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Efficacy Of Constructed Wetlands Of Various Depths For Natural Amphibian Community Conservation

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EFFICACY OF CONSTRUCTED WETLANDS OF VARIOUS
DEPTHS FOR NATURAL AMPHIBIAN COMMUNITY
CONSERVATION

By:

Andrea Drayer

Thesis Approved:



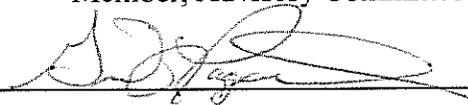
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EFFICACY OF CONSTRUCTED WETLANDS OF VARIOUS
DEPTHS FOR NATURAL AMPHIBIAN COMMUNITY
CONSERVATION

By

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Eastern Kentucky University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
July, 2011

DEDICATION

I dedicate this thesis to my parents, Daniel Alfred Drayer and Debra Lynn Drayer, whose unwavering support has afforded me the opportunities to continue to achieve my goals and aspirations.

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I would like to thank my advisor, Dr. Stephen C. Richter, who has provided unequalled guidance throughout the highs and lows of this project. I would also like to thank my committee members Dr. David Brown and Dr. Sherry Harrel for their comments and support. In addition, Dr. Guenter Schuster provided proposal comments for which I am grateful. This project would not have been possible without the help of Tom Biebighauser (US Forest Service). I thank him for his time, enthusiasm, and help in planning this project. I thank John MacGregor (Kentucky Department of Fish and Wildlife Resources) for kindly commenting on the research proposal draft and for key information during the project. I would like to thank Dr. Alice Jones at Eastern Kentucky University's Environmental Research Institute for the use of a YSI water quality multimeter probe and for calibration assistance. I am indebted to several individuals for their dedicated field assistance: Daniel Douglas, Michelle Guidguli, Stephen Richter, Rob Denton, Chris St. Andre, and Andrew Rumments without whom this project would have been more difficult and less fun. In addition, I would like to thank Mr. Bernall and Mrs. Martha Lewis for their kindness and hospitality. I appreciate the graduate students and faculty of the Department of Biological Sciences, who provided support and assistance and who have enhanced my graduate education. Further, I would like to thank the sources of funding for this project including: The Kentucky Academy of Science, The Kentucky Society of Natural History, and Eastern Kentucky University's Department of Biological Sciences. Lastly, I would like to express gratitude to my family and friends who have encouraged and inspired me throughout this project.

ABSTRACT

Wetlands provide critical habitat for a diverse group of amphibians and provide important ecosystem functions and services to humans. Despite this, most natural wetlands have been lost to land use practices. Consequently, constructing wetlands has become a common practice to mitigate for removed wetlands and to manage for wildlife. There were three primary objectives of this research: 1) to examine whether or not constructed wetlands located on ridge tops in eastern Kentucky in the Daniel Boone National Forest (DBNF) had amphibian communities comparable to natural ephemeral wetlands, 2) to examine amphibian predator-prey relationships within the constructed wetlands, and 3) to determine what wetland characteristics affect species composition. Three types of wetlands were sampled forested natural ephemeral, shallow constructed (< 20 cm minimum depth), and deep constructed wetlands (> 20 cm minimum depth). Within this system, natural wetlands are ephemeral, whereas constructed wetlands typically do not dry. As a result, many species of the natural ridge-top amphibian community were scarce in shallow constructed wetlands and absent in deep constructed wetlands. Additionally, due to constructed wetlands, dominant amphibian predator species, primarily associated with permanent water, *Rana catesbeiana* (American bullfrog) and *Notophthalmus viridescens* (eastern newt), were in greater abundances than would occur naturally. Stomach contents of *R. catesbeiana* contained amphibian remains confirming interspecies predation. Further, in constructed wetlands, water depth, pH, dissolved oxygen, water temperature, and emergent vegetation were greater, whereas canopy closure was lower compared to natural wetlands. These data have influenced

DBNF land managers to revise wetland construction methods and renovate older deep constructed wetlands to attempt to replicate the hydrology of natural ridge-top wetlands.

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I. INTRODUCTION

Background

In recent decades, the scientific community has documented amphibian population declines worldwide (Alford and Richards 1999, Houlihan et al. 2000, Kiesecker et al. 2001, Collins and Storfer 2003, Storfer 2003, Lannoo 2005). In most cases, amphibian declines have been attributed to multiple variables and interactions rather than a single factor or event; these factors include habitat loss and fragmentation, chemical pollution, increased ultraviolet B radiation, increased global temperature, infectious disease, parasitic infection, introduction of exotic species, and commercial amphibian trade (Alford and Richards 1999, Lannoo 2005).

Habitat loss and alteration appear to be two of the most important factors affecting the persistence of amphibian communities (Becker et al. 2007). Many states have lost a large percentage of historical wetland acreage to agricultural conversion. Kentucky sustained a loss of 81% of its historic wetlands (512,332 hectares) between 1780 and 1980, and much of this is attributable to conversion of wetlands for agriculture (Dahl 1990, 2000). Human alteration of wetland hydrology (e.g. deepening an ephemeral pool for cattle watering purposes) changes amphibian community composition. This can be detrimental for amphibian species that have life-history traits specific to ephemeral wetlands.

In 1972, concern for the condition of our federal waters led to the enactment of the Clean Water Act, and for the last four decades, it has been the cornerstone of legislative protection for wetlands within the United States (Clean Water Act of 1972).

The implementation of the Clean Water Act reduced the imprudent destruction of wetland habitat and held developers accountable for mitigation of permitted wetland loss. In 2001, a United States Supreme Court decision changed the course of wetland protection within the United States (*Solid Waste Agency of Northern Cook County vs. U.S. Army Corps of Engineers* 2001). The decision removed hydrologically isolated waters from the jurisdiction of the Clean Water Act (Downing et al. 2003, Zedler 2003). Most states have laws in addition to those outlined in the Clean Water Act; however, most of these additional laws do not protect hydrologically isolated wetlands.

The state of Kentucky is one of only 17 states relying solely on the section 401 water quality certification program (effective under the Clean Water Act) for federal wetland regulation and permitting (Environmental Law Institute 2008). Only six states (Indiana, North Carolina, Ohio, Tennessee, Wisconsin, and Washington) have supplementary wetland protection laws to safeguard protection for these isolated wetlands (Environmental Law Institute 2008). Therefore, under the current laws of Kentucky, small isolated wetlands are not considered jurisdictional wetlands and are not protected. Substantial loss of isolated wetlands will continue in most states because they are not protected under the recent interpretation of the law. This decision is detrimental to many amphibian species relying primarily on isolated wetlands for reproduction.

Isolated wetlands can play a significant role in the maintenance of species diversity within a landscape (Semlitsch and Bodie 1998, Snodgrass et al. 2000b). Pond-breeding amphibians are biphasic, occupying both aquatic and terrestrial habitats during different phases of their lifecycle. As a result of this life-history strategy, amphibians

utilizing isolated wetlands are the source for a large percentage of biomass linking aquatic and terrestrial habitats and are therefore an important contributor to aquatic and terrestrial ecosystem health (Gibbons et al. 2006).

The composition of amphibian communities found within isolated wetlands might be influenced by multiple interacting factors including food availability, water quality, water temperature, hydroperiod, canopy closure, predation, and inter- and intra- species competition (Werner 1986, Skelly et al. 2002, McCoy and Harris 2003, Eagan and Paton 2004, Baldwin et al. 2006, Ryan 2007, Karraker 2007, Karraker et al. 2008, Smith et al. 2007). Canopy closure and hydroperiod, in particular, appear to have influential effects on multiple wetland characteristics and consequently species composition within wetland habitats. A decrease in canopy closure can increase water temperature, decrease hydroperiod, change food availability, and increase dissolved oxygen (Schiesari 2006, Skelley et al. 2002). An increased hydroperiod can allow top amphibian predators to gain a foothold in an otherwise exclusionary habitat, whereas a relatively short hydroperiod can exclude dominant amphibian predators, increase water temperature, and influence development and survival of larvae to metamorphosis (Wellborn et al. 1996, Skelley et al. 2002).

The wetland characteristics that have the greatest influence on amphibian development and survival are temperature (Harkey and Semlitsch 1988, Skelly et al. 2002, Schiesari 2006) and dissolved oxygen content (McIntyre and McCollum 2000, Skelly et al. 2002). Canopy closure reduces water temperature and dissolved oxygen within wetland systems (Schiesari 2006, Skelley et al. 2002). This temperature decrease

can be attributed to a decrease in sunlight reaching the water surface. Skelly et al. (2002) determined an increase in canopy closure decreased water temperature by an average of 5°C. A temperature decrease may depress amphibian larval growth rates, while an increase of 5°C has been shown to double their growth rates (Harkey and Semlitsch 1988). Canopy closure decreases dissolved oxygen content in closed-canopy wetlands by about half of what is observed in open-canopy wetlands (Skelly et al. 2002). Dissolved oxygen might be influential in shaping species composition within wetlands by affecting predator-prey interactions. In a laboratory experiment, McIntyre and McCollum (2000) determined that under hypoxic conditions with no predation risk, ranid tadpoles increased the amount of time spent at the water surface. After the addition of ambystomatid salamander larvae (known tadpole predators), the ranid tadpoles modified their behavior by spending most of their time on the bottom of the tank. At high dissolved oxygen levels, salamander larvae may encounter and prey on tadpoles more regularly; thereby limiting the tadpole population within a system.

Hydroperiod is an important determinant for amphibian community composition and water quality characteristics. For example, a wetland with a short hydroperiod supports amphibian species with short larval periods; while a permanent or long hydroperiod supports amphibian species with long developmental periods (Snodgrass et al. 2000a). While wetlands with a long hydroperiod tend to have higher species richness, wetlands with a short hydroperiod tend to have less common, specialized species (Snodgrass et al. 2000b). This short hydroperiod typically excludes top amphibian predators (e.g. *Rana catesbeiana*, American bullfrogs) and allows for the unimpeded

development of these rare species (Wellborn et al. 1996). Thus, ephemeral wetlands with short hydroperiods are important for maintaining biological diversity (Snodgrass et al. 2000b). However, there is a risk of tadpole mortality during long periods of low precipitation within these temporary habitats (Rowe and Dunson 1995, Seigel et al. 2006).

Amphibian communities have previously been studied in restored, newly created, and mitigation wetland sites (Arntzen and Teunis 1993, Mierzwa 2000, Pechmann et al. 2001, Hazell et al. 2004, Shulse et al. 2010). These studies have assessed amphibian species richness, colonization, and general community assemblages at constructed or restored sites. Presumably due to the lack of natural reference sites, three (Arntzen and Teunis 1993, Mierzwa 2000, Shulse et al. 2010) of the five studies mentioned did not use natural reference wetlands as a comparison. Of the two studies that compared natural and constructed wetlands, one addressed frog communities (Hazell et al. 2004) and the other (Pechmann et al. 2001) considered the entire amphibian assemblage. Both studies found differences in amphibian use of constructed and natural wetlands based on wetland hydrology and amphibian life-history traits dependent on hydrology. For example in Australia, Hazell et al. (2007) found that two stream-dwelling frog species were only present in natural wetlands that had a more dynamic flow than constructed wetlands. This difference in amphibian communities and wetland dynamics is further illustrated by the Pechmann et al. (2001) study in which temporary natural wetlands had more salamander species present than permanent constructed wetlands. Despite their differences, all of

these studies have highlighted the difficulty of replicating natural habitats when attempting to mitigate or create habitat for amphibians.

Statement of Research Objectives

There were three primary objectives of this research: 1) to examine whether or not constructed wetlands located in the Daniel Boone National Forest (DBNF) had amphibian communities comparable to natural ephemeral wetlands, 2) to examine amphibian predator-prey relationships within the constructed wetlands, and 3) to determine what wetland characteristics affect species composition. In particular, this study focused on wetland characteristics that may have management implications, including wetland dimensions, wetland depth, canopy closure, aquatic vegetation, water temperature, and water quality. Identification and quantification of specific characteristics that differ between natural and constructed wetlands would be useful for land managers by giving them information to improve current constructed habitats and increases the success of future amphibian enhancement projects.

II. MATERIALS AND METHODS

Study Sites

Wetlands have been constructed by people on the Daniel Boone National Forest (DBNF) for over 50 years, with many constructed since 1988 for the purpose of wildlife habitat enhancement (T. Biebighauser, pers. comm.). The wetlands used as study sites for this project consisted of ridge-top constructed and natural wetlands located within the Cumberland Ranger District of the DBNF in the Western Allegheny Plateau (EPA 2002). Breaking the Ecoregions down further into Level IV, the northern-most sites (Elk Lick and Big Perry) are located within the Knobs-Lower Scioto Dissected Plateau region, while the remaining sites (Jones' Ridge, Elk Lick, High Energy, Bird Bath, Long Ridge, Pond 5, and HAHA) are located in the Northern Forested Plateau Escarpment region (Woods et al. 2002). All of the study wetlands were hydrologically isolated temporary, permanent, or semi-permanent fishless wetlands more than four years old.

During the first field season March–July 2009, the study sites consisted of five sets of two wetland types, natural ephemeral [Elk Lick Natural Small (ELNS), Elk Lick Natural Large (ELNL), Big Perry Natural (BPN), Jones' Ridge Natural (JRN), and High Energy Natural (HEN)] and constructed [Elk Lick Artificial Small (ELAS), Elk Lick Artificial Large (ELAL), Big Perry Artificial (BPA), Jones' Ridge Artificial (JRA)] (Figure B-1*). In 2010 (May–August), the focus of the project was adjusted from species utilizing constructed vs. natural wetlands to whether species were influenced by wetland depth regardless of wetland type (natural or constructed). To address this question, new wetlands were added to the sampling design as follows: four additional constructed

wetlands [HAHA wetland (HAHA), Long Ridge wetland (LR), Wetland 5 (P5), and Bird Bath wetland (BB)] and one additional natural wetland [Big Perry Complex (BPC)] for a total of 6 natural ephemeral (ELNS, ELNL, BPN, JRN, HEN, BPC), 5 shallow constructed (minimum depth < 20cm) (HAHA, P5, BB, JRA, ELAS), and 4 deep constructed wetlands (minimum depth > 20cm) (LR, HEA, BPA, ELAL) (Figure B-2).

Field Data Collection: Amphibians

During the spring and summer of 2009 and 2010, I surveyed each wetland for amphibians in consecutive one-month intervals for a total of four sampling periods. To incorporate as many breeding amphibians as possible, sampling commenced during peak periods of amphibian breeding. Each amphibian wetland survey included a perimeter visual encounter survey, aural survey, aquatic minnow trapping, and dipnetting (Crump and Scott 1994, Scott and Woodward 1994). Visual encounter surveys started upon arrival at the wetland and consisted of walking the perimeter of the wetland while recording adults, juveniles, larvae, and egg masses observed. In addition, I recorded any anuran calls heard while at the site.

I deployed wire minnow traps along the perimeter of the wetland and distributed them evenly among heterogeneous habitat types. Wire traps were replaced by collapsible mesh minnow traps for the 2010 sampling season. Three minnow traps were set for every 10 x 10 m area (length x width) on the first day of sampling. The number of traps for each wetland was adjusted based on the estimated area of the wetland during each sampling round. The traps were set so that the water reached just above the funnel opening, and each trap was tied to a tree or sturdy piece of vegetation to prevent the trap

from being carried off by mammal predators. Prior to use, the collapsible mesh traps were inspected for tears to prevent loss of amphibians. Within 24 hours of being set, I pulled minnow traps from the water column and checked for amphibians. All species contained in the traps were recorded.

Before dipnetting, a compass was used to visually separate the wetland into quadrants following the cardinal directions, north, south, east, and west. In a 10 x 10 m area, 20 dipnet sweeps (split evenly between the four sections) were performed. The number of dipnet sweeps was scaled up or down based on the estimated size of the wetland during each sampling. Dipnet sweeps per wetland ranged from five to 20. Each dipnet sweep included jabbing a D-frame net into the substrate of the wetland and skimming the bottom of the wetland for approximately a meter before pulling the net straight up out of the water. All habitat types (e.g. emergent vegetation, open water, etc.) were sampled evenly.

During sampling, I identified amphibians to the species level whenever possible. In a few instances (< 5), specimens were collected or multiple macro pictures were taken of amphibians that could not be identified to species. The specimens were analyzed later using a microscope to magnify tooth row morphology. The pictures were analyzed for tooth row and body morphology. Positive identifications were made.

Field Data Collection: Diet Analysis

To assess possible predatory relationships of *R. catesbeiana* and *R. clamitans* (green frogs) on the other amphibian species, I collected stomach contents of these species during the 2010 sampling season. I captured adults for stomach content

processing during the trapping protocol described above. Stomach contents were collected using a non-lethal method, in which I inserted plastic tubing attached to a syringe down the animal's esophagus (Cecala et al. 2007). After the tube was in place, water from the syringe was forced into the frog's stomach and the stomach contents were extracted by flushing. The stomach contents were then strained through a coffee filter. Each coffee filter containing all of the stomach contents was then inserted into a sample tube containing 70% ethanol. Frogs were handled for approximately ten minutes and then released. The sample tubes were then taken back to the lab, placed under a microscope and sorted for amphibian remains only. Invertebrate prey items were not identified. Because amphibian tissue breaks down quickly once in contact with stomach acids, only amphibian tissues and bones that were identifiable and not degraded were included in the analysis.

Field Data Collection: Physical Wetland Characteristics

To understand which factors within natural and constructed wetlands potentially affect amphibian community composition, the following variables were measured at each wetland: percent aquatic vegetation, water quality, depth at 1 meter from shoreline, maximum water depth, minimum water depth, surface temperature at 1 meter from shoreline, surface temperature at maximum depth, and canopy closure.

Aquatic vegetation was systematically surveyed. A compass was used to obtain four azimuths (0° , 90° , 180° , and 270°) from the geometric center of the wetland. A 1 x 1m plot was placed on the edge of the wetland (at each point corresponding to the azimuths) and extended into the wetland 1 meter. In each plot, I recorded percent

vegetation cover in each of 4 categories: emergent, submergent, floating, and open water/none present.

I also collected data on water quality, water depth at 1 meter from shoreline, and percent canopy closure. Conductivity (μmhos), pH, dissolved oxygen (mg/l), surface temperature ($^{\circ}\text{C}$) and water depth measurements (cm) were taken 1 meter out from the wetland edge in each of the cardinal directions with a YSI 556 (Yellow Springs Instruments; Yellow Springs, OH) multi-parameter water quality meter. I recorded maximum water depth during each site visit. Temperature dataloggers (HOBO) (Onset Computer Corporation; Buzzards Bay, MA) were embedded into a 15 x 15 x 2.5 cm styrofoam float and were deployed at the maximum depth location in each of the fifteen wetlands. Surface water temperature readings were then recorded in one-hour intervals. Percent canopy closure was estimated at maximum leaf out with a spherical densiometer at each of the cardinal directions along the perimeter and one point directly above the geometric center of each wetland.

Statistical Analyses

To understand pattern of amphibian communities present within the sampled wetland types, 2009 and 2010 presence-absence data from a comprehensive species list (trap, dipnet, and visual encounter data) were entered into Quantitative Analysis in Ecology (QUANTAN) to obtain measures of similarity, including Jaccard's and Sorensen's coefficients. Because of the change in the overall research question and addition of new study sites during the second year, the two sampling years were analyzed separately.

Individual species abundances were analyzed separately for 2009 and 2010 trapping and dipnetting data. To understand individual species abundances across wetland types, I performed a factor reduction analysis on wetland physical characteristics using principal components analysis. These reduced factors and wetland type were used as predictor variables in regression models in Statistical Package for the Social Sciences (SPSS Inc. Chicago, Illinois). Because these are count data converted to catch per unit effort (CPUE) a compound Poisson (Tweedie) distribution model with a log link function was used (Shono 2008, Shulse et al. 2010). Due to the absence of certain species within wetland types (e.g. *R. catesbeiana* was not observed in natural wetlands for 2009), *Notophthalmus viridescens* (eastern newts) was the only species with sufficient trap data across wetland types to run the analysis in both 2009 and 2010.

An analysis of similarity (ANOSIM), which is a distance-measure analysis of communities with respect to abundances, was run in Paleontological Statistics Software Package for Education and Data Analysis (Hammer et al. 2001) utilizing the 2010 amphibian abundance (CPUE) dipnetting data. I used dipnetting CPUE data because the dataset included more species and was therefore more complete than trapping CPUE data. ANOSIM was performed using Bray-Curtis distance index with 10,000 permutations. To compare amphibian communities across wetland types (natural, shallow constructed, and deep constructed), Bonferroni-corrected pairwise comparisons were performed.

Physical wetland variables were analyzed in SPSS for 2009 and 2010. Prior to analyses, I excluded temperature at 1m data for both years due to the temporal

differences associated with the collection of these data. Conductivity was also excluded from analyses for 2009 due to lack of proper instrumentation early in the season when all wetlands could be sampled. The temperature at maximum depth for one wetland, BPN, was not used in the analysis because the temperature datalogger for this wetland was detached from the wooden stake and lost. For 2010, the temperature at maximum depth measurement was not analyzed. The dataloggers during this year consistently flipped upside-down, exposing the dataloggers to air temperatures rather than water temperatures.

The 2009 wetland characteristic data were analyzed using a one-tailed independent samples t-test for those factors in which variances were equal and a priori predictions were formed including: maximum wetland depth, percent canopy closure, depth at 1 meter from shoreline, and temperature at maximum depth at noon and midnight. For those data for which no a priori predictions were determined (pH and wetland size), a two-tailed t-test was used. A Welch's t-test was used for the two variables with unequal variances, percent emergent vegetation (a priori prediction determined) and dissolved oxygen.

A one-way ANOVA with post-hoc multiple comparisons was used to analyze the 2010 variables with equal variances including: maximum depth, emergent vegetation, depth at 1 meter from shoreline, dissolved oxygen, pH, and wetland size. Two variables, canopy closure and conductivity, did not have equal variances and therefore a Welch's t-test was utilized to analyze them. To further consider the results of the ANOVA, a Tukey

multiple comparison procedure was used to compare the variables between natural and constructed wetland types (natural, shallow constructed, and deep constructed).

III. RESULTS

Amphibian Communities

Of the 16 pond-breeding amphibian species present within the sampling area, 14 were detected during the 2009 and 2010 sampling seasons [Exceptions: *R. sphenoccephala* (southern leopard frogs) and *Acris crepitans* (northern cricket frogs)] (Table A-1*, A-2). When amphibian occurrence at each wetland type for 2009 was examined, the most common amphibian occurring in all natural wetlands was *Ambystoma maculatum* (spotted salamanders). Two other species frequently observed in natural wetlands, *R. sylvatica* (wood frogs) and *Hemidactylium scutatum* (four-toed salamanders), were found in four of the five natural wetlands studied. The constructed wetlands had more species with higher occurrence across all five sites. Five species (*Hyla chrysoscelis* (Cope's gray treefrog), *Pseudacris crucifer* (spring peeper), *R. clamitans*, *N. viridescens* and *A. maculatum*) occurred at all five constructed wetlands. The other species of high occurrence, found in four of five constructed wetlands, were *R. catesbeiana* and *R. sylvatica* (Table A-3).

In 2009, constructed wetlands had higher species richness than natural wetlands (13 species compared to 12); however species composition varied among wetlands. Natural and constructed wetland communities had high similarity (Jaccard's coefficient 0.79, Sorensen's coefficient 0.88). Of the three species that were unique to one wetland type over the other, one species, *Scaphiopus holbrookii* (eastern spadefoot toad), was found only in natural wetlands, whereas *R. catesbeiana* and *R. palustris* (pickerel frogs) were only found in constructed wetlands. In addition, two constructed wetlands (ELAS

and JRA) were recorded to have a shallower minimum depth < 20 cm and more similar amphibian communities to the natural wetlands than the other three constructed wetlands surveyed. ELAS was characterized by an absence of *R. catesbeiana* and a high abundance of *R. sylvatica*; while JRA had the highest species richness of all of the constructed wetlands with 11 species observed. When considering species abundances (CPUE) based on wetland type in 2009, the three most abundant species in natural wetlands were *R. sylvatica*, *S. holbrookii*, and *Bufo* spp. (American/Fowler's toad); while the top three species for constructed wetlands were *N. viridescens*, *R. clamitans*, and *R. catesbeiana* (Figure B-3). Constructed wetlands had significantly more *N. viridescens* than natural wetlands (Wald's $\chi^2 = 13.669$, $df = 1$, $p < 0.001$) (Table A-4).

In 2010, certain species were more often present in constructed wetlands, regardless of depth type: *N. viridescens*, *R. catesbeiana*, *P. crucifer*, *A. maculatum*, *A. jeffersonianum* (Jefferson's salamander), and *R. palustris*. *Rana sylvatica* was more often observed utilizing natural wetlands. Although abundances varied by species, three species were ubiquitous across natural and constructed wetlands: *Hy. chrysozelis*, *R. clamitans*, and *He. scutatum*. The frequency of occurrence at wetland types for each amphibian species differed. Four species were most frequent in natural wetlands, two species (*R. sylvatica* and *R. clamitans*) were observed at five of six natural wetlands and two species (*He. scutatum* and *Hy. chrysozelis*) occurred at four of six natural wetlands. One species was found in all shallow constructed wetlands (*Hy. chrysozelis*); while seven species (*A. jeffersonianum*, *A. maculatum*, *P. crucifer*, *He. scutatum*, *R. catesbeiana*, *R. clamitans*, and *N. viridescens*) were documented in four of the five shallow constructed wetlands.

When considering deep constructed wetlands, four species (*Hy. chrysosecelis*, *He. scutatum*, *R. clamitans*, and *N. viridescens*) were observed in all four of these wetlands, whereas three species (*A. jeffersonianum*, *P. crucifer*, and *R. catesbeiana*) were documented in three of four deep constructed wetlands (Table A-5).

When all survey types (minnow trapping, dipnetting, and visual encounter surveys) were combined, presence of species differed between wetland types (Table A-2). Shallow constructed wetland communities were more similar to natural wetlands compared to deep constructed wetlands. The two constructed wetland types were weakly similar in amphibian community composition (Table A-6). Five species, *S. holbrookii*, *P. brachyphona* (mountain chorus frog), *A. opacum* (marbled salamander), *R. sylvatica*, and *Bufo* spp., were exclusive to natural and shallow constructed wetlands, but not deep constructed wetlands; whereas *R. palustris* was unique to constructed wetlands, regardless of wetland depth (Table A-7).

Individual species abundances, calculated as catch-per-unit-effort (CPUE), varied across wetland types in 2010, as in 2009. Overall, the most abundant species in natural wetlands were, in descending order, *R. sylvatica*, *Hy. chrysosecelis*, *A. maculatum*, and *P. crucifer* (Figure B-4). Again in descending order, the species most abundant in the shallow constructed wetlands were *R. sylvatica*, *N. viridescens*, *Hy. chrysosecelis*, *A. maculatum*, and *Bufo* spp. The dominant species in the deep constructed wetlands consisted of, in descending order, *R. clamitans*, *P. crucifer*, *A. maculatum*, *N. viridescens*, and *Hy. chrysosecelis*. Similarity of amphibian communities was significantly different across wetland types ($p = 0.023$, global $r = 0.291$). Bonferroni-corrected pairwise

comparisons indicated that the amphibian community of natural wetlands was not significantly different than that of shallow constructed wetlands (shallow constructed vs. natural $p = 0.158$); while the amphibian community of deep constructed wetlands was significantly different than that of natural wetlands (deep constructed vs. natural $p = 0.023$). When communities of constructed wetland types were compared, there was no significant difference between them ($p = > 0.99$). Only one of fourteen species, *N. viridescens*, was captured in sufficient numbers in 2010 to conduct analysis of the effects of wetland conditions on abundance. *Notophthalmus viridescens* abundance was significantly predicted by one variable, minimum wetland depth (Wald's $\chi^2 = 9.232$, $df = 2$, $p = 0.010$) (Table A-8). Bonferroni-corrected pairwise comparisons indicated significantly greater *N. viridescens* abundance in shallow constructed wetlands compared to natural ($p = 0.030$).

Interspecies Interactions

During the 2009 sampling season, several instances were noted in which *N. viridescens* adults depredated *R. sylvatica* eggs before hatching. *Rana sylvatica* eggs were observed in 80% of the wetlands (JRN, HEN, BPN, ELNL, JRA, BPA, ELAL, and ELAS) sampled in 2009. Larvae were subsequently observed in 5 of these 8 wetlands, 4 natural (JRN, HEN, BPN, and ELNL) and one constructed (ELAS). ELAS had *R. sylvatica* larvae present, but no *N. viridescens*. The three wetlands (JRA, BPA, and ELAL) where *R. sylvatica* eggs were laid but larvae were not observed were all constructed wetlands and each had *N. viridescens* present. Two of these (JRA and ELAL) had high *N. viridescens* abundances in 2009 (Figure B-5). In 2010, sampling began in

May at least one month after the commencement of *R. sylvatica* egg deposition; therefore *R. sylvatica* egg deposition data were absent for 2010. However, *R. sylvatica* larval presence was noted for that sampling season, and in five (ELAS, HEN, JRN, ELNS and ELNL) of the six wetlands in which *R. sylvatica* larvae were present *N. viridescens* was not observed. Big Perry Complex wetland had one *N. viridescens* capture and was the only wetland during 2010 to have both species present (Figure B-6). Four wetlands (ELAS, HEN, JRN, and ELNL) had *R. sylvatica* larvae present during both years of the study.

Diet Analysis

Twenty-four ranid frogs (13 *R. clamitans* and 11 *R. catesbeiana*) from six different wetlands (HAHA, P5, HEA, JRA, BPA, BPN) were captured in minnow traps in 2010. Of the 13 individual *R. clamitans*, eight stomach content specimens were collected. No amphibian, conspecific or interspecific, tissue or bones were found within the eight *R. clamitans* stomachs. Within the remaining five individuals for which stomach contents were not obtained, two produced no stomach contents when pumped, two had external wounds and were released without being processed, and one was a gravid female that was also released. Of the 11 individual *R. catesbeiana* captured in minnow traps, 9 stomach content specimens were collected. Two of the samples had amphibian tissue or bones. One specimen had an ambystomatid salamander larva (*A. jeffersonianum* or *A. maculatum*) and unidentified adult amphibian bones, and the second had the foot of a ranid (*R. clamitans* or *R. catesbeiana*). The two remaining individuals of the 11 total

captures were processed but did not produce contents and were assumed to have empty stomachs.

Rare Species

In 2009, three species (*A. opacum*, *P. brachyphona*, and *S. holbrookii*) were rare across natural and constructed wetland types. *Ambystoma opacum* was present in three natural wetlands and one constructed wetland. *Pseudacris brachyphona* was observed in three natural wetlands and two constructed wetlands. Further, *S. holbrookii* was found in one natural wetland: a new county record for Rowan County, KY (Table A-3). In 2010, all three of these rare species were again documented at one natural and one shallow constructed wetland (Table A-4). Further detailed information on specific wetlands and sightings for these rare species can be found in Appendix C.

Physical Wetland Characteristics

Three wetland characteristics (canopy closure, wetland depth at 1 meter from shoreline, and pH) were found to be statistically different among wetland types during both years of the study. Four variables were only found to be significant during one year of the study, dissolved oxygen and temperature at midnight for 2009 and maximum depth and emergent vegetation for 2010. Five variables total were considered significant during 2009, four of which were higher for constructed wetlands including: wetland depth at 1 meter from shoreline ($t = -2.691$, $df = 7$, $p = 0.0155$), dissolved oxygen ($t = -5.201$, $df = 5.063$, $p = 0.003$), temperature at midnight ($t = -6.363$, $df = 7$, $p < 0.001$), and pH ($t = -4.952$, $df = 8$, $p < 0.001$) while percent canopy closure was statistically lower in

constructed wetlands ($t = 3.227$, $df = 8$, $p = 0.006$) (Table A-9, Figure B-7). Although not statistically significant in 2009, aquatic vegetation appeared to be more abundant in the constructed open-canopy wetlands sampled ($t = -1.973$, $df = 4.501$, $p = 0.056$). Likewise, five characteristics were found to be significant for 2010: maximum wetland depth ($F = 6.955$, $df = 2$, $p = 0.010$), percent emergent vegetation ($F = 4.988$, $df = 2$, $p = 0.027$), wetland depth at 1 meter from shoreline ($F = 8.277$, $df = 2$, $p = 0.006$), pH ($F = 19.169$, $df = 2$, $p < 0.001$), and percent canopy closure ($t = 6.672$, $df = 2$, $p = 0.043$) (Table A-10, Figure B-8). When assessing the results of the post-hoc pairwise comparison test, three natural wetland variables were lower when compared to deep constructed wetlands: maximum depth ($q = 3.35$, $df = 2$, $p = 0.015$), percent emergent vegetation ($q = -3.15$, $df = 2$, $p = 0.021$), and wetland depth at 1 meter from shoreline ($q = -4.04$, $df = 2$, $p = 0.004$), while pH was higher in deep constructed wetlands ($q = -3.38$, $df = 2$, $p = 0.014$). Natural wetlands differed from shallow constructed wetlands in one characteristic only, pH. They had significantly lower pH when compared with shallow constructed wetlands ($q = -6.13$, $df = 2$, $p < 0.001$). When comparing the constructed wetland types, shallow and deep, maximum wetland depth was higher for the deep constructed wetlands ($q = 3.27$, $df = 2$, $p = 0.017$) (Table A-11).

IV. DISCUSSION

The main objective of this research was to investigate the effectiveness of constructed wetlands for preserving and enhancing amphibian communities within the Appalachian ridge-top wetland ecosystem in eastern Kentucky. By comparing amphibian communities in natural wetlands to constructed wetlands, I found constructed wetlands do not replicate natural pond-breeding amphibian habitat. In fact, it appears that constructed wetlands might be detrimental to natural amphibian communities because these wetlands created suitable source habitat for populations of dominant amphibian predators that would otherwise be absent from the ridge-top ecosystem. In addition, two wetland physical characteristics, wetland drying cycle and canopy closure, were influential in shaping amphibian communities. When constructing wetlands on ridge tops in this region of DBNF, land managers should attempt to replicate the natural wetland communities present in the landscape by creating ponds with an annual drying cycle and a closed canopy.

Amphibian Communities

Two previous US Forest Service studies examined amphibian use of constructed wetlands within Daniel Boone National Forest (D. Dourson unpublished report and M. Toncray unpublished report). Dourson's study included 29 newly constructed wetlands (2 years old) in the Cumberland Plateau region of Powell County near the southern extent of DBNF; while Toncray's study included eight constructed wetlands (1–11 years old) in Menifee and Rowan Counties near the northern extent of DBNF and the field sites for

this study. While these studies are valuable for providing presence/absence data, they do not provide a complete dataset because they lack estimates of abundance and natural wetland comparisons. In addition, both studies included some constructed vernal/autumnal wetlands (Toncray n = 4, Dourson n = 7), but neither specifically grouped them into treatments. The present study provides the first quantified estimates of how amphibian communities in natural wetlands differ from constructed wetlands in the DBNF. During both years of my study 14 species utilized the wetlands sampled. Both Toncray and Dourson documented fewer species (11 and 9 species, respectively). There were three species not recorded during either of the previous studies, *A. opacum*, *He. scutatum*, and *S. holbrookii*. Two of these species (*A. opacum* and *S. holbrookii*) were not common and considered rare in my study. The third species (*He. scutatum*) nests on the periphery of wetlands and accurate estimation of abundance requires a different sampling approach, which may explain the lack of records for this species in these two studies. In addition, Dourson detected two fewer species (*A. jeffersonianum* and *R. catesbeiana*) than Toncray. He attributed the absence of these two species to the young age (1–2 years) of the wetlands he studied. Even though these earlier studies did not record all of the same species as observed in the present study, their findings corroborate my findings for common species found at constructed wetlands in 2009 with the exception of *P. brachyphona*, *P. crucifer*, and *R. catesbeiana*. Toncray's most common species were *Hy. chrysozelis*, *R. clamitans*, and *N. viridescens*, and Dourson's were *R. sylvatica* (based on egg masses), *A. maculatum*, *R. clamitans*, *Hy. chrysozelis*, and *P. brachyphona*. During 2009, in my study wetlands, two additional *P.* species were found to be common (*P.*

crucifer and *R. catesbeiana*) while *P. brachyphona* was rare. Toncray only observed *P. brachyphona* at two of the eight wetlands she studied. One possible reason for this agreement might be that Toncray's study sites were in closer proximity to my study sites, which may indicate locally rather than regionally low *P. brachyphona* abundance. *A. jeffersonianum* and *He. scutatum* were absent from the common species list of the Toncray and Dourson studies but were present in the list of common species observed at my constructed sites. It is likely that these species might have been present and overlooked because *He. scutatum* requires different sampling protocol than used in these studies and *A. jeffersonianum* larval identification can be difficult (J. MacGregor pers. comm.).

The previous published reports for DBNF did not use natural reference wetlands, which are critical for determining the success of constructed wetland habitats. For my study, natural wetlands were included as reference sites to assess the functionality of the constructed wetlands studied. The first year of my project was important for obtaining baseline data for the system. Several key points of information on amphibian communities, wetland types, and wetland characteristics were gathered during 2009.

The similarity measures for 2009 implied amphibian community similarity between constructed and natural wetlands; however, upon close inspection, this appears to be driven by two of the shallow constructed wetlands (ELAS and JRA) having amphibian communities more similar to natural wetlands than the three deeper constructed wetlands. ELAS and JRA had drying properties similar to the natural

wetlands that were studied. ELAS dried completely (albeit temporarily), while JRA was reduced to a depth of <20 cm.

Presence of dominant amphibian predators within a wetland can affect community structure. The absence of *N. viridescens* and *R. catesbeiana*, top amphibian predators, in ELAS most likely allowed for a high abundance of *R. sylvatica* within this small constructed wetland. *N. viridescens* are carnivorous and were observed on several occasions during this study consuming young embryos from inside *R. sylvatica* egg masses. This behavior has been well documented (Hamilton 1932, Anderson et al. 1971, Walters 1975). Further, Werner et al. (1995) corroborated earlier studies by Stewart and Sandison (1972) and McAlpine and Dilworth (1989), which found that *R. catesbeiana* consumed aquatic prey including juvenile frogs more often and in greater quantities than *R. clamitans*. This pattern was supported by the bullfrog diet analysis I performed in which two individual *R. catesbeiana* had consumed other amphibian species. This suggests that *N. viridescens* and *R. catesbeiana* are potential threats to other amphibian species. Wetland depth rather than absence of a predator species may have accounted for the high species richness found at JRA. The intermediate wetland depth at JRA likely provided suitable breeding habitat for species that typically breed in semi-permanent or permanent wetlands, encompassing most species present in the ridge-top ecosystem.

Key information about natural wetlands can be gathered by considering the most abundant species present within the natural wetlands surveyed. These species appear to have adaptations for breeding in these temporary habitats. For example, *R. sylvatica* are apparently adapted to these habitats in that they arrive early to breed at the wetlands in

February and March (Redmer and Trauth 2005). In contrast, *S. holbrookii* and *Bufo* spp. breed later but have short larval periods, which range from 14 to 60 days, to cope with the ephemeral environment (Palis 2005, Green 2005a, Green 2005b). These traits allow these species to flourish within these less predictable habitats. This irregular flux in hydrology of the wetlands reduces the amount of vertebrate and invertebrate predators for these specialized species (Wellborn et al. 1996).

Permanent wetlands, like natural wetlands, have a particular set of species that thrive in the habitat they provide. The three most abundant species (*R. catesbeiana*, *R. clamitans*, and *N. viridescens*) in the permanent constructed wetlands during 2009 can be considered prominent amphibian predators. These species are opportunistic foragers and regularly depredate other amphibian species living within their habitats (Werner et al. 1995). These predators were rare or absent in the ephemeral wetlands studied most likely due to the length of their larval periods of 3 months to 3 years (*R. clamitans* and *R. catesbeiana*) (Casper and Hendricks 2005, Pauley and Lannoo 2005) or, in the case of *N. viridescens*, an adult aquatic life-history stage (Hunsinger and Lannoo 2005), both of which utilize semi-permanent to permanent water.

Of the three species, *S. holbrookii*, *R. catesbeiana*, and *R. palustris*, that were unique to one wetland type over the other in 2009, *S. holbrookii* was found in only one natural wetland (BPN). *Scaphiopus holbrookii* utilizes temporary habitats and is a well-known explosive breeder. Its sporadic breeding events, short larval period (14–60 days), and secretive behavior in its terrestrial environment make it a difficult species to document (Palis 2005). Due to these life-history characteristics, *S. holbrookii* presence at

only one of the wetlands surveyed was not unexpected. The other two exclusive species (*R. catesbeiana* and *R. palustris*) were observed utilizing constructed wetland habitats only. Both have traits more suited for reproduction in semi-permanent or permanent water bodies (Redmer 2005). One adult *R. palustris* was documented utilizing just a single wetland (ELAL). Although the lack of sightings of this species was not predicted, *R. palustris* is secretive and requires specialized sampling protocol to detect them in Kentucky (J. MacGregor, pers. comm.).

After the initial year of this project several patterns became apparent and the focus of the project narrowed. Wetland depth seemed to be a key characteristic for shaping the amphibian communities observed in 2009. In previous studies, hydroperiod gradients have been linked to amphibian community composition and species richness (Wellborn et al. 1996, Snodgrass et al. 2000a, 2000b). Snodgrass and his colleagues (2000a) summarize the general models of community structure that are predicted in lentic systems as follows: “1) a unimodal pattern of species richness with a peak in wetlands with intermediate hydroperiods, 2) reduced species richness in longer hydroperiod wetlands will be correlated with the presence of large predators, and 3) trade-offs in life-history characteristics that maximize fitness along the hydroperiod gradient will produce breaks along the gradient in community structure.” These patterns were generally supported for the wetlands studied in 2010 in eastern Kentucky, with two exceptions. First, on an individual wetland basis, the first pattern held true; however, when the wetlands were grouped according to wetland-depth type, the pattern disappeared. Second,

fish were the primary predator associated with the second model, whereas in this study fish were absent and dominant amphibians were the primary predator species.

When wetlands of different depth types were examined in 2010, species richness varied. Natural wetlands and shallow constructed wetlands both had 13 species utilizing them, whereas the deep constructed wetlands only had nine species. Several species were more common in the constructed wetlands, regardless of construction depth type. These species span a range of different breeding strategies and life-history traits. *Pseudacris crucifer* and *R. palustris* do not have long larval periods like *N. viridescens* and *R. catesbeiana*, but they prefer areas with large amounts of emergent fringe vegetation (Butterfield et al. 2005, Redmer 2005). In the present study, these species were captured more often in constructed wetlands, which had more emergent fringe vegetation than natural wetlands. Ambystomatid salamanders can capitalize on use of permanent wetland habitats by extending their larval periods and overwintering (Cortwright 1988, Phillips 1992). This might explain higher frequency of *A. jeffersonianum* and *A. maculatum* in constructed wetlands that do not seasonally dry.

The 2010 amphibian presence/absence data suggest that shallow constructed wetlands have a similar community to natural wetlands; however, abundance and breeding success differed between these wetland types. Of the five species that occurred in both natural and shallow constructed wetlands, *Bufo* spp. were the only species to have a higher abundance in the shallow constructed wetlands than the natural wetlands. The ANOSIM further supports this trend. Deep constructed wetland communities were

substantially different from natural wetlands; whereas shallow constructed wetland communities were weakly similar to natural wetlands.

Interspecies Interactions

Dominant predator species can negatively affect prey populations (Anderson et al. 1971, Werner et al. 1995). Opportunistic feeding habits of *N. viridescens* might be negatively influencing *R. sylvatica* local breeding success in constructed wetlands. Because *N. viridescens* usually overwinter and remain active in deep permanent water bodies (Pitkin and Tilley 1982), permanent constructed wetland habitats would allow for *N. viridescens* overwintering. *Rana sylvatica* is one of the earliest species arriving at wetlands in late winter/early spring (February or March); therefore their eggs are likely one of the first major food sources for overwintering *N. viridescens*. In addition, high *N. viridescens* abundance observed in constructed wetlands could potentially increase interspecific competition for aquatic prey items, thus amplifying predation on available prey items such as *R. sylvatica* eggs. This predatory interaction was not observed in natural wetlands and seems less likely to occur in temporary habitats where *N. viridescens* are less abundant and tend to move into terrestrial habitat to overwinter (Massey 1990). However, it is important to note that other factors might be limiting *R. sylvatica* persistence within these wetlands. These other factors may include predation by other species, disease, and UV-B radiation damage to embryos (Bradford 2005). Given my data, predation by other species and UV-B radiation are more likely to be the alternate causes of *R. sylvatica* embryo decimation at these sites rather than disease

because no indications of disease (lesions, edema, or obvious mass tadpole mortality) were noted for wood frogs.

Rare Species

Certain rare species, within the suite of ephemeral pond-breeding specialists (*A. opacum*, *P. brachyphona*, and *S. holbrookii*), require breeding wetlands to dry at least semi-annually (Scott 2005, Mitchell and Pauley 2005, Palis 2005). The lack of *A. opacum* presence within most of the constructed wetlands studied is possibly attributable to their specialized breeding strategy (Scott 2005). Although, *A. opacum* were observed at one constructed wetland each year, most of the constructed wetlands studied did not dry seasonally, and therefore they lacked the temporary habitat necessary for successful marbled salamander breeding. In Kentucky, *P. brachyphona* have a short larval period and breed in various types of temporary pools (Mitchell and Pauley 2005). Even though they are typically documented utilizing temporary water bodies, during this study they were found in both constructed permanent wetlands and temporary natural wetlands. As mentioned earlier, *S. holbrookii* are difficult to detect and therefore may seem rare. Earlier accounts have placed them in seven counties in the north east section of Kentucky. There are records from Greenup (2 sites), Lawrence (1 site), Johnson (1 site), Floyd (1 site), Magoffin (2 sites), Wolfe (1 site), and Powell (2 sites) counties (J. MacGregor, pers. comm.). Fortunately, three additional sites were identified in Rowan County during this study suggesting that further research within this region may produce more records if sampled thoroughly.

Physical Wetland Characteristics

Qualitatively, natural and constructed wetlands in this study appeared very different, and measurements of wetland characteristics supported these differences. Canopy closure was higher at natural sites; while dissolved oxygen, temperature at midnight, maximum depth, emergent vegetation, depth at 1 meter from shoreline, and pH were lower in natural wetlands.

Canopy closure was lower at the constructed sites due to the relicts of wetland construction. In order to construct the wetlands, trees are often removed and compaction of the soils by heavy equipment limits tree colonization. In addition, several surrounding trees are removed, reducing existing canopy closure. Certain species may benefit from open-canopy wetlands, such as *R. sevosia*, dusky gopher frogs (Thurgate and Pechmann 2007). However, natural pond-breeding species on ridge tops in eastern Kentucky are adapted to and obligates of closed-canopy wetlands. In light of this, open-canopy constructed wetlands might have detrimental sublethal and lethal effects on these species. One such possibility is the increase in UV-B radiation on the wetland surface caused by an open canopy. UV-B radiation has been implicated in causing amphibian embryo deformities (Blaustein et al. 1997). In addition to UV-B radiation, canopy closure influences several other physical and biological processes within the wetland environment. The absence of substantial canopy closure can also increase dissolved oxygen and water temperature, which in this study were both higher in these wetlands (Schiesari 2006). In addition to these characteristics, low canopy closure can decrease the hydroperiod by increasing evaporation due to solar radiation.

Dissolved oxygen content in the water column is an important characteristic for amphibian larvae, which breathe using gills. The pattern of low dissolved oxygen levels in natural wetlands is similar to two other studies that found dissolved oxygen to be two (Skelley et al. 2002) and three times (Schiesari 2006) higher in open-canopy wetlands. High dissolved oxygen may not be as important as some other wetland characteristics for larval growth. In laboratory experiments, Schiesari (2006) found that growth rates were higher for tadpoles exposed to higher temperatures and food quality but this increase was not observed for higher dissolved oxygen levels. However, anoxic aquatic conditions have been associated with behavioral modifications of amphibian larvae, affecting predator-prey relationships (McIntyre and McCollum 2000). One possible reason for this increase in dissolved oxygen at the open-canopy constructed wetlands is the abundance of aquatic algae and emergent vegetation at these sites. It is important to note, however, that the dissolved oxygen measurements for this project were taken at the surface of the wetlands sampled. Because some of the constructed wetlands studied were deeper than natural wetlands, it is probable that an anoxic region is present at the bottom of the permanent constructed wetlands, presumably due to low light levels, leaf-litter input, and decaying plant materials. Natural wetlands tend to have less oxygen-depleting detritus on the wetland bottom because they dry annually (Colburn 2004).

Because amphibians are ectothermic, temperature of aquatic habitat is a key factor in their growth. Temperature of the constructed wetlands at midnight was observed to be higher than the natural wetlands. This finding is most likely due to the open-canopy feature of the constructed wetlands, which increases solar radiation reaching the water

surface. Thurgate and Pechmann (2007) found photosynthetically active radiation to be 57% higher in open-canopy wetlands when compared to closed-canopy wetlands, and Schiesari (2006) found that open canopies can increase wetland temperatures by 2.5 °C. Additionally, temperature of a larger, deeper body of water will hold heat longer than natural wetlands of fluctuating, shallow depths.

Higher water temperatures have been shown to double growth rates of amphibian larvae (Harkey and Semlitsch 1988, Thurgate and Pechmann 2007, Schiesari 2006). Therefore, in the constructed wetlands studied, it is possible that amphibian larvae are reaching metamorphosis at an earlier date and are larger at metamorphosis allowing for fitness advantages for species with individuals that survive to metamorphosis (Smith 1987, Semlitsch et al. 1988, Scott 1994, Boone 2005). This positive trend, however, is not indicative of all amphibian species. Some species, such as bufonids, spend limited time in aquatic environments and consequently reach metamorphosis at a smaller size. *Bufo* metamorphs may overcome this size difference before overwintering (Boone 2005). Therefore, even though higher water temperatures within constructed wetlands might be positively influencing some amphibian larval growth rates, it is important to note that these differences seem to be species specific.

Several studies have highlighted the importance of pH in amphibian development (Freda and Dunson 1986, Bunnell and Zampella 1999, Rowe et al. 1992, McCoy and Harris 2003, Grant and Licht 1993, Pierce et al. 1984). During both years of this project, pH was found to be significantly higher in constructed wetlands, similar to studies in the Pinelands of New Jersey (Freda and Dunson 1986 and Bunnell and Zampella 1999).

Natural vernal wetlands in the northeastern United States tend to have low pH. Some of the reasons for this include the presence of tannins (a complex organic acid released during the decomposition process of vegetation), acidic soils, and geology (Colburn 2004). The natural wetlands observed in this study had a tea colored appearance, typical of waters with high tannins. Even though the wetlands studied have naturally low pH (4.8–5.5), low pH can have detrimental lethal and sublethal effects on some amphibian embryos and larvae with prolonged exposure (Rowe et al. 1992). In the Freda and Dunson study, Low pH (< 4.31) decreased transplanted embryo survival in Fowler's toads. Pough and Wilson (1977) suggested a pH of 5 and 6 stressed *A. maculatum* embryos and larvae leading to sublethal effects. McCoy and Harris (2003) attempted to measure these sublethal effects by looking at size based fitness correlates for *A. maculatum* larvae. They found that growth during pH treatments was dependent upon the individual wetland the egg masses were derived from. Two species, *R. sylvatica* and *R. catesbeiana* have a high tolerance to lower pH levels. The critical pH limit, at which 50% of the larvae die, for *R. catesbeiana* is 4.0–4.5 for embryos and 4.0 for larvae, while the critical pH level for *R. sylvatica* is lower at 3.75 (Grant and Licht 1993, Pierce et al. 1984). Therefore, responses to pH conditions may vary within and between species at different localities. In my study, natural wetland pH measurements were above these critical limits; however, as Pough and Wilson (1977) suggest some species may still encounter sublethal effects at my observed pH levels.

Shallow littoral zones have been associated with amphibian species richness (Porej and Hertherington 2005). Littoral zone (measured here as wetland depth at 1 meter

from shoreline) was shallower in natural wetlands and shallow constructed wetlands while deep constructed wetlands had deeper littoral zones. Porej and Hetherington (2005), found a positive relationship between shallow littoral zones and a number of species including: *B. americanus*, *P. triseriata* (western chorus frogs), *R. pipiens* (northern leopard frogs), *Hy. versicolor* (gray treefrogs), and *A. texanum* (smallmouth salamanders). The 2010 species richness data from this project corroborates Porej and Hetherington's (2005) findings of higher species richness at wetlands with shallow littoral zones. The occurrence of shallower depths at 1 m may provide basking habitat for developing tadpoles and predator avoidance habitat, specifically for interspecific predator-prey interactions (Porej and Hetherington 2005). Further, littoral zone depth can influence the emergent plant species able to colonize this zone. The emergent vegetation most often associated with deep constructed wetlands was cattails, whereas the emergent vegetation found most often at the natural wetlands were sedges.

The impacts of differences in the above mentioned wetland characteristics, in most cases, are not immediately apparent. Differential response to a decrease in dissolved oxygen or an increase in temperature may lead to changes in species interactions resulting in reduction of individual fitness (McIntyre and McCollum 2000). These sublethal effects may ultimately lead to a decrease in amphibian persistence within a wetland ecosystem with no obvious differences in survival of individual amphibians (Werner and McPeck 1994). Therefore, when intending to replicate natural amphibian habitats, care must be taken to monitor and resolve these differences in water quality.

Management Implications

Historically, the geology in this region of Kentucky likely only supported natural ephemeral or semi-permanent wetlands on ridge tops (J. MacGregor pers. comm.). While historical densities of natural wetlands are difficult to ascertain given the lack of records, it is probable that natural wetlands were not present in high densities across the ridge tops studied. Management of these unique ridge-top ecosystems should reflect historical amphibian habitats and wetland densities. As implied through this research, the construction of multiple permanent wetlands on these ridge tops has led to a shift in the amphibian community composition. To mitigate this shift, future constructed wetlands should mimic natural wetland characteristics. In addition, wetlands should be placed at densities similar to the apparently low historical densities, rather than in large numbers across a ridge top. Further, deep, permanent wetlands already constructed should be filled or renovated to reflect natural functioning wetland characteristics and densities.

The most important physical characteristic identified in this study was the natural wetland drying cycle. All natural wetlands studied were short-cycle, spring filling pools or short-cycle, fall filling pools. This periodic drying allows for the exclusion of dominant unnatural amphibian predators in these sensitive habitats. While decreased wetland depth did enhance the amphibian community richness in the shallow constructed wetlands, abundances of the natural pond-breeding species were limited in these habitats. These shallow constructed wetland habitats support unnatural predatory amphibian species in greater abundances, which might be the limiting factor for many of these rare species. Although the rare species were present, populations in these wetlands might be

sinks. The second most influential wetland characteristic highlighted by this study was canopy closure. Canopy closure can affect many different wetland biological and chemical processes. The species that are historically found in this region of Kentucky breed in closed-canopy wetlands. Therefore, managers should limit tree mortality and soil compaction surrounding the newly constructed wetlands to encourage a dense canopy closure. A closed canopy would likely decrease the type of emergent vegetation observed at the constructed wetlands (cattails) and allow for more natural emergent (sedges and rushes) to colonize successfully.

Recently, due in part to the knowledge gained from this research, changes have been implemented in the design of newly constructed wetlands within Daniel Boone National Forest. These changes reflect a paradigm shift from the purpose of constructed wetland habitats for game species to use by sensitive and non-game species. Newly constructed wetlands reflect modifications including smaller diameter wetlands with shallow maximum/minimum depths and littoral zones. In addition, some wetlands constructed with old design characteristics (deep wetlands with steep littoral zones) have been renovated to reflect a more natural wetland design. A close relationship with the US Forest Service, specifically Tom Biebighauser, has allowed for continual improvement in amphibian habitat in Daniel Boone National Forest. Members of the Molecular Ecology and Conservation of Amphibians Laboratory are continuing research related to these improvements, and we hope to see continued progress in wetland design, restructuring, and densities over the coming years.

Future Research Opportunities

Because this research gathered baseline amphibian data on the use of these constructed habitats, there are threads of research yet to be investigated. There are several specialized or rare species for which predator-prey interactions or general questions of survival were generated, including *R. sylvatica*, *He. scutatum*, *P. brachyphona*, and *A. opacum*. In addition, questions on the unnatural prevalence or densities of *R. catesbeiana*, *R. clamitans*, and *N. viridescens* and their influence on these natural wetland communities have arisen.

One of the most interesting and necessary directions yet to be examined fully is the *N. viridescens* and *R. sylvatica* predator-prey interaction. This relationship needs to be studied in order to determine the extent to which individual *R. sylvatica* survival and subsequent population numbers are impacted by *N. viridescens* in these permanent constructed wetland habitats. Additionally, *He. scutatum* egg clutches were observed at a majority of the sites. Survival to metamorphosis for this species is poorly known at these sites and needs additional research. This research is currently being undertaken by an Eastern Kentucky University graduate student, Susan King. *Pseudacris brachyphona* was at a minority of the sites studied. Little research has been conducted on this species. Additional research into the status of this species in this area of Daniel Boone National Forest would help to close gaps in the knowledge of the life history of this species and help identify habitat characteristics for future management of this species. *Ambystoma opacum* was mostly found at natural wetlands. This species has a specialized life history in which females deposit eggs in dry wetland basins and typically guard them until the

wetland fills (Scott 2005). Therefore, studies investigating the extent to which they are breeding within constructed wetlands, microhabitat use of constructed and natural wetlands, and their survival rates in these environments are necessary. *Rana catesbeiana* prevalence became a major threat within the constructed habitats sampled. These predatory frogs were found in almost all of the constructed wetlands surveyed; conversely they were only found in a minority of the natural wetlands studied. The diet samples collected during this study indicate that bullfrogs are depredating other amphibian species. Further, disease transmission by *R. catesbeiana* involving between-wetland movements is a possible avenue of research. To reduce *R. catesbeiana* prevalence in these ridge-top ecosystems, methods for concentrated localized eradication in sensitive habitat areas (e.g. deep permanent constructed wetlands close to existing natural wetlands) should be investigated.

Wetland hydrology, in terms of depth and duration, was important for shaping amphibian communities. Design features of constructed wetlands other than depth can influence the length of hydroperiod. One such design feature is soil compaction. Soil compaction rates were not measured in this study; however, the permanence of water in very small constructed wetlands implies that soil might be compacted too much while building these constructed habitats. Further research would shed light on the soil compaction necessary to hold water temporarily, rather than permanently.

V. CONCLUSIONS

The constructed wetlands studied here had differences in amphibian communities and physical characteristics when compared with the natural wetlands studied.

Amphibian communities found within the constructed wetlands reflected permanent pond-breeding amphibians, while the natural wetlands studied contained temporary pond-breeding species. Overall, amphibian community composition appeared to be influenced most strongly by habitat requirements of individual species. Permanent water bodies allow for species with long larval stages or aquatic adult stages to thrive, while ephemeral wetlands are essential for explosive breeders with short larval stages.

Adding permanent wetlands to this ridge-top ecosystem presents several potential dangers to populations of species that prefer temporary pool-breeding habitat. Permanent habitats likely increased *R. catesbeiana* and *N. viridescens* predation on eggs, larvae, and juveniles, interspecific competition for food items, and invertebrate predators. In addition, the close proximity of the constructed wetlands to each other and to natural wetlands may provide vectors for amphibian disease transmission. While these constructed permanent habitats might be considered population sinks for some temporary pond-breeding specialists, other adaptable species such as *A. maculatum* and *A. jeffersonianum* might be benefiting from an extended larval period and subsequent larger body size at metamorphosis associated with increased wetland longevity. However, the individual fitness advantage of a larger body size at metamorphosis may only be conveyed to a minority of the hatchlings within these permanent wetlands. Thus,

allowing for a lower survival rate to metamorphosis and a higher survival post metamorphosis.

The U.S. Supreme Court decision implicates continued losses of natural, hydrologically isolated wetlands, increasing the need for research on replacement with constructed wetlands. To alleviate the pressures on amphibian communities utilizing wetlands that are not federally protected, it is imperative for land managers to construct wetlands to provide the best surrogate habitat for hydrologically isolated wetland-dependent species. To provide the best replacement habitat, attention must be given to all characteristics of a wetland ecosystem including: wetland dimensions, canopy closure, wetland depth, water temperature, water quality, amphibian species composition, and quality of surrounding upland habitat.

Land managers should attempt to replicate natural habitats when restoring, replacing, or constructing new wetlands. Wetlands with a temporary hydroperiod, shallow littoral zone, and closed canopy are preferred habitats of natural wetland communities on eastern Kentucky ridge tops. Land managers should strive to include all of these features in design of new wetlands to encourage rare and natural amphibian use. Several avenues of research would considerably increase the knowledge of this ridge-top wetland ecosystem including predator-prey interactions (e.g., *N. viridescens* and *R. sylvatica*), rare species habitat requirements, *R. catesbeiana* prevalence and methods of eradication, and soil compaction studies.

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Appendix A: Tables

Table A-2. A comprehensive amphibian species list for each study wetland for March–July 2009. Observation methods included minnow trapping, dipnetting, aural surveys, and visual encounter surveys. Wetland names: Jones’ Ridge Natural (JRN), High Energy Natural (HEN), Big Perry Natural (BPN), Elk Lick Natural Large (ELNL), Elk Lick Natural Small (ELNS), Jones’ Ridge Artificial (JRA), High Energy Artificial (HEA), Big Perry Artificial (BPA), Elk Lick Artificial Large (ELAL), Elk Lick Artificial Small (ELAS).

| Species | JRN | HEN | BPN | ELNL | ELNS | JRA | HEA | BPA | ELAL | ELAS |
|----------------------------------|-----|-----|-----|------|------|-----|-----|-----|------|------|
| Ambystomatidae | | | | | | | | | | |
| <i>Ambystoma jeffersonianum</i> | X | | X | | | X | | X | | |
| <i>Ambystoma maculatum</i> | X | X | X | X | X | X | X | X | X | X |
| <i>Ambystoma opacum</i> | | X | X | X | | | X | | | |
| Bufonidae | | | | | | | | | | |
| <i>Bufo spp.</i> | X | | X | X | | X | X | X | | |
| Hylidae | | | | | | | | | | |
| <i>Hyla chrysoscelis</i> | X | X | X | | | X | X | X | X | X |
| <i>Pseudacris brachyphona</i> | X | | X | X | | X | X | | | |
| <i>Pseudacris crucifer</i> | X | X | X | | | X | X | X | X | X |
| Pelobatidae | | | | | | | | | | |
| <i>Scaphiopus holbrookii</i> | | | X | | | | | | | |
| Plethodontidae | | | | | | | | | | |
| <i>Hemidactylium scutatum</i> | X | X | X | | X | X | X | | | |
| Ranidae | | | | | | | | | | |
| <i>Rana catesbeiana</i> | | | | | | X | X | X | X | |
| <i>Rana clamitans</i> | X | X | | | X | X | X | X | X | X |
| <i>Rana palustris</i> | | | | | | | | | X | |
| <i>Rana sylvatica</i> | X | X | X | X | | X | | X | X | X |
| Salamandridae | | | | | | | | | | |
| <i>Notophthalmus viridescens</i> | | | X | | | X | X | X | X | X |

Table A-2. A comprehensive amphibian species list for each study wetland for May–August 2010. Observation methods included minnow trapping, dipnetting, aural surveys, and visual encounter surveys. Wetland names: Jones’ Ridge Natural (JRN), High Energy Natural (HEN), Big Perry Natural (BPN), Elk Lick Natural Large (ELNL), Elk Lick Natural Small (ELNS), Jones’ Ridge Artificial (JRA), High Energy Artificial (HEA), Big Perry Artificial (BPA), Elk Lick Artificial Large (ELAL), Elk Lick Artificial Small (ELAS) HAHA wetland (HAHA), Long Ridge wetland (LR), Wetland 5 (P5), Bird Bath wetland (BB).

| Species | HA HA | LR | P5 | BB | HE A | JRA | BPA | ELA S | ELA L | HE N | JRN | BPN | BPC | ELN S | ELN L |
|----------------------------------|----------|----|----|----|---------|-----|-----|----------|----------|---------|-----|-----|-----|----------|----------|
| Ambystomatidae | | | | | | | | | | | | | | | |
| <i>Ambystoma jeffersonianum</i> | X | X | X | | | X | X | X | X | | | | X | | |
| <i>Ambystoma maculatum</i> | X | X | X | | | X | X | X | | | | | X | X | |
| <i>Ambystoma opacum</i> | | | | | | | | X | | X | | | | | |
| Bufonidae | | | | | | | | | | | | | | | |
| <i>Bufo spp.</i> | | | | X | | | | | | | | X | | | X |
| Hylidae | | | | | | | | | | | | | | | |
| <i>Hyla chrysoscelis</i> | X | X | X | X | X | X | X | X | X | | | X | X | X | X |
| <i>Pseudacris brachyphona</i> | | | | X | | | | | | | | X | | | |
| <i>Pseudacris crucifer</i> | | | X | X | X | X | X | X | X | | | X | | | |
| Pelobatidae | | | | | | | | | | | | | | | |
| <i>Scaphiopus holbrookii</i> | | | | | | X | | | | | X | | | | |
| Plethodontidae | | | | | | | | | | | | | | | |
| <i>Hemidactylium scutatum</i> | X | X | X | | X | X | X | | X | X | X | | X | X | |
| Ranidae | | | | | | | | | | | | | | | |
| <i>Rana catesbeiana</i> | X | | X | | X | X | X | X | X | | | X | | | |
| <i>Rana clamitans</i> | X | X | X | | X | X | X | X | X | | X | X | X | X | X |
| <i>Rana palustris</i> | | | X | | | | | X | X | | | | | | |
| <i>Rana sylvatica</i> | | | | | | | | X | | X | X | | X | X | X |
| Salamandridae | | | | | | | | | | | | | | | |
| <i>Notophthalmus viridescens</i> | X | X | X | | X | X | X | | X | | | X | X | | |

Table A-3. Occurrence of amphibian species from March–July 2009 at each wetland type based on all survey methods. The blocks correspond to the number of wetlands within the wetland type for which each species was observed.

| Species | Natural | | | | | Constructed | | | | |
|----------------------------------|---------|---|---|---|---|-------------|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Ambystomatidae | | | | | | | | | | |
| <i>Ambystoma jeffersonianum</i> | ■ | ■ | | | | ■ | ■ | | | |
| <i>Ambystoma maculatum</i> | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| <i>Ambystoma opacum</i> | ■ | ■ | ■ | | | ■ | | | | |
| Bufo | | | | | | | | | | |
| <i>Bufo spp.</i> | ■ | ■ | ■ | | | ■ | ■ | ■ | | |
| Hyla | | | | | | | | | | |
| <i>Hyla chrysoscelis</i> | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | ■ |
| <i>Pseudacris brachyphona</i> | ■ | ■ | ■ | | | ■ | ■ | | | |
| <i>Pseudacris crucifer</i> | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | ■ |
| Pelobatidae | | | | | | | | | | |
| <i>Scaphiopus holbrookii</i> | ■ | | | | | | | | | |
| Plethodontidae | | | | | | | | | | |
| <i>Hemidactylium scutatum</i> | ■ | ■ | ■ | ■ | | ■ | ■ | | | |
| Rana | | | | | | | | | | |
| <i>Rana catesbeiana</i> | | | | | | ■ | ■ | ■ | ■ | |
| <i>Rana clamitans</i> | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | ■ |
| <i>Rana palustris</i> | | | | | | ■ | | | | |
| <i>Rana sylvatica</i> | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | |
| Salamandridae | | | | | | | | | | |
| <i>Notophthalmus viridescens</i> | ■ | | | | | ■ | ■ | ■ | ■ | ■ |

Table A-4. Principle Components Analysis (PCA) loadings of 2009 physical wetland characteristics. The first two axes of the PCA explained 84.9% of the total habitat variation.

| Physical Wetland Characteristics | PC1 | PC2 |
|----------------------------------|--------|--------|
| Maximum Depth | 0.901 | -0.354 |
| Canopy Closure | -0.931 | -0.056 |
| Emergent Vegetation | 0.585 | 0.634 |
| Dissolved Oxygen | 0.969 | 0.214 |
| Temperature | 0.099 | 0.849 |
| pH | 0.902 | 0.007 |
| Wetland Size | 0.527 | -0.762 |

Table A-5. Occurrence of amphibian species from May–August 2010 at each wetland type based on all survey methods. The blocks correspond to the number of wetlands within the wetland type for which each species was observed.

| Species | Natural Wetlands | | | | | | Minimum Depth < 20 cm | | | | | Maximum Depth > 20 cm | | | |
|----------------------------------|------------------|---|---|---|---|---|--------------------------|---|---|---|---|--------------------------|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 |
| Ambystomatidae | | | | | | | | | | | | | | | |
| <i>Ambystoma jeffersonianum</i> | ■ | | | | | | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | |
| <i>Ambystoma maculatum</i> | ■ | ■ | | | | | ■ | ■ | ■ | ■ | | ■ | ■ | | |
| <i>Ambystoma opacum</i> | ■ | | | | | | ■ | | | | | | | | |
| Bufo | | | | | | | | | | | | | | | |
| <i>Bufo spp.</i> | ■ | ■ | | | | | ■ | | | | | | | | |
| Hylidae | | | | | | | | | | | | | | | |
| <i>Hyla chrysoscelis</i> | ■ | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| <i>Pseudacris brachyphona</i> | ■ | | | | | | ■ | | | | | | | | |
| <i>Pseudacris crucifer</i> | ■ | | | | | | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | |
| Pelobatidae | | | | | | | | | | | | | | | |
| <i>Scaphiopus holbrookii</i> | ■ | | | | | | ■ | | | | | | | | |
| Plethodontidae | | | | | | | | | | | | | | | |
| <i>Hemidactylium scutatum</i> | ■ | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ |
| Ranidae | | | | | | | | | | | | | | | |
| <i>Rana catesbeiana</i> | ■ | | | | | | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | |
| <i>Rana clamitans</i> | ■ | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ |
| <i>Rana palustris</i> | ■ | | | | | | ■ | ■ | | | | ■ | | | |
| <i>Rana sylvatica</i> | ■ | ■ | ■ | ■ | ■ | | | | | | | | | | |
| Salamandridae | | | | | | | | | | | | | | | |
| <i>Notophthalmus viridescens</i> | ■ | ■ | | | | | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ |

Table A-6. Similarity measures for 2010 amphibian presence/absence data (compiled using all survey methods).

| Wetland Comparisons | Jaccard's Coefficient | Sorensen's Coefficient |
|---------------------|-----------------------|------------------------|
| N/D<20 | 0.93 | 0.96 |
| N/D>20 | 0.62 | 0.76 |
| D<20/D>20 | 0.57 | 0.73 |

Table A-7. Amphibian species for 2010 unique to one or two wetland types.

| Species | Natural | Depth < 20cm | Depth > 20cm |
|-------------------------------|---------|--------------|--------------|
| <i>Rana sylvatica</i> | X | X | |
| <i>Scaphiopus holbrookii</i> | X | X | |
| <i>Ambystoma opacum</i> | X | X | |
| <i>Pseudacris brachyphona</i> | X | X | |
| <i>Bufo</i> spp. | X | X | |
| <i>Rana palustris</i> | | X | X |

Table A-8. Principle Components Analysis (PCA) loadings of 2010 physical wetland characteristics. The first two axes of the PCA explained 74.7% of the total habitat variation.

| Physical Wetland Characteristics | PC1 | PC2 |
|----------------------------------|--------|--------|
| Maximum Depth | 0.249 | 0.878 |
| Canopy Closure | -0.869 | 0.112 |
| Emergent Vegetation | 0.732 | 0.287 |
| Dissolved Oxygen | 0.773 | 0.466 |
| Conductivity | 0.572 | -0.632 |
| Temperature | 0.731 | 0.054 |
| pH | 0.800 | -0.440 |
| Wetland Size | -0.020 | 0.921 |

Table A-9. Physical wetland characteristics statistics summary table for 2009.

| Physical Wetland Characteristics | A priori Hypotheses | Statistical Test | Mean Difference \pm SE | t | df | p-value |
|----------------------------------|-----------------------|---|--------------------------|---------|----|---------|
| % Canopy Closure | Constructed < Natural | Independent samples t-test (one-tailed) | 25.324 \pm 7.846 | 3.227 | 8 | 0.006 |
| Depth at 1 Meter from Shoreline | Constructed > Natural | Independent samples t-test (one-tailed) | -5.206 \pm 1.935 | - 2.691 | 7 | 0.016 |
| Dissolved Oxygen | Constructed = Natural | Welch t-test (two-tailed) | -6.334 \pm 1.218 | - 5.201 | 5 | 0.003 |
| pH | Constructed = Natural | Independent samples t-test (two-tailed) | -2.250 \pm 0.454 | - 4.952 | 8 | 0.001 |
| Temperature at Midnight | Constructed > Natural | Independent samples t-test (one-tailed) | -2.446 \pm 0.384 | - 6.363 | 7 | <0.001 |

Table A-10. Physical wetland characteristics statistics summary table for 2010.

| Physical Wetland Characteristics | Statistical Test | Test Statistic | df | p-value |
|----------------------------------|------------------|----------------|----|---------|
| % Canopy Closure | Welch test | t = 6.672 | 2 | 0.043 |
| Depth at 1 Meter from Shoreline | One-Way ANOVA | F = 8.277 | 2 | 0.006 |
| % Emergent Vegetation | One-Way ANOVA | F = 4.988 | 2 | 0.027 |
| Maximum Depth | One-Way ANOVA | F = 6.955 | 2 | 0.010 |
| pH | One-Way ANOVA | F = 19.169 | 2 | <0.001 |

Table A-11. Tukey pairwise comparison statistics summary table for 2010 physical wetland characteristics.

| Physical Wetland Characteristics | Wetland Type Pairwise Comparison | Mean Difference \pm SE | q | df | p-value |
|----------------------------------|--|--------------------------|-------|----|---------|
| Depth at 1 Meter from Shoreline | Natural – Deep Constructed | -9.648 \pm 2.386 | -4.04 | 2 | 0.004 |
| % Emergent Vegetation | Natural – Deep Constructed | -22.792 \pm 7.236 | -3.15 | 2 | 0.021 |
| Maximum Depth | Natural – Deep Constructed | 35.958 \pm 10.730 | 3.35 | 2 | 0.015 |
| | Shallow Constructed – Deep Constructed | 36.475 \pm 11.151 | 3.27 | 2 | 0.017 |
| pH | Natural – Shallow Constructed | -1.320 \pm 0.215 | -6.13 | 2 | 0.000 |
| | Natural – Deep Constructed | -0.775 \pm 0.229 | -3.38 | 2 | 0.014 |

Appendix B: Figures

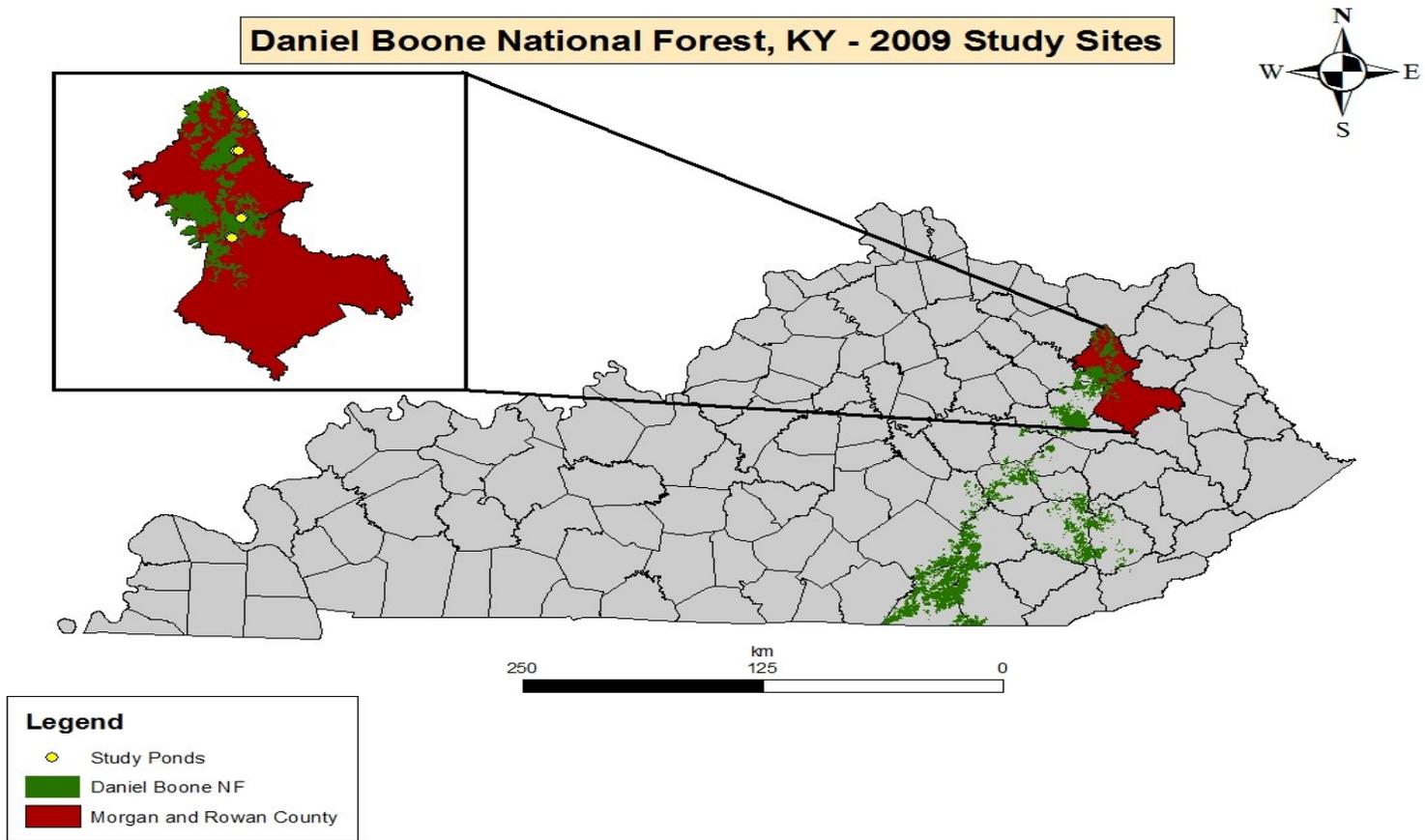


Figure B-3. Map of 2009 study sites in Daniel Boone National Forest, KY. Sites are located in Morgan and Rowan Counties in eastern Kentucky.

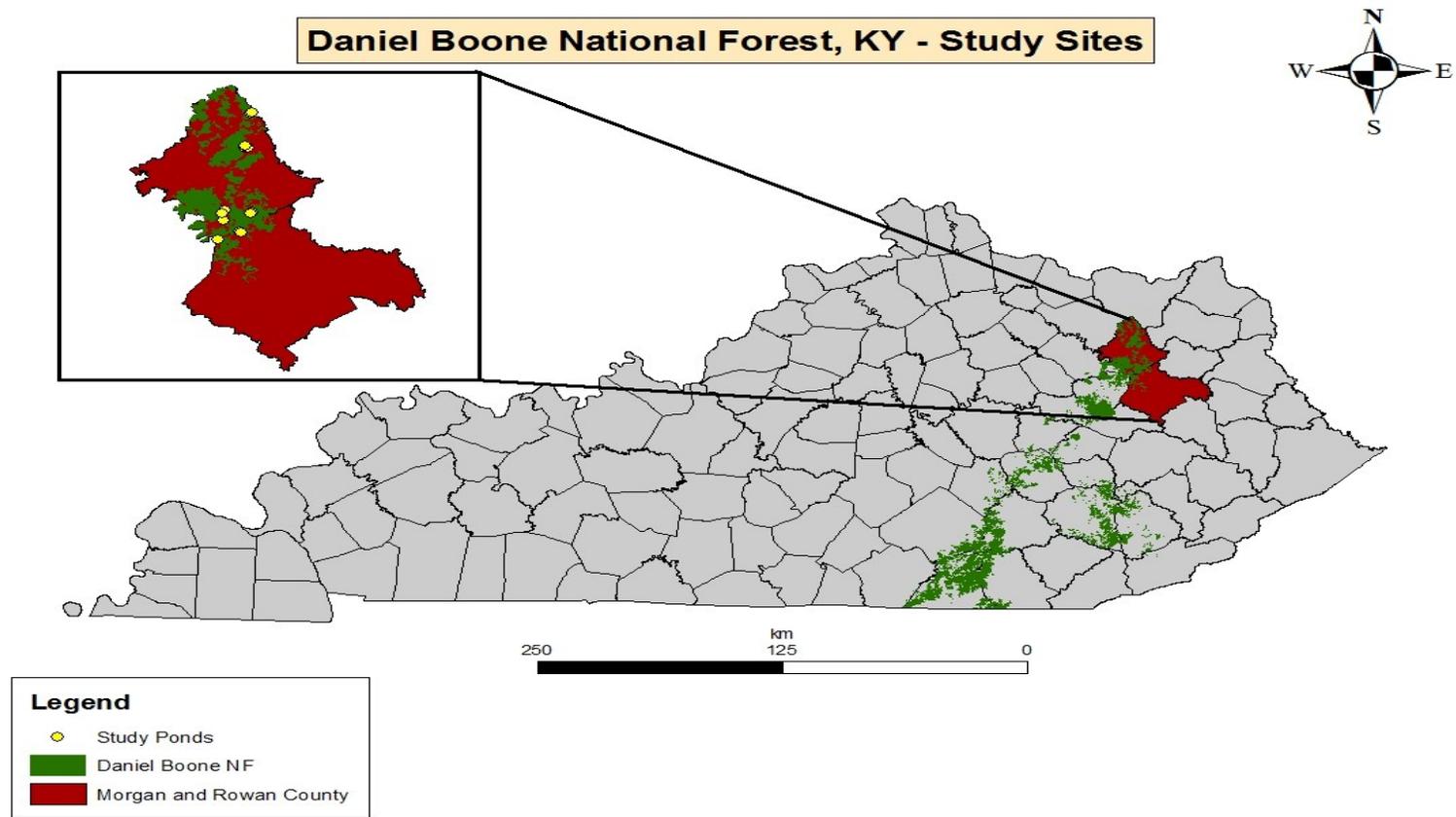


Figure B-4. Map of 2010 study sites in Daniel Boone National Forest, KY. Sites are located in Rowan and Morgan Counties in eastern Kentucky.

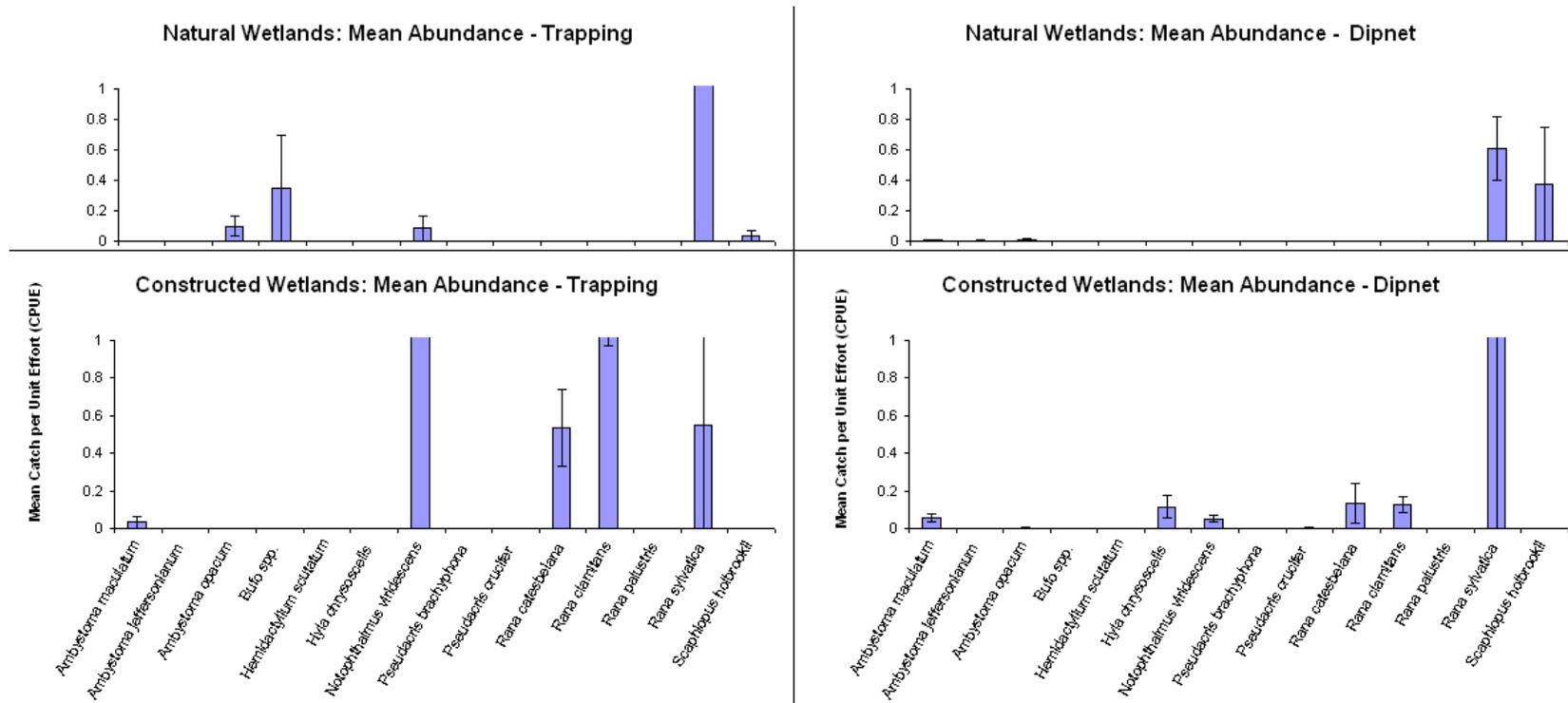


Figure B-3. Amphibian mean species abundances (catch per unit effort; CPUE) for March–July 2009 by wetland and survey type. CPUE numbers for *Rana sylvatica* (constructed dipnet CPUE = 1.48 ± 1.48 SE) (natural trapping CPUE = 9.567 ± 5.782 SE), *Notophthalmus viridescens* (constructed trapping CPUE = 2.382 ± 1.133 SE) and *R. clamitans* (constructed trapping CPUE = 1.306 ± 0.333 SE) continue beyond the y-axis graph limit.

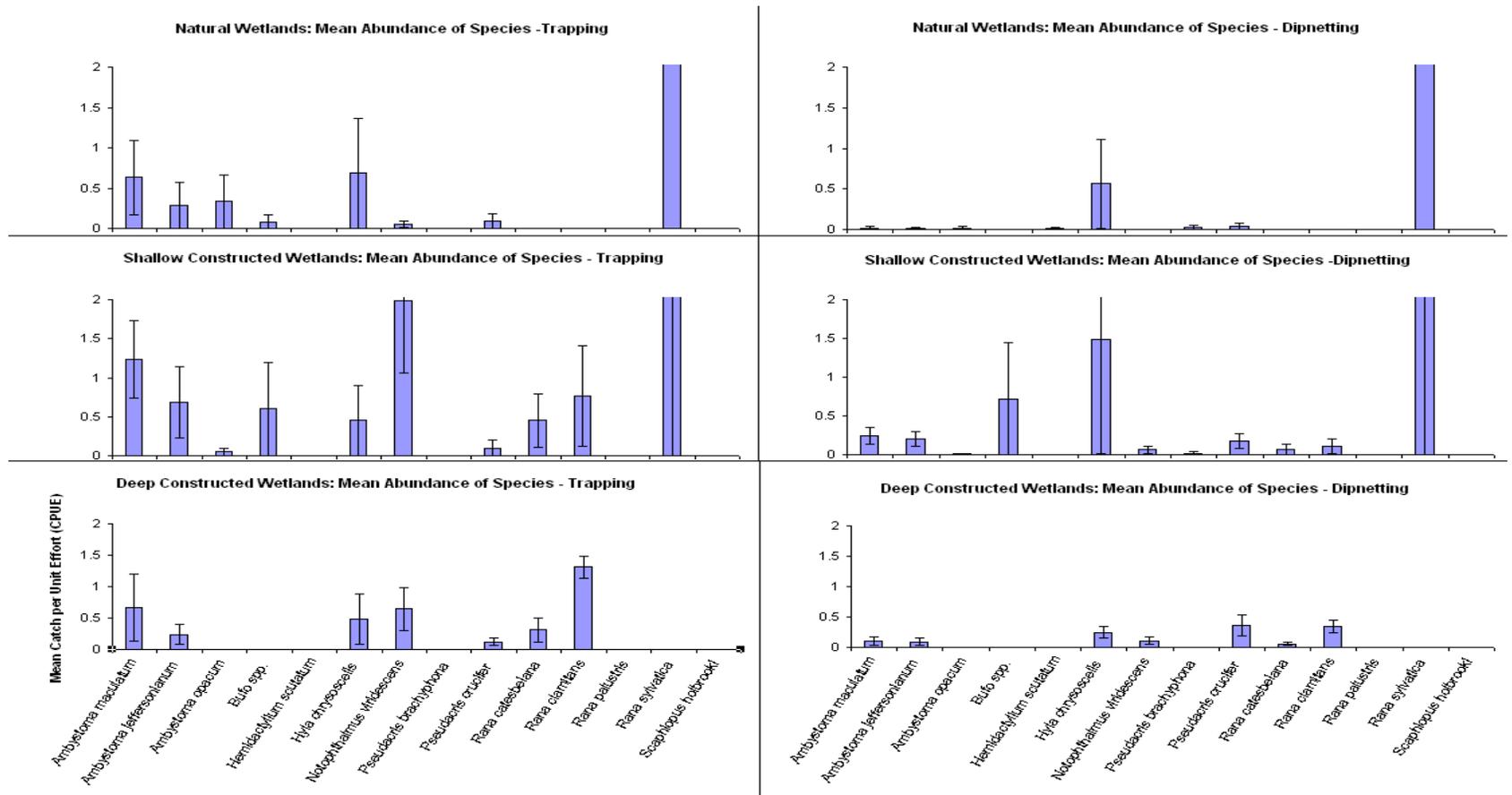


Figure B-4. Amphibian mean species abundances (catch per unit effort; CPUE) for May–August 2010 by wetland and survey type. CPUE numbers for *Rana sylvatica* (natural trapping CPUE = 101.3833 ± 44.142 SE, natural dipnet CPUE = 7.853 ± 3.644 SE, shallow constructed trapping CPUE = 3.9 ± 3.9 SE, shallow constructed dipnet CPUE = 2.04 ± 2.04 SE), *Hyla chrysoscelis* (shallow constructed dipnet CPUE = 1.486 ± 1.466 SE), and *Notophthalmus viridescens* (shallow constructed trapping CPUE = 1.982 ± 0.922 SE) continue beyond the y-axis graph limit.

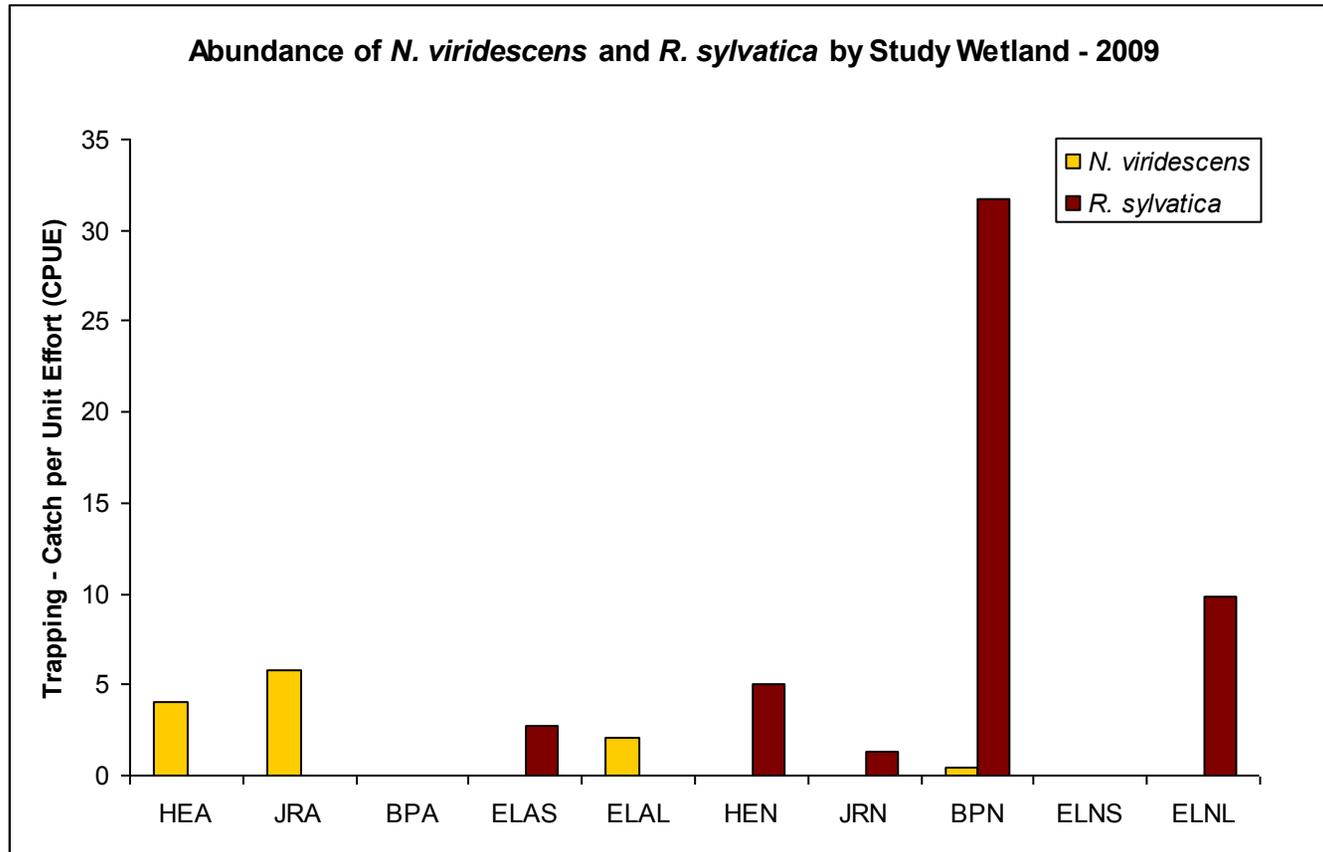


Figure B-5. Abundance (catch per unit effort; CPUE) of *Notophthalmus viridescens* and *Rana sylvatica* by study wetland for 2009.

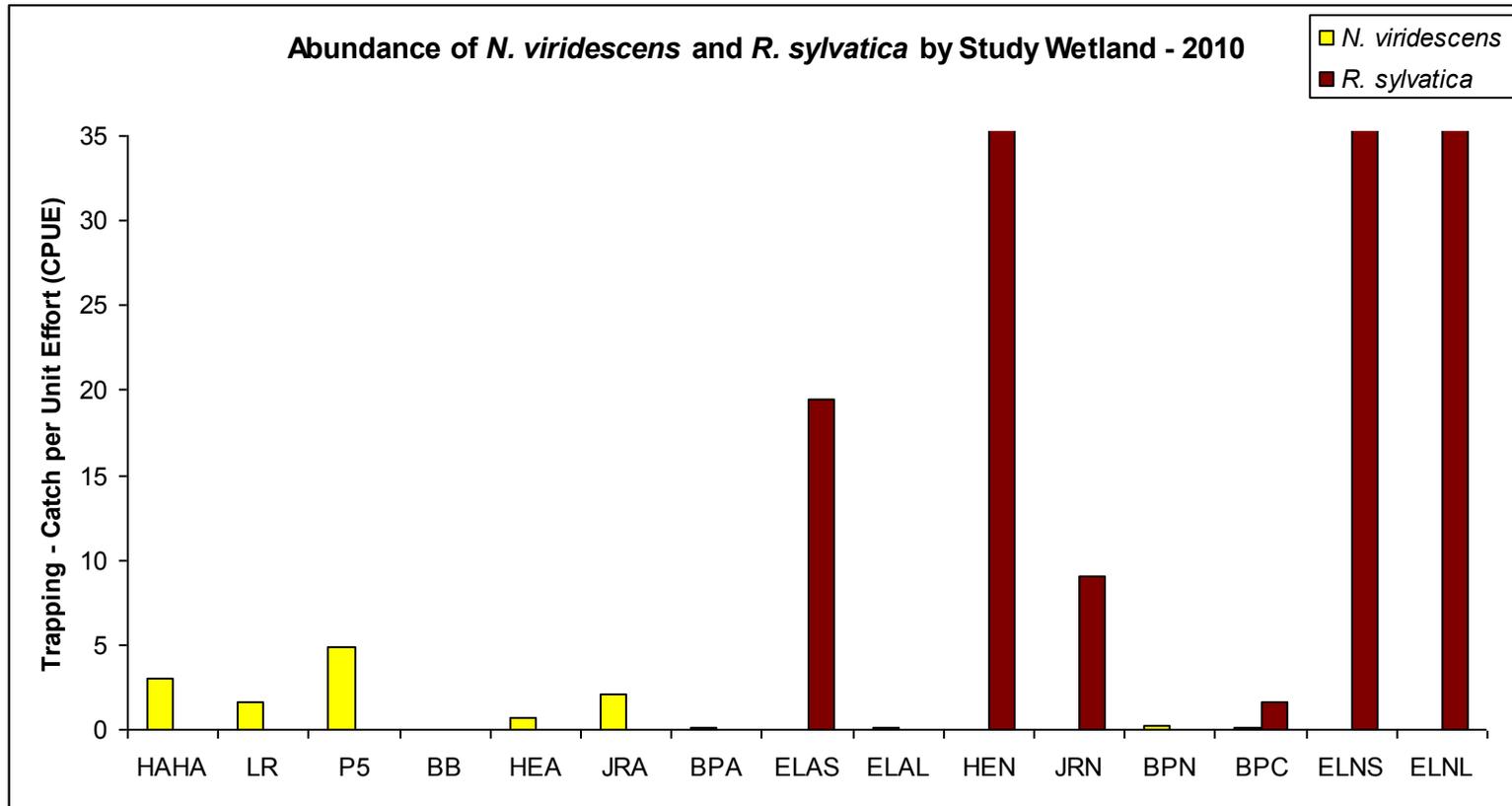


Figure B-6. Abundance (catch per unit effort; CPUE) of *Notophthalmus viridescens* and *Rana sylvatica* by study wetland for 2010.

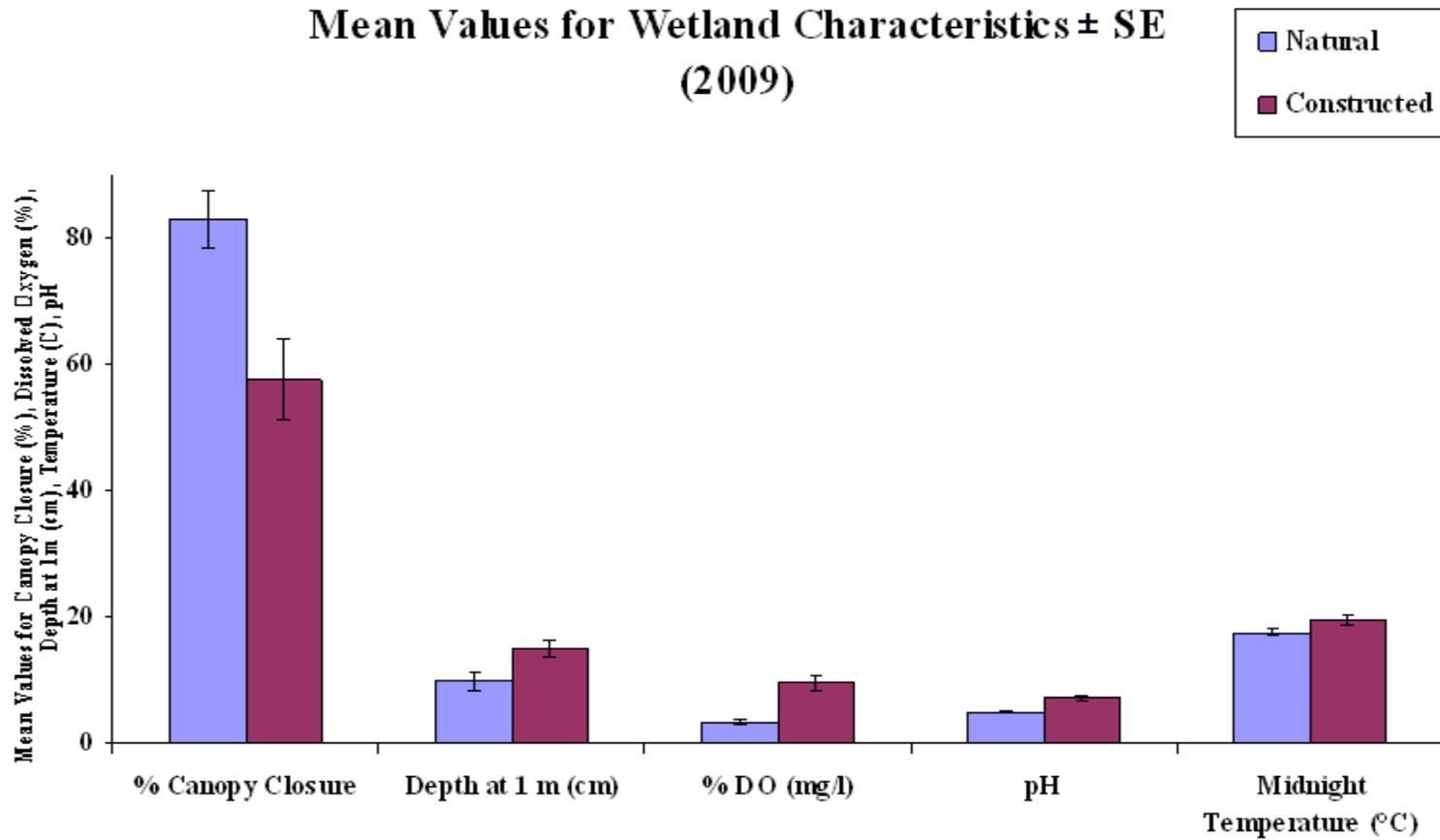


Figure B-7. Mean values for wetland characteristics \pm standard error by wetland type (natural, constructed) for 2009.

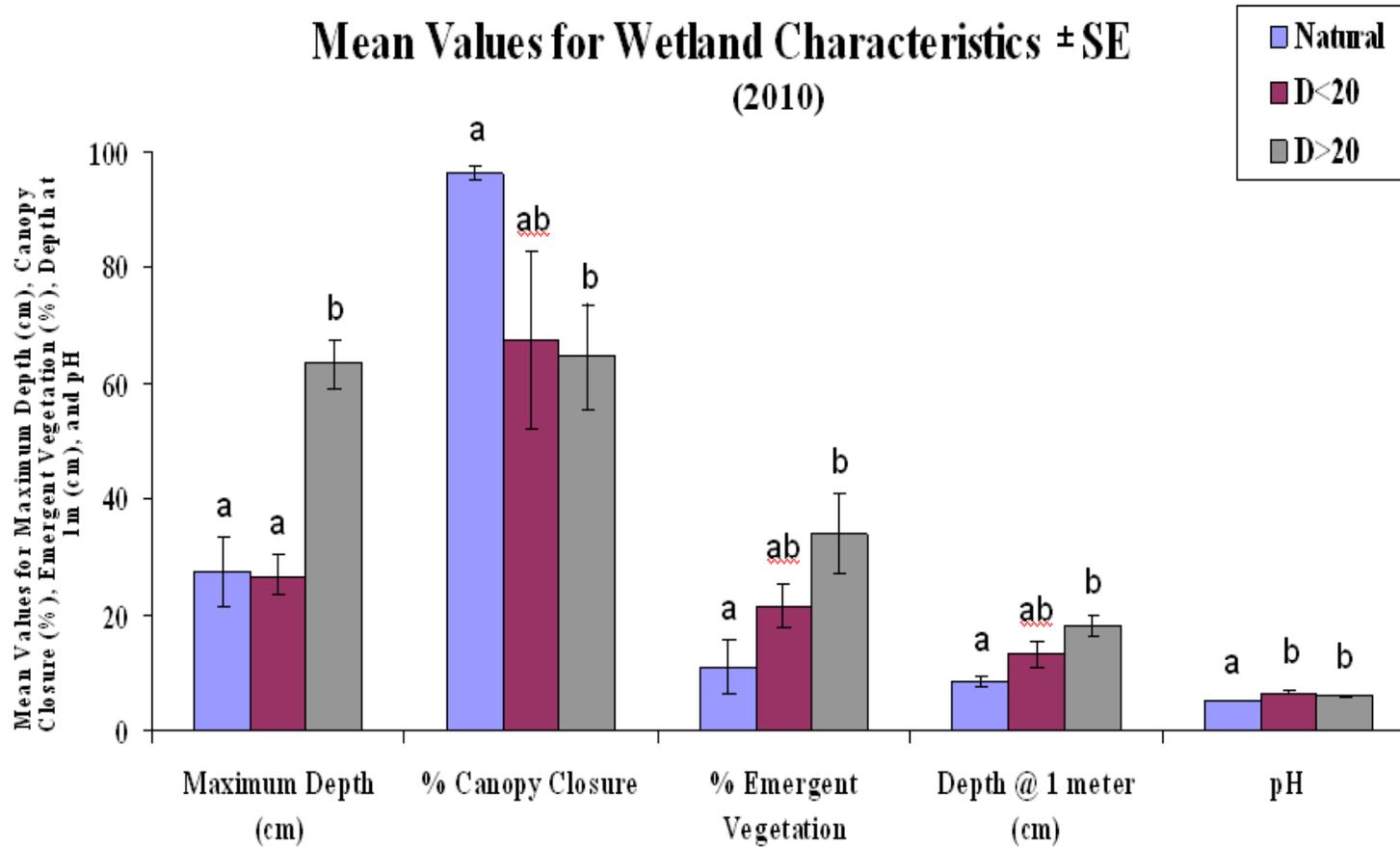


Figure B-8. Mean values for wetland characteristics \pm standard error by wetland type [(natural, shallow constructed (D < 20), deep constructed (D > 20)] for 2010. Different letters indicate statistical significance between groups and shared letters indicate lack of statistical significance between groups.

Appendix C: Rare Species Accounts

Rare Species Notes – 2009

Several species (*Ambystoma opacum*, *Pseudacris brachyphona*, and *Scaphiopus holbrookii*) were rare across both wetland types, natural and constructed. *Ambystoma opacum* was present in three natural wetlands (HEN, BPN, and ELNL) and one constructed wetland (HEA). *Pseudacris brachyphona* was observed in three natural wetlands (JRN, BPN, and ELNL) and two constructed wetlands (JRA and HEA). *Scaphiopus holbrookii* was found in one natural wetland BPN (Lat/Long = -83.3699908, 38.2455861) culminating in a new county record for Rowan County, KY. One adult was captured in a wire minnow trap on 2 May 2009 and subsequently seven adults were captured on 31 May 2009. A small clutch of eggs was observed on 2 May 2009. A larger 3 x ½ m grouping of eggs were observed on 28 May 2009. From 2 June through 16 June 2009 several thousand (estimated) tadpoles were observed in BPN wetland. Several thousand *S. holbrookii* metamorphs (estimated) were exiting the wetland from 27 June through 2 July 2009. In addition to the *S. holbrookii* observed in BPN wetland, a *S. holbrookii* metamorph was observed on 30 June 2009 in the forest adjacent to JRA wetland (Lat/Long = -83.355837, 38.093151).

Rare Species Notes – 2010

All of the rare species aforementioned in the 2009 rare species accounts were only documented in two of the three wetland types, natural and shallow constructed, during the 2010 sampling season. *Ambystoma opacum* was present in one natural (HEN) and one shallow constructed wetland (ELAS). Likewise, *P. brachyphona* was observed in one

natural (BPN) and one shallow constructed wetland (BB). Two new locations were identified for *S. holbrookii* during the 2010 sampling. They were documented at one natural wetland (JRN) and one shallow constructed wetland (JRA).

VITA

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