Figure 1. Map of Kentucky showing locations of 42 riverine wetlands within the Kentucky and Salt river basins for amphibian sampling in 2014 and 2015.

Amphibian Sampling

Amphibian surveys were conducted three times at each wetland between late March and July for both years (March/April, May/June, and June/July). This time frame coincides with the breeding season of most pond-breeding amphibians and accounts for interspecific differences in the timing of breeding (Shulse et al., 2009). Additionally, in 2015, two egg-mass surveys were conducted in January and early March, respectively, at all 42 wetlands to identify the presence of early-breeding amphibians such as spotted salamanders (*Ambystoma maculatum*), Jefferson salamanders (*A. jeffersonianum*), and wood frogs (*Lithobates sylvaticus*), which may have larval periods earlier than the summer breeding of other species. Egg-mass surveys were conducted by walking the perimeter and shallow sections of wetlands and visually inspecting for egg masses.

Wetland length and width were measured during each visit using either an electronic range finder or tape measure. The number of sample locations was determined by length; one sample per 5 m of wetland length, with a maximum of 50 per site. Sampling was conducted within 25-m transects along the length of the wetland, maximum of 5 samples per transect. Sampling methods included both dipnetting and placing mesh minnow traps at 5-m intervals within transects.

Dipnetting was conducted by pushing a d-frame dipnet into the leaf litter and detrital substrate, which larval amphibians use as refuge, in an arc around the sampler, toward the shore (Denton and Richter, 2012). For each dipnet, all amphibians (adults and larvae) were identified to species, counted, and released. A collapsible, unbaited, mesh minnow trap was placed in the same area where dipnetting was performed. Minnow traps were placed so that a portion of the funnels on each end were under water, but with some trap exposed to allow air-breathing organisms to survive. Traps were left overnight (approximately 16–24 hrs) to account for temporal variation in amphibian movements, then checked and removed on the second-day site visit. Collected individuals were identified to species and released unharmed in the same area where they were captured. This work was performed under an existing EKU IACUC protocol (number 03-2014).

Wetland Assessment

Wetland assessments were performed during the first sampling period at each site following the 2013 KY-WRAM protocol (Appendix 1). Some metrics within the KY-WRAM require the use of remote-sensing data (e.g. maps of land-use surrounding wetland at varying radii) and were completed in the lab after the field assessment. While onsite, field staff sketched the wetland assessment area, and walked around the boundary to identify habitat features and assign scores for each sub-metric. Examples of sub-metrics include identification of inputs from water sources (surface, groundwater seeps, or springs), alterations to the hydrological regime (dikes, stream channelization, and dredging or filling activities), and microtopographic features (hummocks, tussocks, large snags) in or near the wetland. When all metric assessments were completed, sub-metrics were tallied for individual metric scores; metrics were then summed to assign a final score to the wetland out of 100 possible points.

Landscape Development Intensity (LDI) index scores were calculated in ArcGIS for each site within a 1-km buffer using data from the 2005 National Land Cover Database (Brown and Vivas, 2005). Scores were calculated by quantifying the proportions of 15 land-use categories within a 1-km radius of the wetland center, and multiplying the proportion by a land-use coefficient for each category (Brown and Vivas, 2005). Proportions were then summed for a final LDI score for each site. This metric was designed to estimate the level of anthropogenic impacts within an area by scoring and weighting land-cover and land-use types based on the fossil-fuel energy use per area. Land-cover types that require more energy inputs (i.e. row crops) have a higher score than natural areas (i.e. forest). Scores range from 1 to 10, and are lower for sites surrounded by natural buffers versus those surrounded by agricultural or urban areas.

Water Sampling

During the 2015 sampling season, water samples were taken at all 42 sites in both March and May, representing possible seasonal variations in water quality. Surface-water sampling followed protocols established by the US Geological Survey (U.S.G.S., 2006). Briefly, grab samples were taken from surface water at the point of outflow and placed into a pre-cleaned bottle. Samples were packed on ice and sent to the Kentucky Division of Water's Laboratory for extraction and analysis**.** Water samples were processed and analyzed following established EPA protocols. Analytes included ammonia (as N), total kjeldhal nitrogen, total phosphorus, nitrate/nitrite (as N), glyphosate, and pesticides via methods 6260 and 6440. Specific conductance, dissolved oxygen (DO), temperature, and pH were measured at each wetland using an YSI probe on the same day as water sampling. Dissolved oxygen was log-transformed to normalize the distribution of the data.

Fish presence/absence was assessed using visual surveys, dipnetting and trapping techniques. Only fish deemed amphibian larval predators (i.e. *Lepomis* sp.) were counted as present (Hecnar and M'Closkey, 1997; Holbrook and Dorn, 2015).

Statistics

I tabulated all data in Microsoft Excel. Amphibian species abundances were calculated by adding together total individuals captured via trapping during each of the three site visits. I calculated a Shannon Diversity Index (H') for each site using abundances from trapping for individual species. I used one-way analysis of variance (ANOVA) followed by Tukey's post-hoc comparisons to test for differences in species richness and diversity (H') among the KY-WRAM score groups. To analyze variation in amphibian species composition, I calculated a Sorensen dissimilarity index, and used a Mantel test to examine the correlation of Euclidian distance and Sorensen's dissimilarity matrices between pairs of sites. Shannon index scores, Sorensen's dissimilarity and the Mantel statistic were calculated in Program R Version 3.2.2 (R Development Core Team 2015) using package vegan (Oksanen et al., 2015).

I examined all possible variables for modeling, and used a Pearson correlation matrix to remove correlated variables ($r > |0.7|$) and determine which individual KY-WRAM metrics, landscape-scale variables, and water quality variables could serve as explanatory variables for species richness. Wetland size was calculated using land-cover maps and information from site visits. Among the water-quality samples, a total of 10 different pesticides were detected at 23 sites during the two rounds of water sampling (Appendix 2). Most analytes were only detected at four or fewer wetlands and were not used in the analyses. Atrazine was the most commonly detected pesticide ($n = 19$), and was used as a variable for AIC modeling. I analyzed species richness using an information theoretic approach to model selection with KY-WRAM scores, landscape-scale variables, and water quality variables as the predictor variables. I used an adjusted Akaike's information criterion to account for small sample sizes (AIC_c). Using 10 covariates, 14 a priori generalized linear regression models were developed, including the global model, which contained all explanatory variables, as well as separate models containing water quality variables, landscape-scale variables, and combinations of these variables (Table 1). Among the top models, only those whose $\Delta AIC_c < 4.0$ are reported. I used model averaging to examine which variables in these top models contributed to explaining species richness, weighted the parameter estimates from each top model, and calculated 85% confidence intervals to make these AIC compatible (Arnold, 2010). AIC modeling was conducted with Program R using package AICcmodavg (Mazerolle, 2015).

Table 1. Candidate models for amphibian species richness in riverine wetlands in the Kentucky and Salt river basins of Kentucky for 2014–2015.

Model Type	Model Parameters
GLOBAL	KYWRAM-score, Atrazine, logDO, Ammonia, Nitrate/nitrite, Total Phosphorus, LDI, Presence of Predatory Fish, Size, Basin
KY-WRAM	KY-WRAM
Water Quality	Ammonia, Nitrate / Nitrite, Phosphorus, logDO
	Atrazine
Landscape Scale	LDI
	LDI, Predatory Fish, Size, Basin
	Basin
	Basin, LDI
	Size
Combined	KY-WRAM, Basin, LDI
	KY-WRAM, Atrazine
	KY-WRAM, LDI
	KY-WRAM, Size
	KY-WRAM, Basin

A presence-absence matrix was generated for each species positively identified, based on data from any of the three sampling techniques. I used contingency tables to determine if species were present in higher proportions of medium and high-category sites than low ones. I used 2 X 3 contingency tables and bootstrapping (1000 permutations) because there were cell frequencies < 5 for each species (Amiri & von Rosen, 2011). When a significant difference was observed, pairwise comparisons among the score groups were completed using 2 X 2 contingency tables. The proportion of species encountered within each group (observed) was compared to the number of wetlands within each score category (expected) using Pearson chi-square. For individual species abundance analyses, catch per unit effort (CPUE) was calculated per wetland by dividing the total number of individuals captured via trapping by the total number of traps (Shono, 2008; Shulse et al., 2010; Denton and Richter, 2013). These data were analyzed for each species separately using the same a priori models as for species richness with an AIC adjusted for small sample sizes (AIC_c). I used a compound Poisson (Tweedie) distribution with log-link function to determine which metrics were associated with abundance of each species. The power parameter value (*p*), which ranges from 1–2 and determines the shape of the distribution of the data (Shono, 2008), was calculated for each species individually using Pearson chi-square. Modeling for individual species was completed in SPSS version 18.

I used redundancy analysis (RDA) to evaluate the effects of KY-WRAM scores, landscape-scale factors, and water quality variables on amphibian species richness in R with package vegan (Oksanen et al., 2015). A Hellinger transformation of the abundance data was used to meet the assumptions of normality (Legendre and Gallagher, 2001). In addition to the RDA, I used a permutation multivariate analysis of variance with a distance matrix (ADONIS) to compare amphibian community composition among the wetland condition categories using 999 permutations.

3. RESULTS

For the 42 wetlands surveyed, KY-WRAM scores ranged from 29 to 84.5 with 7 low, 24 medium, and 11 high category wetlands. I captured and positively identified a total of 11,622 amphibians (adults, juveniles, and larvae) representing 25 species. Two species, *Eurycea lucifuga* and *Pseudotriton ruber*, were only encountered during visual surveys. *Gastrophryne carolinensis* was encountered during visual encounter surveys and dipnetting, but was not caught in traps. All three species were excluded from abundance analyses. Species richness ranged from 2 to 16, and mean richness (± SE) for low, medium and high categories were 4.14 ± 0.77, 7.29 ± 0.52, and 11.64 ± 0.66, respectively, and differed significantly among wetland condition categories ($F_{2,39}$ = 23.99, p <0.001). Using post-hoc Tukey's comparisons I found that species richness was significantly greater in the high category sites compared to the medium and low category sites (low-medium p = 0.009, low-high and medium-high p<0.001). I found a significant positive relationship between amphibian species richness and wetland quality as measured by KY-WRAM scores ($F_{1,40}$ = 64.49, p <0.001, R^2 = 0.62) (Fig. 2). Species richness did not significantly differ between the two basins (p = 0.230).

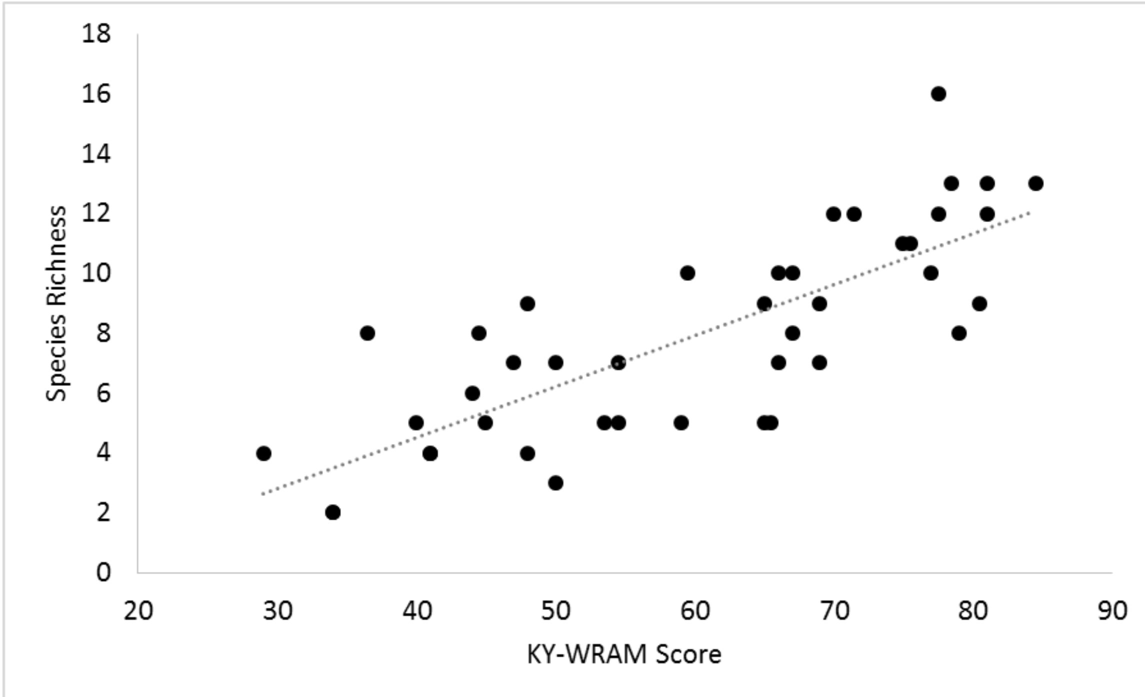


Figure 2. Species richness and KY-WRAM score indicating a significant, positive relationship for 42 riverine wetlands in Kentucky sampled in 2014–2015 ($p < 0.001$, $R^2 = 0.62$).

Mean Shannon diversity index scores (H') (± SE) for low, medium, and high category sites were 0.48 ± 0.21, 0.78 ± 0.09, and 1.31 ± 0.13, respectively, and were significantly different among the three groups ($F_{2,39} = 7.86$, $p = 0.001$). Post-hoc Tukey's comparisons showed that Shannon diversity was significantly greater in the high category sites compared to the medium ($p = 0.009$) and low ($p = 0.002$) category sites, but was not significantly different between the medium and low categories ($p = 0.292$). Results from the Mantel test show a significant relationship between Euclidean distance and Sorensen's dissimilarity between pairs of sites though only a small amount of variation was explained by the relationship ($R^2 = 0.11$; $p = 0.001$).

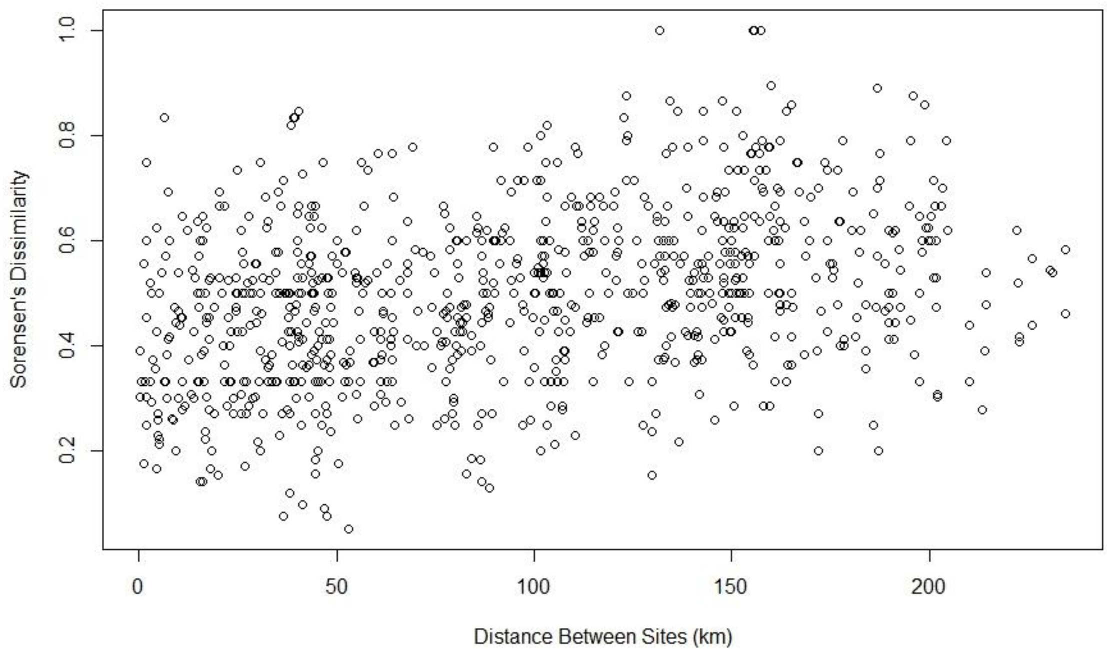


Figure 3. Biplot of Sorensen's dissimilarity scores and distance between pairs of sites (km) for wetlands surveyed in the Kentucky and Salt river basins, 2014-2015. (R^2 = 0.11, p<0.001).

Nutrients were analyzed using the highest level measured for each analyte (peak) between the two sampling periods (March and May), as these values represent water conditions for breeding adults and developing larvae. Mean-peak ammonia, nitrate/nitrite, and total phosphorous (±SD) were 0.42 mg/L (±0.48), 0.28 (±0.50), and 0.41 (±0.77), respectively. Atrazine ranged from 0.01 to 0.33 µg/L, and the mean peak was 0.04 (±0.06).

For species richness, five models had ΔAIC_c <4, and no single model had a model weight above 0.9 (Table 2). The model with only KY-WRAM score was the highest-weighted model, and the parameter was a variable in each of the other top models for species richness. Among these models, only the model-weighted parameter KY-WRAM score was significantly different from zero (Table 3).

Table 2. Linear regression models for species richness in riverine wetlands in the Kentucky and Salt river basins of Kentucky. Only models with a difference in Akaike's Information Criterion corrected for small sample sizes (ΔAICc) below 4.0 are listed.

Model	k[a]	AIC_c	ΔAIC_c	ω_i[b]	Log Likelihood
KY-WRAM	2	188.56	0	0.36	-90.97
KY-WRAM + Atrazine	3	190.01	1.45	0.18	-90.46
KY-WRAM + LDI	3	190.05	1.48	0.17	-90.48
KY-WRAM + Size	3	190.75	2.19	0.12	-90.83
KY-WRAM + Basin	3	190.80	2.24	0.12	-90.86

[a]Number of parameters in model, includes intercept.
[b]Akaike model weight

Table 3. Model-averaged parameter estimates for top species richness models (ΔAICc < 4.0) for 42 riverine wetlands in the Kentucky and Salt river basins of Kentucky for 2014–2015.

Parameter	Model-Averaged Estimate ($\bar{\beta}$)	Unconditional SE	85% CI[a]
KY-WRAM	0.16	0.04	0.10, 0.22
LDI	-0.04	0.06	-0.13, 0.05
Basin	0.04	0.09	-0.09, 0.16
Atrazine	0.99	1.30	-0.89, 2.86
Size	0.00	0.01	-0.01, 0.01

[a]85% Confidence interval was used to make values AIC compatible (Arnold 2010).

I evaluated the same 14 candidate models for individual species abundance (i.e., CPUE) of seven species (Table 4). The abundance of other species was too low to allow statistical analyses. Of the seven species tested, none had a single top model (i.e. ω_i >0.9), so I averaged model-weighted parameter estimates for models with ΔAIC_c <4.0, and reported 85% confidence intervals for each parameter (Table 5). Top AIC_c models for all seven species included KY-WRAM as an important variable, although only two species, *Acris crepitans* and *Pseudacris crucifer,* included KY-WRAM as statistically significant (Table 5).

Table 4. Models with ΔAICc < 4.0 for individual amphibian species abundance caught via minnow traps (CPUE) in 42 riverine wetlands in in the Kentucky and Salt river basins, 2014–2015.

Species	Model (ΔAIC_c <4.0)	AIC_c	ΔAIC_c	ω_i	log likelihood
Acris crepitans	KY-WRAM, Atrazine, log DO, Ammonia, Nitrate / nitrite, Total Phosphorus, LDI, Fish, Size, Basin	95.35	0.00	0.72	-30.29
	LDI, Fish, Size, Basin	97.25	1.91	0.28	-41.43
Hyla chrysoscelis	KY-WRAM, Atrazine, log DO, Ammonia, Nitrate / nitrite, Total Phosphorus, LDI, Fish, Size, Basin	91.07	0.00	0.58	-28.16
	Size, Basin	93.16	2.09	0.21	-39.33
	KY-WRAM, Basin, LDI	93.44	2.36	0.18	-40.88
Lithobates catesbeianus	Size	49.70	0.00	0.56	-21.54
	KY-WRAM, Size	51.26	1.56	0.26	-21.09
Lithobates clamitans	Basin, LDI	146.69	0.00	0.49	-68.81
	KY-WRAM, Basin, LDI	148.47	1.78	0.20	-68.40
	Basin	149.27	2.58	0.14	-71.32
	KY-WRAM, Atrazine, log DO, Ammonia, Nitrate / nitrite, Total Phosphorus, LDI, Fish, Size, Basin	150.49	3.80	0.07	-57.87
Pseudacris crucifer	KY-WRAM, Size	87.00	0.00	0.43	-38.96
	KY-WRAM	88.98	1.98	0.16	-41.18
	KY-WRAM, Basin	90.49	3.49	0.08	-40.71

Table 4 (continued)

Species	Model (ΔAIC_c <4.0)	AIC_c	ΔAIC_c	ω_i	log likelihood
Ambystoma jeffersonianum	Atrazine	79.35	0.00	0.19	-36.36
	Ammonia, Nitrate / Nitrite, Phosphorus, log DO	79.68	0.33	0.16	-32.64
	Basin	80.16	0.81	0.13	-36.77
	LDI	80.41	1.06	0.11	-36.89
	KY-WRAM	81.30	1.95	0.07	-37.33
	KY-WRAM, Atrazine	81.48	2.13	0.07	-36.20
	Size	81.65	2.30	0.06	-37.51
	KY-WRAM, Basin	81.84	2.49	0.06	-36.38
	Basin, LDI	82.04	2.69	0.05	-36.48
	KY-WRAM, Size	82.74	3.39	0.04	-36.83
	KY-WRAM, LDI	82.81	3.46	0.03	-36.86
Ambystoma opacum	Basin, LDI	57.36	0.00	0.57	-24.14
	KY-WRAM, Basin, LDI	58.91	1.55	0.26	-23.62
	LDI, Fish, Size, Basin	60.17	2.81	0.14	-22.88

Table 5. Model-averaged parameter estimates and 85% confidence intervals for individual amphibians captured via minnow traps in riverine wetlands in Kentucky, 2014–2015.

Species	Parameter	Model-Averaged Estimate ($\overline{\beta}$)	Unconditional SE	85% CI
Acris crepitans	KY-WRAM	0.03	0.02	**0.00, 0.06**
	LDI	0.74	0.36	**0.23, 1.23**
	Atrazine	-9.92	5.44	**-17.8, -2.09**
	Size	-0.11	0.10	-0.25, 0.03
	Ammonia	-1.60	0.52	**-2.35, -0.86**
	Nitrate / nitrite	0.67	0.38	**0.12, 1.22**
	Total Phosphorus	-0.34	0.45	-0.99, 0.31
	log DO	2.23	1.20	**0.50, 3.95**
	Fish	-0.97	0.48	**-1.67, -0.27**
Hyla chrysoscelis	KY-WRAM	0.03	0.02	-0.03, 0.05
	LDI	-0.96	0.27	**-1.40, -0.60**
	Basin	-2.40	0.67	**-3.40, -1.40**
	Atrazine	-2.66	4.80	-9.57, 4.25
	Size	-0.04	0.03	**-0.08, -0.00**
	Ammonia	-0.77	0.48	**-1.46, -0.08**
	Nitrate / nitrite	-1.61	5.33	-9.29, 6.07
	Total Phosphorus	0.48	0.43	-0.15, 1.10
	log DO	0.44	0.52	-0.30, 1.19
	Fish	0.56	0.38	**0.01, 1.11**
Lithobates catesbeianus	KY-WRAM	0.00	0.00	-0.01, 0.01
	Size	-0.10	0.04	**-0.11, -0.08**
Lithobates clamitans	KY-WRAM	0.00	0.01	-0.01, 0.00
	LDI	-0.29	0.18	**-0.56, -0.03**
	Basin	-1.78	0.48	**-2.47, -1.09**
	Size	0.00	0.00	-0.00, 0.01
	Atrazine	0.18	0.35	-0.32, 0.68
	Ammonia	-0.09	0.09	-0.26, 0.04
	Nitrate / nitrite	0.03	0.04	-0.03, 0.09
	Total Phosphorus	-0.02	0.04	-0.08, 0.05
	log DO	0.15	0.15	-0.07, 0.37
	Fish	0.03	0.05	-0.03, 0.10
Pseudacris crucifer	KY-WRAM	0.08	0.04	**0.02, 0.14**
	Basin	-0.07	0.09	-0.20, 0.07
	Size	-0.07	0.05	-0.14, 0.00

Table 6 (continued)

Species	Parameter	Model-Averaged Estimate ($\bar{\beta}$)	Unconditional SE	85% CI
Ambystoma jeffersonianum	KY-WRAM	0.01	0.01	-0.01, 0.03
	LDI	-0.09	0.11	-0.24, 0.07
	Basin	0.35	0.39	-0.21, 0.92
	Atrazine	-8.89	8.48	-21.1, 3.32
	Size	-0.01	0.01	-0.02, 0.01
	Ammonia	0.02	0.29	-0.40, 0.43
	Nitrate / nitrite	-4.36	4.44	-10.76, 2.03
	Total Phosphorus	-0.15	0.33	-0.63, 0.32
	log DO	0.63	0.75	-0.46, 1.71
Ambystoma opacum	KY-WRAM	0.01	0.01	-0.01, 0.02
	LDI	-1.18	0.41	-1.77, -0.60
	Basin	1.22	1.53	-0.98, 3.42
	Size	0.00	0.01	-0.12, 0.01
	Fish	-0.07	0.16	-0.31, 0.16

There was a significant relationship between presence of eight of the 25 species and KY-WRAM score groups (Table 6). Of these eight species, pairwise comparisons among KY-WRAM score categories indicated that six species were present in higher proportions in medium and high category sites than lower ones (Table 7). *Acris crepitans* was the only species where the proportion of sites for the low category was not significantly different than the medium or high categories. Bonferroni corrections were used to adjust significance values for multiple pairwise comparisons (n = 3).

Table 7. Relative percentages and wetland counts for amphibian species encountered within the three KY-WRAM score groups for 42 riverine wetlands in the Kentucky and Salt river basins for 2014–2015. Counts (in parenthesis) are positive encounters at sites within each category using visual encounter, dipnetting and minnow trapping.

	Low	Medium	High	Pearson X^2	p
Acris crepitans	57.14 (4)	45.83 (11)	90.91 (10)	6.38	0.041
Anaxyrus americanus	0	45.83 (11)	81.82 (9)	11.55	0.003*
An. fowleri	0	20.83 (5)	63.64 (7)	10.13	0.006*
Gastrophryne carolinensis	14.28 (1)	4.17 (1)	0	1.97	0.374
Hyla chrysoscelis	42.86 (3)	91.67 (22)	100 (11)	13.03	0.001*
H. cinerea	0	4.17 (1)	0	0.77	0.681
Lithobates catesbeianus	100 (7)	75.00 (18)	81.82 (9)	2.20	0.332
L. clamitans	100 (7)	95.83 (23)	100 (11)	0.77	0.681
L. sphenocephalus	0	25.00 (6)	63.64 (7)	9.03	0.011*
L. palustris	0	41.67 (10)	36.36 (4)	4.30	0.117
L. pipiens	0	0	9.09 (1)	2.89	0.236
L. sylvaticus	0	12.50 (3)	45.45 (5)	7.29	0.026
Pseudacris crucifer	28.57 (2)	62.50 (15)	54.55 (6)	2.52	0.284
P. feriarum	14.28 (1)	37.50 (9)	45.45 (5)	1.89	0.389
Scaphiopus holbrooki	0	0	9.09 (1)	2.89	0.236
Ambystoma barbouri	14.28 (1)	37.50 (9)	54.55 (6)	2.95	0.229
Am. jeffersonianum	0	29.17 (7)	54.55 (6)	6.04	0.049
Am. maculatum	14.28 (1)	20.83 (5)	54.55 (6)	5.04	0.080
Am. opacum	28.57 (2)	41.67 (10)	72.73 (8)	4.14	0.126
Am. tigrinum	0	4.17 (1)	18.18 (2)	2.88	0.237
Hemidactylium scutatum	0	0	9.09 (1)	2.89	0.236
Notophthalmus viridescens	14.28 (1)	16.67 (4)	54.55 (6)	6.21	0.045
Eurycea cirrigera	0	12.5 (3)	36.36 (4)	4.77	0.092
E. lucifuga	0	0	18.18 (2)	5.92	0.052
Pseudotriton ruber	0	0	9.09 (1)	2.89	0.236

Bootstrapping 1000 iterations

Low n = 7, medium n = 24, high n = 11

*Significant with Bonferroni correction

Table 8. Pairwise comparisons of amphibian species detections among the three KY-WRAM score groups for riverine wetlands in the Kentucky and Salt river basins for 2014–2015.

	Low to Medium Pearson X^2	p	Low to High Pearson X^2	p	Medium to High Pearson X^2	p
Acris crepitans	0.28	0.598	2.82	0.093	6.39	0.012*
Anaxyrus americanus	4.97	0.026	11.46	0.001*	3.99	0.046
An. fowleri	1.74	0.187	7.29	0.007*	6.13	0.013*
Hyla chrysoscelis	8.27	0.004*	8.08	0.004*	0.97	0.324
L. sphenocephalus	2.17	0.141	7.29	0.007*	4.82	0.028
L. sylvaticus	0.97	0.325	4.41	0.036	4.65	0.031
Am. jeffersonianum	2.64	0.104	5.73	0.017*	2.08	0.149
Notophthalmus viridescens	0.02	0.880	2.92	0.088	5.30	0.021

Bootstrapping 1000 iterations
*Significant with Bonferroni correction

The RDA analysis was significant (p = 0.006), and the KY-WRAM scores, landscape-scale factors and water quality variables accounted for 12% of the variation in amphibian community composition (Figure 4). Among the model parameters, KY-WRAM (F = 3.31) and basin (F = 4.51) both had significant contributions in explaining differences in community composition among sites (p = 0.001) (Figure 4). Vectors for nutrients and dissolved oxygen cluster together, with atrazine, away from KY-WRAM scores (Figure 4A). Landscape disturbance (LDI) and KY-WRAM scores show an opposite association. Bullfrogs (*L. catesbeianus*) clustered near the vector for LDI (Figure 4B). In contrast, the Jefferson's (*A. jeffersonianum*) and marbled salamanders (*A. opacum*) clustered together, along the axis with KY-WRAM scores. Results from the ADONIS procedure indicated that there was a significant difference among the three KY-WRAM score categories ($F_{2,39}$ = 1.42, p = 0.03). Communities in the low and high categories were significantly different (p = 0.01), but differences were not significant between low and medium (p = 0.06) or medium and high (p = 0.49).

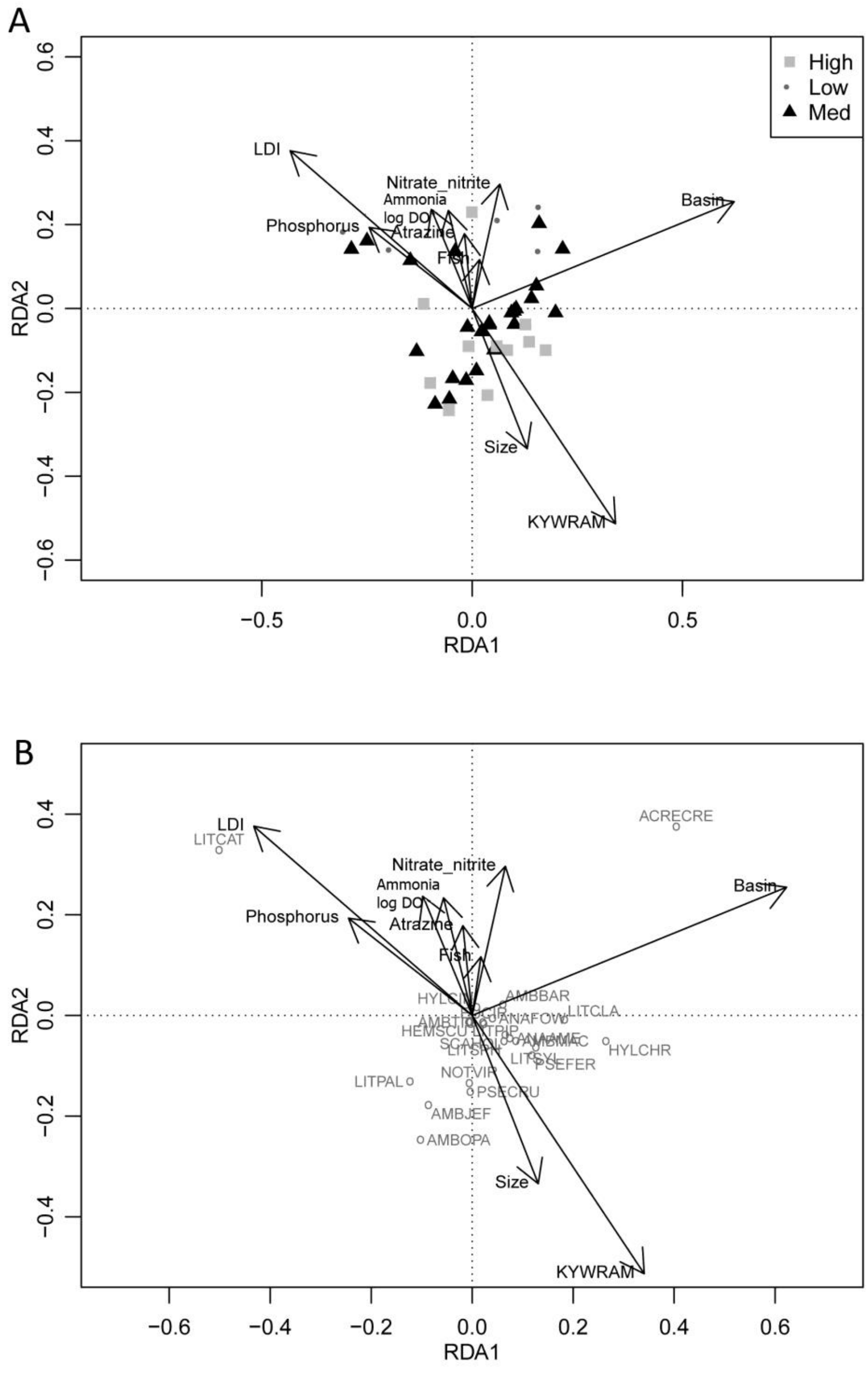


Figure 4. Redundancy analysis (RDA) plots for sites by KY-WRAM score category (A) and species abundance by catch-per-unit effort (B) for 42 riverine wetlands in the Kentucky and Salt river basins for 2014 and 2015.

4. DISCUSSION

Results from both the rapid assessment and intensive survey of amphibians indicate that wetland condition is highest among sites with few anthropogenic impacts to wetland habitats, and that are surrounded by natural landscapes. Additionally, results from both the AIC-selected models and RDA provide support that KY-WRAM scores are an important variable for explaining species richness and abundance of seven species. Among KY-WRAM scoring categories, I found that eight species were present in higher proportions of medium and high-scoring wetlands than low-scoring wetlands. Wetlands are important ecosystems because they not only provide habitat for a variety of organisms, but they provide critical ecosystem functions which are associated with economic values that directly impact humans (Mitsch and Gosselink, 2000). KY-WRAM sub-metrics encompass variation in wetland condition along a human-disturbance gradient and represent both stressors (e.g. habitat modification) and wetland function (e.g. hydrology). Rapid wetland assessments are important management tools that can be utilized to assess restoration and mitigation projects and provide protection for existing resources in the absence of intensive bioassessments (Fennessy et al., 2007).

Intensive bioassessments have been used by multiple states to validate rapid assessments, and validation is confirmed by positive relationships between scores and taxa richness and abundance, across a gradient of condition. KY-WRAM scores are influenced by the various habitat and land-use metrics contained within its scoring methodology, and positively reflect a change in condition. KY-WRAM sub-metrics 4 (habitat alteration and habitat structure development) and 6 (vegetation, interspersion and habitat features) were both positively correlated with amphibian species richness ($r = 0.77$ and 0.78, respectively). These sub-metrics were also correlated with KY-WRAM scores, and so they were not included as separate variables in model selection. These results are similar to those from Ohio, which indicate that metric scores for disturbance are negatively associated with both plant and amphibian communities, and that disturbance within wetlands may decrease the suitability of habitats for amphibians

(Stapanian et al., 2013; Micacchion et al., 2015). Habitat characteristics such as hydroperiod and landscape-scale factors including surrounding land use and buffer sizes have been linked to amphibian richness and community composition in other studies (Babbitt et al., 2003; Semlitsch and Bodie, 2003).

As I anticipated, results show that wetlands and uplands that are heavily impacted by anthropogenic activities have lower richness and abundances of amphibians than wetlands in less disturbed landscapes. Low species richness was likely due to habitat modifications from land-use; low-quality sample sites were riverine wetlands that had been altered to retain water (e.g. cattle or storm water retention ponds), and were surrounded by high-intensity agriculture or urban land-use. In contrast, sites that scored highest under the KY-WRAM have the highest amphibian diversity and support both sensitive and tolerant species. These high-quality wetlands had the lowest levels of habitat disturbance, low levels of invasive plants, and were surrounded by larger areas of buffer. Micacchion and Gara (2008) found that amphibian richness was much lower in urban wetlands, and assemblages were composed of more tolerant species versus reference wetlands, which had higher numbers of sensitive species. Likewise, Lehtinen et al. (1999) found that amphibians responded negatively to urbanization and density of roads at a distance as far as 2500 m from sites.

Results from the RDA provide further support that the KY-WRAM reflects a gradient of condition, and that species respond to habitat impacts differently. Species that are considered habitat generalists (i.e. bullfrog, *L. catesbeianus*) are found among all score groups, whereas species that are considered sensitive (i.e. marbled salamander, *A. opacum*) are found at sites with higher KY-WRAM scores. These results are reflected in their life histories: Bullfrogs are generally considered tolerant to habitat disturbance and prefer ponds with permanent hydrology (Lannoo, 2005); whereas, species such as the Jefferson's (*A. jeffersonianum*) and Marbled salamanders were more closely linked to higher KY-WRAM scores. These two species spend a majority of their adult lives in the terrestrial area surrounding wetlands, and are less tolerant of impacts in these upland areas. Other salamanders such as four-toed (*H. scutatum*), cave

(*Eurycea lucifuga*), and red salamanders (*Pseudotriton ruber*) were only found at high-category wetlands. These species have more specific habitat requirements (e.g. coarse woody debris for refuge, cool clean water, and mossy areas for nesting) and are generally intolerant to habitat disturbance (Petranka, 2010).

Many amphibians are negatively impacted by changes in the landscape surrounding wetlands, which they use for foraging, overwintering and dispersal (Simon et al., 2009). Babbitt et al. (2009) investigated links between surrounding land use, species richness, and wetland health and found that wetlands surrounded by agriculture had significantly lower species richness and abundance than wetlands surrounded by rangeland or woodland. Likewise, other studies have found that wetlands that are closely surrounded by urbanization and agriculture have greater habitat fragmentation and have lower amphibian richness (Houlahan and Findlay, 2003; Riley et al., 2005). Similar to these other studies, I found that species richness had a slight negative relationship with the landscape disturbance intensity index (LDI), though the parameter did not significantly contribute to variation in species richness. LDI scores are higher at sites surrounded by high-intensity land use and lower at sites surrounded by natural landscapes. LDI was included in the top models for the abundance of five species, and was a significant predictor for four. Cope's gray tree frog (*H. chrysoscelis*), green frog (*L. clamitans*), and marbled salamander (*A. opacum*) all have negative relationships with LDI. The abundance of cricket frog (*Ac. crepitans*) was positively associated with LDI, but relationships for this species may be skewed due to one outlier. Shulse et al. (2010) found that salamander (*Ambystoma*) abundance was negatively associated with disturbance, specifically percent cropland and road density within proximity of the wetland.

Presence of predatory fish was a significant factor in models of abundance for two species; the cricket frog had a negative relationship with the presence of fish and Cope's gray tree frog had a positive relationship. Similarly, Lehtinen et al. (1999) found that within the prairie habitats of their study, species richness had a positive relationship with presence of fish. Other studies have found that presence of fish have negative effects on amphibian communities. Boone et al. (2007) found bluegill presence caused a 28% reduction in species richness and larval survival for three amphibians was eliminated or greatly reduced. Likewise, Holbrook and Dorn (2015) found that fish presence and hydroperiod had a negative effect on both species richness and abundance.

Nutrients (i.e. ammonia, nitrate / nitrite, and total phosphorus) were parameters in the top models for abundance (CPUE) of four species. Ammonia was negatively associated with abundances of the cricket frog and Cope's gray tree frog. Cricket frog abundances were positively related to nitrates/nitrites and log-transformed dissolved oxygen (log DO). Although nutrients were not significant in the RDA, they were clustered together, away from KY-WRAM scores. In my samples, mean peak ammonia was measured at levels above those that have been determined to be cold-water lethal concentrations for amphibians. Diamond et al. (1993) found that ammonia acute cold-water (12°C) LC50s for *Lithobates pipiens* and *Pseudacris crucifer* were 0.42 and 0.46 mg/L, respectively. Among my water samples, ammonia concentrations ranged from 0.025 to 2.08 mg/L, with 2 detections above 0.42 mg/L and 15 above 0.46 mg/L. Other studies that have examined effects of nutrients on amphibian richness have been varied. Boone et al. (2007) found positive associations and increased tadpole mass at metamorphosis with addition of 10 mg/L of ammonium nitrate, though this was likely due to increase of periphyton. Hecnar and M'Closkey (1996) found that nutrient ranges of 0.3-4.3 mg/L and 0.1-77.4 mg/L for ammonia and nitrate, respectively, had an overall weak negative effect on amphibian species richness. Similarly, Houlahan and Findlay (2003) found all species except one were negatively correlated with nutrient levels. In their study, species richness was negatively correlated with mean ranges of total Kjeldahl nitrogen (0.61-5.15 mg/L) and total phosphorus (0.02-0.68 mg/L). Although I did not detect strong relationships between water-quality and species richness or individual species abundance, it is likely that pollutants, in conjunction with other anthropogenic stressors (i.e. degraded wetland and upland habitats, and pesticides and other agrochemicals), have negative effects on amphibian populations, including many sites in Kentucky (Brodman et al., 2003; Mann et al., 2009).

The pesticide atrazine was negatively associated with abundance of four species, but only cricket frogs had a significant association. Atrazine was not a significant factor for species richness. This is likely due to the fact that atrazine was detected in water samples at 19 wetlands, and over half of the high-category sites. Atrazine detections (0.01–0.33 ppb) were below EPA water-quality standards (1.5 ppb); however, Hayes et al. (2003) demonstrated that male Northern Leopard frogs (*L. pipiens*) exhibited hermaphroditism at levels as low as 0.1 ppb. Within the state of Kentucky, atrazine is often applied prior to planting or emergence of soybeans and corn to combat broadleaf weeds (farmers, personal communication March 2015). The timing of these applications coincides with the breeding season and developmental period of most pond-breeding amphibians (Hayes et al., 2003). Results from many studies indicate that amphibian exposure to pesticides, usually combined with other stressing factors, negatively influence growth, development, reproductive success and mortality (Boone et al., 2007; Kiesecker, 2011; Baker et al., 2013). In my study, the effect of pesticides on amphibians is difficult to determine, but as in other studies, it is likely that the additive stress of individual parameters contribute to the decline of amphibians.

Besides atrazine, nine additional pesticides were detected at 23 of our sample sites, though none in a high enough frequency for statistical analysis. Among the contaminants detected during our study, four wetlands had detections of hexachlorobenzene, a fungicide historically used to treat against rot in seeds. Three of the wetlands were in the medium category, and one was the highest-scoring of the study sites. Hexachlorobenzene has been listed as a bioaccumulating pollutant with carcinogenic effects in animals, and has a half-life of 3–6 years (ATSDR, 2013). Although the United States EPA banned the use of hexachlorobenzene in 1966, studies have found this chemical is a by-product of the manufacture of other pesticides, namely the fungicide chlorothalonil (Vargyas et al., 2000), although this fungicide was not detected at any of the sites. There are few studies that test the effects of hexachlorobenzene on amphibians, but environmentally-relevant concentrations of chlorothalonil have been linked to larval mortality in several species of amphibians (McMahon et al., 2011). During the spring of 2015, the Kentucky and Salt river basins experienced high snow melt, followed quickly by high rains. The ensuing flooding prevented water sampling at several wetlands, or in some cases forced sampling at alternate locations during the March sampling period. While this water can help flush wetlands of contaminants, samples may not represent the seasonal water conditions that amphibians experience during larval development.

For this study, amphibian surveys were conducted in riverine wetlands of two of the seven basins in Kentucky. Future work should be expanded to include additional basins and other wetland types to continue confirming that KY-WRAM scores reflect wetland condition and changes in amphibian community composition. Future research should also continue to investigate links between KY-WRAM scores, water quality parameters, effects from impacts to the surrounding landscape, and amphibian community composition. Many states have used data from intensive biological surveys to calibrate and validate rapid assessment methods. Further, identifying metrics for each taxon that change in response to disturbance and reflect wetland condition, as scored by the rapid assessment method, can serve as a base for development of taxon-specific indices of biological integrity (IBIs) and can be used to inform regulatory decisions for wetland mitigation and restoration projects (Shulse et al., 2010; Mack and Kentula, 2010; Micacchion et al., 2015). Further work should include development of an amphibian index of biotic integrity (IBI) for the state of Kentucky. This method will be useful to standardize monitoring techniques and amphibian assessments at wetlands throughout the state.

Within my models, the KY-WRAM explained the majority of variation in species richness. This rapid assessment method accounts for factors which have been linked to changes in amphibian communities (e.g. hydrological alterations, impacts within wetland habitats and land-use type in wetland buffers). Similar to other studies, my research demonstrates that amphibians respond negatively to impacted water quality, intensity of surrounding land use (LDI) and impacts to within-wetland habitats (KY-WRAM). Although parameters besides KY-WRAM accounted for small amounts of variation in amphibian community composition, other studies have shown that these factors (e.g. increased nutrients and pesticides) have deleterious effects on amphibians. My models show that a variety of parameters are included in the top models of several species, which indicate that multiple factors interplay to influence richness and abundance at sites. As with other studies, it is likely that the additive effects of multiple stressors compound together to have larger effects on community structure. Overall, these results indicate that among my study sites, wetland condition is reflected in KY-WRAM scores, and strongly relates to amphibian communities. KY-WRAM scores reflect a gradient of wetland condition, and anthropogenic impacts within wetland habitats and surrounding uplands are reflected in these scores. Continued validation and implementation of the KY-WRAM will provide an ecological framework for evaluation of conservation, mitigation and restoration projects throughout a state in which wetlands have suffered massive losses to both wetland area and function. Ultimately this is important for pond-breeding amphibians because of their dependence on wetlands as well as upland habitats at various landscape scales throughout their complex life cycles.

5. REFERENCES

AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY (ATSDR). 2013. Toxicological profile for Hexachlorobenzene. (Draft for Public Comment) Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

AMBURGEY, S., W.C. FUNK, M. MURPHY, and E. MUTHS. 2012. Effects of hydroperiod duration on survival, developmental rate, and size at metamorphosis in Boreal Chorus frog tadpoles (*Pseudacris maculata*). *Herpetologica* 68: 456–467.

AMIRI, S., and D. VON ROSEN. 2011. On the efficiency of bootstrap method into the analysis contingency table. *Computer Methods and Programs in Biomedicine* 104: 182–187.

ARNOLD, T.W. 2010. Uninformative parameters and model selection using Akaike's Information Criterion. *Journal of Wildlife Management* 74: 1175–1178.

BABBITT, K.J., M.J. BABER, D.L. CHILDERS, and D. HOCKING. 2009. Influence of agricultural upland habitat type on larval anuran assemblages in seasonally inundated wetlands. *Wetlands* 29: 294–301.

BABBITT, K.J., M.J. BABER, and T.L. TARR. 2003. Patterns of larval amphibian distribution along a wetland hydroperiod gradient. *Canadian Journal of Zoology* 81: 1539–1552.

BAKER, N.J., B.A. BANCROFT, and T.S. GARCIA. 2013. A meta-analysis of the effects of pesticides and fertilizers on survival and growth of amphibians. *Science of the Total Environment* 449: 150–156.

BEEBEE, T.J.C., and R.A. GRIFFITHS. 2005. The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation* 125: 271–285.

BOONE, M.D., R.D. SEMLITSCH, E.E. LITTLE, and M.C. DOYLE. 2007. Multiple stressors in amphibian communities: effects of chemical contamination, bullfrogs, and fish. *Ecological Applications* 17: 291–301.

BRODMAN, R., J. OGGER, T. BOGARD, A.J. LONG, R.A. PULVER, K. MANCUSO, and D. FALK. 2003. Multivariate analyses of the influences of water chemistry and habitat parameters on the abundances of pond-breeding amphibians. *Journal of Freshwater Ecology* 18: 425–436.

BROWNE, C.L., C.A. PASZKOWSKI, A. LEE FOOTE, A. MOENTING, and S.M. BOSS. 2009. The relationship of amphibian abundance to habitat features across spatial scales in the Boreal Plains. *Ecoscience* 16: 209–223.

BROWN, M.T., and M.B. VIVAS. 2005. Landscape Development Intensity Index. *Environmental Monitoring and Assessment* 101: 289–309.

BRÜHL, C.A., T. SCHMIDT, S. PIEPER, and A. ALSCHER. 2013. Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? *Scientific Reports* 3: .

COLLINS, J.N., E.D. STEIN, M. SUTULA, R. CLARK, A.E. FETSCHER, L. GRENIER, C. GROSSO, and A. WISKIND. 2008. California Rapid Assessment Method (CRAM) for wetlands, v. 5.0. 2. 157 pp. *http://www.cramwetlands.org/documents/2008-09-30CRAM205* 205: .

DAHL, T.E. 1990. Wetland losses in the United States - 1780's to1980's. 1990. U.S. Department of the Interior, Fish and Wildlife Service, Washington,. D.C.

DENTON, R.D., and S.C. RICHTER. 2013. Amphibian communities in natural and constructed ridge top wetlands with implications for wetland construction: Amphibian communities in constructed wetlands. *The Journal of Wildlife Management* 77: 886–896.

DENTON, R., D., and S.C. RICHTER. 2012. A quantitative comparison of two common amphibian sampling techniques for wetlands. *Herpetological Review*44–47.

DIAMOND, J.M., D.G. MACKLER, W.J. RASNAKE, and D. GRUBER. 1993. Derivation of site-specific ammonia criteria for an effluent-dominated headwater stream. *Environmental Toxicology and Chemistry* 12: 649–658.

DIXON, A.D., W.R. COX, E.M. EVERHAM, and D.W. CEILLEY. 2011. Anurans as biological indicators of restoration success in the greater Everglades ecosystem. *Southeastern Naturalist* 10: 629–646.

DODD, C.K., and B.S. CADE. 2008. Movement patterns and the conservation of amphibians breeding in small, temporary wetlands. *Conservation Biology* 12: 331–339.

FENNESSY, M.S., A.D. JACOBS, and M.E. KENTULA. 2007. An evaluation of rapid methods for assessing the ecological condition of wetlands. *Wetlands* 27: 543–560.

GIBBONS, J.W., C.T. WINNE, D.E. SCOTT, J.D. WILLSON, X. GLAUDAS, K.M. ANDREWS, B.D. TODD, ET AL. 2006. Remarkable amphibian biomass and abundance in an isolated wetland: Implications for wetland conservation. *Conservation Biology* 20: 1457–1465.

HAMER, A.J., and K.M. PARRIS. 2011. Local and landscape determinants of amphibian communities in urban ponds. *Ecological Applications* 21: 378–390.

HAYES, T., K. HASTON, M. TSUI, A. HOANG, C. HAEFFELE, and A. VONK. 2003. Atrazine-induced hermaphroditism at 0.1 ppb in American Leopard Frogs (*Rana pipiens*): Laboratory and field evidence. *Environmental Health Perspectives* 111: 568–575.

HECNAR, S.J., and R.T. M'CLOSKEY. 1996. Amphibian species richness and distribution in relation to pond water chemistry in south-western Ontario, Canada. *Freshwater Biology* 36: 7–15.

HECNAR, S.J., and R.T. M'CLOSKEY. 1997. The effects of predatory fish on amphibian species richness and distribution. *Biological conservation* 79: 123–131.

HOLBROOK, J.D., and N.J. DORN. 2015. Fish reduce anuran abundance and decrease herpetofaunal species richness in wetlands. *Freshwater Biology*n/a–n/a.

HOULAHAN, J.E., and C.S. FINDLAY. 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 1078–1094.

KARR, J.R., and E.W. CHU. 1997. Biological monitoring and assessment: using multimetric indexes effectively. University of Washington.

KARR, J.R., and D.R. DUDLEY. 1981. Ecological perspective on water quality goals. *Environmental management* 5: 55–68.

KIESECKER, J.M. 2011. Global stressors and the global decline of amphibians: tipping the stress immunocompetency axis. *Ecological Research* 26: 897–908.

LANNOO, M.J. 2005. Amphibian declines the conservation status of United States species. University of California Press, Berkeley. Available at: http://site.ebrary.com/id/10079955 [Accessed February 9, 2016].

LEGENDRE, P., and E.D. GALLAGHER. 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129: 271–280.

LEHTINEN, R.M., S.M. GALATOWITSCH, and J.R. TESTER. 1999. Consequences of habitat loss and fragmentation for wetland amphibian assemblages. *Wetlands* 19: 1–12.

MACK, J.J. 2006. Landscape as a predictor of wetland condition: an evaluation of the Landscape Development Index (LDI) with a large reference wetland dataset from Ohio. *Environmental Monitoring and Assessment* 120: 221–241.

MACK, J.J. 2001. Ohio Rapid Assessment Method for Wetlands, manual for using version 5.0. Ohio Environmental Protection Agency Technical Report WET/2001-1. Ohio Environmental Protection Agency, Division of Surface Water, Columbus, OH.

MACK, J.J., and M.E. KENTULA. 2010. Metric similarity in vegetation-based wetland assessment methods. EPA/600/R-10/140. U.S. Environmental Protection Agency, Office of Research and Development, Washington, D.C.

MANN, R.M., R.V. HYNE, C.B. CHOUNG, and S.P. WILSON. 2009. Amphibians and agricultural chemicals: Review of the risks in a complex environment. *Environmental Pollution* 157: 2903–2927.

MAZEROLLE, M.J. 2015. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R Package version 2.0-3. http://CRAN.R-project.org/package=AICcmodavg.

MCMAHON, T.A., N.T. HALSTEAD, S. JOHNSON, T.R. RAFFEL, J.M. ROMANSIC, P.W. CRUMRINE, R.K. BOUGHTON, ET AL. 2011. The fungicide chlorothalonil is nonlinearly associated with corticosterone levels, immunity, and mortality in amphibians. *Environmental Health Perspectives* 119: 1098–1103.

MICACCHION, M. 2004. Integrated Wetland Assessment Program. Part 7: Amphibian Index of Biotic Integrity (AmphIBI) for Ohio Wetlands.

MICACCHION, M., and B.D. GARA. 2008. An ecological and functional assessment of urban wetlands in central Ohio. Volume 3: Comparisons of the amphibian communities of urban and reference wetlands using level 1, 2 and 3 assessment tools.

MICACCHION, M., M.A. STAPANIAN, and J.V. ADAMS. 2015. Site-scale disturbance and habitat development best predict an index of amphibian biotic integrity in Ohio shrub and forested wetlands. *Wetlands* 35: 509–519.

MITSCH, W.J., and J.G. GOSSELINK. 2000. The value of wetlands: importance of scale and landscape setting. *Ecological economics* 35: 25–33.

OKSANEN, J., F.G. BLANCHET, R. KINDT, P. LEGENDRE, P.R. MINCHIN, R.B. O'HARA, G.L. SIMPSON, ET AL. 2015. vegan: Community Ecology Package. R package version 2.2-1. Available at: http://CRAN.R-project.org/package=vegan.

PETRANKA, J.W. 2010. Salamanders of the United States and Canada. Pbk. ed. Smithsonian Books, Washington, DC.

RILEY, S.P.D., G.T. BUSTEED, L.B. KATS, T.L. VANDERGON, L.F.S. LEE, R.G. DAGIT, J.L. KERBY, ET AL. 2005. Effects of urbanization on the distribution and abundance of amphibians and invasive species in Southern California streams. *Conservation Biology* 19: 1894–1907.

SALICE, C.J., C.L. ROWE, J.H.K. PECHMANN, and W.A. HOPKINS. 2011. Multiple stressors and complex life cycles: Insights from a population-level assessment of breeding site contamination and terrestrial habitat loss in an amphibian. *Environmental Toxicology and Chemistry* 30: 2874–2882.

SEMLITSCH, R.D. 2000. Principles for management of aquatic-breeding amphibians. *The Journal of Wildlife Management* 64: 615–631.

SEMLITSCH, R.D., and J.R. BODIE. 1998. Are small, isolated wetlands expendable? *Conservation Biology* 12: 1129–1133.

SEMLITSCH, R.D., and J.R. BODIE. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology* 17: 1219–1228.

SHONO, H. 2008. Application of the Tweedie distribution to zero-catch data in CPUE analysis. *Fisheries Research* 93: 154–162.

SHULSE, C.D., R.D. SEMLITSCH, and K.M. TRAUTH. 2009. Development of an Amphibian Biotic Index to evaluate wetland health in Northern Missouri. *World Environmental and Water Resources Congress*.

SHULSE, C.D., R.D. SEMLITSCH, K.M. TRAUTH, and A.D. WILLIAMS. 2010. Influences of design and landscape placement parameters on amphibian abundance in constructed wetlands. *Wetlands* 30: 915–928.

SIMON, J.A., J.W. SNODGRASS, R.E. CASEY, and D.W. SPARLING. 2009. Spatial correlates of amphibian use of constructed wetlands in an urban landscape. *Landscape Ecology* 24: 361–373.

STAPANIAN, M.A., J. MACK, J.V. ADAMS, B. GARA, and M. MICACCHION. 2013. Disturbance metrics predict a wetland vegetation index of biotic integrity. *Ecological Indicators* 24: 120–126.

STAPANIAN, M.A., T.A. WAITE, G. KRZYS, J.J. MACK, and M. MICACCHION. 2004. Rapid assessment indicator of wetland integrity as an unintended predictor of avian diversity. *Hydrobiologia* 520: 119–126.

STEIN, E.D., A.E. FETSCHER, R.P. CLARK, A. WISKIND, J.L. GRENIER, M. SUTULA, J.N. COLLINS, and C. GROSSO. 2009. Validation of a wetland rapid assessment method: use of EPA's level 1-2-3 framework for method testing and refinement. *Wetlands* 29: 648–665.

STUART, S.N. 2004. Status and Trends of amphibian declines and extinctions worldwide. *Science* 306: 1783–1786.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 1995. America's wetlands: our vital link between land and water.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 2002. Methods for evaluating wetland condition: introduction to wetland biological assessment. Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA-822-R-02-014.

U.S. GEOLOGICAL SURVEY. 2006. Collection of water samples (ver. 2.0): U.S. Geological Survey Techniques of water-resources investigations, book 9, chap. A4, September 2006. http://pubs.water.usgs.gov/twri9A4/.

VARGYAS, L.D., G.E. WALLS, W.R. BRAMSTEDT, and G.L. EILRICH. 2000. Simultaneous determination of chlorothalonil and hexachlorobenzene in technical and formulated materials by capillary gas chromatography: collaborative study. *Journal of AOAC International* 83: 1047–1052.

WELSH JR, H.H., and L.M. OLLIVIER. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. *Ecological Applications* 8: 1118–1132.

Appendix 1 – 2013 KY-WRAM Field Form

KY-WRAM Rating Form Version 3.0	Kentucky Wetland Rapid Assessment Method (KY-WRAM) Kentucky Division of Water

Instructions:
The Kentucky Wetland Rapid Assessment Method is intended for use as a tool for functional assessment. The method supplements, but does not replace information used in the existing regulatory process for wetlands, such as delineation. It is intended for use on all types of wetland in Kentucky. This is a rapid assessment method with combined field and office prep time (GIS) of no more than 8 hours. This method does not replace quantitative assessments such as Indices of Biotic Integrity.

The Rater is *STRONGLY URGED* to read the Guidance Manual for using the Kentucky Wetland Rapid Assessment Method (KY-WRAM) for further elaboration and discussion of the questions below prior to using the rating forms.
It is *VERY IMPORTANT* to properly and thoroughly answer each of the questions in the KY-WRAM in order to properly categorize a wetland. To *properly* answer all the questions, the boundaries of the wetland being assessed must be correctly identified. Refer to the Scoring Boundary section in the Guidance Manual for a discussion of how to determine the "scoring boundaries." In some instances, the scoring boundaries may differ from the "jurisdictional boundaries."

The KY-WRAM was developed by a Technical Working Group of state and federal agencies and Eastern Kentucky University. This method is modeled off of the Ohio Rapid Assessment Method (ORAM) with modifications influenced by North Carolina and Michigan's wetland rapid assessment methods.

The total score has been shown to be consistent year round; however, the ideal timeframe for use of this method is during the plant growing season when plant species can be reliably identified. It should be noted that the individual metrics may be scored differently between the seasons because certain metrics are easier to evaluate during the growing season (e.g., highly-invasive plant species coverage, special wetlands, vegetation components) and non-growing season (e.g., substrate/soil disturbance, hydrology).

Although the form may be filled out in a linear manner it is expected that the Rater will make note of wetland characteristics throughout the entire field evaluation. For example, alterations to the hydrology, substrate, or habitat, plant species encountered, and the amount of microtopography features present. This is an important step in evaluating the method properly.

Background Information

Name of wetland:	Evaluator name:
Date of evaluation:	Phone number:
Lat/Long coordinates: (decimal degrees)	Email:
County:	Evaluator affiliation and address:
USACE/WQC Project ID:	
Precipitation within the last 48 hours? Circle: Yes No	

Attachments: Complete and check (√) each box Attach map of wetland location. Use county road map or USGS 7.5 minute topographic map with location indicated. Attach color photographs of wetland including landscape shot of entire wetland (if possible), vegetation components, habitat types, hydrologic features, and other relevant site features. Attach prints of satellite imagery used for buffer and connectivity metrics. This should include multiple prints at appropriate scales. Prints should include labeled marks of the following: site location, Wetland Assessment Area, plant communities within the wetland, streams, 100 year floodplains, ponds, patches of open water, relevant upland features, and location of modification to wetland. Also include north arrow and scale of each print.
Wetland Sketch (include north arrow, hydrologic features, plant communities and other habitat features)

Actual Wetland Size (indicate units):
Wetland Type (indicate NWI & HGM classifications):

Background Information (continued)

Narrative Rating

1. U.S. Fish and Wildlife Service (USFWS) Critical Habitat	
Is any part of the wetland located within the same HUC-12 watershed designated as Critical Habitat? (see Narrative Discussion)	☐ Yes ☐ No
Does any federal (G1/G2) or state-listed T/E plant or animal species (S or S2) occur within the wetland's HUC-12 watershed? (see Narrative Discussion)	☐ Yes ☐ No
Does any S3 (state species of concern) species occur within the wetland's HUC-12 watershed? (see Narrative Discussion)	☐ Yes ☐ No
2. Rare Wetland Community Type	
Does the wetland include a KSNPC rare wetland community? If YES, list the community type, the size of the rare community, and the percent of the wetland area.	☐ Yes ☐ No
3. Scenic, Recreational, and Cultural Value	
Does the wetland have scenic, recreational, or cultural value? (see Narrative Discussion)	☐ Yes ☐ No
Comments:	

Site:	Rater(s):	Date:

Metric 1. Wetland Size and Distribution – Maximum 9 points.

1a. Wetland Size – Maximum 6 points. Using GIS, estimate the size of the wetland (i.e., Wetland Assessment Area). **Select one size class.**			**Score**
Sources/assumptions for size estimate (list):	≥ 50 acres	**6 pts**	
	25 acres to <50 acres	**5 pts**	
	10 acres to <25 acres	**4 pts**	
	3 acres to <10 acres	**3 pts**	
Actual Wetland Size Estimate: ________ acres	0.3 acre to <3 acres	**2 pts**	
	0.1 to 0.3 acre	**1 pts**	
Wetland area proposed to be impacted: ________ %	< 0.1 acre	**0 pts**	

1b. Wetland Scarcity – Maximum 3 points. Use USFWS National Wetlands Inventory (NWI) maps, aerial imagery, and other information to estimate percentage of wetland area remaining within a 2-mile radius from the wetland's center (use ArcGIS or by visual estimate). For this submetric, areas of open water within lakes, streams, rivers, and ponds (PUBX), etc. should be excluded. **Select the most appropriate category below.**		**Score**
0 to 5% of surrounding 2-mile radius is wetland	**3 pts**	
6 to 20% of surrounding 2-mile radius is wetland	**2 pts**	
>20% of surrounding 2-mile radius is wetland	**1 pt**	

Metric 1 Total: add 1a & 1b **(9 points max.)**	

Wetland Size Estimate + Metric to English Conversion							
acres	hectare	$feet^2$	ft on side	$yard^2$	yd on side	m^2	m on side
50	20.2	2,177,983	1,476	241,998	492	202,000	449
25	10.1	1,088,992	1,044	120,999	348	101,000	318
10	4.1	435,596	660	48,340	220	41,000	203
3	1.2	130,679	362	14,520	121	12,000	110
0.3	0.12	13,067	114	1,452	38	1,200	35
0.1	0.04	4,356	66	484	22	400	20

Site:	Rater(s):	Date:

Metric 2. Buffers and Intensity of Surrounding Land Use – Maximum 12 points.
**Use color maps for all metric 2 sub-metrics.

2a. Average Buffer Width around the Wetland's Perimeter – Maximum 4 points.
Draw the cardinal and ordinal lines from the centroid of the wetland and calculate average buffer width. Select only one score.

Buffers Include:	**Non-Buffers Include:**	
☐ shrubland, forest of any age, natural grassland, natural rock outcrops and cobble bars ☐ abandoned row crop field (vegetated & naturalizing) ☐ hay field (non-row crop) ☐ lightly managed forest (selectively logged) ☐ lightly managed parkland ☐ other wetland, lake, or river ☐ Single-track dirt roads (non-motorized vehicle trails that are not sources of sediment)	☐ lawns, golf courses, manicured parkland ☐ residential, commercial, industrial ☐ roadways (including shoulders), parking lots ☐ railroad tracks/beds ☐ active agriculture: row crop field ☐ conservation tillage, grazed pasture, utility right-of ways ☐ clear-cutting or heavily managed forest, mining, construction activity ☐ gravel or double-track dirt roads (includes ATV trails)	**Score**

Wide Buffer Width: 150 feet around the perimeter	**4 pts**	
Medium Buffer Width: 75 to <150 feet around the perimeter	**3 pts**	
Narrow Buffer Width: 25 to <75 feet around the perimeter	**2 pts**	
Very Narrow Buffer Width: 0 (no buffer) to <25 feet around the perimeter	**0 pts**	

2b. Intensity of Surrounding Land Use within 1,000 feet of the Wetland – Maximum 4 points.					
If a land use type in not listed, use the examples below to determine the category. Write in additional land use types here and indicate the land use category you assigned:					
Land Use Category	Estimate the percent coverage comprised by each of the four categories of land use below. Sum the points from all dominant land use categories (i.e., dominant is ≥25% total per category) and then average the score.				
	Land Use Types:	Estimate % of each category	**here ↓**		**Score**
Very Low:	☐ mature growth forest	☐ other wetland, lake, stream, river		**4 pts**	
Low:	☐ shrubland/young forest ☐ hay field (non-row crop) ☐ lightly managed parkland	☐ old field ☐ single track and two track dirt roads ☐ one-lane paved road		**2 pts**	
Moderately High:	☐ residential & lawns ☐ manicured parkland ☐ golf course ☐ grazed pasture ☐ utility right-of-way	☐ conservation tillage ☐ recent logging and clear-cut (<5 years) ☐ two-lane road ☐ railroad ☐ man-made lake		**1 pts**	
High:	☐ commercial, industrial ☐ high-density residential ☐ heavily grazed pasture ☐ row crop field	☐ multi-lane paved roadway ☐ construction activity ☐ parking lot ☐ hazardous areas (mining, landfills, brownfields, etc.)		**0 pts**	

For scores ending in 0.5, round up

2c. Connectivity to Other Natural Areas – Maximum 4 points.
Use GIS with field adjustment if necessary. Evaluate the wetland's connectivity to habitat patches in the greater landscape either contiguously or via a corridor (> 30 ft wide) of natural vegetation. Habitat patches and corridors must be natural terrestrial habitat (i.e., shrubland, forest, natural rock outcrops, cobble bars, wetlands, and etc.). Large streams and rivers, roads, and "non-natural" habitat such as grassland are barriers that end patches and corridors.

Connected at:	**Circle all categories that apply but report only the highest point value**		**Score**
Up to 2500 ft. (can be more)	>50% of area is patch	**4 pts**	
	<50% of area is patch *(minimum patch size requirement = 10 acres)*	**2 pts**	
Up to 1000 ft.	>25% of area is patch	**2 pts**	
	<25% of area is patch	**0 pts**	

Metric 2 Total: add 2a – 2c **(12 points max.)**	**Sub-total:**

Site:	Rater(s):	Date:

Metric 3. Hydrology – Maximum of 29 points.

3a. Input of Water From an Outside Source – Maximum 10 points. Select all that apply.		**Score**
Surface Water: Inundation from a lake, pond, or stream overbank flow at least yearly (in a typical year)	**4 pts**	
Groundwater: Score only if you observe direct evidence of groundwater (e.g. including, but not limited to, a spring or seep)	**4 pts**	
Precipitation: All wetlands receive some portion of their hydrological budget from this	**2 pt**	

3b. Hydrological Connectivity – Maximum 6 points. Select all that apply.		**Score**
100-Year Floodplain or abutting a smaller stream/creek. As defined in FEMA maps or NRCS alluvial soil maps if FEMA maps are unavailable.	**2 pts**	
Between a Stream/Lake/Pond and Human Land Use. The wetland is located between a surface waterbody and any human land use, such that run-off from the adjacent land use could flow through the wetland before it discharges into the surface waterbody.	**2 pts**	
Wetland Complex. The wetland is part of a large scale (10+ acres) complex of *other* wetlands within 2500’ of the assessment area boundary, with small areas of unmanicured/undeveloped vegetated uplands in between.	**2 pts**	

3c. Duration of Inundation/Saturation – Maximum 4 points. Select the option(s) below that best describe(s) the dominant hydrologic characteristic of the wetland. “Dominant” is defined as comprising at least 25% of the wetland area. If separate areas have distinctly different hydrologic characteristics, select all that apply and average the points. Use US ACE hydrology indicators for assistance. Use NRCS growing season criteria to determine the growing season length for the county the wetland is in. If the wetland is in the NWI database, the Rater may consult the hydrology modifiers listed in the Classification Code for assistance.			**Score**
Semi- to Permanently Inundated/ Saturated	(75 – 100% of growing season)	**4 pts**	
Regularly Inundated/ Saturated	(25 – 75% of growing season)	**3 pts**	
Seasonally Inundated	(12.5 – 25% of growing season)	**2 pts**	
Seasonally Saturated in the Upper 12 Inches of Soil	(12.5 – 25% of growing season)	**1 pt**	

3d. Alterations to Natural Hydrologic Regime – Maximum 9 points.

Evaluate the intactness of the natural hydrologic regime of the wetland. Check all forms of observed hydrologic alteration(s) that are potentially influencing the wetland (e.g. alteration may be outside of the wetland). Keep in mind that some alternations do not need to be actively maintained to have permanent negative effects.

A hydrologic alteration may also impact the Substrate/Soil (submetric 4a) and/or Habitat (submetric 4b).

Low	**High**	**Alteration**	**Low**	**High**	**Alteration**
☐	☐	ditch(es) in or near the wetland	☐	☐	stormwater inputs (addition of water)
☐	☐	tile(s) in or near the wetland	☐	☐	non-stormwater discharge(s)
☐	☐	dike(s) in or near the wetland	☐	☐	road bed(s)/RR grades(s) in or near the wetland
☐	☐	weir(s) in or near the wetland	☐	☐	dredging activities in or near the wetland
☐	☐	stream channelization	☐	☐	filling/grading activities in or near the wetland
☐	☐	other(s) (specify)	****only consider anthropogenic alterations (e.g. exclude beaver activity)**		

Select an option below that best describes the extent of wetland hydrology alteration. You may select **adjoining** options and average the points when appropriate.		**Score**
No Hydrologic Alterations Apparent	**9 pts**	
The wetland hydrology appears to have been altered, but the wetland was resilient to alterations and the functions are intact or near optimal level.	**7 pts**	
The wetland hydrology was altered but appears to retain some degree of functions.	**3 pts**	
Alterations are severely impacting the hydrology of the wetland.	**1 pt**	

Metric 3 Total: add 3a – 3d **(29 points max.)**	**Subtotal**

Site:	Rater(s):	Date:

Metric 4. Habitat Alteration and Habitat Structure Development – Maximum 20 Points.

*** A substrate or habitat disturbance may also negatively impact hydrology (Submetric 3d) and substrate/habitat (Submetric 4a/4b).*

4a. Substrate/Soil Disturbance – Maximum 4 points.

Evaluate whether a physical disturbance has occurred to the soil and surface substrates of the wetland. Check all possible forms of observed substrate/soil disturbances **within the wetland** below.

Low	High	Alteration	Low	High	Alteration	Low	High	Alteration
☐	☐	filling	☐	☐	human-induced erosion or exposure	☐	☐	plowing, disking
☐	☐	grading	☐	☐	human-induced sedimentation or burial	☐	☐	intensive grazing (hooves)
☐	☐	logging	☐	☐	dredging (includes excavating)	☐	☐	off-road vehicle use
☐	☐	construction	☐	☐	vehicle use	☐	☐	other(s) (specify)

Select an option below that best describes the extent of wetland soil alteration. You may select **adjoining** options and average the points when appropriate.		**Score**
No Substrate or Soil Disturbance Apparent	**4 pts**	
The wetland substrate or soil appears to have been altered, but the wetland was resilient to alterations	**3 pts**	
The wetland substrate or soil was altered but was somewhat resilient to alterations	**2 pts**	
The wetland substrate or soil was altered and was not resilient to alterations	**1 pt**	

4b. Habitat Alteration – Maximum 9 points.

Evaluate the intactness of the natural habitat and check all possible observed habitat alterations **within the wetland** below.

Utilize aerial photography and field evidence to determine if any habitat alteration has occurred. Determine the approximate pre-disturbance extent of vertical and horizontal habitat attributes (e.g., large woody debris, plant species diversity, hummocks, patchiness, niche diversity, etc.). Disregard changes attributable to wetland community succession or other natural processes.

Low	High	Alteration	Low	High	Alteration	Low	High	Alteration
☐	☐	barriers (e.g. road bed(s)/RR grades(s))	☐	☐	large woody debris (LWD) removal	☐	☐	sedimentation
☐	☐	tree plantation	☐	☐	grazing	☐	☐	dredging
☐	☐	selective cutting	☐	☐	rutting	☐	☐	filling/grading
☐	☐	clearcutting	☐	☐	Herbicide or chemical treatment	☐	☐	plowing/disking/farming
☐	☐	mowing or shrub removal	☐	☐	nutrient enrichment, e.g., nuisance algae	☐	☐	other(s) (specify)

Select an option below that best describes the extent of wetland habitat alteration. You may select **adjoining** options and average the points when appropriate.		**Score**
No Habitat Alterations Apparent	**9 pts**	
The wetland habitat appears to have been altered, but the wetland was resilient to alterations and the functions are intact or near optimal level	**7 pts**	
The wetland habitat was altered but appears to retain some degree of functions	**3 pts**	
The alterations are severely limiting habitat function of the wetland	**1 pt**	

4c. Habitat Reference Comparison – Maximum 7 points.
Determine an overall qualitative rating of the wetland habitat quality in comparison to the best of its type remaining (i.e., any ecologically and/or hydrogeomorphically similar wetland habitat). Do **not** consider the best example for an area (i.e., compare, for example, emergent riverine wetlands to other emergent riverine wetlands). For instances where there is a clear distinction between wetland areas in terms of habitat structure development, the Rater may double-check non-adjoining options, but justification is required. See Guidance Manual for additional assistance.

Select an option below that best describes the wetland habitat structure development. If unclear which of two options is more appropriate, select **adjoining** options and average the points.			**Score**
Excellent:	Wetland appears to represent the best of its type.	**7 pts**	
Good:	Wetland appears to be a good example of its type	**5 pts**	
Fair:	Wetland appears to be a fair example of its type.	**3 pts**	
Poor:	Wetland is a poor example of its type	**1 pt**	

Metric 4 Total: add 4a – 4c **(20 points max.)**	**Subtotal**

Site:	Rater(s):	Date:

Metric 5: Special Wetlands — Maximum of 10 pts.

Metric 5: Special Wetlands — Maximum of 10 pts.
Check all that apply and score as indicated.

Numbers in brackets **[]** indicate point values.

Provide documentation for each selection (photos, checklists, maps, resource specialist concurrence, data sources, references, etc).

5a. Regulatory Protection / Critical Habitat Known occurrence of federally threatened/endangered species or designated critical habitat within a HUC-12 watershed **[10]**. Known occurrence of other rare species with state rank S1 ***[10]**, S2 ***[5]**, S3***[3]**; *use higher rank if there are mixed ranks or qualifiers (i.e., S1/S2 [10] and S2/S3 [5)]. Exclude records which are only "historic" (i.e., surveys have documented that the species is no longer there) within HUC-12 watershed.	**Score**
5b. High Ecological Value / Ranked Communities (See manual and key for ranked list of communities) Appalachian seep/bog (S1S2) **[8]** Bottomland marsh (S1S2) **[8]** Bottomland slough OR Coastal Plain Slough (S2) **[5]** Calcareous seep/bog (S1) **[10]** Coastal Plain forested acid seep (S1) **[10]** Cypress (tupelo) swamp (S1) **[10]** Sinkhole/depression marsh (S1S2) **[8]** Sinkhole/depression pond (S2) **[5]** Wet depression/sinkhole forest (S1S2) **[8]** Wet bottomland hardwood forest (S2) **[5]** Wet meadow (S1) **[10]** Wet prairie (S1) **[10]**	**Score**
5c. Low-Quality Wetland **Check all that apply, but maximum score is -10 points:** Wetland is < 1 acre and has >75% cover of invasive plants **[-10]** Wetland is <1 acre and is nonvegetated mined/excavated land **[-10]** Wetland is <1 acre and is a constructed stormwater treatment pond **[-10]**	**Score**
Metric 5 Total: add 5a – 5c **(10 points max.)***	**Subtotal**

***Score can be negative**

Metric 6. Vegetation, Interspersion, and Habitat Features – Maximum 20 points. **For each Metric 6 sub-metric, do NOT consider the wetland type being assessed, especially for plant species diversity in 6a.

6a. Wetland Vegetation Components – Maximum 9 points.

Determine the Qualitative Cover Score of each Vegetation Component. Using the Scoring Table below, start on the left and proceed to the right, until a point value is obtained for each Component. Vegetation Components may exist in overlapping layers, e.g., significant areas of shrub/sapling and/or herbaceous may exist under a forest canopy. Only groups of trees, clusters of shrubs, or dense patches of herbaceous stems may count toward area coverage. Do not include lone trees, shrub/saplings, or sparse patches of herbaceous stems. See Submetric 6c for list of Kentucky's most invasive wetland species. Check the box on the right to indicate how the score was determined for each Vegetation Component (i.e., F, S or H).

Qualitative Cover Scoring Table

Habitat component - *Check all that apply →*					F	S	H
Vegetation Component is **>0.1** acre	>25% of wetland area	Native species dominate the coverage	High native diversity	3 pts			
			Moderate to low native diversity	2 pts			
		Invasive or non-native species dominate the coverage	Moderate to high native diversity	2 pts			
			Low native diversity	1 pt			
	<25% of wetland area	Native species dominate the coverage	Moderate to high native diversity	2 pts			
			Low native diversity	1 pt			
		Invasive or non-native species dominate the coverage	Moderate native diversity	1 pt			
			Low native diversity	0 pts			
Vegetation Component is **<0.1** acre	>25% of wetland area	Native species dominate the coverage	Moderate to high native diversity	2 pts			
			Low native diversity	1 pt			
		Invasive or non-native species dominate the coverage		0 pts			
	<25% of wetland area			0 pts			

Write in "absent" (don't score it a zero) if habitat is not present.

	Score
Forest Overstory Component (F) – **Maximum 3 points**. Qualitative cover score derived from table. Forested wetland areas are characterized by a group of trees at least 3 inches in DBH, regardless of height.	
Shrub/Sapling Component (S) – **Maximum 3 points**. Qualitative cover score derived from table. Shrub/Sapling wetland areas are dominated by clusters of woody plants less than 3 inches in DBH and greater than 3.28 feet in height. Species include true shrubs, young trees, and stunted trees.	
Herbaceous Component (H) – **Maximum 3 points**. Qualitative cover score derived from table. Herbaceous wetland areas are dominated by dense patches of erect, non-woody plants, regardless of size, and woody plants less than 3.28 feet in height. This component includes the robust-stemmed yellow pond lily (*Nuphar advena*) and American lotus (*Nelumbo lutea*). All floating-leaf species (including *Nymphaea* spp.) are **excluded** from the herbaceous component, and are instead included within the open water component (see Submetric 6b).	

6a. Vegetative Components Score	

Subtotal

Site:	Rater(s):	Date:

6b. Open Water, Mudflat, and Aquatic Bed Habitats – Maximum 3 points.

Open water is an unobstructed, inundated area of water with few or no rooted emergent or non-tree woody plant species. For KY-WRAM, mudflats are considered areas with exposed mud substrate with little to no vegetation. This metric is designed to evaluate habitat for waterfowl, shorebirds, fish, and other wildlife.

This Habitat Component includes combined acreage from any of the following areas:

Small ponds (including farm ponds), streams <u>and/or</u> their floodwaters, pools, saturated sandbars, or other natural or constructed waters

Seasonal standing water areas (e.g., <u>mudflats and dried-down vernal pools</u>) that were inundated long enough during the growing season to support aquatic life. This includes the "understory" below a forest canopy.

Aquatic bed areas (submerged aquatic vegetation). Aquatic bed is dominated by plants growing at or below the surface of the water for most of the growing season in most years. The KY-WRAM includes aquatic bed within the definition of open water, due to the potential difficulty in differentiating the two entities. For the purposes of the KY-WRAM, all floating-leaf aquatic taxa (e.g. water lilies, *Nymphaea* spp.), are included in the definition of aquatic bed (therefore, are included in the definition of open water).

100-foot wide strip of open water along a lake or river (see Wetland Assessment Area guidelines in the *Guidance Manual*). When the Wetland is adjacent to a lake or large river, calculate the acreage of the 100-foot wide open water strip that is included within the Wetland (see KY-WRAM Wetland Assessment Area Boundary Guidelines). Divide the linear feet of shoreline length by 400. For example, if the vegetated portion of the wetland interfaces with 200 linear feet of a lake, then the extent of the lake's open water included within the Wetland would be calculated as: 200/400 = 0.5 acre. Open water ends where water depth is > 6.6 ft; the Rater may use depth charts to establish this, when available.

Shallow pools free of dense shrub canopy (e.g., open area within an inundated shrub swamp).

Shallow pools free of densely-packed herbaceous vegetation (e.g., open area within a marsh or bog).

The Indicators below are intended to provide guidance to determine if open water was present **when the wetland is currently dry.**

If the wetland is currently dry, use the appropriate USACE Wetland Delineation Regional Supplement to determine if indicators of open water are present (appropriate indicators are listed below).

One primary indicator OR two secondary indicators must be present to consider presence of open water. In the section indicated below, describe how you used indicators to determine your score.

Surface Water Present? □ **Yes** – How much? Score below □ **No** – Use indicators below, then assign score

Estimate the total coverage. Choose only 1 category.			**Score**
High:	2.5 acres or more	**3 pts**	
Moderate:	1.0 acre to <2.5 acres	**2 pts**	
Low:	0.25 acre to <1.0 acre	**1 pt**	
Virtually Absent:	<0.25 acre	**0 pts**	

Open Water Hydrology Indicators – Information in parentheses represents US ACE Wetland Delineation Regional Supplement Hydrology Indicators that should be consulted for indicators of open water for the purposes of KY-WRAM.

Check indicators present below:

Primary Indicators (must have 1) OR →	**Secondary Indicators (must have 2)**
☐ Surface Water present on aerial imagery (A1)	☐ Sparsely vegetated concave surface (B8)
☐ Water marks (B1)	☐ Drainage patterns (B10)
☐ Inundation Visible of Aerial Imagery (B7)	☐ Moss trim lines (B16)
☐ Algal mat or crust (B4)	☐ Geomorphic position (D2)
☐ Presence of aquatic fauna (B13)	
☐ Presence of true aquatic plants (B14)	

Describe here how indicators were used to determine score:

Subtotal	

6c. Coverage of Highly-Invasive Plant Species – Maximum 1 point.
Estimate the combined total coverage of **any** invasive species present in the wetland.

Selected invasive plant species. Remember to include any species found on the KY-EPPC list that is within the assessment area.
(Print the complete KY-EPPC list and take into the field)
*These native invasive plants are being included for the purposes of the KY-WRAM (i.e., everything on the KY-EPPC list are exotics)

Alliaria petiolata (Garlic Mustard)
Alternanthera philoxeroides (Alligator Weed)
Conium maculatum (Poison Hemlock)
Euonymus fortunei (Winter Creeper)
Lespedeza cuneata, L. bicolor, L. stipulacea, L. striata, L. thunbergii (non-native *Lespedeza*)
Ligustrum sinense, L. vulgare (Privet)
Lonicera japonica (Japanese Honeysuckle)
Lonicera maackii (Bush Honeysuckle)
Lythrum salicaria (Purple Loosestrife)
Microstegium vimineum (Japanese Stilt Grass)
Myriophyllum aquaticum, M. spicatum (parrotfeather and Eurasion watermilfoil)
Phalaris arundianacea (Reed Canary Grass)*
Phragmites australis (Common Reed)
Polygonum cuspidatum (Japanese knotweed)
Rhamnus cathartica (Common Buckthorn)
Rosa multiflora (Multiflora Rose)
Typha ssp. (Cattail species)*
Other(s): specify below

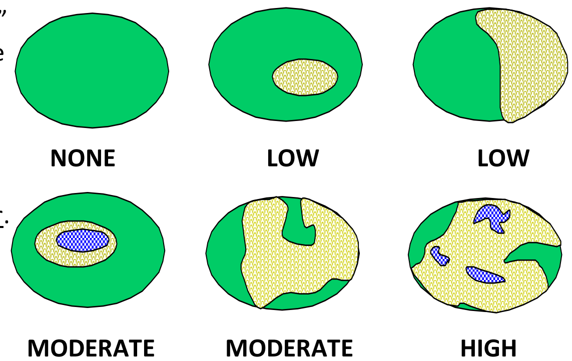
Estimate the total coverage. Choose only 1 category.			**Score**
Virtually Absent:	<1% aerial coverage of invasive species	**1 pt**	
Nearly Absent:	1% to <5% aerial coverage of invasive species	**0 pts**	
Low:	5% to <25% aerial coverage of invasive species	**-1 pt**	
Moderate:	25% to <75% aerial coverage of invasive species	**-3 pts**	
Extensive:	>75% aerial coverage of invasive species	**-5 pts**	

Additional invasive plant species present (list here):

Site:	Rater(s):	Date:

6d. Horizontal (plan view)
Interspersion – Maximum 5 points

Evaluate the wetland from a "plan view," i.e., imagine as if you are hovering above the wetland looking down upon it . The figure shows hypothetical wetlands for estimating the amount of habitat interspersion including growing season vegetation communities and open water. Only include open water that is 6.6 feet deep or less and does not include inundated areas below herbaceous and shrub vegetation. If unclear, select **adjoining** options and average the points.



		Score
Wetland has a high degree of interspersion	**5 pts**	
Wetland has a moderate degree of interspersion	**3 pts**	
Wetland has a low degree of interspersion	**1 pt**	
Wetland has no interspersion	**0 pts**	

Subtotal	

Site:	**Rater(s):**	**Date:**

6e. Microtopographic Features – Maximum 12 points (i.e., 3 points per feature). Choose only one category for each.

1. Hummocks/Tussocks/Tree Mounds, e.g., sedge/grass tussocks, decayed nursery logs (remnants of large logs), root tip-up mounds (uprooted trees), etc. Percent coverage is based on total area of the wetland and includes the depressional matrix within any group of raised features.				**Score**
Absent: 0 pt No features present	Low: 1 pt Present but <1% of the area	Moderate: 2 pts 1% to 5% of the area	High: 3 pts >5% of the area	
2. Large Woody Debris (LWD). per log, average width ≥6 inches (e.g., fallen trees and/or large branches, etc.)				**Score**
Virtually Absent: 0 pt < 1 per acre	Low: 1 pt 1 to 5 per acre	Moderate: 2 pts 6 to 10 per acre	High: 3 pts >10 per acre	
3. Large Snags (≥12 inches DBH).				**Score**
Absent: 0 pt No snags present	Low: 1 pt Present but <1 per acre	Moderate: 2 pts 1 to 5 per acre	High: 3 pts >5 per acre	
4. Amphibian Breeding/Nursery Habitat, e.g., temporary pools with standing water of sufficient duration and depth to support frog and/or salamander reproduction. Permanent areas of vegetated standing water along the edges of ponds, lakes, and some streams also serve as amphibian habitat **(see Manual for description of habitat quality)**.				**Score**
Virtually Absent: 0 pt < 5% of the area	Low: 1 pt Present in small amounts (5% to 10% of the area) but of low to moderate quality	Moderate: 2 pts Present in moderate or greater amounts (>10% of the area) but of low to moderate quality **OR** Present in small amounts (5% to 10% of the area) but of highest quality	High: 3 pts Present in moderate or greater amounts (>10% of the area) and of highest quality	
			6e. Microtopographic Features Score	

Metric 6 Total: add 6a – 6e **(20 points max.)**	**Total Score**

KY-WRAM Summary

Scoring Comments:

HGM definitions:

RIVERINE: Occur in flood plains and riparian corridors in association with stream channels of any flow regime. Dominant water sources are overbank flow or subsurface hydraulic connections.

DEPRESSIONAL: Occur in topographic depressions. Dominant water sources are precipitation, ground water discharge, and water from adjacent uplands. Water moves vertically.

SLOPE: Occur where there is a discharge of ground water to the land surface. Normally occur on sloping land; gradient may be slight to steep. Water does not pool but flows downslope in one direction.

FLAT: Occur most commonly on historic flood plain terraces – where the channel has incised so deeply that it rarely or never floods onto the flood plain. Main source of water is precipitation, and they have poor vertical drainage. They receive no groundwater discharge, which distinguishes them from depressional and slope wetlands.

Appendix 2 – Pesticide and nutrient detections for riverine wetlands in Kentucky and Salt river basins, 2014–2015

	March 2015					
	Methoxychlor	Dichlorovos	Atrazine Desethyl	Atrazine	Metolachlor	Butachlor
Method	6260 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)
KYW11-037	0.00	0.00	0.00	0.00	0.00	0.00
KYW11-045	0.00	0.00	0.00	0.00	0.00	0.00
KYW11-046	0.00	0.00	0.00	0.00	0.00	0.00
KYW12-450	0.00	0.00	0.00	0.00	0.00	0.00
KYW12-453	0.00	0.00	0.00	0.00	0.00	0.00
KYW12-465	0.00	0.00	0.00	0.00	0.00	0.00
KYW12-510	**0.1720**	0.00	0.00	0.00	0.00	0.00
KYW13-212	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-214/273	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-222	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-224	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-228	0.00	0.00	**0.0210**	0.00	0.00	0.00
KYW13-230	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-287 (Dix WMA)	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-288	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-294	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-631	0.00	0.00	0.00	**0.0278**	0.00	0.00
KYW13-632	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-633	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
KYW13-635	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-647	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-649	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-663	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-664	0.00	0.00	0.00	**0.0225**	0.00	0.00
KYW13-664 Adjacent	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-668	0.00	0.00	0.00	0.00	**0.0619**	0.00
KYW13-672	**0.0352**	0.00	0.00	0.00	0.00	0.00
KYW13-676	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-679	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-689	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-690	0.00	0.00	0.00	**0.0476**	0.00	**0.0439**
KYW13-692	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-703	0.00	**0.0463**	0.00	0.00	0.00	0.00
KYW13-BGAD 1	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-Big Bob Slough	0.00	0.00	0.00	0.00	0.00	0.00

Continued

	March 2015					
	Methoxychlor	Dichlorovos	Atrazine Desethyl	Atrazine	Metolachlor	Butachlor
Method	6260 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)
KYW13-I75	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-Powell Sinkhole	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-BGAD Degraded 1	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-BGAD Degraded 2	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-Curry Way	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-Dix Dam Rd	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-Riney B Park	0.00	0.00	0.00	0.00	0.00	0.00

	May 2015									
	Hexachloro benzene	Propachlor	2,4'-DDE	4,4'-DDE	Total DDT	Atrazine Desethyl	Hexachloro benzene	Atrazine	Acetochlor	Metolachlor
Method	6260 (µg/L)	6260 (µg/L)	6260 (µg/L)	6260 (µg/L)	6260 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)
KYW11-037	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.3330**	0.00	0.00
KYW11-045	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW11-046	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW12-450	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW12-453	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW12-465	0.00	0.00	0.00	**0.0118**	**0.0118**	0.00	0.00	**0.1220**	0.00	0.00
KYW12-510	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0532**	0.00	0.00
KYW13-212	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-214/273	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-222	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-224	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-228	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-230	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-287 (Dix WMA)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0350**	0.00	0.00
KYW13-288	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-294	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-631	**0.0293**	0.00	0.00	0.00	0.00	0.00	**0.0253**	0.00	0.00	0.00
KYW13-632	**0.0136**	0.00	0.00	0.00	0.00	**0.0221**	0.00	0.00	0.00	0.00
KYW13-633	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
KYW13-635	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.1720**	0.00	**0.0665**
KYW13-647	0.0128	0.00	0.00	0.00	0.00	0.00	0.00	0.1140	0.0336	0.00
KYW13-649	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0338**	0.00	0.00
KYW13-663	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0585**	**0.0429**	**0.0250**
KYW13-664	**0.0283**	0.00	0.00	0.00	0.00	0.00	**0.0250**	0.00	0.00	0.00
KYW13-664 Adjacent	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0419**	0.00	0.00
KYW13-668	0.00	0.00	0.00	0.00	0.00	**0.0420**	0.00	**0.0361**	0.00	**0.0878**
KYW13-672	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-676	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0649**	**0.0221**	**0.0357**
KYW13-679	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0281**	0.00	0.00
KYW13-689	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0279**	0.00	0.00
KYW13-690	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0811**	0.00	0.00
KYW13-692	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0731**	0.00	0.00
KYW13-703	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.0347**	0.00	0.00
KYW13-BGAD 1	0.00	**0.0375**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-Big Bob Slough	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Continued

	May 2015									
	Hexachloro benzene	Propachlor	2,4'-DDE	4,4'-DDE	Total DDT	Atrazine Desethyl	Hexachloro benzene	Atrazine	Acetochlor	Metolachlor
Method	6260 (µg/L)	6260 (µg/L)	6260 (µg/L)	6260 (µg/L)	6260 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)	6440 (µg/L)
KYW13-I75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-Powell Sinkhole	0.00	0.00	**0.0204**	0.00	0.00	0.00	0.00	**0.0931**	0.00	0.00
KYW14-BGAD Degraded 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-BGAD Degraded 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-Curry Way	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-Dix Dam Rd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW14-Riney B Park	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	March 2015				May 2015			
	Ammonia (as N)	Total Kjeldhal Nitrogen	Nitrate / Nitrite (as N)	Phosphorus, Total	Ammonia (as N)	Total Kjeldhal Nitrogen	Nitrate / Nitrite (as N)	Phosphorus, Total
KYW11-037	**0.2120**	**2.3300**	**0.0179**	**0.3330**	0.00	**1.1100**	**0.0560**	**0.1640**
KYW11-045	**0.0378**	**1.1000**	**0.0114**	**0.1200**	**1.4700**	**8.8100**	0.00	**1.3300**
KYW11-046	0.00	**1.2000**	0.00	**0.2610**	0.00	0.00	0.00	0.00
KYW12-450	**0.0360**	**0.5130**	**0.0480**	**0.0260**	0.00	**1.3800**	0.00	**0.0450**
KYW12-453	**0.0250**	**0.9960**	**0.3350**	**0.1430**	**0.2310**	**1.0600**	0.00	**0.2510**
KYW12-465	0.00	**0.6740**	0.00	**0.1180**	**0.9030**	**2.6700**	**0.0237**	**0.6180**
KYW12-510	**0.1140**	**2.2000**	**0.0231**	**0.2860**	**0.6060**	**1.2900**	**0.0820**	**0.1430**
KYW13-212	0.00	**0.5590**	0.00	**0.1260**	**0.0389**	**1.0500**	0.00	**0.1920**
KYW13-214/273	**0.0979**	**1.1800**	0.00	**0.0490**	**0.4040**	**2.4300**	0.00	**0.0529**
KYW13-222	0.00	**0.7970**	**0.1780**	**0.0576**	**0.2700**	**1.3500**	0.00	**0.1150**
KYW13-224	0.00	**0.3260**	**0.0429**	**0.0672**	0.00	0.00	0.00	0.00
KYW13-228	**0.0284**	**0.5170**	**0.7520**	**0.1880**	**0.2120**	**1.3600**	0.00	**0.3230**
KYW13-230	0.00	0.00	**0.0104**	**0.0358**	0.00	**0.3690**	**0.0154**	**0.1330**
KYW13-287 (Dix WMA)	0.00	**0.8480**	0.00	**0.1090**	0.00	**2.1100**	0.00	**0.1730**
KYW13-288	**0.0274**	0.00	**0.3570**	**0.1300**	**0.1090**	**0.5720**	**1.0800**	**0.3870**
KYW13-294	0.00	0.00	0.00	**0.0304**	0.00	0.00	0.00	0.00
KYW13-631	**0.0477**	**0.5830**	**0.7320**	**0.0662**	**0.2500**	**5.5100**	0.00	**0.3810**
KYW13-632	0.00	**0.7190**	**0.0122**	**0.1540**	**0.0529**	**3.1300**	**0.0121**	**1.1700**
KYW13-633	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KYW13-635	**0.0284**	**0.7040**	**0.6110**	**0.1360**	0.00	**0.8890**	**0.0164**	**0.1170**
KYW13-647	**0.0524**	**0.7160**	**1.3700**	**0.1030**	**1.7700**	**3.2300**	**0.0178**	**0.1190**
KYW13-649	0.00	0.00	0.00	0.00	**0.0445**	**1.4300**	**0.0142**	**0.1090**
KYW13-663	0.00	0.00	0.00	0.00	**0.0250**	**3.4800**	**0.0165**	**0.2930**
KYW13-664	**0.0398**	**0.7780**	0.00	**0.0635**	**0.0500**	**5.0100**	**0.0131**	**0.6940**
KYW13-664 Adjacent	0.00	0.00	0.00	0.00	0.00	**0.5260**	0.00	**0.0290**
KYW13-668	**0.0477**	**0.7900**	**0.2990**	**0.1040**	**0.3920**	0.00	**1.5100**	**0.0910**
KYW13-672	0.00	0.00	0.00	0.00	**0.0439**	**0.9200**	**0.0169**	**0.0910**
KYW13-676	**0.0337**	**0.7010**	0.00	**0.1800**	**0.1160**	**1.9500**	**0.0172**	**0.7550**
KYW13-679	0.00	**1.1800**	**0.4480**	**0.1400**	**0.4980**	**1.6100**	**0.0259**	**0.1600**
KYW13-689	**0.7380**	**1.1200**	**0.0327**	**0.0444**	**0.0790**	**0.5600**	**0.0333**	**0.0287**
KYW13-690	0.00	**2.0800**	**1.8900**	**0.4210**	0.00	**0.7240**	**0.0199**	**0.0611**
KYW13-692	**0.0416**	**0.8450**	**0.4140**	**0.2100**	**1.1300**	**3.2400**	**0.0496**	**0.9650**
KYW13-703	**0.0356**	**0.4080**	**0.0332**	**0.0504**	0.00	**2.6600**	0.00	**0.1710**
KYW13-BGAD 1	**0.0790**	**0.8670**	0.00	**0.0648**	0.00	**1.7800**	0.00	**0.2040**
KYW13-Big Bob Slough	0.00	**0.4100**	0.00	**0.0506**	0.00	**0.7960**	0.00	**0.0762**
KYW13-I75	0.00	**0.5610**	**0.4040**	**0.0912**	**0.1790**	**1.2200**	0.00	**0.2630**

Continued

	March 2015				May 2015			
	Ammonia (as N)	Total Kjeldhal Nitrogen	Nitrate / Nitrite (as N)	Phosphorus, Total	Ammonia (as N)	Total Kjeldhal Nitrogen	Nitrate / Nitrite (as N)	Phosphorus, Total
KYW13-Powell Sinkhole	0.00	0.00	0.00	0.00	**0.4890**	**7.7000**	0.00	**0.4110**
KYW14-BGAD Degraded 1	0.00	**0.3770**	**0.0128**	**0.0679**	0.00	0.00	0.00	0.00
KYW14-BGAD Degraded 2	**0.0264**	**0.6330**	**0.1880**	**0.0807**	**0.0416**	**1.6600**	0.00	**0.2800**
KYW14-Curry Way	0.00	**0.8480**	0.00	**0.2390**	0.00	0.00	0.00	0.00
KYW14-Dix Dam Rd	0.00	**0.4560**	**1.5200**	**0.1780**	**0.2450**	**1.5000**	0.00	**0.8240**
KYW14-Riney B Park	0.00	**0.6780**	0.00	**0.1230**	**0.2780**	**17.4000**	0.00	**4.8500**