BUILDING BETTER WETLANDS FOR AMPHIBIANS: INVESTIGATING THE ROLES OF ENGINEERED WETLAND FEATURES AND MOSQUITOFISH (*GAMBUSIA AFFINIS*) ON AMPHIBIAN ABUNDANCE AND REPRODUCTIVE SUCCESS

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By
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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

BUILDING BETTER WETLANDS FOR AMPHIBIANS: INVESTIGATING THE ROLES OF ENGINEERED WETLAND FEATURES AND MOSQUITOFISH (*GAMBUSIA AFFINIS*) ON AMPHIBIAN ABUNDANCE AND REPRODUCTIVE SUCCESS

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A candidate for the degree of Doctor of Philosophy

And hereby certify that, in their opinion, it is worthy of acceptance.

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Dedicated to my wife, Tracy, and my children, Austin and Ashlee, whose love and support have sustained me through my endeavors.

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ABSTRACT

Compensatory wetland mitigation efforts have been broadly criticized for failing to replace lost ecological functions, even when net gains in wetland area are achieved. Of particular concern is the suitability of constructed wetlands as habitat for vulnerable taxa. Amphibians are vital components of wetland ecosystems, yet one third of all species are threatened. Habitat loss and alteration are primary reasons for these declines so it is imperative that constructed wetlands significantly contribute to amphibian conservation. Nevertheless, few studies have sought to understand how altering engineered wetland features influences amphibian abundance and reproductive success. Results from such studies are necessary to guide wetland planners and managers in their efforts to construct and restore wetlands that aid amphibian conservation.

The aim of my dissertation research was to investigate features that can be altered by wetland planners and managers to enhance wetland suitability for amphibians. I ask two basic wetland planning questions that are essential to providing appropriate habitat:

1) how do aquatic habitat features influence reproductive success and species richness; and, 2) how do features of the core terrestrial habitat influence individual species abundances. The first question addresses how the wetland is designed, whereas the second question addresses where the wetland is placed in the landscape.

First, I surveyed amphibian populations in 49 existing constructed wetlands throughout northern Missouri to investigate influences of both design and placement features on amphibian abundance. Design features typical of open water ponds best explained abundances of cricket frogs (*Acris crepitans*), bullfrogs (*Lithobates catesbeianus*), and green frogs (*L. clamitans*); all commonly captured species. At the placement level, models that included nearby aquatic habitat ranked highest for common species. Salamanders and most hylid frogs were rarely captured and responded positively to aquatic vegetation, but negatively to fish and anthropogenic disturbance-related features in the terrestrial habitat.

Next, I conducted field studies at 18 experimental constructed wetlands to test design feature effects on amphibian metamorph production and species richness. I examined the effects of within-wetland slope, vegetation, and introduced mosquitofish (*Gambusia affinis*). Mosquitofish significantly reduced metamorph production in both survey years. Vegetation cover had significant effects on production the second year. Regression models revealed that total metamorph production was greatest at shallow-sloped, fish-free wetlands during the first year, but shallow-sloped wetlands with high vegetation amounts were best the second year. Species richness was negatively associated with fish and positively associated with vegetation in both years.

Finally, I focused on the role of predators, particularly mosquitofish, in shaping amphibian and invertebrate communities in constructed wetlands. I also investigated the role of predators in increasing the severity of sub-lethal tail injuries to tadpoles, and the role of vegetation in attenuating these injuries. My results indicate that boreal chorus frogs (*Pseudacris maculata*) are intolerant to large populations of aquatic predators, including mosquitofish. Gray treefrogs (*Hyla versicolor/chrysoscelis* complex) were also sensitive to mosquitofish based on larval abundance assessments. Additionally, mosquitofish increased the severity of larval ranid tail injuries, and they reduced both invertebrate abundance and richness. Fish removal increased chorus frog reproduction, bolstered invertebrate populations, and reduced the severity of tadpole tail injuries. Vegetation was important for increasing invertebrate taxa richness, but did not provide larval ranids adequate refuge from mosquitofish based on my tail injury analyses.

The results of my research indicate that constructed wetlands can be effective for amphibian conservation if appropriate habitats for target species are provided at both the design and placement levels. Fish-free, heavily vegetated, shallow-sloped wetlands, placed in landscapes with low anthropogenic disturbance, appear to provide the best habitat for the most uncommon amphibian species captured in my surveys. Mosquitofish should not be introduced into wetlands because of their potential to negatively impact native amphibian and invertebrate communities. Wetlands that attract breeding native salamanders, whose larvae also feed on mosquitoes, are a better alternative and likely more effective for amphibian conservation. Furthermore, designing some wetlands to dry occasionally will reduce populations of fish and predatory aquatic invertebrates that prevent colonization and lower the reproductive success of some amphibian species.

CHAPTER 1

INTRODUCTION

Human societies are organized around continuous conversions of ecosystems for human use (Johns 2010). These conversions include expanding road systems into once inaccessible places, development of rural areas, introducing alien but commercially valuable plants and animals, and the transformation of wild spaces to row-crop agriculture for food and fuel. These transformations are at the core of our society and economy, and they fuel the needs of an ever-growing human population. Nevertheless, these ecosystem conversions also manifest themselves as environmental problems like habitat loss, species extinctions, reduced air and water quality, global climate change, and the introduction and proliferation of invasive species. Many people are increasingly apprehensive about environmental degradation but economic concerns and the pursuit of societal goals lead to inherent conflicts (Deen, 2003). These conflicts have led to a myriad array of mitigation practices that aim to attenuate environmental damage, thereby leading to a myriad of debates regarding the effectiveness of mitigation.

Compensatory wetland mitigation is the act of restoring or creating new wetlands to offset those lost to development. Section 404 of the 1972 Federal Clean Water Act requires mitigation for the deposition of fill into jurisdictional wetlands, and a 1989 federal policy set a goal of "no net loss" of wetland acres and functions. Over half of the pre-European settlement wetlands in the U.S. have been destroyed; and, in some Midwestern states, losses exceed 90% (Dahl 1990, Mitsch and Gosselink 1993). "No net loss" policies and regulations at federal and, in some cases, state levels are attempts to create or restore more wetland area than the area impacted by development. Nevertheless, many have contended that these efforts have been inadequate at replacing lost wetland functions (i.e., Turner et al. 2001, Minkin and Ladd 2003). Furthermore, despite recent net increases in wetland acres (Dahl 2006), many replacement wetlands are not the same type as those impacted (Kihslinger 2008). These shifts in wetland type undoubtedly amplify functional losses. For example, many organisms adapted to large, permanent hydroperiod, open water lakes are unlikely to colonize and successfully reproduce in an ephemeral pool. The result is lost wildlife habitat – one of the ecological functions that compensatory wetlands are intended to replace. However, wildlife habitat, particularly habitat for non-game species, is rarely considered during compensatory wetland planning or monitoring (National Research Council 2001).

Amphibians are ideal organisms to assess the quality of mitigation wetlands as wildlife habitat. Their biphasic life cycles and variable interspecific sensitivities to environmental disturbances reveal information regarding conditions of both the aquatic and nearby terrestrial habitats. Furthermore, the recent attention focusing on global amphibian declines underscores the importance of restoring quality amphibian habitat. Over one third of the approximately 6,000 known species of amphibians are threatened with extinction (Stuart et al. 2004, Wake and Vredenburg 2008). Habitat destruction and alteration are likely the most significant causes of amphibian declines, and designing mitigation wetlands for amphibian species of conservation concern will become increasingly important if these trends continue (Dodd and Smith 2003).

Some of the most important research investigating the effectiveness of mitigation wetlands as amphibian habitat has taken place in Ohio, U.S.A. Micacchion (2004) developed the Ohio Amphibian Index of Biotic Integrity (Ohio AmphIBI) to “score” compensatory wetlands using amphibian communities. The index is based on the habitat requirements and variable disturbance tolerances of wetland-dependent amphibian species (Micacchion 2004). Porej (2004) found that while most impacted wetlands in Ohio were forested, over 85% of compensatory wetlands were placed in locations with less than 25% forest in the surrounding terrestrial habitat; likely explaining the absence of salamanders and wood frogs (*Lithobates sylvaticus*). Porej et al. (2004) focused on the landscape composition within the core terrestrial habitat (see Semlitsch 1998 and Semlitsch and Bodie 2003) surrounding Ohio wetlands and the presence of predatory fish. The authors found strong positive associations between salamanders and the amount of forest in the core terrestrial habitat, and negative associations with predatory fish and the length of paved roads surrounding wetlands. The authors concluded that wetland management cannot be decoupled from the surrounding terrestrial habitat (Porej et al. 2004). In a related study, Porej and Hetherington (2005) examined influences of aquatic features including predatory fish presence, shallow littoral zones, and emergent vegetation on amphibian diversity in compensatory wetlands. Their study revealed a strong positive association between diversity and shallow littoral zones; and a negative association between diversity and predatory fish presence (Porej and Hetherington 2005). Based on their results, the Ohio Environmental Protection Agency implemented a guideline specifying replacement wetland bank slopes of 15:1 or less, and Porej and Hetherington (2005) encouraged other regulatory agencies to adopt this as a standard to avoid negative impacts to amphibian diversity arising from compensatory mitigation.

My dissertation research stems from my experience as an employee of the Missouri Department of Transportation (MoDOT) involved in compensatory wetland mitigation. One of my first assignments was to modify the hydroperiod and increase the aquatic habitat within a recently constructed compensatory mitigation site adjacent to a road maintenance facility. This, and subsequent assignments involving roadside ditch wetlands bordered by row-crops and a wetland stocked with mosquitofish (*Gambusia affinis*) began the process of formulating my questions regarding the value of compensatory wetlands as amphibian habitat. My principal goal has been to improve all constructed wetlands for wildlife, and amphibians in particular.

The previously mentioned research conducted in Ohio has served as a foundation and guide for my dissertation research. However, instead of simply replicating these worthy efforts, my goal was further this research by studying amphibian abundance rather than presence; and to test the effects of selected wetland features on amphibians at replicated experimental wetlands instead of relying solely on the results of observational studies conducted at existing wetlands. This protocol is significant because patterns of abundance can provide reliable indicators of habitat quality (Gardner et al. 2007), and the use of experimental wetlands allowed me to eliminate or reduce many of the confounding variables present in the myriad array of mitigation wetlands while examining the effects of variables of interest.

The first phase of my dissertation research, presented in Chapter 2, is observational and correlates amphibian abundance in existing constructed wetlands with features of the aquatic and core terrestrial habitats (≤ 300 m from the edge of each wetland). Both compensatory and non-compensatory constructed wetlands were surveyed. I use an information theoretic approach to select models that best explain the responses of amphibian species to wetland features at both habitat levels. In a related study, Romero (2010) associated amphibian biotic index scores (see Shulse et al. 2009) to broader landscape context features, beyond the core terrestrial habitat of these same 49 wetlands, to derive equations that better predict how roadway placement impacts amphibian populations. My objective in Chapter 2 is to provide wetland planners and managers with guidance to design and place wetlands that bolster the reproductive success of uncommon amphibian species.

In Chapter 3, I focus on wetland design and present results from replicated experimental wetlands that test the effects within-wetland slope, vegetation, and introduced mosquitofish on amphibian reproductive success and species richness. I test whether the Porej and Hetherington (2005) recommended littoral zone slope bolsters reproductive success and richness compared to a much steeper design. I examine the effects of artificially added and naturally colonizing vegetation to provide wetland planners with guidance to help determine if planted vegetation enhances amphibian habitat, and should therefore be incorporated into wetland designs. Because mosquitofish are sometimes introduced into constructed wetlands to control mosquito-borne diseases, I ask whether they affect amphibian colonization and reproduction. I use multivariate general linear models to examine the effects of these variables on combined amphibian reproductive success. I analyze individual species reproductive success, total reproductive success, and species richness using regression modeling. Again, I employ an information theoretic approach to select models that best explain metamorph production and species richness.

Chapter 4 presents results that describe the influences of mosquitofish and naturally colonizing aquatic predators on amphibian communities in the experimental wetlands. I use data from four years of aquatic habitat sampling and regression modeling to examine relationships between predator abundances and larval gray treefrogs (*Hyla versicolor/chrysoscelis* complex), boreal chorus frogs (*Pseudacris maculata*), and green frogs (*Lithobates clamitans*). I also examine the role of mosquitofish in causing sub-lethal tail injuries to ranid anuran larvae; and the potential role of vegetation cover in attenuating these injuries. I expand my investigations beyond amphibians and investigate the effects of mosquitofish and vegetation on aquatic invertebrate abundance and taxa richness. Finally, in the fifth chapter, I summarize my conclusions and present management recommendations.

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CHAPTER 2

INFLUENCES OF DESIGN AND LANDSCAPE PLACEMENT PARAMETERS ON AMPHIBIAN ABUNDANCE IN CONSTRUCTED WETLANDS

Christopher D. Shulse, Raymond D. Semlitsch, Kathleen M. Trauth,

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ABSTRACT

As natural wetlands disappear, constructed wetlands may play vital roles in amphibian conservation. However, previous investigations have concluded that artificial wetlands do not adequately replace lost wildlife habitat. Nevertheless, constructed wetlands serve as breeding habitat for amphibians where extensive natural wetland loss has occurred. To investigate the roles of engineered wetland features on amphibian abundance, we surveyed 49 constructed wetlands throughout northern Missouri. Cricket frogs (*Acris crepitans*), bullfrogs (*Lithobates catesbeianus*), and leopard frogs (*Lithobates blairi/sphenocephalus* complex) each occurred in over 80% of surveyed wetlands. Salamanders and hylid frogs were rarely encountered. We used an information theoretic approach to examine relationships between individual species and habitat features associated with wetland designs and placements. We found that models incorporating design features of open water ponds best explained abundances of most commonly encountered species. At the placement level, models that included nearby aquatic habitat ranked highest for common species. Salamanders and most hylid frogs responded positively to aquatic vegetative cover but negatively to fish abundance and anthropogenic disturbance-related features in the landscape. Our results indicate that to be effective amphibian conservation tools, constructed wetlands should be fish-free, heavily vegetated, include shallows, and placed within areas of low anthropogenic disturbance.

INTRODUCTION

The effectiveness of constructed and restored wetlands at replacing the ecological functions of those lost to development has been investigated (e.g., Minkin and Ladd 2003; Ambrose and Lee 2004; Mack and Micacchion 2006) and challenged (National Research Council 2001; Kihslinger 2008; Semlitsch 2008a) in multiple reports. The consensus is that the ecological values of artificial wetlands are limited. Nevertheless, wetland trends continue to be measured by area rather than function. For example, although a recent U.S. Fish and Wildlife Service document reports that for the first time net wetland area gains exceeded losses between 1998 and 2004, most gains can be attributed to the creation of artificial freshwater ponds in agriculturally dominated landscapes (Dahl 2006). In fact, nearly 281,500 ha of new freshwater ponds were constructed – an increase of nearly 13%. Over this period, freshwater emergent marshes declined by an estimated 57,720 ha (Dahl 2006). Deep basins and steep slopes of most artificial ponds inhibit vegetation and result in open water systems that are functionally different than vegetated wetlands (Environmental Laboratory 1987; Minkin and Ladd

2003). Additionally, placement of constructed emergent and other vegetated wetlands in degraded landscapes can influence wetland structure resulting in lost function and reduced ecological value (Kettlewell et al. 2008). Because many species of wetland plants and animals are dependent on specific wetland types, constructed wetlands that do not match the ecological requirements of those species equate to lost habitat, and ultimately reduced biodiversity, regardless of area gained.

Missouri has experienced > 80% wetland loss since pre-settlement times (Dahl 1990; Mitch and Gosselink 1993). Most natural wetlands have been cleared, drained, or channelized for agriculture or navigation (Nigh and Schroeder 2002) but constructed ponds are numerous, particularly in northern and western Missouri. Many were created for agriculture, recreation, or wildlife habitat, and densities as high as 1 pond per 50 ha have been estimated (Nigh and Schroeder 2002). Restored wetland area is increasing in Missouri. For example, approximately 300 ha in 70 project-specific sites and three mitigation banks have been restored by the Missouri Department of Transportation (MoDOT) as compensation for approximately 156 ha of wetland impacts (B. Brooks, personal communication).

Amphibians are a threatened vertebrate group (Stuart et al. 2004; Wake and Vredenburg 2008) and habitat alteration and destruction contribute heavily to declines (Gardner et al. 2007). Due to their biphasic life cycle, amphibian assemblages and individual species are influenced by wetland features such as hydroperiod, presence of shallows, vegetation, and predators (Hecnar and M'Closkey 1997; Porej and Hetherington 2005; Hartel et al. 2007); along with the composition, quantity, and quality of surrounding terrestrial habitat (Guerry and Hunter 2002; Harper and Semlitsch 2007;

Harper et al. 2008). Nearby wetlands allow for movement between breeding sites, re-colonization following local extinctions, and “stepping stones” during dispersal (Marsh and Trenham 2001; Johnson et al. 2007; Semlitsch 2008b). Therefore, alteration of the aquatic environment, fragmentation of surrounding uplands, and nearby wetland destruction can result in population declines. Ongoing development, along with corresponding habitat restoration and construction, underscores the importance of properly planning constructed wetlands. Although artificial wetlands can provide surrogate habitat for amphibians (Knutson et al. 2004; Simon et al. 2009; Brand and Snodgrass 2010), their suitability for individual species likely depends on incorporating habitat requirements into wetland plans (Pechmann et al. 2001; Hazell et al. 2004). For wetland planners, aquatic habitat requirements can be viewed as design features and terrestrial habitat requirements can be considered features of placement. The design and placement of wetlands should focus on the habitat requirements of the species of concern (Dodd and Smith 2003).

Our objective was to examine the influences of design and placement on amphibian reproduction in constructed wetlands. In particular, we examined relationships between species abundances and design and placement features to develop models that best explain responses of individual amphibian species or groups. It was not our intent to compare the roles of constructed versus natural wetlands as amphibian habitat. Natural wetlands are scarce and most are functionally impaired in the highly fragmented, largely agricultural landscape of Missouri. Constructed wetlands are likely the primary breeding habitat of many amphibian species in this region (Knutson et al. 2004; Hocking et al. 2008). Thus, we focus on the roles of selected engineered components of wetlands and how they can be altered to improve design and placement for individual amphibian species, especially those that are uncommon.

METHODS

Study Sites

We surveyed amphibians in 49 non-randomly selected constructed wetlands throughout 26 northern Missouri counties (Table 1a,b; Figure 1). We chose wetlands that we had permission to access and that varied widely in design and landscape placement. We selected sites located north of the Missouri River in the Central Dissected Till Plains Ecological Section or along the Missouri River in the Outer Ozark Border Subsection of the Ozark Highlands (Nigh and Schroeder 2002) for ease of logistics and to limit possible confounding of amphibian assemblages. Twenty wetlands were constructed by MoDOT as compensatory mitigation for wetland impacts. Twenty-nine (non-compensatory) sites were located on Missouri Department of Conservation (MDC) public lands; many were built as farm ponds prior to their acquisition and retained by the agency for wildlife use (G. Gardner, personal communication). The entire set ranged from small temporary pools in forests, grasslands, and along roadsides to large permanent hydroperiod open water ponds in agricultural and urban settings. For logistical purposes, wetlands were grouped geographically into 7 sampling zones, each containing 5–9 wetlands and ranging in size from 707–2,932 km^2. Zone sampling order within each survey period was random.

Sampling

To increase the probability of detecting breeding adults, eggs, and larvae of amphibians, we surveyed each wetland during March/April, May/June, and July/August of 2006. Data from all 3 periods were pooled for analysis. Fifteen of the surveyed wetlands were temporary (Table 1) and contained no standing water during 1 or more of the sampling periods; analyses of these sites was based upon 1 or 2 surveys.

Sampling for amphibians consisted of visual and acoustic surveys, area-constrained dip netting, and aquatic funnel trapping (deployed overnight); each conducted once per sampling period. Fish captures occurred concurrently with amphibian dip netting and funnel trapping. Visual and acoustic surveys were not time-constrained and involved walking the perimeter of each wetland upon arrival at the site and recording the number of each species observed or heard calling. Data from these surveys were used only for qualitative analyses of species richness. Captures obtained through standardized dip-netting and aquatic funnel trapping were used in both qualitative and quantitative analyses. Dip-net sweeps were scaled to the size of the wetland (1 sweep/25 m^2 of wetland surface area, 5 sweeps minimum, 200 maximum). Sweeps were ~1.5 m long with the net pressed to the bottom of the wetland and dragged toward the sampler. Two kinds of commercially available minnow traps were used for aquatic funnel trapping. Collapsible nylon mesh traps (3 mm mesh size; 38 x 26 x 26 cm dimensions) had a 6 cm opening at both ends. Galvanized steel wire traps (6mm mesh; 42 cm long) had funnel entrances at both ends that tapered from 20 cm to 2.5 cm diameter. Both traps were used in every wetland at approximately equal proportions. Two traps were placed for every 25 m^2 of wetland surface area and 1 additional trap was placed each time the wetland surface area doubled. For large wetlands (>0.6 ha), 5 additional traps were placed for every additional 0.2 ha of surface area. Traps were distributed proportionally among habitat types. All amphibians and fish were identified in the field and released unharmed at the point of capture.

Vegetation cover within 1.0 m from the shoreline was measured using four 1 m^2 quadrats spaced at cardinal directions around the wetland perimeter. For large wetlands (>0.6 ha) 2 additional quadrats were evaluated and all 6 were spaced evenly around the perimeter. The percentages of open water, emergent, floating, and submerged vegetation were visually estimated within each quadrat. The 3 categories of vegetation were combined and averaged for all quadrats over all sampling periods at each wetland to arrive at an average measure of vegetative cover within the littoral zone of each wetland. Within-wetland slope was measured at each plant quadrat. Depth was measured at 1 m and 3 m along transects perpendicular to the wetland edge. Slope was calculated from these depths and then averaged among quadrats to estimate within-water slope for each wetland.

Wetland area was calculated in the field for the purposes of scaling sampling efforts and sometimes varied between sampling periods if drying or filling occurred. We used a 200 m tape to measure wetland length and width. For large wetlands, a laser range finder (Bushnell Yardage Pro® Sport 450) was also used. Area of maximum pool was verified during GIS analysis of landscape features (below) and these values were $\log_{10}$ transformed and used for statistical analyses.

Landscape Analysis

Landscape analyses were performed using ArcMap 9.2 GIS software (Environmental Systems Research Institute 2006). Surveyed wetlands were located on 2006 National Agricultural Imagery Program (NAIP) orthorectified digital aerial photography (2 m resolution) and their boundaries were delineated to create a polygon representing each wetland within the GIS. Wetland polygons were overlaid on Missouri Resource Assessment Partnership (MoRAP) Landuse and Landcover data (LULC 2005). Statestreams (rivers and streams) and Travelways (roads) line data from MoDOT's Transportation Management System (TMS) were also added as layers within the GIS. National Wetlands Inventory (NWI 2003) data were used to supplement open water pond locations in the GIS because LULC 2005 did not reliably identify these features. We combined woody and herbaceous wetland features from LULC data to create a single wetland feature (excluding open water ponds). We performed a core habitat analysis of the first 300 m of terrestrial habitat surrounding each wetland to quantify percentages of each land cover type and density of roads, streams, and open water ponds within the core habitat. Road and stream density were calculated by dividing the total length of each within the core habitat by the core area (m of road or stream/m^2 core area). Pond density was calculated by dividing the total number of open water ponds by the core area. Because the area of core habitat varied with wetland size, this procedure standardized these features with respect to core area. The 300 m core terrestrial habitat width was chosen because environmental features within this area presumably have the greatest impact on the health of the local breeding population of amphibians (Semlitsch and Bodie 2003; Rittenhouse and Semlitsch 2007).

Data Analysis

Because different species can respond uniquely to the same habitat variables (Gardner et al. 2007), we used abundance of individual species as response variables in regression models. For most amphibian species, standardized counts from dip-net catch-per-unit-effort (CPUE) were used as measures of abundance. Counts at each site were standardized for effort by dividing the number of individuals of each species captured by the number of dip-net sweeps taken. Dip netting resulted in captures at more sites than aquatic funnel trapping for most species (Figure 2). The exception was bullfrog (*Lithobates catesbeianus*), and therefore aquatic funnel trap CPUE (larvae captured per trap) was used as the response variable for this species. Likewise, fish abundance from aquatic funnel trapping was used as a predictor variable for within-wetland analyses due to greater capture frequency than dip-netting. Because some salamander species did not occur throughout the entire range of our study (Daniel and Edmond 2010), we also combined dip-net CPUE for all salamanders to investigate relationships between constructed habitat features and salamander abundance. We did not combine dip net and funnel trap captures into a single measure of CPUE because active and passive sampling efforts together cannot be reliably standardized for effort.

We performed Spearman rank correlation tests on predictor variables of interest at both the design and placement levels (Table 2a,b). Predictor variables were excluded from regression analyses if highly correlated ($r \geq 0.70$). We selected 5 predictor variables for design analyses representing both biotic and abiotic wetland characteristics, and 6 for placement including features of anthropogenic disturbance, grassland habitat, and nearby aquatic habitat (Tables 3 & 4). Selection of variables was based upon previous studies. Percent cropland was negatively correlated with percent forest ($r = -0.68$). Although below our threshold, we chose to only include cropland as the predictor because other regional amphibian studies had examined influences of forest but excluded croplands

(Knutson et al. 2004; Porej et al. 2004) or combined row crop and pasture land covers (Knutson et al. 1999; Knutson et al. 2000).

Regression models with a compound Poisson (Tweedie) distribution and log link function were developed using the generalized linear model in SPSS version 16.0 (2007 SPSS Inc. Chicago, Illinois). Tweedie (1984) distributions are a subset of exponential dispersion models with an index parameter p that determines the shape of the distribution. For continuous data with exact zeros, this parameter can be any value > 1 and < 2. We selected the value of p using test runs of the global models for each species inserting different values and selecting the final value based upon model goodness-of-fit output from SPSS. Tweedie distributions are particularly useful when zeros (no observation) and positively skewed continuous CPUE data make up the dataset. We developed *a priori* models representing biologically relevant combinations including global models containing all predictor variables for design or placement, combinations of 2 or 3 variables, each variable alone, and null intercept-only models (Table 5). We developed 11 models using the 5 within-wetland predictor variables for analyses of design features and we developed 13 for placement analyses using the 6 core habitat variables. Models for design and placement were developed separately, without combining features of each, to avoid comparisons between the aquatic environment and surrounding landscape. We used an information theoretic approach to compare candidate models and avoid over-fitted models (Burnham and Anderson, 2002). Akaike's Information Criterion values corrected for small sample size (AIC_c) were obtained from SPSS output and the model within each category with the smallest value was selected as most supported. The remaining models were ranked according to their differences in AIC_c from the most supported model in the set (ΔAIC). Models with ΔAIC values 0–2 have substantial empirical support within the model set and values > 10 indicate essentially no support.

We calculated Akaike weights (*W*) and individual variable weights (*w*) to make inferences among highly supported candidates and to assess the relative importance of each variable within the entire model set (Burnham and Anderson 2002). We also calculated the percent deviance explained for each model by dividing the reduction in deviance for the full model by the deviance of the null model (Simon et al. 2009).

RESULTS

We detected 16 of 22 species of pond-breeding amphibians (Figure 2) known to occur within our survey range (Daniel and Edmond 2010). Number of species detected per site ranged from 0 to 10 with a mean of 5. The most common species were northern cricket frog (88% of sites), bullfrog (84%), and leopard frog complex (80%). Pond-breeding species known to occur within our survey area but not detected included northern crawfish frogs (*Lithobates areolatus circulosus*), Fowler's toads (*Anaxyrus fowleri*), ringed salamanders (*A. annulatum*), four-toed salamanders (*Hemidactylium scutatum*), plains spadefoot toad (*Spea bombifrons*), and western narrow-mouthed toad (*Gastrophyrne olivacea*). Fish were captured at 43% of the sites, with only green sunfish (*Lepomis cyanellus*, 31% of sites) and mosquitofish (*Gambusia affinis*, 20% of sites) being common.

Cricket Frog

Cricket frogs were the most common species recorded (88% of sites), and dip netted at 57% (0–112 individuals per site CPUE 0–1.1). Global models for both design and placement fit the data (design $\chi^2 = 12.7$, df = 5, $P = 0.027$; placement $\chi^2 = 18.5$, df = 6, $P = 0.005$; n = 49). AIC analysis of within-wetland design features revealed that a negative association with the single variable model THyd (temporary hydrology) best explained abundance of this species (Table 6). Cricket frogs were captured by dip netting in only 18% of the wetlands that dried in 2006. The Aquatic Surroundings model had the highest support in the landscape-level placement analysis, and within the model the variables pond density and stream density were positively associated with cricket frog abundance (Table 7). Stream density had the highest individual variable weight followed by pond density (Table 8).

Bullfrog

Bullfrogs were observed at 84% of sites and dip netted at 45% (0–208 individuals per site, CPUE 0–0.99). Aquatic funnel trapping resulted in captures at 55% of sites (0–114 individuals per site, CPUE 0–8.3). The global models fit the data (design $\chi^2 = 37.7$, df = 5, $P < 0.001$; placement $\chi^2 = 40.4$, df = 6, $P < 0.001$). The global model ranked highest in the AIC analysis of design features, and bullfrogs were positively associated with fish abundance but negatively associated with temporary wetlands. Temporary hydroperiod, within-wetland slope, and fish abundance had variable weights > 0.90. At the landscape placement level, the global model ranked highest and revealed a negative association with the percent of wetland habitat surrounding the sampled wetlands, and a positive association with open water pond density. The more parsimonious Aquatic Surroundings model revealed the same associations and also had high empirical support. A third model, Wildlife Area, strengthened evidence for the positive open water pond density association, and included a positive association with the percent of grassland habitat surrounding the wetlands. Open water pond density had the highest individual variable weight.

Leopard Frog Complex

Leopard frogs (*L. blairi/sphenocephalus* complex) were commonly observed at the survey wetlands (80%) and dip netted at 57% of sites (0–1000 individuals per site, CPUE 0–7.6). The global models fit the data (design $\chi^2 = 24.3$, df = 5, $P < 0.001$; placement $\chi^2 = 31.6$, df = 6, $P < 0.001$). The highest-ranking design model and individual variable was THyd (positive). The Slope model (negative) also had high empirical support (ΔAIC = 1.66) but low model weight ($W = 0.19$) and only modest importance as an individual variable within the set ($w = 0.42$). Placement analysis ranked the Land Cover model highest, and percent grassland and percent wetland within Land Cover were positively associated with leopard frog abundance. Although it had low weight as a single variable model ($W = 0.28$), percent wetland had high empirical support (ΔAIC = 0.81) and the highest relative importance as a variable within the model set ($w = 0.99$).

Green Frog

Lithobates clamitans was found at 55% of sites and dip netted at 37% (0 – 88 individuals per site, CPUE 0 – 0.73). Although green frogs were captured in only 55% of sites, we found green frogs to be extremely common in surveyed sites within their known range in Northeast Missouri. The global models for both design and placement fit the data (design $\chi^2 = 17.2$, df = 5, $P = 0.004$; placement $\chi^2 = 15.0$, df = 6, $P < 0.021$). The Experimental model ranked highest in the analysis of within-wetland design features. Two variables within the model, fish abundance ($w = 0.70$) and within-wetland slope (*w*

= 0.73), were positively associated with green frog abundance. The placement analysis revealed that a negative association with percent wetland habitat within 300 m of the sampled site was the highest ranked model and it had the highest individual variable weight (0.73). The Aquatic Surroundings model was also highly ranked (ΔAIC = 1.55). Like bullfrogs and cricket frogs, green frog abundance was positively associated with pond density in the surrounding landscape but this relationship was not strongly supported by AIC.

Gray Treefrog Complex

Gray treefrogs (*Hyla versicolor/chrysoscelis* complex) were detected at 53% of sites and dip netted at 37% (0 – 181 individuals per site, (CPUE 0 – 7.9). The global models fit the data (design $\chi^2 = 35.6$, df = 5, $P < 0.001$; placement $\chi^2 = 41.8$, df = 6, $P < 0.001$). Wetland size (negative) was the best model for predicting gray treefrog abundance at the design level. This feature also had high importance within the model set as revealed by its individual variable weight ($w = 0.99$). The landscape-level placement analysis revealed that the global model best fit the data; however the more parsimonious Disturbance model containing the variables percent cropland and road density also had high empirical support (ΔAIC = 0.88). Both variables were negatively associated with gray treefrog abundance and had high relative importance within the set of candidate models.

American Toad

American toads (*Anaxyrus americanus*) were detected at 37% of sites and dip netted at 20% (0–650 individuals per site, CPUE 0–1.1). The global models fit the data (design $\chi^2 = 21.7$, df = 5, $P = 0.001$; placement $\chi^2 = 30.7$, df = 6, $P < 0.001$). The

Experimental model ranked highest in the AIC analyses of within-wetland design features. American toad abundance was positively associated with fish abundance and negatively associated with increasing within-wetland slope. Slope was the most influential variable in the model set. Aquatic Surroundings best fit the data in the placement analysis. The variables pond density and percent wetland were negatively associated with toad abundance, but toads were positively associated with high stream densities. Pond density had the highest variable weight and percent wetland and stream density were also relatively important in the model set.

Boreal Chorus Frog

Pseudacris maculata was observed at 31% of wetlands and dip netted at 22% (0–2500 individuals per site, CPUE 0–17.9). Global models fit the data (design $\chi^2 = 108.8$, df = 5, $P < 0.001$; placement $\chi^2 = 42.6$, df = 6, $P < 0.001$). The Experimental model containing the variables fish abundance (negative), vegetative cover (positive), and within-wetland slope (negative) had the highest empirical support in the within-wetland design analysis, and although the global model also had high support, Experimental was most parsimonious. All three variables within Experimental had very high individual weights due to the high combined model weights of both global and Experimental. The global model best fit the data in the landscape-level placement analysis and the variables percent grassland, percent wetland, and stream density were positively associated with chorus frogs.

Spring Peeper

Pseudacris crucifer was detected at 29% of sites and dip netted at 20% (0 – 118 individuals per site, CPUE 0 – 3.9). The global models fit the data (design $\chi^2 = 30.3$, df =

5, $P < 0.001$; placement $\chi^2 = 41.7$, df = 6, $P < 0.001$). The Biotic model containing fish abundance (negative) and vegetation (positive) was best at the design level. Both variables had high relative importance within the candidate set of models. The global model ranked highest in the placement analysis; however Land Cover was also highly supported and more parsimonious (ΔAIC = 1.34). Model effects revealed negative associations with percentages of both cropland and wetland in the core terrestrial habitat. The wetland variable had the highest relative importance and percent cropland was second.

Combined Salamanders

Salamander detection was low within the sampled wetlands. Smallmouth (*Ambystoma texanum*) and eastern tiger (*A. tigrinum*), the two species historically found throughout the range of the study, were found at 18% and 2% of sites, respectively. Other salamander species detected were spotted (*A. maculatum*), marbled (*A. opacum*), and central newt (*Notophthalmus viridescens*). However, these were restricted to wetlands within the Outer Ozark Border Subdivision at the southern limits of our survey range. Collectively, salamanders were detected at 31% of surveyed wetlands and dip netted at 25% (0 – 44 individuals per site, CPUE 0 – 1.5). The global models fit the data (design $\chi^2 = 35.2$, df = 5, $P < 0.001$; placement $\chi^2 = 33.9$, d.f. = 6, $P < 0.001$). At the design level, the global model ranked highest but Experimental was most parsimonious. Salamander abundance was inversely related to fish abundance but positively associated with vegetative cover. Fish abundance had the highest individual variable weight but vegetative cover was also highly weighted within the model set. The best-supported model representing core terrestrial habitat features was Disturbance containing the variables percent cropland and road density. Salamander abundance was negatively associated with both variables; however, the relative importance of percent cropland within the model set was substantially higher than that of road density (0.99 vs. 0.48).

Smallmouth Salamander

Smallmouth salamanders were observed at 18% of sites and dip netted at 14% (0 – 40 individuals/site, CPUE 0 – 0.33). The global models fit the data (design $\chi^2 = 24.8$, df = 5, $P < 0.001$; placement $\chi^2 = 26.9$, df = 6, $P < 0.001$). This species was negatively associated with fish abundance and this feature ranked highest both as a model and an individual variable. The Biotic model containing fish abundance and vegetative cover (positive) also had high support and vegetative cover had modest relative importance within the model set. Percent grassland (positive) ranked highest in the placement analysis but it had low model weight and a modest variable weight. Two models containing stream density (negative) were well supported and this variable had the second highest variable weight.

DISCUSSION

Our results illustrate the diverse habitat preferences of amphibians and the challenges associated with constructing a wetland that benefits all species. While the AIC analyses did not reveal a single shared "best model" for explaining abundances for all species at each habitat level, patterns in model rank, individual variable weights, and responses to individual variables are evident. These patterns are important in wetland planning and will ultimately determine if a constructed wetland will enhance populations of common bullfrogs or rarely encountered salamanders and hylid frogs. Even abundances of species often considered habitat generalists such as cricket frogs, American toads, and leopard frogs clearly were correlated with habitat features supporting the idea that true habitat generalists are rare (Skelly et al. 1999).

Responses to Wetland Design Features

The Experimental model ranked highest for more amphibians than any other design model. However, responses to the component variables of this, and the other candidate models, differed between groups. Most commonly-captured species in our surveys (cricket frogs, bullfrogs, and green frogs) were abundant in wetlands with characteristics of open water ponds such as high fish abundance, increasing slope, and permanent hydrology. These species require permanent wetlands for breeding due to multi-seasonal larval stages or relatively late breeding periods (Johnson 2000; Lannoo 2005). Leopard frogs were the only commonly-captured group that was an exception to this trend. These anurans were often found in compensatory mitigation wetlands that tended to have shallow slopes and were often temporary. Interestingly, other researchers have also observed that leopard frogs tend to avoid habitats that bullfrogs prefer (Porej and Hetherington 2005; Williams 2008). Whether leopard frogs purposely avoid bullfrogs or simply the habitats that bullfrogs prefer is unclear. Bullfrogs have been implicated as a factor in amphibian declines and this native yet invasive species is known to prey on and out-compete other adult and larval amphibians (Adams et al. 2003). Bullfrogs may also serve as unaffected vectors for spreading *Batrachochytrium dendrobatidis* and ranavirus that can cause amphibian die-offs (Gahl et al. 2009; Schloegel et al. 2009). Boone et al. (2008) found that survival of larval southern leopard frogs and two other amphibian species was reduced in the presence of overwintering bullfrog larvae. Others have suggested that leopard frogs preferentially select temporary breeding sites to avoid predators, including bullfrogs (Lannoo 2005). Our observations support this hypothesis and suggest that simply incorporating shallows or reducing hydroperiods can influence the abundance of even comparatively common amphibians in constructed wetlands.

Toads and chorus frogs were uncommon in our surveys but, like leopard frogs, they were often found in high numbers in shallow-sloped compensatory mitigation sites (see also Porej and Hetherington 2005). Shallows provide habitat for calling, foraging, thermoregulation, and refuge from predators (Madison and Farrand 1998; Stratman 2000; Semlitsch 2002). We often observed larval toads congregating in shallow littoral zones that were free of vegetation. Increasing slope and vegetation were only moderately inversely correlated (Table 2a), perhaps because over-compaction during construction prevented re-vegetation of some shallow-sloped sites or many steep-sloped wetlands had narrow edges of emergent vegetation. But while toads were most prolific in wetlands containing fish, chorus frogs, spring peepers, and salamanders were most abundant in heavily vegetated and fish-free wetlands. Although toad larvae are unpalatable to fish, hylid frogs and salamanders are readily consumed and breeding adults may avoid fish-inhabited sites (Kats et al. 1988; Skelly 1996). Although second to fish, vegetation variable weights for salamanders, chorus frogs and spring peepers illustrate the importance of this feature for refuge from predators (Figiel and Semlitsch 1990; Walls 1995; Madison and Farrand 1998), oviposition substrates (Pearl et al. 2005), and/or calling perches (Lannoo 2005). Other attempts to link vegetation to amphibian species richness and reproductive success have yielded contradictory results (e.g., Knutson et al.

2004; Hartel et al. 2007). However, our results indicate that vegetation structure is an important design feature that should be incorporated into wetland plans.

Responses to Wetland Placement Features

The Aquatic Surroundings model ranked highly for commonly detected species suggesting that nearby wetlands, open water ponds, and streams are more important for regulating abundances of these amphibians than terrestrial habitat or anthropogenic disturbance-related features in the matrix. Neighboring aquatic features may be crucial for dispersing metamorphs and inter-pond movements of adult amphibians that are highly aquatic. Newly metamorphosed bullfrogs will rapidly colonize recently constructed ponds (Willis et al. 1956) and cricket frogs will disperse between ponds following rain events (Lannoo 2005), possibly along riparian habitats (Semlitsch, personal observation). Cricket frog populations are highly susceptible to drought (Lannoo 1998) and we found them to be most abundant in permanent wetlands surrounded by high stream densities. Some amphibian abundances were inversely related to the percentage of nearby wetland habitat, especially green frogs and bullfrogs. Additionally, toads were often captured in wetlands near streams but were more abundant in sites surrounded by cropland rather than other wetland habitat (see also Gagné and Fahrig 2007; Williams 2008). Spring peepers require relatively intact core terrestrial habitat (Hecnar and M'Closkey 1997; Knutson et al. 2000), and we rarely captured this species in the bottomland mitigation wetlands where anthropogenic disturbance was high (Tables 1 & 4). While it seems counter-intuitive that abundances of highly aquatic bullfrogs and green frogs would be negatively associated with nearby wetland area, we suspect our results reflect their preference for permanent steep-sloped ponds.

Salamander and most hylid frog abundances were strongly influenced by terrestrial habitat features, including those represented by anthropogenic disturbance. This is not surprising given that amphibian species requiring upland habitat appear to be more sensitive to adjacent land cover than those more closely tied to aquatic habitats (Simon et al. 2009). Our results suggest that wetlands placed near roads or surrounded by cropland will do little to bolster populations of these amphibians and may serve as ecological traps. Both forms of disturbance expose amphibians to elevated levels of pesticides, heavy metals, fertilizers, mechanical disturbance, and increased mortality due to desiccation and vehicular travel (Fahrig et al. 1995; Jorgensen et al. 1997; Findlay and Bourdages 2000; Houlahan and Findlay 2003).

Conclusions and Management Implications

For constructed wetlands to be effective conservation tools, their designs and placements should focus on the requirements of rare or declining species. Unfortunately, most wetlands in our surveys appeared to benefit a few common species. Although many mitigation wetlands sampled incorporated desirable design features such as vegetation and shallows, their placement within disturbed landscapes and vulnerability to fish colonization likely lowered their value for salamanders and hylid frogs. On the other hand, wetlands intended for wildlife use were often situated in relatively undisturbed landscapes but their steep slopes, low vegetation cover, and permanent hydroperiods allowed the persistence of bullfrogs and stocked fish. The average slope of surveyed mitigation wetlands was 14.5:1 whereas that of non-compensatory wetlands was 4.5:1 (Table 4), a reflection of their origin as farm ponds. The recommended within-water slope of open water farm ponds is 3:1 to depths of 0.9 to 1.2 m to discourage growth of aquatic vegetation, and total depths of 2.5 m or more are recommended to ensure fish survival (Deal et al. 1997; Perry n.d.). These are the wetland types credited with reversing the trend in nationwide wetland losses (Dahl 2006) and while they are important for many recreational and agricultural purposes, our results show they are unsuitable for most amphibians.

The proliferation of fish-stocked permanent open water ponds has likely facilitated the spread of bullfrogs. Bullfrog populations have flourished where open water pond construction is common (Willis et al. 1956; Lannoo 2005). Gahl et al. (2009) found that bullfrogs regularly use seasonal pools in the vicinity of permanent breeding ponds, and suggest that temporary wetlands may serve as important non-breeding habitat for bullfrogs, including stepping stones for dispersal and migration. If current wetland trends continue, bullfrogs will gain additional breeding habitat and increased access to temporary natural wetlands, while other amphibians might suffer from increased predation, competition, and exposure to pathogens (Adams et al. 2003; Boone et al. 2008).

Our results indicate that wetland designs should limit the colonization and persistence of fish, incorporate shallow littoral zones, and contain high amounts of vegetation cover. Wetlands should be placed in landscapes with low anthropogenic disturbance and surrounded by appropriate habitat for the target species. Managers should refrain from stocking fish (Walston and Mullin 2007) and steep-sloped permanent ponds can be altered to include shallows and shorten hydroperiods. These efforts may help to discourage establishment of bullfrog populations (Werner and McPeek 1994; Adams et al. 2003; Porej and Hetherington 2005). To prevent fish invasion and increase habitat diversity, designers can incorporate low berms and cells with seasonal hydrology and higher elevation into floodplain wetland complexes. Species such as American toads, chorus frogs, and cricket frogs that are associated with streams (Williams 2008) may benefit from wetland construction coupled with riparian preservation, restoration, and improved stream mitigation practices.

Although compensatory mitigation and pond construction have increased wetland area in agricultural settings (Dahl 2006), these efforts are unlikely to benefit salamanders and many anurans if the surrounding landscape remains cultivated. Similarly, on-site mitigation for highway impacts will not be effective for conserving species sensitive to anthropogenic disturbance. Instead, wetland construction should be coupled with preservation of natural habitats. Developers and agencies that impact wetlands, conservation organizations and agencies, and regulatory authorities should explore collaborative opportunities where wetland construction is tied to preservation of high quality landscapes. While such activities may not result in high net acreage gains in comparison to re-establishment of wetlands in agricultural fields, they will likely be functionally more successful than wetlands placed in degraded landscapes.

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Table 1a. Surveyed MDC wetlands and characteristics. Temporary wetlands are in bold.

Site	Location Description	UTM N	UTM E	Area (m^2)	Depth (m)[a]	Dist. [b]	Forest [c]
MDC01	Redman	4412165.569	556345.437	2200	>1.3	39.0	1.0
MDC02	Redman	4411971.281	555643.622	1200	>1.3	34.0	1.0
MDC03	**Deer Ridge**	**4448674.233**	**598703.286**	**600**	**0.55**	**8.0**	**50.0**
MDC04	**Dunn Ford**	**4415937.382**	**606172.974**	**100**	**0.4**	**5.0**	**41.0**
MDC05	**Rose Pond**	**4466278.933**	**626864.145**	**200**	**0.33**	**14.0**	**2.0**
MDC06	Atlanta - Long Branch	4414278.423	541316.778	1100	>1.3	1.00	71.0
MDC07	Henry Sever	4429796.06	587719.722	900	>1.3	33.0	17.0
MDC08	Diggs	432633.363	617507.536	200	>1.3	40.0	14.0
MDC09	Whetstone Creek	4311055.382	611162.79	300	>1.3	15.0	16.0
MDC10	**Whetstone Creek**	**4311999.613**	**612133.839**	**2000**	**0.63**	**0.0**	**40.0**
MDC11	Blind Pony Lake	4322490.521	470613.369	200	0.71	10.0	55.0
MDC12	Blind Pony Lake	4322466.189	470243.166	1100	>1.3	23.0	36.0
MDC13	Prarie Home	4292948.983	535027.099	400	1.1	23.0	36.0
MDC14	Daniel Boone	4291774.235	639578.337	600	>1.3	1.0	97.0
MDC15	Daniel Boone	4292717.092	639128.982	1700	>1.3	4.0	91.0
MDC16	Bennitt	4345862.927	548703.559	400	>1.3	6.0	79.0
MDC17	Mineral Hills	4472441.042	504735.909	1200	>1.3	3.0	44.0
MDC18	Mineral Hills	4473655.651	503308.591	300	>1.3	0.0	96.0
MDC19	Poosey	4419353.478	441800.217	1800	>1.3	50.0	1.0
MDC20	Elam Bend	4438547.357	391975.497	700	0.81	9.0	68.0
MDC21	Elam Bend	4438613.62	391434.034	300	0.65	24.0	69.0
MDC22	Gallatin	4409456.991	421001.352	300	0.9	5.0	48.0
MDC23	King Lake	4432943.937	377030.699	700	>1.3	20.0	46.0
MDC24	King Lake	4433059.725	376591.925	1400	0.65	23.0	45.0
MDC25	Danville	4303591.223	628306.351	800	>1.3	6.0	20.0
MDC26	Danville	4303380.596	629378.202	200	0.49	1.0	85.0
MDC27	Little Dixie	4307330.451	576252.456	200	>1.3	33.0	29.0
MDC28	White (Robert M II)	4353567.213	597287.756	1000	>1.3	13.0	14.0
MDC29	White (Robert M II)	4353122.813	597116.477	800	>1.3	43.0	18.0

[a] Depth measured at maximum pool. Maximum depth that could be measured was 1.3 meters.

[b] % anthropogenic disturbance based on cropland, roads, and urbanization within 300m core terrestrial habitat.

[c] Percentage of forest within 300m core terrestrial habitat. Includes upland, bottomland, and riparian corridors.

Table 1b. Surveyed MoDOT wetlands and characteristics. Temporary wetlands are in bold.

Site	Location Description	UTM N	UTM E	Area (m²)	Depth (m)[a]	Dist. [b]	Forest [c]
MODOT01	**Center Maintenance Lot**	**4374080.506**	**625894.801**	**9400**	**0.45**	**92.0**	**1.0**
MODOT02	**Route T Black Creek**	**4399011.267**	**590971.285**	**200**	**0.15**	**39.0**	**16.0**
MODOT03	Hwy 15 Davis Fork Salt R.	4338112.57	595812.375	5500	0.7	30.0	31.0
MODOT04	Hwy 136 Fox River	4472181.028	618666.631	20200	>1.3	38.0	48.0
MODOT05	Rte T Elk Fork Chariton R.	4387631.939	540456.395	500	0.4	46.0	35.0
MODOT06	Hwy 36 Mid. Fork Chariton	4401273.158	535005.728	17200	>1.3	59.0	25.0
MODOT07	**Hwy 36 Yellow Creek**	**4401122.009**	**501608.254**	**11900**	**0.7**	**68.0**	**15.0**
MODOT08	**Hwy 36 Muddy Creek**	**4403045.842**	**464191.272**	**100**	**0.08**	**60.0**	**8.0**
MODOT09	**Hwy 36 Beetsma 2**	**4402663.877**	**450661.798**	**1500**	**0.65**	**30.0**	**7.0**
MODOT10	**Hwy 36 Beetsma 4**	**4402697.069**	**451664.19**	**500**	**0.3**	**20.0**	**14.0**
MODOT11	**Hwy 136 Medicine Creek**	**4479804.668**	**476062.525**	**1800**	**0.2**	**24.0**	**24.0**
MODOT12	Hwy 5 Missouri River	4315044.428	521901.893	2500	0.6	93.0	1.0
MODOT13	**Hwy 50/63 Mari-Osa 1**	**4261276.63**	**586932.474**	**200**	**0.13**	**84.0**	**5.0**
MODOT14	Hwy 50/63 Mari-Osa 2	4260426.315	587200.233	10800	>1.3	37.0	21.0
MODOT15	Hwy 65/70 Blackwater R.	4315978.837	482409.473	2900	>1.3	54.0	29.0
MODOT16	Hwy 139 Big Creek Trib.	4361659.337	471549.889	1400	0.55	35.0	42.0
MODOT17	**Hwy 94 Logan Creek**	**4285047.54**	**609125.208**	**100**	**0.13**	**72.0**	**10.0**
MODOT18	Smithville Lake unit 1	4376991.294	373917.826	17000	>1.3	27.0	36.0
MODOT19	**Smithville Lake unit 2**	**4362993.967**	**375817.669**	**6300**	**>1.3**	**27.0**	**23.0**
MODOT20	**Rte 40 Little Blue R.**	**4320581.281**	**383613.674**	**6000**	**0.45**	**57.0**	**23.0**

[a] Depth measured at maximum pool. Maximum depth that could be measured was 1.3 meters.

[b] % anthropogenic disturbance based on cropland, roads, and urbanization within 300m core terrestrial habitat.

[c] Percentage of forest within 300m core terrestrial habitat. Includes upland, bottomland, and riparian corridors.

Table 2. Correlation coefficient matrices for within-wetland design (a) and landscape placement (b) variables used in regression models.

a)

N = 49	Slope	Veg	Size	THyd
Fish	0.067	- 0.029	0.587	- 0.281
Slope		- 0.437	- 0.083	- 0.668
Veg			- 0.084	0.340
Size				- 0.096

b)

N = 49	Grass	Wet	Pond	Stream	Road
Crop	- 0.165	0.202	- 0.293	0.312	0.143
Grass		- 0.268	0.109	- 0.320	- 0.559
Wetland			- 0.147	0.529	0.374
Pond				- 0.071	- 0.059
Stream					0.239

Table 3. Description of within-wetland design and landscape placement variables used in regression models, including literature sources justifying variable selection.

Variable Type	Variable Name	Description	Source
Within Wetland (Design)	Fish	Fish abundance from aquatic funnel trap CPUE (individuals/trap)	Kats et al. 1988, Skelly 1996, Porej and Hetherington 2005
	Veg	Average percentage of all vegetation (emergent, submerged, and floating) within quadrats	Knutson et al. 2004, Hartel et al. 2007
	Size	Log_{10} size of wetland (m^2)	Semlitsch and Bodie 1998
	Slope	Average slope within 3 meters of Shoreline	Adams et al. 2003, Porej and Hetherington 2005
	THyd	Wetland hydrology in 2006. Temporary = 1; Permanent = 0	Skelly 1995, Semlitsch et al. 1996, Snodgrass et al. 2000
Core Terrestrial Habitat Within 300m (Placement)	Grass	% grassland in core terrestrial habitat (within 300m). Includes pasture and prairie.	Porej et al. 2004
	Crop	% cropland in core terrestrial habitat (within 300m)	Gagné and Fahrig 2007
	Wetland	% wetland of all types (excluding open water ponds) in core terrestrial habitat (within 300m)	Marsh and Trenham 2001, Semlitsch 2002
	Pond	Density of open water ponds in core terrestrial habitat (# of ponds/ha within 300m)	Willis et al. 1956, Adams et al. 2003
	Stream	Density of stream length in core terrestrial habitat (m of stream/ha within 300m)	Williams 2008
	Road	Density of road lane meters in core terrestrial habitat (lane m/ha within 300m)	Fahrig et al. 1995, Findlay and Bourdages 2000, Houlahan and Findlay 2003

Table 4. Habitat variables and statistics measured at study wetlands.

		All Wetlands					MDC					MoDOT				
Variable Type	Variable Name	N	Mean	Min	Max	S.D.	N	Mean	Min	Max	S.D.	N	Mean	Min	Max	S.D.
Within Wetland (Design)	Fish	49	1.18	0.00	11.10	2.51	29	0.68	0.00	11.00	2.10	20	1.91	0.00	11.11	2.90
	Veg	49	52.69	0.00	97.00	29.34	29	46.30	1.00	97.00	28.89	20	61.95	0.00	90.00	28.14
	Size	49	3.00	2.00	4.31	0.63	29	2.76	2.00	3.34	0.37	20	3.33	2.00	4.31	0.77
	Slope	49	0.16 (6.25:1)	0.02 (50:1)	0.52 (2:1)	0.11	29	0.22 (4.5:1)	0.04 (25:1)	0.52 (2:1)	0.10	20	0.07 (14.5:1)	0.02 (50:1)	0.15 (6.75:1)	0.04
	THyd	49	0.31	0.00	1.00	0.47	29	0.14	0.00	1.00	0.35	20	0.55	0.00	1.00	0.51
Core Terrestrial Habitat Within 300 m (Placement)	Grass	49	32.92	2.34	73.96	19.87	29	39.50	2.34	73.96	20.83	20	23.40	4.39	50.00	14.00
	Crop	49	21.66	0.00	69.84	19.46	29	13.40	0.00	48.43	15.09	20	33.67	0.00	69.84	19.10
	Wetland	49	8.52	0.00	55.00	12.81	29	3.60	0.00	0.37	8.70	20	15.70	0.00	55.00	14.50
	Pond	49	0.06	0.00	0.19	0.05	29	0.08	0.00	0.19	0.05	20	0.04	0.00	0.15	0.04
	Stream	49	12.12	0.00	34.97	11.11	29	7.42	0.00	25.28	8.80	20	18.93	0.00	34.97	10.70
	Road	49	31.59	0.00	128.88	30.31	29	15.30	0.00	39.55	13.26	20	55.20	5.63	128.88	32.70

Table 5. Models and component variables for design and placement analyses. All models include the intercept.

Model Type	Model Name	Variables in Model
Within Wetland (Design)	Global	All variables
	Abiotic	Size, Slope, THyd
	Experimental [a]	Fish, Veg, Slope
	Biotic	Fish, Veg
	Mitigation [b]	Size, THyd
	Fish	Fish
	Veg	Veg
	Size	Size
	Slope	Slope
	THyd	THyd
	Null (Intercept only)	Null (Intercept only)
Core Terrestrial Habitat Within 300m (Placement)	Global	All variables
	Land Cover	Grass, Crop, Wetland
	Aquatic Surroundings	Wetland, Pond, Stream
	Mitigation [c]	Stream, Road
	Disturbance	Crop, Road
	Wildlife Area [d]	Grass, Pond
	Grass	Grass
	Crop	Crop
	Wetland	Wetland
	Pond	Pond
	Stream	Stream
	Road	Road
	Null (Intercept only)	Null (Intercept only)

[a] Consists of variables tested to examine their effects on amphibian reproductive success at experimental constructed wetlands in northeast Missouri (See Chapter 3).

[b] The two basic design features of compensatory mitigation wetlands are size and hydroperiod.

[c] Many mitigation wetlands in our surveys were placed in floodplains near roads.

[d] Most MDC wildlife areas included areas of warm season grasses and multiple open water ponds.

Table 6. Analysis of Tweedie regression models for amphibian species abundance using AIC$_{c}$·. Only the highest supported models (ΔAIC ≤ 2.0) are displayed. Models selected based on parsimony are in bold.

Species and Index Parameter	Model Name [a]	[Log(£)]	K [b]	AICc	ΔAIC	W [c]	% Deviance [d]
Cricket Frog 1.5	**THyd (D) (−)**	−15.45	2	35.15	0	0.51	16.6
	Abiotic (D)	−14.35	4	37.62	1.07	0.30	20.1
	Aq. Surroundings (P)	−13.38	4	35.67	0	0.48	23.2
	Stream (P) (+)	−16.63	2	37.52	1.85	0.19	12.8
American Toad 1.525	**Experimental (D)**	−26.84	4	62.60	0	0.39	26.5
	Global (D)	−25.09	6	64.17	1.58	0.18	31.6
	Aq. Surroundings (P)	−21.69	4	52.29	0	0.80	41.5
Gray Treefrog complex 1.1	**Size (D) (−)**	−224.98	2	454.22	0	0.57	32.7
	Mitigation (D)	−224.54	3	455.62	1.40	0.28	33.5
	Global (P)	−220.84	7	458.42	0	0.57	40.7
	Disturbance (P)	−226.38	3	459.30	0.88	0.37	30.0
Spring Peeper 1.45	**Biotic (D)**	−28.89	3	64.32	0	0.64	32.4
	Global (P)	−21.02	7	58.77	0	0.49	52.1
	Land Cover (P)	−25.60	4	60.11	1.34	0.25	40.6
Boreal Chorus Frog 1.125	**Experimental (D)**	−78.08	4	165.07	0	0.58	53.5
	Global (D)	−75.85	6	165.70	0.63	0.42	55.8
	Global (P)	−108.92	7	234.57	0	0.96	21.9
Bullfrog 1.1	**Global (D)**	−173.55	6	361.09	0	0.83	32.8
	Global (P)	−172.15	7	361.03	0	0.46	35.2
	Aq. Surroundings (P)	−176.42	4	361.76	0.72	0.32	27.8
	Wildlife Area (P)	−178.10	3	362.72	1.69	0.20	24.9
Green Frog 1.6	**Experimental (D)**	−16.518	4	41.95	0	0.54	21.7
	Wetland (P) (−)	−19.89	2	44.05	0	0.45	12.6
	Aq. Surroundings (P)	−18.34	4	45.59	1.55	0.21	16.8
Leopard Frog complex 1.1	**THyd (D) (+)**	−269.92	2	544.10	0	0.43	25.8
	Slope (D) (−)	−270.75	2	545.76	1.66	0.19	23.7
	Abiotic (D)	−268.48	4	545.86	1.76	0.18	29.6
	Land Cover (P)	−265.88	4	540.68	0	0.42	36.2
	Wetland (P) (+)	−268.62	2	541.49	0.81	0.28	29.2
	Aq. Surroundings (P)	−266.38	4	541.66	0.98	0.26	35.0
Smallmouth Salamander 1.625	**Fish (D) (−)**	−17.26	2	38.79	0	0.54	29.4
	Biotic (D)	−16.74	3	40.00	1.22	0.29	31.0
	Grass (P) (+)	−19.12	2	42.49	0	0.26	24.1
	Stream (P) (−)	−19.63	2	43.53	1.04	0.16	22.6
	Land Cover (P)	−17.32	4	43.55	1.05	0.16	29.3
	Mitigation (P)	−18.51	3	43.56	1.07	0.15	25.9
Combined Salamanders 1.55	Global (D)	−17.83	6	49.66	0	0.57	45.1
	Experimental (D)	−21.36	4	51.64	1.98	0.21	36.1
	Disturbance (P)	−21.48	3	49.50	0	0.43	35.8
	Land Cover (P)	−20.37	4	49.65	0.15	0.40	38.6

[a] D = Design; P = Placement. Single variable model associations are indicated with (+) or (-). Parameter estimates for selected models are shown in Table 7.

[b] *K* = number of estimable parameters in the model, including the intercept.

[c] *W* = Akaike weight can be interpreted as the probability that the model is the best-approximating model in the set.

[d] % Deviance is the reduction in deviance for the model divided by the deviance of the null (intercept-only) model.

Table 7. Parameter estimates of the most parsimonious model for each amphibian.

Species	Parameter	*B*	S.E.	Wald χ^2	*p*-value	95% C.I.	
						Lower	Upper
Cricket Frog	THyd (D)	−2.39	0.77	9.54	0.002	−3.90	−0.87
	Wetland(P)	−2.97	3.35	0.79	0.375	−9.53	3.59
	Pond (P)	12.04	5.16	5.44	0.020	1.92	22.15
	Stream (P)	0.09	0.03	10.23	0.001	0.03	0.14
American Toad	Fish (D)	0.24	0.09	7.75	0.005	0.07	0.42
	Veg (D)	0.85	1.26	0.45	0.500	−1.62	3.32
	Slope (D)	−9.71	4.00	5.90	0.015	−17.54	−1.88
	Pond (P)	−33.23	10.71	9.62	0.002	−54.23	−12.23
	Stream (P)	0.054	0.03	3.65	0.056	−0.001	0.11
	Wetland (P)	−14.36	5.91	5.90	0.015	−25.95	−2.77
Gray Treefrog complex	Size (D)	−2.57	0.53	23.24	0.000	−3.62	−1.53
	Crop (P)	−5.73	1.78	10.32	0.001	−9.23	−2.23
	Road (P)	−0.06	0.02	10.02	0.002	−0.10	−0.02
Spring Peeper	Fish (D)	−2.00	0.65	9.48	0.002	−3.27	−0.73
	Veg (D)	3.50	1.12	9.71	0.002	1.30	5.70
	Crop (P)	−7.72	2.92	7.00	0.008	−13.45	−1.99
	Grass (P)	0.96	1.60	0.36	0.548	−2.12	4.11
	Wetland (P)	−27.13	13.12	4.28	0.039	−52.85	−1.42
Chorus Frog	Fish (D)	−0.67	0.31	4.81	0.028	−1.27	−0.07
	Veg (D)	5.06	1.13	20.26	0.000	2.86	7.27
	Slope (D)	−9.96	3.02	10.90	0.001	−15.87	−4.05
	Crop (P)	0.23	1.20	0.04	0.845	−2.12	2.59
	Grass (P)	6.85	1.54	19.87	0.000	3.84	9.86
	Wetland (P)	4.91	1.29	14.42	0.000	2.38	7.45
	Pond (P)	−7.16	3.84	3.48	0.062	−14.68	0.36
	Stream (P)	0.05	0.02	8.51	0.004	0.02	0.08
	Road (P)	0.013	0.01	1.29	0.256	−0.01	0.04
Bullfrog	Fish (D)	0.17	0.05	13.14	0.000	0.08	0.26
	Veg (D)	1.06	0.61	2.96	0.085	−0.15	2.26
	Slope (D)	3.13	1.79	3.07	0.080	−0.37	6.64
	Size (D)	−0.25	0.37	0.43	0.512	−0.98	0.49
	Thyd (D)	−2.46	1.00	6.01	0.014	−4.42	−0.49
	Wetland (P)	−8.86	3.66	5.87	0.015	−16.03	−1.69
	Pond (P)	10.18	2.89	12.40	0.000	4.52	15.85
	Stream (P)	0.002	0.02	0.01	0.916	−0.04	0.04
Green Frog	Fish (D)	0.24	0.08	8.15	0.004	0.08	0.40
	Veg (D)	0.64	0.90	0.50	0.478	−1.13	2.40
	Slope (D)	6.99	2.43	8.27	0.004	2.23	11.75
	Wetland (P)	−8.21	2.61	9.86	0.002	−13.33	−3.09
Leopard Frog complex	Thyd (D)	1.80	0.42	18.0	0.000	0.97	2.63
	Wetland (P)	5.32	1.03	26.71	0.000	3.30	7.33
Smallmouth Salamander	Fish (D)	−6.06	1.86	10.63	0.001	−9.71	−2.42
	Grass (P)	8.15	2.09	15.22	0.000	4.06	12.25
Combined Salamanders	Fish (D)	−4.67	1.76	7.05	0.008	−8.12	−1.22
	Veg (D)	3.30	1.36	5.84	0.016	0.62	5.97
	Slope (D)	6.82	3.81	3.21	0.073	−0.64	14.28
	Crop (P)	−9.26	2.09	19.70	0.000	−13.36	−5.17
	Road (P)	−0.03	0.02	4.64	0.031	−0.06	−0.003

Table 8. Top three individual variable weights for each amphibian species/group at the design and placement levels. Individual variable weights can be interpreted as the relative importance of each variable within the model set.

Species	Design Parameter	*w*	Placement Parameter	*w*
Cricket Frog	THyd	0.98	Stream	0.86
	Size	0.47	Pond	0.62
	Slope	0.31	Wetland	0.56
American Toad	Slope	0.79	Pond	0.97
	Fish	0.64	Wetland	0.86
	Veg	0.63	Stream	0.85
Gray Treefrog complex	Size	0.99	Crop	0.99
	THyd	0.43	Road	0.94
	Slope	0.15	Grass	0.63
Spring Peeper	Fish	0.99	Wetland	0.89
	Veg	0.98	Crop	0.75
	Slope	0.34	Grass	0.74
Chorus Frog	Fish	0.99	Crop	0.99
	Veg	0.99	Grass	0.99
	Slope	0.99	Wetland	0.99
Bullfrog	THyd	0.93	Pond	0.98
	Slope	0.91	Wetland	0.80
	Fish	0.90	Stream	0.78
Green Frog	Slope	0.73	Wetland	0.73
	Fish	0.70	Pond	0.39
	Veg	0.64	Stream	0.25
Leopard Frog complex	THyd	0.79	Wetland	0.99
	Slope	0.42	Grass	0.47
	Size	0.35	Crop	0.47
Smallmouth Salamander	Fish	0.99	Grass	0.59
	Veg	0.46	Stream	0.49
	Slope	0.18	Wetland	0.33
Combined Salamanders	Fish	0.99	Crop	0.99
	Veg	0.91	Road	0.48
	Slope	0.78	Wetland	0.46

Figure 1. Locations of surveyed wetlands in northern Missouri. Some dots represent two wetlands due to proximity.

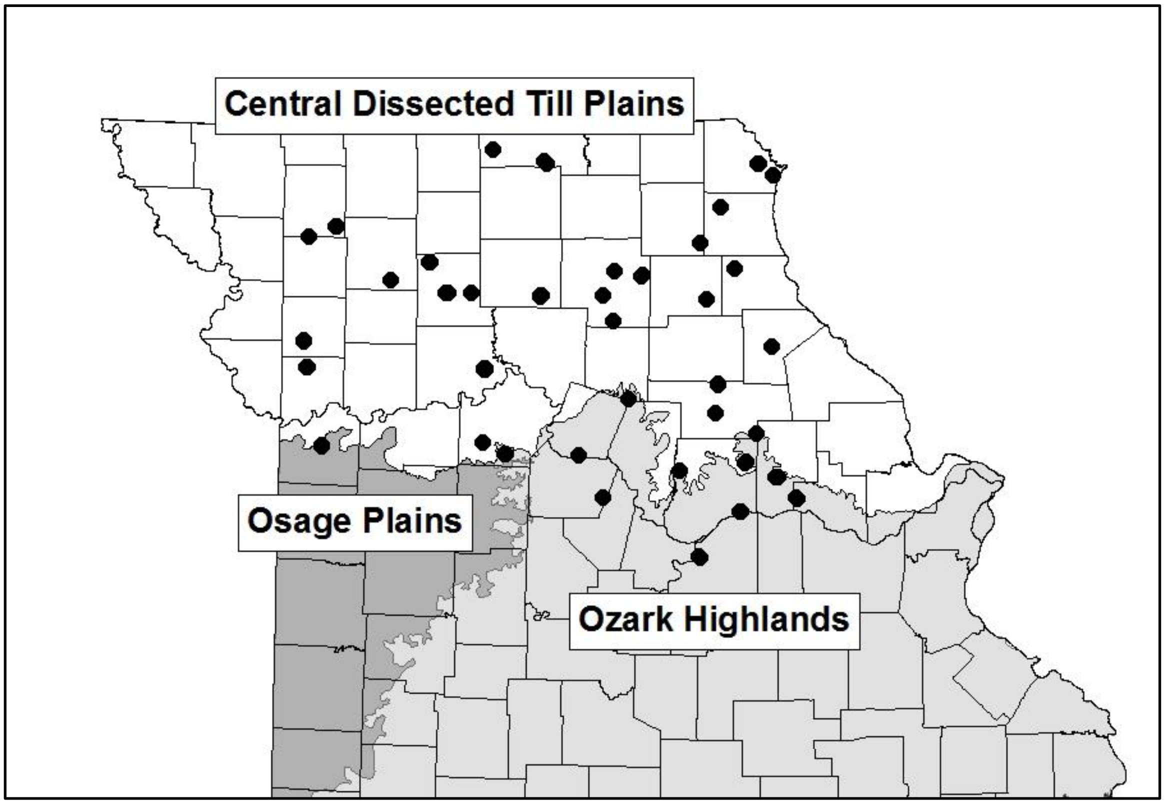
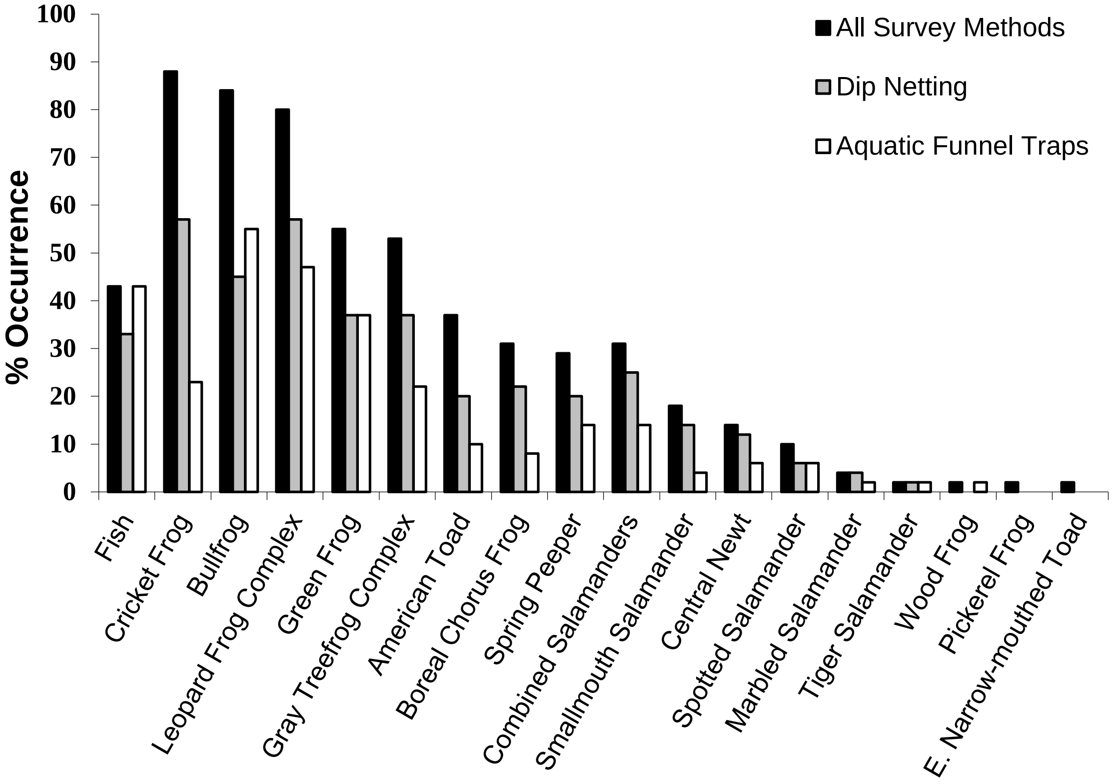

Central Dissected Till Plains
Osage Plains
Ozark Highlands

Figure 2. Total occurrence and capture method comparisons of amphibians and fish in surveyed wetlands. For most species, dip netting resulted in captures at more wetlands than aquatic funnel traps. Pickerel frog and eastern narrow-mouthed toad were only observed during visual or acoustic sampling.



100
90
80
70
60
50
40
30
20
10
0
% Occurrence
All Survey Methods
Dip Netting
Aquatic Funnel Traps
Fish
Cricket Frog
Bullfrog
Leopard Frog Complex
Green Frog
Gray Treefrog Complex
American Toad
Boreal Chorus Frog
Spring Peeper
Combined Salamanders
Smallmouth Salamander
Central Newt
Spotted Salamander
Marbled Salamander
Tiger Salamander
Wood Frog
Pickerel Frog
E. Narrow-mouthed Toad

CHAPTER 3

TESTING WETLAND FEATURES TO INCREASE AMPHIBIAN REPRODUCTIVE SUCCESS AND SPECIES RICHNESS FOR MITIGATION AND RESTORATION

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ABSTRACT

Aquatic habitat features can directly influence the species richness, abundance, and quality of juvenile amphibians recruited into adult populations. We examined the effects of within-wetland slope, vegetation, and stocked mosquitofish (*Gambusia affinis*) on amphibian metamorph production and species richness at 18 constructed experimental wetlands in northeast Missouri grasslands. General linear models revealed mosquitofish significantly affected metamorph production in both survey years. Vegetation structure had significant effects on production the second year. We also used an information theoretic approach (AIC_c) to rank regression models representing total amphibian production, individual species reproduction, and species richness. Total production was greatest at shallow-sloped, fish-free wetlands during the first year, but shallow-sloped wetlands with high vegetation cover were best the second year. Species richness was negatively associated with fish and positively associated with vegetation in both survey

years. Our results indicate that wetland designs should incorporate shallows, high amounts of planted or naturally-established vegetation cover, and should be fish-free.

INTRODUCTION

Restoration ecology by its very nature is manipulative and can provide valuable opportunities to test the effectiveness of various ecological factors in restoring regional biodiversity (Young 2000; Young et al. 2005). Restoration or mitigation often resets successional processes, restarts disturbance regimes, and can favor invasive species proliferation, especially in aquatic ecosystems (Zedler 2000; Young et al. 2001). Predicting ecological succession is challenging in all restoration projects but is particularly important for wetland restorations where regulatory constraints call for specific outcomes on small sites in relatively short time periods (Zedler 2000). While "successful" wetland restoration can be based simply upon establishing a minimum hydroperiod and hydric vegetation, ensuring habitat and species diversities are often overlooked. Once wetland construction is complete, it is often assumed that wildlife will colonize and successfully reproduce. The presence of habitat generalists may reinforce this "build it and they will come" philosophy and their presence can conceal the shortcomings of restoration efforts for habitat specialists that may suffer the most from natural wetland losses.

Constructed wetlands are generally devoid of aquatic topographic and hydroperiod diversity resulting in a predominance of open water lacking more desirable natural wetland characteristics (National Research Council 2001; Porej 2003; Shulse et al. 2010). Aquatic vegetation structure, topography, and hydroperiod are some of the most critical factors influencing the community composition and reproductive success of wetland fauna (Semlitsch et al. 1996; Pechmann et al. 1989, 2001; Brooks 2000; Fairbairn and Dinsmore 2001; Porej and Hetherington 2005). For example, studies have indicated aquatic breeding amphibians benefit from vegetated littoral zones that provide refuge from predators, areas for foraging and thermoregulation, substrates for oviposition, and calling sites for male anurans (Stratman 2000; Semlitsch 2002; Hazell et al. 2004; Hartel et al. 2007). The effects of fish and other predators are also mitigated by vegetation and aquatic features (Babbitt & Tanner 1997; Tarr & Babbitt 2002). However, predators small enough to penetrate dense vegetation, and those that forage in shallows frequented by larval amphibians, will likely reduce reproductive success. These reductions may be especially dramatic when predator density is high.

Mosquitofish (Western - *Gambusia affinis,* Eastern - *G. holbrooki*) are small poeciliids that are native to the southern United States but have been introduced throughout the world for mosquito control. Mosquitofish often occur in high densities and there is a growing body of evidence indicating they negatively impact native ecosystems and their indigenous fauna, including amphibians (Pyke 2008). Mosquitofish reproduce rapidly, they can easily colonize new water bodies through surface water connections from release sites, and they tolerate pollution and low dissolved oxygen (Lawler et al. 1999). Although they forage in shallows and can penetrate dense vegetation, it is unclear if these habits increase their exposure to larval amphibians or if vegetation attenuates predation risk (Webb & Joss 1997; Lawler et al. 1999).

While amphibians are important indicators of the quality of aquatic communities and their associated terrestrial environments (Micacchion 2004), they are often overlooked when designing or monitoring restored or newly constructed wetlands (National Research Council 2001; Porej & Hetherington 2005). Amphibians provide a significant biological nexus between wetland and upland habitats by transferring large amounts of energy, nutrients, and biomass between the two ecosystems (Gibbons et al. 2006; Semlitsch & Skelly 2007). Most species require suitable aquatic and terrestrial habitats to complete their life cycles, posing challenges for both design and placement of wetlands in the landscape (Shulse et al. 2010). While both aquatic and nearby landscape features are necessary to consider during wetland planning, appropriately designed aquatic habitat will directly influence the species, number, and quality of metamorphosing juveniles recruited into terrestrial adult populations (Semlitsch 2002), and the persistence of species within wetland ecosystems (Hamer & Parris 2010).

Here, we examine the assembly of amphibian communities in experimental wetlands constructed *de novo* as can often be the case in wetland restoration and mitigation. Although multiple observational studies have revealed correlations between amphibian populations and the presence or abundance of fish, vegetation cover, or slope (i.e., Knutson et al. 2004; Porej & Hetherington 2005; Shulse et al. 2010), none have explicitly tested these features. Our objective was to test the effects of mosquitofish, littoral zone slope, and vegetation cover on amphibian metamorph production and species richness at replicated constructed wetlands. We hypothesized that both species richness and reproduction would be reduced in wetlands with mosquitofish, but increased by planted vegetation and shallow within-wetland slopes.

METHODS

Study Areas and Design

During October and November 2006, we constructed replicate wetland arrays at three Missouri Department of Conservation (MDC) upland grassland habitats in northeastern Missouri (Figure 1). This region of Missouri has 16 species of pond-breeding amphibians (Daniel & Edmond 2010). Each grassland area had forest nearby, but in varied amounts. We attempted to place wetlands at roughly equal distances from forest cover within each study site to control dispersal distance from potential source populations. We also attempted to place wetlands at roughly equal distances from each other within study sites, but distances varied due to placement limitations (63 – 242 m, mean 117.5 m). We built six wetlands at each study site (n = 18 wetlands) and randomly assigned one of the six combinations of littoral zone slope (steep 4:1 vs. shallow 15:1), mosquitofish (*Gambusia affinis* – stocked vs. un-stocked), and vegetation (planted vs. unplanted; Table 1) to each wetland. Each wetland was round, 23 m diameter, and 0.76 m maximum depth. Shallow-sloped wetlands came to a point at the center where maximum depth was reached. Steep-sloped wetlands had 16 m diameter circular bottoms 16 m at maximum depth (Figure 2). Wetlands were constructed using heavy equipment and a laser level was used to verify depth, slope, and size.

We chose to use 15:1 for the shallow-sloped design based on the definition of "shallows" in Porej and Hetherington (2005); the authors of that study state that the Ohio Environmental Protection Agency incorporated this ratio into replacement wetland designs. The authors also encouraged other regulatory agencies to do the same to avoid negative impacts on amphibians (Porej and Hetherington 2005). We chose 4:1 as the steep-sloped design because this is the steepest bank slopes incorporated into mitigation wetlands constructed by the Missouri Department of Transportation (MoDOT; B. Brooks, personal communication). Additionally, many open water agricultural or wildlife ponds in northern Missouri are constructed with bank slopes of 4.5:1 or steeper (Deal et al. 1997; Perry 2006; Shulse et al. 2010).

We stocked mosquitofish because these fish have been implicated amphibian population declines in California, Australia, and China (Lawler et al. 1999; Pyke & White 2000; Karraker et al. 2010). Although native to southeastern Missouri, *G. affinis* has been expanding in distribution into northern Missouri through natural dispersal and undocumented introductions (Pflieger 1997). In a recent study involving surveys of constructed wetlands in northern Missouri, we captured mosquitofish in 20% of all wetlands surveyed (Shulse et al. 2010) and 45% of compensatory mitigation wetlands (Shulse & Semlitsch, unpublished data). Mosquitofish were purposely introduced into one mitigation wetland for mosquito control in 2003 (B. Brooks, MoDOT, personal communication). We obtained mosquitofish from this wetland and introduced them within the randomly assigned experimental wetlands at a rate of 3,089 fish/ha which is slightly higher than the rate of 2,471 fish/ha (1,000 fish/acre) recommended by Duryea et al. (1996). This rate resulted in a founding population of 125 adult mosquitofish per stocked wetland in March 2007. Fish were re-stocked into wetlands where samples indicated low populations during early spring 2008.

Prairie cordgrass (*Spartina pectinata*) plants were added to the wetlands receiving vegetation treatments. Cordgrass is routinely used to re-vegetate new compensatory mitigation wetlands constructed by MoDOT (B. Brooks, personal communication).

Plants were purchased in 3.8 liter pots and most were split in half prior to planting. Each wetland was planted with 50 divisions spaced approximately evenly apart and radiating from the center, and an un-divided plant was placed in the center (Figure 3). Plants that did not survive the first growing season were replaced during September and October 2007. All other vegetation was allowed to colonize naturally in both planted and unplanted wetlands.

Sampling

Each wetland was completely surrounded by aluminum flashing drift fence (60 cm tall) buried to depths of 10 – 15 cm. Paired pitfall traps were placed 5 m apart along the drift fence. Pitfall traps consisted of plastic pots (23 cm diameter x 39 cm deep) with holes drilled in the bottom for drainage and a sponge for moisture retention. Traps were generally checked every other day from May to August but were checked less frequently (at least twice per week) from mid-August to mid-September. In 2007, traps were checked from 23 April to 16 September and, in 2008, from 12 May to 9 September. Adults captured in outside traps were released inside of the drift fences to facilitate breeding access to the wetlands. Juveniles captured in outside traps were excluded and released outside of fences assuming they were dispersing from other wetlands. All animals captured inside the fences were released outside after recording species, snout-vent length (SVL; 5 – 10 individuals for large cohorts), and sex (adults). Some individuals were captured by hand inside fences and released outside after recording.

Mosquitofish were sampled three times each season using aquatic funnel traps and dip nets. Aquatic funnel trapping occurred for 48 hours per sampling session using two kinds of commercially available minnow traps: collapsible nylon mesh traps (38 x 26 x 26 cm; 3 mm mesh, 6 cm openings) or galvanized steel wire traps (42 cm long; 6 mm mesh, 2.5 cm openings). Two traps of each kind were used per wetland and they were staggered so that traps of each kind were directly across from one another at each cardinal direction. Assignment of pair direction was randomly chosen. One dip net (3mm nylon mesh) sweep (~1.5 m long) was conducted from the water's edge at each cardinal direction with the net pressed to the substrate and pulled toward the sampler. During the second sampling period in 2007, a canvas D-net with 500 micron mesh bottom was added to the sampling protocol to capture macroinvertebrates and zooplankton. Approximately 1.5 m sweeps occurred at each ordinal direction using the canvas net. This resulted in 4 dip net sweeps and 4 canvas net sweeps spaced evenly apart for each wetland during each sampling period. Data from all aquatic sampling methods were combined to calculate mosquitofish abundance and amphibian species richness. Larval and metamorph amphibians captured during aquatic habitat sampling were included in species richness analyses even if no metamorphs of a particular species were captured in pitfall traps.

Within-wetland vegetation cover during 2008 was measured using four 1 m^2 quadrats spaced at cardinal directions around the wetland perimeter. Quadrats were placed at the edge of the wetland to assess vegetation cover within 1 m of the shore and at 3 m from the shore. The percentages of open water, emergent, floating, and submerged vegetation were visually estimated within each quadrat. The three vegetation categories were combined and averaged for all quadrats over all sampling periods at each wetland to calculate an average measure of vegetation cover for each year. Percent vegetation cover was transformed to the arcsine square root of the proportion for analyses.

Data Analysis

All statistical analyses were performed using SPSS version 16.0 (2007 SPSS Chicago, Illinois). We used the cumulative number of metamorphs each year of each species produced at each wetland as dependent variables in multivariate general linear models to test the hypothesis that treatments influence total amphibian reproductive success. We transformed dependent variable data for general linear models by using the base-10 log of the number of individuals captured plus one. We combined data obtained from captures of southern leopard frog (*Lithobates sphenocephalus*) and plains leopard frog (*L. blairi*) because some metamorphs could not be identified definitively. Similarly, we were unable to distinguish between eastern gray treefrogs (*Hyla versicolor*) and Cope's gray treefrogs (*Hyla chrysocelis*). Because all wetland treatment combinations were not possible and our experimental design was not fully factorial, we tested two models for each sampling year (2007 and 2008; four models total). The first model tested the treatments of slope and fish using only the wetlands where no vegetation was added. The second model tested the treatments of fish and added vegetation. This procedure resulted in only 12 wetlands instead of all 18 being included in each model. Therefore, we increased the alpha level to 0.1. Further, in the second year, natural vegetation establishment in some wetlands outpaced those that received the vegetation treatment so we used the continuous variable of vegetation cover as a covariate in our analyses for 2008.

To explain the relationships between total amphibian production, individual species production, and the independent wetland design variables, we developed regression models with a negative binomial distribution and log link function using the generalized linear model option in SPSS. To explain species richness relationships, we developed regression models with a normal distribution and identity link function. Each set contained models with combinations of all three independent variables (global - fish, vegetation, slope), two variable models, single variable models, and an intercept only model. All models included the intercept. We used mosquitofish abundance from aquatic sampling data instead of fish treatment to account for differences in fish populations that may have developed between stocked wetlands. Similarly, for 2008 analyses, we used vegetation cover as an independent variable, as opposed to the vegetation treatment, to account for natural re-vegetation differences. Slope was assumed to remain constant.

We used an information theoretic approach to compare candidate regression models and avoid over-fitting (Burnham & Anderson 2002). Akaike's Information Criterion values corrected for small sample size (AIC_c) were obtained from SPSS output and the model within each category with the smallest value was selected as most supported. The remaining models were ranked according to their differences in AIC_c from the most supported model in the set (ΔAIC). We calculated Akaike weights (W) and individual variable weights (w) to make inferences among highly supported candidates and to assess the relative importance of each variable within the entire model set (Burnham & Anderson 2002). We also calculated the percent deviance explained for each model by dividing the reduction in deviance for the full model by the deviance of the null model (Simon et al. 2009).

Although our primary objective was to examine the effects of the treatment variables on metamorph production and species richness, we conducted limited *post hoc* tests to investigate treatment effects on metamorph quality. We performed univariate analyses of variance using average metamorph size of American toads (*Anaxyrus americanus*), boreal chorus frogs (*Pseudacris maculata*), and leopard frogs as dependent variables. We also performed linear regression analyses for these species to further investigate influences of design variables on metamorph quality. Because cohort density can influence metamorph size (VanBuskirk and Smith 1991), we used hierarchical regression to control for and investigate the effects of individual species metamorph production. Due to limited data for 2007, we restricted size investigations to 2008 data.

RESULTS

Metamorph Production

In 2007, we captured 13,496 metamorphs of five amphibian species exiting the experimental wetlands (Table 2). Most (over 10,700) were American toads that were produced at a single wetland that was shallow-sloped, not stocked with fish, and not planted with vegetation. Gray treefrog and boreal chorus frog metamorphs were almost exclusively captured leaving fish-free wetlands. Leopard frog metamorphs were only captured exiting shallow sloped wetlands and, with the exception of a single individual, only captured in wetlands with planted prairie cordgrass. The only wetland treatment combination that resulted in captures of all five amphibian species detected was the shallow-sloped, no fish, and vegetation planted design (Figure 4a). Over 95% of all metamorphs were produced at shallow-sloped wetlands. Excluding toads, 98% of metamorph production occurred in shallow-sloped wetlands. Vegetation development was slow during 2007. An independent-samples t-test was conducted to compare average annual vegetation cover for planted and non-planted wetlands. There was a significant difference in scores for planted (mean ± s.d. = 0.22 ± 0.02) and non-planted (0.03 ± 0.05; t_{16}= -8.47, $p \leq 0.001$ [two-tailed]).

In 2008, we captured four times more metamorphs (56,617) and twice the number of metamorph amphibian species (10) leaving the experimental wetlands (Table 2). Again, American toads were the most abundant with nearly 29,000 produced. Over 18,000 were again produced at a single wetland (shallow-sloped, fish stocked, vegetation added), but adjacent to the wetland that produced the largest numbers in 2007 (Figure 4b). However, only American toads, boreal chorus frogs, and leopard frogs were captured in enough numbers to include in general linear models and individual species regression models. Natural vegetation establishment in some of the non-planted wetlands outpaced vegetation cover in the planted wetlands during 2008. There was no significant difference in scores for planted (0.55 ± 0.13) and non-planted (0.47 ± 0.29; t_{16} = -0.64, p = 0.53 [two-tailed]). Over 93% of metamorph production in 2008 was in shallow-sloped wetlands (89% with toads excluded).

General Linear Models

The first MANOVA for 2007 contained the treatment variables mosquitofish and slope, and the dependent variables were American toads, gray treefrogs, and boreal chorus frogs. There was no statistically significant effect of slope or the interaction between fish and slope on the combined species (all $p \geq 0.49$). However, mosquitofish stocking significantly reduced the abundance of emerging metamorphs ($F_{3,6}$ = 5.02, p = 0.04; Wilks' Lambda = 0.29).

The second MANOVA for 2007 contained the treatment variables mosquitofish and planted cordgrass and the dependent variables were American toads, gray treefrogs, chorus frogs, and leopard frogs. There were no statistically significant effects of mosquitofish, cordgrass, or the interaction on the combined species (fish $p = 0.27$; vegetation $p = 0.60$; fish x vegetation $p = 0.99$).

Based on the results of our t-test comparing re-vegetation in wetlands during 2008, we used vegetation cover as a covariate in our MANCOVAs. Only toads, chorus frogs, and leopard frogs were captured in enough numbers to include as dependent variables. While the model containing the independent variables mosquitofish and slope did not reveal a significant slope effect ($p = 0.85$), mosquitofish reduced metamorph abundance, but the effect was only marginal ($F_{3,5} = 3.04$, $p = 0.13$; Wilks' Lambda = 0.35; observed power 0.57). Vegetation cover significantly increased abundance ($F_{3,5} = 4.08$, $p = 0.08$; Wilks' Lambda = 0.29; observed power 0.68), but there were no statistically significant effects of the fish x slope interaction ($p = 0.29$). The ANCOVAs revealed no significant effects of mosquitofish or slope on either boreal chorus frog or leopard frog metamorph average size (all $p>0.30$), but the average size of American toads was significantly increased by vegetation cover ($F_{1,4} = 8.52$, $p = 0.04$). The model also revealed a significant fish x slope interaction ($F_{1,4} = 9.92$, $p = 0.04$).

The fish and vegetation MANCOVA for 2008 revealed that fish significantly reduced metamorph production ($F_{3,5} = 5.1$, $p = 0.06$, Wilks' Lambda = 0.25; observed power 0.77) and the interaction of fish and vegetation treatment was also significant ($F_{3,5} = 3.6$, $p = 0.1$, Wilks' Lambda = 0.32; observed power 0.64). There were no significant effects of either vegetation treatment ($p = 0.38$) or vegetation cover ($p = 0.23$) alone. The mosquitofish and vegetation treatment ANCOVA again revealed American toad metamorphs were significantly larger as the covariate vegetation cover increased ($F_{1,5}$ = 6.02, p = 0.06; observed power 0.68), and there was a marginal fish x vegetation treatment interaction ($F_{1,5}$ = 2.68, p = 0.16; observed power 0.41). Additionally, vegetation cover significantly increased leopard frog metamorph size ($F_{1,6}$ = 4.45, p = 0.08; observed power 0.59), but the variables had no significant effects on boreal chorus frog size (all p>0.5).

Regression Models

All of the global models for metamorph production and species richness fit the data and were significant (all P<0.01) except one. During 2008, leopard frog metamorphs were nearly ubiquitous and were captured exiting almost all wetlands. All three variables were included in the leopard frog global model, but the model did not fit the data (χ^2 = 2.1, df = 3, P=0.55). Therefore, we did not analyze any further models for this species using production data from the second year.

Leopard frog metamorphs were only captured leaving shallow-sloped wetlands during the first year. Therefore, only the variables fish abundance and vegetation treatment were included in regression models for these amphibians. The best model explaining leopard frog abundance was VEG. Leopard frogs were positively associated with the planted cordgrass and we found only one metamorph exiting a wetland that was not planted. Our results are reflected in the individual variable weight of 1 for vegetation.

Total metamorph production during the first year post-construction (2007) was best explained by a negative association with littoral zone slope according to AIC analyses (Tables 3 & 4). Two additional models (FISH and FISHSLOPE) also had high empirical support (ΔAICs ≤ 2) and total metamorph production was negatively associated with both variables. Slope also had the highest weight as an individual variable (w = 0.78), but the amount of deviance explained by the SLOPE model, and the other candidate models, was rather low (<16).

During 2008, total production was best explained by the VEGSLOPE model (W = 0.45) but the single variable VEG model almost ranked as high (W = 0.35, ΔAIC = 0.52). Total production was positively associated with vegetation cover, but, as in the first year, negatively associated with slope. As an individual variable, vegetation cover was very important within the set of candidate models (w = 0.99).

Species richness during the first sampling year was best explained by the FISHVEG model. Richness was negatively associated with fish abundance but positively influenced by the presence of planted vegetation. Vegetation had the highest individual variable weight (w = 0.89) followed by fish (w = 0.71). For the 2008 data, FISHVEG was again the highest ranking model explaining species richness. The FISH model was the second highest ranked and also had a high individual variable weight (w = 0.83). Vegetation cover was also relatively important in the 2008 model set (w = 0.66).

Although the percent deviance of the American toad global model for 2007 was low (12.1), their abundance was best explained by the VEGSLOPE model and they were negatively associated with both variables. The SLOPE model also had high support (ΔAIC = 1.5) and was highly ranked as an individual variable (w = 0.77). The VEGSLOPE model was again ranked highest in 2008. However, toads were positively associated with vegetation cover during the second year. Several models had high empirical support within the set, but all contained a positive association with vegetation cover and/or a negative association with slope. As individual variables, both vegetation cover and slope ranked highly (*w* = 0.77 and 0.75 respectively).

Gray treefrogs were only captured in numbers great enough to include in individual species regression models during the first year post-construction and they were negatively associated with mosquitofish. The FISH model best explained their abundance and it was very highly ranked as an individual variable (w = 0.99).

The FISHSLOPE model best explained chorus frog abundance during the first year. Chorus frogs were negatively associated with both variables and no other models received high AIC support. Both fish abundance and slope had individual variable weights of 0.99. During the second year, FISHVEG replaced FISHSLOPE as the best model. Chorus frogs were again negatively associated with fish abundance, but they were also positively associated with vegetation cover. The individual variable weight for fish was again very high (*w* = 0.99) with vegetation cover a close second (*w* = 0.97). The global model for the second year was the second most highly ranked model in the set (*W* = 0.28, ΔAIC = 1.8). Chorus frogs were again negatively associated with steep wetland slopes but this relationship was not highly ranked by our AIC analyses. Only 366 of the 18,362 chorus frog metamorphs captured during the second year emerged from steep-sloped wetlands.

Linear regression analyses revealed little influence of the wetland design features on average American toad and boreal chorus frog metamorph size. Both models had adjusted R-square values < 1 and no significant relationships. The results for leopard frogs were stronger. Mosquitofish abundance, slope, and vegetation cover were entered in Step1 and explained 34.8% of the variance based on adjusted R-square. After adding leopard frog metamorph production in Step 2, the total variance explained by the model was 52.2%, $F_{4,11} = 5.1$, $p = 0.01$). Leopard frog metamorph production explained an additional 17% of the variance, $F_{1,11}$ change = 5.37, $p = 0.04$. In the final model, only slope ($\beta = 0.67$, $p = 0.004$) and leopard frog metamorph production ($\beta = -0.42$, $p = 0.04$) had statistically significant influences on size.

DISCUSSION

Our results provide valuable insight into the alternative pathways of ecological succession when different aquatic features are used by restoration ecologists, and the effects of introducing mosquitofish into wetland construction efforts. Wetland design directly influences amphibian species richness, reproductive success, and assembly of the amphibian community. Incorporating shallow littoral zones and encouraging vegetation maximizes amphibian colonization and bolsters reproductive output. We found that reproductive success of leopard frogs, toads, and chorus frogs was highest in shallow-sloped wetlands during the first year and this trend continued for toads and chorus frogs during the second year. Planted vegetation was also important for bolstering leopard frogs during the first year. Chorus frogs and gray treefrogs were almost exclusively produced at fish-free wetlands, and amphibian diversity was highest in shallow-sloped, fish-free wetlands with high vegetation cover.

While many studies have shown that fish are detrimental to amphibians (e.g., Kats et al. 1988; Snodgrass et al. 2000; Pope 2008; Hamer and Parris 2011), *Gambusia affinis* warrants special caution because of its widespread use, ease of spread, ability to survive a wide range of aquatic conditions including under thick winter ice in northern Missouri

(Shulse & Semlitsch, personal observation); and the widespread perception that it is benign to native wildlife (Pyke 2008). Our results contribute to the growing body of evidence that these invasive fish reduce regional biodiversity, displace native species, and have broad implications for restoration efforts. Adding an alien species (regardless of intention) can dramatically affect ecological succession pathways by excluding some early successional species and reducing the reproductive success of others. Additionally, simply adhering to the “build it and they will come” philosophy of restoration ignores ecological succession and alternative stable states. How restoration sites are built dictates who “they” are, while site maintenance and natural succession determine species that remain and those that later colonize.

Metamorph Production

During the first year post-construction, amphibian production at the wetlands was predictably dominated by early colonizers (Table 2; Figure 4a). These species are adapted to seasonal temporary wetlands that have low predator loads, and their larvae actively search for resources (Skelly 1995). Therefore, at this early stage of wetland development, cover may not be as essential as access to food such as algae and phytoplankton that develop in the shallow, highly illuminated littoral zones. Sunlight is also important for thermoregulation allowing larvae to maximize metabolic activity and growth so that they metamorphose before wetlands dry. Because of their high activity levels, larvae of early colonizers would be vulnerable to predation without some defense mechanism (i.e., unpleasant taste or altered behavior). Unless they are introduced, new constructed wetlands (regardless of their designed hydroperiods) contain few predators during the first year. Some early colonizing amphibians, such as chorus frogs and gray treefrogs, may avoid wetlands containing predators first and then select wetlands that contain features that maximize growth and lower the time to metamorphosis.

Breeding adult amphibians are capable of actively avoiding wetlands with fish (Binckley and Resetarits 2008). During early spring reconnaissance visits, loud diurnal choruses of *P. maculata* were often heard in and around the fish-free experimental wetlands, particularly those with shallow slopes, but none were heard in and around those stocked with fish (Shulse, personal observation). Because some chorus frog metamorphs emerged from fish-stocked sites, low numbers of breeding adults may have selected wetlands with fish during peak breeding activity to avoid intraspecific larval completion (Binkley and Resetarits 2008). American toads, on the other hand, are unpalatable to fish (Kats et al. 1988) and appear to prefer wetlands with shallows regardless of fish presence; a finding consistent with Porej and Hetherington (2005).

The results of our general linear models indicate that there is a significant negative effect of *Gambusia* stocking on amphibian reproductive success. This was also apparent within our regression models but only for the first year. This was likely due to the high numbers of toads produced at a fish-free wetland during the first year. This trend did not continue in the second year as the highest number of toads was produced at a fish-stocked wetland (Table 2 & Figure 4b). The eggs of early colonizing hylid species that usually breed in temporary wetlands may be more palatable to mosquitofish than leopard frogs or toads that often reproduce in both permanent and temporary sites (Grubb 1972).

Although there were no significant effects of SLOPE alone in any of our general linear models, this likely reflects the high variability of metamorph production between wetlands. This was especially apparent during the first year as some wetlands produced no metamorphs but the same combination of variables produced high numbers at other sites. Additionally, the majority of production during both years was attributed to toads whose production ranged from none to very high numbers in single wetlands. This was reflected in no significant effects for toads within our general linear models and rather low % deviance explained in regression models, particularly during the first year. Furthermore, our experimental design made it necessary to analyze groups of 12 wetlands separately in four different models rather than including all 18 in a single model. This lowered sample size and reduced statistical power in our analyses of variance. When all 18 wetlands were included in regression models using total amphibian production as dependent variables, AIC ranked the negative relationship with SLOPE as having the greatest support and FISH as second for the first sampling year.

During the second year post-construction, results from both our MANCOVAs and the AIC analyses of regression models illustrate that vegetation cover increases in importance as a factor driving amphibian production. The refuge provided by shallows alone may become somewhat less important as vegetation structure develops. Nevertheless, from an economic standpoint, constructing wetlands with extensive shallows requires less excavation and labor than steep-sloped ponds and may provide amphibians with important habitat early, particularly prior to the establishment of vegetation.

Species Richness

AIC analyses of species richness regression models gave consistent results for both sampling years. Negative associations with fish and positive associations with vegetation highlight the importance of predator avoidance through breeding site selection and cover. However, the importance of each of these variables reversed between sampling years. During the first year, the planted vegetation clearly bolstered amphibian richness. All detected species produced metamorphs at planted wetlands and leopard frog egg masses were often observed attached directly to prairie cordgrass stems. During the second year, mosquitofish abundance became more important than vegetation cover in driving amphibian species richness (Figure 5). Breeding adult chorus frogs and gray treefrogs continued to largely avoid fish-stocked wetlands keeping their diversity low. However, leopard frogs became nearly ubiquitous during the second year as almost every wetland had developed some vegetation structure (Figure 6).

Previous studies have yielded contradictory results regarding the benefits of vegetation for amphibians leading to differing management recommendations. For example, Knutson et al. (2004) found that multi-species reproductive success was highest in ponds with less emergent vegetation and Porej & Hetherington (2005) found no relationship between species richness and emergent vegetation cover alone. However, Hazell et al. (2004) and Hartel et al. (2007) found that pond occupancy of some amphibians, as well as species richness, were higher in ponds with high vegetation cover. We found that abundances of some amphibians in northern Missouri constructed wetlands were positively associated with vegetation cover (Shulse et al. 2010). The results of our wetland experiments agree with those of the latter field studies that found positive benefits of vegetation for amphibians. While the benefits in the first year appeared to have been strong only for leopard frogs, weaker interactions with other species should not be discounted. Chorus frogs and gray treefrogs also attach their egg masses to twigs and grasses (Johnson 2000). Additionally, our results for toad metamorphs, and leopard frogs to a lesser extent, suggest that vegetation may also improve metamorph quality for some species. The elevated cover and nutritional resources provided by extensive vegetation structure may result in increased size at metamorphosis, but additional studies are needed to investigate these relationships. Furthermore, the amount of vegetation added may be a factor in the number of egg masses deposited during the first year. Planting vegetation may also facilitate faster re-vegetation in future years thereby providing additional cover from predators, bolstering macroinvertebrates (Stewart and Downing 2008) that serve as food for later-colonizing larval caudates, and may help to prevent the establishment of invasive vegetation (Lichko & Calhoun 2003).

Although we did not identify all of the plants that colonized our wetlands, we did not notice any non-native invasive species. Cattails (*Typha sp.*), arrowheads (*Sagittaria sp.*), and spikerushes (*Eleocharis sp.*) appeared to be the most common emergent vegetation; while floating and submerged pondweeds (*Potamogeton sp*) became established in some wetlands. Non-native wetland plants may negatively impact amphibians (Brown et al. 2006), but additional studies are needed to more fully understand how changes to vegetation structure from invasive wetland flora affect amphibians. Nonetheless, the benefits amphibians garner from vegetation structure as refuge from predatory macroinvertebrates and most fish may not translate to effective refuge from mosquitofish. These fish forage in the shallow, heavily vegetated areas of wetlands where many larval amphibians congregate (Baber and Babbitt 2004) exposing larvae to increased levels of predation and injury (Shulse & Semlitsch, unpublished data).

Broader Restoration Implications

Immediately following construction, restoration sites begin the process of ecological succession. Many factors determine the pathways of succession, and efforts to introduce flora or fauna are attempts to speed up processes and meet targeted goals quickly. Even with these efforts, predicting the outcome of restoration is fraught with pitfalls due to a lack of science-based approaches and experimentation (Zedler 2000). Our study examined three features experimentally that past observational studies have shown to be important wetland components for predicting amphibian occurrence, abundance, and species richness (e.g. Hazell et al. 2004; Porej and Hetherington 2005; Hartel et al. 2007; Shulse et al. 2010). Our study goes beyond previous investigations by constructing wetlands that directly examine cause and effect relationships between amphibians and the wetland variables of interest while attempting to control for other confounding variables. However, many factors that we did not alter, and some that we could not control, likely play important roles in shaping the observed amphibian communities and their reproductive success. For example, hydrology is a major characteristic that shapes wetland plant and animal communities. Some species require periodic drying and others require permanent inundation. Our wetlands were permanent for the duration of our two-year study and they have continued to remain permanent, probably because the region experienced higher than normal precipitation (>127 cm) in both 2008 and 2009 (average 89 – 102 cm; National Weather Service). This has likely contributed to the development of amphibian communities in all of the wetlands consisting of large ranid species that require permanent water (see Shulse et al. 2010). Boreal chorus frogs largely abandoned breeding in the wetlands by the third year (2009), probably because of high loads of predatory aquatic macroinvertebrates (see Chapter 4) and potential competition or predation from ranid larvae (Faragher and Jaeger 1998; Boone et al. 2008). If the wetlands dried completely and then re-filled each year, this would likely enhance reproductive success for early colonizing species. This form of disturbance is natural and should be allowed to occur in some portion of restoration wetlands. Diverse wetland habitats provide a range of hydroperiods to sustain diverse communities. Artificially increasing one wetland type, (i.e., permanent open water ponds) over others bolsters populations of species that can tolerate the conditions present in the provided wetlands.

If wetlands are allowed to dry and subsequently re-fill, species requiring temporary wetlands will likely have a "boom" year, while populations of species requiring more permanent water may temporarily suffer. However, these species will likely rebound when conditions turn in their favor. Nevertheless, when adverse conditions continue for multiple years, short lived species, or those with limited dispersal abilities, may experience local extinctions. Amphibian species that require seasonal wetlands may have to find alternative breeding sites such as ditches or swales that have low predator abundances but a higher propensity towards drying on a regular basis. This underscores the importance of providing a diverse array of wetland designs (large and small, temporary and permanent) on small scales to bolster regional diversity and lessen the risks of local extinctions (Semlitsch & Bodie 1998). Furthermore, providing connectivity in the landscape will help to ensure that re-colonization can occur following recruitment failures and eventual local extinction events (Semlitsch 2000).

Invasive species that have been introduced or selected for by humans (either directly or indirectly) are a form of disturbance that native species may not be able to successfully contend with. Such introductions can lead to a “novel ecosystem” with unnatural species combinations that could further complicate restoration efforts (Hobbs et al. 2006). While stocking a biological control agent such as *Gambusia* may initially seem like a reasonable compromise to control or reduce mosquito-borne diseases such as West Nile virus, our results indicate that mosquitofish adversely impact amphibian communities. Furthermore, these fish likely cause reduced macroinvertebrate density and diversity (Blaustein 1991; Schaefer et al. 1994; Lawler et al. 1999). Mosquitofish also have a way of dispersing unchecked into other water bodies. During heavy rains in 2009, mosquitofish escaped of one of our experimental wetlands and colonized a fish-free pond 115 m away downhill. Based on our findings, we recommend that stocking *Gambusia* in mitigation wetlands is a practice that should cease. Wetland managers should not release mosquitofish into water bodies where they are alien, and stakeholders should be educated about their detrimental effects on native ecosystems. We are not suggesting that mosquito control efforts to protect human health should be terminated. Preferable alternatives to *Gambusia* that also feed upon larval mosquitoes are larvae of native salamanders (Brodman et al. 2003, DuRant and Hopkins 2008) and many aquatic invertebrates (Laird 1977). The colonization of these taxa in constructed wetlands should be encouraged through providing appropriate habitats free of *Gambusia*.

Our results illustrate that easy-to-alter wetland design features can help restoration ecologists increase amphibian reproductive success and species richness; and may help predict amphibian community composition. While more research is clearly needed to correlate long-term population trends with successional change in constructed wetlands to better predict outcomes of specific efforts in other regions, it is clear that in the short term, in the Midwest, fish-free wetlands with shallow slopes and high amounts of vegetation cover are best for amphibian reproductive success and species richness.

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Table 1. Experimental wetland treatment combinations and their individual variable components.

Treatment Combination	Independent Variables
1	4:1 slope, fish, not planted
2	4:1 slope, no fish, not planted
3	15:1 slope, fish, not planted
4	15:1 slope, fish, planted
5	15:1 slope, no fish, not planted
6	15:1 slope, no fish, planted

Table 2. Summary of the cumulative number of individuals by species and wetland treatment combination captured during 2007 and 2008.

Treatment Combination	4:1 Fish Not Planted		4:1 No Fish Not Planted		15:1 Fish Not Planted		15:1 Fish Planted		15:1 No Fish Not Planted		15:1 No Fish Planted		Total Species Metamorph Production	
Species	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Acris crepitans Northern Cricket Frog	0	1	0	3	0	2	0	0	0	3	0	1	0	10
Ambystoma texanum Smallmouth Salamander	0	0	0	0	0	1	0	1	0	0	0	0	0	2
Anaxyrus americanus American Toad	611	630	0	265	428	939	398	18746	8097	4181	1223	4212	10757	28973
Hyla versicolor/chrysocelis Gray Treefrog complex	0	0	42	3	1	0	0	0	186	6	265	2	494	11
Lithobates blairi Plains Leopard Frog	0	89	0	416	0	107	48	52	0	193	119	346	167	1203
Lithobates sp Unknown Leopard Frog	0	49	0	55	0	35	173	6	0	8	222	139	395	292
Lithobates catesbeianus American Bullfrog	0	4	0	0	0	6	0	6	0	5	0	1	0	22
Lithobates clamitans Green Frog	0	15	0	2	0	5	0	1	0	1	0	8	0	32
Lithobates sphenocephalus Southern Leopard Frog	0	1141	0	821	0	1873	274	528	1	33	267	3305	542	7701
Pseudacris crucifer Spring Peeper	0	0	0	0	0	0	0	0	0	4	0	2	0	6
Pseudacris maculata Boreal Chorus Frog	0	0	4	362	1	3	4	25	394	12555	736	5417	1139	18362
Unknown	0	2	0	1	0	0	0	0	0	0	2	0	2	3
Total Metamorph Production	611	1931	46	1928	430	2971	897	19365	8678	16989	2834	13433	13496	56617

Table 3. Analysis of regression models for amphibian abundance and species richness using AIC$_c$.

Species	Year	Model[a]	Model Rank	AIC$_c$	ΔAIC$_c$	*W*	Model Likelihood	K[b]	% Deviance	*w*
American Toad	2007	Veg$_{(-)}$+Slope$_{(-)}$	1	204.41	0	0.39	1	3	11.4	Slope 0.77 Veg 0.57 Fish 0.41
		Slope$_{(-)}$	2	205.89	1.48	0.18	0.48	2	7.2	
	2008	Veg$_{(+)}$+Slope$_{(-)}$	1	251.82	0	0.32	1	3	23.5	Veg 0.77 Slope 0.75 Fish 0.40
		FishVegSlope	2	252.76	0.94	0.20	0.62	4	27.5	
		Slope	3	253.42	1.60	0.15	0.45	2	16.1	
		Veg	4	253.67	1.84	0.13	0.40	2	15.7	
Gray Treefrog complex	2007	Fish$_{(-)}$	1	90.88	0	0.43	1	2	51.2	Fish 0.99 Slope 0.46 Veg 0.19
		FishSlope	2	91.08	0.21	0.39	0.90	3	56.8	
Leopard Frog complex[c]	2007	Veg$_{(+)}$	1	86.45	0	0.79	1	2	73.0	Veg 1.00 Fish 0.21
Boreal Chorus Frog	2007	Fish$_{(-)}$+Slope$_{(-)}$	1	109.42	0	0.78	1	3	71.3	Fish 0.99 Slope 0.99 Veg 0.22
	2008	Fish$_{(-)}$+Veg$_{(+)}$	1	166.92	0	0.69	1	3	60.3	Fish 0.99 Veg 0.97 Slope 0.31
		FishVegSlope	2	168.73	1.81	0.28	0.40	4	62.1	
Total Metamorph Production	2007	Slope$_{(-)}$	1	239.25	0	0.45	1	2	12.0	Slope 0.78 Fish 0.53 Veg 0.12
		FishSlope	2	240.43	1.19	0.25	0.55	3	14.1	
	2008	Veg$_{(+)}$+Slope$_{(-)}$	1	311.63	0	0.45	1	3	40.7	Veg 0.99 Slope 0.55 Fish 0.20
		Veg	2	312.15	0.52	0.35	0.77	2	34.3	
Species Richness	2007	Fish$_{(-)}$+Veg$_{(+)}$	1	65.19	0	0.49	1	3	37.6	Veg 0.89 Fish 0.71 Slope 0.29
		Veg	2	66.97	1.78	0.20	0.41	2	25.6	
	2008	Fish$_{(-)}$+Veg$_{(+)}$	1	68.35	0	0.42	1	3	31.9	Fish 0.83 Veg 0.66 Slope 0.18
		Fish	2	69.31	0.96	0.26	0.62	2	22.3	

[a] Veg represents vegetation treatment in 2007 models and vegetation cover in 2008 models.

[b] K = number of estimable parameters in the model, including the intercept.

[c] Leopard frog model contained only the variables vegetation treatment and mosquitofish abundance.

Table 4. Parameter estimates of variables in models ranked highest by AIC_c.

Species	Year	Variables in Model	β	SE	Wald χ^2	*p*-value	95% C.I. Lower	Upper
American Toad	2007	Slope	-14.64	4.19	12.22	0.000	-22.85	-6.43
		Veg	-1.67	0.75	4.86	0.028	-3.14	-0.18
	2008	Slope	-10.06	4.16	5.85	0.016	-18.21	-1.91
		Veg	5.50	2.27	5.88	0.015	1.06	9.95
Gray Treefrog complex	2007	Fish	-0.06	0.01	19.26	0.000	-0.09	-0.04
Leopard Frog complex	2007	Veg	7.70	1.12	47.39	0.000	5.51	9.89
Boreal Chorus Frog	2007	Slope	-27.53	4.68	34.66	0.000	-36.69	-18.36
		Fish	-0.06	0.01	50.07	0.000	-0.08	-0.04
	2008	Fish	-0.04	0.01	36.77	0.000	-0.06	-0.03
		Veg	8.04	2.22	13.05	0.000	3.68	12.40
Total Metamorph Production	2007	Slope	-12.66	3.41	13.80	0.000	-19.35	-5.98
	2008	Slope	-5.67	2.90	3.82	0.051	-11.35	0.01
		Veg	4.80	1.46	10.74	0.001	1.93	7.67
Species Richness	2007	Fish	1.54	0.50	9.43	0.030	0.56	2.52
		Veg	-0.01	0.01	4.69	0.002	-0.02	-0.001
	2008	Fish	-0.01	0.003	5.31	0.021	-0.01	-0.001
		Veg	2.04	1.04	3.87	0.049	0.01	4.07

Figure 1. Experimental wetland locations in Missouri. Six wetlands were constructed at each location.

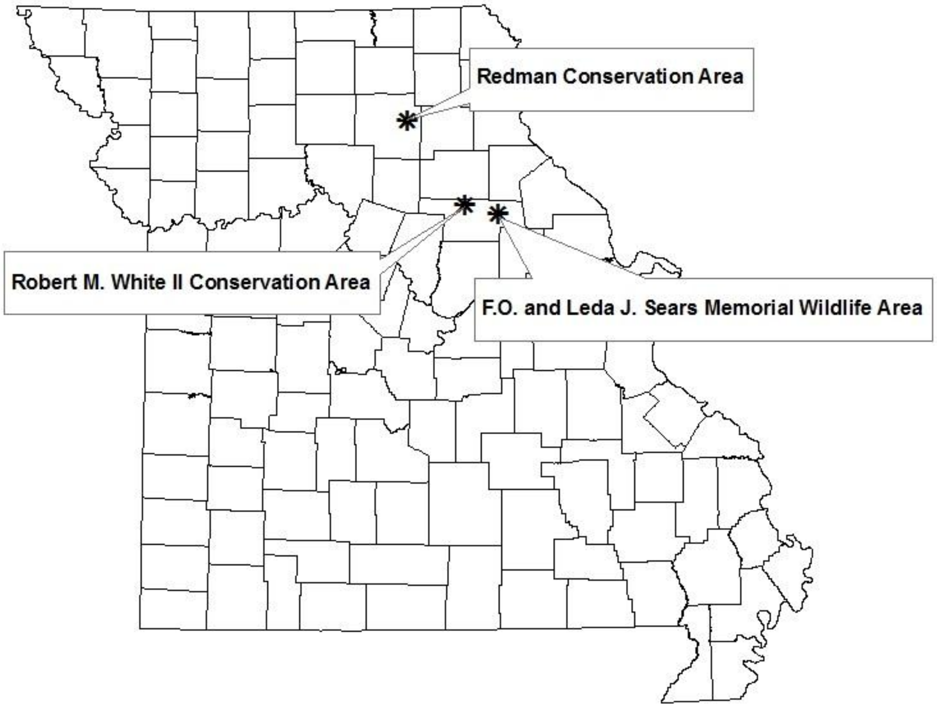

Redman Conservation Area
Robert M. White II Conservation Area
F.O. and Leda J. Sears Memorial Wildlife Area

Figure 2. Experimental wetland designs. Drawings not to scale.

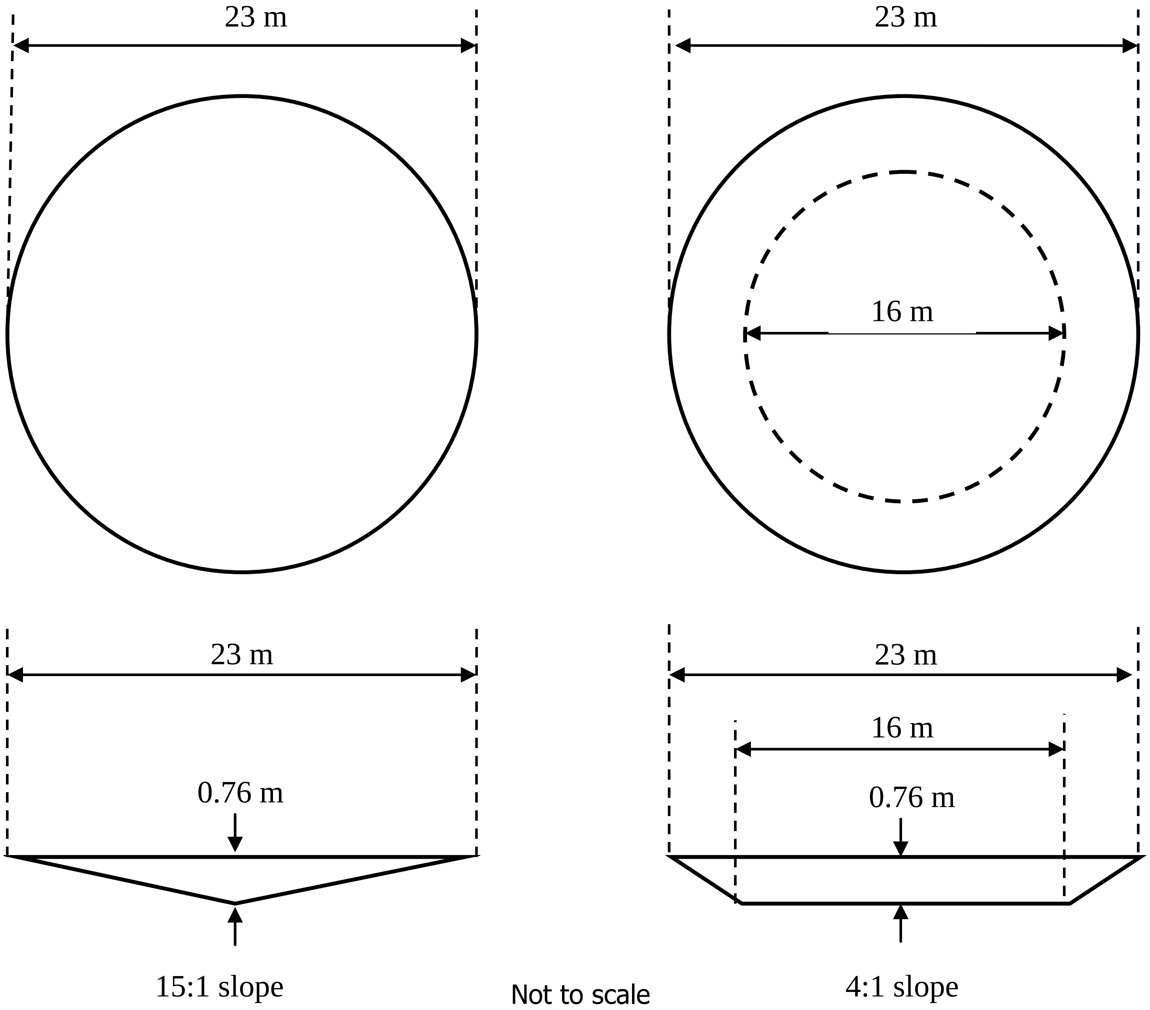
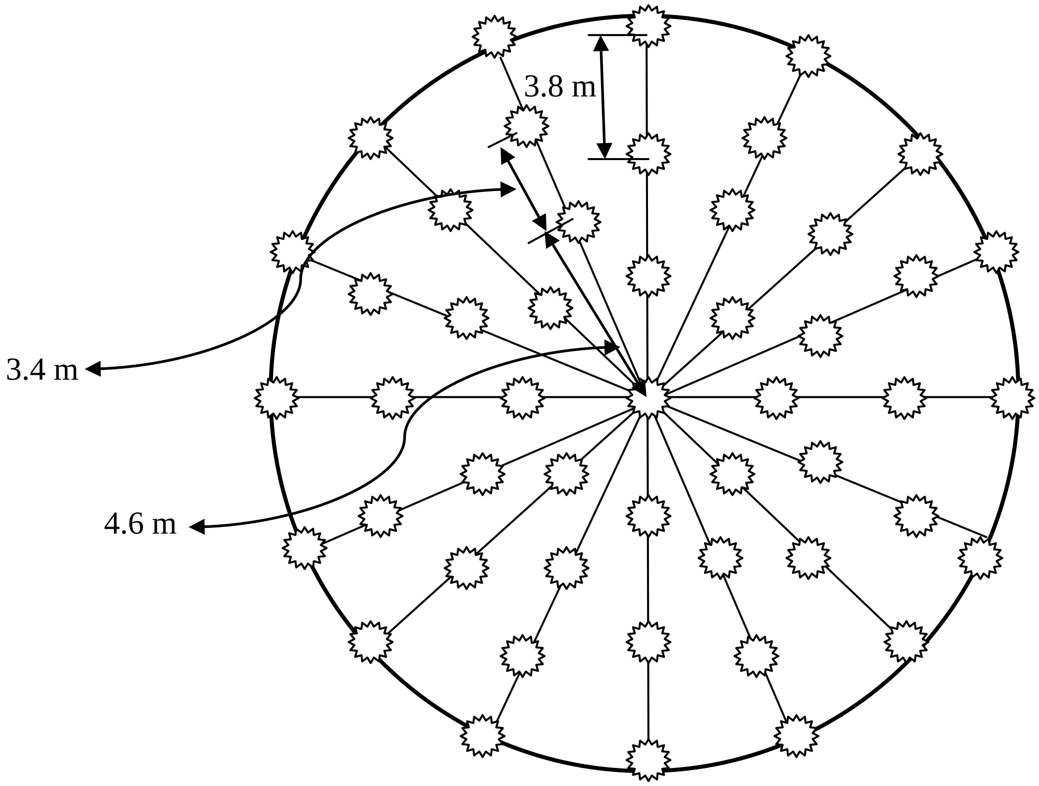

23 m
23 m
16 m
23 m
23 m
16 m
0.76 m
0.76 m
15:1 slope
Not to scale
4:1 slope

Figure 3. Prairie cordgrass (*Spartina pectinata*) placement within wetlands that received the vegetation treatment. Drawing not to scale and lengths between plants are approximate.



Not to scale

Figure 4a. Mean number of metamorphs of each species produced in 2007 based upon treatment combination. Only species captured in quantities high enough to use in individual species regression models are displayed.

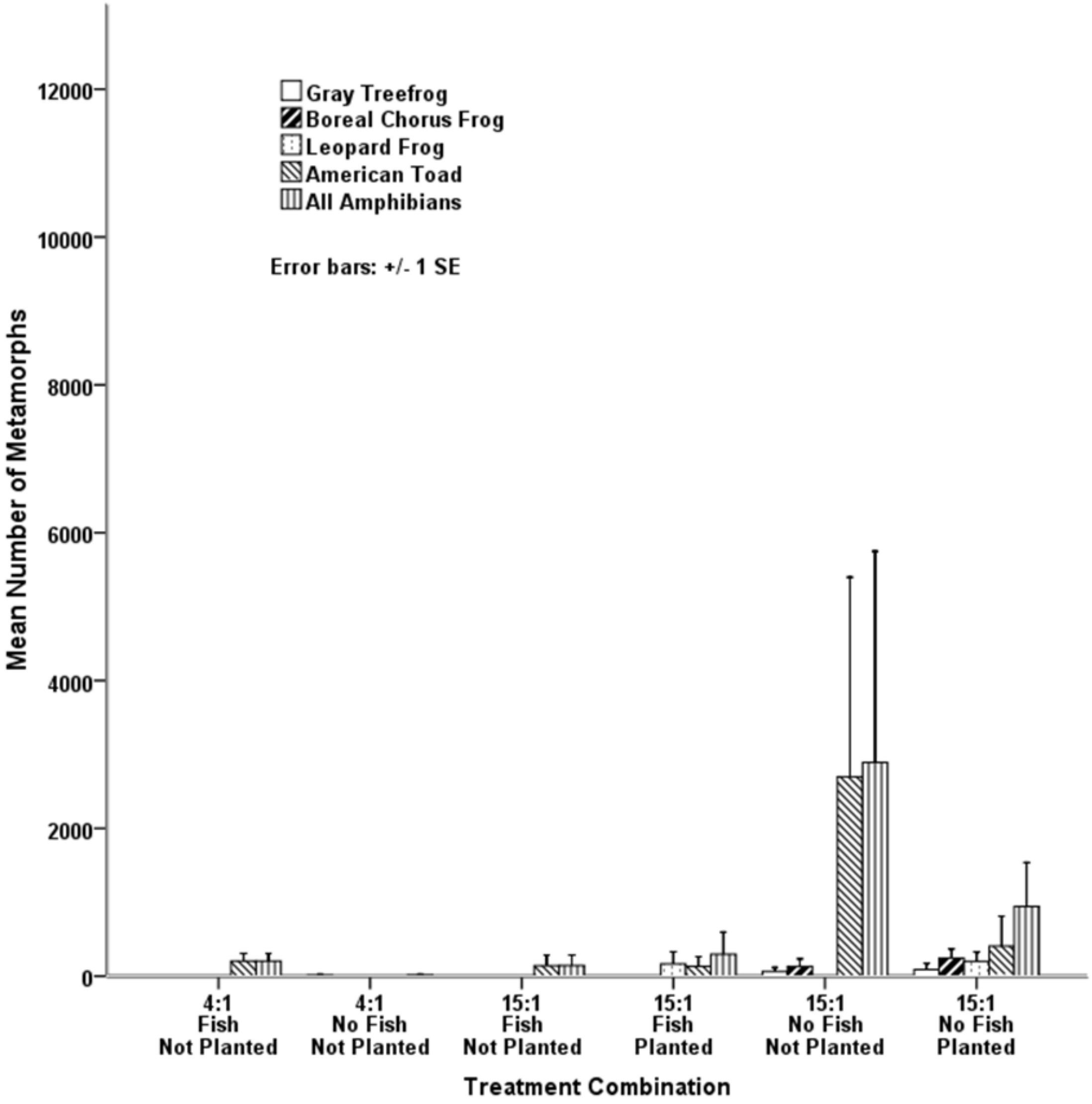

Gray Treefrog
Boreal Chorus Frog
Leopard Frog
American Toad
All Amphibians
Error bars: +/- 1 SE
Mean Number of Metamorphs
12000
10000
8000
6000
4000
2000
0
4:1 Fish Not Planted
4:1 No Fish Not Planted
15:1 Fish Not Planted
15:1 Fish Planted
15:1 No Fish Not Planted
15:1 No Fish Planted
Treatment Combination

Figure 4b. Mean number of metamorphs of each species produced in 2008 based upon treatment combination. Only species captured in quantities high enough to use in individual species regression models are displayed.

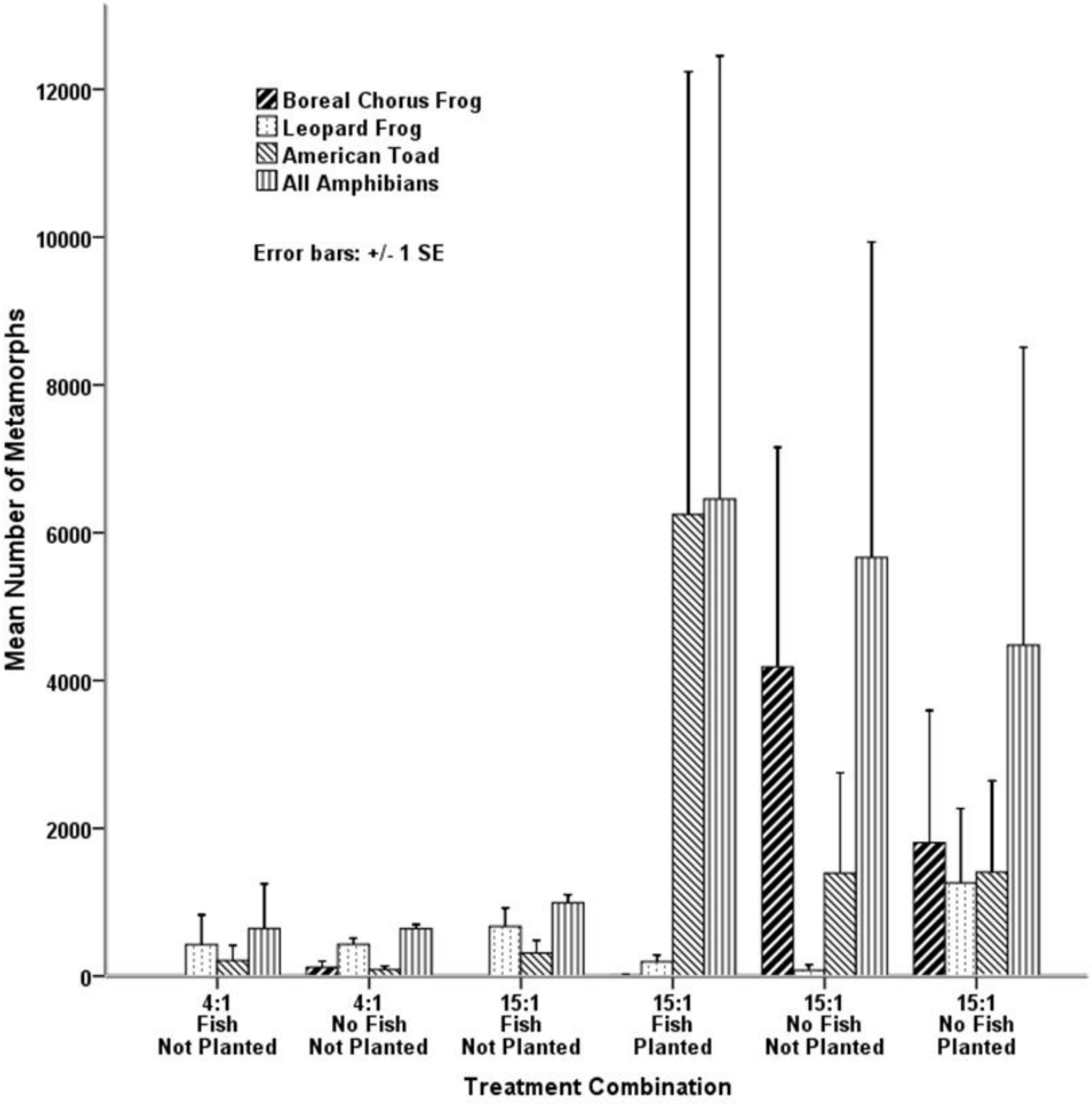
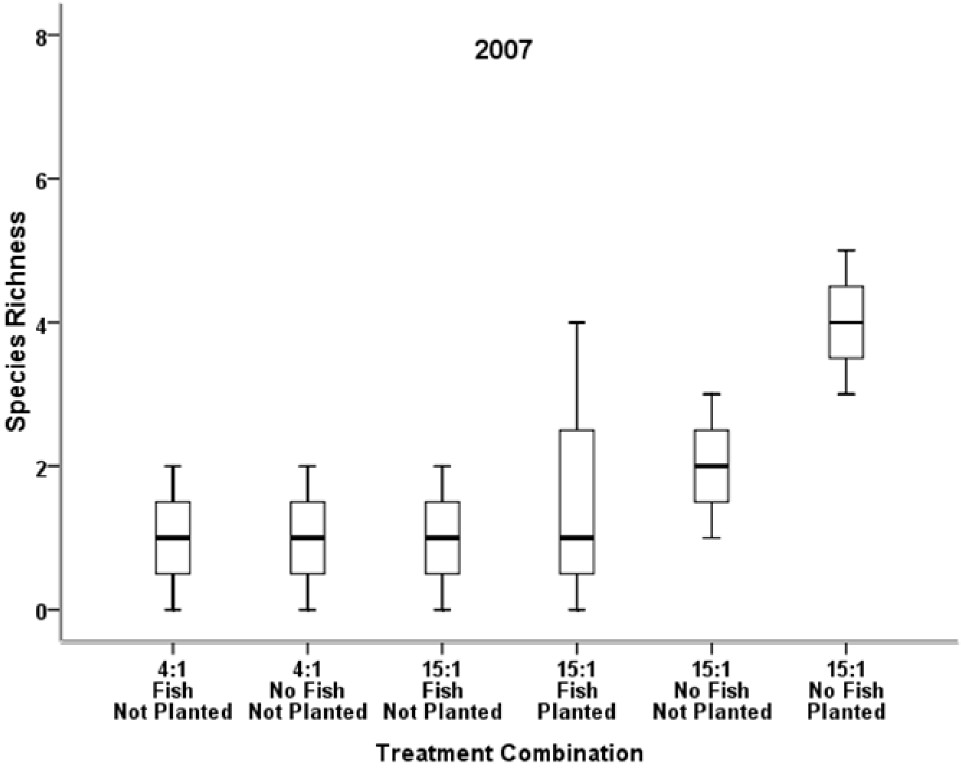

Boreal Chorus Frog
Leopard Frog
American Toad
All Amphibians
Error bars: +/- 1 SE
Mean Number of Metamorphs
12000
10000
8000
6000
4000
2000
0
4:1 Fish Not Planted
4:1 No Fish Not Planted
15:1 Fish Not Planted
15:1 Fish Planted
15:1 No Fish Not Planted
15:1 No Fish Planted
Treatment Combination

Figure 5. Boxplots illustrating species richness for each wetland treatment combination. Boxes represent 50% of the cases and whiskers represent the range.


2007
Species Richness
0
2
4
6
8
4:1 Fish Not Planted
4:1 No Fish Not Planted
15:1 Fish Not Planted
15:1 Fish Planted
15:1 No Fish Not Planted
15:1 No Fish Planted
Treatment Combination

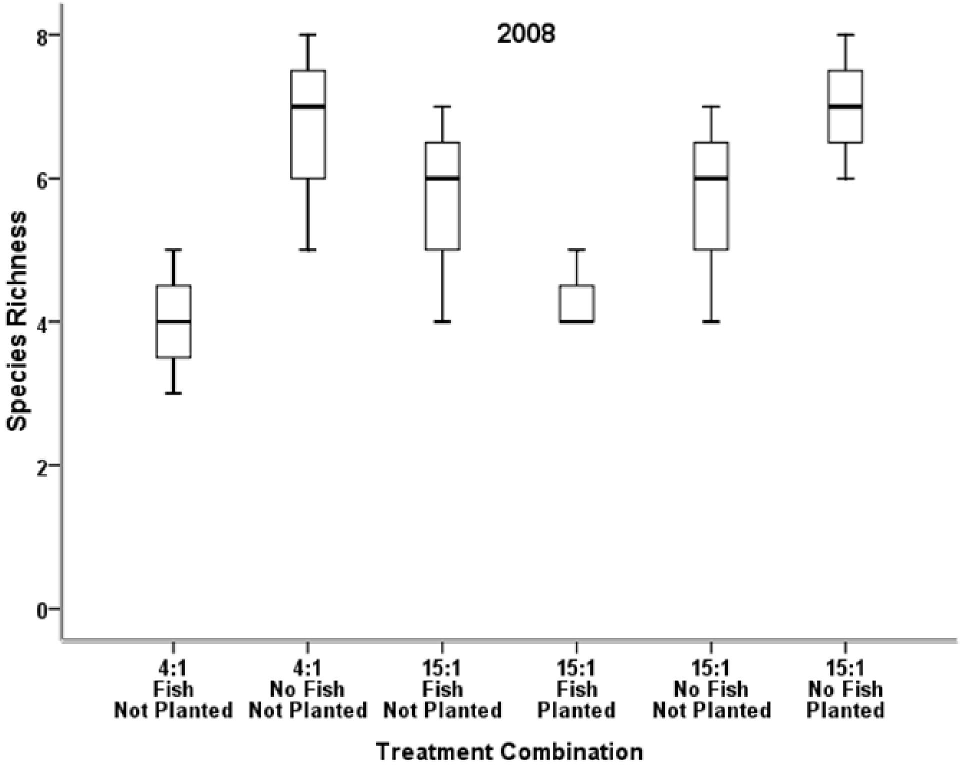
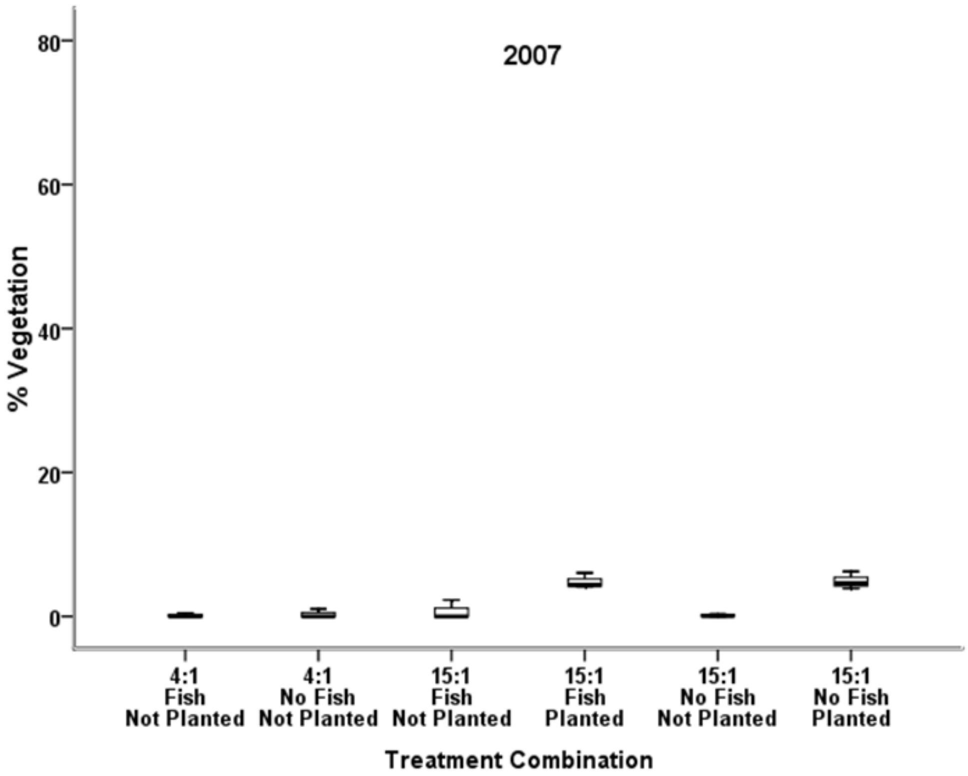
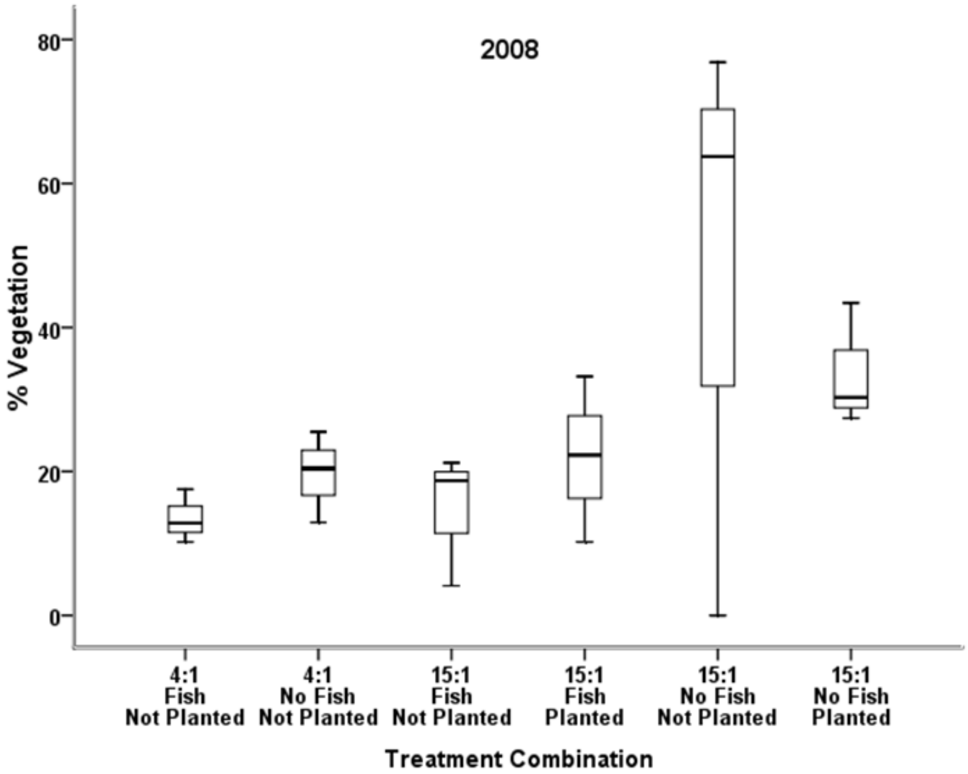

2008
Species Richness
0
2
4
6
8
4:1 Fish Not Planted
4:1 No Fish Not Planted
15:1 Fish Not Planted
15:1 Fish Planted
15:1 No Fish Not Planted
15:1 No Fish Planted
Treatment Combination

Figure 6. Boxplots illustrating % vegetation cover for each wetland treatment combination. Boxes represent 50% of the cases and whiskers represent the range.


2007
% Vegetation
80
60
40
20
0
4:1 Fish Not Planted
4:1 No Fish Not Planted
15:1 Fish Not Planted
15:1 Fish Planted
15:1 No Fish Not Planted
15:1 No Fish Planted
Treatment Combination


2008
% Vegetation
80
60
40
20
0
4:1 Fish Not Planted
4:1 No Fish Not Planted
15:1 Fish Not Planted
15:1 Fish Planted
15:1 No Fish Not Planted
15:1 No Fish Planted
Treatment Combination

CHAPTER 4

MOSQUITOFISH DOMINATE AMPHIBIAN AND INVERTEBRATE COMMUNITY DEVELOPMENT IN EXPERIMENTAL WETLANDS

Christopher D. Shulse, Raymond D. Semlitsch,
and Kathleen M. Trauth

ABSTRACT

Predators are important ecosystem components that play key roles in shaping communities through complex interactions with prey, other predators, and both biotic and abiotic characteristics of their habitat. Introduction of an invasive predator by managers to control pests can also have dramatic effects that may be difficult to predict. We studied the effects of the western mosquitofish (*Gambusia affinis*), an introduced invasive predator, two naturally colonizing predators (crayfish and dragonflies), and vegetation cover on amphibian and invertebrate communities in experimental constructed wetlands. We also investigated the source of sub-lethal tail injuries observed on ranid larvae in the wetlands. We found that mosquitofish reduced abundances of juvenile gray treefrogs (*Hyla versicolor/chrysoscelis* complex) and boreal chorus frogs (*Pseudacris maculata*), reduced aquatic invertebrate abundance and richness, and increased the severity of ranid tail injuries. Furthermore, we found that vegetation cover did not significantly increase amphibian or invertebrate abundances, or attenuate tail injuries. However, vegetation cover did increase invertebrate taxa richness. We also found that chemical removal of

mosquitofish reduced tail injury severity, increased invertebrate abundance, and may have facilitated the re-colonization of chorus frogs into wetlands with low invertebrate predator populations. Our results indicate that mosquitofish are detrimental to aquatic community development, and high dragonfly naiad populations that develop in permanent hydroperiod wetlands are unfavorable to chorus frogs. Therefore, to benefit early colonizing amphibian species, we recommend that managers avoid stocking mosquitofish, and wetland planners design some wetlands with temporary hydroperiods to prevent the persistence of aquatic predators and re-set successional processes.

INTRODUCTION

A major challenge for restoration ecologists involves predicting pathways of ecological succession in the presence of multiple biotic and abiotic conditions. Predators play key roles in shaping natural communities through interactions with both prey and other predators (Van Buskirk 1988, Griffen 2006). These interactions are often complex, thereby making discernment of mechanisms generating natural community patterns and structure difficult (DeWitt and Langerhans 2003). Most prey species are consumed by multiple predators but prey responses to different predators are not the same. The reaction by prey to one predator may make it more vulnerable to another, or it may make it less vulnerable depending on the nature of interactions between the two predators (Sih et al. 1998). These complex interactions are important during restoration activities because as succession proceeds, food webs develop based upon the biotic and abiotic conditions present at a site; some of which can be manipulated by the restoration ecologist. For example, wetland hydroperiod plays a major role in shaping wetland communities (Pechmann et al. 1989) so designing wetlands with temporary or permanent hydroperiods will have a direct impact on the resulting wetland communities (Pechmann et al. 2001). Introducing a predator can also have dramatic consequences on community development, particularly if the predator is invasive and prey species do not possess adaptive traits to reduce predation mortality (Nyström et al. 2001).

Predicting successional pathways is essential to establishing ecological functionality and achieving regulatory goals of wetlands constructed for habitat restoration and compensatory mitigation (Zedler 2000). The addition of flora and fauna to “seed” newly constructed wetlands to speed up ecological succession or to meet some predefined goal may have mixed consequences. For example, adding vegetation to new wetlands can bolster colonization and reproductive success for some amphibians, while the addition of a predator as a biological control for undesirable pests may prevent the successful colonization or reduce the reproductive success of desired species (Shulse et al. 2011). These manipulations can have far-reaching consequences because wetland environments are not ecologically isolated from adjacent terrestrial habitats; aquatic conditions can affect multiple trophic levels in both habitats (Knight et al. 2005). While there are many functional attributes that must be considered during wetland planning, manipulations that alter aquatic predator/prey interactions can influence the biotic integrity of the entire restored wetland ecosystem.

Dragonfly naiads and crayfish are top invertebrate predators in many wetlands. Dragonfly naiads are carnivorous and consume other aquatic invertebrates, small fish, and larval amphibians (Folsom and Collins 1984, Merrill and Johnson 1984, Van Buskirk 1988). Crayfish are omnivorous and consume detritus, vegetation, invertebrates, carrion, fish eggs and young, and amphibian eggs and larvae (Momot 1995, Dorn and Wojdak 2004). Though dragonflies are generalist predators (Wallace et al. 1987) their trophic impact is likely narrower than omnivorous crayfish. The predation effects of crayfish are especially dramatic because they directly impact multiple trophic levels, thereby disrupting trophic cascades and increasing trophic level connections (Dorn and Wojdak 2004). Introduced crayfish have negatively impacted aquatic communities and have been implicated in amphibian declines (Gamradt and Kats 1996, Axelsson et al. 1997). However, results from other studies have suggested that crayfish are inefficient predators of larval amphibians (Holomuzki 1989, Fauth 1990, Lefcort 1996). Nevertheless, reduced vegetation cover caused by crayfish (Axelsson et al. 1997) can reduce habitat complexity needed by amphibians (Hartel et al. 2007) and may indirectly contribute to reductions in amphibian abundance. Dragonfly larvae, on the other hand, are efficient consumers of larval amphibians (Caldwell et al. 1980, Smith 1983) and can induce phenotypic changes in larval anuran tail shape and pigmentation (McCollum and Leimberger 1997). Pigmented tails presumably direct feeding attempts away from the head and towards the less vulnerable tail (Caldwell 1982, Meadows 1993), resulting in injured or missing tail fins and musculature. Dragonflies are also known to inflict sub-lethal injuries to body parts of larval amphibians (Bowerman et al. 2010).

Introduced predatory fish have also been implicated in aquatic community disruptions. Eastern (*Gambusia holbrookii*) and western (*G. affinis*) mosquitofish are small poeciliids that are native to the southern and eastern U.S. but have been introduced throughout the world because of their purported effectiveness at eliminating larval mosquitoes (reviewed in Pyke 2008). Mosquitofish readily consume invertebrates, small fish, and amphibian eggs and larvae (Pyke and White 2000, Richard 2002). Furthermore, they can alter the composition of the aquatic invertebrate community (Hurlbert et al. 1972). Together, the two mosquitofish species are the most widespread fish in the world (Pyke 2008) and the IUCN lists mosquitofish among the 100 worst invasive species (Lowe et al. 2000). Introduced mosquitofish are associated with amphibian declines in California, Australia, and China (Lawler et al. 1999; Pyke & White 2000; Karraker et al. 2010), and negative effects have been recorded in experiments using eggs and larvae of amphibian species within their native range (Grubb 1972, Baber and Babbitt 2004, Stanback 2010).

We investigated the influence of an introduced predator (*G. affinis*), and two naturally colonizing predators (dragonflies and crayfish), on three amphibian species in experimental constructed wetlands. We focused on gray treefrogs (*Hyla versicolor/chrysocelis* complex), boreal chorus frogs (*Pseudacris maculata*), and green frogs (*Lithobates clamitans*). Gray treefrogs and boreal chorus frogs are palatable to fish but green frogs are not (Kats et al. 1988). Furthermore, chorus frogs appear to prefer temporary aquatic habitats, whereas gray treefrogs will reproduce in both temporary and permanent water, and green frogs require relatively permanent aquatic breeding habitat (Kats et al. 1988). Each species employs different mechanisms, including breeding habitat preferences, behavioral changes, or phenotypic plasticity, to adapt to predation stressors (Smith et al. 1983, Van Buskirk et al. 1997, Van Buskirk and McCollum 1999, Van Buskirk 2003). We predicted that introduced mosquitofish would have a greater negative impact on the hylid anurans than on green frogs, and that mosquitofish effects would be greater than those of crayfish and dragonflies. We also examined the effects of mosquitofish on invertebrate communities, along with the influence of vegetation cover on both amphibians and invertebrates. We hypothesized that mosquitofish would reduce invertebrate abundance and richness, and that vegetation cover would attenuate predation mortality for both amphibians and invertebrates, because habitat complexity can provide refuge for prey (Sass et al., 2006; Hartel et al. 2007). During the course of our study, we noted that many larval anurans, particularly ranids, had injuries to their tail fins and (in severe cases) their tail musculature. Mosquitofish and other small, gape-limited fish species have been found to cause similar injuries (Lawler et al. 1999; Hartel et al. 2007; Bowerman et al. 2010). We hypothesized injury severity would be increased by mosquitofish, and that vegetation cover and fish removal would reduce injury severity.

METHODS

Study Areas and Design

During October and November 2006, we constructed replicate wetland arrays at three upland grassland habitats in northeastern Missouri managed by the Missouri Department of Conservation (MDC). Six wetlands were constructed at each location (n = 18). A complete description of the wetland designs used in this study, their locations, and their surroundings, is given in Shulse et al. (2011). One goal of this previous study was to examine the effects of wetland slope on amphibian reproductive success and species richness. Thus, two wetlands at each location were constructed with steep (4:1) slopes and four were constructed with shallow (15:1) slopes. Three wetlands at each location were stocked with mosquitofish and two of the shallow-sloped wetlands were planted with vegetation (prairie cordgrass – *Spartina pectinata*). However, during the course of the study, re-vegetation of the non-planted wetlands, including some with 4:1 slopes, outpaced that of some planted (Appendix Figure 1). Therefore, we used vegetation cover as a continuous covariate within our analyses as opposed to a treatment factor (below).

Although native to southeast Missouri, *G. affinis* has been expanding in distribution into north Missouri through natural dispersal and undocumented introductions (Pflieger 1997). In a recent study involving surveys of constructed wetlands in northern Missouri, we captured mosquitofish in 45% of Missouri Department of Transportation (MoDOT) compensatory mitigation wetlands (Shulse & Semlitsch, unpublished data). For this study, mosquitofish were captured from a MoDOT compensatory mitigation wetland in Audrain County, Missouri and introduced into three randomly chosen experimental wetlands at each location at a rate of 3,089 fish/ha which is slightly higher than the rate of 2,471 fish/ha (1,000 fish/acre) recommended by Duryea et al. (1996). This rate resulted in a founding population of 125 adult mosquitofish per stocked wetland in March 2007. Fish were re-stocked into wetlands where samples indicated low populations during early spring 2008. Reconnaissance sampling in early spring 2009 revealed that mosquitofish populations were healthy in all the stocked wetlands so no re-stocking took place in 2009. Fish were removed from the three stocked wetlands at one location (Redman) on 17 September 2009 using rotenone (chemical restoration). Rotenone was applied to the three stocked wetlands at another site (Sears) on 10 March 2010. Chemical restorations at both locations were conducted by MDC personnel using rates specified on the rotenone label. Dead mosquitofish were noted in the littoral zones of all wetlands prior to personnel leaving both locations. However, mosquitofish re-appeared in one treated wetland (Sears 1) during the second sampling period in 2010. Therefore, although none were captured in the first sampling period, it was assumed that this wetland contained a small number of survivors during the first period so we continued to consider this wetland as fish-stocked in our 2010 analyses. The stocked wetlands at the third locaton (White) were not treated and reconnaissance sampling in early spring 2010 revealed healthy mosquitofish populations.

Sampling

Amphibians, mosquitofish, and invertebrates were sampled three times each season (2007 – 2010; Table 1) using aquatic funnel traps and dip nets. The timing of each period varied slightly over the course of the entire four years. Aquatic funnel traps were deployed for 48 hours during 2007 and 2008, and overnight during 2009 and 2010, using two kinds of commercially available minnow traps: collapsible nylon mesh traps (3 mm mesh size; 38 x 26 x 26 cm dimensions; 6 cm openings) or galvanized steel wire traps (6 mm mesh; 42 cm long; 2.5 cm openings). Two traps of each kind were used per wetland and they were staggered so that traps of the same kind were directly across from one another at each cardinal direction. Assignment of pair direction was randomly chosen. One dip net (3mm nylon mesh) sweep was conducted from the water's edge at each cardinal direction and sweeps were ~1.5 m long with the net pressed to the substrate and pulled toward the sampler. During the second period of sampling in 2007, a zooplankton canvas D-net with 500 micron mesh bottom was added to the sampling protocol to capture very small individuals. Approximately 1.5 m sweeps occurred at each ordinal direction using the canvas net. This resulted in 4 dip net sweeps and 4 canvas net sweeps, spaced evenly apart, for each wetland during each sampling period after 2007-1. Data from all aquatic sampling methods were combined to calculate amphibian, mosquitofish, invertebrate abundance, and invertebrate taxa richness. All organisms were released unharmed at their point of capture immediately after recording. We were unable to distinguish between eastern gray treefrogs (*Hyla versicolor*) and Cope's gray treefrogs (*Hyla chrysocelis*) in the field so gray treefrogs are considered *Hyla versicolor/chrysocelis* complex.

Within-wetland vegetation cover was measured using four 1 m² quadrats spaced at cardinal directions around the perimeter of each wetland. Quadrats were placed at the edge of each wetland to assess vegetation cover within 1 m of the shore and at 3 m from the shore. The percentages of open water, emergent, floating, and submerged vegetation were visually estimated within each quadrat. The three categories of vegetation were combined and averaged for all quadrats over all sampling periods within a season at each wetland to calculate an average measure of vegetation cover for the season. Percent vegetation cover was transformed to the arcsine square root of the proportion for analyses.

Data Analysis

We analyzed each year separately to look for overall patterns in abundance or taxa richness. For all analyses, a single wetland was used as the unit of replication. All statistical analyses were performed using SPSS version 16.0 (2007 SPSS Chicago, Illinois). To explain relationships between abundances of amphibians, predators, and vegetation cover, we developed regression models with a negative binomial distribution and log link function using the generalized linear model option in SPSS. We used mosquitofish abundance, crayfish abundance, dragonfly naiad abundance, and vegetation cover as independent variables. We conducted Spearman Rank Correlation tests between independent variables to avoid including two variables strongly correlated with one another ($r \geq 0.70$) in models. Dragonfly abundance and vegetation cover were highly correlated with one another in 2008 and 2009 (Table 2) therefore, in models for these years, we focused our analyses on predators and excluded vegetation cover. Each regression model contained abundances of gray treefrogs, boreal chorus frogs, or green frogs as dependent variables, and either all four independent variables or the three predator variables (2008 and 2009). Only crayfish, dragonflies, and vegetation cover were included in the model for gray treefrogs in 2010 because no individuals were captured in wetlands containing mosquitofish that year.

To examine the relationship between larval anuran tail injuries, wetland predators, and vegetation cover we developed an index to "score" the severity of damage to the tails of individual tadpoles. We chose to limit our analyses to ranid larvae rather than all anuran species because most larvae observed with tail injuries were ranids. Tail injuries were ranked on a scale of 1 – 5 (damage coefficient) with 1 being no visible injuries to 5 being severely injured (Figure 1). To calculate the "Tail Injury Assessment Index (TIAI)" for each wetland, the numbers of tadpoles at each coefficient level (1 – 5) were multiplied by their respective coefficient to arrive at subtotals for each level. The subtotals were summed and divided by the sum of the total number of individuals. The result is an "average" score of tail damage for each wetland. The TIAI values were non-normally distributed so we $\log_{10}$ transformed the raw scores and then calculated the square root of the resulting value to meet the assumptions of normality and homoscedasticity. The transformed scores were used as dependent variables in hierarchical linear regression models to examine relationships with *a priori* independent variables hypothesized to contribute to (predators) or attenuate (vegetation cover) tail injuries. Hierarchical regression allowed us to examine changes in the explained variance of the model as each independent variable was entered; and to assess the contribution of each variable in explaining the injury scores. Mosquitofish abundance was entered in Step 1, crayfish abundance entered in Step 2, dragonfly abundance entered in Step 3, and finally vegetation cover entered in Step 4. To further test our hypothesis that mosquitofish increase ranid tail injuries and vegetation cover reduces injuries, we performed ANCOVAs using the transformed TIAI scores for each year as dependent variables, the mosquitofish treatment as the factor, and vegetation cover the covariate. Crayfish abundance was also used as a covariate in a *post hoc* ANCOVA for 2010. We also performed a Wilcoxon Signed Rank test to evaluate the TIAI scores of chemically restored wetlands before and after treatment.

To test the hypothesis that mosquitofish reduce invertebrate abundances, we used the cumulative number of invertebrates ($\log_{10}$ transformed) captured during all sampling periods each year at each wetland as dependent variables in general linear models with mosquitofish as a factor and vegetation cover as a covariate. We excluded crayfish, snails, bivalves, and daphniids from analyses of abundance. Data for snails and bivalves were not collected consistently, and daphniids were sometimes challenging to detect. When daphniids were present, they often occurred in very high numbers that were difficult to reliably count in the field. Crayfish grow large enough to escape fish predation (Stein 1977) and even the smallest crayfish we observed in our wetlands were too large for gape-limited mosquitofish to consume.

To test the hypothesis that mosquitofish reduce invertebrate richness, we used the cumulative number of invertebrate taxa captured during all sampling periods each year at each wetland as dependent variables in general linear models and again used mosquitofish as a factor and vegetation cover as a covariate. Invertebrate richness values included daphniids but excluded crayfish, snails, and bivalves. We attempted to identify each invertebrate to family but we were unable to identify some to this level in the field. To achieve normal distribution, invertebrate taxa richness values were $\log_{10}$ transformed for 2007. We also performed Wilcoxon Signed Rank tests to evaluate invertebrate abundance and taxa richness of chemically restored wetlands before and after treatment.

RESULTS

Amphibians

Regression analyses revealed negative associations between gray treefrog abundances and mosquitofish abundances during the first three sampling years (all $p < 0.05$; Table 3). No gray treefrog larvae were captured in wetlands containing mosquitofish during the last sampling year. Gray treefrog abundance was also negatively associated with crayfish abundance in 2007 and 2009; and larval dragonflies in 2008. Gray treefrogs were most abundant during the first year post-construction (Figure 2) but they were captured in only 39% of the wetlands (Figure 3). During subsequent years, they were captured in roughly half of the wetlands but their abundance dropped and remained at relatively low levels.

Boreal chorus frog tadpoles were never captured in large numbers (i.e., >15) in wetlands containing mosquitofish. We did not perform a regression analysis for chorus frogs in 2007 because their larvae were captured in only three wetlands. These wetlands were fish-free, crayfish-free, and no dragonflies were captured in them during the first two sampling periods when chorus frogs were breeding. Chorus frogs were negatively associated with mosquitofish in 2008 ($p = 0.001$) and 2010 ($p = 0.009$) and they were captured in 67% of wetlands in 2008 and 44% in 2010. Only two larval chorus frogs were captured in 2009 and they occurred in a fish-free wetland. Chorus frogs were also negatively associated with dragonflies in 2010 ($p = 0.01$). The peak abundance for chorus frogs occurred during 2008 (Figure 2). Although their larvae were nearly absent in the wetlands in 2009, their abundance and occurrence increased sharply in 2010.

Green frogs did not occur in enough numbers to perform regression analyses in 2007 but their abundance was consistently negatively associated with crayfish during 2008, 2009, and 2010 (all $p < 0.05$). Green frogs were also negatively associated with dragonflies in 2008 ($p = 0.04$). There were no statistically significant relationships between green frog abundance and mosquitofish. Green frog abundance and occurrence increased over the course of the study and peaked during the first sampling period of 2010 (Figures 2 & 3).

Hierarchical linear regression analyses in 2009 revealed that mosquitofish abundance entered in Step 1 explained 45% of the variance in ranid tail quality scores ($F_{1,16} = 12.99$, $p = 0.002$; Table 4) and was positively associated with tail injury scores ($\beta = 0.67$). As the remaining variables were entered in the subsequent four steps, the variance explained by the model increased in each step until the final model explained 57% of the adjusted variance ($F_{4,13} = 4.31$, $p = 0.02$). However, only the beta value for mosquitofish remained statistically significant through each step. The ANCOVA using

TIAI scores as the dependent variable, mosquitofish treatment as the factor, and the vegetation cover covariate revealed that mosquitofish significantly increase tail injuries ($F_{1,15} = 35.39$, $p < 0.001$). Vegetation cover was not statistically significant ($p = 0.23$).

The hierarchical regression analyses for 2010 revealed that mosquitofish abundance entered in Step 1 explained 34% of the variance ($F_{1,15} = 7.67$, $p = 0.01$) and was again positively associated with tail injury scores ($\beta = 0.58$). Crayfish entered in Step 2 were also positively associated with tail injury scores and increased the adjusted variance explained to 51% ($F_{2,14} = 7.28$, p = 0.007). As dragonflies and vegetation cover were entered in the subsequent steps, the variance explained by the model increased in each step until the final adjusted variance explained was 60% ($F_{4,12} = 4.56$, $p = 0.02$). Only mosquitofish had a β value that remained significant in the final model ($\beta = 0.68$, $p = 0.009$). The ANCOVA using vegetation cover as a covariate again resulted in a statistically significant mosquitofish effect ($F_{1,14} = 7.46$, $p = 0.02$) with no significant effect of vegetation cover ($p = 0.17$). To further examine the influences of mosquitofish and crayfish on tail injury scores, we performed a *post hoc* ANCOVA using tail injury scores as the dependent variable, mosquitofish treatment as the factor, and crayfish abundance as the covariate. The test revealed that mosquitofish significantly increased tail damage scores ($F_{1,14} = 13.49$, $p = 0.003$) while the effect of crayfish abundance was much weaker ($p = 0.13$).

A Wilcoxon Signed Rank Test revealed a statistically significant reduction in ranid tail injury scores following rotenone application in fish-stocked wetlands, N = 10, Z = -2.02, $p = 0.04$, with a large effect size (r = 0.64). One wetland that was treated in 2009 was removed from the test because no ranids were captured after treatment, therefore there was no TIAI score for that wetland in 2010. The median tail injury score in the fish-stocked wetlands treated with rotenone was 3.72 prior to treatment and 1.10 after treatment. Of the three wetlands that were stocked with mosquitofish but not treated with rotenone, TIAI scores decreased in two during 2010 while scores increased in the third (Figure 4).

Invertebrates

Analyses of covariance revealed that invertebrate abundance was significantly reduced in the fish-stocked wetlands during all four sampling years (2007: $F_{1,15} = 13.25$, $p = 0.002$; 2008: $F_{1,15} = 21.07$, $p < 0.001$; 2009: $F_{1,15} = 55.15$, $p < 0.001$; 2010: $F_{1,15} = 15.60$, $p = 0.001$). Mosquitofish also significantly reduced invertebrate taxa richness during the first three years (2007: $F_{1,15} = 6.9$, $p = 0.02$; 2008: $F_{1,15} = 19.1$, $p = 0.001$; 2009: $F_{1,15} = 14.61$, $p = 0.002$) but not in 2010 ($p = 0.56$). The vegetation cover covariate had no significant effects on invertebrate abundance during any year but it did significantly increase taxa richness in all years except 2007 when little vegetation cover had developed (2007: $p = 0.48$; 2008: $F_{1,15} = 10.04$, $p = 0.006$; 2009: $F_{1,15} = 12.35$, $p = 0.003$; 2010: $F_{1,15} = 5.05$, $p = 0.04$).

Invertebrate abundance was consistently higher in fish-free wetlands than those stocked with fish throughout the duration of our study (Figure 5). Average invertebrate taxa richness generally increased in both fish-stocked and un-stocked wetlands throughout the duration of the study but most fish-free wetlands were consistently richer during all sampling periods (Figure 6; Appendix Figures 2 – 5).

A Wilcoxon Signed Rank Test revealed a statistically significant increase in invertebrate abundance (excluding crayfish, daphniids, snails, and bivalves) following rotenone application to fish-stocked wetlands, N = 12, Z = -2.20, *p* = 0.03, with a large effect size (r = 0.64). The median invertebrate abundance in fish-stocked wetlands was 50.5 in 2009 prior to treatment. In 2010, after treatment, the median increased to 248.5. Invertebrate abundances were low in the three un-treated fish-stocked wetlands in 2009 and 2010 (2009: mean = 34.0, range 22 – 49; 2010: mean = 28.3, range 17 – 35).

There was also a statistically significant increase in invertebrate taxa richness following rotenone application to fish-stocked wetlands, N = 12, Z = -2.03, *p* = 0.04, with effect size r = 0.59. The median richness in fish-stocked wetlands was 7.5 in 2009 prior to treatment, and in 2010, after treatment, the median increased to 11.5. However, invertebrate taxa richness also increased in the three un-treated fish-stocked wetlands from 2009 to 2010 (2009: mean = 8.7, range 7 – 10; 2010: mean 11.3, range 9 – 13).

Dragonfly abundance in the wetlands spiked during the late summer each sampling season (Figure 7). Dragonflies were negatively correlated with mosquitofish abundance in all but the last sampling year and strongly positively correlated with vegetation cover in all years except 2007 (Table 2). Crayfish were strongly negatively associated with vegetation cover in the last two sampling years (Table 2). Their abundances peaked during late spring each year and were consistently inversely related to dragonfly abundance trends (Figure 7).

DISCUSSION

Our results illustrate that aquatic predators dramatically influence both amphibian and invertebrate communities in constructed wetlands. Furthermore, our results suggest that vegetation cover is less significant than predators as a factor influencing abundances of both communities, although vegetation does appear to bolster invertebrate taxa richness. The results of our tadpole tail injury analyses also suggest that vegetation does not play a significant role in attenuating the effectiveness of the predator at inflicting the injuries. Therefore, predator population crashes induced by natural mechanisms, and manipulations by wetland managers that directly alter predator levels, may be more important drivers of diversity and reproductive success for some wetland species than manipulations of habitat structure. The impacts of mosquitofish were especially dramatic on the experimental wetland communities. Throughout the duration of the study, mosquitofish apparently prevented the successful colonization and lowered the reproductive success of chorus frogs, gray treefrogs, and many invertebrates. Our results from analyses of hylid larval abundances are reinforced by those from metamorph production data recorded at the same wetlands during 2007 and 2008 (Shulse et al. 2011). Furthermore, mosquitofish significantly increased tail injury severity on ranid tadpoles. We suggest that introductions, or merely the tolerance, of these invasive fish in wetlands outside their native range may severely limit the ecological value of restoration efforts.

Our experimental wetlands remained filled with water during all four study years. Therefore, wetlands without fish developed high populations of aquatic invertebrates, and almost all wetlands housed populations of large ranid larvae by the fourth year post-construction. These conditions may explain the near absence of larval chorus frogs by 2009. Ranid larvae may have increased interspecific competition (Faragher and Jaeger 1998, Boone et al. 2008), and larval chorus frogs, which are typically very active, are highly susceptible to invertebrate and other predators (Skelly 1995, Smith and Van Buskirk 1999). Some hylids are able to detect the presence of fish in wetlands, allowing species whose eggs and larvae are palatable to avoid breeding in these habitats (Binkley and Resetarits 2008). We noticed that during diurnal early spring reconnaissance trips to the wetlands during the first two years post-construction, chorus frogs selectively called from fish-free wetlands, but during the third year, chorusing had nearly ceased from all the experimental wetlands. Instead, frogs called from nearby ephemeral swales and ditches (Shulse, personal observation). During the fourth year, chorusing frogs returned in limited numbers to the wetlands, but most were restricted to wetlands that had been chemically restored (Shulse, personal observation). Other studies have shown that fish removal has resulted in increased breeding of fish-sensitive anurans (Brönmark and Edenhamn 1994; Vredenburg 2004) and our chorusing observations were validated by our capture results. In 2010, the highest abundances of larval chorus frogs were captured in wetlands that were either 1) chemically restored prior to the breeding season, or 2) had relatively low predatory insect populations. These results suggest that breeding chorus frogs can detect both the presence of fish and invertebrate predators in aquatic habitats.

Our regression results from 2010 illustrate that chorus frog larval abundance was lower in wetlands with high dragonfly naiad captures during that year. Although this relationship was not noted in 2008, naiad levels may have been too low to trigger avoidance or significantly increase larval mortality. In early 2009, when dragonfly and other aquatic invertebrate populations were higher than in 2008, chorus frogs may have avoided predators by shifting their breeding activity to the alternate habitats previously mentioned. These habitats could dry prior to successful metamorphosis, underscoring the risks associated with trade-offs between predatory stressors and habitat stochasticity. Chorus frogs breed early in the season, when dragonfly populations were always lowest

(Figure 7), but by 2009, the population of overwintering naiads and other predatory insects may have been high enough to exclude breeding adult chorus frogs.

Chorus frogs also appear to prefer wetlands with high vegetation cover (Shulse et al. 2010; Shulse et al. 2011), but dragonfly naiads were also strongly correlated with this feature (Table 2). Based on our observations, chorus frogs may avoid wetlands containing high populations of invertebrate predators, even those with high vegetation cover, if nearby ephemeral aquatic habitat containing few predators is available for breeding. If not, other mechanisms that reduce predation mortality may allow some reproduction to occur in more permanent aquatic habitats. Smith and Van Buskirk (1999) describe larval chorus frogs exhibiting reduced behavior and phenotypic plasticity (increased tail fin and musculature) in the presence of dragonfly larvae resulting in a trade-off between growth rate and predation exposure. Chorus frogs, and other early colonizing amphibians, may have a hierarchy of breeding habitat preferences with fish avoidance as the strongest filter, followed by aquatic invertebrate predators. This hierarchy can be extended below invertebrate predators to include vegetation cover followed by within-wetland slope based on the results of Shulse et al. (2011). Our observations suggest that the primary habitat trade-off for breeding chorus frogs at our wetlands was between predation exposure and breeding site stochasticity.

While larval gray treefrogs were also consistently less abundant in fish-stocked wetlands, they were able to persist in (mostly) fish-free wetlands throughout the duration of the study. Their high numbers during the first year post-construction indicate that they, like chorus frogs, are early colonizers that prefer wetlands with low predation stressors. Adult female gray treefrogs will minimize predation risk to their eggs and larvae by avoiding wetlands containing fish (Binkley and Resetarits 2008). However, larval gray treefrogs will often develop bright red pigment on their tails and altered body shape in the presence of high populations of aquatic invertebrate predators (McCollum and Leimberger 1997). This "dragonfly morph" is apparently less susceptible to invertebrates than the typical morph (McCollum and Van Buskirk 1996) and may indirectly contribute to their ability to continue to breed in permanent, but fish-free, wetlands. We often observed "dragonfly morph" gray treefrog larvae in our wetlands with varying shades and degrees of red tail pigment. Because these anurans appear to be mid-spring to early summer breeders whose larvae emerge during mid to late summer, wetlands that dry by mid-summer would reduce or eliminate reproductive success. However, breeding later in more permanent wetlands may also expose larval gray treefrogs to the highest seasonal levels of dragonfly predators.

The increasing abundance of green frogs over the course of the study, like that of aquatic invertebrates, is a reflection of the permanent hyroperiods of the wetlands. Green frogs, and other large ranids like bullfrogs and late season leopard frogs, overwinter as larvae in their aquatic habitats and therefore require wetlands with permanent or semi-permanent hydroperiods. Green frog abundances were consistently negatively associated with crayfish. Crayfish feed upon amphibian larvae and eggs (Axelsson et al. 1997) but it is not clear if the reduced larval abundances we observed are due to predation or crayfish avoidance by breeding green frogs. Anderson and Brown (2009) observed reduced hatching success of green frog eggs in the presence of crayfish, even when the crayfish had no direct access to the eggs. Green frog abundances were never significantly negatively associated with mosquitofish, a result perhaps reflective of their ability to persist in the presence of fish.

Although our results suggest that vegetation cover appears less important than predators in altering the abundance of the amphibian species we examined, our regression analyses may have missed stronger relationships because we did not include vegetation as an independent variable in two of the four years. Shulse et al. (2011) found strong positive associations between total amphibian metamorph production and vegetation cover during 2008 at the same experimental wetlands used in this study. Additionally, chorus frog metamorph production during 2008 was also positively associated with vegetation cover, but a model that combined mosquitofish abundance and vegetation cover best explained chorus frog reproductive success for that year (Shulse et al. 2011). Comparing metamorph production results from 2007 and 2008 suggests that vegetation cover may increase in importance, surpassing other features such as within-wetland slope, as wetlands age (Shulse et al. 2011). However, our results from the TIAI analyses suggest that vegetation cover provides little protection for larvae against mosquitofish feeding attempts. Ranid larvae attain sizes much larger than mosquitofish and therefore any vegetation concealing a large ranid tadpole may also be penetrated by mosquitofish.

Sub-lethal tail injuries were often common and severe on ranids in fish-stocked wetlands. However, ranid larvae in one fish-stocked wetland (White 1) rarely had severe tail injuries (Figure 4), but many had red lesions on their bodies (Figure 1). These lesions may have been the result of mosquitofish bites, pathogens, or wounds from aquatic invertebrates such as leeches. Segev et al. (2009) recorded damage consistent with biting on the bodies of fire salamanders (*Salamandra infraimmaculata*) in the presence of mosquitofish in both natural ponds and in controlled mescosm experiments. Nevertheless, the authors also observed that damage to the tail fins occurred first, possibly because the tail is the most vulnerable part of fleeing larvae, and damage to other body parts such as gills and limbs occurred later. This suggests that when tail damage progressed to the point of reducing swimming performance, larvae were more vulnerable to attack on other areas of the body (Segev et al. 2009). One of us (Shulse) observed and recorded video of mosquitofish swarming and attacking a green frog tadpole with severe tail injuries (click to view video). While most of the bites were inflicted at the tail end, some bites occurred on the sides and the snout of the tadpole.

The tail injuries observed on ranid larvae in our fish-stocked wetlands were similar to injuries described by Reynolds (2009) that were inflicted upon tadpoles of *Heleioporus eyrei* during repeated attacks by swarming *Gambusia holbrookii* in artificial ponds in Australia. Reynolds (2009) suggested that while small tadpoles of *H. eyrei* and several other anurans were readily consumed by mosquitofish, large tadpoles likely survive attacks by small fish. Lawler et al. (1999) also found that mosquitofish increase tail injuries in California red-legged frog (*Rana aurora*) larvae. Depending on their severity, the wounds sustained by tadpoles could lead to reduced swimming abilities that may, in turn, result in reduced abilities to avoid predators (Figiel and Semlitsch 1991), reduced fitness (Wilbur and Semlitsch 1990), increased susceptibility to infection, and ultimately lower recruitment of quality individuals into adult populations. Over time, these effects may result in population declines and even extinction of local amphibian populations. However, we noted reduced severity of larval tail injuries in wetlands where mosquitofish were removed. Similarly, natural reductions in fish abundance have resulted in reduced injuries to other anuran larvae (Bowerman et al. 2010). These results suggest that wetland managers can improve wetlands for amphibians by removing fish or refraining from fish stocking. Both actions are particularly important when mosquitofish are involved. The combination of predation stressors to breeding hylids and injuries to larvae make mosquitofish a significant threat to amphibian diversity and abundance.

Previous studies (i.e., Hurlburt et al. 1972, Jassby et al. 1977a,b, Lawler et al. 1999) have demonstrated that the threats posed by *Gambusia* are not limited to amphibians but also include aquatic invertebrates. Our results add to this body of evidence. The reduction in invertebrate abundance and taxa richness affect the health of the entire wetland community by disrupting food chains and energy transport. Mosquitofish may alter aquatic communities by selectively feeding on large zooplankton reducing pressure on smaller zooplankton species, phytoplankton, and bacteria (Jassby et al. 1977a,b). Studies have suggested that mosquitofish are primarily zooplanktivores (Garcia-Berthou 1999; Reynolds 2009), and they may prefer this prey to larval amphibians (Reynolds 2009). We captured daphniids only once in a fish-stocked wetland, but they often occurred in high numbers in fish-free sites. The only captures of daphniids in a fish-stocked wetland occurred during the first sampling period after a late winter rotenone treatment that nearly eliminated mosquitofish (Sears 1). Five daphniids were captured, but none were found during subsequent sampling periods when the fish population rebounded. After the fall rotenone treatments in 2009 at other wetlands, daphniid captures increased to the highest levels recorded during the following spring (Shulse and Semlitsch, unpublished data). Because these zooplankton are preyed upon by other aquatic invertebrates, their high abundances following chemical restoration may be due to ideal conditions created by fish-induced low predatory aquatic invertebrate populations, and the removal of the fish themselves; a pattern similar to the conditions that may explain the observed chorus frog breeding pattern and abundances in 2010.

Stewart and Downing (2008) found that both macroinvertebrate richness and abundance were positively associated with vegetation in constructed wetlands. While our results also indicate that invertebrate richness is bolstered by vegetation, we found no evidence that overall invertebrate abundance is significantly increased by this feature. Reynolds (2009) observed that high levels of both aquatic invertebrates and vegetation cover reduced mosquitofish predation on anuran larvae. Nevertheless, our results illustrate that aquatic invertebrate abundance is severely reduced in fish-stocked wetlands, and vegetation cover in our wetlands provided little refuge for either invertebrates or amphibians from mosquitofish. Reynolds (2009) also noted that invertebrate abundances were highest in spring when anuran larval densities were highest, thereby potentially satiating mosquitofish and reducing their predation on anurans. We found invertebrate abundances to be severely limited across all years and sampling periods in fish-stocked wetlands, and these levels are unlikely to provide any satiation effect for larval anurans as evidenced by fish-induced tail injuries on ranids.

Chemical Wetland Restoration and Management Implications

The chemical restoration of selected wetlands revealed that some invertebrates and amphibians that were not present (or present in low numbers) responded positively to both removal of fish and the low existing populations of invertebrate predators. These conditions, while chemically induced, are likely similar to those present after a wetland re-fills after drying completely. However, rotenone can have negative consequences for amphibians and reptiles (Fontenot et al. 1994) so caution is warranted when using this method to eliminate predators. Timing of treatments may be critical to reducing non-target mortality (Bradbury 1986). We did not observe any amphibian mortality when chemical restorations occurred during the fall but we observed freshly killed adult leopard frogs and overwintered ranid larvae, within 8 hours of treatment, when rotenone was applied during late winter. While we cannot state with certainty that rotenone was the cause of this mortality, Hamilton (1941) reports that concentrations of rotenone normally used for fish control are toxic to leopard frog tadpoles.

Our study demonstrates that complex interactions between predators, wetland hydroperiod, and successional processes shape amphibian and invertebrate wetland communities. Natural processes break down with the addition of an introduced invasive predator, and populations of other predators and grazers are altered. Our study provides several lessons for wetland restoration ecologists. First, the introduction of an invasive predator can have drastic consequences for wetland community development. Other predators can be eliminated or reduced and multiple trophic levels can be impacted. Second, it is difficult to design a wetland with a predictable hydroperiod. Our wetlands may dry when annual precipitation levels are lower but they currently function as permanent ponds with high predator populations. These are unsuitable for early colonizing amphibians. It is important to include a diverse array of wetlands of varying sizes and depths to ensure diversity of hydroperiods in the landscape (Petranka et al. 2007, Shoo et al. 2011). This will help ensure that populations of mosquitofish and other aquatic predators that colonize, or invade after flood events, will not persist in some wetlands. Third, chemical restoration of wetlands with rotenone applications can effectively “re-set” aquatic community development in a manner similar to drying, but this option should be a last resort because it may result in die-offs of non-target organisms, and it does not expose nutrients and minerals present in wetland substrates as does periodic drying. Fourth, high vegetation cover is important for increasing aquatic invertebrate taxa richness and dragonfly naiad abundances. While responses to predators were stronger predictors of amphibian abundance in this study, other studies have indicated that high vegetation cover is important for some amphibians and should be encouraged (Hartel et al. 2007; Shulse et al. 2010; Shulse et al. 2011). Vegetation cover increases habitat complexity, and although its effects were not statistically significant in our study, this feature may help to reduce predation mortality. Nevertheless, this benefit appears reduced in the presence of mosquitofish that can penetrate dense vegetation, and that frequent similar habitats as larval amphibians and invertebrates.

Finally, while others have suggested that 3–5 year monitoring periods often associated with meeting regulatory requirements are too short to properly assess amphibian responses to restoration (Petranka et al. 2007), our four year study illustrates that amphibian and invertebrate communities change in relatively short time intervals in response to predators, hydroperiod, and chemical restoration. While we are not suggesting that our results imply that longer term monitoring is not necessary, especially in highly dynamic floodplain wetland ecosystems, it is probable that without natural or anthropogenic alteration of hydroperiod or predator populations, our wetlands would likely continue to remain unsuitable for some amphibian species and invertebrate taxa. These lessons should be kept in mind during wetland planning and envisioning what conditions will define “success” in the finished product.

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Table 1. Dates of sampling periods for each year of the study.

Year	Sampling Period 1	Sampling Period 2	Sampling Period 3
2007	April 25 – April 29	June 7 – June 14	July 27 – August 1
2008	May 22 – May 29	July 15 – July 17	August 18 – August 21
2009	May 31 – June 5	July 20 – July 25	September 2 – September 9
2010	May 12 – May 19	July 8 – July 15	September 2 – September 14

Table 2. Spearman correlation matrix for independent variables in amphibian generalized linear regression models.

N = 18	Mosquitofish	Crayfish	Dragonfly
Vegetation 2007	- 0.13	-0.20	0.14
2008	-0.37	-0.18	0.71
2009	-0.34	-0.56	0.78
2010	-0.44	0.61	0.68
Mosquitofish 2007		0.16	-0.64
2008		0.16	-0.63
2009		0.11	-0.50
2010		0.05	-0.44
Crayfish 2007			- 0.34
2008			-0.39
2009			-0.56
2010			-0.63

Table 3. Parameter estimates for independent variables in amphibian generalized linear regression models.

Species	*Year*	*Parameter*	*β*	*SE*	*Wald* χ^2	*95% CI* **Lower**	**Upper**	*Sig.*
Gray Treefrog complex	2007	Mosquitofish	-0.02	0.01	4.38	-0.05	-0.001	0.04
		Crayfish	-0.03	0.01	7.59	-0.05	-0.009	0.006
		Dragonfly	0.11	0.08	1.87	-0.05	0.28	0.17
		Vegetation	19.81	5.79	11.73	8.47	31.16	0.001
	2008	Mosquitofish	-0.06	0.02	11.68	-0.09	-0.03	0.001
		Crayfish	-0.02	0.01	2.18	-0.05	0.007	0.14
		Dragonfly	-0.11	0.05	5.57	-0.21	-0.02	0.02
	2009	Mosquitofish	-0.03	0.01	8.29	-0.05	-0.009	0.004
		Crayfish	-0.60	0.27	4.97	-1.13	-0.07	0.03
		Dragonfly	-0.05	0.03	2.82	-0.11	0.009	0.09
	2010	Crayfish	-0.01	0.01	0.98	-0.04	0.01	0.32
		Dragonfly	0.003	0.02	0.03	-0.03	0.04	0.87
		Vegetation	2.77	1.84	2.27	-0.84	6.38	0.13
Boreal Chorus Frog	2008	Mosquitofish	-0.02	0.006	10.89	-0.03	-0.009	0.001
		Crayfish	-0.04	0.03	1.61	-0.11	0.02	0.21
		Dragonfly	0.01	0.07	0.02	-.013	0.15	0.88
	2010	Mosquitofish	-0.04	0.01	6.87	-0.07	-0.01	0.009
		Crayfish	0.001	0.02	0.001	-0.04	0.04	0.97
		Dragonfly	-0.12	0.05	6.58	-0.21	-0.03	0.01
		Vegetation	1.46	2.54	0.33	-3.53	6.44	0.57
Green Frog	2008	Mosquitofish	-0.003	0.01	0.06	-0.02	0.02	0.80
		Crayfish	-0.05	0.02	4.91	-0.10	-0.006	0.03
		Dragonfly	-0.12	0.06	4.35	-0.24	-0.008	0.04
	2009	Mosquitofish	0.003	0.003	0.96	-0.003	0.009	0.33
		Crayfish	-0.10	0.03	8.34	-0.17	-0.03	0.004
		Dragonfly	-0.02	-0.02	0.54	-0.06	0.03	0.46
	2010	Mosquitofish	0.008	0.006	2.10	-0.003	0.02	0.15
		Crayfish	-0.07	0.02	13.05	-0.11	-0.03	<0.001
		Dragonfly	0.007	0.01	0.27	-0.02	0.03	0.60
		Vegetation	-0.13	1.54	0.007	-3.15	2.88	0.93

Table 4. Hierarchical regression statistics for ranid TIAI scores.

Model	*Year*	R^2	R^2 *Change*	*F*	*F Change*	*Sig. F Change*	*β*	*Sig.*
1 Mosquitofish	2009	0.45	0.45	12.99	12.99	0.002	0.67	0.002
2 Mosquitofish	2009	0.46	0.01	6.28	0.21	0.65	0.68	0.003
Crayfish							0.09	0.65
3 Mosquitofish	2009	0.54	0.08	5.43	2.49	0.14	0.51	0.03
Crayfish							-0.03	0.86
Dragonfly							-0.35	0.14
4 Mosquitofish	2009	0.57	0.03	4.31	0.97	0.34	0.48	0.04
Crayfish							0.01	0.97
Dragonfly							-0.49	0.09
Vegetation							0.24	0.34
1 Mosquitofish	2010	0.34	0.34	7.67	7.65	0.01	0.58	0.01
2 Mosquitofish	2010	0.51	0.17	7.28	4.90	0.04	0.65	0.004
Crayfish							0.42	0.04
3 Mosquitofish	2010	0.54	0.03	5.09	0.85	0.37	0.75	0.005
Crayfish							0.55	0.04
Dragonfly							0.23	0.37
4 Mosquitofish	2010	0.60	0.06	4.56	1.92	0.19	0.68	0.009
Crayfish							0.45	0.08
Dragonfly							0.37	0.19
Vegetation							-0.33	0.19

Figure 1. Generalized tadpole drawings and representative photos of ranid tadpoles with tail damage. Numbers = TIAI scores. The tadpole in the photo marked with “*”was captured in a fish-free wetland with tail damage that may have been inflicted by an invertebrate. Note the red lesion on the body of the tadpole in the photo illustrating a TIAI score of 1. This lesion is typical of those found on tadpoles in White 1.

1

2

3

3

4

4

5

5

1

2

3

4

4*

5

Figure 2. Mean abundance trends for anurans over all sampling periods. The mean abundance of chorus frogs was 102 in 2008. The scale of the Y-axis has been capped at 30 to more clearly illustrate trends.

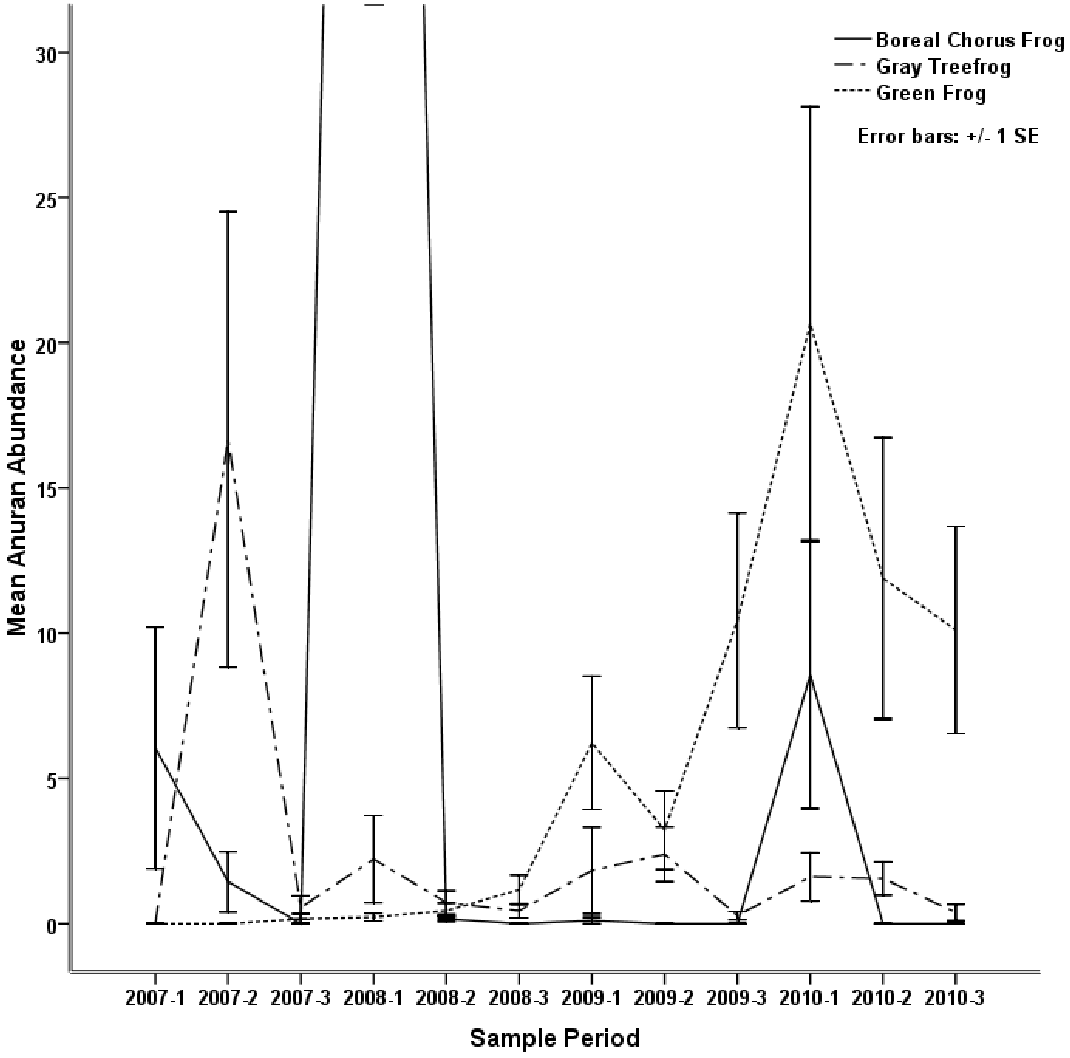

Boreal Chorus Frog
Gray Treefrog
Green Frog
Error bars: +/- 1 SE
Mean Anuran Abundance
30
25
20
15
10
5
0
2007-1
2007-2
2007-3
2008-1
2008-2
2008-3
2009-1
2009-2
2009-3
2010-1
2010-2
2010-3
Sample Period

Figure 3. Occurrence trends for anurans over all four study years. Gray treefrogs did not occur in more than 50% of the wetlands during any year. Chorus frog occurrence peaked in 2008 and plummeted in 2009 before rebounding in 2010. Green frog occurrence increased over the duration of the study.

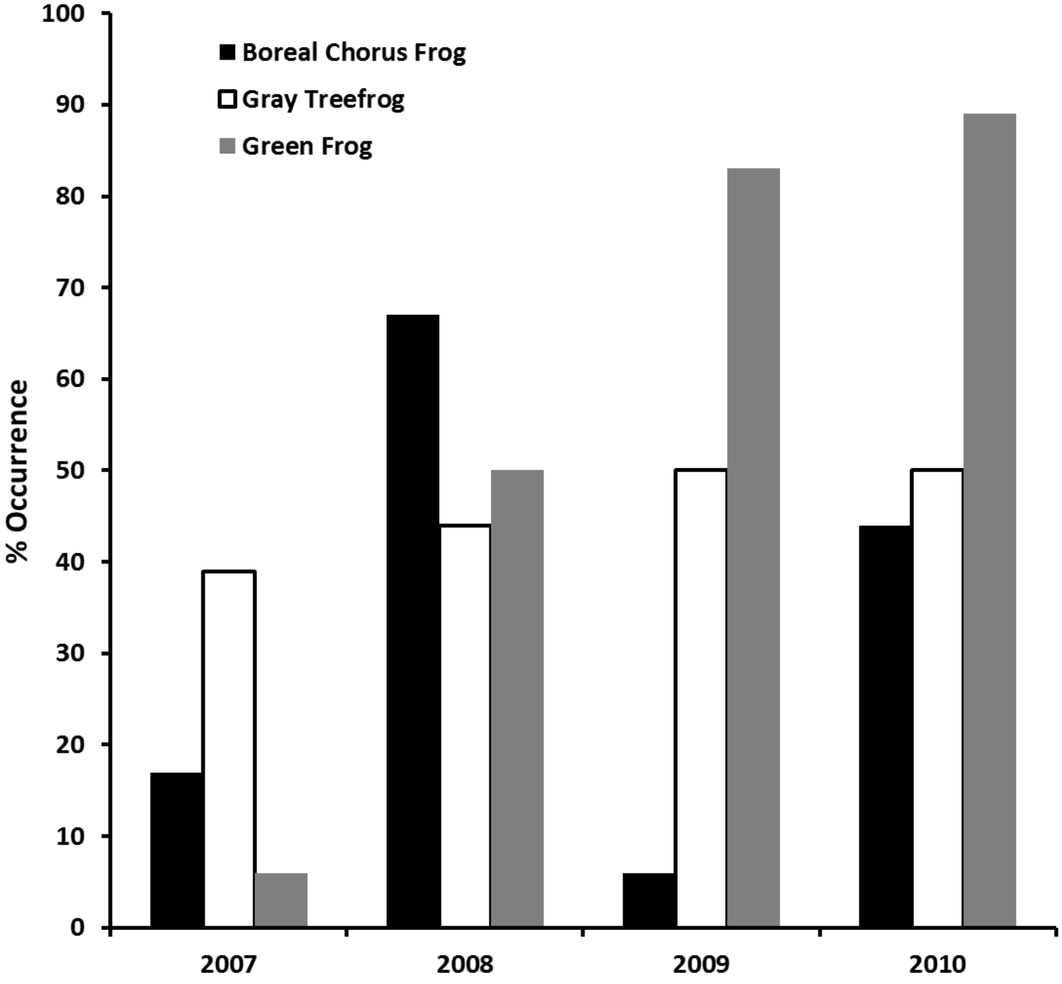

% Occurrence
100
90
80
70
60
50
40
30
20
10
0
■ Boreal Chorus Frog
☐ Gray Treefrog
■ Green Frog
2007
2008
2009
2010

Figure 4. Ranid TIAI scores for each wetland in 2009 and 2010. Wetlands marked with "*" were stocked with mosquitofish. All fish-stocked wetlands at Redman were treated with rotenone on 17 September 2009 and all Sears wetlands were treated on 10 March 2010. Fish re-appeared at Sears 1 during the second sampling period of 2010. None of the White wetlands were treated with rotenone.

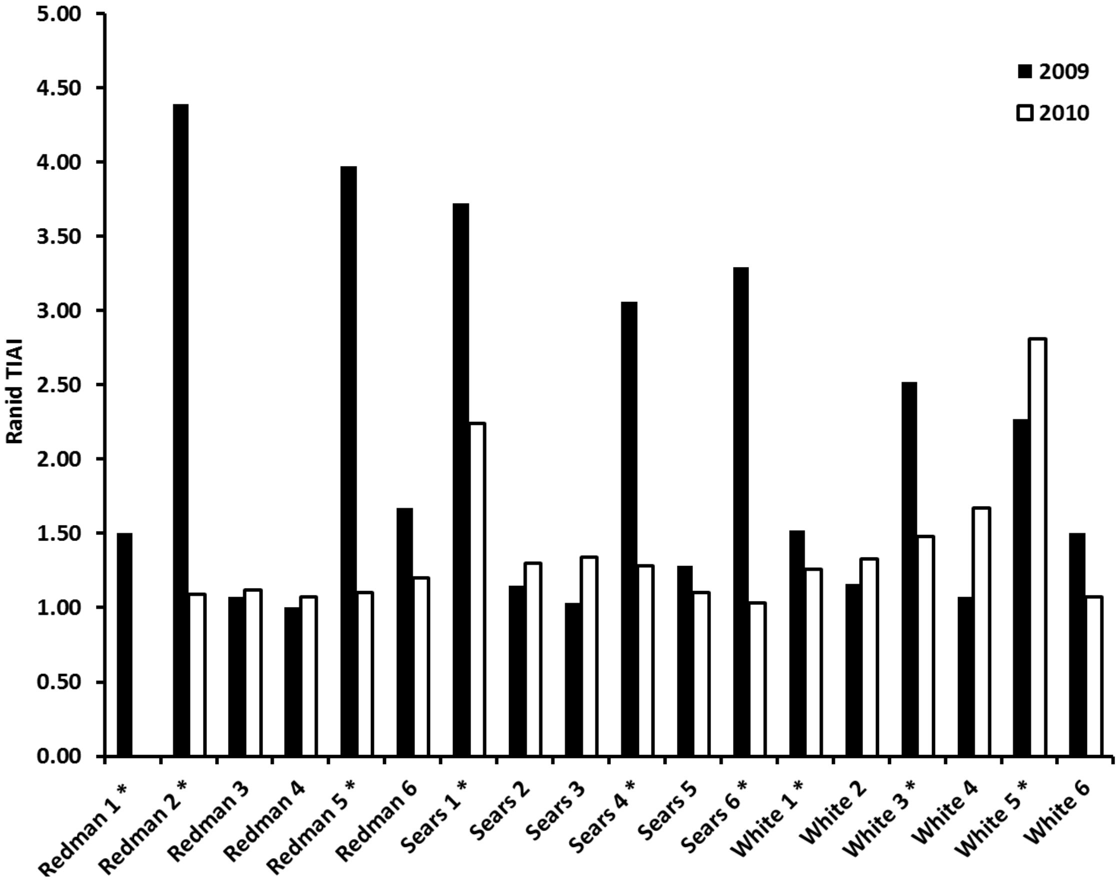

5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Ranid TIAI
■ 2009
□ 2010
Redman 1 *
Redman 2 *
Redman 3
Redman 4
Redman 5 *
Redman 6
Sears 1 *
Sears 2
Sears 3
Sears 4 *
Sears 5
Sears 6 *
White 1 *
White 2
White 3 *
White 4
White 5 *
White 6

Figure 5. Mean invertebrate abundance trends over all sampling periods. Invertebrate abundances were consistently higher in wetlands without mosquitofish. Fish-stocked: N=9 for 2007 – 2009 and N=4 for 2010. Fish un-stocked: N=9 for 2007 – 2009 and N=14 for 2010.

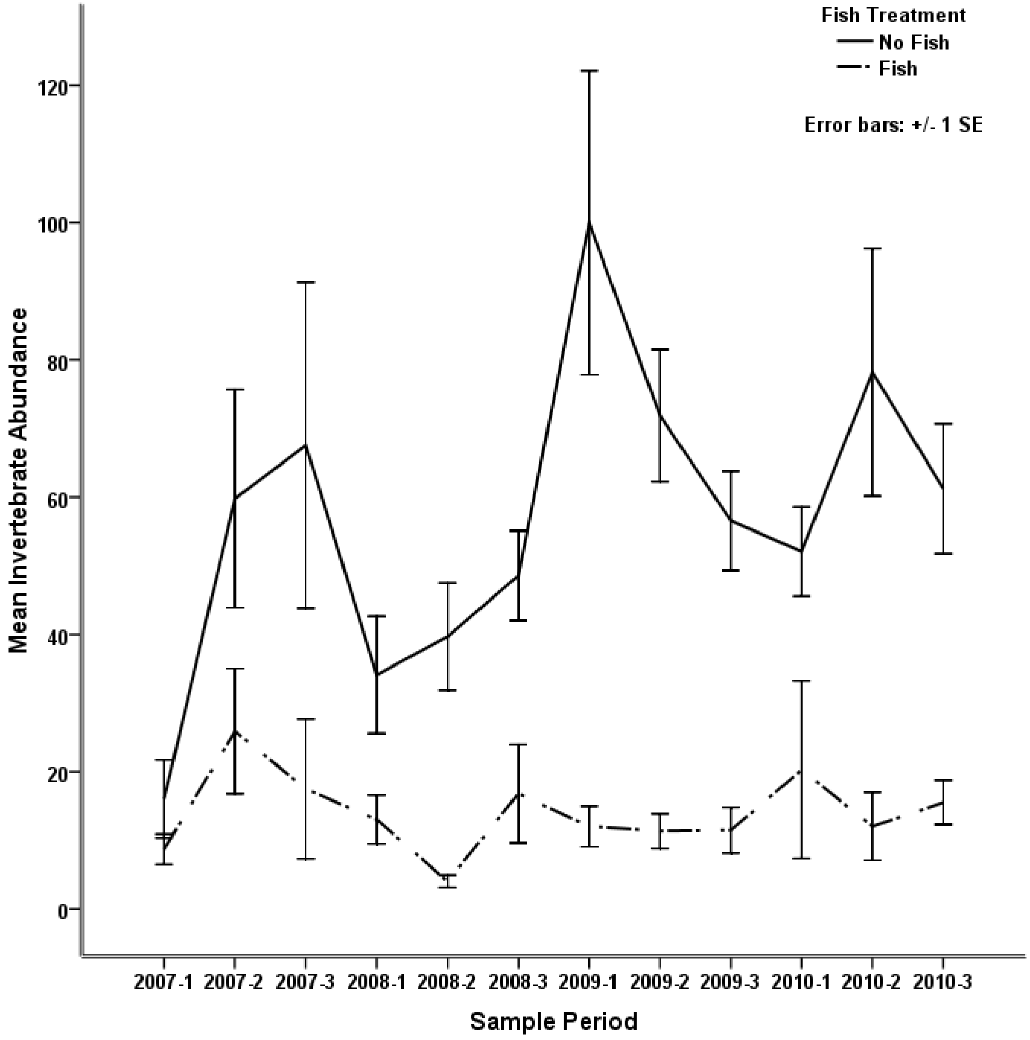

Fish Treatment
No Fish
Fish
Error bars: +/- 1 SE
Mean Invertebrate Abundance
0
20
40
60
80
100
120
2007-1
2007-2
2007-3
2008-1
2008-2
2008-3
2009-1
2009-2
2009-3
2010-1
2010-2
2010-3
Sample Period

Figure 6. Mean invertebrate taxa richness over all sampling periods. While trends for both fish-stocked and un-stocked wetlands generally increased throughout the duration of the study, taxa richness was consistently highest in fish-free wetlands. Fish-stocked: N=9 for 2007 – 2009 and N=4 for 2010. Fish un-stocked: N=9 for 2007 – 2009 and N=14 for 2010.

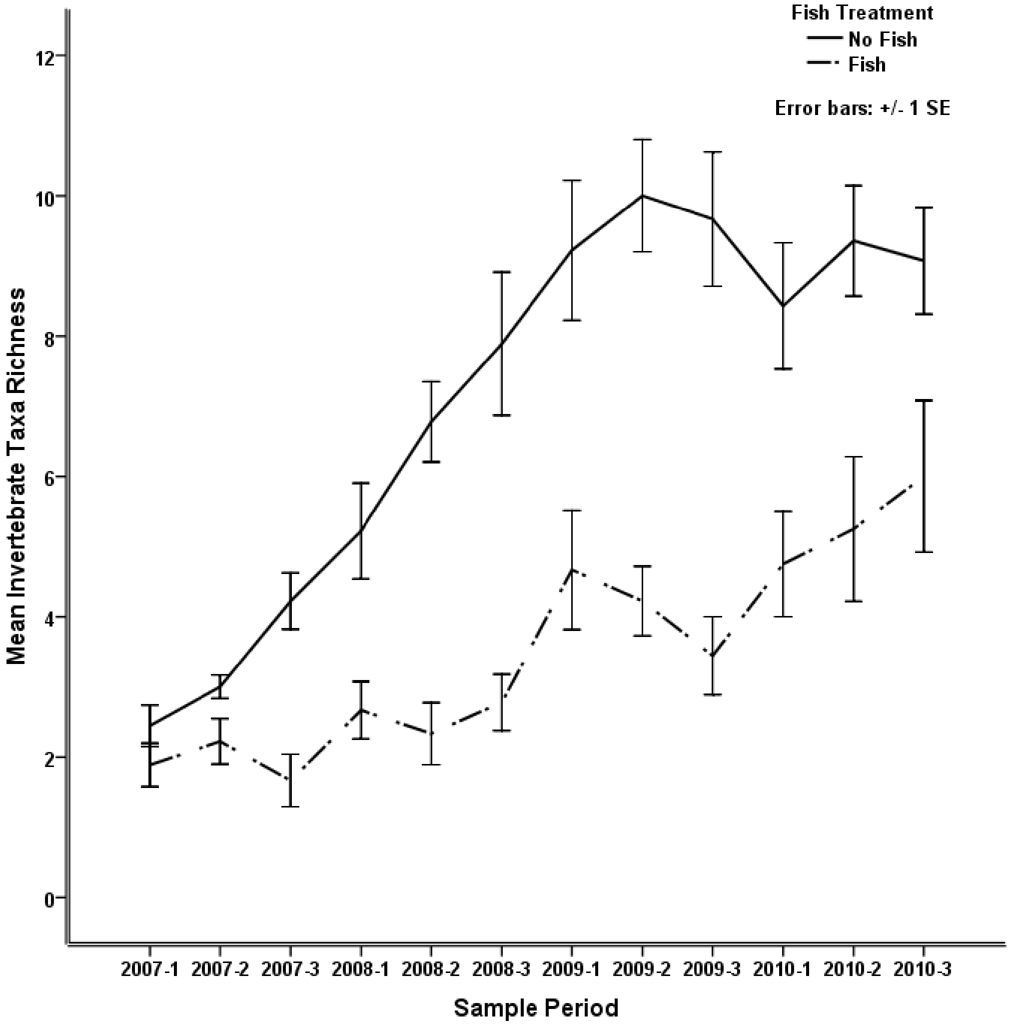
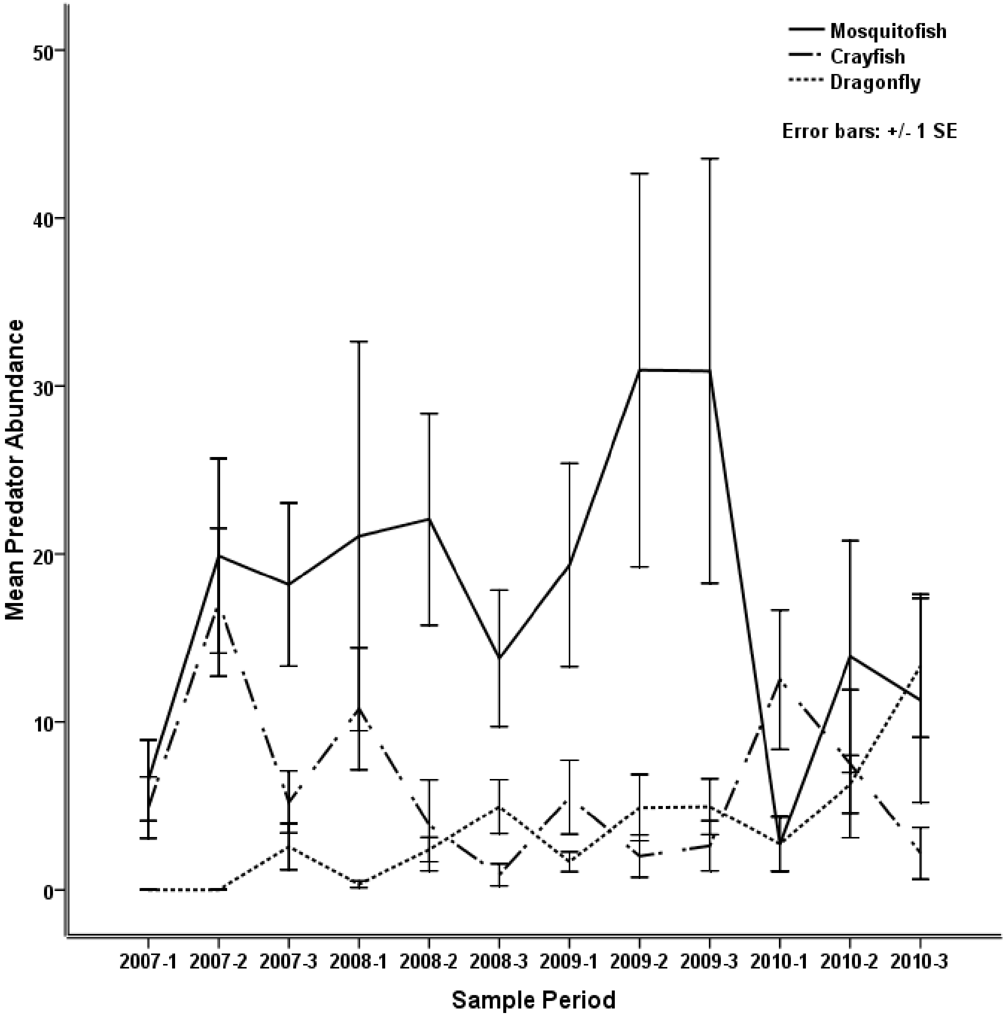

Fish Treatment
No Fish
Fish
Error bars: +/- 1 SE
Mean Invertebrate Taxa Richness
0
2
4
6
8
10
12
2007-1
2007-2
2007-3
2008-1
2008-2
2008-3
2009-1
2009-2
2009-3
2010-1
2010-2
2010-3
Sample Period

Figure 7. Mean predator abundance over all sampling periods. Crayfish abundance peaked during 2007 but rebounded in 2010 and they were most abundant during spring and early summer. Dragonflies peaked in abundance in late summer and became more abundant over the duration of the study. Mosquitofish abundance plummeted after the rotenone treatments in late 2009 and early 2010 but their populations recovered after they re-appeared in Sears 1 and reproduction increased over the summer at the stocked White wetlands.


Mosquitofish
Crayfish
Dragonfly
Error bars: +/- 1 SE
Mean Predator Abundance
50
40
30
20
10
0
2007-1
2007-2
2007-3
2008-1
2008-2
2008-3
2009-1
2009-2
2009-3
2010-1
2010-2
2010-3
Sample Period

APPENDIX

Figure 1. Vegetation development at each wetland. * = 15:1 slope planted wetland. ** = 4:1 slope unplanted wetland. Un-marked = 15:1 un-planted wetland.

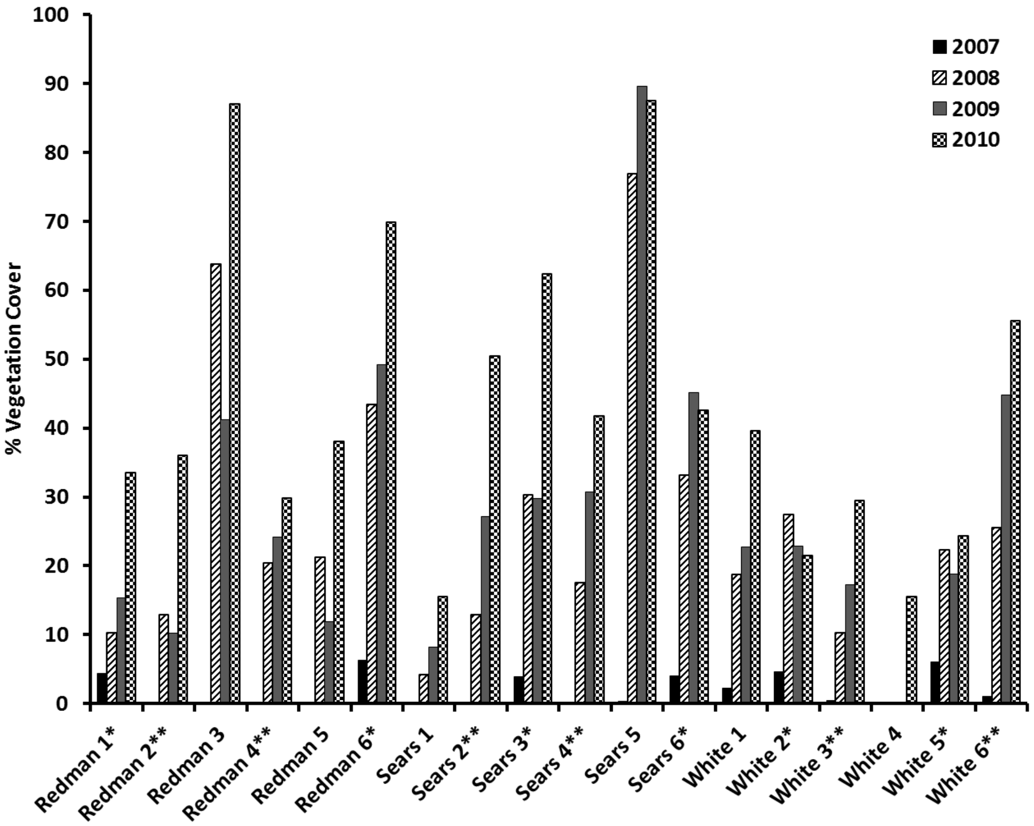
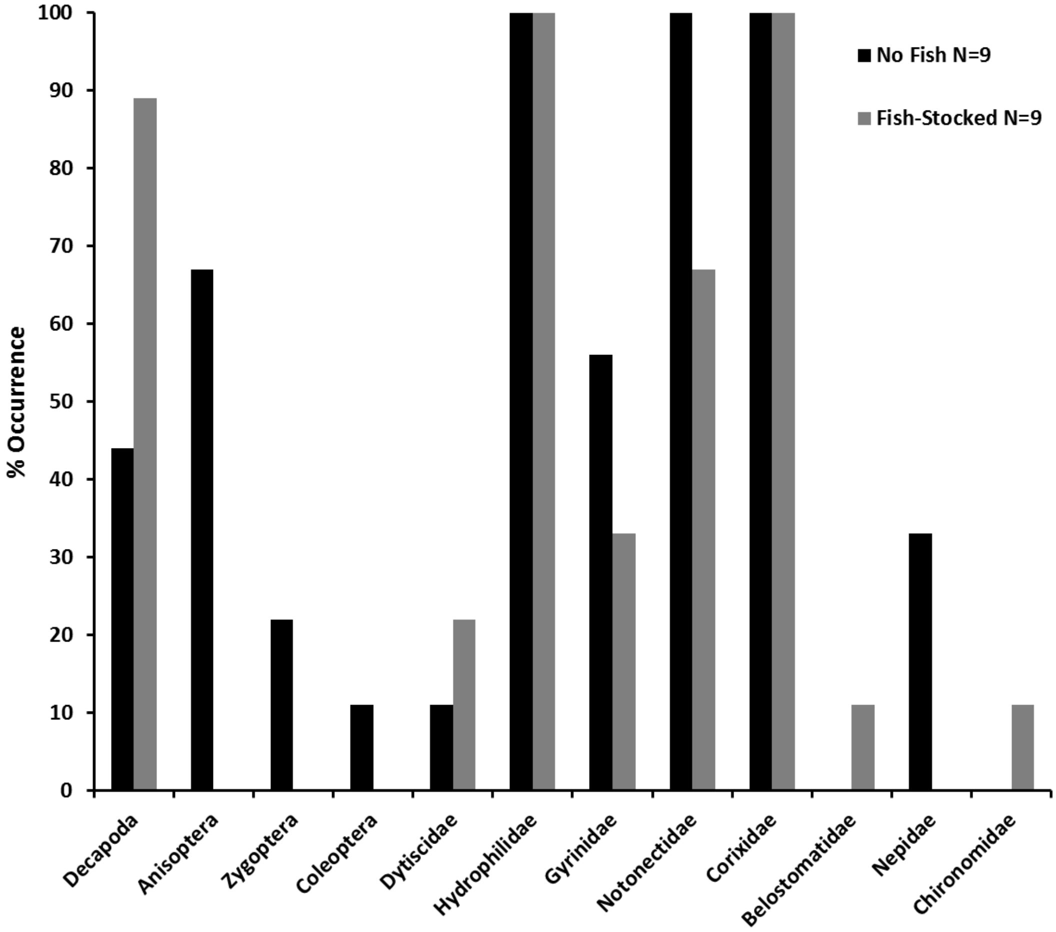

100
90
80
70
60
50
40
30
20
10
0
% Vegetation Cover
■ 2007
▨ 2008
■ 2009
▩ 2010
Redman 1*
Redman 2**
Redman 3
Redman 4**
Redman 5
Redman 6*
Sears 1
Sears 2**
Sears 3*
Sears 4**
Sears 5
Sears 6*
White 1
White 2*
White 3**
White 4
White 5*
White 6**

Figure 2. Invertebrate taxa occurrence in 2007.



No Fish N=9
Fish-Stocked N=9
% Occurrence
100
90
80
70
60
50
40
30
20
10
0
Decapoda
Anisoptera
Zygoptera
Coleoptera
Dytiscidae
Hydrophilidae
Gyrinidae
Notonectidae
Corixidae
Belostomatidae
Nepidae
Chironomidae

Figure 3. Invertebrate taxa occurrence in 2008. * Some notonectids may have been misidentified as corixids in 2008.

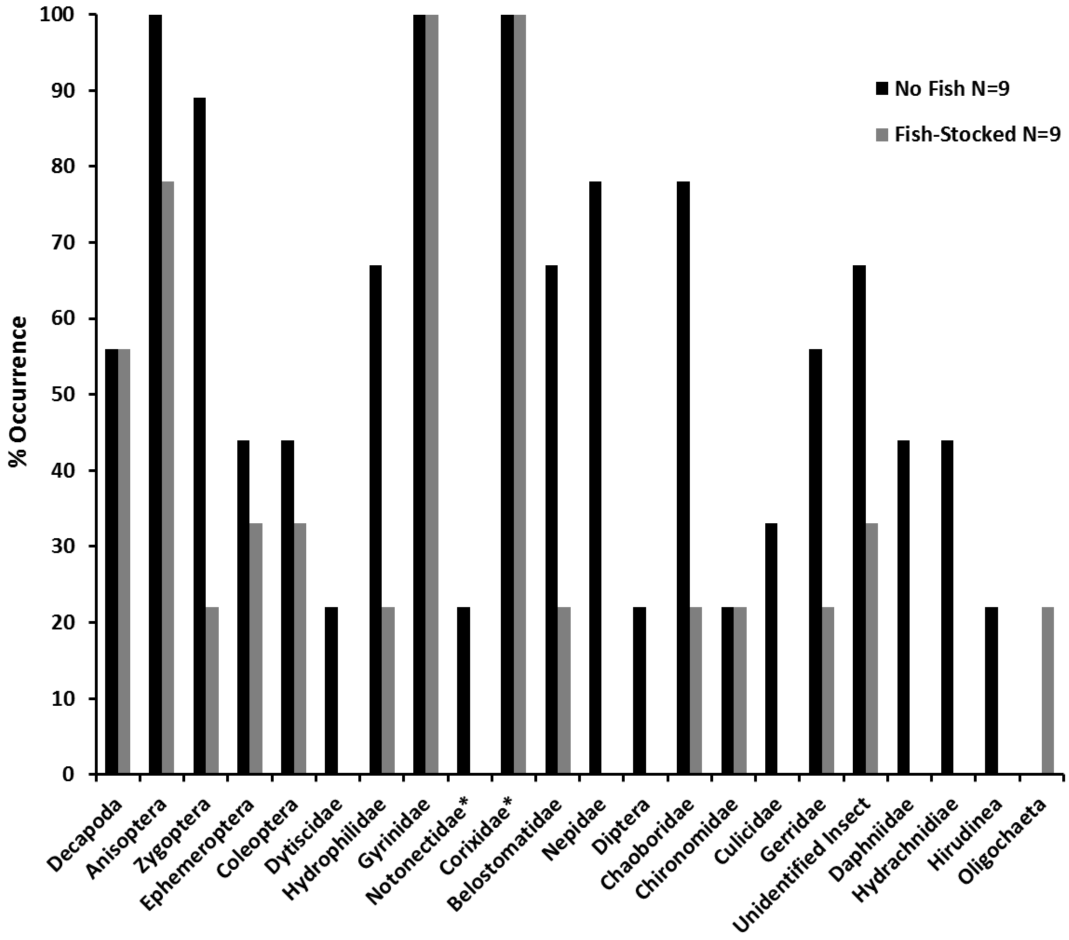

100
90
80
70
60
50
40
30
20
10
0
% Occurrence
■ No Fish N=9
■ Fish-Stocked N=9
Decapoda
Anisoptera
Zygoptera
Ephemeroptera
Coleoptera
Dytiscidae
Hydrophilidae
Gyrinidae
Notonectidae*
Corixidae*
Belostomatidae
Nepidae
Diptera
Chaoboridae
Chironomidae
Culicidae
Gerridae
Unidentified Insect
Daphniidae
Hydrachnidiae
Hirudinea
Oligochaeta

Figure 4. Invertebrate taxa occurrence in 2009.

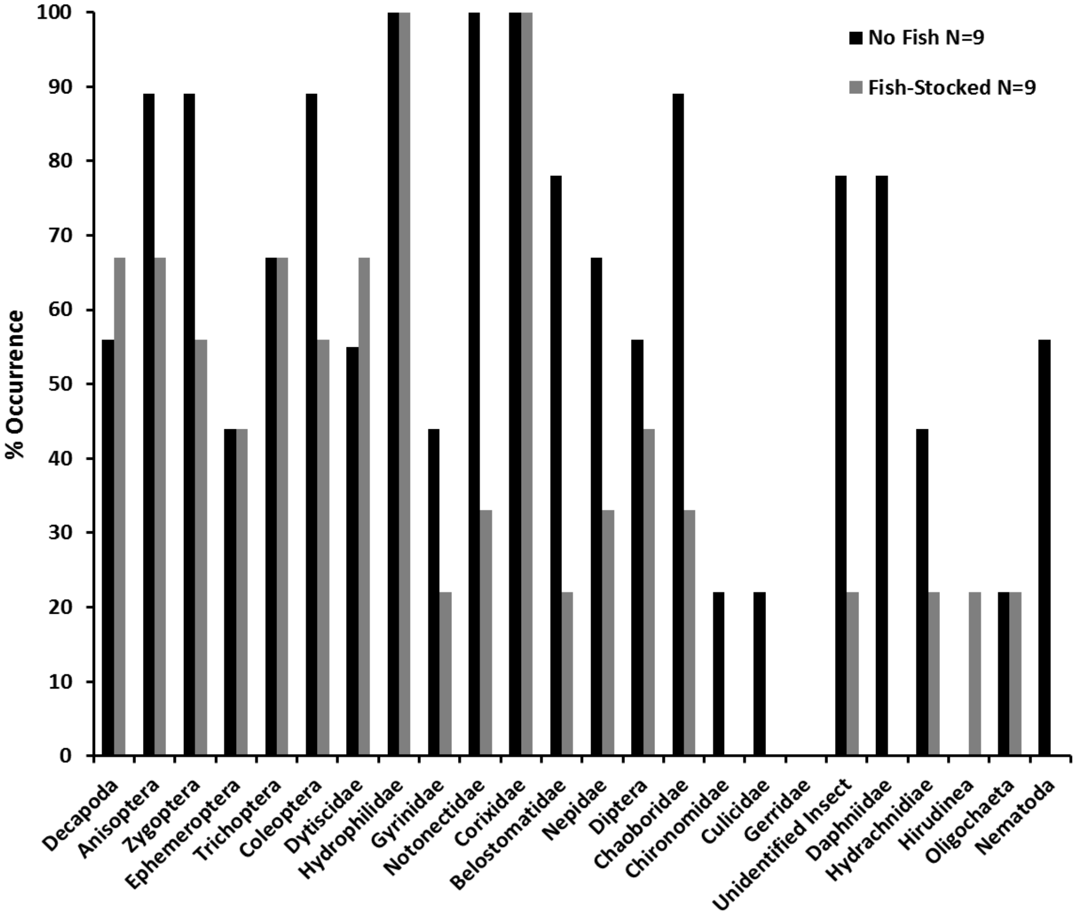
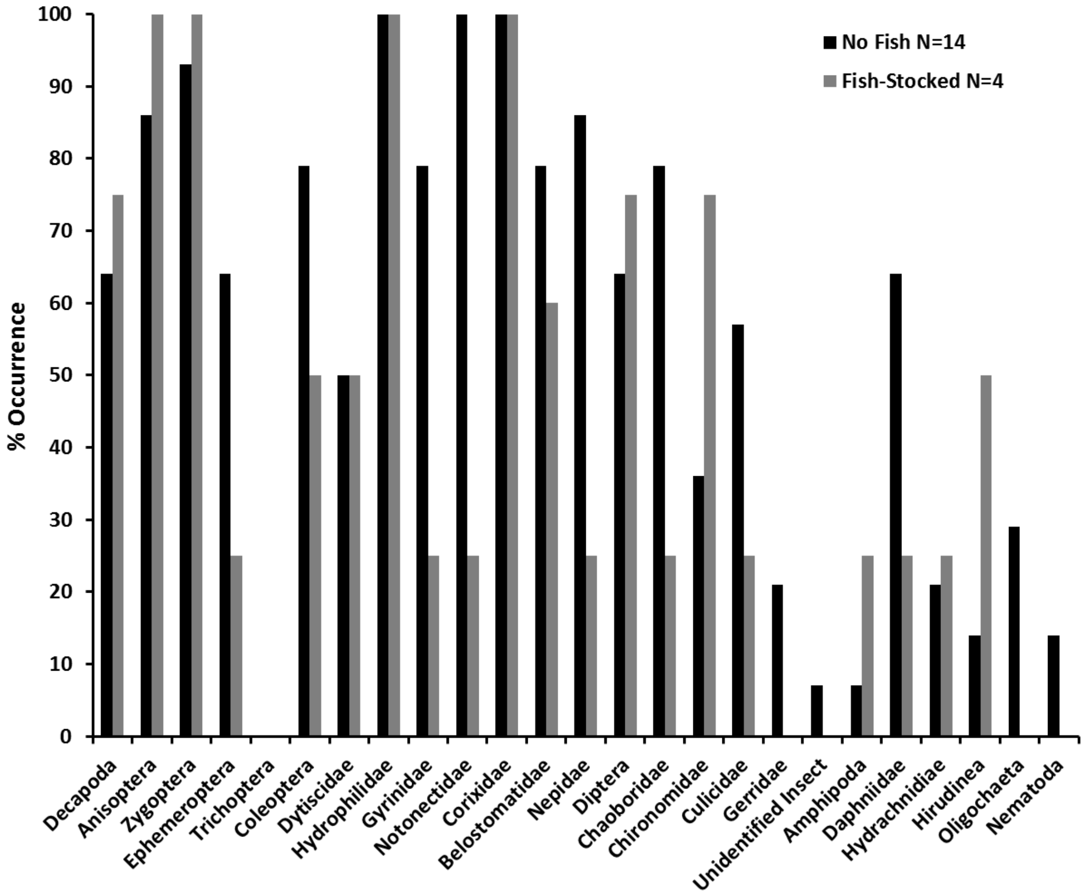

% Occurrence
0
10
20
30
40
50
60
70
80
90
100
No Fish N=9
Fish-Stocked N=9
Decapoda
Anisoptera
Zygoptera
Ephemeroptera
Trichoptera
Coleoptera
Dytiscidae
Hydrophilidae
Gyrinidae
Notonectidae
Corixidae
Belostomatidae
Nepidae
Diptera
Chaoboridae
Chironomidae
Culicidae
Gerridae
Unidentified Insect
Daphniidae
Hydrachnidiae
Hirudinea
Oligochaeta
Nematoda

Figure 5. Invertebrate taxa occurrence in 2010.



% Occurrence
100
90
80
70
60
50
40
30
20
10
0
No Fish N=14
Fish-Stocked N=4
Decapoda
Anisoptera
Zygoptera
Ephemeroptera
Trichoptera
Coleoptera
Dytiscidae
Hydrophilidae
Gyrinidae
Notonectidae
Corixidae
Belostomatidae
Nepidae
Diptera
Chaoboridae
Chironomidae
Culicidae
Gerridae
Unidentified Insect
Amphipoda
Daphniidae
Hydrachnidiae
Hirudinea
Oligochaeta
Nematoda

CHAPTER 5

SUMMARY OF CONCLUSIONS AND MANAGEMENT IMPLICATIONS

For constructed wetlands to be effective for conserving amphibians, they must be designed properly and placed within appropriate landscapes for target species. Failure to provide suitable conditions at both aquatic and core terrestrial habitat levels can prevent colonization or lead to reduced reproductive success. Evaluating habitat feature influences on amphibian reproductive success and species richness provides planners and managers with the information necessary to more effectively replace lost habitat and potentially reverse declines.

Wetland Placement (Chapter 2)

1. Wetlands placed in landscapes with high anthropogenic disturbance can decrease their ability to support breeding populations of conservative species. Salamanders and many hylid frog species require wooded or grassland habitats adjacent to breeding sites. High percentages of cropland and high road densities near wetlands can lower the occurrence and reproductive success of these amphibians. Wetland planning in the region should prioritize placement based on the requirements of salamanders and hylid species that were rarely captured.
2. Bullfrogs were strongly associated with high open water pond densities in the landscape surrounding sampled wetlands. Open water ponds are becoming increasingly common in the landscape of the Midwest, probably facilitating the

spread of bullfrogs away from native habitats. New wetlands placed in areas with high open water pond densities may be subject to bullfrog colonization early in their development, exposing other amphibian colonists to increased predation, competition, and pathogens.

3. Streams and open water ponds were important placement predictors for cricket frogs. This species has suffered declines at the edge of its range and, while apparently common in Missouri, placement of new wetlands near streams and other permanent aquatic habitat can provide additional breeding habitat and aid in dispersal.
4. High percentages of grasslands in core terrestrial habitats were strong predictors for leopard frogs, chorus frogs, and smallmouth salamanders. These amphibians, and species of conservation concern such as the eastern tiger salamander (*Ambystoma tigrinum tigrinum*) and northern crawfish frog (*Lithobates areolatus circulosus*), will likely benefit from prairie wetland restoration.
5. New wetland construction and restoration should be coupled with existing quality habitats. Restoring farmed wetlands will result in net gains in wetland area, but if the surrounding habitat is degraded or in an early successional state, species requiring less disturbed core terrestrial habitat likely will not benefit.
6. Protecting the core terrestrial habitat where wetlands are placed cannot be decoupled from protection of the aquatic habitat. Without both habitats, species that spend a majority of their lives in surrounding uplands will disappear from breeding sites. Current regulations that do not recognize the importance of

surrounding uplands in maintaining wetland ecosystems will lead to a net loss of ecological functions.

Wetland Design

1. Steep within-water slopes increased abundances of bullfrogs and green frogs (Chapter 2) but reduced the reproductive success of leopard frogs, American toads, and boreal chorus frogs (Chapters 2 and 3). Most metamorph production at experimental wetlands occurred in shallow sloped wetlands (Chapter 3) but the effects were strongest for toads and chorus frogs. Furthermore, the advantages for chorus frogs and leopard frogs diminished after the first year post-construction. My results seem to suggest that slope may be less important than the other aquatic features for some amphibians as wetlands develop. However, constructing wetlands with shallow slopes requires less excavation and labor than steep-sloped wetlands providing an economic incentive to design wetlands with shallows.
2. Vegetation cover was an important predictor for increased abundances of chorus frogs, spring peepers, and salamanders in existing wetlands (Chapter 2); and planted vegetation increased both species richness and metamorph production for some amphibians in experimental wetlands (Chapter 3). Although planted vegetation likely provided little effective cover in the first year post-construction, it served as an oviposition substrate (Chapter 3). Vegetation cover surpassed slope in importance for some amphibians during the second year suggesting that as aquatic communities develop, habitat complexity increases in importance. Planting vegetation provides a limited but early boost for amphibian reproduction and natural vegetation colonization and development should be encouraged.

3. Fish were the strongest predictors for reducing abundances of chorus frogs, spring peepers, and salamanders at existing wetlands (Chapter 2) and the reproductive success of chorus frogs and gray treefrogs was also sharply reduced by mosquitofish in the experimental wetlands (Chapter 3 and 4). Furthermore, mosquitofish lowered species richness in both years and was the most important variable driving richness during the second year. The eggs and larvae of many amphibian species are palatable to fish. Wetland designers should incorporate pools into wetland complexes, perched at elevations where fish colonization is unlikely, and low berms can isolate pools from surface connections that disperse fish. Managers should refrain from stocking fish because fish-free wetlands are essential habitat for many salamanders and anurans.
4. Mosquitofish warrant special attention because of their invasiveness and their unwarranted reputation as benign but effective biological control agents of mosquitoes. In addition to reducing the reproductive success of chorus frogs and gray treefrogs (Chapters 3 and 4), these fish also cause sub-lethal injuries to anuran tadpoles much larger than themselves (Chapter 4). Furthermore, they inhibit the development of aquatic communities by reducing both invertebrate abundance and species richness (Chapter 4). Vegetation does not appear to provide adequate cover for amphibians or invertebrates from mosquitofish (Chapter 4). Stocking wetlands and other water bodies with mosquitofish, particularly outside of their native range, is a practice that should cease. Native caudate larvae, invertebrates, and native fish prey upon mosquito larvae and are preferable biological controls.

5. Chemical restoration of wetlands using rotenone was generally effective at eliminating mosquitofish (Chapter 4). Breeding chorus frogs re-colonized restored wetlands, ranid tail injuries were reduced, and invertebrate communities responded positively. However, the treatment was not effective in one wetland, and mortality of ranid adults and larvae were noted at several wetlands after a late winter treatment. Natural drying or pumping wetlands dry to eliminate fish are preferable to chemical restoration, but in large, permanent wetlands, chemical restoration may be the only viable option for eliminating invasive fish species and reestablishing populations of fish-sensitive amphibians.
6. Wetland hydroperiod was a strong predictor for amphibian species requiring permanent or semi-permanent wetlands for larval development (Chapter 2). Populations of early colonizing gray treefrogs and chorus frogs decrease as aquatic invertebrate predator populations rise in wetlands that remain permanent (Chapter 4). Conversely, green frogs and other large ranids with overwintering larvae become more abundant. Wetlands that dry periodically will eliminate populations of aquatic invertebrate predators and large ranid larvae that can eliminate or reduce larvae of early colonizing amphibian species. It is important to include a range of wetlands (large and small, temporary and permanent) into wetland complexes to accommodate diverse amphibian assemblages and buffer the effects of stochastic events.

VITA

Christopher Darren Shulse was born 14 March 1973 in Hannibal, Missouri. He graduated from Hannibal High School in 1991 and earned a B.S. in Biology from Culver-Stockton College in Canton, Missouri in 1995. While at Culver-Stockton, he earned the Dalton Biology Research Award for his research on amphibian cellular physiology. He married Tracy Carpenter in August 1995 and the couple moved to Columbus, Ohio in September 1995 where Chris began his tenure at The Ohio State University under the guidance of Dr. Thomas E. Hetherington. Chris studied neurophysiology and the pathways of airborne sound reception in snakes. He earned a M.S. in Zoology in August 1997. Chris and Tracy moved back to Hannibal, Missouri in June 1997 and Chris began his employment with the Missouri Department of Transportation. He began his tenure at the University of Missouri in 2006 under the guidance of Dr. Raymond D. Semlitsch. He earned his Ph.D. from the Division of Biological Sciences in May 2011. Chris is currently employed by the Missouri Department of Transportation. He plans to continue his employment with the State of Missouri in a profession where he can continue to conduct conservation research. Upon retirement, he plans to pursue a second career as a faculty member at a small, liberal arts college or university. Chris and Tracy have two children - Austin, age 11, and Ashlee, age 9.