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Influences of Cattle on Postmetamorphic Amphibians on the Cumberland Plateau

Elizabeth Carrie Burton
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Elizabeth Carrie Burton entitled "Influences of Cattle on Postmetamorphic Amphibians on the Cumberland Plateau." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

Matthew J. Gray, Major Professor

We have read this thesis and recommend its acceptance:

Debra Miller, Lisa Muller, Ben Fitzpatrick

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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and recommend its acceptance:

Dr. Debra Miller
Debra L. Miller

Dr. Lisa Muller
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Accepted for the Council:

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Carolyn R. Hodges, Vice Provost and
Dean of the Graduate School

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INFLUENCES OF CATTLE ON POSTMETAMORPHIC AMPHIBIANS ON THE CUMBERLAND PLATEAU

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Elizabeth Carrie Burton
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ABSTRACT

Global decline of amphibian populations has been linked to various anthropogenic stressors. Recent studies have quantified the influences of cropland agriculture and deforestation; however, few have examined the impacts of allowing cattle access in wetlands on resident amphibians. I compared four wetlands exposed to cattle grazing for >10 years against four wetlands that had not been grazed for >10 years, at the University of Tennessee Plateau Research and Education Center. At each wetland I measured species richness, diversity, and species-specific relative abundance of postmetamorphic amphibians captured in pitfall traps and during breeding call surveys, amphibian egg mass abundance, shoreline vegetation structure, and soil compaction from March – August 2005 and 2006. Pathogen prevalence and histopathological changes were measured from a subsample of opportunistically collected amphibians. Landscape characteristics were quantified and related to amphibian community structure. Relative abundance of green frog metamorphs was 9.8X greater in 2006 and 2.3X greater in 2005 at non-access wetlands. Relative abundance of American toads was 68X and 76X greater at cattle-access wetlands in 2005 and 2006, respectively. Breeding call abundance of American toad, Fowler's toad, and Cope's gray treefrog was 4 – 25X greater at cattle-access wetlands in 2006. There were 2X more spring peepers and pickerel frogs calling at non-access wetlands in 2005 and 2006, respectively. Species richness, diversity, and egg mass abundance were not significantly different between land-use types each year. In general, body size followed a density-dependent relationship across species. Height and percent horizontal and vertical cover of shoreline vegetation were 74%, 25% and 84% greater, respectively, in non-access wetlands in 2005; trends were similar in 2006.

Soil compaction was 55% greater at cattle-access wetlands. Pathogen prevalence and histopathological changes did not differ between land uses. Landscape analyses revealed species-specific associations related to wetland isolation and geometric complexity of the landscape between wetlands. My results suggest that cattle influence community composition and postmetamorphic body size of amphibians, but effects are species-specific. Differences in postmetamorphic abundance may be related to less vegetation structure and lower water quality at cattle-access wetlands. Fencing cattle from wetlands may be a prudent conservation strategy for some amphibian species.

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CHAPTER I

INTRODUCTION

In 2004, the Global Amphibian Assessment (GAA) released a report indicating that 32% of amphibian species were in decline and in threat of extinction (GAA 2004). Since 1980, 122 known species have gone extinct or have not been found recently and are considered likely extinct (GAA 2004). Tennessee is home to 21 anuran and around 40 salamander species, making it the most species-rich state in the Southeast (TWRA 2004). The Tennessee Wildlife Resources Agency (TWRA) lists 35% of the amphibian species in Tennessee in concern of decline (TWRA 2005). These declines are considered unprecedented, particularly because similar rates of declines are not occurring for bird and mammal species (i.e., 12% and 23% in decline, respectively, GAA 2004). This is concerning because amphibians are important components of aquatic and terrestrial ecosystems (Whiles et al. 2006), and some have potential human medicinal properties (e.g., skin peptides destroying HIV, VanCompernolle et al. 2005). They also are considered sentinels of environmental deterioration, because contaminants can pass easily through their skin, many species depend on both aquatic and terrestrial environments, and they are prone to desiccation following metamorphosis (Alford and Richards 1999). Also due to their typical biphasic life cycle, they are exposed to various pathogens and predators in both aquatic and terrestrial environments. Decline in amphibian populations could signal the onset of environmental degradation (Alford and Richards 1999), and mass extinctions could destabilize the structure of aquatic and terrestrial trophic levels (Whiles et al. 2006). There are several hypotheses for amphibian declines, most which are related to anthropogenic causes. These hypotheses include global climate change,

UV-B radiation (wavelength range 280 – 320 nm), pathogens (bacteria, fungi, viruses and parasites), water contamination, introduction of exotic species, exploitation for food or pets, and habitat deterioration or destruction (Houlahan et al. 2000, Collins and Storfer 2003, Kiesecker et al. 2004, Stuart et al. 2004).

Anthropogenic influences on atmospheric conditions may be contributing to large-scale changes in global distribution and persistence of amphibian populations (Beebee 1995). Rising global temperatures and altered rainfall patterns could change amphibian distributions, reduce water levels, and increase water temperature, possibly leading to local extinctions of amphibian populations over large geographic scales (Kiesecker et al. 2004). Global warming also could change the distribution of pathogens, facilitating infection of naïve populations (Kiesecker et al. 2004). The increased permeability of the ozone layer and subsequent increased exposure to UV-B radiation has been implicated as a cause of amphibian declines (Collins and Storfer 2003, Kiesecker et al. 2004). Increased UV-B radiation likely interacts with other factors such as altered rainfall patterns that lower water levels and reduce shoreline vegetation. Developing amphibian embryos depend on water and intact vegetation to intercept and reduce UV-B intensity. Increased penetration of UV-B radiation in the water can increase embryo mortality, cause delayed development and morphological abnormalities, or increase the susceptibility of amphibian embryos to infection by pathogenic molds, such as *Saprolegnia ferax* (Blaustein and Belden 2003, Kiesecker et al. 2004).

A variety of pathogens have been associated with local amphibian die-offs. The opportunistic bacterium, *Aeromonas hydrophila,* is frequently associated with red-leg disease (Rollins-Smith 2001). This bacterium lives symbiotically with amphibians (Hird

et al. 1981), but becomes pathogenic when individuals are stressed and immunocompetence declines (Carey 1993). The bacterium causes systemic hemorrhaging and can result in mortality of the amphibian host (Cunningham et al. 1996). Similar gross signs of disease are caused by iridoviruses, which are another pathogen associated with amphibian mortality. Iridoviruses can infect an amphibian at any stage of development, compromise the immune system, and facilitate infection by other organisms or be pathogenic themselves (Carey et al. 1999). Chytridiomycosis is a fungal disease that has caused widespread amphibian declines (Rachowicz et al. 2005, Lips et al. 2006). Chytrid epizootics usually occur at higher elevations, and the pathogen (*Batrachochytrium dendrobatidis*) is thought to be transmitted among amphibians in aquatic environments (Daszak et al. 1999, Davidson et al. 2003). Infection by parasitic trematodes (genus *Ribeiroia*) has been linked to many of the malformations observed in amphibians. The eggs of adult *Ribeiroia* worms are located in the esophagus of the primary host, usually waterbirds, and defecated into aquatic environments, where they hatch into mobile miracidium. Miracidium infect Planorbidae snails, where asexual replication occurs. Subsequently, cercariae burrow out of the snail host and infect amphibian larvae (second intermediate host), where they may encyst near developing limb buds. If the cysts are located at a limb bud, they can mechanically disrupt normal growth and cause a malformation (Kiesecker et al. 2004, Johnson et al. 2004). These malformations are thought to increase predation susceptibility of the amphibian to the primary host, thereby facilitating completion of the trematode life cycle (Sessions and Ruth 1990, Johnson et al. 2004). It is hypothesized that humans may increase pathogen prevalence in amphibian populations by degrading their habitat, physiologically stressing

resident individuals, or by facilitating transmission among spatially disjunct populations (Carey et al. 1999).

Introduction of exotic species also can influence amphibian populations. Exotics may be predators of amphibians or introduce pathogens. Exotic predators include fish, birds, mammals or other amphibians. Some exotic amphibians, such as the cane toad (*Bufo marinus*), can competitively exclude indigenous amphibians when niches overlap (Collins and Storfer 2003). Humans who consume amphibians or collect them for the pet trade contribute to amphibian declines in some areas (Collins and Storfer 2003). In one county in Iowa, it was estimated that northern leopard frog (*Rana pipiens*) populations declined from 20 million to 50,000 between 1920 and 1992, with one third of the losses attributed to harvesting (Lannoo et al. 1994). Non-native species released into the environment after being purchased through the pet trade or for bait, could harbor pathogens such as ranaviruses or *Batrachochytrium dendrobatidis* that can be transmitted to native populations of amphibians (Mazzoni et al. 2003).

Finally, the most widespread and influential of all potential human impacts on amphibian populations is direct loss and alteration of aquatic and terrestrial habitat (GAA 2004). Amphibian habitat is destroyed for a variety of human land uses including agriculture, silviculture and urbanization (Collins and Storfer 2003). In many places, habitat loss accounts for most of the decline in amphibian populations (Hecnar and M'Closkey 1998). Even if aquatic environments remain unaltered, changes in land use between amphibian habitat patches can cause population isolation and fragmentation (Marsh and Trenham 2001). Anthropogenic land use also can increase the complexity of the landscape between habitat patches and may influence the probability of successful

dispersal (Gray et al. 2004*b*), which can affect the probability of local extinction (Fahrig and Merriam 1985). Urbanization has been associated with increased exposure to contaminants, eutrophication, changes in geomorphology, and alterations in the hydrology of the landscape (Ehrenfeld 2000, McKinney 2002). Similarly, agricultural cultivation near amphibian aquatic habitats can decrease water quality from chemical run-off, reduce wetland hydroperiods from sedimentation, and influence the terrestrial vegetation composition and structure (Knutson et al. 1999, Gray et al. 2004*a*). Although agriculture can have negative effects on amphibians, low intensity agriculture does not seem to affect populations as dramatically as urban development (Gibbs et al. 2005).

Allowing cattle to graze in wetlands is an agricultural land use that may influence the quality of amphibian habitat (Trimble and Mendel 1995, Hadden and Westbrooke 1996, Belsky et al. 1999, Jansen and Robertson 2001, Line 2003, Knutson et al. 2004). Cattle can increase erosion by trampling the banks and consuming stabilizing shoreline vegetation (Trimble 1994, Trimble and Mendel 1995). Increased nutrient loading from cattle feces further degrades water quality by increasing eutrophication. It is hypothesized that eutrophic conditions increase the abundance of Planorbidae snails by increasing periphyton biomass, which is a food resource for these snails. As mentioned, Planorbidae snails are the first intermediate host for *Ribeiroia* trematodes, thus an increase in snail abundances from eutrophication may increase malformations in amphibians (Johnson et al. 2002, Johnson and Chase 2004). Additionally, cattle defecation and urination in wetlands may provide a source of introduced pathogens in amphibian habitat (Lannoo et al. 2003, Line 2003).

Cattle also may affect amphibian communities by reducing shoreline vegetation (Scrimgeour and Kendall 2002). Cattle grazing reduces vegetation biomass, structure, surface area, and species richness (Trimble and Mendel 1995). Shoreline vegetation is important in wetlands for many amphibians, because it is used for breeding, escape cover, and sites for foraging and oviposition (Jansen and Healey 2002). Cattle also may directly affect amphibian recruitment by trampling egg masses at the bottom of ponds or those that are attached to wetland vegetation (Scrimgeour and Kendall 2002). Additionally, increased turbidity caused by cattle may cause suspended solids to settle on egg masses and reduce oxygen diffusion (Belsky et al. 1999). Finally, trampling by cattle may increase soil compaction (Trimble and Mendel 1995), which may reduce the burrowing ability of some anurans.

In the Cumberland Plateau Region of Tennessee, large ungulates are not new to the landscape. Historically, buffalo, elk and deer were present in this region (Ramsey 1926), and most likely used wetlands containing amphibians (Redmond and Scott 1996). The impacts of these historical ungulates on Tennessee amphibians are unknown. There are some cases where amphibians have been documented using landscape features modified by large ungulates (e.g., bison wallows, Gerlanc and Kaufman 2005). However, in areas where livestock grazing occurs in Tennessee, densities are likely much higher than naturally roaming ungulates. It is hypothesized that human land-use effects, such as cattle grazing on amphibians, will be more severe in small isolated wetlands (Marsh and Trenham 2001, Gray et al. 2004*b*).

Few field experiments have been performed that measured the possible influences of cattle on amphibians. The studies that exist primarily focused on the influences of

cattle on wetland vegetation and amphibian species richness, and are correlative in nature (Healey et al. 1997, Bull and Hayes 2000, Bull et al. 2001, Jansen and Healey 2002, Knutson et al. 2004). Therefore, I performed the following replicated study to quantify the possible impacts of cattle on postmetamorphic amphibians, and to determine how cattle land use may have interacted with landscape structure. I used eight replicate wetlands to perform this research: four had direct cattle access, while the remaining four were fenced off from cattle. My specific research objectives were to examine the influences of cattle on: (1) species-specific postmetamorphic amphibian abundance, (2) amphibian species richness and diversity, (3) amphibian egg mass abundance, (4) shoreline vegetation structure and composition, (5) soil compaction, (6) pathogen (bacteria, viruses, and parasites) and malformation prevalence and type in postmetamorphic amphibians, and (7) to determine the influence of agricultural landscape structure and composition on amphibian community structure. I used a combination of pitfall trap sampling, visual transect surveys, vegetation plot measurements, pathological examinations, and the geographic information system (GIS) to study these objectives. Objectives 1 – 6 are presented in Chapter II and Objective 7 is presented in Chapter III. Chapter IV contains a summary of my findings and thoughts on amphibian conservation. This study represents the first replicated attempt to quantify the impacts of cattle on postmetamorphic amphibian populations in the United States.

CHAPTER II

IMPACTS OF CATTLE ACCESS IN WETLANDS ON POSTMETAMORPHIC AMPHIBIANS

Introduction

There is a vast amount of literature assessing the impacts of anthropogenic stressors on amphibians. However, the direct and indirect effects of cattle grazing on postmetamorphic amphibians has not been explored extensively (Bull et al. 2001), particularly in the southeastern United States. Knutson et al. (2004) assessed 40 agricultural wetlands in Minnesota for their value as amphibian breeding sites. Of wetlands that had direct access by cattle, amphibian species richness and larval and egg mass abundance of some species was lower than in wetlands without direct cattle access. However, Bull and Hayes (2000) found no differences in abundance of Columbia spotted frog (*Rana luteiventris)* eggs between grazed and ungrazed ponds in Oregon. Bull et al. (2001) examined the abundance of Pacific treefrog (*Pseudacris regilla*) and long-toed salamander (*Ambystoma macrodactylum*) larvae in fenced and unfenced wetlands, and detected no difference in relative abundance between cattle land-use types. Pyke and Marty (2005) reported that cattle grazing in natural vernal pools in California may benefit amphibian communities by maintaining suitable hydrologic conditions needed for salamander reproduction. Finally, in Australian billabongs, Healey et al. (1997) and Jansen and Healey (2002) correlated amphibian abundance with wetland characteristics, and suggested that cattle may indirectly negatively influence amphibian abundance by altering wetland vegetation. The regional- and species-specific results of these studies

illustrate the need for additional studies examining the possible impacts of cattle on amphibian populations.

Cattle may influence amphibian habitat by altering aquatic and terrestrial vegetation. Removal of vegetation due to livestock grazing or trampling reduces plant biomass, percent canopy cover, stem density and species richness (Trimble and Mendel 1995, Jansen and Healey 2002, Scrimgeour and Kendall 2002, Ausden 2005). Altering shoreline vegetation can destroy important microhabitat for amphibians (Watson et al. 2003). Postmetamorphic amphibians use emergent vegetation as foraging, breeding, and oviposition sites, shelter, and resting platforms (Hadden and Westbrooke 1996, Healey et al. 1997, Jansen and Healey 2002, Watson et al. 2003).

Survival of larval amphibians is related to water quality (Sparling et al. 1995, Jofre and Karasov 1999), and cattle are known to decrease water quality by increasing erosion as a result of removing shoreline vegetation (Trimble 1994). Further, livestock trampling compacts the soil in the upland, increasing runoff rate into adjacent bodies of water (Trimble and Mendel 1995). Accelerated runoff can increase levels of fertilizer, pesticide, and herbicide contaminants in wetlands if agricultural crops exist nearby (Knutson et al. 1999). Given that cattle are attracted to water to drink and cool themselves, they can spend a substantial amount of time in wetland areas when they are given access (Belsky et al. 1999). Nutrients and bacteria are introduced into wetlands from cattle feces deposited directly into the water or nearby and incorporated into runoff during rainfall (Line 2003). Line (2003) found that continual access of cattle to a stream significantly increased the amount of bacteria in the water. He hypothesized that the cause was likely due to introduced cattle fecal matter. Further, farming techniques, such

as feeding additives, may increase the number of artificial substances in cattle feces and urine, resulting in more than natural byproducts introduced into water systems (Lannoo et al. 2003).

Cattle also have the potential to increase parasitic pathogens in amphibian habitat (Johnson and Chase 2004). Wetlands heavily impacted by cattle have been shown to support dense populations of Planorbidae snails, which are the first intermediate hosts of *Ribeiroia* trematodes (Johnson et al. 2002). Increased nutrient loads from cattle feces and agricultural chemicals in runoff can induce eutrophic conditions in wetlands (Johnson et al. 2002). This process tends to shift the community composition of aquatic snails toward larger species, such as those in the family Planorbidae (Johnson and Chase 2004). Species in the genera *Planorbella, Biomphalaria* and *Helisoma* of the family Planorbidae have been found to be hosts of *Ribeiroia*. *Ribeiroia* trematodes from these snails can form cysts that may result in malformations in developing amphibians (Johnson et al. 1999, Kaiser 1999, Johnson et al. 2002, Kiesecker 2002, Ankley 2004, Johnson et al. 2004).

Susceptibility to trematode infection is increased in the presence of stressors that reduce the immunocompetence of larval amphibians (Kiesecker 2002). Kiesecker (2002) found that wood frog (*Rana sylvatica*) larvae stressed by exposure to pesticides had higher parasite loads than those not exposed to pesticides. Habitat alterations caused by cattle may impose similar stress on amphibians, making them less resistant to infections by trematodes or other pathogens. *Aeromonas hydrophila* is a bacterium often associated with red-leg disease in amphibians (Rollins-Smith 2001). When *Aeromonas* bacteria occur in high abundance in amphibians, it causes reddening of the skin, typically in the

pelvic region, due to petechial hemorrhaging (Cunningham et al. 1996). This bacterium is opportunistic and is found on the skin and in the digestive tract of healthy frogs (Hird et al. 1981). Most mass mortality events prior to 1990 were attributed to red-leg disease however, recent evidence suggests *A. hydrophila* functions as a secondary pathogen, capitalizing on weakened immune systems often due to iridovirus infection (Cunningham et al. 1996).

Iridoviridae is a family of viruses that affects a wide variety of vertebrates. Those that affect amphibians and other cold-blooded vertebrates are members of the genus *Ranavirus* (Williams 1996). The most well-characterized member of this genus is *Frog virus* 3 (FV3; Docherty et al. 2003). Ranaviruses are highly virulent and cause systemic infections in amphibians (Daszak et al. 1999). The virus will invade the kidney, digestive tract and liver of amphibians, and can cause hemorrhaging in skeletal tissue (Cunningham et al. 1996, Daszak et al. 1999). Of the 44 amphibian mortality events (1996 – 2001) studied by Green et al. (2002), 48% were caused by ranaviruses. *Ranavirus* outbreaks have occurred globally but typically in smaller geographical areas, such as single ponds that have been altered (Carey et al. 1999, Daszak et al. 1999). More information is needed on *Ranavirus* transmission, and how anthropogenic and natural stressors may influence its prevalence in amphibian populations (Carey et al. 1999).

In agricultural landscapes, wildlife may act as reservoirs and mobile vectors of pathogens that can infect livestock using the same wetlands. Bacteria such as *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Leptospirosis* spp. and *Mycobacterium paratuberculosis* as well as the pathogenic protozoan *Cryptosporidium* spp. can be shed in cattle excrement (Theon and Johnson 1970, Morse and Duncan 1974,

Shotts 1981, Gray et al. 2004, Olson et al. 2004, Sargeant et al. 2004). Cattle using wetlands defecate and urinate in the water (Johnson et al. 2002), potentially leading to contamination of the entire water body (Morse and Duncan 1974). Few studies have examined the influence of these pathogens on amphibians, and none have found any negative effects yet (Botzler at al. 1973, Murray 1991). Nonetheless, infection of human and bovine pathogens in amphibians remains an area of research interest, as amphibians may function as carriers or spill-over reservoirs of these pathogens and shed them in the environment (Botzler at al. 1973, Everard et al. 1990, Graczyk et al. 1996, Murray 1991, Scherer and Miller 2001, Gray et al. 2007*b*). Thus, as amphibians move among wetlands, they could contaminate water sources, possibly leading to infection of naïve amphibian populations or livestock that drink from contaminated water sources.

To date, few studies have measured the influences of cattle on postmetamorphic amphibian populations. The studies that exist have focused on how cattle may impact wetland vegetation and resident amphibian populations, but they were correlative in nature (Healey et al. 1997, Bull and Hayes 2000, Bull et al. 2001, Jansen and Healey 2002, Pyke and Marty 2005). In addition, no cattle land-use studies have been performed on amphibian communities in the southeastern United States. The objective of my study was to determine the direct and indirect influences of cattle on postmetamorphic amphibian communities and their habitat. Therefore, I quantified species-specific abundance, species richness and diversity, egg mass abundance, emergent wetland vegetation structure and composition, soil compaction, pathogen (bacteria, viruses, and parasites) prevalence, and malformation prevalence and type in postmetamorphic amphibians at wetlands with and without direct cattle access. I hypothesized that cattle

would negatively impact all of these response variables. Eight replicate wetlands were used, four with direct cattle access, while the remaining four were fenced from cattle. As anthropogenic disturbance continues to contribute to the decline of amphibian populations, such studies are imperative for understanding the specific impacts of land-use stressors.

Methods

Study Area

My study was conducted at the University of Tennessee Plateau Research and Education Center (PREC) on the Cumberland Plateau in Crossville, Tennessee (UTM zone 16 [NAD 27], 668310 E, 3987122 N). Sampling occurred from 28 March – 26 August 2005 and 27 March – 25 August 2006. The PREC functions as an outdoor laboratory for crop, orchard, and cattle studies, and has approximately 250 head of Angus, Gelbvieh or Balancer cows, calves and bulls in pastures interspersed throughout the property. The primary source of drinking water for these cattle is constructed wetlands.

Eight PREC wetlands served as experimental units for my study, four had been exposed to grazing (average stocking rate 14 – 46 individuals) for >10 years. The remaining four wetlands were surrounded by fence, preventing direct access by cattle for >10 years. Cattle density around each cattle-access wetland ranged from 39 to 321 cattle per ha of wetland during my study (Table 1, All Tables and Figures appear in Appendix I). Cattle-access treatments remained in place for the duration of the study.

Wetlands ranged in size from 0.143 – 1.037 ha (Figure 1). Cattle-access and non-access wetlands existed in separate watersheds (hence were not hydrologically linked) except for wetlands one and five. Water flowed from wetlands one to five but they were separated by 346 m, thus I assumed changes in water quality in wetland one had minimal impacts on wetland five. Fish were present in all of the wetlands, including four species known to predate on amphibian eggs or larvae: blue gill (*Lepomis macrochirus*), green sunfish (*L. cyanellus*), largemouth bass (*Micropterus salmoides*), and western mosquito fish (*Gambusia affinis*, Schmutzer 2007). Wetlands had emergent non-persistent and persistent herbaceous shoreline vegetation and permanently flooded unconsolidated bottoms (Cowardin et al. 1979). Species composition of herbaceous plants was predominately cattail (*Typha latifolia*), rushes (*Juncaceae*), and sedges (*Cyperaceae*). All wetlands were in relatively close proximity, with inter-wetland distance ranging from 50 – 1300 m. Land use between wetlands was mostly cattle pasture, mowed tall fescue (*Lolium arundinaceum*), and agricultural crop fields. Gravel and paved roads also were present throughout the PREC. Distance from roads to my study wetlands varied from 5 – 150 m. A more thorough description of PREC landscape features and their possible influence on resident amphibians is provided in Chapter III.

Amphibian Species Richness, Relative Abundance, and Body Size

Terrestrial capture.—Species richness and abundance of postmetamorphic amphibians was measured using mark and recapture techniques in pitfall traps and breeding call surveys. In early spring (21 – 25 March 2005), all study wetlands were

partially enclosed (50% of the circumference) with a continuous drift fence that was 60 cm in height (Gray et al. 2004*a*, Figure 2). The drift fence was placed parallel to and approximately 10 m upslope from the shoreline of the wetland. Pitfall traps (19 L buckets) were placed every 10 m on alternate sides of the fence for half the distance of the fence (i.e., 25% of the wetland circumference). Pitfalls were placed every 5 m for the remaining length of the fence. The reason for the difference in spacing was to test for capture-rate differences between bucket-spacing scenarios, which was an ancillary objective of my study not discussed herein. Pitfalls were placed adjacent to the fence and flush with the ground (Dodd and Scott 1994). Vegetation underneath the fence was removed and the bottom of the fence was covered with soil to reduce trespass of amphibians (Gray et al. 2004*b*). An electrical fence surrounded the drift fence and pitfall traps at wetlands with cattle access to prevent cattle from destroying the fence or injuring themselves by stepping in pitfalls. Approximately 3 cm of water and a small sponge were placed in each bucket to prevent desiccation of captured amphibians and drowning or hypothermia of incidentally captured small mammals, respectively (Dodd and Scott 1994). I also took additional precautions to reduce small mammal mortality by attaching a piece of string to nearby vegetation and placing the other end in the pitfall to facilitate escape for trapped rodents (Karraker 2001).

Pitfalls were opened for 24 hrs prior to checking for captures. The order in which pitfalls were opened and processed was the same within a sampling event, but this order sequentially rotated among wetlands between sampling events to randomly distribute potential bias associated with time of day traps were opened or checked. Traps were checked twice per week (Tuesday and Friday) from 28 March – 26 August 2005 and 27

March – 25 August 2006. Following biological processing of captures, the pitfalls were closed.

Biological processing.—Captured individuals were identified by species, age and sex if possible. Individuals <1 year old were classified as metamorphs, and individuals >1 year old but not displaying secondary sexual characteristics were classified as juveniles. Individuals >1 year old were identified as adult females by assessing their body size relative to males (i.e., larger) and inspecting for external reproductive characters, such as unswollen cloacae for salamanders and eggs visible through the skin for anurans. Adult males were identified as being >1 year old if they possessed male reproductive characters, such as enlarged cloacal papillae for salamanders and vocal sacs for anurans (Duellman and Trueb 1986). Nuptial excrescences also were used as a male character for anurans (Duellman and Trueb 1986).

Captured juvenile and adult anurans were individually marked with an alpha-numeric florescent tag (®Northwest Marine Technology, Inc.), and all adult anurans and salamanders were given a unique toe-clipping code as per Hero (1989) using scissors soaked in 0.01% chlorhexidine diacetate (Camper and Dixon 1988). Time necessary to mark individuals, tag retention and infection rates, and visibility of marks were recorded for each technique and recaptured individual as part of an ancillary study not discussed herein. Metamorphs were only toe-clipped because they were too small for tags. Clipping codes for metamorphs were assigned according to the wetland and side of the drift fence they were captured. If metamorphs were captured on the upland side of the fence, it was assumed they were attempting to immigrate to the study wetland (Gray 2002). In contrast, if metamorphs were captured on the wetland side of the fence, it was

assumed they were attempting to emigrate. I batched marked all metamorphs in this fashion, because I anticipated capturing large numbers (e.g., >100 individuals / day, Gray 2002), which would have quickly exceeded toe-clipping code combinations.

To quantify possible impacts of cattle grazing on postmetamorphic body size, I measured mass and snout-vent length (SVL) for the first five individuals caught per species per wetland per sampling event. All individuals also were examined for malformations and other gross indicators of disease, and those with any pathological signs were collected for further examination (see Pathogen Prevalence section, p. 21). For malformed individuals, malformation type was identified using the USGS Field Guide to Malformations of Frogs and Toads (Meteyer 2000). After processing grossly healthy individuals, they were rehydrated (i.e., placed temporarily in a bucket of water) and released on the opposite side of the fence from which they were caught so the direction of their movement was not altered (i.e., emigrating or immigrating, Dodd and Scott 1994). All sampling and marking techniques followed a University of Tennessee Institutional Animal Care and Use Committee approved protocol (#1425).

Breeding call surveys.— Breeding call surveys were performed once per week. Survey methods followed North American Amphibian Monitoring Program (NAAMP) protocol (Weir 2001), except that data were collected for two consecutive time periods (0–5:00 and 5:00–10:00 minutes) and with two observers. Another ancillary objective of my study was to determine if species abundance and richness differed between breeding call surveys lasting five and ten minutes (Burton et al. 2007).

Observers stood at permanent listening stations on opposite sides of each wetland and did not share survey results. Surveys began $\geq$30 minutes after the U.S. Naval

Observatory published time for sunset (U.S. Naval Observatory 2006). Upon arriving at listening stations, researchers waited for 1 minute before beginning surveys to allow observers to acclimate to the surroundings and for anurans to recover from possible disturbances. All species heard were recorded separately for 5- and 10-minute surveys, and species-specific abundance indexed. Following NAAMP protocol, an abundance index of 1 was given when individual calls of a species were distinguished but did not overlap, an index of 2 was assigned when calls overlapped but individuals could be distinguished, and an index of 3 was assigned when there was a full chorus (i.e., calls overlapped and individuals were indistinguishable, Weir 2001). Total number of anuran species heard by both observers and mean abundance index averaged between observers per species were used as response variables.

Egg Mass Abundance

To measure relative egg mass abundance, each wetland was visually surveyed for egg masses once per week. Each wetland was divided into the four cardinal quadrants (Figure 2). One quadrant of each wetland was randomly selected at the beginning of the study for egg mass surveys. The opposing quadrant also was surveyed for egg masses. Within each surveyed quadrant, one of the two cardinal azimuths forming the quadrant was randomly selected. Next, a permanent transect (10 m long) was placed 1 m from the random cardinal azimuth so that it extended into the quadrant. The transect was oriented 2 m from and parallel to the shoreline (Figure 2). All egg masses observed along the transect were counted and identified to one of the following taxonomic groups: American bullfrog (*Rana catesbeiana*) and green frog (*R. clamitans*), pickerel frog (*R. palustris*)

and southern leopard frog (*R. sphenocephala*), Cope's gray treefrog (*Hyla chrysoscelis*), American toad (*Bufo americanus*) and Fowler's toad (*B. fowleri*), and non-amphibian. I combined amphibian species in these groups, because I was unable to distinguish their eggs in the field. Different sets of waders were used for sampling in cattle-access wetlands to avoid possible pathogen transfer, which could have biased my pathogen results (discussed later).

Emergent Shoreline Vegetation

Emergent shoreline vegetation was measured once per month for percent vertical and horizontal cover, height, and plant species richness. Vegetation was measured in a 1-m^2 plot that was placed along a randomly selected azimuth in the two quadrants not used for egg mass surveys (Figure 2). A new azimuth was randomly generated each month per wetland. The plot was placed at the midpoint of the emergent vegetation zone along the azimuth in each quadrant. Vegetation height and vertical structure were measured using a graduated profile board placed at the center of each plot. The board was faced toward the upland and was held such that the bottom of the board was flush with the water surface if water was present. The observer reading the board knelt 2 m upslope from the board and recorded visual obstruction by vegetation. My profile board was divided into four height strata (0 – 0.5 m, 0.5 – 1.0 m, 1.0 – 1.5 m, 1.5 – 2.0 m), with each strata containing 30 alternately colored squares (5 × 5 cm). Percent vertical structure was determined by counting the number of squares that were covered >50% by emergent vegetation in each strata, and dividing by 30 (i.e., the number of squares per section). Maximum and minimum height of the shoreline vegetation covering the profile board

was recorded and averaged for mean vegetation height per sampling location. In the 1-m^2 plot, percent horizontal vegetation and water cover was visually estimated, and plant species richness was enumerated. For each vegetation response variable, all measurements were averaged between sampling locations within wetlands so there was one value per variable per wetland per sampling event.

Soil Compaction

Soil compaction was measured once per month in 2006 with a soil compaction meter (Dickey-john Corporation, Auburn, Illinois, USA). The compaction of upland soil (lbs/in^2) was measured every 5 m along a randomly generated azimuth extending 0 – 20 m from the water line. A new azimuth was randomly generated each month per wetland. These five measurements also were averaged for mean soil compaction per wetland per month. Measurements from the first month (i.e., April 2006) were not used in analyses because two different individuals operated the meter, which could have biased results.

Waterbird Prevalence

Waterbirds, including great blue herons (*Ardea herodias*), green herons (*Butorides virescens*), wood ducks (*Aix sponsa*) and mallards (*Anas platyrhynochos*), have been reported as definitive hosts of the trematode, *Ribeiroia ondatrae* (Johnson and Lunde 2005). Thus, I recorded the presence of these birds upon arriving at each wetland. Observations were recorded four days per week during the sampling periods.

Pathogen (bacteria, viruses and parasitic) Prevalence

Specimen collection.—Five recently metamorphosed green frogs were collected from pitfalls at each wetland (n = 40 individuals) on 15 June 2005 for pathogen analyses. I used green frog metamorphs because they were most abundant at my study wetlands. Metamorphs were transported to the University of Tennessee and housed in terrariums separately by wetland until processed less than 2 days after collection. In addition, I opportunistically collected individuals of various species and age classes with clinical signs suggestive of disease (e.g., abraded skin, reddening of the skin, lethargy, malformations).

Pathological sampling.—Collected amphibians were euthanized by immersion in a benzocaine hydrochloride water bath (250 mg/L), and a complete necropsy was performed. Necropsy protocol followed sterile procedures to prevent specimen contamination. Necropsies began by placing euthanized individuals in dorsal recumbency on a sterilized surgical cutting board. A ventral midline incision was made exposing the coelomic cavity. A swab of the peritoneum was collected, refrigerated at 4ºC, and later tested for *Aeromonas hydrophila, Listeria monocytogenes, Salmonella* spp., and *Escherichia coli* (see Appendix II for all pathological testing procedures).

Sections of brain, heart, skeletal muscle, skin, lung, spleen, liver, kidney, reproductive tract, adrenal glands, bone marrow, stomach, intestines, lymphoid tissues, sinonasal cavity and eye were collected. A subset of these tissues was fixed in 10% buffered formalin for histological examination. Additionally, a partial set of tissues (lung, kidney, spleen, intestines, stomach and liver) was taken for culturing aerobic and anaerobic bacteria. Specific tests were performed for *Leptospira* spp. using liver and

kidney tissues by placing them in a media containing bovine serum albumen (BSA) and refrigerating at 4ºC for later testing (Appendix II). A section of the intestine also was collected and frozen at –20ºC for later testing of *Mycobacterium paratuberculosis*. A partial set of tissues (lung, kidney, spleen, brain, skin, skeletal muscle, heart, intestines, stomach and liver) was frozen at –20ºC and tested for viruses (Appendix II). Feces were collected and refrigerated at 4ºC and tested for parasites and protozoans (including *Cryptosporidium* spp.) using standard fecal analyses, PCR (polymerase chain reaction, for *Cryptosporidium* spp.), and electron microscopy for evidence of viral shedding (Appendix II). All tissues and swabs were transported within 24 hrs of preparation to the University of Georgia, Veterinary Diagnostic and Investigational Laboratory, Tifton, Georgia (UGA VDIL) for pathogen testing (Appendix II).

Malformation and Trematode Prevalence

Each individual captured in pitfalls was visually inspected for malformations. If an individual was malformed, it was opportunistically collected and euthanized by immersion in a benzocaine hydrochloride water bath (250 mg/L). The specimen was fixed in 10% buffered formalin for 24 hrs then transferred to 70% ethanol (Hanken and Wasserug 1981). Specimens were stored in ethanol until they were cleared to determine if malformations were due to trematodes (see Appendix II for clearing procedures).

Statistical Analyses

Amphibian response variables included species-specific relative abundance from pitfalls; body mass and SVL by species, age and sex class; mean breeding call abundance

index by species; egg mass abundance for the taxonomic groups discussed previously; species richness and diversity; and pathogen and malformation prevalence. I used total daily capture of new individuals (i.e., recaptures not included) per wetland as an index of relative abundance. Daily capture was standardized by dividing by the number of pitfall traps at each wetland, because they differed in size (Table 1). Given that wetlands were experimental units, daily captures were subsamples. I was interested in quantifying monthly trends in species-specific abundance; therefore, I averaged captures across days within months for each wetland, which resulted in an 8×6 response matrix corresponding to eight wetlands and six months for each species. Breeding call indices were averaged between observers and across weeks per species per wetland per month to estimate breeding male abundance. Species richness was estimated using pitfall trap captures and breeding call surveys. Total number of species caught or heard at each wetland during a month was used as the response variable. Species diversity was calculated using Shannon-Weiner diversity index from pitfall captures only (Raven and Johnson 1999). Total abundance of egg masses per wetland per month for each taxonomic group and for all species combined were response variables. Pathogen and malformation prevalence was the number of individuals infected or malformed divided by the total number of individuals collected, respectively. Environmental response variables included plant height, plant species richness, percent horizontal and vertical cover of vegetation, soil compaction, and mean daily abundance of waterbirds.

For all response variables, except pathogen and malformation prevalence, and body size, wetlands ($n = 4$ per land-use type) were experimental units and sampling events within months were subsamples for each year. Each of these response variables

also was averaged between years per wetland for a combined estimate over the 2-year duration of my study. There were two main effects of interest (cattle land-use type and month) for all response variables, except body size and waterbird abundance. For these latter variables, I was only interested in testing for a land-use effect. I used an analyses-of-covariance (ANCOVA), with capture date as the covariate, to test for differences in SVL and mass between cattle land uses. Capture date was used as the covariate to partition variation associated with growth. I used a 1-factor analysis-of-variance (ANOVA) to test for differences in waterbird abundance between cattle land-use types.

For all other response variables, I used a used a 2-factor repeated-measures ANOVA with Hunyh-Feldt correction to test for differences between cattle land uses and among months, with the exception of soil compaction (Zar 1999). For this variable, land-use differences were tested using an ANCOVA and monthly differences were tested using a 1-factor (month only) repeated-measures ANOVA. For the ANCOVA, sampling distance from the wetland was used as the covariate. If land-use differences were detected for soil compaction, linear regression models were constructed to determine direction and strength of the relationship between distance from wetland and soil compaction. Normality of all response variables was tested using a Shapiro-Wilk test, and a non-parametric Wilcoxon test used to test for differences between land-use types if violated. If differences were detected in the repeated month effect, Tukey's Honestly Significant Difference (HSD) test was performed to determine pairwise differences. For the 2-factor repeated-measures ANOVAs, analyses were separated by month for land-use tests and by cattle land use for month tests when an interaction between land-use and month effects occurred.

I treated individuals collected for pathogen testing and malformation inspection as experimental units of the cattle land-use main effect. Differences were tested in pathogen prevalence and prevalence of each malformation type between cattle land uses using 2-sample *Z*-tests for proportions. I used a one-sample *Z*-test for proportions to test for deviance in overall malformation rates from 0.5 between cattle land uses (Zar 1999).

I also was interested in identifying possible environmental co-factors of cattle land use that explained significant variation in amphibian abundance. Thus, I built multiple linear regression models using stepwise selection (entry and stay $\alpha = 0.10$, Meyers 1990), with species-specific relative abundance as the response variable. Possible explanatory variables for these models included mean number of cattle per ha of wetland (Table 1), four vegetation variables (plant height, percent horizontal cover, percent vertical structure, and plant species richness), and soil compaction. In addition, I included eight water quality variables (NO_2, NO_3, NH_3, PO_4, pH, temperature, turbidity, and specific conductivity), and relative daily abundance of larvae per species from a concurrent study (Schmutzer 2007). For details on water quality and larval sampling methods, please see Schmutzer (2007).

For the final models, I presented un-standardized and standardized parameters (Meyers 1990). Un-standardized parameters can be used to predict species-specific relative abundance given values of explanatory variables in the model. I used standardized estimates to interpret the magnitude and direction of the relationship between relative abundance and an explanatory variable. I also presented variance inflation factors (VIF); values of VIF > 10 are suggestive of multicollinearity (Freund and Littell 2000). Finally, I provide overall and partial coefficients of determination for a

measure of the variation explained in relative abundance by the final model and each significant explanatory variable, respectively (Meyers 1990). All statistical analyses were performed using the SAS® system (Littell et al. 1991, Stokes et al. 2003). Due to the small sample size for the majority of my statistical analyses (n = 8 experimental units / wetlands), I used $\alpha = 0.10$ as the level of statistical significance. I did so to increase the likelihood that meaningful biological trends would be detected. Although I recognize there was a 10% chance of committing a Type I error, I consider this error rate to be reasonable for interpreting biological trends (Tacha et al. 1982). Other wildlife studies have supported the use of $\alpha = 0.10$ as a level of statistical significance when samples sizes are small (Tacha et al. 1982, Peterman 1990, Thompson et al. 1992, Stevens et al. 2003, Kaminski et al. 2006).

Results

Cattle Land-use Effect

Mean daily abundance of green frogs at non-access wetlands was 8.7X greater than at cattle-access wetlands in 2006 (Wilcoxon $Z = 1.9$, $P = 0.06$, Table 2); however, land-use and month effects interacted ($F_{5,30} = 3.86$, $P = 0.07$). By monthly tests revealed that green frog abundance at non-access wetlands was 16X and 21X greater than at access wetlands in May and July 2006, respectively (Wilcoxon $Z \geq 1.9$, $P \leq 0.05$). Significant differences did not exist in 2005 ($F_{5,30} = 2.15$, $P = 0.19$) or in the combined analysis (Wilcoxon $Z = 1.6$, $P = 0.11$), but the same trend existed for green frogs. Green frogs were 2.4X and 4.1X more abundant at non-access wetlands than at access wetlands in 2005 and in combined years (Table 2).

Age-sex class tests revealed that green frog metamorphs were the demographic group driving the aforementioned trends. Abundance of green frog metamorphs at non-access wetlands was 10X greater than at access wetlands in 2006 (Wilcoxon $Z = 1.9$, $P = 0.06$, Table 3). Additionally, green frog metamorphs were 3X and 5X more abundant at non-access wetlands in 2005 and in combined years, although statistical differences were not detected ($F_{1,6} = 2.27$, $P = 0.18$ [2005]; Wilcoxon $Z = 1.6$, $P = 0.11$ [combined]). In 2006, green frog juveniles also were 3.4X more abundant at non-access wetlands, despite that statistical differences were not detected (Wilcoxon $Z = 1.2$, $P = 0.22$, Table 3).

Mean relative abundance of American toads at cattle-access wetlands was 70X greater than at non-access wetlands over the 2 years (Wilcoxon $Z \geq 2.2$, $P \leq 0.03$, Table 2). In 2005 and 2006, mean relative abundance at cattle-access wetlands was 68X and 76X greater than at non-access wetlands (Wilcoxon $Z \geq 2.2$, $P \leq 0.03$). Land-use and month effects interacted in 2006 ($F_{5,30} = 5.01$, $P = 0.03$). By monthly tests revealed that American toads were more abundant at cattle-access wetlands in April 2006 (Wilcoxon $Z = 2.3$, $P = 0.02$); no individuals were captured at non-access wetlands that month. This trend was driven by all age-sex classes, but only mean abundance of adult female and male American toads at access wetlands was significantly greater than at non-access wetlands in combined years (Wilcoxon $Z \geq 2.2$, $P \leq 0.03$). In 2005, mean abundance of adult females was significantly greater, while in 2006, mean abundance of adult males was significantly greater at cattle-access wetlands (Wilcoxon $Z \geq 2.3$, $P \leq 0.02$, Table 3).

For all other species, no differences were detected in mean daily abundance between cattle-access and non-access wetlands ($F_{5,30} \leq 3.35$, $P \geq 0.12$, Table 2). However, there was a trend that Fowler's toads were more abundant at cattle-access

wetlands. Relative abundance of most other species tended to be greater at non-access wetlands (Table 2).

Mean breeding call index of American toad, Fowler's toad and Cope's gray treefrog was 4 – 25X greater at access wetlands than at non-access wetlands in 2006 and across both years (Wilcoxon $Z \geq 1.8$, $P \leq 0.07$, Table 4). Land-use and month effects interacted for these three species when years were combined ($F_{5,30} \geq 3.2$, $P \leq 0.05$). Monthly tests for combined years indicated that the call index for American toad was 4X greater at access wetlands in April, Fowler's toad was 5X greater at access wetlands in May and June, and Cope's gray treefrog was 10X and 11X greater at access wetlands in May and June, respectively (Wilcoxon $Z \geq 1.9$, $P \leq 0.06$). In 2006, land-use and month effects interacted for Fowler's toad and Cope's gray treefrog ($F_{5,30} \geq 3.7$, $P \leq 0.02$). Monthly tests revealed that the call index for Fowler's toad and Cope's gray treefrog was 4 – 9X greater at access wetlands in May and June 2006 ($F_{1,6} \geq 5.9$, $P \leq 0.05$). In 2005, land-use and month effects also interacted for Cope's gray treefrog ($F_{5,30} \geq 5.4$, $P \leq 0.02$), and monthly tests indicated that its call index was 4X greater at access wetlands in June (Wilcoxon $Z = 1.7$, $P = 0.08$). In contrast, the call index was 2X greater at non-access wetlands for spring peepers in 2005 and for pickerel frogs in 2006 ($F_{1,6} \geq 5.06$, $P \leq 0.07$); however, land-use and month effects interacted for spring peepers in combined years and in 2005, and for pickerel frogs in combined years and in 2006 ($F_{5,30} \geq 4.7$, $P \leq 0.01$). Monthly tests revealed that the call index of spring peeper was 3X and 12X greater in March at non-access wetlands in combined years and in 2005, respectively ($F_{1,6} \geq 7.6$, $P \leq 0.03$). The call index of pickerel frog at non-access wetlands was 3X and 5X greater than at access wetlands in combined years and in 2006, respectively ($F_{5,30} \geq 7.7$, $P \leq$

0.03). No other differences were detected in call indices between land uses (Wilcoxon $Z \leq 1.6$, $P \geq 0.11$, Table 4).

Mean relative abundance of RAPA-RASP egg masses in cattle-access wetlands was significantly greater than at non-access wetlands across years (Wilcoxon $Z = 1.8$, $P = 0.07$, Table 5); no egg masses were observed at non-access wetlands. However, land-use and month effects interacted ($F_{5,30} = 4.04$, $P = 0.08$). By monthly tests revealed that mean abundance of RAPA-RASP eggs was greater at cattle-access wetlands only in April (Wilcoxon $Z = 1.8$, $P = 0.07$). No other differences were detected between land uses in relative abundance of egg masses (Wilcoxon $Z \leq 1.6$, $P \geq 0.11$, Table 5).

Differences were not detected in species diversity between land-use types in combined years or for each year separately ($F_{1,6} \geq 2.07$, $P \leq 0.20$, Table 6). Similarly, mean species richness in pitfalls and breeding call surveys was not different between cattle land uses in 2005 ($F_{1,6} \leq 0.3$, $P \geq 0.60$, Table 6). However, in combined years and in 2006, month and land-use effects interacted for mean species richness in pitfalls and call surveys ($F_{5,30} \geq 2.51$, $P \leq 0.05$). When years were combined, monthly tests indicated that pitfall species richness was 3X greater at non-access wetlands in August ($F_{1,6} \geq 11.72$, $P \leq 0.01$). In 2006, pitfall species richness was 3X and 5X greater at non-access wetlands in July and August, respectively ($F_{1,6} \geq 4.57$, $P \leq 0.08$). In contrast, pitfall species richness at access wetlands was 4X greater than at non-access wetlands in April 2006 ($F_{1,6} = 13.5$, $P = 0.01$). Monthly tests for breeding call species richness revealed that species richness at cattle-access wetlands was 37% and 62% greater than at non-access wetlands in June for both years combined and in 2006, respectively ($F_{1,6} = 8.73$, $P = 0.03$, Table 6).

Species composition of amphibians captured in pitfall traps was different between cattle land uses both years. Species composition at cattle-access wetlands was more evenly distributed than at non-access wetlands (Figure 3). In 2005, cattle-access wetlands were dominated by American toads (37%) and green frogs (32%), whereas non-access captures were mostly green frogs (78%). In 2006, green frogs were dominant (62%) at non-access wetlands, while southern leopard frogs (33%), green frogs (18%), and Fowler's toad (18%) were most common in cattle access (Figure 3).

Mass and SVL at non-access wetlands were 20 – 185 % greater than at access wetlands for metamorph and juvenile Fowler's toad (Wilcoxon $Z \geq 1.7$, $P < 0.10$, Tables 7 and 8). Snout-vent length at non-access wetlands also was 11% greater than at access wetlands for adult male Fowler's toad ($F_{1,28} = 12.48$, $P < 0.01$). In contrast, mass and SVL at cattle-access wetlands were 7 – 36% greater than at non-access wetlands for green frog, southern leopard frog, and pickerel frog metamorphs (Wilcoxon $Z \geq 2.31$, $P \leq 0.02$). Snout-vent length at cattle-access wetlands also was 14% greater than at non-access wetlands for adult male American toad ($F_{1,32} = 20.58$, $P < 0.01$). No other differences were detected (Wilcoxon $Z \leq 1.5$, $P \geq 0.14$), although there was a trend of greater body size in cattle-access wetlands for ranids (Tables 7 and 8).

Across both years, height and percent vertical structure of vegetation at non-access wetlands were 56% and 60% greater, respectively, than at access wetlands ($F_{1,6} \geq 7.11$, $P \leq 0.04$, Table 9). Height, percent horizontal cover, and percent vertical structure of vegetation at non-access wetlands were 74%, 25%, and 84% greater, respectively, than at cattle-access wetlands in 2005 ($F_{1,6} \geq 4.79$, $P \leq 0.07$). Percent vertical structure also was 41% greater at non-access wetlands in 2006 (Wilcoxon $Z = 2.2$, $P = 0.03$). No

additional differences were detected (Wilcoxon $Z \leq 1.6$, $P \geq 0.11$), however in general, vegetation structure and richness were greater at non-access wetlands compared to cattle-access wetlands.

Soil compaction was 55% greater at access wetlands than at non-access wetlands ($F_{2,37} \geq 15.85$, $P \leq 0.01$, Table 9). At non-access wetlands there was a strong positive relationship between soil compaction and distance from the shoreline, and 70% of the variation in soil compaction was explained by distance (Figure 4a). At access wetlands there was a moderate positive relationship between soil compaction and distance from the shoreline, but only 10% of the variation in soil compaction was explained by this variable (Figure 4b).

Mean daily abundance of green herons was 6X and 10X greater at non-access wetlands across years and in 2005, respectively (Wilcoxon $Z \geq 2.2$, $P \leq 0.03$, Table 10). In contrast, abundance of mallards was 20X greater at cattle-access wetlands across years (Wilcoxon $Z = 2.2$, $P = 0.03$); however, treatment and month effects interacted ($F_{5,30} = 7.18$, $P = 0.01$). Monthly tests revealed that mallard abundance was greater at access wetlands in March (Wilcoxon $Z = 2.3$, $P = 0.02$); there were no mallard observations at non-access wetlands during this month. No other differences in waterbird abundance between land-use types were detected ($F_{5,30} \leq 3.21$, $P \geq 0.12$, Table 10).

Month Effect

Mean daily abundance differed among months for American bullfrog, green frog, spring peeper and mole salamander across years ($F_{5,30} \geq 2.41$, $P \leq 0.08$, Table 11). Mean daily abundance of green frogs was 3 – 130X greater in June than in all other months for

combined years and each year separately ($F_{5,30} \geq 6.73$, $P \leq 0.02$). Spring peeper abundance was greatest in April when years were combined and in 2005 ($F_{5,30} \geq 6.42$, $P \leq 0.03$). Tukey's HSD test did not detect monthly differences for American bullfrog and mole salamander in combined years, although the overall monthly test was significant ($F_{5,30} \geq 2.41$, $P \leq 0.08$). Mean daily abundance differed among months in 2006 for American toad and American bullfrog ($F_{5,30} \geq 5.01$, $P \leq 0.03$). American toad abundance was 12 – 17X greater in April than all other months, except June ($F_{5,30} = 5.01$, $P = 0.03$). American bullfrog abundance was 2 – 31X greater in July than March – June 2006 ($F_{5,30} = 4.58$, $P = 0.02$). No other differences were detected in mean daily abundance among months ($F_{5,30} \leq 2.25$, $P \geq 0.14$, Table 11).

Mean breeding call index was different among months for northern cricket frog, American toad, Fowler's toad, Cope's gray treefrog, spring peeper, American bullfrog, green frog, pickerel frog and southern leopard frog across years ($F_{5,30} \geq 4.02$, $P \leq 0.03$, Table 12). Mean breeding call index of northern cricket frog was 40 – 68X greater in June than in March and April in combined years and in 2005 ($F_{5,30} \geq 6.39$, $P \leq 0.02$). In 2006, mean index was significantly different among months for northern cricket frog ($F_{5,30} = 4.29$, $P = 0.02$); however, Tukey's HSD test did not detect differences. Across years, mean call index for Fowler's toads was greater in June than in March and August ($F_{5,30} = 4.02$, $P = 0.03$), while in 2006, mean index was greater in May and June than in March and August ($F_{5,30} = 5.95$, $P < 0.01$). Mean calling index of Cope's gray treefrog was 11 – 29X greater in May – July than all other months across years and in 2006 ($F_{5,30} \geq 17.90$, $P \leq 0.01$). In 2005, calling index of Cope's gray treefrog was 9 – 11X greater in June and July than in all other months ($F_{5,30} = 8.89$, $P < 0.01$). Similarly, in combined

years and in 2006, mean calling index of American bullfrog was 2 – 182X greater in June and July than in all other months ($F_{5,30} \geq 49.63$, $P \leq 0.01$). In 2005, mean calling index of American bullfrog was 2 – 180X greater in July than in all other months, except June ($F_{5,30} = 26.39$, $P < 0.01$). Mean calling index of green frogs was greater in June – August than in all other months when years were combined ($F_{5,30} = 78.80$, $P < 0.01$). In 2005, mean calling index of green frogs was 1 – 39X greater in July than in all other months except August ($F_{5,30} = 82.84$, $P < 0.01$), while in 2006, green frog calling index was 2 – 14X greater in June and July than in all other months except August ($F_{5,30} = 42.99$, $P < 0.01$). In contrast, American toad mean calling index was greater in April than in all other months across years and in 2005 ($F_{5,30} \geq 11.86$, $P \leq 0.01$). Similarly, mean breeding call index of pickerel frog was 1 – 21X greater in April than in all other months across years and in 2006 ($F_{5,30} \geq 40.63$, $P \leq 0.01$), while in 2005, mean breeding call index was 1 – 7X greater in March and April than in all other months ($F_{5,30} = 17.89$, $P < 0.01$). Spring peeper calling index was 2 – 17X greater in March and April than in all other months across years and in 2005 ($F_{5,30} \geq 35.66$, $P \leq 0.01$), while in 2006, mean index was 3 – 49X greater in April than in all other months except March ($F_{5,30} = 18.62$, $P < 0.01$). Across years, mean calling index of southern leopard frogs was greater in April than in July and August ($F_{5,30} = 5.49$, $P = 0.02$), while in 2005, calling index was greater in April than in June – August ($F_{5,30} = 4.96$, $P = 0.03$). No other differences were detected in breeding call indices among months ($F_{5,30} \leq 1.67$, $P \geq 0.2$, Table 12).

Mean estimates of pitfall and breeding call species richness were different among months across years ($F_{5,30} \geq 10.17$, $P < 0.01$, Table 13); however, month and land-use effects interacted ($F_{5,30} \geq 2.51$, $P \leq 0.05$). Within land-use tests for combined years

revealed that pitfall species richness was 3 – 12X greater in June and July than in all other months for non-access wetlands ($F_{5,18} = 8.89$, $P < 0.01$); no differences were detected among months for access wetlands ($F_{5,18} = 0.95$, $P = 0.47$). Within land-use tests for combined years revealed that mean breeding call species richness was 2 – 5X greater in May than in March, July and August in access wetlands ($F_{5,18} = 16.31$, $P < 0.01$), and 1 – 2X greater in May than in all other months except April in non-access wetlands ($F_{5,18} = 17.62$, $P < 0.01$). Mean pitfall and breeding call species richness was different among months each year ($F_{5,30} \geq 4.29$, $P < 0.01$); however, month and land-use effects interacted for pitfall richness in both years and breeding call richness in 2006 ($F_{5,30} \geq 2.43$, $P \leq 0.06$). Within land-use tests revealed that pitfall species richness in July 2005 was 5X greater than in March for non-access wetlands ($F_{5,18} = 3.95$, $P = 0.01$). In 2006, pitfall species richness was 8 – 14X greater in June, July and August than in all other months for non-access wetlands ($F_{5,18} = 11.14$, $P < 0.01$). Breeding call richness in 2006 was 69 – 175% greater in May than in all other months for non-access wetlands ($F_{5,18} = 9.40$, $P < 0.01$). In cattle-access wetlands, breeding call richness was 3 – 5X greater in May and June 2006 than it was in March and August ($F_{5,18} \geq 11.01$, $P < 0.01$). Breeding call species richness in 2005 was 2 – 3X greater in May than it was in March, July and August ($F_{5,30} = 15.04$, $P < 0.10$); no month and land-use interaction existed (Table 13).

Mean species diversity also differed among months in combined years ($F_{5,30} = 4.60$, $P = 0.01$, Table, 13); however, month and treatment effects interacted ($F_{5,30} = 2.61$, $P = 0.07$). Within land-use tests indicated that mean species diversity was 15X greater in July than in April for non-access wetlands ($F_{5,18} = 4.07$, $P = 0.01$), while no differences

were detected among months in access wetlands ($F_{5,18} = 0.41$, $P = 0.83$). Mean species diversity also differed among months each year ($F_{5,30} \geq 2.77$, $P \leq 0.04$). In 2006, species diversity was greater in July than in March and May months ($F_{5,30} = 5.40$, $P = 0.02$). In 2005, Tukey's HSD test did not detect differences among months despite significance of the overall test ($F_{5,30} = 2.77$, $P = 0.04$). No additional differences were detected in species richness or diversity among months ($F_{5,18} \leq 1.87$, $P \geq 0.15$, Table 13).

Mean abundance of pickerel frog and southern leopard frog egg masses was greater in April than all other months across years ($F_{5,30} = 4.04$, $P = 0.08$, Table 14). No differences were detected in egg mass abundance among months for within year tests ($F_{5,30} \leq 2.41$, $P \geq 0.16$). In general, egg masses of American bullfrog, green frog, and Cope's gray treefrog were more abundant in June, July and August, whereas pickerel frog and southern leopard frog egg masses were more abundant in April and May (Table 14).

Species composition of pitfall captures was different among months both years (Figures 5 and 6). In 2005, southern leopard frogs and pickerel frogs were most common in March, while American toads were most common in April and May. Fowler's toads also were fairly common in May 2005. On the other hand, green frogs were dominant from June – August in 2005. American bullfrogs also were common in August 2005. In 2006, there were no captures in March. American toads and Fowler's toads were dominant in April 2006. American toads and green frogs were most common in May 2006. Green frogs were dominant from June – August 2006, southern leopard frogs were common in June and July 2006, and American bullfrogs were captured often in July and August 2006 (Figures 5 and 6).

Mean height, percent horizontal cover, percent vertical structure of shoreline vegetation and plant species richness differed among months in across years ($F_{4,24} \geq 5.41$, $P < 0.01$, Table 15). Mean height and percent horizontal cover were 50 – 132% greater in August than in April or May in combined years and in 2005 ($F_{4,24} \geq 6.69$, $P < 0.01$, Table 15). Across years, vertical structure was 78 – 135% greater in June and August than in April and May ($F_{4,24} = 12.68$, $P < 0.01$), although Tukey's HSD test did not detect any differences among months for plant species richness. In 2006, mean height, percent horizontal cover, and vertical structure were 60 – 111% greater in July and August than in April ($F_{4,24} \geq 6.83$, $P < 0.01$). Plant species richness was 59% greater in May than in April in 2006 ($F_{4,24} = 5.37$, $P < 0.01$). No differences in plant species richness were detected in 2005 ($F_{4,24} = 2.12$, $P = 0.14$). Soil compaction was 98% greater in June than in May ($F_{3,21} = 7.76$, $P < 0.01$, Table 15).

Prediction Models

Substantial variation (44 – 99%) was explained in mean relative abundance by final models for six amphibian species both years (Tables 16 and 17). The greatest variation in relative abundance of American toad was explained by cattle density (83%) and turbidity (90%) in 2005 and 2006, respectively. Both variables were positively related with American toad abundance. Most of the variation in Fowler's toad abundance was explained by un-ionized ammonia (NH_3, 77%) and vertical structure of vegetation (85%) in 2005 and 2006, respectively. Ammonia was positively related and vertical structure was negatively related with Fowler's toad abundance. For spring peepers in 2005, 64% of the variation in their abundance was explained by specific conductivity. A

negative relationship existed between spring peeper abundance and this water quality variable. The greatest variation in American bullfrog abundance was explained by mean daily capture of bullfrog tadpoles (92%) and specific conductivity (77%) in 2005 and 2006, respectively. American bullfrog abundance was positively and negatively related with tadpole abundance and specific conductivity. Most of the variation in pickerel frog abundance was explained by abundance of pickerel frog tadpoles both years (98% in 2005 and 88% in 2006). Un-ionized ammonia explained 78% and 52% of the variation in mean abundance of southern leopard frogs in 2005 and 2006, respectively. This variable was positively related with southern leopard frog abundance (Tables 16 and 17). Finally, specific conductivity explained 82% of the variation in green frog abundance in 2006, which was negatively related (Table 17).

Pathology

Prevalence of eosinophilic infiltrates in the kidney of green frog metamorphs at non-access wetlands was greater than at cattle-access wetlands ($Z = 2.42$, $P = 0.02$, Table 18). No other differences in prevalence of histopathological changes were detected between cattle land uses (Fisher's $Z \leq 1.46$, $P \geq 0.15$, Table 18). Differences were not detected either in bacterial prevalence (Fisher's $Z \leq 1.76$, $P \geq 0.23$, Table 19), parasite prevalence (Fisher's $Z \leq 1.49$, $P \geq 0.23$, Table 20), or parasite load (Wilcoxon $Z \leq 1.15$, $P \geq 0.25$, Table 21) between cattle land uses.

Four pathogenic bacteria were isolated from green frog metamorphs: *Acinetobacter lwoffi, Aeromonas hydrophila, Chryseobacterium meningosepticum* and *Pseudomonas* spp. (Table 19). Extramedullary hematopoiesis was found in the liver of

32% and 15% of green frogs sampled in cattle-access and non-access wetlands, respectively (Table 18). Myxospridia were identified in the kidneys of 32% and 25% of green frogs sampled in cattle-access and non-access wetlands, respectively (Table 18). Other parasites including trematodes were identified in the kidneys of 21% and 15% of the sampled green frogs in cattle-access and non-access wetlands, respectively. Trematodes also were identified in granulomas at the base of the lungs of one green frog metamorph sampled from a cattle-access wetland, and cutaneously in another sampled green frog metamorph from a non-access wetland. Parasites including *Ichthyophonus* spp. and trematodes were identified in the skeletal muscle of 11% and 10% of the sampled green frogs in cattle-access and non-access wetlands, respectively (Table 18). In addition, one enterovirus was detected in the feces of a green frog metamorph sampled at a non-access wetland. The GenBank BLAST search (NCBI 2005) on sequences obtained by PCR of Ranavirus revealed that *Frog virus* 3 was detected in three green frog metamorphs at non-access wetlands and one malformed bullfrog metamorph with microphthalmia captured at a cattle-access wetland.

Thirty-six individuals (2%) of all individuals captured in pitfall traps were malformed, and 11 malformation types were documented (Table 22). Brachydactylyl malformations and malformations due to injuries were more prevalent in cattle-access wetlands (Fisher's $Z \geq 2.23$, $P \leq 0.05$). No other differences were detected in the prevalence of other malformation types between cattle-access treatments (Fisher's $Z \leq 1.44$, $P \geq 0.27$, Table 22). Overall, malformation rates of individuals did not differ between cattle-access (42%) and non-access (58%) wetlands ($Z = 1$, $P = 0.317$).

Trematode metacercariae were identified in three individuals; one of these individuals was captured at cattle-access wetlands and two were captured at non-access wetlands.

Five individuals were opportunistically collected due to overt signs of disease: four of these were from cattle-access wetlands and one was from a non-access wetland (Table 23). An American toad with an irregular black focus of ecchymosis on the head was collected from a cattle-access wetland (BUAM 1, Table 23). The organisms *Penicillium* spp. and *Trichoderma* spp. were identified from the facial lesions of this individual along with seven bacterial species (Table 23). Twelve bacterial species were isolated from the liver, kidney, intestine and abdominal swab of this individual (Table 23). The bacterial species *Brevibacterium* spp. and *Delftia acidovorans* were isolated from a cutaneous lesion and the abdominal swab of this individual (Table 23). Histological examination found cestodes in the gastrointestinal tract, liver, heart and mesentery, but no inflammation or degenerative changes were noted.

Another American toad (BUAM 2, Table 23) was opportunistically collected from a cattle-access wetland due to a 5 mm swelling at the midpoint of the right rear tibia-fibula region. This lesion was found to be a space filled with clear serous fluid. Histological examination of the lesion revealed dilated vascular spaces but no inflammatory cells or other pathological findings. The bacteria *Pseudomonas fluorescens* and *P. stutzeri* were isolated from the kidneys and abdominal swab, respectively (Table 23). Two fungi, *Candida albicans* and *C. guilliermondii,* were isolated from the intestines and abdominal swab, respectively. The differential white blood cell count of heart blood was 8% segmented neutrophils, 26% lymphocytes, 12% monocytes, 53% basophils, and 1% metamyelocytes. Due to time constraints, white blood cell counts

were not taken for all individuals. Abnormal morphological changes were not noted for any of the blood cells. Parasitological examination of a green frog (RACL 1, Table 23) collected from a cattle-access wetland due to swollen regions on a hind limb found low intensity infection by parasitic nematodes and moderate intensity of flagellate protozoans in the feces of the specimen. *Aeromonas hydrophila* was isolated from the lesion (Table 23). Histological examination of a southern leopard frog (RASP 1, Table 23) with swellings on the right rear limb collected from a cattle-access wetland found that the swellings contained serous fluid and had no inflammatory cell infiltrates. Trematodes and nematodes were identified in the kidneys and lungs, respectively. *Citrobacter freundii* was isolated from the intestines of this individual (Table 23). Parasitic examination also found nematodes and flagellated protozoans in the feces.

A third American toad (BUAM 3, Table 23) collected from a non-access wetland was lethargic and the skin on the feet was black. Gross examination revealed dermal lesions, diffuse eroded excoriations, and numerous parasitic cysts throughout the coelomic cavity, on the serosa and throughout the parenchyma of all organs. Histological examination reported cestodes with granulomatus inflammation in the kidney, liver, gastrointestinal tract and heart, and multifocal edema and vacuolar degeneration in the skeletal muscle. The bacteria *Chyseobacterium indologenes* and *Staphylococcus epidermidis* were isolated from a leg lesion on this individual (Table 23).

Discussion

The results of my study suggest that cattle may have species-specific effects on postmetamorphic amphibians that lead to changes in community composition. Mean

relative abundance of green frog metamorphs in non-access wetlands was greater than in cattle-access wetlands. However, American toads were more abundant in cattle-access wetlands than in wetlands where cattle were excluded. A variety of environment co-factors of cattle land use may be responsible for these trends. I documented that cattle negatively impact shoreline vegetation in wetlands. In addition, a concurrent study (Schmutzer 2007) found that water quality and detrital biomass were lower in cattle-access wetlands. I also found that postmetamorphic body size generally followed density-dependent trends. A discussion of possible mechanisms driving species-specific abundance and body size trends follows. I also discuss some trends observed in pathogen prevalence between cattle land uses. Finally, a discussion on trends in monthly relative abundance and species richness is provided.

Species-specific Abundance

Shoreline vegetation.—Height, percent horizontal cover, and percent vertical structure of vegetation in non-access wetlands were greater than in cattle-access wetlands both years. It is well-known that cattle reduce vegetation in wetlands through mechanical trampling and herbivory (Trimble and Mendel 1995, Belsky et al. 1999, Jansen and Robertson 2001, Ausden 2005). This is a concern for many amphibian species because emergent shoreline vegetation provides cover from predators and inclement weather, protection from desiccation, and sites for amplexus, oviposition and foraging (Duellman and Trueb 1986, Hazell et al. 2001, Jansen and Healey 2002, Dodd 2004). A reduction in vegetation at amphibian breeding sites results in a decrease in relative abundance (Healey et al. 1997, Joly et al. 2001, Houlahan and Findlay 2003). For example, Jansen and

Healey (2002) found that adult frog abundance in New South Wales, Australia, was positively correlated with the amount of emergent shoreline vegetation. However, responses to reductions in vegetation from cattle may be species-specific, depending on habitat preferences governed by species-specific life history traits and adaptations. I found that green frogs were more abundant in non-access wetlands, which had greater shoreline vegetation. Others have reported high abundance of green frogs associated with shoreline vegetation (Woodford and Meyer 2003, Lichtenberg et al. 2006). Although green frogs are habitat generalists (Minton 1972, Hecnar 1997, Conant and Collins 1998), adults typically spend most of the time during the growing season near the shoreline of wetlands in areas with emergent vegetation (Minton 1972). Green frog tadpoles also spend most of the day in shoreline emergent vegetation, presumably hiding from predators and foraging (Warkentin 1992). Hence, this suggests that green frogs may be attracted to wetlands with greater amounts of shoreline vegetation, as occurred in non-access wetlands.

In contrast, I found a greater abundance of American toads in cattle-access wetlands, and the same trend was observed in Fowler's toads. American and Fowler's toads also are considered habitat generalists (Semlitsch and Bodie 2003), but they may be more adapted for areas with less vegetation. True toads commonly inhabit xeric environments (Conant and Collins 1998), and are able to withstand a greater loss of body water than ranids (Thorsen 1955, Schmid 1965, Duellman and Trueb 1986). Although I did not measure differences in light intensity or ground temperature, it is reasonable to assume that these abiotic factors were greater along shorelines at cattle-access wetlands due to increased exposure to solar radiation associated with less vegetation. Vegetation

also was shorter in the uplands at cattle-access wetlands (Burton, *personal observation*). Thus, the terrestrial micro-climate at cattle-access wetlands and their associated uplands may have been less hospitable for green frogs than for American or Fowler's toads.

Greater abundance of toads at cattle-access wetlands also may have been related to differential predation rates. Adult toads have numerous glanular glands that produce toxins making them unpalatable to most vertebrate predators (Duellman and Trueb 1986, Wright and Whitaker 2001). In addition, toad tadpoles are unpalatable to fish predators (Kats et al. 1988). Thus, reduction in shoreline vegetation likely does not increase their probability of predation. In fact, Woodward (1983) suggested that *Bufo* tadpoles are primarily predated by aquatic invertebrates, which are found in shoreline vegetation. Correspondingly, *Bufo* tadpoles frequently avoid vegetated areas where invertebrate predation may be high (Denton and Beebee 1997, Swart and Taylor 2004). On the other hand, ranids have fewer glanular glands as adults, and green frog tadpoles frequently avoid predation by aquatic vertebrates in shoreline vegetation (Warkentin 1992).

Another possibility for higher *Bufo* abundance at cattle-access wetlands may be related to morphology and locomotion. Toads have short legs, which reduces their jumping capability. Thus, toad saltatorial locomotion is composed of several short hops compared to ranids which jump farther (Duellman and Trueb 1986). It is possible that toads spend less energy traversing areas with shorter vegetation, because there are fewer obstructions. In previous studies, American toads have been found in high association with less vegetated open areas (Guerry and Hunter 2002), and they are common in terrestrial environments that are human modified (Kolozsvary and Swihart 1999, Lehtinen et al. 1999, Waldick et al. 1999).

Toads also may be taking advantage of less competition with and predation by ranids at cattle-access wetlands. Toads comprised over 1/3 of captures at cattle-access wetlands and 5% of captures at non-access wetlands, whereas green frogs comprised over 60% of captures in non-access wetlands and less than 1/3 of captures at cattle-access wetlands. Breeding call index of American and Fowler's toads also was greater at access wetlands. American and Fowler's toads have short larval stages (i.e., approximately 2 months, Dodd 2004), and are thought to be inferior competitors to larger ranid species, such as green frogs and American bullfrogs (Alford and Wilbur 1985, Wilbur and Fauth 1990). In addition, ranid tadpoles, particularly those individuals that overwinter, can be macrophagous and predate on eggs and tadpoles of other species including toads (Petranka et al. 1994, Petranka et al. 1998, Petranka and Kennedy 1999). More studies are needed exploring competitive exclusion and predation interactions of ranids and bufonids.

Several other common species exhibited trends in abundance between cattle land uses. In general, American bullfrogs were more abundant at non-access wetlands. This species, similar to the green frog, is considered to be associated more strongly with aquatic systems (Thorson 1955, Conant and Collins 1998), and thus they may be unable to tolerate desiccation as much as species associated more with the terrestrial environment (i.e., toads, Schmid 1965). As a result, bullfrogs may more adapted to wetland systems with greater amounts of shoreline vegetation that affords protection from the sun. In fact, bullfrog abundance has been positively associated with amount of woody litter along shorelines (Lichtenberg et al. 2006). American bullfrog adults and tadpoles are known to be good competitors, therefore it is unlikely that this species would

be competitively displaced by other species from cattle-access wetlands (Alford 1989*b*, Lannoo 2005).

Although statistical differences were not detected, mean abundance of pickerel and southern leopard frogs was greater at cattle-access wetlands. This is contradictory to trends for the other two ranids that I captured: green frogs and American bullfrogs. I hypothesize that this may be related to differences in their life histories. Pickerel and southern leopard frogs at my study site bred earlier in the growing season and their larvae metamorphosed usually in 3 months compared to American bullfrogs and green frogs which bred in the summer and their larvae often overwintered. In addition, juvenile and adult pickerel and southern leopard frogs are known to migrate large distances to terrestrial foraging habitats during summer (Conant and Collins 1998). Green frogs and American bullfrogs rarely travel far from breeding sites except during dispersal events (Lannoo 2005). Thus, because these pickerel and southern leopard frogs spend less time at wetlands, perhaps potential negative effects associated with reduced shoreline vegetation at cattle-access wetlands are less important.

Unfortunately, capture rates of other species were small, which reduced my ability to document possible additional cattle land-use trends. However, in general, I would hypothesize that reduction in shoreline emergent vegetation would negatively impact other species such as caudates. Female caudates, especially Ambystomatids, are known to attach fertilized egg masses to submersed vegetation (Lannoo 2005). While my results suggest reduced shoreline vegetation caused by cattle has species-specific effects, they also support the hypothesis that shoreline vegetation is important for some species and should be considered in conservation (Semlitsch and Bodie 2003). Appendix III

contains all the amphibian species currently documented in Cumberland County. Of these, there are two salamander species (four-toed salamander [*Hemidactylium scutatum*], spotted salamander [*Ambystoma maculatum*]) that use permanently flooded wetlands but were not found in my study wetlands. The four-toed salamander in particular is listed as a species in need of management in Tennessee (TWRA 2004). It is possible that cattle grazing on the Cumberland Plateau creates inhospitable conditions for these species. Previous studies on salamanders highlight the importance of connectivity between intact upland forests and wetland breeding sites (Semlitsch 1998, Guerry and Hunter 2002).

Water quality.—Differences in water quality between cattle-access and non-access wetlands may have contributed to observed amphibian trends by impacting larval populations and postmetamorphic recruitment. The larvae of all species that I documented in my study, except two (Ocoee salamander, *Desmognathus ocoee* and slimy salamander, *Plethodon glutinosus*), spend a portion of their life cycle developing in lentic systems. The permeability of amphibian embryos and tadpole skin makes them sensitive to changes in water chemistry (Vitt et al. 1990). Cattle use of wetlands decreases water quality (Belsky et al. 1999, Jansen and Healey 2002, Scrimgeour and Kendall 2002, Line 2003, Collins 2004, Knutson et al. 2004, Schmutzer 2007). The reduction in shoreline (and upland) vegetation that I observed at cattle-access wetlands also can increase erosion and run-off into wetlands, leading to higher sediment loads and turbidity (Trimble and Mendel 1995, Belsky et al. 1999, Scrimgeour and Kendall 2002, Line 2003, Knutson et al. 2004). Cattle defecation and urination in and around wetlands also increases nutrient inputs in the water (Hooda et al. 2000, Schmutzer 2007). Increased nitrate and ammonia levels are known to increase eutrophication in wetlands (Mitsch and Gosselink 2000),

and eutrophic wetlands have higher temperatures and lower dissolved oxygen (Cole 1994, Boyer and Grue 1995, Schmutzer 2007).

Poor water quality in agricultural landscapes has been shown to reduce amphibian diversity (Bonin et al. 1997, Lehtinen et al. 1999, Schmutzer 2007). Field and laboratory studies have shown that pH (Freda 1986, Sparling et. al 1995, Laposata and Dunson 2000, Gerlanc and Kaufman 2005), temperature (Smith-Gill and Berven 1979, Laposata and Dunson 2000), conductivity (Laposata and Dunson 2000), dissolved oxygen (Laposata and Dunson 2000), and increased nutrient levels (nitrates; Berger 1989, Laposata and Dunson 2000, Smith et al. 2006 and ammonia; Jofre and Karasov 1999) can affect development and survival of larval and embryonic amphibians. A study that ran concurrent with mine at the same wetlands found greater specific conductivity and turbidity and lower dissolved oxygen at cattle-access wetlands (Schmutzer 2007). Similarly, trends suggest that temperature, as well as nitrate and ammonia levels, were higher at cattle-access wetlands, indicating that cattle reduced water quality at these wetlands (Schmutzer 2007).

Differences in tolerance to reductions in water quality and developmental life history traits of amphibian larvae may be drivers contributing to species-specific postmetamorphic abundance trends at cattle-access and non-access wetlands. Species that overwinter in sediment at the bottom of wetlands, such as green frogs and American bullfrogs, have been found to be more sensitive to elevated nutrient levels than other species, possibly due to longer exposure to stressors in the aquatic environment (Houlahan and Findlay 2003). For example, American toad embryos have been found to tolerate higher ammonia levels than green frog embryos (Jofre and Karasov 1999).

Ammonia absorbed by amphibians is detoxified in the liver, however if levels are too high, it cannot be detoxified rapidly enough and oxidative metabolism is disrupted (Wright and Whitaker 2001). In a laboratory study, green frog embryos exhibited a decline in survival at ammonia levels 0.6 – 0.9 mg/L, whereas there was no increase in mortality of American toad embryos up to 0.9 mg/L (i.e., the highest concentration in the study, Jofre and Karasov 1999). Schmutzer (2007) found that mean ammonia levels were 0.4 mg/L in non cattle-access wetlands and 0.7 mg/L in cattle-access wetlands. High ammonia levels at cattle-access wetlands may have caused reduced embryonic hatching of green frogs and American bullfrogs, thus reducing postmetamorphic recruitment. American toads, on the other hand, may not have experienced any population reductions due to ammonia levels. Moreover, *Bufo* larvae at cattle-access wetlands may have been able to take advantage of reduced competition for resources (Schmutzer 2007), leading to greater postmetamorphic recruitment.

Other studies suggest that the less common species I captured also could be negatively impacted by a decrease in water quality at cattle-access wetlands. Diamond et al. (1993) reported negative effects of ammonia >0.9 mg/L on spring peepers. Low pH has been shown to cause embryonic or larval mortality in each of the anuran species that I captured (Gosner and Black 1957, Freda and Dunson 1985, Sparling et al. 1995) as well as some *Ambystoma* species (Pough and Wilson 1977, Freda 1986, Rowe et al. 1992). Eastern red-spotted newts (*Notophthalmus viridescens*) abundance has been negatively correlated with turbidity (Brodman et al. 2003). More water quality studies are needed to improve our understanding of species-specific tolerances, and how various water quality variables may affect amphibian species distributions. Nonetheless, these previous studies

support the hypothesis that decreased water quality observed at cattle-access wetlands may be contributing to decreases in postmetamorphic amphibian abundance for some species.

Breeding call surveys.—For toads and spring peepers, trends in breeding call indices followed larval and postmetamorphic abundance. Call indices at cattle-access wetlands for American and Fowler's toads were greater than at non-access wetlands, while the opposite relationship existed for spring peepers. As discussed previously, these trends may be a consequence of differences in shoreline vegetation structure and water quality. Interestingly, breeding call indices for American bullfrogs and green frogs did not differ between land uses despite lower larval and postmetamorphic abundance. This result provides additional evidence that cattle-access wetlands may function as ecological traps for breeding American bullfrogs and green frogs.

Egg Mass Abundance

Considering the trends in postmetamorphic abundance, one might have expected greater abundances of toad, pickerel and southern leopard frog egg masses observed at cattle-access wetlands, and more American bullfrog and green frog egg masses at non-access wetlands. However, there was an overall trend for greater egg mass abundance, regardless of species, in cattle-access wetlands. First, it is important to note that very few egg masses were observed (i.e., 20 total across 8 wetlands in 2 years), so inferences from these data should be interpreted cautiously. It possible that micro-habitat conditions for breeding amphibians were better for all species at cattle-access wetlands, and that decreases in abundance of green frog and American bullfrog metamorphs was a

consequence of high embryo and larval mortality. However, breeding call indices for green frogs and American bullfrogs did not support this suggestion. I believe differences in egg mass abundance were impacted by differences in detectability. I believe that greater amounts of vegetation at non-access wetlands reduced my ability to locate eggs. In contrast, I had very little difficulty seeing egg masses at cattle-access wetlands, because there was very little vegetation to conceal them (Burton, *personal observation*). Lastly, for toads and gray treefrogs, the greater numbers of breeding adults heard at cattle-access wetlands may have contributed to higher egg masses abundance for these species.

Amphibian Community Metrics

I did not detect any differences between cattle land-use types in postmetamorphic amphibian species richness and diversity in 2005 and combined years, respectively. In 2006 and both years combined, there were land-use effects for species richness, but it varied by month and sampling method (i.e., pitfalls versus breeding call surveys). Pitfall species richness was greater at cattle-access wetlands in April, but greater in non-access wetlands in July and August 2006. Trends were the same when years were combined. On the other hand, breeding call species richness was greater in cattle-access wetlands in June in 2006 and across years. The lack of trends between land-use types suggests that cattle may not strongly influence amphibian species richness or diversity. This result is consistent with Gray et al. (2004*a*) that reported no differences existed in postmetamorphic species diversity between amphibian communities at wetlands surrounded by agricultural cultivation compared to those at undisturbed grassland

wetlands. Homyack and Giuliano (2002) also found that excluding cattle from a stream did not increase amphibian species richness. However, others have found negative associations between cattle grazing and amphibian species richness (Jansen and Healey 2002, Knutson et al. 2004). The inconsistencies in these studies further emphasize the likelihood of species-specific and perhaps regional differences in the relative impacts of cattle on amphibians. Also, in some cases, stocking rates were not reported (Knutson et al. 2004), while for other studies (e.g., Homyack and Giuliano 2002, Jansen and Healey 2002), cattle grazing intensity was calculated based on pasture size instead of wetland size. This makes direct comparisons among studies tenuous.

Even though species richness and diversity did not differ between cattle land uses, species composition was altered. Species composition was more evenly distributed at cattle-access wetlands, with a greater percentage of bufonids compared to non-access wetlands. At non-access wetlands, captures in pitfalls were dominated by ranids. Again, these differences in community composition likely reflect species-specific tolerances to stressors and habitat preferences related to vegetation structure.

Habitat Models

Habitat models that I built explained substantial variation (44 – 99%) in relative abundance of postmetamorphic amphibians for several species at my study wetlands. Significant explanatory variables included ammonia, specific conductivity, turbidity, vertical vegetative structure, cattle density and species-specific abundance of tadpoles. Models were developed separately for 2005 and 2006, because I anticipated yearly variations in postmetamorphic abundance and explanatory variables. Indeed, one finding

that I made was that within species, significant explanatory variables often differed between years. This result suggests that environmental stressors may impact species differently depending on yearly circumstances, and lends support to the hypothesis that stressors are not independent (Hecnar and M'Closkey 1996, Laposata and Dunson 2000, Brodman et al. 2003, Gerlanc and Kaufman 2005, Loman and Lardner 2006). Below are discussions of the final models and the relationships between significant explanatory variables and species-specific postmetamorphic abundance.

Significant variation in relative abundance of American toads was explained by cattle density and nitrite concentration in the water in 2005. Cattle density explained 83% of the variation in American toad abundance. The standardized parameter estimate for cattle density was 1.2, indicating a strong positive relationship. In 2006, turbidity and un-ionized ammonia explained 90% and 7% of the variation in American toad relative abundance, respectively. Abundance was strongly positively related to turbidity and weakly negatively related to ammonia. Similar final models existed for Fowler's toad. In 2005, un-ionized ammonia and nitrite explained 77% and 17% of the variation in relative abundance. Ammonia was strongly positively related and nitrite moderately negatively related with abundance. In 2006, vertical structure of vegetation explained 85% of the variation in abundance, and this variable was negatively correlated with abundance. Water temperature explained an additional 12% variation in Fowler's toad abundance, and it was positively related.

These results provide further evidence that toads are associated with areas that are poorer in water quality and have less shoreline vegetation. Interestingly, nitrite was negatively associated with relative abundance of both toads. Nitrite absorbed by tadpoles

oxidizes iron in hemoglobin to form methemoglobin, which cannot bind to oxygen (Johnson et al. 1987, Hecnar 1995), leading to low oxygen carrying capacity of the blood and a corresponding decrease in health of the individual. In undisturbed aquatic systems, levels of nitrites are usually low, but in areas with high organic matter concentrations, nitrite levels can be >1 mg/L (McCoy 1972). Schmutzer (2007) found that mean nitrite at cattle-access and non-access wetlands were 0.11 and 0.07 mg/L, respectively. Nitrite quickly oxidizes to nitrate in aquatic environments (Rouse et al. 1999), thus may not reach critical levels. However, nitrates may interact with other stressors and have negative synergistic effects on developing tadpoles. Indeed, this hypothesis needs to be tested for toads and other amphibian species.

The greatest variation in spring peeper abundance in 2005 was explained by specific conductivity (64%). No explanatory variables were significant in 2006. A standardized parameter of –0.78 indicated a strong negative relationship between specific conductivity and spring peeper abundance. In 2006, the greatest variation in relative abundance of American bullfrogs (77%) and green frogs (82%) was explained by specific conductivity. Relative abundance of these species also was negatively related. Specific conductivity is a measure of total dissolved solids in the water, thus an index of the concentrations of nutrients, metals, and sediment. Laposata and Dunson (2000) found that American bullfrog egg hatching success was negatively correlated with specific conductivity in temporary ponds with wastewater effluent inputs. Brodman et al. (2003) reported that spring peeper abundance was negatively correlated with concentrations of detergents and chloride. As mentioned earlier, high levels of nutrients (specifically nitrogen compounds) have been shown to increase American bullfrog mortality and

decrease green frog growth (Smith et al. 2006). Knutson et al. (2004) reported that green frog and spring peeper abundance was negatively associated with nutrients and sediment. Nutrient and sediment inputs increase ions in the water, leading to a rise in conductivity, and apparently a negative effect on some amphibian species.

The greatest variation in American bullfrog abundance (92%) in 2005 and pickerel frogs both years (98% and 88% in 2005 and 2006) was explained by relative abundance of their tadpoles. A strong positive relationship existed between species-specific postmetamorphic abundance and tadpole abundance in all cases. These results suggest that larval recruitment may have been more important than other possible abiotic or biotic drivers of postmetamorphic abundance for these particular years and species. Finally, in both years, southern leopard frog abundance was strongly positively associated with ammonia (78% and 52% of variation explained in 2005 and 2006, respectively), suggesting that this species may be able to tolerate higher levels of ammonia. To my knowledge, no toxicology studies have yet examined the effects of ammonia on southern leopard frogs. Future controlled studies are needed to support or refute hypotheses related to species-specific tolerances to water quality variables as they may be highly variable even within a genus.

Monthly Trends

Species richness in pitfall traps was greatest in June and July at cattle-access and non-access wetlands, respectively, in 2006 and across years. The majority of my captures (96%) during these months were metamorphs, which reflects the months when most amphibians are metamorphosing in Tennessee. At my wetlands, toads began

metamorphosing in May and continued through June, whereas most ranid tadpoles metamorphosed in June and July. Also, all five salamander species were caught in June or July, which contributed to richness estimates. Similar trends existed in a study in Rhode Island that included six of the same species in my study; they reported peak metamorph emigration in June (Paton and Crouch 2002). An important trend that was found both years was that the greatest number of metamorph captures in June and July occurred in cattle-access wetlands and non-access wetlands, respectively. Earlier metamorphosis in cattle-access wetlands may reflect accelerated development due to greater levels of stress in tadpoles inhabiting these wetlands (Newman 1992). This hypothesis needs to be tested.

The greatest species richness for call surveys occurred in May both years and for both land uses. This probably reflects the overlap in breeding season for most of the species at my study site. All *Bufo, Hyla,* and *Rana* species were heard calling in May in at least one year of my study. These results reflect the typical breeding life history of these species in Tennessee (Dodd 2004). Similarly, the greatest species richness of calling amphibians in Texas occurred when the breeding seasons of *Bufo, Hyla,* and *Rana* species overlapped (Saenz et al. 2006). Monthly species composition also reflects species-specific breeding seasons and metamorph emergence. In general, toads were heard and captured earlier in the year, whereas green frogs and American bullfrogs at my study site bred and metamorphs emerged later in the year.

Egg mass abundance of pickerel frogs and southern leopard frogs was greater in April than all other months across years. Toads also followed this trend. American bullfrogs, green frogs and Cope's gray treefrogs showed a trend for greatest egg mass

abundance in July. Overall, egg mass abundance corresponded to the typical breeding season of these species (Lannoo 2005). I also found that vegetation structure was always greatest in August, which was expected as per the growing season. Soil compaction tended to be less during April, which may have corresponded with spring rains.

Postmetamorphic Body Size

In general, postmetamorphic body size was greater for individuals captured at cattle-access wetlands for all species, except Fowler's and American toads. This trend was especially noticeable for metamorphs. These results are directly correlated with relative abundance of postmetamorphic and larval amphibians at my study wetlands (This Study, Schmutzer 2007). These results echo previous studies on postmetamorphic body size at agricultural wetlands, which reported density-dependent relationships with body size (Oldham 1985, Gray and Smith 2005). Density-dependent relationships build on the premise that fewer individuals imply a greater amount of resources for each individual, thus lower competition (Wilbur 1976, 1977*a*, *b*, Collins 1979, Semlitsch and Caldwell 1982, Goater 1994). It has been reported that density of conspecifics in the terrestrial and aquatic environments is negatively related to postmetamorphic body size (Wilbur 1977*a*, Goater 1994, Morey and Reznick 2001).

The only species that did not follow a postmetamorphic density-dependent trend were pickerel frogs and southern leopard frogs. Mean postmetamorphic abundance of these species was greater at cattle-access wetlands and so was body size of metamorphs. However, Schmutzer (2007) found that abundance of pickerel frog and southern leopard frog tadpoles was less at my cattle-access wetlands compared to the non-access wetlands.

Therefore, body size of metamorphs may have been strongly influenced by larval density, which has been reported in controlled studies (e.g., Wilbur and Collins 1973, Brady and Griffiths 2000).

Pathology

Green frog metamorphs.—I detected no statistical differences in prevalence of bacteria, viruses or parasites in green frog metamorphs collected at cattle-access and non-access wetlands. This may reflect a true lack of biological trends or be a consequence of small samples sizes (n = 19 and 21 metamorphs collected for access and non-access wetlands). If the former explanation is true, this may be a result of immunocompromised individuals may not be surviving through metamorphosis, hence resulting in only clinically "normal" individuals being collected. Indeed, all amphibians undergo a temporary reduction in immune function during metamorphosis as their larval immune system is being dismantled and restructured for terrestrial life (Rollins-Smith 1998), which increases their susceptibility to infection (Wright and Whitaker 2001). Thus, the greatest mortality associated with diseases may occur during this developmental stage (Rachowicz et al. 2006). Moreover, infections of amphibians by pathogens may be greatest during this period (Gray et al. 2007*b*), but eliminated from the body during the juvenile stage as the immune system redevelops. Indeed, additional research is needed comparing pathogen infection in amphibians exposed to developmental and environmental stressors during different life stages.

I did observe some histological changes in the green frog metamorphs that I collected. Eosinophilic infiltrates (EOS) in the kidneys were 2.5X more prevalent in

individuals collected from non-access wetlands. This is an inflammatory reaction often suggestive of parasite presence. In fact, 33% of individuals with parasites in their kidneys also had EOS in their kidneys. Although amphibians are normally hosts to a variety of parasites and show no signs of disease, parasites can become pathogenic depending on the species of parasite, the intensity of parasite infection, and presence of other stressors (Fox et al. 2002). Myxospridia were identified in the kidneys of 28% of the sampled green frogs. Myxospridia infection in the renal tubules is common in green frogs (Wright and Whittaker 2001). However, no specific disease has been attributed to this organism, and usually histological changes associated with Myxospirdia are minimal in the affected renal tubes (Wright and Whittaker 2001). Other parasites in the kidneys may or may not have been negatively affecting the collected green frogs. Prevalence of parasitic cysts in the kidneys was 40% greater in cattle-access wetlands. The identity of these parasites is unknown, but they may have been trematodes because they are common in amphibians (Wright and Whitaker 2001). Some trematode species use amphibians as an intermediate host, while others use them as their final host (Smyth 1994). Histological changes associated with renal trematodes include inflammation around cysts, which could lead to renal dysfunction (Martin and Conn 1990). Parasites in the respiratory tract, however, are not typical of healthy amphibians. One green frog from a cattle-access wetland had granulomas that contained trematodes in the base of the lungs. In this location, this type of lesion could potentially be life threatening if it blocked the bronchial lumen or if opportunistic bacteria (e.g., *Pseudomonas* spp.) gained entry to these areas.

Another individual collected from a non-access wetland had cutaneous trematodes. The most common trematodes found in the skin of amphibians are in the

class Digenea (Wright and Whittaker 2001). Some species, such as *Clinostomum attenuatum*, are easily visible in live amphibians (Miller et al. 2004, Sutherland 2005, Gray et al. 2007*c*). In fact, Gray et al. (2007*c*) reported that metacercariae of *C. attenuatum* could be seen in the skin of Great Plains toads (*Bufo cognatus*), barred tiger salamanders (*Ambystoma tigrinum mavortium*) and New Mexico spadefoots (*Spea multiplicata*) without any magnification. The species of trematode in my specimen was not identified, but considering its location, may have been *C. attenuatum*. Metacercariae of *C. attenuatum* have not been linked to amphibian malformations (Sutherland 2005), but pathological changes may occur. Miller et al. (2004) noted that in heavily infected amphibians, metacercariae may be found near organs, where they may negatively influence survival and reproduction. No pathological changes associated with the cutaneous trematode, however, were noted in my specimen.

Two parasites, *Haplometra* spp. and *Glypthelmins* spp., are known to infect the intestines following penetration of the epidermis of many species of North American ranids (Wright and Whittaker 2001). As previously mentioned, most amphibian-parasite relationships do not degrade the health of the amphibian. However, parasites have the potential to become pathogenic in immunocompromised individuals (Fox et al. 2002). The skin of amphibians is the first line of defense against infection from foreign pathogens given it produces antibacterial and antifungal secretions, yet it also may contain many bacteria and parasites in healthy amphibians (Wright and Whittaker 2001, Rollins-Smith 2001, Nicholas and Mor 1995). Immunocompromised individuals could become internally infected with the parasites that normally inhabit their skin or induce stress such that opportunistic bacteria or fungi may become pathogenic.

Trematodes and *Ichthyophonus* spp. were identified in the skeletal muscle of five sampled individuals, with approximately equal prevalence between cattle land-use types. There are over 20 genera of trematodes that have been reported in the skeletal muscle of amphibians (Flynn 1973). Infection from all of these trematodes results in the formation of granulomas (Wright and Whitaker 2001). Infection by *Ichthyophonus* in wild amphibians appears to cause the same response (Mikaelian et al. 2000). Mikaelian et al. (2000) reported *Ichthyophonus*-like organisms in skeletal muscle of six different amphibian species, including green frogs. The effect of small numbers of *Ichthyophonus* spp. on the host likely is minimal; however, high loads could potentially impair the mobility of the infected individual, which would increase the probability of predation or decrease foraging efficiency. In my study, two of the three sampled green frogs that had *Ichthyophonus* in the skeletal muscle also were infected with fungal organisms or bacteria that can be pathogenic. Dual infection by potentially pathogenic organisms can suggest impaired immune function (Wright and Whitaker 2001). One of these individuals was collected at a cattle-access wetland and the other at a non-access wetland.

Trematodes, nematodes, amoeba-protozoan, and flagellated protozoans also were identified from the feces of collected green frog metamorphs. Three of four individuals reported to have fecal parasites at access and non-access wetlands typically also showed signs of inflammation in the kidneys, parasites in the skeletal muscle, FV3, or potentially pathogenic bacteria elsewhere in the body. These cases of multiple infection suggest that either individuals were stressed due to the presence of other pathogens, and the parasites opportunistically invaded, or the parasites were causing immunosuppression facilitating infection by other pathogens.

Mild to occasionally moderate histopathological changes (e.g., cellular degeneration, inflammatory cell infiltrates) were noted in 98% of the green frog metamorphs, and suggest they were being pathogenically challenged. All of the bacteria present in the metamorphs are typically found in water, soil, on plants, or are part of the normal intestinal flora of amphibians (Buchanan and Gibbons 1974, Igra-Siegman et al. 1980, Lloyd 1994, Palumbo 1993, Murray 2003). Four of these bacteria, however, are known to be potentially pathogenic to amphibians: *Acinetobacter lwoffi, Aeromonas hydrophila, Chryseobacterium meningosepticum* and *Pseudomonas* spp. The bacterium *Acinetobacter lwoffi* has been reported to cause local infections in immunocompromised individuals (Li and Lipman 1995, Glorioso et al. 1974), while *C. meningosepticum, A. hydrophila* and *Pseudomonas* spp. have been associated with bacterial septicemia or red-leg disease in immunocompromised individuals (Gibbs 1963, Glorioso et al. 1974, Brodkin et al. 1992, Mauel et al. 2002). Signs of red-leg disease include edema and systemic hemorrhaging in internal organs and on the skin of the pelvic region causing petechial reddening that led to its name (Gibbs 1963, Köhler 2006). Recent investigations have surmised that many of the older reports of amphibian die-offs that were attributed to red-leg disease likely were incorrect (Green et al. 2002). Rather, iridovirus infection probably reduced immunocompetence and allowed for secondary infection by opportunistic bacteria (Green et al. 2002, Cunningham et al. 1996). Dual infection by FV3 and *Aeromonas hydrophila* occurred for one of three iridovirus-infected individuals in my study. Previous studies determined that granulomatus inflammation in the muscle, enlargement of the liver, spleen and kidneys, and hemorrhagic gastrointestinal tracts have been associated with the opportunistic bacteria found in my

study (Cunningham et al. 1996, Hubbard 1981, Mauel et al 2002). In these studies, inflammation was not attributed to other pathogens, suggesting that these individuals may have been responding to infection from these bacteria.

Enterovirus or FV3 were documented from four of my collected green frogs. Parasites were found in the kidney, feces or skeletal muscle of three of these individuals, and *Aeromonas hydrophila* was isolated from the fourth individual. Fat necrosis also was documented in the fourth individual. Each of these cases suggests that the virus may have been compromising the immune system of the green frog, allowing for parasitic or bacterial invasion. Enteroviruses are ubiquitous throughout the world and are typically transmitted by the fecal-oral route (CDC 2007). Amphibians are known reservoirs of these viruses (Wilson and Sande 2001), although the effects of enteroviruses on the amphibians are not well understood.

Frog virus 3 belongs to the *Ranavirus* genus and is known to cause systemic necrosis and hemorrhage in tissues of amphibians (Converse and Green 2005). Ranaviruses have been implicated in almost half of reported amphibian die-offs in the United States, and their prevalence has been associated with anthropogenic disturbance (Daszak et al. 1999 and Converse and Green 2005). A concurrent study (Gray et al. 2007*a*) reported that FV3 prevalence was greater in green frog tadpoles from cattle-access wetlands compared to those inhabiting non-access wetlands. In contrast, prevalence of FV3 in American bullfrog tadpoles did not differ between cattle land uses. Given that I captured fewer green frog metamorphs in cattle-access wetlands, this suggest that green frog tadpoles infected with FV3 may not have survived to the postmetamorphic stage, thus FV3 may have contributed to observed postmetamorphic

abundance trends. In addition, the results of Gray et al. (2007*a*) suggest that green frogs may be more susceptible to pathogens than American bullfrogs. Specifies-specific susceptibilities to FV3 need to be quantified in the lab, especially for less common species.

Other pathological signs that were documented included extramedullary hematopoiesis (EMH), lymphocyte aggregates and inflammatory cells in the intestines. Extramedullary hematopoiesis was common in the liver (24%) and kidneys (70%) of tested individuals. Also, prevalence of EMH in the liver was 2X greater in individuals collected from cattle-access wetlands than in those from non-access wetlands. Extramedullary hematopoiesis is considered normal in amphibian metamorphs (Wright and Whitaker 2001), thus may not have been a pathological concern. Aggregates of lymphocytes were present in over 60% of collected green frog metamorphs. Similar to EMH, lymphoid aggregates are common in many organs, though they have not been studied in detail (Barrutia et al. 1983, Manning and Horton 1982, Saad and El Masri 1995). Over 80% of the sampled individuals also had minimal numbers of inflammatory cells in the small and large intestine. This may have been caused by the dramatic internal changes that metamorphs undergo as they convert from omnivory to carnivory (Hoff et al. 1999) and their intestinal tract shortens (Wright and Whittaker 2001).

Opportunistic captures.—Opportunistic captures of two additional species and one green frog that were morbid provided additional evidence of disease occurring at my wetlands. Three American toads were opportunistically captured during field investigations because of observed lesions: two were caught at cattle-access wetlands and one at a non-access wetland. A wide variety of bacteria were cultured from the American

toad with facial ecchymosis that was captured at a cattle-access wetland. Interestingly, none of the bacteria (*Chryseobacterium indolgenes and Pseudomonas* spp.) cultured from the liver and kidney of this individual were cultured from facial lesions. Thus, these internally cultured bacteria may have represented a separate and perhaps systemic infection, and suggest that this individual may have been immunologically stressed. The bacterium *Chryseobacterium meningosepticum* was cultured from the facial lesion and has the potential to be pathogenic in immunocompromised individuals (Mauel et al. 2002). It is also a human pathogen, reported most often in neonates, and targets the brain resulting in meningitis (Bloch et al. 1997). Fungal cultures from each lesion also revealed *Penicillium* spp. and *Trichoderma* spp. Both of these fungi are ubiquitous in the environment and can be pathogenic in severely immunocompromised humans and animals (Rippon 1988). Their role in amphibian disease is not well documented, though a toxin (OTA) produced by *Penicillium* has been reported to cause cranial malformations and reduced embryonic growth in *Xenopus laevis* (O'Brian et al. 2005). *Penicillium* also has been implicated in amphibian hepatitis (Griner 1983). Numerous cestode-containing cysts were identified in the walls of the intestines and stomach as well as the liver, heart and mesentery of this American toad. Many cestode species, such as *Mesocestodes* spp., use amphibians as intermediate hosts, and their larvae can encyst in various tissues (Goater and Goater 2001). Adult cestodes have protrusions that allow them to attach to the walls of the small intestine, potentially penetrating the gastrointestinal lining (Goater and Goater 2001). These lesions at the cestode attachment sites may allow opportunistic bacteria and fungi to enter the circulatory system resulting in septicemia (Wright and Whittaker 2001). Nonetheless, no histopathological changes were associated with the

numerous cestode-containing cysts in this individual, thus it is unlikely these parasites were significantly compromising the individual. However, if the immunocompetence level of this individual dropped, it could have led to bacterial and fungal infection, especially considering the species isolated from the external lesions.

The other American toad from a cattle-access wetland was collected because of a midshaft swelling on the right rear limb, which was found to be a fluid (serous) filled structure. This individual also had inflammatory reactions in the kidney, liver, gastrointestinal tract and heart, possibly due to cestode-containing cysts present in these tissues. Additionally, two potentially pathogenic bacteria, *Chyseobacterium indologenes* and *Staphylococcus epidermidis* were isolated from the leg lesion, though no inflammatory cells were found. Other than the leg swelling, this individual was alert prior to euthanizing and had no other gross signs of illness. It is possible though that the aforementioned histopathological changes were significant enough to compromise the individual, and perhaps allow future parasite and bacterial invasion if it had not been collected for my study.

The final opportunistically collected American toad was collected from a non-access wetland, because it had multiple dermal lesions. The bacterium *Pseudomonas* was cultured from the kidney. Fungal isolates from the intestines and abdominal swab revealed *Candida albicans* and *C. guilliermondii*, respectively. *Candida* spp. are opportunistic pathogens that are part of the normal bacterial fauna of many animals (Rippon 1988). The fungus *C. guilliermondii* has been reported previously as a potential pathogen in anurans (Mok and de Carvalho 1985). Infections by *C. albicans* are typically found in the alimentary or respiratory tract, while *C. gulliermondii* is typically associated

with endocarditis (Rippon 1988). Relative differential counts of blood cells found in this individual were suggestive of an acute immune-mediated process (i.e., proportion of heterophils was low, and basophils were high compared to normal proportions for American toads, Forbes et al. 2006). Changes in blood cell counts could be explained by reallocation of cells to fight infection or the release of corticosterone due to stress. Basophils are involved in processing surface immunoglobulins and histamine release, and are found to increase in response to parasite load and viral infections (Jacobson 2007). Elevated basophil counts in heart blood also may suggest a cardiac infection, because basophils are recruited to sites of infection (Guyton and Hall, 2000). Evidence of endocarditis was not seen during histological examination of this individual, but early (peracute) recruitment may have been initiated if the cultured *C. gulliermondii* became systemic and targeted the heart. Heterophils, being the first line of immune defense, may have been low if the toad was fighting infection for some time or if the infection was becoming systemic. Additionally, release of corticosterone reduces inflammation by decreasing the allocation of white blood cells, thus potentially decreasing the percent of heterophils. Although if this explanation were true, I should have seen a decrease in eosinophils and lymphocytes (Guyton and Hall 2000), which I did not. Regardless, these findings suggest that the individual was stressed, particularly because fungal infections are usually secondary to stress or disease (Fox et al. 2002).

One southern leopard frog with swellings on the right rear limb was opportunistically collected from a cattle-access wetland. Similar to the second American toad mentioned above, these swellings contained serous fluid with no inflammatory cell infiltrates, which suggested an acute traumatic event. A potentially pathogenic

bacterium, *Citrobacter freundii* was isolated from the intestines of the animal. However, given this bacterium is part of the normal gut flora of amphibians (Gibbs et al. 1966) and infections were not found in other organs or the cysts, bacteria cultured from the intestines were likely commensals. Nematodes and flagellated protozoans were found in the feces of this individual, and were likely incidental findings or may be suggestive of low-grade immune suppression.

The final opportunistically collected individual was a green frog metamorph at a cattle-access wetland, because it had immobile joints on the right rear limb. The bacterium *Aeromonas hydrophila* was isolated from the leg, and may have been an opportunistic invader. However, neither inflammatory cells nor bacteria were found during histological examination. Histological examination of affected joints did not reveal obvious anatomical changes that would explain the gross finding. However, a potentially significant finding was the presence of myositis in the skeletal muscle in the retroperitoneal area of the affected leg. This inflammatory reaction was suggestive of parasites, although none were discernible. If parasites were present during limb development, it could explain the gross malformation. Other findings included granulomatus inflammation at the base of the lungs (suggestive of parasite presence) and a nematode within the mesentery.

Malformed individuals.—I also collected 36 malformed anurans, identified malformation types, compared malformations prevalence between cattle land uses, and determined whether malformations were caused by the trematode *Ribeiroia*. No differences were detected in malformation prevalence due to trematodes between cattle land uses. Malformation rates (2%) were typical of normal amphibian populations (Tyler

1998), and several malformation types were consistent with trematode presence (Sessions and Ruth 1990, Johnson et al. 2002, Johnson and Chase 2004), such as amelia, ectromelia and polymelia. My results do not provide evidence that cattle increase prevalence of *Ribeiroia* or other trematodes that cause malformations, such as *Manodistomum* and *Telochis* spp. (Kiesecker 2002, Sutherland 2005). This is contradictory to previous studies (e.g., Johnson and Chase 2004, Johnson et al 2001.), which suggest that eutrophied conditions associated with cattle ponds facilitate *Ribeiroia* infections. I offer some explanations below.

In order for *Ribeiroia* infection to occur, all three hosts must co-occur. I observed four waterbirds known to be definitive hosts (i.e., the primary host) of *Ribeiroia* at my study wetlands, but there was no noticeable trend in relative abundance of all species combined between cattle land uses. In a concurrent study, Schmutzer (2007) found that abundance of Planorbella snails was greater in cattle-access wetlands in only one month, which was likely was due to more eutrophied conditions (Chase 2003, Johnson and Chase 2004). Green frog tadpoles (i.e., second intermediate host) were present in cattle-access wetlands, but around 3X more abundant in non-access wetlands (Schmutzer 2007). In addition, overall relative abundance of tadpoles (species combined) was around 3X greater in non-access wetlands. Infection by *Ribeiroia* likely increases as relative abundance of all hosts increases (Johnson et al. 2004), which was not the case at cattle-access wetlands. Infection of tadpoles by *Ribeiroia* also is positively correlated with cercariae density in the water (Johnson et al. 2002), which is a function of the number of primary and secondary hosts. Although I did not measure density of *Ribeiroia* cercariae, I found no evidence to suggest they would have been elevated because all three hosts

either showed no trend in abundance between cattle land uses or there were fewer individuals in cattle-access wetlands.

Another possibility is that *Ribeiroia*-infected individuals experienced mortality as tadpoles or during metamorphosis, so that true trends in postmetamorphic prevalence were not documented. High mortality rates of tadpoles infected with *Ribeiroia* have been documented during the pre-limb bud stage (Gosner 24–25, Schotthoefer et al. 2003). Also, it is hypothesized that predation rates of malformed metamorphs are higher than for normal individuals (Johnson and Chase 2004), which may have been greater at cattle-access wetlands because of less vegetation for escape cover. However, in two years of research, I rarely observed malformed frogs near my wetlands. Therefore, I believe that either mortality is occurring prior to metamorphosis, cercariae are low, or the cattle land-use hypothesis for *Ribeiroia* is false. Indeed, more research is needed to discern mechanisms driving trends in *Ribeiroia* infection.

Abnormalities due to injury and one malformation type, brachydactyly, were more prevalent at cattle-access wetlands. Injuries may have been increased at cattle-access wetlands due to trampling by cattle. Brachydactyly (shortened toes) may have been greater at cattle-access wetlands due to lower water quality (Schmutzer 2007). Some studies have suggested that poor water quality reduces growth and may be an underlying mechanism of amphibian malformations (Fort et al. 1999*a*, *b*; Jofre and Karasov 1999, Sparling 2000; but also see Lannoo et al. 2003). However, if water quality was the cause, it is unclear why this particular malformation type (brachydactyly) would have been only been observed in one limb of the individual and why it would have been the only malformation type to show a significant difference in prevalence between

land uses. Potentially, a chemical or combination of chemicals found in cattle-access wetlands may cause developmental problems in the feet of amphibians. Alternatively, my sample size (n = 15 access and n = 21 non-access) may not have been sufficient to detect statistical differences for other malformation types or were sufficient to obtain unbiased estimates. I recommend additional research exploring the roles environmental stressors, especially water quality, in causing the different types of malformations.

Pathological conclusions.—Pathological examinations revealed that amphibians at these wetlands are subjected to numerous potential pathogens and stressors in the environment that may be influencing infection rates. However, cattle do not appear to be increasing the prevalence of the pathogens that I documented in postmetamorphic individuals. Nonetheless, pathogens may still contribute to shaping the amphibian communities at cattle-access and non-access wetlands by influencing survival of individuals, particularly as they go through metamorphosis, thus influencing postmetamorphic recruitment.

Conclusions and Conservation Recommendations

My results suggest that the potential effects of cattle in wetlands on amphibians are species-specific, which appeared to alter the structure of the resident amphibian community at my study wetlands. In general, American toads may be positively influenced by environmental changes associated with cattle grazing at wetlands, while green frogs (and perhaps other ranids or less common species) may be negatively impacted by cattle in wetlands. Similar trends may exist elsewhere in Tennessee. Environmental co-factors of cattle land use responsible for these postmetamorphic trends

are unknown, but probably include a combination of reduced shoreline vegetation and lower water quality at cattle-access wetlands. Differences in shoreline vegetation may have served as proximate cues for habitat selection or increased mortality of ranids through accelerated water loss or increased predation. Differences in species-specific tolerances to water quality also may have influenced ranid larval survival, and thus postmetamorphic abundance. In general, controlled studies (e.g., Jofre and Karasov 1999) suggest that toad tadpoles are influenced less by lower water quality, perhaps contributing to their greater abundance at cattle-access wetlands. In addition, Gray et al. (2007*a*) documented that green frog tadpoles inhabiting cattle-access wetlands were 4X more likely to be infected with *Frog Virus 3* (FV3) than in non-access wetlands. Thus, FV3 infection may have been an important driver of green frog postmetamorphic abundance. Pathological findings in my study revealed that amphibians at these wetlands are subjected to numerous potential pathogens, and that stressors in the environment may be influencing infection rates. However, cattle did not appear to increase prevalence of the pathogens that I documented in postmetamorphic individuals. Nonetheless, pathogens may still contribute to shaping the amphibian communities by influencing survival of individuals, particularly at tadpole stages and as they go through metamorphosis, thus influencing postmetamorphic populations in Tennessee wetlands.

To reduce the impact of cattle on amphibian populations in Tennessee, I recommend that water tanks be supplied for cattle, and ideally that cattle be excluded from wetlands and from adjacent terrestrial habitat. Terrestrial buffers of 30 m generally are considered sufficient to protect water resources (Houlahan and Findlay 2004). However, during the non-breeding season, most adult amphibians are found 30 – 200 m

from wetlands (Rittenhouse and Semlitsch 2007). Previous studies recommend buffers 160 – 200 m to conserve amphibians (Semlitsch 1998, Regosin et al. 2005). Partially fencing cattle from portions of a wetland or limiting cattle density in space or time also may minimize impacts of amphibians and be a viable conservation alternative. Partial exclusion of cattle from wetlands has been shown to have positive impacts on red-legged frog (*Rana aurora*) and tiger salamander (*Ambystoma tigrinum*) populations in the western United States (Contra Costa Water District 2005). Future research directions need to quantify the benefits of partial exclosures, regulating cattle density, and rotational grazing on amphibians.

One study that I recommend is a controlled grazing intensity experiment. Seemingly, there exists a cattle density where the negative impacts of cattle are minimized. Jansen and Healy (2002) suggested that amphibian abundance and species richness increased with decreasing grazing intensity. Mean number of cattle per ha of wetland was 86 at my study site. Therefore, a hypothetical threshold for negative cattle impacts exists somewhere between 0 and 86 head / ha. I recommend a field experiment with four grazing intensity treatments (0, 25, 50, and 100 head / ha) replicated at four wetlands per treatment. Variables measured in Schmutzer (2007) and my study should be measured in addition to larval and postmetamorphic survival in mesocosm enclosures (e.g., Rothermel 2004, Todd and Rothermel 2006). If possible, postmetamorphic population density should be estimated using Program Mark and a Jolly-Seber model to estimate population size (Williams et al. 2001). I was unable to estimate population size in my study (only relative abundance), because recapture rate was 4.2 – 4.8% among wetlands. It is recommended that recapture rates exceed 60% for precise Jolly-Seber

estimates (Sandercock 2006). This could be accomplished by increasing sampling intensity and marking all captured individuals uniquely. Results from such a study could be used directly in conservation incentive programs for cattle farmers. Additional studies that I recommend include testing buffer size widths, effectiveness of partial cattle exclosures and rotational grazing, quantifying the impacts of cattle land use on amphibian home-range and dispersal movements, and lab experiments on species-specific tolerances to water quality and how water quality interacts with pathogen infectivity. Such studies are necessary to better understand the impacts of anthropogenic stressors on amphibians, and to formulate conservation recommendations that counteract amphibian declines.

Finally, for conservation recommendations to be effective, farmers should be made aware of cost-share opportunities that are available to them through USDA Natural Resources Conservation Service (NRCS) programs. Currently, the NRCS Environmental Quality Incentives Program (EQIP) and the Wildlife Habitat Incentives Program (WHIP) provide up to 75% cost-share for conservation projects that reduce non-point source pollution and soil erosion in agricultural watersheds, and create quality wildlife habitat (NRCS 2007). Cattle farmers also should be educated on the potential economic benefits of excluding cattle from wetlands. Allowing cattle access in wetlands can reduce water quality by increasing nutrient inputs through fecal deposition (Trimble and Mendel 1995, Hooda et al. 2000, Schmutzer 2007). Poor water quality can lead to a loss in live weight of beef cattle and a reduction in milk production in dairy cows (Willms 2002, Looper and Waldner 2002). Fencing cattle from wetlands also may be benefit herd health (e.g. *Listeria* spp., Botzler et al. 1973; *Salmonella* spp., Daniels et al. 2003; *Leptospira* spp., Shotts 1981; *Mycobacterium* spp., Beard et al. 2001). Pathogens

can be easily transmitted among herd members in water via the fecal-oral route (Theon and Johnson 1970, Shotts 1981). Callaway et al. (2005) estimated that *Salmonella* spp. prevalence was 27 – 31% in U.S. dairy herds. In cattle, this pathogen can cause diarrhea, dehydration, abortion, and death if left untreated (Glaser et al. 1994, NADIS 2002). The pathogen *Mycobacterium paratuberculosis* also is a concern to cattle farmers, because it can cause Johne's disease (Olsen et al. 2002). Cows with Johne's disease may produce 700 kg less milk per cow than uninfected cows (Ott et al. 1999). Farmers with herds that test positive for *M. paratuberculosis* also have reported lower cull-cow revenues and greater cow mortality rates than farmers with herds that were not infected (Ott et. al 1999). Overall, these economic losses due to Johne's disease have been estimated to cost the United States $200 – $250 million annually (Ott et. al 1999). Many cattle pathogens also can survive and reproduce for long durations outside the host. For example, *Salmonella* spp. can survive in pond water and pasture soil for around 120 days, and it can remain viable in cattle manure for up to three weeks (Morse and Duncan 1974, Himathongkham 1999). Thus, if an infected herd member defecates in a communal water source, the pathogen may be maintained in the aquatic system and facilitate infection of other herd members, even after potential treatment of the infected individual with antibiotics. Maintenance of cattle pathogens in wetlands also may occur in wildlife reservoirs (Botzler et al. 1973, Morse and Duncan 1974, Beard et al. 2001). For example, *Salmonella* spp., *Mycobacterium* spp., and *Escherichia. coli* have been isolated from the intestines of tadpoles (Hird et al. 1983, Monzon et al. 1995, Hoop 1997). Although these studies did not determine whether the bacterial isolates were pathogenic to cattle, the possibility exists that tadpoles as well as other wildlife may

serve as spill-over reservoirs of cattle and human pathogens (Gray et al. 2007*b*). Thus, I suggest that fencing cattle from wetlands also is a prudent agricultural practice that likely will reduce pathogen transmission among herd members and between wildlife and cattle.

Cattle also are primary reservoirs of zoonotic pathogens that cause human illness. Zoonotic pathogens can be transmitted from cattle to humans via food products or in contaminated water. Mead et al. (1999) estimated there are 76 million cases of foodborne illnesses in the United States every year, resulting in 5,000 deaths annually. Listeriosis and salmonellosis are some of the most common zoonotic diseases associated with cattle-human interactions (Lynch 2006). It has been estimated that illnesses due to salmonellosis cost the U.S. economy $2.4 billion annually (Mead et al. 1999), and many of the outbreaks have been linked to consumption of cattle food products (Hedberg et al. 1992). Cattle farms also are hotspots for *Listeria monocytogenes*, a foodborne pathogen associated with meningitis in humans (Gray et al. 2004). In addition to foodborne risks, livestock waste can contaminate ground water and surface waters, which can infect drinking water and water sources used for recreation. For example, runoff from a cattle farm was suspected as the source of *Cryptosporidium* that contaminated drinking water in Milwaukee, Wisconsin (EPA 2001). This outbreak caused 403,000 cases of cryptosporidiosis and 50 human deaths (EPA 2001). Allowing cattle to access wetlands also can contaminate irrigation water, which could result in zoonotic pathogens being transmitted to humans on vegetable products. In fall 2006, an *E. coli* O157:H7 outbreak in the United States was connected to spinach, probably contaminated with irrigation water containing infected cattle feces (CDC 2006). Further, Gray et al. (2007*b*) provided

lab evidence that American bullfrog metamorphs are suitable hosts of *E. coli* O157:H7. It is possible that if an infected herd member released this or other zoonotic pathogens into a wetland that they could be maintained by resident wildlife, allowing re-infection of cattle. Thus, fencing cattle from wetlands would reduce the likelihood of contaminating water or infecting wildlife hosts in wetlands with zoonotic pathogens by preventing direct deposition of infected feces. Feces contaminated runoff into wetlands also may be reduced when cattle are excluded, as shoreline vegetation establishes and serves as a buffer (EPA 2001, 2006).

CHAPTER III

RELATIONSHIPS BETWEEN AGRICULTURAL LANDSCAPE CHARACTERISTICS AND AMPHIBIAN COMMUNITY STRUCTURE

Introduction

The most widespread and influential of all possible anthropogenic stressors on amphibians is the loss and alteration of aquatic and terrestrial habitat. Amphibian habitat is destroyed or degraded for a variety of human land uses including agriculture, silviculture and urbanization (Collins and Storfer 2003). Anthropogenic land uses can increase the complexity of the landscape and distance between habitat patches (Gibbs 1993, Gray et al. 2004*b*). This may reduce the probability of successful dispersal by creating a greater number of edges and potential barriers to movement, such as roads or disturbed land (Gibbs 1998, Hecnar and M'Closkey 1998, Rothermel and Semlitsch 2002). In most temperate landscapes, wetlands represent isolated patches of amphibian habitat separated by inhospitable upland terrain. Wetland isolation influences the tendency of amphibians to undergo colonization or extinction events (Skelly et al. 1999). Populations at isolated wetlands usually have lower rates of immigration than emigration, and have a greater probability of extinction (Laan and Verboom 1990).

Wetlands surrounded by agriculture have been considered to be more isolated than those in undisturbed landscapes (Vos and Chardon 1998). Agricultural cultivation decreases suitable terrestrial habitat for amphibians by reducing natural vegetative cover, increasing exposure to the sun, and creating disturbed soils (Mazerolle and Desrochers 2005). Undisturbed terrestrial habitat is important for amphibians, because these sites are

used for foraging, overwintering and estivation (Semlitsch and Bodie 2003). Removal or reduction in natural vegetative cover can decrease food resources and increase the probability of desiccation and predation. This may explain why amphibians typically avoid agriculturally cultivated areas (Mazerolle and Desrochers 2005). Thus, agricultural fields adjacent to wetlands may function as barriers to movement (Gibbs 1998, Gray et al. 2004*a*).

Roads and urbanization also may decrease connectivity of spatially disjunct amphibian populations (Gibbs 1998). Roads may deflect movement (Marsh et al. 2005), reduce terrestrial habitat (Semlitsch et al. 2007), and be a direct cause of mortality (Orlowski 2007). Thus, roads may increase the extinction probability for local amphibian populations (Vos and Chardon 1998). Similarly, urbanization can decrease amphibian habitat and be impermeable to movement (Richter and Azous 1995).

Spatial isolation may increase the nestedness of certain amphibian species. Site fidelity of male Túngara frogs (*Physalaemus pustulosus*) increased with inter-pond distance (Marsh et al. 2000). Kolozsvary and Swihart (1999) found that amphibians showed strong nestedness in an agricultural landscape. Gray et al. (2004*a,b*) supported these findings reporting an increase in abundance of spadefoots in wetlands surrounded by geometrically complex cropland landscapes. They hypothesized that cropland landscapes may be perceived by spadefoot toads (*Spea multiplicata, S. bombifrons*) as viscous environments and reflect movement back to their natal wetland (Gray et al. 2004*a*). These studies illustrated that although isolation may decrease species richness (MacArthur and Wilson 1967), some species might increase in abundance in isolated ponds because emigration is reduced. On the other hand, other species that depend more

on the terrestrial environment to meet life-cycle needs may decrease in abundance if terrestrial and aquatic habitats become separated due to human disturbance (Loman 1988, Vos and Stumpel 1995).

The complexity and physical structure of anthropogenically modified landscapes can reduce permeability to amphibians, especially metamorphs (Rothermel and Semlitsch 2002), although it appears some species are better than others at traversing disturbed landscapes (Stevens et al. 2004). In general, anuran dispersal ability is positively correlated with body size and leaping abilities (Taigen and Pough 1981, John-Alder and Morin 1990, Beck and Congdon 2000). Body size also may influence the perception of an individual to landscape permeability (Wiens et al. 1997, Gray et al. 2004*a*). Other factors such as desiccation resistance, temperature tolerance, and seasonal requirements for breeding can influence the vulnerability of amphibians to fragmentation (Kolozsvary and Swihart 1999). Traversing a complex landscape can be energetically costly and may increase mortality, particularly if the inter-patch landscape matrix is unsuitable amphibian habitat (Ims and Yoccoz 1997). Consequently, inter-patch geometric complexity can affect species composition of amphibian communities across a fragmented landscape (Gray et al. 2004*a*). In forested and prairie landscapes, the presence of trees and native grass may increase connectivity between habitat patches and help maintain undisturbed amphibian demographics (Laan and Verboom 1990, Waldick 1997, Gray et al. 2004*b*).

In addition to the composition of the inter-patch matrix and the distance between amphibian habitats, wetland size also can affect amphibian communities. Vos and Chardon (1998) reported a positive relationship between pond size and probability of

occupancy by anurans. Wetland size and depth can be important predictors of amphibian species richness (Kolozsvary and Swihart 1999). In general, larger wetlands have greater depth stratification, which creates more diverse habitat and increases species richness (Laan and Verboom 1990). On the other hand, small wetlands can be very important in maintaining amphibian metapopulations (Semlitsch and Bodie 1998). Gibbs (1993) illustrated that removing small wetlands (<4 ha) in Maine increased inter-wetland distance by 67%. Semlitsch and Bodie (1998) reported that removing small wetlands (< 4 ha) in South Carolina increased inter-wetland distance by 136%, and that small wetlands can function as source populations (Pulliam 1988).

Several researchers have reported that landscapes dominated by agricultural cultivation can influence amphibian communities (Knutson et al. 1999; Kolozsvary and Swihart 1999; Gray et al. 2004*a*,*b*; Knutson et al. 2004). However, no studies have examined the relationship between landscape structure and amphibian community composition in agricultural landscapes dominated by cattle grazing. At the University of Tennessee Plateau Research and Education Center (PREC), approximately 24% of the landscape is pasture, with permanent wetlands interspersed. In Chapter II, I provided evidence that cattle land use can impact amphibian community structure; however, this effect may interact with landscape features. Thus, my objective for Chapter III was to quantify geo-spatial metrics of landscape structure and composition, and relate these to amphibian community composition using relative abundance estimates from Chapter II for my most common species. Based on previous studies, I hypothesized that geographic isolation would negatively influence amphibian abundance (Loman 1988, Laan and Verboom 1990, Vos and Stumpel 1995, Lehtinen et al. 1999), geometric complexity of

the landscape would be positively related to amphibian abundance due to hindered movement and increasing population nestedness (Knutson et al. 1999, Kolozsvary and Swihart 1999), and possible landscape effects would be species dependent (Gray et al. 2004*b*). Understanding the relationships of agricultural landscape structure on amphibian communities is important when developing conservation strategies for amphibians (Marsh and Trenham 2001, Semlitsch and Bodie 2003).

Methods

Amphibian abundance was quantified in pitfall traps at eight wetlands on the PREC from March – August 2005 and 2006. Sampling procedures followed those outlined in Chapter II. Mean species-specific abundance was calculated for each wetland over the two years for the following species: American toad (*Bufo americanus*), Fowler's toad (*B. fowleri*), American bullfrog (*Rana catesbeiana*), green frog (*R. clamitans*), pickerel frog (*R. palustris*), and southern leopard frog (*R. sphenocephala*). American toad and Fowler's toad abundance were combined under *Bufo* species abundance, because of their similar life history traits and morphology (Dodd 2004). I used the aforementioned species because they were the most abundant at my study wetlands (Chapter II). Infrequently caught species were not used, because the multivariate techniques that I used for analyses are sensitive to zeroes in the response matrix (discussed below).

Landscape structure and composition was quantified using remote sensing techniques, the geographic information system, and spatial analysis software. The 2004 digital orthophoto quadrangle (DOQQ) of Cumberland County, Tennessee, that contained

the PREC was downloaded from the Tennessee Spatial Data Server (http://www.tngis.org/). I imported this coverage into ESRI® ArcGIS 9.1, and designated landscapes for each study wetland. Due to the differences in habitat requirements and dispersal ability of my species, I designated landscapes at two different scales: 1 km and 0.5 km around each wetland (Vos and Chardon 1998, Lehtinen et al. 1999). These landscape sizes are based on biological criteria. Semlitsch and Bodie (2003) and Rittenhouse and Semlitsch (2007) provided evidence that the majority of temperate amphibian species use terrestrial habitat within 500 m of wetland breeding sites. Others have used 1-km scale (e.g., Knutson et al. 1999, Houlahan and Findlay 2003), because this landscape size is near the maximum dispersal distance of many amphibian species (Sinsch 1990, Semlitsch and Bodie 2003, Rittenhouse and Semlitsch 2007), hence may be a better representation of landscape influences on interdemic movement. I digitized the following land cover types for all my landscapes: wetland, stream, forest, cattle pasture, cropland, mowed grass not being grazed, gravel road, paved road used for primarily for local residential traffic, two-lane highway, parking lot, and building. All ArcGIS® shapefiles associated with cover types were merged together and converted to a raster image (Figure 7). Finally, each landscape within the extent of each buffer (1 km and 0.5 km) was extracted by mask to create landscape plots for spatial analyses.

Fragstats® software was used to quantify landscape structure and composition at three levels: patch, aggregate properties of the patches (class), and the landscape (McGarigal and Marks 1995). Spatial metrics calculated at the patch level were wetland shape index (SI), wetland area (WA), and nearest-neighbor distance from each study

wetland to surrounding wetlands (WNN). At the class level, calculated metrics were mean nearest-neighbor distance from all wetlands in a landscape to surrounding wetlands (MNN), percent land cover of wetlands (PLC), number of wetlands (NW), and an interspersion-juxtaposition index of wetlands (IJI). The patch and class level metrics were used to quantify isolation and spatial positioning (McGarigal and Marks 1995). At the landscape level, metrics were edge density (ED, m edge/ha), mean number of edges to cross from each study wetland to other wetlands in the landscape (ME), landscape shape index (LSI), patch richness (PR), Shannon evenness index of land cover types (SEI), and Shannon diversity index of land cover types (SDI, McGarigal and Marks 1995). Each of the landscape level metrics was used to measure landscape complexity (McGarigal and Marks 1995). Unity was assigned to cover types and permeability of edges, because the relative vagility among my species was unknown (Compton et al. 2007).

I used canonical correspondence analyses (CCA) to determine the relationship between landscape structure and amphibian community composition (McGarigal et al. 2002, Gray et al. 2004*b*). The response matrix (8 × 6) was mean daily abundance of each species over two years (i.e., 6 columns) for each wetland (i.e., 8 rows). I natural-log transformed all mean abundances, because CCA is sensitive to outliers and bimodally distributed data (ter Braak 1995). I used Program CANOCO® (version 4.5) to perform CCA analyses (Lepŝ and Ŝmilauer 2003). Global Monte Carlo permutation tests were used to determine if a significant relationship ($\alpha = 0.10$ existed between landscape metrics and species composition (ter Braak 1995). I created a dimensionless species-landscape metric biplot (i.e., ordination) for each landscape size to examine the

relationship between species abundance and landscape metrics. The biplot was composed of triangles and arrows representing species abundances and landscape metrics, respectively. The length and direction of each arrow corresponded to the eigenvalue and eigenvector, respectively, for the particular landscape metric. Metrics with larger eigenvalues (hence longer arrow lengths) were associated more strongly with amphibian abundance. Also, species that were more closely positioned to a landscape-metric arrow were more strongly correlated with it. To further illustrate the relationships among landscape metrics and species-specific amphibian abundance, I also created an inferred ranking diagram. This diagram was created by extending the blunt end of each eigenvector through the origin of the biplot and drawing orthogonal lines from each species to the eigenvector. Species closer to the arrowhead and blunt end of the eigenvector were more positively and negatively correlated, respectively, with the landscape metric. Species positions along the inferred ranking also can be interpreted as positive and negative associations (ter Braak 1995).

I also was interested in constructing multiple linear regression models to use as conservation tools for predicting univariate species-specific relative abundance using significant landscape metrics. The response variable was mean abundance for a species (i.e., one column from the multivariate response matrix), and explanatory variables included all aforementioned landscape metrics. I followed the identical protocol for regression analyses outlined in Chapter II. All statistical analyses were performed using the SAS® system (Littell et al. 1991, Stokes et al. 2003).

Results

Multivariate analyses using global Monte Carlo permutation tests based on 499 permutations revealed that 92% of variation in amphibian community composition was explained by landscape structure and complexity for both landscape sizes. For 1-km landscapes, WNN, ED, and PLC explained significant variation in amphibian community composition ($F \geq 3.35$, $P \leq 0.04$ Figure 8a). For the 0.5-km landscape, WNN, ED, and MNN explained significant variation in amphibian community composition ($F \geq 2.42$, $P \leq 0.07$, Figure 9a). Other variables were retained in the final multivariate model for both landscape sizes, but they did not explain significant variation ($F \leq 5.66$, $P \geq 0.11$, Figures 8a and 9a).

Orthogonal inferred ranking of species along significant eigenvectors indicated that *Bufo* spp. and southern leopard frogs were positively and negatively associated with WNN and ED, respectively, for both landscape scales (Figures 8b and 9b). Southern leopard frogs and *Bufo* spp. were positively and negatively associated, respectively, with PLC in 1-km landscapes. In 0.5-km landscapes, southern leopard frogs and *Bufo* spp. were negatively and positively associated with MNN, respectively. Other anurans (pickerel frog, American bullfrog and green frog) generally showed negative associations with *Bufo* spp. and southern leopard frogs, hence exhibited opposite relationships with landscape metrics (Figures 8b and 9b).

Multiple linear regression models for the 1-km landscapes explained 59 – 99% of the variation in mean amphibian abundance using landscape metrics as predictor variables (Table 24). Approximately 60% and 21% of the variation in *Bufo* spp. was explained by ME and WNN, respectively. For American bullfrogs, around 65% of the

variation in abundance was explained by PLC. The majority of variation in green frog abundance was explained by WNN (61%) and IJI (26%). Mean nearest neighbor distance and PLC explained 61% and 28% of the variation in mean abundance of pickerel frogs. For southern leopard frogs, MNN and WA explained 53% and 22% of the variation in mean abundance, respectively (Table 24).

Final univariate landscape-metric models for 0.5-km landscapes explained 45 – 99% of the variation in mean amphibian abundance (Table 25). Fifty-two percent of the variation in *Bufo* abundance was explained by LSI. Mean number of edges to cross from the study wetland to adjacent wetlands and PLC explained 68% and 29% of the variation in mean American bullfrog abundance. Most of the variation in green frog abundance was explained by WNN (61%) and PLC (23%). Approximately 75% of the variation in pickerel frog abundance was explained by SEI. Finally, SEI explained 78% of the variation in southern leopard frog abundance of RASP (Table 25).

Discussion

My results suggest that landscape metrics, representing landscape structure and composition, were positively and negatively associated with species-specific amphibian abundance. Multivariate ordinations and Monte Carlo simulation tests for both landscape sizes revealed that *Bufo* spp. and southern leopard frogs were positively associated with distance from study wetlands to adjacent wetlands and negatively associated with edge density (i.e., meters of edge / ha). These results suggest that these species inhabited more isolated wetlands on my study site, but they were potentially negatively impacted by increasing geometric complexity of the landscape. The opposite trend existed for

pickerel frogs, American bullfrogs and green frogs. Few individuals of these species were found at isolated wetlands, while landscape complexity did not seem to negatively impact their populations.

I believe the aforementioned trends are related to specific-specific vagility and life history. American and Fowler's toads may be influenced less by wetland isolation, because they are considered good dispersers partially due to their relatively large body size (Conant and Collins 1998), and they are able to tolerate greater water loss than other amphibians (Schmid 1965). Adult American and Fowler's toads have been reported traveling up to 6 and 34 km, respectively (Smith and Green 2005). Similarly, southern leopard frogs are known to travel far distances from breeding sites to forage in terrestrial habitats (Martof et al. 1980, Conant and Collins 1998). Although no studies exist on dispersal capability of southern leopard frogs, northern leopard frogs (*Rana pipiens*) are known to travel up to 5.2 km (Dole 1971). In contrast, the other ranids in my study may be less vagile (Raney 1940, Carr and Fahrig 2001). A study in Missouri indicated that American bullfrogs rarely traveled between wetlands, and those that did moved only 0.16 – 2.8 km (Willis et al. 1956). Similarly, green frogs have been shown to rarely travel more than 1 m from water unless under ideal conditions (e.g., rainy nights, Minton 1972).

Despite potentially high dispersal capability, movement of *Bufo* spp. and southern leopard frogs may be reflected by edges. Rothermel and Semlitsch (2002) found that forest edges reflected movement of recently metamorphosed American toads. A similar study determined that pasture adjacent to forest may be substantial barrier to dispersing American toad metamorphs because of the lack of suitable terrestrial habitat (Rothermel 2004). Highly fragmented uplands also may reduce successful toad dispersal given low

survival of American toads in clearcuts (Todd and Rothermel 2006). No studies have been performed quantifying the influences of edges on pickerel frog, green frog, and American bullfrog movement; however, it is possible that these species are influenced less by edges due to more restricted home ranges and lower maximum dispersal distance compared to *Bufo* spp. or southern leopard frogs. Indeed, this hypothesis needs to be tested.

An alternative explanation for increased abundance of pickerel frogs, American bullfrogs and green frogs in more geometrically complex landscapes may be related to their life history. Typically, these species remain relatively close to more permanent wetlands to meet their life cycle needs (McAtee 1921, Raney 1940, Oldham 1967, Shroeder 1976). Hence, edges in a landscape may have less impact on these ranids, because they are traversed less due to stronger association with permanent wetlands and smaller home ranges. Increased nestedness in geometrically complex landscapes also could represent the return of dispersing individuals to breeding sites due to inhospitable conditions in the surrounding terrestrial environment (Kolosvary and Swihart 1999, Gray et al. 2004*a*).

The multivariate ordination and inferred ranking also indicated that mean abundance of southern leopard frogs was positively related with increasing wetland area in 0.5-km landscapes. Wetland size also was a significant predictor variable in the final univariate model for this species in the 1-km landscapes. Thus, this species may be attracted to larger wetlands. Larger wetlands are typically deeper and more stratified, thus providing more habitat for competing larval amphibian species (Laan and Verboom 1990). Larger wetlands may also provide more habitat along shorelines, and reduce

inter-and intra-specific competition for breeding territories. Finally, adult southern leopard frogs may use larger wetlands with longer hydroperiods to provide sufficient time for their larvae to develop (ca. 3 months). In contrast, toads were negatively related with wetland size, and are known to use shallow, temporary wetlands as breeding habitat (Conant and Collins 1998). Use of smaller, wetlands may allow toads to exploit habitats not used by longer developing ranids given their larvae can complete development in <1 month. Smaller wetlands also may have poorer water quality, especially in agricultural landscapes where nutrients and contaminants become concentrated. As discussed in Chapter II, toads appear to tolerate poorer water quality than other species (Jofre and Karasov 1999).

At the 0.5-km scale, *Bufo* spp. were positively associated with mean nearest neighbor distance among all wetlands in a landscape, indicating that as distance between wetlands increased, their abundance increased. This provides further evidence that *Bufo* spp. may be less impacted by wetland isolation than other species. Interestingly, relative abundance of southern leopard frogs was negatively related with this variable. This result is opposite of the 1-km landscape, which suggested positive associations for this species with increasing inter-wetland distance. This scale dependency raises question about the relationship between wetland isolation and southern leopard frog abundance. It may suggest that southern leopard frogs benefit from closely juxtaposed wetlands for short distance migratory movements (<500 m), but also are able to reach isolated wetlands (e.g., >1 km) and maintain viable populations.

Significant variables in univariate prediction models generally corresponded with multivariate ordinations. In general, *Bufo* spp. and southern leopard frogs were positively

and negatively related to predictor variables that represented wetland isolation and inter-wetland geometric complexity, respectively. The other amphibian species generally had opposite relationships with these variables. I did not interpret each significant variable directly, because the intent of these models was a conservation tool (discussed later). In addition, univariate models ignore interdependencies among species in a community. Thus, the relationships observed in univariate models usually are less realistic than those observed in multivariate ordinations (McGarigal et al. 2002).

An additional observation that can be made from the multivariate ordinations and inferred rankings is the general negative relationship between *Bufo* and southern leopard frogs and other anuran species. This may be related to relative competitive ability between these groups of species as well as the possible negative impact of predation by ranids. For example, the small body size of bufonid species larvae and metamorphs may prevent them from competing as effectively with ranids in the aquatic and terrestrial environment. In my study, recently emerged *Bufo* metamorphs were, on average, 15.13 mm long and 1.6 g, while green frog and bullfrog metamorphs were at least double in size (Chapter II). Larger body sizes have been associated with an increased ability to acquire food resources (Newman 1999), thus ranids may negatively impact postmetamorphic *Bufo* abundance by outcompeting them for food resources. This has not been tested in postmetamorphs, but has been shown in larval ranid and bufonids (Alford 1989*a*). Although postmetamorphic bullfrogs are known to eat smaller amphibians, it is believed they do not predate on postmetamorphic toads (Tucker and Sullivan 1975). However, substantial ranid predation on toad tadpoles has been documented in larval studies (Petranka et al. 1994).

Conservation Recommendations and Future Research

My prediction models can be used to guide management and conservation strategies to improve existing agricultural landscapes for amphibians or to predict the impact of planned agricultural modifications on amphibians. My CCA results and multivariate ordinations suggested that landscape characteristics up to 1 km from amphibian breeding sites potentially could impact amphibian community structure. However, landscape influences appear to be species-specific. For species present at my wetlands, bufonids and ranids appeared to be positively and negatively associated with wetland isolation, respectively. The same respective associations existed for inter-wetland geometric complexity. Thus, amphibian conservation plans should consider landscape characteristics and be cognizant of species-specific dependencies. For example, I documented negative associations of ranids with wetland isolation. Although the ranids in my study are common, if conservation for a similar species of concern (e.g., *Rana capito*) was an interest, conservation efforts could focus on increasing wetland connectivity through strategies, such as restoration of riparian and upland corridors or creation of wetlands between existing breeding sites.

Predictions in amphibian response to land-use changes for a particular landscape can be done by: (1) creating an ESRI® ArcGIS coverage for cover types outlined in my methods, (2) calculating significant metrics in species-specific models using Fragstats, (3) making abundance predictions by solving the species-specific models in Tables 24 and 25 given calculated landscape-metric values, (4) creating a new coverage with the proposed land-use change, and (5) repeating steps 2 and 3. The predicted percent change in species-specific abundance can be calculated as the quotient of predictions before and

after the proposed land-use change. If the interest is to determine the potential impacts of the land-use change on relative abundance due to short-distance migratory movements, I recommend using models associated with the 0.5-km analyses. If the interest is to determine potential impacts on amphibian abundance due to long-distance dispersal movements, I recommend using the models associated with the 1-km analyses.

I recommend that future studies test the usefulness of my landscape models in predicting relative abundance of amphibians using manipulative experiments. This could be done by estimating species-specific relative abundance following methods in Chapter II, and comparing predictions before and after a land-use change. In addition, I suggest that recommendations provided by Semlitsch and Bodie (2003) and others (e.g., Rittenhouse and Semlitsch 2007) for buffer size widths around wetlands be tested. Conservation recommendations from these studies are based on home-range movements of various amphibian species. In general, they recommend buffer widths ranging from 30 – 500 m. A replicated experiment comparing larval and postmetamorphic amphibian abundance for five buffer-size treatments (0, 30, 100, 300, 500 m) would be useful. I also recommend additional studies on understanding the impacts of cover types and edges on juvenile and adult amphibian movements. Gray (2002) recommended landscape-scale experiments similar to those performed by With (1994) and others (e.g., McIntyre 2000) to examine the effects of landscape structure and configuration on amphibian movements. In addition, studies that displace individuals and examine movements following release (e.g., Marsh et al. 2005) would be useful. Relative differences in movement patterns (represented as probabilities) associated with different cover types and edges can be used as permeability estimates, which can be incorporated into landscape analyses in Fragstats

or other special analysis programs (e.g., Ramas® GIS, Akcakaya 1994) to perform least-cost path analyses. Such estimates of permeability also can be use to assess the functional connectivity of wetlands at local and regional scales, and the connectivity of wetlands to upland habitat using resistant-kernel estimator models (Compton et al. 2007).

Lastly, understanding factors that contribute to postmetamorphic survival in the terrestrial environment is critical. Enclosure experiments (e.g., Rothermel 2004) are one possible approach to determine micro-habitat characteristics (e.g., vegetation structure, invertebrate abundance) positively associated with survival. The relative difference in postmetamorphic survival should be compared between fields dominated by native warm-season grass (e.g., big bluestem, *Andropogon gerardii*) versus those covered by exotic cool-season grasses (e.g., tall fescue, *Lolium arundinaceum*), which the later are common in agricultural landscapes. Native warm-season grasses may benefit amphibians by providing greater structural complexity, which might reduce predation by avian predators and decrease incidence with the sun. Ultimately, native warm season grasses may provide more suitable habitat conditions for amphibians and increase connectivity among spatially disjunct habitats. Such studies will improve our understanding of the effectiveness of conservation strategies on minimizing the impacts of anthropogenic stressors on amphibians.

CHAPTER 4

CONCLUSIONS

My study provided evidence that allowing cattle access in wetlands influences resident amphibian communities on the Cumberland Plateau, Tennessee. Specifically, green frog metamorph abundance was reduced at cattle-access wetlands. American bullfrogs also followed this trend. On the other hand, American and Fowler's toad abundance was positively associated with cattle-access wetlands, and these trends were generally observed across all age classes. In general, no differences in species richness and diversity were detected between cattle-access and non-access wetlands, although there was a tendency for species richness to be greatest at cattle-access wetlands in May and greatest at non-access wetlands in June and July. Total postmetamorphic abundance also differed among months, and generally was greatest in June and July at cattle-access and non-access wetlands, respectively. This trend was primarily driven by metamorphs. Postmetamorphic body size of bufonids was greater at non-access wetlands, whereas ranids typically were bigger at cattle-access wetlands. This trend followed a density-dependent relationship. Percent horizontal and vertical cover and plant height of shoreline vegetation were less at cattle-access wetlands. In addition, a concurrent study (Schmutzer 2007) found that water quality was substantially lower at cattle-access wetlands. Pathological examination of opportunistically collected postmetamorphic amphibians revealed that individuals were exposed to a variety of potential pathogens, but in general, a trend of histological changes representative of a morbid condition were not noted between cattle land-use types. Sample sizes associated with these analyses were small and most of the individuals I collected did not exhibit gross signs of disease.

Hence, the lack of trend with my pathology results needs to be interpreted cautiously, and further investigation of cattle impacts on diseases in postmetamorphic amphibians is warranted. Finally, landscape analyses revealed that wetland positioning and geometric complexity of the landscape between wetlands is important in structuring amphibian communities on the Cumberland Plateau. Overall, bufonids and southern leopard frogs were positively associated with wetland isolation but negatively associated with increasing inter-wetland geometric complexity. Green frogs, American bullfrogs and pickerel frogs generally showed opposite relationships compared to these species.

It is likely that several abiotic and biotic mechanisms were responsible for trends observed in amphibian communities between cattle-access and non-access wetlands. Species-specific differences in abundance probably were related to different habitat preferences between bufonids and ranids. In general, bufonids can tolerate open areas with little vegetation, and ranids prefer aquatic habitats with more vegetation where there may less of a risk of desiccation (Conant and Collins 1998, Lannoo 2005). In addition, differences in species-specific tolerances to water quality may have contributed to abundance trends. Controlled studies suggest that bufonid tadpoles can tolerate poorer water quality than ranid tadpoles (Jofre and Karasov 1999). Thus, poor water quality may have positively influenced postmetamorphic recruitment of bufonids at cattle-access wetlands. Density of conspecific larval and postmetamorphic amphibians at my wetlands probably was the primary driving force behind postmetamorphic body size trends between land uses. In general, amphibian abundance and postmetamorphic body size were negatively related across species. Schmutzer (2007) also reported similar species-specific abundance and body size trends in amphibian larvae between land-use types at

my study wetlands. Differences in species-specific vagility (i.e., maximum dispersal capability and home range size), habitat preference, and competitive interactions are possible mechanisms driving the trend that bufonids were positively related with wetland isolation and negatively related with most ranids. My results collectively indicate that allowing cattle access in wetlands affects the structure of the amphibian community in Cumberland Plateau wetlands. Some ranids appear to be negatively impacted, while cattle access in wetlands does not appear to negatively impact bufonids. Unfortunately, my results are limited to common species in Tennessee. Less commonly caught species that use lentic wetland systems for breeding, such as ambystomatid salamanders and hylids, may be negatively impacted, but few captures prevented documentation of any trends. Indeed, futures studies need to ascertain the potential influences of cattle on amphibian species of concern in Tennessee and elsewhere.

Given that negative associations of cattle and some amphibian species were documented, I recommend that some level of cattle exclusion from agricultural wetlands should occur. Agricultural wetlands are important habitats for amphibians (Knutson et al. 2004), and often are the only breeding sites remaining in anthropogenically disturbed landscapes. Fencing cattle from wetlands completely and establishing conservation buffers (e.g., 160 – 200m; Regosin et al. 2005, Rittenhouse and Semlitsch 2007) is an ideal scenario. Partial exclosures, rotational grazing, or moderating grazing density (number of head per ha of wetland) may be alternatives but need to be tested with research.

Most farmers on the Cumberland Plateau and elsewhere in Tennessee do not have the funds necessary to voluntarily implement such conservation strategies. Thus, I

recommend that conservation strategies be implemented through cost-share programs, such as those provided by USDA NRCS (e.g., Environmental Quality Incentives Program [EQIP], Wetlands Reserve Program [WRP], Wildlife Habitat Incentives Program [WHIP]). The EQIP program currently provides 75% cost share and technical assistance to farmers for fencing off cattle from wetlands and riparian areas, and for purchasing water tanks and pipes necessary to distribute water to pastures (NRCS 2004). Regulating cattle access in wetlands will reduce negative impacts on shoreline vegetation and increase water quality, which will benefit resident amphibians. In California, controlling grazing intensity in wetlands has improved habitat for two federally listed species: the red-legged frog (*Rana aurora*) and California tiger salamander (*Ambystoma californiense*, Contra Costa Water District 2005). Given that beef farming in Tennessee generates $514 million annually in revenue (Livestock, Dairy and Poultry Outlook 2006), implementing prudent conservation practices that are sensitive to the needs of the beef industry also is important. I believe that plans to partially or completely exclude cattle from wetlands, in conjunction with cost-share incentives, will have the greatest likelihood of acceptance by Tennessee beef farmers.

Excluding cattle from wetlands also may benefit herd health. Pathogens, such as *Cryptosporidium* spp., *Leptospira* spp., *Salmonella* spp., *Mycobacterium* spp. and *Listeria* spp., have either been documented or have the potential to be transmitted from wildlife to livestock via surface water (Botzler et al. 1973, Shotts 1981, Mahon and Manuselis 1995, Beard et al. 2001, Daniels et al. 2003). In addition, cattle infected by these or other pathogens can transmit them to other herd members via fecal-oral route when they defecate and urinate in communal water sources such as wetlands (Theon and

Johnson 1970, Botzler et al. 1973, Shotts 1981, Katz et al. 1982, Daniels et al. 2003). Reducing the risk of transmitting pathogens between wildlife and cattle, or within cattle herds, has financial and public health benefits. For example, cattle infected with *Mycobacterium paratuberculosis* have reduced daily milk production (USDA 1999). Similarly, a study reported that 50% of dairy farms that tested positive for *Cryptosporidium* spp. experienced economic losses in milk production and cattle mortality (Anderson 1988). Infection of cattle by zoonotic pathogens also can impact human health, because they can be transferred to consumers in food products (Callaway et al. 2005). For example, the pathogen *Escherichia*. *coli* O157:H7 is frequently passed to humans via undercooked or uncooked beef and vegetables (Lynch 2006). Humans that contract the pathogen experience severe, bloody diarrhea and intestinal hemorrhaging (CDC 2002). In immunocompromised individuals, the pathogen may cause hemolytic uremic syndrome (HUS), which results in renal failure (CDC 2002). Humans also can contract zoonotic pathogens in the environment when water contaminated with infected cattle feces is used for drinking, recreation or irrigation (EPA 2001). For example, there was an outbreak of *E. coli* O157 in Walkerton, Ontario, in 2000 that resulted in hundreds of cases of diarrhea, five deaths, and an estimated $155 million in health care costs (PHAC 2000). It was determined that cattle manure that washed into the city water supply after heavy rains was the source of contamination (PHAC 2000). Indeed, excluding cattle from wetlands will decrease fecal loads in surface water and reduce the likelihood of human infection by zoonotic pathogens. Therefore, I suggest that fencing cattle from wetlands is a prudent public health strategy in addition to being beneficial to amphibians. Sharing results from my study and Schmutzer (2007) with farmers as well

as alerting them of the agro-economic and public health benefits of excluding cattle from wetlands will be important in ensuring the success of cattle-exclusion conservation strategies.

As alluded, research is needed on the benefits of regulating cattle use in wetlands on amphibians. Additional studies should include laboratory and field experiments that evaluate species tolerances to herbicides, pesticides, antibiotics and fertilizers, and how they may interact with pathogen infectivity and endocrine disruption. Quantifying the impacts of different land cover types and edges on species- and age-specific movements as well as quantifying maximum dispersal distances for Tennessee amphibians is needed.

Studies such as Schmutzer (2007) and mine help contribute to our understanding of the impacts of anthropogenic stressors on amphibian populations and global declines. However, research should not be restricted to documenting impacts. Researchers and biologists must endeavor to conceive and test the effectiveness of reasonable conservation strategies that minimize human impacts, while compromising with human needs. I also think future efforts need to focus on less common species, because they are most at risk of extinction. Lastly, waiting to document all possible impacts and interactions of human stressors on amphibians prior to making conservation recommendations will likely be too late for many rare species found in the United States and elsewhere. Thus, I encourage researchers to make conservation recommendations as data are acquired, and aggressively evaluate conservation strategies to document benefits for amphibians and other wildlife.

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APPENDIX I

TABLES AND FIGURES

Table 1. Mean cattle[a] abundance and density at four cattle-access wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Wetland	Size (ha)	Year	Mean Abundance	Density[b]
1	0.1433	2005	46	321.00
		2006	31	216.33
2	0.2830	2005	39	137.81
		2006	43	151.94
3	0.6091	2005	24	39.40
		2006	25	41.04
4	0.2248	2005	19	84.52
		2006	14	62.28

[a]Cattle included black angus cows, calves and bulls.

[b]Density = $\bar{x}$ cattle/ ha of wetland.

Table 2. Relative daily abundance[a] of amphibians between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[b]	Year[c]	Land-use Type			
		Access		Non-access	
		$\bar{x}$ [d,e,f]	SE	$\bar{x}$	SE
ACCR	Combined	0.00011 A	0.00011	0.00015 A	0.00010
	2005	0.00022 A	0.00022	0.00030 A	0.00021
	2006	NT	NT	NT	NT
AMTA	Combined	0.00027 A	0.00016	0.00053 A	0.00031
	2005	0 A	0	0.00097 A	0.00056
	2006	0.00054 A	0.00032	0.00009 A	0.00009
BUAM	Combined	0.01909 A	0.01279	0.00027 B	0.00016
	2005	0.03204 A	0.02555	0.00047 B	0.00027
	2006	0.00613 A	0.00284	0.00008 B	0.00008
BUFO	Combined	0.01126 A	0.00771	0.00162 A	0.00036
	2005	0.01190 A	0.01026	0.00303 A	0.00060
	2006	0.01063 A	0.00538	0.00022 A	0.00022
DEOC	Combined	0.00012 A	0.00012	0.00033 A	0.00033
	2005	0 A	0	0.00044 A	0.00044
	2006	0.00024 A	0.00024	0.00022 A	0.00022
HYCH	Combined	0.00007 A	0.00007	0 A	0
	2005	NT	NT	NT	NT
	2006	0.00014 A	0.00014	0 A	0
NOVI	Combined	0.00034 A	0.00034	0.00012 A	0.00012
	2005	0 A	0	0.00023 A	0.00023
	2006	0.00068 A	0.00068	0 A	0
PLGL	Combined	0 A	0	0.00070 A	0.00070
	2005	0 A	0	0.00094 A	0.00094
	2006	0 A	0	0.00047 A	0.00047
PSCR	Combined	0.00037 A	0.00024	0.00071 A	0.00024
	2005	0.00050 A	0.00050	0.00119 A	0.00044
	2006	0.00024 A	0.00024	0.00023 A	0.00023
PSMO	Combined	0 A	0	0.00004 A	0.00004
	2005	0 A	0	0.00007 A	0.00008
	2006	NT	NT	NT	NT
RACA	Combined	0.00624 A	0.00564	0.01115 A	0.00303
	2005	0.00827 A	0.00738	0.00659 A	0.00142
	2006	0.00421 A	0.00390	0.01572 A	0.00492

Table 2 (continued).

Species[b]	Year[c]	Land-use Type			
		Access		Non-access	
		$\bar{x}$ [d,e,f]	SE	$\bar{x}$	SE
RACL	Combined	0.01945 A	0.01165	0.08019 A	0.02756
	2005	0.02742 A	0.01662	0.06394 A	0.01854
	2006	0.01149 A	0.00679	0.09643 B	0.03735
RAPA	Combined	0.00797 A	0.00697	0.00403 A	0.00073
	2005	0.00780 A	0.00633	0.00119 A	0.00018
	2006	0.00814 A	0.00766	0.00688 A	0.00137
RASP	Combined	0.01542 A	0.01404	0.00708 A	0.00661
	2005	0.00773 A	0.00723	0.00137 A	0.00106
	2006	0.02311 A	0.02087	0.01279 A	0.01218

[a]Relative abundance was mean daily capture in pitfall traps standardized by wetland size and number of days sampled per month.

[b]ACCR = northern cricket frog (*Acris crepitans*), AMTA = mole salamander (*Ambystoma talpoideum*), BUAM = American toad (*Bufo americanus*), BUFO = Fowler's toad (*B. fowleri*), DEOC = Ocoee salamander (*Desmognathus ocoee*), HYCH = Cope's gray treefrog (*Hyla chrysoscelis*), NOVI = eastern red-spotted newt (*Notophthalmus viridescens*), PLGL = northern slimy salamander (*Plethodon* glutinosus), PSCR = spring peeper (*Pseudacris crucifer*), PSMO = mud salamander (*Pseudotriton montanus*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

[c]Combined = data averaged across years.

[d]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance for combined RACL, RACL in 2005 and RACA in 2006; Wilcoxon two-sample test was used for all other tests (i.e., normality was violated; Shapiro-Wilk test, $P \leq 0.01$).

Table 2 (continued).

[e]NT = no test was performed because capture = 0.

[f]There was a significant month × land-use interaction for RACL in 2006; land-use differences existed only in May and July.

Table 3. Relative daily abundance[a] for each age and sex class of amphibian species that differed significantly in relative daily abundance between cattle land uses (see Table 2) at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[b]	Age and Sex Class[c]	Year[d]	Land-use Type			
			Access		Non-access	
			$\bar{x}$ [e]	SE	$\bar{x}$	SE
BUAM	meta	Combined	0.0057 A	0.0050	0.0005 A	0.0002
		2005	0.0054 A	0.0052	0.0007 A	0.0004
		2006	0.0061 A	0.0050	0.0002 A	0.0002
	juv	Combined	0.0013 A	0.0010	0.0001 A	0.0001
		2005	0.0010 A	0.0006	0.0002 A	0.0002
		2006	0.0016 A	0.0016	0 A	0
	AF	Combined	0.0017 A	0.0003	0.0001 B	0.0001
		2005	0.0025 A	0.0005	0 B	0
		2006	0.0010 A	0.0004	0.0001 A	0.0001
	AM	Combined	0.0045 A	0.0020	0.0002 B	0.0001
		2005	0.0055 A	0.0035	0.0003 A	0.0002
		2006	0.0036 A	0.0013	0 B	0
RACL	meta	Combined	0.0170 A	0.0104	0.0772 A	0.0273
		2005	0.0244 A	0.0145	0.0602 A	0.0189
		2006	0.0096 A	0.0063	0.0942 B	0.0366
	juv	Combined	0.0011 A	0.0005	0.0020 A	0.0008
		2005	0.0015 A	0.0012	0.0018 A	0.0007
		2006	0.0006 A	0.0006	0.0022 A	0.0009
	AF	Combined	0.0011 A	0.0004	0.0006 A	0.0003
		2005	0.0016 A	0.0011	0.0011 A	0.0006
		2006	0.0006 A	0.0004	0.0001 A	0.0001
	AM	Combined	0.0003 A	0.0003	0.0004 A	0.0004
		2005	0 A	0	0.0008 A	0.0007
		2006	0.0007 A	0.0007	0 A	0

[a]Relative abundance was mean daily capture in pitfall traps standardized by wetland size and number of days sampled per month.

[b]BUAM = American toad (*Bufo americanus*), RACL = green frog (*Rana clamitans*).

Table 3 (continued).

[c]meta = metamorph (<1 yr old), juv = juvenile (>1 yr but not displaying secondary sexual characteristics), AF = adult female (>1 yr and possessing female reproductive characteristics such as eggs), AM = adult male (>1 yr and possessing male reproductive characteristics such as vocal sacs).

[d]Combined = data averaged across years.

[e]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance for RACL meta and juv in combined years and in 2005; Wilcoxon two-sample test was used for all other tests (i.e., normality was violated; Shapiro-Wilk test, $P \leq 0.06$).

Table 4. Mean breeding call index[a] of amphibians between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[b]	Year[c]	Land-use Type			
		Access		Non-access	
		$\bar{x}$ [d,e,f]	SE	$\bar{x}$	SE
ACCR	Combined	0.574 A	0.304	0.333 A	0.096
	2005	0.640 A	0.316	0.428 A	0.130
	2006	0.507 A	0.293	0.239 A	0.067
BUAM	Combined	0.084 A	0.0008	0.022 B	0.015
	2005	0.112 A	0.015	0.044 A	0.030
	2006	0.055 A	0.028	0 B	0
BUFO	Combined	0.207 A	0.069	0.013 B	0.008
	2005	0.133 A	0.075	0 B	0
	2006	0.282 A	0.075	0.025 B	0.016
GACA	Combined	0.002 A	0.002	0 A	0
	2005	NT	NT	NT	NT
	2006	0.004 A	0.004	0 A	0
HYCH	Combined	0.506 A	0.060	0.038 B	0.013
	2005	0.385 A	0.104	0.050 A	0.024
	2006	0.627 A	0.124	0.025 B	0.011
PSCR	Combined	0.547 A	0.197	0.957 A	0.105
	2005	0.452 A	0.161	0.959 B	0.111
	2006	0.643 A	0.237	0.954 A	0.120
PSTR	Combined	0.028 A	0.010	0.005 A	0.005
	2005	0.023 A	0.013	0.010 A	0.010
	2006	0.031 A	0.020	0 A	0
RACA	Combined	0.677 A	0.170	0.741 A	0.065
	2005	0.738 A	0.215	0.855 A	0.118
	2006	0.616 A	0.135	0.628 A	0.044
RACL	Combined	1.228 A	0.149	1.228 A	0.181
	2005	1.232 A	0.118	1.255 A	0.157
	2006	1.225 A	0.181	1.201 A	0.209
RAPA	Combined	0.330 A	0.038	0.485 A	0.075
	2005	0.301 A	0.094	0.419 A	0.122
	2006	0.358 A	0.061	0.552 B	0.060
RASP	Combined	0.164 A	0.024	0.118 A	0.047
	2005	0.235 A	0.076	0.160 A	0.061
	2006	0.092 A	0.046	0.076 A	0.057

Table 4 (continued).

[a]The following indices were assigned to male breeding choruses:1 = individuals can be distinguished and calls do not overlap, 2 = calls overlap but individuals can be distinguished, 3 = calls overlap and individuals cannot be distinguished (full chorus).

[b]ACCR = northern cricket frog (*Acris crepitans*), BUAM = American toad (*Bufo americanus*), BUFO = Fowler's toad (*B. fowleri*), GACA = Eastern narrowmouth toad (*Gastrophryne carolinensis*), HYCH = Cope's gray treefrog (*Hyla chrysoscelis*), PSCR = spring peeper (*Pseudacris crucifer*), PSTR = upland chorus frog (*Pseudacris triseriata*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

[c]Combined = data averaged across years.

[d]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance for ACCR, PSCR, RACA,RACL,RAPA,RASP in 2005 and BUAM, BUFO, PSCR, RACA, RACL, and RAPA in 2006, and combined ACCR, PSCR, RACA, RACL, RAPA, RASP; Wilcoxon two-sample test was used for all other tests (i.e., normality was violated; Shapiro-Wilk test, $P \leq 0.09$).

[e]There was a significant month × land-use interaction for combined BUAM, BUFO, HYCH, PSCR, and RAPA, for BUFO, HYCH, and RAPA in 2006 and for HYCH and PSCR in 2005; land-use differences existed only in April for combined BUAM, in May and June for combined BUFO and in 2006, in June for HYCH in 2005, and in May and June for combined HYCH and in 2006, in March for combined PSCR and in 2005, and March for combined RAPA and in 2006.

[f]NT = no test was performed because index = 0.

Table 5. Abundance of amphibian egg masses between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[a,b]	Year[c]	Land-use Type			
		Access		Non-access	
		$\bar{x}$ [d,e,f]	SE	$\bar{x}$	SE
RACA-RACL	Combined	0.25 A	0.20	0.13 A	0.10
	2005	0.29 A	0.18	0.21 A	0.21
	2006	0.21 A	0.21	0.04 A	0.04
RAPA-RASP	Combined	0.31 A	0.15	0 B	0
	2005	0.33 A	0.24	0 A	0
	2006	0.29 A	0.20	0 A	0
HYCH	Combined	0.35 A	0.33	0 A	0
	2005	NT	NT	NT	NT
	2006	0.71 A	0.65	0 A	0
BUFO	Combined	0.02 A	0.02	0 A	0
	2005	0.04 A	0.04	0 A	0
	2006	NT	NT	NT	NT
All Species	Combined	0.94 A	0.64	0.13 A	0.10
	2005	0.67 A	0.42	0.21 A	0.21
	2006	1.21 A	0.91	0.04 A	0.04

[a]RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*), HYCH = Cope's gray treefrog (*Hyla chrysoscelis*), BUFO = American toad (*Bufo americanus*) and Fowler's toad (*B. fowleri*), All Species = all species combined.

[b]RACA-RACL, RAPA-RASP and BUFO groups were used because eggs of these species were indistinguishable in the field.

[c]Combined = data averaged across years.

[d]Wilcoxon two-sample test was used for all tests (i.e., normality was violated; Shapiro-Wilk test, $P \leq 0.06$).

Table 5 (continued).

[e]NT = no test performed because observations = 0.

[f]There was a significant month × land-use interaction for combined RAPA-RASP; land-use differences existed only in April.

Table 6. Amphibian species richness and diversity between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Metric[a]	Year[b]	Month[c]	Land-use Type			
			Access		Non-access	
			$\bar{x}$ [d]	SE	$\bar{x}$	SE
RP	Combined	March	0.38 A	0.24	0.13 A	0.13
		April	0.47 A	0.08	0.26 A	0.11
		May	0.53 A	0.17	0.40 A	0.14
		June	1.04 A	0.44	1.33 A	0.22
		July	0.67 A	0.35	1.57 A	0.35
		August	0.33 A	0.13	1.10 B	0.18
	2005	NI	0.68 A	0.29	0.80 A	0.09
	2006	March	0 A	0	0 A	0
		April	0.50 A	0.05	0.13 B	0.09
		May	0.36 A	0.11	0.17 A	0.03
		June	1.11 A	0.54	1.50 A	0.28
		July	0.56 A	0.40	1.78 B	0.43
		August	0.25 A	0.05	1.28 B	0.26
RC	Combined	March	1.25 A	0.25	2.13 A	0.38
		April	4.50 A	0.35	4.00 A	0.20
		May	6.00 A	0.84	5.25 A	0.14
		June	5.13 A	0.31	3.75 B	0.32
		July	3.63 A	0.24	2.88 A	0.43
		August	2.38 A	0.31	2.13 A	0.13
	2005	NI	4.30 A	0.45	3.80 A	0.08
	2006	March	1.25 A	0.48	2.00 A	0.41
		April	4.50 A	0.65	3.25 A	0.25
		May	6.00 A	0.71	5.50 A	0.50
		June	5.25 A	0.48	3.25 B	0.48
		July	4.00 A	0.58	2.75 A	0.48
		August	2.00 A	0.41	2.25 A	0.25
Diversity	Combined	NI	0.09 A	0.07	0.14 A	0.05
	2005	NI	0.11 A	0.10	0.11 A	0.05
	2006	NI	0.07 A	0.04	0.17 A	0.05

[a]RP = species richness from pitfall traps, RC = species richness from breeding call surveys, Diversity = Shannon-Weiner Diversity Index.

[b]Combined = data averaged across years.

Table 6 (continued).

[c]There was a significant month × land-use interaction when analyses are separated by months; NI = no interaction was detected.

[d]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance for RP and RC in 2005, and diversity in 2006; analysis-of-variance was used for RP in combined years and in 2006 for RP and RC; Wilcoxon two-sample was performed for diversity in combined years and in 2005, and RC in combined years (i.e., normality was violated; Shapiro-Wilk test, $P = 0.04$).

Table 7. Mass (g) of postmetamorphic amphibians between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[a]	Age and Sex Class[b,c]	Land-use Type					
		Access			Non-access		
		n	$\bar{x}$ [d]	SE	*n*	$\bar{x}$	SE
BUAM	juv*	13	3.02 A	0.98	2	4.75 A	2.0
	AM*	33	26.31 A	1.45	2	19.50 A	3.25
BUFO	meta*	56	0.85 A	0.44	3	2.42 B	1.80
	juv*	10	2.45 A	0.67	9	5.19 B	0.73
	AF	6	30.75 A	3.75	2	31.0 A	25.0
	AM*	28	19.75 A	0.94	3	22.53 A	2.73
PSCR	AF*	2	1.75 A	1.0	3	3.42 A	0.08
RACA	meta*	33	5.03 A	0.56	88	4.38 A	0.25
	juv	5	12.45 A	0.67	4	12.50 A	2.43
	AF*	7	29.0 A	6.60	6	20.88 A	3.86
RACL	meta*	105	3.79 A	0.17	422	2.78 B	0.06
	Juv*	8	8.49 A	1.49	23	8.15 A	0.62
	AF	7	27.96 A	4.34	6	27.08 A	3.01
RAPA	meta*	28	1.78 A	0.09	32	1.44 B	0.09
	AF*	6	15.67 A	0.42	2	22.50 A	11.0
RASP	meta*	57	1.73 A	0.07	68	1.38 B	0.06

[a]BUAM = American toad (*Bufo americanus*), BUFO = Fowler's toad (*B. fowleri*), PSCR = spring peeper (*Pseudacris crucifer*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

[b]meta = metamorph (<1 yr old), juv = juvenile (>1 yr but not displaying secondary sexual characteristics), AF = adult female (>1 yr and possessing female reproductive characteristics such as eggs), AM = adult male (>1 yr and possessing male reproductive characteristics such as vocal sacs).

[c]If an age-sex class is missing, analyses were not performed due to insufficient data.

Table 7 (continued).

[d]Means within rows followed by unlike letters are different by analysis-of-covariance with date of capture as the covariate; "*" = Wilcoxon two-sample test was performed (i.e., normality was violated; Shapiro-Wilk test, $P \leq 0.03$).

Table 8. Snout-vent length (mm) of postmetamorphic amphibians between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[a]	Age and Sex Class[b,c]	Land-use Type					
		Access			Non-access		
		n	$\bar{x}$ [d]	SE	*n*	$\bar{x}$	SE
BUAM	juv	13	26.78 A	2.83	2	34.39 A	4.49
	AM	33	60.93 A	0.96	2	53.24 B	0.64
BUFO	meta*	56	12.75 A	0.40	3	17.5 B	1.48
	juv*	10	28.73 A	1.92	9	34.42 B	1.75
	AF	6	62.42 A	2.84	2	55.66 A	16.37
	AM	28	53.82 A	0.79	3	59.96 B	1.27
PSCR	AF*	2	25.31 A	3.11	3	30.37 A	0.18
RACA	meta*	33	37.36 A	1.26	88	35.02 A	0.63
	juv	5	50.13 A	0.85	4	50.91 A	2.97
	AF	7	63.82 A	5.21	6	59.47 A	3.80
RACL	meta*	105	32.47 A	0.39	422	29.78 B	0.23
	juv*	8	42.89 A	1.88	23	44.07 A	1.03
	AF	7	64.56 A	3.47	6	63.56 A	2.35
RAPA	meta*	28	27.38 A	0.68	32	25.55 B	0.63
	AF	6	58.75 A	0.87	2	60.51 A	12.30
RASP	meta*	57	26.36 A	0.27	68	24.59 B	0.35

[a]BUAM = American toad (*Bufo americanus*), BUFO = Fowler's toad (*B. fowleri*), PSCR = spring peeper (*Pseudacris crucifer*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

[b]meta = metamorph (<1 yr old), juv = juvenile (>1 yr but not displaying secondary sexual characteristics), AF = adult female (>1 yr and possessing female reproductive characteristics such as eggs), AM = adult male (>1 yr and possessing male reproductive characteristics such as vocal sacs).

[c]If an age-sex class is missing, analyses were not performed due to insufficient data.

Table 8 (continued).

[d]Means within rows followed by unlike letters are different by analysis-of-covariance with day of capture as a covariate for snout-vent length; "*" = Wilcoxon two-sample test was performed (i.e., normality was violated; Shapiro-Wilk test, $P \leq 0.09$).

Table 9. Emergent shoreline vegetation characteristics and soil compaction between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Metric[a]	Year[b]	Land-use Type			
		Access		Non-access	
		$\bar{x}$[c]	SE	$\bar{x}$	SE
Hgt (m)	Combined	0.51 A	0.09	0.80 B	0.06
	2005	0.42 A	0.08	0.73 B	0.08
	2006	0.60 A	0.11	0.86 A	0.03
HC	Combined	53.70 A	5.37	66.49 A	2.34
	2005	47.48 A	4.33	59.42 B	3.31
	2006	59.93 A	6.69	73.56 A	3.54
VS	Combined	27.06 A	4.94	43.43 B	3.12
	2005	24.48 A	4.40	44.94 B	6.14
	2006	29.65 A	5.79	41.92 B	0.92
Richness	Combined	4.27 A	0.65	4.60 A	0.28
	2005	4.23 A	0.98	4.03 A	0.38
	2006	4.31 A	0.46	5.18 A	0.36
SC (lbs/in^2)	2006	514.78 A	34.61	332.77 B	29.85

[a]Hgt = mean plant height, HC = percent horizontal cover, VS = percent vertical structure, Richness = plant species richness, SC = soil compaction.

[b]Combined = data averaged across years.

[c]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance for all tests on vegetation variables, except Hgt and VS in 2006 and combined HC, where Wilcoxon two-sample test was used because normality was violated (Shapiro-Wilk test, $P \leq 0.09$); analysis-of-covariance was for soil compaction tests with sampling distance from the wetland as the covariate.

Table 10. Relative daily abundance of definitive avian hosts of *Ribeiroia ondatrae* between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[a]	Year[b]	Land-use Type			
		Access		Non-access	
		$\bar{x}$ [c,d]	SE	$\bar{x}$	SE
Great blue heron	Combined	0.027 A	0.004	0.019 A	0.011
	2005	0.037 A	0.005	0.034 A	0.019
	2006	0.017 A	0.005	0.005 A	0.005
Green heron	Combined	0.007 A	0.003	0.043 B	0.018
	2005	0.005 A	0.003	0.049 B	0.023
	2006	0.010 A	0.004	0.038 A	0.016
Mallard	Combined	0.074 A	0.028	0.004 B	0.004
	2005	0.115 A	0.067	0 A	0
	2006	0.033 A	0.014	0.007 A	0.007
Wood duck	Combined	0.005 A	0.005	0.009 A	0.007
	2005	0.005 A	0.005	0.014 A	0.014
	2006	0.005 A	0.005	0.005 A	0.005
All Species	Combined	0.113 A	0.031	0.076 A	0.034
	2005	0.161 A	0.072	0.096 A	0.050
	2006	0.064 A	0.013	0.055 A	0.029

[a]Great blue heron (*Ardea herodias*), Green heron (*Butorides Virescens*), Mallard (*Anas platyrhynochos*), Wood duck (*Aix sponsa*), All Species = all species combined.

[b]Combined = data averaged across years.

[c]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance for great blue heron in 2005 and 2006; Wilcoxon two-sample test was used for all other tests (i.e., normality was violated; Shapiro-Wilk test, $P \leq 0.02$).

[d]There was a significant month × land-use interaction for combined mallard; land-use differences existed only in March.

Table 11. Relative daily abundance[a] of amphibians among months at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[b]	Year[c]	Month											
		March		April		May		June		July		August	
		$\bar{x}$ [d,e]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
ACCR	Combined	0 A	0	0 A	0	0.0004 A	0.0003	0.0003 A	0.0003	0 A	0	0 A	0
	2005	0 A	0	0 A	0	0.0009 A	0.0007	0.0007 A	0.0007	0 A	0	0 A	0
	2006	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
AMTA	Combined	0 A	0	0.0012 A	0.0006	0.0008 A	0.0004	0.0001 A	0.0001	0 A	0	0.0003 A	0.0003
	2005	0 A	0	0.0015 A	0.0001	0.0009 A	0.0007	0.0002 A	0.0002	0 A	0	0.0003 A	0.0003
	2006	0 A	0	0.0001 A	0.0001	0.0007 A	0.0007	0 A	0	0 A	0	0.0003 A	0.0003
BUAM	Combined	0 A	0	0.0166 A	0.0091	0.0256 A	0.0222	0.0144 A	0.0112	0.0007 A	0.0004	0.0006 A	0.0004
	2005	0 A	0	0.0204 A	0.0134	0.0503 A	0.0444	0.0250A	0.0223	0.0015A	0.0008	0.0005 A	0.0005
	2006	0 B	0	0.0129 A	0.0064	0.0011 B	0.0008	0.0040 AB	0.0040	0 B	0	0.0007 B	0.0007
BUFO	Combined	0 A	0	0.0061 A	0.0029	0.0156 A	0.0117	0.0037 A	0.0022	0.0096 A	0.0060	0.0036 A	0.0029
	2005	0 A	0	0.0052 A	0.0004	0.0139 A	0.0091	0 A	0	0.0185 A	0.0121	0.0073 A	0.0057
	2006	0 A	0	0.0071 A	0.0045	0.0173 A	0.0145	0.0074 A	0.0043	0.0007 A	0.0007	0 A	0
DEOC	Combined	0 A	0	0.0004 A	0.0004	0.0003 A	0.0003	0.0007 A	0.0007	0 A	0 A	0 A	0
	2005	0 A	0	0 A	0	0.0007 A	0.0007	0.0007 A	0.0007	0 A	0	0 A	0
	2006	0 A	0	0.0007 A	0.0007	0 A	0	0.0007 A	0.0007	0 A	0	0 A	0
HYCH	Combined	0 A	0	0 A	0	0.0002 A	0.0002	0 A	0	0 A	0	0 A	0
	2005	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	2006	0 A	0	0 A	0	0.0004 A	0.0004	0 A	0	0 A	0	0 A	0
NOVI	Combined	0 A	0	0 A	0	0.0002 A	0.0002	0.0004 A	0.0003	0.0004 A	0.0004	0.0004 A	0.0004
	2005	0 A	0	0 A	0	0.0005 A	0.0005	0.0002 A	0.0002	0 A	0	0 A	0
	2006	0 A	0	0 A	0	0 A	0	0.0006 A	0.0006	0.0007 A	0.0007	0.0007 A	0.0007
PLGL	Combined	0 A	0	0 A	0	0.0010 A	0.0010	0 A	0	0.0007 A	0.0007	0.0004 A	0.0004

Table 11 (continued).

Species[b]	Year[c]	Month											
		March		April		May		June		July		August	
		$\bar{x}$ [d,e]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
PLGL	2005	0 A	0	0 A	0	0.0013 A	0.0013	0 A	0	0.0007 A	0.0007	0.0007 A	0.0007
	2006	0 A	0	0 A	0	0.0007 A	0.0007	0 A	0	0.0007 A	0.0007	0 A	0
PSCR	Combined	0 B	0	0.0029 A	0.0009	0 B	0	0.0003 B	0.0003	0 B	0	0 B	0
	2005	0 B	0	0.0051 A	0.0020	0 B	0	0 B	0	0 B	0	0 B	0
	2006	0 A	0	0.0007 A	0.0007	0 A	0	0.0007 A	0.0007	0 A	0	0 A	0
PSMO	Combined	0 A	0	0 A	0	0 A	0	0.0001 A	0.0001	0 A	0	0 A	0
	2005	0 A	0	0 A	0	0 A	0	0.0002 A	0.0002	0 A	0	0 A	0
	2006	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
RACA	Combined	0.0015 A	0.0015	0.0018 A	0.0009	0.0027 A	0.0014	0.0105 A	0.0048	0.0216 A	0.0101	0.0141 A	0.0052
	2005	0.0030 A	0.0030	0.0027 A	0.0011	0.0046 A	0.0026	0.0057 A	0.0042	0.0153 A	0.0092	0.0133 A	0.0083
	2006	0 B	0	0.0010 B	0.0010	0.0009 B	0.0009	0.0153 B	0.0059	0.0279 A	0.0129	0.0148AB	0.0065
RACL	Combined	0 B	0	0.0016 B	0.0008	0.0098 B	0.0036	0.1938 A	0.0720	0.0583 B	0.0229	0.0354 B	0.0174
	2005	0 B	0	0.0015 B	0.0015	0.0091 B	0.0045	0.1659 A	0.0450	0.0632 B	0.0220	0.0345 B	0.0147
	2006	0 B	0	0.0017 B	0.0009	0.0106 B	0.0067	0.2216 A	0.0986	0.0535 B	0.0266	0.0364 B	0.0209
RAPA	Combined	0.0054 A	0.0040	0.0030 A	0.0019	0 A	0	0.0153 A	0.0100	0.0104 A	0.0051	0.0019 A	0.0008
	2005	0.0108 A	0.0080	0.0054 A	0.0038	0 A	0	0.0043 A	0.0043	0.0052 A	0.0029	0.0012 A	0.0010
	2006	0 A	0	0.0007 A	0.0005	0 A	0	0.0262 A	0.0159	0.0156 A	0.0074	0.0026 A	0.0014
RAUT	Combined	0.0078 A	0.0078	0.0011 A	0.0010	0.0009 A	0.0009	0.0393A	0.0257	0.0177 A	0.0101	0.0008 A	0.0008
	2005	0.0156 A	0.0156	0.0020 A	0.0020	0.0017 A	0.0017	0.0020 A	0.0017	0.0058 A	0.0027	0.0003 A	0.0003
	2006	0 A	0	0.0003 A	0.0003	0 A	0	0.0766 A	0.0498	0.0296 A	0.0183	0.0013 A	0.00130

[a]Relative abundance was mean daily capture in pitfall traps standardized by wetland size and number of days sampled per month.

Table 11. (continued)

[b]ACCR = northern cricket frog (*Acris crepitans*), AMTA = mole salamander (*Ambystoma talpoideum*), BUAM = American toad (*Bufo americanus*), BUFO = Fowler's toad (*B. fowleri*), DEOC = Ocoee salamander (*Desmognathus ocoee*), HYCH = Cope's gray treefrog (*Hyla chrysoscelis*), NOVI = eastern red-spotted newt (*Notophthalmus viridescens*), PLGL = northern slimy salamander (*Plethodon glutinosus*), PSCR = spring peeper (*Pseudacris crucifer*), PSMO = mud salamander (*Pseudotriton montanus*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

[c]Combined = data averaged across years.

[d]Means within rows followed by letters are different by repeated-measures analysis-of-variance and Tukey's HSD test.

[e]NT = no test was performed because capture = 0.

Table 12. Mean breeding call index of amphibians among months at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species	Year[b]	Month											
		March		April		May		June		July		August	
		$\bar{x}$ [c,d]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
ACCR	Combined	0 B	0	0.016 B	0.016	0.656 AB	0.198	1.094 A	0.363	0.815 AB	0.360	0.141 AB	0.073
	2005	0 B	0	0.031 B	0.031	0.788 AB	0.209	1.250 A	0.371	0.917 AB	0.417	0.219 AB	0.110
	2006	0 A	0	0 A	0	0.525 A	0.193	0.938 A	0.404	0.713 A	0.348	0.063 A	0.063
BUAM	Combined	0 B	0	0.227 A	0.060	0.075 B	0.030	0.016 B	0.016	0 B	0	0 B	0
	2005	0 A	0	0.344 A	0.100	0.125 B	0.049	0 B	0	0 B	0	0 B	0
	2006	0 A	0	0.109 A	0.093	0.025 A	0.025	0.031 A	0.031	0 A	0	0 A	0
BUFO	Combined	0 B	0	0.047 AB	0.047	0.231 AB	0.076	0.273 A	0.142	0.108 AB	0.095	0 B	0
	2005	0 A	0	0 A	0	0.075 A	0.062	0.219 A	0.145	0.104 A	0.104	0 A	0
	2006	0 B	0	0.094 AB	0.094	0.388 A	0.127	0.328 A	0.146	0.113 AB	0.088	0 B	0
GACA	Combined	0 A	0	0 A	0	0 A	0	0 A	0	0.006 A	0.006	0 A	0
	2005	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	2006	0 A	0	0 A	0	0 A	0	0 A	0	0.013 A	0.013	0 A	0
HYCH	Combined	0 B	0	0.023 B	0.023	0.350 A	0.118	0.656 A	0.218	0.602 A	0.250	0 B	0
	2005	0 B	0	0 B	0	0.063 B	0.042	0.578 A	0.193	0.667 A	0.302	0 B	0
	2006	0 B	0	0.047 B	0.047	0.638 A	0.220	0.734 A	0.290	0.538 A	0.258	0 B	0
PSCR	Combined	1.594 A	0.395	2.039 A	0.275	0.763 B	0.194	0.117 BC	0.076	0 C	0	0 C	0
	2005	1.563 A	0.513	1.797 A	0.235	0.688 B	0.188	0.188 BC	0.108	0 C	0	0 C	0
	2006	1.625 AB	0.451	2.281 A	0.336	0.838 BC	0.229	0.047 C	0.047	0 C	0	0 C	0
PSTR	Combined	0.031 A	0.031	0.047 A	0.033	0.019 A	0.019	0 A	0	0 A	0	0 A	0
	2005	0 A	0	0.063 A	0.041	0.038 A	0.038	0 A	0	0 A	0	0 A	0
	2006	0.063 A	0.063	0.031 A	0.031	0 A	0	0 A	0	0 A	0	0 A	0
RACA	Combined	0 C	0	0.008 C	0.008	0.738 B	0.121	1.384 A	0.129	1.458 A	0.156	0.667 B	0.172

Table 12 (continued).

Species[a]	Year[b]	Month											
		March		April		May		June		July		August	
		$\bar{x}$ [c,d]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
RACA	2005	0 C	0	0.016 C	0.016	0.863 B	0.153	1.360 AB	0.176	1.730 A	0.282	0.813 B	0.217
	2006	0 C	0	0 C	0	0.613 B	0.136	1.409 A	0.119	1.188 A	0.088	0.521 B	0.135
RACL	Combined	0 C	0	0.102 C	0.054	1.075 B	0.181	1.938 A	0.206	2.229 A	0.200	2.026 A	0.205
	2005	0 D	0	0.063 D	0.047	0.988 C	0.223	1.797 B	0.206	2.458 A	0.188	2.156 AB	0.173
	2006	0 C	0	0.141 C	0.069	1.163 B	0.156	2.078 A	0.231	2.000 A	0.236	1.896 AB	0.276
RAPA	Combined	0.938 B	0.220	1.383 A	0.127	0.125 C	0.034	0 C	0	0 C	0	0 C	0
	2005	0.813 A	0.249	1.172 A	0.180	0.175 B	0.075	0 B	0	0 B	0	0 B	0
	2006	1.063 B	0.333	1.594 A	0.124	0.075 C	0.053	0 C	0	0 C	0	0 C	0
RASP	Combined	0.313 AB	0.155	0.383 A	0.078	0.119 ABC	0.054	0.031 AB	0.017	0 C	0	0 C	0
	2005	0.500 AB	0.250	0.563 A	0.140	0.125 AB	0.053	0 B	0	0 B	0	0 B	0
	2006	0.125 A	0.125	0.203 A	0.078	0.113 A	0.072	0.063 A	0.033	0 A	0	0 A	0

[a]ACCR = northern cricket frog (*Acris crepitans*), BUAM = American toad (*Bufo americanus*), BUFO = Fowler's toad (*B. fowleri*), GACA = Eastern narrowmouth toad (*Gastrophryne carolinensis*), HYCH = Cope's gray treefrog (*Hyla chrysoscelis*), PSCR = spring peeper (*Pseudacris crucifer*), PSTR = upland chorus frog (*Pseudacris triseriata*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

[b]Combined = data averaged across years.

[c]Means within rows followed by letters are different by repeated-measures analysis-of-variance and Tukey's HSD test.

Table 12 (continued).

[d]NT = no test was performed because index = 0.

Table 13. Amphibian species richness and diversity among months at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Metric[a]	Year[b]	Land-use Type[c]	Month											
			March		April		May		June		July		August	
			$\bar{x}$ [d]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
RP	Combined	Access	0.38 A	0.24	0.47 A	0.08	0.53 A	0.17	1.04 A	0.44	0.67 A	0.35	0.33 A	0.13
		Non-access	0.13 C	0.13	0.26 BC	0.11	0.40 BC	0.14	1.33 A	0.22	1.57 A	0.35	1.10 AB	0.18
	2005	Access	0.75 A	0.48	0.44 A	0.14	0.69 A	0.25	0.97 A	0.34	0.78 A	0.33	0.44 A	0.25
		Non-access	0.25 B	0.25	0.39 AB	0.14	0.64 AB	0.25	1.16 AB	0.16	1.36 A	0.33	1.00 AB	0.16
	2006	Access	0 A	0	0.50 A	0.05	0.36 A	0.11	1.11 A	0.54	0.56 A	0.37	0.25 A	0.05
		Non-access	0 B	0	0.13 B	0.09	0.17 B	0.03	1.50 A	0.28	1.78 A	0.43	1.28 A	0.26
RC	Combined	Access	1.25 D	0.25	4.50 AB	0.35	6.00 A	0.84	5.13 AB	0.31	3.63 BC	0.24	2.78 DC	0.31
		Non-access	2.13 C	0.38	4.00 AB	0.20	5.25 A	0.14	3.75 B	0.32	2.88 BC	0.43	2.13 C	0.13
	2005	NI	1.75 C	0.37	4.63 AB	0.38	5.50 A	0.73	4.63 AB	0.32	3.13 BC	0.23	2.38 C	0.18
	2006	Access	1.25 C	0.48	4.50 AB	0.65	6.00 A	0.71	5.25 A	0.48	4.00 AB	0.58	2.00 BC	0.41
		Non-access	2.00 B	0.41	3.25 B	0.25	5.50 A	0.50	3.25 B	0.48	2.75 B	0.48	2.25 B	0.25
Diversity	Combined	Access	0.08 A	0.08	0.06 A	0.01	0.07 A	0.04	0.19 A	0.16	0.12 A	0.11	0.03 A	0.03
		Non-access	0 B	0	0.03 B	0.03	0.08 AB	0.05	0.18 AB	0.09	0.38 A	0.12	0.19 AB	0.06
	2005	NI	0.08 A	0.08	0.04 A	0.03	0.13 A	0.05	0.12 A	0.07	0.23 A	0.09	0.09 A	0.04
	2006	NI	0 C	0	0.04 ABC	0.01	0.03 BC	0.01	0.25 AB	0.10	0.27 A	0.11	0.13 ABC	0.06

[a]RP = species richness from pitfall traps, RC = species richness from breeding call surveys, Diversity = Shannon-Weiner Index .

[b]Combined = average results of both years combined.

Table 13 (continued).

[c]There was a significant month × land-use interaction when analyses were performed by land-use; NI = no interaction was detected.

[d]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance and Tukey's HSD test.

Table 14. Abundance of amphibian egg masses among months at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Species[a,b]	Year[c]	Month											
		March		April		May		June		July		August	
		$\bar{x}$ [d,e]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
RACA–RACL	Combined	0 A	0	0 A	0	0 A	0	0.06 A	0.06	0.94 A	0.63	0.13 A	0.08
	2005	0 A	0	0 A	0	0 A	0	0.13 A	0.13	1.25 A	0.73	0.13 A	0.13
	2006	0 A	0	0 A	0	0 A	0	0 A	0	0.63 A	0.63	0.13 A	0.13
RAPA–RASP	Combined	0 B	0	0.88 A	0.52	0.06 B	0.06	0 B	0	0 B	0	0 B	0
	2005	0 A	0	1 A	0.76	0 A	0	0 A	0	0 A	0	0 A	0
	2006	0 A	0	0.75 A	0.53	0.13 A	0.13	0 A	0	0 A	0	0 A	0
HYCH	Combined	0 A	0	0 A	0	0 A	0	0.75 A	0.68	0.31 A	0.31	0 A	0
	2005	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	2006	0 A	0	0 A	0	0 A	0	1.5 A	1.36	0.63 A	0.63	0 A	0
BUFO	Combined	0 A	0	0.06 A	0.06	0 A	0	0 A	0	0 A	0	0 A	0
	2005	0 A	0	0.13 A	0.13	0 A	0	0 A	0	0 A	0	0 A	0
	2006	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
All Species	Combined	0 A	0	0.94 A	0.50	0.06 A	0.06	0.81 A	0.67	1.25 A	0.93	0.13 A	0.08
	2005	0 A	0	1.13 A	0.86	0 A	0	0.13 A	0.13	1.25 A	0.73	0.13 A	0.13
	2006	0 A	0	0.75 A	0.53	0.13 A	0.13	1.5 A	1.36	1.25 A	1.25	0.13 A	0.13

[a]RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*), HYCH = Cope's gray treefrog (*Hyla chrysoscelis*), BUFO = American toad (*Bufo americanus*) and Fowler's toad (*B. fowleri*), All Species = all species combined.

Table 14 (continued).

[b]RACA-RACL, RAPA-RASP and BUFO groups were used because eggs of these species were indistinguishable in the field; All Species = total egg mass abundance across all species groups.

[c]Combined = data averaged across years.

[d]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance.

[e]NT = no test performed because observations = 0.

Table 15. Emergent shoreline vegetation characteristics and soil compaction among months at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Metric[a]	Year[b]	Month									
		April		May		June		July		August	
		$\bar{x}$ [c]	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Hgt (m)	Combined	03.38 C	0.08	0.52 BC	0.08	0.75 AB	0.09	0.74 AB	0.08	0.87 A	0.10
	2005	0.30 C	0.07	0.42 BC	0.09	0.71 AB	0.11	0.5 AB	0.09	0.80 A	0.11
	2006	0.45 B	0.11	0.61 AB	0.08	0.80 AB	0.09	0.84 A	0.11	0.95 A	0.12
HC	Combined	44.74 C	6.61	51.15 BC	5.98	58.36 ABC	4.21	69.54 AB	3.27	76.69 A	3.79
	2005	41.70 BC	7.15	40.11 C	5.84	52.27 ABC	6.14	62.57 AB	4.73	70.61 A	3.69
	2006	47.78 B	7.17	62.20 AB	6.72	64.45 AB	3.67	76.50 A	4.26	82.77 A	5.58
VS	Combined	19.54 C	4.21	25.86 BC	4.91	43.03 A	5.71	41.86 AB	4.96	45.93 A	5.47
	2005	16.72 B	4.62	23.91 AB	7.65	45.68 A	8.37	41.12 A	7.08	46.13 A	6.02
	2006	22.37 B	5.61	27.81 AB	3.75	40.39 AB	3.99	42.60 A	5.69	45.73 A	5.46
Richness	Combined	3.47 A	0.44	4.86 A	0.45	4.72 A	0.31	4.70 A	0.37	4.42 A	0.39
	2005	3.53 A	0.82	4.28 A	0.67	4.38 A	0.49	4.66 A	0.38	3.78 A	0.43
	2006	3.41 B	0.14	5.44 A	0.46	5.06 AB	0.58	4.75 AB	0.42	5.06 AB	0.50
SC (lbs/in^2)	2006	.	.	263.25 B	51.95	520.85 A	55.3	460.09 AB	92.01	450.90 AB	56.95

[a]Hgt = mean plant height, HC = percent horizontal cover, VS = percent vertical structure, Richness = plant species richness, SC = soil compaction.

[b]Combined = data averaged across years.

Table 15 (continued).

[c]Means within rows followed by unlike letters are different by repeated-measures analysis-of-variance and Tukey's HSD test for all tests, except soil compaction where analysis-of-covariance was used with distance from the wetland as the covariate.

Table 16. Multiple linear regression models predicting mean daily capture of postmetamorphic amphibians using various environmental co-factors of cattle land use and larval abundance at eight wetlands on the University of Tennessee Research and Education Center on the Cumberland Plateau, Crossville, Tennessee, March – August 2005.

Species[a]	Metric[b,c]	Parameter Estimates		*t*	*P*	VIF[d]	Partial R^2
		Un-standardized	Standardized				
BUAM	Intercept	0.02263	0	12.68	0.001	0	.
	Cattle	0.00040	1.20	39.49	<0.001	2.13	0.826
	NO_2	-0.39402	-0.38	-12.14	0.001	2.27	0.136
	Turbidity	-0.00019	-0.28	-9.18	0.003	2.07	0.031
	PO_4	-0.02826	-0.11	-3.57	0.038	2.27	0.006
BUFO	Intercept	0.13548	0	6.09	0.009	0	.
	NH_3	0.02891	0.89	17.50	<0.001	1.70	0.769
	NO_2	-0.22427	-0.57	-11.87	0.001	1.54	0.170
	PH	-0.02021	-0.35	-6.12	0.009	2.12	0.048
	BUFOL	0.00032	0.11	2.36	0.099	1.37	0.008
PSCR	Intercept	-0.00826	0	-1.75	0.140	0	.
	SC	-0.00002	-0.78	-4.25	0.008	1.00	0.638
	PH	0.00171	0.44	2.39	0.063	1.00	0.193
RACA	Intercept	-0.00785	0	-2.00	0.116	0	.
	RACAL	0.00074	1.08	17.49	<0.001	1.23	0.916
	HC	0.00026	0.25	4.04	0.016	1.23	0.053
	SC	-0.00004	-0.14	-2.43	0.072	1.01	0.018
RAPA	Intercept	-0.00353	0	-3.19	0.033	0	.
	RAPAL	0.00006	0.93	40.14	<0.001	1.26	0.976
	Turbidity	0.00004	0.23	6.38	0.003	3.10	0.019
	VS	0.00006	0.11	2.89	0.045	3.11	0.004
RASP	Intercept	-0.1306	0	-25.45	0.002	0	.
	NH_3	0.1569	1.34	77.08	<0.001	5.15	0.782
	PO_4	-0.0305	-0.45	-32.93	<0.001	3.16	0.169
	Hgt	0.1569	0.06	25.30	0.002	5.43	0.038
	NO_2	-0.0295	-0.16	-8.31	0.014	2.77	0.010
	PRich	0.0004	0.06	5.95	0.027	1.63	0.002

[a]BUAM = American toad (*Bufo americanus*), BUFO = Fowler's toad (*B. fowleri*), PSCR = spring peeper (*Pseudacris crucifer*), RACA = American bullfrog (*Rana castesbeiana*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

Table 16 (continued).

[b]Metrics retained by stepwise selection using entry and stay significance levels at $\alpha = 0.1$; all overall *F*-tests on final models were significant ($P \leq 0.012$) and coefficients of determination adjusted for number of variables in the model (i.e., R^2_{adj}) were 0.997, 0.990, 0.763, 0.979, 0.997, and 0.999 for BUAM, BUFO, PSCR, RACA, RAPA, and RASP, respectively.

[c]Retained water quality variables were NO_2, NH_3, PO_4, pH, turbidity, and specific conductivity (SC); Cattle = mean number of heard / ha of wetland and BUFOL, RACAL, RAPAL were BUFO, RACA, and RAPA larval abundance; vegetation variables were plant height, mm (Hgt), percent horizontal cover (HC), percent vertical structure (VS), and plant species richness (PRich).

[d]VIF = variance inflation factor where VIF >10 was suggestive of a linear dependency between ≥1 variables (Freund and Littell 2000).

Table 17. Multiple linear regression models predicting mean daily capture of postmetamorphic amphibians using various environmental co-factors of cattle land use and larval abundance at eight wetlands on the University of Tennessee Research and Education Center on the Cumberland Plateau, Crossville, Tennessee, March – August 2006.

Species[a]	Metric[b,c]	Parameter Estimates		*t*	*P*	VIF[d]	Partial R^2
		Un-standardized	Standardized				
BUAM	Intercept	-0.04016	0	-3.45	0.026	0	.
	Turbidity	0.00010	1.2961	20.16	<0.001	2.51	0.903
	NH_3	-0.00575	-0.3557	-6.79	0.002	1.67	0.071
	Temp	0.00194	0.1855	3.45	0.026	1.75	0.020
BUFO	Intercept	-0.11230	0	-3.71	0.014	0	.
	VS	-0.00099	-1.1094	-13.73	<0.001	1.28	0.854
	Temp	0.00748	0.3940	4.88	0.005	1.28	0.121
RACA	Intercept	0.03167	0	5.89	0.002	0	.
	SC	-0.00043	-1.2918	-6.66	0.001	2.19	0.771
	NO_2	0.18056	0.5609	2.89	0.034	2.19	0.143
RACL	Intercept	0.25476	0.0400	6.37	<0.001	0	.
	SC	-0.00199	0.0004	-5.22	0.002	1.00	0.819
RAPA	Intercept	0.01305	0	4.28	0.008	0	.
	RAPAL	0.00153	0.9695	11.05	<0.001	1.01	0.884
	SC	-0.00009	-0.2803	-3.20	0.024	1.01	0.078
RASP	Intercept	-0.2588	0	-1.35	0.225	0	.
	NH_3	0.07585	0.7215	2.55	0.043	1.00	0.521

[a]BUAM = American toad (*Bufo americanus*), BUFO = Fowler's toad (*B. fowleri*), RACA = American bullfrog (*Rana castesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

[b]Metrics retained by stepwise selection using entry and stay significance levels at $\alpha = 0.1$; all overall *F*-tests on final models were significant ($P \leq 0.043$) and coefficients of determination adjusted for number of variables in the model (i.e., R^2_{adj}) were 0.989, 0.964, 0.880, 0.789, 0.947, and 0.441 for BUAM, BUFO, RACA, RACL, RAPA, and RASP, respectively.

Table 17 (continued).

[c]Retained water quality variables were NO_2, NH_3, turbidity temperature (Temp), and specific conductivity (SC); RAPAL = RAPA larval abundance; vegetation variables were percent vertical structure (VS).

[d]VIF = variance inflation factor where VIF >10 was suggestive of a linear dependency between ≥1 variables (Freund and Littell 2000).

Table 18. Prevalence of histological changes in green frog (*Rana clamitans*) metamorphs[a] collected at cattle-access and non-access wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, June 2005.

Organ	Histological Change	Land-use Type	
		Access[b]	Non-access
Cutaneous	Parasitic cysts	0 A	0.05 A
Liver	Lymphoid Aggregates	0.53 A	0.75 A
Liver	Granulomas	0.05 A	0.05 A
Liver	Eosinophilic Infiltrates	0 A	0.1 A
Liver	Extramedullary hematopoiesis	0.32 A	0.15 A
Spleen	Lymphoid Depletion	0.05 A	0 A
Kidney	Tubular Epithelium Degeneration (droplets)	0.05 A	0.25 A
Kidney	Eosinophilic Infiltrates	0.26 A	0.65 B
Kidney	Extramedullary Hematopoiesis	0.63 A	0.75 A
Kidney	Myxosporidia	0.32 A	0.25 A
Kidney	Parasitic cysts	0.21 A	0.15 A
Pancreas	Vacuolation	0.05 A	0 A
Small Intestine	Inflammatory cells	0.79 A	0.85 A
Large Intestine	Inflammatory cells	0.79 A	0.85 A
Skeletal Muscle	Parasitic cysts	0.11 A	0.1 A
Skeletal Muscle	Ichthyophonus	0.05 A	0.1 A
Lungs	Granulomas	0.05 A	0 A
Lungs	Parasitic cysts	0.05 A	0 A
Cloacal	Parasites in lumen	0.05 A	0 A
Fat	Steatitis	0.05 A	0.05 A

[a]Total sample size was $n = 40$ metamorphs; access $n = 19$ and non-access $n = 21$.

[b]Proportions within rows followed by unlike letters are different ($P < 0.02$) by *Z*-tests for liver granulomas, kidney eosinophilic infiltrates, kidney extramedullary hematopoiesis, and kidney myxosporidia; Fisher's Exact test was used on all other tests (i.e., expected frequency was <5).

Table 19. Prevalence of bacteria isolates associated with green frog (*Rana clamitans*) metamorphs[a] collected at cattle-access and non-access wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, June 2005.

Bacteria[b]	Respiration Type	Land-use Type	
		Access[c]	Non-access
Achromobacterxylosoxidans	Aerobic	0.05 A	0.05 A
Acinetobacter spp. baumannii	Aerobic	0 A	0.10 A
Acinetobacter lwoffi	Aerobic	0.05 A	0 A
Aeromonas hydrophila	Facultatively anaerobic	0.32 A	0.15 A
Chyseobacterium meningosepticum	Aerobic	0 A	0.15 A
Delftia acidovorans	Aerobic	0.11 A	0 A
Enterobacter amnigenus	Facultatively anaerobic	0 A	0.15 A
Hafnia alvei	Facultatively anaerobic	0.11 A	0 A
Ochrobactrum anthropi	Aerobic	0.05 A	0 A
Pantoea agglomerans	Facultatively anaerobic	0 A	0.05 A
Pseudomonas spp.	Aerobic	0.21 A	0.05 A
Ralstonia pickettii	Aerobic	0 A	0.05 A
Yokenella regensburgei	Aerobic	0 A	0.05 A

[a]Total sample size was $n = 40$ metamorphs; access $n = 19$ and non-access $n = 21$.

[b]Bacteria were isolated from abdominal swabs and pooled organs.

[c]Proportions with rows followed by unlike letters are different by Fisher's Exact test.

Table 20. Prevalence of parasites in tissues from green frog (*Rana clamitans*) metamorphs[a] collected at cattle-access and non-access wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, June 2005.

	Land-use Type	
Taxa[b]	Access[c]	Non-access
Parasite	10.53 A	0 A
Cestode	26.32 A	25.0 A
Ichthyophonus	5.26 A	10.0 A
Trematode	5.26 A	0 A

[a]Total sample size was $n = 40$ metamorphs; access $n = 19$ and non-access $n = 21$.

[b]Parasite = parasites that were unable to be identified to taxa because only remnants remained.

[c]Proportions within rows followed by unlike letters are different by Fisher's Exact test.

Table 21. Parasitic load in feces from green frog (*Rana clamitans*) metamorphs[a] collected at cattle-access and non-access wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, June 2005.

Taxa	Land-use Type			
	Access		Non-access	
	$\bar{x}$ [b]	SE	$\bar{x}$	SE
Amoeba	0.05 A	0.23	0 A	0
Nematode	0.05 A	0.23	0.05 A	0.22
Protozoan	0 A	0	0.05 A	0.22

[a]Total sample size was $n = 40$ metamorphs; access $n = 19$ and non-access $n = 21$.

[b]Mean fecal loads within rows with unlike letters are different by Wilcoxon two-sample test (i.e., normality was violated; Shapiro-Wilk test).

Table 22. Prevalence of malformation types in malformed amphibians[a,b] captured in pitfall traps between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Malformation Type[c]	Land-use Type	
	Access[d]	Non-Access
Amelia	0.11 A	0.04 A
Anophthalmia	0.11 A	0.04 A
Brachydactyly	0.17 A	0 B
Ectrodactyly	0 A	0.14 A
Ectromelia	0 A	0.14 A
Hemimelia	0.06 A	0 A
Iris Abnormal	0 A	0.11 A
Micrognathia	0 A	0.07 A
Microphthalmia	0.06 A	0.18 A
Polydactyly	0 A	0.04 A
Polymelia	0 A	0.04 A
Miscellaneous	0.28 A	0.14 A
Injury	0.17 A	0 B

[a]Total sample size was $n = 36$ malformed individuals; access $n = 15$ and non-access $n = 21$.

[b]Species collected were American bullfrogs (*Rana catesbeiana*), American toads (*Bufo americanus*), Fowler's toads (*Bufo fowleri*), green frogs (*Rana clamitans*), pickerel frogs (*R. palustris*), and southern leopard frogs (*R. sphenocephala*).

[c]Malformations were classified as per the USGS Field Guide to Malformations of Frogs and Toads (Meteyer 2000); miscellaneous malformations were lack of thigh or calf muscles, immobile joints, bone projections and missing webbing between hind digits.

[d]Proportions within rows followed by unlike letters are different by Fisher's Exact test.

Table 23. Bacteria cultured from five injured amphibians opportunistically collected from cattle-access and non-access wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006.

Bacteria[a]	Respiration Type	Specimens[b,c,d]				
		Access				Non-access
		BUAM 1	BUAM 2	RACL 1	RASP 1	BUAM 3
Aeromonas hydrophila	Facultatively anaerobic			X		
Brevibacterium spp.*	Aerobic	⊗				
*Chryseobacterium indologenes**	Aerobic	X				X
Chyseobacterium meningosepticum	Aerobic	X				
*Citrobacter freundii**	Aerobic				X	
*Clostridium perfringens Type A**	Anaerobic					X
*Corynebacterium species**	Aerobic	X				
Delftia acidovorans	Aerobic	⊗				
*Empedobacter brevis**	Aerobic	X				
*Escherichia coli**	Facultatively anaerobic	X				
Hafnia alvei	Facultatively anaerobic	X				
*Leifsonia aquatica**	Aerobic					X
*Moraxella osloensis**	Aerobic	X				
*Morganella morganii**	Aerobic	X				
Pantoea agglomerans	Facultatively anaerobic					
*Pseudomonas fluorenscens**	Aerobic	X	X			
*Pseudomonas mendocina**	Aerobic	X				
*Pseudomonas stutzeri**	Aerobic	X	X			
*Psychrobacter phenylpyruvica**	Aerobic	X				
*Ralstonia pickettii**	Aerobic	X				
*Shewanella putrefaciens**	Anaerobic	X		X		
*Staphylococcus epidermidis**	Aerobic					X
*Stenotrophomonas maltophilia**	Aerobic	X				
Yokenella regensburgei	Aerobic				X	

[a]Bacteria marked with an "*" were not previously found in pathogen sampling of green frog metamorphs in June 2005 (Table 19).

Table 23 (continued).

[b]BUAM = American toad (*Bufo americanus*), RACL = green frog (*R. clamitans*), and RASP = southern leopard frog (*R. sphenocephala*); BUAM 1, BUAM 2, RACL 1, and RASP 1 were collected at cattle-access wetlands and BUAM 3 was collected at a non-access wetland.

[c]BUAM 1 had facial ecchymosis, BUAM 2 had a swollen midshaft right rear distal limb, BUAM 3 had multiple dermal lesions, RACL 1 had immobile joints in the right rear limb, RASP 1 had 2 swellings on the right rear limb.

[d]⊗ = Bacteria was isolated from cutaneous lesions and either internal organs or abdominal swab.

Table 24. Multiple linear regression models predicting total abundance of postmetamorphic amphibians using landscape[a] metrics of a 1-km buffer surrounding each of eight wetlands on the University of Tennessee Research and Education Center on the Cumberland Plateau, Crossville, Tennessee, March – August 2005 and 2006.

Species[b,c]	Metric[d,e]	Estimates		*t*	*P*	VIF[f]	Partial R^2
		Un-standardized	Standardized				
BUFO	Intercept	506.20	0	4.83	0.005	0	.
	ME	-50.57	-0.98	-4.52	0.006	1.20	0.595
	WNN	-0.24	-0.50	-2.33	0.068	1.20	0.210
RACA	Intercept	-122.09	0	-2.83	0.030	0	0
	PLC	70.48	0.81	3.33	0.016	1.00	0.649
RACL	Intercept	-2250.72	0	-8.73	0.003	0	.
	WNN	-1.27	-1.12	-20.70	<0.001	1.35	0.608
	IJI	26.71	0.75	12.82	0.001	1.59	0.256
	MNN	5.21	0.30	5.46	0.012	1.42	0.082
	WSI	89.54	0.24	4.69	0.018	1.18	0.048
RAPA	Intercept	-339.27	0	-16.87	0.004	0	.
	MNN	1.34	0.61	14.86	0.005	2.09	0.607
	PLC	47.60	0.66	18.42	0.003	1.61	0.284
	NW	-3.64	-0.60	-9.29	0.011	5.30	0.065
	ME	5.26	0.33	7.13	0.019	2.71	0.035
	WA	8.33	0.17	2.97	0.097	4.03	0.007
RASP	Intercept	-1005.78	0	-2.58	0.049	0	.
	MNN	5.08	0.59	2.54	0.052	1.08	0.529
	WA	95.11	0.49	2.13	0.087	1.08	0.224

[a]Landscapes (n = 8) were plots extending 1-km from the perimeter of each study wetland.

[b]BUFO = American toad (*Bufo americanus*) and Fowler's toad (*B. fowleri*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

Table 24 (continued).

[c]American toads (*Bufo americanus*) and Fowler's toad (*B. fowleri*) were combined under the species BUFO because metamorphs of these two species were indistinguishable in the field.

[d]Landscape metrics retained by stepwise selection process using entry and stay significance levels at $\alpha = 0.1$; all overall *F*-tests on final models were significant ($P \leq 0.030$) and coefficients of determination adjusted for number of variables in the model (i.e., R^2_{adj})= 0.728, 0.590, 0.985, 0.994, 0.654 for BUFO, RACA, RACL, RAPA, and RASP, respectively.

[e]ME = Mean number of edges to cross from the study wetland to surrounding wetlands, WNN = nearest-neighbor distance from study wetland to surrounding, PLC = percent land cover of wetlands, IJI = interspersion/juxtaposition index of wetlands, MNN = mean nearest-neighbor distance from each wetland to all others, NW = number of wetlands, WA = area of wetland (ha).

[f]VIF = variance inflation factor where VIF >10 is suggestive of a linear dependency between ≥1 variable (Freund and Littell 2000).

Table 25. Multiple linear regression models predicting total abundance of postmetamorphic amphibians using landscape[a] metrics of a 0.5-km buffer surrounding each of eight wetlands on the University of Tennessee Research and Education Center on the Cumberland Plateau, Crossville, Tennessee, March – August 2005 and 2006.

Species[b,c]	Metric[d,e]	Estimates		*t*	*P*	VIF[f]	Partial R^2
		Un-standardized	Standardized				
BUFO	Intercept	257.00	0	2.90	0.027	0	.
	LSI	-24.42	-0.72	-2.57	0.042	1.00	0.524
RACA	Intercept	289.80	0	11.56	<0.001	0	.
	ME	16.15	1.08	14.66	<0.001	2.83	0.683
	PLC	-9.28	-1.10	-15.51	<0.001	2.59	0.293
	SEI	144.20	0.21	2.90	0.044	2.64	0.016
RACL	Intercept	-1854.68	0	-2.37	0.064	0	.
	WNN	-1.66	-1.47	-4.71	0.005	3.04	0.608
	PLC	45.39	0.84	2.70	0.043	3.04	0.233
RAPA	Intercept	342.32	0	4.42	0.005	0	.
	SEI	-495.66	-0.87	-4.25	0.005	1.00	0.751
RASP	Intercept	892.17	0	4.19	0.009	0	.
	SEI	3.24	-0.78	-6.72	0.001	1.07	0.783
	IJI	-1749.39	0.40	3.47	0.018	1.07	0.153

[a]Landscapes (n = 8) were plots extending 0.5-km from the perimeter of each study wetland.

[b]BUFO = American toad (*Bufo americanus*) and Fowler's toad (*B. fowleri*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*).

[c]American toads (*Bufo americanus*) and Fowler's toad (*B. fowleri*) were combined under the species BUFO because metamorphs of these two species were indistinguishable in the field.

Table 25 (continued).

[d]Landscape metrics retained by stepwise selection process using entry and stay significance levels at $\alpha = 0.1$; all overall *F*-tests on final models were significant ($P \leq 0.042$) and coefficients of determination adjusted for number of variables in the model (i.e., R^2_{adj})= 0.445, 0.987, 0.776, 0.710, 0.911 for BUFO, RACA, RACL, RAPA, and RASP, respectively.

[e]LSI = landscape shape index, ME = mean number of edges to cross from the study wetland to surrounding wetlands, PLC = percent land cover of wetlands, SEI = Shannon evenness index of landcover, WNN = nearest-neighbor distance from study wetland to surrounding wetlands, WA = area of wetland (ha), IJI = interspersion/juxtaposition index of wetlands.

[f]VIF = variance inflation factor where VIF >10 is suggestive of a linear dependency between ≥1 variable (Freund and Littell 2000).

Figure 1. Cattle access (1 – 4) and non-access (5 – 8) wetlands at the University of Tennessee Plateau Research and Education Center, Cumberland County, Tennessee, USA, 2004.

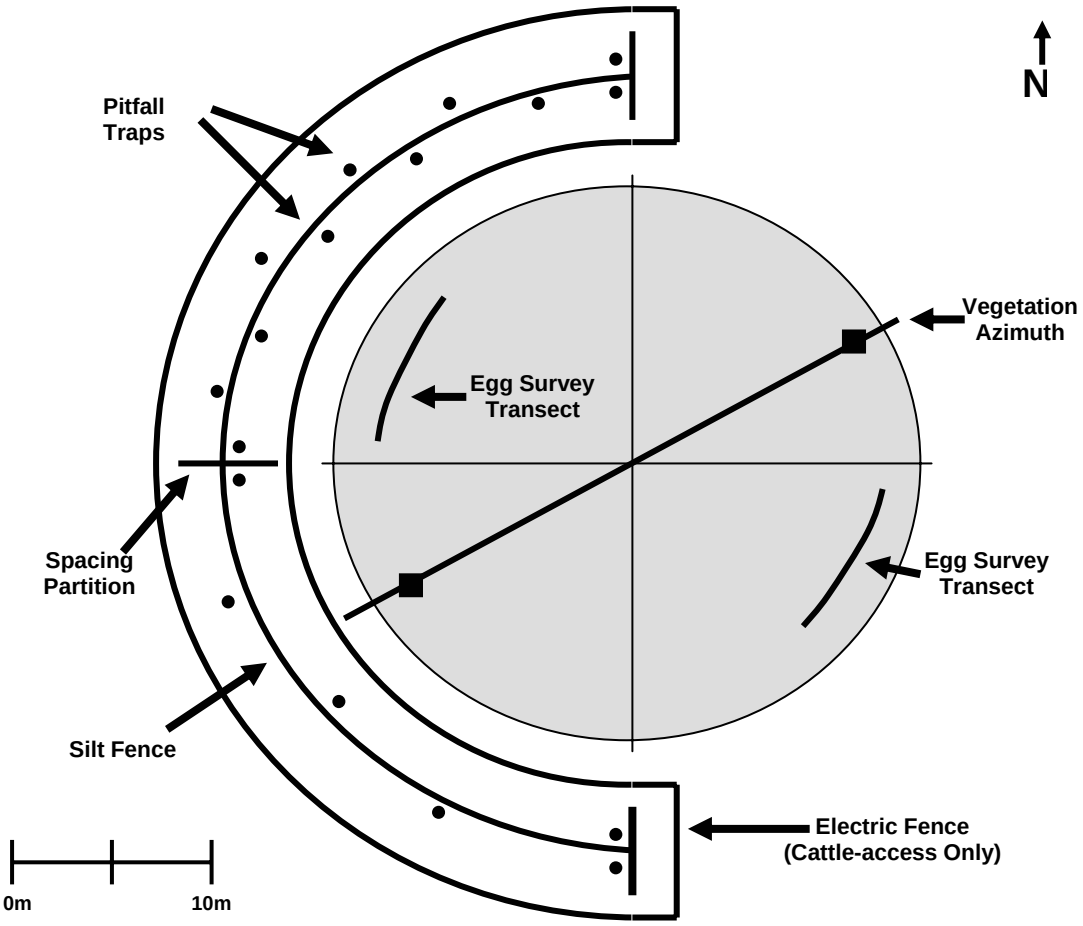
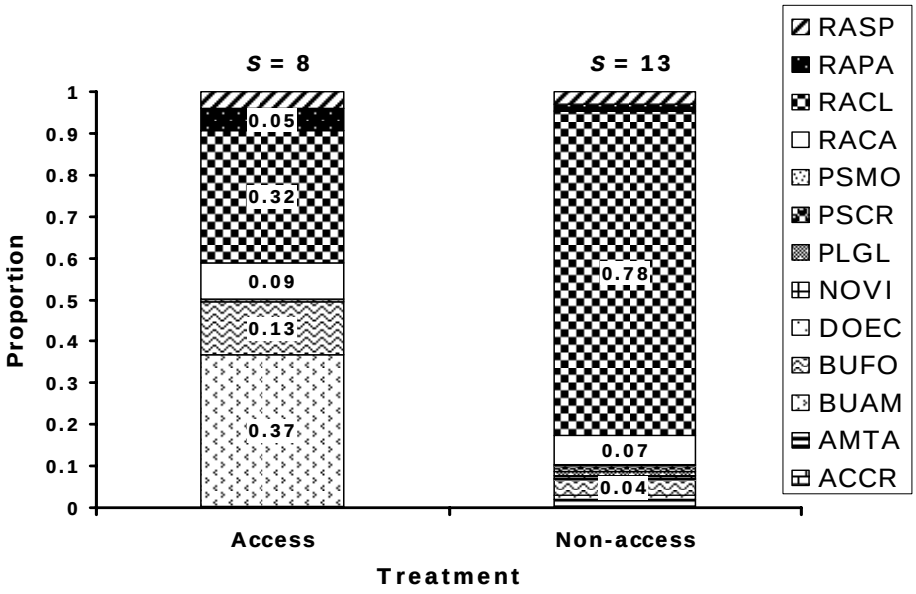


Figure 2. Schematic of postmetamorphic amphibian sampling at study wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, 2005 – 2006.

a)



b)

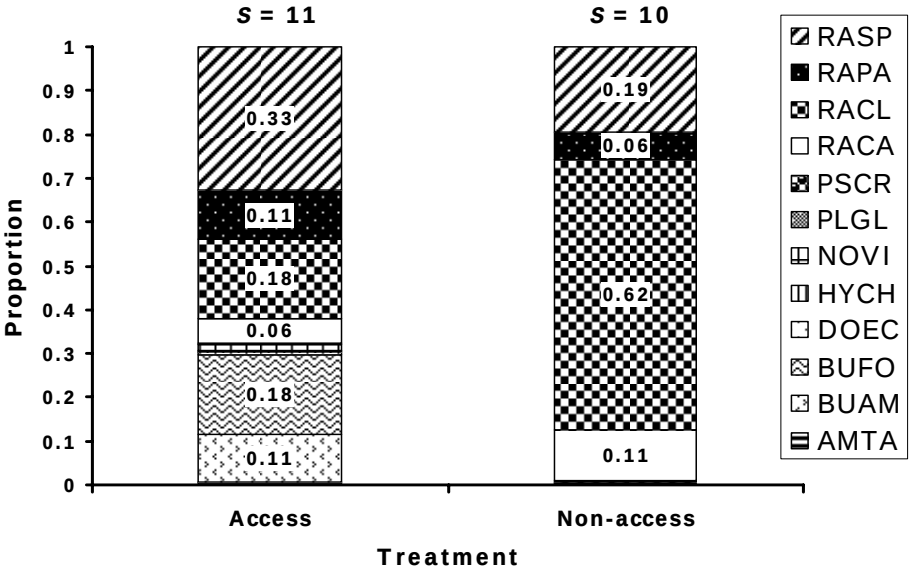
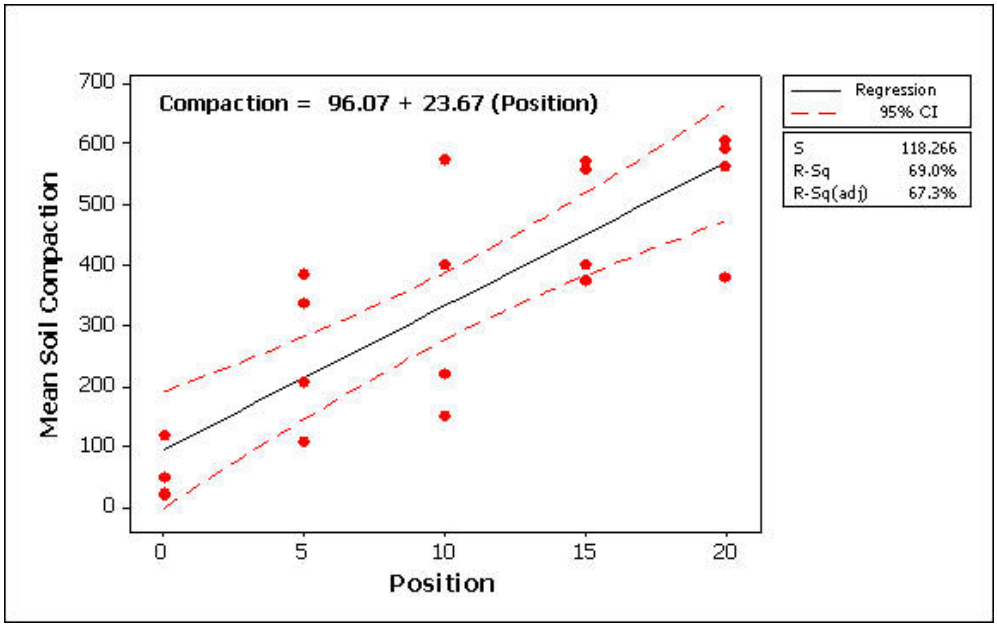


Figure 3. Species composition and total richness (*S*) of amphibians captured in pitfalls between cattle land uses at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 (a) and 2006 (b).

a)



b)

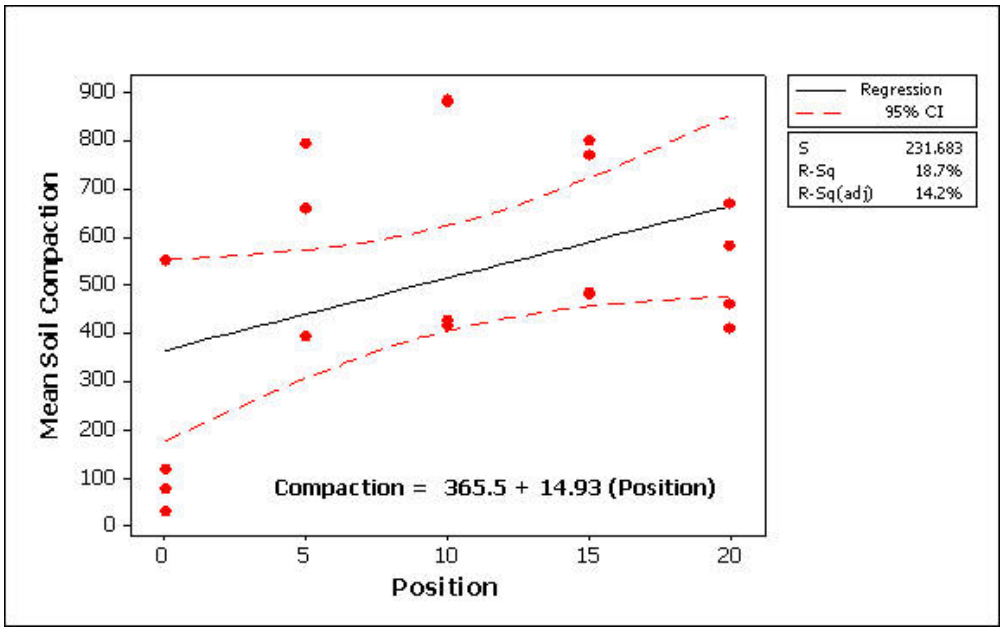


Figure 4. Relationship between mean soil compaction and position (i.e., distance, m) that measurements were taken from the shoreline at four non-access (a) and four cattle-access

Figure 4 (continued).

(b) wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2006.

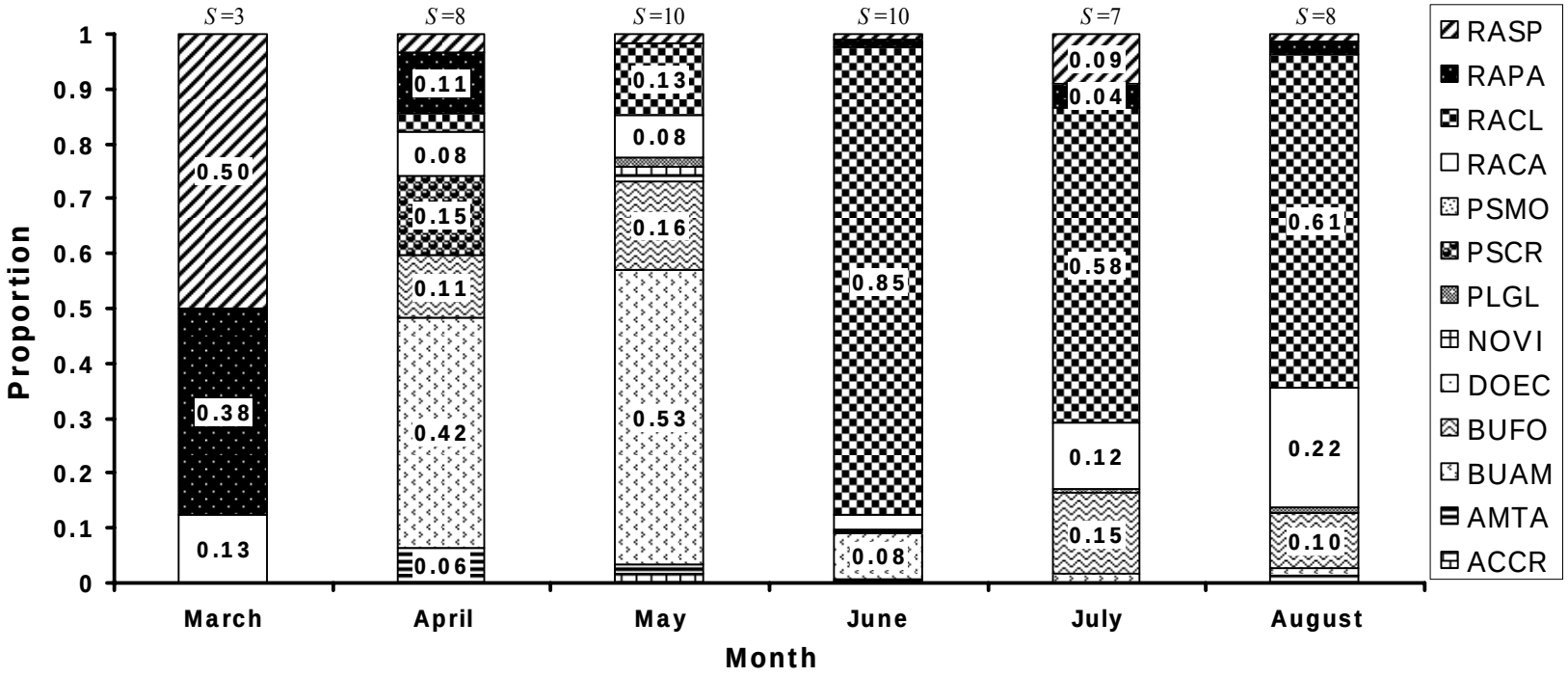


Figure 5. Species composition and total richness (S) of amphibians captured in pitfalls among months at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005.

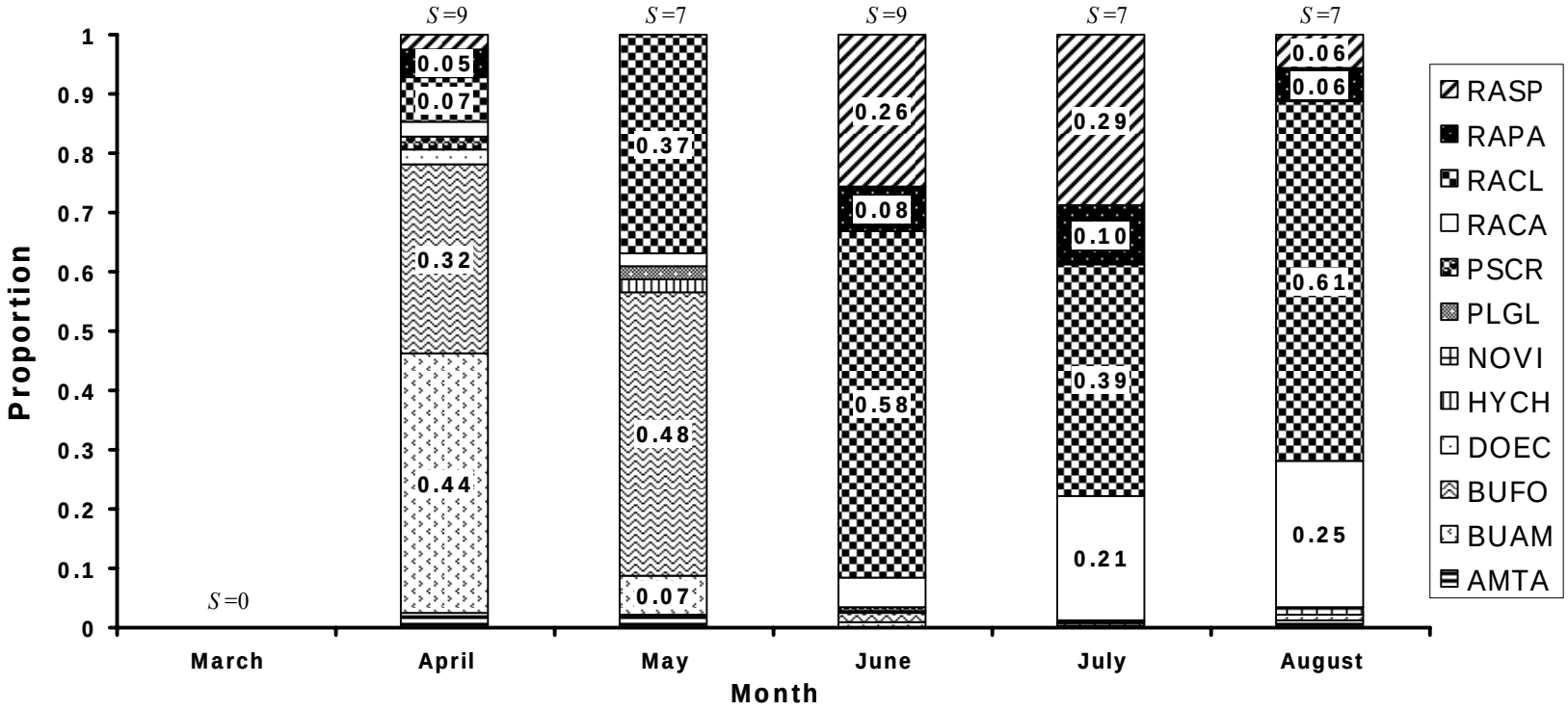


Figure 6. Species composition and total richness (S) of amphibians captured in pitfalls among months at eight wetlands on the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee March – August 2006.

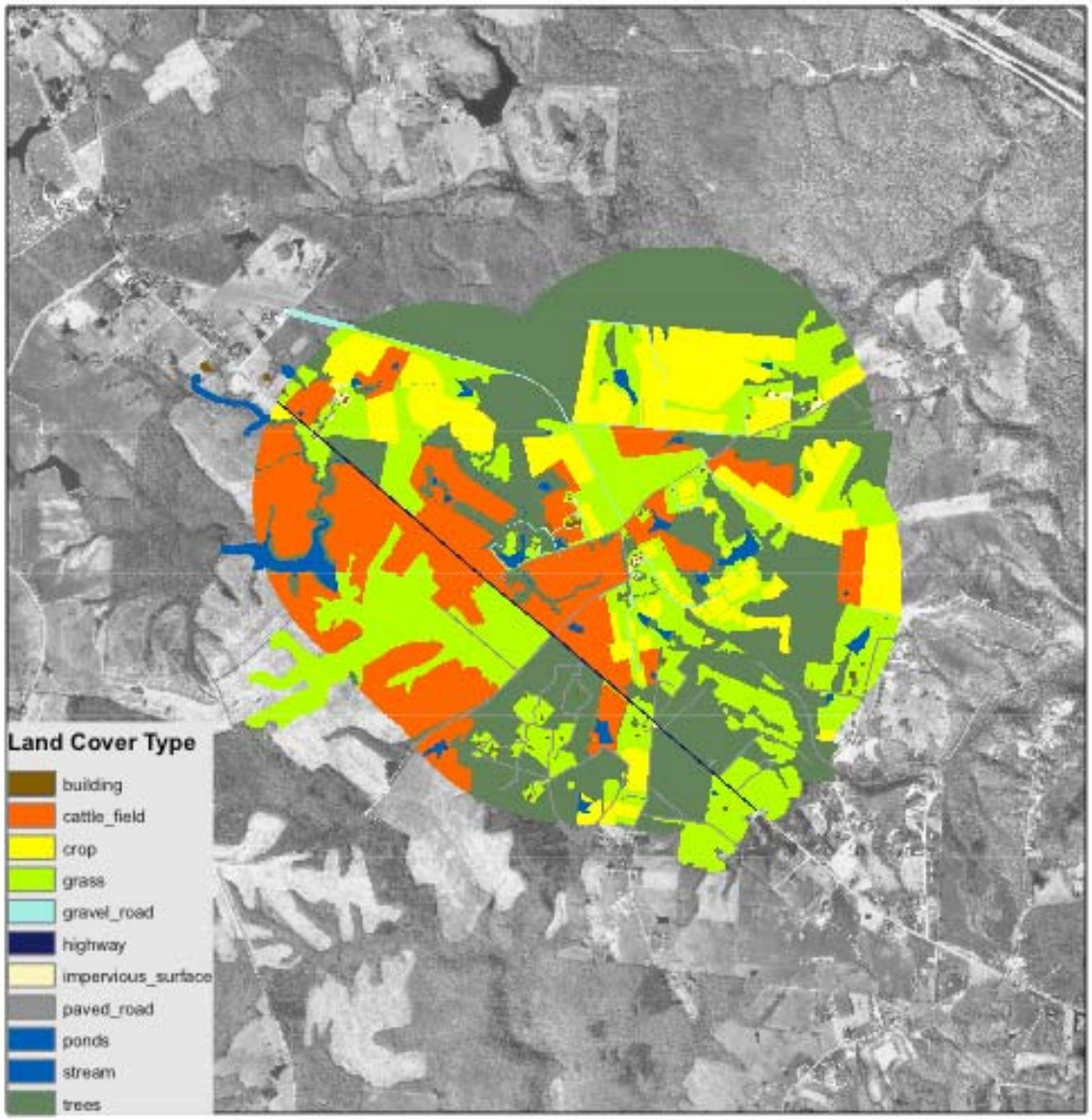


Figure 7. Rasterized land cover types of the landscape extent used in amphibian analyses overlaid on the digital orthophoto quadrangle for Cumberland County, Tennessee, 2004.

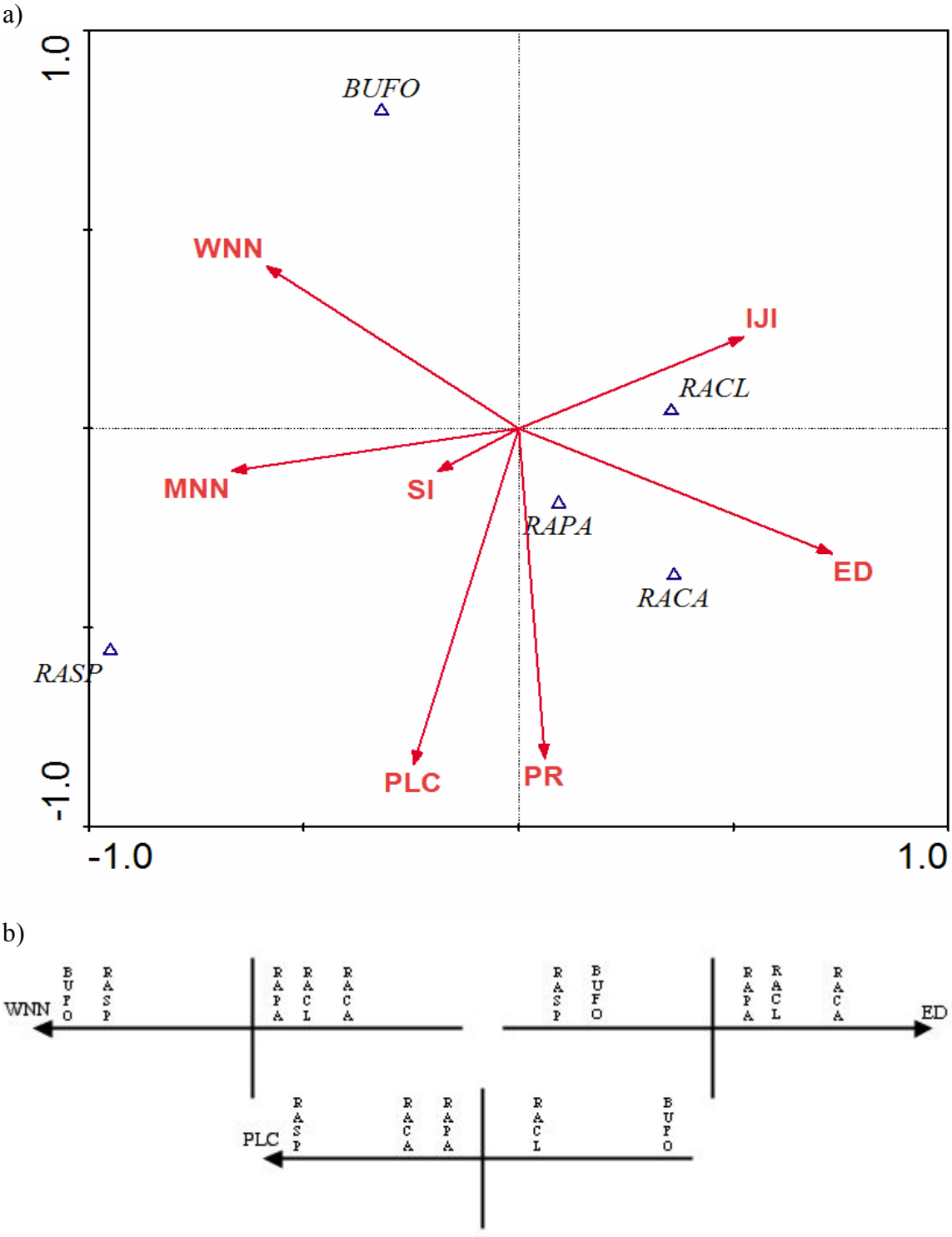
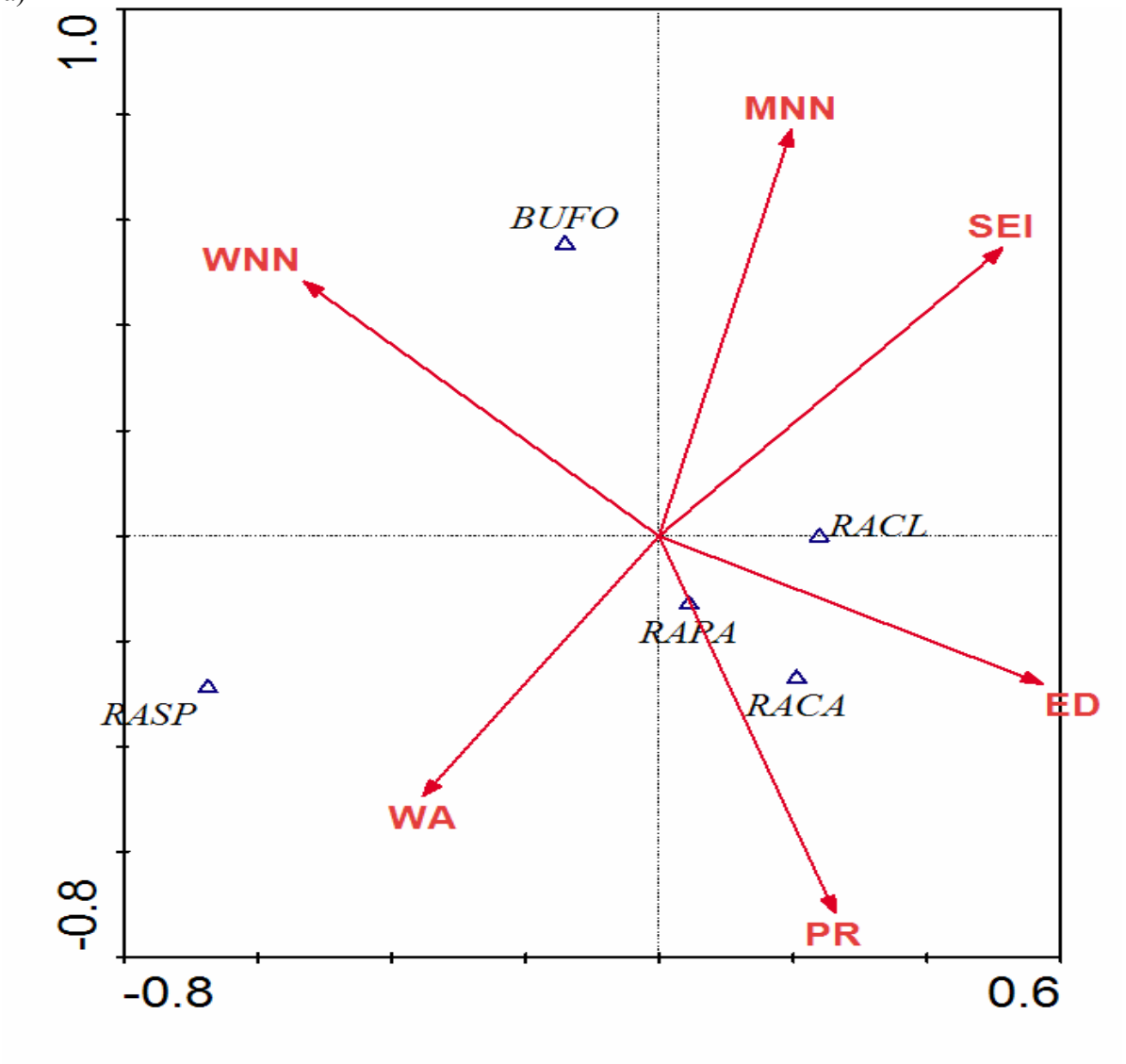


Figure 8. Canonical correspondence analysis of relative amphibian abundance (natural-log transformed) and landscape metrics of a 1-km landscape around each of eight

Figure 8 (continued).

wetlands at the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006. (a) Species-environmental biplot where the length of eigenvectors represents the strength of the correlation between each landscape variable and the pattern of amphibian community composition; species closest to an eigenvector are most strongly associated with the corresponding landscape metric; ED = edge density, IJI = interspersion/juxtaposition index of wetlands, MNN = mean nearest-neighbor distance from each wetland to all others, PLC = percent land cover of wetlands, PR = patch richness, SI = wetland shape index, WNN = nearest-neighbor distance from study wetland to surrounding wetlands; Amphibian species were: BUFO = American toad (*Bufo americanus*) and Fowler's toad (*B. fowleri*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*). (b) Inferred ranking of species with significant landscape variables based on interpretation from biplots (see Figure 7 part a); the ranking was determined after extending the end of each eigenvector through the origin of the biplot and drawing intersecting orthogonal lines from each species to the eigenvector; the vertical segment bisecting each inferred ranking represents the origin of the biplot; species closer to the arrowhead end of the eigenvector are more positively correlated to with that landscape metric; conversely, species closer to the blunt end of the eigenvector are more negatively related with the landscape metric.

a)



b)

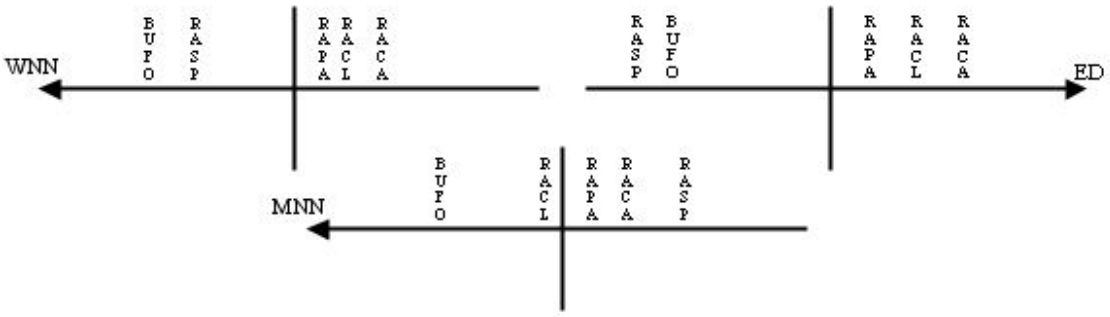


Figure 9. Canonical correspondence analysis of relative amphibian abundance (natural-log transformed) and landscape metrics of a 0.5-km buffer around each of eight wetlands

Figure 9 (continued).

at the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, March – August 2005 and 2006. (a) Species-environmental biplot where the length of eigenvectors represents the strength of the correlation between each landscape variable and the pattern of amphibian community composition; species closest to an eigenvector are most strongly associated with the corresponding landscape metric; ED = edge density, IJI = interspersion/juxtaposition index of wetlands, MNN = mean nearest-neighbor distance from each wetland to all others, PR = patch richness, SEI = Shannon evenness index of landcover, WA = area of wetland (ha), WNN = nearest-neighbor distance from study wetland to surrounding wetlands; Amphibian species were: BUFO = American toad (*Bufo americanus*) and Fowler's toad (*B. fowleri*), RACA = American bullfrog (*Rana catesbeiana*), RACL = green frog (*R. clamitans*), RAPA = pickerel frog (*R. palustris*), RASP = southern leopard frog (*R. sphenocephala*). (b) Inferred ranking of species with significant landscape variables based on interpretation from biplots (see Figure 8 part a); the ranking was determined after extending the end of each eigenvector through the origin of the biplot and drawing intersecting orthogonal lines from each species to the eigenvector; the vertical segment bisecting each inferred ranking represents the origin of the biplot; species closer to the arrowhead end of the eigenvector are more positively correlated to with that landscape metric; conversely, species closer to the blunt end of the eigenvector are more negatively related with the landscape metric.

APPENDIX II

PATHOGEN TESTING PROCEDURES

Bacterial, viral, and parasitic testing procedures. All sections below were written under the guidance of Dr. Debra Miller.

Histology.—These analyses were performed by Dr. Debra Miller and the UGA VDIL staff. Formalin-fixed tissues were routinely processed and embedded in paraffin blocks. One or more 5 μm sections were cut from each block and placed on glass slides. The slides were stained with hemotoxylin and eosin, Gram, and Kinyoun's acid-fast, and examined using light microscopy for evidence of histological changes suggestive of disease (Prophet et al. 1994).

Bacterial cultures.—These analyses were performed by Dr. Sreekumari Rajeev, Cindy Watson and Jill Johnson of UGA VDIL. Bacterial culture and identifications were performed using standard operating protocols outlined in Isenberg (1998), Murray et al. (2003) and Quinn et al. (1994). Sections of internal organs (≤1 cm in diameter) were pooled and homogenized to test for all bacterial pathogens. For isolation of aerobic bacteria, the samples were inoculated onto Tryptic Soy Agar with 5% sheep blood (Remel Inc., Lenexa, Kansas, USA) and incubated at 29°C for 18 – 24 hrs. Colonies of target species were subcultured to obtain a pure culture and identified using light microscopy. Primary inoculation plates were maintained for at least 48 – 72 hrs for possible detection of slower growing bacteria.

For isolation of anaerobic bacteria, the samples were inoculated onto Phenylethyl Alcohol Agar with 5% sheep blood. Inoculated plates were incubated at 37ºC in a Forma Scientific 1024 Anaerobic System (Thermo Fisher Inc., Waltham, Massachusetts, USA). The cultures were maintained for 5 days and observed each day for the presence of any bacteria.

For detection and isolation of *Salmonella* spp., the samples were inoculated onto Hektoen Enteric Agar (HE, Remel Inc., Lenexa, Kansas, USA) and into Tetrathionate broth (Remel Inc., Lenexa, Kansas, USA). Inoculated HE plates were incubated in an aerobic incubator at 29°C, and after 18 – 24 hrs they were examined for the presence of *Salmonella* spp. colonies. Inoculated Tetrathionate broth also was subcultured onto additional HE plates, incubated under the same conditions, and checked for *Salmonella* colonies after 18 – 24 hrs. Suspect colonies were subcultured onto Tryptic Soy Agar with 5% sheep blood to obtain a pure culture for identification using light microscopy.

Additionally, tissues were tested for *Listeria monocytogenes*. Samples were inoculated onto Polymyxin B-acrivlavine-lithium chloride-ceftazidime-esculin-mannitol (PALCAM) agar (The Oxoid group, Basingstoke, Hampshire, United Kingdom), which is a selective media for *Listeria* spp. Cultures were incubated in a CO_2 incubator at 29^{o}C for 24 – 48 hours then examined for *Listeria* colonies. Any suspect colonies were subcultured onto Tryptic Soy Agar with 5% sheep blood and identified. All isolates were speciated either by using an automated bacterial identification system (Sensititer, Trek Diagnostic Systems, Westlake, Ohio, USA) or conventional biochemical testing including RapID systems (Remel, Inc., Lenexa, Kansas, USA) and API systems (BioMerieux., Inc., Durham, North Carolina, USA).

Pooled tissue samples also were cultured to determine the presence of *Leptospira* spp. The samples were serially diluted in bovine serum albumin buffer (Fisher Scientific International, Inc., Pittsburgh, Pennsylvania, USA) and inoculated into Ellinghausen-McCullough-Johnson-Harris (EMJH) semisolid media containing fluorouracil as a decontaminant (Bectron, Dickinson & Company, Franklin Lakes, New Jersey, USA).

The cultures were incubated at 29°C for 8 weeks. The tubes were monitored throughout the incubation period for the presence of a "dinger zone," which commonly occurs with *Leptospira* spp. The dinger zone can be described as a discrete band that develops ca. 3 mm below the surface of the medium (Murray et al. 2003). Colonies associated with dinger zones were inspected using darkfield microscopy for *Leptospira* spp.

To detect *Mycobacterium paratuberculosis*, the intestinal tissue of each metamorph was mixed with 35 ml of water, and 5 ml of the resulting supernatant was transferred to a 0.9% hexadecylpyridinium chloride (HPC) solution (Sigma–Aldrich, Atlanta, Georgia, USA) prepared with half strength (i.e. diluted in distilled water) brain-heart infusion (BHI) broth (Bectron, Dickinson & Company, Franklin Lakes, New Jersey, USA). After overnight incubation and centrifugation at 3000 x g, samples were transferred to an antibiotic solution prepared with BHI. After overnight incubation, samples were inoculated into ESP *para*-JEM broth (Trek Diagnostics, Westlake, Ohio, USA) containing supplements as recommended by the manufacturer, then placed in the ESP Culture System II instrument (Trek Diagnostics, Westlake, Ohio, USA). Samples were incubated until the instrument detected gas production by any bacteria able to survive the growth media, indicating a positive signal, or until 42 days had passed. All signal positive samples were acid-fast stained and confirmed for the pathogen using PCR as per UGA Tifton Veterinary Diagnostic and Investigational Laboratory standard operating procedure for *Mycobacterium paratuberculosis*. Similarly, all signal negative samples were acid-fast stained after 42 days of incubation, and all acid-fast positive samples were confirmed by PCR.

Virus isolation.—These analyses were performed by Dr. Charles Baldwin and the UGA VDIL staff. The subset of lung, kidney, spleen, brain, skin, skeletal muscle, heart, intestines, stomach and liver samples that were frozen for virus isolation were used to make a 10% tissue homogenate in minimal essential medium (MEM) containing 1% gentamycin (Sigma–Aldrich, Atlanta, Georgia, USA). The homogenate was centrifuged at 2000 x g for 15 minutes at 4°C. The supernatant was collected and filtered through a 0.2 μ filter (Fisher Scientific, Pittsburgh, Pennsylvania, USA) directly onto confluent monolayers of a variety of cell lines, including fathead minnow (FHM), white sturgeon skin (WSSK), Chinese catfish ovary (CCO), and epithelioma papilloma cyprini cells (EPC). Inoculated cultures were incubated at 22.5°C, and examined microscopically daily for two weeks for viral cytopathic effect (CPE). At the end of the two weeks, material from the first inoculation was transferred to a second confluent monolayer of cells and examined daily for an additional 2 weeks. Cultures that did not demonstrate CPE at the end of the 4-week period were deemed negative. Cultures showing CPE were harvested and amplified further by inoculating small tissue culture flasks (25 cm^2) containing MEM. Random isolates were verified by electron microscopy (procedures below).

Electron microscopy of fecal samples.—These analyses were performed by Dr. Eloise Styer of the UGA VDIL. Fecal samples were examined using negative stain electron microscopy. Samples were diluted with distilled water to make a 15 – 20% suspension. The suspension was subjected to two cycles of freezing and thawing with liquid nitrogen. Each freeze-thaw cycle was followed by homogenization. The suspension was centrifuged for 8 minutes at 12,000 x g, and the resulting supernatant was

centrifuged for 30 minutes at 23,000 x g. The 23,000 x g pellet was resuspended and diluted in distilled water until a 25 µl drop was lightly opalescent. A drop of the diluted pellet was mixed with an equal volume of 1.5% phosphotungstic acid (pH = 6.8) and placed on Formvar-coated 400 mesh grids (SPI supplies, West Chester, Pennsylvania, USA). Any excess liquid was removed with filter paper, and the grids were allowed to air dry briefly (<5 minutes). The grids were examined for any viruses or virus-like particles using a Zeiss EM 900 TEM (Carl Zeiss SMT, Inc., Thornwood, New York, USA) at a magnification of 12,000X or greater.

PCR for Cryptosporidium spp.—These analyses were performed by Dr. Debra Miller and Lisa Whittington of the UGA VDIL. Paraffin-embedded, fresh tissues or fecal samples were used for isolation of *Cryptosoridium* spp. If fresh tissues were used, approximately 1 ml of fresh tissue homogenate was centrifuged to a pellet. The pellet was processed using the QIAamp DNA mini kit (QIAGEN, Valencia, California, USA) according to manufacturer specifications, except that 100 µl rather than 200 µl of Buffer AE (elution buffer) was used in the final step to minimize dilution of the DNA.

Paraffin-embedded tissues were used for supportive documentation of *Cryptosporidium* spp. Genomic DNA was extracted from these samples following the protocol of Kattenbelt et al. (2000), which are briefly outlined hereafter. Five to ten 10-µm serial sections were taken from blocks, placed in a microcentrifuge tube, and xylene (1.5 ml) added. The sample was vortexed and incubated for 15 minutes at room temperature, then centrifuged for 5 minutes and the xylene decanted. Twice, 1.5 ml of 100% ethanol was added, and the solution vortexed, centrifuged and the liquid decanted. Finally, 1.5 ml of 95% ethanol was added, and the sample vortexed, centrifuged, and

liquid decanted. The residual alcohol was allowed to evaporate by placing the tube in a 37°C incubator for 15 minutes. Sterile water (250 µl) was added to the sample, and it was subjected to five replications of a freeze-thaw procedure (i.e., 5 min liquid nitrogen, 5 min boiling water). DNA was extracted from the sample using the QIAmp DNA Mini Kit. Feces were subjected to the five replications of 5-minute freeze-thaw procedure with liquid nitrogen and boiling water. After this procedure, 1 ml of DNA STAT 60 (Tel-Test "B," Inc., Friendswood, Texas, USA) was added, the sample mixed, and 200 µl of chloroform added. The sample was vortexed, incubated at room temperature for 3 minutes, centrifuged for 15 minutes at 12,000 x g, and the supernatant removed and transferred to a new tube. This sample was incubated for 10 minutes at room temperature with 500 µl of isopropanol, centrifuged for 10 minutes, decanted, and 500 µl 75% ethanol added. Samples were centrifuged and the liquid removed with pipetting. The pellet was dried and resuspended in 50 µl of 100 mM Tris. The sample was then boiled for 5 minutes.

Conserved primers were used for detecting the acetyl coenzyme A synthetase gene (390 bp) *Cryptosoridium* spp. as described by Morgan et al. (2000). For this reaction, 5 µl gDNA (from above extraction protocols) were added to a PCR reaction mixture to make a 50 µl total reaction volume containing: 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.0 mM $MgCl_2$, 200 µM of each doxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP), 2.5 U Taq DNA polymerase (Promega Corporation, Madison, Wisconsin, USA), 5 µl of each primer, and sterile ddH_2O. Primer sequences were GGA CCT ATT

GAA TTT GTC AAG G (forward) and GAG TAA TTC TGT GTC TCT CCA C (reverse). PCR products (10 µl) were resolved via electrophoresis on a 1.5% agarose gel.

Fecal flotation.—These analyses were performed by Dr. Debra Miller, Anita Merrill, and others in the UGA VDIL staff. Fecal samples were used to test for parasites and protozoans, including *Cryptosporidium* spp. In brief, a mixture of 1 g of feces and 5 ml of water was strained and mixed with Sheather's sugar solution (RICCA Chemical Company, LLC., Arlington, TX, USA) then allowed to sit for 1 hr with a cover slip covering the test tube. The cover slip was placed on a slide and examined for parasite ova and oocysts using light microscopy. If inadequate fecal material was available to perform the flotation, a thin film of fecal material was smeared directly onto a glass slide, a drop of Sheather's solution added, the slide cover-slipped, and examined by light microscopy.

PCR for Ranavirus.—These analyses were performed by Dr. Debra Miller and Lisa Whittington of the UGA VDIL. Initial procedures for *Ranavirus* PCR followed those for *Cryptosporidium*, except that it was a hemi-nested procedure, with different incubation times and primers. In brief, the first-round reaction mixture (25 µl, total volume) contained 50 – 100 pmol of primers FV3-991 (5' – CGCAGTCAAGGCCTTGATGT) and FV3-1571R (5' – AAAGACCCGTTTTGCAGCAAAC), 1X PCR buffer (50 mM KCl, 10mM TRIS-HCl 3nM $MgCl_2$), 0.2 mM of dATP, dCTP, dGTP and DTTP, 1.25 U Taq polymerase (Promega Corporation, Madison, Wisconsin, USA), and 2.5 µl of template. The thermal cycler program was 35 cycles with an initial denaturization step of 5 minutes at 94°C

followed by 35 cycles of 1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C. The final cycle was followed by a 10-minute elongation step at 72°C.

The second round reaction (25 µl, total volume) contained the same materials as the first round with the exception of the primers. The primers were P1050N (5;TCAAGAGCGCCACGCTGGTGTA) and FV3-1571R. Only 0.5 µl of the first-round product was carried over to the second-round PCR. For the second round, the thermal cycler program was 25 cycles with an initial denaturization step of 10 minutes at 94°C, followed by 25 cycles of 1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C. The final cycles was followed by a 10-minute elongation at 72°C.

Ten microlitres of PCR products were resolved via elecrophoresis on a 1.5% agarose gel. Additionally, the resulting amplicons were prepared for sequencing with the Stratagene Clearcut Mini-Prep Kit (Stratagene, La Jolla, California, USA) according to the manufacturer instructions, and submitted to SeqWright DNA Technology Services, Houston, Texas, U.S.A. for automated sequencing. The reverse primer (FV3-1571R) was used for obtaining the reverse sequence; however to obtain the forward sequence, it was necessary to develop a second primer that was 17bp, FV3-E5778 (5' – ACTATGCCACCTCCATC). This primer was developed by SeqWrightDesign, DNA Technology Services, Houston, Texas USA (S603624,UGA-1,2,3-CP3).

Individual sequences were assembled using SeqMan program in the LasterGene Sequence Analysis Package (DNASTAR, Inc, Madison, Wisconsin, USA). A GenBank BLAST search was performed (NCBI 2005) on the consensus sequence. A phylogenetic tree and alignment of the consensus sequence and the sequences obtained from the

BLAST search were obtained using BLAST tree Widget View. This search reveals a percent identity with the capsid protein gene and the genome.

Fungal cultures for opportunistically collected individuals.—These analyses were performed by Dr. Sreekumari Rajeev, Cindy Watson and Jill Johnson of UGA VDIL. Fungal culture and identifications were performed using standard operating protocols outlined in Isenberg (1998), Murray et al. (2003) and Quinn et al. (1994). Swabs were taken of gross lesions and inoculated onto Sabouraud Dextrose Agar (Becton, Dickinson & Company, Franklin Lakes, New Jersey, USA). Plates were incubated at room temperature for 30 days with daily examination for fungal growth. If growth was present, the fungal colonies were examined microscopically using a Lactophenol Cotton Blue stain (Becton, Dickinson & Company, Franklin Lakes, New Jersey, USA) to determine morphology for identification.

White blood cell count of an opportunistically collected individual.—These analyses were performed by Anita Merrill. A drop (ca. 10 µl) of heart blood was placed at one end of the glass slide and the drop spread longitudinally via capillary action using another glass slide. Blood slides were air dried, stained with Wrights and Giemsa (Bennett 1970), and the blood smear was examined under oil emersion using light microscopy. A total of 100 white blood cells were counted and the percentage of each cell type was calculated. Morphology of red and white blood cells also was observed for any indication of disease.

Malformation and trematode testing procedures

Clearing Procedure.—I performed the following procedures after Hanken and Wasserug (1981). Specimens were skinned, eviscerated and placed in a solution of Alcian Blue cartilage stain (Fisher Scientific International, Inc., Pittsburgh, Pennsylvania, USA) for 24 hrs. Specimens were transferred to an ethanol-acetic acid solution (70:30) for 1 hr then placed in 100% ethanol for 24 hrs. Following this, specimens were soaked in distilled water overnight and transferred to Trypsin enzyme solution (MP Biomedicals, Solon, Ohio, USA) for an additional 24 hrs. Specimens were transferred from the Trypsin solution to Alizarin Red-S bone stain (Fisher Scientific International, Inc., Pittsburgh, Pennsylvania, USA) for 24 hrs then rinsed at least twice with 1% KOH to remove excess Alizarin Red. Specimens then were treated with a graded series of 2:1, 1:1, and 1:2 of 1% KOH:glycerin. Specimens remained in each KOH:glycerin solution for 4 days. If the tissues of specimens in the final solution were not clear after 4 days, specimens remained in the final solution until tissues were clear. Specimens were stored in 100% glycerin with one crystal of thymol.

Each cleared specimen was examined for trematode metacercariae using stereo-microscopy. Two metamorphs with unilateral ocular malformations were further analyzed by removal of the structures from the orbit and examining them using electron microscopy (procedure below). Further, if the electron microscopic examination revealed viral particles, PCR was performed using gel electrophoresis to identify the genus followed by sequencing to determine the viral species.

Electron microscopy for orbit structures.—These analyses were performed by Dr. Eloise Styer of the UGA VDIL. Structures were removed from the orbits of two

malformed metamorphs and examined by electron microscopy to determine their contents. Each structure was transferred to McDowell and Trump's modified Karnovsky's fixative (Dykstra 1993), and allowed to stand overnight at room temperature. The following day, the structure was washed in a 2% osmium tetroxide in 0.1M phosphate buffer (pH 7.3) solution, fixed in 2% osmium tetroxide, dehydrated through a graded series of acetone solutions (50 – 100%), and infiltrated with Spurr's resin. These steps were performed in a laboratory microwave oven (Giberson 2001). The resin was polymerized overnight at 70ºC, and sections 0.5 µm and 70 nm thick were cut with a diamond knife on a Leica UC6 ultramicrotome (Leica, Wetzlar, Germany) for light microscopy and transmission electron microscopy (TEM), respectively. Light microscopic sections were stained with toluidene blue-O (Mikel 1994), whereas sections for TEM were stained with uranyl acetate (Hayat 1972) followed by Reynold's lead citrate (Hayat 1972). Transition electron microscope sections were examined using a Zeiss EM 900.

APPENDIX III

REPORTED AMPHIBIAN SPECIES IN CUMBERLAND COUNTY

Common name	Scientific name
northern cricket frog	*Acris crepitans*
American toad	*Bufo americanus*
Fowler's toad	*Bufo fowleri*
eastern narrowmouth toad	*Gastrophryne carolinensis*
Cope's gray treefrog	*Hyla chrysoscelis*
mountain chorus frog	*Pseudacris brachyphona*
spring peeper	*Pseudacris crucifer*
upland chorus frog	*Pseudacris triseriata*
eastern spadefoot	*Scaphiopus holbrookii*
American bullfrog	*Rana castesbeiana*
green frog	*Rana clamitans*
southern leopard frog	*Rana sphenocephala*
pickerel frog	*Rana palustris*
spotted salamander	*Ambystoma maculatum*
marbled salamander	*Ambystoma opacum*
mole salamander	*Ambystoma talpoideum*
green salamander	*Aneides aeneus*
hellbender	*Cryptobranchus alleganiensis*
dusky salamander	*Desmognathus fuscus*
mountain dusky salamander	*Desmognathus ochrophaeus*
Ocoee salamander	*Desmognathus ocoee*
black mountain salamander	*Desmognathus welteri*
southern two-lined salamander	*Eurycea cirrigera*
longtail salamander	*Eurycea longicauda*
cave salamander	*Eurycea lucifuga*
spring salamander	*Gyrinophilus porphyriticus*
four-toed salamander	*Hemidactylium scutatum*
eastern red-spotted newt	*Notophthalmus viridescens*
zigzag salamander	*Plethodon dorsalis*
slimy salamander	*Plethodon glutinosus*
mud salamander	*Pseudotriton montanus*
red salamander	*Pseudotriton ruber*

VITA

Elizabeth Carrie Burton was born in Chicago, IL on August 20, 1978. She was raised in Deerfield, IL and went to grade school at Kipling Elementary School. She went to junior high school at Shepard Jr. High School and began high school at Deerfield High School. She moved to Kirkwood, MO in the middle of her sophomore year and finished high school at Kirkwood High School in 1996. From there, she went to Grinnell College in Grinnell, IA and received her B.A. in Anthropology and Biology in 2000. Elizabeth went on to the University of Tennessee to receive her M.S. in Wildlife and Fisheries Science in 2007.