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THE ECOLOGY OF THE ENDANGERED DUSKY GOPHER FROG (*RANA SEVOSA*) AND
A COMMON CONGENER, THE SOUTHERN LEOPARD FROG (*RANA SPHENOCEPHALA*)

A Dissertation

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

Doctor of Philosophy
in
Conservation Biology

by

Nicole Y. Thurgate

BSc(hons.) James Cook University, 1997

May 2006

DEDICATION

This dissertation is dedicated to my parents, Helen Mueller and the late Ian Mansfield Thurgate, without whose support and encouragement I would never have completed it. It is also dedicated to my sister Mia Thurgate who has helped me in more ways than I can name.

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ABSTRACT

Many amphibian populations are rapidly disappearing throughout the world. An important issue for ecologists is why some amphibian species are more susceptible to decline than others. Here I present five experiments that compare the performance of an endangered (*Rana sevosa*) and a common (*Rana sphenoccephala*) frog in changing habitats, to determine why these two species differ in their persistence. I include additional studies investigating the habitat requirements and behavior of *R. sevosa*. I found that habitat change in the form of canopy closure over breeding ponds negatively affects both species, making them smaller as tadpoles and at metamorphosis. The magnitude of size differences was greater for *R. sevosa* and this species was less likely to survive in closed canopy ponds. Larval survival was not affected in *R. sphenoccephala* and this is likely a key reason for the persistence of this species in habitats where *R. sevosa* has been extirpated. The introduction of fish to breeding ponds would also differentially affect the two species. *R. sevosa* did not display behavioral defenses to the threat of fish predation while *R. sphenoccephala* did. *R. sevosa* displayed a preference for certain characteristics in its habitat including open canopy ponds, grassy terrestrial habitats and an abundance of burrows. An association with the chemical cues of burrow making organisms suggests that these organisms may be important for *R. sevosa*. Therefore, specialized habitat requirements and behaviors which may be contributing to its decline. I found some evidence of asymmetric competition in the larval stage with *R. sevosa* negatively affecting *R. sphenoccephala*. It does not appear that larval competition with *R. sphenoccephala* has contributed to the decline of *R. sevosa*. The primary difference between the two species was in responses to habitat change. *R. sevosa* appears to be rigid in its habitat requirements and behavior and its inability to respond and adapt to change is a key component of its rarity. By contrast *R. sphenoccephala* showed an ability to

cope with habitat changes. Conservation of *R. sevosia* will require suitable management of the aquatic and terrestrial habitats, primarily through the instigation of an appropriate fire regime.

CHAPTER ONE INTRODUCTION

INTRODUCTION

Extinction, declines and persistence

Human activities are altering the biota of the planet in unprecedented ways. In the past 300 years scientists have documented a massive increase in the extinction of a variety of species worldwide (Benton 2003). Estimates place current extinction rates at 1,000 to 10,000 times greater than the background rates of extinction inferred from the fossil record, and ecologists are still unsure what the impact of this species loss will be (Singh 2002). We do know that a range of ecosystem processes relies significantly on biodiversity and that biodiversity provides useful products to humans such as food, medicine or shelter (Callicott 1997, Hooper et al. 2005). There is also an intrinsic worth to species, regardless of their usefulness to humans, and many would argue that we have moral obligations concerning the preservation of biodiversity (Rolston 1997). Therefore, conservation of biodiversity is of great importance and the current rate of extinction is cause for concern. Effective conservation however, requires a deeper understanding of the causes of these extinctions and declines.

Less frequently discussed are the numerous species that are surviving in spite of enormous changes to their environment. Why do some species go extinct while others appear to thrive in the same habitat? This question is fundamental to the basic problem of ecology: to determine the *causes* of the distribution and abundance of organisms (Krebs 2001). Understanding the reasons for both extinction and survival is an essential step in halting the global loss of biodiversity.

Worldwide amphibian declines and extinctions have received significant attention (Gibbons et al. 2000). Concern for these declines stems from the perceived importance of

amphibians as indicators of environmental quality (Blaustein & Kiesecker 2002). Amphibians may be especially sensitive to environmental changes because their complex life cycles expose them to both aquatic and terrestrial disturbances, and because their highly permeable skin and eggs make them more susceptible to fluctuations in the external environment (Pough et al. 1998). Additionally, amphibians are important predators, prey and grazers in a variety of ecosystems and their loss from these systems may affect other organisms (Blaustein & Kiesecker 2002).

Postulated explanations for amphibian declines are complex and are generally thought to result from the interaction of a number of factors, including increases in ultraviolet radiation, habitat destruction and alteration, introduction of exotic or novel predators and competitors, changes in acidity, exposure to toxicants and diseases, and global climate change (reviewed in Alford & Richards 1999, Blaustein & Kiesecker 2002). Explaining amphibian declines in relatively undisturbed habitats is more problematic and further research is needed to understand this phenomenon (Pough et al. 1998).

Habitat alteration and destruction has been directly linked to amphibian population declines and changes to amphibian community structure (Blaustein & Kiesecker 2002). In North America the widespread clearing of native forests, expansion of silviculture and rapidly increasing urbanization have been associated with amphibian declines (Skelly 2001). In places where forests remain intact, or where second growth forest has been planted or regenerated, other subtle processes are changing the composition of amphibian communities. Forest succession, fire suppression and selective logging can all cause alterations to the forest habitat and the ecology of animal communities within them (Halverson et al. 2003).

Competition is recognized as a ubiquitous component of natural systems however, no clear consensus exists regarding its importance relative to other regulatory mechanisms such as

disturbance and environmental change (Pianka 2000). Competition may be of particular importance in structuring communities containing rare species (Parris and Semlitsch 1998). If rare species are competitively inferior this can contribute to population declines and local extinctions (Jaeger 1972). Habitat change can further disadvantage rare species as changes to habitat may favor other species to the exclusion of the rare species (Bardsley and Beebee 2001). As anthropogenic habitat change is a feature of most landscapes at present, understanding the ecology of rare species in these habitats is important to their conservation. Additionally, understanding how rare and common species interact and knowing the outcomes of these interactions, provides fundamental information about the potential causes of commonness and rarity.

The behavior of organisms plays a role in many ecological processes that affect population structure and dynamics, such as competition, dispersal and reproduction (Blaustein & Walls 1995). There can be a strong genetic basis to behavior, particularly where such behaviors are adaptive and confer some fitness benefit (Murray et al. 2004). Changes to the habitat of an organism require the organism to have some flexibility within its behavioral repertoire, particularly where former behaviors become maladaptive. Research into the behavior of declining species can provide important information about the reasons for its decline.

Study organisms

The dusky gopher frog, *Rana sevosa* (Goin and Netting 1940) is arguably the most endangered amphibian in the United States. In January 2001 it was added to the federal endangered species list as a distinct population segment of the gopher frog *R. capito* (USFWS 2001). However, there is evidence that warrants the elevation of *R. sevosa* to the species level. Young and Crother (2001) used allozyme electrophoresis to examine the relationships between

gopher frogs (*R. capito* and *R. sevososa*) and crawfish frogs (*R. aereolata*) and found evidence to support the separation of the two groups. Additionally, they found a single allozyme allele that was always present in *R. sevososa* and always absent in other gopher frogs (*R. capito* ssp) providing evidence for the original designation of Goin and Netting (1940). A recent publication suggests changes to both the genus and species names of *R. sevososa* to *Lithobates sevosus* (Frost et al. 2006). To avoid confusion between published and unpublished portions of this dissertation however, I will continue to use the better known *R. sevososa*.

Dusky gopher frogs are in the family Ranidae. The original species distribution was from west of the Mobile River Basin in Alabama to east of the Mississippi River in Louisiana (Goin and Netting 1940). Although once common (Allen 1932), there are presently only two confirmed extant populations of *R. sevososa*, one consisting of less than 100 adults and the other with even fewer (Richter et al. 2003, M. Sisson, Mississippi Museum of Natural Science, personal communication). Researchers have conducted numerous surveys in Louisiana, Mississippi and Alabama in recent years but no further populations have been located (Bailey 1992, Seigel and Doody 1992, Karsen 2000, Leonard et al. 2003).

The typical non-breeding season habitat of *R. sevososa* is upland and flatland longleaf pine (*Pinus palustris*) forests with a well-developed grassy understory (Dundee and Rossman 1989). Historic records show they may have originally utilized a wider variety of habitats including some wetter and lower areas (Seigel and Doody 1992). *R. capito* generally appears to prefer a similar longleaf habitat but has been observed breeding in wetlands surrounded by lowlands in Georgia (Wright 1932) and Tennessee (Miller & Campbell 1996). The loss of approximately 97% of the non-breeding habitat to agriculture, urbanization and forestry is likely to have been one cause of the decline of *R. sevososa* (Smith et al. 2000).

Rana sevosa and *R. capito* are often found in association with the gopher tortoise (*Gopherus polyphemus*) and some reports suggest that they spend the majority of their lives within gopher tortoise burrows (Allen 1932, Palis 1997). Franz (1986) found that *R. capito* preferred active tortoise burrows to inactive tortoise burrows. Bailey (1992) also found that individuals returned to the same burrows across years following breeding events. Conversely, Lee (1973) found that gopher frogs were more common in rodent (*Peromyscus* spp.) burrows than tortoise burrows. He suggested that this was because the density of ground vegetation was much lower around rodent burrows (Lee 1973). Recently metamorphosed individuals have been found in tortoise burrows suggesting that burrows are sought out as soon as an animal leaves its larval pond (Braid et al. 2000). Gopher frogs also use other available shelter such as stump holes and root mounds of fallen trees (Richter et al. 2001).

R. sevosa and *R. capito* breed in semi-permanent or ephemeral wetlands that regularly dry completely (Semlitsch et al. 1995, USFWS 2001, Jensen et al. 2003). Ponds are generally hard-bottomed, open canopy and contain emergent and submerged vegetation (USFWS 2001). Ponds fill in late winter or early spring when gopher frog breeding occurs and dry in mid to late summer (Palis 1998).

Broad-scale threats to the continued survival of *R. sevosa* populations include logging and conversion of longleaf pine forests to silviculture, agriculture, and urban use, habitat fragmentation, draining of and introduction of fish to breeding ponds, pond canopy closure, disease and road mortality (Bailey 1992, Richter et al. 2001, Green et al 2002, personal observations). Additional threats include the planned construction of housing developments close to the only known breeding ponds (Richter et al. 2003, Linda LaClaire, United States Fish and Wildlife Service, personal communication). Multiple breeding sites (metapopulation) may

be important in sustaining *R. capito* populations (Greenburg 2001); however, it appears no suitable alternative ponds exist near the current breeding sites. Surveys throughout the DeSoto National Forest and southern Mississippi have found that almost all extant ponds have closed canopies, or have been altered to become permanent or to drain rapidly. Options for the translocation of gopher frogs to other sites, to create a metapopulation, are therefore limited.

The southern leopard frog (*Rana sphenocephala*) breeds at approximately the same time and under the same conditions as *R. sevosia* (personal observation). The two species lay almost identically sized egg masses and tadpoles hatch within days of one another (Bailey 1988, personal observation). The southern leopard frog can tolerate a wide range of habitats, and adults are found near most fresh water habitats (Dundee and Rossman 1989) although it is less commonly observed in permanent water, or in water containing fish (Babbitt and Tanner 1998). *R. sphenocephala* is the only congener of *R. sevosia* observed breeding at Glen's Pond and as such southern leopard frog tadpoles are the most likely to be in competition with those of the dusky gopher frog. *R. sphenocephala* appears to have remained relatively common throughout this area of Mississippi in spite of changes that have driven *R. sevosia* to the point of extinction. Therefore *R. sphenocephala* and *R. sevosia* provide ideal model organisms for examining ecological questions pertaining to rarity and commonness.

Overarching dissertation questions

The current status of the dusky gopher frog is bleak. Without aggressive management action the extinction of this species, in the near future, is certain. However, translocation and recovery efforts that are not based on sound ecological knowledge of a species are more likely to fail than succeed (Seigel and Dodd 2002). Research involving endangered species is often logistically difficult; however studying the mechanisms involved in their decline is crucial. The

dusky gopher frog provides a model organism for examining the mechanisms involved in extinction and comparing its ecology to that of a common species provides valuable information relevant to all declining species.

The research within my dissertation has a number of broad themes or questions. I have attempted to maintain at least one of these themes in each experiment or study to provide a cohesive body of knowledge. These questions are:

1. Has habitat change in the aquatic environment contributed to the decline of *R. sevosa*?
2. Has habitat change in the terrestrial environment contributed to the decline of *R. sevosa*?
3. Has larval competition with *R. sphenoccephala* contributed to the decline of *R. sevosa*?
4. Are there identifiable features of the ecology of *R. sevosa* that make it particularly vulnerable to extinction?
5. Why is *R. sevosa* declining while *R. sphenoccephala* remains common in the same habitat?
6. Can my research provide information that is useful to managers of *R. sevosa* and other declining species?

The research conducted in my dissertation is presented here as 5 manuscripts. In Chapter 2 I use a laboratory experiment to address the question of whether chemical cues assist post-metamorphic *R. sevosa* in finding suitable burrows. The research in this chapter addresses questions 4, 5 and 6 above. Chapter 3 contains a field experiment that explores the behavior of *R. sevosa* and *R. sphenoccephala* larvae in the presence of two predators. The research in this chapter addresses questions 1, 4, 5 and 6 above. Chapter 4 is an observational study that looks at features of the terrestrial and aquatic habitats at sites where *R. sevosa* is present and absent. This chapter focuses on questions 1, 2, 4 and 6 above. Chapters 5 and 6 contain a series of field experiments designed to explore the performance of larval *R. sevosa* and *R. sphenoccephala* in

response to habitat change in the form of canopy closure and competition. This research addresses questions 1, 3, 4, 5, and 6. In Chapter 7 these broad research questions are examined in light of the results obtained.

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CHAPTER 2 CHEMICAL CUES AND BURROW CHOICE IN NEWLY METAMORPHOSED DUSKY GOPHER FROGS (RANA SEVOSA)

NICOLE Y. THURGATE AND JOSEPH H. K. PECHMANN

ABSTRACT: The dusky gopher frog (*Rana sevosa*) spends the majority of its juvenile and adult life in burrows, including those of the gopher tortoise (*Gopherus polyphemus*) and small mammals such as rodents (*Peromyscus* spp.). We tested the hypothesis that chemical cues from burrow associates assist newly-metamorphosed *R. sevosa* in the location of suitable burrows. Individuals were offered a choice between an artificial burrow containing chemical cues of *G. polyphemus* and a control burrow. In a second set of trials, the choice was between a burrow with *P. gossypinus* cues and a control. We found that *R. sevosa* spent significantly more time in the treatment burrows than control burrows, which suggests that *R. sevosa* has an innate response to the chemical cues of these species. This ability may allow juvenile *R. sevosa* to rapidly find high-quality burrow sites and thereby minimize their exposure to predation, desiccation, and fire. This, to our knowledge, is the first study to show innate knowledge of the chemical cues of non-predatory species in any adult amphibian. Maintaining populations of *G. polyphemus* and *P. gossypinus* or other species that provide burrows may aid the conservation and recovery of the endangered *R. sevosa*.

INTRODUCTION

Chemically Mediated Interactions

There is increasing recognition of the importance of chemically mediated interactions for population dynamics, community structure and species coexistence (Vet 1999). Chemoreception can provide essential information to an organism about its environment, including chemical cues about the availability of food, mates, abiotic factors (e.g. shelter), competitors and predators (Dicke & Grostal 2001). In many instances appropriate responses to these cues, such as avoiding an encounter with a predator or locating a breeding site, are adaptive behaviors (Murray et al. 2004). Individuals best able to use this chemical information may therefore be favored by natural selection (Waldman & Bishop 2004). Knowledge of chemical interactions has the potential to provide ecologists with a rich understanding of not just how an organism interacts with its environment, but also why certain responses are seen (Vet 1999). This in turn can give us information about how a species may respond to changes in its environment, a question of crucial concern in a time where extinctions are becoming all too common (Rittenhouse et al. 2004).

The importance of chemical cues to amphibians, particularly larval forms, is well established. Larval amphibians of certain species have been shown to aggregate in kin or conspecific groups based on chemical recognition of group members (Dawley 1998). These aggregations potentially benefit group members by enhancing foraging efficiency, swamping potential competitors, increasing the efficiency of thermoregulation and minimizing exposure to predation (Blaustein & Walls 1995). In addition, most larval amphibians exhibit a wide variety of adaptive physiological and behavioral responses to chemical cues from predators (Marquis et al. 2004; Moore et al. 2004), damaged tissues of conspecifics (Gallie et al. 2001, Marquis et al.

2004) and cues based on the presence of conspecifics in predator diets (Belden et al. 2000, Wirsing et al. 2005). The juveniles and adults of certain amphibian species also use chemical cues to mediate predation risk, which suggests this response is retained after metamorphosis and into adulthood (Chivers et al. 2001). Whether these responses are learned or innate varies from species to species and remains a subject of considerable debate (Murray et al. 2004).

Mate selection, territoriality, kin and neighbor recognition, and competition have been shown to have chemical components in some salamanders (e.g. Dawley 1998, Martin et al. 2005). There is much less information about the importance of such signals to anuran sociality however the available evidence suggests that chemical signaling may be widespread in anuran amphibians (Lee & Waldman 2002, Waldman & Bishop 2004). Chemical cues can also play a part in homing and orientation to breeding and home sites for amphibians, although their relative importance compared to visual and other cues remains debatable (Ishii et al. 1995). At least one species, *Bufo japonicus*, has been shown to rely almost entirely on chemical cues for homing and orientation (Ishii et al. 1995).

Burrow Choice in *Rana sevos*

A number of amphibian species rely on quite different taxa to provide shelter to adults and juveniles. Dusky gopher frogs (*Rana sevos*) and Florida gopher frogs (*R. capito*) are often found in association with the gopher tortoise (*Gopherus polyphemus*) and some reports suggest that they spend the majority of their adult lives within gopher tortoise burrows (Allen 1932, Palis 1997). Recently metamorphosed individuals have been found in and around tortoise burrows suggesting that burrows are sought out as soon as an animal leaves its larval pond (Braid et al. 2000). Franz (1986) found that *R. capito* preferred active tortoise burrows to inactive tortoise burrows. Conversely, Lee (1973) found that gopher frogs were more common in rodent

(*Peromyscus* spp.) burrows than tortoise burrows. He suggested that this was because ground vegetation was much less dense around rodent burrows (Lee 1973). Gopher frogs also use other accessible shelter such as stump holes and root mounds of fallen trees (Richter et al. 2001).

On average adult *R. sevosa* only move short distances from the breeding pond (< 300m; Richter et al. 2001). Individual gopher frogs tend to emigrate out of breeding ponds in the direction they immigrated from, suggesting that individuals return to the same burrows after breeding (Bailey 1992, Palis 1998, Richter & Seigel 2002). Once in the burrow animals rarely change location and site fidelity appears high (Richter et al. 2001). This suggests that burrows are of vital importance to this species.

How *R. capito* and *R. sevosa* locate their burrows is unknown. In addition, the relative importance of gopher tortoises and rodents to the survival of these species remains unclear. Given evidence that *R. capito* is declining in its range (Greenburg 2001) and that *R. sevosa* has been listed as an endangered species (USFWS 2001), it is vital that we better understand their terrestrial ecology, including burrow usage.

We suggest that chemical cues may be important to *R. sevosa* in locating burrows. To test this assumption we asked the following question: do newly metamorphosed *R. sevosa* use chemical cues to find the burrows of *Gopherus polyphemus* and/or *Peromyscus gossypinus*? We hypothesized that we would see a positive response to chemical cues from both species.

METHODS

Newly metamorphosed *R. sevosa* were tested for a response to the chemical cues of *G. polyphemus* and *P. gossypinus*. We collected *R. sevosa* eggs from Glen's Pond in Harrison County, Mississippi and reared hatchlings to metamorphosis in cattle watering tanks adjacent to the pond using standard methods (Chapter 5, Thurgate and Pechmann, in press). Frogs were

removed from the tanks upon metamorphosis (defined as forelimb emergence) and placed in clean 10 x 10 x 6cm plastic containers lined with paper towels dampened with well water. Animals were then held under controlled environmental conditions in a nearby laboratory until the tail was absorbed (when tail was no longer than wide). We used 20 newly metamorphosed *R. sevosia* in each trial (40 total).

To prepare the *G. polyphemus* chemical cue, we placed paper towels in a 120cm x 60 cm plastic container containing a single gopher tortoise for 24 hours prior to the start of the experiment. Two different gopher tortoises served as donors. We discarded any paper towels that had become soiled by feces, food or other contaminant. We collected *P. gossypinus* cues in the same manner as the gopher tortoise stimulant. However, six different mice, all trapped within the vicinity of Glen's Pond served as donors for these trials.

Trials were conducted at four different times, beginning on September 4, 11, 18 and 25, 2005. For each trial a 55 x 38 x 15cm plastic container was completely lined with paper towels. We placed two 15cm lengths of 3.81cm diameter PVC pipe 15 cm apart at one end of the container. Each end of each pipe was blocked with 4 unbleached paper towels. One pipe received blank towels having no chemical cue and the other received trial towels having either tortoise or mouse cues, depending on the trial. Each paper towel was saturated with well water before being placed in the pipe. We determined the position of the blank (left or right side of the container) by a coin toss. After the experimental apparatus was correctly positioned, we saturated the paper towels within each container with well water using a spray bottle, to ensure that any observed avoidance was not attributable to differences in moisture level (Chivers et al. 2001). Containers were sprayed again after 24 hours to ensure constant moisture levels.

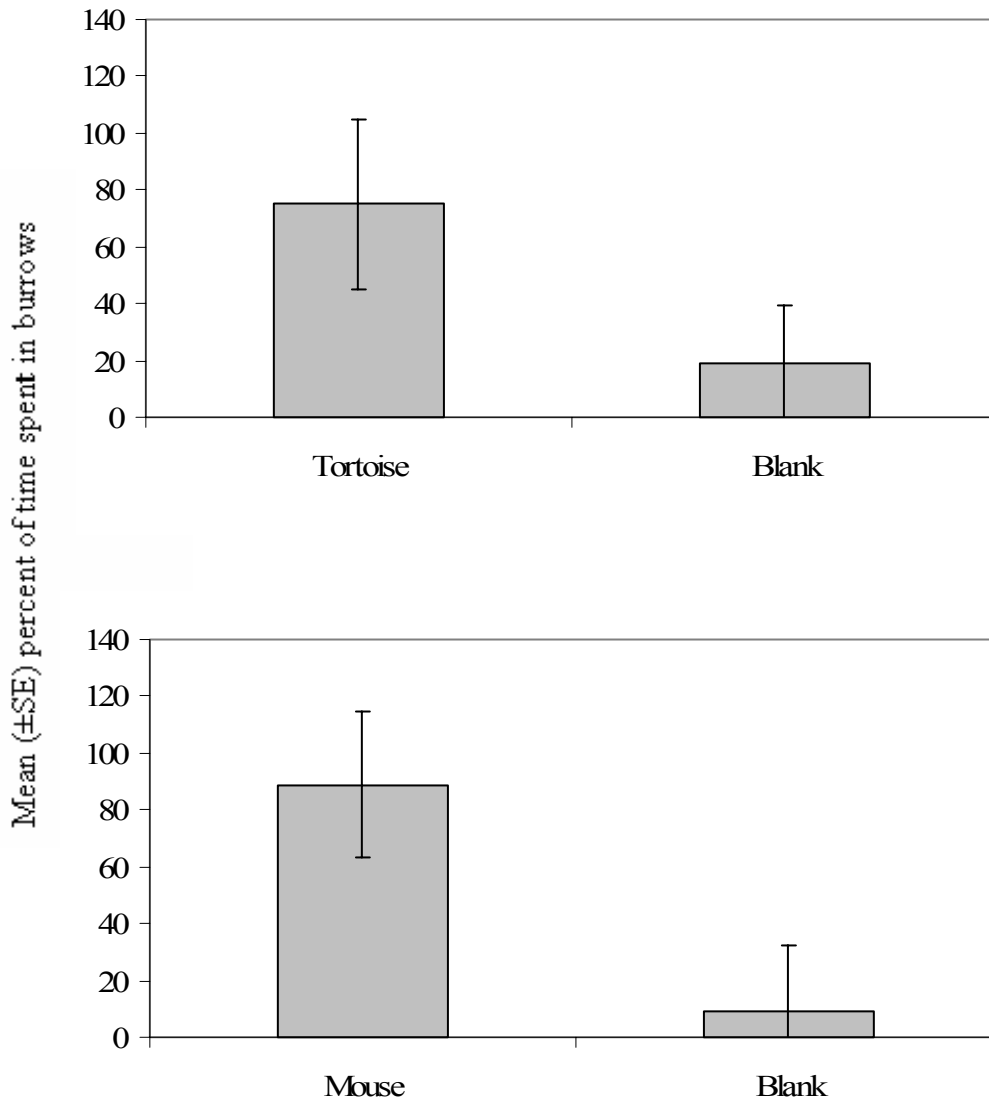
At the start of each trial, we introduced a single animal to the opposite side of the container from the pipes. We noted the position of the animal within the container every 6 hours for 48 hours. As gopher frogs live in burrows post-metamorphosis we assumed they would prefer the pipes (artificial burrows) to sitting exposed in the container. Each individual gopher frog was used in a single trial and released at Glen's Pond when the trial was completed. All experimental apparatus was cleaned in a dilute bleach solution, rinsed with well water and allowed to dry between trials to minimize chemical contamination between trials.

For each trial we counted the number of times each individual was found in each pipe (out of a possible 8 observations). Using a Wilcoxon Signed Rank test, we tested whether gopher frogs spent significantly more time in the treatment pipe than in the control pipe (Chivers et al. 2001).

RESULTS

Newly metamorphosed *R. sevos*a responded to the cues of both *G. polyphemus* and *P. gossypinus* (Figure 2.1). *R. sevos*a spent significantly more time in artificial burrows containing the chemical cues of *G. polyphemus* than in control burrows ($Z=2.053$, $P=0.04$). *R. sevos*a also spent significantly more time in the artificial burrows containing *P. gossypinus* cues than in those containing blanks ($Z=3.397$, $P=0.0007$).

Figure 2.1 –Mean percent of time *R. sevos*a spent in treatment and control burrows.



DISCUSSION

The results of this study suggest that when *R. sevos*a metamorphose from Glen's Pond they have an innate knowledge of the chemical cues of *P. gossypinus* and *G. polyphemus*. This, to our knowledge, is the first study to show innate knowledge of the chemical cues of non-

predatory species in any adult amphibian. Response to the cues suggests that *P. gossypinus* and *G. polyphemus* burrows are an important resource for *R. sevosia*. *R. sevosia* metamorphose with the ability to seek out *G. polyphemus* even though there are few tortoises near Glen's Pond. The gopher tortoise is federally listed as threatened in the western part of its range, including Mississippi. There appear to be many *P. gossypinus* living in the vicinity of this pond, however.

This information has implications for the conservation and recovery of *R. sevosia*. Where managers are attempting to improve habitat or create translocation sites, it may be helpful to ensure that one or both of these species are present and abundant in the habitat surrounding the breeding site. *Gopherus polyphemus* is also declining in the same habitat as *R. sevosia* primarily because of habitat destruction and fire suppression (Van Lear et al. 2005). Maintaining an appropriate fire regime is vital to providing the diverse, herbaceous under-story gopher tortoises require for forage. An appropriate fire regime also helps provide stump and root holes as additional *R. sevosia* burrows. *P. gossypinus*, conversely, does not appear to be declining in the same habitats (although its status has not been studied) and is not listed as endangered or threatened in any state. There is, however, evidence to suggest that inappropriate forest management in a similar habitat would also have negative effects on this species (Mitchell et al. 1995). Management burns may increase the abundance of *P. gossypinus* as has been found for other *Peromyscus* sp. in short-leaf pine grasslands (Masters et al. 1998). Therefore, sensitive management of the forest habitat of *R. sevosia* is needed to ensure its survival and the availability of those species that help create and maintain the burrows in which it lives. Other species we did not test, e.g., other rodents or crayfish, may also provide burrows for *R. sevosia*. If they do, we expect that *R. sevosia* would also respond to their chemical cues in a similar fashion.

Why is recognition of *P. gossypinus* and *G. polyphemus* innate? It is generally assumed that innate responses to chemical cues are adaptive and confer a selective advantage (Murray et al. 2004). There are several possible advantages of recognizing burrow associates at metamorphosis. The ability to take advantage of chemical cues upon leaving a pond would allow an individual to find a suitable burrow more rapidly, thereby minimizing exposure to predation and desiccation. Another possibility is that *R. sevosae* respond to the chemical cues from these two species because they provide especially high-quality burrows. Rodents, such as *P. gossypinus*, are unlikely to be sharing refuges with predators such as shrews or snakes and so their chemical cues may signal a safe burrow. Alternatively, the large size of *G. polyphemus* burrows may provide adequate space, environmental conditions and shelter for a newly metamorphosed individual, and insect burrow associates may provide a potential source of prey (Franz 1986). Being able to locate a high-quality burrow may have a selective advantage, as individuals with superior burrows might have enhanced survival, fecundity, or growth rates (Regosin et al. 2003). Burrows can also provide amphibians protection from fires. In ecosystems where fire is a regular feature of the landscape, the proportion of burrowing amphibians is high (Friend 1993). The longleaf pine habitat of *R. sevosae* is historically a fire-dominated ecosystem with the majority of fires being ignited through lightning strike in the summer, when newly metamorphosed *R. sevosae* would be leaving the pond (Van Lear et al. 2005). Therefore, rapid location of a burrow may provide protection from fire for juvenile *R. sevosae*.

Our study did not address whether *R. sevosae* shows a preference for the chemical cues of one potential burrow associate over another. Additional questions concern *R. sevosae*'s ability to recognize the chemical cues of potential predators, conspecifics, and other possible burrow

occupants. Understanding habitat selection in this species is of vital importance to its conservation. Understanding habitat selection in any species, particularly with reference to chemical cues, provides insights on the importance of species interactions.

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CHAPTER 3- ANTI-PREDATOR BEHAVIOR AND COMPETITION IN LARVAL DUSKY GOPHER FROGS AND SOUTHERN LEOPARD FROGS.

NICOLE Y. THURGATE AND JOSEPH H. K. PECHMANN

ABSTRACT: Aquatic predators play an important role in regulating the structure of larval amphibian communities. Tadpoles exhibit inducible physiological and behavioral defenses to help overcome these predation risks. The presence of competitors can influence these responses and elicit their own suite of inducible changes. The ability to respond appropriately to risks of predation and competition is a key component of larval amphibian fitness. We conducted experiments on the behavioral responses of the coexisting dusky gopher frog (*Rana sevosa*) and southern leopard frog (*Rana sphenoccephala*) under the threat of predation by two types of predators (the green sunfish *Lepomis cyanellus* and the giant water-bug *Lethocerus americanus*). We also examined how responses change when each species was alone versus when in the presence of the congener. We found that both species responded to the presence of *L. americanus* by decreasing activity and increasing refuge use. *R. sphenoccephala* exhibited identical behaviors when exposed to the green sunfish but *R. sevosa* had no response. The presence or absence of potential competitors had no observable effect on the behavior of either species. While it is not uncommon to find species differences in response to predation risk, the lack of response in *R. sevosa* suggests it lacks inducible defenses against fish predation and may be more vulnerable to fish introductions than other species. The capacity to respond to multiple predators may be a key component in the ability of *R. sphenoccephala* to exploit multiple habitats.

INTRODUCTION

Aquatic predators play an important role in shaping tadpole communities. Their presence or absence can influence the abundance and persistence of amphibian species within

communities (Semlitsch and Gavasso 1992). In addition, predators can mediate interactions among prey species by promoting coexistence among competitors or selectively reducing one species more than others (Walls et al. 2002). Evolution has favored a plastic response in amphibian larvae, giving many species a wide repertoire of defenses against predation (Van Buskirk 2000). The ability of prey species to respond appropriately to predation risk is a key component of its fitness in a given environment (Relyea 2001).

Predators can induce a range of behavioral, morphological, and physiological responses in amphibian larvae (Pearl et al. 2003). Morphological and physiological responses include rapid hatching of eggs, smaller size throughout larval development, slower larval development, darker pigmentation, brighter tail pigmentation, deeper tail fin and muscle development and decreases in body depth (McIntyre et al. 2004, Teplitsky et al. 2003, Saenz et al. 2003, Smith et al. 2005). Behavioral responses include low swimming activity, increased refuge use, spatial avoidance of predators and shifts to nocturnal feeding (Marquis et al. 2004, McIntyre et al. 2004, Pearl et al. 2003, Walls et al. 2002).

The relative importance of behavioral shifts remains uncertain, however, it may relate to the foraging mode of the predator (McIntyre et al. 2004). Where predators are gape limited, e.g. a fish, prey escapes predation once prey size exceeds the gape of the predator (Walls et al. 2002). In response to gape limited predators many larval amphibians have inducible defenses, e.g. increased head or tail size, that assist in exceeding predator gape (Relyea and Werner 2000, Van Buskirk and Schmidt 2000). Where predators are not gape limited, as in many invertebrates, prey may benefit more from other forms of predator avoidance such as behavior modification (Walls et al. 2002).

There are potential costs of anti-predator defenses (Van Buskirk 2000). Increased refuge use and decreased activity, for example, inhibit the ability of a tadpole to feed and so may slow growth and increase larval period. Small larval size and slow development may cause low juvenile survival, later age and smaller size at first reproduction, and reduced fecundity (Smith 1987, Berven 1990). Therefore, there is a tradeoff between size and timing of metamorphosis and responses to predators (Barnett and Richardson 2002).

Individual prey species may respond to the same predator differently. In one experiment, of six species of larval amphibians studied, all exhibited different responses to the same predator species (Relyea 2001). Conversely, individual species may have a range of anti-predator responses to different predators, suggesting a plasticity of response and the ability to distinguish between predators (Van Buskirk 2000). This implies that responses to a particular predator may not serve as a general defense to all predators (Relyea 2001). The introduction of new or rarely encountered predators can have consequences for species interactions and community structure. Where a prey species is very susceptible to novel predators, localized extinctions may occur (Vrendenburg 2004). Those species better able to adapt to and defend against such predators may increase in abundance or range (Pearl et al., 2003).

Amphibian larvae also have traits induced by the presence of competitors. These are generally opposite to those induced by predators: higher activity levels, shallower tails and longer bodies, (Relyea 2004). Therefore, tadpoles must also fine-tune a range of inducible responses in such a way as to respond to both competition and predation when both are present.

We examined the influence of the threat of predation by two types of predators, the gape-limited green sunfish, *Lepomis cyanellus*, and the non gape-limited giant water-bug, *Lethocerus americanus*, on the behavior of dusky gopher frog, *Rana sevosa*, and southern leopard frog, *Rana*

sphenocephala, larvae. *Rana sevosa* breed only in temporary ponds, *R. sphenocephala* and *Lethocerus americanus* are in both temporary and permanent ponds, and *Lepomis cyanellus* is found only in permanent ponds and streams except when heavy flooding connects these to temporary waters. *Rana sevosa* and *R. sphenocephala* can breed on the same nights at the same pond, and tadpoles can coexist during development and potentially compete for resources. *R. sphenocephala* has a relatively wide distribution throughout the south-eastern United States, while *R. sevosa* has declined to less than 100 adults in Mississippi, making it critically endangered (Richter et al. 2003). Both predators are common in southern Mississippi where we conducted our study.

The purpose of this study was first, to examine whether *R. sevosa* and *R. sphenocephala* differed in their behavioral responses to two kinds of predators. Behavioral differences between the two species could indicate differences in their relative vulnerability to predation (Walls et al. 2002). The second objective was to examine how each species responded to caged predators when alone and in the presence of the congener. The presence of competitors can alter responses to the risk of predation (Relyea 2004).

METHODS

Tadpole trials

R. sevosa and *R. sphenocephala* eggs were collected from Glen's Pond in Harrison County, Mississippi, on 7 March 2003, and reared in predator-free, single species cattle watering tanks as part of another experiment (Chapter 5; Thurgate and Pechmann, in press). All tadpoles were hatched and free swimming for a maximum of 50 days. Tadpoles reared in these tanks had a larval period of 175.57 (± 29.55 SD) days for *R. sevosa* and 110.88 (± 10.60 SD) days for *R.*

sphenocephala (Thurgate and Pechmann, in press). All tadpoles used in trials were smaller than 25.0mm total length (mean 22.71mm \pm 1.04).

Trials were conducted on 27, 28, 29 April and 4, 5, 6 May, 2003. We caught *Lepomis cyanellus* in a local stream about 1km from Glen's Pond using dip-nets immediately prior to trials beginning. Two individuals, most likely juveniles, were used in these trials. One, caught on 27 April measured 65.3 mm total length and the other, caught on 4 May measured 58.5 mm. We collected three *Lethocerus americanus* from Glen's pond and another nearby pond immediately prior to trials beginning. One individual was collected from Glen's pond on April 27 and one each from Glen's and a nearby pond on 4 May.

For each trial we filled a 37 L glass aquarium with water taken from the predator-free cattle watering tanks where tadpoles were being raised (Thurgate and Pechmann, in press). The cattle watering tanks contained well water to which we had added terrestrial tree leaves and plankton collected from Glen's Pond and another nearby pond. The water in the aquaria was changed at each trial and aquaria were cleaned with a dilute bleach solution and rinsed thoroughly with well water to minimize contamination between trials. At the start of each trial 50 g of wet pine needles, sweet gum and oak leaves were placed in each aquarium to provide refuge sites for tadpoles. The sides of each aquarium were covered with cardboard and the top of the aquarium was covered with 70% fiberglass fly-screen to minimize external visual stimuli.

We measured the level of activity and refuge use of *R. sevosa* and *R. sphenocephala* in the presence and absence of one another and in the presence and absence of each predator. At the start of each trial two tadpoles were introduced to the aquarium and left undisturbed for one hour. After one hour the two tadpoles were observed for 10 minutes. The amount of time, in seconds, individuals spent swimming, feeding (actively scraping leaves or aquarium surfaces

with mouth parts), hiding (actively burrowing under leaves and remaining still) and resting (not moving but exposed) was recorded. Once trials were complete tadpoles were returned to cattle tanks. Each tadpole was only used in a single trial only.

Trials were run in three combinations: two gopher frogs together, two leopard frogs together and one individual of each species together. Pairs of tadpoles were placed in predator free tanks for observation. The process was then alternated in the presence of *L. cyanellus* and *L. americanus*. Predators were suspended in sealed fiberglass screen cages within aquaria to provide chemical and visual cues but preclude actual predation. Each trial was replicated 5 times for a total of 45 trials, and tested 90 tadpoles.

Statistical analysis

Data were square root transformed to meet assumptions of homogeneity of variance. The analysis was done using MANOVA and ANOVA (GLM procedure, SAS 9.1 software, SAS Institute Inc., Cary, North Carolina, USA). As response variables were not independent we used MANOVA with species, predator treatment and competition (intra- or interspecific) as predictor variables and trial number as a covariate. We also used three-way ANOVA to test for differences in individual response variables. Tests were for differences in mean time spent swimming, feeding, hiding and resting for mixed and single species treatments, without predators present and in the presence of *L. cyanellus* and *L. americanus*. Initially all variables were included in the model. To ensure that experimental order was not having an effect on results, trial number was included as a variable in a preliminary analysis. Trial number was not shown not to have a significant effect ($F < 1.3$, $p > 0.15$) and subsequently excluded from further analysis. The mean of the two individuals of the same species in each trial was used in analysis,

rather than individual measurements. Where trials contained individuals of two species, each individual was considered an independent data point.

RESULTS

MANOVA showed that species, predator and the interaction between species and predator differed significantly (Table 3.1). Whether individuals were in mixed or single species treatments had no significant effect on any response variable or their interactions (Table 3.1).

Table 3.1. Summary of multiple analysis of variance for the effects of species differences, non-lethal predation, competition and their interactions.

Test	df	Wilks' F	P
Species	8,71	11.02	<0.0001
Predator	16,142	10.92	<0.0001
Competition	8,71	1.04	0.4763
Species*predator	8,71	11.02	<0.0001
Species*competiton	8,71	0.56	0.8061
Predator*competition	16,142	1.14	0.3268
Species*predator*competition	16,142	1.22	0.2602

ANOVA showed significant main effects of tadpole species and predator treatment on the amount of time spent swimming, feeding, hiding and being still, although the effect of species on feeding was only marginally significant (Table 3.2). There was also a significant interaction

between species and response to predators for all variables (Table 3.1). This interaction indicates that the two species responded differently to the two predators.

The main difference between the two species was in the response to *L. cyanellus*. When *L. cyanellus* were present *R. sevosia* did not differ in the amount of time-spent active (Figure 3.1) or the amount of refuge use and inactivity (Figure 3.2) from trials where no predator was present. *R. sevosia* showed a marked response to the presence of *L. americanus*, reducing activity level and increasing refuge use and the amount of time spent still. By contrast *R. sphenoccephala* reduced activity levels and increased time spent hiding or still in the presence of both predators (Figures 3.1 and 3.2).

Table 3.2. Summary of analysis of variance for the effects of non-lethal predation and species differences and their interactions. Only variables found significant in MANOVA are reported.

Test	Response	Df	MS	Test df	Test MS	F	P
Species	Time swimming	1	37.83	54	6.85	5.52	0.02
Predator	Time swimming	2	241.81	54	6.85	35.3	<0.0001
Species*predator	Time swimming	2	119.2	54	6.85	17.4	<0.0001
Species	Time feeding	1	29.24	5	7.92	3.69	0.058
Predator	Time feeding	2	454.81	54	7.92	57.45	<0.0001
Species*predator	Time feeding	2	174.23	54	7.92	22.01	<0.0001
Species	Time still	1	7.27	54	1.77	4.11	0.046
Predator	Time still	2	69.96	54	1.77	39.51	<0.0001
Species*predator	Time still	2	29.01	54	1.77	16.39	<0.0001
Species	Time hidden	1	591.98	54	10.63	55.66	<0.0001
Predator	Time hidden	2	668.31	54	10.63	62.84	<0.0001
Species*predator	Time hidden	2	298.98	54	10.63	28.11	<0.0001

Figure 3.1 Mean amount of time (\pm SE) spent A. Swimming and B. feeding by all combinations of *R. sevos*a and *R. sphenoc*ephala in the three predator treatments.

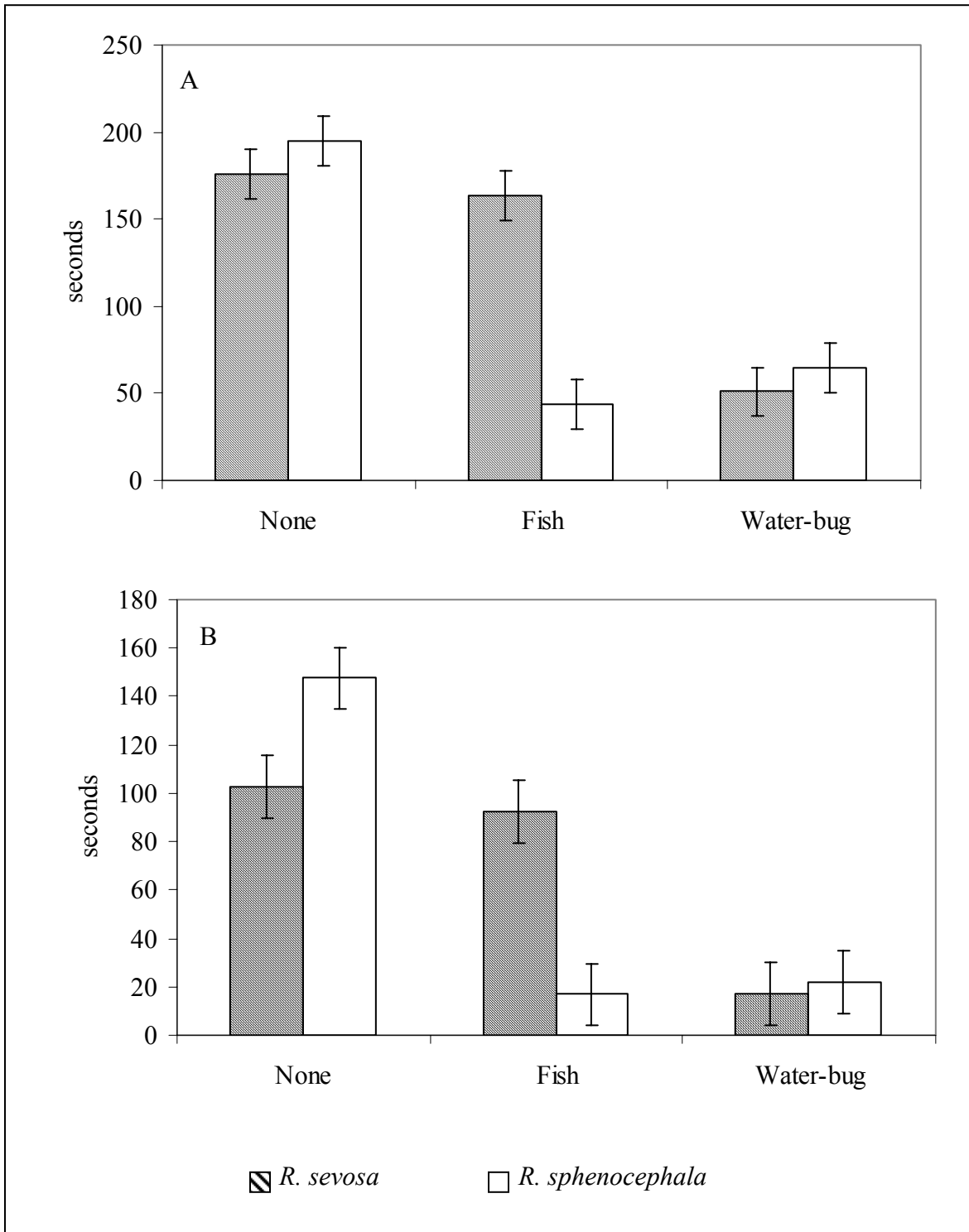
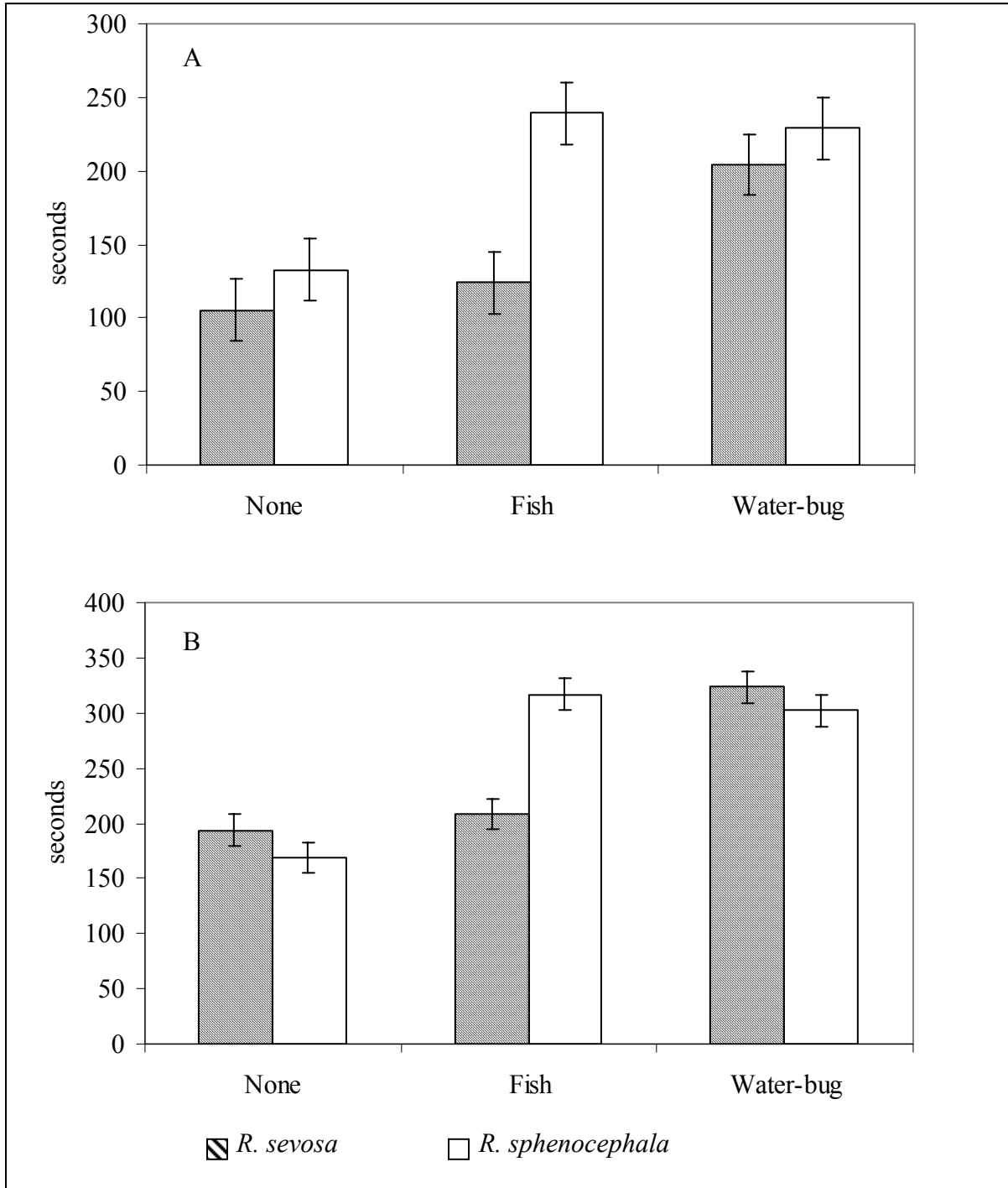


Figure 3.2A Mean amount of time (\pm SE) spent A. hiding and B. still (\pm SE) by all combinations of *R. sevos*a and *R. sphenoc*ephala in the three predator treatments.



DISCUSSION

Predation

Rana sphenoccephala exhibited strong behavioral responses to the presence of both predators, whereas *R. sevosa* responded only to *L. americanus*. There are a number of possible explanations for the lack of response to the fish predator by *R. sevosa*. Late in development, *R. sevosa* become very large tadpoles (total length > 74 mm) and may be too large for predation by all but the largest fish (Volpe 1957). If *R. sevosa* tadpoles were large enough they may no longer perceive fish as a threat (Pearl et al 2003). Van Buskirk (2001) showed that tadpoles generally stop hiding from predators at late stages in development. Swart and Taylor (2004) showed that earlier stage tadpoles exhibited a much stronger behavioral predator response than late stage tadpoles, which tended to rely more on morphological and physiological defenses. The fish used in this experiment were smaller than a late stage *R. sevosa* tadpole but the tadpoles used in this experiment never exceeded 25 mm in length. Therefore, the possibility for predation by fish presumably existed, as shown by the response of *R. sphenoccephala*.

Most tadpoles show a combination of defenses against predators that vary with predator abundance and type (Relyea 2004). Had the experiment been conducted over a longer period of time it is possible that *R. sevosa* may have exhibited morphological and physiological predator defenses, which were not measured in this experiment. These cannot be induced instantaneously and it is common for tadpoles to exhibit a behavioral defense component while other changes are taking place (Pearl et al. 2003). The most common response to the introduction of fish in other ranid species is a reduction in activity and an increase in refuge use (Laurila 2000, Parris et al. 2001, Richardson 2001, Van Buskirk 2001), as exhibited by *R. sphenoccephala* here. Therefore,

future studies may show that *R. sevosia* will exhibit a similar response to the presence of a fish, if they recognized fish as a threat.

Since all gopher frog species breed in temporary ponds that are generally fish free habitats (Bailey, 1991) the lack of response in *Rana sevosia* tadpoles here suggests that it evolved outside the context of fish predators. Therefore, if gopher frog larvae rarely encounter fish, except during times of exceptional flooding, *R. sevosia* may lack inducible defenses against fish predators. Selection is unlikely to foster inducible defenses against a predator that is almost never encountered (Schmidt and Amezcuita 2001). In an experiment using the gopher frog species *R. capito*, Braid et al. (1994) observed that when larvae were raised in the presence of fish, none survived past three weeks due to predation. This suggests that *R. sevosia* may also be palatable to fish and vulnerable to fish predation.

The distribution of *R. sphenoccephala* however, suggests it can tolerate a wide range of habitats. It has been observed breeding in most fresh water ponds, although it is most commonly found in temporary ponds (Dundee and Rossman 1989, Parris et al. 2001). *Rana sphenoccephala* tadpoles have occasionally been found in ponds containing fish (Babbitt and Tanner 1998). This suggests that *R. sphenoccephala* has at least some evolutionary history with fish as predators, and so selection may have favored some inducible defenses to this kind of predator. Parris et al. (2001) found low survival for *R. sphenoccephala* tadpoles in experimental pools containing fish, but some did survive suggesting at least partial ability to co-exist with fish.

The strong response of both prey species to *L. americanus* was expected given that this species would be commonly encountered by amphibian larve. One of the main advantages to using temporary ponds for breeding is the lack of predators when ponds first fill. The insect *L. americanus* however, can fly among newly filled ponds to exploit resources. *L. americanus* is

not gape limited, instead it feeds by injecting poison through a sharp pointed rostrum and then sucking out prey body fluids (Ohba et al. 2006). Predatory species within the same family have been observed feeding on prey much larger than themselves, including fish (Swart and Feigenhauer 2003) and so even the largest tadpoles would be at risk of predation. Here selection might favor a strong response to a commonly encountered predator that can be lethal throughout tadpole development.

Competition

There was no detectable change in behavior due to the presence of competitors. In other experiments, we found evidence of competition between tadpoles of *R. sevosia* and *R. sphenoccephala* (Thurgate and Pechmann, in press, Chapter 6 this volume). Competition appears to be asymmetrical, favoring *R. sevosia* under some conditions. The lack of response observed here may be because responses to competitors are not behavioral and thus were not measured here. Changes to behavior in the presence of competitors generally occur when food resources are reduced under high density (Relyea 2004) conditions not replicated in the present experiment.

Conservation implications

Throughout much of the world it is common for temporary ponds to be deepened and made permanent, and for fish to be introduced either intentionally or accidentally (Ortubay et al. 2006, Beja and Alcazar 2003, Baber et al. 2002, Hamer et al. 2002, Wood et al. 2001). Some amphibian species, that have a long evolutionary history with fish, can recognize and respond to even novel fish predators and so can successfully utilize ponds with fish (Pearl et al. 2003). Other species however, do not appear to display any predator defenses when fish are present and fish introductions have been linked to the decline and near extinction of a number of amphibian

species (Adams 2000, Wood et al. 2001, Hamer et al. 2002, Vredenburg 2004). Removal of introduced fish has been shown to lead to the rapid recovery of a declining frog, *R. mucosa* (Vredenburg, 2004) and so may be important to amphibian conservation.

Our experimental results suggest that larval *R. sevosia* do not exhibit behavioral defenses toward a local fish. Therefore, the introduction of fish to ponds where the endangered *R. sevosia* is present has the potential to severely inhibit or halt larval recruitment. Managers need to ensure that remaining breeding sites and potential translocation sites remain fish free. *Rana sphenoccephala* may be less strongly effected by fish introductions and their ability to respond to multiple predator types may have contributed to its continued success in habitats where *R. sevosia* has been eliminated.

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CHAPTER 4 THE HABITAT ATTRIBUTES OF THE ENDANGERED DUSKY GOPHER FROG (RANA SEVOSA)

NICOLE Y. THURGATE AND JOSEPH H. K. PECHMANN

ABSTRACT: We used an observational approach to identify habitat attributes associated with the presence of the critically endangered dusky gopher frog (*Rana sevosa*). We measured a number of attributes in the terrestrial and aquatic environments of 8 ponds in southern Mississippi; 3 where gopher frogs are present and 5 potential translocation sites where gopher frogs are absent. We used principal components analysis and ANOVA to compare attributes among sites. Results suggest that dusky gopher frogs are more likely to be present in ponds with abundant emergent vegetation and terrestrial environments with a well-developed grassy understory, numerous burrows and plentiful coarse woody debris. This highlights the importance of maintaining a well burned habitat for *R. sevosa* and identifies potential factors important in the management of the species. In addition we identified one potential translocation site with attributes that suggest it may be suitable habitat for a gopher frog population.

INTRODUCTION

All organisms rely on a suite of characteristics within its habitat to ensure its survival. Habitat characteristics determine, in part, the presence, size and persistence of a population at any location (Oldham et al. 2000). Identifying these characteristics for a particular species is challenging given the multiple potentially important factors and their interactions (Welch and MacMahon 2005). For amphibians and other species with multi-stage lifecycles, this difficulty is increased by the necessity of identifying stage-specific factors in multiple habitats (Semlitsch 2000). Experimental approaches, such as transplant experiments or habitat manipulations, can

be especially helpful in providing insight into the relative importance of various factors (Nyström et al. 2002). However, with secretive, rare or endangered species, applying these methods can be logistically, ethically and politically difficult (Welch and MacMahon, 2005).

An alternative approach is to use survey data that does not rely on direct manipulation of individuals (Welch and MacMahon 2005). Many researchers have identified associations between a species and its habitat by comparing characteristics of occupied and unoccupied sites (Lecis and Norris 2004). This approach can reveal helpful information about specific habitat attributes associated with species presence or absence, and knowledge of these attributes can be applied directly to species management and conservation.

We used survey data based on the presence and absence of the critically endangered dusky gopher frog (*Rana sevosa*) to identify habitat associations for this species. There are approximately 100 breeding adults of *R. sevosa* extant in southern Mississippi (Richter et al. 2003) so direct experimental manipulation of remaining adult individuals is unfeasible. There are only three known populations of *R. sevosa* left in its former range and all remaining populations are isolated from one another within a highly fragmented landscape. Therefore the primary management focus for this species is the restoration or creation of suitable habitat for translocation, and appropriate management of existing populations. Unless additional populations of *R. sevosa* can be created and current populations maintained, the future of this species is very bleak.

Only when we understand the factors that regulate populations of threatened species can we understand why they decline, or what may limit their distributions and further expansion, and so manage them appropriately (Nyström et al. 2002). Little is known about the habitat requirements of *R. sevosa* therefore making management decisions is difficult. For managers of

*R. sevos*a, knowledge of habitat requirements is crucial to avoiding time consuming and biologically risky mistakes in occupied sites and those being considered for translocations (Semlitsch 2000). Recent studies on *R. sevos*a have provided information about the structure of the breeding population (Richter et al. 2003) and some aspects of larval habitat requirements (Thurgate and Pechmann in press). However, little is known of its habitat requirements outside of breeding ponds.

The primary goal of this study was to compare the terrestrial and aquatic habitats of sites occupied by *R. sevos*a to sites where the species is absent. We hoped to identify specific habitat attributes associated with the presence of *R. sevos*a that may be of use to managers. In addition, in order to locate potentially suitable areas for restoration and translocation, we wanted to identify absence sites where *R. sevos*a is absent but with similar habitat characteristics to sites where *R. sevos*a is present.

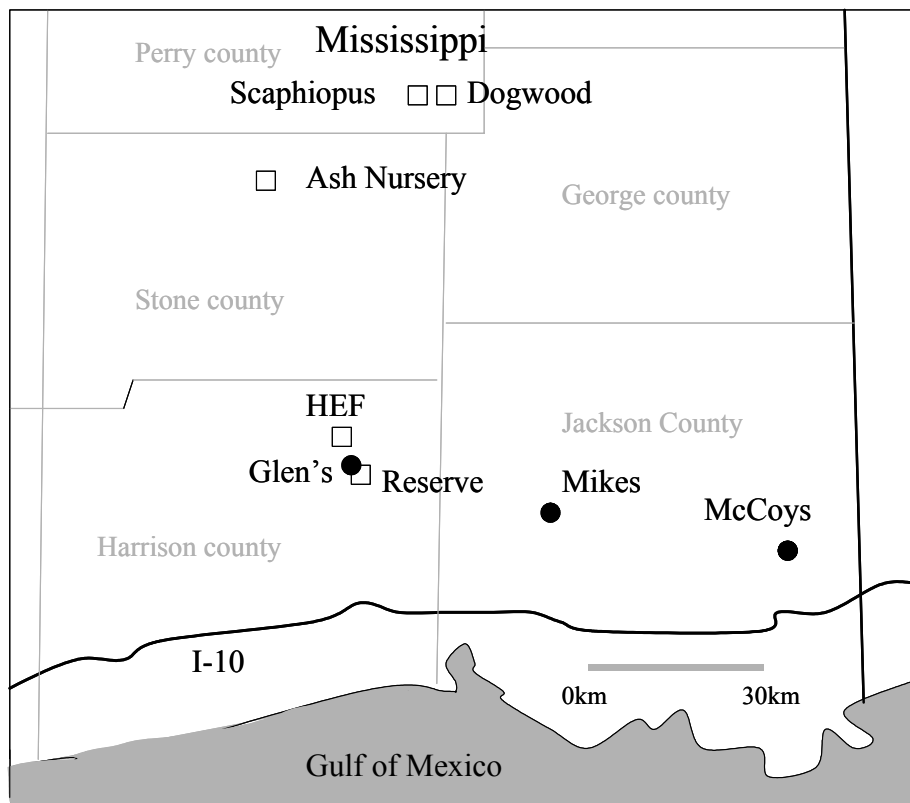
METHODS

Site selection

This study surveyed the physical characteristics of 8 temporary pond basins and their adjacent terrestrial habitats. *R. sevos*a had been observed at three of these ponds within the last 5 years. One site was Glen's Pond, Harrison County, MS, which has been monitored for *R. sevos*a breeding activity since 1988 (Richter et al. 2003). Currently less than 100 adults are thought to be breeding at this site and larval recruitment is very low in most years (Richter et al. 2001). Glens' Pond is located approximately 40km north of Gulfport, in Harrison County, MS on the DeSoto National Forest (Figure 4.1). The second occupied study site was McCoy's Pond, Jackson County, MS (Figure 4.1) where a single male *R. sevos*a was heard calling in 2004. We

were unable to locate more individuals or signs of breeding activity (tadpoles or eggs) at this site, suggesting this population may be extinct. Until this site has been intensively monitored we cannot discount the possibility of a larger population. The third occupied site was Mike’s Pond, in Jackson County, MS where *R. sevosa* eggs and tadpoles have been found in low numbers (Mike Sisson, Mississippi Museum of Natural Science, personal communication). Exact numbers of adults using this pond are unknown however the extant population appears to be small.

Figure 4.1. Location of study ponds in southern Mississippi, USA.



We chose 5 additional sites on the DeSoto National Forest, in consultation with the U. S. Fish and Wildlife Service, Gopher Frog Recovery Group, based on their potential as translocation sites for *R. sevosa*; Reserve, HEF, Scaphiopus, Dogwood and Ash Nursery ponds

(Figure1) have all been considered potentially suitable restoration and translocation sites by managers. The hydroperiod, size and depth of all ponds (with the exception of HEF pond which has a longer hydroperiod) are similar to Glen's pond. Other characteristics of the ponds and their surrounding habitat were not necessarily similar to sites where gopher frog eggs are present, but the Recovery Group assumed they could all be made similar through restoration and management.

No evidence of *R. sevosia* (eggs, tadpoles, calling males or adults) has been found at any of these 5 sites at any time and the consensus of managers is that *R. sevosia* is absent from them. At 3 of the 5 unoccupied sites managers have begun attempting to restore pond habitat. Based on suggestions from previous experiments (Thurgate and Pechmann in press) Scaphiopus pond had several trees removed from the pond basin in 2003. Ash Nursery pond had approximately 20% of trees removed from within the pond basin in 2004. Reserve Pond had trees removed from within the pond basin in 1997. HEF and Dogwood ponds have not been intentionally altered in any way to the best of our knowledge and retain completely closed canopies.

The terrestrial habitat around all ponds is a mix of replanted and naturally regenerated pines, primarily longleaf (*Pinus palustris*), slash pine (*P. eliottii*) and loblolly (*P. taedus*). The understory consists of shrubs (primarily *Ilex vomitoria* and *I. glabra*), grasses and herbs. The relative abundance of these components at each site is largely dependent on physical characteristics and historical management of the surrounding forest.

Pond habitat surveys

We identified potentially important habitat characteristics for *R. sevosia* in the terrestrial and aquatic environments based on the available published literature. The habitat of each pond was sampled between May 1 and June 26, 2005. The area of each study pond was calculated by

drawing polygons around ponds on aerial photographs from 1987-1996 using the program Mapcard (www.mapcard.com).

Coarse woody debris (CWD) and grassy vegetation within a pond provide a important services to tadpoles, such as refuges from predators and increased water oxygenation (Skelly et al. 2002). CWD and grassy vegetation cover were measured along two perpendicular axes in each pond, running north-south and east-west. The axes ended at the pond edge (defined as the boundary between aquatic and terrestrial vegetation). The abundance of CWD larger than 5cm in diameter was counted in a 2m-wide strip along each axis (Leonard et al. 2003). Items near the pond center were only counted once if they lay on both axes. At nine evenly spaced points along each axis (only once at the pond center, 17 sample points total) we estimated the percentage ground cover of bare ground, grasses and sedges, shrubs and leaf litter in a 1m² quadrat. Estimates were assessed by eye and could only add up to 100% in each quadrat. Counts were then averaged to obtain a percent coverage of each variable for each pond.

As the amount of canopy closure above a pond affects larval *R. sevosia* growth and survival, we also counted the number of trees within 5 meters of each transect sample point as a measure of tree abundance (Thurgate and Pechmann in press). In addition we calculated percent canopy closure at 5 locations within each pond; at the pond center and halfway between the pond center and the pond edge along each major axis (north, south, east and west) using a spherical densiometer. The five readings were then averaged to provide an average value of canopy closure for each pond (Skelly et al. 2002).

Terrestrial habitat surveys

The terrestrial habitat surrounding ponds was surveyed concurrently with pond basins. At each pond the sampling axes were extended 300 meters from the ponds edge into surrounding

forest. We chose 300m based on the average movements of *R. sevosia* at Glen's Pond (Richter et al. 2001). The structure and complexity of vegetation surrounding breeding ponds has been shown to be of great importance to a number of amphibian species (Griffin and Case 2001, Hamer et al. 2002). Therefore, we chose variables within the terrestrial habitat that would reflect vegetation architecture. At 20m intervals along each axis we estimated the percent cover of grasses, herbs, leaf litter, shrubs and bare ground inside a 1m² quadrat as described above. The number of trees (over 2m tall) and bushes within 5m of the sampling point and the diameter at breast height (dbh) of the closest tree to the sampling point were also measured.

Adult and juvenile *R. sevosia* are thought to spend the majority of their lives in the burrows of gopher tortoises (*Gopherus polyphemus*) and small mammals or stump holes and similar structures (Lee 1976, Palis 1997, 1998, Allen 1932, Richter & Seigel 2002). Therefore, we counted the number of potential burrows (greater than 3cm diameter) and stumps within 5m of each sampling point. Average canopy closure at each sampling point was also measured with a spherical densiometer. Measurements were then averaged along each axis (north, south, east and west) for analysis.

Although little research has addressed the potential impact of invasive fire ants (*Solenopsis invicta*) on amphibian species, they have the potential to impact *R. sevosia* through direct mortality and occur at all study sites. We included counts of the number of active fire ant nests within 5 meters of each sampling point.

Statistical analysis

The average of the 4 axes (north, south, east and west) at each site was used as the unit of observation. We used principal components analysis (PCA) to look for groupings of ponds (terrestrial and aquatic habitats) and examined whether these groupings corresponded to the

presence and absence of *R. sevosia* (data reduction module, SPSS 11.0 software, SPSS inc., Chicago, Illinois, USA). The two data sets were analyzed separately to see if patterns differed based on requirements in different stages of the life-cycle. Percentage data were arcsine square root transformed and count data were log transformed to meet assumptions of homogeneity of variance. Transformed variables were then z-score transformed to standardize measurement scales (Ramsey and Schaffer, 1997). Eigenvectors with eigenvalues greater than 1 were retained. Eigenvectors were then rotated with a Varimax rotation to simplify interpretation of resulting plots (Abdi, 2003). One limitation of PCA is that ecological interpretation of the principal components may be difficult. We attempted to overcome this limitation by selecting 0.70 as the minimum loading coefficient required to include a habitat variable in the interpretation of a given principal component (Summerville et al. 2005).

We used one-way ANOVA to test for differences between ponds occupied and not occupied by gopher frogs for all variables measured in the terrestrial and aquatic habitats (GLM procedure, SAS 9.1 software, SAS Institute Inc., Cary, North Carolina, USA). Data were arcsine square root and log transformed as described above. We also conducted tests on principal component scores from pond basins and surrounding terrestrial habitat to compare ponds with and without *R. sevosia*.

RESULTS

Pond habitats

The first three components in our principal components analysis (eigenvalues >1) explained 84.854% of the total variance in our data set. Principal component 1 (which explained 49.172% of variance) was primarily influenced by the ground cover and canopy cover of ponds

(Table 4.1). Sites loading positively on this PCA axis had high proportions of shrub cover and leaf litter at ground level and a high percentage canopy cover over the pond basin. Sites with a high proportion of vegetation cover loaded negatively on this axis. The second principal component (which explained 22.275% of the total variance) was influenced by the number of trees and the amount of CWD. Sites loading positively on this axis had a large number of trees and a high abundance of CWD in the pond basin. The third principal component (which explained 14.583% of the total variance) was primarily influenced by the amount of bare ground and the area of pond basins. Sites that loaded positively on this PC had a large proportion of bare ground and were small ponds. ANOVA of the first three principal components did not show a significant difference in these components between sites where gopher frogs were present or absent (all $F < 2.763$, all $p > 0.4166$).

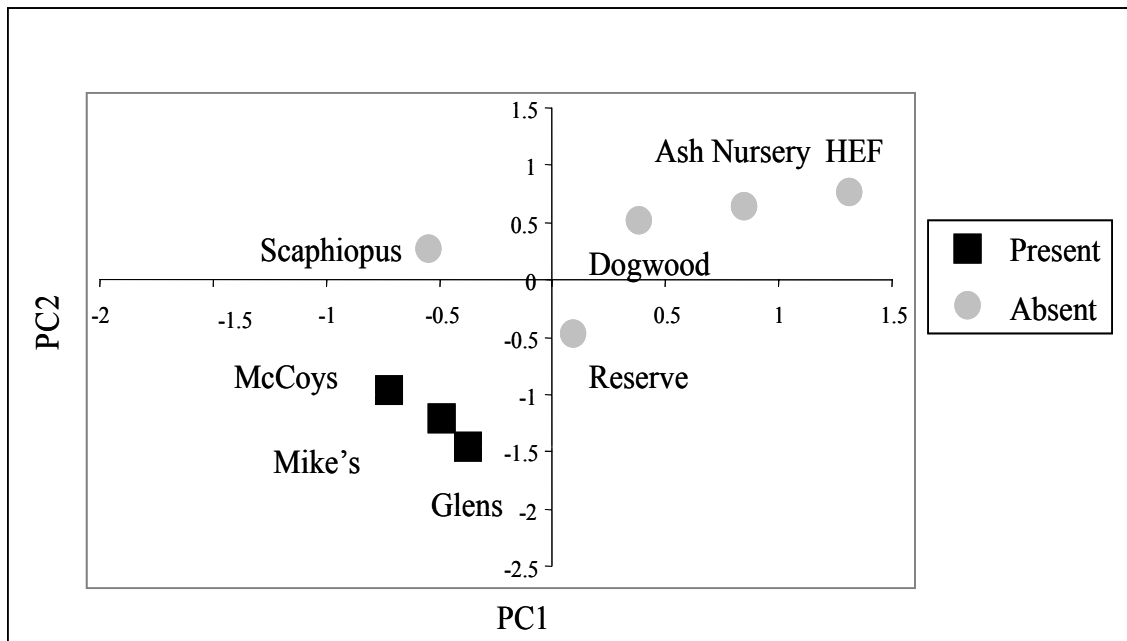
Table 4.1. Results of PCA for 8 environmental variables measured in 8 temporary ponds in Mississippi. Variables in bold were considered important in the interpretation of each principal component.

	Component 1	Component 2	Component 3
Shrub cover (%)	0.910	0.057	0.323
Vegetation cover (%)	-0.913	0.186	0.022
Leaf litter (%)	0.929	-0.211	-0.006
Bare ground (%)	0.419	0.144	0.768
Trees	-0.331	0.786	0.287
Canopy cover (%)	0.879	-0.314	-0.294
Pond area	0.221	0.093	-0.794
CWD	-0.066	0.892	-0.185

A plot of the scores of each pond across PC1 and 2 (which explained the majority of data variance) showed that the three ponds where *R. sevosia* are present grouped together based on

negative loadings on PC1 and 2 (Figure 4.2). Two other ponds, Reserve and Scaphiopus, grouped near sites where *R. sevosa* were present by having negative loadings on one of the two principal components. Dogwood, Ash Nursery and HEF ponds (the three closed canopy ponds) formed a third group with positive loadings on both PC1 and 2.

Figure 4.2. Distribution of the 8 pond basins relative to the site scores of the first two principal components for aquatic variables.



ANOVA showed that only the percentage cover of vegetation in pond basins was significantly different between sites where gopher frogs were present and absent ($F_{1,6} = 8.30$, $p = 0.028$). There was significantly more emergent vegetation at sites where gopher frogs were present ($63.8\% \pm 13.79$) than sites where they were absent ($40.78\% \pm 10.08$).

Terrestrial habitat surveys

The first 3 principal components (eigenvalues >1) explained 83.291% of the variance in our terrestrial habitat data set. Principal component 1 (which explained 46.33% of variance) was primarily influenced by the percentage cover of shrubs, bare ground, number of trees, canopy

cover and number of stumps (Table 4.2). Sites that had positive loadings with PC1 had a large proportion of the ground surface covered with shrubs, high percentage canopy cover and a high number of trees. Sites with negative loadings on PC1 had more bare ground and a high number of stumps. There was a tendency for the percent cover of grass to be important in the interpretation of this PC, sites with a high percent cover of grass loaded negatively on PC1. PC2 (which explained 22.275% of variance) was influenced by the percent ground cover of leaf litter and the presence of burrows (Table 4.2). Sites that loaded positively on this PC had a small percent coverage of leaf litter and many burrows. There was a tendency for grass to load positively on PC2, and sites that loaded positively on this PC had a high percent grass cover. The third principal component (which explained 14.583% of the variance) was influenced by the percent coverage of herbs, the dbh of trees and the availability of CWD (Table 4.2). Sites with positive loadings on PC3 had high a high percent ground cover of herbs, large trees and abundant CWD. ANOVA of the first three principal components did not show a significant difference in these components between sites where gopher frogs were present or absent (all $F < 1.99$, all $p > 0.37$).

Table 4.2. Results of PCA for environmental variables measured in the terrestrial habitat within 300m of 8 temporary ponds in Mississippi. Variables in bold were considered important in the interpretation of each principal component.

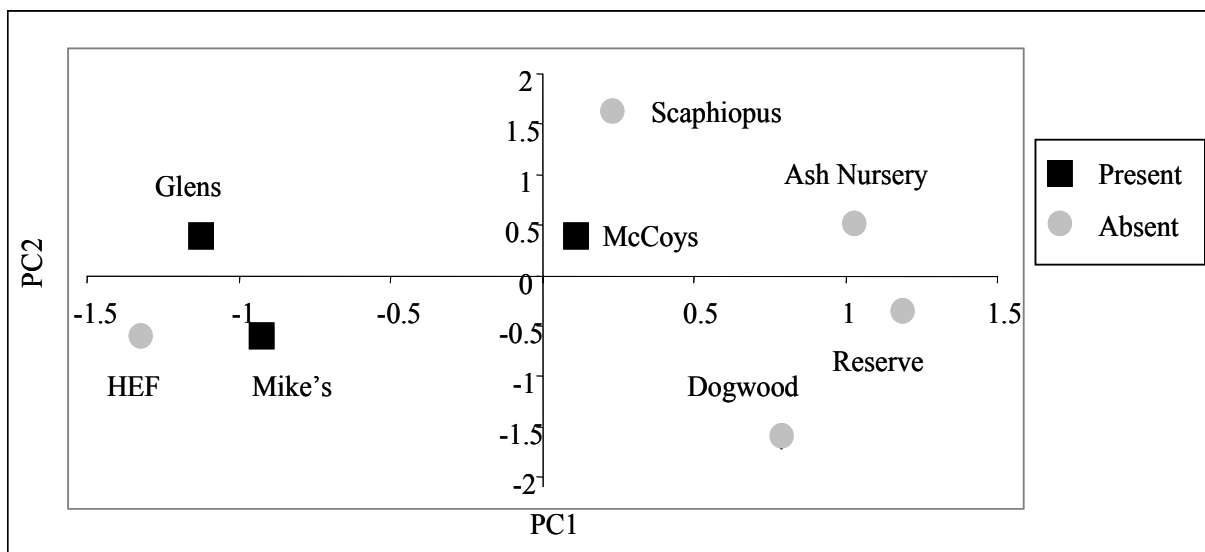
	Component 1	Component 2	Component 3
Grass cover (%)	-0.645	0.637	0.170
Herb cover (%)	0.311	0.016	0.844
Shrub cover (%)	0.966	-0.068	-0.195
Leaf litter (%)	0.181	-0.913	0.268
Bare ground (%)	-0.705	-0.217	-0.464

	Component 1	Component 2	Component 3
Trees	0.866	-0.352	0.153
Tree dbh	0.079	-0.159	0.874
Canopy cover	0.770	-0.507	0.153
Burrows	-0.247	0.925	0.237
CWD	-0.191	0.061	0.736
Stumps	-0.709	0.586	-0.014
Ant-mounds	0.043	0.612	-0.564

(Table 4.2 cont.)

A plot of the scores of each pond across PC1 and 2 (which explained the majority of data variance) showed that two of the ponds where *R. sevosia* are present (Glens and Mikes Ponds) grouped together based on negative loadings on PC1 (Figure 4.3). The two ponds grouped with a third pond, HEF Pond, based on similarity of scores on PC1. The remaining 5 ponds had positive scores on PC1. Three of these ponds, Scaphiopus, Ash nursery and McCoys Ponds grouped together based on positive scores on PC2. Dogwood and Reserve Ponds grouped together based on negative scores on PC2.

Figure 4.3. Distribution of the 8 ponds relative to the site scores of the first two principal components for terrestrial habitats.



ANOVA of all variables showed that the percent ground cover of grass ($F_{1,6} = 8.62$ $p = 0.0261$), number of burrows ($F_{1,6} = 16.02$, $p = 0.0071$) and the amount of CWD ($F_{1,6} = 6.77$ $p = 0.0406$) were significantly different between sites where gopher frogs were present and absent. There was significantly more grass cover at sites where *R. sevosa* was present ($57.39\% \pm 9.59\%$) than absent ($31.57\% \pm 5.38$). There were significantly more burrows within 5m of each survey point in presence sites (1.065 ± 0.0102) than absence (0.45 ± 0.079). There were significantly more pieces of CWD within 5m of each survey point in sites where gopher frogs were present (2.24 ± 0.22) than at absence sites (1.5 ± 0.17). No other surveyed variables differed significantly among sites.

DISCUSSION

Pond habitats

Based on variables measured within pond basins the 3 sites occupied by *R. sevosa* all shared similar characteristics. These ponds all had a high percent coverage of grassy vegetation within the pond basin and low values for variables associated with heavy tree cover (i.e. a high percent coverage of shrubs and leaves, dense canopy cover, large numbers of trees and CWD). The percentage cover of emergent vegetation was the only variable significantly related to gopher frog presence in ponds in this study; and this combined with the results of the PCA suggests that grassy vegetation within ponds may be an important habitat attribute for *R. sevosa*.

Female *R. sevosa* always attach their egg masses to vegetation when breeding in Glen's Pond and Mike's Pond (Richter et al. 2001, pers. ob.). *R. sevosa* egg masses are large and dense and so sufficient oxygen diffusion to all eggs is a key component of survival of larvae to

hatching. Keeping egg masses close to the better oxygenated water surface may contribute to increased egg oxygenation and improved light penetration, which is important for symbiotic algae (Pinder and Friet 1994, Seymour and Bradford 1995). Potential translocation sites with low amounts of vegetation within the pond basin (such as the closed canopy ponds) may therefore offer limited egg deposition sites and so be unsuitable for *R. sevosia* reproduction. This unsuitability could be based on female site choice or direct egg mortality.

An increase in the amount of shade over ponds has been shown to be an important limiting factor to the distribution of a number of amphibian species (Skelly et al. 2002, Werner and Glennemeier 1999). The size and survival of *R. sevosia* are negatively affected by high canopy cover over ponds (Thurgate and Pechmann in press, Chapters 5 and 6). Canopy closure can affect tadpoles by lowering temperature, dissolved oxygen and food availability within ponds (Thurgate and Pechmann in press, Skelly et al. 2002) and can reduce or eliminate emergent vegetation within the pond basin (Werner and Glennemeier 1999). Given that all ponds where *R. sevosia* are present have open canopies we suggest that canopy cover is an important limiting factor in the distribution of *R. sevosia*.

Based on groupings within the PCA it appears that Scaphiopus and Reserve ponds are the most similar to the sites where gopher frogs are present. This suggests that, based on pond data only, these sites may be currently the most suitable for *R. sevosia* translocation. Reserve pond however had a slightly positive loading on PC1, suggesting higher amounts of trees or shrubs within the pond, decreasing its suitability for *R. sevosia*. Given that Reserve pond is in close proximity to Glen's Pond (Figure 4.1), it is also likely that if this site were suitable for use by the species it would already be occupied. Scaphiopus Pond had a similar loading on PC1 to occupied ponds and so may be a more suitable candidate for translocation. Although Scaphiopus

Pond separates slightly from the occupied ponds on PC2, this axis explains less variation than PC1.

Terrestrial habitats

Based on our analyses the three sites occupied by *R. sevosia* had some similar characteristics in the terrestrial habitat. PC1, which was largely explained by the presence of many trees, high shrub and canopy cover and a low occurrence of stumps separated McCoy's pond from Glen's and Mike's ponds, however. McCoy's pond is the site where only a single male *R. sevosia* has been found. An increase in the tree canopy and dominance of the understory by shrubs suggests that McCoy's pond may be fire suppressed as high proportions of these variables are an indirect indicator of fire suppression in longleaf pine savanna (Bishop and Haas 2005, Smith et al. 2000). The presence of *R. sevosia* has been anecdotally associated with upland (higher elevation, drier areas) and flatland longleaf pine forests with a well-developed grassy understory (Dundee and Rossman 1989). In this study the percent cover of grass was significantly different between sites where gopher frogs were present and absent. This suggests regular burning of the terrestrial habitat is necessary to provide suitable habitat for *R. sevosia*.

The number of burrows in the terrestrial habitat was also significantly different between terrestrial habitats where gopher frogs were present and absent. Burrows were also associated with a positive loading for sites on PC2. *R. sevosia* appear to spend the majority of the terrestrial portion of the life cycle within burrows so their importance at sites associated with *R. sevosia* is not surprising (Lee 1972, Palis 1998, Richter & Seigel 2002). The importance of CWD in this analysis is likely representative of a similar shelter requirement. Mike's pond had a negative association with PC2 which suggests it has fewer burrows than Glen's or McCoy's ponds. Mike's Pond has more houses located around it than the other occupied ponds, and is adjacent to

a recent clear-cut. It is possible that burrows are being destroyed by road building or site preparation near Mike's Pond, or that these activities reduce the abundance of burrowing animals. Restoration of this site to improve burrow availability may be difficult but could be crucial to the long term persistence of this population.

Based on loadings on PC1 HEF pond is the most similar to two of the locations where *R. sevosia* is present. This suggests that this location may be the most suitable of available terrestrial locations for translocation. However, this site had a slightly negative association with burrow availability which suggests that some restoration to increase the number of burrows at this site may be needed. Scaphiopus pond had the highest positive loading of any pond on PC2 suggesting high burrow availability. This suggests that the terrestrial habitat around Scaphiopus pond may also be suitable for translocation.

Management Recommendations

No candidate translocation site grouped with sites where *R. sevosia* was present based on both terrestrial and aquatic habitats. Scaphiopus Pond did have certain qualities such as a high abundance of burrows within the terrestrial habitat and low numbers of trees within the pond basin suggesting it may be a suitable translocation site with some restoration. This pond did have a slightly negative loading on PC1 in the terrestrial habitat suggesting a low abundance of grass cover. The introduction of an appropriate fire regime may improve grass cover and increase site suitability. Based on terrestrial habitat data, HEF pond also appears to be an appropriate translocation site. However the aquatic habitat at this site was indicative of a heavily closed canopy and so may be unsuitable for breeding. Managers could eliminate trees from within the pond and burn the pond basin to remove leaves and promote the growth of emergent vegetation. The long hydroperiod and low landscape position of this pond suggests that natural

fire frequency within the pond basin is low, however, and that it would be difficult to conduct management burns there.

When assessing the suitability of potential translocation sites managers must ensure an open canopy pond containing an abundance of emergent vegetation to meet habitat requirements for breeding. Open canopy ponds may also be important for the growth and survival of larvae. In the terrestrial habitat managers should look for a well burnt grassy understory, containing many burrows and much CWD. It is also crucial managers make certain these features continue to be available at currently occupied sites for long-term persistence of these populations. The use of an appropriate fire regime is the most likely way of ensuring the suitability of habitat for *R. sevosa*.

Translocation of amphibians is a difficult process and previous attempts with a variety of amphibian species show that it is more likely to fail than succeed (Seigel and Dodd 2002). Therefore attempts at translocation of *R. sevosa* should be approached cautiously and experimentally to ensure that extant populations are not negatively affected. In addition, recovery plans for any amphibian species need to consider not only site suitability and specific habitat requirements but ultimately the creation of self-sustaining networks of interconnected populations (Semlitsch 2000). Managers must also monitor translocation sites and consider how to measure a successful translocation and maintain habitat suitability at these sites with a long term habitat management plan (Semlitsch 2002).

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CHAPTER 5 CANOPY CLOSURE, COMPETITION, AND THE ENDANGERED DUSKY GOPHER FROG

NICOLE Y. THURGATE AND JOSEPH H. K. PECHMANN

ABSTRACT: A major challenge facing wildlife biologists is understanding why some species go extinct while others persist in the same habitat. To address this question we explored whether tree canopy closure over ponds affects growth and survival of tadpoles within ponds and mediates competitive interactions among species. We conducted two experiments to test whether canopy closure and competition may have contributed to the decline of the endangered dusky gopher frog (*Rana sevosa*) but allowed the persistence of the southern leopard frog (*R. sphenoccephala*). The response of both species to canopy closure was explored in single species and mixed (1:1) species treatments of identical total tadpole density. An experiment using aquatic enclosures in natural temporary ponds showed that canopy closure reduced tadpole growth approximately 20% for both species. Survival of *R. sevosa* tadpoles was higher in mixed species enclosures than in single species enclosures. In a complementary experiment using artificial ponds, *R. sevosa* had lower survival to and reduced size at metamorphosis, and produced a lower total biomass of metamorphosed juveniles in shaded ponds. *R. sphenoccephala* exhibited reduced body size at metamorphosis only when shaded. These studies suggest that pond canopy closure, not larval competition, may be contributing to the decline of the dusky gopher frog. The different responses to canopy closure suggest a potential mechanism for the loss of *R. sevosa* and the persistence of *R. sphenoccephala*. Careful removal of trees from historically open canopy ponds may help facilitate the recovery of *R. sevosa* and benefit similar species.

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INTRODUCTION

Globally there is increasing evidence for the accelerating loss or decline of species from nearly all taxonomic groups (Dirzo and Raven 2003). In spite of widespread interest in understanding and halting these losses, their causes are often poorly understood, especially for amphibians (Blaustein and Kiesecker 2002, Stuart et al. 2004). Factors causing changes in local population sizes are multiple and complex, vary among species and operate at diverse spatial and temporal scales (Holmes and Sherry 2001). Habitat loss and alteration are seen as the major cause of population declines and losses for many species (Semlitsch 2000, Dirzo and Raven 2003). Less frequently discussed are the species that survive in spite of enormous changes to their environment. An underevaluated question in ecology is why some species decline in abundance while sympatric species persist. Understanding the mechanisms behind both extinction and persistence of species is an essential step in halting the global loss of biodiversity.

In southeastern North America, the extensive loss of native forests is often linked to the decline of a range of native species, including amphibians (Smith et al. 2000). Where forests remain intact or have been replanted or regenerated, other processes have changed the composition of local communities. Forest succession, fire suppression and selective logging all affect light penetration through the forest canopy (Halverson et al. 2003). A change to the forest canopy and light penetration affects organisms within that forest in a variety of ways (Saunders et al. 1991).

Many amphibian species are dependent on small, temporary ponds for reproduction and larval development, and changes in forest structure can affect these ponds (Skelly et al. 2002). Historically, these ponds were heterogeneous in their habitat characteristics fostering varied community structure within each pond (Blaustein et al. 1999, Wellborn et al. 1996). Modern

land use practices have changed the characteristics of many ponds, potentially disrupting the communities within them. Some ponds within the southeastern United States contained trees or were heavily shaded while others were treeless depending on local conditions (Folkerts 1997). Fire suppression appears to have altered this landscape by encouraging the growth of trees in and around historically treeless wetlands (Kushlan 1990, Kirkman et al. 2000, De Steven and Toner 2004). If the pond is small, growth of surrounding vegetation can reduce the amount of sunlight reaching the water (Skelly et al. 1999). Alternatively, trees can colonize the pond basin itself, shading it when they become mature (Halverston et al. 2003).

Shading potentially lowers the pond water temperature, which can slow the growth and development of amphibian larvae dependent on open canopy ponds (Blaustein et al. 1999, Werner and Glennmeier 1999, Skelly et al. 2002). The closure of the tree canopy may also lower dissolved oxygen availability in ponds (Skelly et al. 2002), placing behavioral and physiological demands on aquatic species (Ultsch et al. 1999). In addition, shaded ponds may contain limited algal communities, which are the main diet of most larval anurans (Skelly and Golon 2003). The presence of trees within or near a pond may also shorten the hydroperiod of that pond by increasing evapotranspiration, although this is offset to some degree by a reduction in evaporation from the pond's water surface. Where hydroperiod is shortened, tadpoles may be unable to successfully reach metamorphosis before pond drying (Skelly 2004). Canopy closure thus has the potential to reduce the growth, survival, and size at metamorphosis of amphibian larvae dependent on open canopy ponds (Blaustein et al. 1999). This in turn may cause low juvenile survival, later age and smaller size at first reproduction, and poor physiological terrestrial performance for these amphibians (Smith 1987, Berven 1990).

With changes to aquatic and terrestrial habitats there has also been a decline in a number of animal species within the southeastern U. S. Coastal Plain region (Smith et al. 2000). One of these, the dusky gopher frog (*Rana sevosa*, Goin and Netting 1940), is arguably the most endangered amphibian in the United States. In January 2001 it was added to the federal endangered species list as a distinct population segment of the gopher frog *R. capito* (USFWS 2001). Recent genetic evidence suggests that the original species designation is appropriate and as such will be used here (Young and Crother 2001). There are only two confirmed extant populations of *R. sevosa*, one consisting of less than 100 adults and the other with even fewer (Richter et al. 2003, M. Sisson, Mississippi Museum of Natural Science, personal communication). Basic ecological information about this species is poorly known and is crucial to its recovery and management. Other species living in the same region do not appear to be suffering such a dramatic decline, although the population status of many amphibians remains unknown. The southern leopard frog, *R. sphenocephala*, is one of a few species still found widely throughout the Southeast in spite of enormous changes to its habitat. In addition, adults of this species are found breeding on the same nights and at the same locations as *R. sevosa*.

The importance of competition in shaping ecological communities is well documented; however, no clear consensus exists regarding its importance relative to other mechanisms such as disturbance and habitat change (Pianka 2000). As changes to the canopy cover over a pond affect a variety of abiotic variables important to larval amphibians, as well as the availability of resources such as food, the potential exists that these changes may also affect the outcome of competitive interactions within ponds.

Competition may be of particular importance for rare species (Parris and Semlitsch 1998). If rare species are competitively inferior, this can contribute to population declines and

local extinctions (Jaeger 1972, Griffies and Jaeger 1998). Habitat change can further disadvantage rare species if the changes favor their competitors (Bardsley and Beebee 2001).

The aims of this study were to explore how the larvae of a common amphibian species and a rare amphibian species survive, grow, and interact in both closed and open canopy ponds. We used the southern leopard frog and the dusky gopher frog to ask the following questions: 1) How do the larvae of these two species perform when reared in open and closed canopy ponds? 2) Has competition with *R. sphenocephala* contributed to the decline and near extinction of *R. sevosia*? 3) Do the outcomes of competitive interactions change in open and closed canopy ponds? 4) Could a decrease in the relative frequency of open canopy ponds, due to pond canopy closure or other processes, have contributed to the decline and near extinction of *R. sevosia*?

METHODS

We conducted two experiments, one using enclosures in natural ponds and the other using artificial ponds.

Aquatic enclosures

Approximately 110 eggs were gently removed from each of 5 *R. sevosia* and 5 *R. sphenocephala* egg masses in Glen's Pond, Harrison County, MS, on October 3, 2002, 3 days after the eggs were laid. Each partial clutch of eggs was placed in a separate plastic container containing pond water and covered with fiberglass screen. Containers were floated in a shallow, shaded section of Glen's Pond until the tadpoles hatched and were free swimming. Tadpoles were placed in enclosures one week after egg collection, following a stratified random design in which each clutch was equally represented in each enclosure having that species.

Nine enclosures were placed in Glen's Pond, an open canopy pond with a firm bottom dominated by *Eleocharis* spp. and other sedges, grasses, and rushes. Glen's Pond is approximately 1.5 ha in area and fills to a maximum depth of about 1.1m. The other 9 enclosures were in Middle Pond, a closed canopy pond dominated by water tupelo (*Nyssa sylvatica*), located approximately 550m west of Glen's Pond. Middle Pond is approximately 1.1 ha in area and fills to a maximum depth of about 90cm. The bottom of Middle Pond is covered in a thick layer of decaying leaves and other organic matter with little or no emergent vegetation. The hydroperiod of the two ponds is of almost identical length with the two ponds drying within days of one another. Both ponds typically fill in winter and hold water until late summer (June or July). The terrestrial habitat surrounding these ponds is primarily replanted longleaf pine (*Pinus palustris*) with an understory of shrubs (primarily *Ilex vomitoria* and *I. glabra*) and grasses.

Enclosures were 0.36m², constructed of a PVC pipe frame completely covered with 1.5 mm mesh fiberglass screen. The top of the enclosures could be opened and closed so tadpoles could be monitored. Dry pine needles (300g) were put in each enclosure. All enclosures in each pond were placed close together at 45 cm initial depth to ensure similar conditions. Enclosures were arranged in spatial blocks containing one enclosure of each treatment. In 3 enclosures per pond *R. sevosa* were raised alone at a density of 10 tadpoles per enclosure (28 tadpoles per m²). Three enclosures per pond contained 5 *R. sevosa* and 5 *R. sphenocéphala*. The last 3 enclosures each contained 10 *R. sphenocéphala*. Tadpoles were monitored biweekly and the experiment was terminated after 114 days, when Middle Pond dried on January 29, 2003. At the end of the experiment tadpoles were removed from the enclosures and measured to the nearest 0.1 mm

from the tip of the snout to the tip of the tail (total length) and from the tip of the snout to the vent (body length).

A strong thunderstorm with high wind gusts knocked over several enclosures in both ponds on December 29, 2002. Two enclosures (one of gopher frogs alone and one of both species together) in Glen's Pond were found empty after this. One enclosure (leopard frogs alone) was found empty in Middle Pond. The number of tadpoles remaining in the other enclosures suggested that few or none had escaped from them, although we could not rule out the possibility. The empty enclosures were excluded from data analyses.

Light transmission to the water's surface was measured as an index of canopy closure using a LI-COR, LI-191SA Quantum Line sensor. Five measurements were taken in Middle Pond on November 29, at 12pm on a clear day, one within the approximate center of the pond and four 15m away in the four cardinal directions. Most tree leaves abscise during November at this site. Measurements were repeated on May 12, 2003 after trees had fully leafed out. Measurements were taken in Glen's Pond 10 minutes later on both dates using identical procedures.

Artificial ponds

For this experiment 450 eggs of *R. sphenocéphala* and *R. sevosa* were collected from Glen's Pond on March 7, 2003. Eggs were collected and held as described above. Eighteen plastic cattle watering tanks (1.8 m diameter, 0.6 m high, total capacity 1325 liters) were filled to a depth of 45cm (~1000 liters) with water from a well located at Glen's Pond on March 7, 2003. The tanks were fitted with screened standpipes adjusted to keep the water depth from exceeding 45 cm. Well water was added to the tanks whenever water levels dropped below 40 cm (except during the last month of the experiment- see below). Tanks were covered with 1.5 mm mesh

fiberglass screen lids throughout filling and the subsequent experiment to keep out potential predators and competitors. An artificial canopy of 70% shade cloth was suspended 5cm above the top of 9 of the tanks. This was representative of the average amount of shade within Middle Pond.

Air-dried leaves (approximately 1/3 pine, 1/3 sweetgum and 1/3 oak by volume) weighing 1.5 kg were added to each tank upon filling. Zooplankton and phytoplankton were collected and concentrated from Glen's Pond and Middle Pond and 800ml aliquots (400ml from each pond) were randomly assigned to each tank, two days after filling. Tanks received additional 800ml aliquots of plankton on March 23. Due to a disease outbreak (D. E. Green, National Wildlife Disease Center, personal communication), which caused massive rapid tadpole mortality in Glen's Pond however, no further plankton was added until July. After this 800 ml plankton aliquots from Reserve Pond, a nearby open canopy pond, and Middle Pond were added on July 6 and 21, August 11 and 30 and September 21.

Tadpoles were placed in tanks on 14 March 2003 following a stratified random design where each clutch was equally represented in each tank that contained that species. *R. sevosia* tadpoles were randomly assigned to 12 cattle tanks. Six contained 50 *R. sevosia* tadpoles per tank (20 tadpoles per m²). Half of these tanks were shaded and half were unshaded. In another 6 tanks *R. sevosia* tadpoles were raised in competition with *R. sphenoccephala* tadpoles (i.e., 25 tadpoles of each species). To assess whether competitive interactions change depending on canopy type, 3 tanks were placed in full sun and 3 were shaded. *R. sphenoccephala* tadpoles were also raised in 6 tanks at a density of 50 per tank, 3 tanks shaded and 3 in sun. Tanks were arranged in 3 spatial blocks with 6 tanks per block (one tank of each treatment in each block). Shaded and unshaded tanks were grouped together, following a split plot design.

Tanks were monitored biweekly throughout the experiment and daily once tadpoles developed large rear limbs. Tadpoles were removed from the tanks upon metamorphosis (defined as forelimb emergence) and placed in clean plastic containers lined with damp paper towels. Animals were then held under controlled environmental conditions at the Southern Institute of Forest Genetics, Harrison Experimental Forest, until the tail was absorbed (when tail was no longer than wide) for a period not exceeding 5 days. Individuals were then weighed to the nearest 0.01g, measured (snout-vent length) to the nearest 0.1mm with calipers, and cohort marked by treatment (sun or shade) with elastomer dyes before being released at Glen's Pond.

The water level in tanks was gradually lowered from 45 to 5 cm in late summer to mimic natural pond drying and encourage metamorphosis of remaining tadpoles. Standpipes were lowered in 5 cm increments on September 1, 5, 10, 15, 20, 23, 27 and 30 September. All remaining tadpoles were removed from the tanks and released in Glen's Pond on October 1, 2003.

Statistical analyses

Aquatic enclosures. Analysis of variance (ANOVA) was used to test for differences in mean total length, body length, and survival between the two ponds, between single and mixed species treatments, and their interaction (GLM procedure, SAS 9.1 software, SAS Institute Inc., Cary, North Carolina, USA). Where data from enclosures were missing, all analyses excluded those enclosures. Spatial block was not incorporated into the analyses because the missing data resulted in multiple incomplete blocks. Preliminary analyses on subsets of the data suggested that block had an inconsequential effect.

The two species were analyzed separately. Size data were log-transformed and survival data were arcsine square root transformed to meet assumptions of homogeneity of variance. The

enclosure was used as the unit of replication rather than the individual, because individuals within enclosures are not independent (Wilbur 1987). The interaction of pond, treatment (species alone or in competition), and enclosure was used as the error term. As the experimental design was unbalanced (the number of surviving individuals measured varied among enclosures and ponds), it was necessary to include a proportion of the individual mean square in the error term to obtain an appropriate expected error mean square for analyses of size (Littell et al. 1991). This was done using a Satterthwaite approximation (Milliken and Johnson 1984).

Artificial Ponds. ANOVA was used to analyze the data from the artificial ponds following procedures analogous to those described above. Tests were conducted for differences in mean size at metamorphosis (mass and snout-vent length), adjusted tank biomass (calculated as total mass of surviving individuals divided by the starting number of tadpoles in each tank), length of larval period, survival to metamorphosis and overall survival (including tadpoles surviving after tanks were dried, only *R. sevosia* tadpoles remained) between shaded and unshaded tanks, intra- and interspecific competition, and the interaction of both treatments. Size variables (mass and snout-vent length) were log-transformed, average biomass per tank and length of larval period were square root transformed, and survival was arcsine square root transformed to meet assumptions of homogeneity of variance. Shade treatment by block was used as the error term for tests of shading (main plot) effects whereas the block by treatment interaction was used as the error term for tests of competition effects (subplot) and for tests of the interaction between competition and shading. Block had no significant main effects ($F_{2,6} \leq 0.16$, $p \geq 0.86$) in preliminary analyses and was excluded from further analyses (results were similar whether or not block was included).

RESULTS

Aquatic enclosures

Survival of gopher frog tadpoles did not differ significantly between ponds (Table 5.1). The least square mean of survival in the open canopy pond was 76%, and in the closed canopy pond it was 70%. More gopher frogs survived when in competition with leopard frogs (l_smean 90%) than when reared with conspecifics (l_smean 56.7%; Table 5.1). There was no significant interaction between shading and competition for gopher frog survival. Mean body length and total length of gopher frog tadpoles were both significantly greater in the open canopy pond than in the closed canopy pond (Table 5.1, Figure 5.1A and B). There were no significant effects of competition or interaction between competition and shading on gopher frog size (Table 5.1, Figure 5.1A and B).

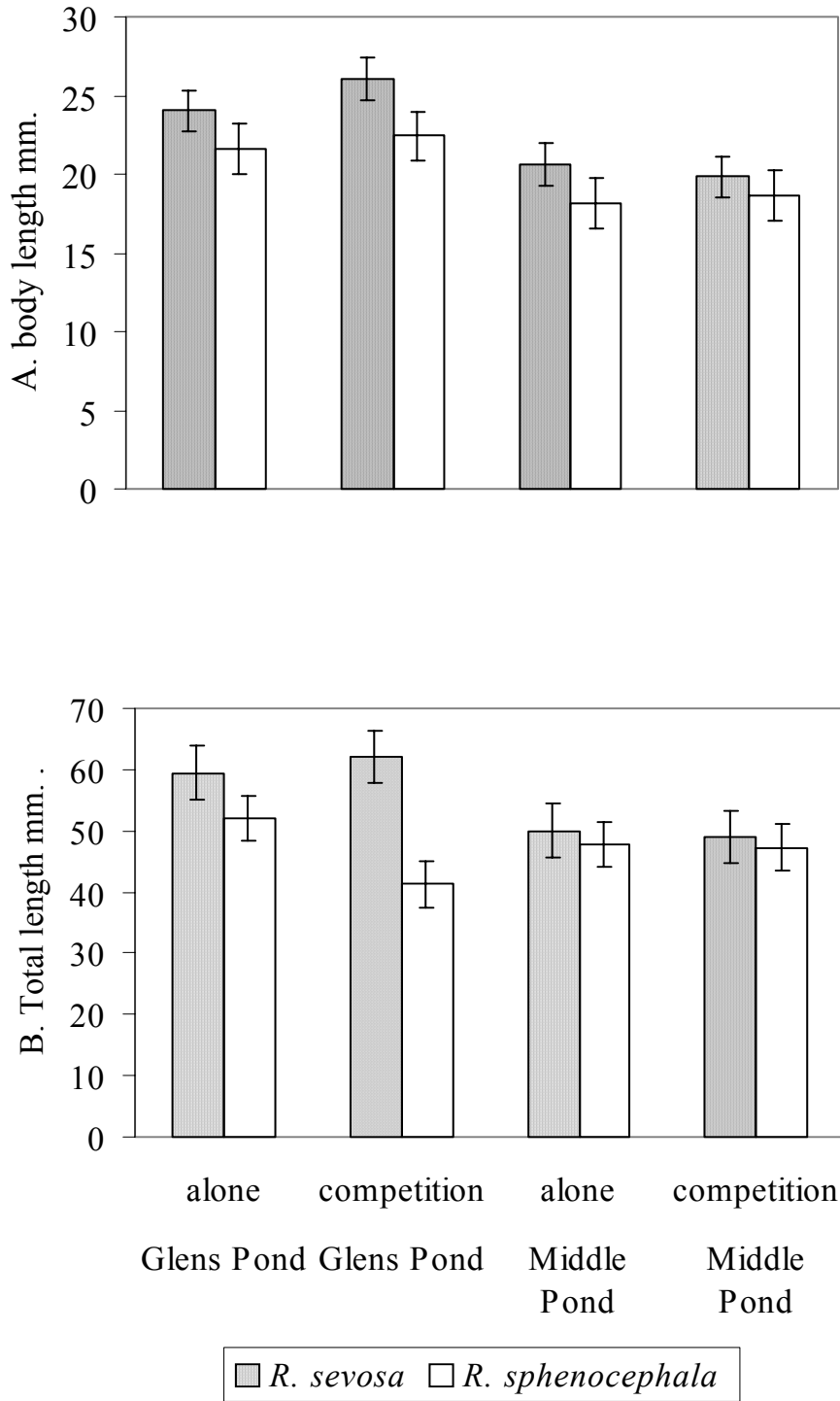
Survival of leopard frog tadpoles did not differ significantly between ponds (Table 5.1). The least square mean of survival was 30% in the open canopy pond and 52.5% in the closed canopy pond. There were no significant differences in survival between leopard frogs raised only with conspecifics (l_smean 47.5%) and those raised with gopher frogs (l_smean 35%, Table 5.1). There was no significant interaction between shading and competition for leopard frog survival.

There was a significant difference between ponds in body length but not total length of leopard frog tadpoles (Table 5.1). Mean body length was greater in the open canopy pond (Figure 5.1A). There was no significant effect of competition or interaction between shading and competition on leopard frog size (Table 5.1, Figure 5.1).

Table 5.1 ANOVAs of growth variables (log transformed) and survival (arcsine square root transformed) for gopher and leopard frog larvae in single species and mixed species (competition) enclosures in an open (Glen's) and a closed (Middle) canopy pond.

Test	df	MS	Test df	Test MS	F	P
<i>R. sevosia</i>						
Survival (pond)	1	0.006	4	0.02	0.30	0.605
Survival (competition)	1	0.103	4	0.02	5.39	0.0593
Survival (interaction)	1	0.004	4	0.02	0.2	0.68
Body length (pond)	1	0.407	8.93	0.023	15.53	0.0035
Body length (competition)	1	0.006	8.65	0.026698	0.21	0.6603
Body length (interaction)	1	0.034	8.54	0.027	1.25	0.2941
Total length (pond)	1	0.4069	8.96	0.038	10.65	0.0098
Total length (competition)	1	0.001493	8.83	0.039	0.04	0.8497
Total length (interaction)	1	0.008	16.37	0.153	0.05	0.95
<i>Rana sphenoccephala</i>						
Survival (pond)	1	0.108	4	0.0359	3.01	0.16
Survival (competition)	1	0.0347	4	0.0359	0.97	0.381
Survival (interaction)	1	0.0347	4	0.0359	0.97	0.381
Body length (pond)	1	0.1534	6.43	0.0195	7.86	0.029
Body length (competition)	1	0.0059	6.43	0.0195	0.30	0.6
Body length (interaction)	1	0.0002	6.43	0.0195	0.01	0.92
Total length (pond)	1	0.0028	5.14	0.0254	0.11	0.752
Total length (competition)	1	0.08	5.14	0.0254	3.14	0.135
Total length (interaction)	1	0.06	5.14	0.0254	2.21	0.196

Figure 5.1 A. Mean body length (mm) and B. Mean total length (mm) for both species at the end of the enclosure experiment (least square means (\pm SE)).



The average (\pm SD) photosynthetically active radiation (PAR) at the surface of the closed canopy pond on November 29 was $828.95 (\pm 175.89) \mu\text{mol s}^{-1} \text{m}^{-2}$, and in the open canopy pond it was $1936.5 (\pm 5.48) \mu\text{mol s}^{-1} \text{m}^{-2}$. All Glen's Pond measurements were in full sun, therefore, at this time the closed canopy pond received approximately 57% less PAR than an open canopy pond. The spring measurements in the closed canopy pond averaged $342.71 (\pm 124.83) \mu\text{mol s}^{-1} \text{m}^{-2}$, 82% less than in the open canopy pond, $1944.4 (\pm 7.02) \mu\text{mol s}^{-1} \text{m}^{-2}$. The average PAR reduction at the closed canopy pond for the two seasons (70%) was used as a guide for shading the artificial ponds.

Artificial Ponds

The two species had very different larval periods. *R. sevosia* tadpoles in shaded tanks had a mean larval period of $175.57 (\pm 29.55 \text{ SD})$ days while those in the unshaded tanks had a mean larval period of $165.58 (\pm 31.18 \text{ SD})$ days. For *R. sphenoccephala* the mean larval period in shaded tanks was $122.35 (\pm 19.51 \text{ SD})$ days and in unshaded tanks it was $110.88 (\pm 10.60 \text{ SD})$ days. Differences in larval period between shading and competition treatments and their interaction were not significant for either species (Table 5.2).

Significantly more *R. sevosia* tadpoles survived to metamorphosis in unshaded tanks than in shaded tanks (Table 5.2, Figure 5.2A). Shading had a marginally significant effect on the total proportion of *R. sevosia* surviving to the end of the experiment, which included unmetamorphosed tadpoles remaining in the dried tanks as well as metamorphosed individuals (Table 5.2). Total survival of *R. sevosia* averaged 55% (including an average of 10% unmetamorphosed) in unshaded tanks and 20.5% (including an average of 16.8% unmetamorphosed) in shaded tanks. Competition and the interaction between competition and shading had no significant effect on either measure of survival for *R. sevosia* (Table 5.2). *Rana*

sphenocephala survival to metamorphosis was not significantly different in any treatment, and all surviving *R. sphenocephala* metamorphosed by the end of the experiment (Table 5.2, Figure 5.2A).

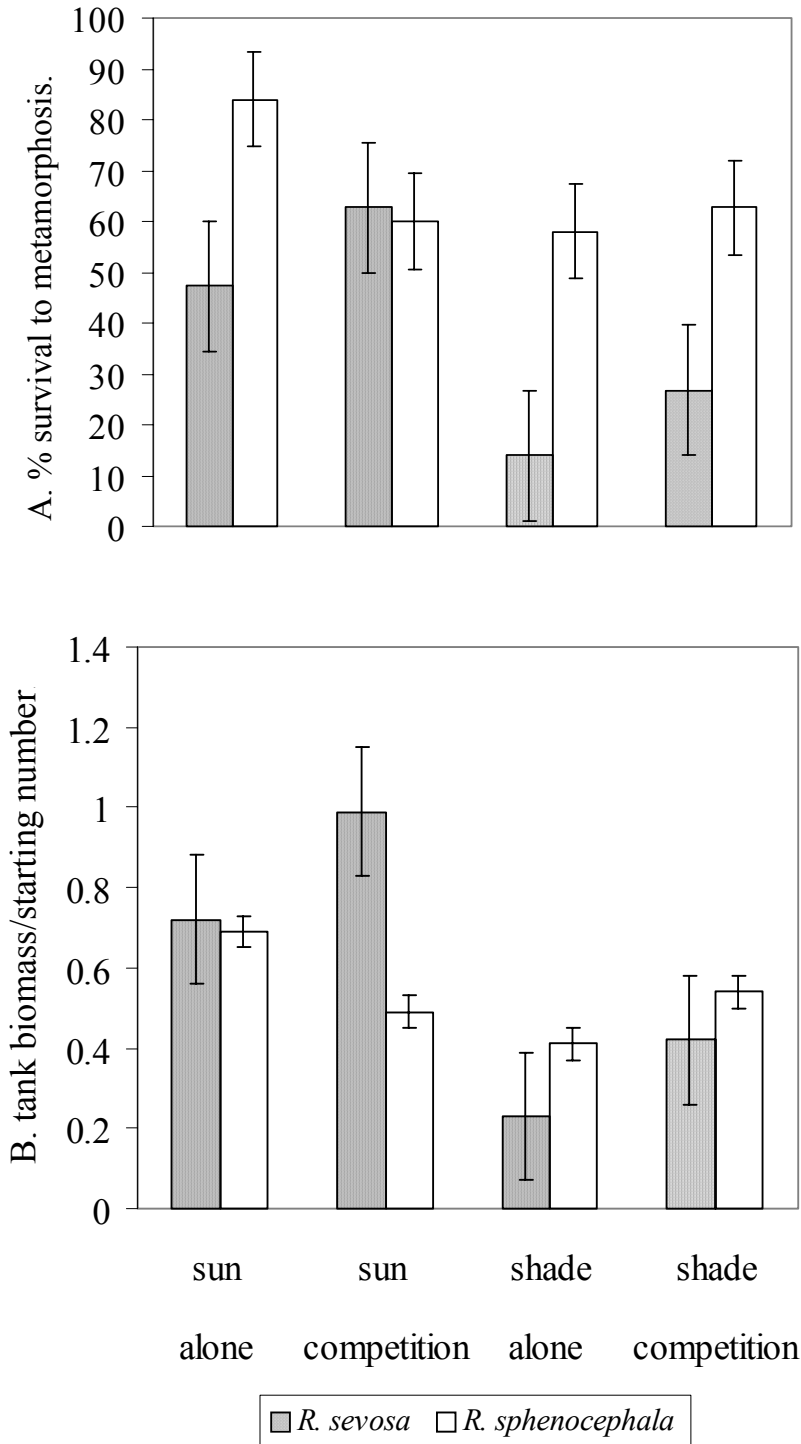
Table 5.2 ANOVA of growth variables (log transformed), average tank biomass (square root transformed) and survival (arcsine square root transformed) for gopher and leopard frog larvae in single species and mixed species (competition) artificial ponds with and without a shade-cloth canopy.

Test	df	MS	Test df	Test MS	F	P
<i>R. sevosia</i>						
Larval period (canopy)	1	3.83	4.18	12.002	0.32	0.6
Larval period (competition)	1	0.38	6.02	3.67	0.1	0.7
Larval period (interaction)	1	4.44	6.2	3.54	1.25	0.3
Survival (canopy)	1	0.3106	4	0.039	7.94	0.048
Survival (competition)	1	0.05	4	0.044	1.11	0.35
Survival (interaction)	1	0.0003	4	0.044	0.01	0.93
Survival including tadpoles (canopy)	1	0.194	4	0.032	6.04	0.07
Survival including tadpoles (competition)	1	0.01	4	0.04	0.27	0.63
Survival including tadpoles (interaction)	1	0.03	4	0.04	0.78	0.43
Snout-vent length (canopy)	1	0.09	6.72	0.01	9.16	0.02
Snout-vent length (competition)	1	0.0032	7.12	0.02	0.16	0.7
Snout-vent length (interaction)	1	0.0007	7.5	0.02	0.03	0.86
Mass (canopy)	1	0.832	5.36	0.12	6.85	0.04
Mass (competition)	1	0.009	7.08	0.15	0.06	0.82
Mass (interaction)	1	0.012	7.36	0.14	0.08	0.78
Tank biomass (canopy)	1	0.56	4	0.07	8.1	0.04
Tank biomass (competition)	1	0.08	8	0.07	1.01	0.37
Tank biomass (interaction)	1	0.00001	8	0.07	0.0	0.99

Test	df	MS	Test df	Test MS	F	P
<i>Rana sphenoccephala</i>						
Larval period (canopy)	1	17.67	4.005	12.87	1.37	0.31
Larval period (competition)	1	3.85	4.006	11.78	0.33	0.59
Larval period (interaction)	1	4.32	4.006	11.78	0.36	0.57
Survival (canopy)	1	0.28	4	0.007	3.73	0.13
Survival (competition)	1	0.017	4	0.03	0.6	0.48
Survival (interaction)	1	0.043	4	0.03	1.42	0.3
Snout-vent length (canopy)	1	0.324	4.01	0.06	5.17	0.08
Snout-vent length (competition)	1	0.0003	4.02	0.031	0.01	0.93
Snout-vent length (interaction)	1	0.0015	4.02	0.031	0.05	0.84
Mass (canopy)	1	2.48	4.01	0.43	5.77	0.07
Mass (competition)	1	0.02	4.02	0.28	0.06	0.82
Mass (interaction)	1	0.004	4.02	0.28	0.01	0.91
Tank biomass (canopy)	1	0.022	4	0.01	2.09	0.22
Tank biomass (competition)	1	0.0005	4	0.004	0.32	0.74
Tank biomass (interaction)	1	0.043	4	0.004	7.29	0.03

(Table 5.2 cont.)

Figure 5.2 A. Mean percent survival to metamorphosis and B Mean biomass per tank (g)/starting number of individuals of the species in that tank (least square means (\pm SE)) in the artificial pond experiment for both species



Shading reduced snout-vent length and mass at metamorphosis of both species (Table 5.2). For *R. sevosia* mean snout-vent length was 26.54 (± 0.24) mm in unshaded tanks and 24.45 (± 0.61) mm in shaded tanks, and mean mass was 1.68 (± 0.4) g in unshaded tanks and 1.30 (± 0.11) g in shaded tanks. For *R. sphenoccephala* mean snout-vent length was 22.7 (± 0.4) mm in unshaded tanks and 20.76 (± 0.4) mm in shaded tanks, and mean mass was 0.82 (± 0.4 SE) g in unshaded tanks and 0.69 (± 0.04) g in shaded tanks. Competition and the interaction between competition and shading had no significant effect on size at metamorphosis for either species.

Survival and the average size of the survivors are not independent. For example, where survival is low, competition may be reduced, and surviving animals may grow larger. Therefore, average adjusted (for initial number of individuals) biomass of metamorphs per tank was analyzed as a measure that incorporated both survival and size. *R. sevosia* biomass was significantly lower in shaded tanks (Table 5.2, Figure 5.2). There was no significant effect of competition or interaction between competition and shading on *R. sevosia* biomass. *R. sphenoccephala* did not show a significant effect of shading or competition on biomass, however, there was a significant interaction between the two treatments (Table 5.2, Figure 5.2). This interaction was due to higher average biomass when the species was reared alone in unshaded tanks, and higher average biomass when the species was reared in competition with *R. sevosia* in shaded tanks (Figure 2).

DISCUSSION

Canopy cover

We expected that an increase in canopy cover would have negative effects on both species studied and especially on *R. sevosia*. The few known current and historical breeding sites

of *R. sevosa* are all open canopy ponds. *R. sphenoccephala* breeds in both open and closed canopy ponds in southern Mississippi, although it is far more common in open canopy ponds. Growth in body length of both species was reduced by a similar amount (20%) in the closed canopy natural pond compared to the open canopy pond, although only *R. sevosa* showed a significant reduction in length when the tail was included (total length). Depressed larval growth rates have been found for other ranid species in closed canopy ponds (Werner and Glennemeier 1999, Skelly et al. 2002).

We found that shading affected the two species differently in our artificial pond experiment where larvae could be followed to metamorphosis. Shading reduced survival to metamorphosis, the total biomass of metamorphosed juveniles and body size at metamorphosis of *R. sevosa* but reduced only (and with marginal significance) body size at metamorphosis of *R. sphenoccephala*. The lower survival of shaded *R. sevosa* was due to both lower tadpole survival and the failure of more individuals to metamorphose before the ponds dried. In contrast, all surviving *R. sphenoccephala* tadpoles, even those in shaded ponds, were able to metamorphose by the end of the experiment. This was apparently because *R. sphenoccephala* can metamorphose after a shorter larval period and at a smaller body size than *R. sevosa*.

Canopy closure thus had both lethal and sub-lethal effects on *R. sevosa* but only sub-lethal effects on *R. sphenoccephala* as measured at metamorphosis. This may explain, in part, how canopy closure could eliminate *R. sevosa* from ponds but allow *R. sphenoccephala* to persist. Average survival to metamorphosis of *R. sevosa* is very low even at the open canopy Glen's Pond, because the pond often dries before *R. sevosa* can complete its long larval period (Richter and Seigel 2002). Further reduction in survival to metamorphosis from canopy closure could reduce juvenile recruitment below replacement levels.

The small size at metamorphosis of *R. sphenoccephala* and *R. sevosa* in shaded ponds may affect their demographics. Small size can lower juvenile survival, delay first reproduction and reduce size and fecundity at first reproduction (Smith 1987, Berven 1990, Alford 1999). These effects alone may eliminate populations from shaded ponds. Where both survival to and size at metamorphosis are low, as for *R. sevosa*, local extinctions are especially likely. Therefore, while canopy closure affects both species, its effects have more severe consequences for *R. sevosa*.

Canopy closure may have contributed to the range-wide decline of *R. sevosa*. Many temporary ponds within the range of *R. sevosa* that are now dominated by hardwoods appear to have had an open canopy in the early 1950s based on aerial photographs (M. Sisson, Mississippi Museum of Natural Science, personal communication). Research in Florida suggests a similar trend across much of the Gulf coastal plain (Kusklan 1990). Conceptual models of wetland vegetation dynamics (Kirkman et al. 2000, De Steven and Toner 2004) suggest that hardwood invasion may result from hydroperiod reduction or fire suppression. Drainage ditches and other alterations have reduced hydroperiods at a few ponds in this region. Fire suppression is widespread in the southeastern U. S. Even in those areas where fire is used as a management tool, burns have traditionally been conducted in the winter, when temporary ponds usually contain water and do not burn.

A number of factors besides pond canopy closure threaten *R. sevosa*, including logging and conversion of longleaf pine to pine plantations, habitat fragmentation, draining of or introduction of fish to breeding ponds, and mortality on roads (Richter et al., 2001, Bailey, 1991). Additional threats include the planned construction of a housing development 250m from one of the known breeding ponds and two diseases recently found at that pond (Green et al., 2002, Richter et al., 2003). Determining what causes decline of one species while another

persists is crucial to effective conservation (Julliard et al. 2003). Understanding these differences provides key insights into the management strategies needed to help stop species decline and extinction. Inappropriate habitat management however, affects some species more than others. Certain species lack the flexibility to respond to habitat change, and even relatively minor changes may cause these species to decline or become extinct.

Competition

The hypothesis that gopher frogs are rare because they are out-competed by leopard frogs was not supported. Rather, survival of gopher frog tadpoles was higher when with this species than alone in the aquatic enclosures experiment. Gopher frogs may be more strongly affected by the presence of conspecifics than by congeners due to the greater size of conspecifics or niche partitioning (Morin and Johnson 1988). This result was not found in the artificial ponds experiment, which may be an effect of different experimental venue or because the two experiments had different timeframes.

The biomass data for the artificial ponds experiment indicated that the effect of *R. sevosia* on *R. sphenoccephala* differed with shading treatment. This was due primarily to the low survival of *R. sevosia* in shaded artificial ponds. In shaded competition treatments *R. sphenoccephala* were released from the effects of competition with *R. sevosia*, while in unshaded treatments they were not. Shading did not change the outcome of competition between the two species in any other detectable way. Overall it does not appear that the two species have a strong competitive effect on one another under either open or closed canopy conditions.

Competition from *R. sphenoccephala* also has been found to have no detectable effects on a close relative of *R. sevosia*, the northern crawfish frog *R. areolata* (Parris and Semlitsch 1998). Prior to 1991 *R. sevosia* was included as a subspecies of *R. areolata* (Young and Crother 2001),

so we expected to find similar results for the two studies. Asymmetric competition with another species, *R. blairi*, however, may contribute to the rarity of *R. areolata* (Parris and Semlitch 1998), and it is possible that competition from other species affects *R. sevosa*. Asymmetric competition and habitat change have also been cited in the decline of other species of amphibians (Bardsley and Beebee 2001) and other taxa (e. g., Wijesinghe and Brook 2004).

Management implications

Canopy closure over temporary ponds is not unique to the Gulf coastal plain. A historical comparison of ponds at a research site in Michigan 30 years ago with present conditions showed that canopy closure is occurring at most ponds while no ponds are becoming more open (Skelly et al. 1999). Canopy closure may be contributing to localized extinctions of even historically common amphibian species in Michigan (Skelly et al. 1999). Other studies have shown that the adults of certain species avoid closed canopy ponds for breeding and that those that do breed at these sites pay a cost in larval performance (Skelly et al. 1999, Werner and Glennmeier 1999, Palik et al. 2001, Skelly 2001, Skelly et al. 2002, Halverston et al. 2003, Skelly and Golon 2003, but see Skelly 2004). Our study, together with these others, suggests that canopy closure over temporary ponds is an issue that requires further attention from researchers and managers.

One of the primary goals of managers of *R. sevosa* is to create self-sufficient populations at additional sites. Our study indicates that translocation sites should be open canopy temporary ponds, but these are all but non-existent in southern Mississippi. Implementation of an appropriate fire regime may be needed to remove pond canopy where closure has occurred. Other methods of tree removal, e.g., mechanical may also be necessary where trees cannot be eliminated by fire. The effects of tree removal in ponds should be explored. Removal of invasive aquatic vegetation from ponds and pond excavation and redefinition resulted in an

increase in pond usage and breeding by the endangered natterjack toad (*Bufo calamita*) in England (Phillips et al. 2002). Similar management action may be required in southern Mississippi. These actions would best be attempted in ponds that could be identified as historically open canopy, with the aim of restoring a landscape including temporary ponds with various degrees of canopy closure.

Removal of trees from ponds should be approached experimentally and cautiously. Modified hydrological dynamics, increased siltation, and decreased coarse woody debris in the pond basin could result from tree removal and negatively affect some species. Effects of tree removal may also vary with characteristics of the pond. Construction of new open canopy ponds may be an alternative method of providing additional larval habitat for *R. sevosia*, although creating temporary ponds with the desired hydroperiod can be a challenge (Pechmann et al. 2001).

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CHAPTER 6 THE EFFECTS OF CANOPY CLOSURE AND COMPETITION ON THE LARVAL PERFORMANCE OF A RARE AND A COMMON AMPHIBIAN.

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ABSTRACT: Small wetlands can become shaded by the growth of trees in or around the pond basin, e. g., if natural fires are suppressed. The amount of shade over ponds can affect the distribution and abundance of pond breeding amphibians. In southern Mississippi the dusky gopher frog (*Rana sevosa*) has declined while a congener, the southern leopard frog (*Rana sphenoccephala*) has remained common. We conducted two experiments to examine the larval performance of these species alone and in competition, in open and closed canopy ponds. We also assessed biotic and abiotic differences between these habitats. Survival, growth, and body size at metamorphosis of *R. sevosa* larvae were significantly lower in closed canopy ponds. *R. sphenoccephala* did not exhibit a similar reduction in survival in closed canopy ponds although tadpoles and recently metamorphosed individuals were also smaller. We also found evidence of asymmetrical competition between the two species. *Rana sevosa* appeared to be a superior competitor in open canopy ponds. Temperature, dissolved oxygen and the abundance of periphyton were all lower in closed canopy ponds, which may explain reduced larval performance of anurans in this habitat. These studies suggest that pond canopy closure, but not larval competition, could have contributed to the decline of the dusky gopher frog. Opening of the tree canopy at potential breeding sites may contribute to the recovery of this species.

INTRODUCTION

Mechanisms determining the distribution and abundance of natural populations remain in many cases, poorly understood. Studies have shown dramatic changes in the distribution and

abundance of a variety of species, primarily due to alterations in their habitat (Werner and Glennemeier 1999, Semlitsch 2000, Dirzo and Raven 2003). Some changes in amphibian populations may result from alterations in the vegetation canopy above small wetlands used for breeding (Skelly et al. 1999, 2002, Werner and Glennemeier 1999, Halverson et al. 2003, Schiesari 2006, Thurgate and Pechmann in press). Where forest succession is occurring, shade over ponds increases when the forest canopy grows over them or trees colonize the pond basin (Skelly et al., 2002).

Recent studies suggest that the degree of shading over ponds exerts a strong control over the distribution of pond breeding amphibians (Skelly et al. 1999, Werner and Glennemeier, 1999). Where canopy closure has occurred, there have been localized extinctions of amphibian species that appear to breed only in unshaded or open canopy ponds (Skelly et al. 1999, 2002, Werner and Glennemeier 1999, Halverson et al. 2003). Relatively few species have been identified that are able to persist in closed canopy ponds (Skelly et al. 2002). Identifying the attributes of species that contribute to their success or failure in different environments can increase our understanding of how some species persist in altered environments whereas others are eliminated.

The observed relationships between canopy closure and the distribution of species is likely the result of two broad mechanisms (Skelly et al. 2002). Firstly, adult amphibians display selectivity in the choice of breeding sites and may be deliberately avoiding sites where canopy closure has occurred, although this remains to be tested (Laurila and Aho 1997, Halverston et al. 2003). Secondly, differences in the biotic and abiotic environments of closed canopy ponds may make them unsuitable for growth and survival of larvae (Skelly, 2001).

Canopy closure appears to affect amphibian larvae in a number of ways. For the majority of species studied, the growth of larvae is slowed and individuals raised in closed canopy ponds are smaller at metamorphosis than counterparts raised in open canopy ponds (Werner and Glennemeier, 1999, Skelly et al., 2002, Schiesari 2006, Thurgate and Pechmann, in press). Differences in growth rates have been found even for species that appear quite tolerant of canopy closure (Werner and Glennemeier, 1999, Skelly et al. 2002, Halverson et al. 2003). Additionally, larval survival is lower in closed canopy ponds (Halverson et al. 2003, Lauck et al. 2005, Thurgate and Pechmann, in press.), although there are exceptions (Werner and Glennemeier, 1999, Skelly et al. 2002).

Canopy closure may also influence species interactions, with the outcome changing in different habitats. Werner and Glennemeier (1999) showed that apparent asymmetrical competitive effects apparent in open canopy ponds were reduced or eliminated in closed canopy ponds. However, competitive effects have been largely overlooked in studies of canopy closure, or where it has been tested, results have been inconclusive (Schiesari 2006, Thurgate and Pechmann in press).

In a previous study we used the endangered dusky gopher frog (*Rana sevosa*) and a common congener the southern leopard frog (*Rana sphenoccephala*) to examine the associations between larval performance and canopy closure (Thurgate and Pechmann in press). We were interested in discovering whether canopy closure may have contributed to the decline of the dusky gopher frog while allowing the southern leopard frog to persist in the same habitat. The dusky gopher frog had not been observed breeding in local closed canopy ponds, while the southern leopard frog had, and we hypothesized that the two species would respond to canopy closure differently. We also explored competitive interactions between these two species, as

larval competition can exhibit a strong control on the distribution and abundance of species (Bardsley and Beebee 2001) We found that the growth of both species was negatively affected by canopy closure and that the dusky gopher frog had lower survival in shaded treatments. We did not find any strong evidence of competition in these experiments.

We were unable to speculate on the mechanisms that may have contributed to the effects observed in our first experiments, e.g. temperature, because we measured few environmental variables. In the experiments here we include information about changes to biotic and abiotic variables in shaded and unshaded artificial and natural ponds to explore associations connecting larval performance and canopy cover rather than testing the function of a precise causal factor. However, the measurement of biotic and abiotic variables allows us to suggest potential mechanisms for observed patterns (Skelly et al. 2002).

We asked the following questions. 1. Does survival and/or growth of *R. sevosa* and *R. sphenoccephala* differ between open and closed canopy ponds? 2. Does competition affect growth or survival of *R. sevosa* and *R. sphenoccephala*? Do the effects of competition vary with canopy closure? 3. Does water temperature, dissolved oxygen or pH differ between open and closed canopy ponds? 4. Does the abundance of food resources in the form of periphyton differ between open and closed canopy ponds?

METHODS

Aquatic enclosures

Approximately 300 eggs were removed from 5 *R. sevosa* egg masses in Glen's Pond, Harrison County, MS, on April 2, 2005, 3 days after eggs were laid. The same numbers of *R. sphenoccephala* eggs were also removed from nearby Carr Bridge Road Pond, Harrison County,

Mississippi on the same day. Only two pairs of *R. sphenoccephala* bred at Glen's Pond during this breeding event so it was necessary to obtain eggs of that species from another site. Each partial clutch of eggs was placed in a separate plastic container containing pond water and covered with fiberglass screen. Containers were placed in a screened, shaded room at the Southern Institute of Forest Genetics, Harrison County, Mississippi until tadpoles were hatched and free swimming. Tadpoles were placed in aquatic enclosures one week after egg collection, following a stratified random design in which each clutch was equally represented in enclosures containing that species.

Twelve aquatic enclosures were placed in both Glen's and Scaphiopus Ponds; two open canopy ponds. Twelve more enclosures were also placed in both HEF and Dogwood Ponds; two closed canopy ponds. Glen's Pond and HEF Ponds are both approximately 20 kilometers north of Gulfport, MS and approximately 2 km apart. Scaphiopus and Dogwood Ponds are approximately 40 kilometers north of Glen's Pond and approximately 1 km apart. Ponds were chosen based on similarity in hydroperiod (all ponds filled and dried within days of each other) and availability of open canopy ponds (there are very few open canopy ponds in southern Mississippi). All ponds were a similar size (between 1.21 and 1.63 hectares) and depth (maximum depths between 94 cm and 118 cm).

Enclosures were 0.4 m², constructed of a PVC-pipe frame and completely enclosed with 1.5 mm mesh fiberglass screen. The enclosures could be opened and closed so tadpoles could be monitored. Dry pine needles (300 g) were put in each enclosure to provide refugia for tadpoles. All enclosures were placed close together at 50 cm initial depth to ensure starting similarity. Enclosures were spatially blocked, with each block containing one enclosure of each experimental treatment. In 4 enclosures per pond *R. sevosia* tadpoles were raised alone at a

density of 10 tadpoles per enclosure (26 tadpoles m²). Four enclosures per pond contained 5 *R. sevos*a and 5 *R. sphen*ocephala. The last 4 enclosures each contained 10 *R. sphen*ocephala.

Tadpoles growth and survival were monitored biweekly and the experiment was terminated after 33 days, when pond depth reached 5 cm on May 4, 2005 and drying was imminent. At the time of removal each tadpole was measured to the nearest 0.1 mm from the tip of the snout to the tip of the tail (total length) and from the tip of the snout to the vent (body length).

Dissolved oxygen, temperature and pH were measured at HEF and Glen's Ponds on April 16 between 12-1 pm and at Scaphiopus and Dogwood Ponds on April 17 between 12-1 pm. Both days were cloudless. Further measurements were planned but could not be completed due to the rapidity of pond drying. Measurements were taken at the deepest point of each pond using a calibrated Hydrolab with the sensor placed 15 cm below the water surface. We attempted to measure periphyton in each pond (as described below) but the rapidity of drying precluded this (slides had to be removed after 5 days when water levels began to fall rapidly).

Artificial ponds

Approximately 1100 eggs (~550 of each species) were removed from 5 *R. sevos*a and 5 *R. sphen*ocephala egg masses in Glen's Pond on March 29, 2004, 2 days after the eggs were laid. Partial clutches of eggs were then placed in large plastic containers containing pond water and covered with fiberglass screen. Containers were floated in a shaded part of Glen's Pond until tadpoles were hatched and free-swimming at which time they were taken to the laboratory for counting. Tadpoles were placed in artificial ponds ten days after egg collection, following a stratified random design.

Twenty four plastic cattle watering tanks (1.8 m diameter, 0.6 m high, total capacity 1325 liters) were filled to a starting depth of 30cm with water from a well located at Glen's Pond.

Water levels were raised in 5cm increments weekly thereafter to a total depth of 45cm (~1000 litres). Blocks 1 and 2 (see below) were filled on March 29, 2004 and blocks 3 and 4 were filled on March 30. Each tank was fitted with a screened standpipe to ensure water depth did not exceed 45 cm. We readjusted water levels to 45 cm by adding well water when levels dropped below 40 cm (except during the last month of the experiment- see below). Tanks were covered with 1.5 mm mesh fiberglass screen lids throughout filling and the ensuing experiment to exclude potential predators and competitors. An artificial shade canopy of 70% shade-cloth was suspended 5cm above the top of 12 of the 24 tanks. This was representative of the amount of shading at a local closed canopy pond (Thurgate and Pechmann, in press).

Air-dried leaves (1 kg) that had been raked from the bottom of a dry, closed-canopy pond were added to each tank during filling. Tanks also received air-dried pine needles (500 g) for a total of 1.5 kilograms of leaves. Zooplankton and phytoplankton were collected and concentrated from 2 local, closed canopy ponds (Middle Pond and HEF Pond) and 800 ml aliquots (400 ml from each pond) were randomized and added to shaded tanks one day after all tanks were filled (March 31). Plankton was also collected from 2 local, open canopy ponds (Reserve Pond and Glen's Pond) and 800 ml aliquots (400 ml from each pond) were randomized and added to unshaded tanks. Additional 800 ml aliquots were collected from the same ponds and added to tanks on April 12 and April 21. After this 800 ml aliquots of plankton were added monthly (except in July when ponds were dry).

Closed canopy temporary ponds usually contain little herbaceous vegetation while open canopy ponds generally contain emergent sedges, grasses, and rushes. To approximate the vegetation structure found in open canopy temporary ponds, 95 cm lengths of 1-inch yellow polypropylene rope were completely frayed by hand after being tied to a clay brick. Each brick

had 4 sections of rope tied on to it, with the middle of the rope tied through holes in the brick and the frayed ends floating at the water surface. Two such bricks were placed equidistant from the center of each of the unshaded tanks, 15 cm apart. Two bricks without ropes were placed in each of the shaded tanks to control for any effects of bricks.

Tanks were arranged in 4 spatial blocks with 6 tanks per block, with each block receiving one tank of each treatment. Eight tanks contained 40 *R. sevosia* tadpoles per tank (16 tadpoles m⁻²). Half of these tanks were shaded and half were unshaded. In another 8 tanks *R. sevosia* tadpoles were raised in competition with *R. sphenoccephala* tadpoles, 20 tadpoles of each species per tank. To assess whether competitive interactions changed depending on canopy type, 4 tanks were placed in full sun and 4 were shaded. *R. sphenoccephala* tadpoles were also raised in 8 tanks at a density of 40 per tank, 4 tanks shaded and 4 in sun. Shading was the main plot in a split plot design whereby the three shaded tanks in each block were placed together under the same shade cloth and the three unshaded tanks were also placed together.

Tanks were monitored biweekly throughout the experiment and daily once tadpoles neared metamorphosis. Tadpoles were taken from tanks upon metamorphosis (defined as forelimb emergence) and placed in plastic containers (10 x 10 x 6cm) lined with moist paper towels. Animals were kept under controlled environmental conditions at the Southern Institute of Forest Genetics, Harrison Experimental Forest, MS, until tail absorption was complete. Individuals were then weighed (mass) to the nearest 0.01g, measured (snout-vent length) to the nearest 0.1mm with calipers, and cohort marked by treatment (sun or shade) with subcutaneous elastomer dyes before being released at Glen's Pond.

The water level in tanks was progressively lowered from 45 to 5 cm in late summer to mimic natural pond drying and promote metamorphosis of remaining tadpoles. Standpipes were

lowered in 5 cm increments on September 1, 5, 10, 15, 20, 23 and 28. All remaining tadpoles were removed from the tanks and released in Glen's Pond on September 30.

Periphyton measurements

Three frosted end glass microscope slides were placed in each tank on April 11. Slides were suspended from the tank edge by cotton rope and placed at 15cm below the water surface. Each slide was secured to the rope by a wooden clothespin attached at the frosted end of the microscope slides. Slides were placed at the north, east and west edges of tanks. Slides were removed after 14 days and placed in screw top histological vials filled with water from the tank for transportation (Skelly et al. 2002). This process was repeated beginning on June 1.

To determine the average biomass per tank, periphyton was scraped from two slides from each date (north and east slides) onto pre-weighed filter paper, one week after removal from tanks (Skelly et al. 2002). Periphyton was only removed from the clear glass portion of slides. Filter papers that periphyton had been scraped onto were then re-dried at 80°C and reweighed to the nearest milligram. Productivity was expressed as the number of milligrams of dry mass accumulated per square centimeter per day (Skelly et al. 2002). The species composition of the periphyton was to be measured from the remaining slides. Unfortunately, these slides were lost during hurricane Katrina and this analysis could not be completed.

Water measurements

Water temperature, pH and dissolved oxygen were measured 15cm below the water surface in each tank on 9 days during May and June using a calibrated Hydrolab©. Measurements were taken between 10.00am and 5pm on cloudless days. The average values for each tank were used in analyses (Werner and Glennemeier, 1999).

Statistical analysis

Aquatic enclosures. Analysis of variance (ANOVA) was used to test for differences in mean total length, body length, and survival between open and closed canopy ponds, in single and mixed species treatments, and for interactions between competition and canopy (GLM procedure, SAS 9.1 software, SAS Institute Inc., Cary, North Carolina, USA). Preliminary analyses suggested that block had an inconsequential effect ($F > 1.43$, $p < 0.26$) and block was excluded from further analysis.

The two species were analyzed separately. Size data were log-transformed and survival data were arcsine square root transformed to meet assumptions of homogeneity of variance. The enclosure was used as the unit of replication rather than the individual, because individuals within enclosures are not independent (Wilbur 1987). The interaction of pond, treatment (species alone or in competition), and enclosure nested within canopy was used as the error term for tests of canopy (main plot) whereas pond within canopy was used as the error term for tests of competition (subplot) and the interaction between shading and competition. As the experimental design was unbalanced (the number of surviving individuals measured varied among enclosures and ponds), it was necessary to include a proportion of the individual mean square in the error term to obtain an appropriate expected error mean square for analyses of size (Littell et al. 1991). This was done using a Satterthwaite approximation (Milliken and Johnson 1984).

Artificial ponds. Analysis of variance (ANOVA) was used to test for differences in mean mass and snout-vent length at metamorphosis, survival to metamorphosis, overall survival (including tadpoles alive at the end of the experiment), length of larval period and adjusted tank biomass (calculated as total mass of metamorphosed individuals divided by the starting number of

tadpoles in each tank) between the shaded and unshaded tanks, between single and mixed species treatments, and their interactions. Tank means were used as the unit of replication. Size variables (mass and snout-vent length) were log-transformed, average biomass per tank and larval period were square root transformed, and survival was arcsine square root transformed to meet assumptions of homogeneity of variance. Shade treatment by block was used as the error term for tests of shading (main plot) effects whereas the block by treatment interaction was used as the error term for tests of competition effects (subplot) and for tests of the interaction between competition and shading. Block had no significant main effects ($F_{2,6} \leq 0.15$, $p \geq 0.72$) in preliminary analyses and was excluded from further analyses (results were similar whether or not block was included). As the number of surviving individuals measured varied among tanks, it was necessary to include a proportion of the individual mean square in the error term to obtain an appropriate expected error mean square for analyses of size (Littell et al. 1991). This was done using a Satterthwaite approximation (Milliken and Johnson 1984).

Other variables. ANOVA was also used to test for differences in periphyton productivity, temperature, dissolved oxygen, and pH between shaded and unshaded tanks. Spatial (main plot) blocks of 3 shaded or 3 unshaded tanks were treated as replicates rather than individual tanks as individual tanks are not independent. Analysis originally included a full split-plot model however species composition was not significant and excluded from further analyses. No analysis was conducted on these variables in ponds due to lack of replication within each pond.

RESULTS

Aquatic enclosures

Significantly more *R. sevosia* survived in unshaded ponds than shaded ponds (Table 6.1, Figure 6.1). There was no significant effect of competition, or interaction between competition and canopy, for *R. sevosia* survival. Mean total length and mean body length of *R. sevosia* were both significantly greater in open canopy ponds (Table 6.1, Figure 6.2). There was also a trend towards significance for competition and the interaction of competition and canopy for body length, but not total length in *R. sevosia* (Table 6.1, Figure 6.2A and B). *R. sevosia* tadpoles had larger bodies when reared in single species enclosures than when in competition in the shade but not in the sun.

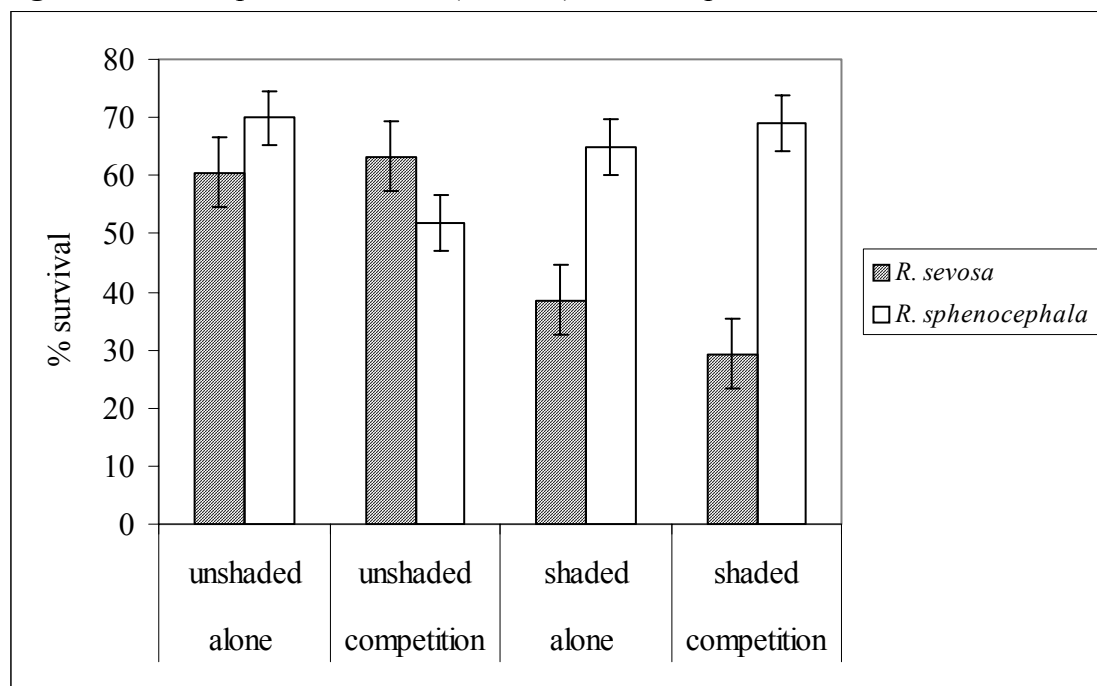
Table 6.1 ANOVAs of survival (arcsine square root transformed) and growth variables (log transformed) for gopher and leopard frog larvae in single species and mixed species (competition) enclosures in open and closed canopy ponds.

Test	df	MS	Test df	Test MS	F	P
<i>R. sevosia</i>						
Survival (canopy)	1	0.5597	26	0.0282	220.02	0.0045
Survival (competition)	1	0.0183	26	0.0302	0.61	0.443
Survival (interaction)	1	0.0164	26	0.0302	0.54	0.4678
Body length (canopy)	1	1.893	10.59	0.00523	362.08	<0.0001
Body length (competition)	1	0.1713	39.31	0.0478	3.58	0.0657
Body length (interaction)	1	0.1617	39.31	0.0478	3.38	0.0735
Total length (canopy)	1	1.028	13.0372	0.0139	74.19	0.0031
Total length (competition)	1	0.09	39.003	0.0425	2.14	0.1517
Total length (interaction)	1	0.0208	39.003	0.0425	0.49	0.4883
<i>Rana sphenoccephala</i>						
Survival (canopy)	1	0.034	26	0.0354	0.96	0.4299
Survival (competition)	1	0.035	26	0.0168	2.02	0.1667

Test	df	MS	Test df	Test MS	F	P
Survival (interaction)	1	0.0894	26	0.0168	5.32	0.0294
Body length (canopy)	1	0.8256	10.993	0.2001	4.12	0.01798
Body length (competition)	1	0.0241	38.231	0.0309	0.78	0.3818
Body length (interaction)	1	0.0001	38.247	0.0308	0.00	0.9448
Total length (canopy)	1	0.9693	10.9897	0.0807	12.01	0.0747
Total length (competition)	1	0.0005	35.245	0.02383	0.02	0.8828
Total length (interaction)	1	0.00892	35.245	0.0238	0.37	0.5445

(Table 6.1 cont.)

Figure 6.1 Mean percent survival (lsmeans) for both species in shaded and unshaded ponds.

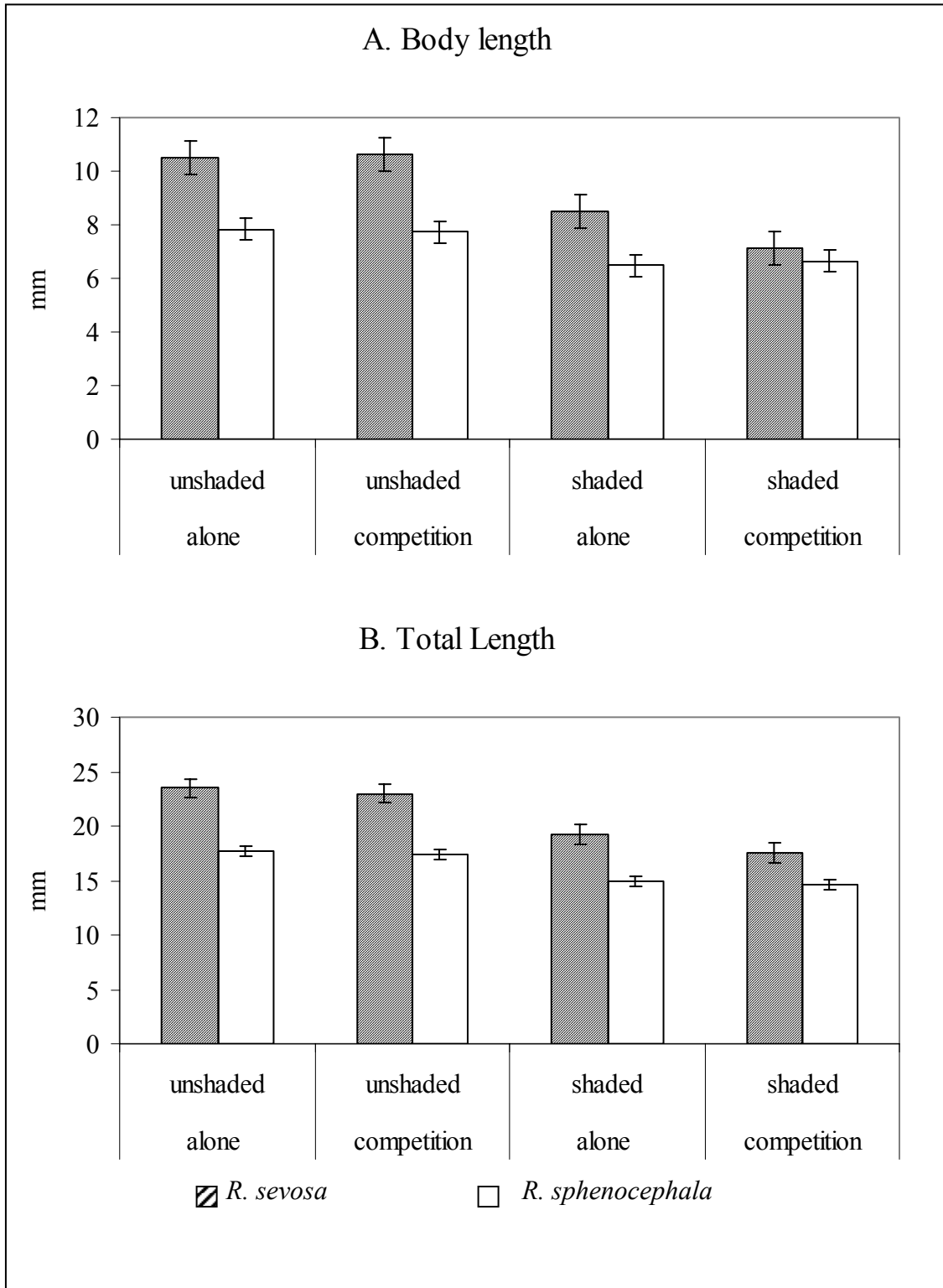


Survival of *R. sphenoccephala* was not significantly affected by canopy or competition, however, there was a significant interaction between the two (Table 6.1, Figure 6.1). More *R. sphenoccephala* survived in unshaded ponds in single species treatments than in competition; however this trend was not exhibited in shaded ponds. Mean total length of *R. sphenoccephala*

was nearly significantly greater under open canopies and mean body length was significantly higher in open canopy ponds (Table 6.1, Figure 6.2A and B). There was no significant effect of competition, or interaction between canopy and competition, on size of *R. sphenoccephala* tadpoles.

Dissolved oxygen in Glen's pond was 11.8% and in Scaphiopus pond it was 12.2%. In the two closed canopy ponds dissolved oxygen was lower, although this was not tested due to lack of within pond replication. In HEF pond it was 5.8% and in Dogwood pond it was 8.9%. Temperature in Glen's Pond was 26.8°C and in Scaphiopus pond it was 28.4°C. In HEF pond temperature was 23.5°C and in Dogwood pond it was 24.2°C. The pH of the open canopy ponds was 6.2 in Glen's pond and 6.4 in Scaphiopus pond. The pH of the closed canopy ponds was 5.3 in HEF pond and 5.9 in Dogwood pond.

Figure 6.2 A. Mean body length (mm) and B. mean total length (mm) for both species at the end of the enclosure experiment (least square means (\pm SE)).



Artificial ponds

The larval period of *R. sevosa* was considerably longer than that of *R. sphenoccephala* in all treatments (Figure 6.3). There was no significant effect of any treatment on larval period for *R. sevosa*, however, larval period was significantly affected by canopy cover, competition and the interaction of these variables for *R. sphenoccephala* (Table 6.2). *R. sphenoccephala* had a significantly longer larval period in shaded treatments and the larval period of this species was significantly reduced when in competition with *R. sevosa* (Figure 6.3 and Table 6.2). This change in larval period when in competition was more pronounced in shaded than unshaded treatments (Figure 6.3).

Figure 6.3 Mean larval period in days (least square means \pm SE) for both species in the artificial ponds.

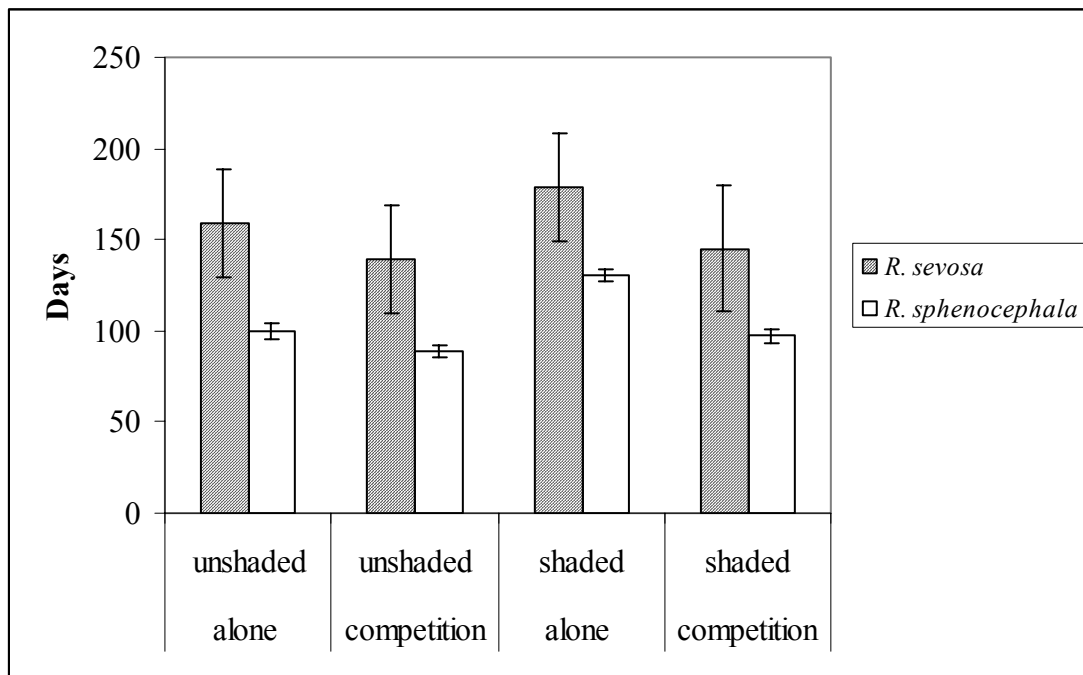


Table 6.2 ANOVA of growth variables (log transformed), larval period and average tank biomass (square root transformed) and survival (arcsine square root transformed) for both species in single species and mixed species artificial ponds with and without a shade-cloth canopy.

Test	df	MS	Test df	Test MS	F	P
<i>R. sevosia</i>						
Larval period (canopy)	1	0.432	3.5371	4.116	0.01	0.92
Larval period (competition)	1	10.312	6	18.3	0.56	0.48
Larval period (interaction)	1	2.877	6	18.3	1.25	0.71
Survival (canopy)	1	0.214	3.15	0.019	11.01	0.042
Survival (competition)	1	0.078	6	0.026	2.98	0.14
Survival (interaction)	1	0.005	6	0.026	0.21	0.67
Survival with tadpoles (canopy)	1	0.227	3.12	0.018	12.72	0.035
Survival with tadpoles (competition)	1	0.01	6	0.018	0.78	0.42
Survival with tadpoles (interaction)	1	0.036	6	0.036	1.99	0.21
Snout-vent length (canopy)	1	99.494	3.728	12.738	7.81	0.043
Snout-vent length (competition)	1	34.57	6	74.312	0.47	.52
Snout-vent length (interaction)	1	20.697	6	74.312	0.28	0.62
Mass (canopy)	1	0.366	3.274	0.024	15.42	0.025
Mass (competition)	1	0.068	6	0.056	1.2	0.32
Mass (interaction)	1	0.00002	6	0.056	0.00	0.98
Tank biomass (canopy)	1	0.34	3.967	0.014	25.05	0.008
Tank biomass (competition)	1	0.048	6	0.101	0.47	0.52
Tank biomass (interaction)	1	0.00009	6	0.101	0.0	0.98
<i>Rana sphenoccephala</i>						
Larval period (canopy)	1	3.102	3.04	0.305	10.14	0.049
Larval period (competition)	1	4.131	6	0.108	38.3	0.0008
Larval period (interaction)	1	0.91	6	0.108	8.44	0.0272
Survival (canopy)	1	0.478	3.008	0.064	7.48	0.0815
Survival (competition)	1	0.0142	6	0.005	2.96	0.14
Survival (interaction)	1	0.096	6	0.005	20.04	0.0042

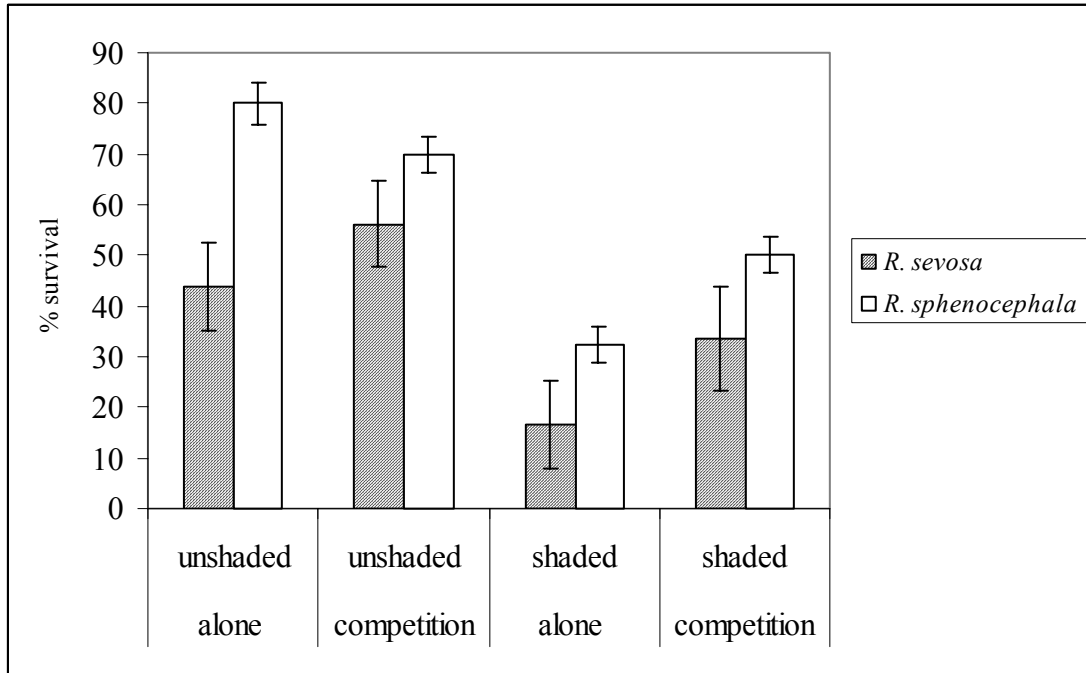
Test	df	MS	Test df	Test MS	F	P
Survival with tadpoles (canopy)	1	0.384	3.014	0.05	7.62	0.0797
Survival with tadpoles (competition)	1	0.009	6	0.007	1.39	0.28
Survival with tadpoles (interaction)	1	0.082	6	0.007	12.47	0.0124
Snout-vent length (canopy)	1	0.003	4.2	0.0001	32.83	0.004
Snout-vent length (competition)	1	0.3622	6	0.0009	0.97	0.36
Snout-vent length (interaction)	1	0.0001	6	0.0009	0.15	0.71
Mass (canopy)	1	0.03	5.67	0.0006	49.06	0.0005
Mass (competition)	1	0.002	6	0.01	0.19	0.68
Mass (interaction)	1	0.004	6	0.01	0.04	0.85
Tank biomass (canopy)	1	0.346	3.01	0.04	8.80	0.079
Tank biomass (competition)	1	0.003	6	0.004	0.79	0.41
Tank biomass (interaction)	1	0.077	6	0.004	19.48	0.0045

(Table 6.2 cont.)

At the end of the experiment there were 114 (23.75%) *R. sevosa* tadpoles remaining in tanks and 9 (1.8%) *R. sphenoccephala*. Of the 114 *R. sevosa* 68.6% were in shaded tanks, while 100% of *R. sphenoccephala* were in shaded tanks. Survival to metamorphosis and overall survival (including tadpoles) were significantly lower for *R. sevosa* in shaded treatments than unshaded (Table 6.2, Figure 6.4). Survival to metamorphosis was $50.1\% \pm 6.71$ in unshaded treatments and $24.2\% \pm 6.13$ in shaded treatments. Overall survival was $58.13\% \pm 4.78$ in unshaded treatments and $33.13\% \pm 5.23$ in shaded treatments. There was no effect of competition or interaction between competition and shade for survival of this species. There was a trend towards fewer *R. sphenoccephala* surviving to metamorphosis in shaded than in unshaded treatments (Table 6.2, Figure 6.4). While there was no significant effect of competition there was a significant interaction between shading and competition for survival in *R. sphenoccephala*. When in competition with *R. sevosa* in unshaded treatments, fewer *R. sphenoccephala* survive to

metamorphosis (Figure 6.4) and this pattern is reversed in shaded treatments (Figure 6.4). When *R. sphenoccephala* tadpoles surviving to the end of the experiment were included in the analysis, the same major patterns remain (Table 6.2).

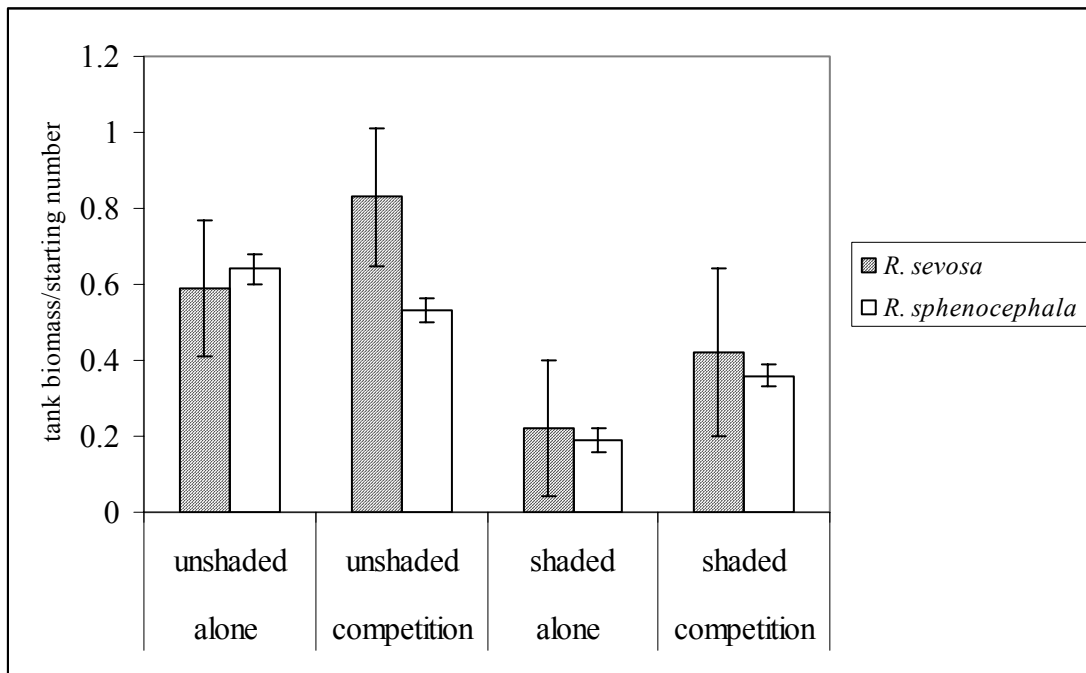
Figure 6.4 Mean percent survival to metamorphosis (least square means (\pm SE)) in artificial ponds for both species.



Shading significantly reduced snout-vent length and mass at metamorphosis for both species (Table 6.2), although much less so for *R. sphenoccephala*. For *R. sevosia* mean snout-vent length was 28.64 (\pm 3.05) mm in unshaded tanks and 23.4 (\pm 3.34) mm in shaded tanks, and mean mass was 2.07 (\pm 0.22) g in unshaded tanks and 1.4 (\pm 0.24) g in shaded tanks. For *R. sphenoccephala* mean snout-vent length was 22.44 (\pm 0.26) mm in unshaded tanks and 21.77 (\pm 0.24) mm in shaded tanks, and mean mass was 0.78 (\pm 0.03) g in unshaded tanks and 0.72 (\pm 0.026) g in shaded tanks. Competition and the interaction between competition and shading had no significant effect on size at metamorphosis for either species.

Survival and size were not independent. Where survival is low, competition for food resources may be reduced, and surviving animals may grow larger. Therefore, average adjusted (for starting number of individuals) biomass of individuals metamorphosing per tank was analyzed as a measure that integrated both size and survival. *R. sevosa* biomass was significantly lower in shaded tanks (Table 6.2, Fig. 5). There was no significant effect of competition or interaction between competition and shading on *R. sevosa* biomass. *R. sphenoccephala* biomass showed a trend towards significance with shading but no influence of competition. There was a significant interaction between the two treatments however, for this species (Table 6.2, Fig. 6.5). This interaction was due to higher average biomass when the species was reared alone in unshaded tanks, and higher average biomass when the species was reared in competition with *R. sevosa* in shaded tanks (Figure 6.5).

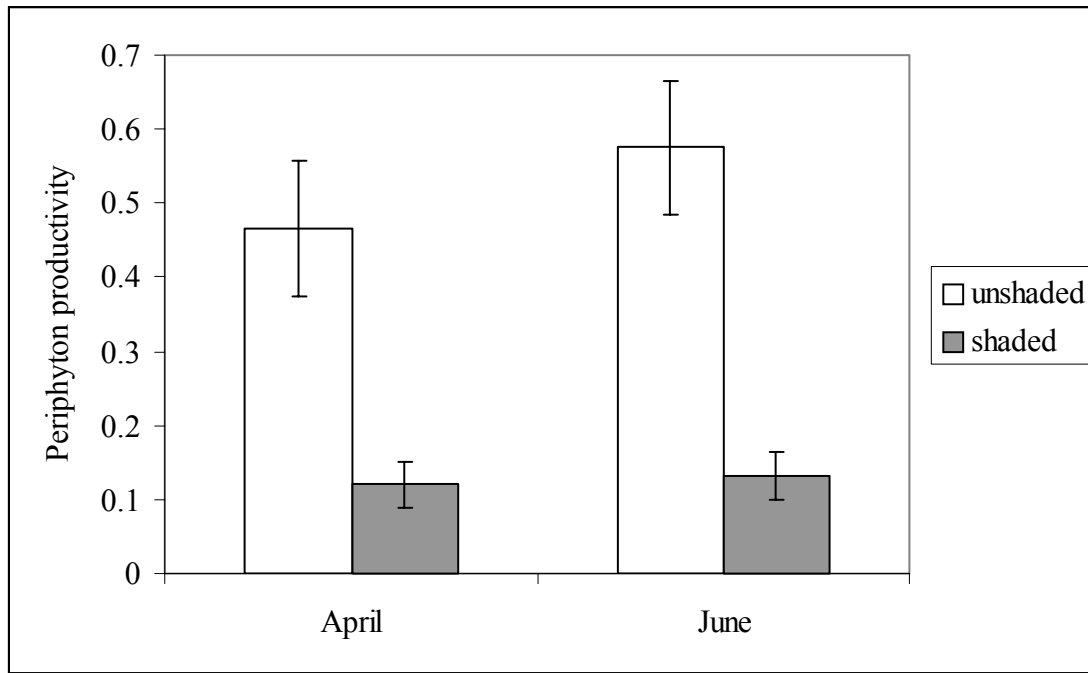
Figure 6.5 Mean biomass per tank (g)/starting number of individuals of that species in that tank (least square means (\pm SE)).



Periphyton and water measurements

There was significantly less periphyton produced in shaded than unshaded tanks (Figure 6.6) in during both April ($F_{1,6.5} = 351.66$ $p < 0.0001$) and June ($F_{1,8.05} = 664.13$ $p < 0.0001$).

Figure 6.6 Productivity of periphyton measured as the dry mass accumulated per day per slide over a two-week period.

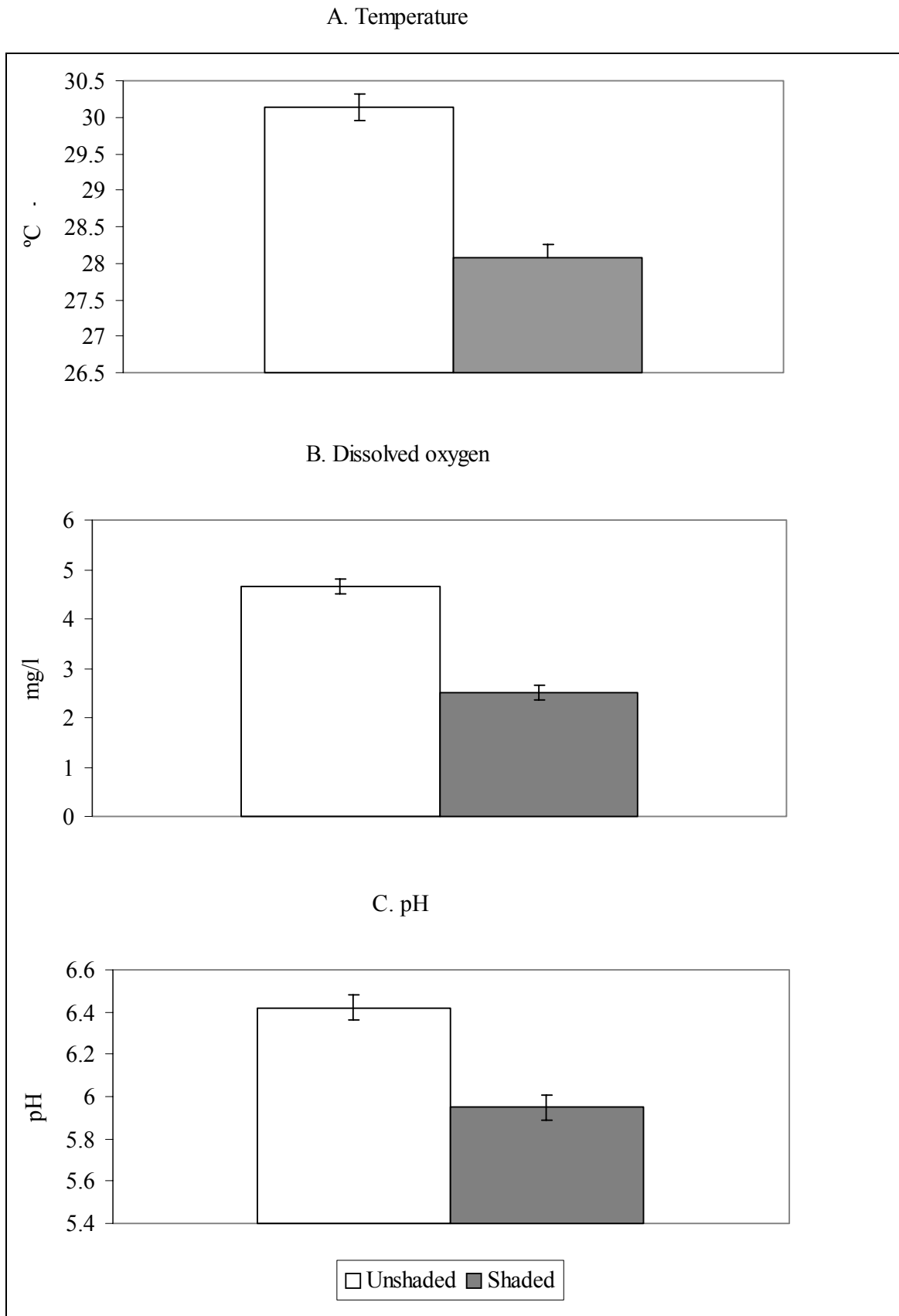


Measurements for temperature, dissolved oxygen and pH were significantly different between shaded and unshaded tanks (Table 6.3). The mean temperature in unshaded tanks was on average over 2°C higher than in shaded tanks (Figure 6.7A). Dissolved oxygen was halved in shaded tanks (Figure 6.7B) and pH was lower in shaded tanks (Figure 6.7C).

Table 6.3 ANOVA of water measurements between shaded and unshaded tanks.

Test (shaded vs. unshaded)	df	MS	Test df	Test MS	F	P
Temperature °C	1	17.92	12.461	0.0789	227.09	<0.0001
Dissolved oxygen mg/l	1	24.95	8.237	0.0819	304.73	<0.0001
pH	1	0.973	9.8783	0.036	26.97	0.0004

Figure 6.7 Means (\pm SE) of A. Temperature B. Dissolved Oxygen and C. pH in shaded vs. unshaded tanks



DISCUSSION

Larval performance and canopy closure

Patterns of forest cover are constantly shifting, especially in fire dominated ecosystems such as the longleaf pine savanna favored by *R. sevosia* (Lauck et al. 2005, Van Lear et al. 2005). However, recent research suggests that human activities have fostered a shift in many forests away from a dynamic system towards increasingly closed canopies in terrestrial habitats and ponds (Skelly et al. 1999, 2002, Halverson et al. 2003, Lauck et al. 2005). The results of the present and similar research suggest that canopy closure has a major influence on amphibian distribution and abundance. The consequences of canopy closure may be more severe for some species than others, and so may be a topic of conservation concern in certain ecosystems.

We found that the two study species were negatively affected by canopy closure although their responses differed. The survival of *R. sevosia* was significantly reduced by canopy closure in both experiments, whether tadpoles were included or not. While there were trends towards lower survival for *R. sphenoccephala* under closed canopies, the reduction was not significant for either experiment. This is a key difference in the performance of these two species in open and closed canopy ponds. The ability of *R. sphenoccephala* to survive in closed canopy ponds, albeit at a reduced size, may be a key factor in its continued persistence in an environment where most ponds appear to be becoming closed canopy. Conversely, the poor survival of *R. sevosia* in the same ponds hints at one possible mechanism for its severe, range-wide reduction.

The growth of *R. sevosia* was reduced in both natural and artificial closed canopy ponds. The growth of *R. sphenoccephala* was also negatively affected by canopy closure, but to a lesser degree. When growth and survival were incorporated into a single variable (biomass), *R. sevosia* exhibited a stronger response to canopy closure than *R. sphenoccephala*. Size at metamorphosis

is an important component of amphibian fitness. Individuals that are small at metamorphosis may exhibit delayed reproduction, smaller clutch size and reduced survival to adulthood (Smith 1987, Berven 1990, Alford 1999). Survival in *R. sevososa* is already low in closed canopy ponds. The potential fitness consequences of reduced growth, which persist into the adult portion of the life cycle, may further disadvantage this species.

The bulk of studies on larval amphibian performance in closed canopy wetlands have found reduced growth for the majority of larval amphibians and reduced survival for some species (Werner and Glennemeier, 1999, Skelly et al., 2002, Lauck et al. 2005, Schiesari 2006, Thurgate and Pechmann, in press). In our study, effects were more severe for *R. sevososa*, which suggests that this species is reliant on open-canopy ponds for breeding. The less severe effects exhibited by *R. sphenoccephala* suggest that this species may be able to tolerate at least some degree of canopy closure. Pond canopy is not confined to just open or closed categories, as forest succession progresses there is a gradient from completely open to heavily shaded (Werner and Glennemeier 1999). There also appears to be a species specific light boundary, below which species are eliminated from ponds (Halverson et al. 2003). While this study did not assess light boundaries for *R. sevososa* and *R. sphenoccephala* the better performance of the latter at a high level of shading suggest it may have a greater tolerance to shading than *R. sevososa* and may persist longer at a pond that is becoming shaded.

Competition and the interaction with canopy closure

Previous experiments failed to find any strong evidence of competition between *R. sevososa* and *R. sphenoccephala* (Thurgate and Pechmann in press). The experiments here suggest that asymmetrical competition is occurring between the two species, and that this is influenced

by canopy. We found interactions between canopy and competition in the artificial pond experiment. More *R. sphenoccephala* survived in single species treatments in sun and more survived in mixed species treatments in shade. The same pattern was observed in the biomass measure (which incorporates size and survival) for *R. sphenoccephala*. This was due to the low survival and smaller size of *R. sevosa* in shaded artificial ponds. In shaded competition treatments *R. sphenoccephala* were released from the effects of competition with larger *R. sevosa*, while in unshaded treatments they were not.

The larval period of *R. sphenoccephala* decreased when in competition with *R. sevosa* in both treatments and this difference was more pronounced in shaded treatments. This reduction in the larval period of *R. sphenoccephala* corresponds to the predictions of the Wilbur-Collins model of amphibian metamorphosis (Wilbur and Collins, 1973). In this model, when growth conditions are favorable, amphibian larvae delay time to metamorphosis to enhance size at metamorphosis (Wilbur and Collins, 1973). When growth conditions are poor however, individuals metamorphose quickly after reaching the minimum size threshold for metamorphosis (Day and Rowe, 2002). This suggests that competition with *R. sevosa*, at least as measured by the timing of metamorphosis, creates poor conditions for *R. sphenoccephala*. We did not find similar results in the size data of this species. Therefore, only the larval period of *R. sphenoccephala* appears to correspond with this model.

Competition may be of great importance in structuring communities containing rare species (Parris and Semlitsch, 1998). If rare species are competitively inferior this can contribute to population declines and local extinctions (Jaeger, 1972, Griffies and Jaeger 1998). Habitat change can further disadvantage rare species as changes to habitat may favor other species to the exclusion of the rare species (Bardsley and Beebee, 2001). In this study however, we could find

little evidence that *R. sevosia* is a competitively inferior species, in either of the habitats tested. In fact data suggest it is a superior competitor, at least in open canopy ponds. We found that habitat change did not change competitive outcomes for *R. sevosia* although it did affect the common species, *R. sphenoccephala*. We conclude that other mechanisms have been of greater influence in the decline of *R. sevosia* than competition.

Water characteristics and periphyton

A variety of factors may contribute to the unsuitability of closed canopy ponds for certain species. Canopy closure lowers the water temperature of ponds through shading. Water temperature has been shown to have a very strong effect on larval amphibian developmental and growth rates, and is an important predictor of amphibian presence or absence in ponds (Newman, 1998). However, not all species exhibit the same responses to temperature changes and some exhibit broader thermal tolerances than others (Halverson et al. 2003). Laboratory experiments have shown that an increase in water temperature of 5°C can lead to a doubling of larval growth rates for certain species (Smith-Gill & Berven, 1979). We found average temperature difference of 2.06°C which may be a large enough change to affect larval growth rates and at least in part explain the results found in our experiments. Previous studies have found average temperature differences of 1.7°C to 5°C between open and closed canopy ponds (Blaustein et al., 1999, Werner and Glennmeier, 1999, Skelly et al., 2002). Given the difficulty of isolating temperature changes from other affects of canopy closure however, the consensus appears to be that the effects of temperature are important within a combination of other features of closed canopy ponds.

Our study and that of Skelly et al. (2002) found that the dissolved oxygen content of open canopy ponds was twice that of closed canopy ponds on average. The dissolved oxygen

concentration in both open (4.66 mg/l) and closed (2.51 mg/l) canopy artificial ponds was very low in our experiments. Hypoxic conditions have been known to cause mortality in tadpoles, although its effects have been studied in very few species (Crowder et al., 1998). Where dissolved oxygen is very low, tadpole growth rates may be retarded and survival may be compromised (Plenet et al. 1998, Werner and Glennemeier, 1999). In response to hypoxia, tadpoles increase swimming and air breathing which may expose them to an increased risk of predation and be energetically expensive (Plenet et al. 1998, McIntyre and McCollum 2000). Therefore dissimilarity in dissolved oxygen concentration potentially contributed to the performance differences observed in larval amphibians in open and closed canopy ponds. However, further research is needed to adequately address larval amphibian response to oxygen stress.

The pH of pond water can strongly limit the distribution of amphibian species (Skelly, 2001). Low pH can cause increased mortality and reduced growth in many amphibian species (Glos et al., 2003). Additionally, changes to pH can alter the outcome of competitive interactions within the pond community, favoring some species over others (Kiesecker, 1996). Differences in pH have not been reported for the majority of studies examining amphibian response to canopy closure. One study found that the pH in closed canopy ponds was significantly lower than in open canopy; however the pH difference was not considered great enough to affect amphibian larvae (Werner and Glennmeier, 1999). While pH was significantly different in our study we do not believe the differences were large enough to contribute to the results observed in tadpoles.

The biotic differences between open and closed canopy ponds may also affect tadpole performance and strongly influence abiotic factors. Closed canopy ponds contain fewer

macrophytes than open canopy ponds and in many cases have a substrate of decomposing leaves, which contributes to lowering the dissolved oxygen and pH of water (Holomuzki, 1998, Werner and Glennmeier, 1999, McIntyre and McCollum 2000). By contrast open canopy ponds have a substrate of living aquatic grasses and sedges, which could potentially provide benefits to tadpoles such as increased water oxygenation, refuge from predators or improved growth surfaces for periphyton (Werner and Glennemeier 1999, Skelly and Golon 2003).

Closed canopy ponds have lower algal productivity than open canopy ponds and algae are the primary food source of most larval anurans (Holomuzki 1998). Skelly et al. (2002) found that closed canopy ponds had 3 to 4 times less periphyton than open canopy ponds. Our results were similar. Food availability has a very strong influence on survival to, size at and timing of metamorphosis in larval amphibians (Newman 1998). Where food resources are very low tadpoles can exhibit up to 50% smaller size at metamorphosis, up to a 20% greater larval period and up to 12 times greater mortality than tadpoles that have abundant food resources (Newman 1994, Audo et al. 1995, Kupeferberg 1997). The addition of food to tadpoles being reared in closed canopy ponds partly mitigated depressed growth rates, suggesting that food limitation plays an important role in larval performance in closed canopy ponds (Skelly et al. 2002). It is likely that food resource limitations contributed to the effects we observed in these experiments.

Given the difficulty of testing the role of each individual component of the biotic and abiotic differences between open and closed canopy ponds on larval amphibian performance, most researchers discuss the synergistic effects of these factors. Until experiments are conducted that pry apart each individual mechanism discussed here and test the response of all species to changes in them, we will be restricted to discussion of possible impacts on natural populations (Skelly 2001). However, given what we do know about potential changes between open and

closed canopy ponds, and the growing body of literature that shows impacts on larval amphibians, the results of our experiments suggest that canopy closure may have an important impact on the distribution and abundance of both *R. sevosia* and *R. sphenoccephala* through physiological and developmental consequences.

The decline of the dusky gopher frog

The results of this study suggest that canopy closure may well have contributed to the severe, range-wide decline of *R. sevosia*. Many temporary ponds within the range of *R. sevosia* that are now dominated by *Nyssa biflora* and other hardwoods had an open canopy in the early 1950s based on aerial photographs (Michael Sisson, Mississippi Museum of Natural History, *personal communication*). Theoretical models of wetland vegetation dynamics (Kirkman et al. 2000; De Steven and Toner 2004) propose that hardwood invasion may result from hydroperiod reduction or fire suppression. Drainage ditches and other alterations have reduced hydroperiods at a few ponds in this region; however, fire suppression is widespread in the southeastern U. S. Even in those areas where fire is used as a management tool, burns have traditionally been conducted in the winter, when temporary ponds usually contain water and do not burn (Bishop and Haas, 2005). Furthermore, open canopy ponds are more likely to occur on ridgetops and other high ground (Kirkman et al. 2000; De Steven and Toner 2004), areas highly desirable for agriculture and development in the coastal plain. Open canopy ponds may therefore have been subjected to draining, conversion to permanent farm ponds, and alteration of the surrounding terrestrial habitat more often than closed canopy ponds, reducing their relative frequency across the landscape (Michael Sisson, Mississippi Museum of Natural History, *personal communication*).

A contrast between the historical and present conditions of ponds at a research site in Michigan 30 years ago revealed that canopy closure is happening at most ponds while no ponds are becoming more open (Skelly et al. 1999). This canopy closure appears to be contributing to localized extinctions of even historically common species. Other research suggests that adults of certain species do not use closed canopy ponds for breeding and that those that do breed at these sites show reduced larval performance (Skelly et al. 1999; Werner and Glennmeier 1999; Palik et al. 2001; Skelly 2001; Skelly et al. 2002; Halverston et al. 2003; Skelly & Golon 2003). This, coupled with the results of our studies, suggests that canopy closure over temporary ponds may be a serious conservation problem for many amphibian species. For species like *R. sevosa* the effects of canopy closure may be serious enough to contribute to extinction. As so few open canopy ponds remain in southern Mississippi managers may have to alleviate this problem by removing trees from ponds which historically had an open canopy, and implementing a fire regime that includes burning of temporary pond basins.

Reversing habitat change can in some instances help reverse population declines. The removal of invasive aquatic vegetation from ponds and pond excavation and redefinition resulted in an increase in pond usage and breeding by the endangered natterjack toad (*Bufo calamita*) in England (Phillips et al. 2002). Similar management action, in the form of tree removal and increased burning, may be needed to ensure the continued survival of *R. sevosa*.

Changes to habitat have been implicated in the decline of a vast number of species and changes in the structure and function of communities (Holmes & Sherry 2001, Skelly 2001, Blaustein and Kiesecker 2002, Halverston et al. 2003, Julliard et al. 2003, Wijesinghe & Brook 2004). Taxa that are unable to exploit new habitats, or cope with changes to existing habitats are more likely to become rare, decline or go extinct (Cadotte and Lovett-Doust, 2002). The current

status of *R. sevos*a is extremely bleak and the species remains poised at the brink of extinction. Less than 100 breeding adults remain in southern Mississippi and continued threats to remaining populations call for drastic management action (Richter et al. 2003, Thurgate and Pechmann in press). If the trend towards canopy closure continues in the habitat of *R. sevos*a, the results of our research suggest this process may ultimately contribute to the loss of the species.

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CHAPTER 7 CONCLUSIONS

In chapter 1 of my dissertation I posed 6 broad questions or themes central to my research. These questions related to the ecology and conservation biology of *R. sevosia* and *R. sphenoccephala* and, based on the results obtained, I will address each question below.

1. Has habitat change in the aquatic environment contributed to the decline of *R. sevosia*?

Habitat change in the form of closure of the tree canopy over breeding ponds is likely to have contributed to the decline of *R. sevosia*. In the survey study (Chapter 4) we found that *R. sevosia* was more likely to be present in ponds with abundant grassy vegetation, a feature of open canopy ponds. In four experiments (Chapters 5 and 6) we found a strong negative response to canopy closure in the form of larval performance. *R. sevosia* was smaller, both as tadpoles and at metamorphosis, in closed canopy ponds and survival was very low in these habitats.

The small size of tadpoles would affect *R. sevosia* populations at closed canopy ponds. Small tadpoles take longer to mature and this can influence how long it takes to obtain the minimum size needed to achieve metamorphosis (Wilbur and Collins 1973). If tadpoles fail to reach this minimum size before ponds dry, then there is no recruitment into the adult population. Small size at metamorphosis also has negative implications for populations of *R. sevosia*. Animals that are small at metamorphosis can have lower juvenile survival, delayed first reproduction and reduced size and fecundity at first reproduction (Smith 1987, Berven 1990, Alford 1999). These effects alone may eliminate populations from closed canopy ponds.

Low survival of *R. sevosia* larvae in closed canopy ponds may be especially problematic. There has only been successful natural recruitment in the Glen's pond (open canopy) population in 3 of the last 12 years (Richter et al., 2003, personal observation). Even in years where

recruitment is successful, numbers of recently metamorphosed individuals are very low and estimates of survival from egg to metamorphosis are between 0.35% and 5.36% (Richter et al., 2003). If survival as tadpoles and to metamorphosis is further lowered due to canopy closure, recruitment will almost certainly be insufficient to sustain populations. This would be especially true if surviving tadpoles and post-metamorphic animals are small and less likely to survive. Given the overwhelming abundance of closed canopy ponds in the former range of the dusky gopher frog, it is highly likely the multiple effects of canopy closure on *R. sevosia* populations have contributed to the decline of the dusky gopher frog. Habitat change in the form of fish introductions may also have contributed to the decline of *R. sevosia*. In Chapter 3 we showed that *R. sevosia* does not respond appropriately to the presence of a potentially lethal predator. In an experiment using *R. capito*, Braid et al. (1994) observed that when larvae were raised with fish, none survived beyond three weeks after hatching. It is likely that *R. sevosia* would be similarly affected by the introduction of fish into breeding habitats.

Changes to the aquatic habitat of *R. sevosia* can be readily observed in Mississippi. Finding open canopy, fish-free temporary ponds containing abundant emergent vegetation has been one of the greatest challenges facing managers of the species. The empirical data presented in my dissertation suggest that restoration of such habitats will be necessary if we wish to increase the abundance of *R. sevosia* and halt the decline of remaining populations.

2. Has habitat change in the terrestrial environment contributed to the decline of *R. sevosia*?

In Chapter 4 we showed differences between terrestrial habitats where *R. sevosia* was present and absent. Sites where they were present were more likely to have abundant grasses in the forest understory and have low amounts of shrubs and canopy cover. This implies an association

with sites that are regularly burned (Bishop and Haas 2005, Smith et al. 2000). In addition, the presence of *R. sevosa* was associated with a high number of burrows. This suggests a need for burrow-making organisms such as gopher tortoises (*Gopherus polyphemus*) or small mammals (e.g. *Peromyscus* spp.) in its habitat. The importance of these burrowing animals is also highlighted by the results of Chapter 2 which showed that *R. sevosa* respond positively to the chemical cues of these organisms. The burrowing animals in turn are reliant on open understory vegetation to provide adequate food and are dependent on fire to maintain these conditions (Dickman et al. 2000, Mushinsky et al. 2003). Fire also creates potential burrows by burning out roots and stumps of dead trees. The loss of these important features of *R. sevosa* terrestrial habitat would likely have contributed to its decline.

3. Has larval competition with *R. sphenoccephala* contributed to the decline of *R. sevosa*?

We explored competition between *R. sevosa* and *R. sphenoccephala* larvae in 5 experiments in Chapters 3, 5 and 6. We could not find any evidence that larval competition with *R. sphenoccephala* has contributed to the decline of *R. sevosa*. Instead we found that the common species *R. sphenoccephala* was negatively affected by the presence of the endangered *R. sevosa*, especially in open canopy ponds. Competition from *R. sphenoccephala* has been found to have no detectable effects on a close relative of *R. sevosa*, the northern crawfish frog, *R. areolata* (Parris and Semlitsch 1998). Prior to 1991 *R. sevosa* was included as a subspecies of *R. areolata* (Young and Crother 2001) and so we were not surprised to find similar results in our studies. Asymmetric competition with another ranid species, *R. blairi* however, may have contributed to the decline of *R. areolata* (Parris and Semlitch 1998).

4. Are there identifiable features of the ecology of *R. sevosia* that make it particularly vulnerable to extinction?

A number of ecological traits appear to increase the vulnerability of *R. sevosia* to extinction. The primary traits of extinction-prone species are endemism, large body size, long life spans, low fecundity, less adaptable behavior patterns, high trophic level, specialized habitat needs, restricted range and poor dispersal (McKinney 1997, Lips et al. 2002). In this dissertation, research primarily examined the habitat needs and behavior patterns of *R. sevosia*.

It is clear that *R. sevosia* has specialized habitat needs during both the aquatic and terrestrial portions of the life cycle. As larvae, *R. sevosia* requires open canopy ponds with abundant emergent vegetation (Chapters 5 and 6) and ponds must be fish free to protect larvae from a potentially lethal predator (Chapter 3). The behavior of *R. sevosia* suggests it lacks the ability to respond appropriately to the threat of predation by fish. A preference for open canopy, fish free ponds is not a unique feature of *R. sevosia*. Other amphibian species have similar preferences (see for example Skelly et al. 2002, Relyea 2004) but may cope better when these conditions are not available.

The ecological requirements of *R. sevosia* in the terrestrial habitat are also very specialized. There was an association with the grassy open understorey typical of a well burned longleaf pine savanna (Chapter 4). Loss of approximately 97% of the preferred terrestrial habitat and fire suppression in remaining remnants has likely contributed to the decline of the dusky gopher frog (Smith et al. 2000). There was also an association with burrow abundance for *R. sevosia* in the terrestrial habitat (Chapter 4). Experimental results suggest that this need for burrows may be an innate part of the behavior of *R. sevosia* (Chapter 2). In a habitat where burrow making organisms such as gopher tortoises are declining and fire, which can also create suitable shelter,

is being suppressed, the reliance on external factors to fulfill such an important habitat requirement is likely to be contributing to the decline of *R. sevosa*. Other factors such as long larval period, low and stochastic levels of natural recruitment and poor dispersal ability in a fragmented habitat presumably increase the likelihood of extinction of *R. sevosa* in synergy with specialized habitat requirements.

5. Why is *R. sevosa* declining while *R. sphenoccephala* remains common in the same habitat?

As discussed above the specialized habitat and less adaptable behavior of *R. sevosa* are a likely reason for its decline. *R. sphenoccephala* by contrast does not seem to be as specialized in its requirements. The ability of *R. sphenoccephala* to survive as tadpoles and to metamorphosis in closed canopy habitats (Chapters 5 and 6) is a key difference between it and *R. sevosa*. While canopy closure negatively affects performance of its larvae, the ability of *R. sphenoccephala* to eke out an existence, even under adverse conditions, likely contributes to its persistence. *R. sphenoccephala* also displayed behavioral plasticity in response to predatory fish. This ability to respond to change would also contribute to its persistence in habitats where fish were introduced.

6. Can my research provide information that is useful to managers of *R. sevosa* and other declining species?

The results of my research have provided managers of *R. sevosa* with important information that can be directly applied to the management of the species in Mississippi. Restoration of new populations and maintenance of existing populations will require an emphasis on appropriate fire as a management tool, as fire is the key component to *R. sevosa* ecology. Fire maintains open canopy ponds with abundant emergent vegetation; it creates burrows and preserves the open grassy understory in terrestrial habitats. In addition fire is essential to burrow

creating organisms such as gopher tortoises. Managers must also ensure that new and existing larval habitats remain fish free and that no other novel predators encroach on the habitat of *R. sevosia*.

The results of my research may also apply to management of other declining species. Understanding why a species may be prone to extinction provides a proactive basis for conservation instead of current approaches of inconsistent reaction (McKinney 1997). If we can identify common traits that make a species likely to become extinct we can identify those species that need protection before serious declines occur. Where species have already declined the results of this research may help highlight some processes that may be contributing to declines and suggest potential ways to identify them.

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APPENDIX 1. IACUC permit

University of New Orleans

Institutional Animal Care and Use Committee (IACUC)

DATE: April 26, 2001
TO: Joseph H.K. Pechmann, Ph.D.
FROM: Gerald J. LaHeste, Ph.D. *G. LaHeste*
Chairman
RE: *IACUC Protocol No. 027*
Entitled: Complex Life Cycles and Amphibian Conservation

Your revised application for the use of animals in research (referenced above) has been received and found to be acceptable. The approval date is May 2, 2001 and the expiration date is May 2, 2004. Annual renewal forms (which do not require re-submission or re-review) will be sent to you at yearly intervals until the expiration date.

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

VITA

Nicole Thurgate was born in Sydney, New South Wales, Australia and received her BSc (hons) from James Cook University, Townsville, Queensland.