8-8-2007

The decline of Fowler's Toad (Bufo fowleri) in southern Louisiana: molecular genetics, field experiments and landscape studies

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Recommended Citation

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The decline of Fowler's Toad (*Bufo fowleri*) in southern Louisiana: molecular genetics, field experiments and landscape studies

A Dissertation

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Conservation Biology

by

Laura Sanders Vogel

B.S. Howard University, 2000

August 2007

Acknowledgements:

I wish to thank the following people for their love and support, without which this dissertation would not have been completed. My father, A. Philip Vogel, imbued me with the strength and determination that he applied to every aspect of his life. My mother, Ann Ford is my staunchest ally, cherished parent and dearest friend; she is the most wonderful mom that any daughter has ever had. I am very glad she married my stepfather, Jeffrey Ford, as he is a wonderful addition to the family. Charles S. Brewton is a wonderful, caring and generous uncle and I am lucky to be his favorite niece. My late great-aunt, Faye W. Sanders and my great-uncle, Dr. Fred B. Sanders have been like grandparents to me and I am proud to have their name. My friend, Will Curry, steadfastly supported me through the daily trials and triumphs of my doctoral career, and my appreciation for this can never be adequately expressed.

I would also like to thank the members of my committee: Dr. John Utley, Dr. Karen Kandl, Dr. Nicola Anthony, Dr. Mary Clancy and especially Dr. Joe Pechmann. Joe’s unwavering support and assistance with everything from amphibian ecology to experimental design was critical to my success.

Finally, I wish to thank my brilliant and dedicated adviser, Dr. Steven G. Johnson, who honed my persistence and critical-thinking and writing skills. Steve is an impressive biologist and a wonderful person who has been a great source of inspiration and support for me.

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Abstract:

Two of the most pervasive threats to species biodiversity are invasive species and habitat loss and degradation. Invasive species are often relatively insensitive to disturbance and many expand their range into disturbed and fragmented habitats. This dissertation uses an interdisciplinary approach to investigate how anthropogenic habitat disturbance is precipitating a range expansion in an invasive toad species, *Bufo nebulifer*, which is driving a decline in its native congener, *B. fowleri*. I employed a remote sensing and GIS study using historical data to compare changes in the two species distributions and habitat changes, a molecular genetic study to identify interspecific hybrids and their potential effects on the parental species, and an experimental ecology study to look at the effects of competition and predation on the two species. The results of the landscape level analyses of species' distributional changes in different disturbance levels showed that both species' distributions have changed significantly. The distributions of the two species are inversely affected by habitat disturbance; the distribution of *B. fowleri* in highly degraded habitat has contracted while the expansion of *B. nebulifer* increased substantially. The molecular genetic study successfully demonstrated the use of nuclear and mitochondrial markers to identify cryptic hybrids and their maternal lineage. Three hybrids were detected using nuclear introns and a morphologically cryptic hybrid was identified using mitochondrial DNA as the progeny of a cross that was previously thought to be inviable. Although relatively few hybrids were currently found, the identification of a cryptic hybrid implies that the rate of historical hybridization may have been drastically underestimated. Ecological studies showed that competition with *B. nebulifer* tadpoles had a negative effect on both body size measures and survival to metamorphosis for *B. fowleri* tadpoles. The addition of predators to experiment did not favor the survival of *B. fowleri* over *B. nebulifer*. *Bufo fowleri*'s inability to compete with its invasive congener could be a driving mechanism for the decline of *B. fowleri* and the expansion of *B. nebulifer*. The methods discussed in this dissertation offer promising and practical new approaches for evaluating and managing changes in the distribution of species of conservation concern.

Keywords: *Bufo fowleri, Bufo nebulifer*, invasive species, hybrids, ranges, nuclear DNA, introns, SNPs, mtDNA, remote sensing, GIS, museum data, competition, predation, disturbance

CHAPTER 1: DISSERTATION INTRODUCTION

INTRODUCTION

Declining amphibian biodiversity

The loss of biodiversity is of great concern to resource managers, scientists and legislators. Although considerable resources are expended to prevent the loss of species in acute danger, the protection of a relative few species does not address the larger issues that threaten many others. As disruption of ecological processes and loss of critical habitat become more prevalent, many common species will gradually be lost from large portions of their ranges. Most often, a limited number of charismatic species that are in dire need of management demand the majority of funding; however, conservationists and managers must also remain aware of other "common" species so they do not enter a crisis state unnoticed.

A well-known example of the rapid and unforeseen imperilment of an entire class of animals stems from the recent reports of amphibian disappearances, declines and deformities worldwide (for reviews see Barinaga, 1990; Blaustein and Wake, 1990; Alford and Richards, 1999; Houlahan et al., 2001; Alford et al., 2001, Collins and Storfer, 2003). These reports have alarmed scientists and incited a barrage of studies aimed at understanding the causes of the amphibians' decline. Identifying and mitigating the factors that are negatively affecting amphibian health is crucial for many reasons. Amphibians are key members of most ecosystems, both as prey and as predators, and their decline may strongly affect other organisms within those systems (Duellman and Trueb, 1986). Amphibians are also strong indicators of the state of the environment and the status of global biodiversity (Blaustein and Wake, 1990).

Scientists have postulated a variety of causes for the widespread decline of amphibians. Collins and Storfer (2003) categorized them into those that are general threats to overall biodiversity including habitat destruction and fragmentation, introduction of non-native species and overharvesting (Drost and Fellers, 1996; Fisher and Shaffer, 1996), and those that appear to be more specific to amphibians such as climate change, ultraviolet radiation, agricultural pollutants, and pathogens (Blaustein et al., 1994; Davidson et al., 2002).

Natural fluctuations in the cycles of breeding activity and local population persistence confound the study of amphibian declines (Pechmann et al., 1991). Precipitation and other climatically influenced factors, as well as intra- and interspecific interactions such as competition and predation, contribute to natural variation in annual population dynamics (Pechmann et al., 1991, Wake, 1991). It is often difficult to distinguish natural population fluctuation from anthropogenically-induced declines because spatial and temporal shifts in abundance are difficult to monitor, interpret and analyze (Shaffer et al., 1998, Pechmann and Wilbur, 1994). An alternative method for evaluating a perceived decline is to assess whether a species' current range has contracted from its former range using information about past and current distribution (Drost and Fellers, 1996; Fisher and Shaffer, 1996).

Unfortunately, most studies of amphibian declines have been, for practical and logistical purposes, short-term relative to the cycles of fluctuation (Pechmann and Wilbur, 1994). Few amphibian species have been studied long enough to demonstrate a convincing argument that range contraction represents a decline in the species. Long-term monitoring programs with standardized methodologies are optimal, but are often unfeasible because of financial or time constraints. Studies that integrate hypothesis testing from various disciplines, including spatial, molecular and community ecology, are a promising alternative to disentangle the influence of synergistic abiotic and biotic influences on changes in species' distribution and abundance (Storfer, 2003).

Species distributions and the effects of disturbance and fragmentation

With the exception of obvious physical range limitations such as oceanic margins that confine a vast variety of terrestrial and marine organisms, geographic barriers to dispersal and local environmental niche requirements are important, though usually not the ultimate, limiting factors of a species' distribution (Gaston, 2003). The boundaries of species' ranges are temporally and spatially variable, often expanding, contracting and shifting in response to environmental changes and shifts in community structure such as the arrival of competitors or parasites (Holt and Keitt, 2005). Most species have at least moderate genetic variation in niche requirements, dispersal ability and other traits that influence range limits; therefore, environmental and demographic stochasticity as well as local population dynamics also influence the extent of colonization and localized extinction of peripheral populations (Kirkpatrick and Barton, 1997; Gaston, 2003; Holt, 2003).

Anthropogenic destruction and modification of habitat impede the natural processes that structure species' ranges. Habitat loss and alteration can cause immediate extinctions of rare, sessile and/or sensitive species; however, an equally urgent dilemma is the range contraction of species that do not immediately go extinct, but experience local and regional declines in abundance and subsequent fragmentation of populations (Hobbs and Mooney, 1998). Population fragmentation disrupts natural population and genetic structure and often results in the loss of small, isolated populations (Segelbacher et al., 2003).

Dissolution of metapopulation structure from habitat loss and fragmentation disrupts normal population dynamics such as random migration between populations and colonization of adjacent unoccupied patches, and can lead to a subsequent reduction in gene flow (Gaston, 2003). Genetic consequences of population fragmentation, resulting from inbreeding depression and the loss of genetic variation through genetic drift in small populations, become more pronounced as dispersal between isolated populations decreases (Quinn and Hastings, 1987). Various mechanisms that reduce fitness at low population densities (i.e. Allee effects) also increase the likelihood of extinction for many fragmented and isolated populations and diminish the probability of successful colonization of new habitats (Keitt et al., 2001).

Invasive species effects

The advancement of native and non-native invasive species into degraded habitat can exacerbate declines in native species initiated by anthropogenic disturbance. Exotic or introduced species are generally the most prevalent invaders; however, native species also can become invasive when their range expansion into areas where they were not previously native coincides with anthropogenic alteration of the habitat (Mack et al., 2000). Adaptations of some invasive species to human-altered urban, suburban or agricultural habitats can hasten their colonization of altered habitat, and a fragmented landscape structure can actually benefit the dispersal preferences of many invasive species that are good dispersers and relatively insensitive to disturbance (Case and Taper, 2000; With, 2001; Brown et al., 2006). When species preferentially occupy or invade disturbed habitat or areas of secondary growth, clearing of forests and other native vegetation for lumber, urban development or agriculture not only provides prime new habitat, but highly exploitable corridors for invasion as well (McDonnell et al., 1978; Harrison and Arnold, 1982; Sullivan and Lamb, 1988; Mendelson, 1998; 1999).

Genetic variation is beneficial for the continuation of the range expansion, and invasions often stall because genetic drift in colonizing populations reduces genetic diversity (Sakai et al., 2001.) Continued dispersal from the source area can both benefit and hinder further range expansion because, although dispersal increases genetic variation, it can also constrain adaptation to local habitats (Kirkpatrick and Barton, 1997.) Lag times between colonization and expansion often reflect the time necessary for species to purge deleterious genes or evolve adaptations to a new environments or invasive life-history characteristics (Sakai et al., 2001).

Once they have become established in their new habitat, many invasive species pose a serious threat to native species; often species that share the same or a similar ecological niche with the invasive species are most heavily impacted. Many invasive species exhibit superior ability in exploiting disturbed habitat and can precipitate decline and extirpation when they encounter native congeners and other species (Petren and Case, 1996; Kupferberg, 1997; Sakai et al., 2001.) Competition with invasive species can result in a significant negative impact on native species, particularly since many invasive species have been shown to be superior competitors (Kupferberg, 1997; Holway, 1999). Interspecific hybridization between related invasive and native species can also quickly result in extinction through genetic admixing or outbreeding depression (Sakai et al., 2001.) Locally advantageous genes from native populations can also be introduced into invasive species and promote continued invasion (Rhymer and Simberloff, 1996.)

Study organisms

Species that can tolerate a wide range of ecological conditions usually persist across a large environmental gradient. Fowler's Toad (*Bufo fowleri*) is a habitat generalist, widely distributed across a large range of environmental conditions throughout the United States. Open woodlands and meadows, and sandy dunes are among its preferred habitats, though it can also inhabit vegetated suburban and urban areas (Cory and Manion, 1955; Volpe, 1955; Dundee and Rossman, 1989; Hecnar and M'Closkey, 1997; Conant and Collins, 1998; Green, 2000; Green and Parent, 2003; Green, 2005). The distribution of the species, from the Midwest to the Northeast coast and into Canada down to the Gulf Coast, is one of the most widespread of all the toads in North America (Fig. 1.1).

Bufo fowleri belongs to the *B. americanus* species group of North American toads that includes several closely related taxa, variously treated as species or subspecies depending on author and context. Another member of the *B. americanus* complex and a species closely related to *B. fowleri*, *B. velatus* (East Texas Toad) shares a similar ecological niche. Although the two species are shown to be partially sympatric in some parts of Louisiana on the USGS distribution maps (Figs.1.1 & 1.2), the two species' ranges actually overlap very little (Dundee and Rossman, 1989; personal comm. with LADWF herpetological expert Dr. Jeff Boundy). Regional herpetologists generally agree that *B. fowleri* predominates in the Atchafalaya River Basin and east into the Florida Parishes above Lake Pontchartrain and *B. velatus* occurs farther west in Louisiana and into eastern Texas.

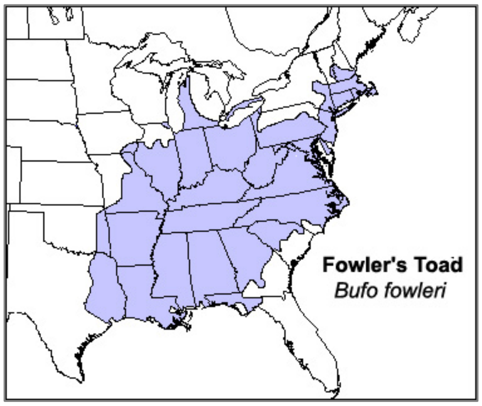

Figure 1.1: Range map of *B. fowleri* from: from http://www.npwrc.usgs.gov/narcam/idguide/bfowl.htm

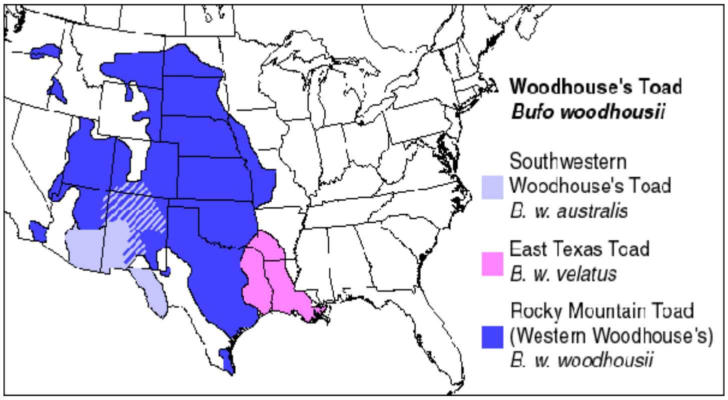

Figure 1.2: Range map of the *B. woodhousii* complex http://www.npwrc.usgs.gov/narcam/idguide/bwood.htm

Anecdotal evidence from area herpetologists and Louisiana Amphibian Monitoring Program (LAMP) data indicate that both *B. fowleri* and *B. velatus* are currently absent from many historical locations in Louisiana where they were formerly present. Historical collections and field notes in the Louisiana State University Museum of Natural Science and the Tulane Museum of Natural History herpetological collections further support this assertion. Currently, *B. fowleri* is found breeding solely in forested areas in southern Louisiana, though historical museum and field records indicate that it bred and thrived in suburban and vegetated urban areas, including the Baton Rouge and Lafayette metropolitan areas less than 50 years ago.

Although historically widespread and abundant throughout most of its range in the eastern United States and the Midwest, *B. fowleri* has been listed as "vulnerable" in its northernmost range in Ontario since 1984 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), and was listed as "threatened" in 2000 because it has been extirpated from all but three of its historical breeding locations along the northern shore of Lake Erie. Green (1999) attributes the population decline to environmental stochasticity due to periodic severe winter storms and floods, and fluctuating lake levels. A population viability analysis indicated that Fowler's Toads in Canada have a 20.3% chance of being entirely extirpated from Canada in the next fifty years (Green, 2000). In Ontario, *B. fowleri* is at the far northern edge and Green (1999, 2000) attributes the decline in their abundance in Canada to a range shift caused by increasing severity of ecological factors related to global climate change.

Although the northernmost populations of *B. fowleri* in Ontario appear to have declined due to a combination of ecological factors, the scenario of unfavorable climatic changes is less plausible for the decline of the species along the southernmost boundaries of its range, where it has been historically a widespread and abundant toad species in Louisiana (Dundee and Rossman, 1989). The pattern of northern populations experiencing a decline due to abiotic variables versus southern populations declining due to biotic variables is supported by the paradigm that distribution at higher latitudes is limited by physiological tolerance to environmental factors such elevation, temperature and aridity, whereas distributions at lower latitudes are impacted by increasing numbers of competitors and predators (Hersteinsson and Macdonald, 1992; Brown et al., 1996; Richter et al., 1997). While southeastern Louisiana has undergone significant industrial and developmental changes in the past century, the cause of the decline in *B. fowleri* also may be linked to interspecific interactions with a congener, *B. nebulifer*, which is sympatric with *B. fowleri* throughout southeastern Louisiana (Figs. 1.2 & 1.3).

Another species of *Bufo* found in southeastern Louisiana, *B. nebulifer* (Coastal Plain Toad) (Fig. 1.3) has been identified as a northern clade of the Mesoamerican *B. valliceps* (Gulf Coast Toad) species and granted taxonomic status as an independent species (Mulcahy and Mendelson, 2000). *Bufo valliceps* prefers urban and agricultural areas and areas of secondary growth and rapidly colonizes disturbed and degraded areas (Mendelson 1998, 1999, 2005; Mulcahy and Mendelson, 2000). During the breeding season, *B. nebulifer* is usually found in open, cleared and/or degraded areas such as marshes, roadside ditches, and urban, suburban and agricultural areas, but can successfully breed in forested areas as well (Dundee and Rossman, 1989; Conant and Collins, 1998; Mendelson, 1998, 1999, 2005).

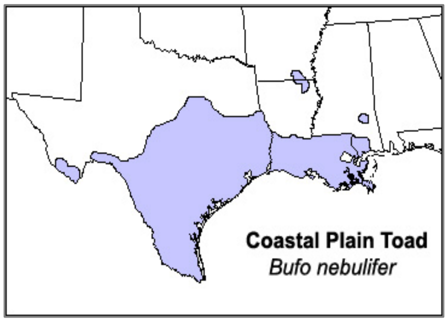

Figure 1.3: Range map *of B. nebulifer* modified from the U.S.G.S. web site http://www.npwrc.usgs.gov/narcam/idguide/bvall.htm

Study Area

The southern Louisiana regions of the study area are located within the Mississippi River Alluvial Plain in the uplands and wetlands that comprise the fertile Prairie Complex above the Mississippi River Deltaic Plain. It is bounded by Lafayette just to the west of the Atchafalaya River Floodway and by Baton Rouge on the eastern bank of the Mississippi River. The forested wetlands that comprise the natural areas of the research study are composed mainly of bald cypress-tupelo swamps and bottomland hardwood species such as various oak and ash species, black gum, red maple, sweetgum and elm (McNab and Avers, 1994).

Due to alteration and containment of the path of the Mississippi River, urbanization and extensive conversion of forested areas to cropland, only 18,000 square kilometers of bottomland hardwood forest of an original 100,000 square kilometers still remain in Louisiana (Dugan, 1993). A period of intensive logging from the mid 1800’s century into the 1920’s, followed by government subsidized agricultural conversion of vast areas of forested wetland to soybean and cotton crops into the 1980’s, would have made southern Louisiana ideal territory for colonization by a species such as *B. nebulifer* that thrives in disturbed habitat (Dugan, 1993).

Survey and collection sites in southern Louisiana (Fig. 1.4) span diverse ecological and geographical regions, and landscape variables found within disturbed, moderately disturbed and undisturbed habitat are well-represented. The research areas are located in the Baton Rouge metropolitan area including portions of West Baton Rouge, Iberville and East Baton Rouge parishes (Region 1), the Atchafalaya River Floodway including sites in Point Coupee, St. Martin and St. Landry parishes (Region 2), and the Lafayette metropolitan area (Region 3). The northern Louisiana sites, in which *B. fowleri* is still allopatric with *B. nebulifer*, are located in Ouachita parish, in the Monroe city limits and surrounding areas (Region 4).

Due to its proximity to Louisiana State University, East Baton Rouge parish contains most of the historical locations. An urban to rural gradient radiates outward from Baton Rouge, the epicenter of urbanization, industrialization and development within the parish. Since the Standard Oil Company established a refinery along the banks of the Mississippi River in 1909, Baton Rouge has been a hub of commerce and industry in southeastern Louisiana (Goins and Caldwell, 1995). Today, the Port of Baton Rouge is second in tonnage only to New Orleans among state ports, and the petrochemical industry in the parish is mainly responsible for the enormous growth of the city and its environs since the 1920's and 30's. From the 1940's, when the historical collections of *B. fowleri* and *B. nebulifer* first showed the former to be the dominant toad species in the parish, to 1990, when *B. nebulifer* had clearly overtaken *B. fowleri* in distribution and abundance, the population of the East Baton Rouge parish grew from 88,415 to 350,105 inhabitants (Goins and Caldwell, 1995).

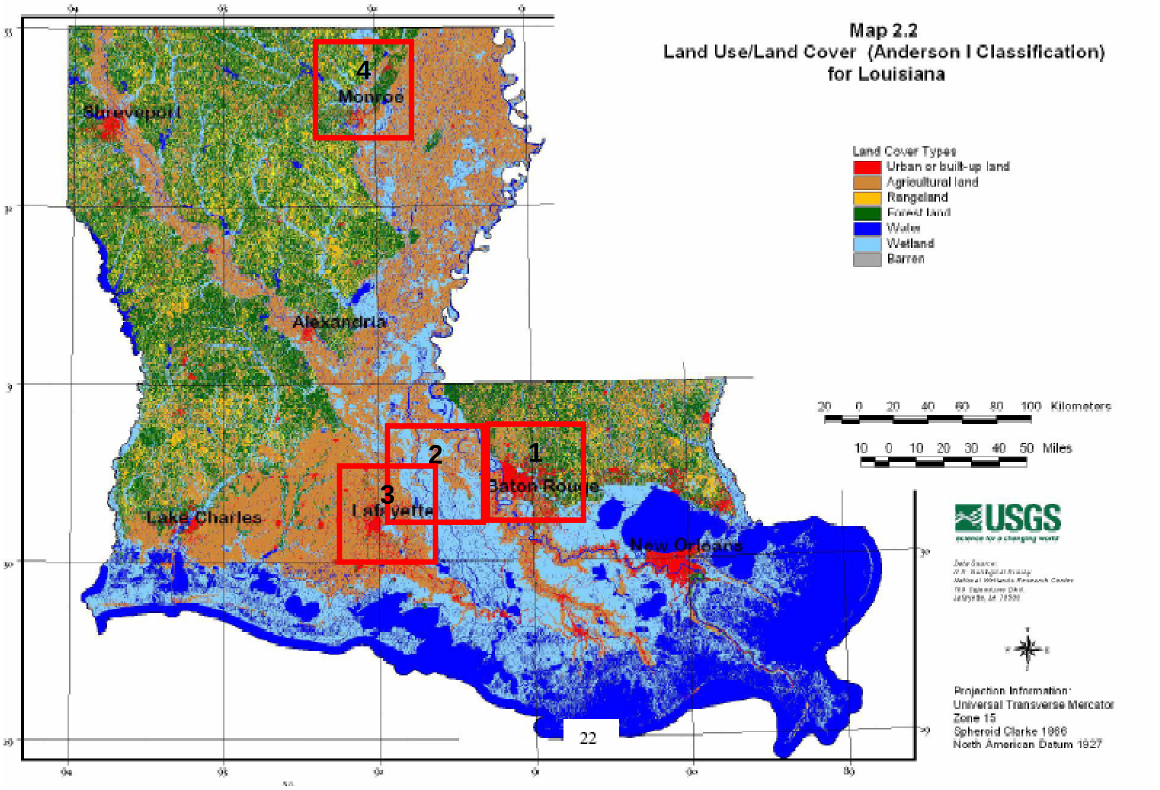

Figure 1.4: USGS map with study regions in Louisiana delineated by red squares. Region 1 encompasses East Baton Rouge, West Baton Rouge, Ascension, and Iberville parishes. Region 2 encompasses Point Coupee, St. Landry and St. Martin parishes. Region 3 encompasses Lafayette parish and Region 4 encompasses Ouachita parish.

By contrast, neighboring Iberville parish abuts the bottom of East Baton Rouge parish and has remained relatively unaltered. The western half of the parish lies across the Mississippi River within the Atchafalaya River Basin and is marked by oil and gas fields that have relatively minor immediate impact on the surrounding terrestrial environment. Because the area is primarily wetlands and subject to periodic inundation by rising waters, limited urban development has occurred, with a minimal amount of soybean farming and recreation as primary land uses (Goins and Caldwell, 1995). The eastern half of the parish is adjacent to East Baton Rouge parish and is primarily low-impact residential areas.

Within the Atchafalaya River floodway region, St. Martin, St. Landry and Pointee Coupee parishes are relatively undisturbed and composed mainly of forested wetland. However, channel dredging, oil and gas extraction and agricultural use result in significant anthropogenic impacts to the area. Many human activities occur on or adjacent to the Atchafalaya River and the Whiskey Bay Pilot Channel. The Lafayette metropolitan area also has a significant number of historical records due to its proximity to the University of Louisiana at Lafayette. Alternating rice and crawfish fields depending on the growing season, as well as soybean, sugarcane and cotton are the main crops of the Cajun swamplands surrounding the city.

The study sites in Ouachita parish are located at the convergence of the Upper West Gulf Coastal Plain and the Mississippi Alluvial Valley Ecoregions in northeastern Louisiana (McNab and Avers, 1994). The city of Monroe is the urban center of Ouachita parish and home to the University of Louisiana at Monroe. Agricultural and crop land is steadily encroaching on the already fragmented bottomland hardwood forest that formerly surrounded the city of Monroe and its outskirts (Goins and Caldwell, 1995). Numerous bayous, lakes and ponds, as well as the Ouachita River traverse the landscape of Ouachita parish in and around the city of Monroe.

Research Approach and Questions:

Numerous threats to global biodiversity have been posited and examined in the past twenty-five years including habitat loss and degradation, exotic species, UV-B radiation, global climate change, agrochemicals and pathogens (Blaustein et al., 1994; Drost and Fellers, 1996; Fisher and Shaffer, 1996; Davidson et al., 2002; Collins and Storfer, 2003). Blaustein and Kiesecker (2002) emphasize that differential susceptibility of species to biotic and physical variables can result in widely varying responses to environmental changes. Synergistic effects of many threats have been recognized, such as an increase in disease vulnerability resulting from environmental stressors, and the interactions between multiple factors has become an important research focus for many conservation biologists, particularly in the field of amphibian conservation (Kiesecker et al., 2001; Blaustein and Kiesecker, 2002; Taylor et al., 2004, Navas, 2006).

Two topics of great interest to conservation biologists and managers are the impact of habitat disturbance and fragmentation on species' distributions and the effects of invasive species on the decline of native species. The overarching focus of this research is to examine the effects of landscape degradation on species' distributions independently and as a compounding factor of species' invasive potential. The broadly-stated hypothesis of this dissertation is that *B. fowleri* historically thrived in moderately disturbed (i.e. urban and suburban habitat in the 1950's and 60's) and undisturbed habitat, but widespread anthropogenic habitat alteration in southern Louisiana over the past fifty years has favored dispersal and colonization by *B. nebulifer* and displacement of *B. fowleri* from much of its former distribution. Specifically, the questions addressed within the broad framework of this dissertation are:

1. Is *B. fowleri* undergoing a range contraction (i.e. decline) in southern Louisiana and is *B. nebulifer* expanding its range in southern Louisiana?
2. Is a decline in *B. fowleri* and an increase in *B. nebulifer* concurrent with an increase in habitat disturbance?
3. Is *B. fowleri* undergoing a decline in disturbed or undisturbed habitat in northern Louisiana where it is allopatric with *B. nebulifer*?
4. Is interspecific hybridization with *B. nebulifer* contributing to the decline of *B. fowleri*?
5. Is interspecific larval competition with *B. nebulifer* in breeding sites characteristic of disturbed habitat contributing to a decline in *B. fowleri*?

My dissertation uses an interdisciplinary approach to address these questions in the following three chapters. Chapter Two uses historical museum collection records and several years of current advertisement calls to examine changes in each species' historic and current distribution. I then use geographic information system and remote sensing techniques to observe temporal changes in species' ranges related to landscape alteration in areas of sympatry and allopatry. In Chapter Three, I use both nuclear and mitochondrial molecular genetic markers to examine the potential for deleterious interspecific hybridization between the two species and detect putative cryptic hybrids. Chapter Four presents two experiments that mimic natural conditions of larval competition between the two species to determine whether interspecific competition is a possible mechanism driving a decline in *B. fowleri*. Chapter Five concludes with a discussion of the questions posed by this introduction and, through the examination of the results as a whole, the acceptance of the foundational hypothesis that anthropogenic habitat disturbance is precipitating a range expansion in *B. nebulifer* that is driving a decline in *B. fowleri*.

CHAPTER 2: USE OF HISTORICAL MUSEUM DATA WITH REMOTE SENSING AND GEOGRAPHIC INFORMATION SYSTEMS TO EXAMINE SPECIES DISTRIBUTIONAL CHANGES AND THE INFLUENCE OF LANDSCAPE DISTURBANCE

INTRODUCTION

Comparison of original and current distributions using museum data and contemporary surveys

Abiotic factors such as anthropogenic landscape alteration as well as biotic interactions between species such as interspecific competition can drive changes in species distributions (Holt, 2003; Holt et al., 2005). Identifying species' range contractions and expansions is important in conservation biology because of the critical need to understand, manage, and potentially intervene, in cases of swift contractions of imperiled species and rapid expansions of non-native species (Hobbs and Mooney, 1998). Distributional changes that may signal a species decline have been inferred via surveys of presence and absence of amphibians in or around appropriate terrestrial and breeding habitat (Drost and Fellers, 1993; Ernst et al., 1995). However, this approach, and predictions based on local breeding habitat characteristics, have proven challenging because natural population fluctuations of amphibians may be misinterpreted as absences or declines (Munger et al., 1997; Knutson et al., 1999). A promising alternative is to use historical data from the natural history collections of museums and universities to establish a foundation for comparison with current surveys of species at known collection sites (Shaffer et al., 1998; Wilson, 2000; Kress et al., 2001; Graham et al., 2004).

Historical accounts, vouchered specimens, natural history collections, ledgers and field notes from museums and universities are often excellent sources of long-term data for comparison with contemporary field data (Graham et al., 2004). However, there are limitations inherent in the data of museum and natural history collections related to issues with sampling bias, as well as spatial and temporal variation in observer effort (Ponder et al., 2001; Graham et al., 2004). Despite these limitations, the current rate of biodiversity loss provides compelling incentive to devise methods to reconcile these shortcomings and incorporate historical data into sampling schemes, experimental designs and critical conservation decisions (Graham et al., 2004)

Various studies have successfully compared baseline data of known historical occurrences of arthropods (Light et al., 1995), fishes (Frissel, 1993; Reznick et al., 1994), birds

(Herkert, 1991) and plants (Drayton and Primack, 1996) with contemporary censuses of the original locations to examine population changes and document species' declines. Use of historical data is also effective for detecting declining amphibian populations and differentiating declines from naturally occurring population variation (Drost and Fellers, 1996; Fisher and Shaffer, 1996; Skelly et al., 2003). Unfortunately, recognition of species' declines and range changes, without identification of the causal factors, offers limited and often conflicting management and recovery recommendations for vulnerable species (Drost and Fellers, 1996, Lips, 1998, 1999; Gibbs et al., 2005).

Using remote sensing and GIS techniques to examine how landscape changes affect distributions

Within the past decade, scientists, conservation managers and policy makers have recognized the enormous potential of remote sensing and geographic information system (GIS) frameworks to aid a variety of conservation and management objectives (Munger et al., 1997, Davidson et al., 2002; Marjokorpi and Otsamo, 2006; Martinez et al., 2006; Barbaro et al., 2007; Falcucci et al., 2007). Species distributional data integrated with spatial habitat data has an enormous capacity to elucidate abiotic and biotic factors contributing to a species declines (Graham et al., 2004). Recently, remotely sensed and GIS data have been used in conjunction with historical data to incorporate a landscape-level investigation of environmental variables shape a species' distribution (Fisher and Shaffer, 1996; Carroll et al., 1999; Davidson et al., 2001, 2002; Gibbs et al., 2005).

Studies have suggested that the landscape and regional scale habitat surrounding the breeding habitat of amphibians have much greater predictive power of species' distributions than local environmental variables of the breeding sites (Beebee, 1985; Pavignano et al., 1990; Hecnar and M'Closkey, 1996). Knutson et al. (1999) found associations between amphibian species richness and abundance and landscape-scale environmental variables and Rubbo and Kiesecker (2005) found deleterious effects of habitat fragmentation and loss on amphibian distributions. Most previous studies combining GIS and historical data have focused on environmental correlates of amphibian decline without addressing indirect impacts of environmental change such as alteration of biotic interactions (Carey et al., 2001; Davidson et al., 2001, 2002; Gibbs et al., 2005; but see Riley et al., 2005).

Remote sensing, GIS techniques and historical data also can be used to examine how alteration of landscape factors affects biotic interactions, for example, by shifting competitive balances among species (Case et al., 2005). Anderson et al. (2002) utilized GIS and ecological niche modeling to demonstrate that a species of pocket mouse utilized its entire predicted distribution in allopatry, but was competitively excluded by its congener in areas of sympatry. Furthermore, anthropogenic disturbance frequently benefits the habitat preferences of non-native organisms and promotes dispersal and range expansion (With, 2002; Case et al., 2005). Alteration of competitive advantages among species following environmental disturbance can cause native species to retreat from highly competitive invasive species or alter their resource usage to minimize competition (With, 2001).

Critical alteration of environmental gradients and their effects on biotic interactions can be identified using remote sensing and GIS techniques, and considered primarily in developing predictions and hypotheses to test plausible mechanisms of species distribution change (Guisan and Zimmerman, 2000). My study incorporates historical and contemporary distributional data of *B. fowleri* and *B. nebulifer* with remote sensing data spanning more than 50 years to evaluate whether landscape alteration alone is causing a decline in *B. fowleri,* or if *B. nebulifer* has exploited an ability to colonize degraded habitat and subsequently displaced its native congener.

Historical records indicate that *B. fowleri* originally thrived across a wide range of disturbed and undisturbed habitat in southern Louisiana, but is currently extirpated from urbanized areas (personal observation, communication with LADWF state herpetologist Dr. Jeff Boundy) and persists only in rural, forested areas that are the less preferred habitat of *B. nebulifer*. I hypothesize that anthropogenic habitat alteration over the past fifty years has favored dispersal and colonization by *B. nebulifer* and subsequent displacement of *B. fowleri* from much of its former distribution.

I predict that in southern Louisiana, *B. fowleri* will be absent from the historical collecting sites designated as urban and suburban where it was formerly present, but it will still be present at permanent breeding sites in rural habitat. Furthermore, I predict that *B. fowleri*'s range has contracted in southern Louisiana and that overall the species has retracted into forested areas and away from human-altered habitat; therefore, *B. fowleri* will be associated with a higher percentage of forested habitats and a far lower percentage of agricultural and urban habitats. In addition, I predict an overall decline in the distribution of *B. fowleri* in southern Louisiana.

However, because *Bufo nebulifer* is not found in northwestern Louisiana *B. fowleri* will maintain its former distribution across all habitat disturbance classes in this region.

By contrast, *B.nebulifer* will be present at almost every historical collecting site where it was formerly present, and will have expanded its range into the collecting localities where *B. fowleri* is now absent. Although *B. nebulifer* will be present in rural locations, it will be more prevalent in urban and suburban areas and strongly associated with a lower percentage of forested cover.

If *B. fowleri* currently breeds in highly disturbed areas in the absence of *B. nebulifer*, this supports the hypothesis that the presence of *B. nebulifer* is a critical factor in the decline of its native congener. A significant decline in *B. fowleri* in disturbed habitat where it was formerly present and an expansion of *B. nebulifer* concurrent with anthropogenic disturbance into those areas where it where it was formerly absent will the hypothesis of displacement caused by an invasive species and exacerbated by anthropogenic habitat disturbance. If no decline in *B. fowleri* is found in comparable habitat in northwestern Louisiana, where it is allopatric with *B. nebulifer*, it will further support the displacement hypothesis.

MATERIALS AND METHODS

Compilation of historical collection sites and identifying historic versus current distribution

Ninety-two collection sites in southern and northern Louisiana where *B. nebulifer* and *B. fowleri* were historically present and absent for the designated 18-year period from 1950 to 1968 were compiled from the *Bufo* spp. collection of the Louisiana State University Natural History Museum's Herpetological Collection (Appendix 2.1). Multiple collection records for a single location were grouped together and assigned a collection date and collector. Historical collection sites were only used for resurvey if their location could be pinpointed to within a 1 km radius, although most survey sites were located to within 500 m. In most cases, site locations were identified and scouted during the daytime and then revisited at night during advertisement vocalizations. To maximize the likelihood that present species would be detected, surveys of male breeding calls were conducted during the spring breeding season; both species are explosive breeders and breed during the first heavy spring rains at temperatures above 18° Celsius (Volpe, 1956). Surveys were only undertaken on nights when males of both species were heard calling at one or more sites.

Male breeding calls for these species are distinctive and can be used to identify species presence at a site. Surveying male breeding calls is considered an accurate method for assessing the presence of aggregating amphibian species (Heyer et al., 1994; Knutson et al., 1999). Any site at which one or both species was not heard vocalizing was also visually searched for males or females of either species using a high-intensity flashlight and LED headlamp to a radius of approximately 500 m or as far as the site would allow.

Presence and absence data are significantly more useful than “presence-only” data (Ponder et al., 2001); therefore, with a few exceptions, historical sites where one species originally was not found were used only if the collector verified that the “absent” species would have been collected if it had been heard or seen. Surveys of population status at historical sites in East Baton Rouge and Iberville parishes (Region 1) were performed beginning in the spring 2004 breeding through spring 2006 season (Fig. 2.1). Resurveys of previously visited sites were conducted if one or both species was not detected during an earlier visit. Surveys of sites in Regions 2, 3, & 4 were conducted during the spring 2006 breeding season.

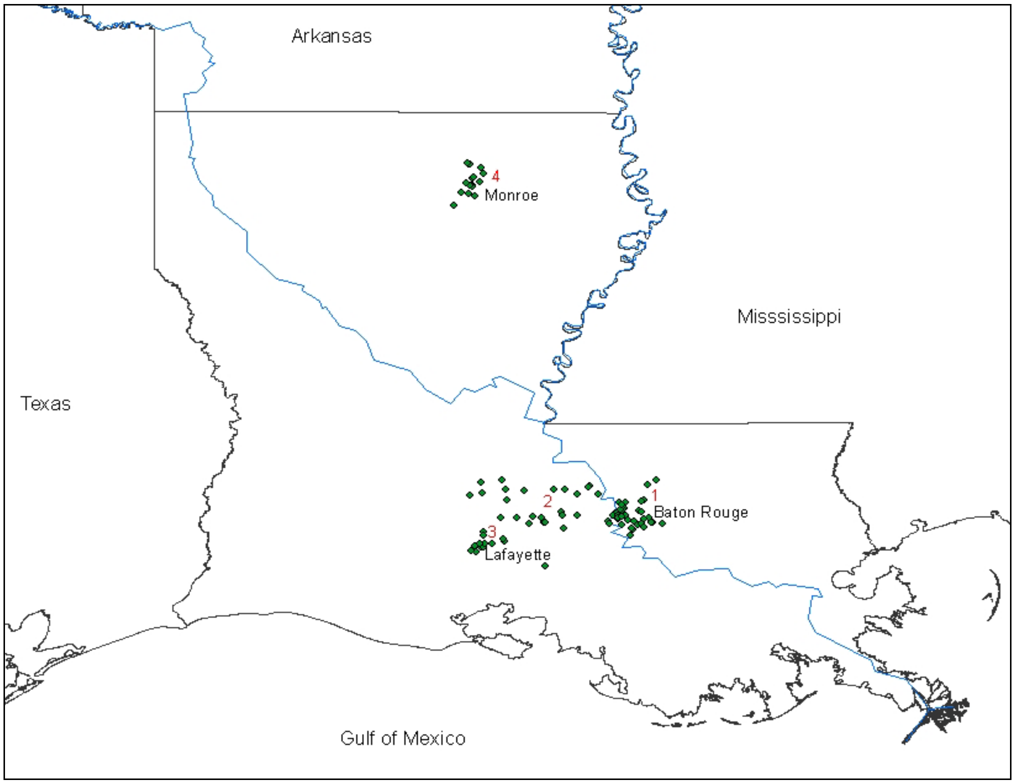

Figure 2.1: All survey sites in Louisiana with 4 study regions denoted by red numbers.

Using GIS to process historical aerial photographs and contemporary DOQQs

Coordinates of the historical collection localities were recorded at each site using a handheld global positioning system (Garmin GPS eTrek). The GPS data were differentially corrected, projected to UTM coordinates and converted to GIS format. Current aerial photographs known as Digital Orthophoto Quarter Quadrangle (DOQQs) are available as digitized, compressed, MultiResolution Seamless Image Database (MrSid) files on the Louisiana Geospatial Database (Atlas – www.atlas.lsu.edu). DOQQs are high resolution, small scale (1:24,000) color infrared aerial photographs adjusted to make each point appear as though it is directly below the camera.

The 2004 DOQQs in Mr Sid format were converted to GeoTIFF format using a proprietary LizardTech (www.lizardtech.com) command line utility that was modified for batch conversion in UNIX. GeoTIFF files are TIFF (Tagged Image File Format) files that utilize geospatial tags imbedded within the TIFF file. ArcGIS and other spatial software read these internal tags and automatically import a file's spatial coordinates, as well as any additional spatial reference information (e.g. map projections, datums) found within the tag. DOQQ coverage of each study region was created via mosaic of converted GeoTIFF files in ArcGIS® 9.1 (ESRI, Redlands, CA) in North American Datum 1983 UTM projection.

Eighty-three total aerial photographs were obtained from the historical aerial photograph database at the Louisiana State University Cartographic Information Center (CIC). Appropriate aerial photographs for each collection site were identified from photomosaic indexes at the CIC, and historic topographic maps and recent aerial photographs obtained from the U.S.G.S.-Microsoft Terraserver that were used to create a mosaic coverage of the study regions. Dates for aerial photographs varied depending on availability for each parish (Appendix 2.2). Hardcopy aerial photographs were scanned and converted to raster format as .tif files at a photo scale of 1:20,000 at 500 dpi.

Several steps were performed in ArcGIS to transform the historical aerial photographs for overlay onto the DOQQs. First, the aerial photographs were added to a data layer as .tifs in ArcGIS® 9.1 for georeferencing with the DOQQs. The process of georeferencing each aerial photograph to its respective DOQQ spatial references was relatively simple given the flat terrain of southern Louisiana. The process of georeferencing essentially ties the XY-coordinates (latitude and longitude) from the pre-referenced DOQQ to the unreferenced aerial photograph pixels. The Georeferencing toolbar in ArcGIS was used to attach ground control points on the DOQQ to the aerial photograph; a minimum of 6 ground control points were used (usually multiple points were attempted and discarded in the meantime).

An affine transformation was used to establish the geometric location between the original input pixel location (row and column) of the aerial photograph with the associated DOQQ map coordinates (Verbyla, 2002). This process of rectifying the aerial photograph with the DOQQ was accomplished with a maximum Root Mean Square (RMS) error value no greater than 2.0 pixels. The RMS error value is the sum of the residuals, or the deviations (measured in image pixels) of the aerial photograph from the DOQQ coordinates (Verbyla, 2002). Finally, the aerial photograph was clipped and aligned to fit the collection site using the Spatial Analyst extension of ArcGIS. The original coordinates of the collection sites were added onto the aerial photograph and DOQQ layers and buffered to a distance of 500 meters using the Analysis extension of ArcGIS.

Using GIS to plot changes in species distributions and habitat disturbance over the past 50 years

Historic and current species distributions (i.e. presence and absence at the original collection locations) in each study region were plotted onto the maps of each study region composed of DOQQs and overlaid historical photographs made in ArcGIS. Although sites will be referred to as historical and current sites, they are the same sites, just temporally separated. In other words, a ‘historic’ site and a ‘current’ site are plotted at the same geographic location, but the historic site is defined by the land use/ land cover attributes of the historical aerial photographs, whereas the current site is defined by its respective habitat attributes on the current DOQQ (Fig.2.2)

Figure 2.2 Illustration of “current” and “historic” sites. Both are in the same geographic location, but the current site is plotted on a DOQQ and the historic site is plotted on a historical aerial photograph that has been georeferenced and rectified.

The seven land use/land cover classes from the Anderson I classification system (Anderson et al., 1976) shown on the map of Louisiana in the first chapter (Fig. 1.4) were generalized into 3 habitat disturbance classes relevant for use in this study (Pearce et al., 2001). Because increased disturbance, and not unique habitat variables, was hypothesized to promote the spread of *B. nebulifer*, the 3-level disturbance scale of low, medium and high disturbance were thought to provide the most accurate and meaningful results. The percentage of each disturbance class (low =1, medium=2, and high=3) within the 500 meter radius core terrestrial habitat was visually interpreted for each collection site. The size of the terrestrial habitat zone was determined by time constraints and the availability of aerial photographs, though Clarke (1973) found that 526 meters is an average annual dispersal distance for the Fowler’s Toad.

Areas of each type of land-use/land-cover at each of the 92 sites were created as new feature class polygons for both the historical and current sites (Fig. 2.3). Forested areas and wetlands were classified as low disturbance (class 1), agricultural fields and pastures and low density residential areas (e.g. suburban) were classified as medium disturbance (class 2) and high density residential areas (e.g. urban) commercial and industrial areas were designated as high disturbance (class 3). The high resolution of the aerial photographs and DOQQs (~1m) makes visual interpretation ideal, whereas the comparatively low resolution of satellite imagery (~ 30m) renders this method less ideal (Lu et al., 2004). The use of visual interpretation is considered a powerful method for detecting change with high resolution data (Loveland et al., 2002; Lu et al., 2004). In this case, prior ground-truthing of many of the collection sites lends support to the interpretation.

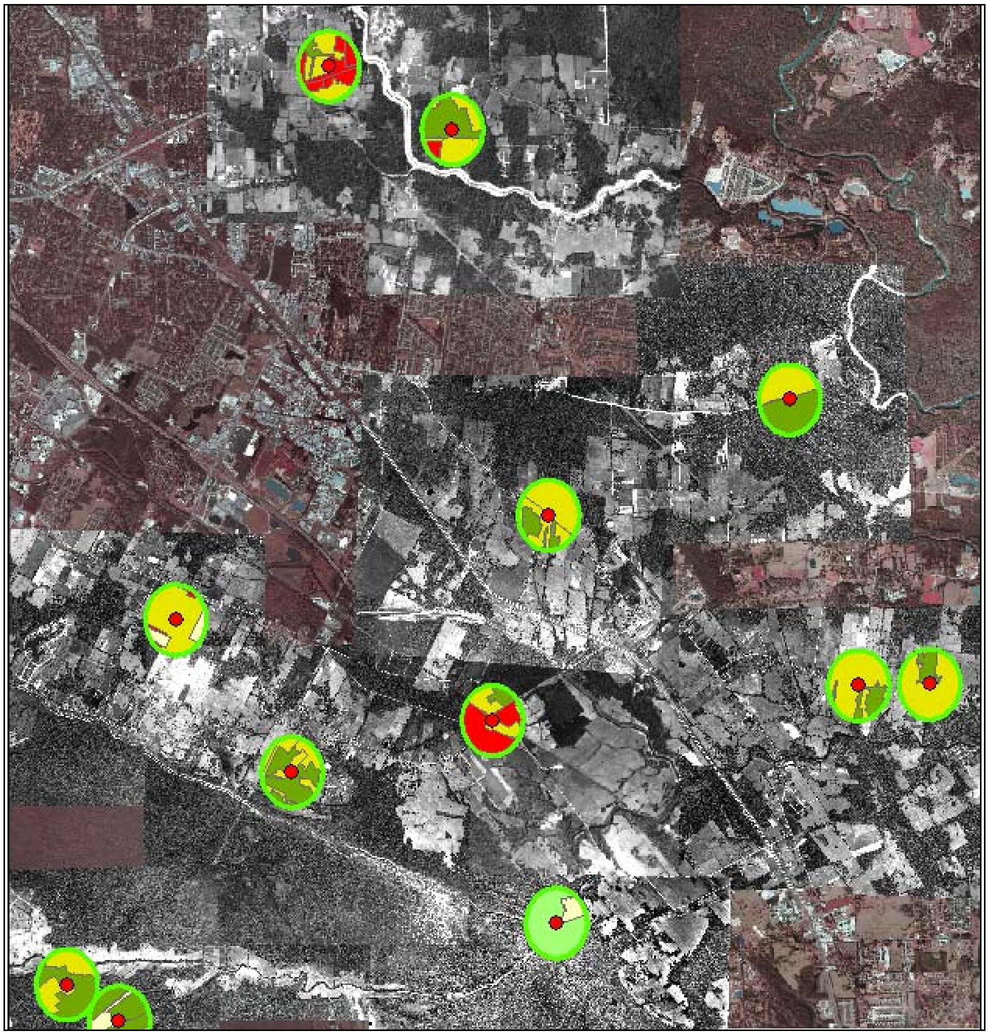

Figure 2.3: Aerial photographs (of suburban Baton Rouge) overlaid onto the DOQQs, with polygons of disturbance classes identified within each buffer zone.

Attribute tables of the polygons for each of the 3 disturbance classes at each site for both the aerial photograph and DOQQ layers were converted in ArcGIS from new shapefiles into personal database files in ArcCatalog. The area of each polygon of all 3 classes of habitat disturbance was recorded by the personal database files for each polygon in meters, and the percentage of each of the 3 classes for the historical and current distributions of both species was calculated for each site. For the historical and current time periods each site was classified as low, medium or high disturbance based on the percentage of each disturbance class within the buffer zone. Sites with < 33% medium or high disturbance were designated class 1, sites with 33 – 66% disturbance were designated were designated class 2, and sites with >66% disturbance were designated as class 3. Sites with > 40% high disturbance were also designated as class 3.

The interpretation of disturbance class for the historical and current species distributions was further supported by overlaying maps of wetland and upland habitat classifications at a 1:24,000 scale that were created by the U.S. Fish and Wildlife Service's National Wetlands Inventory (NWI) using color infrared aerial photographs from 1988 and ground truthing. Wetlands were categorized by the NWI using the Cowardin et al. (1979) classification scheme, and a customized classification scheme was developed for upland habitat by the U.S. Geological Survey (U.S.G.S.) National Wetlands Research Center (NWRC) with cross-referencing to the Anderson et al. (1976) classification system.

Statistical analyses:

Associations between the historic and current distributions of *B. nebulifer* and *B. fowleri* in low, medium and high disturbance classes were tested with log-linear modeling of the frequencies of three variables: species, time period, and disturbance class (low, medium and high) (Table 2.1). No variables could be specifically identified as dependent or independent; therefore, log-linear modeling was used to analyze interactions between the discrete, categorical variables using the natural logarithm of the cell frequencies within a multi-way contingency table format (Gotelli and Ellison, 2004). An analysis of changes in distribution for *B. fowleri* in sympatry and allopatry was also performed. A saturated model with partial associations was performed and the best model was identified by likelihood-ratio tests (Gotelli and Ellison, 2004).

Species	**Time period**	**Disturbance classes**		
		Low (1)	**Medium(2)**	**High (3)**
B. nebulifer	**historic**			
B. fowleri				
B. nebulifer	**current**			
B. fowleri				

Table 2.1: an example of the data tabulation for the log-linear method

RESULTS

The log-linear analysis of *B. nebulifer* and *B. fowleri* in sympatry in each of the 3 habitat disturbance classes for both time periods found that all three variables were significant and the best model was a 3-way interaction between species, the period, and disturbance classes (Table 2.2, Fig. 2.4). The current distribution of *B. fowleri* has declined significantly compared to its historical distribution while the current distribution of *B. nebulifer* has increased significantly (Fig. 2.4). Furthermore, the near total disappearance of *B. fowleri* coincident with an increase in *B. nebulifer* in high disturbance sites suggests that the spread of *B. nebulifer* in disturbed habitat is driving a decline in *B. fowleri*.

Bufo fowleri declined across all habitat disturbance classes, particularly in highly disturbed habitat (Fig. 2.4). There were 29% (n= 24 historic, 17 current) fewer sites where *B. fowleri* was present in low disturbance habitat in southern Louisiana from the historic to the current time period. In moderate disturbance, *B. fowleri* was found at 30% (n= 10 historic, 7 current) fewer sites currently than historically. An almost complete decline of *B. fowleri* occurred at highly disturbed sites in sympatry with *B. nebulifer*, *B. fowleri* is absent at 96% (n= 45 historic, 2 current) of the high disturbance sites where it was formerly present.

Bufo nebulifer individuals had minor declines in low (n=19 historic, 18 current) and moderate (n=13 historic, 12 current) disturbance sites of 5.2% and 8.3%, respectively (Fig. 2.4). A major increase of 57% in *B. nebulifer* individuals was found in highly disturbed areas. The increase of *B. nebulifer* in highly disturbed areas is inversely related to the decline of *B. fowleri* in highly altered habitat. The slight declines of *B. nebulifer* in low and moderately disturbed habitat probably reflect a decrease in these habitat classes and an increase in highly disturbed sites. The increase in highly disturbed sites occupied by *B. nebulifer* also indicates that as habitat alteration and fragmentation increase, the decline of *B. fowleri* is likely to continue.

The log-linear analysis compared changes in the distribution of *B. fowleri* in sympatry and allopatry in each of the 3 disturbance classes for both time periods, and again found the best model included the 3-way interaction between the species, the time period and the disturbance classes (Table 2.3, Fig. 2.5).

Effect	**df**	**Partial Chi-square**	**Significance**
Species x Time	1	25.814	0.000
Species x Disturbance	2	9.113	0.039
Time x Disturbance	2	1.759	0.256
Species x Time x Disturbance	2	0.000	<0.0001

Table 2.2: Results of log-linear analysis of *B. fowleri* and *B. nebulifer* in 3 different habitat classes from the historic to the current time period.

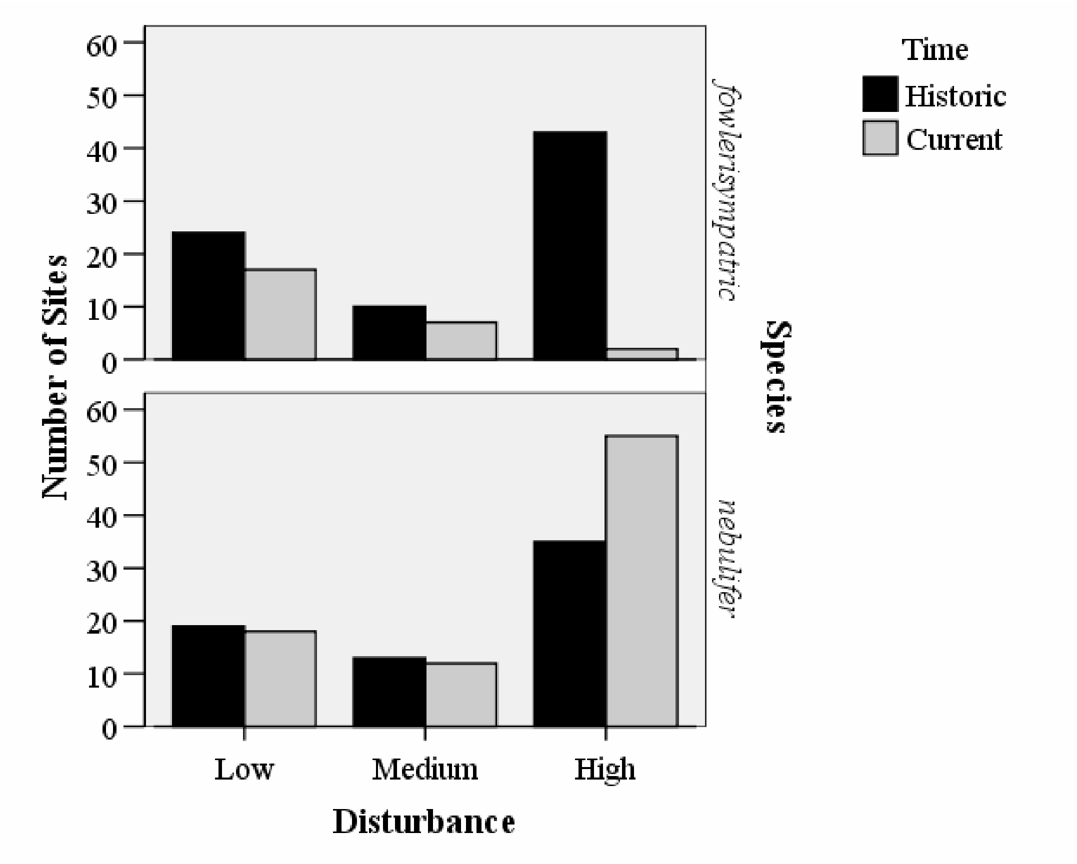

Figure 2.4: Number of sites at which each species was found in each disturbance class in the historic and current time periods.

Effect	**df**	**Partial Chi-square**	**Significance**
Species x Time	1	4.997	0.025
Species x Disturbance	2	7.499	0.024
Time x Disturbance	2	9.428	0.009
Species x Time x Disturbance	2	0.000	<0.0001

Table 2.3: Results of log-linear analysis of *B. fowleri* in allopatry in northern LA in 3 different habitat classes from the historic to the current time period

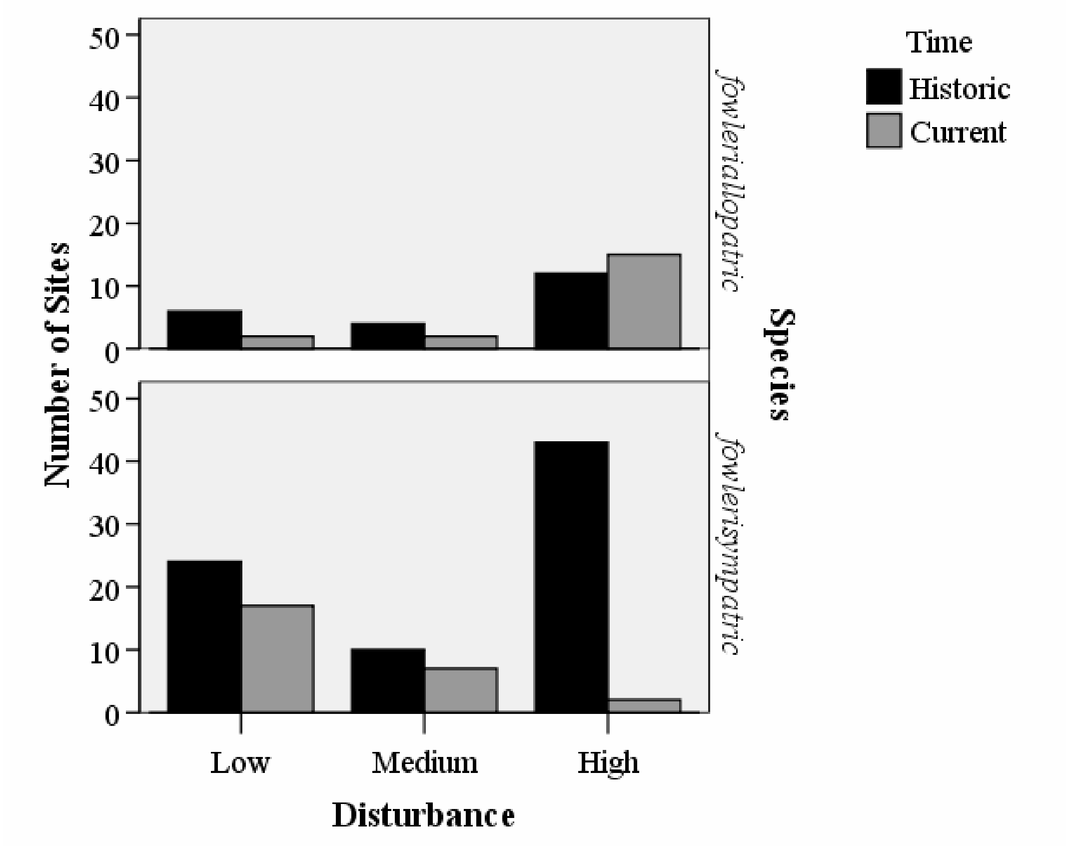

Figure 2.5: Comparison of the number of sites at which allopatric and sympatric populations of *B. fowleri* were found in each disturbance class in the historic and current time periods

In northern Louisiana where *B. fowleri* is allopatric with *B. nebulifer*, 67% (n =6 historic, n=2 current) fewer sites were occupied in low disturbance areas and 50% (n=4 historic, 2 current) fewer sites were occupied in moderately disturbed areas. However, a 25% (n=12 historic, 15 current) increase in presence at highly disturbed sites was found. The decline in occupied sites in low and medium disturbance classes reflects the low sample sizes in Region 4, as well as the transition from lower to higher disturbance classes. This result supports the prediction that *B. fowleri* would be found in all disturbance levels in allopatric environments.

DISCUSSION

The results of the log-linear modeling show that both species' distributions have changed significantly from the original collection period to the present, driven by an increasing transition from low and moderate disturbance to high disturbance sites. This study supports the hypothesis that increased disturbance is contributing to a steep decline in *B. fowleri* via the expansion of its invasive congener, *B. nebulifer*. These findings also demonstrate the utility of historical data integrated with the remote sensing and GIS methods to identify the effects of biotic interactions on a species decline that might otherwise have been thought to result exclusively from changes in landscape-level habitat variables.

An enormous decline in *B. fowleri* has occurred between the historic and current survey periods, concomitant with a surge in the distribution of *B. nebulifer*. These results are particularly pronounced in areas of high disturbance where the two species are sympatric. As predicted, the distribution of *B. fowleri* has contracted significantly in areas of high disturbance and the species is currently found in areas of low to moderate disturbance almost exclusively. No decline was noted in *B. fowleri* in areas of allopatry with *B. nebulifer*, in fact, a current increase in *B. fowleri* in disturbed habitat was observed. This finding strongly supports the conclusion that the decline in *B. fowleri* in sympatry is being driven by the increasing presence of its invasive congener in southern Louisiana.

. As expected, the distribution of *B. nebulifer* in highly degraded habitat has expanded drastically and it maintains its distribution in low and moderately disturbed habitats as well. Although slightly fewer individuals were currently found in these habitat classes; this reflects an increase in landscape disturbance rather than a decrease in their distribution. Though some studies have reported that toads are generally more impervious to the effects of disturbance than other amphibian species (Hecnar and M'Closkey, 1996; Knutson et al., 1999; but see Gibbs et al., 2005), the demonstrated superiority of *B. nebulifer* in disturbed habitat may be more related to its invasive nature. Brown et al. (2006) found that radio-tracked individuals of the highly invasive South American species *B. marinus* preferentially uses cleared habitat and open corridors, such as roads and fencelines to disperse. A study conducted by Petren and Case (1998) of interspecific competition between the invasive common house gecko and the native mourning gecko showed that competitive displacement of the latter species by the former was much more severe in the absence of topographically complex habitat. Petren and Case attributed this finding to differential foraging and agonistic behavior between the species in variably structured habitat.

Although interspecific interactions appear to be contributing greatly to the drastic decline in *B. fowleri*, abiotic factors can influence changes in species' distributions if two species exhibit differences in sensitivity to environmental stressors, or if physical factors affect the outcomes of interspecific interactions. Enhanced susceptibility to years with lower rainfall could result in lower juvenile recruitment for *B. fowleri* than for *B. nebulifer* (Shaffer et al., 1998). Several years of field observations suggests that this explanation is unlikely because the abundance of *B. fowleri* individuals at sites where they were present was equal to the abundance of *B. nebulifer* individuals (L. Vogel, unpublished data).

Habitat alteration resulting from forest clearing may also favor the breeding strategy of tadpoles of *B. nebulifer* over tadpoles of *B. fowleri*. Changes in canopy cover over aquatic breeding sites can have significant effects on amphibian assemblages via diminished light penetration, lower temperatures, lower dissolved oxygen content, and changes in available resources (Werner and Glennemeier, 1999; Skelly et al., 2002; Halverson et al., 2003). In an experiment that compared the survivorship and growth rates of open- and closed-canopy breeding amphibian species, Werner and Glennemeier (1999) found that altering the degree of shading strongly influenced breeding habitat success via interspecific competition in different canopy levels. In a common garden experiment using open-canopy specialists and canopy generalists, Skelly et al. (2002) found the open-canopy species grew substantially faster under open canopy conditions than the canopy generalist, while the canopy generalist species grew much faster in the closed canopy treatment. As a species that thrives in highly ephemeral aquatic habitat, *B. nebulifer* tadpoles may have a competition advantage in disturbed habitat and subsequently diminished canopy cover.

Although studies suggest that landscape factors are more indicative of toad distribution and abundance than local aquatic breeding habitat (Hecnar and M'Closky, 1996; Knutson et al., 1999), aquatic habitat characteristics also may have influenced the decline of *B. fowleri* in degraded habitat. Degeneration of water quality often accompanies disturbance due to increased runoff water from roads, parking lots and sidewalks causing heavy metals, inorganic acids, agrochemicals and other toxic substances to accumulate in nearby water bodies (Davidson et al., 2001; Gibbs et al., 2005, but see Lips, 1998). High concentrations of many toxins are known to impair normal amphibian development, behavior, fitness and survivorship (Hecnar et al., 1995; Davidson et al., 2002).

Contaminants and agricultural chemicals have also been found to differentially affect biotic interactions between amphibians and their predators. Boone and Semlitsch (2001) found significant variability in survival to and mass at metamorphosis for the toad species, *B. woodhousii*, and two species of treefrogs to carbaryl, a common insecticide. Susceptibility to carbaryl affected subsequent competitive interactions between the amphibians and their ability to evade a predatory newt species. Although toad species may differ in their tolerance for contaminants, Rubbo and Kiesecker (2005) found that general water quality did not significantly predict the distribution of either *B. fowleri* or *B. americanus.* Lack of detailed data on water quality at each site precluded including breeding site characteristics in the analyses.

Other causal factors that may significantly affect these two species' distributions, global warming and UV-B radiation, were outside the scope of this research and thus were not tested. Both factors affect amphibian behavior, growth, development and survival, and are expected to shift amphibian distributions toward the poles and higher elevations (Davidson et al., 2002, Araujo et al., 2006; Navas, 2006). Louisiana is the southernmost margin of *B. fowleri*'s range and may represent a physiological impediment to continued dispersal; the current range contraction of *B. fowleri* may simply reflect a response to an increase in global temperature and UV-B radiation. Conversely, *B. nebulifer* may be exploiting a superior tolerance to a rise in temperature and UV-B levels by expanding its range northward. This hypothesis is supported by the findings of Kiesecker et al. (2001) that *B. bufo* has much higher embryonic mortality than its congener, *B. calamita*, at the same level of UV-B radiation. However, the propensity of *B. nebulifer* to breed in shallower sites that are more susceptible to higher levels of UV-B, and the short distance between southern and northern Louisiana, where *B. fowleri* continues to thrive, does not support climate change or increased UV-B levels as primary causes of decline.

This research is novel in several aspects, especially in its incorporation of historical data with remote sensing and GIS to test the hypothesis that environmental disturbance is causing a decline via an interspecific interaction. Former research that has integrated historical and geographic approaches has attempted to disentangle numerous landscape-level environmental and climatic correlates of decline, but has rarely considered biotic factors (Shaffer et al., 1998; Davidson et al., 2001, 2002, Gibbs et al., 2005). Few studies that have addressed spatial components of change in species' distributions have evaluated biotic correlations with abiotic factors (Knutson et al., 1999; Rubbo and Kiesecker, 2005; but see Riley et al., 2005). The recent use of ecological niche modeling to predict species' ranges has only recently introduced biotic variables to landscape level studies (Guisan and Zimmerman, 2000). Consideration of biotic interactions is critical to examining species' distributional changes and mechanisms of decline as demonstrated in this study.

The approach to detecting spatial and temporal change in landscape variables is another novel aspect of this study. The use of multi-source data remote sensing data is becoming increasingly common; however, the use of various types of satellite imagery (e.g. Landsat, SPOT, Quickbird) is currently favored for change detection because of its wide availability and broad coverage (Turner et al., 2003). One serious drawback to using satellite imagery is that data availability is discouragingly recent for scientists and managers who need long-term regional data to contrast historical and present-day land use/land cover with spatial patterns of species distributional changes. The relatively short history of satellite remote sensing permits change detection only within the past 30 years, while historical aerial photographs are available as far back as the 1940's depending on the coverage area. Furthermore, the superior resolution and applicability of aerial photographs for visual interpretation of change detection analysis has been acknowledged, but is underutilized due to the relative difficulty of data acquisition, processing and analysis (Carey et al., 2001).

Finally, successful integration of historical data with landscape-level remote sensing techniques to detect the highly deleterious expansion of an invasive species and subsequent decline of a native species has important conservation applications for threatened and endangered species. Adequate time to conduct critical field and laboratory experiments pertaining to potential mechanisms of decline is critical to halting the spread of the invasive and the loss of the native species. In addition, focusing on changes in the spatial patterns of both biotic and abiotic factors narrows the pool of potential mechanisms of decline to be evaluated.

More thorough determination of the precise mechanism or mechanisms for the displacement of *B. fowleri* by *B. nebulifer* required developing additional predictions and hypotheses that focused on the influence of disturbance and invasiveness. The results of two hypotheses regarding unproductive interspecific hybridization and competitive exclusion are reported in the following two chapters of this dissertation.

Appendix 2.1: Survey sites, species, years and collectors by parish and region (collectors marked with an asterisk were not located or contacted). Descriptions marked in bold are personal observations of particular sites for easier identification.

Region 1: **Baton Rouge metropolitan area**

East Baton Rouge Parish

	Original Species	**Site Location**	**Year**	**Collector**
1	Both species	LSU, Greek Theater	1951	Gandy, B.E.
2	Both species	Melrose Sub. Div	1963	Delahoussaye, A.J.
3	Both species	City Park Lake **east side**	1965	Wilson, L.D.
4	Both species	Campus Lake **south end**	1965	Wilson, L.D.
5	*B. fowleri*	½ mi E on Starring Rd., 5 mi. S of LSU (**Worthington Lake**)	1961	Delahoussaye, A.J.
6	*B. fowleri*	614 Sunset Blvd	1951	*Glasgow, L.E.
7	*B. fowleri*	LSU, Nicholson Apartments	1951	Gandy, B.E.
8	Both species	2 mi. S of LSU on Lee Dr.	1951	Gandy, B.E.
9	*B. fowleri*	Junct. River Road & S Campus Dr	1951	Gandy, B.E.
10	*B. fowleri*	Sherwood Forest Subdivision	1951	Gandy, B.E.
11	*B. fowleri*	Ben Hur Swamp, **LSU Central Research Station**	1951	Dahlquest, W.W.
12	Both species	Hoo Shoo Too Road, 3 mi E La. Hwy. 73	1965	Hahn, D.E.
13	*B. fowleri*	Lee Rd Country Club (**Webb Park & Golf Course**)	1961	Delahoussaye, A.J.
14	*B. fowleri*	Bayou Fountain at Highland Lake	1951	Dahlquest, W.W.
15	*B. fowleri*	Lee High Rd	1963	Wilson, L.D.
16	*B. fowleri*	University Terrace Elementary School	1965	Sanford, G.E.
17	*B. fowleri*	366 Carriage Way	1963	*Keiser, E.D.
18	*B. fowleri*	S Tiger Bend Rd. at bridge (**at Babin Rd**.)	1966	Wilson, L.D.
19	*B. fowleri*	387 Brookhaven Dr. off Highland Rd.	1965	Wilson, L.D.
20	Both species	5 mi NE Indian Mound (**at Amite River**)	1965	Wilson, L.D.
21	Both species	Brookstown Drive, Baton Rouge	1965	Wilson, L.D.
22	Both species & hybrid	University Lake Peninsular	1967	Hahn, D.E.
23	Both species & hybrid	Dawson Cr. at Quail Drive, **behind LDWF building**	1959	McCreedy, E.A.
24	*B. fowleri*	Between Frenchtown Rd and Comite River, 1 mi S, 0.1 mi E of Comite	1952	Dahlquest, W.W.
25	Both species & hybrid	1 mi SW LSU on levee	1951	Gandy, B.E.
26	Both species	1.1 mi. N. 1.3 mi W of Indian Mound	1967	Hollander, P.J.
27	*B. nebulifer*	Wyandotte St. (**Powhatan St.)**	1965	*Dumin, J.

28	*B. nebulifer*	1 mi E of Baton Rouge on **E.** Perkins Rd	1964	Mooney, D.E.
29	*B. nebulifer*	Jct. W. Parker & Gourrier Sts	1961	Reece, R.
30	*B. nebulifer*	2838 Terrace Ave	1959	Harwood, M.
31	Both species	3 mi S of L. S. U on River Rd	1951	Gandy, B.E.
32	Both species	4.0 mi S of L. S. U on River Rd.	1951	Gandy, B.E.
33	*B. nebulifer*	Cocodrie Ave, Old Jefferson Subdivision, Baton Rouge	1968	Wilson, L.D.
34	*B. nebulifer*	Intersection of Brightside Dr. and Nicholson Dr., Baton Rouge	1967	Wilson, L.D.
35	*B. nebulifer*	Forest Park, Baton Rouge	1963	*Keiser, E.D.
36	*B. nebulifer*	S. Tiger Bend Road at Hoo Shoo Too Road	1967	Hahn, D.E.
37	*B. nebulifer*	1812 Potwin Drive	1965	Wilson, L.D.
38	*B. nebulifer*	Gardere Ln. betw. Nicholson Rd. and River Rd.	1967	Hahn, D.E.
39	*B. nebulifer*	620 College Hill Dr.	1951	Dahlquest, W.W.
40	Both species	5 mi SW University - Conrad Pt	1951	Gandy, B.E.
41	Both species	Waddill Wildlife Refuge	1967	Sandford, G.E.
42	Both species	Kendalwood Road within 0.5 miles of Hoo Shoo Too Road	1968	Rekas, A.
43	*B. nebulifer*	St. Gerard Majella School (3655 Majella Street)	1966	Hahn, D.E.
44	Both species	365 Centenary Dr.	1963	*Keiser, E.D.

West Baton Rouge Parish

	Original Species	Site Location	Year	Collector
45	Both species	1.4 mi. W of jct. 623 on La 415	1968	Burke, C.
46	Both species	2 mi. SW Chamberlin on La 983	1968	Rekas, A.
47	Both species	LA 415 b/n US 190 and LA 76 (Rosedale Rd.)	1966	Wilson, L.D.
48	Both species	6 mi. W of Port Allen, 2 mi. E of Rosedale, Hwy 1	1968	Burke, C.

Iberville Parish

	Original Species	Site Location	Year	Collector
49	*B. fowleri*	5.1 mi N of Ramah on inner E levee	1968	Rekas, A.
50	*B. fowleri*	1 mi S of Whiskey Bay Hunt Club	1968	Rekas, A.
51	Both species	Whiskey Bay exit from I-10, in ditch	1965	Kirton, M.P.
52	*B. fowleri*	5 mi N of Whiskey Bay turnoff on I-10, Sherbourne WMA	1968	Rekas, A.
53	*B. fowleri*	Whiskey Bay exit, 0.5 mi S I-10, R. H. Miller Hunting Club	1969	Eberle, G.W.
54	*B. fowleri*	Cypress Flats at Spanish Lake	1964	Wilson, L.D.
55	*B. fowleri*	Bayou Paul Rd, 0.2 mi SE of Pecan Drive	1964	Wilson, L.D.

56	Both species	1631 Pecan Dr., 3.7 mi N St. Gabriel	1964	Wilson, L.D.
57	*Both species*	St. Gabriel Research Station, 1.6 mi N, 0.3 mi E of St. Gabriel	1968	Rekas, A.
58	*B. nebulifer*	Bayou Grosse Tete Rd in Ramah	1964	Wilson, L.D.
59	Both species	0.2 mi N of I-10 on E levee of Atchafalaya basin	1965	Kirton, M.P.
60	Both species	8.4 mi. S of 1-10 at Ramah exit on side of levee	1965	Kirton, M.P.

Region 2: **Atchafalaya River floodway**

Pointe Coupee Parish

	Original Species	Site Location	Year	Collector
61	Both species & hybrid	1.1 W of jct. La. 979 & La. 978	1968	Burke, C.
62	*B. fowleri*	3.7 mi. N of Livonia on La 77	1968	Burke, C.
63	Both species	2 mi. N of US 190 on E. inner levee	1968	Burke, C.

St. Landry Parish

	Original Species	Site Location	Year	Collector
64	Both species	Krotz Springs, 8 mi. NW on LA 71	1950	Shaw, G.
65	Both species	4 mi NW Washington, Thistlethwaite Boy Scout Camp	1967	Boettcher, J.W.
66	Both species	17 mi N Henderson on W levee of Atchafalaya spillway	1965	Wilson, L.D.
67	Both species	957 N. Market, Opelousas	1968	Dyer, A.
68	Both species	US 190 0.5 mi E Port Barre	1961	Dugas, L.J.
69	Both species	9 mi N I-10 Butte La Rose Exit, inner W levee	1964	Gannon, M.J.
70	Both species	5.2 mi N of US 71 on W outer levee	1975	Kirton, M.P.
71	Both species	3.7 mi W Krotz Springs on old US 190	1966	Hahn, D. E.

St. Martin Parish

	Original Species	Site Location	Year	Collector
72	Both species	0.7 mi. from jct LA 351 on LA 321-AOR	1950	Shaw, G.
73	Both species	4.9 mi. E jct. LA 94 on LA 353 (**at St. Martin parish line**)	1957	Boettcher, J.W.
74	Both species	Bayou Teche along bank; 2 mi. E of Breaux Bridge (**off LA 347**)	1955	Wilson, L.D.
75	Both species	1.5 mi. S Lake Dautervive	1968	Rekas, A.
76	Both species	Under I-10 bridge at Butte LaRose exit	1964	Wilson, L.D.
77	Both species	2.4 mi. N jct Henderson levee and LA 347	1965	Eberle, G.W.

Region 3: **Lafayette metropolitan area**
Lafayette Parish

	Species	**Site Location**	**Year**	**Collector**
78	Both species	Girard Park	1965	Thomas, B.
79	Both species	107 & 110 N. Beverly Dr., Lafayette	1963	Benett, R.P.
80	Both species	Cypress Lake, USL campus	1968	Conzelmann, P.J.
81	*B. nebulifer*	111 Deshotel St., Lafayette	1964	Rabeaux, J.
82	*B. nebulifer*	4.2 mi. S of Hwy 90 on Hwy 3095	1965	Thomas, B.
83	*B. fowleri*	Pond N of Municipal Airport, Lafayette	1961	Delahoussaye, A.J.
84	Both species & hybrid	300 St. Julian St., Lafayette	1961	Delahoussaye, A.J.
85	*B. nebulifer*	100 yds. W of Vermillion R. brdg on Hwy 3073	1969	Arceneaux, D.J.
86	*B. fowleri*	303 Marie Antoinette St., Lafayette	1962	Conzelmann, P.J.
87	*B. fowleri*	0.7 mi. N. jct. US 90 on La. 93	1968	Delahoussaye, A.J.
88	*B. fowleri*	Off Moss St. ext., 0.4 mi. S of Mouton Switch Rd.	1968	Sonnier, J.C.
89	Both species	1700 Johnston St., Lafayette	1967	Thomas, B.
90	*B. nebulifer*	Lake Martin, 1.5 mi. SE jct. US 167 on Hwy 3073	1969	Conzelmann, P.J.
91	*B. fowleri*	Pond at rear of 415 N Mall St., Lafayette	1961	Delahoussaye, A.J.
92	*B. fowleri*	Teche Dr., Lafayette	1962	Morgan, E.C.

Region 4: **Monroe metropolitan area**

Ouachita Parish

	Species	**Site Location**	**Year**	**Collector**
93	*B. fowleri*	Swartz-Fairbanks Rd., NW of Swartz	1965	Thomas, R.A.
94	*B. fowleri*	2 mi. off US 165 on LA 553	1963	Tarver, J.W.
95	*B. fowleri*	4 mi. W of jct. 165 & 553 on 553	1968	Conzelmann, P.J.
96	*B. fowleri*	703 Victoria St., Monroe (Clements' residence)	1964	Rabeaux, J.
97	*B. fowleri*	Hwy 134, between Kline and Hwy 165 intersection	1965	Thomas, R.A.
98	*B. fowleri*	Hwy 134 N of Swartz-Fairbanks Rd.	1961	Tarver, J.W.
99	*B. fowleri*	Hwy 134 at Kline (**at Bark Ave.**)	1961	Tarver, J.W.
100	*B. fowleri*	5107 Blank St. Lot 44, Monroe (**at Ransom**)	1969	Tarver, J.W.
101	*B. fowleri*	Southside High School (LTI), in front of "C" cottage, Monroe	1962	Tarver, J.W.
102	*B. fowleri*	Moon Lake, off Moon Lake Rd.	1968	Tarver, J.W.
103	*B. fowleri*	LWFC Office N of Monroe on US 165	1968	Tarver, J.W.
104	*B. fowleri*	Hwy 557 from Hwy34 to Luna	1967	Thomas, B.
105	*B. fowleri*	1004 S. 5th St., Monroe (**at Beuregard**)	1967	Tarver, J.W.
106	*B. fowleri*	College Ave., near NLSC (**Univ. @ McGuire**)	1961	Delahoussaye, A.J.
107	*B. fowleri*	32-D Louis Lock Homes (**at Winnsboro**)	1962	Morgan, E.C.
108	*B. fowleri*	ULM campus		
109	*B. fowleri*	OPJHS on Nutland Rd.		
110	*B. fowleri*	W of Bayou Oaks Dr., Bayou Desiard		
111	*B. fowleri*	Bon Air Dr., Monroe		
112	*B. fowleri*	6 mi. S of jct. LA 841 1-1.5 mi. E of New Light Church		
113	*B. fowleri*	Loop Rd., Monroe		
114	*B. fowleri*	324 Kentucky St., Monroe		

Appendix 2.2: Survey and geographical information for each site. Use numbers to refer to Appendix 2.1 for exact location. Information was inadvertently lost for sites with missing geographical locations. Refer to author for geographical coordinates of each site.

Aerial photos	**Index number**	**DOQQ ID & Quad**	**NWI ID**	**yrs. surv.**	***fowleri*** **historical**	***nebulifer*** **historical**	***fowleri*** **current**	***nebulifer*** **current**
East Baton Rouge								
1.CQF-7T-8	EBR (3) - 1957	West B.R. SE		2005-2006	x	x		x
2. CQF-4T-148	EBR (3) - 1957	West B.R. NE	West B.R.	2005-2006	x	x		x
3. CQF-7T-9	EBR (3) - 1957	West B.R. SE	West B.R.	2004-2006	x	x		x
4. CQF-7T-8	EBR (3) - 1957	West B.R. SE	West B.R.	2004-2006	x	x		x
5. CQF-4T-153	EBR (3) - 1957	St. Gabriel NW/ EBR SW	St. Gabriel/ EBR	2005-2006	x			x
6. CQF-4T-165	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006	x			x
7. CQF-7T-8	EBR (3) - 1957	West B.R. SW	West B.R.	2005-2006	x			x
8. CQF-7T-6	EBR (3) - 1957	West B.R. SW	West B.R.	2005-2006	x	x		x
9. CQF-7T-8	EBR (3) - 1957	West B.R. SW	West B.R.	2005-2006	x			x
10. CQF-4T-51	EBR (4) - 1957	East B.R. SE	East B.R.	2005-2006	x			x
11. CQF-7T-6	EBR (3) - 1957	Plaquemines NE/ WBR SE	Plaquemines/ WBR	2005-2006	x			x
		Prairieville NW	Prairieville	2005-2006	x	x		x
12. CQF-4T-169	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006	x			x
13. CQF-4T-58	EBR (4) - 1957	St. Gabriel NE/NW	St. Gabriel	2004-2006	x			x
14. CQF-4T-167	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006	x			x
15. CQF-7T-9	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006	x			x
16. CQF-4T-166	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006	x			x
17. CQF-3T-110	EBR (4) - 1957	Denham Springs SW	Denham Springs	2005-2006	x			x
18. CQF-4T-166	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006	x			x
19. CQF-3T-42	EBR (1) - 1957	Watson NW/NE	Watson	2005-2006	x	x	x	x
20. CQF-4T-146	EBR (3) - 1957	West B.R. NE	West B.R.	2005-2006	x	x		x
21. CQF-7T-8	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006	x	x		x
22. CQF-4T-152	EBR (3) - 1957	West B.R. SE	West B.R.	2004-2006	x	x		x
23. CQF-3T-164	EBR (1) - 1957	East B.R. NE	East B.R.	2004-2006	x		x	x
24. CQF-5T-71	EBR (3) - 1957	West B.R. SW	West B.R.	2004-2006	x	x		x
25. CQF-6T-62	EBR (1) - 1957	Watson NW	Watson	2005-2006	x	x	x	x

26. CQF-7T-13	EBR (3) - 1957	West B.R. NE	West B.R.	2005-2006		x		x
27. CQF-3T-155	EBR (4) - 1957	St. Gabriel NE	St. Gabriel	2004-2006		x		x
28. CQF-7T-7	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006		x		x
29. CQF-7T-10	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006		x		x
30. CQF-5T-69	EBR (3) - 1957	Plaquemines NW	Plaquemines	2004-2006	x	x		x
31. CQF-5T-68	EBR (3) - 1957	Plaquemines NW	Plaquemines	2004-2006	x	x		x
32. CQF-3T-156	EBR (4) - 1957	St. Gabriel NE/East B.R. SE	St. Gabriel/EBR	2005-2006		x		x
33. CQF-7T-6	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006		x		x
34. CQF-3T-160	EBR (4) - 1957	East B.R. SE	East B.R.	2005-2006		x		x
35. CQF-3T-113	EBR (4) - 1957	Prairieville NW	Prairieville	2004-2006		x		x
36. CQF-4T-66	EBR (4) - 1957	St. Gabriel NW	St. Gabriel	2005-2006		x		x
37. CQF-4T-163	EBR (3) - 1957	Plaquemines NE/ West B.R. SE	Plaquemines	2005-2006		x	x	x
38. CQF-4T-166	EBR (3) - 1957	West B.R. SE	West B.R.	2005-2006		x		x
39. CQF-5T-68	EBR (3) - 1957	Plaquemines NW	Plaquemines	2004-2006	x	x	x	x
41. CQF-4T-48	EBR (4) - 1957	East B.R. NE	East B.R.	2005-2006	x	x	x	x
42. CQF-3T-137	EBR (4) - 1957	Prairieville NE	Prairieville	2004-2005	x	x	x	x
43.								
44.								
West Baton Rouge								
45. CQN-5T-161	WBR - 1959	Walls SE	Walls	2006	x	x	x	
46. CQN-1T-48	WBR - 1959	Walls NW	Walls	2006	x	x	x	x
47. CQN-1T-78	WBR - 1959	Erwinville SE/SW	Erwinville	2006	x	x		x
48.				2005-2006		x		
Iberville								
48. CQH-3T-79	Iberville(1)-1957	Maringouin SE	Maringouin	2004-2006	x		x	x
50. CQH-3T-143	Iberville(1)-1957	Butte LaRose NE	Butte LaRose	2005-2006	x		x	x
51. CQH-3T-143	Iberville(1)-1957	Butte LaRose NE	Butte LaRose	2004-2006	x	x		x
52. CEL-8H-68	St. Martin (1)-1951	Maringouin NW SE	Maring NW	2004-2006	x		x	x
53. CQH-3T-143	Iberville(1)-1957	Butte LaRose NE/Cow Bayou NW	Butte LaRose/Cow Bayou	2005-2006	x		x	x
54. CQE-1T-119	Iberville(2)-1957	St. Gabriel NE	St. Gabriel	2005-2006	x		x	x
55. CQH-1T-162	Iberville(2)-1957	St. Gabriel NW	St. Gabriel	2005-2006	x		x	x
56. CQH-1T-162	Iberville(2)-1957	St. Gabriel NW/SW	St. Gabriel	2005-2006	x	x		x
57. CQH-1T-197	Iberville(2)-1957	St. Gabriel SW	St. Gabriel	2005-2006	x	x		x
58. CQH-3T-3	Iberville(1)-1957	Gross Tete SE	Gross Tete	2005-2006		x		x

59. CQH-3T-80	Iberville(1)-1957	Maringouin SE	Maringouin	2005-2006	x	x	x	x
60. CQH-3T-85	Iberville(1)-	Cow Bayou NE/SE	Cow Bayou	2005-2006	x	x	x	x
Point Coupee								
61. CQL-2HH-270	PC (4) - 1966	Fordoche NE/SE	Fordoche	2006	x	x	x	x
62. CQL-1HH-249	PC (4) - 1966	Fordoche NW/SW	Fordoche	2006	x	x	x	x
63. CQL-1HH-49	PC (3) - 1966	Lottie NW/SW Krotz Springs NE/SE	Lottie/Krotz Springs	2006	x	x	x	x
St. Landry								
64. CEK-1DD-61	St. Landry(1)-1963	Port Barre NE	Port Barre	2006	x	x	x	x
65. CEK-4DD-54	St. Landry(2)-1963	Opelousas NE	Opelousas	2006	x	x	x	x
66. CEK-1DD-127	St. Landry(5)-1963	Portage NW/ Krotz Springs SW	Portage/ Krotz Springs	2006	x	x	x	
67. CEK-4DD-123	St. Landry(4)-1963	Opelousas SW	Opelousas	2006	x	x	x	x
68. CEK-4DD-50	St. Landry(4)-1963	Opelousas SE	Opelousas	2006	x	x	x	x
69. CEL-6H-20	St. Martin(2)-1951	Arnaudville SE	Arnaudville	2006	x	x		x
70. CEL-7H-155	St. Martin(1)-1951	Maringouin NW SW	Maring NW	2006	x	x	x	x
71. CEK-1DD-99	St. Landry(5)-1963	Krotz Springs NW/SW	Krotz Springs	2006	x	x	x	x
St. Martin								
72. CEL-6H-93	St. Martin(3)-1951	Broussard NE/Parks NW	Broussard/Parks	2006	x	x	x	x
73. CEL-11H-171	St. Martin(3)-1951	Broussard NW	Broussard	2006	x	x		x
74. CEL-6H-95	St. Martin(3)-1951	Breaux Bridge SE/Broussard NE	Breaux Bridge/Broussard	2006	x	x		x
75. CEL-8H-120	St. Martin(4)-1951	Jackass Bay NW SW/ Loreauville NE SE	Jackass Bay/Loreauville	2006	x	x	x	x
76. CEL-7H-158	St. Martin(1)-1951	Butte LaRose NW	Butte LaRose	2006	x	x		x
77. CEL-7H-39	St. Martin(2)-1951	Portage SE/SW	Portage	2006	x	x	x	x
Lafayette								
78. CEI-2DD-94	Lafayette (3) - 1963	Lafayette NE	Lafayette	2006	x	x		x
79. CEI-2DD-93	Lafayette (3) - 1963	Lafayette NE/SE	Lafayette	2006	x	x		x
80. CEI-2DD-94	Lafayette (3) - 1963	Lafayette NE	Lafayette	2006	x	x		x
81. CEI-2DD-116	Lafayette (1) - 1963	Carencro SE/ Breaux Bridge SW	Carencro/Breaux Bridge	2006		x		x

82. CEI-2DD-123	Lafayette (3) - 1963	Lafayette NE/SE	Lafayette	2006		x		x
83. CEI-2DD-122	Lafayette (3) - 1963	Broussard NW/Lafayette NE	Broussard/Lafayette	2006	x			x
84. CEI-2DD-94	Lafayette (3) - 1963	Lafayette NE	Lafayette	2006				x
85. CEI-2DD-91	Lafayette (3) - 1963	Lafayette SE	Lafayette	2006		x		x
86. CEI-2DD-36	Lafayette (3) - 1963	Lafayette NE	Lafayette	2006	x			x
87. CEI-2DD-38	Lafayette (3) - 1963	Lafayette SW	Lafayette	2006	x			x
88. CEI-2DD-118	Lafayette (1) - 1963	Carencro SE/ Breaux Bridge SW	Carencro/Breaux Bridge	2006	x			x
89.				2006	x	x		
90.				2006	x	x		
91.				2006	x			
92.				2006	x			
Ouachita								
93. CQK-9G-116	Ouachita (2) -1951	Monroe N NE	Monroe N	2006	x		x	
94. CQK-8G-196	Ouachita (2) -1951	Monroe N NE/NW	Monroe N	2006	x		x	
95.				2006	x			
96. CQK-8G-200	Ouachita (2) -1951	Monroe N SE	Monroe N	2006	x		x	
97. CQK-8G-189	Ouachita (2) -1951	Rocky Branch SE/ Sterlington SW	Rocky Branch/Sterlington	2006	x			
98. CQK-9G-118	Ouachita (2) -1951	Monroe N NE	Monroe N	2006	x		x	
99. CQK-8G-190	Ouachita (2) -1951	Rocky Branch SE/ Sterlington SW	Rocky Branch/Sterlington	2006	x		x	
100. CQK-11G-15	Ouachita (4) - 1951	W Monroe S NE/ W Monroe N SE	W Monroe S & N	2006	x		x	
				2006	x			
101. CQK-8G-68	Ouachita (2) -1951	Rocky Branch SE	Rocky Branch	2006	x		x	

102. CQK-8G-195	Ouachita (2) -1951	Monroe N NE/NW	Monroe N	2006	x	x
103. CQK-12G-67	Ouachita (3) -1951	W Monroe S SE/SW	W Monroe S	2006	x	x
104. CQK-8G-202	Ouachita (4) -1951	Monroe S NW	Monroe S	2006	x	x
105. CQK-8G-199	Ouachita (2) -1951	Monroe N NE/NW	Monroe N	2006	x	x
106. CQK-8G-202	Ouachita (4)-1951	Monroe S NW	Monroe S	2006	x	x
107. CQK-8G-200	Ouachita (2) -1951	Monroe N SE	Monroe N	2006	x	x
108. CQK-9G-24	Ouachita (4) -1951	Monroe S NW/NE	Monroe S	2006	x	x
109. CQK-9G-112	Ouachita (2) -1951	Monroe N NE/ Swartz NW	Monroe N/Swartz	2006	x	x
110.				2006	x	
111.				2006	x	
112. CQK-8G-199	Ouachita (2) -1951	Monroe N NE/NW	Monroe N	2006	x	x
113. CQK-8G-77	Ouachita (2) -1951	W Monroe N NW/SW	W Monroe N	2006	x	x
114.				2006	x	x

CHAPTER 3: ESTIMATION OF HYBRIDIZATION AND INTROGRESSION FREQUENCY IN TOADS (GENUS: BUFO) USING DNA SEQUENCE VARIATION AT MITOCHONDRIAL AND NUCLEAR LOCI

INTRODUCTION

Hybridization that occurs as a result of human activities may contribute to the loss of rare species through interbreeding with common ones (Rhymer and Simberloff, 1996; Allendorf et al., 2001). Dissolution of ecological barriers to gene exchange and the decrease of suitable breeding habitat sites can predispose amphibian species to hybridization resulting from human habitat disturbance (Blair, 1941; Lamb and Avise, 1986; Schlefer et al., 1986; Sullivan, 1986; Gollman, 1996; Riley et al., 2003). Anthropogenic hybridization between amphibian species has been implicated in the decline of at least one of the parental species in several instances, and is particularly harmful when mating between males of one species and females of the other species occurs more frequently than does the reciprocal pairing (Lamb and Avise, 1986; Sullivan, 1986, Malmos et al., 2001). Wastage of reproductive effort results when hybrid offspring are inviable, sterile or maladapted to the parental niches (Kruuk et al., 1999; Burke and Arnold, 2001).

Intermediate characteristics have been used to identify putative hybrids; however, hybrids that are indistinguishable from the parental forms or the products of frequent backcrossing can render these methods ineffective (Jones, 1973; Gerhardt et al., 1980, Masta et al., 2002). Molecular methods combining both nuclear and mitochondrial markers can accurately identify cryptic individuals and their maternal ancestry, and detect the extent and direction of introgression (Belfiore et al., 2003; Kulikova et al., 2004).

Hybridization between Fowler's Toad (*Bufo fowleri*) and the Coastal Plain Toad (*B. nebulifer*) has been reported in museum records and literature since the mid-twentieth century throughout their sympatric range in southeastern Louisiana (Orton, 1951; Liner, 1954; Volpe, 1956, 1960). *Bufo fowleri* is among the *B. americanus* complex that includes several taxa that are variously treated as species or subspecies (Meacham, 1962; Blair, 1963; Jones, 1973; Sullivan et al., 1996, Masta, 2002). Several members of this complex, including *B. fowleri, B. terrestris, B. woodhousii* and *B. velatus*, have partially overlapping ranges in the central and southeastern United States (Fig. 3.1 A & B), and hybridization has been documented between these species despite apparent mechanisms of intrinsic and extrinsic reproductive isolation (Blair, 1941; Cory and Manion, 1955; Volpe, 1956; Meacham, 1962). Different habitat preferences are a strong ecological barrier to hybridization between sympatric species of toads, but may be disrupted by anthropogenic habitat modification (Cory and Manion, 1955; Blair, 1963; Brown, 1971; Malmos et al. 2001). In general, Fowler's Toad is associated with open meadows and

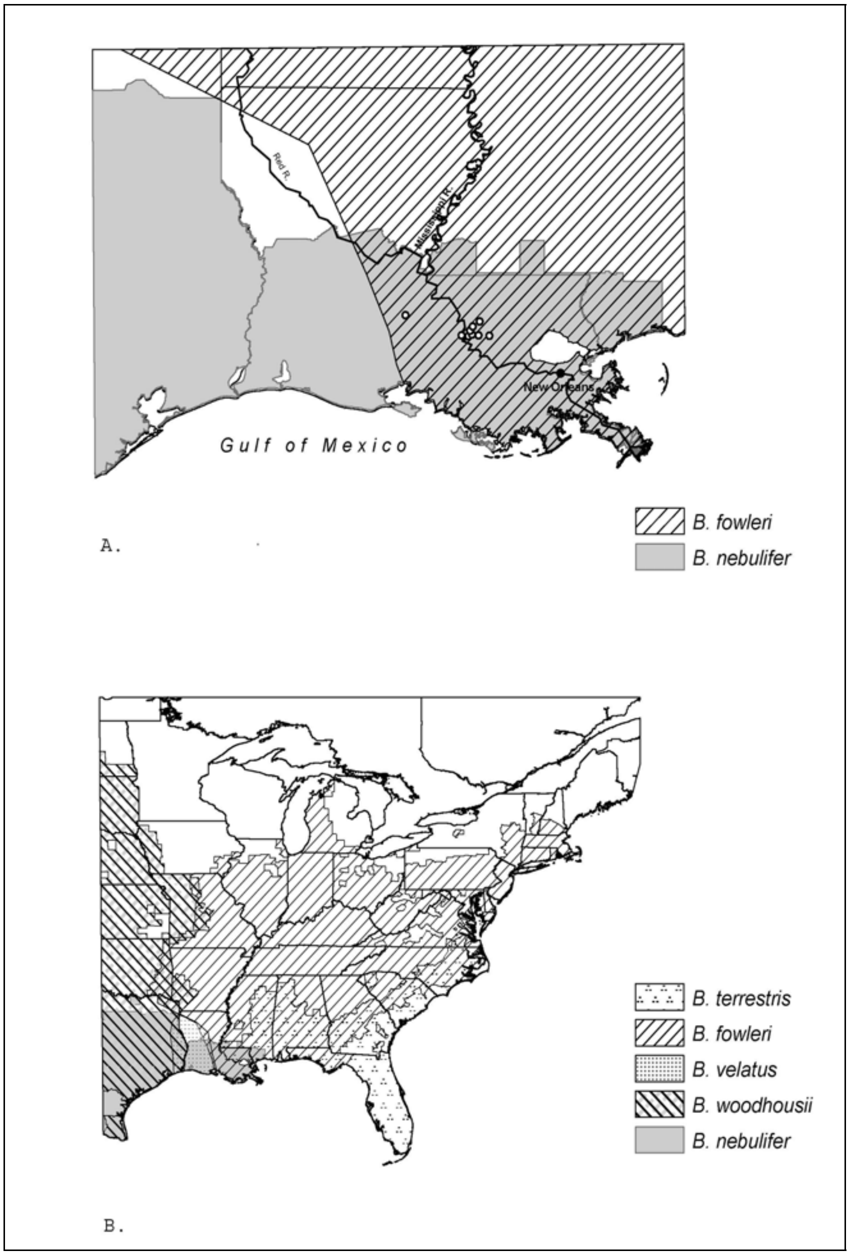

Figure 3.1: A. Ranges of *B. nebulifer* and *B. fowleri* in Louisiana; circles represent collecting localities. B. Ranges of five *B. americanus* group species in the eastern United States

deciduous woodlands with loose, sandy soils, though it is capable of breeding in moderately disturbed habitat as well (Cory and Manion, 1955; Volpe, 1955; Dundee and Rossman, 1989; Sullivan et al., 1996; Conant and Collins, 1998).

The Coastal Plain Toad (*B. nebulifer,* formerly *B. valliceps*) is the sister species of the Mesoamerican species, *B. valliceps* (Mulcahy and Mendelson, 2000). *Bufo nebulifer* shares many ecological attributes with its sister species, including a habitat preference for urban and agricultural areas and areas of secondary growth, and the ability to rapidly colonize disturbed areas and expand its range in the wake of anthropogenic deforestation (Mendelson 1998, 1999; Mulcahy and Mendelson, 2000). Ecological isolation due to habitat preferences of *B. fowleri* and *B. nebulifer* probably limited historical hybridization between the two species. However, habitat alteration of southern Louisiana over the past century has provided an ideal corridor and habitat for a rapid, large-scale expansion of *B. nebulifer*, resulting in increased contact and hybridization between the native *B. fowleri* and the putatively invasive *B. nebulifer*.

Unproductive hybridization precipitated by the range expansion of *B. nebulifer* into the human-altered habitat of southeastern Louisiana may have contributed to the disappearance of *Bufo fowleri* in the New Orleans metropolitan area and neighboring parishes. Historical collections of vouchered specimens at the Louisiana State University Museum of Natural Science (LSUMZ) indicate that apparent hybrids composed almost 8% of the pre-1960 *Bufo* populations in Orleans and Jefferson Parishes. *Bufo fowleri* composed 31% of the same population; however, no *B. fowleri* individuals are present in any of the state herpetological collections for either parish since 1969 (personal communication with J. Boundy). Volpe (1960) observed the frequency of hybrids and interspecific mating in New Orleans from 1956 to 1958 and found that while hybrids composed about 2% of the total population, 31% of female *B. fowleri* were mispaired with male *B. nebulifer* or hybrids compared to only 7% of mispaired female *B. nebulifer*. Furthermore, Volpe observed that F_1 hybrids were more often mated with females of *B. fowleri* than with females of *B. nebulifer*. Asymmetric hybridization between male *B. nebulifer* or F_1 hybrids and female *B. fowleri* may have occurred more frequently as habitat disturbance facilitated range expansion of *B. nebulifer*, and the concomitant difficulty of finding conspecific mates by female *B. fowleri*. The effects of unproductive mating between male *B. nebulifer* and female *B. fowleri* would have been especially deleterious to Fowler's Toads because males are capable of fertilizing multiple clutches per breeding season, while females may potentially waste their entire reproductive effort for that breeding season (Volpe, 1960).

Laboratory crosses of male *B. nebulifer* and female *B. fowleri* (and, apparently, other species of the *B. americanus* complex) resulted in no female offspring and males that are presumed to be sterile, yet are larger than both parental species and active participants in breeding choruses (Blair, 1959; Volpe, 1956, 1960). Hybrids are therefore expected to be almost entirely F_1 males, though a negligible possibility of introgression exists since complete sterility of male hybrids cannot be confirmed. Reciprocal crosses between female *B. nebulifer* and male *B. fowleri* are thought to be completely inviable because no offspring of this pairing survived through early development in the laboratory (Blair, 1959; Volpe, 1956, 1960).

Conflicting descriptions of putative bufonid hybrids as being readily identifiable (Volpe, 1956, 1960) or virtually indistinguishable from one of the parental species (Thornton, 1955; Jones 1973; Masta et al., 2002) provide an ideal opportunity to apply molecular methods to identify hybrids between *B. fowleri* and *B. nebulifer.* Single nucleotide polymorphisms (SNPs) are abundant in many species' genomes and there is enormous potential for application in ecological, evolutionary and conservation research (Brumfield et al., 2003; Morin et al., 2004). Because SNPs are almost always bi-allelic genetic markers, they can be used to identify hybrids based on the presence of two different alleles at loci where the species are polymorphic (Gaskin and Schaal, 2002; Belfiore et al., 2003).

A primary objective of this study was to determine whether a fragment of a nuclear intron would provide adequate sequence variation to identify cryptic hybrids of *B. nebulifer* and *B. fowleri*. In contrast to the biparentally inherited SNPs, the non-recombining mitochondrial DNA (mtDNA) genome is maternally-inherited and can be used to determine the maternal ancestry of hybridizing organisms. Therefore, we used mitochondrial sequence variation to identify the female lineage of hybrids. Furthermore, hybridization and introgression involving a directional mating bias can result in the disproportionate acquisition of one species' mtDNA by F_1 and later-generation hybrids, without a simultaneous co-occurrence in the nuclear DNA. If such a bias is occurring or has occurred historically, the mtDNA of one species could appear in an individual that phenotypically resembles another species or an indistinguishable backcross (Lamb and Avise, 1986). For this reason, we also examined mtDNA sequence variation to determine whether frequency or asymmetry of hybridization and introgression between female *B. fowleri* and male *B. nebulifer* would be discernable at levels that could have contributed to a decline in Fowler's Toad. Finally, this study sought to resolve phylogenetic relationships among *B. fowleri* and its close relatives in the *B. americanus* species complex using the mtDNA sequence variation.

MATERIALS AND METHODS

Taxon sampling

Tissue samples from 72 individuals of *B. nebulifer* and *B. fowleri* were collected from 9 breeding populations in East Baton Rouge and Iberville parishes during the 2004 mating season from April to early July (Appendix 3.1). The sampling encompassed metropolitan Baton Rouge, the largest urban area where both species are still sympatric in Louisiana, and surrounding suburban and rural habitat as well. Of these 72 individuals, 40 specimens (2 females, 38 males) were identified as *B. nebulifer* in the field, 1 was identified as a male hybrid and 31 (6 females, 25 males) were identified as *B. fowleri*.

Identification of species and putative hybrids was made in the field by observing the presence or absence of several diagnostic morphological characteristics (Fig. 3.2 A-H). Traits used to distinguish *B. fowleri* were: dark blotches with three or more warts per blotch on the back and elongated, kidney-shaped parotoid glands (Dundee and Rossman, 1989; Conant and Collins, 1998). Diagnostic characteristics of *B. nebulifer* were: triangular parotoid glands, high cranial crests that create a deep valley between the eyes and a distinctive light-colored lateral line (Dundee and Rossman, 1989; Conant and Collins, 1998). Both species have distinctive advertisement calls that also were used to support morphological identifications in many cases. Putative hybrid individuals were distinguished by abnormal cranial crest morphology and a heavily pigmented ventral side. The middle toe on the left foot was clipped from each specimen collected in the field for a DNA sample and kept frozen until used for molecular analyses.

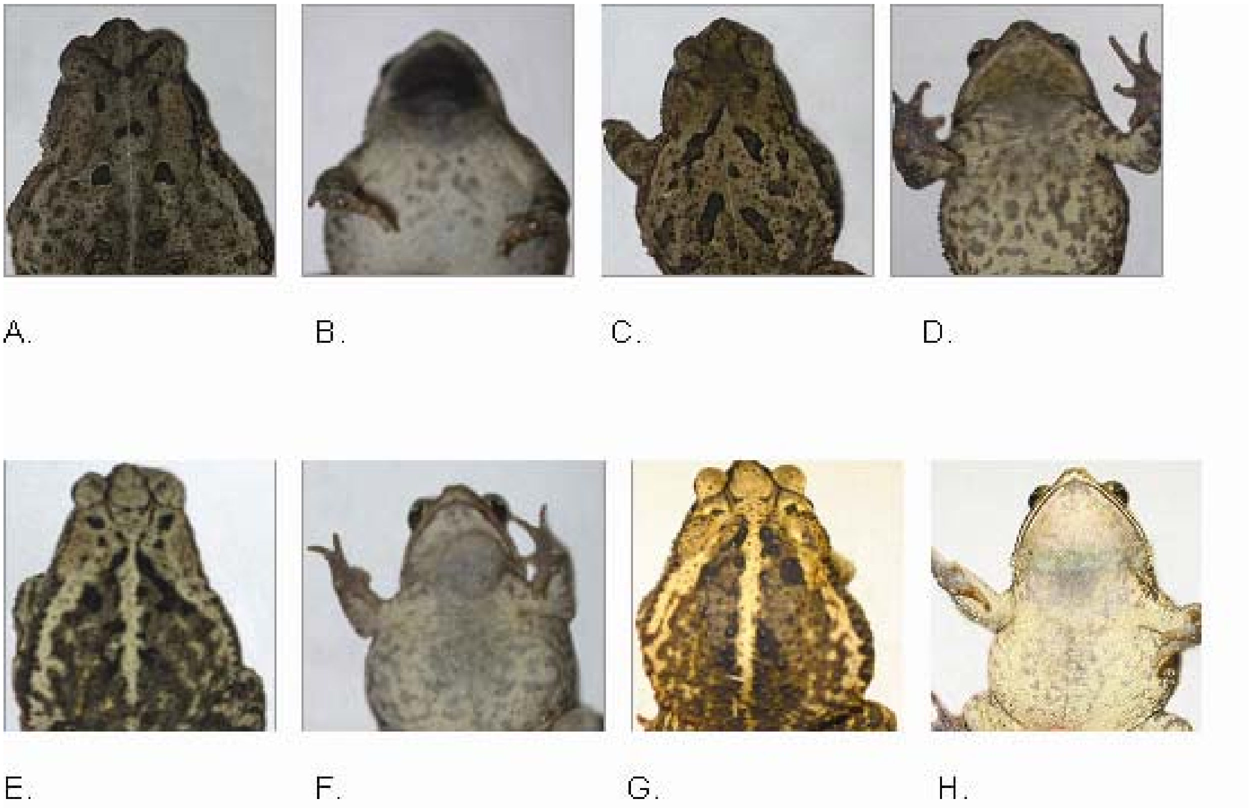

Figure 3.2 (l-r): A**.** Dorsal side of a male *B. fowleri* collected in East Baton Rouge Parish (EBR) B. Ventral side of a male *B. fowleri* C. Dorsal side of a male hybrid, female *B. fowleri* x male *B. nebulifer*, collected in EBR D. Ventral side of a male hybrid E. Dorsal side of a male *B. nebulifer* collected in EBR F. Heavily-spotted ventral side of a male *B. nebulifer* G. Dorsal side of a male hybrid, female *B. nebulifer* X male *B. fowleri*, collected in Iberville Parish H. Ventral side of a male hybrid

Specimens of *B. fowleri*, *B. nebulifer*, *B. terrestris*, *B. velatus*, and *B. woodhousii* from three other sources were used to expand the geographic range of the sampling area and to examine relationships among taxa in the *B. americanus* group complex (Appendix 3.1). Sixteen frozen heart and liver tissue samples collected from specimens in Louisiana, Alabama, Florida and Mississippi were obtained from the Herpetological Collection at the Museum of Natural Science at Louisiana State University (LSUMZ). Muscle tissue preserved in 70% EtOH from *B. velatus* and *B. woodhousii* from east Texas was provided by B. Fontenot. Five *B. terrestris* specimens collected in Louisiana during the 2003 breeding season were also included in the analyses.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from frozen toe, heart and liver tissue and EtOH-preserved muscle tissue with the DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA). Primers that were originally developed to amplify a rhodopsin intron 1 in *Hyla chrysocelis* were used to amplify 333 base pairs (bp) in all five *Bufo* species. Amplification using the forward primer, I1-U, modified from Hoegg et al. 2004 (5'- AACGGAACAGAAGGCCCAAACTT- 3') and an unpublished reverse primer I1-L (5'- GCCAAAGCCATGATCCAGGTGA- 3') developed by Holloway and Cannatella, was performed using the following thermocycle protocol: 94^{o}C for 2 min; 30 cycles of 94^{o}C for 1 min; 59^{o}C for 45 sec; 72^{o}C for 1.5 min; and a final elongation step at 72^{o}C for 8 min. Amplified products were purified from 1% agarose gel slices using a Geneclean II kit (Qbiogene, Irvine, CA). Cycle sequencing reactions were completed with ABI Prism BigDye Terminator chemistry Version 1 (Applied Biosystems, Foster City, CA), purified with Sephadex G-50 (S-6022 Sigma, St. Louis, MO) in Centrisep columns (CS-901 Princeton Separations, Princeton, NJ) and analyzed with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequences were aligned and edited using the program SEQUENCHER 4.1 (Gene Codes, Ann Arbor, MI) and then rechecked by eye.

Extraction and amplification of the mtDNA sequence followed the same protocol that was used for the intron, with the exception of the primers and a change in the annealing temperature from 58^{o}C to 59^{o}C. We amplified 449 bp of the mitochondrial 12S and16S rRNA genes using primers that correspond to positions 2968-2988 and 3623-3642 of *Xenopus laevis* (Pauly et al., 2004). Sequences were aligned and edited using SEQUENCHER 4.1 and rechecked by eye.

Sequence analysis

A 333 bp sequence of the rhodopsin intron was generated from 91 individuals. Of the total specimens that were sequenced, 39 specimens of *B. nebulifer*, 2 putative hybrids and 30 specimens of *B. fowleri* were collected from nine populations represented during active breeding choruses; four of these were mixed choruses of both *B. nebulifer* and *B. fowleri*. Seven *B. fowleri*, 7 *B. velatus*, 1 putative hybrid, 3 *B. terrestris*, 1 *B. nebulifer* and 1 *B. woodhousii* specimens from sources mentioned previously were included in the 91 individuals that were sequenced.

SNPs in the intron were used to identify putative F_1 hybrids based on heterozygosities at species-specific sites; the additional specimens were sequenced to insure that species-specific polymorphisms from *B. nebulifer* and *B. fowleri* are geographically conserved and not unique to the collection area in the two Louisiana parishes. Reconstruction of gametic phase for the twelve heterozygous sites using known haplotypes was performed using PHASE 2.1 (Stephens et al., 2001; Stephens and Donnelly, 2003). The 91 sequences were collapsed to five unique haplotypes using the program Collapse 1.2 (available from http://darwin.uvigo.es). Among-population nucleotide diversity was calculated for *B. fowleri* and *B. nebulifer* using DnaSP 3.0 (Rozas and Rozas, 1999). A haplotype network was constructed using TCS 1.13 (Clement et al., 2000) to show the relative frequencies of and relationships between haplotypes of *B. nebulifer* and *B. fowleri* and the other *B. americanus* group species.

A 449 bp mtDNA fragment was sequenced from 43 individuals. Eight overlapping sequence fragments from published GenBank sequences (Pauly et al., 2004) of *B. velatus, B. woodhousii, B. fowleri* and *B. terrestris* were also included in the analysis to expand the geographical sampling. In total, sequences from 51 individuals were analyzed.

Phylogenetic relationships among mitochondrial haplotypes were reconstructed using maximum likelihood in PAUP* 4.01b (Swofford, 2001). Selection of GTR+I+G as the best-fitting, simplest model of sequence evolution was based on hierarchical likelihood-ratio tests performed in MrModeltest 2.2 (Nylander, 2004). This model of sequence evolution and its parameter estimates were used to perform a heuristic maximum-likelihood search with 10 stepwise addition sequence replicates and TBR branch swapping in PAUP*. MrBayes (Huelsenbeck, 2000) was used to estimate the clade support by running four Markov chains for 5,000,000 generations, sampling once every 5,000 generations. A burn-in of 500,000 generations was used because maximum likelihood tree scores stabilized around this number of generations. The remaining trees were imported into PAUP*, and summarized using a majority-rule consensus tree to estimate the posterior probabilities of particular clades.

RESULTS

A total of twelve SNPs were found between *B. nebulifer* and *B. fowleri*. Three individuals from East Baton Rouge (RR3-7 and 19044) and Iberville (SH-10) parishes were conclusively identified as hybrids due to presence of two different nucleotides, manifested as double peaks, at the species-specific sites (Fig. 3.3). Reconstruction of gametic phase for the twelve heterozygous sites yielded unambiguous haplotypes for the three hybrids. The two divergent sequences found in each diploid hybrid represent a different haplotype inherited from each parental species.

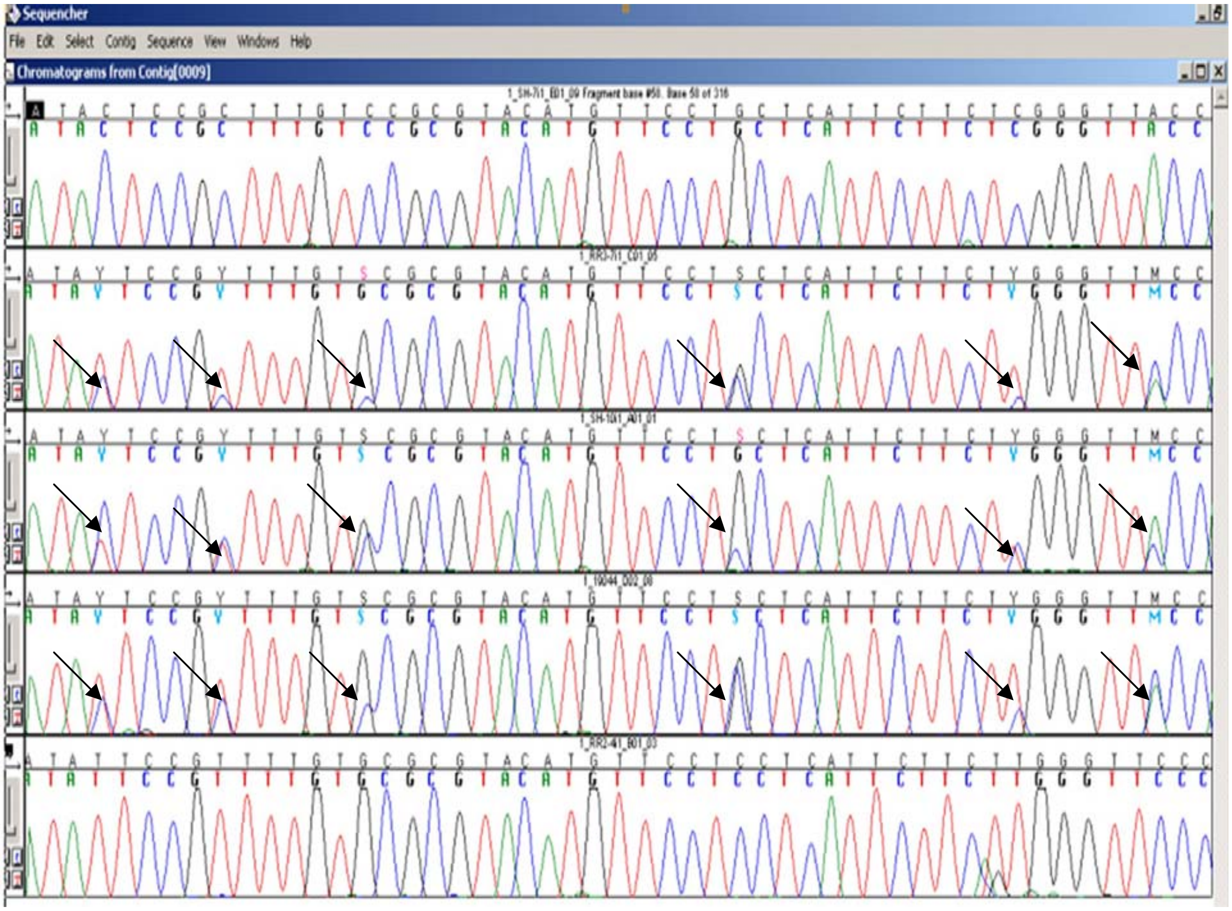

Figure 3.3: Screenshot of intron sequence alignments in Sequencher to demonstrate the method for identification of hybrids. The individual at the top (SH-7) is a *B. nebulifer*, the three individuals in the middle (RR3-7, SH-10 and 19044, in descending order) are hybrids and the individual at the bottom (RR3-4) is a *B. fowleri*. The hybrids are heterozygous for the two parental alleles at multiple species-specific polymorphic sites (six sites are pictured here).

The haplotype network illustrates an 11 bp divergence between the sole haplotype of *B. nebulifer* and the closest haplotype of *B. fowleri* (Fig. 3.4). A total of three haplotypes were detected for 43 *B. fowleri* and were shared by other species of the *B. americanus* group. The most common haplotype was shared by all four *B. americanus* group species that were sequenced and two of the hybrids. The second and third most common haplotypes were divergent only with respect to a single C to T transition from the other haplotypes. The final haplotype was unique to a single *B. terrestris* from Florida and differed from the other

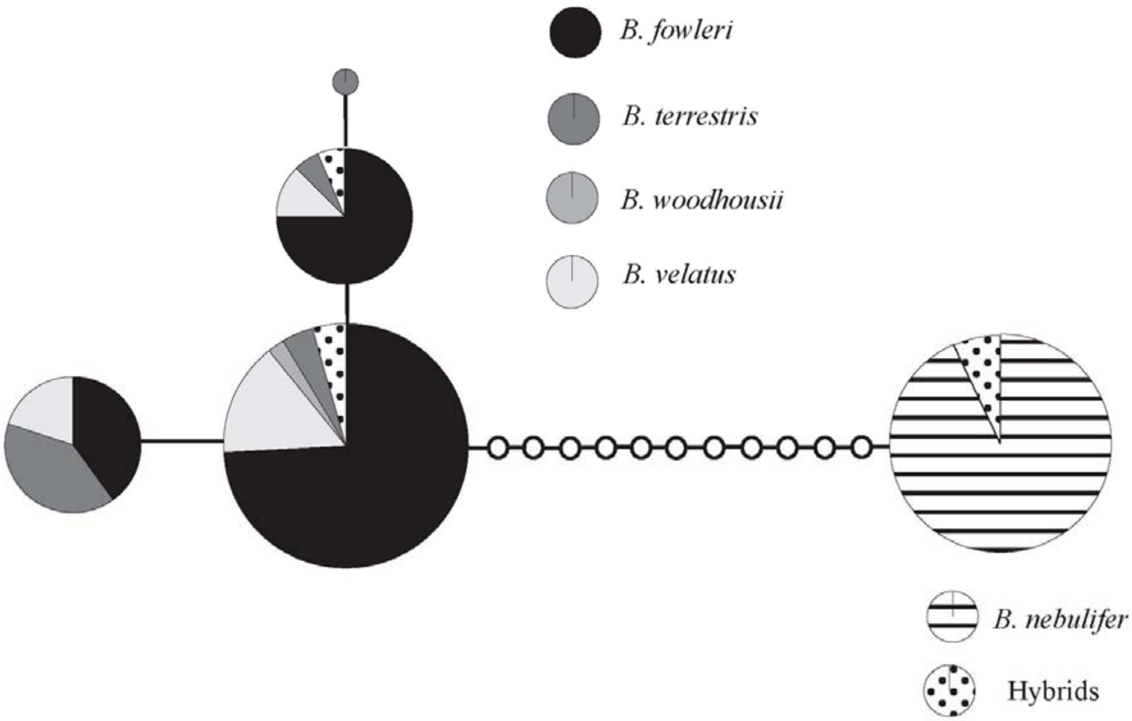

Figure 3.4: Haplotype network showing the relative frequencies and relationships between haplotypes of *B. nebulifer* and *B. fowleri*, *B. velatus*, *B. terrestris* and *B. woodhousii*. The open circles represent missing haplotypes. The haplotype of *B. nebulifer* is obviously quite divergent from the haplotypes of the *B. americanus* complex species.

haplotypes by a T to A transversion. Nucleotide diversity (π) was 0 among *B. nebulifer* populations and .00131 among populations of *B. fowleri* for the intron as calculated by DnaSP. Uncorrected sequence divergence between *B. nebulifer* and *B. fowleri* was 3.6% for the intron.

A total of fifty-one individuals were included in the mtDNA sequence analysis, including specimens from all five species and three hybrids. Results for the mtDNA segment resembled the results obtained for the intron. In total, sequences from 51 individuals collapsed to 18 unique haplotypes. All fourteen *B. nebulifer* sequences had the same haplotype. The 37 individuals of the *B. americanus* species group represented 16 different haplotypes, with different species sharing some haplotypes. The most common haplotype was shared by 6 *B. fowleri* individuals from Louisiana and 5 *B. velatus* from Louisiana and Texas. None of the haplotypes belonging to *B. fowleri* or other *B. americanus* complex species were found in *B. nebulifer* individuals. Nucleotide diversity (π) was 0.0049 and haplotype diversity (*h*) was 0.8390 ± .040 for *B. fowleri*; both diversity indices were 0 for *B. nebulifer*. Substantial sequence divergence of 10.5% for the mtDNA sequence was found between *B. nebulifer* and *B. fowleri*.

The consensus tree of the 12S and 16S mitochondrial genes again demonstrates a significant genetic divergence between *B. nebulifer* and *B. fowleri* (Fig. 3.5). The tree also demonstrates that two of the hybrids were crosses of a female *B. fowleri* with a male *B. nebulifer*. A third cryptic hybrid, morphologically designated as a male *B. nebulifer*, was identified as a product of the interspecific cross of a female *B. nebulifer* and a male *B. fowleri* – a cross that was previously posited as inviable (Blair, 1941; Volpe, 1956, 1960). The clade containing the *B. nebulifer* haplotype and this hybrid haplotype has 100% support and is completely divergent from *B. fowleri* and the other *B. americanus* species. Paraphyly of *B. terrestris* and *B. fowleri*, previously reported by Masta et al. (2001) and Pauly et al. (2004), is shown here by a well-supported clade of *B. fowleri* individuals that are the sister taxon to *B. terrestris* individuals. Another group of *B. fowleri* forms a well-supported clade that includes individuals from Louisiana and North Carolina, as well as one hybrid, and clearly indicates that *B. fowleri* is the maternal ancestor of this hybrid.

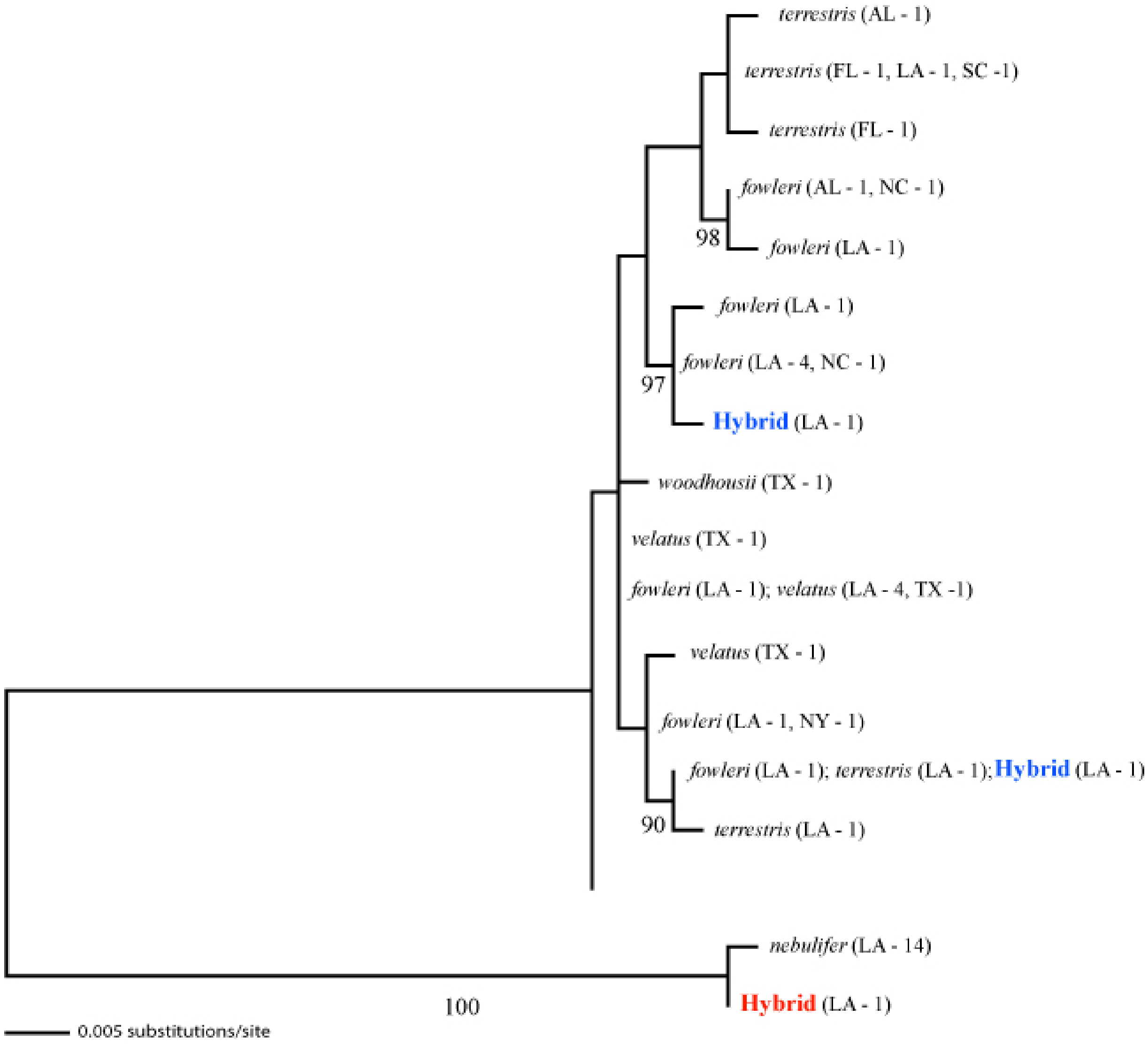

Figure 3.5: Maximum likelihood topology for 12S and16S mtDNA gene. The numbers are the Bayesian posterior probabilities from 500,000 sampled trees. The hybrids in blue represent crosses of female *B. fowleri* by male *B. nebulifer* and the hybrid in red is the reciprocal cross.

DISCUSSION

The use of both nuclear SNPs and mtDNA sequence variation to identify morphologically cryptic amphibian hybrids and their matrilineal inheritance was successfully demonstrated in this study. The use of both types of DNA is important because not only can a specific hybrid individual and the maternal parent be identified, current or historical introgression across entire geographic regions can leave a characteristic signature in the DNA of hybridizing species. Although directional introgression of mtDNA was not observed by this study, the method described herein could be useful for species in which hybridization is more common and hybrid vigor results in significant introgression. Finally, nuclear and mitochondrial DNA markers were used to examine genetic diversity and relatedness between the two species, and between members of the *B. americanus* species complex, to which *B. fowleri* belongs.

Sequence divergence made identification of hybrids straightforward; three hybrids were detected based on the presence of two nucleotides at the 12 nuclear intron sites fixed for alternate nucleotides in the parental species. Although less than 4% of the sampled populations were hybrids, this finding does not preclude the hypothesis that hybridization contributed to a decline in *B. fowleri*. Hybridization has historically been reported in mixed breeding populations of *B. fowleri* and *B. nebulifer* at rates as high as 8% (Volpe, 1960); however, the identification of a cryptic hybrid implies that the rate of historical hybridization may have been drastically underestimated since as many as half of F1 hybrids could be indistinguishable from *B. nebulifer* males. The discovery of cryptic hybridization using molecular methods is especially significant because the cross of male *B. fowleri* and female *B. nebulifer* was previously thought to be completely inviable.

Volpe (1956, 1960) argued that directional hybridization was occurring based on field observations that males of *B. nebulifer* males were much more often mismated with females of *B. fowleri* than males of *B. fowleri* with females of *B. nebulifer*. Male toads are notoriously indiscriminate in their choice of mate, and a growing majority of male *B. nebulifer* may have interfered with the mate choice of female *B. fowleri* and contributed to the directional hybridization proposed by Volpe. Hybrids between the species have also shown a preference for mating with female *B. fowleri* (Volpe, 1960). If directional hybridization did occur historically and was responsible for the decline in *B. fowleri*, two explanations might account for the low levels of hybridization that were detected by this study.

Historic hybridization could have occurred at a much greater rate while *B. fowleri* was still abundant in East Baton Rouge (and neighboring parishes) but is now undetectable because most, if not all, hybrids are sterile males and hybridization and introgression therefore would be impossible to detect. Also, hybridization occurs much less frequently now simply because there are far fewer *B. fowleri* in southern Louisiana. It is interesting to note that the cryptic hybrid from a cross of a male *B. fowleri* and a female *B. nebulifer* was found in forested habitat that is preferred more by *B. fowleri* than by *B. nebulifer*. The other two hybrid crosses of *B. nebulifer* males with *B. fowleri* females were found in urban habitat within the city limits of Baton Rouge, where *B. nebulifer* currently far outnumbers *B. fowleri*. The lack of availability of conspecific mates may favor directionality of hybridization between these two species.

Another scenario to explain the paucity of hybrids is that selection may favor differences in breeding times or female preference for specific breeding calls in areas where *B. nebulifer* and *B. fowleri* are sympatric (Arnold, 1997; Noor, 1999). Reinforcement of pre-mating isolating barriers has been proposed as a mechanism to prevent unproductive interspecific mating within bufonids (Jones, 1973) and other anuran species (Hostert, 1997; Loftus-Hills and Littlejohn, 1992) when inviable or inferior hybrids result. Although the two species have distinctive breeding calls, female preference for conspecific breeding calls may have strengthened in areas of sympatry.

Bufo nebulifer and *B. fowleri*, including closely related species, form two highly divergent groups based on mitochondrial and nuclear sequences. The lack of variation within the mtDNA and intron sequences of *B. nebulifer* was striking, but is supported by a study using mitochondrial markers. Mulcahy and Mendelson (2000) sequenced a fragment of 16S and cytochrome *b* in their study of the phylogeography of *B. valliceps* and found similar results in the clade they eventually differentiated into the northern clade, and separate species, *B. nebulifer*. Sampling of multiple individuals from eight localities, from Mexico to Louisiana, only yielded four haplotypes for *B. nebulifer*, whereas the southern clade of *B. valliceps* had almost four times as many haplotypes (Mulcahy and Mendelson, 2000). The authors attribute the lack of genetic variation in *B. nebulifer* haplotypes to a historical vicariance event that isolated a small number of individuals, followed by northerly range expansion into a relatively uniform habitat along the Gulf Coast from Mexico through Texas and into Louisiana. A relatively recent and rapid colonization is consistent with the lack of sequence variation we observed in this study.

The interpretation of sequence variation for the species in the *B. americanus* complex was less straightforward. Systematics of Louisiana bufonids has long been complicated by the presence of four putative species or subspecies: *B. terrestris*; *B. velatus, B. fowleri*, and *B. americanus* of the *B. americanus* complex species (Conant and Collins, 1998, Dundee and Rossman, 1989). The ranges of the species overlap minimally in Louisiana, but species in the *B.*

americanus complex are known to hybridize in sympatry and to produce viable, fertile hybrid offspring (Volpe, 1956; Blair, 1959; Jones, 1973; Masta et al., 2002). Prior inference of these species' phylogenies using molecular methods has been complicated by historical hybridization within this group and introgression among species (Masta et al., 2002; Pauly et al., 2004).

Hybridization of *B. fowleri* with its close relatives in the *B. americanus* species complex may have confounded the hybridization study. *Bufo fowleri* individuals that were used in this study possessed morphological and advertisement call characteristics that are unique to the species; however, they shared nuclear haplotypes with *B. terrestris*, *B. woodhousii* and *B. velatus* and mitochondrial haplotypes with *B. terrestris* and *B. velatus*. The shared haplotypes raises the question of whether past hybridization of *B. fowleri* with other members of the *B. americanus* complex dilutes isolating mechanisms and increases the likelihood that it will hybridize more readily with *B. nebulifer*. The effects of differential selection acting on various *B. fowleri* haplotypes may result in unequal fitness and survivorship of *B. fowleri* x *B. nebulifer* intergrades. Finally, extensive hybridization between *B. americanus* complex species with different ecological adaptations, such as spatial and temporal breeding preferences, may be contributing to a decline in *B. fowleri* irrespective of hybridization with *B. nebulifer*.

The mtDNA consensus tree strongly supports two clades that are composed entirely of sequences from *B. fowleri* individuals and one hybrid. Grouped as the sister taxon to one of these two clades of *B. fowleri* individuals, but with no support, is a clade comprised of sequences from *B. terrestris*. Haplotypes from *B. terrestris* and *B. fowleri* from an area in Louisiana where they are sympatric with each other also form a well-supported clade that includes another hybrid. This paraphyly of *B. terrestris* and *B. fowleri* was also recovered by Masta et al. (2002) and Pauly et al. (2004).

Two hypotheses would explain the lack of sequence divergence and the sharing of haplotypes between *B. terrestris*, *B. fowleri* and *B. velatus*. Recent speciation and incomplete lineage sorting can result in individuals from several species sharing an ancestral haplotype. Another likely hypothesis for the lack of diversity between *B. terrestris*, *B. fowleri* and *B. velatus* is that secondary contact in Louisiana and, potentially, a much larger geographic area has led to hybridization and extensive introgression. However, while there is very strong support for two clades of *B. fowleri* sequences and one clade containing both *B. fowleri* and *B. terrestris,* the sequences belonging to *B. velatus* formed a diverse and unsupported group. The taxonomic status of *B. velatus* as a discrete species from *B. fowleri* is primarily based on morphological characteristics and should potentially be reconsidered due to the lack of molecular distinction.

The haplotype network estimated from intron sequence variation demonstrated an even smaller number of shared haplotypes within the *B. americanus* group species than the mtDNA consensus tree. Because it takes four times longer for nuclear genes to coalesce than mitochondrial genes and given the mitochondrial haplotype similarity, the complete lack of geographic or species structure was not unexpected for the nuclear sequence (Avise, 2000). Despite the appearance of preliminary divergence between species in the mtDNA sequence, the nuclear data show no differentiation between species. This pattern could be attributable to either extensive hybridization or incomplete lineage sorting.

In conclusion, analyses of mitochondrial and nuclear loci both unambiguously show that *B. nebulifer* and *B. fowleri* are differentiated species that are not threatened by genetic admixture. Both reciprocal crosses produce viable, yet likely sterile, offspring that do not provide evidence of historical introgression. The molecular methods that were used by this project are optimal for identifying hybrids; however, the fragment of 12S and 16S mtDNA genes used to examine introgression in this study lacks the genetic variation required to resolve what appears to be very recent divergences among the *B. americanus* species group. In addition to hybridization and extensive introgression, historical and contemporary range expansion as well as incomplete lineage sorting could account for the appearance of the same haplotypes in different geographic regions and the paraphyly observed for *B fowleri* and *B. terrestris*. A more rapidly evolving gene such as the mtDNA control region and additional SNPs in unlinked loci would be ideal complements for further explorations of hybridization and relatedness within *B. americanus* complex species.

Acknowledgments.- We are grateful to Alisha Holloway and Greg Pauly for generously providing the primers that made the nuclear analyses possible. We thank the following people for their comments and contributions to this study: J.H.K. Pechmann, N. Thurgate, J. Boundy, B. Thomas, G. Pauly, J. Mendelson, J. Utley, A. Ford and W. Curry. We also thank the Museum of Natural Science at Louisiana State University and Brian Fontenot of the University of Texas at Arlington for providing additional tissue samples for this study. Funding for this project was provided by the Louisiana Board of Regents, and the University of New Orleans' College of Sciences. Toads were handled in accordance with ASIH-HL-SSAR Guidelines for the Use of Live Amphibians and Reptiles in Field Research and regulations of the Louisiana Department of Wildlife & Fisheries scientific collecting permit number: LNHP-05-061.

Appendix 1: Sources, collection localities and coordinates (where available) for all specimens used in intron and mtDNA sequence analyses. Multiple GenBank accession numbers represent mtDNA (s) and intron sequences. Specimens with two unique intron haplotypes have two accession numbers per intron.

Taxa	***Source***	***Specimen #***	***Collection Locality & Coordinates (decimal degrees)***	***Sequence***		***GenBank #***
				intron	mtDNA	
Bufo nebulifer	field	QU-5	Louisiana, East Baton Rouge (EBR) Parish: Dawson Creek at Quail Drive behind LDWF – N30.40060 W091.12827	✓	✓	EF372081(s) EF372185
Bufo nebulifer	field	QU-15	EBR Parish: Dawson Creek at Quail Drive behind LDWF – N30.40060 W091.12827	✓		EF372186
Bufo nebulifer	field	RR2-5	EBR Parish: River Road at Gardere Lane N30.34269 W091.14499	✓		EF372187(1a) EF372213(1b)
Bufo nebulifer	field	RR2-6	EBR Parish: River Road at Gardere Lane N30.34269 W091.14499	✓		EF372214
Bufo nebulifer	field	RR3-2	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓	✓	EF372078 (s) EF372189
Bufo nebulifer	field	RR3-3	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372190
Bufo nebulifer	field	RR3-4	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372191
Bufo nebulifer	field	RR3-5	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372194
Bufo nebulifer	field	RR3-6	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372195
Bufo nebulifer	field	RR3-8	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372196
Bufo nebulifer	field	RR3-9	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372197
Bufo nebulifer	field	RR3-10	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372193
Bufo nebulifer	field	RR3-11	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372215
Bufo nebulifer	field	RR3-12	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372216
Bufo nebulifer	field	RR3-15	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372188
Bufo nebulifer	field	RR3-16	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372217
Bufo nebulifer	field	RR3-19	EBR Parish: River Road at Conrad Point N30.34919 W091.16288	✓		EF372218
Bufo nebulifer	field	RR4-1	EBR Parish: River Road – 0.8 mi. S of Brightside Drive N30.35062 W091.22565	✓	✓	EF372088(s) EF372198
Bufo nebulifer	field	RR4-2	EBR Parish: River Road – 0.8 mi. S of Brightside Drive N30.35062 W091.22565	✓		EF372202
Bufo nebulifer	field	RR4-3	EBR Parish: River Road – 0.8 mi. S of Brightside Drive N30.35062 W091.22565	✓		EF372192
Bufo nebulifer	field	RR4-6	EBR Parish: River Road – 0.8 mi. S of Brightside Drive N30.35062 W091.22565	✓		EF372203
Bufo nebulifer	field	KW2-8	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓	✓	EF372087(s) EF372209
Bufo nebulifer	field	KW2-19	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓	✓	EF372080(s) EF372210
Bufo nebulifer	field	KW2-22	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372211
Bufo nebulifer	field	KW2-23	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372212
Bufo nebulifer	field	KW3-3	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓	✓	EF372089(s) EF372199
Bufo nebulifer	field	KW3-8	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372201
Bufo nebulifer	field	WB-1	EBR Parish: Wright Babin Road/private road N30.639 W091.09611	✓	✓	EF372086(s) EF372204
Bufo nebulifer	field	WB-6	EBR Parish: Wright Babin Road/private road N30.639 W091.09611	✓	✓	EF372079(s) EF372205

Bufo nebulifer	field	FT-1	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓	✓	EF372077(s) EF372206
Bufo nebulifer	field	FT-4	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓		EF372207
Bufo nebulifer	field	FT-5	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓		EF372208
Bufo nebulifer	field	HR1-1	EBR Parish: Highland Road at E. Perkins Rd. (train tracks) N30.34969 W091.02590	✓	✓	EF372082(s) EF372220
Bufo nebulifer	field	HR1-2	EBR Parish: Highland Road at E. Perkins Rd. (train tracks) N30.34969 W091.02590	✓	✓	EF372083(s) EF372221
Bufo nebulifer	field	NC-15	EBR Parish: Fountain Bayou at Seigen Lane (Normco Cons.) N30.35927 W091.10392	✓	✓	EF372084(s) EF372222
Bufo nebulifer	field	NC-3	EBR Parish: Fountain Bayou at Seigen Lane (Normco Cons.) N30.35927 W091.10392	✓		EF372223
Bufo nebulifer	field	SH-7	Louisiana, Iberville Parish, LA: Sherbourne W.M.A. N30.38473 W091.64767	✓	✓	EF372085(s) EF372219
Bufo nebulifer	field	SH-8	Iberville Parish: Sherbourne W.M.A. N30.38473 W091.64767	✓		EF372200
Bufo nebulifer	field	SH-9	Iberville Parish: Sherbourne W.M.A. N30.38473 W091.64767	✓		EF372225
Bufo nebulifer	LSUMZ	H-18871	Louisiana, St. Landry Parish: Thistlewaite N.M.A.	✓	✓	EF372076 (s) EF372224
Bufo fowleri	field	FT-6	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓	✓	EF372094(s) EF372121(1a) EF372122(1b)
Bufo fowleri	field	FT-7	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓	✓	EF372097(s) EF372123
Bufo fowleri	field	FT-8	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓	✓	EF372106(s) EF372124(1a) EF372125(1b)
Bufo fowleri	field	FT-9	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓		EF372126
Bufo fowleri	field	FT-10	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓		EF372127
Bufo fowleri	field	FT-11	EBR Parish: Frenchtown Road at Comite River N30.49760 W091.01575	✓	✓	EF372096(s) EF372128
Bufo fowleri	field	KW2-1	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓	✓	EF372095(s) EF372130
Bufo fowleri	field	KW2-2	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓	✓	EF372107(s) EF372131
Bufo fowleri	field	KW2-3	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372132
Bufo fowleri	field	KW2-4	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372133
Bufo fowleri	field	KW2-5	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372134
Bufo fowleri	field	KW2-10	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓	✓	EF372098(s) EF372129

Bufo fowleri	field	KW2-11	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111		✓	EF372099(s)
Bufo fowleri	field	KW2-12	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372135(1a) EF372136(1b)
Bufo fowleri	field	KW2-13	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372137(1a) EF372138(1b)
Bufo fowleri	field	KW2-14	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372139
Bufo fowleri	field	KW2-15	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372140(1a) EF372141(1b)
Bufo fowleri	field	KW2-16	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372142(1a) EF372143(1b)
Bufo fowleri	field	KW2-17	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372144(1a) EF372145(1b)
Bufo fowleri	field	KW2-18	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372178
Bufo fowleri	field	KW2-20	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372146
Bufo fowleri	field	KW2-24	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372148
Bufo fowleri	field	KW2-26	EBR Parish: Kendalwood Road at Amite River N30.34711 W090.09111	✓		EF372149
Bufo fowleri	field	SH-2	Iberville Parish: Sherbourne W.M.A. N30.38473 W091.64767	✓		EF372150
Bufo fowleri	field	SH-3	Iberville Parish: Sherbourne W.M.A. N30.38473 W091.64767	✓		EF372151(1a) EF372152(1b)
Bufo fowleri	field	SH-4	Iberville Parish: Sherbourne W.M.A. N30.38473 W091.64767	✓	✓	EF372101(s) EF372153
Bufo fowleri	field	RR2-1	EBR Parish: River Road at Gardere Lane N30.34269 W091.14499	✓	✓	EF372108(s) EF372154
Bufo fowleri	field	RR2-3	EBR Parish: River Road at Gardere Lane N30.34269 W091.14499	✓		EF372155
Bufo fowleri	field	RR2-4	EBR Parish: River Road at Gardere Lane N30.34269 W091.14499	✓		EF372156
Bufo fowleri	field	RR4-4	EBR Parish: River Road – 0.8 mi. S of Brightside Drive N30.35062 W091.22565	✓	✓	EF372102(s) EF372157
Bufo fowleri	field	RR4-5	EBR Parish: River Road – 0.8 mi. S of Brightside Drive N30.35062 W091.22565	✓	✓	EF372100(s) EF372158
Bufo fowleri	LSUMZ	H-2952	Mississippi, Pike County: Percy Quin State Park	✓		EF372174(1a) EF372175(1b)
Bufo fowleri	LSUMZ	H-3356	Louisiana, East Feliciana Parish	✓		EF372179(1a) EF372180(1b)
Bufo fowleri	LSUMZ	H-16037	Louisiana, St. Tammany Parish	✓	✓	EF372114(s) EF372160
Bufo fowleri	LSUMZ	H-18650	Louisiana, Tangipahoa Parish: Sandy Hollow W.M.A.	✓	✓	EF372103(s) EF372164

Bufo fowleri	LSUMZ	H-18787	Alabama, Tallapoosa County: Lake Martin	✓	✓	EF372104(s) EF372162
Bufo fowleri	LSUMZ	H-18939	Louisiana, Washington Parish: Pushpatapa Creek	✓		EF372173
Bufo fowleri	LSUMZ	H-18945	Louisiana, St. Tammany Parish: Five Lakes Camp	✓	✓	EF372105(s) EF372165(1a) EF372166(1b)
Bufo fowleri			Pauly et al., 2004		✓	AY680224
Bufo fowleri			Pauly et al., 2004		✓	AY680223
Bufo fowleri			Pauly et al., 2004		✓	AY680212
Bufo terrestris	field	TS5-2	Louisiana, St. Tammany Parish: Money Hill development	✓		EF372181(1a) EF372182(1b)
Bufo terrestris	field	TS5-3	Louisiana, St. Tammany Parish: Money Hill development		✓	EF372092(s)
Bufo terrestris	field	TS6-2	Louisiana, St. Tammany Parish: Weyerhaueser Co. pine plantation	✓		EF372183(1a) EF372184(1b)
Bufo terrestris	field	TS7-1	Louisiana, St. Tammany Parish: Weyerhaueser Co. pine plantation		✓	EF372093(s)
Bufo terrestris	field	TS1-3	Louisiana, St. Tammany Parish: Weyerhaueser Co. pine plantation		✓	EF372031(s)
Bufo terrestris	LSUMZ	H-18293	Florida, Okaloosa County: Blackwater River S.F., South Hurricane Lake	✓	✓	EF372117 (s) EF372119 (1a) EF372120 (1b)
Bufo terrestris			Pauly et al., 2004		✓	AY680222
Bufo terrestris			Pauly et al., 2004		✓	AY680221
Bufo terrestris			Pauly et al., 2004		✓	AY680220
Bufo velatus	B. Fontenot	BF033	Texas, Henderson County: 10 km south of Athens N32.28444 W095.96500	✓	✓	EF372110(s) EF372176
Bufo velatus	LSUMZ	H-2695	Louisiana, Webster Parish: Parish Rd. 190	✓	✓	EF372109(s) EF372163
Bufo velatus	LSUMZ	H-16075	Louisiana, Bossier Parish	✓		EF372161
Bufo velatus	LSUMZ	H-18136	Louisiana, Union Parish: Union W.M.A.	✓	✓	EF372115(s) EF372169(1a) EF372170(1b)
Bufo velatus	LSUMZ	H-18143	Louisiana, Grant Parish	✓	✓	EF372116(s) EF372167(1a) EF372168(1b)
Bufo velatus	LSUMZ	H-18195	Louisiana, Natchitoches Parish: Kisatchie National Forest	✓		EF372159
Bufo velatus	LSUMZ	H-18502	Louisiana, Allen Parish	✓	✓	EF372118(s) EF372171(1a) EF372172(1b)

Bufo velatus			Pauly et al., 2004		✓	AY680210
Bufo velatus			Pauly et al., 2004		✓	AY68209
Bufo woodhousii	B. Fontenot	BF095	Texas, Tarrant County: RidgeNorth and Benbrook Aledo Road N32.68250 W097.50839	✓	✓	EF372111(s) EF372177
hybrid	field	RR3-7	EBR Parish: River Road at Conrad Point N30.34919 W091.1628	✓	✓	EF372113(s) EF372226(1a) EF372227(1b)
hybrid	field	SH-10	Iberville Parish: Sherbourne W.M.A. N30.38473 W091.64767	✓	✓	EF372090(s) EF372230(1a) EF372231(1b)
hybrid	LSUMZ	H-19044	Louisiana, EBR Parish: Quail Drive, Baton Rouge	✓	✓	EF372112(s) EF372228(1a) EF372229(1b)

CHAPTER 4: EFFECTS OF COMPETITION, HYDROPERIOD AND PREDATION ON A NATIVE ANURAN AND ITS INVASIVE CONGENER

INTRODUCTION

A primary goal of conservation biology is to understand how habitat disturbance and fragmentation affect changes in species interactions and distributions, particularly when the increase of an invasive species coincides with a decline in a native species (Holway, 1999; Kiesecker et al., 2001; Collins and Storfer, 2003). When habitat degradation favors the expansion of invasive species that specialize in disturbed habitats, the detrimental effect on native species is further magnified (Kupferberg, 1997; Mack et al., 2000; With, 2001). Freshwater ecosystems are particularly vulnerable to disturbance from numerous human activities that may also precipitate invasion by non-native species (Moyle and Light, 1996; Saunders et al., 2002, Dominguez-Dominguez et al., 2006). Landscape-level activities such as drainage, channelization, and clearing of forested areas have profound effects on larval amphibian species because they alter the natural hydroperiod and community structure of freshwater habitats used for breeding (Whittier et al., 2002; Lichter et al. 2006).

Studies of community structure in lentic habitats demonstrate that numerous taxa including insects (Law, 1979; McLachlan, 1985), snails (Brown, 1982; Brown and DeVries, 1985) crustaceans (Wiggins et al. 1980, Mahoney et al., 1990) fishes (Rahel, 1984) and amphibians (Werner and McPeek, 1994; Leips et al., 2000; Skelly, 2001) are primarily distributed by physiological tolerance to variance in abiotic factors (e.g. pond duration, temperature, dissolved oxygen) along a hydroperiod gradient from ephemerality to permanence. Biotic interactions, mainly predation and competition, concomitant with these changes in abiotic pressures ultimately constrain species that might otherwise occupy a broader range of the gradient (Wellborn et al., 1996). Broadly speaking, freshwater animals exhibit a fitness trade-off between desiccation avoidance at the ephemeral end of the spectrum that gradually shifts toward predator avoidance at the permanence end.

Larval amphibians are a well-studied and broadly arrayed assemblage that generally conforms well to the predictions about aquatic organisms (Wellborn et al., 1996). The larval stage of the amphibian dual life cycle strongly influences fitness in the adult stage (Skelly and Werner, 1990; Wilbur, 1996). Among larval anurans, it is advantageous to achieve the largest size possible at metamorphosis, while avoiding mortality from desiccation, because large size at metamorphosis confer significant advantages in adult survival and fecundity (Wilbur, 1996; Morey and Reznick, 2004). Fitness trade-offs between size at and swiftness to metamorphosis reflect species' ability to withstand levels of abiotic and biotic variables in their larval habitat (Werner, 1986).

Predation is a major cause of tadpole mortality, and predator composition along the hydroperiod continuum has a strong impact on species' distributions (Werner and McPeek, 1994; Wilbur, 1997). Distinct shifts in anuran species composition are imposed by the succession of predator abundance and diversity from rapidly-drying temporary habitats with few predators, to temporary habitats that dry periodically and support invertebrate but not fish predators, to permanent habitats that contain invertebrate and fish predators (Skelly, 1995; Wellborn et al., 1996). Different levels of desiccation and predation across hydroperiod gradients favor phenotypes in tadpoles that maximize fitness in one area of the permanence spectrum to the detriment of fitness at the opposite extreme. Selection for alternate phenotypes is driven by trade-offs between acquiring resources required for growth and the suppression of foraging activity due to mortality risk by visually-orienting predators (Morin, 1983; Skelly, 1995). Interspecific differences in anuran species' life history characteristics such as behavior, body size and shape, and developmental and growth rates reflect differential susceptibility across the hydroperiod and predator transitions (Morin, 1983, Skelly, 1995; Wellborn et al., 1996).

Interspecific competition can also have widespread and varied impacts on species' distributions when the ranges of closely related species coincide; for instance, a stable boundary can form at the interface of two strongly competitive species (Case and Taper, 2000; Keitt et al., 2001). Competitive exclusion occurs when one species is a stronger competitor for an important limited resource such as food, shelter or breeding habitat in the presence of a similar species that utilizes the same or a similar ecological niche. Exploitative competition between two species with overlapping niches can result in local extinctions of the inferior competitor, or divergence of resource use via character displacement can permit coexistence in sympatry (Colwell and Futuyma, 1971).

In freshwater habitats, interspecific competition is an important force in structuring the aquatic community across both hydroperiod and predator gradients (Brockelman, 1969; Wellborn et al., 1996; Bardsley and Beebee, 2001). Asymmetry in competitive strength, often due to exploitative competition between species of different sizes, can exclude the inferior competitor (Brockelman, 1969; Morin, 1983; Morin and Johnson, 1988; Richter-Boix et al., 2004). When anuran species share breeding habitat, particularly ephemeral sites, a density effect results where larger and/or more aggressive members of a community dominate and outcompete smaller individuals for food resources (Wilbur, 1977). Food limitation, both in quantity and quality, has been found to be the mechanism of density-dependent effects on growth and survival in various anuran species, including *Bufo americanus* (Brockelman, 1969; Wilbur, 1977; Wilbur, 1980, Alford and Harris, 1988.) Several studies of *Bufo* and other anuran species with differing growth rates that were raised together support the assertion that interference competition among amphibian larvae is strong and instances of larger tadpoles monopolizing food resources are common (Bardsley and Beebee, 1998, 2001; Richter-Boix et al., 2004).

The range expansion of highly competitive non-native species is often closely linked to destruction and alteration of local and regional habitats that favor the species' continued invasion (Sakai et al., 2001). Environmental disturbance can also alter competitive advantages among species and cause native species to retract into less disturbed areas of the range (Bardsley and Beebee, 1998, 2001). Native species may retreat from highly competitive invasive species or alter their resource usage to minimize competition (Case et al., 2005). Bullfrogs (*Rana catesbiana*) were first introduced into California in 1896 and have dramatically increased their distribution within the last century, aided greatly by utilizing anthropogenically disturbed areas (Kiesecker et al., 2001) to the detriment of native species. Bullfrog larvae have significant negative effects on survival and size at metamorphosis of native *Rana* and *Hyla* larvae even in unaltered habitats. Kupferberg (1997) demonstrated that large bullfrog tadpoles quickly deplete algal food resources and even lower the quality of remaining algae. Conversely, there were no significant negative effects of native tadpoles on bullfrog larvae.

A study by Smith (2005) considered the effects of interspecific competition of two non-native anuran species, the Cane Toad, *Bufo marinus*, and the Cuban Treefrog, *Osteophilus septentionalis*, on the growth and development rate of two native anurans, the Southern Toad, *B. terrestris*, and the green treefrog, *Hyla cinerea.* Delayed metamorphosis for both native species and a reduced growth rate of *B. terrestris* tadpoles in the presence of *O. septentionalis* tadpoles demonstrates a strong negative impact of invasive anuran larvae on the fitness of native anuran species (Smith, 2005).

Interactions between interspecific competition and predation also significantly influence community structure and competitive superiority over a broad range of conditions (Wilbur, 1987; Wilbur and Fauth, 1990). Interspecific competition may alter the community structure created by pond duration and predator composition; however, predation can also mediate the effects of interspecific competition (Relyea, 2000). Because few predators are found in highly ephemeral sites, species that inhabit these rapidly drying habitats are often superior competitors at the cost of increased susceptibility to predation. Larval anurans that utilize temporary breeding sites must have higher foraging levels in order to facilitate growth and development to metamorphosis before desiccation. The threat of predation rather than desiccation drives the morphology and behavior of tadpoles that inhabit permanent breeding sites. Species in permanent sites generally exhibit characteristics that allow them to coexist with predators and, subsequently, have diminished activity levels, lower growth rates and inferior competitive abilities (Skelly, 1995; Wellborn et al., 1996; Relyea, 2000).

Throughout its range in the eastern United States, *B. fowleri* breeds in various habitats including permanent and temporary ponds, roadside ditches, flooded fields, and the shores and shallows of lakes and slow-moving rivers (Dundee and Rossman, 1989; Green, 2005). However, in southern Louisiana, *B. fowleri* no longer breeds in the temporary sites where museum records, field notes and literature indicate it was formerly found, and now breeds solely in entirely permanent water bodies (personal observation, personal communication with J. Boundy). Conversely, the cleared and degraded habitat in which the Coastal Plain Toad specializes often contains its preferred ephemeral, artificial and disturbed breeding sites (Mendelson, 2005). Increasing habitat disturbance and alteration in southern Louisiana promotes the rapid increase in distribution and abundance of *B. nebulifer* into habitat that formerly supported *B. fowleri* as well.

Two experiments were conducted to examine whether interspecific competition with a non-native specialist in disturbed habitat is driving the decline of a native species that is tolerant of moderate anthropogenic habitat disturbance. The first ‘competition’ experiment examined the effects of competition in simulated permanent and temporary breeding habitats to determine whether either species is a superior competitor in a particular habitat. The second ‘predator’ experiment investigated the potential mediation of interspecific competition by an invertebrate predator in both hydroperiods and also examined the interaction between competition and predation.

The addition of a predator tested whether trade-offs and plasticity in the growth and development strategies of *B. fowleri* and *B. nebulifer* would be evident. Because *B. fowleri* tadpoles appear to breed exclusively in permanent sites in southern Louisiana, the length of their larval period may reflect a slow growth rate and poor competitive ability related to a predator avoidance strategy. *Bufo nebulifer*'s growth rate may reflect a lack of predation in its normal breeding habitat and selection for high growth and activity rates and good competitive ability, and may result in high mortality in the presence of a predator. These experiments addressed several questions regarding competition and predation between the two species under simulated permanent and temporary breeding habitat conditions: Is *B. nebulifer* better adapted to utilize temporary breeding habitat? Is competition with *B. nebulifer* in temporary breeding habitat contributing to a decline in *B. fowleri*? Will *B. nebulifer* or *B. fowleri* alter their growth strategies due to a mortality risk from a predator? Will *B. fowleri* outcompete *B. nebulifer* in permanent breeding habitats in the presence of a predator?

MATERIALS AND METHODS

Experimental design

Competition Experiment

To test the hypothesis that *B. nebulifer* larvae have a competitive advantage over *B. fowleri* larvae in temporary breeding habitat, both species were raised under intra- and interspecific conditions in artificial pools (plastic cattle tanks) under different drying regimes in 2005. Tadpoles of each species were raised separately and at equal density of the other species. Water levels in the simulated permanent habitat were kept constant while the drying habitat was designed to mimic rapid drying of a temporary breeding site. The distribution of the three species combinations in the tanks (*B. nebulifer, B. fowleri,* both species) resulted in an intraspecific treatment (each species alone) and an interspecific treatment (species together). The species treatments were crossed with the drying and constant water level treatments to result in a 3 x 2 factorial design (Table 4.1). Each of the six treatments was replicated three times for a total of 18 experimental units. Tanks were grouped in 3 spatial blocks with 6 tanks per block in a randomized block design; one tank of each treatment was randomly assigned to each block.

		Species treatment		
		B. fowleri	*B. fowleri & B. nebulifer*	*B. nebulifer*
Water level treatment	Drying	3x	3x	3x
	Constant	3x	3x	3x

Table 4.1: Experimental 3x2 factorial design of 3 species ‘competition’ treatments crossed with 2 water level ‘drying’ treatments

Eighteen cattle watering tanks (1.8m diameter, 0.6m height) were filled with de-chlorinated water to a depth of 35 cm. Tanks were covered with screened lids to decrease accessibility to competitors and predators. Each tank contained a mesh-covered standpipe that was adjusted to manipulate water levels. A natural substrate of 1.5 kg of mixed pine, oak and sweet gum leaves was added to each tank after it was filled. Zooplankton and phytoplankton were collected from natural breeding sites, mixed together and added to the artificial pools in aliquots of approximately 1 liter per tank.

Eggs from two amplexing pairs of *B. nebulifer* and *B. fowleri* were collected in the field on two consecutive nights after fertilization occurred. Egg masses were brought to the lab and raised separately in plastic containers until the tadpoles hatched and were able to swim freely. Tadpoles were counted and assigned to ponds following a stratified random design that equally represented each egg mass in each artificial pool designated to contain that species. *Bufo nebulifer* and *B. fowleri* tadpoles were randomly assigned to 12 cattle tanks. Six tanks contained a total of 140 *B. nebulifer* tadpoles per tank, six tanks contained a total of 140 *B. fowleri* tadpoles per tank, and six tanks contained 70 of both tadpole species, totaling 140 tadpoles.

To assess the ability of tadpoles to respond to drying conditions after the tadpoles had acclimated for a day, the water levels were lowered 3 cm per day for the first 7 days and then 2 cm/day for the next 7 days the standpipe was lowered to 0 cm on the 15th. Tadpoles were monitored daily until metamorphosis, which began at eleven days (for *B. nebulifer*) after tadpoles were put into the tanks. Tadpoles were removed from the tanks once they metamorphosed (defined as forelimb emergence) and were held in the laboratory until the tail was completely absorbed. Size at metamorphosis (snout-vent length in mm and mass in g), length of larval period, and the proportions of tadpoles that survived to metamorphosis were measured separately for each species.

Predation Experiment

An additional artificial pond experiment was conducted in 2006 following similar protocols. Eighteen cattle tanks were manipulated and analyzed similarly to the experiment that was previously described, with several exceptions. First, an initial increase in the drying rate was made to the ponds receiving the drying treatment. The tanks were filled to 35 cm and tadpoles were allowed to acclimate for a day before the water level was lowered. However, the water level was lowered more rapidly at a rate of 3.5 cm/day for 8 days and then 2 cm/day for 3 days until the water level was at 0 cm on the 11th day. On the 12th day, the moist leaf litter in the 18 designated drying treatment tanks was removed from the tanks and thoroughly examined to determine whether significant tadpole mortality was caused by failure to metamorphose prior to pond drying. In the previous year's experiment, tadpoles that had not metamorphosed by the final drying date were allowed to metamorphose in the small amount of remaining water.

Second, predatory dragonfly larvae *Pachydiplax longipennis* (Order Odonata: Family Libelluidae) and *Anax junius* (Order Odonata: Family Aeshnidae) were added to another eighteen cattle tanks randomly chosen as an additional treatment. Odonate predators were captured within one week of the egg collection from one site where both species of amplexing pairs were found and also from a nearby roadside ditch where amplexing pairs of *B. nebulifer* were captured. These odonate species were chosen because they are known predators of *B. americanus* tadpoles, which many vertebrate and invertebrate species find distasteful (Skelly and Werner, 1990; Van Buskirk, 2001). The consumption of both prey species by both predator species in the lab confirmed that odonate species find *B. fowleri* or *B. nebulifer* palatable. Both predators are commonly found in tadpole breeding sites in both temporary and permanent habitat in southern Louisiana, though *P. longipennis* larvae are smaller and have a shorter larval period than the large *A. junius,* and tend to occur more often in temporary sites (Crumrine, 2005). Two mid-instar *A. junius* larvae and four mid-instar *P. longipennis* larvae were placed into each of the 18 predator ‘presence’ tanks after the tadpoles were allowed to acclimate for 24 hours.

The same protocol from the previous year was followed; however, eggs from 3 amplexing pairs of both species were collected on the same night. The tadpoles were held until free-swimming and randomly assigned to the 36 tanks using a stratified random design. The predation treatment was crossed with the species treatments and the drying and constant water level treatments to result in a fully crossed 3 x 2 x 2 factorial design (Table 4.2). Each of the twelve treatments was replicated three times for a total of 36 experimental units. Tanks were grouped in 3 spatial blocks with 12 tanks per block in a randomized block design; one tank of each treatment was placed in each block. A total of 5040 tadpoles of both species were used for both these experiments.

	Species treatment			
Water level treatment	*B. fowleri*	*B. fowleri &* *B. nebulifer*	*B. nebulifer*	**Predation treatment**
Drying	3x	3x	3x	Presence
Constant	3x	3x	3x	Absence
Drying	3x	3x	3x	Presence
Constant	3x	3x	3x	Absence

Table 4.2: Experimental 3x2x2 factorial design of 3 species ‘competition’ treatments crossed with 2 water level ‘drying’ treatments and 2 predation treatments.

Statistical analyses:

Competition Experiment

Analysis of variance (ANOVA) was used to test for differences between species mean mass and snout-vent length at metamorphosis, length of larval period and proportion of tadpoles that survived to metamorphosis under intra- and interspecific competition in drying and constant hydroperiods and their interactions (Gotelli and Ellison, 2004). Data for individuals were used in

the analyses of snout-vent length, mass and larval period analyses and were log transformed to meet assumptions of homogeneity of variance. Because the experimental design was unbalanced due to variation in the number of surviving individuals among the tanks, a proportion of the individual mean square was included in the error term to obtain an appropriate expected error mean square for the analyses of size (Littell et al., 1991). This was done using a Satterthwaite approximation (Milliken and Johnson, 1984). Tank within treatment was the error term;
tanks were used as the unit of replication for the survival analysis and were arcsine square root transformed to meet assumptions of homogeneity of variance.

Predation Experiment

An ANOVA was also used to test for differences between species in size (mass and snout-vent length) at metamorphosis, length of larval period, and proportion of tadpoles that survived to metamorphosis. Tank means were used as the unit of replication for all variables. Mass, snout-vent length and length of larval period were log transformed and the proportion of individuals surviving to metamorphosis was arcsine square root transformed to meet assumptions of homogeneity of variance (Gotelli and Ellison, 2004).

Bufo fowleri was excluded from further analyses because it was eliminated from most of the treatments containing predators. Because an ANOVA cannot be performed for treatments with values of zero, a logistic regression was performed to evaluate the relationships between survival to metamorphosis and predation, competition, and drying treatments for each species. The interaction terms used - predator treatment by species, and predation treatment x species x water treatment - test whether these two bufonids differ in their susceptibility to odonate predation, and whether the susceptibility of species depends on the drying regime (ephemeral versus permanent). Logistic regression analysis is appropriate when the dependent variable is discrete (dead or alive). Predation also was used as a variable in the analysis of *B. nebulifer* size measurements, length of larval period and survival to metamorphosis.

Results:

Competition Experiment

There were significant effects of drying and competition on mean snout-vent length and mass for *B. fowleri* metamorphs. *Bufo fowleri* metamorphs were smallest in length and in mass in tanks where they were in interspecific competition under drying conditions, but the treatment interaction was nonsignificant. There were no significant effects for either treatment or interaction between treatments for *B. nebulifer*.

Snout-vent length was greatest for *B. fowleri* metamorphs when they were alone and in a non-drying tank and was significantly decreased when they were in competition in drying tanks (Table 4.3, Fig. 4.1). The least square mean of snout-vent length for *B. fowleri* metamorphs in the drying treatment alone was 9.67 (± 0.03) mm and 8.95 (±0.09) mm in competition. For *B. fowleri* in the non-drying tanks alone, the least square mean was 10.22 (± 0.04) mm and it was 9.75 (± 0.14) mm in competition with *B. nebulifer*. No significant effects of interaction between competition and drying treatments were found for either species.

Significant differences in mass of *B. fowleri* tadpoles were found in the competition and drying treatments, but again there were no significant effects on mass for *B. nebulifer* tadpoles for either treatment (Table 4.4, Fig. 4.2). Mass was greatest for *B. fowleri* tadpoles when they were alone and in a non-drying tank. Least square means of mass for *B. fowleri* alone under drying conditions was 0.093 (±0.0008) g and 0.077 (±.002) g for *B. fowleri* in competition in drying conditions. The least square mean of mass for *B. fowleri* metamorphs under non-drying conditions was 0.115 (±0.001) g and 0.0962 (0.004) g when *B. fowleri* that were in competition with *B. nebulifer*. There was also no interaction effect between competition and drying treatment for mass in either species.

Larval period was not significantly affected by the drying or the competition treatment for either species (Table 4.5, Fig. 4.3). There was no interaction between competition and drying treatments for larval period in either species. The difference in larval period between species across drying and competition treatments was striking (Fig. 4.9). In every tank but one (a not drying competition tank), the first date of emergence was 11 days for *B. nebulifer* tadpoles. *Bufo fowleri* tadpoles' earliest emergence ranged from day 13 to day 15 and emergence date was not affected by treatment. Least squares means for larval period in *B. fowleri* alone in drying and not drying tanks was 15.74 (± 0.099) days and 17.20 (± 0.117) days, respectively. In competition tanks, *B. fowleri*'s larval period was 16.19 (± 0.251) days in the drying treatment and 16.72 (± 0.378) in the not drying treatment.

Survival to metamorphosis in *B. fowleri* tadpoles was significant for the competition treatment, but only showed a trend toward significance (P=0.0761) for the drying treatment

(Table 4.6, Fig. 4.4). Survival to metamorphosis was significantly greater for *B. fowleri* tadpoles when they were alone in a drying tank and was lowest in competition with *B. nebulifer* tadpoles in a non-drying tank. The least square mean of survival to metamorphosis for *B. fowleri* tadpoles alone in a drying tank was 83%. For *B. fowleri* tadpoles in competition with *B. nebulifer* in a drying tank the least square mean of survival to metamorphosis was 46%. The least square mean of survival to metamorphosis for *B. fowleri* tadpoles alone in a non-drying tank was 61% and for *B. fowleri* tadpoles in competition with *B. nebulifer* tadpoles in a non-drying tank survival to metamorphosis was 17%.

The drying treatment was significant for survival in *B. nebulifer* tadpoles, but competition was not. Survival to metamorphosis was greatest for *B. neublifer* tadpoles in a drying tank in competition with *B. fowleri* tadpoles. In the drying treatment without competition, the least mean square of *B nebulifer*'s survival to metamorphosis was 53%, but in the drying treatment in competition with *B. fowleri* tadpoles, its least square mean of survival to metamorphosis rose to 81%. In the non-drying treatment without competition, *B. nebulifer*'s survival to metamorphosis was 41% and in the non-drying treatment in competition with *B. fowleri*, the least square mean of survival to metamorphosis for *B. nebulifer* was 43%. There was no interaction effect between competition and drying treatment for survival in either species.

Table 4.3: ANOVA of log-transformed snout-vent length for both species from the 2005 competition experiment. Significant P-values are in bold.

Response variable	Species	Treatment	df	Type III Mean Squares	F	P
S-v length	B. fowleri					
		drying	1	0.261184	13.55	**0.0060**
		competition	1	0.192471	9.99	**0.0131**
		interaction	1	0.012502	0.65	0.4435
		block	2	0.005839	0.28	0.7647
		Error	8.1352	0.019274		
	B. nebulifer					
		drying	1	0.128711	2.22	0.1808
		competition	1	0.088352	1.53	0.2577
		interaction	1	0.000506	0.01	0.9282
		block	2	0.047829	0.83	0.4774
		Error	6.7889	0.057879		

Table 4.4: ANOVA of log-transformed mass for both species from the 2005 competition experiment. Significant P-values are in bold.

Response variable	Species	Treatment	df	Type III Mean Squares	F	P
mass	B. fowleri					
		drying	1	2.489332	13.79	**0.0070**
		competition	1	1.613647	8.94	**0.0193**
		interaction	1	0.000061	0.00	0.9858
		block	2	0.057800	0.28	0.7560
		Error	7.2855	0.180465		
	B. nebulifer					
		drying	1	0.487131	3.05	0.1305
		competition	1	0.263867	1.65	0.2453
		interaction	1	0.295051	1.85	0.2222
		block	2	0.246401	1.54	0.2870
		Error	6.1136	0.159782		

Table 4.5 ANOVA of log-transformed larval period for both species from the 2005 competition experiment. Significant P-values are in bold.

Response variable	Species	Treatment	df	Type III Mean Squares	F	P
larval period	B. fowleri					
		drying	1	0.150001	2.51	0.1553
		competition	1	0.000953	0.02	0.9029
		interaction	1	0.024033	0.00	0.5454
		block	2	0.038528	0.28	0.5867
		Error	7.3444	0.059828		
	B. nebulifer					
		drying	1	0.141646	0.90	0.3781
		competition	1	0.026392	0.17	0.6957
		interaction	1	0.029923	0.19	0.6774
		block	2	0.012802	0.08	0.9227
		Error	6.122	0.156955		

Table 4.6 ANOVA of arcsine square root-transformed survival to metamorphosis for both species from the 2005 competition experiment. Significant P-values are in bold.

Response variable	Species	Treatment	df	Type III Mean Squares	F	P
survival	B. fowleri					
		drying	1	0.213373	4.58	0.0761
		competition	1	0.592747	12.73	**0.0118**
		interaction	1	0.003917	0.08	0.7816
		block	2	0.048305	1.04	0.4103
		Error	6	0.046569		
	B. nebulifer					
		drying	1	0.240196	7.16	**0.0368**
		competition	1	0.089814	2.68	0.1530
		interaction	1	0.075084	2.24	0.1854
		block	2	0.004253	0.13	0.8833
		Error	6	0.033563		

Figures 4.1 – 4.8: Least square means graphs of size variables, larval period and survival to metamorphosis. Figures 1-4 (left side of the page) are from the 2005 competition experiment. Figures 5-8 (right side of the page) are results for the same response variable from the 2006 predation experiment.

2005

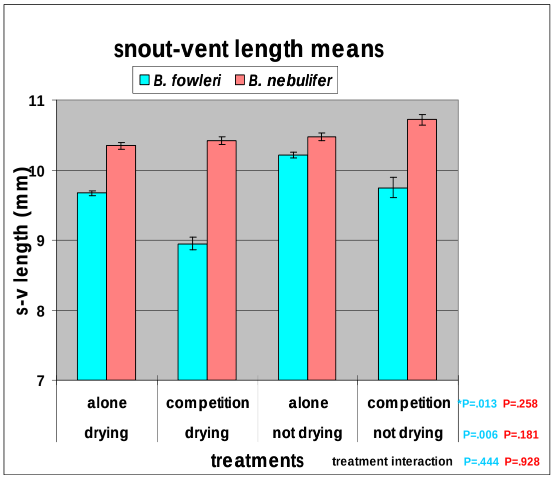

Figure 4.1 – Mean snout-vent length (least square means ± 2 S.E.) for both species.

2006

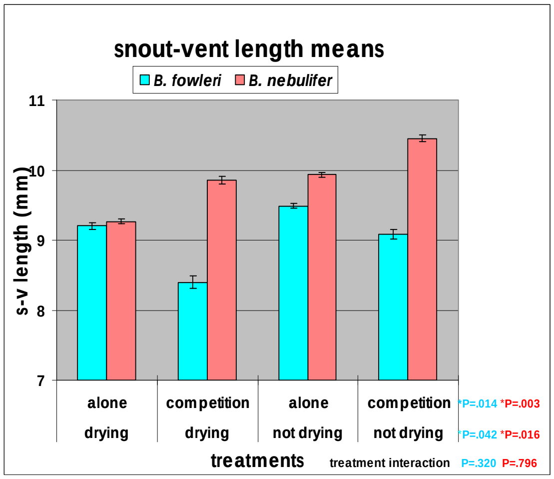

Figure 4.5 –Mean snout-vent length (least square means ± 2 S.E.) for both species.

2005

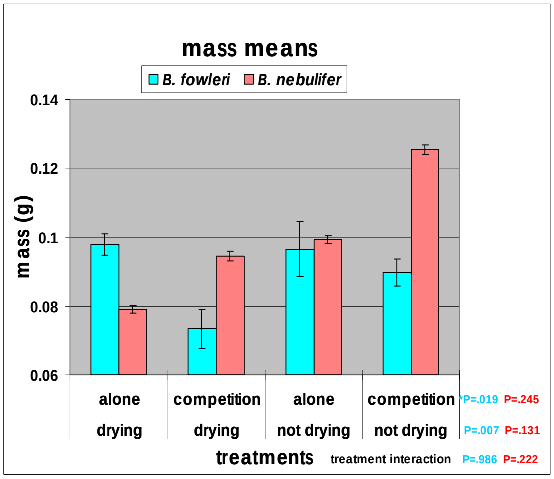

Figure 4.2- Mean mass (least square means ± 2 S.E.) for both species.

2006

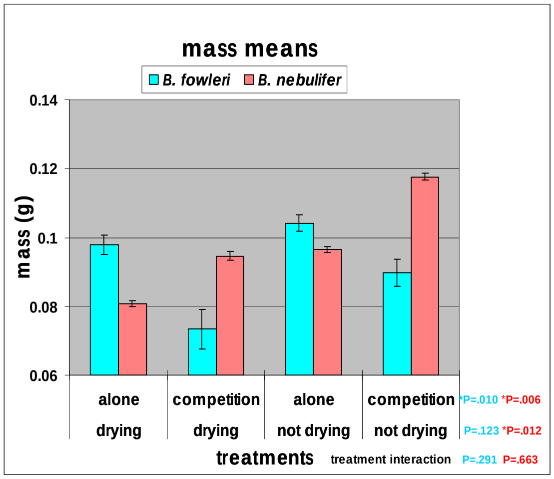

Figure 4.6 – Mean mass (least square means ± 2 S.E.) for both species.

Figures 4.1 – 4.8 continued

2005

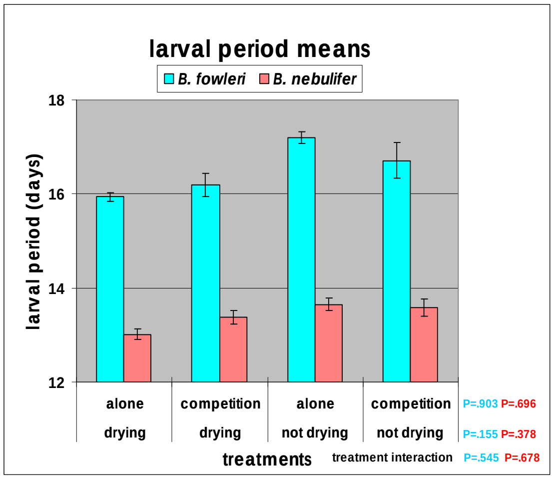

Figure 4.3 – Mean larval period (least squares means ± 2 S.E.) for each species.

2006

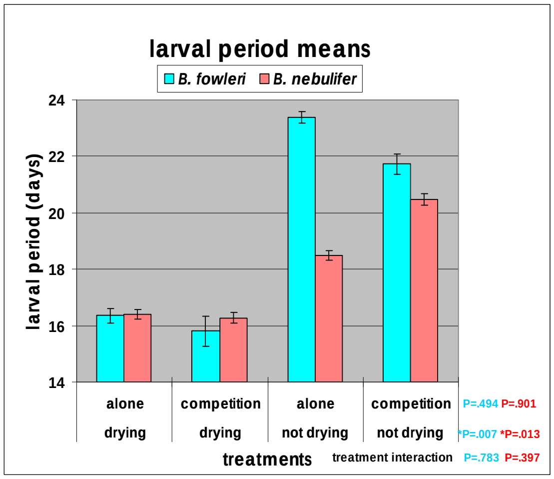

Figure 4.7 – Mean larval period (least squares means ± 2 S.E.) for each species.

2005

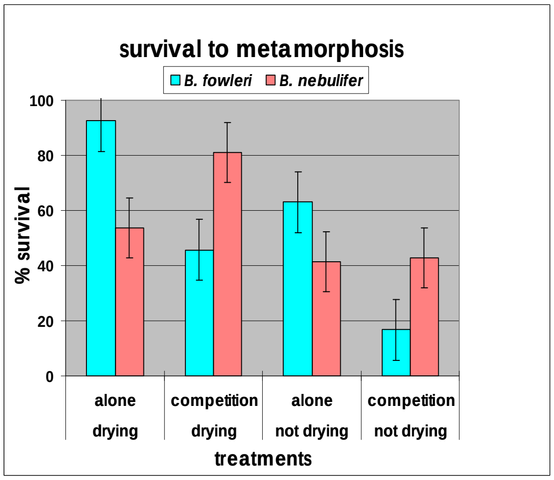

Figure 4.4 - Mean survival (least squares means ± 2 S.E.) for both species.

2006

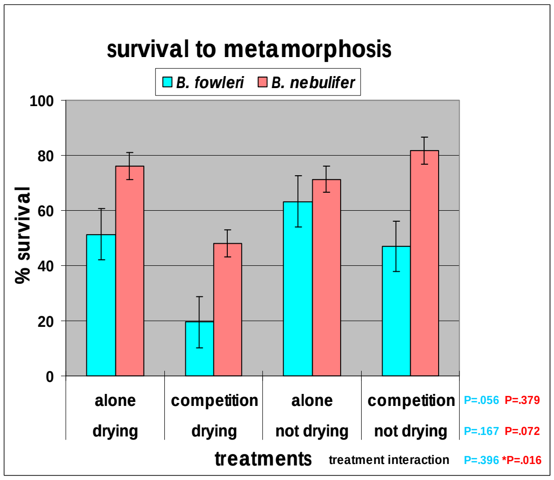

Figure 4.8 - Mean survival (least squares means ± 2 S.E.) for both species.

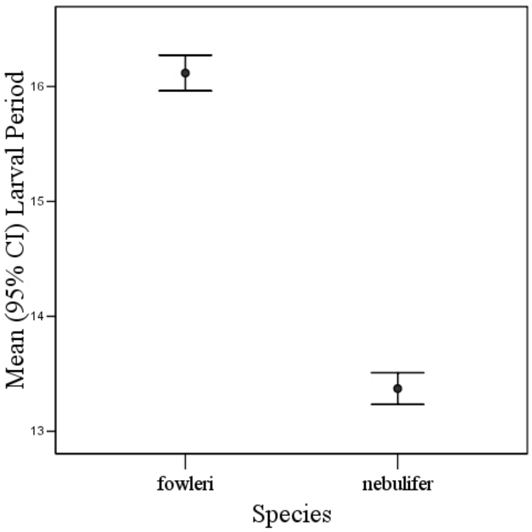

Figure 4.9 – Mean differences between species' larval periods (in days) from competition experiment.

Predation Experiment

ANOVA of both species without predation

ANOVA was used to analyze the mean differences between *B. fowleri* and *B. nebulifer* in snout-vent length (mm), mass (g), larval period (number of days) and percent survival to metamorphosis by removing all predator treatment data (including treatments where there were survivors) for both species and discarding predator as a predictor variable. The least square mean differences in body size from the predation experiment strongly resembled the results from the previous year's competition experiment.

Snout-vent length and mass of *B. nebulifer* and *B. fowleri* tadpoles were significantly affected by the competition and drying treatments. In competition with *B. nebulifer* tadpoles, the snout-vent length (Table 4.7, Fig. 4.5) and mass (Table 4.8, Fig. 4.6) of *B. fowleri* tadpoles was significantly lower. In non-drying, intraspecific competition the least-square mean snout-vent length for *B. fowleri* was 9.488 (±0.0361) mm and least square mean mass was 0.1042 (± 0.0023) g and in drying, interspecific treatment the least square mean snout-vent length was 8.395 (±0.09) mm and least square mean mass was .0734 (±0.0057). The competition and drying treatments significantly affected *B. nebulifer* tadpoles in both mass and snout-vent length also; the least square mean snout-vent length of 9.265 (±0.0324) mm and least square mean mass of 0.1176 (±0.001) was highest in interspecific competition in non-drying conditions and the least square mean snout-vent length of 0.0808 (±0.0008) mm and the least square mean mass of 9.26 (±0.0324) g was lowest in the drying treatment under intraspecific competition

Larval periods for both species were significantly affected by the drying treatment (Table 4.9, Figs. 4.7 &. 4.9). Both tadpoles had much longer larval periods in the competition experiment. There was an approximately seven day increase in mean larval period for *B. fowleri* in the non-drying, intraspecific competition treatment compared to the 2005 experimental results. The increase in mean larval period for both species is probably attributable to the almost 2 month difference (late April vs. early June) in when the eggs were collected for the 2005 and 2006 experiments. The difference in larval periods in the drying treatment from 2006 to the previous year reflects the change in protocol that precluded further metamorphosis from the tanks after the conclusion of the drying treatment.

There were no significant affects of either treatment on percent survival to metamorphosis for either species, though there was a significant interaction between treatments for tadpoles of *B. nebulifer* (Table 4.10, Fig. 4.8). There was also an extremely strong trend toward a negative affect of the competition treatment on *B. fowleri* tadpoles. Mean survival to metamorphosis for *B. fowleri* tadpoles was similar to the results of the previous year and was much greater 63.33% (±0.091) in non-drying tanks when they were not in competition with *B. nebulifer* tadpoles than mean percent survival 19.52% (± 0.091) in drying, interspecific competition tanks. Larvae of *B. nebulifer* were not affected by the individual affects of either treatment, but did show a significant affect of the interaction of the treatments. Percent mean survival to metamorphosis for *B. nebulifer* was highest 85.71% (± 0.0485) in competition in the not-drying tanks and percent mean survival was lowest 48.10 (± 0.0485) in competition in the drying tanks.

Logistic regression of both species in the predator treatment

A logistic regression of the main effects of toad species, predation, water level and their interactions was used to examine differences in the effect of predation on the two species (Fig. 4.10). There was a highly significant predator x species interactions (Wald statistic = 56.58, df =1, p <0.001). It is clearly evident that *B. fowleri* was completely excluded from all predator treatments. There was also a highly significant three-way interaction (species x water treatment x predation treatment; Wald statistic = 25.62, df =1 p < 0.001). Interestingly, *B. fowleri* has higher survivorship than *B. nebulifer* in predator-free, non-drying conditions and the opposite is true in the drying treatments.

Table 4.7 ANOVA of log-transformed snout-vent length for both species for the 2006 predation experiment. Significant P-values are in bold.

Response Variable	Species	Treatment	df	Type III Mean Squares	F	P
S-v length	B. fowleri					
		drying	1	0.236204	6.42	**0.0421**
		competition	1	0.414197	11.25	**0.0139**
		interaction	1	0.042792	1.16	0.3200
		block	2	0.027842	0.76	0.5074
		Error	6.3977	0.036818		
	B. nebulifer					
		drying	1	0.677984	10.85	**0.0163**
		competition	1	0.488255	7.81	**0.0310**
		interaction	1	.004563	0.07	0.7959
		block	2	0.114797	0.1.83	0.2395
		Error	6.0726	.062498		

Table 4.8 ANOVA of log-transformed mass for both species for the 2006 predation experiment. Significant P-values are in bold.

Response variable	Species	Treatment	df	Type III Mean Squares	F	P
mass	B. fowleri					
		drying	1	1.003628	3.16	0.1227
		competition	1	4.176534	13.15	**0.0098**
		interaction	1	0.421357	1.33	0.2906
		block	2	0.180397	0.57	0.5931
		Error	6.4103	0.317911		
	B. nebulifer					
		drying	1	6.356786	17.08	**0.0060**
		competition	1	4.740830	12.74	**0.0116**
		interaction	1	0.078034	0.21	0.6630
		block	2	0.891960	2.38	0.1724
		Error	6.0732	0.374550		

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Table 4.9 ANOVA of log-transformed larval period for both species for the 2006 predation experiment. Significant P-values are in bold.

Response variable	Species	Treatment	df	Type III Mean Squares	F	P
larval period	B. fowleri					
		drying	1	8.676780	16.20	**0.0066**
		competition	1	0.282067	0.53	0.4947
		interaction	1	0.044464	0.08	0.7827
		block	2	0.053207	0.10	0.9069
		Error	6.1396	0.535460		
	B. nebulifer					
		drying	1	3.924682	11.72	**0.0139**
		competition	1	0.005589	0.02	0.9014
		interaction	1	0.426676	1.27	0.3018
		block	2	0.362777	1.08	0.3965

Table 4.10 ANOVA of arcsine square root-transformed survival to metamorphosis for both species for the 2006 predation experiment. Significant P-values are in bold.

Response variable	Species	Treatment	df	Type III Mean Squares	F	P
survival	B. fowleri					
		drying	1	0.129823	2.85	0.1669
		competition	1	0.199524	7.08	0.0563
		interaction	1	0.025478	0.90	0.3956
		block	2	0.045616	1.62	0.3261
		Error	6	0.046569		
	B. nebulifer					
		drying	1	0.103427	5.91	0.0719
		competition	1	0.010680	0.98	0.3785
		interaction	1	0.177842	16.30	**0.0156**
		block	2	0.017507	1.60	0.3290
		Error	6	0.010910		

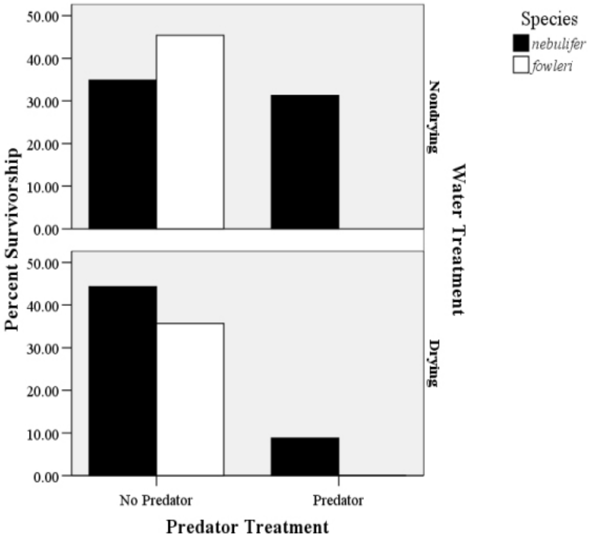


Figure 4.10 – Logistic regression of predation and drying treatments on % survivorship of both species.

ANOVA of B. nebulifer with predation

An analysis of predation on *B. fowleri* was not possible using ANOVA because there were zero survivors from every replicate of 4 of the 6 treatments containing predators, including every interspecific competition treatment. An ANOVA was performed on mean snout-vent length, mass, larval period, and proportion of individuals that survived to metamorphosis using *B. nebulifer* tadpoles since predation did not completely eliminate every tadpole from any replicate of any of the treatments. The competition treatment was significant for both snout-vent length (Table 4.11, Fig. 4.11) and mass (Table 4.12, Fig. 4.12) means and *B. nebulifer* tadpoles were largest under interspecific competition in the non-drying treatments in the tanks containing predators. Neither treatment was significant for larval period means or for survival to metamorphosis (Table 4.13, Fig. 4.13). The predation treatment was not significant for any of the response variables, except the proportion of individuals surviving to metamorphosis where it was extremely significant (Table 4.14, Fig. 4.14). There was 68.6% decline in *B. nebulifer* tadpole survival in the competition non-drying tanks with a predator present rather than absent.

Table 4.11 ANOVA of log-transformed snout-vent length for *B. nebulifer* for 2006 predation experiment. Significant P-values are in bold.

Treatment	df	Type III Mean Squares	F	P
drying	1	0.032428	7.55	0.1108
competition	1	0.023459	10.74	**0.0066**
predator	1	<0.00001	0.01	0.9419
drying x competition	1	<0.00001	0.00	0.9767
drying x predator	1	0.00045	0.21	0.6559
competition x predator	1	0.000305	0.14	0.7151
water x competition x predator	1	0.000194	0.09	0.7709
block	2	0.004294	1.97	0.1826
Error	12	.002184		

Figure 4.11 Mean snout-vent length (least squares means ± 2 S.E.).

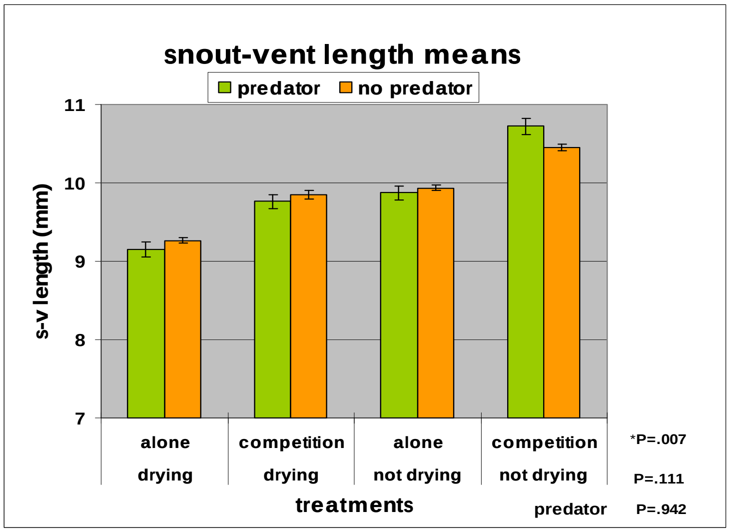


Table 4.12 ANOVA of log-transformed mass for *B. nebulifer* for 2006 predation experiment. Significant P-values are in bold.

Treatment	df	Type III Mean Squares	F	P
drying	1	0.254476	9.29	0.0929
competition	1	0.296001	14.84	**0.0023**
predator	1	0.018115	0.91	0.3594
drying x competition	1	0.003194	0.16	0.6961
drying x predator	1	0.000321	0.02	0.9012
competition x predator	1	0.014834	0.74	0.4054
water x competition x predator	1	0.012469	0.63	0.4445
block	2	0.027404	1.37	0.2902
Error	12	.002184		

Figure 4.12 Mean mass
(least squares means ± 2 S.E.).

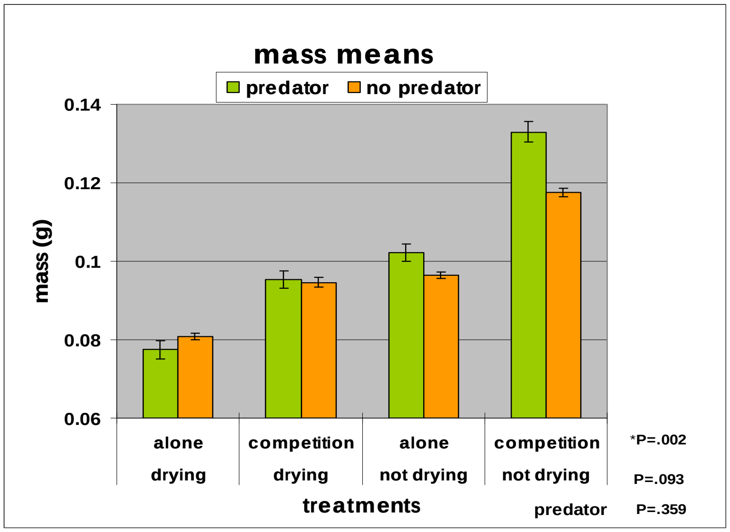


Table 4.13 ANOVA of log-transformed larval period for *B. nebulifer* for 2006 predation experiment.

Treatment	df	Type III Mean Squares	F	P
drying	1	0.166029	12.33	0.0724*
competition	1	0.009657	1.25	0.2855
predator	1	0.003756	0.49	0.4990
drying x competition	1	0.014875	1.92	0.1906
drying x predator	1	0.002119	0.27	0.6100
competition x predator	1	0.012523	1.62	0.2271
water x competition x predator	1	<0.00001	0.00	0.9752
block	2	0.026936	1.74	0.2165
Error	12	.007728		

Figure 4.13 Mean larval period (least squares means ± 2 S.E.).

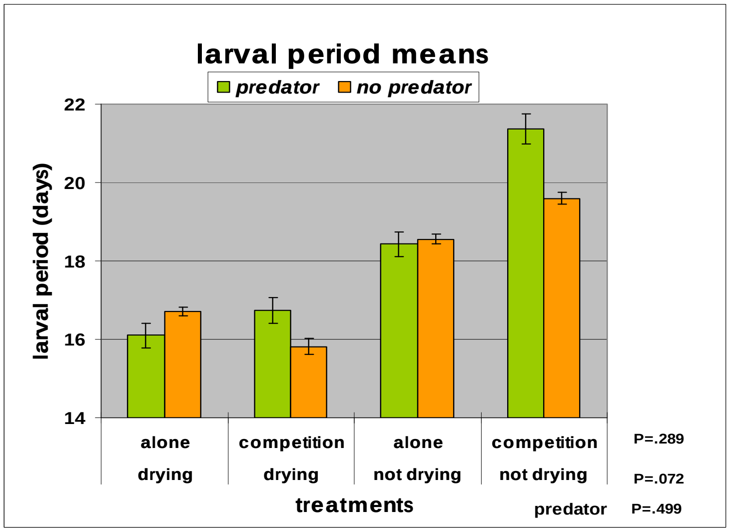
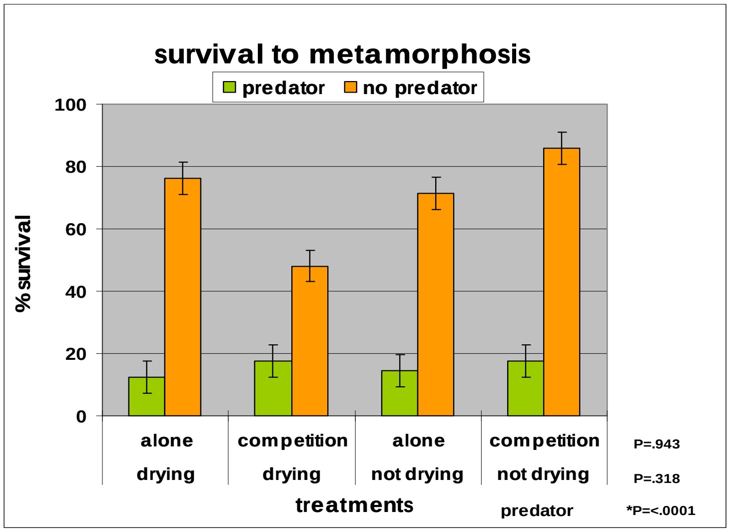


Table 4.14 ANOVA of arcsine square root-transformed survival to metamorphosis for *B. nebulifer* for 2006 predation experiment.

Treatment	df	Type III Mean Squares	F	P
drying	1	0.054855	1.74	0.3183
competition	1	0.000072	0.01	0.9434
predator	1	2.281546	165.58	<**0.0001**
drying x competition	1	0.077435	5.62	**0.0354**
drying x predator	1	0.048665	3.53	0.0847*
competition x predator	1	0.023919	1.74	0.2123
water x competition x predator	1	0.101201	7.34	**0.0190**
block	2	0.063173	2.29	0.1435
Error	12	.002184		

Figure 4.14 Mean survival to metamorphosis (least squares means ± 2 S.E.).



Discussion:

Competition Treatment

Bufo nebulifer demonstrated that is a much better competitor than *B. fowleri*, both in the drying and non-drying treatments, and this result confirms the hypothesis that competition between the larvae of the two species may be largely contributing to the decline of *B. fowleri*. Competition with *B. nebulifer* tadpoles had a very negative effect on both body size measures (i.e. snout-vent length and mass) and survival to metamorphosis for *B. fowleri* tadpoles.

That competition with *B. nebulifer* tadpoles would have such a detrimental affect on *B. fowleri* tadpoles was unexpected. Based on field observations of *B. fowleri*'s current breeding distribution throughout southern Louisiana over 3 breeding seasons (personal observation), the species breeds solely in permanent breeding habitat and it was predicted that *B. fowleri* may have had equal competitive footing in the non-drying tanks. Although tadpoles of *B. fowleri* should be able to compete with *B. nebulifer* tadpoles, *B. fowleri* was outcompeted under both drying and non-drying conditions. Interspecific competition was the driving force in the small size and lower percentage of survival to metamorphosis. *Bufo fowleri*'s intolerance of interspecific competition could be a driving mechanism for the decline of *B. fowleri* and the expansion of *B. nebulifer*. A small size at metamorphosis can result in lower juvenile survival and diminished reproductive success (Werner, 1986).

The drying treatment was also significant for *B. fowleri* for body size measures, but only marginally significant for survival to metamorphosis. The magnitude of the species' differences in body size measures is most pronounced in the tanks where the two species are in competition and are under drying conditions. *Bufo fowleri* tadpoles were largest in non-drying tanks in the absence of interspecific competition. Conversely, *B. nebulifer* tadpoles were, in size, unaffected by the drying and the competition treatments, though they were slightly larger in the drying treatments. Furthermore, *B. nebulifer* was more successful in interspecific tanks than in intraspecific tanks, indicating that at higher densities of tadpoles, it is easier for it to outcompete congenerics than conspecifics. There was no treatment interaction between competition and drying, demonstrating that *B. fowleri* tadpoles are adversely affected by both drying and competition individually.

Asymmetric competition between *Bufo* species has resulted in decreased survival, increased larval period and decreased size at metamorphosis of the inferior competitor (Gomez-

Mestre and Tejedo, 2002). Bardsley and Beebee (1998) documented deleterious asymmetric competition when they raised *B. bufo*, a range-expanding generalist, in sympatry and allopatry with *B. calamita*, a sand dune specialist. Because *B. bufo* tadpoles spawn earlier than *B. calamita* tadpoles they are competitively superior and when the two formerly ecologically isolated species breed in the same habitat *B. calamita* suffers increased mortality, a longer larval period and a smaller size at metamorphosis. Kiesecker et al. (2001) demonstrated that a competitive advantage of invasive bullfrog tadpoles (*Rana catesbiana*) over native, threatened red-legged frog tadpoles (*R. aurora*) was intensified in clumped- versus scattered-resource ponds, suggesting that interference in human-altered habitat may intensify competitive interactions of invasive and native larval amphibians.

The superior competitive ability of *Bufo nebulifer*, particularly in ephemeral wetlands may have resulted in the ecological displacement and subsequent decline of regionally sympatric populations of *B. fowleri* in human created or altered breeding habitats. *Bufo nebulifer* is likely to possess a competitive advantage over *B. fowleri* in disturbed habitats because it breeds primarily in non-natural temporary sites and is therefore adapted to actively forage and metamorphose faster and at a larger size. Due to their slower developmental rate, *B. fowleri* tadpoles may be unable to compete for food resources with *B. nebulifer* tadpoles in temporary breeding situations, and may prefer permanent ponds where their protracted larval period allows them to reach adequate size for metamorphosis. An inverse relationship between competitive strength and the ability to evade predators has been demonstrated among larval anuran species and supports this theory (Wilbur, 1987).

Amphibian larvae can vary rates of growth (i.e. size) and development (i.e. metamorphic stage) depending on factors such as imminent desiccation or, at the opposite extreme, the favorableness of ecological conditions at the breeding site (Werner and McPeek, 1994; Loman and Claesson, 2003; Morey and Reznick, 2004). Variability in pond hydroperiod induces contradictory selective pressures on development and growth rate, though pressure to be phenotypically plastic is stronger on ephemeral site breeders because an increase in development rate in response to drying cues (e.g. temperature, increased density of conspecifics, decreased food availability) directly affects survival (Leips et al., 2000; Loman and Claesson, 2003). Neither species responded to the drying treatment by decreasing time to metamorphosis, although the water level was not lowered quickly enough to threaten either species. *Bufo* *nebulifer* simply has a much faster developmental rate than *B. fowleri* under all four treatments. Larval period was slightly longer for *B. fowleri* in predator-free, non-drying treatments, possibly to take advantage of additional growth in a relatively optimal and predator-free environment.

Survival for *B. fowleri* was highest in drying tanks without competition. *Bufo fowleri* tadpoles had very low survival in drying tanks with *B. nebulifer* tadpoles; this may be attributable to the fact that *B. nebulifer* tadpoles were largest in these tanks and were competing fiercely. *Bufo nebulifer* had significantly higher survival (but were somewhat smaller) in drying than in non-drying tanks and this may be attributable to *B. nebulifer* also diverting resources to development rather than growth. The highly negative effect of competition on the survival of *B. fowleri* suggests that *B. fowleri* juvenile recruitment may be decreased each year, perhaps directly causing the decline in *B. fowleri*.

This experiment thoroughly supports the hypothesis that *B. nebulifer* is capable of outcompeting *B. fowleri* in drying and non-drying conditions. Only when *B. fowleri* tadpoles were in a non-drying tank without competition were they larger than *B. nebulifer* tadpoles and this result may partially explain why *B. fowleri* persists in undisturbed areas near large, permanent water bodies. However, the survival to metamorphosis of *B. fowleri* in the drying tanks indicates that it can successfully utilize temporary breeding habitat when it is not in competition with *B. nebulifer* tadpoles. The strong negative influence of interspecific competition with *B. nebulifer* tadpoles on *B. fowleri* tadpoles clearly indicates why *B. fowleri* may have rapidly disappeared from disturbed breeding habitat in southern Louisiana.

Predation Treatment

The addition of odonate predators to the original competition experiment did not favor the survival of *B. fowleri* over *B. nebulifer* as was originally hypothesized. In fact, the predators consumed every *B. fowleri* tadpole from all treatments except those in which the tadpoles were not competing with *B. nebulifer* or in danger of desiccation, and only a handful survived to metamorphosis from those tanks. The original hypothesis was constructed from *B. fowleri*'s choice of permanent breeding habitat in southern Louisiana, and the well-researched idea that growth is sacrificed under risk of predation; therefore, superior competitors are usually inversely susceptible to predation and vice versa (Morin, 1983; Werner and McPeek, 1994; Skelly, 1995; Skelly and Werner, 1990; Wellborn et al. 1996.; Relyea, 2000, 2001).

There are several explanations for the exclusion of *B. fowleri* tadpoles from the predator tanks. The sit and wait foraging strategy of *A. junius* and *P. longipennis* in and above the leaf litter at the bottom of the tanks may have foiled the attempts of *B. fowleri* to avoid them. Size at metamorphosis is strongly correlated with adult survival and reproduction, thus selection favors tadpoles that have a strong incentive acquire resources for rapid metamorphosis at a large size. Not only do *B. fowleri* tadpoles forage in and above the leaf litter in the odonates primary hunting territory but, because they were placed in the tanks before the predators, they may not have perceived or reacted to water-borne chemical signals rapidly. Chemical cues have been shown to be as important as or more important detecting and responding to predation risk in anuran larvae. (Kiesecker et al., 1996, Pearl et al., 2003). *Bufo nebulifer* tadpoles may be more responsive to invertebrate chemical cues because their preferred ephemeral breeding habitat is less likely to contain fish predators of invertebrates than the permanent breeding habitat of *B. fowleri*.

Anuran larvae can employ a suite of anti-predator defenses that are generally categorized as behavioral, life history, morphological or physiological, though most inducible morphological and physiological reactions are species-specific responses to particular predator species (Relyea, 2000; Relyea and Werner, 2000; Skelly and Werner, 1990). Skelly and Werner (1990) showed that larval American Toads (*B. americanus*) reduced activity and metamorphosed at a smaller size, in the presence of an odonate predator. In a study of larval Western Toads (*B. boreas*) raised in the presence of chemosensory stimuli from aquatic predators (*Notonecta* spp.) and aquatic non-predators (Corixidae), Chivers et al. (1999) found that Western Toad tadpoles metamorphose in a significantly shorter time in the presence of predators than in the absence of predators. Relyea (2001) found that *B. americanus* responded to predators, including *Anax* spp., by significantly reducing activity and developing a shallower and longer tail. Because a shorter larval period and reduced activity results in a smaller size at metamorphosis and lowered adult fitness, and there is a significant cost associated with inducible defense, responding only to aquatic invertebrates that pose a predation risk is probably adaptive (Van Buskirk, 2000, 2001).

The breeding sites used by *B. fowleri* in southern Louisiana are usually completely permanent and most contain fish (personal observation). Although most fish species find bufonid tadpoles entirely unpalatable, they do consume macroinvertebrates including dragonfly larvae. Snakes, bullfrogs and giant waterbugs (Genus: *Belastoma*) may be more dangerous than odonate predators in completely permanent breeding sites than in fishless sites that dry sporadically (Wellborn et al., 1996). Tadpoles of *B. fowleri* may not recognize odonate predators as a mortality risk in their native habitats.

In mean snout-vent length and mass, *Bufo nebulifer* tadpoles significantly benefited from the interspecific competition in the presence of the predator. Tadpoles were largest at metamorphosis in competition with *B. fowleri* in the non-drying tanks in the presence of predators. Release from competition with *B. fowleri* in the predation treatments seems to be the most plausible answer for the large size of *B. nebulifer* metamorphs in those treatments (Morin, 1983). Since the predators consumed every *B. fowleri* tadpole in the tanks, *B. nebulifer* foraged at much lower densities of competitors. The longer larval periods and larger size in the presence of competitors suggest that *B. nebulifer* did not reduce its activity or shorten its larval period to escape predation.

Predation resulted in a highly significant effect on the proportion of *B. nebulifer* tadpoles that survived to metamorphosis. However, a mean survival of 15.5% in the predation treatments compared to the total decimation of *B. fowleri* tadpoles is a substantial advantage. Although *B. nebulifer* tadpoles are certainly not adept at avoiding odonate predators they do have an advantage over the larvae *B. fowleri*. The advantage of *B. nebulifer* may lie in a higher toxicity level, though odonate preference between the two species was not obvious in laboratory trials. The significantly shorter larval period of *B. nebulifer* tadpoles also may be the key to its advantage over *B. fowleri*. Predation becomes less severe as tadpoles grow larger, and *B. nebulifer*'s rapid growth may allow some individuals to attain a substantially higher burst-swimming speed escape predation (Dayton et al., 2005).

Although further study is needed to ascertain the mechanism(s) by which *B. nebulifer* tadpoles survive predation by odonates and *B. fowleri* tadpoles do not, this experiment provides further evidence of the superiority of *B. nebulifer* tadpoles in breeding habitat shared by both species. The ability of *B. nebulifer* tadpoles to escape predators in both drying and non-drying habitats provides another advantage over B. *fowleri* tadpoles in the process of displacement of the latter species by the former.

CHAPTER 5: DISSERTATION CONCLUSIONS

Widespread and rapid declines of amphibian species around the world, even in areas that remain relatively pristine, have served as an inducement to act on behalf of not simply amphibians but other species and ecosystems as well. The effects of habitat fragmentation and invasive species have been studied in great detail and are arguably the most severe causes of worldwide biodiversity loss (Collins and Storfer, 2003). However, it is becoming increasingly apparent that there are many causes of amphibian decline and loss that require much more investigation, including the effects of climate change, UV-B radiation, pesticide and herbicide use, and disease (Collins and Storfer, 2003; Shaffer et al., 1998; Davidson, 2002). One area that requires much further consideration is the synergistic effects of known stressors, for instance, how the accumulation of agricultural toxins in tissues of the body affects disease resistance (Kiesecker et al., 2001). The focus of this dissertation is to integrate multiple disciplines to investigate how anthropogenic habitat disturbance is precipitating a range expansion in an invasive species, *B. nebulifer,* which is driving a decline in a native species, *B. fowleri*.

As Storfer (2003) states, identifying amphibian declines and disentangling potential synergistic causes can be extremely complex, but is highly necessary for the continued management, conservation and restoration of amphibian species. He emphasizes the utility of a multi-factorial approach to elucidate mechanisms of decline by integrating landscape-level molecular genetics, and empirical studies. This research used a landscape-level approach to identify changes in habitat disturbance over the past half century, combined with an examination of concurrent changes in species' occurrences to detect a decline in the species of concern and the concomitant increase in its invasive congener. This broad-scale analysis of species' distributional changes in different disturbance levels provided a framework within which to narrow the search for underlying mechanisms of decline and advance in the two species.

From the broader examination of the two species, two hypotheses were generated to test probable mechanisms by which an invasive amphibian species might displace a native (Sakai et al., 2001; With, 2001). A priori questions and predictions were developed primarily using historical museum collection data, as well as field observations, literature and anecdotal reports of local herpetologists. The first primary hypothesis was tested using two different molecular markers (nuclear and mitochondria) to identify cryptic hybrids and their maternal lineages. The second hypothesis was tested with two years of larval competition experiments in the presence and absence of predators over different drying regimes. Five questions were posed in the introductory chapter and will be answered based on the results on the research conducted in the course of this dissertation.

The first question posed was whether *B. fowleri* is undergoing a range contraction (i.e. decline) in southern Louisiana and if *B. nebulifer* expanding its range in southern Louisiana? The second question asked whether the decline in *B. fowleri* and increase in *B. nebulifer* were concurrent with an increase in habitat disturbance. Finally, the third question asked whether *B. fowleri* has undergone a decline in disturbed or undisturbed habitat in northern Louisiana where it is allopatric with *B. nebulifer*. These three questions will be answered simultaneously because there is a strong correlation between them.

Both species' distributions have changed significantly from the original collection period to the present, correlated with increasing disturbance levels at historical sites. An enormous decline in *B. fowleri* has occurred between the historic and current survey periods, concomitant with a surge in the distribution of *B. nebulifer*. As predicted, the decline of *B. fowleri* is most significant in areas of high disturbance, and the species is currently found in areas of low to moderate disturbance almost exclusively. The distributions of the two species are inversely affected by habitat disturbance; the distribution of *B. fowleri* in highly degraded habitat has contracted while the expansion of *B. nebulifer* increased substantially.

Although slightly fewer individuals were currently found in low and moderate habitat disturbance classes, *B. nebulifer* maintains its distribution in these habitats as well. The slight decline probably reflects an increase in landscape disturbance rather than a decrease in their distribution. Though some studies have reported that toads are generally more impervious to the effects of disturbance than other amphibian species (Hecnar and M'Closkey, 1996; Knutson et al., 1999; but see Gibbs et al., 2005), the demonstrated superiority of *B. nebulifer* in disturbed habitat may be more related to its invasive nature. Brown et al. (2006) found that radio-tracked individuals of the highly invasive South American species *B. marinus* preferentially uses cleared habitat and open corridors, such as roads and fencelines to disperse. A study conducted by Petren and Case (1998) of interspecific competition between the invasive common house gecko and the native mourning gecko showed that competitive displacement of the latter species by the former was much more severe in the absence of topographically complex habitat.

No decline was found in *B. fowleri* in areas of allopatry with *B. nebulifer*, in fact, a current increase in presence of *B. fowleri* in disturbed habitat was observed. This finding suggests that an increase in the distribution of *B. nebulifer* is the driving force in the decline of *B. fowleri* in sympatry, and not simply a result of habitat disturbance itself.

The fourth question asked whether interspecific hybridization between the two species could be detected using nuclear and mitochondrial markers and whether it contributed to a decline in *B. fowleri*. The use of both types of DNA is a unique method and its ability to identify morphologically cryptic amphibian hybrids and their matrilineal inheritance was successfully demonstrated. Sequence divergence made identification of hybrids straightforward; three hybrids were detected based on the presence of two nucleotides at the 12 nuclear intron sites fixed for alternate nucleotides in the parental species. Although less than 4% of the sampled populations were hybrids, this finding does not preclude the hypothesis that hybridization contributed to a decline in *B. fowleri*. Hybridization has historically been reported in mixed breeding populations of *B. fowleri* and *B. nebulifer* at rates as high as 8% (Volpe, 1960); however, the identification of a cryptic hybrid implies that the rate of historical hybridization may have been drastically underestimated since as many as half of F1 hybrids could be indistinguishable from *B. nebulifer* males. The discovery of cryptic hybridization using molecular methods is especially significant because the cross of male *B. fowleri* and female *B. nebulifer* was previously thought to be completely inviable.

Historic hybridization could have occurred at a much greater rate while *B. fowleri* was still abundant in East Baton Rouge (and neighboring parishes) but is now undetectable because most, if not all, hybrids are sterile males and hybridization and introgression therefore would be impossible to detect. Also, hybridization occurs much less frequently now simply because there are far fewer *B. fowleri* in southern Louisiana. It is interesting to note that the cryptic hybrid from a cross of a male *B. fowleri* and a female *B. nebulifer* was found in forested habitat that is preferred more by *B. fowleri* than by *B. nebulifer*. The other two hybrid crosses of *B. nebulifer* males with *B. fowleri* females were found in urban habitat within the city limits of Baton Rouge, where *B. nebulifer* currently far outnumbers *B. fowleri*. The lack of availability of conspecific mates may favor directionality of hybridization between these two species but is well beyond the scope of this study.

The final question asked if interspecific larval competition with *B. nebulifer* in ephemeral breeding sites characteristic of disturbed habitat contributing to a decline in *B. fowleri*? And, as a secondary focus to the main question, whether *B. fowleri* would outcompete *B. nebulifer* in permanent breeding habitats in the presence of a predator.

Bufo nebulifer demonstrated that is a much better competitor than *B. fowleri*, both in the drying and non-drying treatments, and this result confirms the hypothesis that competition between the larvae of the two species may be largely contributing to the decline of *B. fowleri*. Competition with *B. nebulifer* tadpoles had a very negative effect on both body size measures (i.e. snout-vent length and mass) and survival to metamorphosis for *B. fowleri* tadpoles.

Although tadpoles of *B. fowleri* should be able to compete with *B. nebulifer* tadpoles in permanent breeding sites where it breeds exclusively in southern Louisiana (L. Vogel, unpubl. data), *B. fowleri* was outcompeted under both drying and non-drying conditions. Interspecific competition was the driving force in the small size and lower percentage of survival to metamorphosis. *Bufo fowleri*'s inability to compete with its invasive congener could be a driving mechanism for the decline of *B. fowleri* and the expansion of *B. nebulifer*.

Asymmetric competition between *Bufo* species has resulted in decreased survival, increased larval period and decreased size at metamorphosis of the inferior competitor (Gomez-Mestre and Tejedo, 2002). Bardsley and Beebee (1998) documented deleterious asymmetric competition when they raised *B. bufo*, a range-expanding generalist, in sympatry and allopatry with *B. calamita*, a sand dune specialist. The competitive advantage of invasive bullfrog tadpoles (*Rana catesbiana*) over native, threatened red-legged frog tadpoles (*R. aurora*) demonstrated by Kiesecker et al. (2001) in clumped- versus scattered-resource ponds, suggests a possible mechanism by which *B. nebulifer* competitively displaces *B. fowleri*.

The superior competitive ability of *Bufo nebulifer*, particularly in ephemeral wetlands may have resulted in the ecological displacement and subsequent decline of regionally sympatric populations of *B. fowleri* in human created or altered breeding habitats. *Bufo nebulifer* is likely to possess a competitive advantage over *B. fowleri* in disturbed habitats because it breeds primarily in non-natural temporary sites and is therefore adapted to actively forage and metamorphose faster and at a larger size. Due to their slower developmental rate, *B. fowleri* tadpoles may be unable to compete for food resources with *B. nebulifer* tadpoles in temporary breeding situations, and may prefer permanent ponds where their protracted larval period allows them to reach adequate size for metamorphosis.

This experiment thoroughly supports the hypothesis that *B. nebulifer* is capable of outcompeting *B. fowleri* in drying and non-drying conditions. Only when *B. fowleri* tadpoles were in a non-drying tank without competition were they larger than *B. nebulifer* tadpoles and this result may partially explain why *B. fowleri* persists in undisturbed areas near large, permanent water bodies. However, the survival to metamorphosis of *B. fowleri* in the drying tanks indicates that it can successfully utilize temporary breeding habitat when it is not in competition with *B. nebulifer* tadpoles. The strong negative influence of interspecific competition with *B. nebulifer* tadpoles on *B. fowleri* tadpoles clearly indicates why *B. fowleri* may have rapidly disappeared from disturbed breeding habitat in southern Louisiana.

The addition of odonate predators to the original competition experiment did not favor the survival of *B. fowleri* over *B. nebulifer* as was originally hypothesized. In fact, the predators consumed every *B. fowleri* tadpole from all treatments except those in which the tadpoles were not competing with *B. nebulifer* or in danger of desiccation, and only a handful survived to metamorphosis from those tanks. The original hypothesis was constructed from *B. fowleri*'s choice of permanent breeding habitat in southern Louisiana, and the well-researched idea that growth is sacrificed under risk of predation; therefore, superior competitors are usually inversely susceptible to predation and vice versa (Morin, 1983; Werner and McPeek, 1994; Skelly, 1995; Skelly and Werner, 1990; Wellborn et al. 1996.; Relyea, 2000, 2001).

There are several explanations for the high mortality of *B. fowleri* tadpoles from the predator tanks. The sit and wait foraging strategy of the odonates in and above the leaf litter at the bottom of the tanks may have foiled the attempts of *B. fowleri* to avoid them. Not only do *B. fowleri* tadpoles forage in and above the leaf litter in the odonates primary hunting territory but, because they were placed in the tanks before the predators, they may not perceived or reacted to water-borne chemical signals rapidly. Chemical cues have been shown to be as important as or more important detecting and responding to predation risk in anuran larvae. (Kiesecker et al., 1996, Pearl et al., 2003). *Bufo nebulifer* tadpoles may be more responsive to invertebrate chemical cues because their preferred ephemeral breeding habitat is less likely to contain fish predators of invertebrates than the permanent breeding habitat of *B. fowleri*.

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Although further study is needed to ascertain the mechanism(s) by which *B. nebulifer* tadpoles survive predation by odonates and *B. fowleri* tadpoles do not, this experiment provides further evidence of the superiority of *B. nebulifer* tadpoles in breeding habitat shared by both species. The ability of *B. nebulifer* tadpoles to escape predators in both drying and non-drying habitats provides another advantage over B. *fowleri* tadpoles in the process of displacement of the latter species by the former.

This research is novel in numerous respects. The incorporation of historical data with remote sensing and GIS is beginning to be recognized as a powerful combination in conservation biology. Use of both types of molecular markers is an extremely recent and underutilized method to identify hybrids and their maternal ancestors. Multi-year empirical studies to test mechanisms of decline based on field observations and remote sensing and historical data are an uncommon, yet pragmatic, approach to identifying mechanisms of decline.

The complementary design of this research project employs a variety of traditional and innovative techniques to answer several different hypotheses that are related to the central research question of whether environmental disturbance intensifies invasiveness to result in the loss of a native species. The methods discussed in this dissertation offer promising and practical new approaches for evaluating and managing changes in the distribution of species of conservation concern.

Literature Cited:

Alford, R. A. and R.N. Harris. 1988. Effects of larval growth history on larval metamorphosis. American Naturalist 131:91-106.

Alford, R.A. and S.J. Richards. 1999. Global amphibian declines: a problem in applied ecology. Annual Review of Ecology and Systematics 30:133-165.

Alford, R.A., Dixon, P.M. and Pechmann, J.H.K. 2001. Global amphibian population declines. Nature 412:499-500.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenberg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution 16:613-622.

Anderson, J. R., E. E. Hardy, J. T. Roach, and R. E. Witmer. 1976. A land use and land cover classification system for use with remote sensor data. U.S. Geological Survey Professional Paper 964. 28 pp.

Anderson, R. P., Peterson, A. T. and M. Gomez-Laverde. 2002. Using niche-based GIS modeling to test geographic predictions of competitive exclusion and competitive release in South American pocket mice. Oikos 98:3-16.

Araujo, M.B., Thuiller, W. and R. G. Pearson. 2006. Climate warming and the decline of amphibians and reptiles in Europe. Journal of Biogeography 33:1712-1728.

Arnold, M. L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York.

Avise, J. C. 2000. Phylogeography. Harvard University Press, Cambridge, MA.

Barbaro, L., Rossi, J., Vetillard, F., Nezan, J. and H. Jactel. 2007. The spatial distribution of birds and carabid beetles in pine plantation forests: the role of landscape composition and structure. Journal of Biogeography 34:652-664.

Bardsley, L. and T.J.C. Beebee. 1998. Interspecific competition between *Bufo* larvae under conditions of community transition. Ecology 79:1751-1759.

-------------------------------------. 2001. Strength and mechanisms of competition between common and endangered anurans. Ecological Applications 11:453-463.

Barinaga, M. 1990. Where have all the froggies gone? Science 247:1033-1034.

Beebee. T. J. C. 1985. Discriminant analysis of amphibian habitat determinants in South-east England. Amphibia-Reptilia 6:35-43.

Belfiore, N. M., F. G. Hoffman, R. J. Baker, and J. A. Dewoody. 2003. The use of nuclear and mitochondrial single nucleotide polymorphisms to identify cryptic species. Molecular Ecology 12:2011–2017.

Blair, A. P. 1941. Variation, isolation mechanisms and hybridization in certain toads. Genetics 26:398-417.

Blair, W. F.1959. Genetic compatibility and species groups in U.S. toads (*Bufo*). Texas Journal of Science 11:427-453.

---------- 1963. Intragroup genetic compatibility in the *Bufo americanus* species group of toads. Texas Journal of Science 13:163-175.

Blaustein, A. R. and Wake, D.B. 1990. Declining amphibians: a global phenomenon? Trends in Ecology and Evolution 5:203-204.

Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinction. Conservation Biology 8:60-71.

Blaustein, A. R. and J. M. Kiesecker. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters 5:597-608.

Boone, M. D. and R. D. Semlitsch. 2001. Interactions of an insecticide with larval density and predation in experimental amphibian communities. Conservation Biology 15:228-238.

Brockelman, W. Y. 1969. An analysis of density effects and predation in *Bufo americanu*s tadpoles. Ecology 50:632-644.

Brown, G. P., Phillips, B. L., Webb, J. K. and R. Shine. 2006. Toad on the road: use of roads as dispersal corridors by cane toads (*Bufo marinus*) at an invasion front in tropical Australia. Biological Conservation 133:88-94.

Brown, J.H., Stevens, G.C. and D.M. Kaufman. 1996. The geographic range: size, shape, boundaries and internal structure. Annual Review of Ecology and Systematics 27:597-623.

Brown, K. M. 1982. Resource overlap and competition in pond snails: an experimental analysis. Ecology 63:412-422.

Brown, K. M. and D. R. DeVries. 1985. Predation and the distribution and abundance of a pulmonate pond snail. Oecologica 66:93-99.

Brown, L. E. 1971. Natural hybridization and reproductive ecology of two toad species in a disturbed environment. American Midland Naturalist 86:185-199.

Brumfield, R., P. Beerli, D. A. Nickerson, and S. V. Edwards. 2003. The utility of single nucleotide polymorphisms in inferences of population history. Trends in Ecology and Evolution 18:249-257.

Burke, J. M. and M. L. Arnold. 2001. Genetics and the fitness of hybrids. Annual Review of Genetics 35:31-52.

Carey, C., Heyer, W. R., Wilkinson, J., Alford, R. A., Arntzen, J. W., Halliday, T., Hungerford, L., Lips, K. R., Middleton, E. M., Orchard, S. A. and A. S. Rand. 2001. Amphibian declines and environmental change: use of remote sensing data to identify environmental correlates. Conservation Biology 15:903-913.

Carroll, C., Zielinski, W. J. and R. F. Noss. 1999. Using presence-absence data to build and test spatial habitat models for the fisher in the Klamath region, U.S.A. Conservation Biology 13:1344-1359.

Case, T. J., Holt, R.D., McPeek, M. A. and T. H. Keitt. 2005. The community context of species' borders: ecological and evolutionary perspectives. Oikos 108:28-46.

Case, T.J. and M.L. Taper. 2000. Interspecific competition, environmental gradients, gene flow and the coevolution of species' borders. American Naturalist 155:583-605.

Chivers, D. P., Kiesecker, J. M., Marco, A., Wildly, E. L. and A. R. Blaustein. 1999. Shifts in life history as a response to predation in Western Toads (*Bufo boreas*). Journal of Chemical Ecology 25:2455-2463.

Clarke, R. 1973. Activity and movement patterns in a population of Fowler's Toad, *Bufo woodhousei fowleri*. American Midland Naturalist 92:257-273.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657-1660.

Collins, J. P., and A. Storfer. 2003. Global amphibian declines: Sorting the hypotheses. Diversity & Distributions 9:89-98.

Colwell, R.K. and D.J. Futuyma. 1971. On the measurement of niche breadth and overlap. Ecology 52:567-576.

Conant, R. and J. T. Collins. 1998. Peterson Field Guide to Reptiles and Amphibians: Eastern/Central North America. Houghton Mifflin Company, New York, NY.

Cory, L. and J.J. Manion. 1955. Ecology and hybridization in the genus *Bufo* in the Michigan-Indiana region. Evolution 9:42-51.

Cowardin, L. M., V. Carter, F. C. Golet, E. T. LaRoe. 1979. Classification of wetlands and deepwater habitats of the United States. U. S. Department of the Interior, Fish and Wildlife Service, Washington, D.C. Jamestown, ND: Northern Prairie Wildlife Research Center Online.

Crumrine, P. W. 2005. Size structure and substitutability in an odonate intraguild predation system. Oecologia 145:132-139.

Davidson, C., Shaffer, H.B. and M.R. Jennings. 2001. Declines of the California red-legged frog: climate, UV-B, habitat and pesticide hypotheses. Ecological Applications 11:464-479.

--. 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. Conservation Biology 16:1588-1601.

Dayton, G. H., Saenz, D., Baum, K. A., Langerhans, R. B. and T. J. DeWitt. 2005. Body shape, burst speed and escape behavior of larval anurans. Oikos 111:582-591.

Dominguez-Dominguez, O., Martinez-Meyer, E., Zambrano, L. and G. Perez-Ponce de Leon. 2006. Using ecological-niche modeling as a conservation tool for freshwater species: live-bearing fishes in central Mexico. Conservation Biology 20:1730-1739.

Drayton, B. and R. B. Primack. 1996. Plant species lost in an isolated conservation area in metropolitan Boston from 1894 to 1993. Conservation Biology 10:30-39.

Drost, C. A. and G. M. Fellers. 1996. Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, USA. Conservation Biology 10:414-425.

Duellman, W. E. and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill, New York.

Dugan, Patrick, ed. 1993. Wetlands in Danger: A World Conservation Atlas. Oxford University Press. New York, NY.

Dundee, H. A. and D. A. Rossman. 1989. The Amphibians and Reptiles of Louisiana. Louisiana State University Press. Baton Rouge, LA.

Ernst, C. H., Boucher, T. P., Sekscienski, S. W., and J. C. Wilgenbush. 1995. Historical distribution, current status and a range extension of *Bufo boreas* in Utah. Herpetological Review 26:187-189.

Falcucci, A., Maiorano, L. and L. Boitani. 2007. Changes in land- use/land-cover patterns in Italy and their implications for biodiversity conservation. Landscape Ecology 22:617-631.

Fisher, R.N. and Shaffer, H.B. 1996. The decline of amphibians in California's great central valley. Conservation Biology 10:1387-1397.

Frissell, C. A. 1993. Topology of extinction and endangerment of native fishes in the Pacific northwest and California (U.S.A.) Conservation Biology 7:342-354.

Gaskin, J. F. and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. Proceedings of the National Academy of Science 99:11256-11259.

Gaston, K.J. 2003. The structure and dynamics of geographic ranges. Oxford University Press. New York, NY.

Gerhardt, H. C., S. I. Guttman, and A. A. Karlin. 1980. Natural hybrids between *Hyla cinerea* and *Hyla gratiosa*: morphology, vocalizations and electrophoretic analysis. Copeia 1980:577-584.

Gibbs, J.P., Whiteleather, K.K. and Schueler, F.W. 2005. Changes in frog and toad populations over 30 years in New York state. Ecological Applications 15:1148-1157.

Goins, C.R. and Caldwell, J.M. 1995. Historical atlas of Louisiana. University of Oklahoma Press. Norman, OK.

Gollman, G. 1996. Structure and dynamics of a hybrid zone in *Bombina* (Amphibia: *Anura: Discoglossidae*). Israel Journal of Zoology 42:121-133.

Gomez-Mestre, I. and Tejedo, M. 2002. Geographic variation in asymmetric competition: a case study with two larval anuran species. Ecology 83:2102-2111.

Gotelli, N.J. and A.M. Ellison. 2004. A primer of ecological statistics. Sinauer Associates, Inc. Sunderland, Massachusetts.

Graham, C.H., Ron, S.R., Santos J.C., Schneider J.C., and C. Moritz. 2004. New developments in museum-based informatics and applications in biodiversity analysis. Trends in Ecology and Evolution 19:497-503.

Green, D.M. 1999. Status Report Update on Fowler's Toad Bufo fowleri in Canada. Committee on the Status of Endangered Wildlife in Canada.

----------------.2000. Addendum to COSEWIC Status Report Update. A Population Viability Analysis of Fowler's Toads, Bufo fowleri, in Canada. Committee on the Status of Endangered Wildlife in Canada.

---------------. 2005. *Bufo fowleri* Hinckley. *In* Amphibian declines: the conservation status of United States species. pp. 408-412. Michael Lannoo (ed.). University of California Press.

Green, D.M., and C. Parent. 2003. Variable and asymmetric introgression in a hybrid zone in the toads, *Bufo americanus* and *B. fowleri*. Copeia 2003:34-43.

Guisan, A. and N. E. Zimmerman. 2000. Predictive habitat distribution models in ecology. Ecological Modelling 135:147-186.

Halverson, M. A., Skelly, D. K., Kiesecker, J. M. and L. K. Freidenburg. 2003. Forest mediated light regime linked to amphibian distribution and performance. Oecologia 134:360-364.

Harrison, R.G. and J. Arnold. 1982. A narrow hybrid zone between closely related cricket species. Evolution 36:535-552.

Hecnar, S. J. 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians in southern Ontario. Environmental Toxicology and Chemistry 14:2131-2137.

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

--------------------------------------. 1997. Patterns of nestedness and species association in a pond-dwelling amphibian fauna. Oikos 80:371-381.

Herkert, J. R. 1991. Prairie birds of Illinois: population response to two centuries of habitat change. Illinois Natural History Survey, Bulletin 34:393-399.

Hersteinsson P. and D.W. Macdonald. 1992. Interspecific competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus*. Oikos 64:505-515.

Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. C. and M. S. Foster. 1994. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institute Press. Washington D.C.

Hobbs, R.J. and H.A. Mooney. 1998. Broadening the extinction debate: population additions and deletions in California and Western Australia. Conservation Biology 12:271-283.

Hoegg, S., M. Vences, H. Brinkmann, and A. Meyer. 2004. Phylogeny and comparative substitution rates of frogs inferred from sequences of three nuclear genes. Molecular Biology and Evolution 71:1188-1200.

Holt, R.D. 2003. On the evolutionary ecology of species' ranges. Evolutionary Ecology Research 5:159-178.

Holt, R.D. and T.H. Keitt. 2005. Species' borders: a unifying theme in ecology. Oikos 108:3-6.

Holt, R. D., Keitt, T. H., Lewis, M. A., Maurer, B. A. and M. L. Taper. 2005. Theoretical models of species' borders: single species approaches. Oikos 108:18-27.

Holway, D. A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. Ecology 80:238-251.

Hostert, E. E. 1997. Reinforcement: a new perspective on an old controversy. Evolution 51:697-702.

Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, A. H., S. L. Kuzmin. 2001. Quantitative evidence for global population declines. Nature 412:499-500.

Huelsenbeck, J. P. 2000. MrBayes: Bayesian inference of phylogeny. Distributed by the author. Department of Biology, University of Rochester.

Jones, J. M. 1973. Effects of thirty years of hybridization on the toads *Bufo americanus* and *Bufo woodhousii fowleri* at Bloomington, Indiana. Evolution 27:435-448.

Keitt, T. H., Lewis, M. A. and R. D. Holt. 2001. Allee effects, invasion pinning, and species' borders. American Naturalist 157:203-216.

Kiesecker, J. M., Blaustein, A.R. and C.L. Miller. 2001. Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfrogs. Ecology 82:1964-1970.

Kiesecker, J.M., Blaustein, A.R. and L.K. Belden. 2001. Complex causes of amphibian population declines. Nature 410:681-684.

Kiesecker, J. M., Chiver, D. P. and A. R. Blaustein. 1996. The use of chemical cues in predator recognition by western toad tadpoles. Animal Behavior 52:1237-1245.

Kirkpatrick, M. and N.H. Barton. 1997. Evolution of a species' range. American Naturalist 150: 1-23.

Knutson, M. G., Sauer, J. L., Olsen, D. A., Mossman, M. J., Hemesath, L. M. and M. J.Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, U.S.A. Conservation Biology 13:1437-1446.

Kress, W. J., Miller, S.E., Krupnick, G.A. and T. E. Lovejoy. 2001. Museum collections and conservation efforts. Science 291:828-829.

Kruuk, L. E. B., J. S. Gilchrist, and N. H. Barton. 1999. Hybrid dysfunction in the fire-bellied toads (*Bombina*). Evolution 53:1611-1616.

Kulikova, I. V., Y. N. Zhuravlev, and K. G. McCracken. 2004. Asymmetric hybridization and sex-biased gene flow between eastern spot-billed ducks (*Anas zonorhyncha*) and mallards (*A. platyrhynchos*) in the Russian Far East. The Auk 121:930-49.

Kupferberg, S. J. 1997. Bullfrog (*Rana catesbiana*) invasion of a California river: the role of larval competition. Ecology 78:1736-1751.

Lamb, T. and J. C. Avise. 1986. Directional introgression of mitochondrial DNA in a hybrid population of treefrogs: the influence of mating behavior. Proceedings of the National Academy of Sciences 83:2526-2530.

Law, R. 1979. Optimal life histories under age specific predation. American Naturalist 114:399-417.

Leips, J., McManus, M. G. and J. Travis. 2000. Response of treefrog larvae to drying ponds: comparing temporary and permanent pond breeders. Ecology 81:2997-3008.

Lichter, J., Caron, H., Pasakarnis, T. S., Rodgers, S.L., Squiers Jr., T. S. and C. S. Todd. 2006. The ecological collapse and partial recovery of a freshwater tidal ecosystem. Northeastern Naturalist 13:153-178.

Light, T., Erman, D. C., Myrick, C. and J. Clarke. 1995. Decline of the Shasta Crayfish (*Pacifastacus fortis* Faxon) of Northeastern California. Conservation Biology 9:1567-1577.

Liner, E. A. 1954. The herpetofauna of Lafayette, Terrebonne and Vermillion Parishes, Louisiana. Proceedings of the Louisiana Academy of Sciences 17:65-85.

Lips, K. R. 1998. Decline of a tropical montane amphibian fauna. Conservation Biology 12:106-117.

Lips, K. R. 1999. Mass mortality and population declines of anurans at an upland site in western Panama. Conservation Biology 13:117-125.

Littell, R.C., Freund, R.J. and P.C. Spector. 1991. SAS system for linear models, 3rd edition. SAS Institute, Cary, N.C.

Loftus-Hills, J. J. and M. J. Littlejohn. 1992. Reinforcement and reproductive character displacement in *Gastrophryne carolinensis* and *G. olivacea* (*Anura: Microhylidae*): a reexamination. Evolution 46:896-906.

Loman, J. and D. Claesson. 2003. Plastic response to pond drying in tadpoles *Rana temporia*: tests of cost models. Evolutionary Ecology Research 5:179-194.

Loveland, T. R., Sohl, T. L., Stehman, S. V., Gallant, A. L., Sayler, K. L., and D. E. Napton. 2002. A strategy for estimating the rates of recent United States land-cover changes. Photogrammetric Engineering and Remote Sensing 68:1091-1099.

Lu, D., Mausel, P., Brondizios, E. and E. Moran. 2004. Change detection techniques. International Journal of Remote Sensing 25:1265-2407.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. and F.A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences and control. Ecological Applications 10:689-710.

Mahoney, D. L., Mort, M. A. and B. E. Taylor. 1990. Species richness of calanoid copepods, cladocerans and other branchiopods in Carolina bay temporary ponds. American Midland Naturalist. 123:244-258.

Malmos, K. B., B. K. Sullivan, and T. Lamb. 2001. Calling behavior and directional hybridization between two toads (*Bufo microscaphus* x *B. woodhousii*) in Arizona. Evolution 55:626-630.

Marjokorphi, A. and R. Otsamo. 2006. Prioritization of target areas for rehabilitation: a case study from West Kalimantan, Indonesia. Restoration Ecology 14:662-673.

Martinez, I.. Carreno, F., Escudero, A. and A. Rubio. 2006. Are threatened lichen species well-protected in Spain? Effectiveness of a protected areas network. Biological Conservation 133:500-511.

Masta, S. E., B. K. Sullivan, T. Lamb, and E. J. Routman. 2002. Molecular systematics, hybridization, and phylogeography of the *Bufo americanus* complex in Eastern North America. Molecular Phylogenetics and Evolution 24:302-314.

McDonnell, L.J., Gartside, D.F., and M.J. Littlejohn. 1978. Analysis of narrow hybrid zone between two species of *Pseudophryne* (Anura: Leptodactylidae) in south-eastern Australia. Evolution 32:602-612.

McLachlan, A .J. 1985. What determines the species present in a rain pool? Oikos 45:1-7.

McNab, W. H., and P. E. Avers. 1994. Ecological subregions of the United States:section descriptions. U.S. Forest Service WO-WSA-5. Washington, D.C.

Meacham, W. R. 1962. Factors affecting secondary intergradation between two allopatric populations in the *Bufo woodhousei* complex. American Midland Naturalist 67:282-304.

Mendelson, J.R., III. 1998. Geographic variation in *Bufo valliceps* (Anura: Bufonidae), a widespread toad in the United States and Middle America. Scientific Papers of the Natural History Museum, University of Kansas 8:1-12.

---------------- 1999. Natural history notes on the *Bufo valliceps* group (Anura: Bufonidae): description of the tadpole of *Bufo tutelarius*, and a key to the tadpoles of the group. Journal of Herpetology 33:327-332.

----------------2005. *Bufo nebulifer* Girard. *In* Amphibian declines: the conservation status of United States species. pp. 408-412. Michael Lannoo (ed.). University of California Press.

Milliken, G.A. and D.E. Johnson. 1984. Analysis of messy data. Volume 1: designed experiments. Van Nostrand Reinhold Company. New York, NY

Morey, S.R. and Reznick, D.N. 2004. The relationship between habitat permanence and larval development in California spadefoot toads: field and laboratory comparisons of developmental plasticity. Oikos 104:172-190.

Morin, P. A., G. Luikart, R. K. Wayne, and the SNP workshop group. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology and Evolution 19:208-216.

Morin, P. J. 1983. Predation, competition and the composition of larval anuran guilds. Ecological Monographs 53:119-138.

Morin, P.J. and E.A. Johnson. 1988. Experimental studies of asymmetric competition among anurans. Oikos 53:398-407.

Moyle, P.B. and T. Light. 1996. Biological invasions of fresh water: empirical rules and assembly theory. Biological Conservation 78:149-161.

Mulcahy D. M. and J. R. Mendelson, III. 2000. Phylogeography and speciation of the morphologically variable, widespread species *Bufo valliceps,* based on molecular evidence from mtDNA. Molecular Phylogenetics and Evolution 17:173-189.

Munger, J. C., Gerber, M., Madrid, K., Carroll, M., Peterson, W., and L. Heberger. 1997. U.S. National Wetland Inventory classifications as predictors of the occurrences of Columbia

Spotted Frogs (*Rana luteiventris*) and Pacific Treefrogs (*Hyla regilla*). Conservation Biology 12:320-330.

Navas, C. A. 2006. Patterns of distribution of anurans in high Andean tropical elevations: insights from integrating biogeography and evolutionary physiology. Integrative and Comparative Biology 46:82-91.

Noor, M. A. F. 1999. Reinforcement and other consequences of sympatry. Heredity 83:503-508.

Nylander, J. A. 2004. MrModeltest vers. 2.2. Distributed by the author. Evolutionary Biology Centre, Uppsala University.

Orton, G. L. 1951. An example of interspecific mating in toads. Copeia 1:78.

Pauly, G. B., D. M. Hillis, and D. C. Cannatella. 2004. The history of a Nearctic colonization: molecular phylogenetics and biogeography of the Nearctic toads (*Bufo*). Evolution 58:2517-2535.

Pavignano, I., Giacomo, C. and S. Castellano. 1990. A multivariate analysis of amphibian habitat determinants in north western Italy. Amphibia-Reptilia 11:311-324.

Pearce, J. L., Cherry, K., Drielsma, M., Ferrier, S. and G. Whish. 2001. Incorporating expert opinion and fine-scale vegetation mapping into statistical models of faunal distribution. Journal of Applied Ecology 38:412-424.

Pearl, C. A., Adams, M. J. Schuytema, G. S. and A. V. Nebeker. 2003. Behavioral responses of anuran larvae to chemical cues of native and introduced predators in the Pacific Northwestern United States. Journal of Herpetology 37:572-576.

Pechmann, J. H. K., Scott D. E., Semlitsch, R. D., Caldwell, J. P., Vitt, L. J. and J. W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. Science 253:892-895.

Pechmann, J.H.K. and H.M. Wilbur. 1994. Putting declining amphibian populations in perspective: natural fluctuations and human impacts. Herpetologica 50:65-84.

Petren, K. and T.J. Case. 1996. An experimental demonstration of exploitation competition in an ongoing invasion. Ecology 77:118-132.

----------------------------. 1998. Habitat structure determines competition intensity and invasion success in gecko lizards. Proceedings of the National Academy of Sciences 95:11739-11744.

Ponder, W.F., Carter, G.A., Flemons, P. and R.R. Chapman. 2001. Evaluation of museum collection data for use in biodiversity assessment. Conservation Biology 15:648-657.

Quinn, J.F. and A. Hastings. 1987. Extinction in subdivided habitats. Conservation Biology 1: 198-208.

Rahel, F. J. 1984. Factors structuring fish assemblages along a bog successional gradient. Ecology 65:1276-1289.

Relyea, R. A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. Ecology 81:2278-2289.

---------------. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology 82:523-540.

Relyea, R. A. and E. E. Werner. 2000. Morphological plasticity in four larval anurans distributed along an environmental gradient. Copeia 2000:178-190.

Reznick, D., Baxter, R.J. and J. Endler. 1994. Long-term studies of tropical stream fish communities: the use of field notes and museum collections to reconstruct communities of the past. American Zoologist 34:452-462.

Rhymer, J. M. and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27:83-109.

Richter, T.A., Webb, P.A. and J.D. Skinner. 1997. Limits to the distribution of the southern African Ice Rat (*Otomys sloggetti*): thermal physiology or competitive exclusion? Functional Ecology 11:240-246.

Richter-Boix, A., Llorente, G. A. and A. Montori. 2004. Responses to competition effects of two anuran tadpoles according to life-history traits. Oikos 106:39-50.

Riley, S .P. D., Busteed, G. T., Kats, L. B., Vandergon, T. L., Lee, L. F. S., Dagit, R. S., Kerby, J.L., Fisher, R.N. and R.M. Sauvajot. 2005. Effects of urbanization on the distribution and abundance of amphibians and invasive species in southern California streams. Conservation Biology 19:1894-1907.

Riley, S. P. D., H. B. Shaffer, S. R. Voss, and B. M. Fitzpatrick. 2003. Hybridization between a rare, native tiger salamander (*Ambystoma californiese*) and its introduced congener. Ecological Applications 13:1263-1275.

Rozas, J. and R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174-175.

Rubbo, J.M. and J.M. Kiesecker. 2005. Amphibian breeding distribution in an urbanized landscape. Conservation Biology 19:504-511.

Sakai, A.K., et al. 2001. The population biology of invasive species. Annual Review of Ecology and Systematics 32:305-332.

Saunders, D. L., Meeuwig, J.J. and A. C. J. Vincent. 2002. Freshwater protected areas: strategies for conservation. Conservation Biology 16:30-41.

Schlefer, E. K., M. A. Romano, S. I. Guttman, and S. B. Ruth. 1986. Effects of twenty years of hybridization in a disturbed habitat on *Hyla cinerea* and *Hyla gratiosa*. Journal of Herpetology 20:210-221.

Segelbacher, G., Hogland, J. and I. Storch. 2003. From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. Molecular Ecology 12:1773-1780.

Shaffer, H.B., Fisher, R.N. and C. Davidson. 1998. The role of natural history collections in documenting species declines. Trends in Ecology and Evolution 13:27-30.

Skelly, D. K. 1995. A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. Ecology 76:150-164.

----------------. 2001. Distributions of pond-breeding anurans: an overview of mechanism. Israel Journal of Zoology 47:313-332.

Skelly, D. K. and E. E. Werner. 1990. Behavioral and life-historical responses of larval American Toads to an odonate predator. Ecology 71:2313-2322.

Skelly, D.K., Freidenburg, L.K., and J. M. Kiesecker, J.M. 2002. Forest canopy and the performance of larval amphibians. Ecology 83:983-992.

Skelly, D. K., Yurewicz, K. L., Werner, E. E. and R. A. Relyea. 2003. Estimating decline and distributional change in amphibians. Conservation Biology 17:744-751.

Smith, K. G. 2005. Effects of nonindigenous tadpoles on native tadpoles in Florida: evidence of competition. Biological Conservation 123:433-441.

Stephens, M., and P. Donnelly. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. American Journal of Human Genetics 73:1162-1169.

Stephens, M., N. Smith, and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. American Journal of Human Genetics 68:978-989.

Storfer, A. 2003. Amphibian declines: future directions. Diversity and Distributions 9:151-163.

Sullivan, B. K. 1986. Hybridization between the toads *Bufo microscaphus* and B*ufo woodhousii* in Arizona: morphological variation. Journal of Herpetology 20:11-21.

Sullivan, B.K. and T. Lamb. 1988. Hybridization between the toads *Bufo microscaphus* and *Bufo woodhousii* in Arizona: variation in release calls and allozymes. Herpetologica 44:325-333.

Sullivan, B. K., K. B. Malmos, and M. F. Given. 1996. Systematics of the *Bufo woodhousii* complex (Anura: Bufonidae); advertisement call variation. Copeia 1996:274-280.

Swofford, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony* (and other methods). Sinauer Associates, Sunderland, MA.

Taylor, C. N., Oseen, K. L. and R. J. Wassersug. 2004. On the behavioural response of Rana and Bufo tadpoles to echinostomatoid cercariae: implications to synergistic factors influencing trematode infections in anurans. Canadian Journal of Zoology 82:701-706.

Thornton, W. A. 1955. Interspecific hybridization in *Bufo woodhousei* and *Bufo valliceps*. Evolution 9:455-468.

Turner, W., Spector, S., Gardiner, N. Fladeland, M. Sterling, E., and M. Steininger. 2003. Remote sensing for biodiversity science and conservation. Trends in Ecology and Evolution 18:306-314.

Van Buskirk, J. 2000. The costs of an inducible defense in anuran larvae. Ecology 81:2813-2821.

-----------------. 2001. Specific induced responses to different predator species in anuran larvae. Journal of Evolutionary Biology 14:482-489.

Verbyla, D. L. 2002. Practical GIS analysis. Taylor and Francis, New York, NY.

Volpe, E. P. 1955. Intensity of reproductive isolation between sympatric and allopatric populations of *Bufo B. americanus* and *Bufo fowleri*. American Naturalist 89:303- 318.

--------------. 1956. Experimental F_1 hybrids between *Bufo valliceps* and *Bufo fowleri*. Tulane Studies in Zoology. 4:59-76.

-------------- 1960. Evolutionary consequences of hybrid sterility and vigor in toads. Evolution 14:81-193.

Wake, D.B. 1991. Declining amphibian populations. Science 253:860.

Wellborn, G. A., Skelly, D. K. and E. E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27:337-363.

Werner, E.E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. The American Naturalist 128:319-341.

Werner, E.E. and M.A. McPeek.1994. Direct and indirect effects of predators on two anuran species along an environmental gradient. Annual Review of Ecology and Systematics 27:337-363.

Werner, E. E. and K. S. Glennemeier. 1999. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. Copeia 1:1-12.

Whittier, T. R., Larsen, D. P., Peterson, S. A. and T. M. Kincaid. 2002. A comparison of impoundments and natural drainage lakes in the Northeast USA. Hydrobiologica 470:157-171.

Wiggins, G. B., Mackay, R. J. and I. M. Smith. 1980. Evolutionary and ecological strategies of animals in annual temporary ponds. Archiv fuer Hydrobiologie. 58:97-206.

Wilbur, H.M. 1977. Density-dependent aspects of growth and metamorphosis in *Bufo americanus*. Ecology 58:196-200.

----------------. 1980. Complex life cycles. Annual Review of Ecology and Systematics 11:67-93.

----------------. 1987. Regulation of structure in complex systems: experimental temporary pond communities. Ecology 68:1437-1452.

----------------.1996. Multi-stage life cycles. *In* Spatial and temporal aspects of population processes. pp. 177-219. O. E. Rhodes, Jr. R. K. Chesser and M. H. Smith (eds). University of Chicago Press.

---------------.1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology 78:2279-2302.

Wilbur, H. M. and J. E. Fauth. 1990. Experimental aquatic food webs: interactions between two predators and two prey. American Naturalist 135:176-204.

Wilson, E.O. 2000. Museum collections and conservation efforts. Science 289:2279.

With, K. A. 2001. The landscape ecology of invasive spread. Conservation Biology 16:1192-1203.

Appendix:

Animal Subjects approval form

University of New Orleans

Institutional Animal Care and Use Committee (IACUC)

DATE: June 15, 2007

TO: Laura S. Vogel

FROM: Gerald J. LaHoste, Ph.D.
Chairman

RE: *IACUC Protocol No.* **091**
Entitled: **The decline of Fowler's Toad (*Bufo fowleri*) in southern Louisiana: molecular genetics, field experiments and landscape studies**

Your revised application for the use of animals in research (referenced above) was approved from the period of September 1, 2004 through August 31, 2007.

◆ A Member of the Louisiana State University System Committed to Equal Opportunity ◆

Vita:

Laura S. Vogel was born in Washington D.C. and received her B.S. in biology from Howard University, Washington D.C. She will pursue a postdoctoral research career working with endangered butterflies at North Carolina State University, Raleigh, NC.