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Amphibian Community Similarity Between Natural Ponds And Constructed Ponds Of Multiple Types In Daniel Boone National Forest, Kentucky

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AMPHIBIAN COMMUNITY SIMILARITY BETWEEN NATURAL PONDS AND CONSTRUCTED PONDS OF MULTIPLE TYPES IN DANIEL BOONE NATIONAL FOREST, KENTUCKY

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Chair, Advisory Committee

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By

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DEDICATION

This thesis is dedicated to my mother, Kari Bragg, and my grandfather, Robert Denton. Without the support of my family, I would not have had the opportunity to pursue my passions. I am beyond fortunate to have been surrounded by the highest level of encouragement and love.

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ABSTRACT

Amphibians are in a worldwide decline. Among the many causes for amphibian declines, habitat loss and alteration remains one of the most significant. A lack of federal protection for isolated wetlands that provide habitat for unique species has resulted in the loss of breeding habitat and unregulated mitigation practices. Ponds built for mitigation purposes often do not replicate the lost ponds in structure or ecological processes. A lack of general monitoring has produced a void in knowledge of what long-term role constructed ponds play in shaping amphibian communities. My objective was to compare amphibian communities of natural ponds and multiple types of constructed ponds in the Daniel Boone National Forest, Kentucky. A suite of habitat variables including canopy cover, hydroperiod, upland coarse woody debris, aquatic vegetation, maximum depth, Ohio Wetland Rapid Assessment Score (ORAM), and pond type were recorded to examine relationships between amphibian species and habitat variables. Community comparisons were made using canonical correspondence analysis (CCA) and one-way analysis of similarity (ANOSIM). Stepwise regression models were developed to predict individual species abundance based on the habitat variables. Amphibian communities differed significantly between ponds types (natural, new construction method, old construction method). Additionally, wood frogs (*Rana sylvatica*) and marbled salamanders (*Ambystoma opacum*) were almost exclusively found in natural, ephemeral ponds, whereas large ranid frogs (*R. clamitans*, *R. catesbeiana*, *R. palustris*) were only found breeding in permanent, constructed ponds.

Habitat predictors for some species showed differing preferences for hydroperiod, canopy cover, maximum depth, ORAM score, and amount of upland coarse woody debris. New construction methods were intermediate between old construction method and natural ponds in terms of habitat variables and amphibian community composition. As amphibian conservation and management become increasingly important in light of rapid declines, the ability to construct habitat and monitor it efficiently will be crucial in preservation of species. The results of this research underscore the need for monitoring of constructed wetlands in order to verify if goals are met and to assess ecological condition.

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CHAPTER 1

I. INTRODUCTION

Over the past decade, scientists have been evaluating, quantifying, and interpreting the climbing rate of amphibian decline and extinction (Alford and Richards 1999, Houlahan et al. 2000, Kiesecker et al. 2001, Mendelson et al. 2006, McCallum 2007). One in three of the world's amphibian species are listed as threatened on the IUCN Red List of Threatened Species (Baillie et al. 2004). Although extinctions are a natural component of amphibian evolution, current extinction rates may be over 200 times the natural background average rate based on paleontological evidence (McCallum 2007); and recent extinctions may be the product of long-term declines over the past 50 years (Houlahan et al. 2000).

Evidence supports amphibian declines stemming from a range of factors. These include non-native species, overexploitation, land alteration, global climate change, infectious disease, chemical contamination, and synergistic effects of multiple factors (Collins and Storfer 2003). Habitat loss is one of the main contributors to a global decline in biodiversity and an increase in extinction rates of many taxa (Brooks et al. 2002, Cushman 2005). Amphibians' relatively limited mobility, breeding site fidelity, and physiological limitations suggest an inability to recover from habitat loss (Blaustein et al. 1994). Consequences of land use change, including isolation, fragmentation, and lessened habitat connectivity, have negatively affected amphibian dispersal and survival

(Cushman 2005). Habitat loss and alteration is especially important for species that prefer forested habitats. Globally, 82% of rapidly declining amphibian species are associated with forested habitat (Stuart et al. 2005).

Due to their biphasic life history, most amphibians depend on the health of both the terrestrial and aquatic environments around them. Even amphibians without aquatic eggs require water to some extent in order to reproduce. In forested habitats, this source of water is most often from streams or vernal pools (Wells 2007). Vernal pools are of high value in light of habitat alteration due to their variability from year to year and sensitivity to disturbance (Semlitsch and Bodie 1998). These bodies of water function as connections between amphibian populations as well as habitat for unique, endemic species (Egan and Paton 2004, Zedler 2003). Many amphibians utilize isolated vernal pools and other temporary bodies of water due to their lack of fish predators (Wellborn et al. 1996). The biomass contributed by amphibian populations in these habitats can be relatively large (Calhoun et al. 2003, Gibbons et al. 2006).

Kentucky has lost ca. 81% of its historical wetlands (Dahl 2000). The current 121,400 hectares of wetlands are mostly palustrine forested wetlands characterized by hydrophytic trees, shrubs, and herbaceous plant species (Environmental Law Institute 2007). Among these, ridge-top vernal pools have long been described as unique habitats in Kentucky for plant species (Braun 1937). These small, isolated pools are common to the Cumberland Plateau, and are documented as having relatively high amphibian species richness (Corser 2008). Despite vernal pools and temporary bodies of water being part of the forested landscape and significant in the conservation of biodiversity, laws protecting their status are lacking. Geographically isolated waters were removed from the Clean Water Act in 2001 via a Supreme Court decision, "Solid Waste Agency of Northern Cook County vs. U.S. Army Corps of Engineers" (Zedler 2003). The majority of U.S. states have enacted legislation aimed at supplementing the Clean Water Act, but only six states (Indiana, Ohio, Tennessee, Virginia, Washington, and Wisconsin) regulate activities in hydrologically isolated wetlands. Kentucky is one of 17 states that regulates and grants permits based only on Clean Water Act legislation guidelines. This policy allows for no legal protection of small, isolated wetlands.

Thirty-six states, including Kentucky, provide guidelines for the mitigation of bodies of water that do not meet Clean Water Act criteria (Environmental Law Institute 2008). Nationwide, in 2003, 17,624 hectares of mitigation wetlands were required to balance the approximate 10,522 hectares of permitted lost wetlands (Environmental Law Institute 2007, Martin et al. 2006). Although mitigation is aimed at replacing the functions of lost wetlands, constructed wetlands often fail to duplicate natural functions of lost wetlands and mitigation projects suffer from a lack of monitoring, poor record keeping, and lack of consistency (Turner et al. 2001, Lichko and Calhoun 2003, Minkin and Ladd 2003, Mack and Micacchion 2006, *reviewed in* Kihslinger 2008). In 2008, the U.S. Army Corps of Engineers issued revised regulations to unify the regulations for all three types of mitigation (permittee-responsible compensatory mitigation, mitigation banks, and in-lieu fee mitigation) and provide more organization for monitoring and record keeping (Environmental Protection Agency 2008), but these regulations do not address the need for improvement of construction methods.

Mitigation wetlands and ponds that are constructed for habitat enhancement provide habitat for amphibians (Monello and Wright 1999, Pechmann et al. 2001, Knutson et al 2004, Balcombe et al. 2005, Porej and Hetherington 2005, Vasconcelos and Calhoun 2006). Although constructed ponds provide amphibian breeding habitat, the amphibian communities present in constructed ponds may not be similar to nearby removed ponds (Pechmann et al. 2001); and may be acting as ecological sinks where larval survival is greatly reduced (DiMauro and Hunter 2002) or as areas of low amphibian diversity (Porej and Hetherington 2005). One of the difficulties in comparing constructed and natural ponds is that both the habitat qualities of ponds and amphibian populations are not static (Skelly et al. 1999; Pechmann et al. 2001). Studies involving the succession of wetland plant communities in mitigation areas have been conducted in the short term (Niering 1990, Kusler and Kentula 1990) and the long term (Atkinson et al. 2005); demonstrating that plant communities change in composition over time. Shifts in habitat characteristics coincide with changes in amphibian communities (Hermann et al. 2005, Petranka et al. 2003, Purrenhage and Boone 2009, Snodgrass et al. 2000). Constructed and natural ponds can also vary widely in terms of hydroperiod, the length of time water is present (Gamble and Mitsch 2008). Hydroperiod affects amphibian community composition, with many species only found in ephemeral pools that typically dry at least once a year (Snodgrass et al. 2000).

Due to the natural succession of pond characteristics over time and the complex breeding cycles of amphibians, it is difficult to determine when to evaluate the success of constructed ponds in terms of amphibian population status. Amphibians utilize both the aquatic and surrounding terrestrial habitat and have predictable breeding cycles, making them potential indicators of wetland function. Researchers have suggested many different minimum ages at which constructed ponds have reached equilibrium; ranging from 2 years to > 25 (Confer and Niering 1992, Petranka et al. 2003, D'Avanzo 1990). This lack of precision is most likely a result of an insufficient number of constructed wetlands of sufficient age to study. Studies of amphibian communities in constructed ponds that have been established for ten years or more are lacking. This includes surveying artificial ponds that are older than ten years as well as studies in which artificial ponds have been surveyed for periods of time greater than ten years. Published studies to date have monitored amphibian communities in constructed ponds for 1-8.5 years, with an average survey period of 3.2 years (Monello and Wright 1999, Babbitt and Tanner 2000, Hazell et al. 2004, Lehtinen and Galatowitsch 2001, Pechmann et al. 2001, DiMauro and Hunter 2002, Petranka et al. 2003, Vasconcelos and Calhoun 2006, Shulse et al. 2010). Although these studies are important, they have been limited by a lack of constructed ponds older than nine years.

More than 400 ponds have been constructed over the past 22 years within the Daniel Boone National Forest (DBNF) for habitat enhancement, game use, and Indiana bat (*Myotis sodalis*) conservation (T. Biebighauser, pers. comm.), but very few have been monitored after their construction. Since 2004, construction protocols were adjusted in an effort to create more natural ponds using shallower depths, increased upland coarse woody debris, and smaller total sizes. Understanding differences in amphibian communities between pond types could aid managers in establishing a chronology to best indicate when constructed ponds best duplicate natural ponds in terms of amphibian community structure and provide an evaluation of the success of the new construction method. The objective of my research was to determine if amphibian communities differ between natural and constructed ponds of different ages and construction methods in the Daniel Boone National Forest (DBNF), Kentucky. Specifically, the following questions were addressed: (1) Do natural ponds differ from constructed ponds of multiple construction types and ages in amphibian community composition?, and (2) What habitat variables associated with constructed and natural ponds predict the presence and abundance of each amphibian species?

CHAPTER 2

II. MATERIALS AND METHODS

Study Sites

All study sites were located within the DBNF, eastern Kentucky. This study focused on the Cumberland District, the northernmost district of the DBNF; encompassing areas of Bath, Estill, Lee, Menifee, Morgan, Powell, Rowan, and Wolfe counties. The Cumberland District was chosen due to its high densities of constructed ponds and consistency with which they have been built since 1988. The majority of the ponds constructed in the DBNF are surrounded by deciduous forest, on ridge tops, fishless, and isolated. All constructed ponds were built in areas following a selective timber harvest. From 1988 to 2003, ponds were constructed with dams to hold water permanently. Since 2004, ponds constructed in the DBNF have been shallower and contain more coarse woody debris than the ponds constructed 1988-2003. The density of the constructed ponds in the DBNF and the consistency with which they have been built provides an opportunity for monitoring many ponds across multiple age classes within the same physiographic region, the Western Allegheny Plateau (Woods et al. 2002).

The ponds used in this study were chosen by ground truthing as many ponds as possible from a GIS database of constructed ponds in the DBNF. Ponds were categorized by age based on year of construction and each assigned a number. Constructed ponds were separated into two study groups: ponds built 2004-2008 and those built 1988-2003. These groups were used to separate the two different styles of pond construction. The pond "Kidney88" was the exception. This pond was built in 1988, but was more indicative of the newer construction method; therefore it was shifted to the newer construction method group. Natural ponds were placed in a third category (n = 5). Natural ponds were limited within Bath and Menifee Counties; therefore all natural ponds located were selected. Study sites were then randomly selected from each constructed pond study group. The total sample size was based on an estimate of how many ponds could be potentially surveyed within a 24-hour period in order to keep temporal consistency between ponds when sampling. Nineteen ponds were surveyed for amphibians and habitat characteristics (Table 1).

Amphibian Surveys

Two methods were used to survey for amphibians and were chosen on the basis of comparative studies of common techniques (e.g., Smith et al. 2006, Gunzburger 2007). Collapsible mesh minnow traps (4-mm mesh size, 46 x 26 x 26 cm dimensions, 6-cm openings at each end) [Promar, Gardena, CA] were installed at each pond in order to capture both larvae and aquatic adults (Adams et al. 1997, Heyer et al. 1994). Traps were attached to a silt fence that extended perpendicularly from the shore of the pond two meters into the pond within the littoral zone. The traps were tied to the end post of the silt fence and rested against the substrate, with at least three cm of space above the water in order to provide access to air. The use of silt fences as supplements to the

Table 1. A summary of ponds surveyed for amphibians May-August 2010 in Daniel Boone National Forest, Kentucky.

Pond Name	Year Constructed	Location	Type
60/70s	approx. 1970	N38 03.742 W83 32.618	Old Construction Method
Kidney88	1988	N38 03.304 W83 31.679	New Construction Method
040-90	1990	N38 04.521 W83 31.924	Old Construction Method
2009rework	1992	N38 04.573 W83 31.509	Old Construction Method
42-93	1993	N38 02.130 W83 34.096	Old Construction Method
95NEW	1995	N38 00.310 W83 35.059	Old Construction Method
060-96	1996	N38 01.847 W83 36.232	Old Construction Method
35-97	1997	N38 02.441 W83 30.927	Old Construction Method
04A	2004	N38 03.916 W83 34.503	New Construction Method
05A	2005	N38 03.972 W83 33.417	New Construction Method
06A	2006	N38 04.702 W83 32.719	New Construction Method
06C	2006	N38 04.709 W83 32.887	New Construction Method
06D	2006	N38 04.687 W83 32.917	New Construction Method
06E	2006	N38 04.551 W83 33.114	New Construction Method
DC2	-	N38 00.737 W83 33.541	Natural
DC5	-	N38 00.531 W83 33.424	Natural
DC6	-	N38 00.513 W83 33.138	Natural
DC0	-	N38 00.625 W83 33.773	Natural
Booth	-	N37 54.089 W83 34.855	Natural

minnow traps increases the amount of captures compared to using minnow traps alone (Willson and Dorcas 2004). All but two study ponds had one trap array (one silt fence with two attached traps); the two largest ponds (DC2 and Booth) were both more than twice as large as the overall third largest pond, and two trap arrays were installed at these sites. The remaining constructed and natural ponds were similar in size. Dipnetting protocols were implemented at each site (Shaffer et al. 1994). Dipnet sweeps were taken every five meters while walking the edge of the pond. A sweep consisted of guiding the d-frame net in a 180° arc from the shoreline while jabbing the net into the substrate. All amphibians captured were released immediately after being identified to species and life stage (Conant and Collins 1998, Dodd 2004). The individuals used for statistical analyses were all larvae with the exception of eastern red-spotted newts (*Notopthalmus viridescens*), which were all adults. This species has a complex life cycle that includes an adult, aquatic breeding phase. I interpreted the abundance of these adults as a measure of breeding within the pond.

Each study pond was surveyed during one sampling period per month, May-August 2010 (Table A - 1). Survey dates were chosen in order to maximize detectability for the amphibian species of the region (Dodd 2004). In each sampling period, a pond was surveyed for amphibians on three consecutive trap nights. Due to the travel distance between ponds, ponds were split into two groups, each group to be surveyed during separate three days spans. Four of the ponds did not hold water during one or more of the sampling periods, and amphibian data from these ponds were only based on one to three sampling events.

Habitat Characteristics

Habitat characteristics including canopy cover, upland coarse woody debris availability, plant cover, and maximum depth were measured for all ponds in order to compare pond parameters that may change over time or differ between construction methods. My objective was to compare these habitat features along with the amphibian communities present to provide data that determines the effectiveness of constructed ponds in replicating the functional amphibian communities of natural ponds in the DBNF. Aquatic vegetation was surveyed using a 1 m^2 quadrat placed on the edge of the pond at each of the cardinal directions (Shulse et al. 2010) and at the center of the pond. The percentage of vegetation cover was estimated and placed into four categories: emergent, submergent, floating, and open water/none present. Angular canopy closure was estimated directly above each aquatic vegetation quadrat and from the center of the pond with a spherical densiometer. The percentage of angular canopy closure was averaged across the five sample points. Pond depth was taken at the deepest point of each pond during each sampling period. Each site was scored for wetland quality according to the Ohio Rapid Wetland Assessment Method (ORAM; Mack 2001). Upland coarse woody debris (CWD) was measured according to an adapted line-intersect sampling protocol from Waddell (2002), in which 50-m transects were taken from each cardinal direction perpendicular to the pond border into the surrounding uplands (Warren and Olsen 1964). Upland coarse woody debris with a diameter ≥ 10 cm at its narrowest end that intercepted each transect was recorded (DiMauro and Hunter 2002). Each piece of CWD was measured for total length, and diameter at the narrowest and widest end (Waddell 2002). These measurements were used to calculate an estimate of the cubic volume of CWD per hectare (Husch et al. 1972, Waddell 2002 *after* DeVries 1973). Each habitat variable was chosen based on its associations with amphibians (Table 2).

Table 2. Description of habitat variables determined for each pond and literature sources justifiying selection for use in regression models.

Predictor Variable	Description	Source
Hydroperiod	Wetland hydrology (ephemeral = 0, permanent = 1)	Semlitsch et al. 1996, Snodgrass et al. 2000
Canopy Cover	Average of angular canopy cover percentage.	Werner and Glennemeier 1999
Upland coarse woody Debris	Estimated volume of coarse woody debris within 50 m of pond greater than 10 cm in diameter on ground	DiMauro and Hunter 2002
Size	Approximate area of pond (m^2)	Semlitsch and Bodie 1998, Snodgrass et al. 2000
Maximum Depth	Maximum depth of pond at the deepest point (cm)	Snodgrass et al. 2000, Skidds and Golet 2005
ORAM	Ohio Rapid Assessment Method for wetlands	Mack 2001
Vegetation	Average percentage of aquatic vegetation within five quadrats sampled	Knutson et al. 2004, Mazerolle et al. 2006, Shulse et al. 2010

Data Analyses

In order to prevent counting individual larva multiple times, one sample event per species from both the aquatic minnow trap and dipnet technique was selected per month to represent that month's sampling. This decision was made based on the highest abundance of each species on the given trap night. The selected values for each species were then totaled across the four sampling periods to provide a total abundance of each species for the breeding season. For the dipnet data, capture-per-unit-effort (CPUE) was calculated and used for analyses (Shulse et al. 2010). The abundance of each species at each pond during the separate months was divided by the number of dipnet sweeps per pond. Because the number of aquatic trap arrays was adjusted for the size of the ponds and variation in size was low (1-2 arrays), the amphibian data from aquatic trapping was analyzed using abundance values. Each habitat variable was compared between groups using a one-way analysis of variance (ANOVA) with a Tukey's post-hoc comparison test. If the assumption of equal variance was not met, a Welch's ANOVA was used with a Tukey's post-hoc comparison test.

(1) Do natural ponds differ from constructed ponds of multiple construction types and ages in amphibian community composition?

Amphibian community data and all habitat variables for constructed and natural ponds were examined using canonical correspondence analysis (CCA; Ter Braak 1986) in Program R with package VEGAN (R Development Core Team 2005, Oksanen et al. 2011). CCA is a constrained ordination technique that utilizes a linear regression to relate species community structure to a matrix of environmental variables. The linear regression step constrains the ordination axes to describe variation within the amphibian assemblage related to habitat variables. This method is used to visually assess the associations between amphibian species, habitat variables, and pond type. Permutation tests using the "anova.cca" command in Program R were used to examine significance of both the terms and axes used in CCA plots (Oksanen 2011).

To test for statistical differences in amphibian community composition between the construction types and natural ponds, a one-way analysis of similarity (ANOSIM; Clarke and Warwick 1994) was conducted using the program PAST (Hammer et al.

2001). This test examines a pairwise distance matrix to compare distances between and within groups. The Bray-Curtis Similarity Index was chosen as the distance measure (Bray and Curtis 1957), using 10,000 permutations. Sequential Bonferroni corrected p-values were used to compare the three groups (Rice 1989).

(2) What habitat variables associated with constructed and natural ponds predict the presence and abundance of each amphibian species?

Different amphibian species vary in response to the same suite of habitat variables (Gardner et al. 2007). To address this, I used stepwise regression models with amphibian abundance as the response variable. Statistical models were employed for the aquatic minnow trap and dipnet data separately because of the possibility of bias between the active and passive sampling methods. Because I expected some redundancy in the predictor variables (Table 2) and because the number of observations ($n = 19$) was low relative to the number of predictor variables, I conducted a principle component analysis (PCA) using SPSS in order to reduce the six variables to a smaller number of principal components that would account for the variance in amphibian abundances. The component scores for each site produced by the PCA procedure were then used as predictor variables along with pond type (Natural, New Construction Method, Old Construction Method) in all regression models. To interpret the most meaningful variables within the principal components, I counted communalities that had a value greater than 0.60, as recommended by Stevens (1986).

Dipnet statistical models - Using the generalized linear modeling in SPPS version 17.0 (SPSS Inc. 2008), I selected a regression model with a compound Poisson (Tweedie) distribution and log link function for the dipnet data (Shono 2008, Shulse et al. 2010). The Tweedie distribution was chosen based on its ability to utilize discrete to continuous data and large numbers of zeroes within the data set and because count data become more continuous when converted to CPUE. The index parameter value (p), which is the parameter in the model that varies depending on how continuous the data are, can be any value >1 and <2 for CPUE data and determines the distribution shape (Shono 2008, Shulse et al. 2010). Models were initially run with parameter values within this range, and a parameter value of 1.1 was supported based on the lowest Akaike's Information Criterion values (AIC_c) for each species. For the regression analyses that followed, each predictor variable with the largest p value that was >0.10 was removed in a stepwise fashion and the model was repeated until all factors remaining had p <0.10.

Aquatic trapping models - Abundance data from aquatic minnow trapping was first used to select species-specific distributions followed by a stepwise regression in SAS version 9.2 (SAS Institute Inc. 2008). Using the COUNTREG procedure for count data in SAS, I conducted stepwise regressions for each species with >25 individuals captured within each pond type. The type of distribution employed was determined using likelihood ratio tests. Distributions considered were Poisson, negative binomial, zero-inflated Poisson (ZIP), and zero-inflated negative binomial (ZINB). Each predictor variable that had the largest Type III effect significance >0.10 was removed in a stepwise fashion and the model repeated until all factors remaining had a p <0.10.

CHAPTER 3

III. RESULTS

Habitat Variation Between Pond Types

All natural ponds dried during the summer (DC5/DC0: June; DC6: August; DC2/Booth: September; Figure 1). Two of the new construction method ponds dried in June and July; Kidney88 and 06C, respectively. All old construction method ponds had permanent hydrology. One-way ANOVA tests showed that old construction method ponds had significantly higher maximum pond depth than new construction method ($p = 0.003$) and natural ponds ($p = 0.002$) ponds (Table 3, Figure 2). Natural ponds had significantly higher average ORAM scores than both old construction method ($p < 0.001$) and new construction method ($p < 0.001$) ponds (Table 3, Figure 3). Using a Welch's ANOVA test, canopy closure was significantly higher at natural ponds compared to the new construction method ponds ($p < 0.001$; Table 3, Figure 3). The amount of upland coarse woody debris surrounding the ponds and the percent of total vegetation did not differ significantly between pond types (Figures 2 and 4).

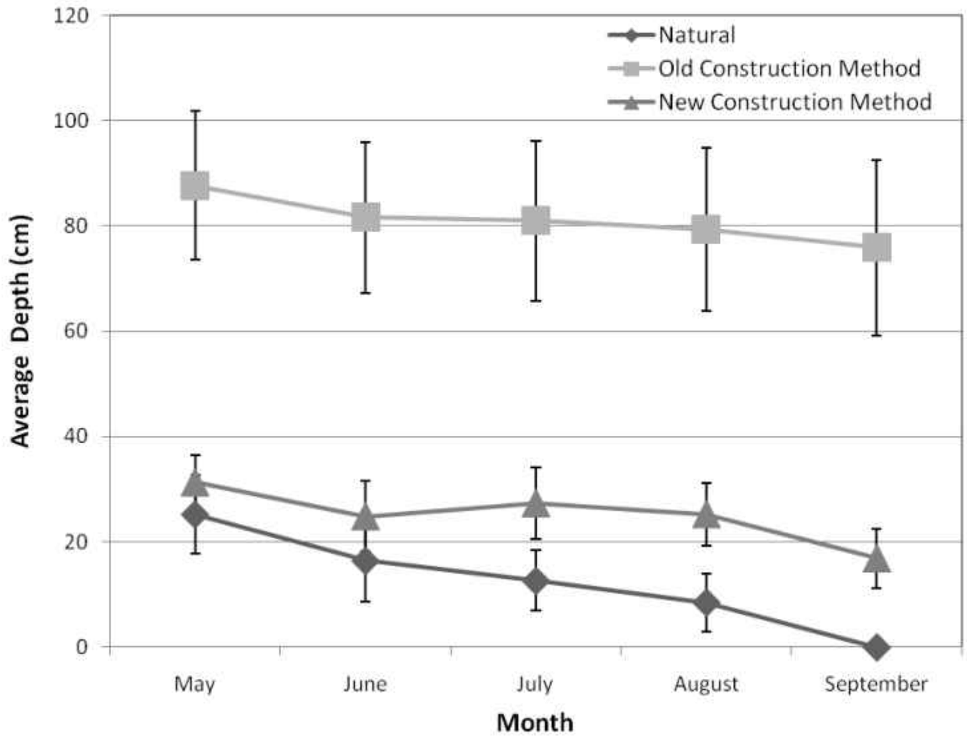

Figure 1. Average depth of each pond type in the Daniel Boone National Forest (KY) over May-August sampling period and September 2010. Depth was measured at deepest point.

Table 3. Results of one-way analysis of variance of Ohio Rapid Wetland Assessment Method (ORAM) score and maximum depth between pond types. Post-hoc Tukey pairwise comparisons are presented for the three pond types. For the variable canopy cover, Welch's robust test of equality of means was used.

Pond Type Comparison	Mean Difference	F	df	*p*
ORAM score	-	34.77	2	**< 0.001**
Natural - New Construction Method	27.43	-	-	**< 0.001**
Natural - Old Construction Method	26.43	-	-	**< 0.001**
New - Old Construction Method	1.00	-	-	0.951
Maximum Depth	-	11.54	2	**0.001**
Natural - New Construction Method	-7.09	-	-	0.886
Natural - Old Construction Method	-62.51	-	-	**0.002**
New - Old Construction Method	-55.43	-	-	**0.003**

Habitat Variable and Pond Type Comparison	Mean Difference	Welch	df	*p*
Canopy Cover	-	20.03	2	**< 0.001**
Natural - New Construction Method	25.82	-	-	**0.048**
Natural - Old Construction Method	25.43	-	-	0.052
New - Old Construction Method	0.29	-	-	0.999

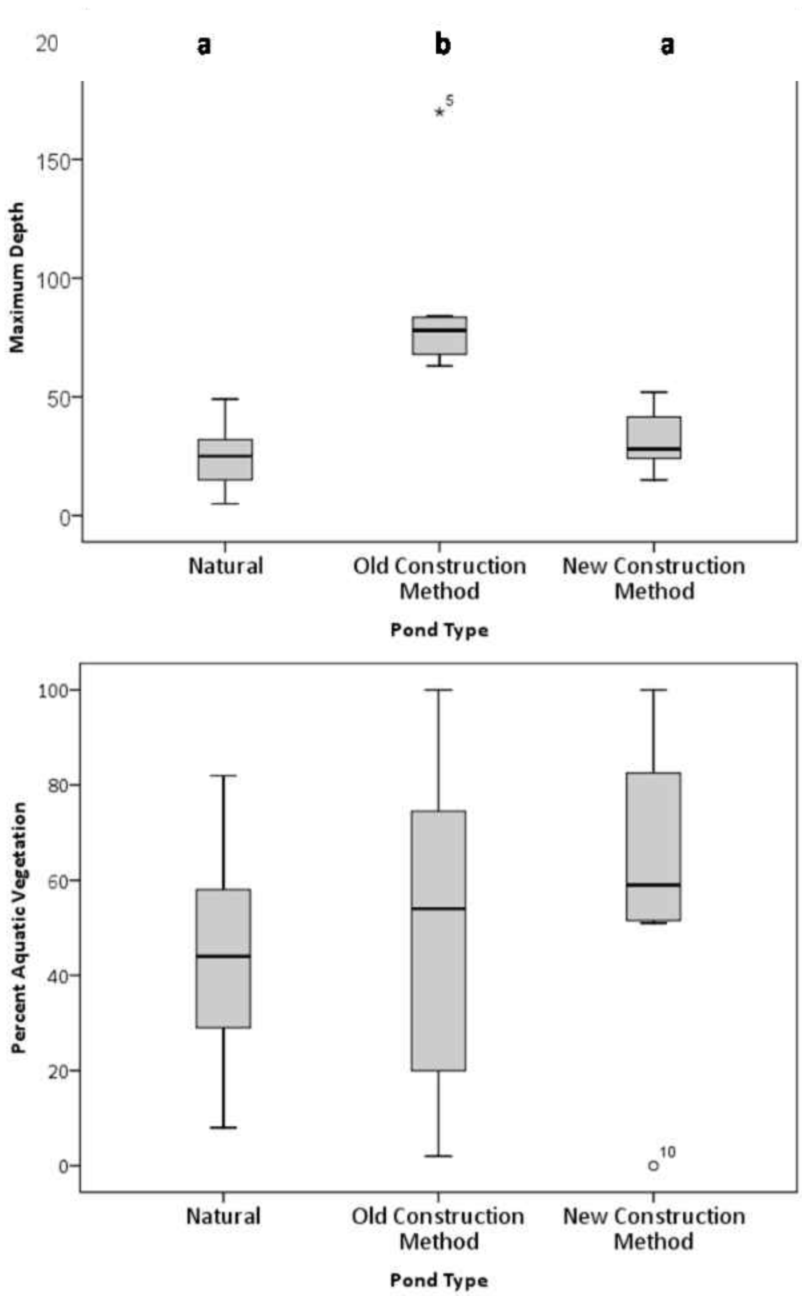

Figure 2. A comparison of mean maximum depth (top) and percent aquatic vegetation (bottom) between the three pond types in Daniel Boone National Forest (KY). Boxplots represent 25th and 75th percentiles and boxplot stems represent 95% confidence interval. Different letters in the maximum depth boxplot indicate results of post-hoc Tukey multiple comparisons.

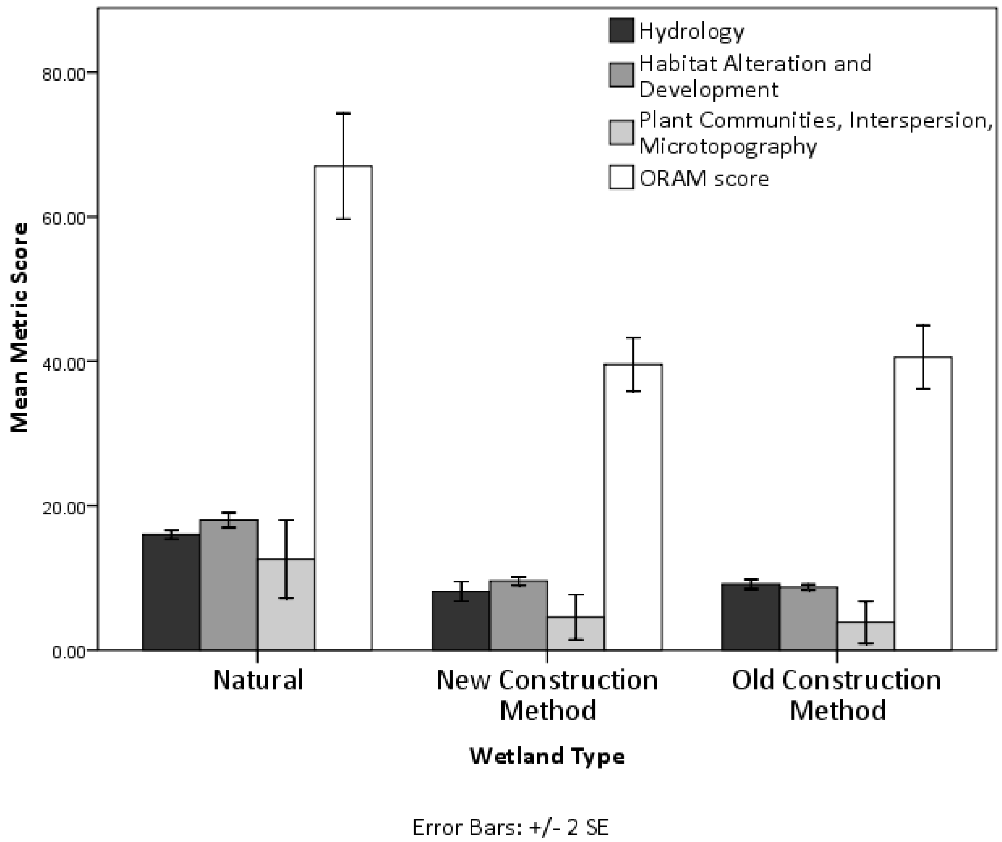

Figure 3. Mean (± 2 SE) metric scores (hydrology, habitat alteration and development, plant communities/interspersion/microtopography) and total Ohio Rapid Wetland Assessment Method (ORAM) score compared between three pond types in the Daniel Boone National Forest (KY).

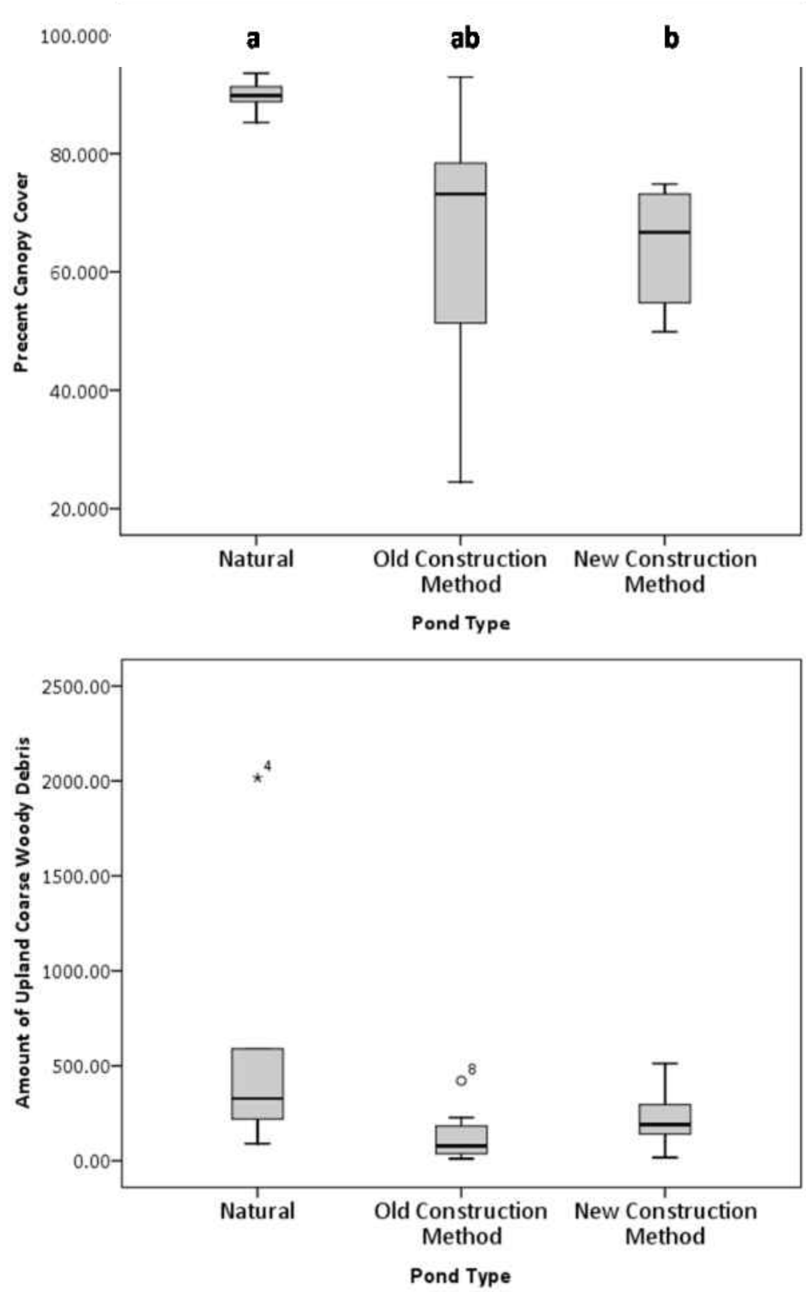

Figure 4. A comparison of mean percent canopy cover (top) and mean volume of upland coarse woody debris (bottom) between three pond types in the Daniel Boone National Forest (KY). Different letters in the canopy cover boxplot indicate post-hoc Tukey comparisons.

Amphibian Community Composition

I captured 9,716 individuals (5,435 from aquatic trapping; 4,281 from dipnetting) representing 13 species (Table 4). The only pond-breeding species known to occur in the area that were not detected were the eastern spadefoot toad (*Scaphiopus holbrookii*) and mountain chorus frog (*Pseudacris brachyphona*). After choosing representative trap events from each month, 3,425 and 2,372 individuals were used for statistical analyses from aquatic trapping and dipnetting procedures, respectively (Tables A - 2, A - 3). Natural ponds had the largest number of captured individuals (5,222) and the highest total species richness (12), but the lowest overall Shannon-Wiener diversity index score (Table 5). All ponds were used for breeding by multiple species; except for a single pond (06C) where only red-spotted newts were detected.

Table 4. Amphibian species found during surveys of constructed and natural ponds in the Daniel Boone National Forest (KY), May-August 2010.

Scientific Name	Common Name	Total # Individuals	# of Ponds Where Present
Bufo americanus	American toad	192	2
B. fowleri	Fowler's toad	31	3
Hyla chrysoscelis	Cope's gray tree frog	1283	9
Pseudacris crucifer	spring peeper	104	6
Rana sylvatica	wood frog	2367	4
R. clamitans	green frog	238	11
R. catesbeiana	bull frog	130	10
R. palustris	pickerel frog	7	2
Hemidactylium scutatum	four toed salamander	11	6
Ambystoma opacum	marbled salamander	44	5
A.maculatum	spotted salamander	486	16
A.jeffersonianum	Jefferson salamander	133	15
Notopthalmus viridescens	eastern red-spotted newt	771	17

Table 5. Comparison of total abundance, species richness, and mean (± 2 SE) Shannon-Wiener Diversity Index scores for three types of ponds in the Daniel Boone National Forest (KY), May-August 2010.

		Constructed Ponds	
	Natural	Old Method	New Method
Total Number of Captured Amphibians	5,222	1,857	1,130
Total Species Richness	12	10	10
Shannon-Wiener Index Score (Traps)	0.70 ± 0.30	1.03 ± 0.17	0.90 ± 0.22
Shannon-Wiener Index Score (Dipnet)	0.91 ± 0.33	1.39 ± 0.14	1.06 ± 0.19

I produced CCA plots for both dipnetting and aquatic trapping survey methods (Figures 5 and 6). Prior to the CCA analysis, the variable "Coarse Woody Debris" was log transformed because of extreme outliers in the raw data. In addition, site "06C" was removed from the CCA analysis for dipnetting due to zero individuals being captured at this site using dipnetting. The first two axes of the CCA procedure using dipnetting data accounted for 51.2% of the explained variation and both axes were found to significantly explain the variation in the dataset (CCA1: χ^2= 0.80, df=1, p = 0.005; CCA2: χ^2= 0.50, df=1, p = 0.037). Hydroperiod (dipnetting) was a significant vector term (χ^2= 0.37, df=1, F=3.90, p = 0.02). The first two axes of the CCA procedure using aquatic trap data accounted for 41.0% of the explained variation and the both axes significantly explained variation in the data set (CCA1: χ^2= 0.83, df=1, p = 0.005; CCA2: χ^2= 0.30, df=1, p = 0.028). All combined constrained axes of the CCA procedures explained 59.2% and 47.8% of the total variation possible, from dipnetting data and aquatic trapping, respectively.

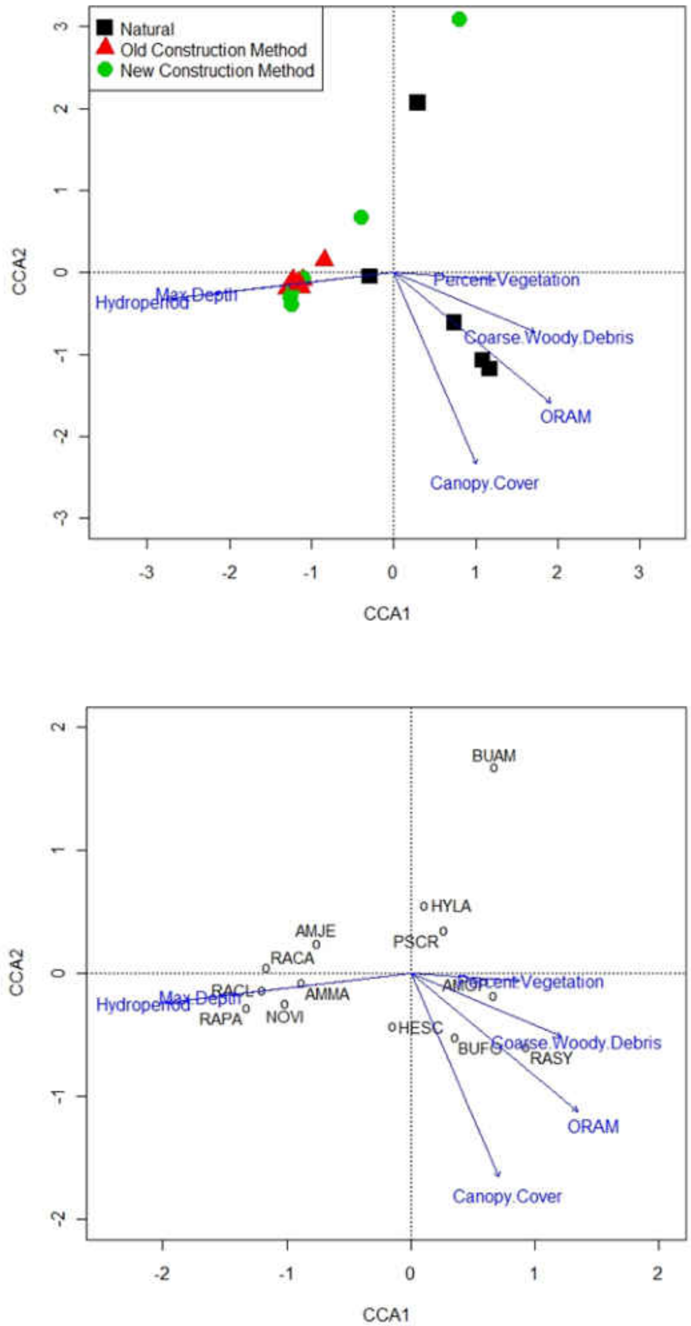

Figure 5. Canonical Correspondence Analysis (CCA) biplots for ponds (top) and species (below) collected by dipnetting three pond types in Daniel Boone National Forest (KY). Pond types are designated by shape (squares = natural, triangles = old construction method, circles = new construction method). Species are abbreviated using the first two letters of their genera and species names. Vectors represent habitat variable scores and the direction of gradients. ORAM = Ohio Rapid Wetland Assessment Method Score.

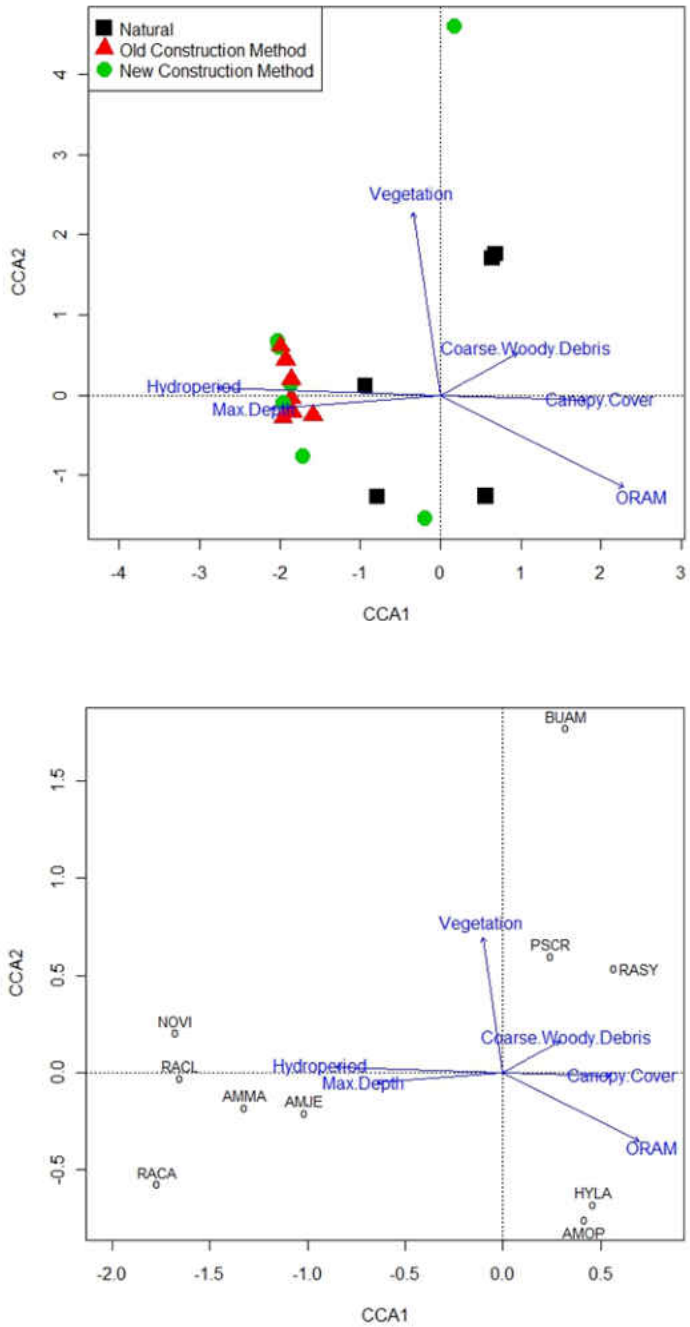

Figure 6. Canonical Correspondence Analysis (CCA) biplots for ponds (top) and species (below) collected by trapping three pond types in Daniel Boone National Forest (KY). Pond types are designated by different shapes (squares = natural, triangles = old construction method, circles = new construction method). Species are abbreviated using the first two letters of their genera and species names. Vectors represent habitat variable scores and the direction of habitat gradients. ORAM = Ohio Rapid Wetland Assessment Score.

Using the ANOSIM procedure, I found significant differences between pond types in terms of amphibian community composition (aquatic minnow traps: Global R = 0.3786, $p < 0.001$; dipnetting: Global R = 0.2245, $p = 0.009$). Pairwise comparisons of groups show that natural ponds were significantly different from old construction method ponds in terms of amphibian community (Table 6). Abundance data from aquatic trapping showed significantly different amphibian communities between natural ponds and new construction method ponds.

Table 6. Results of one-way analysis of similarity (ANOSIM) with 10,000 permutations using abundance or presence/absence data for each capture method. Global R values and sequential Bonferroni corrected pairwise p-values are displayed for the two amphibian survey methods. Bold text indicates significant *p* values at the $\alpha = 0.05$ level.

	Aquatic Minnow Traps		**Dipnetting CPUE**	
	Global R	*p*-value	Global R	*p*-value
Abundance data (Bray-Curtis distance values)	0.379	**<0.001**	0.225	**0.007**
Natural - New Construction Method	-	**0.007**	-	0.280
Natural - Old Construction Method	-	**0.004**	-	**0.002**
New - Old Construction Method	-	0.199	-	1.000
Presence/absence data (Jaccard's distance values)	0.246	**0.003**	0.188	**0.012**
Natural - New Construction Method	-	0.053	-	0.358
Natural - Old Construction Method	-	**0.008**	-	**0.020**
New - Old Construction Method	-	0.720	-	0.338

Individual Species Habitat Associations

A linear regression showed no trend for any species in regards to pond age alone. The PCA procedure extracted two components (Table 7). Bartlett's test of sphericity supported the validity of the component loadings ($\chi^2 = 26.88$, df = 15, p = 0.30). For dipnetting data, only species with >25 individuals captured within each pond type were used for regression models, resulting in regression models for three species (CPUE: Table 8; Trap Count: Table 9). For some species, patterns of abundance and presence were clearly distinct (i.e. a species only present in natural ponds). Even though these species were drivers of community differences, they were precluded from individual species analyses because they were only found in one pond type. For example, wood frogs (*Rana sylvatica*) were only captured in natural, ephemeral ponds. Due to their large abundance in these ponds, wood frogs were drivers in the community differences between the natural and constructed ponds. However, because they were only found in one pond type, they could not be compared for habitat associations across all pond types.

Two species (*Ambystoma maculatum* and *A. jeffersonianum*) were combined based on similar life histories and the precedence set by Shulse et al. (2010). Eleven and eight species were detected in multiple pond types by dipnetting and aquatic minnow trapping (Figures B - 1, B - 2, B - 3, B - 4).

Table 7. Principle Components Analysis (PCA) loadings of habitat variables measured at the 19 ponds surveyed for amphibians. The first two axes of the PCA explained 66.5% of the total habitat variation. The major axis loadings for each component is in bold print.

Habitat Variable	PC1	PC2
Canopy Cover	**0.773**	-0.062
Upland coarse woody Debris	0.402	**0.731**
Pond Size	**0.769**	-0.330
Maximum Depth	-0.172	**-0.758**
ORAM score	**v0.968**	-0.001
% Vegetation	-0.190	**0.645**

ORAM = Ohio Rapid Wetland Assessment Score

Table 8. Results of selected stepwise linear regression models using dipnet capture-per-unit-effort (CPUE) data. Only species with >25 individuals captured per wetland type were used for modeling. Pond type is indicated by number (1 = natural, 2= Old construction method, 3 = new construction method).

Species	Factor*	Beta Coefficient ± SE	Wald's χ2	*p*
Rana clamitans	Pond Type	1 vs. 3: -8.73 ± 2.71 2 vs. 3: -1.18 ± 0.72	11.46	0.003
	Coarse Woody Debris (-)	-1.29 ± 0.41	9.67	0.002
	Maximum Depth (+)	-	-	-
	% vegetation (-)	-	-	-
Combined Ambystoma	Coarse Woody Debris (-)	-0.92 ± 0.26	12.96	<0.001
	Maximum Depth (+)	-	-	-
	% vegetation (-)	-	-	-
Notopthalmus viridescens	Pond Type	1 vs. 3: -5.25 ± 0.44 2 vs. 3: -0.11 ± 0.50	14.18	0.001
	Canopy Cover (-)	1.52 ± 0.53	8.19	0.004
	Pond Size (+)	-	-	-
	ORAM (-)	-	-	-

AIC_c = Akaike's Information Criterion value, ORAM = Ohio Rapid Wetland Assessment score. *Factors other than Pond Type represent the loadings within each principal component communality >0.60 that was found to be significant in the model. Each variable within these components was described as positively (+) or negatively (-) associated with the abundance of each species based on scatterplots.

Table 9. Results of selected stepwise linear regression models using aquatic trapping abundance data. The type of distribution chosen was based on likelihood ratio tests. Only species with >25 individuals captured per wetland type were used for modeling. Pond type is indicated by number (1 = natural, 2= Old construction method, 3 = new construction method).

Species	Factor*	Beta Coefficient ± SE	Wald's χ2	*p*
Rana clamitans (Poisson)	Pond Type	1 vs. 3: -4.84 ± 1.24 2 vs. 3: -0.75 ± 0.33	25.67	<0.001
	Coarse Woody Debris (-)	-0.69 ± 0.17	16.04	<0.001
	Maximum Depth (+)	-	-	-
	% vegetation (-)	-	-	-
Combined	Coarse Woody Debris (-)	-1.30 ± 0.35	13.86	<0.001
Ambystoma	Maximum Depth (+)	-	-	-
(Poisson)	% vegetation (-)	-	-	-
Notopthalmus Viridescens (Negative Binomial)	Pond Type	1 vs. 3: -2.36 ± 0.99 2 vs. 3: -0.97 ± 0.46	9.75	0.007

AIC_c = Akaike's Information Criterion value, ORAM = Ohio Rapid Wetland Assessment score.

*Factors other than Pond Type represent the loadings within each principal component communality >0.60 that was found to be significant in the model. Each variable within these components was described as positively (+) or negatively (-) associated with the abundance of each species based on scatterplots.

American Toad

American toads were detected in 10.5% of total ponds surveyed (0-17 individuals/site trapped, 0-24.83 CPUE). This species was only located in one natural pond and one pond of the new construction type, both of which dried during the June sampling period. The two ponds where larval American toads were located were tied for the second lowest maximum depth (15 cm). Because of the low number of ponds in which this species was located, it was not included in the modeling procedures.

Fowler's Toad

Fowler's toads were detected in 15.8% of the total ponds surveyed (2-4 individuals/site, 0.34-1.31 CPUE). Two of the ponds where Fowler's toads were detected were new construction method ponds and the third was a natural pond. All ponds where this species occurred as larvae dried during the sampling season. Due to a small sample size for this species, it was not included in any regression modeling procedures.

Cope's Gray Tree Frog

Gray tree frogs were detected in 47.4% of the ponds surveyed (0-1093 individuals/site, 0.13-3.50 CPUE) and was detected in all three pond types. Larval Cope's gray tree frogs were most abundant in natural ponds, where one pond (DC2) had especially high abundance (1092 individuals, 2.80 CPUE). Due to the high amount of variation and low sample number between ponds types, gray tree frogs were not included in any regression analyses.

Spring Peeper

Spring peeper larvae were found in 31.6% of ponds (0-5 individuals, 0.17-2.59 CPUE). This species was detected in all three pond types in relatively low abundances and was not included in any regression analyses.

Wood Frog

Wood frogs were found in 21.1% of ponds surveyed (12-606 individuals/site, 1.01-42.26 CPUE). Wood frogs were only found in natural, ephemeral ponds and were therefore not considered for regression models. However, this species was captured in high numbers where it was detected (Trap Mean = 344 ± 124 SE; CPUE Mean = 19.07 ± 8.56 SE).

Green Frog

Green frogs were found in 57.9% of ponds surveyed (2-13 individuals/site, 0.05-6.60 CPUE). Except for <5 larvae found in a single natural pond (Booth), all green frog larvae were detected in both types of constructed ponds. The natural pond in which green frogs were detected dried in September. A Tweedie regression model for green frog CPUE found that pond type (χ^2 = 9.67, df = 2, p = 0.008) and principal component two (upland coarse woody debris, maximum depth, % vegetation) significantly (χ^2 = 15.50, df = 1, p < 0.001) predicted the abundance of larvae (Table 8). Pairwise comparisons between pond types show that green frogs were in significantly higher abundances in permanent ponds, regardless of construction type (Table 10). Aquatic trap abundance models for green frogs produced by the COUNTREG procedure using a Poisson distribution also found pond type ($\chi^2 = 25.67$, df = 2, $p < 0.001$) and principal component two ($\chi^2 = 16.04$, df = 1, $p < 0.001$; Table 8) to significantly explain larval abundance. Pairwise comparisons between pond types for trap count data indicated that green frog larvae were in significantly higher abundances in old construction method permanent ponds ($\chi2 = 15.69$, df = 1, Pr > $\chi2 < 0.001$) and new construction method ponds ($\chi2 = 23.60$, df = 1, Pr > $\chi2 <0.001$) compared to natural ponds. Old construction method ponds had significantly higher green frog abundance than new construction method ponds ($\chi2 = 5.24$, df = 1, Pr > $\chi2 = 0.022$; Table 11).

Bullfrog

Bullfrog larvae were detected in 52.6% of ponds surveyed (0-17 individuals/site, 0.14-1.90 CPUE). Except for <5 larvae found in a single natural pond (Booth), all bullfrog larvae were detected in constructed ponds and were not included in regression analyses. The natural pond in which bullfrogs were detected dried in September.

Pickerel Frog

Pickerel frog larvae were found in 10.5% of the ponds surveyed (0.14-0.53 CPUE). These larvae were exclusively detected in old method construction ponds. Because of the small sample size for this species, it was not included in any regression analyses.

Table 10. Pairwise comparisons of red spotted newt (*Notopthalmus viridescens*) and green frog (*Rana clamitans*) abundance between pond types from stepwise Tweedie regression procedure. Only comparisons with significant ($p < 0.05$) differences are shown.

Species	Pond Type 1	Pond Type 2	Mean Difference	Standard Error	df	*p*
Red spotted newt	Natural	New Construction Method	-3.13	1.21	1	**0.009**
	Natural	Old Construction Method	-3.51	1.55	1	**0.024**
Green frog	Natural	New Construction Method	-0.35	0.19	1	**0.035**
	Natural	Old Construction Method	-1.01	0.44	1	**0.011**

Table 11. Pairwise comparisons of red spotted newt (*Notopthalmus viridescens*) and green frog (*Rana clamitans*) aquatic trap abundance between pond types from stepwise regression procedure. Only comparisons with significant (p < 0.05) differences are shown.

Species	Pond Type 1	Pond Type 2	χ2	df	Pr > χ2
Red spotted newt	Natural	New Construction Method	4.64	1	**0.031**
	Natural	Old Construction Method	8.08	1	**0.045**
	New Construction Method	Old Construction Method	3.90	1	**0.048**
Green frog	Natural	New Construction Method	23.60	1	**<0.001**
	Natural	Old Construction Method	15.69	1	**<0.001**
	New Construction Method	Old Construction Method	5.24	1	**0.022**

Four-Toed Salamander

Four-toed salamander larvae were detected in 31.6% of the total ponds surveyed, but were captured in small numbers where they were present (0.09-0.24 CPUE). Four-toed salamanders were only detected by dipnetting surveys, and were found in all three types of ponds. However, due to the rarity of this species, it was not including in regression analyses.

Marbled Salamander

Marbled salamander larvae were detected in 26.3% of the ponds surveyed (0-8 individuals/site, 0-1.90 CPUE). All but three individuals (93.2%) were captured in natural ephemeral ponds. Marbled salamanders were not used in regression analyses due to small sample size.

Spotted and Jefferson Salamander

Spotted and Jefferson salamander larvae were combined in analyses due to their similar life histories (Shulse et al. 2010). Spotted salamanders were detected in 84.2% of all surveyed ponds (1-24 individuals/site, 0.11-5.69 CPUE). Jefferson salamanders were found in 78.9% of surveyed ponds (0-7 individuals/site, 0-2.28 CPUE). Both species were found in all pond types. Using Tweedie regressions from CPUE data (Table 11), I found that the combined ambystomatid abundance was significantly ($\chi^2 = 12.96$, df = 1, $p < 0.001$) predicted by principal component two (upland coarse woody debris, maximum depth, % vegetation). Trap count models produced in COUNTREG using a Poisson distribution also indicated that principal component two was significant ($\chi^2 = 13.86$, df = 1, $p < 0.001$) in predicting ambystomatid larvae abundance (Table 12).

Red-Spotted Newt

Red-spotted newts were detected in 89.5% of all ponds surveyed (2-103 individuals/site, 0-4.93 CPUE). Newts were found breeding in all pond types, and were excluded only from the two natural ephemeral ponds that dried the earliest (DC5, DC0).

Using the Tweedie regression model for red-spotted newt larvae, I found that pond type (χ^2 = 14.18, df = 3, p = 0.001) and principal component one (canopy closure, pond size, ORAM; χ^2 = 8.19, df = 1, p = 0.004) significantly predicted abundance (Table 8). Pairwise comparisons between pond types showed significantly higher abundance of red-spotted newts in both types of constructed permanent ponds compared to natural ponds (Table 10). Trap count models produced in COUNTREG using a negative binomial distribution also indicated that pond type significantly (χ^2 = 9.75, df = 3, p = 0.007) predicted red-spotted newt abundance (Table 9). Pairwise comparisons between pond types for trap count data indicated that red-spotted newts were in significantly higher abundances in old construction method permanent ponds ($\chi2$ = 8.08, df = 1, Pr > $\chi2$ = 0.045) and new construction method ponds ($\chi2$ = 4.64, df = 1, Pr > $\chi2$ <0.031) compared to natural ponds. Old construction method ponds had significantly higher green frog abundance than new construction method ponds ($\chi2$ = 3.90, df = 1, Pr > $\chi2$ = 0.048; Table 11).

CHAPTER 4

IV. DISCUSSION

Habitat Variation Between Pond Types

A greater maximum depth resulted in permanent hydroperiods for ridge-top ponds constructed in the DBNF using the old construction method. Even though the new construction method ponds were significantly shallower than the old construction method ponds (Table 4), their general lack of drying contributed to lower ORAM scores in the hydrology metric (Figure 3). Natural ponds scored higher in plant communities, interspersion, and microtopography (see metric descriptions in Mack 2001). These categories scored higher in the natural ponds, which had higher amounts of vegetated mounds and standing snags. While new construction method ponds had more aquatic structure in the form of large coarse woody debris that had been added to the ponds, it was not in sufficient amount to garner higher metric scores from the ORAM. The differences between pond types in percent canopy closure could be related to the year of pond construction. The new construction method ponds had the lowest amount of canopy cover and were the most recently disturbed by construction equipment. Natural ponds had a lower influence of anthropogenic disturbance. Most constructed ponds were built near forest roads, while the majority of natural ponds were relatively secluded.

Amphibian Community Composition

The analyses reported here reinforce the complex gradients of habitat variables that predict amphibian presence and abundance (Skelly et al. 1999, Shulse et al. 2010). The CCA procedures used here showed two different groups of species that associate most closely with old construction method ponds or natural ponds, and a gradient of species that used all three types of wetlands but showed preferences towards one end of the hydroperiod gradient. Wood frogs and marbled salamanders were especially associated with ephemeral natural ponds, while green frogs and bullfrogs associated strongly with permanent constructed ponds. It is unknown whether wood frogs are excluded from constructed ponds due to egg predation from green frog larvae (Vasconcelos and Calhoun 2006), red-spotted newt adults (Andrea Drayer, unpublished data), or some other factor. Other species that were found in all three pond types exhibited higher abundances either in ephemeral or in permanent ponds. Species that were found in higher abundances in ephemeral ponds (constructed and natural) included spring peepers, gray tree frogs, American toads, Fowler's toads, and four-toed salamanders. Although typically associated with ephemeral wetlands (Petranka 1998), spotted and Jefferson salamanders were in higher abundances in permanent constructed ponds along with red-spotted newts. Because all of the study ponds were fishless, the abundance of these ambystomatid salamanders might be more affected by fish presence than wetland hydroperiod (Porej and Hetherington 2005, Shulse et al. 2010). These species preferences were the drivers behind the significant difference between the old construction method ponds (all permanent) and the natural ponds (all ephemeral). New construction method ponds included permanent (n=5) and ephemeral (n=2) hydroperiods, but the ephemeral constructed ponds were not used by wood frogs or marbled salamanders. However, these ephemeral constructed ponds excluded the large ranid species from breeding, making them more similar in amphibian community composition to the natural ponds than the old construction method ponds. In other words, the amphibian community similarity between the new construction method and natural ponds is primary the result of the mutual exclusion of the large ranid frogs and not the mutual occurrence of species that are primarily ephemeral breeders such as wood frogs and marbled salamanders (*Ambystoma opacum*).

Individual Species Habitat Associations

The three most commonly captured amphibians (green frogs, spotted and Jefferson salamanders combined, red-spotted newts) showed similar habitat preferences. Both green frogs and red-spotted newts preferred old construction method ponds. Green frogs and bullfrogs require permanent bodies of water due to their overwintering larvae and late breeding periods (Conant and Collins 1998, Lannoo 2005); while red-spotted newt's affinity for deep ponds has been previously documented (Gates and Thompson 1982). Green frog and ambystomatid salamander abundance was negatively correlated with the amount of coarse woody debris around the pond and amount of aquatic vegetation. Because green frogs are predominately aquatic, it is unlikely this species utilizes coarse woody debris in the surrounding uplands. Low amounts of coarse woody debris and aquatic vegetation were common traits of old construction method ponds. Adult spotted salamanders showed no significant preference for amount of coarse woody debris, which corroborates the results of Patrick et al. (2008). However, spotted salamander's negative association with increased aquatic vegetation is counter to what was found by Calhoun et al. (2003), Egan and Paton (2004), and Shulse et al. (2010). The selection for permanent ponds by ambystomatid salamanders in this study could be due to their preference for longer hydroperiods (Egan and Paton 2004) and ability to tolerate the presence of green frogs (Vasconcelos and Calhoun 2006). Red-spotted newts were negatively associated with percentage of canopy cover and ORAM score, but positively associated with pond size. Constructed ponds with more open canopies were generally given lower ORAM scores, and newts were in high abundance in these pond types. Because of the skin toxicity exhibited by red-spotted newts, this species has shown the ability to occupy habitat shared with predatory fish (Gates and Thompson 1982). Therefore, newts were likely found in higher abundances in the deeper constructed ponds because of a tolerance for larger ranid predators and the avoidance of the energy requirements needed for migration after a pond dries (Hunsinger and Lannoo 2005). Although red-spotted newts were observed actively feeding on spotted and Jefferson salamander eggs in the spring, there was no clear relationship between the presence and abundance of the three species.

Implications for Wetland Construction and Planning

Old construction method ponds in the DBNF fail to duplicate the functions of natural, ephemeral ponds. Because natural ridge-top ponds are scarce in the DBNF, creating ponds that are more natural in function has become a priority. A new method of pond construction implemented 2004-2007 has failed in consistently constructing ephemeral ponds, which is necessary to exclude green frogs and bullfrogs from breeding as well as reduce the abundance of red spotted newts. Even though these permanent-water breeding amphibians are endemic to the DBNF, they were historically most likely confined to lowland basins where permanent marshes, oxbows, and natural lakes provided breeding habitat. The large ranid frogs, especially bullfrogs, are known to be invasive in altered aquatic habitats with permanent water (Fuller et al. 2010). My results indicate that, in the DBNF, ephemeral-breeding specialists such as wood frogs and marbled salamanders are predominately confined to the few natural, ephemeral ponds. Even though the ephemeral specialist species are in high abundances in natural, ephemeral ponds, being isolated to only natural wetlands could lead to long-term negative consequences from genetic isolation. The propagation of permanent ponds over the last twenty years in the DBNF has likely provided avenues of dispersal and migration for green frogs and bullfrogs; which may expose naturally occurring ridge-top amphibian species to direct predation and disease, e.g. amphibian Chytrid fungus (*Batrachochytrium dendrobatidis*) and ranavirus (Daszak et al. 2004, Gahl 2007, Gahl et al. 2009). Additionally, the speeds at which these species may disperse are predicted to be higher in the low-resistance matrix of the continuous forest (Rothermel and Semlitsch 2002), hence the high density of constructed ponds within the DBNF could provide "stepping stones" for dispersal. Removing or altering old construction method ponds may lessen the isolation of ephemeral breeding amphibian species. As a consequence of studies from our research group, the U.S. Forest Service began altering these ponds in the Fall of 2010 (T. Biebighauser, per. comm.).

Results of this study underscore the importance of using constructed wetland habitat as a conservation strategy for amphibians. Due to growing concerns surrounding amphibian declines and the current inability of mitigated wetlands to replace removed wetlands, producing quality constructed wetlands is requisite in conservation of amphibians and in reviving declining populations. However, replacing wetland function requires extensive knowledge of the natural types of regional wetlands. For ridge-top ponds in the DBNF, constructed wetlands should be ephemeral and placed in areas where canopy cover is maximized. Any future developments by the U.S. Forest Service in construction techniques for ephemeral wetlands could be used to improve construction protocols in the eastern United States, where legal protection of ephemeral ponds is lacking (*see* Environmental Law Institute 2008). As suggested by Semlitsch (2008), wetlands constructed for mitigation or otherwise should be built with consideration to function and quality, not quantity exclusively. Building wetlands that replace the function of previously removed natural wetlands is difficult, as shown in this study, but efforts to do so could ultimately aid in developing more efficient conservation strategies.

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APPENDIX A

Table A - 1. Amphibian sampling dates for study ponds in the Daniel Boone National Forest, KY, May-August 2010.

	Sampling Dates																	
Pond Name	11-May	12-May	13-May	15-May	16-May	17-May	15-Jun	16-Jun	17-Jun	18-Jun	19-Jun	20-Jun	16-Jul	17-Jul	18-Jul	13-Aug	14-Aug	15-Aug
60/70s				x	x	x				x	x	x	x	x	x	x	x	x
Kidney88				x	x	x												
040-90				x	x	x				x	x	x	x	x	x	x	x	x
2009rework				x	x	x				x	x	x	x	x	x	x	x	x
42-93	x	x	x				x	x	x				x	x	x	x	x	x
95NEW	x	x	x				x	x	x				x	x	x	x	x	x
060-96	x	x	x				x	x	x				x	x	x	x	x	x
35-97	x	x	x				x	x	x				x	x	x	x	x	x
04A				x	x	x				x	x	x	x	x	x	x	x	x
05A				x	x	x				x	x	x	x	x	x	x	x	x
06A				x	x	x				x	x	x	x	x	x	x	x	x
06C				x	x	x				x	x	x						
06D				x	x	x				x	x	x	x	x	x	x	x	x
06E				x	x	x				x	x	x	x	x	x	x	x	x
DC2	x	x	x				x	x	x				x	x	x	x	x	
DC5	x	x	x															
DC6	x	x	x				x	x	x				x	x	x			
DC0	x	x	x															
Booth	x	x	x				x	x	x				x	x	x	x	x	x

Table A - 2. Abundance of each species captured from all study ponds using aquatic minnow traps in Daniel Boone National Forest, KY, May-August 2010.

	Pond Name and Type																		
	Natural					Old Construction Method							New Construction Method						
Species	DC2	DC5	DC6	DC0	Booth	95new	696	42-93	35-97	2009	490	60/70	04A	05A	06A	06C	06D	06E	88
Bufo americanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
Bufo fowleri	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	4	0	0
Hyla chrysoscelis	1093	0	0	0	11	0	10	1	0	0	1	0	5	0	0	0	3	36	0
Pseudacris crucifer	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Rana sylvatica	345	0	606	411	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rana clamitans	0	0	0	0	2	13	6	5	0	3	12	0	9	6	3	0	7	3	0
Rana catesbeiana	0	0	0	0	0	3	5	7	0	16	0	0	17	10	0	0	5	2	0
Rana palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemidactylium scutatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ambystoma opacum	5	8	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ambystoma maculatum	6	10	2	0	18	22	24	12	15	3	23	1	15	6	1	0	1	0	0
Ambystoma jeffersonianum	5	5	1	0	2	2	1	2	7	2	4	2	2	3	0	0	1	1	0
Notopthalmus viridescens	6	0	7	0	24	27	38	21	103	30	58	62	12	29	41	2	37	9	2

Table A - 3. Abundance (capture-per-unit-effort) of each species captured from all study ponds using standardized dipnetting protocol in Daniel Boone National Forest, KY, May-August 2010.

	Pond Name and Type																		
	Natural					Old Construction Method							New Construction Method						
Species	DC2	DC5	DC6	DC0	Booth	95new	696	42-93	35-97	2009	490	60/70s	04A	05A	06A	06C	06D	06E	88
Bufo americanus	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	24.8
Bufo fowleri	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Hyla Chrysoscelis	2.8	0.0	0.0	0.0	1.2	0.0	0.4	0.7	0.0	0.1	0.0	0.0	1.4	0.0	0.0	0.0	0.8	3.5	0.0
Pseudacris crucifer	2.6	0.0	0.0	0.0	0.5	0.0	0.8	0.9	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	2.6
Rana sylvatica	15.7	0.0	17.3	42.3	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rana clamitans	0.0	0.0	0.0	0.0	0.0	6.6	2.7	0.6	0.0	0.9	1.0	0.0	3.8	0.6	0.1	0.0	2.3	0.2	0.0
Rana catesbeiana	0.0	0.0	0.0	0.0	0.1	1.7	0.2	0.8	0.0	0.9	0.3	0.0	1.9	0.8	0.0	0.0	1.1	0.0	0.0
Rana palustris	0.0	0.0	0.0	0.0	0.0	0.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hemidactylium scutatum	0.1	0.0	0.1	0.0	0.2	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Ambystoma opacum	0.1	0.4	1.9	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ambystoma maculatum	1.3	1.2	0.3	0.0	1.1	4.8	1.2	1.4	4.6	1.3	5.6	0.1	5.7	2.2	0.4	0.0	0.8	1.5	0.0
Ambystoma jeffersonianum	0.5	0.2	0.0	0.0	0.2	0.6	0.6	0.5	2.3	0.5	1.1	0.4	0.5	0.0	0.0	0.0	0.0	1.2	0.0
Notopthalmus viridescens	0.2	0.0	0.1	0.0	1.1	1.8	4.9	1.1	2.7	1.0	1.3	0.9	2.4	3.8	1.4	0.0	4.2	0.0	0.0

APPENDIX B

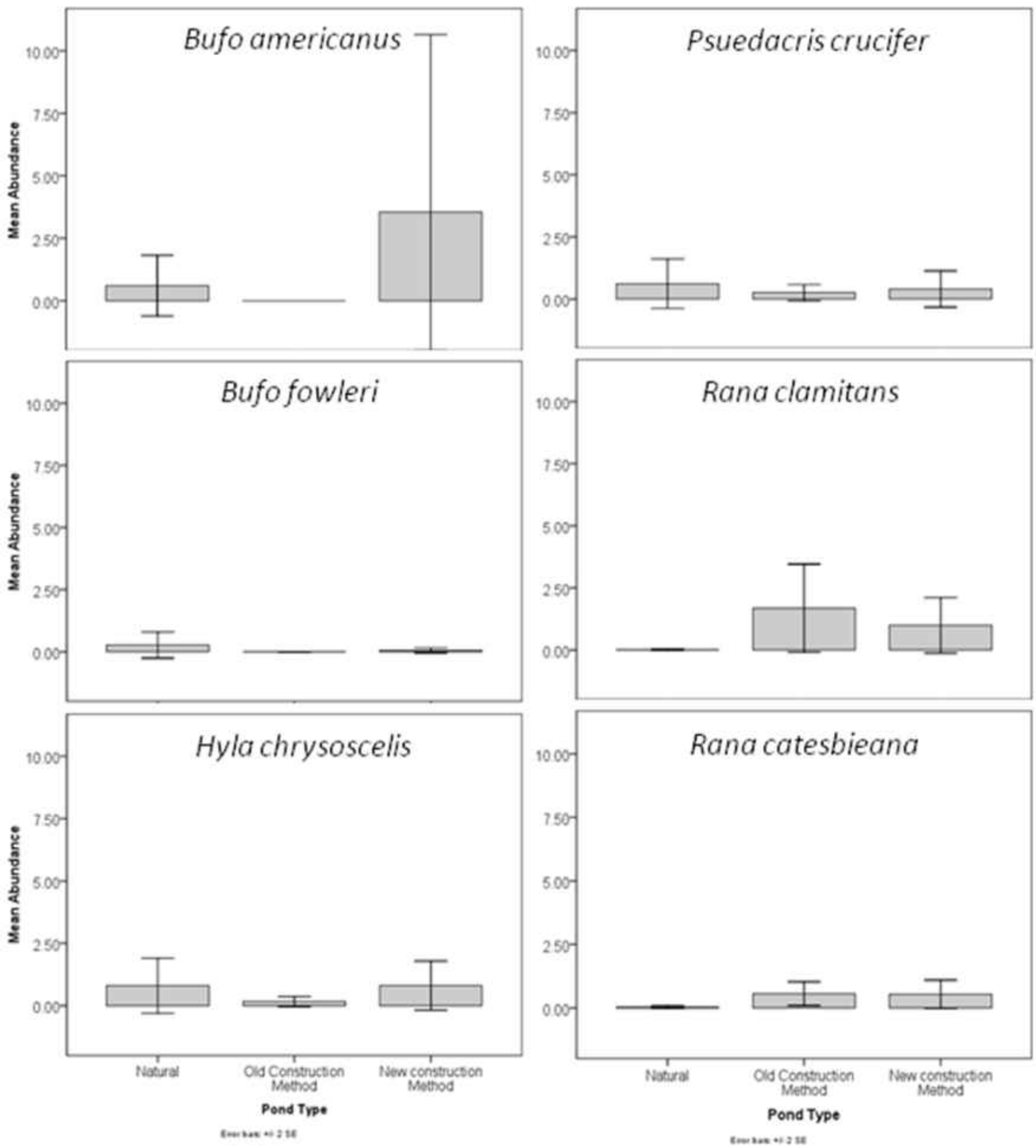

Figure B - 1. Bar charts comparing amphibian species' abundance [mean (± 2 SE) dipnet catch-per-unit-effort] across pond types (Natural, New Construction Method, Old Construction Method) in the Daniel Boone National Forest, KY, May-August 2010.

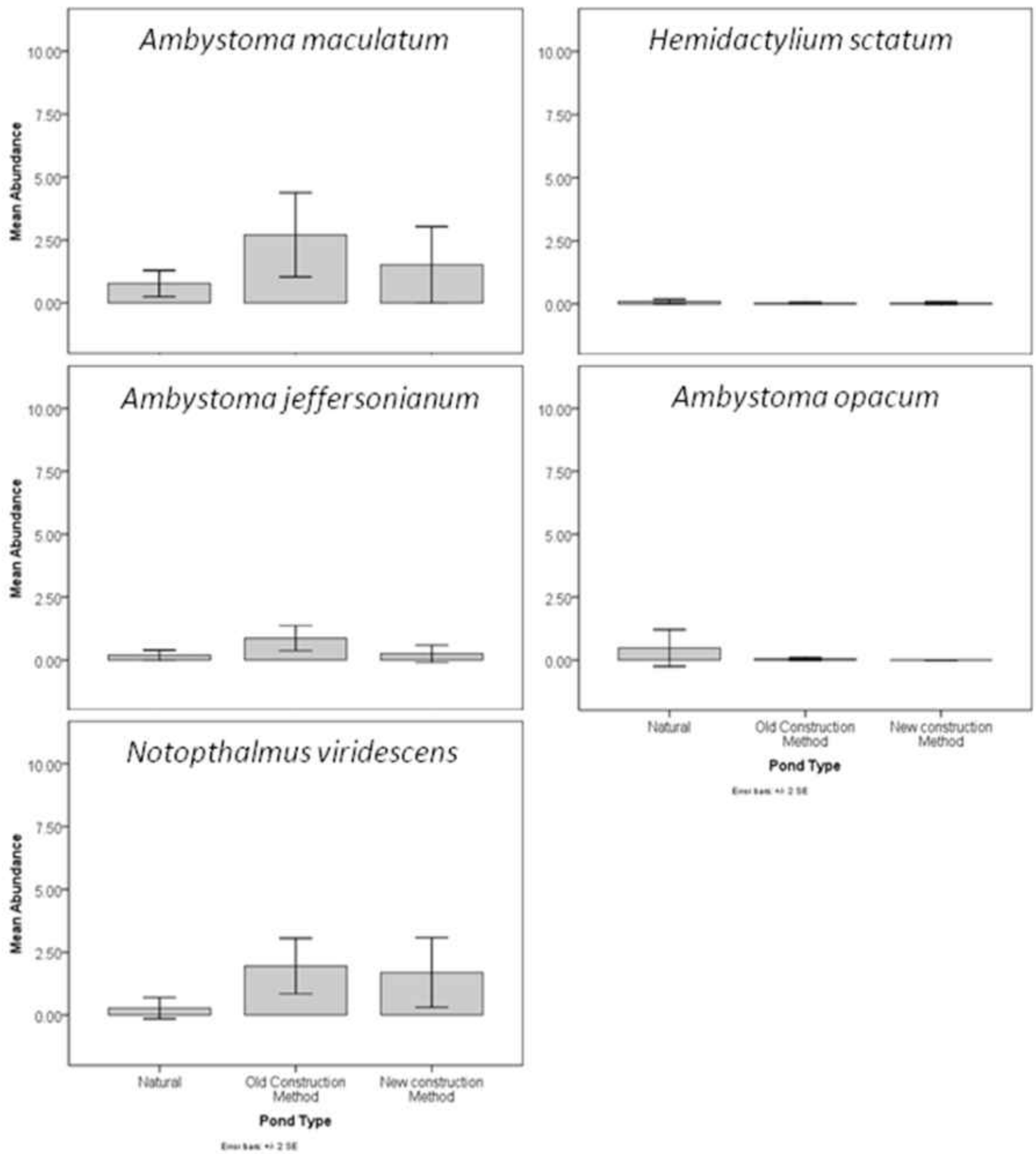

Figure B - 2. Bar charts comparing amphibian species' abundance [mean (± 2 SE) dipnet catch-per-unit-effort] across pond types (Natural, New Construction Method, Old Construction Method) in the Daniel Boone National Forest, KY, May-August 2010.

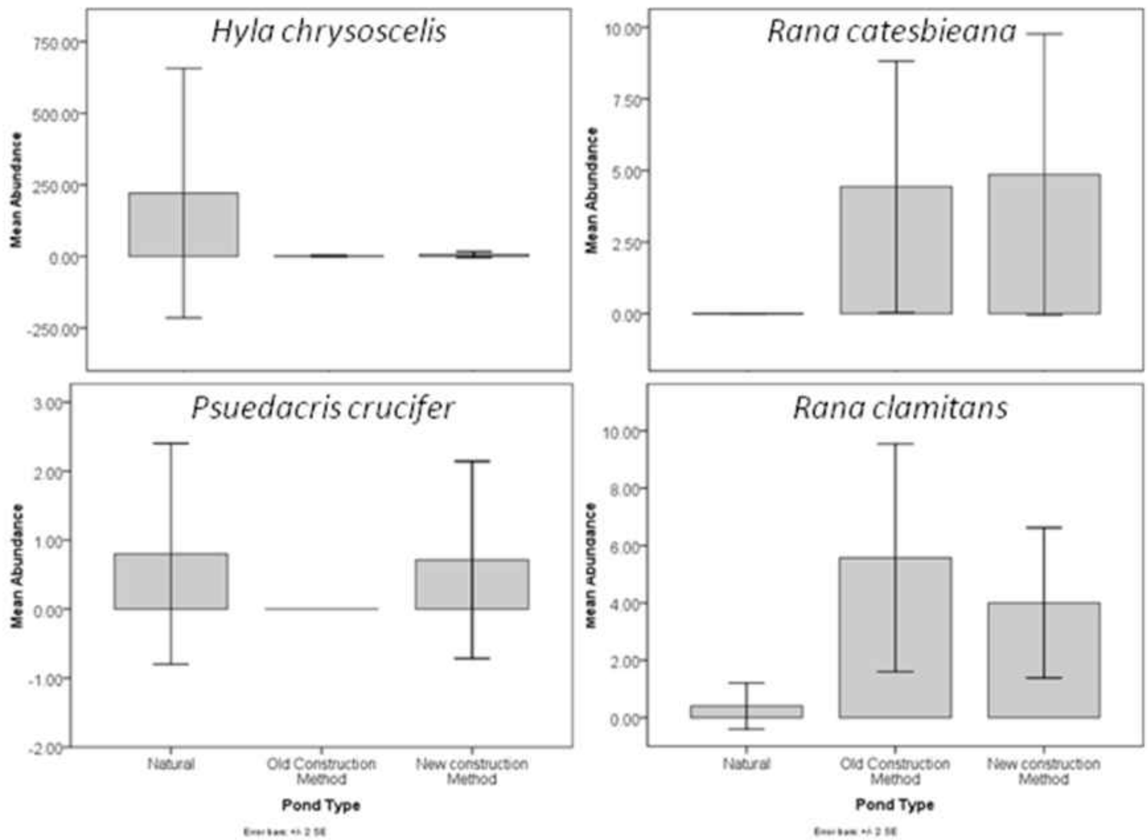

Figure B - 3. Bar charts comparing amphibian species' abundance [mean (± 2 SE) aquatic trapping] across pond types (Natural, New Construction Method, Old Construction Method). All axes are different in scale.

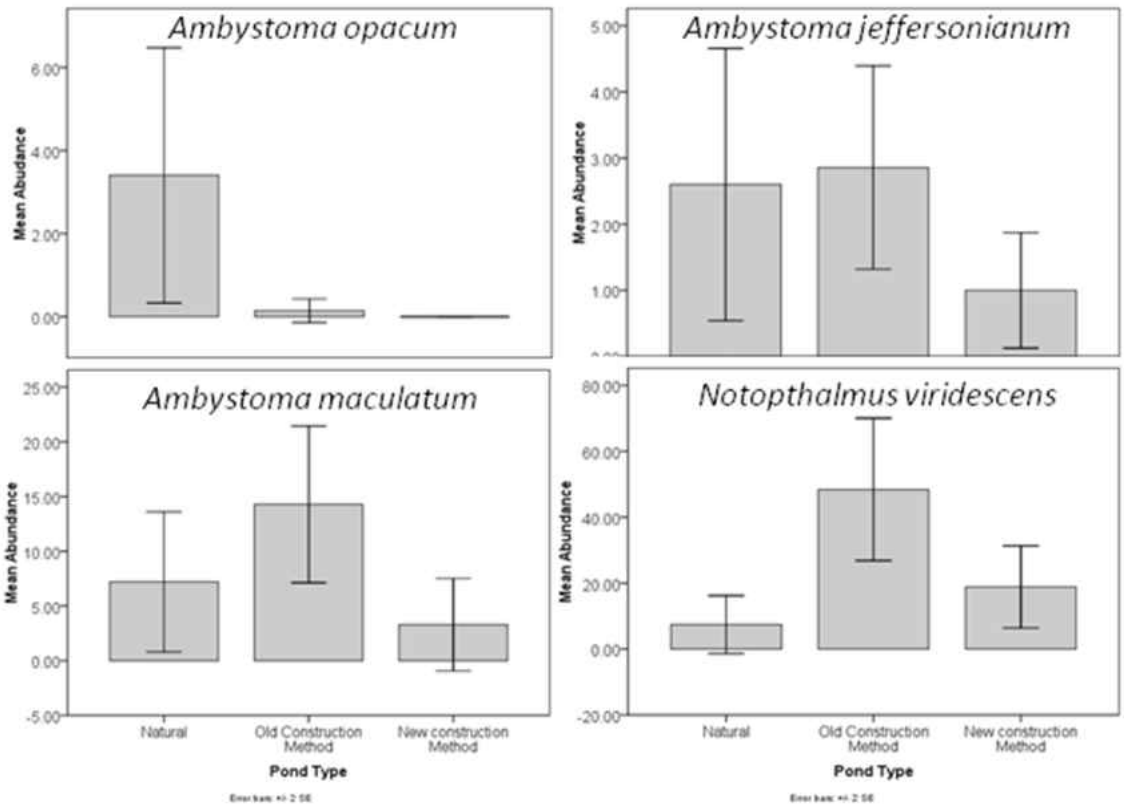

Figure B - 4. Bar charts comparing amphibian species' abundance [mean (± 2 SE) aquatic trapping] across pond types (Natural, New Construction Method, Old Construction Method). All axes are different in scale.