AN ABSTRACT OF THE THESIS OF

Joseph M. Kiesecker for the degree of Doctor of Philosophy in Zoology presented on May 29, 1997.

Title: The Effects of Pathogens, UV-B Radiation, and Introduced Species on Amphibians in the Pacific Northwest

Redacted for Privacy

Abstract approved: _____

Andrew R. Blaustein

I examined two amphibian communities to assess factors that may impact amphibian biodiversity. The results suggest that the potential factors which influence the maintenance of amphibian biodiversity are multi-faceted and thus, attempts to understand these factors must reflect these complexities.

I investigated factors that influenced the susceptibility of western toad (*Bufo boreas*), Cascades frog (*Rana cascadae*), and Pacific treefrog (*Hyla regilla*) embryos to infection with the fungal pathogen *Saprolegnia ferax*. I found that there were considerable interspecific differences in susceptibility of anuran embryos to infection with *Saprolegnia*. Interspecific differences can be attributed to differences in egg-laying behavior and sensitivity to ambient levels of ultraviolet radiation.

I studied the effect of *Saprolegnia* on competitive interactions between larval *R. cascadae* and *H. regilla*. The presence of *Saprolegnia* differentially affected larval

recruitment of the two species and mediated competitive interactions. These results suggest that pathogens may have strong effects on species interactions and thus, when present may have strong influences on community composition.

I examined population differences in response of native red-legged frogs (*R. aurora)* to introduced bullfrogs (*R. catesbeiana*). Syntopic *R. aurora* tadpoles reduced their activity and increased their refuge-use when presented with the chemical cues of *R. catesbeiana*, whereas allotopic *R. aurora* did not. Predation by *R. catesbeiana* was lower for syntopic *R. aurora* compared with animals from allotopic populations. Individuals that are unfamiliar with novel, introduced organisms may not possess adaptations that would prevent a negative encounter. In field experiments I demonstrated that introduced *R. catesbeiana*, and smallmouth bass *Micropterus dolomieui*, influenced the microhabitat use, growth, and survival of larval and metamorphic *R. aurora*. These results illustrate the potential complexities of interactions between native and exotic species. These results also stress the importance of understanding the mechanisms of interactions between native and exotic species to allow for the persistence of native biodiversity.

The Effects of Pathogens, UV-B Radiation, and Introduced Species on Amphibians in the Pacific Northwest

by

Joseph M. Kiesecker

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Doctor of Philosophy

Presented May 29 1997

Commencement June 1998

Doctor of Philosophy thesis of Joseph M. Kiesecker presented on May 29, 1997.

APPRO Redacted for Privacy

Major Professor, representing Zoology

Redacted for Privacy

Chair of Department of Zoology

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Joseph M. Kiesecker, Author

ACKNOWLEDGEMENTS

The work in this thesis would clearly not have been possible without the support and help of numerous people. I first wish to acknowledge the help and guidance of my graduate committee: Andrew Blaustein, Bruce Menge, Joseph Beatty, Dede Olson, Gordon Reeves, and Douglas Derryberry. During my time at Oregon State University they have provided me with a great deal of guidance and constructive critism.

Much of the credit should be given to my "impresentable" major advisor Andy Blaustein. Andy has contributed greatly to my development as a scientist. Andy sees the world in a manner different from that of the average person, and it is because of this that I attribute his success as a scientist and a major advisor. Andy has conferred more than just his scientific advice, he has given his friendship (and provided material for numerous stories) and for that I am indebted to him, and glad to have been a part of the Blaustein lab.

Bruce and Gordon have provided a rigorous background in experimental design and have helped in many ways to focus my ideas. Dede has helped develop many of my research ideas as well as providing funding when needed. Joe has taught me much about the natural history of amphibians and has provided the opportunity for numerous field excursions.

During my time at OSU I have had the unique opportunity of overlapping with a variety of graduate students, postdocs and visiting professors. The "early group" of Grant Hokit,

Susan Walls and Brian Edmond added much to my work at OSU. Both Grant and Susan were a constant sounding board for ideas, scientific or otherwise. They both greatly influenced my research and their presence improved the quality of time I spent in Oregon. The "later group" of Erica Wildy, Mike Anderson, Doug Chivers, Don Juan Adolfo Marco, Jill DeVito, Lisa Belden and Joseph Riesecrer also contributed much to my time at OSU. Doug Chivers has read several of my manuscripts and collaborated on a number of projects. Erica has tirelessly listened to many discussions about my work and has been a excellent addition to the lab.

Thanks, also to my family who have provided a great deal of support throughout my life and particularly during my time in graduate school.

Thanks to my wife Cheri Kiesecker whose support was paramount, and who without, this thesis would not have been completed. Cheri was both a scientific collaborator and the main driving force in my life, clearly this thesis is as much hers as it is mine.

TABLE OF CONTENTS

(Table of Contents continued)

LIST OF FIGURES

(List of Figures continued)

LIST OF TABLES

(List of Tables continued)

The Effects of Pathogens, UV-B Radiation, and Introduced Species on Amphibians in the Pacific Northwest

CHAPTER 1

GENERAL INTRODUCTION

Global biodiversity loss is currently of international concern. A recent report by a U.S. National Science Foundation task force on the "biodiversity crisis" suggests that one quarter to one half of the earth's species may become extinct in the next 30 years (National Science Board 1989). Continued loss of biodiversity can have long-term ecological, social, and economic consequences (Soule 1986, Ehrlich and Ehrlich 1981, Wilson 1988). Conservation programs that attempt to understand the factors involved in diversity loss typically concentrate on the direct effects of single factors. This is despite the fact that ecological experiments and theory demonstrate that species interact in complex ways with both abiotic and biotic factors (e.g. Abrams 1992, Schoener 1993, Woodward and Kiesecker 1994, Menge 1995, Kiesecker 1996, Werner and Anholt 1996). Therefore, to understand and predict the impact of environmental degradation (e.g. habitat alteration, ozone depletion, global warming), ecologists must consider the interaction of multiple causal factors. While habitat alteration is responsible for much of the diversity loss, it likely is accompanied by complex changes, such as the spread of exotic species and increased prevalence of disease, which

could also impact biodiversity (Soule 1990, Orth et al. 1990, Hobbs 1991, Almendares 1993, Lodge 1993a, Loevinsohn 1994, Stone 1995, Moyle and Light 1996). However, the impact of these changes on biodiversity are poorly understood.

Both pathogens and introduced species have been implicated as potentially important, but poorly understood factors in the loss of native biodiversity (e.g. Soule 1986, Mooney and Drake 1986, Dobson and May 1986, Lodge 1993a, Lodge 1993b). For example, numerous case studies have documented the decline of native species after the arrival of an exotic (see reviews in Elton 1958, Bennett 1990, Lodge 1993b, Krebs 1994). However, the mechanisms that enable exotic species to thrive at the expense of native species are often unclear (Lodge 1993b). Competition or predation is frequently proposed to explain losses of native species after the arrival of an exotic. Rarely are such mechanisms isolated and tested in an experimental setting (but see Petren et al. 1993). Nevertheless, understanding these mechanisms will provide insights into factors involved in determining community structure and may be used to implement management programs (Vitousek 1989; Vitousek 1990; Lodge 1993b, Wormington & Leach 1992; Holland 1993; Nicholis & Hopkins 1993, Zaret & Paine 1973; Groves & Burdon 1986; Savidge 1988; Haag et al. 1993; Petren et al. 1993; Kupferburg 1995; Petren & Case 1996).

Few quantitative data are available on how pathogens influence host abundance in nature (see Hudson 1986, Washburn et al. 1991, Hudson et al. 1992, Fuller and Blaustein 1996). Although numerous studies suggest that pathogens likely play important roles in determining species performance and the outcome of species interactions (e.g. Park 1948, Anderson and May 1979, May and Anderson 1979, Scott and Anderson 1984, Price et al. 1986, Scott 1987, Price et al. 1988). Few experimental studies examine how pathogens may affect species interactions and influence community dynamics (Price et al. 1986). To date, ecological studies at the community level are dominated by studies that examine the role of competition, predation, and disturbance (e.g. Wilbur 1972, Menge and Sutherland 1976, Connell 1983, Morin 1983, Wellborn et al. 1996). Currently, the role that pathogens play in influencing community composition is poorly understood.

Attempts to understand the impact of introduced species and the role of pathogens in communities has often been hampered by the inability of researchers to perform experimental manipulations. It is typically difficult to isolate and control introduced and pathogenic species under field conditions. In this thesis I employed experimental manipulations to understand the interrelationships between introduced species, disease and ultraviolet radiation and the impact that these factors may have on native amphibians.

Anuran amphibians provide a model system often used in studies of vertebrate community ecology (e.g. Wilbur 1972, Morin 1983, Werner and Anholt 1996). There are several advantages of using anurans for studies involving pathogens and introduced species. Amphibian embryos and larvae are contained in an easily definable environment (pond or lake) thus species composition and environmental factors can be readily manipulated. Also, embryos and larvae of many species can be reared under both laboratory and field conditions without difficulty. Many pond breeding anurans lay definable egg masses that can be easily divided for use in experiments. Consequently, it is relatively easy to minimize variation arising from differences in genetic origin. Because amphibians can be assigned to discrete aquatic environments, it is possible to manipulate the presence of pathogens and introduced species to assess effects on survival, growth, and development. Larval anurans also display a series of definable anti-predator behaviors that allow quantification of their responses to introduced species.

Under some situations amphibians may be good indicators of environmental change (Wake 1991, Blaustein et al. 1994a, Blaustein 1994). Embryos of some species are laid where they are exposed to both physical and chemical changes of the environment. The jelly matrix of embryos and the skin of both larvae and adult stages are often highly permeable and

may make them extremely sensitive to changes in environmental conditions. Also, because of the complex lifecycles of many amphibian species, they may be subjected to changes in both aquatic and terrestrial environments.

Amphibians are key components of many ecosystems, both on small (Dickman 1973, Seale 1982) and large scales (Hairston 1987, Petranka et al. 1993, Stewart 1995). For example, in mesic forests of North America, salamanders may be the most abundant group of vertebrates in both numbers and biomass (Burton and Likens 1975a, 1975b). The various lifestages of amphibians serve as both predator and prey to numerous organisms (e.g. Duellman and Treub 1986, Stewart 1995). Larval amphibians can also play important roles in the regulation of nutrient flow and primary productivity in aquatic systems (e.g. Dickman 1973, Seale 1982). Even though they are important to many systems, amphibians have often been neglected in ecosystem management studies (Pough et al. 1987, Petranka et al. 1993). Historically, amphibians have not received the conservation concern given to groups such as birds or large mammals. This may be due, in part, to less popular interest in amphibians, but also to poor understanding of population processes and trends for most amphibian species (Blaustein et al. 1994a). Furthermore, amphibians are of particular interest because of the

numerous reports suggesting that many species are undergoing population declines and range reductions (see Blaustein 1994).

AMPHIBIAN DECLINES

Amphibians in many regions of the world have recently experienced population declines and/or reductions in range. Many of the losses are clearly related to environmental factors such as habitat loss and degradation (Blaustein and Wake 1990, Vitt et al. 1990, Wyman 1990, Wake 1991, Blaustein 1994, Blaustein et al. 1994a, Corn 1994). Losses of amphibians from areas lacking overt habitat degradation are more difficult to understand (e.g. Corn et al. 1989, La Marca and Reinthaler 1991, Crump et al. 1992, Carey 1993, Fellers and Drost 1996). Species that occur in these habitats often have disappeared over a portion of their historical range and their ability to recover after declines is a matter of concern (Blaustein et al. 1994a).

Losses have been reported from virtually every continent where amphibians occur. For example, in Australia, many amphibian species have experienced dramatic declines and even apparent extinctions (e.g. Tyler 1991, Richards et al. 1993). The most alarming of these Australian declines has occurred among endemic frogs confined to montane rain forests of eastern Australia (Czechura and Ingram 1990, McDonald 1990, Ingram and McDonald 1993, Richards et al.

1993, Laurance et al. 1996, Laurance In Press). Since the 1970's, fourteen frog species have declined, including the two species of gastric brooding frogs, *Rheobatrachus spp.*, which are believed to be extinct (Richards et al. 1993). In the Monteverde Cloud Forest of Costa Rica, intensive surveys have recorded the decline of the harlequin frog, *Atelopus varius* and the apparent extinction of the golden toad, *Bufo periglenes* (Crump et al. 1992, Pounds and Crump 1994). Continued monitoring of this Costa Rican site indicated that of the fifty species which had previously inhabited the area, twenty species of frogs from six separate families appear to have disappeared (Pounds et al. In Press). During the same time period La Maraca and Reinthaler (1991) documented the decline of five species within the genus *Atelopus* from the Venezuelan Andes.

In western North America numerous species of amphibians have experienced serious losses. Dramatic losses have occurred for the western toad, *Bufo boreas*, in the Rocky Mountains (Corn et al. 1989, Carey 1993, Corn 1994). In Colorado, this species is found at only 17% of its former sites (Corn et al. 1989, Corn 1994). The spotted frog, *Rana pretiosa*, has virtually disappeared from all of its sites west of the crest of the Cascade Mountains in both Washington and Oregon (McAllister and Leonard 1990, McAllister et al. 1993). The red-legged frog, *Rana aurora,* a once abundant species (Stebbins 1985, Nussbaum et al. 1983),

has disappeared from much of its former range in California and the subspecies (*R.a.draytonii*) found there is now listed as federally endangered (Hayes and Jennings 1986, Fisher and Schaffer 1996). Initial surveys of 42 historical sites in Oregon indicate that *R. aurora* is gone from 69% of those sites (Kiesecker et al. In Prep.). Populations of the mountain yellow-legged frog, *Rana muscosa* have disappeared from over 75% of historical sites in the Sierra Nevada Range of California (Bradford et al. 1994). Declines of the northern leopard frog, *Rana pipiens*, have been reported across the species range in the western U.S. (Corn and Fogleman 1984, Hayes and Jennings 1986, Clarkson and Rorabaugh 1989, Corn 1994, Fisher and Schaffer 1996). However, of the 79 species of amphibians in the western United States (Stebbins 1985) only four species (desert slender salamander, *Batrachoseps aridus*; Santa Cruz long-toed salamander, *Ambystoma macrodactylum croceum*; Wyoming toad, *Bufo hemiophrys baxteri*; and California red-legged frog, *Rana aurora draytonii*) are listed as either threatened or endangered by the U.S. Fish and Wildlife Service. Although, information on range reductions has, in many cases, resulted in listing species on regional threatened lists. The Pacific Northwest, specifically Oregon, is a particular concern because of the 33 species of amphibians (Leonard et al. 1993) that occur there, 54% are listed as sensitive (Walls et al. 1992, Blaustein et al. 1994a)

The reports of amphibian declines have been met with some skepticism. Because populations fluctuate naturally, an apparent decline could be an artifact of short-term observations. Pechmann et al. (1991) point to large fluctuations in the abundance of several species at a breeding pond in lowland South Carolina over 12 years. A species was sometimes rare or absent one year and then relatively abundant the next. They suggested that natural and random processes may play an important role in the dynamics of amphibian populations (Pechmann et al 1991, Pechmann and Wilbur 1994, Semlitsch et al. 1996), and these may result in false interpretations of population declines. However, when a common species disappears over a wide area, for example *Bufo periglenes* in Costa Rica (Crump et al. 1992, Pounds and Crump 1994), *Bufo boreas* in the Southern Rocky Mountains (Corn et al. 1989, Carey 1993), and *Taudactylus spp.* in northern Queensland, Australia (McDonald 1990, Czechura and Ingram 1990, Richards et al. 1993), natural and random events are unlikely explanations and anthropogenic causes must be investigated. Moreover, if amphibian populations naturally undergo large scale fluctuations (Pechmann et al. 1991), then this stocastic variation may be exacerbated by human induced changes (Olson 1992, Blaustein et al. 1994a).

There has also been considerable confusion regarding the phenomenon referred to as "amphibian declines". Much of the debate has centered on how we can determine if population declines exceed natural population fluctuations (Pechmann et al. 1991, Pechmann and Wilbur 1994, Blaustein 1994, Blaustein et al. 1994a). This is despite the fact that the most convincing evidence regarding "amphibian declines" comes from the loss of species across wide geographic areas and not the study of single populations (e.g. Richards et al. 1993, Drost and Fellers 1996, Pounds et al. In Press). It is important to establish whether these declines are affected by human-induced disturbances, or whether they represent natural population fluctuations. Documentation of an actual decline in a single species will involve monitoring several populations for more than one generation (Pechmann and Wilbur 1994). Thorough censuses of long lived species could take several decades. If populations are declining, census data would most likely be too late for conservation measures to benefit many species. Thus, it is critical to experimentally identify human-influenced disturbances that could exacerbate or cause these declines.

While the issue of amphibian declines still remains controversial, several recent studies have documented the real and serious nature of amphibian losses (e.g. Richards et al. 1993, Drost and Fellers 1996, Fisher and Shaffer 1996, Laurance et al. 1996, Semlitsch et al. 1996, Pounds et

al. In Press). In fact, some reports suggest that within a given geographic area, losses of amphibians may exceed losses experienced by other vertebrate groups (Pounds et al. In Press).

Undoubtedly, habitat alteration and destruction is the main contributor to the loss of amphibian biodiversity (Blaustein et al. 1994a); however, for many of the declines, causes remain unknown. Hypothesized causes for the losses have included acidification, global warming, ozone depletion, introduced exotic species, drought, increased disease prevalence, and natural fluctuations (e.g. Corn et al. 1989, Wake 1990, Wyman 1990, Blaustein and Wake 1991, Crump et al. 1992, Crawshaw 1992, Carey 1993, Blaustein 1994, Pechmann and Wilbur 1994, Blaustein et al. 1994b, Pounds and Crump 1994, Beebee 1995, Kiesecker 1996). While there has been no shortage of hypotheses for the losses, few tests, especially experimental tests of hypotheses have been conducted.

Although, for most of the declines, specific causes have not been identified, some tentative patterns have emerged: 1) reported declines have been mainly for anuran (frogs) species (Blaustein et al. 1994a), with most of the reports concerning true toads of the family Bufonidae and true frogs of the genus *Rana* (Wake 1991, Blaustein and Wake 1991, Blaustein et al. 1994a, Drost and Fellers 1996); 2) certain geographic areas appear to be more affected than others

(Blaustein and Wake 1990, Wake 1991, Richards et al. 1993, Pounds et al. In Press). For example, the overwhelming majority of reported declines in North America come from western regions (e.g. Wake 1991, Blaustein 1994, Blaustein et al. 1994a, Corn 1994, Fisher and Schaffer 1996, Drost and Fellers 1996). 3) Declines appear to have occurred recently, with many of the well-documented declines occurring within the last 20 years (Beiswenger 1986, Bradford 1989, Crump et al. 1992, Richards et al. 1993, Fellers and Drost 1993, Kagarise Sherman and Morton 1993, Pounds and Crump 1994, Fisher and Schaffer 1996, Drost and Fellers 1996, Pounds et al. In Press); 4) In areas where declines have been reported, not all species of amphibians have been affected (Richards et al. 1993, Fisher and Schaffer 1996, Pounds et al. In Press). For example, in the Pacific Northwest region of North America several species (e.g. *R. cascadae*, *R. aurora*, *B. boreas*) have experienced losses while populations of other sympatric species (e.g. *Hyla regilla*) appear robust (Blaustein and Wake 1990, Blaustein and Olson 1991, Olson 1992, Blaustein 1994, Blaustein et al. 1994a, 1994b). While the interspecific differences are puzzling, they provide a unique opportunity to assess the causes of the declines and may provide insights into what ecological factors influence these amphibian assemblages.

THESIS ORGANIZATION

The work conducted in this thesis took place in two distinctly different amphibian communities. In the Oregon Cascades I focused on interactions between pathogenic fungus, *Saprolegnia ferax*, and *B. boreas*, *R. cascadae* and *H. regilla*. My work in the Willamette Valley of Oregon focused on interactions between introduced bullfrogs, *R. catesbeiana* and native *R. aurora*. The research in this thesis is conceptually divided into five parts, each with its own rationale. While in some cases the results obtained in one part had profound implications on the questions addressed in other sections, other sections are independent from one another. However, all chapters are linked by the common theme of attempting to understand the factors contributing to amphibian losses in the Pacific Northwest.

Oregon Cascades Study System

In the Oregon Cascade Range increased embryo mortality has been observed for some species (Blaustein and Olson 1991, Blaustein et al. 1994b, 1994c). Amphibian embryo mortality is often associated with the presence of *S. ferax* (Blaustein and Olson 1991, Blaustein et al. 1994c, Kiesecker and Blaustein 1997). Amphibian embryo mortality is also associated with exposure to ambient levels of ultraviolet-B radiation (Blaustein et al. 1994b, Blaustein et al. 1995, Kiesecker and Blaustein 1995). While it is apparent that

certain species experience high mortality (e.g. *Rana cascadae*, *Bufo boreas*), other species (e.g. *Hyla regilla*) appear unaffected (Kiesecker and Blaustein 1997).

The work presented in chapters 2 through 4 include a series of comparative studies involving field observations and experiments to understand the impacts of *Saprolegnia* on amphibian communities in the Oregon Cascade Range. I attempted to stress two major themes: 1) understanding the role that *Saprolegnia* may play in amphibian losses in the Pacific Northwest and 2) understanding how pathogens, in a general sense, may influence amphibian communities. The fact that *Saprolegnia* may be introduced during fish stocking is also considered in each chapter.

In chapter 2 (Kiesecker and Blaustein 1997) I examined the prevalence of *S. ferax* on amphibian embryos at natural oviposition sites. First, I observed embryo mortality over four years, at a variety of sites, and quantified interspecific differences in susceptibility to infection with *Saprolegnia*. Then, through a series of field experiments, I examined how differences in egg laying behavior contributed to susceptibility to infection with *Saprolegnia*.

In chapter 3 (Kiesecker and Blaustein 1995), I examined the synergistic effects of exposure to *S. ferax* and ambient levels of UV-B radiation. Recent reports have shown that amphibians have differential sensitivity to ambient levels

of ultraviolet-B radiation (UV-B 290-320 nm), which in some species causes embryonic mortality in the field (Blaustein et al. 1994b, Blaustein et al. 1995, Blaustein et al. 1996, Blaustein and Kiesecker In Press). In Oregon, amphibian embryo mortality is also associated with the presence of *Saprolegnia ferax* (Blaustein and Olson 1991, Blaustein et al. 1994c). Because stresses may make an individual (or population) more susceptible to disease (e.g. Munck et al. 1984, Bateman et al. 1989, Carey 1993), it is possible that amphibians stressed by UV-B (or other factors) may be more susceptible to infection with *Saprolegnia*.

In chapter 4 I build on results from the previous two chapters to explore how *Saprolegnia* infection can alter the outcome of competitive interactions. The presence of pathogens that effect species differentially would likely result in alterations of species interactions. In chapter 4, I examine how the outcome of competitive interactions could be altered, if a superior competitor is also more susceptible to disease.

Willamette Valley Study System

Interactions with introduced bullfrogs are continually invoked as a primary cause for losses of ranid frogs native to the western United States (e.g. Moyle 1973; Bury & Luckenbach 1976; Bury et al. 1980; Nussbaum et al. 1983; Hayes & Jennings 1986; Blaustein 1994). Several studies have

documented the decline of native ranid frogs after the introduction of bullfrogs (Moyle 1973; Bury & Luckenbach 1976; Green 1978; Hammerson 1982; Clarkson & DeVos 1986; Fisher & Shaffer 1996). However, these studies only suggest a negative association between bullfrogs and other frog species. Few studies have attempted to experimentally examine the mechanism in which introduced bullfrogs may impact ranid frogs (but see Kupferberg 1995). The specific impacts of bullfrogs on native frog populations is often unclear because at many sites their introductions have occurred simultaneously with the introduction of predatory fish. Here I experimentally examined the combined effects of introduced bullfrogs (*R. catesbeiana*) and smallmouth bass (*Micropterus dolomieui*) on native red-legged frogs (*R. aurora*).

In chapters 5 and 6 I examined how the presence of introduced species cause changes in the behavior, life history and habitat use of native red-legged frogs. In both chapters I stress the importance of understanding the mechanism by which introduced species impact natives.

Chapter five (Kiesecker and Blaustein In Press) compared population differences in behavioral responses of red-legged frogs to introduced bullfrogs. I then conducted laboratory and field experiments to assess how naive and experienced larvae differ in their susceptibility to introduced predators.

In chapter 6 I assessed the combined effects of introduced bullfrogs and introduced predatory fish on the habitat use, growth, development and survival of native red-legged frogs. Finally, in chapter 7, I summarize results from chapters 2 through 6 and make recommendations for future research involving amphibian declines.

CHAPTER 2

INFLUENCES OF EGG LAYING BEHAVIOR ON PATHOGENIC INFECTION OF AMPHIBIAN EGGS.

JOSEPH M. KIESECKER

AND

ANDREW R. BLAUSTEIN

PUBLISHED IN CONSERVATION BIOLOGY **11**:214-220, 1997.

ABSTRACT

Mass mortality of developing amphibian eggs and larvae from pathogenic infection has been recently documented in some amphibian populations. For example, the pathogenic fungus, *Saprolegnia ferax*, has been linked with amphibian embryo mortality in the Pacific Northwest. Continued mortality in early life history stages may ultimately contribute to a population decline. We document the prevalence of *S. ferax* on embryos of three anuran species (*Bufo boreas*, *Rana cascadae*, and *Hyla regilla*) common to the Pacific Northwest. These species differ in key aspects of their behavior and ecology, and these differences may lead to differential susceptibility to *S. ferax*. *Rana cascadae* often lays its eggs communally and *B. boreas* usually deposits its eggs communally. We observed embryos at natural oviposition sites. Eggs laid communally had higher mortality than those laid away from other egg masses. Field experiments that manipulated both the spatial position and timing of egg laying demonstrated that eggs laid later and in closer proximity to communal masses had higher mortality. Our results suggest that eggs in communal masses are highly susceptible to infection with *S. ferax*.

INTRODUCTION

Pathogens and parasites, though often overlooked, are among the most important aspects of conservation biology. Many factors may influence how pathogens spread in natural populations. Global or local environmental changes may stress organisms, making them more prone to disease (e.g. Snyder 1976; Kripke 1984; Munck et al. 1984; Bateman et al. 1989; Orth et al. 1990; Kripke et al. 1992; Carey 1993; Tevini 1993; Blaustein et al. 1994a). The distribution and density of a population may also influence its susceptibility to disease (Dobson & May 1986). An increase in the size of aggregations or of the density of a population can increase the chance of disease transmission (Freeland 1976; Anderson & May 1979; Hoogland 1979; Plowright 1982; Brown & Brown 1986; Dobson & May 1986; Rubenstein & Hohmann 1989).

In amphibians, there is great potential for pathogens to cause population reductions (Smith et al. 1986; Hunter et al. 1989; Worthylake & Hovingh 1989; Bradford 1991; Aho 1990; Gruia-Gray & Desser 1992; Crawshaw 1992). Several reports have suggested that diseases may play a role in the decline of some amphibian populations (Beebee 1977; Hunter et al. 1989; Blaustein & Wake 1990; Bradford 1991; Wake 1991; Crawshaw 1992; Carey 1993; Richards et al. 1993; Blaustein et al. 1994a; Laurance et al. 1996). Pathogens, however, have been largely neglected and not carefully

documented with regard to amphibian population declines (Blaustein et al. 1994a). Numerous amphibian species possess behavioral or life history traits that may facilitate the spread of disease. Many species have large breeding leks, form large communal egg masses, or their larvae can be found in high densities (Duellman & Treub 1986; Stebbins & Cohen 1995), all conditions that can facilitate the transmission of disease.

In some regions, declines of certain amphibian populations have been puzzling because 1) they seem unrelated to habitat destruction, 2) they are apparently not the result of natural population fluctuations, and 3) populations of sympatric species seem to be robust (Blaustein et al. 1994b). For example, in the western United States, western toads (*Bufo boreas*) and Cascades frogs (*Rana cascadae*) have undergone significant population declines (Corn et al. 1989; Federal Register 1991; Carey 1993; Fellers & Drost 1993) whereas population declines of sympatric Pacific treefrogs (*Hyla regilla*) have not been reported.

Several factors have recently been documented that are responsible for egg mortality of amphibians and could potentially contribute to a population's decline. Amphibians have differential sensitivity to ambient levels of ultraviolet-B radiation (UV-B 290-320 nm) (Blaustein et al. 1994c; Long et al. 1995), which in some species causes

embryonic mortality in the field (Blaustein et al.1994c). In Oregon, amphibian embryo mortality is also associated with the presence of the pathogenic fungus *Saprolegnia ferax* (Blaustein et al. 1994c). Although either UV-B radiation or *Saprolegnia* alone may contribute to embryonic mortality, field experiments have shown that there is a synergistic effect between these two factors that enhances mortality (Kiesecker & Blaustein 1995). Pathogens such as *S. ferax* may be present in nature at low densities, but their effects may be enhanced when potential hosts are weakened by stress that can be caused by such agents as UV-B radiation or when hosts occur at high densities.

In this paper, we compare the prevalence of *S. ferax* on embryos of *B. boreas*, *R. cascadae*, and *H. regilla* in natural populations. In some vertebrates, parasite load or mortality due to disease may increase with breeding group size (Hoogland 1979; Brown & Brown 1986; Rubenstein & Hohmann 1989). Therefore, we hypothesized that eggs deposited communally would have higher infection rates than eggs laid further away from communal egg masses. Furthermore, we hypothesized that eggs laid late in the breeding season would have a greater chance of infection than those laid earlier due to the accumulation of fungal spores. To test these hypotheses, we conducted field experiments

examining 1) the rates of infection by *S. ferax* in relation to egg dispersion and 2) how the timing of egg laying influences infection with *S. ferax*.

METHODS AND MATERIALS

Observations

Since 1979 ARB and his students have been monitoring the breeding activity of *B. boreas*, *R. cascadae*, and *H. regilla* at several locations in the Oregon Cascade Range (e.g. O'Hara 1981; Olson et al. 1986; Olson 1988; Blaustein and Olson 1991; Blaustein et al. 1994a; Blaustein et al. 1994c). Since 1993, we have monitored in detail (both macroscopically and under a microscope) the development of eggs at several natural oviposition sites (Appendix A.) We estimated the total number of eggs laid by either counting the number of egg masses or by counting the number of breeding pairs. The mode of egg laying was classified at each breeding site as either communal or non-communal. Communal sites were considered to be those sites where $\geq$ 75% of the egg masses were laid in contact with one another. Non-communal sites had $\geq$ 75% of the egg masses laid separately.

The eggs of *B. boreas* are laid in gelatinous strands several meters in length (Nussbaum et al. 1983). For *B. boreas*, we estimated the total number of eggs laid by multiplying the number of breeding pairs by 12,000, the average number of eggs laid per female per breeding period (Blaustein 1988). The eggs of *R. cascadae* are deposited as a rounded mass, approximately 15 cm in diameter (Nussbaum et al. 1983) For *R. cascadae*, we estimated the number of eggs

laid by multiplying the number of egg masses by 500, the average number of eggs per clutch (Nussbaum et al. 1986; pers. obs.). Females of *H. regilla* can deposit several hundred eggs per season, but they fasten them to vegetation in packets that are approximately 35 mm in length and average about 25 eggs (Nussbaum et al.1983). For *H. regilla*, we estimated the number of eggs laid by multiplying the number of egg packets by 25. The infection of eggs with *S. ferax* is readily observable. Infected eggs become covered with a visible crown of white hyphal filaments, and they generally do not hatch (Smith et al. 1985; Blaustein et al. 1994b).

The percent mortality of eggs at each site was estimated by placing a $1m^2$ grid, containing squares with an area of 0.1^2m over egg masses. We counted the total number of dead and healthy eggs in each square. The percentage of egg mortality was averaged for each square to get an estimate for each grid. The grid was moved to 5 different areas of the egg masses for an estimate of percent mortality for that site.

Field Experiments

To assess the effects of distance from the communal egg mass and the timing of egg deposition on *B. boreas* and *R. cascadae* egg mortality, we conducted two field experiments from 13 March to 22 April 1994. Experiments were conducted

with these two species because they deposit eggs in communal masses thus making it possible to manipulate the spatial position of eggs. Experiments were conducted at natural oviposition sites of *B. boreas* (Lost Lake, see Appendix A) and *R. cascadae* (Parrish Lake, see Appendix A).

We used a factorial design with five spatial regimes and two temporal regimes. There were five replicates for each treatment, for a total of 50 enclosures per experiment. One hundred newly deposited eggs (< 24 hr old) from 5 clutches were placed in each enclosure, for a total of 500 eggs/enclosure. Plastic enclosures (27 cm x 16 cm x 11.5 cm) were covered with 1mm^2 fiberglass mesh screen that prevented eggs from moving in or out but allowed water flow and fungal transmission. The 50 enclosures were placed in five consecutive linear arrays, parallel to the communal egg mass. The first array was placed within the communal egg mass, the other arrays were placed at 1m, 2m, 3m, and 4m respectively from the communal mass.

Each array contained 10 enclosures; 5 enclosures that had eggs of early egg layers and 5 with eggs of late egg layers. Fresh eggs were collected from animals laying eggs during the formation of the communal eggs mass. These eggs were placed into 25 of the enclosures, five at each distance. Fresh eggs were again collected four days later, after the formation of the communal mass and were placed into the remaining 25 enclosures.

All enclosures were placed in approximately 25.5 cm of water. Temperatures were monitored daily at each enclosure. The experiment was terminated when all of the original embryos either hatched or died. Survival was measured as the proportion of hatchlings produced per enclosure. Data on the percentage surviving to hatching were analyzed using an ANOVA with the factors of distance from mass and time of ovipositing. For all experiments, parametric assumptions were met and no data transformations were necessary.

RESULTS

The percentage of mortality associated with the fungus varied between species and across sites (Figure 2.1a,b, & c). *Bufo boreas* laid eggs in communal masses at all sites and consistently had 50% or more of its eggs infected with *Saprolegnia* (Figure 2.1a). Egg mortality for *R. cascadae* ranged from 8% to 80% (Figure 2.1b). Eggs of *R. cascadae* in communal masses had at least 40 % mortality. At sites where eggs were laid non-communally, mortality was 15% or less. (Figure 2.1b). *Hyla regilla* never laid eggs in communal masses and egg mortality never exceeded 6% at any site (Figure 2.1c).

The two factors (proximity to communal mass and time of egg laying) interacted significantly, with the temporal effect being more pronounced in the regimes that were closer to the communal egg mass (Figure 2.2, Table 2.1). In general, the closer eggs were to the communal egg mass, the greater their infection with *S. ferax* (Figure 2.2, Table 2.1). Eggs laid late had significantly increased infections of *S. ferax* except when laid away from the communal mass. There were no significant temperature differences between treatments for either *B. boreas* ($F_{9,40}$ = 0.042, P = 0.947) or *R. cascadae* ($F_{9,40}$ = 0.077, P = 0.813).

Table 2.1 Analysis of variance (ANOVA) on percent survival for *Bufo boreas* and *Rana cascadae* eggs in field enclosures for different distances from the communal egg mass "space" and two temporal regimes "time:early or late".

Source of variation	df	ms	F	P
Bufo boreas				
Time	1	2290.29	161.643	<.0001
Space	4	8698.436	613.915	<.0001
Time x Space	4	84.643	5.974	.001
Error	40	14.169		
Rana cascadae				
Time	1	2410.957	516.929	<.0001
Space	4	8392.871	1799.501	<.0001
Time x Space	4	338.867	72.656	<.0001
Error	40	4.664		

Figure 2.1 Observations of egg mortality associated with *Saprolegnia* infection for *Bufo boreas* (a), *Rana cascadae* (b) and *Hyla regilla* (c) at various sites. Sites where *R. cascadae* had not laid in a communal mass are marked with an asterisk.

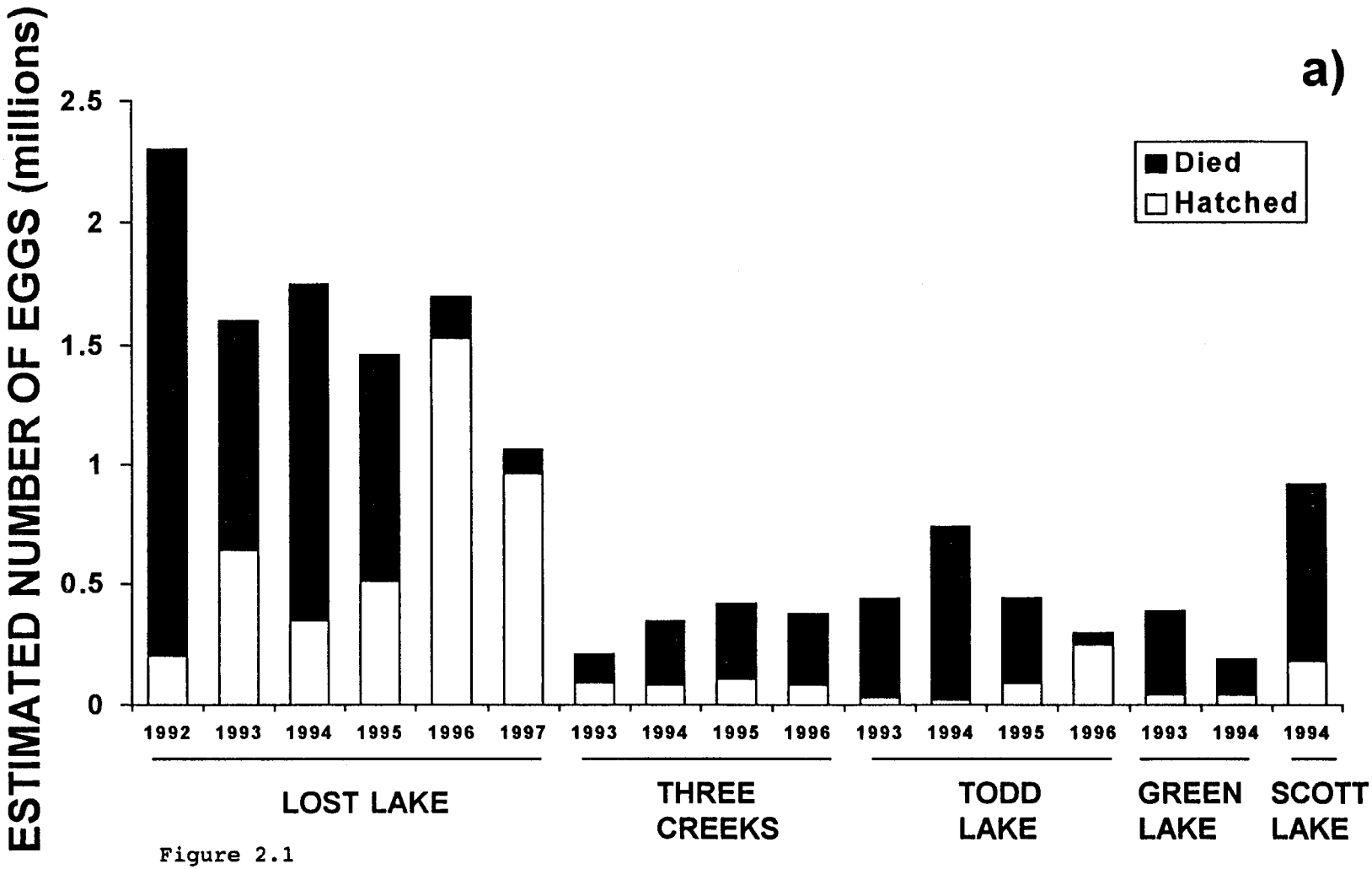

a)
ESTIMATED NUMBER OF EGGS (millions)
2.5
2
1.5
1
0.5
0
Died
Hatched
1992
1993
1994
1995
1996
1997
1993
1994
1995
1996
1993
1994
1995
1996
1993
1994
1994
LOST LAKE
THREE CREEKS
TODD LAKE
GREEN LAKE
SCOTT LAKE

Figure 2.1

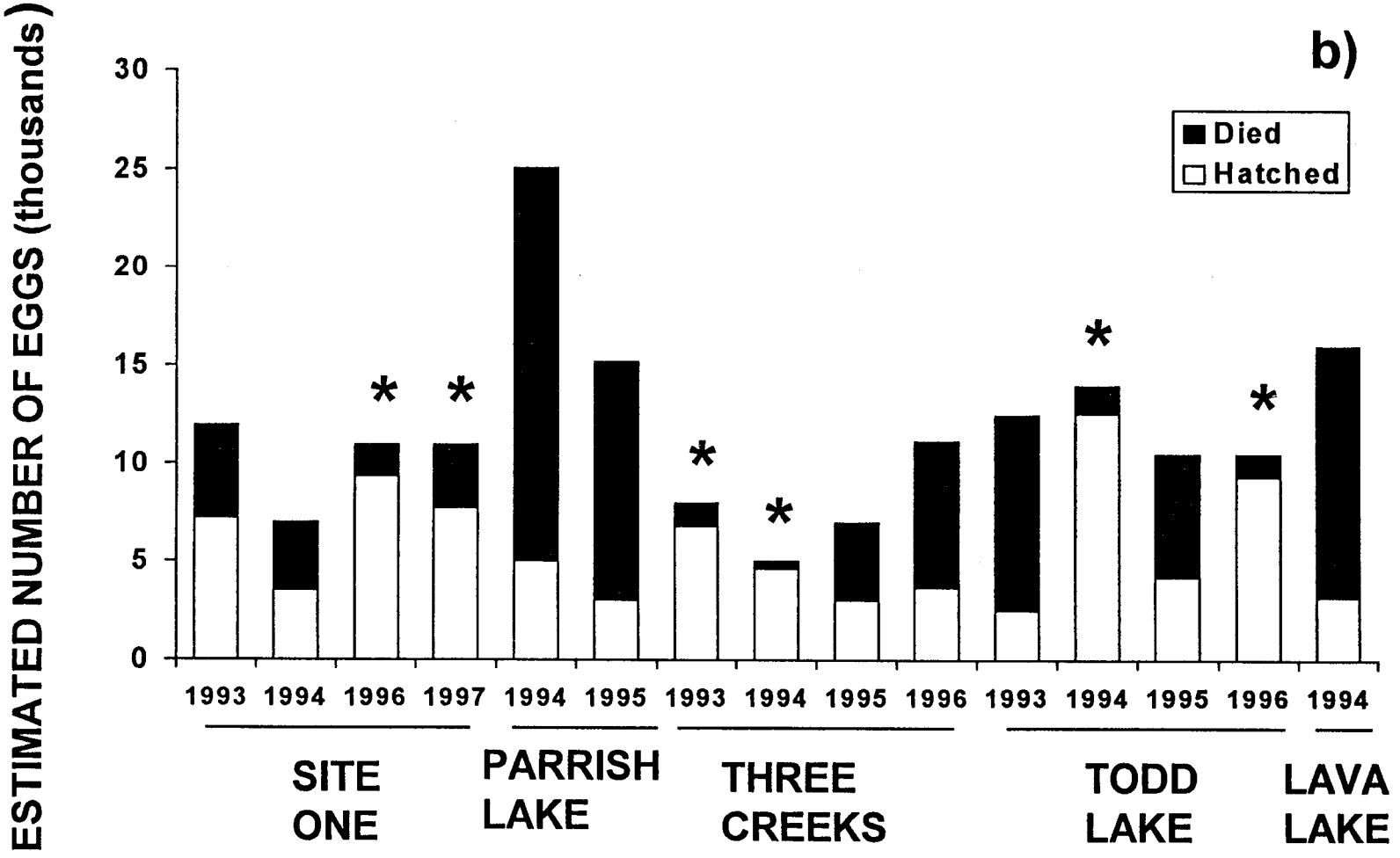


Figure 2.1 Continued

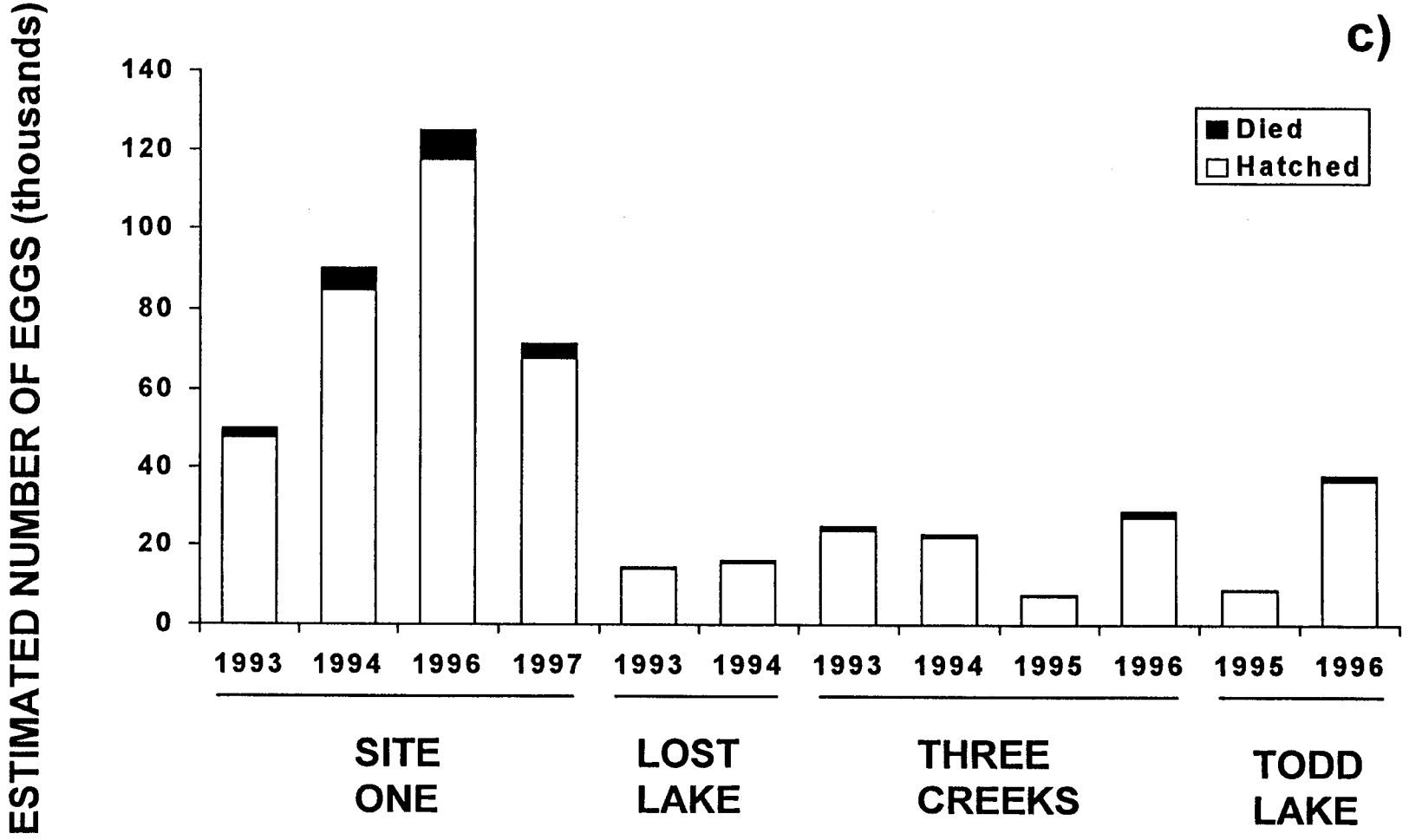


Figure 2.1 Continued

Figure 2.2 Effects of distance from the communal egg mass and time of egg deposition on hatching success (x +/- SE) for *Bufo boreas* (a) and *Rana cascadae* (b).

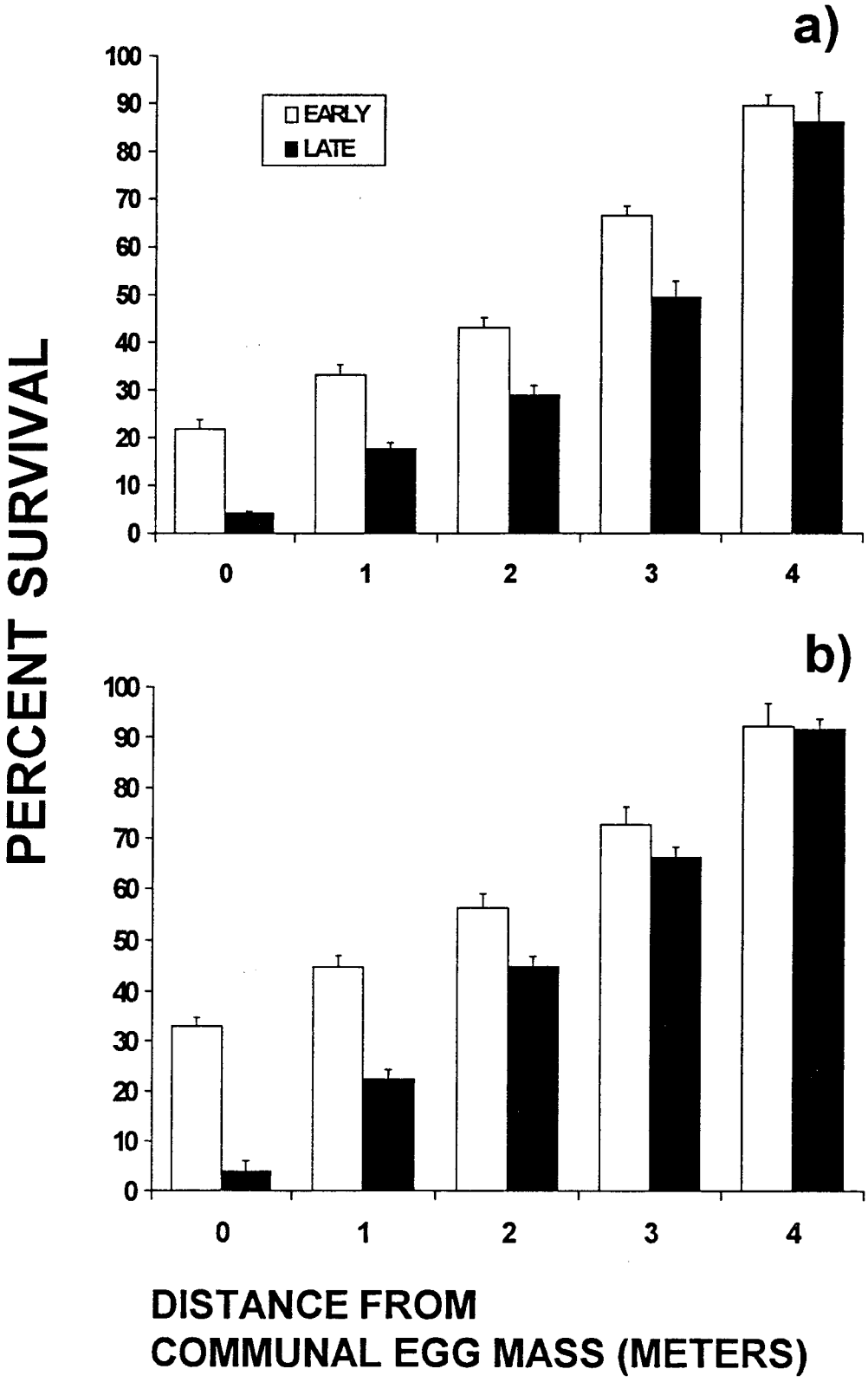

a)
EARLY
LATE
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
b)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
PERCENT SURVIVAL
DISTANCE FROM
COMMUNAL EGG MASS (METERS)

Figure 2.2

DISCUSSION

We demonstrated a relationship between the prevalence of *Saprolegnia* and the egg laying behavior of three amphibian species. Observations on embryo mortality at natural oviposition sites showed that species that lay eggs in communal egg masses had higher mortality rates than species that lay eggs non-communally. Field experiments demonstrated that eggs had increased mortality when in the proximity of the communal egg mass. Our data corroborate previous observations (Blaustein et al. 1994a) that *S. ferax* is one important factor associated with the mortality of amphibian embryos in the Pacific Northwest. Continued mortality in early life history stages may ultimately contribute to a population decline. Thus, it is possible that *S. ferax* contributes to population declines, of *B. boreas* and *R. cascadae*.

We suggest that interspecific differences in species egg laying behaviors are important determinants of infection rate. Infection by *Saprolegnia* can spread through either growth of hyphae by direct contact or by colonization by the freeswiming zoospore stage (Smith et al. 1985; Wood and Willoughby 1986). Thus, communal egg layers such as *B. boreas* and *R. cascadae* are probably more prone to infection than species that do not lay their eggs communally, such as *H. regilla*.

Saprolegnia is a common fish pathogen and may be introduced by fish into lakes and ponds during fish stocking (Seymour 1970; Richards and Pickering 1978; Srivastava and Srivastava 1978; Pickering and Wiloughby 1982; Wood and Willoughby 1986; Blaustein et al. 1994a). Many of the species of fish that are stocked into lakes in the Oregon Cascades (e.g. *Salmo spp., Salvelinus spp., Oncorhynchus spp.*) are prone to *Saprolegnia* infection (Seymour 1970; Wood and Willoughby 1986). *Saprolegnia* may be reintroduced with each stocking event or may become established with repeated stocking.

Although *Saprolegnia* seems to be a major factor contributing to egg mortality in Oregon, there may be complex interactions between *Saprolegnia* infection and environmental stress. Individuals may be especially susceptible to *Saprolegnia* infection if they are under stress (Schaefer et al. 1981; Pickering and Willoughby 1982). In amphibians, *Saprolegnia* infection has been observed to occur more readily under conditions such as low temperature and low pH that are considered stressful to developing embryos (Banks and Beebee 1988; Beattie et al. 1991).

One source of stress, UV-B radiation, has effects that weaken disease defense systems (Kripke 1984; Orth et al. 1990; Kripke et al. 1992; Tevini 1993). Increasing mortality rates of amphibian embryos in Oregon over the past decade

may be the result of several interacting agents including UV-B radiation and *Saprolegnia*. For example, mortality rates of *R. cascadae* and *B. boreas* in Oregon appear to have been no more than 10% from the 1950's to mid 1980's (A.R.B. pers. obs. and unpublished field notes of R.M. Storm and R.K. O'Hara; Blaustein and Olson 1991). Recent field experiments have shown that embryos of *R. cascadae* and *B. boreas* are more susceptible to *Saprolegnia* infection when exposed to ambient UV-B radiation (Kiesecker and Blaustein 1995). Conversely, embryos of *H. regilla* were not affected by exposure to UV-B radiation (Kiesecker and Blaustein 1995).

Possible increases in UV-B radiation (see Worrest and Grant 1989; Kerr and McElroy 1993; Zurer 1993) may induce a more pronounced affect of pathogens on species whose defense systems are compromised by UV-B radiation. *Bufo boreas* and *R. cascadae* lay eggs in open shallow water in high density communal egg masses, and this increases the likelihood that solar radiation and fungal infection will damage their embryos. Further, the embryos of these species have a relatively low capacity to repair UV damage to their DNA that can result in cell death (Blaustein et al. 1994c). In contrast, *H. regilla* may be less prone to UV-B damage and *Saprolegnia* infection because it does not lay eggs in communal masses and has a relatively high capacity to repair UV induced damage to its DNA (Blaustein et al. 1994c).

Selective pressure over evolutionary time may have favored laying eggs in a communal mass because eggs in communal masses have increased developmental rates over those in non-communal masses (e.g. Sype 1975; O'Hara 1981). However, our results suggest that for some species, egg laying in communal masses may no longer be beneficial.

ACKNOWLEDGEMENTS

We thank Dr. George Muller for providing information on fungal culture techniques and for identifying *Saprolegnia* samples; the USDA Forest Service, Pacific Northwest Research Station and especially Deanna H. Olson for use of vehicles and other assistance; D.H. Olson also helped in obtaining breeding population sizes estimates for *B. boreas*; Brian Edmond, Grant Hokit, Susan Walls, Henry Hill, Karen Hill, James Conway, Tommy Devito, and Paul Sicero for the help they provided in the field. Doug Chivers, Erica Wildy, and Jill DeVito provided helpful suggestions on earlier versions of this manuscript. Special thanks to Cheri Miller for all of her help in the field and lab. This work was supported by a ZoRF grant (Zoology Research Funds) to JMK and NSF grant #DEB-942-333 to ARB.

Appendix A. Amphibian breeding sites surveyed for embryo mortality in the Oregon Cascade Mountain Range, USA.

Site	Location
Lost Lake	Linn County, Oregon
	97 km east of Albany, Oregon
	Elevation 1220 m
Three Creeks	Deschutes County, Oregon
	43 km west of Bend, Oregon
	Elevation 2000 m
Todd Lake	Deschutes County, Oregon
	46 km west of Bend, Oregon
	Elevation 2000 m
Green Lake	Deschutes County, Oregon
	48 km west of Bend, Oregon
	Elevation 2600 m
Scott Lake	Lane County, Oregon
	95 km east of Springfield, Oregon
	Elevation 1500 m
Site One	Linn County, Oregon
	92 km east of Albany, Oregon
	Elevation 1190 m
Parrish Lake	Linn County, Oregon
	90 km east of Albany, Oregon
	Elevation 1190 m
Lava Lake	Linn County, Oregon
	91 km east of Albany, Oregon
	Elevation 1150 m

CHAPTER 3

SYNERGISM BETWEEN UV-B RADIATION AND A PATHOGEN MAGNIFIES AMPHIBIAN EMBRYO MORTALITY IN NATURE

Joseph M. Kiesecker

and

Andrew R. Blaustein

PUBLISHED IN THE PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES **92**:11049-11052, 1995.

ABSTRACT

Previous research has shown that amphibians have differential sensitivity to ultraviolet-B (UV-B) radiation. In some species, ambient levels of UV-B radiation causes embryonic mortality in nature. The detrimental effects of UV-B alone or with other agents, may ultimately affect amphibians at the population level. Here, we experimentally demonstrate a synergistic effect between UV-B radiation and a pathogenic fungus in the field that increases the mortality of amphibian embryos compared with either factor alone. Studies investigating single factors for causes of amphibian egg mortality or population declines may not reveal the complex factors involved in declines.

INTRODUCTION

In the Pacific Northwest, high mortality of eggs in certain amphibian species has recently been attributed to two main agents, ultraviolet-B (UV-B; 290-320 nm) radiation and a pathogenic fungus, *Saprolegnia ferax* (Blaustein et al. 1994b, Blaustein et al. 1994c, Blaustein et al. 1995). For example, the western toad (*Bufo boreas*) has experienced from 50 to nearly 100% egg mortality at several sites in the Oregon Cascade Range (Blaustein and Olson 1991, Blaustein et al. 1995). Conversely, Pacific treefrog (*Hyla regilla*) eggs in the same ponds and lakes as *B. boreas* have not experienced unusual mortality (Blaustein et al.1994b). Recent research showing that amphibians have differential sensitivity to ambient UV-B radiation is consistent with these observations. When shielded from UV radiation *B. boreas* and Cascades frog (*Rana cascadae*) eggs showed much higher hatching success than unshielded eggs (Blaustein et al. 1994b). Hatching success in *H. regilla* was unaffected by shielding (Blaustein et al. 1994b).

Potential detrimental effects of UV-B radiation acting with other agents (Blaustein et al. 1994c) may enhance egg mortality in nature and ultimately affect amphibian populations. Indeed, populations of amphibians in widely distributed locations, including *B. boreas* and *R. cascadae*, appear to have undergone declines and range reductions in recent times (Wake 1991, Federal Register 1991, Carey 1993,

Fellers and Drost 1993, Blaustein et al. 1994a), with some, perhaps, becoming extinct (Richards et al. 1993, Pounds and Crump 1994). Habitat destruction and natural population fluctuations undoubtedly play a role in the declines of some populations (Blaustein 1994, Pechmann and Wilbur 1994). However, several investigators have suggested that UV radiation may be one factor contributing to the declines of certain populations in relatively remote regions where there are no other obvious explanations (La Marca and Reinthaler 1991, Richards et al. 1993, Blaustein et al. 1994b).

The detrimental effects of UV-B radiation on amphibian embryos acting alone or synergistically with other agents are poorly understood in natural systems. For example, in nature, it is possible that the effects of pathogens, such as *Saprolegnia*, may be enhanced when defense systems are weakened by stressors (Carey 1993, Blaustein et al. 1994c). One source of stress, UV-B radiation, has well documented effects that weaken disease defense systems (Orth et al. 1990, Tevini 1993).

To test the hypothesis that UV-B radiation and *Saprolegnia* interact synergistically to enhance amphibian egg mortality, we conducted field experiments on *R. cascadae*, *B. boreas*, and *H. regilla* to assess the hatching success of their eggs. All three species lay their eggs in open, shallow water, exposed to UV-B radiation and *Saprolegnia*.

METHODS AND MATERIALS

Experiments were conducted at three natural oviposition sites in the central Oregon Cascade Range (USA). Tests of all species were conducted at Three Creeks Lake (43 km west of Bend, Deschutes Co., Oregon, elevation 2000 m). Additionally, tests of *B. boreas* were conducted at Lost Lake (Linn Co., Oregon, 97 km east of Albany, elevation 1220 m) and *R. cascadae* and *H. regilla* at Small Lake (Linn Co., Oregon, 92 km east of Albany, Oregon, elevation 1190 m). Enclosures (38 X 38 X 7 cm) were placed in small plastic pools (110 cm diameter, 18 cm deep) so that we could control *Saprolegnia* densities. Within the pools, eggs were emersed in 5-10 cm of natural lake water, a depth at which eggs are naturally laid (O'Hara 1981). Pools with enclosures were placed in a linear array parallel to the water's edge in a 2 X 3 randomized block design (Zar 1984) with three sunlight treatments crossed with two fungal treatments. There were four replicates for each treatment. Thus, there were 24 enclosures at each site. Enclosures had clear plexiglass frames with floors of 1 mm^2 fiberglass mesh screen. For *R. cascadae* and *B. boreas*, 25 eggs from each of six different clutches (total = 150 eggs per enclosure) were placed in each enclosure. Because of their small clutch size, for *H. regilla* we used eggs from more than six clutches and randomly assigned 25 eggs from at least six clutches to each enclosure (total = 150 eggs per enclosure).

A UV-B blocking filter (50 X 50 X 7 cm) made of mylar was placed over one third of the enclosures. An acetate filter that transmitted UV-B (a control for using a filter over the eggs) was placed over another third of the enclosures. The remaining enclosures had no filters. Analyses with an Optronics 752 spectroradiometer showed that the mylar blocked 100% of UV-B. The acetate allowed about 80% UV-B transmission. *Saprolegnia* was cultured in the laboratory on 20 ml corn meal agar in standard petri dishes. Using the standardized culture protocol, boiled hemp seeds (Laskin and Lechevalier 1978) were added to cultures and cultures were allowed to incubate at 20°C for approximately 168 hours. In the pools where *Saprolegnia* was added, we introduced three hemp seeds laden with *Saprolegnia* (approximately 3000 - 5000 zoospores/liter).

In experiment 1, we placed 150 newly deposited eggs (< 24 hr old) in each of 24 enclosures at natural oviposition sites of each species. The enclosures at each site were randomly assigned to unfiltered sunlight, sunlight filtered to remove UV-B and shorter wavelengths, and sunlight filtered to remove wavelengths shorter than UV-B. We randomly added *S. ferax* to half of the enclosures in each sunlight treatment (Fig. 1). To the remainder of the enclosures, we added the antifungal agent, Malachite green (Mattison 1988, Coborn 1992), to remove any

Saprolegnia that may be present naturally. Enclosures were placed in plastic pools. Within the pools, eggs were emersed in natural lake water.

Experiment 2 was conducted at Three Creeks Lake simultaneously with the tests conducted for experiment 1. In experiment 2, we used procedures identical to those in experiment 1 except that enclosures were placed directly into the lake. Thus, in this experiment, embryos were exposed to all three sunlight regimes and natural levels of *Saprolegnia*.

The experiments ended when all embryos either hatched or died. Survival was measured as the proportion of hatchlings produced per enclosure. The proportion of hatchlings produced per enclosure (survivorship through hatching) was assessed using Analysis of Variance (ANOVA) to test for differences among the treatments.

RESULTS

In experiment 1, the ANOVA indicated a significant UV-B effect by itself. However, the effect is secondary to the interaction effect of UV-B radiation and fungus. All three species had reduced hatching success in the presence of *Saprolegnia*. However, with *Saprolegnia* present, UV-B enhanced this effect in *Bufo* and *Rana* (Figure 3.1; Table 3.1). *Hyla regilla* hatching success was not affected by UV-B and its hatching success was only reduced in the presence of Saprolegnia.

In experiment 2, the hatching success of *B. boreas* and *R. cascadae* was also greater in regimes shielded from UV-B (Figure 3.2; Table 3.2). *Hyla regilla* hatching success did not differ among the regimes.

Table 3.1 ANOVA of hatching success for experiment 1.

Source of variation	F	df	P
B. boreas			
Lost Lake			
UV	55.920	2,18	<0.001
Fungus	938.024	1,18	<0.001
UV x Fungus	69.484	2,18	<0.001
Three Creeks			
UV	16.958	2,18	<0.001
Fungus	363.966	1,18	<0.001
UV x Fungus	18.455	2,18	<0.001
R. cascadae			
Small Lake			
UV	7.544	2,18	0.004
Fungus	360.660	1,18	<0.001
UV x Fungus	10.079	2,18	<0.001
Three Creeks			
UV	15.879	2,18	<0.001
Fungus	368.494	1,18	<0.001
UV x Fungus	13.529	2,18	<0.001
H. regilla			
Small Lake			
UV	0.977	2,18	0.396
Fungus	266.734	1,18	<0.001

(Table 3.1 continued)

UV x Fungus	2.185	2,18	0.141
Three Creeks			
UV	0.300	2,18	0.746
Fungus	150.834	1,18	<0.001
UV x Fungus	0.018	2,18	0.982

A preliminary analysis indicated no significant block effects (i.e. no differences between temperature or other variables among blocks). Therefore, the block and error terms were pooled for remaining tests (Zar 1984). Post hoc comparisons (Tukey Test)(Zar 1984) were performed to test for differences between means among the six regimes. Temperatures were taken within enclosures for each species in each treatment. Mean temperatures (and ANOVAs) are given for each species at each site for the unfiltered, UV-B transmitting, and UV-B blocking regimes with and without the fungus respectively: Bufo at Lost Lake = 8.3, 8.7, 8.8, 9.1, 8.6, and 8.5°C, $F_{5,18}$ = 0.822, MS = 0.034, P = 0.649; Bufo at Three Creeks = 12.0, 12.9, 13.2, 12.1, 12.8, and 12.6°C, $F_{5,18}$ = 1.294, MS = 0.039, P = 0.160; Rana at Small Lake = 10.8, 11.3, 10.7, 11.3, 10.7, and 10.3°C, $F_{5,18}$ = 1.061, MS = 0.034, P = 0.464; Rana at Three Creeks = 10.9, 12.6, 11.5, 11.4, 11.6, and 12.5°C, $F_{5,18}$ = 2.237, MS = 0.043, P = 0.227; Hyla at Small Lake = 11.2, 10.9, 11.6, 11.4, 10.7, and 11.9°C, $F_{5,18}$ = 1.561, MS = 0.005, P = 0.271; Hyla

(Table 3.1 continued)

at Three Creeks = 12.3, 13.8, 14.3, 13.6, 13.7, and 12.9°C, $F_{5,18}$ = 0.798, MS = 0.006, P = 0.667. MS = Mean-Square; F = F statistic (with degrees of freedom); P = probability.

Table 3.2 ANOVA of hatching success for experiment 2.

Source of variation	F	df	P
B. boreas			
Treatment	19.97	2,6	0.002
R. cascadae			
Treatment	12.98	2,6	<0.001
H. regilla			
Treatment	3.43	2,6	0.723

A preliminary analysis indicated no significant block effects. Therefore, the block and error terms were pooled for remaining tests (Zar 1984). Post hoc comparisons (Tukey Test)(Zar 1984) were performed to test for differences between means among the three regimes. Temperatures were taken within enclosures for each species in each treatment. Mean temperatures (and ANOVAs) are given for each species for the unfiltered, UV-B transmitting, and UV-B blocking regimes respectively: Bufo = 13.9, 14.3, and 14.1°C, $F_{2,6}$ = 0.497, MS = 0.011, P = 0.786; Rana = 11.6, 12.1, and 11.3°C, $F_{2,6}$ = 4.599, MS = 12.671, P = 0.345; Hyla = 14.8, 15.3, and 15.6°C, $F_{2,6}$ = 0.175, MS = 0.001, P = 0.952. MS = Mean-Square; F = F statistic (with degrees of freedom); P = probability.

Figure 3.1 Results of experiment 1 showing the effects of UV-B radiation and manipulated amounts of *Saprolegnia ferax* on hatching success (mean +/- 1 standard error of the mean) in *Bufo boreas* (a), *Rana cascadae* (b) and *Hyla regilla* (c).

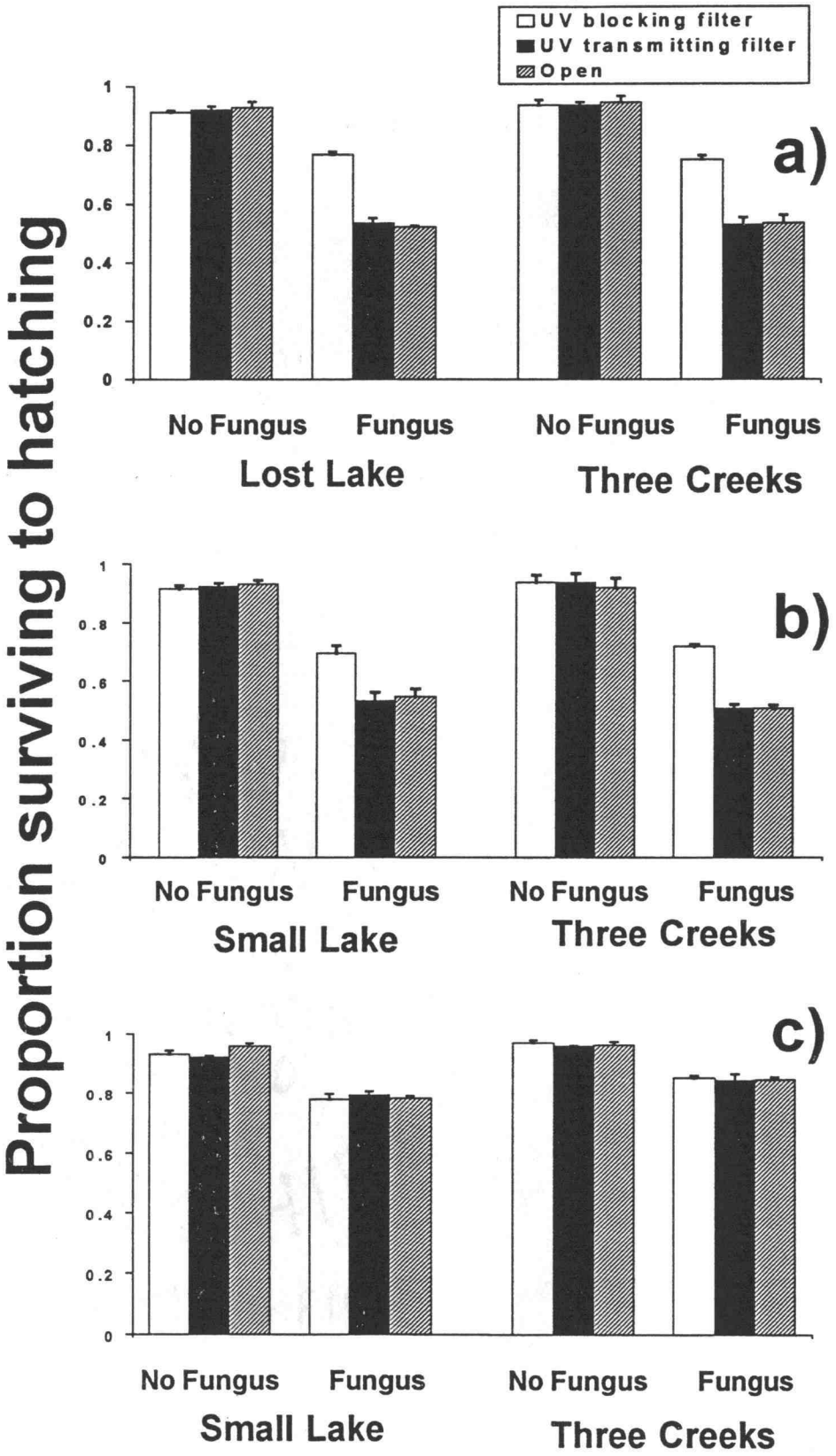

UV blocking filter
UV transmitting filter
Open
Proportion surviving to hatching
a)
b)
c)
0
0.2
0.4
0.6
0.8
1
No Fungus
Fungus
Lost Lake
Three Creeks
Small Lake
Three Creeks
Small Lake
Three Creeks

Figure 3.1

Figure 3.2 Results of experiment 2 showing the effects of UV-B radiation on hatching success (mean +/- 1 standard error of the mean) of eggs emersed directly into a lake. This experiment was conducted at Three Creeks Lake simultaneously with the tests conducted at this site described in Figure 1.

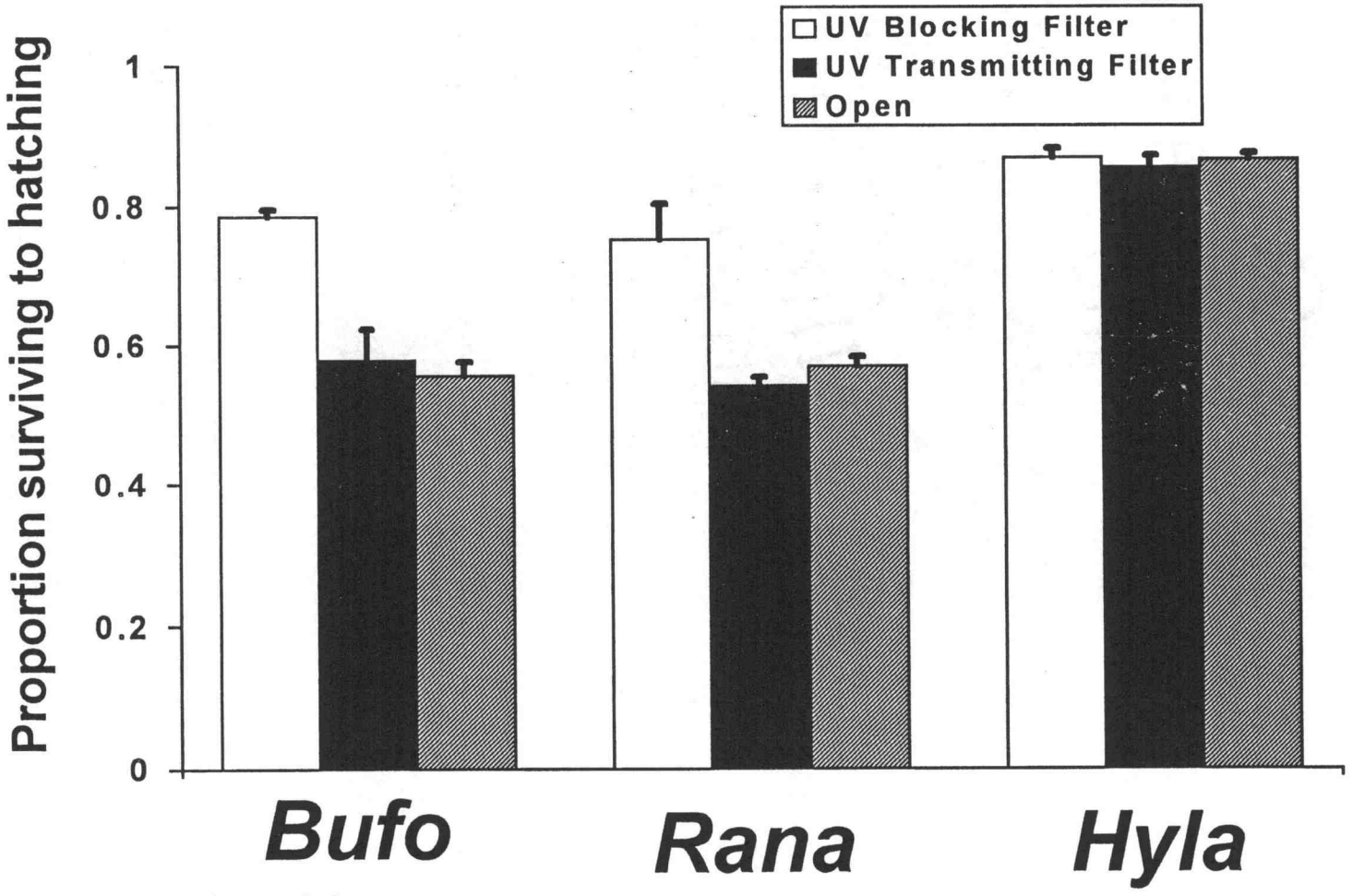

UV Blocking Filter
UV Transmitting Filter
Open
Proportion surviving to hatching
1
0.8
0.6
0.4
0.2
0
Bufo
Rana
Hyla

Figure 3.2

DISCUSSION

We showed a synergistic interaction between a pathogen and UV-B radiation that is killing amphibian embryos in nature. Although UV-B radiation and *Saprolegnia* alone may damage *Rana* and *Bufo* embryos, the results of experiment 1 showed that UV-B radiation and *Saprolegnia* together produced an effect that was greater than the effect of UV-B radiation or *Saprolegnia* alone. Thus, complex interactions among several factors may affect amphibians in nature that could potentially lead to a population decline. *Rana cascadae* populations have virtually disappeared from the southern portion of their range in California and have shown declines in Oregon (Blaustei ad Wake 1990, Federal Register 1991, Fellers and Drost 1993). *Bufo boreas*, a previously ubiquitous species in western North America (Stebbins 1985), has undergone
drastic declines in numbers throughout its range and has exhibited unusually high egg mortality at certain montane sites (Federal Register 1991, Carey 1993, Blaustein et al. 1994c).

Saprolegnia is a well-known pathogen of amphibians and fishes (Blaustein et al. 1994c). Yet, it has been largely overlooked in the context of amphibian declines (Blaustein et al. 1994c). *Saprolegnia* occurs in lakes and ponds with amphibians in Oregon. The origin of *Saprolegnia* at our study

sites is unknown but one likely source is infected hatchery-reared fishes that are stocked throughout the Cascade Range (Blaustein et al. 1994c).

UV-B radiation probably does not contribute to the declines of all amphibian populations. For example, UV-B radiation is less likely to affect species that lay their eggs in relatively deep water or under dense foliage, shielded from solar radiation (Richards et al. 1993, Pounds and Crump 1994). However, we suggest that the anticipated progressive expansion of UV-impacted areas to lower latitudes (Worrest and Grant 1989, Kerr and McElroy 1993 Zurer 1993) could potentially lead to increased mortality of amphibian embryos as they develop in nature. Moreover, individuals not directly exposed to UV-B radiation or *Saprolegnia* may become contaminated as *Saprolegnia*-infected individuals disperse.

Multifactor studies investigating stressors that may compromise disease defense mechanisms are warranted (Tevini 1993, Blaustein et al. 1994c). Furthermore, our results suggest the importance of multifactor studies when investigating mortality factors in early life stages that could eventually lead to a population decline.

ACKNOWLEDGEMENTS

We thank C. Miller, D. G. Hokit, S. Walls, P. Raven, E. Graham, M. Crane and W. Gates for helping with field work and reviewing the manuscript. H. Whisler and G. Mueller advised us on all aspects of *Saprolegnia* biology. Support was provided by the National Science Foundation (USA) and the U. S. Forest Service through the cooperation of Deanna H. Olson.

CHAPTER 4

PATHOGEN MEDIATED COMPETITION BETWEEN *RANA CASCADAE* AND *HYLA REGILLA*

Joseph M. Kiesecker

and

Andrew R. Blaustein

ABSTRACT

Few experimental studies have documented-pathogen mediated interactions under natural conditions. We studied the effect of pathogenic water mold, *Saprolegnia ferax* on competitive interactions bewteen the Cascades frog, *Rana cascadae* and the Pacific treefrog, *Hyla regilla*. The presence of *Saprolegnia* differentially affected larval recruitment of the two species; *Rana cascadae* survival was significantly decreased in the presence of *Saprolegnia*, whereas *H. regilla* was not affected. In the absence of *Saprolegnia*, *R. cascadae* had strong negative effects on the growth, development and survival of *H. regilla*. However, in the presence of *Saprolegnia* the outcome of the interaction between the two species was reversed. These results suggest that pathogens may have strong effects on species interactions and thus, when present may have strong influences on community structure.

INTRODUCTION

Despite the prevalence of pathogens and the diseases they cause, few quantitative data are available on how pathogens influence host fitness in nature (see Hudson 1986, Washburn et al. 1991, Hudson et al. 1992, Fuller and Blaustein 1996). Even less common are experimental studies that examine how pathogens may affect species interactions and influence community dynamics (Price et al. 1986). Ecological studies at the community level have consistently focused on the role of competition, predation, and disturbance (e.g. Wilbur 1972, Menge and Sutherland 1976, Connell 1983, Morin 1983, Wellborn et al. 1996). Numerous studies suggest however that pathogens are also likely to play important roles in determining species performance and the outcome of species interactions (e.g. Park 1948, Anderson and May 1979, May and Anderson 1979, Scott and Anderson 1984, Price et al. 1986, Scott 1987, Price et al. 1988). Thus, a key step in the further development of community theory is to document the role of pathogens at the community level.

The strength and the direction of pairwise species interactions often change in the presence of other species (e.g. Vandermeer 1969, Levine 1976, Holt 1977, Yodzis 1988, Abrams 1991, 1992, Schoener 1993, Menge 1995). Such indirect effects are almost always present in multispecies systems (e.g. Levine 1976, Abrams 1987, Yodzis 1988). However, documentation of indirect effects involving pathogens are

uncommon (but see Park 1948, Price et al. 1988). The presence of pathogens that effect species differentially could result in alterations of species interactions. For example, pathogens may act as keystone species (Power et al. 1996) by diminishing performance of dominate competitors, and inturn allowing for coexistence of competitively inferior species.

Ideally ecologists should conduct controlled field experiments to examine pathogen-mediated interactions. However, it is typically difficult to identify, isolate and control pathogens under field conditions. These difficulties probably underlie the general lack of ecological studies that have explored the role of pathogens. Although, experimental manipulation of pathogens under field conditions have been accomplished in a few systems (e.g. Hudson 1992, Lehmann 1992, Fuller and Blaustein 1996). Criteria needed to examine pathogen-mediated interactions include: (1) the effects of pathogens on hosts should be readily observable and (2) potential host species should show interspecific variation in susceptibility to infection. Many pathogenic water molds of the family Saprolegniaceae meet these criteria. *Saprolegnia*-infected embryos become covered with a visible crown of white hyphal filaments, and usually do not hatch. Infection can spread through either direct contact from growing hyphae or by colonization by freeswimming zoospores (Wood and Willoughby 1986).

The genus *Saprolegnia* is cosmopolitan in distribution, occurring in most freshwater habitats (Seymour 1970, Wood and Willoughby 1986, Blaustein et al. 1994a, Kiesecker and Blaustein 1997). *Saprolegnia* is a well known pathogen of amphibians and fishes, yet species show strong interspecific variation in their susceptibility to infection with *Saprolegnia* (Bragg 1958, Seymour 1970, Richards and Pickering 1978, Smith et al. 1985, Wood and Willoughby 1986, Kiesecker and Blaustein 1997). The ease with which *Saprolegnia* infection can be identified and manipulated under experimental conditions (Kiesecker and Blaustein 1995) make it a model system for examining the influence of pathogens on species interactions.

In the Pacific Northwest region of the United States massive amphibian embryo mortality is associated with the presence of *Saprolegnia ferax* (Blaustein et al. 1994a, Kiesecker and Blaustein 1997). Amphibian embryo mortality is also associated with exposure to ambient levels of Ultraviolet-B radiation (Blaustein et al. 1994b, Kiesecker and Blaustein 1995, Blaustein et al. 1996). Although either UV-B radiation or *Saprolegnia* alone may contribute to embryonic mortality, field experiments have shown that there is a synergistic effect between these two factors that enhances mortality (Kiesecker and Blaustein 1995). While it is apparent that certain species experience high mortality

from these factors (e.g. *Rana cascadae*), other species (e.g. *Hyla regilla*) appear unaffected (Kiesecker and Blaustein 1997).

In this study we explored the effect of *S. ferax* during embryonic development on larval competitive interactions between *R. cascadae* and *H. regilla*. Embryos of both species are deposited in open shallow water where they are exposed to infection with *Saprolegnia*. Larvae of both species feed on periphyton, phytoplankton, and detritus (Nussbaum et al. 1983). Both species have larval periods of similar duration, and frequently breed in the same ponds in the Oregon Cascade Range in spring (Nussbaum et al 1993, pers. obs.). The presence of pathogens like *Saprolegnia*, that are known to differentially influence larval recruitment, may affect the outcome of larval interactions, and hence community structure.

METHODS AND MATERIALS

We manipulated the presence of pathogen, *S. ferax* and embryonic *H. regilla* and *R. cascadae* (high and low densities) into replicated artificial ponds. Artificial ponds were located in a field adjacent to a natural breeding site of *R. cascadae* and *H. regilla* in the Deschutes National Forest (15 miles south of Sisters, Oregon, Deschutes County, OR). Field experiments were conducted during the natural breeding season, from 20 June 1996 to 6 August 1996. We created pond communities in plastic pools that were 1.5 meters wide and were filled to a depth of approximately 20 cm. Ponds contained approximately 150 liters of water. In order to provide food for developing larvae, we added 55 g of leaf litter and macrophytes, and 15 grams of Purina Trout Chow. This method provided conditions for growth that were at least as good as conditions in natural ponds. Mean masses of metamorphs in our experiment were at the high end of the range of masses from metamorphs collected from natural ponds.

We used a fully factorial design where we crossed the presence of *Hyla* (alone or with *Rana*) and the presence of *Rana* (alone or with *Hyla*) at two natural densities, low (30 animals per pool) and high (60 animals per pool). We also crossed these treatments with *Saprolegnia* (present or absent), and the resulting twelve treatments were replicated four times for a total of 48 pools. We controlled for

density of embryos between the single species and the combined species treatments to ensure that effects were due to interspecific effects and not increased density (see Connell 1980, Underwood 1986).

Densities of both species were comparable to densities observed at field sites (Hokit and Blaustein In Press, J. Kiesecker *unpublished data*). All embryos used in the experiment were collected within 12 hours of fertilization and were matched (Gosner Stage 1- 4, Gosner 1960) for developmental stage. For *Rana* we added eggs from each of six different clutches into each pond. Because of the small clutch size, for *Hyla* we used eggs from more than six clutches and randomly assigned eggs from at least six clutches to each pond. Initially all embryos were rinsed in a dilute (2ppm) solution of malachite green, to eliminate any *Saprolegnia* that may have been present on the embryos (Kiesecker and Blaustein 1995).

Using the standardized culture protocol, *Saprolegnia* was cultured in the laboratory on 20 ml corn meal agar in standard Petri dishes. Boiled hemp seeds were added to cultures and cultures were allowed to incubate for approximately 240 hours. In ponds where *Saprolegnia* was added, we introduced three hemp seeds laden with *Saprolegnia*. Control pools receiving no *Saprolegnia* received three clean boiled hemp seeds.

Ponds were left uncovered during the embryonic period of *Rana* and *Hyla* so that embryos were exposed to ambient levels of UV-B. After embryos had hatched larval predators that had entered ponds were removed (a total of 3 Notonectids) and screen lids, designed to prevented predators from colonizing, were placed over the tops of each pond.

During the embryonic period of *Hyla* and *Rana* we monitored ponds daily and recorded mean survivorship to hatching and to metamorphosis per pond. We terminated the experiment when all tadpoles had either metamorphosed or died. Our criterion for metamorphosis was front limb emergence (Gosner stage 42, Gosner 1960). We checked for metamorphs daily. We removed individuals from the enclosures as they metamorphosed, and recorded mass (to the nearest mg) at, and time (in days) to metamorphosis.

Statistical Analyses We used multivariate analysis of variance (MANOVA) to test for the effects of independent factors including density (high or low), *Saprolegnia* (present or absent), and association (alone or with competitor) on the dependent variables mean time in days, mean mass and mean survivorship to metamorphosis (Tabachnick & Fidell 1989). After MANOVA, we used a Bonferroni adjusted univariate analysis of variance (ANOVA) on each response variable to assess which variables were responsible for significant main effects.

Because individuals in ponds were not independent of one another these measures were analyzed as pond means. We arc-sin transformed the data on survivorship before the analysis. For all other dependent variables parametric assumptions were met and no data transformations were necessary.

RESULTS

Rana Responses: Neither the presence of *Hyla* nor density had a significant effect on *Rana* growth, development or survivorship (Table 4.1, Figure 4.1). There were however, strong effects of *Saprolegnia* on the responses of *Rana* (Table 4.1, Figure 4.1). Both survival and time to metamorphosis were decreased for *Rana* in the presence of *Saprolegnia* (Figure 4.1). Unexpectedly, mean mass at metamorphosis was increased for *Rana* in the presence of *Saprolegnia* (Figure 4.1).

Hyla Responses: Density, *Rana*, and *Saprolegnia* all had significant effects on *Hyla* growth, development and survivorship (Table 4.2). However, the main effects of *Rana* and *Saprolegnia* are secondary to the interaction effect between the two factors (Table 4.2). When the effects of *Rana* without *Saprolegnia* are examined, *Rana* reduced survivorship to, and mass at metamorphosis, and increased time to metamorphosis of *Hyla* (Table 4.2, Figure 4.2). In contrast, *Saprolegnia* alone had little effect on Hyla responses. However, the effect that *Rana* had on *Hyla* was dependent on whether *Saprolegnia* was present or not. In the presence of *Saprolegnia*, the outcome of the interaction between *Rana* and *Hyla* was reversed (Table 4.2, Figure 4.2).

Table 4.1 Results of MANOVA for overall effects of *Hyla regilla*, *Saprolegnia* and density on *Rana cascadae* survival, growth and time to metamorphosis and ANOVAs for each response variable. Response variables are proportion surviving (survival), mass at metamorphosis (mass) and time to metamorphosis (time).

MANOVA	F	D.F.	P
Constant	1713.605	3,22	< 0.001
Hyla	0.186	3,22	0.905
Saprolegnia	85.104	3,22	< 0.001
Density	1.302	3,22	< 0.299
Hyla x *Saprolegnia*	0.330	3,22	0.804
Hyla x Density	0.060	3,22	0.980
Saprolegnia x Density	1.442	3,22	0.258
Hyla x *Saprolegnia* x Density	0.111	3,22	0.953
ANOVAs	F	D.F.	P
Mass			
Hyla	0.306	1,24	0.585
Saprolegnia	21.592	1,24	< 0.001
Density	0.712	1,24	0.407
Hyla x *Saprolegnia*	0.027	1,24	0.870
Hyla x Density	0.059	1,24	0.810

(Table 4.1 continued)

Saprolegnia x Density			
	4.111	1,24	0.054
Hyla x *Saprolegnia* x Density			
	0.245	1,24	0.625
Time			
Hyla	0.199	1,24	0.659
Saprolegnia	4.982	1,24	0.035
Density	2.270	1,24	0.145
Hyla x *Saprolegnia*			
	0.526	1,24	0.475
Hyla x Density	0.112	1,24	0.741
Saprolegnia x Density			
	1.009	1,24	0.325
Hyla x *Saprolegnia* x Density			
	0.153	1,24	0.700
Survival			
Hyla	0.211	1,24	0.650
Saprolegnia	268.502	3,22	< 0.001
Density	1.317	1,24	0.262
Hyla x *Saprolegnia*			
	0.539	1,24	0.470
Hyla x Density	0.001	1,24	0.994
Saprolegnia x Density			
	0.103	1,24	0.751
Hyla x *Saprolegnia* x Density			

(Table 4.1 continued)

0.001	1,24	0.998

Significance level for univariate tests is 0.0125 (Bonferroni-adjusted for three response variables).

Table 4.2 Results of MANOVA for overall effects of *Rana cascadae*, *Saprolegnia* and density on *Hyla regilla* survival, growth and time to metamorphosis and ANOVAs for each response variable. Response variables are proportion surviving (survival), mass at metamorphosis (mass) and time to metamorphosis (time).

MANOVA	F	D.F.	P
Constant	1728.63	3,22	< 0.001
Rana	7.808	3,22	0.001
Saprolegnia	8.513	3,22	< 0.001
Density	5.677	3,22	< 0.005
Rana x *Saprolegnia*	10.271	3,22	< 0.001
Rana x Density	0.978	3,22	0.421
Saprolegnia x Density	0.921	3,22	0.447
Rana x *Saprolegnia* x Density	0.848	3,22	0.482
ANOVAs	F	D.F.	P
Mass			
Rana	1.116	1,24	0.301
Saprolegnia	9.767	1,24	0.005
Density	11.030	1,24	0.003
Rana x *Saprolegnia*	14.608	1,24	0.001
Rana x Density	0.308	1,24	0.584
Saprolegnia x Density	0.001	1,24	0.991

(Table 4.2 continued)

Rana x *Saprolegnia* x Density			
	0.199	1,24	0.659
Time			
Rana	1.263	1,24	0.272
Saprolegnia	1.262	1,24	0.273
Density	6.498	1,24	0.018
Rana x *Saprolegnia*			
	2.273	1,24	0.145
Rana x Density	0.548	1,24	0.466
Saprolegnia x Density			
	0.019	1,24	0.892
Rana x *Saprolegnia* x Density			
	0.169	1,24	0.685
Survival			
Rana	22.896	1,24	< 0.001
Saprolegnia	24.865	1,24	< 0.001
Density	1.989	1,24	0.171
Rana x *Saprolegnia*			
	27.440	1,24	< 0.001
Rana x Density	1.342	1,24	0.258
Saprolegnia x Density			
	2.438	1,24	0.132
Rana x *Saprolegnia* x Density			
	1.461	1,24	0.238

(Table 4.2 continued)

Significance level for univariate tests is 0.0125 (Bonferroni-adjusted for three response variables).

Figure 4.1 Summary of the effects of density (low or high) *Saprolegnia* (absent or present) and *Hyla* on the mean time (days to metamorphosis), mean mass (mass at metamorphosis) and mean survivorship (survivorship to metamorphosis) of *Rana cascadae*.

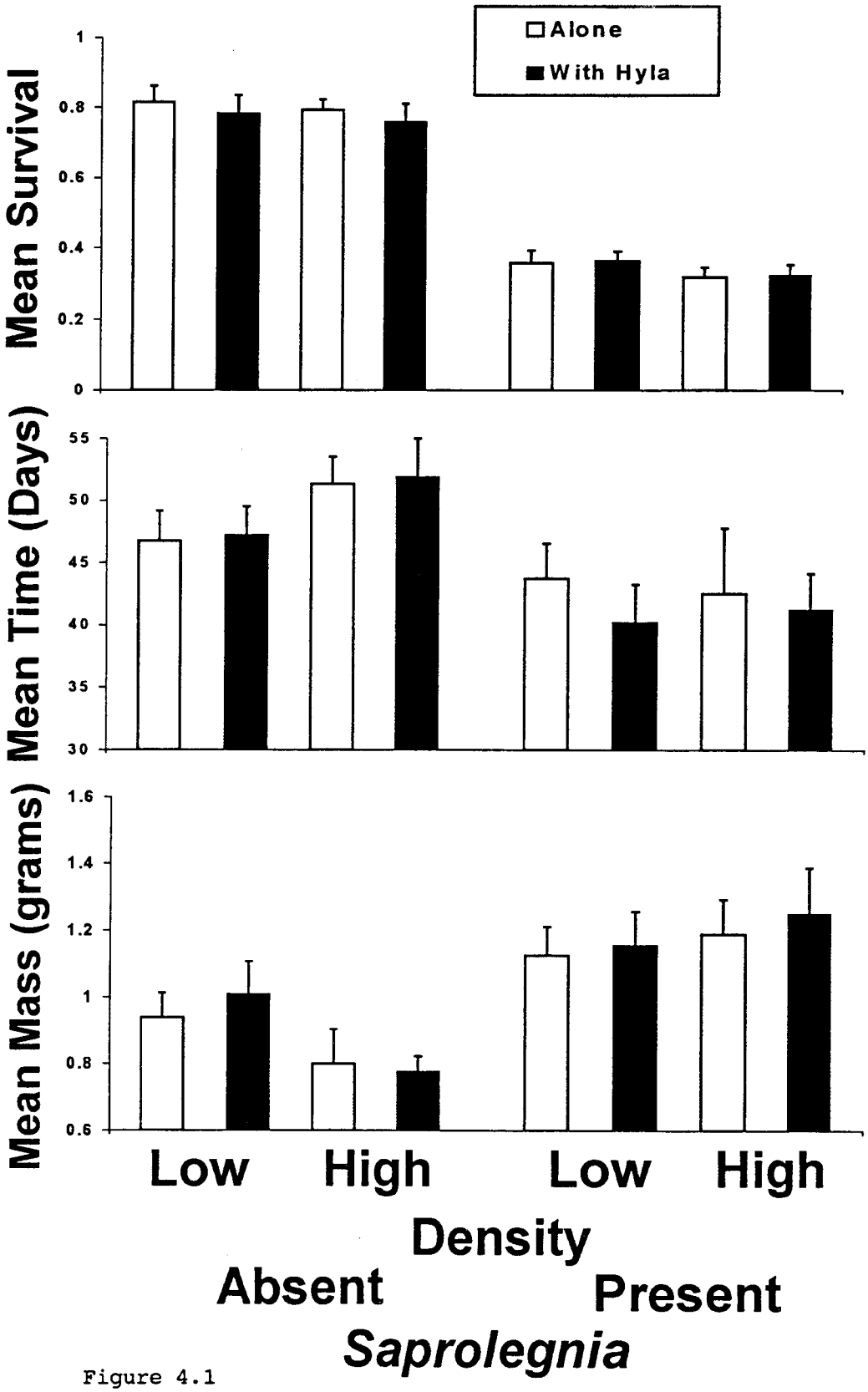

Alone
With Hyla
Mean Survival
1
0.8
0.6
0.4
0.2
0
Mean Time (Days)
55
50
45
40
35
30
Mean Mass (grams)
1.6
1.4
1.2
1
0.8
0.6
Low
High
Low
High
Density
Absent
Present
Saprolegnia

Figure 4.1

Figure 4.2 Summary of the effects of density (low or high) *Saprolegnia* (absent or present) and *Rana* on the mean time (days to metamorphosis), mean mass (mass at metamorphosis) and mean survivorship (survivorship to metamorphosis) of *Hyla regilla*.

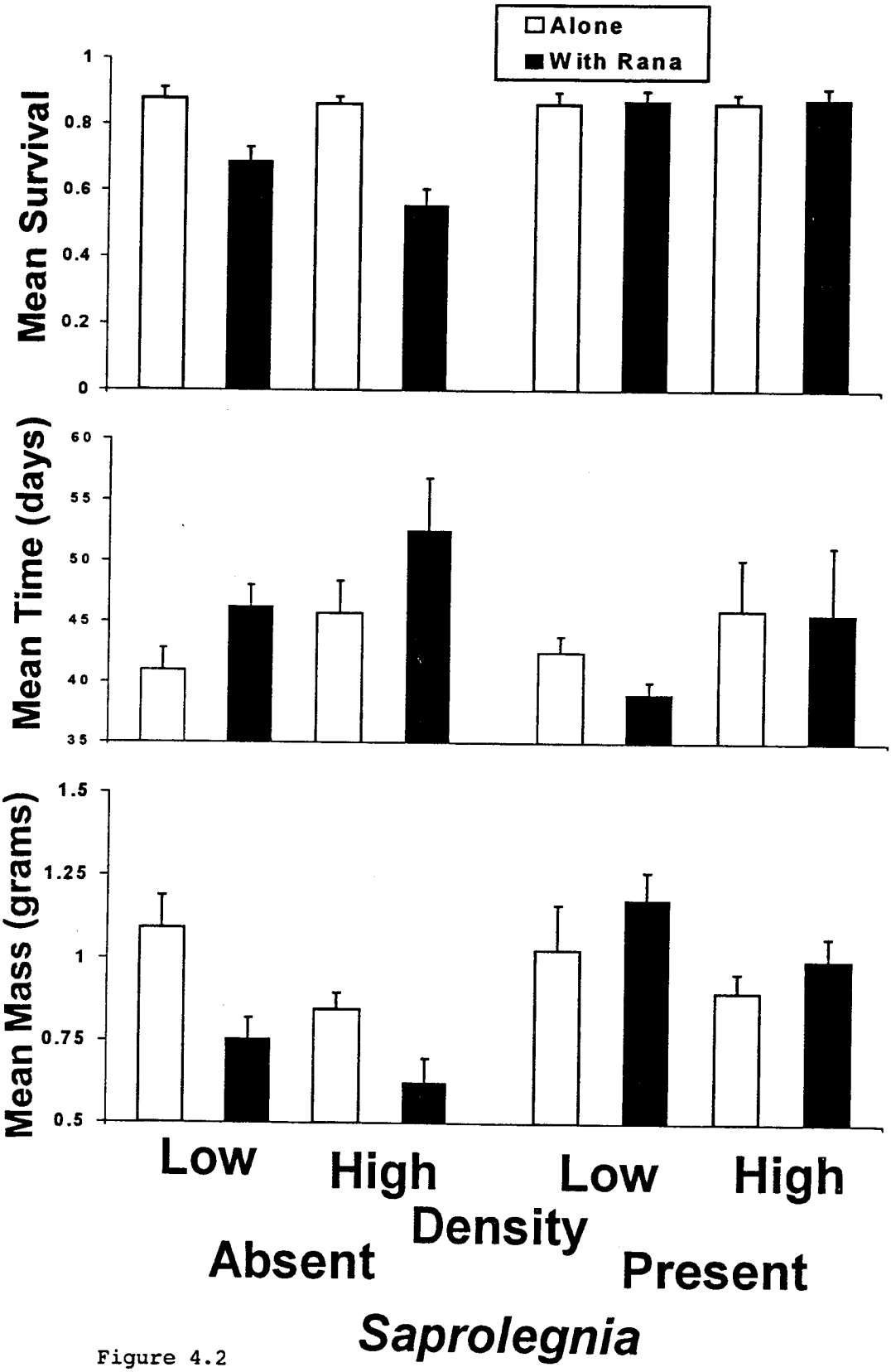

Alone
With Rana
Mean Survival
1
0.8
0.6
0.4
0.2
0
Mean Time (days)
60
55
50
45
40
35
Mean Mass (grams)
1.5
1.25
1
0.75
0.5
Low
High
Low
High
Density
Absent
Present
Saprolegnia

Figure 4.2

DISCUSSION

The results of this study clearly show that disease and competition can interact to determine patterns of relative abundance in communities. *Saprolegnia* differentially affected larval recruitment of *R. cascadae* and *H. regilla*. In the absence of *Saprolegnia*, *R. cascadae* had strong negative effects on the performance of *H. regilla*. Increased mortality of *R. cascadae*, resulting from *Saprolegnia* infection of embryos, led to a reversal of the outcome of interactions bewteen *Rana* and *Hyla*. However, the overall effect of *Saprolegnia* on *Rana* is more difficult to predict. Survivorship of *Rana* exposed to *Saprolegnia* was decreased. However, *Rana* that survived *Saprolegnia* infection, developed faster and were larger at metamorphosis. This suggests that *Saprolegnia* may have positive effects on both *Hyla* and *Rana*. First, by decreasing *Rana* survivorship, *Hyla* is released from interspecific competition. Second, by decreasing larval densities of *Rana*, surviving larvae are released from intraspecific competition. These results indicate that pathogens may have strong effects on species interactions and thus, when present may have strong influences on determining species assemblages.

The influence that disease has on host populations can be cryptic. Hosts populations may appear healthy for long periods until conditions that favor the pathogen result in an outbreak. When species differ in their response to

changing conditions, such changes could result in interspecific differences in susceptibility to disease. The shift in the interaction between *Rana* and *Hyla* resulted from increased *Saprolegnia* infection of *Rana* embryos. *Hyla* appears resistant to infection with *Saprolegnia* (Kiesecker and Blaustein 1995, Kiesecker and Blaustein 1997). This divergence may result from differences in capacity to repair UV induced damage to their DNA (Blaustein et al. 1994b). *Hyla* has a relatively high capacity to repair UV induced DNA damage compared to *Rana* (Blaustein et al. 1994b). Moreover, field experiments demonstrated that *Rana* embryos are more susceptible to *Saprolegnia* infection when exposed to ambient UV-B radiation (Kiesecker and Blaustein 1995). In contrast, *Hyla* embryos were not affected by UV-B exposure (Kiesecker and Blaustein 1995).

In the western United States, as elsewhere, many amphibian species have experienced dramatic population declines and reduction in range (e.g. Crump et al. 1992, Richards et al. 1993, Fellers and Drost 1993, Blaustein 1994, Fisher and Schaffer 1996). For example, the disappearance of *R. cascadae* from portions of its range has made it a candidate for listing as an endangered species (Fellers and Drost 1993). Several studies have suggested that amphibian declines may be the result of an exotic pathogen (Carey 1993, Blaustein et al 1994a, Laurance et al 1996). The introduction of a highly virulent strain of

Saprolegnia may explain recent outbreaks of *Saprolegnia* infection that have been observed in the Pacific Northwest (Blaustein and Olson 1991, Blaustein et al. 1994a, Kiesecker and Blaustein 1997). While *Saprolegnia* is common in most freshwater habitats, new species or strains may be introduced during fish stocking. Many species of hatchery reared fish (e.g. *Salmo spp, Salvelinus spp. Oncorhynchus spp*) are prone to *Saprolegnia* infection (Richards and Pickering 1978, Pickering and Willoughby 1982).

Saprolegnia may act as a keystone species by reducing the density of competitively superior species (Power et al. 1996). Numerous studies have documented the fact that predators can alter the outcome of interspecific competition among their prey (e.g. Paine 1966, Morin 1981, Morin 1983, Morin 1986, Wilbur 1972, Werner and Anholt 1996). Predators, by selectively consuming competitively superior species provide a competitive release for competitively inferior species. The presence of pathogens that cause differential mortality among competitors could result in patterns similar to those observed for keystone predators. Any general predictive theory of community ecology must incorporate the importance of pathogens and their ability to alter the outcome of interactions. While numerous descriptive and observational studies suggest the importance of pathogens (e.g. Anderson and May 1978, May and Anderson 1979, Scott and Anderson 1984, Price et al. 1986, Scott 1987, Price et

al. 1988, Schmitz and Nudds 1994), few empirical studies have manipulated pathogen levels under natural or semi-natural conditions. Only when we increase the number of experimental manipulations of pathogens under natural conditions will we understand their importance to communities.

ACKNOWLEDGEMENTS

We thank Cheri Kiesecker for providing technical assistance, and D. Grant Hokit, Susan Walls, Bruce Menge, Vincent Vega, Jules Winnfield, Marsellas Wallace and Wendle Wolf for helpful discussions regarding experimental design. We thank the U.S. Forest Service, Pacific Northwest Research Station and especially Deanna H. Olson for the use of vehicles. Doug Chivers, Erica Wildy, Jill DeVito and Adolfo Marco provided helpful suggestions on this manuscript. Funding was provided by the Department of Zoology Research Funds, Sigma Xi and the National Science Foundation (Grant Number DEB-9423333).

CHAPTER 5

POPULATION DIFFERENCES IN RESPONSES OF RED-LEGGED FROGS (*RANA AURORA*) TO INTRODUCED BULLFROGS (*RANA CATESBEIANA*).

JOSEPH M. KIESECKER

AND

ANDREW R. BLAUSTEIN

IN PRESS FOR ECOLOGY.

ABSTRACT

We studied eight populations of the red-legged frog, *Rana aurora*, to examine responses of allotopic and syntopic tadpoles to bullfrogs, *R. catesbeiana* an introduced predator of *R. aurora*. We also assessed predation rates by *R. catesbeiana* on syntopic and allotopic populations of *R. aurora*. Syntopic *R. aurora* tadpoles significantly reduced their activity and increased their refuge use when presented with the chemical cues of both tadpoles and adult *R. catesbeiana*. In contrast, allotopic tadpoles did not significantly alter their behavior in the presence of either *R. catesbeiana* adults or larvae. Predation by *R. catesbeiana* was lower in syntopic populations of *R. aurora* tadpoles compared with tadpoles from allotopic populations. Our results show differential responses of syntopic and allotopic *R. aurora* tadpoles to larval and adult *R. catesbeiana*. Syntopic tadpoles avoid predation by *R. catesbeiana* more efficiently than tadpoles from allotopic populations. This suggests that individuals that are unfamiliar with novel, introduced organisms may not possess adaptations that would prevent a negative encounter.

INTRODUCTION

There are numerous examples of losses of native species after the introduction of exotic predators (e.g. Elton 1958; Morton 1990; Lodge 1993). Naive, native prey animals may be susceptable to predation by introduced predators because of their inability to recognize new predators and execute anti-predatory behaviors (Maloney and McLean 1995). Population declines of many ranid frogs native to the western United States have been reported by several workers (e.g. Hayes and Jennings 1986; Blaustein and Wake 1990; Blaustein 1994). Although there are many possible causes contributing to ranid declines, several studies have documented the decline of native ranid frogs after the introduction of bullfrogs (e.g. Moyle 1973; Bury and Luckenbach 1976; Bury et al. 1980; Nussbaum et al. 1983; Blaustein and Wake 1990; Blaustein 1994). However, these studies only suggest a negative association between bullfrogs and other frog species. Few studies have attempted to examine the mechanisms by which introduced bullfrogs may impact ranid frogs (but see Kupferberg 1995). The recent listing of *R. aurora draytonii*, a subspecies of the red-legged frog, as "threatened" by the U.S. Fish and Wildlife Service (Federal Register 1996), emphasizes the importance of understanding the impact of introduced predators on native red-legged frogs.

Failure of a prey animal to recognize and respond to a predator increases the likelihood that it will be captured during an interaction with a predator. However, a prey animal will waste time and energy that could be used for other activities, such as feeding and reproduction, if it exhibits anti-predator behaviors upon encountering a non-predator (Milinski 1986; Werner and Hall 1988; Lima and Dill 1990). Many prey animals respond only to predators with which they share prior experience (Kats et al. 1988; Mathis et al. 1993; Chivers and Smith 1994). As predators expand their ranges or are introduced into areas outside of their historical geographic ranges, they may interact with prey animals that are unfamiliar with them. Thus, naive prey may not exhibit anti-predator behaviors when exposed to new predators. The ability to recognize predators may have both a genetic (Curio 1975; Mueller & Parker 1980; Hobson et al. 1988; Riechert & Hedrick 1990) and a learned basis (Curio et al. 1978; Conover 1987; Thornhill 1989; Chivers and Smith 1994). The time it takes for prey animals to acquire anti-predator behaviors to a novel predator may indicate the impact that the new predator can have on populations of prey animals (Maloney & McLean 1995). Once the behavior has been acquired, the speed with which it spreads within a population may be indicative of the intensity of predation pressure by the new predator.

Adult bullfrogs are highly aquatic predators that are known to feed on a wide variety of invertebrate and vertebrate prey, including other amphibians (Bury and Whelan 1986; Beringer and Johnson 1995; Werner et al. 1995). Larval bullfrogs in Oregon typically take 1-3 years to reach metamorphosis (Nussbaum et al. 1983). Thus, the larvae of native species of frogs such as *R. aurora* are exposed to larger older bullfrog tadpoles. Both tadpoles and adults of *R. catesbeiana* are known to prey on tadpoles of other species (e.g. Ehrlich 1979, Bury and Whelan 1986; Werner et al. 1995), although the extent to which this occurs and the factors that may influence its occurrence, are unknown.

Eight populations of the red-legged frog, *R. aurora*, were studied to examine responses of predator-naive tadpoles to a new predator (the bullfrog, *R. catesbeiana*) and to assess if any of the populations have acquired anti-predator behaviors since the introduction of bullfrogs into Oregon in the early 1930's (Nussbaum et al. 1983). Four of the populations studied are allotopic with *R. catesbeiana* and thus have no prior experience with bullfrogs. The other four *R. aurora* populations are syntopic with *R. catesbeiana* and have had exposure to bullfrogs for not more than 60 years (Nussbaum et al. 1983). To assess differences between populations, we first compared the response of *R. aurora* larvae from syntopic and allotopic populations to chemical cues of *R. catesbeiana*. Second, we compared predation rates and

survivorship between syntopic and allotopic *R. aurora* in the presence of *R. catesbeiana* adults and tadpoles. We also assessed factors that may influence predation of *R. aurora* by larval *R. catesbeiana*.

METHODS AND MATERIALS

Collection and Maintenance

Red-legged frog eggs were collected from four populations that are syntopic (hereafter S1, S2, S3 and S4) and four populations that are allotopic (hereafter A1, A2, A3 and A4) with bullfrogs (sites are located in Benton, Douglas, Lane, Lincoln, Linn, and Tillamook Counties, Oregon USA). Eggs were transported to our laboratory for rearing and testing . *Rana aurora* considered syntopic were collected from ponds that had a breeding population of R. catesbeiana present. Thus, larval *R. aurora* were exposed to both larval and adult forms of *R. catesbeiana*. Allotopic *R. aurora* were collected from counties (Lincoln and Tillamook) where *R. catesbeiana* does not occur (Nussbaum et al. 1983). Thus, these R. aurora have no prior exposure to *R. catesbeiana*. Bullfrog tadpoles and adults were collected from the E.E. Wilson Wildlife Refuge (Benton County, Oregon, USA), a site where *R. aurora* historically occured but is no longer found.

All animals were maintained on a 14:10 light-dark photoperiod and at 15-20°C. *Rana aurora* eggs from each population were kept separate and placed into aerated 38 L aquaria filled with dechlorinated water. After hatching, tadpoles were fed ground rabbit chow ad libitum. Water was changed every 5-7 days. *Rana catesbeiana* tadpoles were

reared in 38 L aquaria and were maintained on a diet of ground rabbit chow. Adult *R. catesbeiana* were reared in 38 L aquaria and fed earthworms ad libitum.

Experiment 1: Detection of larval *Rana catesbeiana* chemical cues by larval *Rana aurora*.

The ability of syntopic and allotopic *R. aurora* tadpoles to detect *R. catesbeiana* was evaluated by exposing them to chemical cues of *R. catesbeiana*. Testing occurred from 11 January 1995 to 26 March 1995. We tested tadpoles in a gravitational flow-through system modified from Petranka et al. (1987) that was composed of three 25-liter plastic tubs 51 x 37 x 21 cm deep. The tubs were placed at different heights so that water flowed from one to another at 0.6 liters/min. The two lower most tubs had both input and output openings and never contained more than 12 liters of water. The upper container was filled with 23 liters of water. We thoroughly rinsed the tank with tap water before each test and used clean dechlorinated tap water for each test. We added stimulus animals (*R. catesbeiana* tadpoles for experimental treatments, nothing for controls) to the middle tub. From the middle tub water flowed to the lower most tub which contained five *R. aurora* tadpoles, and a line that divided the tub into width-wise halves. Refuge made of opaque plexiglass, measuring 25 x 37 cm, was present on the output side of the lower tub. The water in the lower tank

was at a depth of 10 cm. The refuge was placed at a depth of 5 cm. Thus, tadpoles had a choice of swimming above or below the refuge.

A test began 10 minutes after flow was initiated. Each test included two five minute trials (an initial and final response) separated by a five minute pause. An observer concealed behind an opaque blind measured tadpole activity and distribution. As a measure of activity level, we counted the number of times a test animal crossed the center line during each trial. To assess avoidance of stimulus animals, we counted the number of test individuals under refugia at 30 second intervals. These 30 second counts were then averaged for each test. The treatments were presented in random order. Twenty tests were conducted with animals from the 4 allotopic and 4 syntopic populations, 10 controls and 10 experimentals (for a total of 160 tests in this experiment). Test individuals were never used more than once.

Stimulus animals used in tests were arbitrarily drawn from stock tanks and then returned after testing. During testing, all stimulus animals were size matched between tests (mean mass, +/- standard error of the mean: 6.3 +/- .033 g, range 4.27g to 8.47g). In all tests three stimulus animals were used. Test animals (*R. aurora* tadpoles) used in this experiment were all of the same developmental stage

(Gosner stage 25, Gosner 1960) and approximate size (mean mass in grams, +/- 1 standard error of the mean: = 0.07g +/- .0044, range from 0.043g to 0.095g).

Experiment 2: Detection of adult *Rana catesbeiana* chemical cues by larval *Rana aurora*.

We tested whether *R. aurora* tadpoles would respond to the chemical cues of *R. catesbeiana* adults. Testing occurred from 15 January 1995 to 29 March 1995. Testing took place in a manner similar to experiment 1, except that *R. catesbeiana* adults instead of tadpoles were used as stimulus animals. In this experiment syntopic S1, S2, S3 and allotopic A1, A2, A3 populations were used. We used animals from each population in 20 tests with 10 controls and 10 experimentals for a total of 120 tests.

During testing all stimulus animals were size matched between tests (mean mass, +/- standard error of the mean: 96.3 +/- 2.733 g, range 78.27g to 108.47g). Test animals (*R. aurora* tadpoles) used in this experiment were all at the same developmental stage (Gosner stage 25, Gosner 1960) and approximate size (mean mass in grams, +/- 1 standard error of the mean: = 1.07g +/- .0134, range from 1.33g to 0.97g). Test individuals were never used in more than one test.

Experiment 3: Predation by *Rana catesbeiana* tadpoles on *Rana aurora* tadpoles in the laboratory.

We measured survival of *R. aurora* in the presence of *R. catesbeiana*. Because many larval anurans increase activity in the presence of food (Duellmann and Trueb 1986; pers. obs.), the presence of food may increase *R. aurora* encounter rate with *R. catesbeiana*. Thus, by varying the food levels we were able to assess how *R. catesbeiana* feeding rates may affect predation on *R. aurora* larvae. We used tadpoles from two populations where individuals were of the same developmental stage and size (S4 and A4) to assess how tadpoles that are syntopic (S4) survive compared with those that are allotopic (A4).

We used a 2 by 3 by 2 factorial design, replicated 3 times with treatments being the presence or absence of bullfrog tadpoles crossed with three food levels (0, 1, or 5 grams food; rabbit chow pellets) crossed with red-legged frog tadpoles that were either syntopic or allotopic with bullfrogs. The experiment was conducted from 18 March to 21 March 1995. Thirty six 20-liter aquaria were placed on a lab table, where the air temperature was maintained at 17° C and lights were on a 12L:12D schedule. Treatments were randomly assigned to aquaria. *Rana catesbeiana* tadpoles were added to the aquaria simultaneously with food and were given one hour

to acclimate, after which the *R. aurora* tadpoles were added. After three days, aquaria were checked and the percentage of *R. aurora* surviving in each aquarium was determined.

Rana aurora tadpoles from both populations were matched for developmental stage (Gosner stage 25, Gosner 1960) and size (mean mass in grams +/- 1 S.E. = 0.05g +/- .0024, range from 0.043g to 0.056g). Only first year *R. catesbeiana* tadpoles were used and they were matched for size across each treatment (mean +/- 1 SE = 5.9g +/-.0023, range from 4.93g to 7.37g).

Experiment 4: Predation by *Rana catesbeiana* adults on *Rana aurora* tadpoles in field experiments.

We experimentally examined predation by *R. catesbeiana* adults on *R. aurora* tadpoles. We used tadpoles from two populations (S4 and A4) to assess how tadpoles that are syntopic survive compared with those that are allotopic when exposed to *R. catesbeiana* adults.

We used a 2 by 2 factorial design, replicated three times with treatments being the presence or absence of adult bullfrogs crossed with red-legged frog tadpoles that are either syntopic or allotopic with bullfrogs. The experiment was conducted from March 22 to March 30 1995 in the south marsh at E.E. Wilson Wildlife Refuge. We used twelve rectangular open bottom pens 8m by 4m ($32m^2$) constructed of 1 mm^2 mesh fiberglass screen that was pressed approximately

20 cm into the soft mud substrate. Enclosures were placed in a linear array parallel to the water's edge in a randomized block design. The depth of water in each enclosure ranged from approximately 10 cm to 1.5 meters. Enclosures were cleared of their macrofauana (e.g. corixids, notonectids, salamanader larvae) by repeated sweeping with a net prior to experimentation. To minimize potential spatial gradients (e.g. temperature, depth) from confounding treatments effects, enclosures were blocked for assignment of treatment. Treatments were randomly assigned to enclosures.

Rana aurora tadpoles from both populations were matched for developmental stage (Gosner stage 25, Gosner 1960) and similar size (mean mass in grams +/- 1 S.E. = 1.57g +/- .247, range from 1.077g to 1.83g). *Rana catesbeiana* adults used were matched for size across each treatment (mean +/- 1 SE = 103.4 +/- 3.733 g, range 88.27g to 126.13g). One *R. catesbeiana* adult was added to each enclosure and was given 24 hrs to acclimate, after which 50 *R. aurora* tadpoles were added. Other than the *R. aurora* larvae, the adult bulfrog was the only vertebrate in the enclosures. After 7 days, enclosures were checked and the percentage of *R. aurora* surviving in each enclosure was determined. At the end of the experiment we also captured and flushed the stomach of each bullfrog.

Statistical Analyses

The number of times tadpoles crossed the center line was summed for experiment 1 and 2 for each test. We also calculated the mean number of individuals under refuge for each 30 second reading. For experiments 1 and 2 we initially tested whether there was a difference in the tadpole initial responses (first five minutes) and final responses (last five minutes) using paired t-tests. However, given that there was no difference between the intial and final responses for any population tested ($P > 0.13$ for all comparisons) the data from intial and final responses were combined for further analyses. This resulted in only one observation of activity and refuge use for each trial. We then used multivariate analysis of variance (MANOVA) to test for stimulus effects on the dependent variables activity (mean number of line crosses) and shelter use (mean number under refuge) (Tabachnick and Fidell 1989). We evaluated the independent significance of activity and shelter use with the total structure coefficients from a discriminant function analysis (Reznick 1990). Total structure coefficients are the correlations between an individual's discriminant function score and its value for the dependent variable; a significant correlation indicates that the variable contributed significantly to the discrimination among treatments.

We used ANOVA to test for statistical differences in survival between treatments in experiment 3 and 4. For experiment 4 a preliminary analysis indicated no significant block effects. Therefore, the block and error terms were pooled for remaining tests (Zar 1984). For all experiments, parametric assumptions were met and no data transformations were necessary.

RESULTS

Experiment 1

Chemical cues from *R. catesbeiana* tadpoles affected *R. aurora* tadpole behavior (Table 5.1, Figure 5.1). Both activity (number of center line crosses) and use of shelter was influenced by the presence of *R. catesbeiana* tadpoles (Table 5.1). Only syntopic *R. aurora* tadpoles significantly reduced their activity and increased their use of refuge when presented with the chemical cues of *R. catesbeiana* tadpoles (Table 5.1, Figure 5.1).

Experiment 2

Chemical cues of *R. catesbeiana* adults affected *R. aurora* tadpole behaviour (Table 5.2). Both activity (number of center line crosses) and use of shelter was influenced by the presence of *R. catesbeiana* adults (Table 5.2, Figure 5.2). Only syntopic *R. aurora* tadpoles significantly reduced their activity and increased their use of refuge when presented with the chemical cues of *R. catesbeiana* adults (Table 5.2, Figure 5.2).

Experiment 3

Survivorship of *R. aurora* was significantly effected by food level, population status, and presence of bullfrogs (Table 5.3). Significant interaction effects were also obtained for bullfrog presence and food level as well as for

Table 5.1 Results of MANOVA for overall effects of *Rana catesbeiana* tadpoles and *Rana aurora* population on *R. aurora* behavior. Response variables are number of times a tadpole crossed the center line (activity) and the number of tadpoles under the shelter (shelter) (A). Results of Discriminant Function Analysis of activity and shelter use (B).

Experiment 1

A)

	F	D.F.	P
Constant	5365.63	2, 143	< 0.001
Population	7.14	14, 286	< 0.001
Bullfrog	89.60	2, 143	< 0.001
Bullfrog x Population	9.86	14, 286	< 0.001

B)

	Standardized Discriminant Function Coefficient	Total Structure Coefficient	P
Activity	0.908	0.746	< 0.001
Shelter	0.882	0.962	< 0.001

Table 5.2 Results of MANOVA for overall effects of *Rana catesbeiana* adults and *Rana aurora* population on *R. aurora* behavior. Response variables are number of times a tadpole crossed the center line (activity) and the number of tadpoles under the shelter (shelter) (A). Results of Discriminant Function Analysis of activity and shelter use (B).

Experiment 2

A)

	F	D.F.	P
Constant	8314.08	2, 107	< 0.001
Population	13.68	10, 214	< 0.001
Bullfrog	137.63	2, 107	< 0.001
Bullfrog x Population	18.09	10, 214	< 0.001

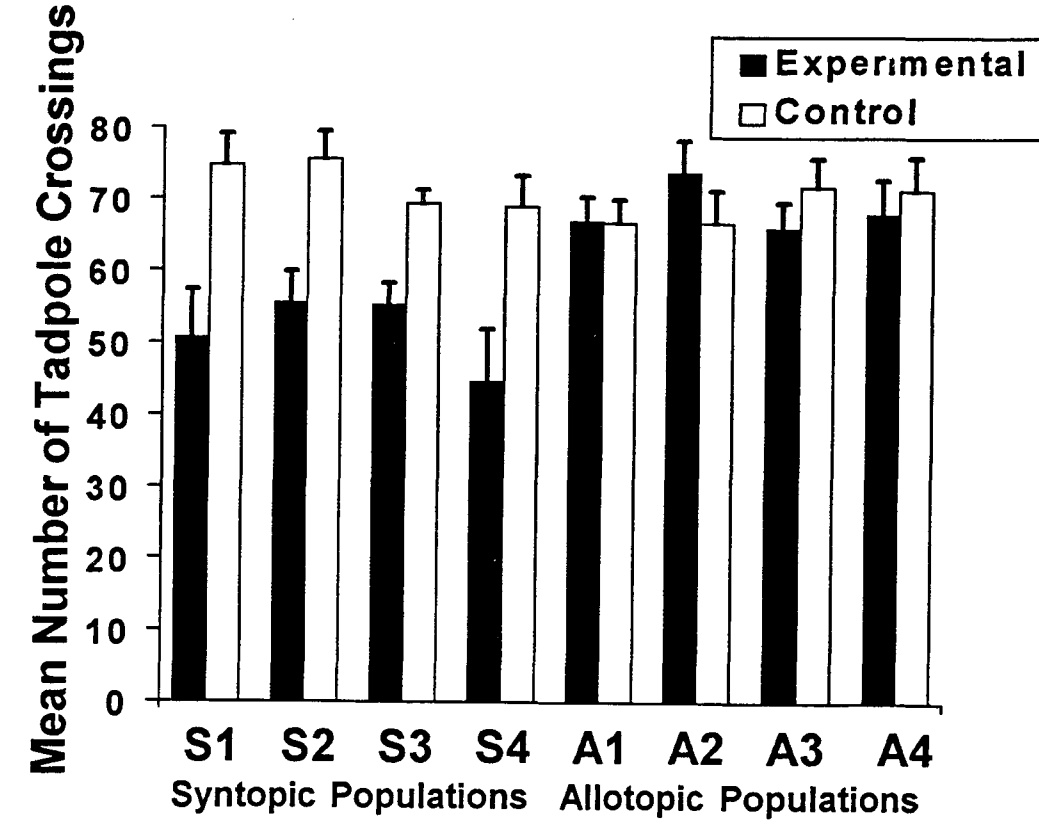
B)

	Standardized Discriminant Function Coefficient	Total Structure Coefficient	P
Activity	0.776	0.882	< 0.001
Shelter	0.812	0.325	< 0.001

Table 5.3 ANOVA results on the effects of food level, presence of *Rana catesbeiana* tadpoles and population status (syntopic/allotopic) on the survival of red-legged frog tadpoles.

Experiment 3			
Source	d.f.	F-ratio	p value
MAIN EFFECTS			
A) Food Level	2,24	47.0	<0.0001
B) Bullfrog	1,24	113.01	<0.0001
C) Population	1,24	5.47	0.0253
INTERACTIONS			
AB	2,24	42.21	<0.0001
AC	2,24	0.90	0.4122
BC	1,24	18.03	0.0002
ABC	2,24	0.533	0.5347

Figure 5.1 Mean Number of tadpole crossings (activity) and mean number of animals under shelter +/- SE for *Rana aurora* tadpoles from syntopic (S1, S2, S3 and S4) and allotopic (A1, A2, A3 and A4) populations exposed to chemical cues of larval *Rana catesbeiana*.


Experimental
Control
Mean Number of Tadpole Crossings
0
10
20
30
40
50
60
70
80
S1 S2 S3 S4 A1 A2 A3 A4
Syntopic Populations
Allotopic Populations

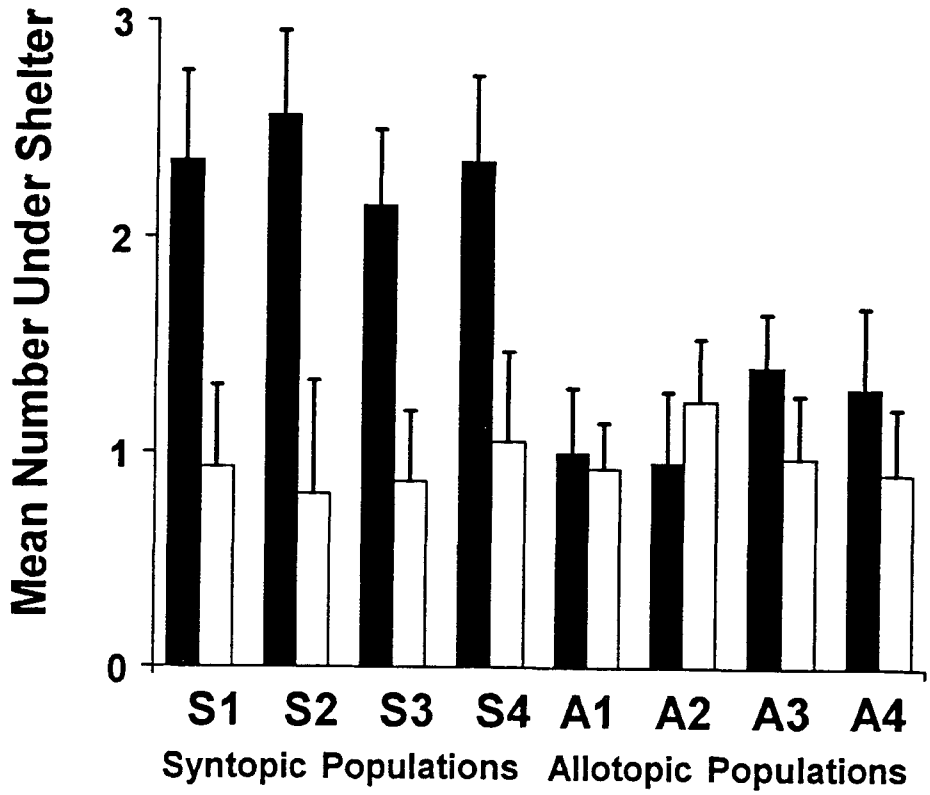

Mean Number Under Shelter
0
1
2
3
S1 S2 S3 S4 A1 A2 A3 A4
Syntopic Populations
Allotopic Populations

Figure 5.1

Figure 5.2 Mean Number of tadpole crossings (activity) and mean number of animals under shelter +/- SE for *Rana aurora* tadpoles from syntopic (S1, S2, and S3) and allotopic (A1, A2, and A3) populations exposed to chemical cues of adult *Rana catesbeiana*.

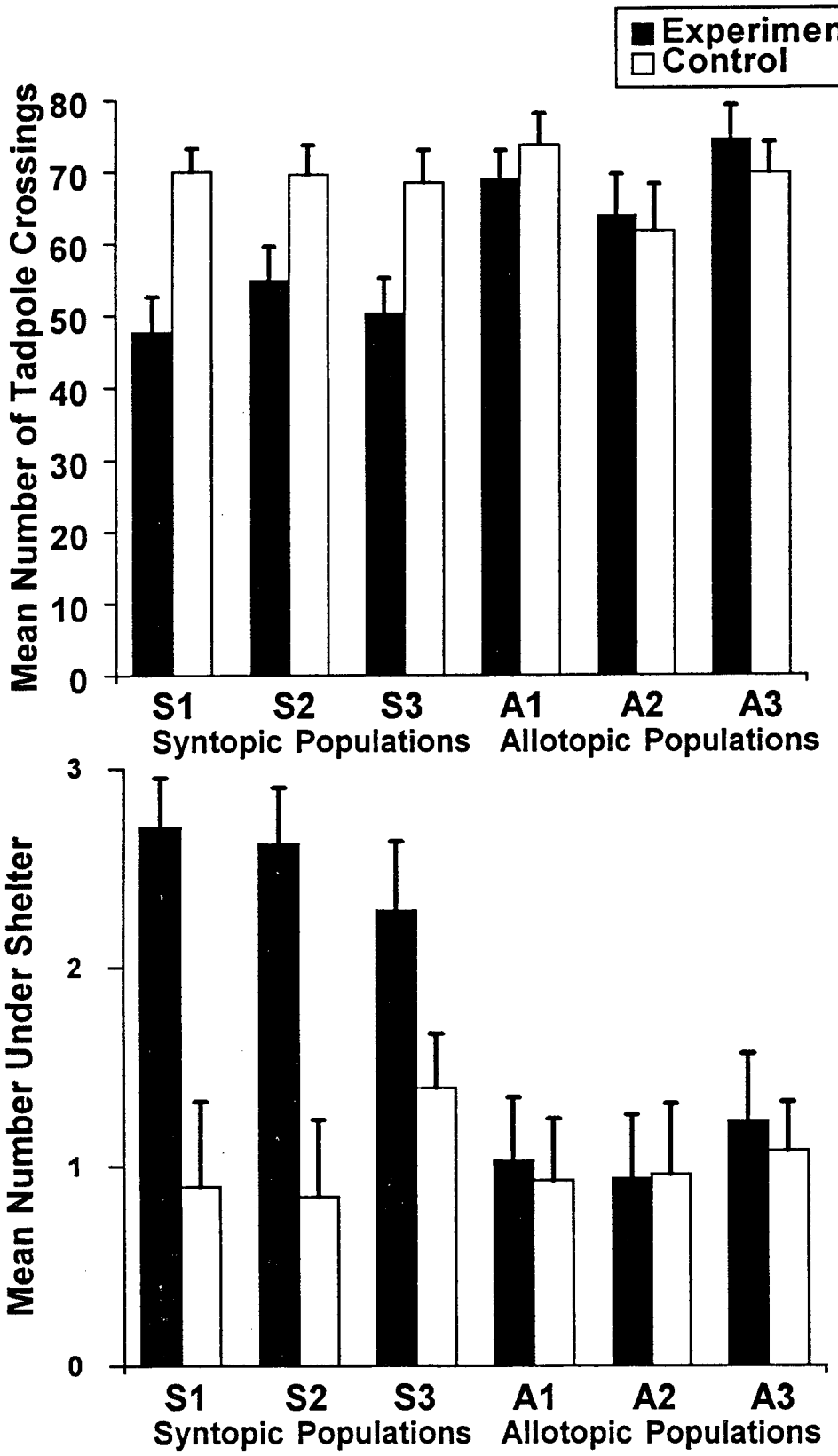

Experimental
Control
Mean Number of Tadpole Crossings
0
10
20
30
40
50
60
70
80
S1
S2
S3
A1
A2
A3
Syntopic Populations
Allotopic Populations
Mean Number Under Shelter
0
1
2
3
S1
S2
S3
A1
A2
A3
Syntopic Populations
Allotopic Populations

Figure 5.2

bullfrog presence and *R. aurora* population status (Table 5.3, Figure 5.3). It is only when food is present that *R. catesbeiana* tadpoles consume *R. aurora* tadpoles (Figure 5.3). There was differential survival of *R. aurora* tadpoles from different populations when exposed to tadpoles of *R. catesbeiana*. Survival of *R. aurora* tadpoles from the syntopic population (S4 = 87.7%) was greater than the allotopic naive population (A4 = 64.7%) (Table 5.3, Figure 5.3), when exposed to *R. catesbeiana* tadpoles. Survivorship was 100% for *R. catesbeiana* in treatments.

Experiment 4

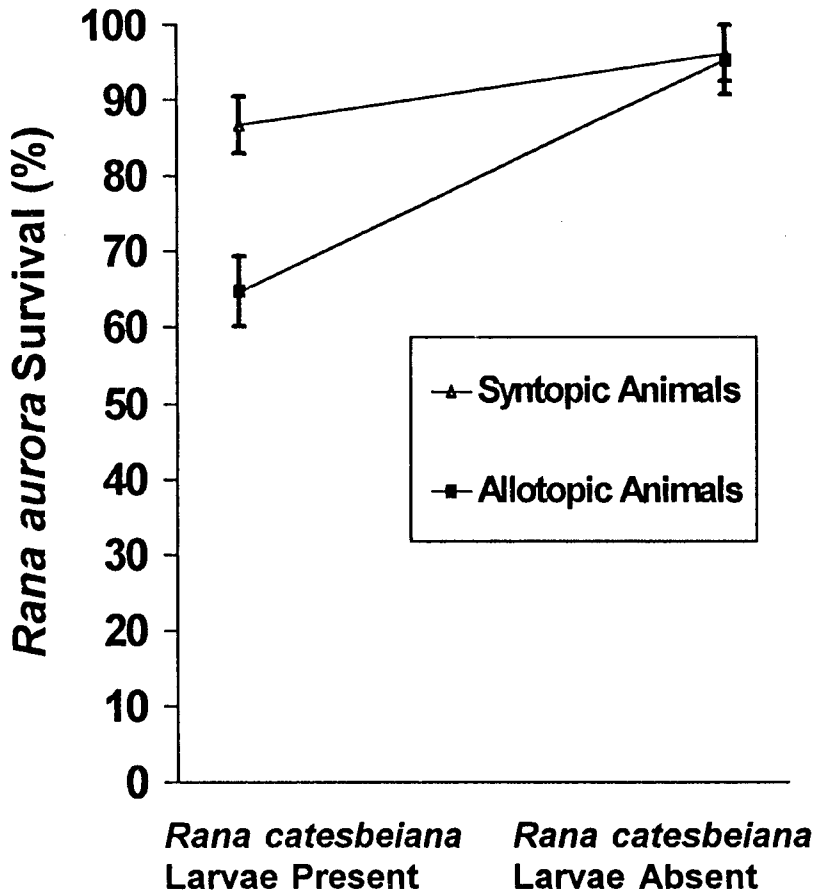
Survivorship of *R. aurora* was significantly effected by bullfrog presence in the field experiment (Table 5.4). There was also a significant interaction bewteen bullfrog presence and *R. aurora* population status (Table 5.4, Figure 5.4). *Survivorship* was differential bewteen *R. aurora* tadpoles from different populations when exposed to adult *R. catesbeiana*. Survival of *R. aurora* the syntopic population (S4 = 90.7%) was greater than in the allotopic naive population (A4 = 43.5%) (Table 5.4, Figure 5.4), when exposed to *R. catesbeiana* adults. All bullfrogs from enclosures with allotopic *R. aurora* had tadpoles in their stomachs. Whereas, only 1 out of the 3 bullfrogs from enclosures with syntopic *R. aurora* had tadpoles in the stomach.

Table 5.4 ANOVA results on the effects presence of *Rana catesbeiana* adults and population status (syntopic/allotopic) on the survival of red-legged frog tadpoles.

Experiment 4

Source	d.f.	F-ratio	p value
MAIN EFFECTS			
A) Bullfrog	1,8	177.34	<.001
B) Population	1,8	142.18	<.001
INTERACTIONS			
AB	1,8	144.18	<.001

Figure 5.3 Interaction plot for the effects of bullfrog presence and *Rana aurora* population, and bullfrog presence and food level. *Rana catesbeiana* tadpoles are either present or absent; *R. aurora* are from either syntopic (S4) or allotopic (A4) populations. food levels are: no food, 1 gram of food, 5 grams food.


100
90
80
70
60
50
40
30
20
10
0
Rana aurora Survival (%)
Syntopic Animals
Allotopic Animals
Rana catesbeiana
Larvae Present
Rana catesbeiana
Larvae Absent

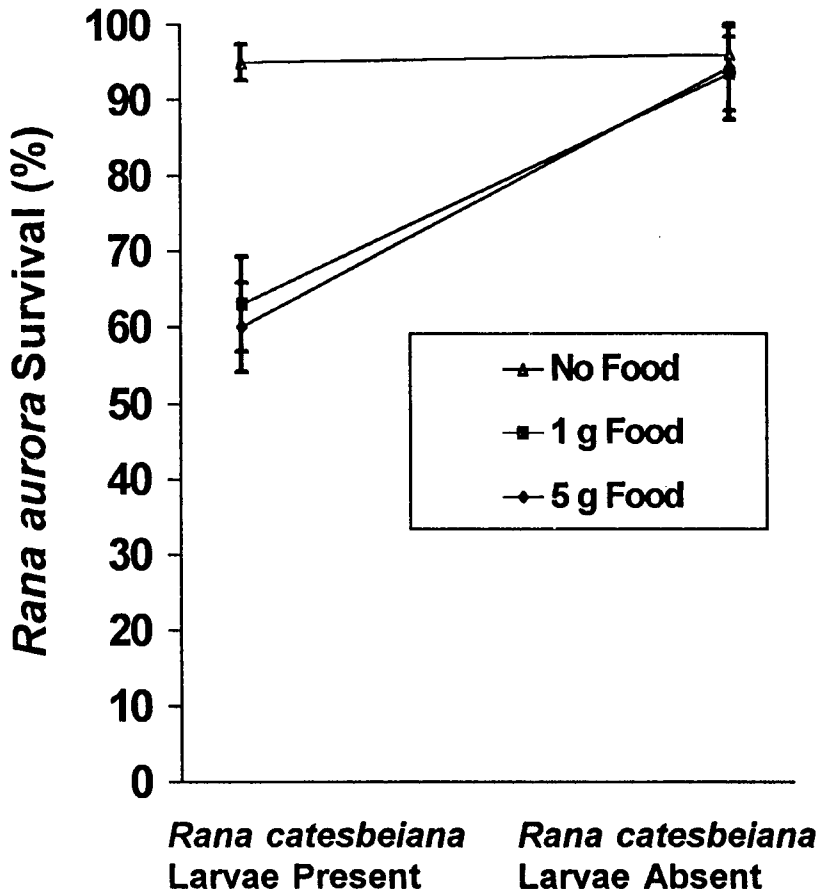

100
90
80
70
60
50
40
30
20
10
0
Rana aurora Survival (%)
No Food
1 g Food
5 g Food
Rana catesbeiana
Larvae Present
Rana catesbeiana
Larvae Absent

Figure 5.3

Figure 5.4 Survival of *Rana aurora* tadpoles from different populations, exposed to *Rana catesbeiana* adults in field enclosures.

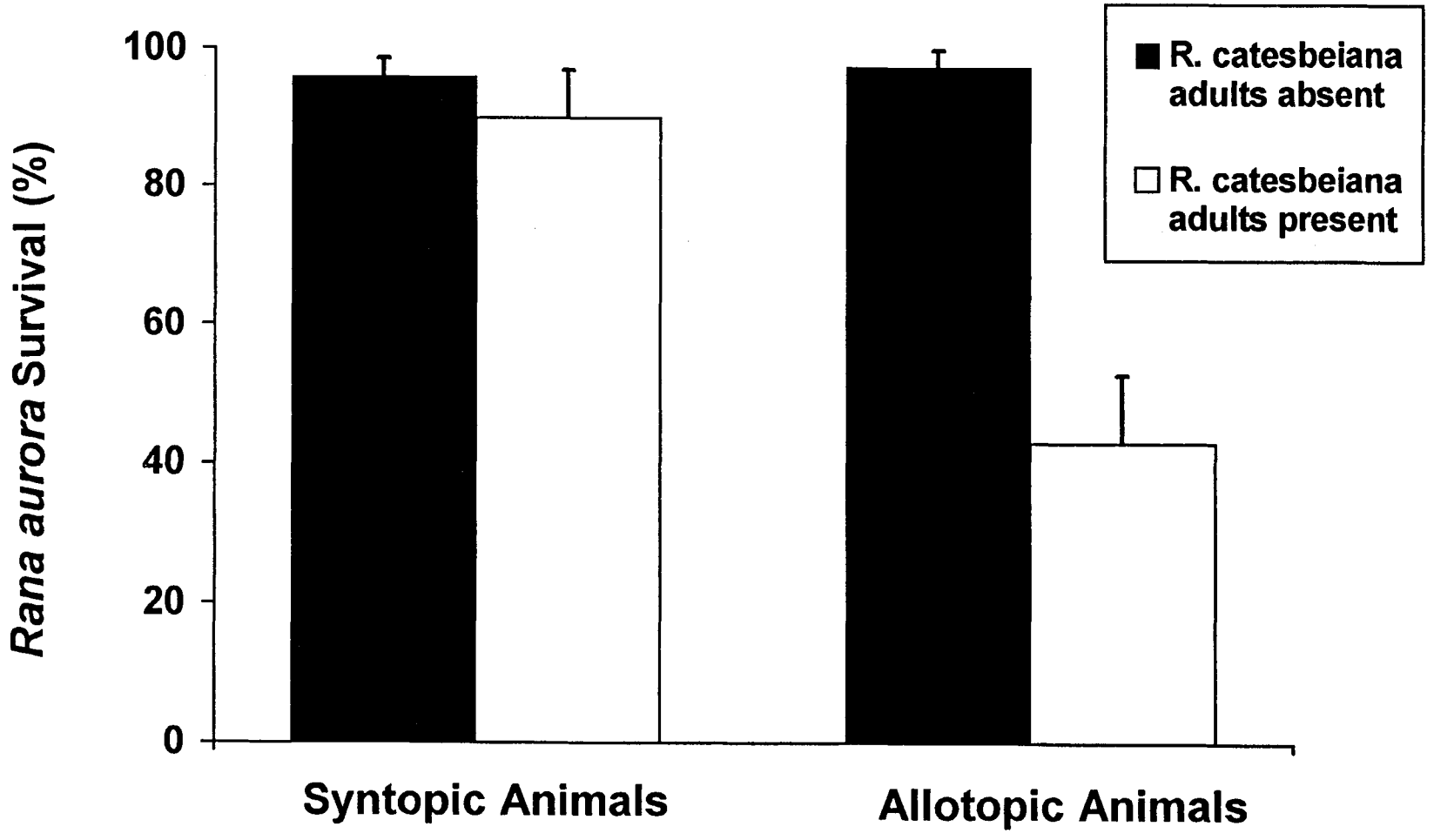

Rana aurora Survival (%)
100
80
60
40
20
0
R. catesbeiana adults absent
R. catesbeiana adults present
Syntopic Animals
Allotopic Animals

Figure 5.4

DISCUSSION

The results of this study clearly show that tadpoles of *R. aurora* from syntopic populations exhibit anti-predator behaviors when exposed to the chemical cues of both *R. catesbeiana* larvae and adults. *Rana aurora* tadpoles that are allotopic with *R. catesbeiana* did not respond to chemical cues of either *R. catesbeiana* larvae or adults. However, *R. aurora* tadpoles from these same allotopic populations exhibit anti-predator behaviors when exposed to chemical cues of native predators (Wilson and Lefcort 1993; Kiesecker and Blaustein unpublished data). Thus, the absence of the behavioral response by allotopic tadpoles in the presence of *R. catesbeiana* was not due to a lack of these types of anti-predator behaviors. The anti-predator responses exhibited by syntopic tadpoles included reduction in movement and an increased use of shelter. Both of these prey responses are frequently reported in the presence of predators (Lima and Dill 1990; Sih 1987). Increased use of shelter and decreased activity may be adaptive against predators that locate their prey by detecting the prey's movement.

We also found increased mortality for allotopic *R. aurora* tadpoles compared to that of syntopic tadpoles when in the presence of both *R. catesbeiana* larvae and adults. Adult bullfrogs are established predators of tadpoles (e.g. Bury and Whelan 1986; Werner et al. 1995). However, most anuran larvae, including *R. catesbeiana*, are thought to be

primarily herbivorous filter feeders or grazers on algae or detritus (Wright and Wright 1949, Dickman 1968, Thrall 1972, Wassersug 1980, Corse and Metter 1980, Duellman and Trueb 1986). Except for the specialized larvae of some cannibalistic species (e.g. Bragg 1964, Pomeroy 1981, Crump 1983), predation by tadpoles on other species has been rarely reported (e.g. Heyer et al. 1975, Kluge 1981, Duellman and Trueb 1986). Yet, *R. catesbeiana* tadpoles have been observed preying on tadpoles of other species (e.g. Ehrlich 1979). In our tests, it is only when food is present that we observed predation of *R. aurora* by *R. catesbeiana* tadpoles. The presence of food and feeding behavior may elicit predation on *R. aurora*. Observations during the experiment suggest that predation occurs when the placement of *R. aurora* and *R. catesbeiana* tadpoles overlap near food. The much larger tadpoles of *R. catesbeiana* consume food resources and may accidently consume smaller R. aurora tadpoles that do not move away. The presence of food increases activity of both *R. aurora* and *R. catesbeiana* and thus increases encounter rates. We believe it is the differences in response of syntopic and allotopic *R. aurora* tadpoles to *R. catesbeiana* that explains the differential survival. Syntopic tadpoles respond to *R. catesbeiana* and thus can avoid predation by *R. catesbeiana*. Predation by *R.*

catesbeiana larvae under field conditions may be rare, however, the potential for it to occur may influence interactions between larvae of the two species.

Alternative sources of mortality may exist for *R. aurora* larvae in the presence of *R. catesbeiana* larvae. For example, bullfrog larvae may transmit pathogens, stress larvae through chemical interference, or alter food resources. However, given the short term nature of experiment # 3 we feel these are unlikely explanations for the observed mortality.

Priority effects have been found to be extremely important in shaping the structure of larval amphibian communities (Alford and Wilbur 1985, Alford 1989, Lawler and Morin 1993). In Oregon, introduced *R. catesbeiana* breed during July and August and tadpoles overwinter until reaching metamorphosis in one to three years (Nussbaum et al. 1983, pers. obs.) *Rana aurora* breed in December and January, and their tadpoles are exposed to larger, older bullfrog tadpoles. The large size difference that exists between *R. catesbeiana* and *R. aurora* may make it possible for *R. catesbeiana* to consume *R. aurora*. Thus, it is likely that only hatchling or small *R. aurora* larvae are susceptible to predation from *R. catesbeiana* tadpoles.

Individuals that are unfamiliar with novel, introduced predators may not possess behavioral adaptations that would prevent predation. Knowledge of how individuals vary in

their response to introduced organisms may be extremely important in understanding the dynamics of biological invasions. In the short time (< 60 years, Nussbaum et al. 1986) that syntopic *R. aurora* have been exposed to *R. catesbeiana*, they appear to have adapted to their presence. The speed in which these populations have acquired these anti-predatory behaviors may be indicative of the intense predation pressure by *R. catesbeiana* (see Chivers and Smith 1995).

Differences in the anti-predator behavior of populations within a single species have been observed for a variety of animals (e.g. Owings and Coss 1977; Riechert and Hedrick 1990; Ducey and Brodie 1991; Mathis et al. 1993; Matity et al. 1993; Maloney and McLean 1995). These differences are generally assumed to have resulted from natural selection, because the responses of prey are more pronounced in populations experiencing the greatest predation pressure. However, natural selection can only operate on anti-predator behavior if it has a genetic basis. Few studies have investigated the role of heredity in determining anti-predator behaviors (Breden et al. 1987; Garland 1988; Riechert and Hedrick 1990).

Given that we used test animals in our experiments that were collected as embryos, and had no prior exposure to *R. catesbeiana*, we consider the ability of *R. aurora* to recognize *R. catesbeiana* as predators to have some genetic

basis. Few studies have attempted to examine the role of genetic or learned factors in predator recognition in amphibians. Kats et al. (1988) demonstrated that several larval amphibians, collected as embryos from ponds with predatory fish, responded to chemical cues of these predators even in the absence of prior exposure. A similar finding has been reported by Sih and Kats (1994). However, many species of amphibian larvae collected as embryos from ponds without predatory fish, do not exhibit anti-predatory behaviors when exposed to chemical cues of these predators (Kats et al. 1988). Thus, the ability of larval amphibians to recognize predators has been suggested to be a genetic trait, based on the population's exposure to that particular predator (Kats et al. 1988).

There are numerous reports describing a negative relationship between the distribution of introduced *R. catesbeiana* and ranid frogs native to the western U.S. (e.g. Moyle 1973; Bury and Luckenbach 1976, Bury et al. 1980, Nussbaum et al. 1983; Hayes and Jennings 1986). In fact, it has been suggested that *R. catesbeiana* may be in part responsible for observed declines of *R. aurora* in Oregon (Nussbaum et al. 1983). Predation by *R. catesbeiana* on *R. aurora* larvae may contribute to the observed declines of *R. aurora* in Oregon. However, predators may also influence survival of prey by altering their activity levels and habitat use (Lima and Dill 1990; Skelly and Werner 1990;

Skelly 1991). Thus, *R. catesbeiana* may directly influence *R. aurora* survival through predation, but also may indirectly influence their survival through the anti-predator behaviors that they elicit.

The spread of exotic species is a global phenomenon that poses critcal problems for many ecosystems. This study demonstrates the importance of understanding the behavioral and context dependent responses of prey to predators and how this can influence the outcome of interactions between species. This is especially true in systems where species are in decline and where there have been introductions of exotic species.

ACKNOWLEDGEMENTS

We thank Cheri Miller and Brian Edmond for providing technical assistance, and D. Grant Hokit, Will Kane, Amy Kane, Jonas Henderson, Harvey Pell and Frank Miller for helpful discussions regarding experimental design. Doug Chivers, Erica Wildy, Jill DeVito, Adolfo Marco, David Reznick, David Skelly and an anonymous reviewer provided helpful suggestions on this manuscript. Thanks to Dave Debou and the staff at E.E. Wilson Wildlife Refuge for all the help they provided. Funding was provided by the Department of Zoology Research Funds and the National Science Foundation (DEB-9423333 to ARB).

CHAPTER 6

EXPERIMENTAL ANALYSIS OF THE EFFECT OF INTRODUCED BULLFROGS AND SMALLMOUTH BASS ON NATIVE RED-LEGGED FROGS (*RANA AURORA*).

JOSEPH M. KIESECKER

AND

ANDREW R. BLAUSTEIN

SUBMITTED TO CONSERVATION BIOLOGY.

ABSTRACT

We examined the direct and indirect effects of two introduced species, the bullfrog (*Rana catesbeiana*) and smallmouth bass (*Micropterus dolomieui*), on the microhabitat use, growth, development and survival of larval and metamorphic red-legged frogs, (*Rana aurora*). In field enclosure experiments, tadpoles of *R. aurora* altered their microhabitat use in the presence of bullfrog larvae and adults. The shift in microhabitat use by *R. aurora* corresponded with increased activity of adult *R. catesbeiana*. Time to metamorphosis increased and mass at metamorphosis decreased when *R. aurora* tadpoles are exposed to either larval or adult *R. catesbeiana*. In contrast, smallmouth bass alone had little effect on the growth and development of *R. aurora*. In all experiments survivorship of *R. aurora* was only significantly affected when *R. aurora* were exposed to the combined effects of bullfrog larvae and adults or bullfrog larvae and smallmouth bass. Thus, the interaction between stages (larval/adult) or species (bullfrog/smallmouth bass) produced indirect effects that were greater than when each factor was considered separately. Tests that examine multiple factors provide needed insights to the impacts of exotic species on native species.

INTRODUCTION

The introduction and spread of exotic species is a global phenomenon (Elton 1958) that poses critical problems for many natural ecosystems (Drake et al. 1989; Krebs 1994). The mechanisms that enable exotic species to thrive at the expense of native species are often unclear (Lodge 1993). While there are many examples of the decline of native species after the arrival of an exotic species (see reviews in Elton 1958; Bennett 1990; Lodge 1993; Krebs 1994), the mechanism underlying the decline is often unknown. Competition or predation is frequently proposed to explain population declines or habitat shifts of native species after exotic introductions. However, such mechanisms are rarely isolated and tested in an experimental setting. The importance of understanding the mechanisms that allow exotic species to thrive, often at the expense of native species takes on new urgency as invasions of alien species alter ecosystems (Vitousek 1989; Vitousek 1990; Lodge 1993), modify trophic structure (Wormington & Leach 1992; Holland 1993; Nicholis & Hopkins 1993) and displace species (Zaret & Paine 1973; Groves & Burdon 1986; Savidge 1988; Haag et al. 1993; Petren et al. 1993; Kupferburg 1995; Petren & Case 1996, Gamradt and Kats 1996, Gamradt et al. In Press).

Understanding the mechanisms that facilitate the success of exotic species can be particularly difficult when interactions between natives and exotics involve more than a

single developmental stage. Thus, the overall interaction between the two species may comprise a web of interactions containing both direct and indirect effects. For example, many organisms exhibit marked niche shifts and trophic level changes throughout their ontogeny. These ontogenetic shifts may influence interactions between species (Werner & Gilliam 1984; Stein et al. 1988; Olson et al. 1995). This may be especially true for many anuran amphibians which undergo a shift from herbivorous larvae to carnivorous adults (Duellman & Trueb 1986). Thus, to fully understand the overall impact that introduced species have on native species, experimental tests should include the various life stages of an exotic organism that may influence natives.

We present experimental evidence suggesting that introduced bullfrogs (*Rana catesbeiana*) in combination with smallmouth bass (*Micropterus dolomieui*) have negative impacts on native red-legged frogs (*Rana aurora*). Several studies have documented the decline of native ranid frogs after the introduction of bullfrogs (Moyle 1973; Bury & Luckenbach 1976; Green 1978; Hammerson 1982; Clarkson & DeVos 1986; Fisher & Shaffer 1996). However, these studies only suggest a negative association between bullfrogs and other frog species. Few studies have attempted to experimentally examine the mechanism by which introduced bullfrogs affect ranid frogs (but see Kupferberg 1995). We investigated: (1) the influence of non-native bullfrogs and

smallmouth bass on habitat use of *R. aurora* larvae in field enclosures; (2) the influence of bullfrogs and smallmouth bass on growth, development and survival of *R. aurora* larvae; (3) the separate and combined effects of stages (bullfrog larvae and adult) and species (bullfrog and smallmouth bass) on larval *R. aurora*.

Natural History and Study System

Rana aurora occurs west of the Cascade-Sierra Nevada Ranges from British Columbia, Canada to northern Baja California, USA (Stebbins 1985). Breeding habitats vary from small ephemeral ponds to large lakes. In Oregon *R. aurora* breed from December to February, and larvae reach metamorphosis in 2 to 3 months (Nussbaum et al. 1983). After breeding, adult red-legged frogs are highly terrestrial and can be found far from aquatic habitats (Nussbaum et al, 1983; pers. obs.). Like some other species, *R. aurora* has exhibited marked range contractions and population declines (e.g. Crump et al. 1992; Richards et al. 1993; Blaustein et al. 1994a; Pounds et al. In Press) and is currently a candidate for federal endangered species status (Nussbaum et al. 1983; Federal Register 1996; Stebbins & Cohen 1995; Fisher & Shaffer 1996).

Interactions with introduced bullfrogs are continually invoked as a primary cause for losses of red-legged frogs (e.g. Moyle 1973; Bury & Luckenbach 1976; Bury et al. 1980;

Nussbaum et al. 1983; Hayes & Jennings 1986; Blaustein 1994). *Rana catesbeiana* is native to the eastern United States, occurring naturally as far west as the great plains (Nussbaum et al. 1983; Stebbins 1985). However, bullfrogs have been extensively introduced throughout much of the western United states (Hayes & Jennings 1986; Stebbins & Cohen 1995). In Oregon, bullfrogs were first introduced in the late 1920s or early 1930s and now occur in much of Oregon west of the Cascades mountains (Nussbaum et al. 1983).

Adult bullfrogs feed on a variety of aquatic prey, including other amphibians (Corse & Metter 1980; Bury & Whelan 1986; Beringer & Johnson 1995; Werner et al. 1995). Both tadpoles and adults of *R. catesbeiana* are known to prey on tadpoles of other species (e.g. Ehrlich 1979; Bury & Whelan 1986; Werner et al. 1995; Kiesecker and Blaustein 1997b). In Oregon, bullfrogs typically breed from June to August and larval bullfrogs take 1-3 years to reach metamorphosis (Nussbaum et al. 1983). Thus, the larvae of native species of frogs such as *R. aurora* may be exposed to larger, older bullfrog tadpoles.

The introduction of several species of non-native predatory fish, including smallmouth bass (*Micropterus dolomieui*), may contribute to population declines of ranid frogs (Hayes & Jennings 1986). Smallmouth bass are known to prey on larval amphibians, including red-legged frog larvae

(Scott & Crossman 1973; Kruse & Francis 1977, pers. obs.). Historically, smallmouth bass were restricted to central and eastern North America, but have since been introduced throughout western North America (Lee et al. 1980; Minckley & Deacon 1991). Predation by non-native fish has negative effects on native frog populations (Bradford 1989; Bradford et al. 1993). Furthermore, exotic fish may have indirect effects by introducing pathogens that can be transmitted to amphibians (Blaustein et al. 1994b; Kiesecker and Blaustein 1995, Kiesecker & Blaustein 1997a).

The specific impacts of bullfrogs or fish on native frog populations is often unclear because at many sites their introductions have occurred simultaneously. Potentially the influence that one introduced species has may vary in the presence of the other. For example, *R. aurora* larvae are known to alter their behavior in the presence of bullfrog adults and larvae (Kiesecker & Blaustein 1997b). Changes in behavior in the presence of bullfrogs may make *R. aurora* larvae more susceptible to predation by predatory fish. Also, changes in behavior may influence microhabitat use which may in turn influence growth and development.

METHODS AND MATERIALS

Collection and Maintenance

All red-legged frog larvae used in experiments were collected as embryos (20 clutches; 12 in 1994, 8 in 1995) from a marsh adjacent to Dorena Lake (12 miles south of Springfield, Lane County, Oregon, USA) and transported to our laboratory in Corvallis, Oregon. Bullfrog adults and tadpoles used in experiments were collected from the north marsh on E.E. Wilson Wildlife Refuge (Benton County, Oregon).

We kept *R. aurora* eggs in aerated 38 L aquaria filled with dechlorinated tap water. After hatching, tadpoles were transported to field enclosures for use in experiments # 1 and # 3 (see below). Approximately 600 tadpoles remained in the laboratory until metamorphosis, at which time they were transported to field enclosures for use in experiment # 2 (see below). *Rana catesbeiana* tadpoles were reared for approximately 7 days in 38 L aquaria, after which time they were transported to field enclosures. While in the laboratory, all animals were maintained on a 14L:10D photoperiod, at a temperature of approximately 15°C, and were maintained on a diet of ground rabbit chow.

Field studies took place at E.E. Wilson Wildlife Area (18 km north of Corvallis, Benton Co., Oregon USA. The site previously contained several breeding populations of *R. aurora* (R.M. Storm pers. comm.) but currently *R.*

catesbeiana, Pacific Treefrog (*Hyla regilla*), long-toed salamamder (*Ambystoma macrodactylum*), Northwestern salamander (*A. gracile*) and roughskinned newt (*Taricha granulosa)* are the only amphibians known to breed there (pers. obs.). Several marshes occur at the site; experiments were conducted in the south marsh area, approximately 2 hectares in area.

Experiment 1

We assessed the impact of larval and adult bullfrogs on *R. aurora* habitat use, growth, development and survival in experiments at the south marsh in E.E. Wilson Wildlife Refuge. We used rectangular open bottom pens (8 x 4m, $32m^2$) constructed of 1 mm^2 mesh fiberglass screen that was pressed approximately 20 cm into the soft mud substrate. Each enclosure was placed perpendicular to the shore line. The depth of water in each enclosure ranged from approximately 10 cm near the shore to approximately 1.5 meters away from the shore. Before tadpoles were added, enclosures were cleared of their macrofauna (e.g. corixids, notonectids) by repeated sweeping with a net. After tadpoles were added, invertebrates were allowed to colonize the enclosures naturally. Other than the experimental animals no vertebrates were observed in the enclosures. To minimize

potential spatial gradients (e.g. temperature, vegetation) from confounding treatment effects, enclosures were blocked and treatments were assigned randomly within the blocks.

We used a fully factorial design with all combinations of the presence and absence of larval and adult bullfrogs. Four bullfrog treatments, each replicated three times for a total of 12 enclosures, were + adult (1 bullfrog adult, 150 R. aurora larvae); + larvae (50 bullfrog larvae, 100 *R. aurora* larvae); +adult/+larvae (1 bullfrog adult, 50 bullfrog larvae, 100 *R. aurora* larvae); - adult/- larvae (150 *R. aurora* larvae). We controlled for overall density of larvae (150) to ensure that negative effects of *R. catesbeiana* larvae on *R. aurora* were due to interspecific effects and not increased density (see Connell 1980, Underwood 1986). Densities of both species were comparable to densities observed at other field sites (J. Kiesecker *unpublished data*). All hatchling *R. aurora larvae* (0.01 +/- 0.001 g, mean +/- 1 S.E.; Gosner Stage 25, Gosner 1960; n = 100) were matched for developmental stage and size, as were 1st year *R. catesbeiana* larvae (7.8 +/- 1.1 g, mean +/- 1 S.E.; Gosner stage 25, Gosner 1960, n = 300). *Rana catesbeiana* adults were also matched for size. (114.7 +/- 13.1 g, mean +/- 1 S.E.; n = 6).

To reflect the natural breeding phenology in ponds where the two species are found, *R. catesbeiana* adults and larvae were present in the enclosures prior to the addition of *R.*

aurora larvae (Nussbaum et al. 1983). Adult and larval *R. catesbeiana* were added to enclosures on 27 October 1993 and 16 January 1994 respectively. Hatchling *R. aurora* were added on 30 January 1994.

Once every seventh day the position of tadpoles within the enclosures was determined by using funnel traps placed at 2, 4 and 6 m from the shore line. Traps were placed in enclosures for 24 hrs and we identified the species and counted the number of tadpoles captured at each trap. We consistently captured 80% or more of the tadpoles in each enclosure during sampling. Traps measured 72 x 55 x 20 cm and were constructed of fiberglass screen and wire. Animals entered traps via an inward directed cone 23 cm long at each end of the trap. Mouth and apical openings were 15 and 5 cm in diameter, respectively. In each enclosure, the average position of tadpoles of each species was estimated by ranking the number of tadpoles at each distance and dividing this by the total number of tadpoles caught in the traps to give the average ranked tadpole position. Water temperature was measured at each trap 3 times a day (6 am, 12 pm and 6 pm) using a Barnant 115 thermocoupler. Adult bullfrog activity was assessed with daily visual surveys.

We terminated the experiment when all *R. aurora* had either metamorphosed or died. Our criterion for metamorphosis was front limb emergence (Gosner stage 42, Gosner 1960). We checked for metamorphs daily. Individuals

were removed from the enclosures as they metamorphosed, and mass (to the nearest mg) at and time (in days) to metamorphosis was recorded.

Experiment 2

We assessed the impact of adult *R. catesbeiana* presence on survival of *R. aurora* metamorphs (Gosner stage 44). Enclosures used in this experiment were identical to those used in experiment 1, except that one half of each enclosure was in water and one half was on land. The depth of water in the deep end of each enclosure was approximately 1.0 m. This allowed metamorphs of *R. aurora* to complete metamorphosis and move on to land. A line of pitfall traps (4 m long, 20 cm wide) were placed on the terrestrial portion of the enclosures, 2 m from the shore line. This ensured that any of the metamorphs would be captured as they moved on to land. Enclosures were blocked and treatments were randomly assigned within blocks.

In six of the enclosures, a single adult *R. catesbeiana* was added on 20 March 1994. On 27 March 1994, 50 *R. aurora* metamorphs were added to all twelve enclosures. Metamorphs were animals raised (see above) in our lab for this experiment. Densities of both species were comparable to densities observed at other field sites (J. Kiesecker *unpublished data*). All *R. aurora* (1.11 +/- 0.41 g, mean +/- 1 S.E.; Gosner Stage 44, Gosner 1960; n = 600) metamorphs

were matched for developmental stage and size. *Rana catesbeiana* adults were also matched for size (124.3 +/- 17.1 g, mean +/- 1 S.E.; n = 6).

The experiment was terminated when all *R. aurora* had either metamorphosed or died. We removed individuals from the pitfall traps each day and recorded the number surviving in each enclosure. Funnels over the top of the pitfall traps prevented bullfrog adults from eating *R. aurora* once they were inside the traps.

Experiment 3.

We evaluated the combined effects of larval bullfrogs and introduced fish on *R. aurora* habitat use, survival growth and development with a field experiment. Enclosures and procedures used in this experiment were identical to those used in experiment 1.

In a fully factorial design all combinations of the presence or absence of smallmouth bass and larval bullfrogs were crossed. The resulting four treatments were each replicated three times for a total of twelve enclosures. Smallmouth bass and *R. catesbeiana* larvae were both added on 1 February 1995, while *R. aurora* larvae were added to enclosures on 15 February 1995.

Densities of both species were comparable to densities observed at other field sites (J. Kiesecker *unpublished data*). All hatchling *R. aurora* (0.02 +/- 0.004 g, mean +/- 1

S.E.; Gosner Stage 25, Gosner 1960; n = 100) larvae were matched for developmental stage and size, as were 1st year *R. catesbeiana* larvae (6.9 +/- 1.1 g, mean +/- 1 S.E.; Gosner stage 25, Gosner 1960, n = 300) *Micropterus dolomieui* were also matched for total length (113.7 +/- 10.1 mm, mean +/- 1 S.E.; n = 6).

Statistical Analyses

Multivariate analysis of variance (MANOVA) was used to test for differences in independent factors (larval bullfrogs and adult bullfrog, experiment 1 and larval bullfrogs and smallmouth bass, experiment 3) on the dependent variables time (mean days to metamorphosis), mass (mean mass at metamorphosis) and survivorship (mean survivorship to metamorphosis) (Tabachnick & Fidell 1989). After MANOVA, we used Bonferroni adjusted univariate analysis of variance (ANOVA) on each response variable to assess which variables were responsible for significant main effects. Post hoc comparisons (Tukey Test) were performed to test for differences between means among the treatments (Zar 1984). For experiments 1 and 3 we also tested for differences in *R. aurora* microhabitat use by using a two-way repeated measures ANOVA. The average ranked tadpole position of each enclosure from each sampling period was used in

statistical tests. For experiment 2 we tested for differences in survival between treatments with a Student's t test.

For all experiments a preliminary analysis indicated no significant block effects. Therefore, the block and error terms were pooled for remaining tests (Zar 1984). Because individuals in enclosures are not independent of one another these measures were analyzed as enclosure means. For all experiments, parametric assumptions were met and no data transformations were necessary.

RESULTS

Experiment 1

The presence of *R. catesbeiana* adults and tadpoles significantly interacted to effect *R. aurora* growth, development and survivorship (Table 6.1). Below we interpret these effects for each response variable employing the univariate tests and post hoc comparison (Tukey tests).

Mass - Exposure to both adult and larval bullfrogs influenced *R. aurora* mass at metamorphosis (Table 6.1, Figure 6.1). Mass was greatest for *R. aurora* when alone, compared to any of the other treatments (Tukey HSD $p < 0.001$, Figure 6.2). Larval bullfrogs decreased the mass at metamorphosis of *R. aurora* more so than did adult bullfrogs (Tukey HSD $p < 0.01$, Figure 6.1). Red-legged frogs with only adult bullfrogs had a higher mass than red-legged frogs with both adult and larval bullfrogs (Tukey HSD $p = 0.02$). Mass at metamorphosis for *R. aurora* exposed only to larval bullfrogs did not differ from mass in the combined treatment (Table 6.1, Tukey HSD $p \leq .098$), suggesting that larval bullfrog had the stronger affect on red-legged frogs.

Time - Both adult and larval bullfrogs increased time to metamorphosis of red-legged frogs (Figure 6.1, Table 6.1). Time to metamorphosis was fastest for *R. aurora* when alone, compared to any of the other treatments (Tukey HSD $p < 0.01$, Figure 6.1). There was no difference in time to metamorphosis between *R. aurora* exposed to either adult or

Table 6.1 Results of MANOVA for overall effects of *Rana catesbeiana* adults and tadpoles on *Rana aurora* survival, growth and time to metamorphosis and ANOVAs for each response variable. Response variables are proportion surviving (survival), mass at metamorphosis (mass) and time to metamorphosis (time).

MANOVA	F	D.F.	P
Constant	5827.785	3,6	< 0.001
Adult	95.357	3,6	< 0.001
Larvae	43.594	3,6	< 0.001
Adult x Larvae	15.023	3,6	0.003
ANOVAs	F	D.F.	P
Mass			
Adult	79.852	1,8	< 0.001
Larvae	20.136	1,8	0.002
Adult x Larvae	2.767	1,8	0.135
Time			
Adult	66.057	1,8	< 0.001
Larvae	30.229	1,8	0.001
Adult x Larvae	0.914	1,8	0.367
Survival			
Adult	51.682	1,8	< 0.001
Larvae	49.960	1,8	< 0.001
Adult x Larvae	49.723	1,8	< 0.001

Significance level for univariate tests is 0.0125 (Bonferroni-adjusted for three response variables).

Figure 6.1 Mean (+/- 1 S.E) mass at, time to, and survival to metamorphosis for *Rana aurora* larvae exposed to larval and adult bullfrogs (*Rana catesbeiana)* in field enclosures.

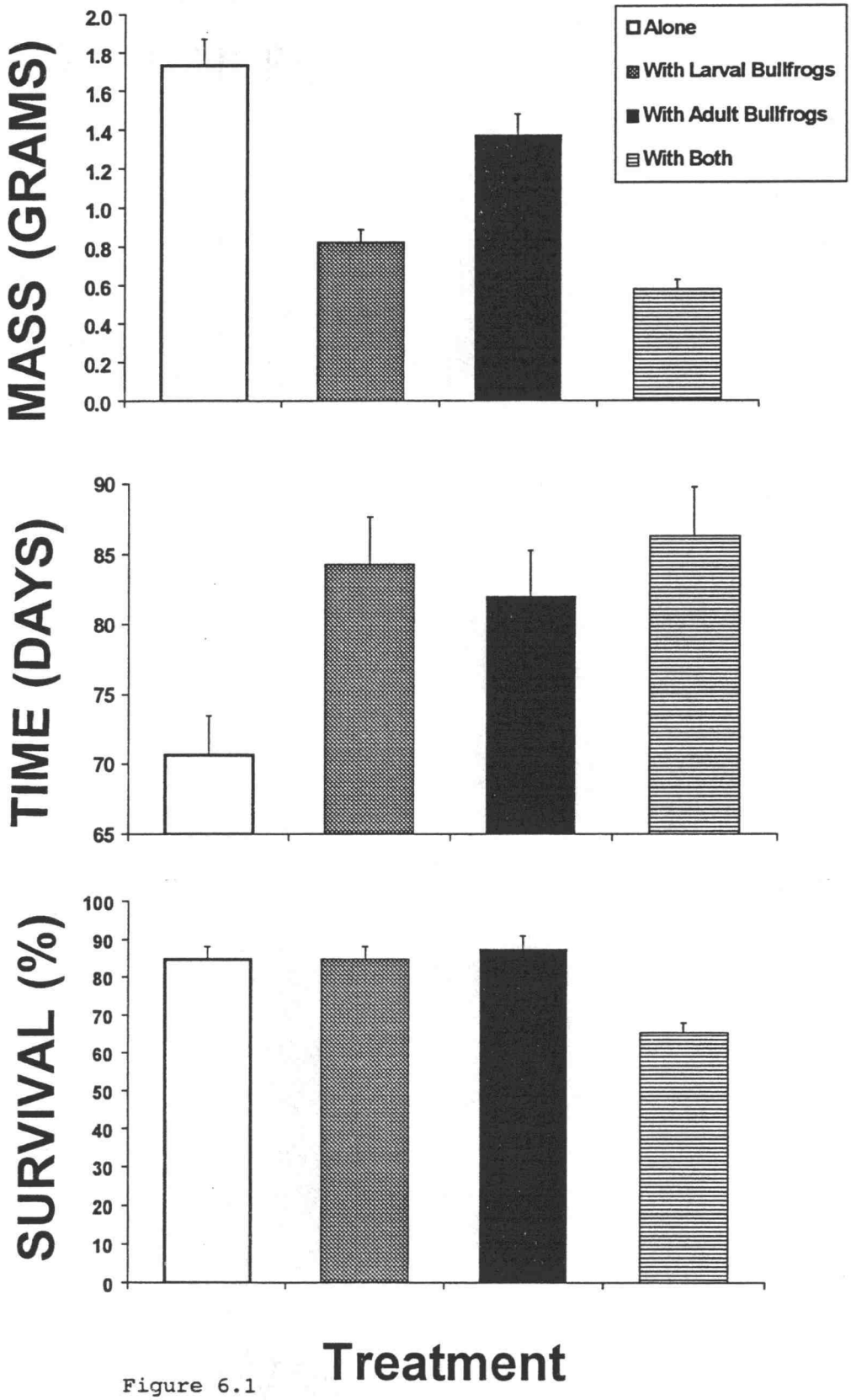

MASS (GRAMS)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Alone
With Larval Bullfrogs
With Adult Bullfrogs
With Both
TIME (DAYS)
90
85
80
75
70
65
SURVIVAL (%)
100
90
80
70
60
50
40
30
20
10
0
Treatment

Figure 6.1

Figure 6.2 Mean ranked position (+/- 1 S.E.) of *Rana aurora* tadpoles when alone (A), with *R. catesbeiana* larvae (B), with *R. catesbeiana* adults (C), or with both *R. catesbeiana* larvae and adults (D). Arrows indicate the start of adult activity.

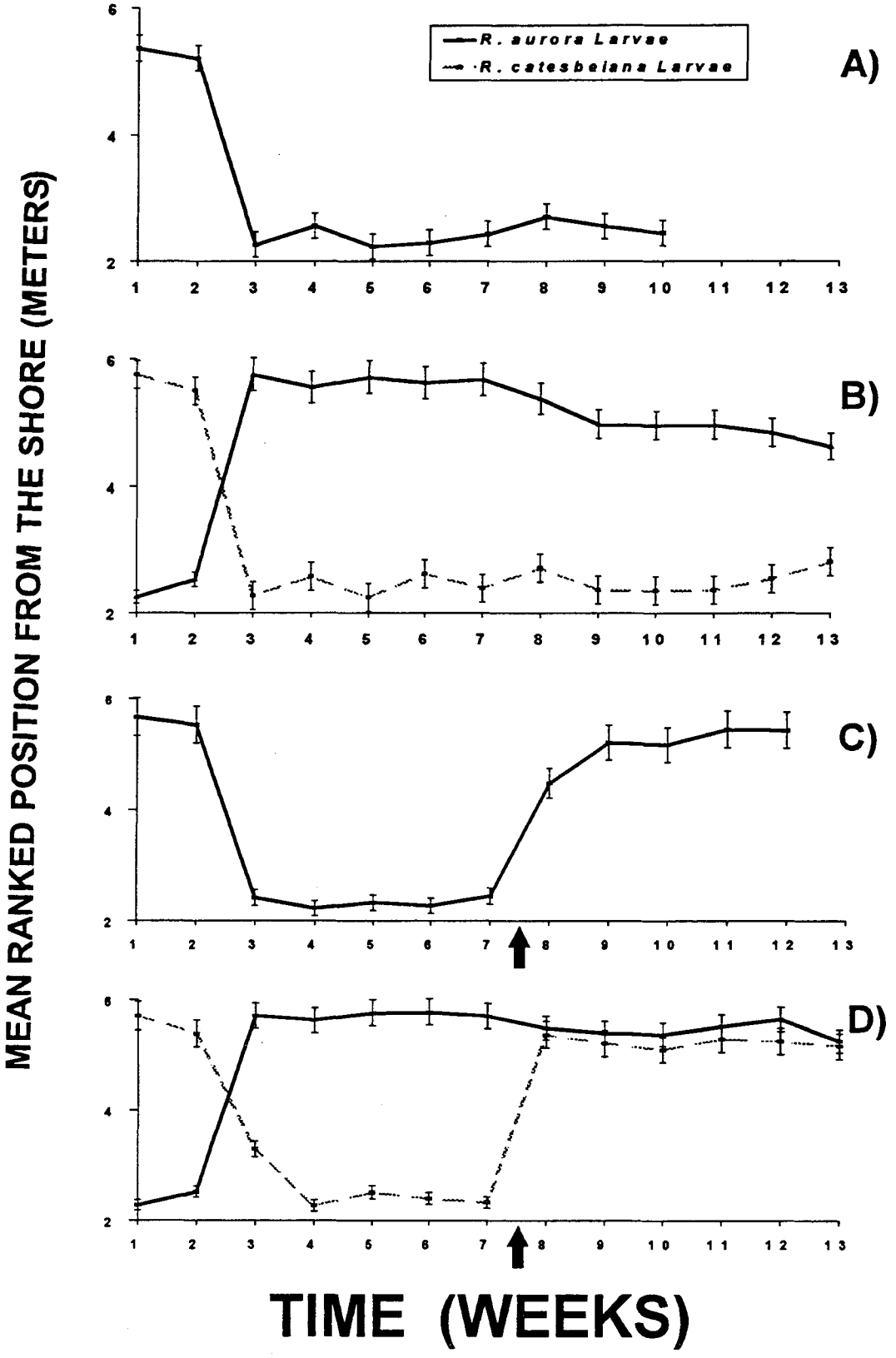

R. aurora Larvae
R. catesbeiana Larvae
A)
B)
C)
D)
MEAN RANKED POSITION FROM THE SHORE (METERS)
TIME (WEEKS)

Figure 6.2

larval bullfrogs only (Tukey HSD p = .67), suggesting that both adult and larval bullfrog had similar effects on *R. aurora* developmental time.

Survivorship - The combined effects of both adult and larval bullfrogs influenced survival to metamorphosis of *R. aurora* (Figure 6.1, Table 6.1). Survivorship was generally high for *R. aurora* when alone, or either with bullfrog tadpoles only, or bullfrog adults only, averaging 84.3%, 86.1% and 82.3% respectively. However, the survivorship of *R. aurora* was (Tukey HSD $p < 0.01$) decreased to 69.3% when in the presence of both larval and adult bullfrogs. Survivorship for adult and larval bullfrogs was 100% and 94.7 % respectively.

Habitat Use

Microhabitat use by larval *R. aurora* changed during the experiment and was significantly altered by the presence of adult and larval bullfrogs (Table 6.2). When *R. aurora* larvae were alone they were found in the warmest areas of the enclosures (Figure 6.2a, Table 6.3). This was also true for larval bullfrogs when they were only with *R. aurora* larvae (Figure 6.2b, Table 6.3). Overall, average water temperatures increased during the experiment (Table 6.3). The warmest water temperatures were found in the deep end of the enclosures for the first two weeks and then in the shallow ends for the remainder of the experiment (Table

Table 6.2 Repeated measures ANOVA results on the effects of presence of *Rana catesbeiana* larvae and adults on the space use of red-legged frog tadpoles.

BETWEEN SUBJECTS			
Source	d.f.	F-ratio	p value
Adult	1	34.118	< 0.0001
Larvae	1	141.492	< 0.0001
Adult x Larvae	1	87.102	< 0.0001
Error	8		
WITHIN SUBJECTS			
Source	d.f.	F-ratio	p value
Time	9	21.785	< 0.0001
Time x adult	9	21.902	< 0.0001
Time x Larvae	9	348.772	< 0.0001
Time x Adult x Larvae	9	25.169	< 0.0001
Error	72		

Table 6.3 Mean water temperature (°C) taken from January 30 1994 to May 1, 1994 during the 24 hour trapping period for experiment 1.

	Distance from the shore		
	2 meters	4 meters	6 meters
Week			
1	6.8	10.1	11.4
2	7.8	8.9	11.3
3	13.6	10.4	11.7
4	13.9	12.6	12.9
5	15.6	12.7	13.2
6	16.8	13.7	14.2
7	18.6	12.5	13.6
8	18.3	13.6	13.2
9	18.6	14.2	13.3
10	19.4	14.6	13.4
11	21.7	14.5	13.2
12	22.9	14.3	13.6
13	23.3	14.2	13.0

6.3). When exposed to bullfrog larvae, *R. aurora* larvae used a different portion of the enclosures compared to when they were alone (Figure 6.2a,b). Adult bullfrogs also influenced the habitat use of *R. aurora* larvae. Adult bullfrogs remained active for two weeks (27 October to 10 November) after being introduced into the enclosures, and activity was not observed again until 20 March 1994 (week 7). When adult bullfrogs became active, the mean ranked distance from shore of both bullfrog and *R. aurora* larvae shifted from the shallow end of the enclosures to the deep end (Figure 6.2). *Rana aurora* larvae in the combined treatment had a mean ranked distance from the shore similar to when they were with bullfrog larvae only. However, bullfrog larvae also shifted position in response to adult activity. Thus, in the combined treatment, the mircohabitat use of both species of larvae overlapped for the last six weeks of the experiment.

Experiment 2

There were significant effects of adult bullfrogs on the survival of *R. aurora* metamorphs ($t_{11} = 4.7$, $p < 0.001$). Survival of metamorphs in the presence and absence of adult bullfrogs was 27.7% and 84.7%. All adult *R. catesbeiana* survived the experiment.

Experiment 3.

Both *R. catesbeiana* larvae and smallmouth bass influenced *R. aurora* growth, development and survival (Table 6.4). Below we interpret these effects for each response variable employing the univariate tests and post hoc comparison (Tukey tests).

Mass - As in experiment 1, bullfrog larvae reduced mass at metamorphosis of *R. aurora* (Figure 6.3, Table 6.4). However, smallmouth bass did not influence mass of *R. aurora*, either when alone or with bullfrog larvae (Tukey HSD $p = 0.47$). Mass was greatest for *R. aurora* when alone, or with smallmouth bass alone compared to any of the other treatments (Tukey HSD $p < 0.01$, Figure 6.3).

Time - Time to metamorphosis was similarly affected by bullfrog larvae and smallmouth bass. (Figure 6.3, Table 6.4). Time to metamorphosis was fastest for *R. aurora* when alone or exposed to smallmouth bass only, compared to any of the other treatments (Tukey HSD $p < 0.01$, Figure 6.3). There was no significant difference in time to metamorphosis for *R. aurora* between the bullfrogs only treatment, and the combined treatment (Tukey HSD $p = .483$).

Survivorship - The combined effects of both larval bullfrogs and smallmouth bass influenced survival to metamorphosis of *R. aurora* (Figure 6.3, Table 6.4). Survivorship was generally high for *R. aurora* when alone, or either with

Table 6.4 Results of MANOVA for overall effects of bullfrog larvae and smallmouth bass on *Rana aurora* tadpole growth, development and survival, and ANOVAs for each response variable. Response variables are proportion surviving (survival), mass at metamorphosis (mass) and time to metamorphosis (time).

MANOVA	F	D.F.	P
Constant	9657.726	3,6	< 0.0001
Bass	73.692	3,6	< 0.0001
Bullfrog Larvae	91.316	3,6	< 0.0001
Bass x Bullfrog	53.573	3,6	0.003
ANOVAs	F	D.F.	P
Mass			
Bass	0.071	1,8	0.796
Bullfrog	89.623	1,8	< 0.0001
Bass x Bullfrog	0.210	1,8	0.659
Time			
Bass	1.47	1,8	0.197
Bullfrog	40.186	1,8	< 0.0001
Bass x Bullfrog	0.496	1,8	0.501
Survival			
Bass	272.283	1,8	< 0.0001
Bullfrog	304.713	1,8	< 0.0001
Bass x Bullfrog			

(Table 6.4 continued)

195.709	1,8	< 0.0001

Significance level for univariate tests is 0.0125

(Bonferroni-adjusted for three response variables).

Figure 6.3 Mean (+/- 1 S.E.) mass at, time to, and survival to metamorphosis for *Rana aurora* larvae exposed to larval *Rana catesbeiana* and smallmouth bass in field enclosures.

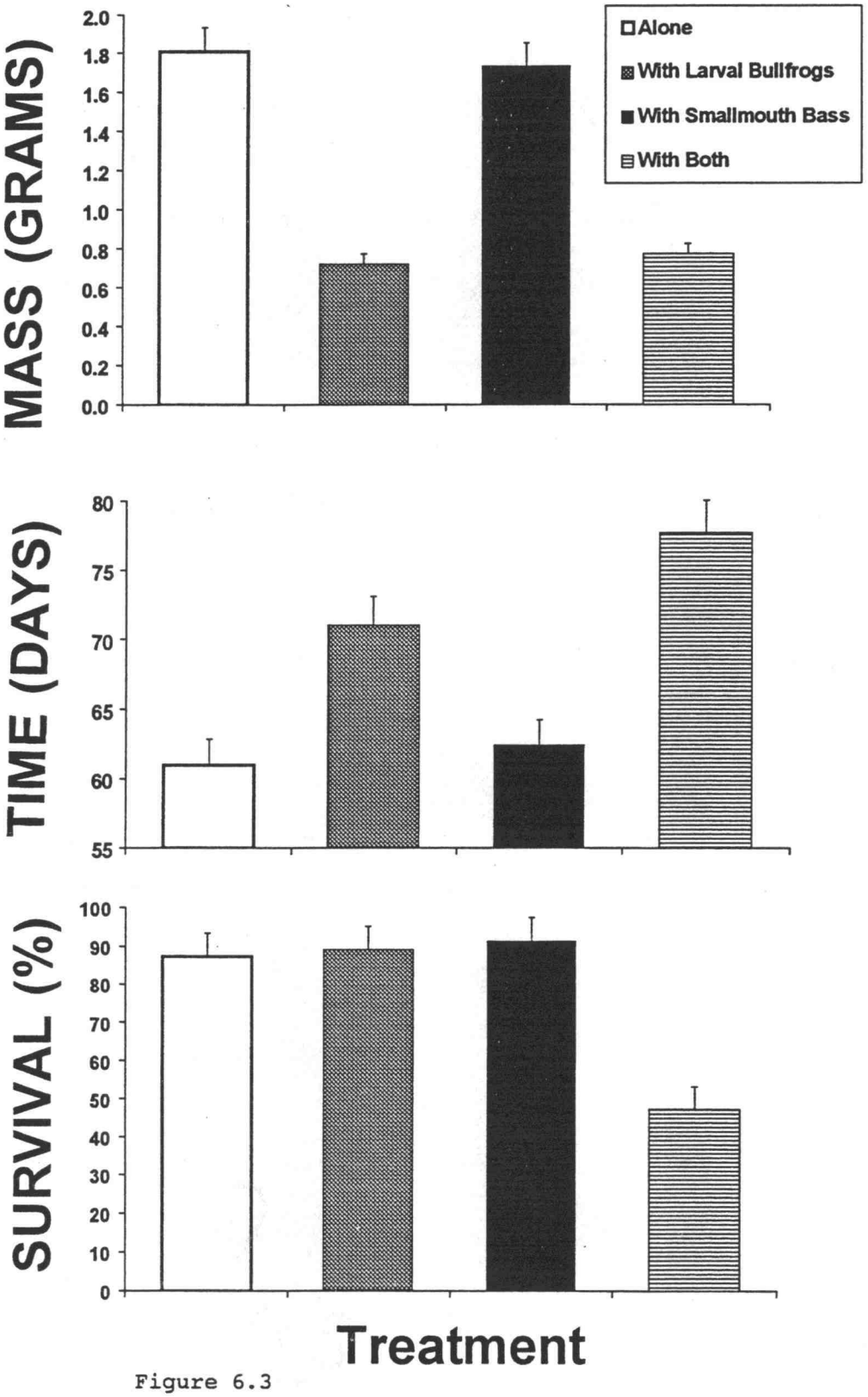

MASS (GRAMS)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Alone
With Larval Bullfrogs
With Smallmouth Bass
With Both
TIME (DAYS)
80
75
70
65
60
55
SURVIVAL (%)
100
90
80
70
60
50
40
30
20
10
0
Treatment

Figure 6.3

bullfrog tadpoles only, or smallmouth bass only, averaging 87.3%, 89.1% and 91.3%. However, the survivorship of *R. aurora* was decreased to 47.3% (Tukey HSD $p < 0.01$) when in the presence of both larval bullfrogs and smallmouth bass. Survivorship for smallmouth bass and *R. catesbeiana* larvae was 100% and 97.4% respectively.

Habitat Use

Microhabitat use by larval *R. aurora* was significantly altered by the presence of larval bullfrogs and smallmouth bass (Table 6.5). Overall, microhabitat use by *R. aurora* larvae when alone or with bullfrog larvae was similar to that observed in experiment 1. When alone, *R. aurora* tended to be found in the warmest areas of the enclosure, which was also true for larval bullfrogs (Figure 6.4, Table 6.6). *Rana aurora* exposed only to smallmouth bass, used a microhabitat that was similar to when they were alone. However, their use of microhabitat changed when in the presence of both bullfrogs and bass compared to bullfrogs only (Table 6.5). The mean ranked distance from the shore of *R. aurora* in the combined treatment was not as high compared to the bullfrog only treatment (Figure 6.4). This suggests that red-legged frog larvae responded to smallmouth bass, but only after they have moved into deeper water in the presence of bullfrogs.

Table 6.5 Repeated measures ANOVA results on the effects of presence of *Rana catesbeiana* larvae and smallmouth bass on the space use of red-legged frog tadpoles.

Experiment 3

BETWEEN SUBJECTS

Source	d.f.	F-ratio	p value
Bass	1	6.698	0.032
Bullfrog	1	733.223	< 0.0001
Bass x Bullfrog	1	7.713	0.024
Error	8		

WITHIN SUBJECTS

Source	d.f.	F-ratio	p value
Time	8	1.117	0.364
Time x Bass	8	1.246	0.287
Time x Bullfrog	8	1.220	0.302
Time x Bass x Bullfrog	8	0.673	0.713
Error	64		

Table 6.6 Mean water temperature (°C) taken from February 1 1995 to May 10, 1995 during the weekly 24 hour trapping period for experiment 3.

	Distance from the shore		
	2 meters	4 meters	6 meters
Week			
1	14.3	11.1	12.4
2	14.8	9.7	11.3
3	15.6	10.4	11.7
4	16.9	13.6	12.9
5	17.6	14.7	13.2
6	17.8	14.9	13.8
7	18.6	13.5	13.6
8	20.3	14.6	13.2
9	21.6	14.2	13.3
10	22.4	14.6	13.4

Figure 6.4 Mean ranked position (+/- 1 S.E.) of *Rana aurora* tadpoles when alone (A), with *R. catesbeiana* larvae (B), with smallmouth bass (C), or with both *R. catesbeiana* larvae and smallmouth bass (D).

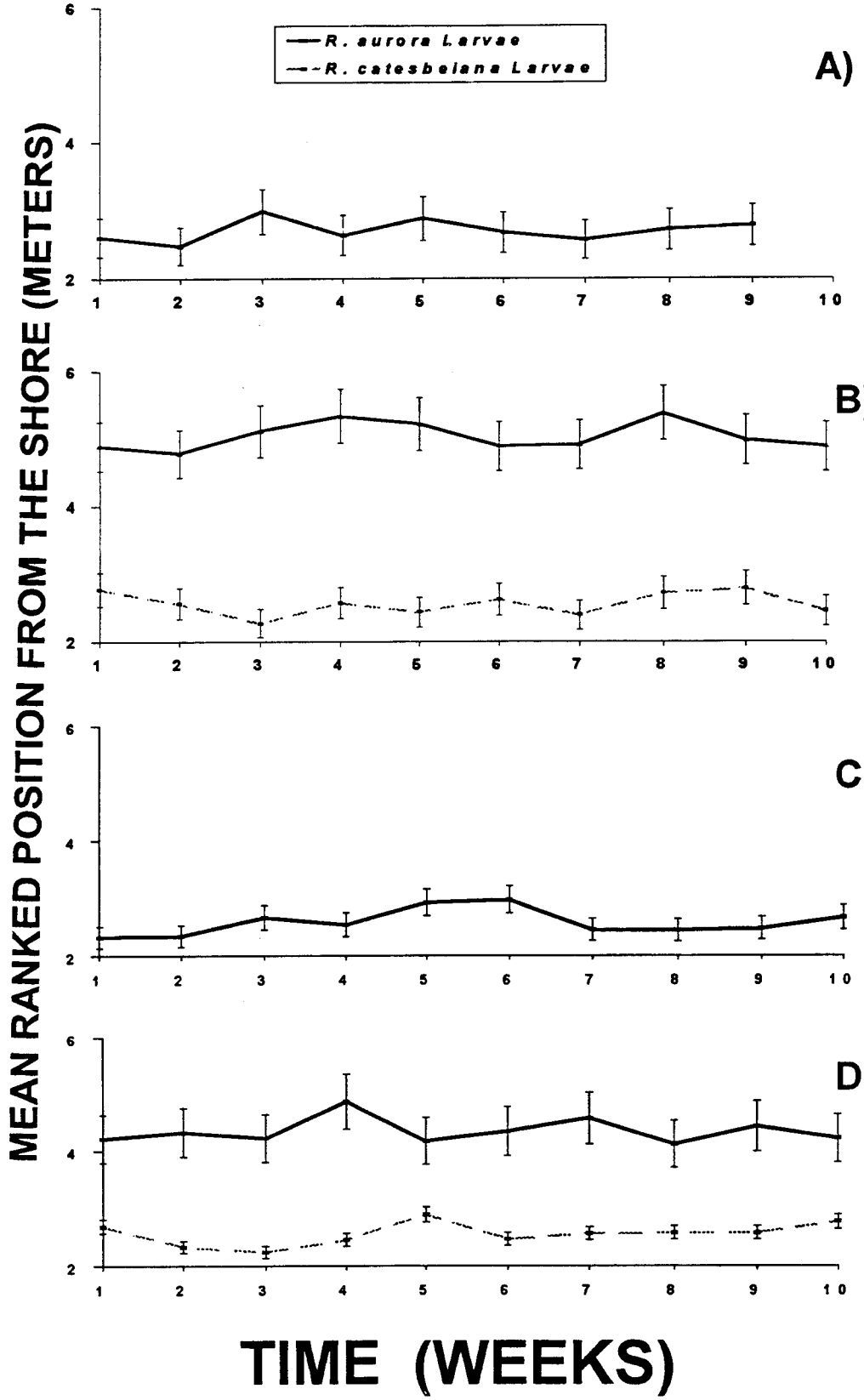

R. aurora Larvae
R. catesbeiana Larvae
A)
B)
C)
D)
MEAN RANKED POSITION FROM THE SHORE (METERS)
TIME (WEEKS)

Figure 6.4

DISCUSSION

The results of this study clearly show the negative impacts of bullfrogs in conjunction with smallmouth bass on red-legged frogs. The presence of these species resulted in alteration of microhabitat use, slower growth, reduced development and effects on survival. Our experiments stress the importance of understanding the context-dependent nature of interactions between native and exotic species. For example, in experiment 3, smallmouth bass alone appeared to have little effect on *R. aurora*. Yet, in the combined treatment, there was indeed an effect of bass on *R. aurora*. Similarly, in experiment 1, the combined treatments had the strongest effect on *R. aurora* larvae. Thus, the interaction between stages (larval/adult) or species (bullfrog/smallmouth bass) may produce indirect effects that are greater than when each factor is considered separately.

Amphibians have complex life cycles, which exposes them to a diversity of possible interactions with invading species, and hence a diversity of ways to be adversely affected by invaders. This is especially true if the introduced species also has a complex life cycle. Thus, introduced species may produce a series of direct and indirect effects that can impact natives. Below we will discuss the direct and indirect interactions between red-legged frogs and the introduced species involved in this study.

Microhabitat Use

The key feature in the interaction of bullfrogs with red-legged frogs appears to be the alteration of microhabitat use. Many studies have documented the behavioral responses of prey to the presence of their predators (see Sih 1987; Lima & Dill 1990; Petranka et al. 1987; Wilson & Lefcort 1993), indicating that such behavioral effects may be common. The mechanism responsible for the shift in microhabitat use are likely based on behavioral responses. In fact, we have observed that *R. aurora* react to the presence of *R. catesbeiana* by retreating and reducing activity levels (Kiesecker & Blaustein 1997b).

Several hypotheses may account for the negative associations between bullfrog larvae and red-legged frog larvae. First, habitat partitioning may be a result of competition. Although tadpoles of different species may have similar feeding morphology and behavior (Duellmann & Trueb 1986), they may differ considerably in their rates of removal of food resources (Seale & Wassersug 1979). Tadpoles that forage more efficiently could cause those that forage less efficiently to leave an area. Several studies have shown that larger larvae can monopolize clumped food resources and prevent smaller tadpoles from obtaining food through vigorous swimming and butting movements (e.g. Savage 1952; Wilbur 1977). These types of behaviors may lead to tadpoles segregating by size into different microhabitats

(Alford 1986; Alford & Crump 1982). Second, tadpoles may release substances that may inhibit the growth and development of other larvae (Steinwascher 1978; Beebee & Wong 1992; Hayes et al. 1993; Griffiths et al. 1993). Thus, *R. aurora* tadpoles may avoid areas where chemical substances could hamper their growth. Third, larvae of *R. catesbeiana* consume tadpoles of other species (Erhlich 1979). We have observed bullfrog larvae consuming hatchling *R. aurora* under laboratory conditions (Kiesecker & Blaustein 1997b). If this occurs under natural conditions, it would provide an obvious reason for the observed avoidance behavior.

Exposure to adult *R. catesbeiana* also caused a shift in microhabitat use by both *R. aurora* and *R. catesbeiana* larvae, that corresponded with increased adult activity. At the start of experiment 1, when temperatures were relatively cool (6.8 to 11.4 °C), adult *R. catesbeiana* were not active, and were presumably over-wintering in the mud (Nussbaum et al. 1983). Activity of adult bullfrogs paralleled an increase in water temperature during the experiment. Larvae of *R. aurora* likely shifted position to avoid predation by adult *R. catesbeiana*. Adult *R. catesbeiana* are aquatic predators consuming a broad diversity of prey, including other amphibians (Corse & Metter 1980; Bury & Whelan 1986; Clarkson & DeVos 1986; Schwalbe & Rosen 1988; Werner et al.

1995; Beringer & Johnson 1995). The use of deep water by *R. aurora* in the presence of adult *R. catesbeiana* may be an effective way for tadpoles to avoid predation.

In contrast, *R. aurora* did not alter habitat use when exposed to smallmouth bass only, probably because smallmouth bass were only found in water deeper than 1 meter. Thus, the emergent vegetation in the shallow end of the enclosures appeared to provide suitable refuge for *R. aurora* larvae. However, the response of *R. aurora* to bullfrogs was dependent on the presence of bass. This suggests that *R. aurora* larvae respond to bass when in the situations where bass are capable of preying them.

Growth and Development

Introduced aquatic predators and competitors may have strong effects on developmental and growth rates of larval amphibians. Increased developmental time and decreased mass at metamorphosis can influence individual fitness and thus may ultimately affect populations. Aside from increasing the time that larvae are subjected to aquatic predators (Morin 1983), an extended larval period also can affect the post-metamorphic stage by leaving inadequate time to store fat for winter survival (Berven & Gill 1983). Smaller post-metamorphoic size can decrease both survival and

reproductive success in the terrestrial environment (Woodward 1983; Berven & Gill 1983; Woodward 1987; Smith 1987; Berven 1990; Scott 1994).

Larval *R. aurora* exhibited increased time to metamorphosis and decreased mass at metamorphosis when exposed to either larval or adult *R. catesbeiana*. These effects on *R. aurora* may be due to the shift in *R. aurora* habitat use induced by bullfrogs. In the presence of active adult bullfrogs, *R. aurora* larvae were found in the cooler deep water of the enclosures. Temperature may have strong effects on the growth and development of larval amphibians (Duellman & Trueb 1986), and cooler temperatures experienced by *R. aurora* may explain increases in developmental time. Reduced activity also is common for prey in the presence of predators (Sih 1987; Lima & Dill 1990; Sih & Kats 1994) and can result in reduced growth and increased developmental time (Skelly & Werner 1990; Skelly 1992). Also, microhabitats may differ in more ways than just temperature. For example, the quality and quantity of food present were also likely to be different. This difference in resources between microhabitats also may influence *R. aurora* larvae because tadpoles are sensitive to diet quality as well as quantity (Kupferberg et al. 1994).

The influence of larval bullfrogs on *R. aurora* is difficult to interpret. Interspecific competition with bullfrog larvae can decrease survivorship and growth of

native tadpoles (Kupferburg 1995). However, in both experiment 1 and 3, *R. aurora* shifted habitat use in the presence of *R. catesbeiana* larvae. Thus, the reduced growth and increased time to metamorphosis experienced by *R. aurora* may be due to competition between the two species or a result of habitat alteration and the less favorable conditions associated with the alternative microhabitat.

Survival

Populations of native species may decline if introduced species affect their recruitment. Amphibians, because of their complex life cycles can potentially be influenced in both the aquatic and terrestrial environments. Survival of native amphibians could be affected in numerous ways by the concurrent introduction of both bullfrogs and predatory fish. Attempts to assess any of these factors in isolation may bypass potential interactions that ultimately may explain native species losses.

Survivorship of *R. aurora*, in both experiments #1 and #3, was only affected in the combined treatments. In experiment 1 we do not know the specific causes of this mortality, but we presume that it was due to the synergistic effects of reduced activity in the presence of adult bullfrogs and low food resources due to overlap with larval bullfrogs. Both of these effects may lead to poorer conditions and increased chance of starvation. However, although we cannot rule out

predation by adult *R. catesbeiana,* we consider this unlikely since survival of red-legged frog larvae was not affected when they were exposed to adult bullfrogs only. However, the results of experiment 2 suggest that the influence of adult *R. catesbeiana* can be largely underestimated if the experiments are terminated at metamorphosis because survival of metamorphic *R. aurora* was greatly reduced in the presence of *R. catesbeiana* adults.

In experiment 3, the habitat shift of *R. aurora* in the presence of bullfrog larvae likely led to increased predation by smallmouth bass. Smallmouth bass were mainly observed in deeper water of the enclosures. The shift in microhabitat use by *R. aurora* likely led to increased overlap between *R. aurora* and smallmouth bass. Bass (*Micropterus sp.*) are efficient predators of larval amphibians (Scott & Crossman 1973; Kruse & Francis 1977) and under laboratory conditions smallmouth bass readily consume *R. aurora* larvae (pers. obs).

Predicting Invasion Impact

Biological invasions pose a threat to ecological communities and global biodiversity (Elton 1958; Mooney & Drake 1986; Bennett 1990; Drake et al. 1989; Lodge 1993). In particular, biological invasions poses a serious threat to freshwater biodiversity (Taylor et al. 1984; Master 1990; Allan & Flecker 1993). For example, introduced organisms

have been associated with 68% of the forty North America fish extinctions that have occurred since the turn of the century (Miller et al. 1989). Bullfrogs may be of special interest because of their widespread introduction throughout the western United States, and have been implicated in losses of native ranid frogs (Moyle 1973; Hammerson 1982; Green 1978; Clarkson & DeVos 1986). Bullfrogs have also been introduced into other regions of the world, including Italy (Albertini & Lanza 1987), and the Netherlands (Stumpel 1992), where they may potentially have similar influences on other native species.

Knowledge of the mechanisms of how introduced species thrive at the expense of native organisms through field experiments under natural conditions may provide a means to identify critical interactions that determine invasion success (Lodge 1993). This may help in facing the challenge posed by the ever-growing rate at which organisms are introduced beyond their natural ranges and also provide us with a better understanding of factors that are involved in community structure.

ACKNOWLEDGEMENTS

We thank Cheri Miller, Michael Anderson, and Brian Edmond for providing technical assistance, and D. Grant Hokit, Susan Walls, Sam Rothstein, Ginger McKenna, Nicky Santoro, and Billy Sherbert for helpful discussions regarding experimental design. We thank the U.S. Forest Service, Pacific Northwest Research Station and especially Deanna H. Olson for the use of vehicles. Bruce Menge, Dede Olson, Doug Chivers, Erica Wildy, and Adolfo Marco provided helpful suggestions on this manuscript. Thanks to Dave Budeau and the staff at E.E. Wilson Wildlife Refuge for all the help they provided. Funding was provided by the Department of Zoology Research Funds and the National Science Foundation (Grant Number DEB-9423333).

CHAPTER 7

SUMMARY

The results of the work in this thesis clearly stress the importance of attempting to examine multiple factors when assessing amphibian declines. Although, conservation programs regularly concentrate on the direct impacts of environmental change on a single species, ecological experiments and theory demonstrate that species are affected in complex ways by other species, changes in abiotic factors, and disturbance (e.g. Abrams 1987, Abrams 1992, Schoener 1993, Woodward and Kiesecker 1994, Menge 1995, Kiesecker 1996, Werner and Anholt 1996). Therefore, to understand and predict the impact of environmental change, ecologists should consider the interaction of multiple causal factors. This fact is clear when we examine the results of chapters 3, 4 and 6. In chapter 3, the effect of exposure to ultraviolet radiation was influenced by pathogenic fungus *S. ferax*. In chapter 4, the outcome of competitive interactions between *R. cascadae* and *H. regilla* depended on *S. ferax*. In chapter 6, the interaction between life stages (bullfrog: larval/adult) or species (bullfrog/smallmouth bass) produced indirect effects which were greater than when each factor was considered separately.

Results in chapters 2 through 4 clearly denote the importance of including disease as a factor in ecological theory. The influence of pathogens and the diseases they cause have been largely unstudied by ecologists. Community level ecological studies consistently focus on the role of competition, predation, and disturbance (e.g. Wilbur 1972, Menge and Sutherland 1976, Connell 1983, Morin 1983, Wellborn et al. 1996). However, numerous studies suggest that pathogens can play important roles in determining species performance and the outcome of species interactions (e.g. Park 1948, Anderson and May 1979, May and Anderson 1979, Scott and Anderson 1984, Price et al. 1986, Scott 1987, Price et al. 1988).

Although *Saprolegnia* seems to have a major influence on amphibian communities in the Pacific Northwest, there is a complex interaction between *Saprolegnia* infection and UV radiation. Individuals may be especially susceptible to *Saprolegnia* infection if they are under stress (Schaefer et al. 1981; Pickering and Willoughby 1982, Orth et al. 1990). UV-B radiation has well-documented effects that weaken disease defense systems (e.g. Kripke 1984; Orth et al. 1990; Kripke et al. 1992; Tevini 1993). Possible increases in UV-B radiation, resulting from decreased stratospheric ozone or consequences of climate change (see Worrest and Grant 1989; Kerr and McElroy 1993; Zurer 1993, Schindler et al. 1996, Yan et al. 1996), may induce a more pronounced effect of

pathogens on species whose defense systems are compromised by UV-B radiation. The effects of global or regional environmental degradation, such as global climate change, ozone depletion or habitat alteration, may influence biodiversity by increasing the incidence of disease. In fact, several studies suggest that environmental degradation will likely result in increased incidence of disease (e.g. Loevinsohn 1994, Stone 1995). Thus, studies that investigate stressors (e.g UV-B, global warming) that may compromise disease defense mechanisms are warranted.

My findings in chapters 5 and 6 extend our understanding of interactions between native and exotic species. The key feature in the interaction of bullfrogs and smallmouth bass with red-legged frogs appears to be the change in behavior and alteration of microhabitat use. Many studies have documented the behavioral responses of prey to their predators (e.g. Sih 1987; Lima & Dill 1990; Wilson and Lefcort 1993, Kiesecker et al. 1996), indicating that such behavioral effects may be common in interactions between native species and introduced predators.

The results of these chapters also stress the importance of understanding the mechanism of interactions between native and exotic species. Understanding this mechanism may allow for the sustainable management of the red-legged frog. For example, preliminary surveys in Oregon indicate that *R. aurora* is missing from 69% of its historical breeding sites

(Kiesecker et al. In Prep.). At many of the sites where *R. aurora* is absent, *R. catesbeiana* is now found. However, there are several sites where the two species currently co-exist. Analysis of these sites indicate that they contain more shallow water (< 20 cm) with emergent vegetation compared to sites where *R. aurora* is now absent (Kiesecker et al. In Prep.). This difference in microhabitat between sites may explain why *R. aurora* is able to co-exist with *R. catesbeiana*. This seems likely considering that experiments have identified the importance of microhabitat use in the interaction of *R. aurora* with introduced species. In fact, other studies have suggested that the amount of habitat alteration can be an important factor in determining the outcome of interactions between native and exotics (e.g. Hobbs 1991, Lodge 1993a, Fisher and Schaffer 1996).

The results of this thesis also suggest future areas of study for amphibian decline research as well as potential guidelines that may facilitate understanding of causal factors. I recommend that more monitoring effort be placed on regional surveys. While it is important to establish long-term trends within individual populations, evidence of declines in a single population tell us little about the status of a species across its range. Numerous recent studies have demonstrated the benefits of conducting historical site surveys (Fellers and Drost 1993, Richards et al. 1993, Lannoo et al. 1994, Fisher and Schaffer 1996,

Drost and Fellers 1996, Pounds et al. In Press). Such studies not only assess the status of a species, but may also generate potential hypotheses regarding losses (see Drost and Fellers 1996, Fisher and Schaffer 1996). Data from historical site surveys can then be used in spatially-explicit models to evaluate the mechanism involved in the distributional change (Skelly and Meir 1997). Once the status of a species has been established, a subset of populations can be monitored on a regular basis. This monitoring would not have to be as intensive as that given to long-term population monitoring (i.e. mark-recapture efforts). For example, estimates of population size can be inferred by making counts of egg masses or measuring larval densities.

More effort should be placed on experimentally examining causal factors for declines. While there have been numerous hypotheses generated (see Blaustein 1994) actual tests of hypotheses have been few. Once testable hypotheses have been established, I advocate that experiments be conducted at several levels, including laboratory, mesocosm, and field experiments. The scale at which an experiment is conducted largely determines the applicability of the results to complex conditions in nature (Frost et al. 1988, O'Neil 1989). Important results from the simplest levels of study should be integrated into experiments at the next level, until experiments are conducted at the highest level,

preferably in the field. Such hierarchial experimentation, built on results from single-factor and single-species studies, comprise what is likely to be an effective mechanistic approach to communities. Some systems may be difficult to manage. For example, it may be difficult to control factors at a landscape level. Most amphibians, because of their small size and ease in which they can be manipulated, will allow some form of experimentation.

Each of the systems studied in this thesis involve understanding the factors that may threaten the maintenance of native amphibian biodiversity. In every chapter the importance of conducting multifactor studies when investigating potential factors involved in amphibian losses is apparent. The potential factors that influence the maintenance of amphibian biodiverity are varied and thus, attempts to understand these factors must aspire to reflect these complexities. While the loss of biodiversity is troubling, by studying systems currently undergoing change, not only can we understand the factors responsible for species loss but we may also better understand factors that are responsible for generating species assemblages.

BIBLIOGRAPHY

Abrams, P. 1987. Indirect interactions between species that share a predator: varieties of indirect effects. Pages 38-54 in W.C. Kerfoot and A. Sih editors. Predation: direct and indirect impacts on aquatic communities. University Press of New England, Dartmouth, New Jersey, USA.

Abrams, P. 1991. Strengths of indirect effects generated by optimal foraging. Oikos **59**:147-156.

Abrams, P. 1992. Predators that benefit prey and prey that harm predtors: unusal effects of interacting foraging adaptations. American Naturalist **140**:573-600.

Aho, J.M. 1990. Helminth communities of amphibians and reptiles: Comparative approaches to understanding patterns and processes. Pages 157-195 in G. Esch, A. Bush and J.M. Aho, editors. Parasite communities: patterns and processes. Chapman and Hall, New York, New York, USA.

Albertini, G. and B. Lanza. 1987. *Rana catesbeiana* Shaw, in Italy. Alytes **6**:117-129.

Alford, R.A. 1986. Habitat use and positional behavior of anuran larvae in a northern Florida temporary pond. Copeia **1986**:408-423.

Alford, R.A. 1989. Variation in predator phenology affects predator performance and prey community composition. Ecology **70**:206-219.

Alford, R.A., and M.L. Crump. 1982. Habitat partitioning among size classes of larval southern leopard frogs, *Rana utricularia*. Copeia **1982**:367-373.

Alford, R.A., and H.M. Wilbur. 1985. Priority effects in experimental pond communities: competition between *Bufo* and *Rana*. Ecology **66**:1097-1105.

Allan, J.D., and A.S. Flecker. 1993. Biodiversity conservation in running waters. BioScience **43**:32-43.

Almendares, J.M., Sierra, P.K., Anderson, P.R., and P.R. Epstein. 1993. Critical regions, a profile of Honduras. The Lancet **342**:1400-1402.

Anderson, R.M, and R.M. May. 1979. Population biology of infectious diseases: part I. Nature **280**:361-367.

Banks, B, and T.J.C. BeeBee. 1988. Reproductive success of natterjack toads, *Bufo calamita* in two contrasting habitats. Journal of Animal Ecology **57**:475-492.

Bateman, A., A. Singh, T. Kral, and S. Solomon. 1989. The immune-hypothalamic-pituitary adrenal axis. Endocrinology Review **10**:92-112.

Beattie, R.C., R.J. Aston, and A.G.P. Milner. 1991. A field study of fertilization and development in the common frog *Rana temporaria* with particular reference to acidity and temperature. Journal of Applied Ecology **28**:346-357.

Beebee, T.J.C. 1977. Environmental change as a cause of natterjack toad (*Bufo calamita*) declines in Britain. Biological Conservation **11**:87-102.

Beebee, T.J.C. 1995. Amphibian breeding and climate. Nature **374**:219-220.

Beebee, T.J.C. and A.L.C. Wong. 1992. Protheca-mediated interference competition between anuran larvae operates by resource diversion. Physiological Zoology **65**:815-831.

Beiswenger, R.E. 1986. An endangered species, the Wyoming toad *Bufo hemiophyrs baxteri*-The importance of an early warning system. Biological Conservation **37**:59-71.

Bennett, W.A. 1990. Biological Invasions. American Naturalist **135**:725.

Beringer, J., and T.R. Johnson. 1995. *Rana catesbeiana* (Bullfrog): Diet. Herpetological Review **26**:98.

Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology **71**:1599-1608.

Berven, K.A., and D.E. Gill 1983. Interpreting geographic variation in life-history traits. American Zoologist **23**:85-97.

Blaustein, A.R. 1988. Ecological correlates and potential functions of kin recognition in anuran larvae. Behavior Genetics **41**:1079-1083.

Blaustein, A.R., and D.H. Olson. 1991. Declining amphibians. Science **253**:1467.

Blaustein, A.R., Edmond, B., Kiesecker, J.M, Beatty, J.J, and D.G. Hokit. 1995. Ambient ultraviolet radiation causes mortality in salamander eggs. Ecological Applications **5**:740-743.

Blaustein, A.R., P.D. Hoffman, D.G. Hokit, J.M. Kiesecker, S.C. Walls, and J.B. Hays. 1994b. UV-repair and resistance to solar UV-B in amphibian eggs: A link to population declines? Proceedings of the National Academy of Science **91**:1791-1795.

Blaustein, A.R., P.D. Hoffman, J.M. Kiesecker, and J.B. Hays. 1996. DNA repair activity and resistance to solar UV-B radiation in eggs of the red-legged frog. Conservation Biology **10**:1398-1402.

Blaustein, A.R., D.G. Hokit, R.K. O'Hara, and R.A. Holt. 1994c. Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. Biological Conservation **67**:251-254.

Blaustein, A.R. and J.M. Kiesecker. The effects of Ultraviolet-B radiation on amphibians in natural ecosystems. In. D. Hader editor. Effects of Ozone Depletion on Aquatic Ecosystems. R.G. Landes Publishing, Georgetown, Texas, USA. In Press.

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

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

Bradford, D.F. 1989. Allotopic distribution of native frogs and introduced fishes in high Sierra Nevada lakes of California: Implications of the negative effect of fish introductions. Copeia **1989**:775-778.

Bradford, D.F. 1991. Mass mortality and extinction in a high elevation population of *Rana muscosa*. Journal of Herpetology **25**:174-177.

Bradford, D.F., F. Tabatabai, and D.M. Graber. 1993. Isolation of remaining populations of the native frog, Rana muscosa, by introduced fishes in Sequoia and Kings Canyon National Parks, California. Conservation Biology **7**:882-888.

Bragg, A.N. 1958. Parasitism of spadefoot tadpoles by *Saprolegnia*. Herpetologica **14**:34.

Breden, F., M. Scott, and E. Michel. 1987. Genetic differences for anti-predator behaviour in the Trinidad guppy, *Poecilla reticulata*. Animal Behaviour **35**:618-620.

Brown, C.R., and M.B. Brown 1986 Ectoparasitism as a cost of coloniality in cliff swallow (*Hirundo pyrrhonota*). Ecology **67**:1206-1218

Burton, T.M. and G.E. Likens. 1975a Energy flow and nutrient cycling in salamander populations in the Hubbard Brook experimental forest, New Hampshire. Ecology **56**:1068-1080.

Burton, T.M. and G.E. Likens. 1975b Salamander populations and biomass in the Hubbard Brook experimental forest, New Hampshire. Ecology **56**:1068-1080.

Bury, R.B., and R.A. Luckenbach. 1976. Introduced amphibians and reptiles in California. Biological Conservation **10**:1-14.

Bury, R.B, C.K. Dodd Jr., and G.M. Fellers. 1980. Conservation of the amphibia of the United States: a review. U.S. Fish and Wildlife Service, Resource Publication **134**:1-34.

Bury, R. B., and J.A. Whelan. 1986. Ecology and Management of the Bullfrog. U.S. Fish and Wildlife Service, Resource Publication **155**:1-24.

Carey, C. 1993. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. Conservation Biology **7**:355-362.

Chivers, D.P., and R. J. F. Smith. 1994. The role of experience and chemical alarm signalling in predator recognition by fathead minnows, *Pimephales promelas*. Journal of Fish Biology **44**:273-285.

Chivers, D.P., and R.J.F. Smith. 1995. Free-living fathead minnows rapidly learn to recognize pike as predators. Journal of Fish Biology **46**:949-954.

Clarkson, R.W. and J.C. De Vos, Jr. 1986. The bullfrog, *Rana catesbeiana* Shaw, in the lower Colorado River, Arizona, California. Journal of Herpetology **20**:42-49.

Clarkson, R.W. and J.C. Rorabaugh. 1989. Status of leopard frogs (*Rana pipiens* complex Ranidae) in Arizona and southeastern California. Southwestern Naturalist **34**:531-538.

Coborn, J. 1992. The Proper Care of Amphibians. TFH Publications, Neptune City, New Jersey, USA.

Connell, J.H. 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos **35**:131-138.

Connell, J.H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. American Naturalist **122**:661-696.

Conover, M.R. 1987. Acquisition of predator information by active and passive mobbers in ring-billed gull colonies. Behaviour **102**:41-57.

Corn, P.S. 1994. What we know and don't know about amphibian declines in the west. Pages 59-67 in W.W. Covington and L.F. DeBano editors. Sustainable ecological systems: Implementing an ecological approach to land management. Technical Report RM-247 USDA Forest Service, Fort Collins Colorado, USA.

Corn, P.S. and J.C. Fogleman. 1984. Extinction of montane populations of the northern leopard frog (Rana pipiens) in Colorado. Journal of Herpetology **18**:147-152.

Corn, P.S., W. Stolzenburg and R.B. Bury. 1989. Acid precipitation studies in Colorado and Wyoming: interim report of surveys of montane amphibians and water chemistry. Air pollution And Acid Rain Report No. 26, U.S. Fish And Wildlife Service, Fort Collins, Colorado, USA.

Corse, W.A. and D.E. Metter. 1980. Economics, adult feeding and larval growth of *Rana catesbeiana* on a fish hatchery. Journal of Herpetology **14**:231-238.

Crawshaw, G.J. 1992. The role of disease in amphibian declines. Declines in Canadian Amphibian populations: designing a national monitoring strategy. Canadian Wildlife Service. **76**:60-62.

Crump, M.L. 1983. Opportunistic cannibalism by amphibian larvae in temporary aquatic environments. American Naturalist **121**:281-289.

Crump, M.L., F.R. Hensley and K.L. Clark. 1992. Apparent decline of the golden toad: Underground or extinct? Copeia **1992**:413-420.

Curio, E. 1975. The functional organization of anti-predator behaviour in the pied flycatcher: a study of avian visual perception. Animal Behaviour **23**:100-115.

Curio, E., U. Ernst, and W. Vieth. 1978. Cultural transmission of enemy recognition: one function of mobbing. Science **202**:899-901.

Czechura, G.V. and G.S. Ingram. 1990. *Taudactylus diurnus* and the case of the disappearing frogs. Memoirs of the Queensland Museum **29**:361-365.

Dickman, M. 1968. The effect of grazing by tadpoles on the structure of a periphyton community. Ecology **49**:1188-1190.

Dobson, A.P., and R.M. May. 1986. Diseases and conservation. Pages 345-365 in M.E. Soule, editor. Conservation biology:the science of scarcity and diversity. Sinauer, Sunderland, Massachusetts, USA.

Drake, J., F.Di Castri, R. Groves, F. Kruger, H. Mooney, M. Rejmanek, and M. Williamson, editors. 1989. Biological invasions: a global perspective. Wiley & Sons, Chichester, England.

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

Ducey, P.K., and E.D. Brodie Jr. 1991. Evolution of antipredator behaviour: individual and population variation in a neotropical salamander. Herpetologica **47**:89-95.

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

Ehrlich, D. 1979. Predation by bullfrog tadpoles (*Rana catesbeiana*) on eggs and newly hatched larvae of the plains leopard frog (*Rana blairi*). Bulletin of the Maryland Herpetological Society **15**:25-26.

Ehrlich, P. and A. Ehrlich. 1981. Extinction. Ballantine Books, New York, New York, USA.

Elton, C.S. 1958. The ecology of invasions by animals and plants. Chapman and Hall, London, England.

Federal Register. 1991 Endangered and threatened wildlife and plants; animal candidate review listing as endangered or threatened species proposed rule. Department of the Interior, Fish and Wildlife Service. Part VIII Washington, D.C., USA.

Federal Register. 1996. Endangered and threatened wildlife and plants; Determination of threatened status for the California red-legged frog. **61**:25813-25833.

Fellers, G.M., and C.A. Drost. 1993. Disappearance of the Cascades frog (*Rana cascadae*) from the southern end of its range, California. Biological Conservation **65**:177-181

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

Freeland, W.J. 1976. Pathogens and the evolution of primate sociality. Biotropica **8**:12-24.

Frost, T.M., D.L. Deangelis, S.M. Bartell, D.J. Hall and S.H. Hulbert. 1988. Scale in the design and interpretation of aquatic community research. Pages 229-258 in S.R. Carpenter editor. Complex Interactions in Lake Communities. Springer-Verlag, New York, New York, USA.

Fuller, C.A., and A.R. Blaustein. 1996. Effects of the parasite *Eimeria arizonensis* on survival of deer mice (*Peromyscus maniculatus*). Ecology **77**:2196-2202.

Gamradt, S.C., and L.B. Kats. 1996. Effect of introduced crayfish and mosquitofish on California newts. Conservation Biology **10**:1155-1162.

Gamradt, S.C., L.B. Kats, and C.B. Anzalone. Aggression by nonnative crayfish deters breeding in California newts. Conservation Biology In Press.

Garland, T. 1988. Genetic basis of activity metabolism. I. Inheritance of speed, stamina, and antipredtor displays in the garter snake *Thamnophis sirtalis*. Evolution **42**:335-350.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**:183-190.

Green, D.M. 1978. Northern leopard frogs and bullfrogs on Vancouver Island. Canadian Field Naturalist **92**:78-79.

Griffiths, R.A., J. Denton, and A.L.C. Wong. 1993. The effects of food level on competition in tadpoles: interference mediated by prothecan algae? Journal of Animal Ecology **62**:274-279.

Groves, R.H., and J.J. Burdon, editors. 1986. Ecology of biological invasions: an Australian perspective. Australian Academy of Science, Canberra, Australia.

Gruia-Gray, J., and S.S. Desser. 1992. Cytopathological observations and epizootiology of frog erythrocytic virus in bullfrogs (*Rana catesbeiana*). Journal of Wildlife Diseases **28**:34- 41.

Haag, W.R., D.J. Berg, D.W. Garton, and J. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Drissena polymorpha*) in western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences **50**:13-19.

Hairston, N.G. Sr. 1987. Community ecology and salamander guilds. Cambridge University Press, New York, New York, USA.

Hammerson, G.A. 1982. Bullfrog eliminating leopard frogs in Colorado? Herpetological Review **13**:115-116.

Hayes, M.P., and M.R. Jennings. 1986. Decline of ranid frog species in Western North America: Are bullfrogs (*Rana catesbeiana*) responsible? Journal of Herpetology **20**:490-509.

Hayes, T.B., R. Chan, and P. Licht. 1993. Interaction of temperature and steroids on larval growth, development, and metamorphosis in a toad (*Bufo boreas*). The Journal of Experimental Biology **266**:206-215.

Hobbs, R.J. 1991. Disturbance as a precursor to weed invasion in native vegetation. Plant Protection Quarterly **6**:99-104.

Hobson, K.A., M.L. Bouchart, and G.S. Sealy. 1988. Responses of naive yellow warblers to novel nest predators. Animal Behaviour **36**:1223-1230.

Hokit, D.G., and A.R. Blaustein. The effects of kinship in interactions between tadpoles of *Rana cascadae*. Ecology In Press.

Holland, R.E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island area, Western Lake Erie since the establishment of the zebra mussel. Journal of Great Lakes Research **19**:617-624.

Hoogland, J.L. 1979. Aggression, ectoparasitism, and other possible costs of prairie dog (Sciuridae, *Cynomys* spp.) coloniality. Behaviour **69**:1-35.

Hudson, P.J. 1986. The effect of a parasitic nematode on the breeding production of red grouse. Journal of Animal Ecology **55**:85-92.

Hudson, P.J., A.P. Dobson, and D. Newborn. 1992. Do parasites make prey vulerable to predation? Red grouse and parasites. Journal of Animal Ecology **61**:681-692.

Hunter, B.R., D.L. Carlson, E.D. Seppanen, P.S. Killian, B.K. McKinnell. and R.G. McKinnell. 1989. Are renal carcinomas increasing in *Rana pipiens* after a decade of reduced prevalence? American Midland Naturalist **122**:307-312.

Ingram, G.J., and K.R. McDonald. 1993. An update on the decline of Queenslands frogs. Pages 297-303 in D. Lunney and D. Ayers editors. Herpetology in Australia: a diverse discipline. Royal Zoological Society of New South Wales, Mosman, New South Wales, Australia.

Kagarise Sherman, C. and Morton M.L. 1993. Population declines of Yosemite toads in the eastern Sierra Nevada of California. Journal of Herpetology. **27**:186-198.

Kats, L.B., J.W. Petranka, and A. Sih. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. Ecology **69**:1865-1870.

Kerr, J.B. and C.T. McElroy. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science **262**:1032-1034.

Kiesecker, J.M. 1996. pH induced growth reduction and its effects on predator-prey interactions between *Ambystoma tigrinum* and *Pseudacris triseriata*. Ecological Applications. **6**:1325-1331.

Kiesecker, J.M. and A.R. Blaustein. 1995. Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proceedings of the National Academy of Sciences **92**:11049-11052.

Kiesecker, J.M., and A.R. Blaustein. 1997a. Influences of egg laying behavior on pathogenic infection of amphibian eggs. Conservation Biology **11**:214-220.

Kiesecker, J.M., and A.R. Blaustein. 1997b. Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs. Ecology In press.

Kiesecker, J.M., Chivers, D.P. and A.R. Blaustein. 1996. The use of chemical cues in predator recognition by western toad (*Bufo boreas*) tadpoles. Animal Behaviour. **52**:1237-1245.

Kluge, A.G. 1981. The life history, social organization and parental behavior of *Hyla rosebergi* Boulenger, a nest building gladiator frog. Miscellaneous Publications of the Museum of Zoology at the University of Michigan **160**:1-170.

Krebs, C.J. 1994. Ecology, Fourth Edition. Harper Collins Publishers, New York, USA.

Kripke, M.L. 1984. Immunological unresponsiveness induced by ultraviolet radiation. Immunology Review **80**:87-102.

Kripke, M.L., P.A. Cox, L.G. Alas and D.B. Yarosh. 1992. Pyrimidine dimers in DNA initiate systemic immunosuppression in UV-irradiated mice. Proceedings of the National Academy of Sciences **89**:7516-7520.

Kruse, K.C., and M.G. Francis. 1977. A predation deterrent in larvae of the bullfrog, *Rana catesbeiana*. Transactions of the American Fisheries Society. **106**:248-252.

Kupferberg, S.J. 1995. The ecology of native tadpoles (*Rana boylii* and *Hyla regilla*) and the impacts of invading bullfrogs (*Rana catesbeiana*) in a northern California river. Dissertation, University of California, Berkeley, California, USA.

Kupferberg, S.J., J.C. Marks, and M.E. Power. 1994. Effects of variation in natural algal and detrital diets on larval anuran (*Hyla regilla*) life history traits. Copeia **1994**:446-457.

LaMarca, E. and Reinthaler, H.P. 1991. Population changes in *Atelopus* species of the Cordeillera de Merida, Venezuela. Herpetological Review **22**:125-128.

Lannoo, M.J., K. Lang, T. Waltz, And G.S. Phillips. 1994. An altered amphibian assemblage: Dickinson County, Iowa, 70 years after Frank Blanchards Survey. American Midland Naturalist **131**:311-319.

Laskin, A. I. & Lechevalier, H. A. 1978 Handbook of Microbiology, Volume II: Fungi, Algae, Protozoa, and Viruses. CRC Press, West Palm Beach, Florida, USA.

Laurance, W.F. 1996. Catastrophic declines of Australian rainforest frogs: is unusual weather responsible? Biological Conservation In Press.

Laurance, W.F., K.R. McDonald and R.S. Speare. 1996. Epidemic disease and the catastrophic decline of Australian rain forest frogs. Conservation Biology **10**:406-413.

Lawler, S.P., and P.J. Morin. 1993. Temporal overlap, competition and priority effects in larval anurans. Ecology **74**:174-182.

Lee, D.S., C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr. 1980. Atlas of North American freshwater fishes. North Carolina State Museum of Natural History, Raleigh, North Carolina, USA.

Lehmann, T. 1992. Ectoparasite impacts on *Gerbillus andersoni allenbyi* under natural conditions. Parasitology **104**:479-488.

Leonard, W.P., H.A. Brown, L.L. Jones, K.R. McAllister, R.M. Storm. 1993. Amphibians of Washington and Oregon. Seattle Audubon Society, Seattle, Washington, USA.

Levine, S.H. 1976. Competitive interactions in ecosystems. American Naturalist **110**:903-910.

Lima, S.L., and L.M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology **68**:619-640.

Lodge, D.M. 1993a Species invasions and deletions: community effects and responses to climate and habitat change. Pages 367-387 in P.M. Kareiva, J.G. Kingsolver, and R.B. Huey editors. Biotic interactions and global change. Sinauer, Sunderland, Massachusetts, USA.

Lodge, D.M. 1993b. Biological invasions: Lessons for ecology. Trends in Ecology and Evolution **8**:133-137.

Loevinsohn, M.E. 1994. Climatic warming and increased malaria incidence in Rwanda. The Lancet **343**:714-718.

Long, L.E., L. Saylor and M.E. Soule'. 1995. A pH/UV-B synergism in amphibians. Conservation Biology **9**:1301-1304.

Maloney, R.F, and L.G. McLean. 1995. Historical and experimental learned predator recognition in free-living New Zealand robins. Animal Behaviour **50**:1193-1201.

Master, L. 1990. The imperiled status of North American aquatic animals. Biodiversity Network News **3**:1-8.

Mathis, A., D.P. Chivers, and R.J.F. Smith. 1993. Population differences in response of fathead minnows (*Pimephales promelas*) to visual and chemical stimuli from predators. Ethology **93**:31-40.

Matity, J.G, D.P. Chivers, and R.J.F. Smith. 1994. Population and sex differences in anti-predator responses of breeding fathead minnows (*Pimephales promelas*) to chemical stimuli from garter snakes (*Thamnophis radix* and *T. sirtalis*). Journal of Chemical Ecology **20**:2111-2121.

Mattison, C. 1988. The Care of Reptiles and Amphibians in Captivity. Sterling Publishing, New York, New York, USA.

May, R.M. and R.M. Anderson. 1979. Population biology of infectious diseases: Part II. Nature **280**:455-461.

McAllister, K.R. and B. Leonard. 1991. 1990 progres report-Past distribution and current status of the spotted frog in western Washington. Washington Department of Wildlife.

McAllister, K.R., W.P. Leonard, and R.M. Storm. 1993. Spotted frog (*Rana pretiosa*) surveys in the Puget Trough of Washinfgton, 1989-1991. Northwest Naturalist **74**:10-15.

McDonald, K.R. 1990. *Rheobatrachus* Liem and *Taudactylus* Straughan & Lee (Anura Leptodactylidae) in Eungella National Park, Queensland: distribution and decline. Transactions of the Royal Society of South Autralia **11**:187-194.

Menge, B.A. 1995. Indirect effects in marine rocky intertidal interaction webs: patterns and importance. Ecological Monographs **65**:21-74.

Menge, B., and Sutherland, J.P. 1976. Species diversity gradients: synthesis of the role of predation, competition, and temporal heterogeneity. American Naturalist **110**:351-369.

Milinski, M. 1986. Constraints placed by predators on feeding behaviour. Pages 236-252 *in* T.J. Pitcher editor. The behaviour of Teleost Fishes. Croom Helm, London, England.

Minckley, W.L., and J.D. Deacon, editors. 1991. Battle Against Extinction: Native Fish Management in the American West. The University of Arizona Press, Tucson, Arizona, USA.

Miller, R.R., J.D. Williams, and J.E. Williams 1989. Extinctions of North American fishes during the past century. Fisheries **14**:22-38.

Mooney, H.A., and J.A. Drake, editors. 1986. Ecology of biological invasions of North America and Hawaii. Springer-Verlag, New York, New York, USA.

Morin, P.J. 1981. Predatory salamanders reverse the outcome of competition among three species of anurans tadpoles. Science **212**:1284-1286.

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

Morin, P. J. 1986. Interactions between intraspecfic competition and predation in an amphibian predator-prey system. Ecology **67**: 713-720.

Morton, S.R. 1990. The impact of European settlement on the vertebrate animals of arid Australia: a conceptual model. Proceddings of the Ecological Society of Australia **16**:201-213.

Moyle, P.B. 1973. Effects of introduced bullfrogs, *Rana catesbeiana*, on the native frogs of the San Joaquin Valley, California. Copeia **1973**:18-22.

Moyle, P.B. and T. Light. 1996. Fish invasions in california: Do abiotic factors determine success? Ecology **77**:1666-1670.

Mueller, H.C., and P.G. Parker. 1980. Naive ducklings show different cardiac response to hawk than to goose models. Behaviour **74**:101-113.

Munck, A., P.M. Guyre, and N.J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrinology Review **5**:25-44.

National Science Board. 1989. Loss of biological diversity: A global crisis requiring international solutions. Report NSB-89-171. National Science Foundation, Washington, D.C., USA

Nicholls, K.H. and G.J. Hopkins. 1993. Recent changes in Lake Erie (North Shore) phytoplankton: Cumulative impacts of phosphorus loading reductions and the zebra mussel introduction. Journal of Great Lakes Research **19**:637-647.

Nussbaum, R.A., E.D. Brodie, JR., and R.M. Storm. 1983. Amphibians and Reptiles of the Pacific Northwest. Northwest Naturalist Books, The University of Idaho Press, Moscow, Idaho, USA.

O'Hara, R.K. 1981. Habitat selection behavior in three species of anurans larvae: environmental cues, ontogeny and adaptative significance. Ph.D. Thesis, Oregon State University, Corvallis, Oregon, USA.

Olson, D.H. 1988. The ecological and behavioral dynamics of breeding in three sympatric anuran amphibians. Dissertation, Oregon State University, Corvallis, Oregon, USA.

Olson, D.H. 1989. Predation on breeding western toads (Bufo boreas). Copeia **1989**:391-397.

Olson, D.H. 1992. Ecological susceptibility of amphibians to population declines. In H.M. Kerner editor. Proceedings of the symposium on biodiversity of northwestern California. Wildland Research Center, University Of California, Berkeley, California, USA.

Olson , D.H., A.R. Blaustein, and R.K. O'Hara. 1986. Mating pattern variability among western toad *Bufo boreas* populations. Oecologia **70**:351-356.

Olson, M.H., G.G. Mittelbach, and C.W. Osenberg. 1995. Competition between predator and prey: Resource-based mechanisms and implications from stage-structures dynamics. Ecology **76**:1758-1771.

O'Neill, R.V. 1989. Perspectives in hierarchy and scale. Pages 140-156 in J. Roughgarden, R.M. May and S.A. Levin editors. Perspectives in Ecological Theory. Princeton University Press, Princeton, New Jersey, USA.

Orth, A.B., A.H. Teramura, and H.D. Sisler. 1990. Effects of ultraviolet-B radiation on fungal disease development in *Cucumis sativus*. American Journal of Botany **77**:1181-1192.

Owings, D.H, and R.G. Coss. 1977. Snake mobbing by California ground squirrels: adaptive variation in ontogeny. Behaviour **62**:50-69.

Paine, R.T. 1966. Foodweb complexity and species diversity. American Naturalist **100**:65-75.

Park, T. 1948. Experimental studies of interspecific competition. I. Compettion between populations of the flour beetles, *Tribolium confusum* Duval and *Tribolium castaneum* Herbst. Ecological Monographs **18**:265-308.

Pechmann, J.H.K., Scott, D.E., Semlitsch, R.D., Caldwell, J.P., Vitt, L.J., and W. Gibbons. 1991. Declining amphibian populations: The problem of seperating human impacts from natural fluctuations. Science **153**:892-895.

Pechmann, J.H.K., and H.M., Wilbur. 1994. Putting amphibian population declines in perspective: natural fluctuatuions and human impacts. Herpetologica **50**:65-84.

Petranka, J.W., M.E. Eldridge, and K. E. Haley. 1993. Effects of timber harvesting on southern Appalachian salamanders. Conservation Biology **7**:363-370.

Petranka, J.W., L.B. Kats, and A. Sih. 1987. Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. Animal Behaviour **35**:420-425.

Petren, K., D.T. Bolger, and Case, T.J. 1993. Mechanisms in the competitive success of an invading sexual gecko over an asexual native. Science. **259**:354-358.

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

Pickering, A.D., and L.G. Willoughby. 1982. *Saprolegnia* infections in Salmonid fish. Special Publications of the Society for General Microbiology **9**:271-297.

Plowright, W. 1982. The effects of rinderpest and rinderpest control on wildlife in Africa. Animal disease in relation to animal conservation. Symposia of the Zoological Society of London **50**:1-28.

Pomeroy, L.V. 1981. Developmental polymorphism in the tadpoles of the spadefoot toad. Dissertation. University of California, Riverside, Califonia, USA.

Pough, H.F., E.M. Smith, D.H. Rodes, and A. Collazo. 1987. The abundance of salamanders in forest stands with different histories of disturbance. Forest Ecology and Management **20**:1-9.

Pounds, J.A. and M.L. Crump. 1994. Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. Conservation Biology **8**: 72-85.

Pounds, J.A., M.P. Fogden, J.M. Savage, and G.C. Gorman. Amphibian declines on a neotropical mountain: patterns and tests of null models. In Press Conservation Biology.

Power, M.E., D. Tilman, J.A. Estes, B.A. Menge, W.J. Bond, L.S. Mills, G. Daily, J.C. Castilla, J. Lubchenco and R.T. Paine. 1996. Challenges in the quest for keystones. BioScience **46**:609-620.

Price, P.W., M. Westoby, B. Rice, P.R. Atsatt, R.S. Fritt, J.N. Thompson, and K. Mobley. 1986. Parasite mediation in ecological interactions. Annual Reveiw of Ecology and Systematics **17**:487- 505.

Price, P.W., M. Westoby, and B. Rice. 1988. Parasite-mediated competition: some predictions and tests. American Naturalist **131**:544-555.

Reznick, D.N. 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): An experimental evaluation of alternative models of development. Journal of Evolutionary Biology **3**:185-203.

Richards, R.H., and A.D. Pickering. 1978. Frequency and distribution patterns of *Saprolegnia* infection in wild and hatchery-reared brown trout *Salmo trutta* and char *Salvelinus alpinus*. Journal of Fish Diseases **1**:69-82.

Richards, S.J., K.R. McDonald, and R.A. Alford. 1993. Declines in populations of Australia's endemic tropical rainforest frogs. Pacific Conservation Biology **1**:66-77.

Riechert, S.E., and A.V. Hedrick. 1990. Levels of predation and genetically based anti-predator behaviours in the spider, *Agelenopsis aperta*. Animal Behaviour **40**:679-687.

Rubenstein, D.I. and M.E. Hohmann 1989. Parasites and the social behaviour of island feral horses. Oikos **55**:312-320.

Savage, R.M. 1952. Ecological, physiological, and anatomical observations on some species of anuran tadpoles. Proceedings of the Zoological Society of London **122**:467-514.

Savidge, J.A. 1988. Food habits of *Boiga irregularis*, an introduced predator of Guam (West Pacific). Journal of Herpetology **22**:275-282.

Schaefer, W.F., R.A. Heckmann, and W.A. Swenson. 1981. Postspawning mortality of rainbow smelt in western Lake Superior. Journal of Great Lakes Research **7**:37-41.

Schindler, D.W., P. Jefferson Curtis, B.R. Parker and M.P. Stainton. 1996. Consequences of climate warming and lake acidification fro UV-B penetration in North American Boreal lakes. Nature **379**:705-708.

Schmitz, O.J. and T.D. Nudds. 1994. Parasite-mediated competition in deer and moose: how strong is the effect of meningeal worn on moose. Ecological Applications **4**:91-103.

Schoener, T.W. 1993. On the relative importance of direct versus indirect effects in ecological communities. Pages 365-411. In H. Kawanabe and J.E. Cohen, editors. Mutualism and organization in natural communities. Oxford University Press, Oxford, England.

Schwalbe, C.R., and P.C. Rosen. 1988. Preliminary report on effect of bullfrogs on wetland herpetofaunas in southeastern Arizona. Pages 166-173 *in* Management of Amphibians, Reptiles, and Small Mammals in North America, Proceedings of the Symposium, General Technical Report RM-166. USDA Forest Service, Fort Collins, Colorado, USA.

Scott, D.E. 1994. The effects of larval density on adult demographic traits in *Ambystoma opacum*. Ecology **75**:1383-1396.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fish of Canada. Fisheries Research Board of Canada, Bulletin **184**:1-996.

Scott, M. E. 1987. Regulation of mouse colony abundance by *Heligmosomoides polygyrus*. Parasitology **95**:111-124.

Scott, M.E. and R.M. Anderson. 1984. The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecillia reticulata*. Parasitology **89**:159-194.

Seale, D.B., and R.J. Wassersug. 1979. Suspension feeding dynamics of anuran larvae related to their functional morphology. Oecologia **39**:259-272.

Semlitsch, R.D., D.E. Scott, J.H. Pechmann, and J.W. Gibbons. 1996. Structure and dynamics of an amphibian community: Evidence from a 16-year study of a natural pond. Pages 217-248 in M.L. Cody and J.A. Smallwood editors. Long-term studies of vertebrate communities. Academic Press, San Diego, California, USA.

Seymour, R.L. 1970. The genus *Saprolegnia*. Nova Hedwigia **30**:1-124.

Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. Pages 203-224 *in* A. Sih and W.C. Kerfoot, editors. Predation Direct and Indirect Impacts on Aquatic Communities. University Press of New England, Hanover, New Hampshire, USA.

Sih, A.,and L.B. Kats. 1994. Age, experience, and the response of streamside salamander hatchlings to chemical cues from predatory sunfish. Ethology **96**:253-259.

Skelly, D.K. 1992. Field evidence for a cost of behavioral antipredator response in a larval amphibian. Ecology **73**:704-708.

Skelly, D.K. and E. Meir. Rule-based models for evaluating mechanisms of distributional change. Conservation Biology **11**:531-538.

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

Smith, A.W., M.P. Anderson, D.E. Skilling, J.E. Barlough, and P.K. Ensley. 1986. First isolation of calcivirus from reptiles and amphibians. American Journal of Veterinary Research **47**:1718-1721.

Smith, D.C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology **68**:344-350.

Smith, S.N., R.A. Armstrong, J. Springate and G. Barker 1985. Infection and colonization of trout eggs by Saprolegniaceae. Transactions of the British Mycological Society **85**:719-724.

Snyder, R.L. 1976 The biology of Population Growth. St Martin's Press, New York, New York, USA.

Soule, M.E. 1986. Conservation Biology. Sinauer, Sunderland, Massachusetts, USA.

Soule, M.E. 1990. The onslaught of alein species, and other challenges in the coming decades. Conservation Biology **4**:233-239.

Srivastava, R.C., and G.C. Srivastava. 1978. Fungi associated with the diseases of freshwater fishes. Mycopathologia **63**:121-126.

Stebbins, R.C. 1985. A Field Guide to Western Reptiles and Amphibians. Houghton Mifflin, Boston, USA.

Stebbins, R.C. and N.W. Cohen. 1995. A Natural History of Amphibians. Princeton University Press, Princeton, New Jersey, USA.

Stein, R.A., S.T. Threlkeld, C.D. Sandgren, W.G. Sprules, L. Persson, E.E. Werner, W.E. Neill, and S.I. Dodson. 1988. Size-structured interactions in lake communities. Pages 161-180 In S.R. Carpenter, editor. Complex interactions in lake communities. Springer-Verlag, New York, New York, USA.

Steinwachser, K. 1978. The effects of coprophagy on the growth of *Rana catesbeiana* tadpoles. Copeia **1978**:130-134.

Stewart, M.M. 1995. Climate driven population fluctuation in rain forest frog. Journal of Herpetology **29**:437-446.

Stone, R. 1995. If the mercury soars, so may health hazards. Science **267**:957-958.

Stumpel, A.H.P. 1992. Successful reproduction of introduced bullfrogs, *Rana catesbeiana* in northwestern Europe: a potential threat to indigenous amphibians. Biological Conservation **60**:61-62.

Sype, W.E. 1975. Breeding Habitats, Embryonic Thermal Requirements and Embryonic and Larval Development of the Cascade Frog, *Rana cascadae* Slater. Dissertation, Oregon State University, Corvallis, Oregon, USA.

Tabachnick, B.G., and L.S. Fidell. 1989. Using multivariate statistics. Harper Collins, New York, New York, USA.

Taylor, J.N., Courtenay, W.R., and McCann, J.A. 1984. Known impacts of exotic fishes in the continental United States. Pages 322-373. In W.R. Curtenay and J.R. Stauffer editors. Distribution, Biology and Management of Exotic Fishes. John Hopkins Press, Baltimore, Maryland, USA.

Tevini, M. 1993. UV-B radiation and ozone depletion: Effects on Humans, Animals, Plants, Microorganisms, and Materials. Lewis, Boca Raton, Florida, USA.

Thornhill, R. 1989. Nest-defence by red jungle fowl, *Gallus gallus spadiceus*, hens: the roles of renesting potential, parental experience and brood reproductive value. Ethology **83**:31-42.

Thrall, J. 1972. Food, Feeding, and digestive physiology of the larval bullfrog, *Rana catesbeiana*. Copeia **1972**:751-752.

Tyler, M.J. 1991. Declining amphibian populations-a global phenomemon? An Australian perspective. Alytes **9**:43-50.

Underwood, A.J. 1986. The analysis of competition by field experiments. Pages 240-268 in D.J. Anderson and J. Kikkawa editors. Community ecolgy:pattern and process. Blackwell Scientific, Oxford, England.

Vandermeer, J. 1969. The competitive structure of communities: An experimental approach with protozoan communities. Ecology **50**:362-371.

Vitousek, P.M. 1989. Biological invasion by *Myrica faya* in Hawaii (USA): Plant demography, nitrogen fixation, ecosystem effects. Ecological Monographs **59**:247-266.

Vitousek, P.M. 1990. Biological invasions and ecosystem processes: Toward an integration of population biology and ecosystem studies. Oikos **57**:7-13.

Vitt, L.J., Caldwell, J.P., Wilbur, H.M., and D. C. Smith. 1990. Amphibians as harbingers of decay. BioScience **40**:418.

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

Walls, S.C., A.R. Blaustein and J.J.Beatty. 1992. Amphibian biodiversity of the Pacific Northwest with special reference to old growth stands. Northwest Environmental Journal **8**:53-69.

Washburn, J.O.,D.R. Mercer. and J.R. Anderson. Regulatory role of parasites: Impact on host population shifts with resource availibity. Science **253**:185-188.

Wassersug, R.J. 1980. Internal oral features of larvae from eight anuran families: functional, systematic, evolutionary and ecological considerations. Miscellaneous Publications of the Museum of Natural History at the University of Kansas 68:1-146.

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

Werner, E.E., and B.R. Anholt. 1996. Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. Ecology **77**:157-169.

Werner, E.E., and J.F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics **15**:393-426.

Werner, E.E. and D.J. Hall. 1988. Ontogenetic habitat shifts in the bluegill sunfish (*Lepomis macrochirus*): the foraging rate-predation risk trade-off. Ecology **69**:1352-1366.

Werner, E.E., Wellborn, G.A. and M.A. McPeek. 1995. Diet composition in postmetamorphic bullfrogs and green frogs: implications for interspecific predation and competition. Journal of Herpetology **29**:600-607.

Wilbur, H. M. 1972. Competition, predation, and the structure of the *Ambystoma-Rana sylvatica* community. Ecology **53**:3-21.

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

Wilson, D.J. & H. Lefcort. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora* tadpoles. Animal Behaviour **46**:1017-1019.

Wilson, E.O. 1988. Biodiversity. National Academy Press. Washington, D.C., USA.

Wood, S.E., and L.G. Willoughby. 1986. Ecological observations on the fungal colonization of fish by *Saprolegniaceae* in Windermere. Journal of Applied Ecology **23**:737-749.

Woodward, B.D. 1983. Predator-prey interactions and breeding-pond use of temporary pond species in a desert anuran community. Ecology **64**:1549-1555.

Woodward, B.D. 1987. Interactions between woodhouse's toad tadpoles (*Bufo woodhousii*) of mixed sizes. Copeia **1987**:380-386.

Woodward, B.D., and J.M. Kiesecker. 1994. Ecological factors and the Notonectid-Fairy shrimp interaction. Southwest Naturalist **39**:160-165.

Wormington, A., and J.H. Leach. 1992. Concentrations of migrant diving ducks at Point Pelee National Park, Ontario, in response to invasion of zebra mussels, *Dreissena polymorpha*. Canadian Field-Naturalist **106**:376-380.

Worrest, R.C. and Grant, L.D. 1989. Effects of Ultraviolet-B radiation on terrestrial plants and marine organisms. Pages 197-206 in R.R. Jones and T. Wigley editors. Ozone depletion: Health and environmental consequences, John Wiley and Sons, New York, New York, USA.

Worthylake, K.M., and P. Hovingh. 1989. Mass mortality of salamanders (*Ambystoma tigrinum*) by bacteria (*Acinetobacter*) in an oligotrophic seepage mountain lake. Great Basin Naturalist **49**:364-372.

Wright, A.H., and A.A. Wright. 1949. Handbook of frogs and toads of the United States and Canada. Comstock Publishing, New York, New York, USA.

Wyman, R.L. 1990. Whats happening to the amphibians? Conservation Biology **4**:350-352.

Yan, N.D., W. Keller, N.M. Skully, D.R.S. Lean and P.J. Dillon. 1996. Increased UV-B penetration in a lake owing to drought-induced acidification. Nature **381**:141-143.

Yodzis, P. 1988. The indeterminacy of ecological interactions as perceived through perturbation experiments. Ecology **69**:508-515.

Zaret, T.M. and R.T. Paine. 1973. Species introduction in a tropical lake. Science **182**:449-455.

Zurer, P.S. 1993. Ozone depletion's recurring surprises challenge atmospheric scientists. Chemical and Engineering News **71**:8-18.