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Nonindigenous Herpetofauna of Florida: Patterns of Richness and Case Studies of the Impacts of the Tadpoles of Two Invasive Amphibians, Osteopilus septentrionalis and Bufo marinus

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To the Graduate Council:

I am submitting herewith a dissertation written by Kevin G. Smith entitled "Nonindigenous Herpetofauna of Florida: Patterns of Richness and Case Studies of the Impacts of the Tadpoles of Two Invasive Amphibians, Osteopilus septentrionalis and Bufo marinus." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Arthur C. Echternacht, Major Professor

We have read this dissertation and recommend its acceptance:

James A. Drake, Michael L. McKinney, Daniel Simberloff

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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

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Arthur C. Echternacht
Major Professor

Christine R.B. Boake
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We have read this dissertation
and recommend its acceptance:

James A. Drake

Michael L. McKinney

Daniel Simberloff

Acceptance for the Council:

Anne Mayhew
Vice Chancellor and Dean of Graduate Studies

(Original signatures are on file with official students records)

NONINDIGENOUS HERPETOFAUNA OF FLORIDA: PATTERNS OF RICHNESS AND CASE STUDIES OF THE IMPACTS OF THE TADPOLES OF TWO INVASIVE AMPHIBIANS, *OSTEOPILUS SEPTENTRIONALIS* AND *BUFO MARINUS*

A Dissertation Presented for the
Doctor of Philosophy Degree
The University of Tennessee, Knoxville

Kevin G. Smith
August 2005

DEDICATION

This dissertation is dedicated to the natural areas of the world, large and small, that inspired my interest in biology, ecology, and conservation and to the individuals who enjoyed these places with me and helped me to enjoy them.

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The contributors to the completion of this dissertation are a diverse and numerous bunch. The small ways in which some of these individuals helped me were as influential and significant as the larger ways of others. All of these people contributed significantly, and this dissertation would be a very different—and certainly lesser—document were it not for them.

I could not possibly have come across an advisor better suited to my needs than Sandy Echternacht. Sandy is an expert at giving the advice that is needed while withholding the advice that isn't. His actions define him as an advisor who is loyal and proud and protective of his students, and I benefited greatly from his character is these respects. His record shows his success with providing the perfect environment for motivated students to succeed, and I'm honored to have been given the opportunity to work in such an environment.

Ken Dodd deserves a great deal of credit for his role as an unofficial, but *de facto*, co-advisor. As my former boss, Ken steered me toward the path I'm on now by providing me with the opportunities I needed to prepare for and succeed in a PhD program. As a collaborator, Ken, like Sandy, showed great restraint and trust by letting me run a show for which he was ultimately responsible. I thank him equally for the chances he gave me to fail and to succeed. I learned from them all.

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In Gainesville, Jamie Barichivich and Jennifer Staiger gave incredibly generously of their time and space by providing me with a place to stay for almost a cumulative year. As if that weren't enough, they helped with many of the day-to-day tasks and major and minor hurdles of my research in Florida. They're also great friends and excellent mountain biking partners.

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The U.S. Geological Survey provided me with significant funding for most of the research described in this dissertation though their Cooperative Ecosystem Study Unit agreement program. In a time of reduced funding to the natural sciences, this grant was essential to the success of my research. Ken Dodd alerted me to the existence of this program and was the in-house sponsor of the research. Phyllis Bice (UT) and Christine Fadeley (USGS) provided invaluable assistance with the administration of this grant. The University of Tennessee Graduate School and Department of Ecology and Evolutionary Biology also provided a great deal of financial support in the form of travel grants, assistantships, fellowships, awards, and research funds. This support enabled me to apply a great deal of focus directly to my research. Additional support was offered by the Explorer’s Club, The Bermuda Zoological Society, The Chicago Herpetological Society and The University of Tennessee Graduate Researchers in Ecology, Behavior, and Evolution.

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ABSTRACT

Although invasive species are considered to be a significant threat to native biodiversity, the impacts of very few nonindigenous species are well known. In this dissertation I describe the results of several studies evaluating the impacts of the presence of nonindigenous amphibians and reptiles in Florida. I conducted an analysis to assess the patterns and effects on biodiversity of the establishment of nonindigenous amphibians and reptiles in Florida at the county level. The richness of the 40 established nonindigenous amphibians and reptiles is not distributed evenly across the state, but instead is significantly greater in the southern part of the state and in counties with large human populations. These trends likely reflect the recent breakdown of historical barriers to invasion between Florida and the Caribbean region and the influence of human activities in the establishment of nonindigenous species. I also conducted several experimental case studies of the effects of the presence of the tadpoles of two invasive amphibians, the cane toad (*Bufo marinus*) and the Cuban treefrog (*Osteopilus septentrionalis*), in native Floridian tadpole communities. These studies indicated that *B. marinus* does not significantly affect native tadpoles through competition, while *O. septentrionalis* tadpoles outcompete and adversely affect native tadpoles, both in laboratory microcosms and naturalistic outdoor mesocosms. Larval *O. septentrionalis* also prey on native tadpoles, but this effect is probably not significant under natural conditions when alternative food is present. A mechanistic laboratory study indicated that the competitive effects of *O. septentrionalis* were mediated through exploitation competition with no evidence of interference competition. When keystone predators, eastern newts (*Notophthalmus viridescens*), were included in the experimental tadpole communities, the negative effects of *O. septentrionalis* tadpoles on native tadpoles were reduced significantly. Higher mortality of *O. septentrionalis* larvae suggests that newts preyed selectively on *O. septentrionalis* tadpoles, supporting their role as keystone predators. If general, this result suggests that keystone predators are important to the maintenance of diversity in invaded communities. Collectively, these results suggests that *O. septentrionalis* larvae may affect native amphibian populations in Florida through larval interactions, but these impacts may be limited by the presence of keystone predators.

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INTRODUCTION

It is generally agreed that invasive species are one of the most significant threats to biological diversity (e.g., Wilcove et al. 1998, Ricciardi and Rasmussen 1999, Mack et al. 2000, but see Gurevitch and Padilla 2004, cf. Clavero and Garcia-Berthou 2005). Consequently, the study of invasive species has recently become a major focus of both conservation biology and ecology (Simberloff 1997) as scientists in these fields are faced with assessing, predicting, or mitigating the environmental and economic impacts of thousands of potentially injurious nonindigenous species (Pimentel et al. 2000). As the major impacts of invasive species are frequently the result of a relatively small proportion of the many nonindigenous species present in a given region, the identification of these relatively few potentially injurious invasive species is essential to the successful implementation of conservation programs (Gurevitch and Padilla 2004).

In the United States, the state of Florida is emblematic of the magnitude of the ecological threat posed by nonindigenous species. Florida is one of the most invaded regions of the United States (Mooney and Drake 1986, Simberloff et al. 1997) and is also home to a unique, but highly threatened, biota (Stein et al. 2000). The past and potential future consequences of biological invasions in Florida are therefore as significant as almost anywhere, as evidenced by the substantial economic and ecological impacts of a number of highly invasive species (Schardt 1997, Schmitz et al. 1997). Among the many established nonindigenous species in Florida are at least 40 species of amphibian and reptile (Meshaka et al. 2004), the majority of which have only been recorded as established since 1960 (Butterfield et al. 1997). The recent trend in the rates of establishment of these taxa closely matches human population growth in the state, and has yet to reach a plateau (Smith *In press*). Furthermore, few nonindigenous amphibians and reptiles in Florida have been studied with regard to their extralimital ecology or impacts, and only one species (*Anolis sagrei*) has been studied thoroughly, though not exhaustively (Brown 1988, e.g., Campbell 2000, Gerber and Echternacht 2000, Vincent 2002). The ecological impacts of the nonindigenous amphibians and reptiles of Florida thus remain largely unknown, despite the rapidly increasing rate of their establishment.

The impacts of individual nonindigenous species notwithstanding, large assemblages of nonindigenous species can also influence biodiversity, especially when the similarity of biotas is compared among locations. For example, because successful invasive species can become established in many locations or can spread rapidly once established, the presence of many widely-distributed nonindigenous species in a region can rob the region of its ecological distinctiveness, resulting in biotic homogenization (McKinney and Lockwood 1999, Lockwood and McKinney 2001). Although introduced species can also result in biological differentiation, especially when relatively few nonindigenous species are present (e.g., McKinney 2004), the worst-case combination may be the establishment of cosmopolitan exotic species in combination with the extinction of rare native species of restricted distribution, resulting in significant homogenization (Olden and Poff 2003). Furthermore, if such widespread, invasive species themselves contribute to the extinction or extirpation of rare native taxa, then native biodiversity will be substantially altered. Although there is some evidence to suggest that invasive species are a leading threat to threatened and endangered taxa and contribute to extinctions (Wilcove et al. 1998, Clavero and Garcia-Berthou 2005), there is still a great need for studies assessing the impacts of invasive species at the level of community ecology, thereby allowing the integration of species-specific impacts with the overall effects of nonindigenous species on biological diversity.

Within this framework, the goals of this dissertation are to describe broad patterns in the establishment of nonindigenous amphibians and reptiles across Florida and to evaluate the potential effects of these species on the herpetofaunal diversity of Florida. First, I conduct a descriptive analysis of the nonindigenous herpetofauna as a group, also evaluating the influence of these species on the biodiversity of the herpetofauna of Florida. Second, I describe several case studies in which I focus on the ecology and impacts of the larvae of two nonindigenous anurans, the cane toad (*Bufo marinus*: Bufonidae) and the Cuban treefrog (*Osetopilus septentrionalis*: Hylidae) in Florida.

In Part I, I conduct an analysis to identify correlates of nonindigenous herpetofaunal richness in Florida at the county level. Because of the relatively large number of established nonindigenous amphibians and reptiles in Florida, and the relatively clustered nature of the distributions, one goal of this analysis is to determine if particular geographic or anthropogenic factors can be used to predict invasion "hot spots." Similar studies of other taxa at larger spatial scales suggest that some factors may contribute to the establishment of nonindigenous species in a general way (reviewed in Smith *In press*), so the results of this study are potentially applicable across regions and taxa. Furthermore, the effects on biodiversity of the presence of a relatively rich nonindigenous herpetofauna in Florida have not previously been assessed. As introductions of nonindigenous species are continuing at a high rate, the establishment of a diversity benchmark against which past and future changes can be measured is overdue.

In Parts II and III, I describe the results of two microcosm studies designed to assess the ecological impacts of larval *B. marinus* and *O. septentrionalis* on amphibian larvae native to Florida. These studies jointly address the competitive interactions of *B. marinus* and *O. septentrionalis* tadpoles with those of two native species, the potential role of *O. septentrionalis* as a predator of native and nonindigenous tadpoles, and the potential for interactions between *O. septentrionalis* and *B. marinus* that may modify their impacts. These topics are particularly relevant to conservation of Florida's anurans as competition and predation are significant factors in structuring larval anuran communities (Wilbur 1980, Alford 1999). Furthermore, intraspecific predation (i.e., "cannibalism") has been observed in *O. septentrionalis* larvae in laboratory conditions (Crump 1986), suggesting that this species may also prey on heterospecific tadpoles. Because *B. marinus* larvae are toxic to a number of vertebrate and invertebrate predators (Crossland and Alford 1998, Crossland and Azevedo-Ramos 1999), the presence of *B. marinus* may modify the impacts of *O. septentrionalis* where these two species co-occur.

In Part IV I present the results of a mechanistic study in which I evaluate whether the competitive impacts of *O. septentrionalis* documented in Part II are the result of exploitation or interference competition. Information on the specific mechanisms of competition is useful to the prediction of the outcome of competitive interactions among unstudied or novel species groups (Tilman 1987). This is especially useful given that it is impossible to assess the impacts of the presence of *O. septentrionalis* in all communities in which this species does or may occur. Furthermore, some forms of competition among anuran larvae may occur in laboratory microcosms but are not important in natural systems (Petranka 1989). For this reason, information on the mechanisms of competition by *O. septentrionalis* is essential to the assessment of the relevance of the results from Parts II to natural tadpole communities.

The research presented in Part V extends the evaluation of the impacts of nonindigenous anuran larvae to naturalistic, outdoor mesocosms. This study asks if the competitive effects of *O. septentrionalis* on native anuran larvae persist in a more realistic, complex aquatic community and in the presence of a native keystone predator of larval anuran guilds, the eastern newt (*Notophthalmus viridescens*: Salamandridae). Because the primary mechanism of keystone predation is selective predation on competitively superior prey species (Paine 1966, Morin 1983), keystone predators may play an important role in limiting impacts of invasive species This research therefore is relevant not only to the impacts of *O. septentrionalis* in tadpole communities, but also to broader issues in conservation and invasion biology.

I conclude this dissertation with a brief summary and overview of the major findings of this research.

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PART I

PATTERNS OF NONINDIGENOUS HERPETOFAUNAL RICHNESS AND BIOTIC HOMOGENIZATION AMONG FLORIDA COUNTIES

The following section is a slightly modified version of a paper to be published in the journal *Biological Conservation*, as part of a special issue on urbanization and homogenization (edited by M.L. McKinney):

Smith, K.G. *In press*. Patterns of nonindigenous herpetofaunal richness and biotic homogenization among Florida counties. Biological Conservation.

As the sole author of the original paper, I selected the topic of study, designed the study, collected and compiled the data, conducted all statistical analyses, and wrote the accepted manuscript.

INTRODUCTION

The study of invasive species has come to be one of the primary foci of conservation biology (Simberloff et al. 1997a, Mack et al. 2000). Much of the current research on invasion biology focuses on evaluating, mitigating, or predicting the impacts of particular nonindigenous species in their surrogate habitats, partly because of the severe impacts rendered by some highly injurious invasive species (e.g., Savidge 1987, Ogutu-Ohwayo 1993, Williamson 1996). The importance of this type of crisis-based, idiopathic research notwithstanding, research on a broader scale is also important, as such research can contribute to a more general understanding of the dynamics, predictors, and perhaps consequences of the establishment of nonindigenous species in native communities. This premise is supported by the success of recent searches for broad ecological patterns in biological invasions, which collectively suggest that relatively simple predictors can explain much of the regional variation in nonindigenous species richness (e.g., Lonsdale 1999, McKinney 2001, Sax 2002).

These studies illustrate an important goal of invasion biology: the identification of the roles played by *a priori* biogeographic factors in the establishment and richness of nonindigenous organisms. The relatively limited research on this topic suggests that measures of propagule pressure, land area, disturbance, and native species richness variously predict nonindigenous species richness (Lonsdale 1999, McKinney 2001, 2002b, Sax 2002). However, these studies are restricted to only those taxa (e.g., fish, plants) and spatial scales (e.g., state/province or country) for which data are available. Whether the patterns documented in these studies are general and apply to other taxa or at other, especially smaller, spatial scales is an unanswered but fundamental question.

Another important goal of invasion biology is the determination of how assemblages of nonindigenous species affect overall biodiversity (McKinney and Lockwood 1999). Although the impacts of certain individual invasive species is a frequent topic of study, a general understanding of the consequences of the integration of many nonindigenous species into regional biotas is lacking. One potential result of increases in nonindigenous species richness is biotic homogenization (i.e., the increase in similarity of regional biotas (McKinney and Lockwood 2001)), a phenomenon with significant, global conservation implications (Olden et al. 2004). Although it is accepted that the establishment of nonindigenous species is an important mechanism of biotic homogenization, species introductions alone do not necessarily result in homogenization (Olden and Poff 2003). In fact, the establishment of nonindigenous species has led to biotic differentiation in some cases and homogenization in others, perhaps owing to differences in scale and taxon of study (cf., Rahel 2000, Marchetti et al. 2001, Rahel 2002, McKinney 2004b, a, Olden and Poff 2004). Because of this variation and the current emphasis placed on the preservation of biological diversity, a better understanding of the relationship between nonindigenous species richness and biotic homogenization is needed.

In this paper I address these two aspects of invasion biology: correlates of geographical patterns in nonindigenous species richness and the influence of these patterns on biodiversity. First, I assess the relationship of seven ecological and anthropogenic factors to nonindigenous amphibian and reptile richness at a relatively fine spatial scale, the county level, using recent data from Florida. The second goal of this study is to use these data to determine if the establishment of nonindigenous amphibians and reptiles has resulted in significant homogenization of Florida's herpetofauna at the county level. Because of the large number of vertebrate introductions in Florida, it is expected that the future biota of this state will be drastically different from current and past biotas (Forys and Allen 1999). It is consequently important to determine if the current trend is toward a homogenized herpetofauna as a result of species introductions in Florida, a state with remarkable, but threatened, biodiversity (Stein et al. 2000).

MATERIALS AND METHODS

Patterns of nonindigenous herpetofaunal richness

I compiled counts of nonindigenous amphibians and reptiles for each of the 67 counties of Florida from a summary of the established nonindigenous herpetofauna of Florida, assembled from literature and museum records, personal observations, and voucher specimens (data in Meshaka et al. 2004). Meshaka et al. (2004) restricted their summary to established (i.e., persistent and reproducing) nonindigenous species, including only those species for which reproduction was documented or could be inferred from population data or museum specimens.

I used the following seven variables for analysis as potential predictors of established nonindigenous herpetofaunal species richness (hereafter, “NI richness”): county population, county land area, county central latitude, % population growth from 1990-2000, presence/absence of a metropolitan statistical area (as defined by the U.S. Census Bureau), presence/absence of a four-year college or university larger than 2500 students, and number of native herpetofaunal species per county. I compiled data for the first five variables from the U.S. Census Bureau online database for the year 2000 census (www.census.gov). Data on university presence or absence were from an online database of accredited colleges and universities (www.princetonreview.com). I tallied counts of non-marine native herpetofaunal species for each county from the distribution maps found in three field guides (Ashton and Ashton 1981, 1985, 1988), which represent locality data compiled from the collections of 17 natural history museums. Although these survey data may not be complete for all counties, I assume that they are a representative index of actual species richness per county.

I conducted univariate and bivariate analyses to ensure that the data used in this study met the assumptions of multiple regression. I $\log_e$ transformed county population and county land area, square-root transformed NI richness, and arcsine-square-root transformed % population growth (Sokal and Rohlf 1995). I also created correlation and bivariate scatterplot matrices to confirm the linearity of relationships between the predictor and response variables and to test for multicollinearity among the predictor variables. Because multicollinearity with the two binary variables (presence/absence of a metropolitan area and presence/absence of a university) could not be evaluated using these methods, I also calculated variance inflation factors (VIF), where VIF > 10 suggests multicollinearity (Neter et al. 1996). There was no strong evidence of multicollinearity among the predictor variables (all VIF < 5), so I retained all seven predictor variables for regression analysis.

I used simple and multiple regression of the seven predictor variables to explain variation in NI richness across Florida's 67 counties. I first used simple linear regression to assess the independent relationship of each continuous variable with NI richness. Because simple linear regression against binary variables is not meaningful I used the Wilcoxon rank sum (Mann-Whitney U) test to evaluate the null hypothesis that there is no difference in NI richness between counties with and without metropolitan areas. I repeated this test for counties with and without universities.

I next used stepwise multiple regression to identify the predictor variables that best explained NI richness after taking into account the influence of all other variables. This procedure reduced the full model (all seven predictor variables) to only those that contribute significantly to the explanation of NI richness once the influence of other variables was considered. Finally, I used residual plots to confirm that variables excluded from the final model were not associated with residual variation in NI richness and also to reconfirm the validity of the assumptions of multiple linear regression. I used JMP 5.1 (SAS 2004), NCSS (Hintze 2001) and PC-ORD 4.01 (McCune and Mefford 1999) and conducted all statistical analyses at $\alpha = 0.05$.

Homogenization of the Floridian herpetofauna

To determine if the establishment of nonindigenous amphibians and reptiles in Florida has led to changes in herpetofaunal similarity among counties I used a common measure of similarity between sites, the Jaccard similarity index (JI):

$$\mathrm{JI} = c / (n_1 + n_2 - c)$$

where c is the number of species common to both sites in a comparison, and n_1 and n_2 are the respective site-specific species richness values. To create a matrix of representative comparisons, I randomly selected 12 counties and calculated JI of the native herpetofaunal assemblages ($\mathrm{JI}_{\mathrm{native}}$) for all 66 unique pairwise combinations of these counties. I then calculated JI of the total (i.e., native + nonindigenous) herpetofaunal assemblages ($\mathrm{JI}_{\mathrm{all}}$) for each comparison. Because the 66 pairwise comparisons are non-independent owing to the repeated use of the same 12 counties, I compared mean $\mathrm{JI}_{\mathrm{all}}$ and $\mathrm{JI}_{\mathrm{native}}$ with a Monte Carlo randomization test (10,000 iterations) to determine if biotic similarity between counties differed owing to the presence of nonindigenous species. Comparatively high values of $\mathrm{JI}_{\mathrm{all}}$ indicate increased similarity owing to the presence of nonindigenous species (homogenization), while low $\mathrm{JI}_{\mathrm{all}}$ values indicate decreased similarity (differentiation).

I also calculated the mean ratio of $\mathrm{JI}_{\mathrm{all}} / \mathrm{JI}_{\mathrm{native}}$ from all 66 county pairs; a ratio > 1 indicates overall homogenization, while a value < 1 indicates overall differentiation within the Floridian herpetofauna. I also used this ratio to determine if a pattern exists in homogenization among the 12 sampled counties by regressing mean values of $\mathrm{JI}_{\mathrm{all}} / \mathrm{JI}_{\mathrm{native}}$

for each county against total population, mean population density, and difference in latitude of those counties. Only latitudinal distance related significantly with JI_{all} / JI_{native} in this multiple regression. Therefore, to assess the relationship of geographic distance and homogenization I also conducted a more conservative Mantel test (10,000 Monte Carlo randomizations) to evaluate the null hypothesis that faunal dissimilarity is not associated with distance between counties (Sokal and Rohlf 1995).

RESULTS

Patterns and predictors of nonindigenous herpetofaunal richness

There are 40 species of established nonindigenous reptile and amphibian in Florida (Meshaka et al. 2004), including 36 reptiles and 4 amphibians. Miami-Dade Co. has the greatest number of established species (36), while 16 counties contain only one species (mean ± SD = 5.3 ± 5.8). The most widespread species are the greenhouse frog (*Eleutherodactylus planirostris*) and the brown anole (*Anolis sagrei*). Approximately 130 native herpetofaunal species are present in Florida (Ashton and Ashton 1981, 1985, 1988), with a range of 16 (Flagler Co.) to 95 (Alachua Co.) documented non-marine native species per county (mean ± SD = 54.1 ± 17.6).

Several of the continuous predictor variables used in this study are significantly correlated with NI richness (Table I-1[1]). NI richness is positively related to county land area and county human population, but strongly negatively related to county central latitude. These results indicate that, excluding the influence of other variables, southerly

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[1] All tables and figures appear in Appendix I-A

counties, counties with large human populations, and counties of large land area will harbor a greater richness of nonindigenous amphibians and reptiles. There is not a significant relationship between NI richness and either native herpetofaunal richness or county population growth rate.

Simple linear regressions of population, land area, and latitude also show that latitude is the best predictor of NI richness (Table I-1); this variable alone explains nearly two thirds of the variation in NI richness. Metropolitan area presence and university presence also significantly affect NI richness (U-test on metropolitan area: $n_{present} = 38$, $n_{absent} = 29$, $Z = -3.23$, $P = 0.001$; university: $n_{present} = 15$, $n_{absent} = 52$, $Z = 4.17$, $P < 0.0001$). Excluding the influence of other variables, mean numbers of NI species per county are significantly greater in counties with a metropolitan area and counties with a college or university.

In the multiple regression analysis county latitude, human population, and university presence together best explain NI richness (Table I-2). Although land area significantly predicted NI richness in a simple linear regression (Table I-1), this variable was not selected by stepwise variable selection. Forcing this variable into the model results in an increase in r^2 of only 0.003. Similarly, the forced inclusion of metropolitan area presence also increases r^2 by only 0.003. The multiple regression model otherwise confirms the results of the simple regression analyses: latitude contributes the most information to NI richness, again explaining about two thirds of the variation in this variable. Population and university presence also explain significant variation in NI richness in both the simple and multiple regression analyses to lesser degrees.

Similarity of the Floridian herpetofauna between counties

Overall, the mean similarity of the total Floridian herpetofauna (JI_{all}) among the 12 randomly-selected counties did not differ significantly from the native similarity (JI_{native}) (mean $JI_{all} = 0.347$; $JI_{native} = 0.355$; Monte Carlo randomization $P = 0.651$). The mean ratio of JI_{all} / JI_{native} in this study is 0.986 (± 0.07 SD) indicating a slight differentiating effect of nonindigenous amphibians and reptiles among the 12 counties. However, this ratio was not significantly different from 1.00 (Monte Carlo randomization $P = 0.104$), also suggesting that nonindigenous species have no net effect on the similarity of the sampled counties.

Despite the lack of a net homogenizing effect, JI_{all} / JI_{native} is significantly related to latitudinal distance between counties (Monte Carlo Mantel test, $r = 0.568$, $P = 0.027$). The positive correlation indicates that the presence of nonindigenous herpetofauna tends to decrease the similarity of distant county pairs while increasing the similarity of nearby counties (Fig. I-1). The homogenizing effect on nearby counties in this sample is small, however: JI_{all} / JI_{native} of adjacent counties (i.e., latitudinal distance = 0) is only slightly greater than 1.0.

DISCUSSION

Interpretation of observed patterns of nonindigenous herpetofaunal richness

The results of this analysis suggest that the pattern of NI richness in Florida is strongly structured by a few key factors, as indicated by the high r^2 value of the final, three-variable regression model. Latitude, population, and presence of a university contribute to explain approximately 80 % of the variation in NI richness at the county level across Florida.

The importance of human population to nonindigenous species richness has been documented previously and is mechanistically related to propagule pressure (Williamson 1996, McKinney 2001). Although the "nonindigenous" classification of non-native species includes both species introduced by humans as well as those dispersing on their own (sensu Simberloff 1997), the majority of nonindigenous species established within the United States have been introduced by humans, either intentionally or inadvertently (Mack et al. 2000). Consequently, a positive relationship between human population and number of nonindigenous species has repeatedly been documented in plants and fishes (McKinney 2001, 2002a, b, Espinosa-Garcia et al. 2004, Gido et al. 2004). An informal comparison of human population and NI richness in Florida since 1900 also suggests the presence of a strong relationship, as the recent accumulation of nonindigenous amphibians and reptiles over time closely follows the rapid growth of the human population of Florida (Fig. I-2).

Several other potentially influential factors are related to human population, but may have effects on nonindigenous species richness distinct from those of population itself. For example, urbanized metropolitan areas may include features that enhance nonindigenous species establishment (e.g., higher habitat diversity, increased disturbance, presence of animal importers) regardless of the human population of the area (Rebele 1994). That presence of a metropolitan area was not a significant predictor of NI richness in this analysis suggests that any contribution of urbanization to NI richness is subsumed within the effects of human population (but see Olden et al., this issue). However, because human population is itself a strong predictor of metropolitan area presence, the relationship between urbanization *per se* and NI richness is difficult to assess.

Percent population growth also was not significantly related to NI richness in this study. In this study I used % population growth as a surrogate variable for recent anthropogenic habitat disturbance following the assumption that rapidly growing counties experience greater ecological disturbance relative to counties experiencing little growth. This assumption may not be valid, however, and absolute population growth, rather than proportional growth, may be a more valid measure of habitat disturbance. Additionally, because even highly invasive species sometimes exhibit a “lag” period following introduction (Schmitz et al. 1997, Mack et al. 2000), the effects of recent disturbance caused by population growth may not be observed for a number of years or even decades. However, % population growth may be useful as a predictor of future increases in nonindigenous species richness since high absolute populations will always be preceded by population growth.

The most influential explanatory variable in this analysis was county central latitude. The negative effect of latitude on native species richness is a well-documented biogeographic phenomenon (reviewed in Rosenzweig 1995, Brown and Lomolino 1998), but the importance of latitude to patterns of nonindigenous richness has been assessed rarely (cf. Lonsdale 1999, Sax 2001). In this study, latitude related negatively with NI richness, explaining approximately two-thirds of the variation in this variable (Tables I-1, I-2). Although this result is consistent with general patterns of native species richness, the pattern of NI richness in Florida is opposite that of the native herpetofaunal richness, which shows a positive relationship with latitude (Fig. I-3). This unusual trend in native species richness may be a consequence of the "peninsula effect," which in Florida is putatively caused by lower habitat diversity in the tipward peninsula of Florida (Means and Simberloff 1987). Although the ecological constraint of reduced habitat diversity should theoretically apply to nonindigenous species as well as native species, the pattern of NI richness in Florida clearly violates this premise. One possible explanation for this pattern is that very few nonindigenous amphibians and reptiles have invaded undisturbed natural habitats in south Florida; most occur in the numerous anthropogenic habitats of urbanized areas (Wilson and Porras 1983, Butterfield et al. 1997, Bartlett and Bartlett 1999). As a result, most nonindigenous amphibians and reptiles in south Florida are commensally related to humans, which may free them from the typical habitat constraints imposed on native species.

A distinct but nonexclusive explanation for the effect of latitude in this study is that the establishment of nonindigenous species in northern Florida is precluded by competition with the relatively rich indigenous herpetofauna in that part of the state (Elton 1958). There is no evidence to support this biotic resistance hypothesis, however, as there was not a significant relationship between native and nonindigenous richness in this study (*P* = 0.3574 for native species richness in the full model and Table I-1). Furthermore, most other taxa for which there are data show a positive, not negative, relationship between regional native and nonindigenous species richness, which agrees with the null expectation for spatial scales larger than local communities (Fridley et al. 2004). In sum, there is presently little empirical support for the limitation of nonindigenous species richness by competition with native species (Simberloff 1995, D'Antonio and Levine 1999, Lonsdale 1999, Levine 2000, McKinney 2001, 2002b).

Finally, another nonexclusive explanation is related to the relative permeability of the geographic barriers that surround Florida. Few historical physiographic barriers separated the indigenous faunas of southeastern North America and Florida, while tropical and subtropical amphibians and reptiles have been separated from Florida by a significant marine barrier. For this reason, the herpetofaunas of Florida and the Caribbean region are relatively dissimilar, allowing for a greater number of invasion opportunities. In contrast, relatively few novel invasions can originate from north of Florida due to historic homogenization of the southeastern and Floridian faunas. Thus, the greater NI richness of south Florida may be a function of both invasion source and historical biogeography. This explanation is supported by a comparison of the geographic origins of the 36 nonindigenous amphibians and reptiles considered to be established in Florida as of 1995, 34 of which are of tropical or subtropical climatic origin (Butterfield et al. 1997). However, that this pattern simply arises from an importation bias that intentionally or inadvertently favors tropical species cannot presently be discounted.

One novel result in this study is that presence of a four-year college or university was positively associated with NI richness. The contribution of this variable to the overall explanation of NI richness was small but significant, even after accounting for the effects of population and latitude. This positive effect of university presence could be mediated through one or both of two mechanisms. First, the presence of a university may result in greater opportunities for transport of nonindigenous species, resulting in greater propagule pressure and greater rates of establishment (e.g., Lockwood 1999). Second, the presence of a university could result in greater detection of nonindigenous species due to the association of biologists or ecologists with colleges and universities.

If the effect of university presence is due to the latter mechanism of increased detection probability, then a similar relationship may exist with native amphibian and reptile richness patterns, as well. A multiple regression of native herpetofaunal species richness against the remaining six regressors used in this study shows that university presence is also positively associated with native herpetofaunal species richness, explaining a significant proportion of the variance after other significant variables are considered (partial $r^2 = 0.14$, $P = 0.008$). This concordant result suggests that sampling bias is at least partially responsible for the relationship between university presence and NI richness in Florida. This result is not surprising, since biogeographic analyses at fine spatial scales require survey data with relatively high resolution and it is known that errors of omission can influence apparent patterns in biogeography (e.g., Kodric-Brown and Brown 1993) . Future analyses of species richness patterns at relatively small spatial scales may benefit from the inclusion of variables that explicitly address sampling bias to account for the reduced resolution of survey data as such scales.

Finally, although the effect of area on native species richness is a well-known phenomenon and is one of a small set of ecological “laws” (Rosenzweig 1995, Lawton 1999), county land area was not significantly related to NI richness in the multiple regression model in this study. This result differs from the results of one analysis of nonindigenous herpetofaunal richness among states (Lonsdale 1999) and two studies on introduced fishes (McKinney 2001, Gido et al. 2004). Even when a nonindigenous species-area relationship has been detected, however, the causal mechanism has sometimes been more closely allied with the whims of anthropogenic introductions, rather than the effects of area *per se* (McKinney 2001). In this study, habitat homogeneity among counties may have precluded a species-area relationship. Additionally, although it has been hypothesized that all organisms in an ecological community should be constrained by the same environmental factors (Sax 2001), nonindigenous biotas are not assembled naturally over ecological or evolutionary time, so they may appear to violate ecological laws over short time scales (e.g., prior to relaxation and extirpation of nonreproductive propagules). Additional theoretical and empirical research on the relationship of area and nonindigenous species richness is needed, especially with regard to the effect of time.

Homogenization and differentiation of the Floridian herpetofauna

The results of this analysis suggest that the presence of nonindigenous herpetofauna in Florida has not resulted in an overall effect on the similarity of the herpetofauna. However, the presence of a relationship between similarity and county distance suggests that the scale of comparison influences the perception of homogenization, with no effect or slight homogenization at small scales (neighboring counties) and differentiation at larger scales (distant counties). A similar pattern has also been observed in nonindigenous plants (McKinney 2004b, a) and is not unexpected given the strong relationship of latitude to NI richness in this study. This relationship apparently results in different subsets of established nonindigenous species in north and south Florida, whereas counties within latitudinal subregions tend to share similar species. This trend generally agrees with the expectation that the effect of nonindigenous species on diversity is scale-dependent, with increases in diversity at relatively small spatial scales and decreases at large (e.g., regional or global) scales (Marchetti et al. 2001, McKinney 2002a, Sax et al. 2002, Sax and Gaines 2003).

It is tempting to regard the lack of significant homogenization in the Floridian herpetofauna optimistically, but several factors suggest that such an outlook is premature. First, although few researchers have specifically tracked the spread of nonindigenous species in Florida (but see Campbell 1996), the ranges of several nonindigenous amphibians and reptiles in Florida clearly continue to expand (citations in species accounts in Meshaka et al., 2004). Therefore, even disregarding future introductions, the mean number of shared species among counties is likely to increase, resulting in increased similarity and biotic homogenization. Additionally, 27 of the 66 county pairs showed evidence of homogenization ($JI_{all} / JI_{native} > 1$), so the absence of an overall trend toward homogenization should not be misconstrued as a complete lack of homogenization. Furthermore, there is no reason to expect that species gains from introductions and species losses caused by extinctions will occur on the same temporal scale. Recent increases in herpetofaunal richness may only be the precursor to the extirpation of native species, perhaps as a result of "invasion meltdown" scenarios (Simberloff and Von Holle 1999), in which increases in nonindigenous diversity are but one step in a positive feedback loop caused by mutualisms and facilitation among introduced species, with potentially severe costs to native species (Marchetti et al. 2001). In fact, experimental evidence of adverse effects of nonindigenous amphibians and reptiles in Florida suggests that future impacts are possible (Campbell 2000, Smith 2005).

Finally, disregarding the potential impacts of invasive species on native taxa, extinctions of rare species owing to any cause will tend to enhance the effects of homogenization as a result of species introductions.

CONCLUSIONS AND IMPLICATIONS

This study extends the results of previous research on geographical patterns of nonindigenous species richness to little-studied taxa and to a relatively small spatial scale. Not surprisingly, cumulative evidence, including that presented here, suggests that the effect of human population on nonindigenous species richness is nearly universal and that the roles played by humans in the introduction and establishment of nonindigenous species are manifold. For example, even the strong effect of latitude in this study may be a consequence of the source of introduction (as dictated by human transport) and not solely due to biogeographic factors. Additionally, the minor effect of university presence on NI richness in this study suggests the existence of an important distinction between county-level analyses and those conducted at larger spatial scales. This result is a reminder that fine-scale inventories of nonindigenous species are likely to be incomplete or biased in some cases.

Although the generality of several patterns discussed here remains unresolved, examination of the patterns of nonindigenous species richness should continue toward the ultimate goals of explanation and prediction. A popular truism among invasion biologists is that the costs of preventing the establishment of nonindigenous species are quite reasonable when compared to the costs of control or eradication (Simberloff et al. 1997b, Mack et al. 2000). Therefore, even if the natural laws that limit the distribution and richness of nonindigenous species are few and elusive, their pursuit is worthy because the potential benefits are so great. However, the overwhelming contribution of human activities to the establishment of nonindigenous species presents a caveat in the search for these laws: it is important to recognize that natural laws may bend or break under the influence of human activities and that the resulting "unnatural laws" may be equally or more important.

Finally, there is presently no evidence for an overall homogenizing effect of nonindigenous amphibians and reptiles in Florida. This result should not be confused with an absence of the effects of nonindigenous species in Florida, however. Because the effects of nonindigenous species on biodiversity and biotic similarity have only begun to be explored, the results of this study are best used as a benchmark to assess future changes in diversity in Florida as a result of changes in native and nonindigenous herpetofaunal richness.

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APPENDIX I-A

TABLES AND FIGURES

Table I-1. Correlation coefficients, associated *P*-values, and r^2 of potential explanatory variables ("regressor") of nonindigenous herpetofaunal species richness among Florida counties.

Regressor	**Correlation (r)**	***P***	**r^2**
Log(Land Area)	0.4583	0.0001	0.2101
Latitude	-0.8100	< 0.0001	0.6562
Native Species Richness	0.0154	0.9018	-
Log(Population)	0.6979	< 0.0001	0.4867
Population Growth	-0.0943	0.4479	-

Table I-2. Parameter estimates and contribution to r^2 of significant variables in the final multiple regression model for nonindigenous species richness in Florida counties.

Variable	**Parameter Estimate**	**Partial r^2**	***P***
Latitude	-0.4295	0.66	< 0.0001
Log(Population)	0.3849	0.13	< 0.0001
University Presence	0.2511	0.02	0.01
		Model r^2 = 0.80[a]	

[a] Discrepancy with total of partial r^2 values used in this table (0.81) is due to rounding error.

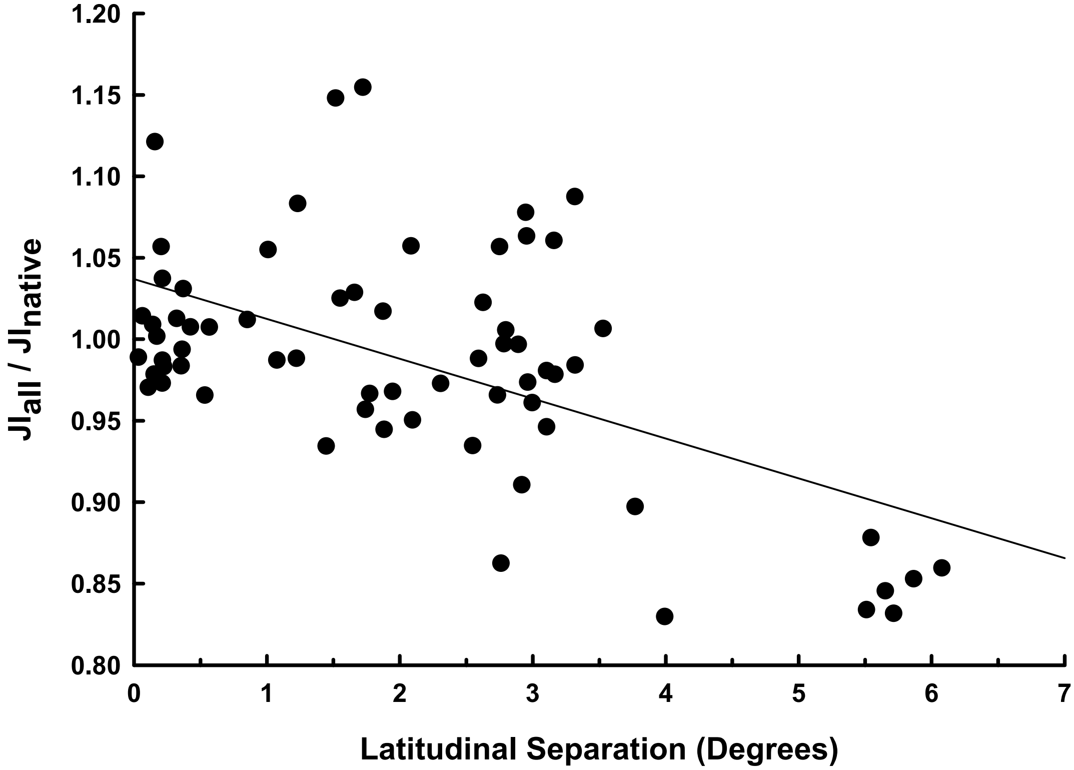

Fig. I-1. Ratio of Jaccard similarity index of the entire herpetofaunal community (JI_{all}) to that of the native herpetofaunal community (JI_{native}) compared among all unique pairs of 12 randomly-selected Florida counties and plotted against county latitudinal separation. Ratio values > 1 indicate biotic homogenization and values < 1 indicate biotic differentiation as a result of nonindigenous species presence. A least-squares regression line is provided to illustrate the trend only; for statistical significance see the Mantel test results.

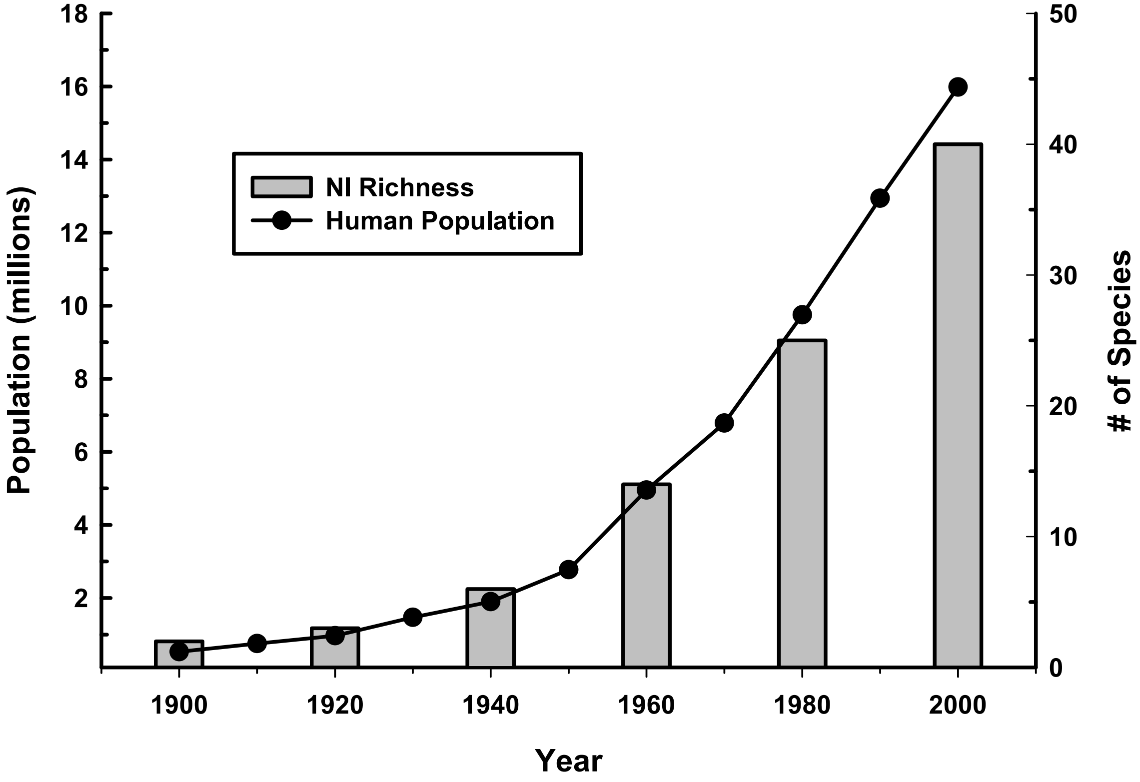

Fig. I-2. Accumulation of nonindigenous herpetofaunal richness (NI Richness) and human population in Florida since 1900. Population data are from the U.S. Department of the Census (www.census.gov) and NI richness data are from Butterfield et al. (1997) and Meshaka et al. (2004).

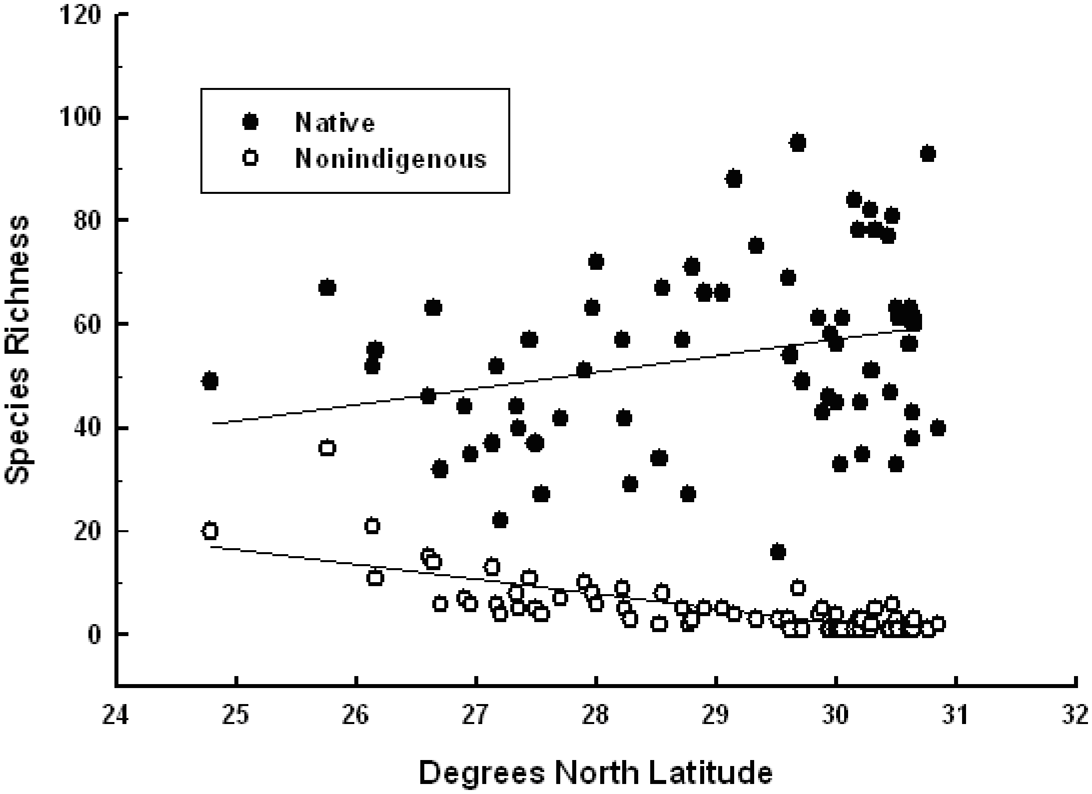

Fig. I-3. Linear relationships of native and nonindigenous herpetofaunal species richness with latitude in Florida. Individual points are species richness counts for each county (*n* = 67). For native species, slope = 3.144 and $r^2 = 0.08$ ($P = 0.02$) ; for nonindigenous species slope = -2.820 and $r^2 = 0.56$ ($P < 0.0001$).

PART II

EFFECTS OF NONINDIGENOUS TADPOLES ON NATIVE TADPOLES IN FLORIDA: EVIDENCE OF COMPETITION

The following section is a slightly modified version of a paper published in the journal *Biological Conservation*:

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

As the sole author of the original paper, I selected the topic of study, designed the study, conducted the study and collected and compiled the data, conducted all statistical analyses, and wrote the accepted manuscript.

INTRODUCTION

Invasive species are widely considered to be a significant threat to native biodiversity (Wilcove et al. 1998, Mack et al. 2000). Consequently, the biology of invasive and nonindigenous species has become a focus of conservation biology (e.g., Mooney and Drake 1986, Simberloff et al. 1997). Despite this focus, evidence of the ecological impacts of nonindigenous species on native species is frequently absent or is strictly anecdotal (Vitousek et al. 1987, Simberloff 1995, 1997). As a result, biologists often lack the information necessary to prioritize conservation and management recommendations when faced with the presence of many nonindigenous species. Furthermore, not all nonindigenous species become invasive or injurious to native communities. Research that contributes to the correct identification of potentially injurious nonindigenous species is therefore essential, as is evidenced by the sometimes ecologically or economically severe impacts of such species (Diamond 1984, Savidge 1987, Wilcove et al. 1998, Pimentel et al. 2000).

Nonindigenous species may affect native taxa through several mechanisms, one of which is interspecific competition. Though frequently studied in native communities (reviewed in Schoener 1983), interspecific competition is relatively difficult to demonstrate unequivocally in invaded communities (Vitousek et al. 1987, Simberloff 1997). As a result, studies assessing the role of interspecific competition as a mechanism of impact of nonindigenous species are few, especially among vertebrates (but see Petren et al. 1993, Petren and Case 1996, Kupferberg 1997, Kiesecker and Blaustein 1998, Gurnell et al. 2004). A better understanding of the significance of competition to the impacts of invasive species is needed.

Larval anuran (tadpole) communities are ideal model communities for the study of competition between native and nonindigenous taxa. Such communities may be particularly vulnerable to competition with exotic species because they are frequently characterized by high population densities in temporally and spatially restricted aquatic habitats. As a result, competition is an important ecological factor in tadpole communities (reviewed in Wilbur 1980, Alford 1999). Despite the importance of competition in tadpole communities and the potential for competition between nonindigenous and native species, there has been little research on the impacts of nonindigenous tadpoles on native tadpoles from competition *per se* (but see Kupferberg 1997, Kiesecker and Blaustein 1998).

In this study, I designed laboratory microcosm experiments to address the possibility of competition among native and exotic anuran larvae. I combined larvae of two nonindigenous anurans, the Cuban treefrog, *Osteopilus septentrionalis*, and the cane toad, *Bufo marinus*, with larvae of one of two anurans native to Florida, USA (southern toad, *Bufo terrestris*, and green treefrog, *Hyla cinerea*). The goal of this study was to determine whether *B. marinus* and *O. septentrionalis* larvae affect the growth, development, and survivorship of larval *B. terrestris* and *H. cinerea*.

STUDY SYSTEM

Both *B. marinus* and *O. septentrionalis* have occurred in Florida for more than 50 years. *Bufo marinus* is native to South and Central America (Zug and Zug 1979) but became established in Florida prior to 1957 as the result of several intentional and accidental introductions beginning around 1936 (Easteal 1981, Wilson and Porras 1983). Since its initial establishment in the Miami-Dade Co. area, *B. marinus* has expanded its range throughout much of southern peninsular Florida (Lever 2001). *Osteopilus septentrionalis* is native to Cuba and adjacent islands (Duellman and Crombie 1970) and was established in mainland Florida by 1951 (Schwartz 1952). *Osteopilus septentrionalis* now occurs in much of peninsular Florida (Meshaka 2001) and both species are syntopic with several native anurans, including *B. terrestris* and *H. cinerea*.

Bufo terrestris and *H. cinerea* are both locally abundant, widespread species that occur throughout Florida and the southeastern coastal plain of the United States. Although populations of both native species are apparently stable throughout most of their range (Bartlett and Bartlett 1999), several ecologists have expressed concern regarding the impacts of *B. marinus* and *O. septentrionalis* on native anurans in south Florida and suggest that declines of native species have occurred (Bartlett 1967, Rossi 1981, Bartlett and Bartlett 1999). The role of larval interactions in these putative declines has been almost entirely ignored, however (but see Rossi 1981).

Several characteristics of larval *B. marinus* and *O. septentrionalis* suggest that these species may affect native tadpole communities. Both *B. marinus* and *O. septentrionalis* larvae occur in temporary or ephemeral bodies of water and develop rapidly in these habitats, which may suggest the potential for competition with native temporary pond species such as *B. terrestris* and *H. cinerea*. Additionally, carnivory and cannibalism are traits of some ephemeral pond species (Hoff et al. 1999) and *O. septentrionalis* is known to be cannibalistic when reared in high densities (Crump 1986) and will prey on heterospecific anuran larvae under certain conditions (low food, high density Smith 2005). Larvae of *B. marinus* are not carnivorous but are toxic to many vertebrate and invertebrate predators (Crossland 1998, Crossland and Alford 1998, Crossland and Azevedo-Ramos 1999), including some anuran larvae (Crossland 2000). Although few native anuran larvae in Florida are carnivorous, most species will scavenge dead tadpoles, so dead *B. marinus* larvae may be a toxic food source for native tadpoles.

MATERIALS AND METHODS

Tadpole collection

I collected freshly laid clutches of *B. terrestris* and *H. cinerea* from wild-caught colonies held in vinyl tanks at the U.S. Geological Survey Florida Integrated Science Center in Alachua Co., Florida (hereafter, FISC) on 24 May 2003 Freshly laid clutches of *B. marinus* and *O. septentrionalis* were collected from a flooded field in Dade Co., Florida on the morning of 25 May. When available, multiple egg clutches were used for each species to reduce genetic effects on competitive ability (Travis 1980). Limited availability of eggs and time constraints precluded the use of multiple clutches for all species, however (*B. terrestris* and *B. marinus*, one clutch each). All eggs were hatched in captivity and hatchlings were fed tadpole chow *ad libitum* until the experiments began on 30 May. Tadpole chow was a 3:1 ratio by mass of finely ground rabbit chow pellets (primary ingredient = alfalfa) and TetraFin™ goldfish food and was similar to that used in similar research (e.g., Alford 1994).

Experimental design

I simultaneously conducted two randomized block experiments to assess the competitive effects of *B. marinus* and *O. septentrionalis* tadpoles on a single native species; one experiment measured effects on *B. terrestris* and one measured effects on *H. cinerea*. Native and nonindigenous tadpoles were randomly assigned to one of four experimental treatments: native control (36 native larvae with no nonindigenous larvae), *B. marinus* treatment (18 native larvae with 18 *B. marinus* larvae), *O. septentrionalis* treatment (18 native larvae with 18 *O. septentrionalis* larvae), and *B. marinus* + *O. septentrionalis* treatment (12 native larvae with 12 *B. marinus* larvae and 12 *O. septentrionalis* larvae). Initial density of all treatments was 36 tadpoles per aquarium (2 tadpoles / L) and is within the highly variable range of naturally occurring densities of these species. All four treatments were included in one of four spatial blocks (four treatments per block X four replicates = 16 aquaria per experiment) and the location of each treatment within a block was randomly assigned with the restriction that no treatment would be adjacent to the same treatment in another block.

All experimental microcosms were housed in the North Wet Lab of FISC. Microcosms were 10 gal (37.85 L; 25.5 cm X 50 cm) glass aquaria fitted with standpipe drains and filled with aerated well water to a depth of 14.5 cm (approximately 18 L). All aquaria were flushed with fresh well water for 15 min every 1-3 d to prevent the accumulation of lethal levels of metabolic products without completely removing potentially allelopathic substances from the microcosms. Ten grams of oven-dried oak leaves were added to each aquarium prior to the start of each experiment to provide microhabitat complexity. Aquaria were arranged on a pallet rack shelving system with an entire experiment (16 aquaria) on one shelf. Artificial light was provided on a 12 h:12 h light:dark cycle from a fluorescent light source directly above the rack system. Food was added to all aquaria at a ration of 2.7 g tadpole chow per aquarium every three days throughout the experimental period.

Response variables, data collection and statistical analysis

At the outset of the experiments, a haphazard sample of larvae of each native species (n = 30) was collected for developmental stage determination (Gosner 1960), body length measurement to nearest 0.1 mm (as described in Altig and McDiarmid 1999), and wet body mass measurement to nearest 0.1 mg (as described in Alford 1994).

Differences in size and developmental stage of tadpoles at the start of the experiments were the result of natural differences in hatching and developmental rates (Table II-1[2]).

Because both nonindigenous species develop and reach metamorphosis rapidly, data on growth and development of focal species (*B. terrestris* and *H. cinerea*) were collected twice during the experimental period. All native tadpoles were weighed when the first tadpole in an experiment reached metamorphosis (experiment days 15-16; "early larval period"), which reflected the effects of the initial densities of each treatment. Each tadpole was weighed again individually at metamorphosis, which reflected the effects of decreasing densities during the experiment as competitors transformed. Metamorphosis was defined as the emergence of the second forelimb (Gosner stage 42) at which time the individual was permanently removed from the aquarium. Data collected from transforming individuals were date of transformation and wet mass to nearest 0.1 mg. These data allowed for comparison of relative larval growth and developmental rates, which are positively correlated with adult anuran survival and fitness (Berven and Gill 1983, Smith 1987). These data also allowed for the calculation of time to metamorphosis and proportion surviving to metamorphosis for focal species in each treatment group.

I used multivariate analysis of variance (MANOVA) to detect the effects of independent factors (presence of nonindigenous tadpoles) on the growth and development of native anuran larvae. Data from both the early larval period and metamorphosis were combined in a single MANOVA for each native species to test for the overall effects of the presence of nonindigenous tadpoles. Response variables used for the early larval period were body mass and Gosner stage and those used for metamorphosis were body

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[2] All tables and figures appear in Appendix II-A

mass and time to metamorphosis. Proportion surviving to metamorphosis (arcsine transformed) was analyzed in a separate ANOVA. I used treatment means in all statistical analyses to avoid pseudoreplication. Data from one microcosm in the B. terrestris experiment was eliminated from analysis due to contamination and failure. If MANOVA results indicated a significant treatment effect I then used individual univariate ANOVAs to determine the response variables in which significant effects occurred. The use of a multivariate test such as MANOVA prior to multiple ANOVAs avoids problems associated with experimentwise error rate inflation and replaces the use of more subjective error control methods such as Bonferonni corrections (Cabin and Mitchell 2000, Moran 2003). If MANOVA and ANOVA results showed significant differences among groups, I then used post hoc contrasts (Tukey-Kramer Multiple-Comparison Tests) to detect the presence of significant differences among specific treatment groups within significant response variables as determined by ANOVA. In all analyses, "replicate" was used as a random effects blocking factor. I used NCSS (Hintze 2001) and a significance level of $\alpha = 0.05$ for all statistical analyses.

RESULTS

Effects of nonindigenous tadpoles on larval *Bufo terrestris*

The presence of nonindigenous larvae significantly reduced the growth and developmental rates of *B. terrestris* tadpoles over the entire larval period (MANOVA, treatment effect $F_{12,14} = 11.87$, $P < 0.001$; block effect $F_{12,14} = 1.33$, $P = 0.307$).

Specifically, larvae of *O. septentrionalis* alone or in combination with *B. marinus* inhibited the growth of *B. terrestris* tadpoles as measured by body mass during both the early larval period (ANOVA, $F_{3,11} = 15.76$, $P < 0.01$) and at metamorphosis (ANOVA, $F_{3,11} = 81.43$, $P < 0.001$; Fig. II-1). In contrast, body mass of *B. terrestris* was not significantly affected by the presence of *B. marinus* and did not differ between the *O. septentrionalis* treatment and the *B. marinus* + *O. septentrionalis* treatment (Tukey-Kramer test; Fig. II-1), suggesting that the main effect on *B. terrestris* was driven by the presence of O. *septentrionalis*.

Only the presence of *O. septentrionalis* larvae affected the developmental rate of *B. terrestris* during the early larval period. After 15 days, *B. terrestris* larvae reared with *O. septentrionalis* larvae were significantly less developed as measured by Gosner stage when compared to the control group (ANOVA, $F_{3,11} = 28.96$, $P < 0.001$; Table II-2). This depressed developmental rate persisted to metamorphosis, as *B. terrestris* tadpoles also transformed later when reared with *O. septentrionalis* larvae (ANOVA, $F_{3,11} = 11.68$, $P < 0.01$). The magnitude of this effect was the addition of 1.9 days (12.1%) to the larval period of *B. terrestris* (Table II-2).

Low mortality of *B. terrestris* in all treatments resulted in no significant difference in survivorship of *B. terrestris* among treatments (ANOVA, $F_{3,11} = 2.04$, $P = 0.1874$). The mean proportion surviving to metamorphosis across all treatments was 0.9756 ± 0.02 for this species.

Effects of nonindigenous tadpoles on larval *Hyla cinerea*

The presence of nonindigenous tadpoles also affected the growth and developmental rates of *H. cinerea* tadpoles during the entire larval period in this study (MANOVA, treatment effect $F_{12,16} = 10.31$, $P < 0.001$; block effect $F_{12,16} = 1.28$, $P = 0.317$). After 16 days, *H. cinerea* tadpoles exposed to *O. septentrionalis* larvae were significantly smaller than *H. cinerea* tadpoles in the control group as measured by body mass (ANOVA, $F_{3,12} = 79.03$, $P < 0.001$; Fig. II-1). The effects of *O. septentrionalis* larvae alone were not significantly different from the effects of *B. marinus* + *O. septentrionalis* larvae and *B. marinus* tadpoles had no significant effect on body mass of *H. cinerea* (Tukey-Kramer Test; Fig. II-1).

The presence of nonindigenous anuran larvae also affected body mass of *H. cinerea* at metamorphosis. However, in contrast to the early larval period, *H. cinerea* tadpoles reared with both nonindigenous species transformed at larger body mass than in the control group (ANOVA, $F_{3,12} = 7.49$, $P < 0.01$; Fig. II-1). This result suggests that *H. cinerea* larvae benefited from the presence of nonindigenous tadpoles during late larval development. However, in this experiment both nonindigenous species reached metamorphosis more rapidly than did the native *H. cinerea*. The mean time to metamorphosis (± 1 S.E.) of *H. cinerea* in this study was 37.5 (± 0.37) days, compared to 17.4 (± 0.32) days and 17.4 (± 0.21) days for the nonindigenous *B. marinus* and *O. septentrionalis*, respectively. Consequently, *H. cinerea* tadpoles reared with nonindigenous tadpoles experienced lower densities during the latter part of the study period (12 or 18 *H. cinerea* per aquarium) than did those in the control group (36 *H. cinerea* per aquarium), owing to metamorphosis of nonindigenous larvae. Individuals in the control group were therefore exposed to higher tadpole densities—and greater intraspecific competition—for a longer period of time.

If these results were caused by lower levels of density-dependent intraspecific competition, then change in mass during the late larval period of *H. cinerea* should be inversely correlated with the number of *H. cinerea* tadpoles in each treatment. This prediction is confirmed; a strong negative relationship exists between increase in body mass during the late larval period and number of *H. cinerea* tadpoles per treatment group (Spearman rank correlation, $r_s = -0.8245$, $n = 16$, $P < 0.001$). This result suggests that differences in mean metamorphic body mass of *H. cinerea* were related to larval density rather than to the presence of nonindigenous tadpoles.

The developmental rate of *H. cinerea* was also significantly affected by the presence of *O. septentrionalis* throughout the larval period (Table II-2). *Hyla cinerea* tadpoles exposed to either the *O. septentrionalis* or the *B. marinus* + *O. septentrionalis* treatment were less developed after 16 days than were *H. cinerea* tadpoles in the control group (ANOVA, $F_{3,12} = 50.80$, $P < 0.001$). Despite experiencing decreased competition during the late larval period, *H. cinerea* tadpoles took significantly longer to reach metamorphosis when reared with *O. septentrionalis* larvae (ANOVA, $F_{3,12} = 13.18$, $P < 0.01$). The magnitude of this effect was the addition of 4.1 days (11.2%) to the larval period of *H. cinerea*. The presence of *B. marinus* tadpoles alone had no effect on the developmental rate of *H. cinerea* tadpoles, suggesting that the main effect on the development of *H. cinerea* was due to the presence of *O. septentrionalis*.

A trend toward decreased survivorship in *H. cinerea* reared with *O. septentrionalis* larvae was detected, but this result was not statistically significant

(ANOVA, $F_{3,12} = 3.03$, $P = 0.086$). Mean proportion surviving to metamorphosis across all treatments was 0.8775 ± 0.03 for this species.

Metamorphosis and survivorship of nonindigenous species

Neither *B. marinus* nor *O. septentrionalis* exhibited significant variation in time to metamorphosis or proportion surviving across treatments in either experiment in this study (Table II-3).

DISCUSSION

Ecological interactions among anuran larvae can lead to several outcomes that may negatively affect the fitness of competing tadpoles. Interactions may result in mortality, resulting in decreased recruitment to the breeding population. Interspecific predation among tadpoles (e.g., Heyer et al. 1975), toxicity of tadpoles (e.g., Crossland 2000), or interspecific competition (e.g., Wilbur and Fauth 1990) are three mechanisms that may result in decreased survivorship of tadpoles. Alternatively, interactions among tadpoles may also have sublethal—but still deleterious—effects on the growth and developmental rates community members. In such cases, survivorship to metamorphosis may not be significantly affected, but the subsequent fitness of juveniles or adults may be compromised as a result of interactions among tadpoles (Smith 1987).

Survivorship of native tadpoles

In this study, the proportion of native tadpoles surviving to metamorphosis was not significantly affected by the presence of nonindigenous tadpoles, although a trend toward reduced survival was seen in *H. cinerea*. This result allows for the elimination of several potential mechanisms of impacts of *B. marinus* and *O. septentrionalis* on *B. terrestris* and *H. cinerea*. Although *O. septentrionalis* tadpoles will prey on heterospecific larvae when reared in high densities (approx. 60 tadpoles / L) and held without other food (Smith 2005), significant predation of native tadpoles by *O. septentrionalis* did not occur in this study, perhaps because densities in this study were moderate (2 tadpoles / L) and food was readily available. The conditions of high tadpole densities and low food availability may exist during the drying of natural ephemeral pools, however, so predation by *O. septentrionalis* on native tadpoles may yet be a significant impact in natural settings.

The toxicity of *B. marinus* also did not affect survivorship of either native species, despite evidence that both *B. terrestris* and *H. cinerea* are susceptible to *B. marinus* toxin (Punzo and Lindstrom 2001). Since survivorship of *B. marinus* was high in this study (Table II-3), few potentially toxic carcasses of this species were available for scavenging by *B. terrestris* or *H. cinerea*.

A trend toward decreased survivorship of *H. cinerea* was evident in this study; marginally fewer *H. cinerea* tadpoles survived to metamorphosis when reared with *O. septentrionalis* or both *B. marinus* and *O. septentrionalis*. This trend suggests that were *B. marinus* and *O. septentrionalis* larvae present for the entire larval period of *H. cinerea*, survival to metamorphosis of *H. cinerea* might have been significantly compromised.

Additional experiments in which transforming nonindigenous tadpoles are replaced would have greater resemblance to natural systems and can be used to evaluate the trend reported here.

Delayed metamorphosis of native tadpoles

Both *B. terrestris* and *H. cinerea* use a variety of breeding habitats including temporary bodies of water with short hydroperiods, which are the preferred breeding habitat of *O. septentrionalis*. In these habitats, the addition of two to four days to the larval period (the increase in the larval period for *B. terrestris* and *H. cinerea*, respectively, as a result of the presence of *O. septentrionalis* larvae) may significantly decrease larval survivorship to metamorphosis owing to desiccation or increased predation. Desiccation caused by pond drying is a significant source of mortality in some tadpoles (Seale 1982, Newman 1987) and in habitats in which desiccation is not a factor, longer larval periods can also decrease survival by increasing the duration of exposure to aquatic predators (Pritchard 1965, Heyer et al. 1975, Caldwell et al. 1980, Wilbur 1980). Risk of desiccation or predation were not factors in this microcosm study, so survivorship of *B. terrestris* and *H. cinerea* was not adversely affected by delayed metamorphosis. One or both of these factors are expected to be present in natural tadpole communities, however, where an increase in the duration of the larval period of *B. terrestris* and *H. cinerea* as a result of the presence of *O. septentrionalis* may lead to reduced recruitment of the native species. Additional experiments that include predators or implement a drying regime can more rigorously evaluate the importance of delayed metamorphosis to larval survivorship.

Body size at metamorphosis

In addition to experiencing delayed metamorphosis, larvae of *B. terrestris* also transformed at significantly smaller sizes when reared with *O. septentrionalis* larvae. Tadpoles that transform at small body mass are generally less likely to survive to reproduction and those that do survive grow to smaller adult body sizes (Berven and Gill 1983, Smith 1987, Berven 1990). Furthermore, mating success and clutch size are positively correlated with adult body size in some anurans (e.g., Howard 1978, Berven 1981, Woolbright 1983), so even small decreases in growth or developmental rates of larval anurans may affect fitness if such decreases result in smaller reproductive body size. If the results from this study are representative of the impacts of *O. septentrionalis* on native anuran larvae in natural settings, the fitness and survival of adult native anurans such as *B. terrestris* may be affected by the presence of this nonindigenous species, even if larval survival *per se* is not affected.

In contrast to *B. terrestris*, the size of larval *H. cinerea* at metamorphosis was not negatively affected by the presence of nonindigenous larvae. However, *H. cinerea* larvae experienced decreased growth and developmental rates during the early larval period as a result of exposure to larval *O. septentrionalis*. These results suggest that *H. cinerea* larvae may be able to recover from the competitive effects of the presence of nonindigenous tadpoles if they are released from competition by the transformation of more rapidly developing *B. marinus* and *O. septentrionalis* larvae. This type of competitive release has been documented for related species (e.g., as a result of predation on competitors, Morin 1983), but because the length of the larval period may be determined during early growth and development (Travis 1984), delayed metamorphosis may still result as it did in this study. Additionally, ecological release and compensatory growth may not occur in natural populations of *H. cinerea* where adult *O. septentrionalis* reproduce continuously throughout the breeding season and repeatedly re-establish their larvae as potential competitors. Finally, these results may also suggest that native species with shorter larval periods will be more sensitive to the competitive effects of nonindigenous tadpoles, as their will be greater temporal overlap between the competing species.

CONSERVATION IMPLICATIONS

The results of this study strongly suggest that nonindigenous anuran larvae, in this case those of *O. septentrionalis*, may impact native anuran larvae through interspecific competition. Although the presence of *B. marinus* did not significantly affect the native tadpoles in this study, the potential toxicity of the eggs and larvae of *B. marinus* to aquatic predators warrants further examination of the impacts of this species on native aquatic taxa.

Although the results of this study are striking, the impacts of the presence of *O. septentrionalis* in natural tadpole communities remain unknown. Declines of some native anurans in south Florida have been reported and are anecdotally correlated with the arrival of *O. septentrionalis* (Bartlett 1967). These declines have been attributed to predation by adult nonindigenous anurans on native species (Bartlett 1967) and, perhaps more probably, to concurrent effects of habitat destruction (Krakauer 1968, Wilson and Porras 1983). Whatever the cause of past declines, the ability of *O. septentrionalis* to disperse (Meshaka 1996) and to penetrate relatively undisturbed, protected habitats such as Everglades National Park (Butterfield et al. 1997, Meshaka 2001) suggests that future impacts are possible since most habitats in peninsular Florida will eventually be invaded by *O. septentrionalis*. Additional research, including that conducted in more natural settings, will help determine the importance of larval competition with *O. septentrionalis* to the population status of native anurans in Florida.

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APPENDIX II-A

TABLES AND FIGURES

Table II-1. Range of Gosner stage and mean (± 1 SD) of mass and body length of tadpoles at day 0 of this study. For all species $n = 30$.

Species	**Gosner Stage**	**Mass (mg)**	**Body Length (mm)**
Bufo terrestris	25-27	7.18 ± 0.728	3.6 ± 0.15
Hyla cinerea	25	7.05 ± 2.651	2.9 ± 0.34
Bufo marinus	24-27	3.34 ± 0.968	2.9 ± 0.29
Osteopilus septentrionalis	25-26	13.74 ± 3.653	4.0 ± 0.44

Table II-2. Measures of development (Gosner stage or time to metamorphosis) of *Bufo terrestris* and *Hyla cinerea* tadpoles when reared in the presence and absence of nonindigenous tadpoles. "Early larval period" data were collected on days 15 and 16 for *B. terrestris* and *H. cinerea*, respectively. Values are means ± 1 S.E. and boldface type indicates a significant difference between that value and the control group (α = 0.05).

	Treatment	***Gosner Stage***	
		B. terrestris	H. cinerea
Early Larval Period	***Control***	41.6 ± 0.22	34.6 ± 0.20
	B. marinus	41.6 ± 0.10	33.9 ± 0.25
	O. septentrionalis	**40.2 ± 0.16**	**30.8 ± 0.27**
	B. marinus + O. septentrionalis	41.0 ± 0.03	**31.6 ± 0.38**
		Time to Metamorphosis (d)	
Metamorphosis	***Control***	15.7 ± 0.43	36.7 ± 0.51
	B. marinus	15.5 ± 0.14	35.5 ± 0.46
	O. septentrionalis	**17.6 ± 0.21**	**40.8 ± 0.71**
	B. marinus + O. septentrionalis	16.9 ± 0.26	38.1 ± 0.92

Table II-3. ANOVA results and proportion survivorship and time to metamorphosis of *Bufo marinus* and *Osteopilus septentrionalis* in the two experiments in this study. *F* and *P* values are from an ANOVA across treatments for each species within an experiment. Values are pooled means (± 1 S.E.) of all treatments and replicates.

	***B. terrestris* experiment**		***H. cinerea* experiment**	
	Survivorship	**Time to Metamorphosis (d)**	**Survivorship**	**Time to Metamorphosis (d)**
B. marinus	$F_{1,6} = 0.02$, $P = 0.9075$	$F_{1,6} = 2.58$, $P = 0.2067$	$F_{1,6} = 0.40$, $P = 0.5712$	$F_{1,6} = 0.88$, $P = 0.4178$
	0.981 ± 0.013	18.3 ± 0.41	0.955 ± 0.024	17.4 ± 0.32
O. septentrionalis	$F_{1,6} = 0.36$, $P = 0.5931$	$F_{1,6} = 0.25$, $P = 0.6498$	$F_{1,6} = 3.56$, $P = 0.1555$	$F_{1,6} = 1.07$, $P = 0.3771$
	0.945 ± 0.017	18.2 ± 0.25	0.936 ± 0.019	17.4 ± 0.21

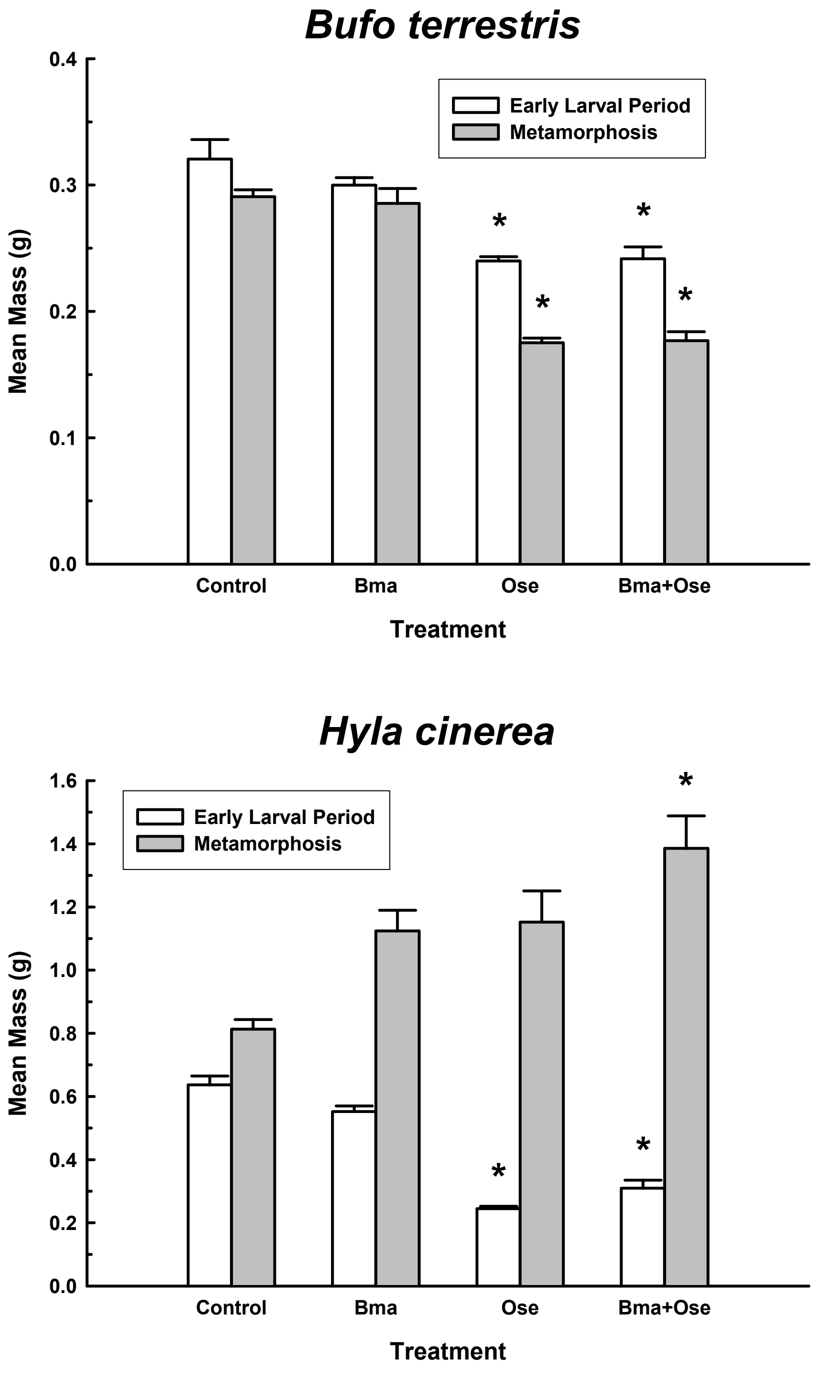

Fig. II-1. Effects of the presence of nonindigenous tadpoles (Bma = *Bufo marinus*; Ose = *Osteopilus septentrionalis*) on body mass of larval *Bufo terrestris* and *Hyla cinerea* after early larval development and at metamorphosis. "Early larval development" data were collected on days 15 and 16 for *B. terrestris* and *H. cinerea*, respectively. Error bars show +1 S.E.. Asterisks (*) indicate a significant difference from the control group (Tukey-Kramer Multiple Comparison Test, α = 0.05).

PART III

AN EXPLORATORY ASSESSMENT OF CUBAN TREEFROG (*OSTEOPILUS SEPTENTRIONALIS*) TADPOLES AS PREDATORS OF NATIVE AND NONINDIGENOUS TADPOLES IN FLORIDA

The following section is a slightly modified version of a paper published in the journal *Amphibia-Reptilia*:

Smith, K.G. *In Press*. An exploratory assessment of Cuban treefrog (*Osteopilus septentrionalis*) tadpoles as predators of native and nonindigenous tadpoles in Florida. Amphibia-Reptilia.

As the sole author of the original paper, I selected the topic of study, designed the study, conducted the study and collected and compiled the data, conducted all statistical analyses, and wrote the accepted manuscript.

INTRODUCTION

The introduction and establishment of nonindigenous species is a significant threat to native biodiversity worldwide (Vitousek et al. 1996, Mack et al. 2000). The adverse ecological impacts of such species on native taxa are generally attributed to several basic mechanisms, perhaps the most obvious of which is the direct predation of native organisms by a nonindigenous species (Simberloff 1997). In the most extreme cases, predation by nonindigenous species has caused the extinction of native and endemic faunas (e.g., Honegger 1981, Savidge 1987, Atkinson 1989). Although the impacts of nonindigenous predators are not always this severe, they are nonetheless worrisome if native community structure is significantly altered. For these reasons the study of the impacts of potentially injurious invasive predators is an essential component of biodiversity conservation.

The nonindigenous Cuban Treefrog, *Osteopilus septentrionalis*, has been established in mainland Florida, USA since at least 1951 (Schwartz 1952) and has since spread to occupy much of peninsular Florida where it occurs syntopically with several native anurans (Meshaka 2001). Anecdotal reports of local declines of these native species coincided roughly with the appearance of *O. septentrionalis* (Bartlett 1967), leading several researchers to consider the effect of predation on native anurans by adult *O. septentrionalis* (Bartlett 1967, Lee 1968, Wilson and Porras 1983, Meshaka 2001). In contrast, the interactions among larval *O. septentrionalis* and native tadpoles have been essentially neglected. Consequently, one important topic that has yet to be addressed is the role of larval *O. septentrionalis* as a predator of native tadpoles. There are no published reports of predation by larval *O. septentrionalis* on heterospecific anuran larvae, but Crump (1986) observed that the larvae of *O. septentrionalis* readily consume conspecific larvae ("cannibalism") when crowded. Because cannibalism is merely a special case of predation, a reasonable prediction is that *O. septentrionalis* larvae will prey on heterospecific anuran larvae, as well.

The primary objective of this study was to conduct exploratory research (sensu Jaeger and Halliday 1998) to determine if larval *O. septentrionalis* will prey on native (Squirrel Treefrog, *Hyla squirella*) and nonindigenous (Cane Toad, *Bufo marinus*) tadpoles. The eggs and/or larvae of *B. marinus* are toxic to many aquatic predators (Crossland and Azevedo-Ramos 1999, Crossland 2000) including *O. septentrionalis* (Punzo and Lindstrom 2001), so a secondary objective of this study was to determine if the consumption of *B. marinus* larvae resulted in mortality of *O. septentrionalis* tadpoles.

MATERIALS AND METHODS

I collected freshly-laid eggs of *B. marinus* and *O. septentrionalis* from a flooded field in Dade Co., Florida on the morning of 25 May 2003. I collected eggs from several clutches of *O. septentrionalis* and from one clutch of *B. marinus*. The eggs of both species were hatched and larvae reared at the U.S. Geological Survey Florida Integrated Science Center in Gainesville, Florida (hereafter, FISC). On 5 June I collected *H. squirella* larvae from vinyl tanks outside of FISC. These tanks were used as breeding habitat by several pairs of *H. squirella* during recent rainstorms. Tadpoles of all species were fed supplementary food *ad libitum* (3:1 ratio by mass of finely ground rabbit chow pellets (primary ingredient = alfalfa) and TetraFin™ goldfish food) until 24 h prior to the beginning of the experimental trials on 5 June (*B. marinus* trial) and 8 June (*H. squirella* trial). On 5 June a subsample of tadpoles was haphazardly collected for developmental stage determination (Gosner, 1960) and body length measurement (as described in Altig and McDiarmid 1999) (Table III-1[3]).

I designed two separate experimental trials to assess larval *O. septentrionalis* as a predator of *H. squirella* and *B. marinus*. I randomly assigned *H. squirella* or *B. marinus* to one of two treatments in each trial: a control treatment (five *H. squirella* or five *B. marinus* larvae alone) and an Osteopilus treatment (five *H. squirella* or five *B. marinus* larvae plus one *O. septentrionalis* larva). A third treatment, an Osteopilus control (one *O. septentrionalis* larva alone), was also included in the *B. marinus* trial to test for the effects of *B. marinus* toxin on *O. septentrionalis*. Treatments in the *H. squirella* trial were replicated 10 times and treatments in the *B. marinus* trial were replicated five times.

Experimental microcosms for both trials were small, hexagonal plastic weigh boats (ca. 75 mm wide X 17 mm deep) filled with 100 mL well water. All microcosms were arranged in a systematic design (2 X 10 for the *H. squirella* trial and 3 X 5 for the

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[3] All tables and figures appear in Appendix III-A

O. septentrionalis trial) on a laboratory table in the North Wet Lab of FISC. Artificial light was provided by overhead fluorescent lights on a 14 h light : 10 h dark schedule and ambient temperatures during the trials fluctuated between 23.9 and 29.3°C. The *B. marinus* trial began on 5 June and I collected data on survivorship of tadpoles on 6 June, while the *H. squirella* trial began on 8 June and I collected data on survivorship on 9 June. No food was provided during the trials. Behavioral observations were made every 2-8 h during the experimental period. At the end of the *B. marinus* trial on 6 June I transferred all *O. septentrionalis* larvae to new microcosms with fresh well water and food and observed them for an additional 24 h.

I used the nonparametric Mann-Whitney U Test to test for the effects of the presence of *O. septentrionalis* on number of surviving *H. squirella* and *B. marinus* after each experimental trial. I used NCSS statistical software (Hintze 2001) and a significance level of $\alpha = 0.05$ for analyses. I conducted no statistical analysis on the effects of the toxicity of *B. marinus* on *O. septentrionalis* survival because all *O. septentrionalis* in both trials survived the entire experimental period.

RESULTS AND DISCUSSION

The presence of *O. septentrionalis* tadpoles significantly affected the number of surviving *H. squirella* (Mann-Whitney *U*-test, $Z = 1.99$, $P = 0.047$, $n = 10$) and *B. marinus* (Mann-Whitney *U*-test, $Z = 2.68$, $P = 0.007$, $n=5$). Significantly fewer *H. squirella* and *B. marinus* larvae survived the experimental trial when they were combined with one *O. septentrionalis* tadpole than when they were housed with conspecifics only

(Fig. III-1). Although the cause of mortality was not observed for all missing *H. squirella* and *B. marinus* tadpoles, on several occasions another individual and I observed *O. septentrionalis* consuming struggling live tadpoles of both species. In one instance I observed an *O. septentrionalis* tadpole catch a live *H. squirella* tadpole by the tail and consume it. Surviving tadpoles of both *B. marinus* and *H. squirella* appeared healthy at the end of the trial and no uneaten, dead tadpoles of any species were observed. Although adult *O. septentrionalis* are distasteful to some predators (Meshaka 2001), the larvae of this species are not known to be toxic or allelopathic to heterospecific anuran larvae. These observations are consistent with the conclusion that all deaths were the result of predation by *O. septentrionalis* and were not caused by some other factor.

Based on the conclusion that all missing or dead tadpoles were preyed upon by *O. septentrionalis*, most individuals of *O. septentrionalis* preyed on heterospecific tadpoles. Four of five *O. septentrionalis* housed with *B. marinus* and nine of 10 *O. septentrionalis* housed with *H. squirella* consumed at least one tadpole. *Osteopilus septentrionalis* tadpoles consumed significantly more *B. marinus* tadpoles than *H. squirella* tadpoles (Mann-Whitney *U*-test, $Z = 2.49$, $P = 0.013$). The mean number of tadpoles eaten by *O. septentrionalis* (± 1 SE) was 3.8 (± 0.58) *B. marinus* tadpoles and 1.3 (± 0.42) *H. squirella* tadpoles.

All *O. septentrionalis* larvae survived the experimental period; consumption of *B. marinus* and *H. squirella* larvae did not affect the survival of *O. septentrionalis*.

These results provide the first evidence that larval *O. septentrionalis* will prey on heterospecific anuran larvae. Fewer *H. squirella* and *B. marinus* survived the experimental period when housed with one *O. septentrionalis* than when housed in the control treatment. Since little mortality occurred in the *H. squirella* control treatment and no mortality occurred in the *B. marinus* control treatment (Fig. III-1), it is unlikely that the disappearance of tadpoles in the *O. septentrionalis* treatments was due to cannibalism by *H. squirella* or *B. marinus*. Furthermore, observations of predation by *O. septentrionalis* on both *H. squirella* and *B. marinus* suggest that the disappearance of tadpoles in this study was caused by direct predation and not by the scavenging of dead individuals.

The degree of predation documented in this study suggests that predation by *O. septentrionalis* tadpoles may reduce the survivorship of heterospecific tadpoles in natural ponds. However, the very high density and controlled nature of this study (e.g., no alternative food available), while necessary for the documentation of predation, make the extension of these results to natural systems problematic. For example, predatory behavior in this species may be conditional based on tadpole density and resource availability. In other, longer-term studies I have observed *O. septentrionalis* preying on heterospecific tadpoles (*B. marinus*, *Bufo terrestris,* and *Gastrophryne carolinensis*) at various overall densities (between 0.5 and 2 tadpole / L). In another laboratory study specifically designed to assess interspecific competition, however, the survivorship of *B. terrestris*, *Hyla cinerea*, and *B. marinus* was not affected by the presence of *O. septentrionalis*. In this latter study, alternative food was available and tadpole densities were moderate (2 tadpoles / L vs. 60 tadpoles / L, in this study). This comparison suggests that predatory behavior by *O. septentrionalis* larvae is facultative and either profitable or possible only when resources are limited and/or when tadpole densities are very high. High tadpole densities and low resource levels are conditions that are frequently met during the drying of ephemeral pools favored by *O. septentrionalis* (Crump 1986, Meshaka 2001), so predation of heterospecific anuran larvae by *O. septentrionalis* in natural water bodies may occur under some circumstances. The impact of such predation on the survivorship of natural populations of native tadpoles in Florida is unknown, however. Other factors (e.g., the presence of competitors or other predators) may also modify the effect of *O. septentrionalis* predation. More naturalistic confirmatory studies on the effects of predation by *O. septentrionalis* larvae on native tadpoles are needed, particularly to determine the significance of this phenomenon in natural populations.

Surprisingly, *O. septentrionalis* consumed significantly more *B. marinus* larvae than *H. squirella* larvae in this study. This result is difficult to explain since *B. marinus* larvae are distasteful, toxic, or both to many vertebrate predators in the introduced range of *B. marinus* (Crossland and Alford 1998, Crossland and Azevedo-Ramos 1999, Crossland 2000, 2001). *Osteopilus septentrionalis* larvae are susceptible to the toxin contained in *B. marinus* eggs and consumption of eggs resulted in 30% mortality of larval *O. septentrionalis* in one study (Punzo and Lindstrom 2001). In the present study, *O. septentrionalis* did show symptoms of what is assumed to be *B. marinus* intoxication (general lethargy and lack of response to stimuli), although these symptoms subsided completely within 24 h. Even if the small sample size in this study (five *O. septentrionalis*, of which only four consumed *B. marinus* larvae) is responsible for the absence of observations of mortality, this does not explain why *O. septentrionalis* larvae consumed more of the larger *B. marinus* than the smaller *H. squirella* larvae, which are not known to be significantly toxic or distasteful. There was a noticeable, though not quantified, difference in microhabitat choice between the two prey species, however. *Bufo marinus* tadpoles generally remained on the bottom of the containers while *H. squirella* stayed near the surface or were more pelagic. Whether this difference in microhabitat can explain the difference in rates of predation is unknown, however.

In conclusion, *O. septentrionalis* larvae will prey on heterospecific tadpoles under certain conditions (high tadpole density, low resource availability). The effect of such predation on the survival and recruitment of native tadpoles is unknown and will depend in part on whether the mortality is compensatory or additive. Even small increases in additive mortality may significantly affect recruitment in those tadpole species with very high mortality (e.g., as high as 92-100% mortality for *Rana sylvatica* larvae (Berven 1990)). In contrast, compensatory mortality will have no effect on recruitment. Additionally, aquatic predators can significantly alter the outcome of the competitive interactions among tadpoles (e.g., Morin 1983, Wilbur and Fauth 1990), so the addition of a significant nonindigenous predator to native tadpole communities may alter native tadpole community structure. Although the results of this study also suggest that the consumption of *B. marinus* larvae does not significantly affect survivorship of *O. septentrionalis* larvae as a result of the toxicity of the former species, this conclusion is weakened by low statistical power owing to a small sample size. Further experimentation on the ecological interactions of *O. septentrionalis* and *B. marinus* is needed since these two species frequently occur syntopically in south Florida in combination with the larvae of native anurans.

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APPENDIX III-A

TABLES AND FIGURES

Table III-1. Approximate age, range of Gosner stage, and mean (± 1 SE) of body length of tadpoles on 5 June. Exact age of *Hyla squirella* larvae was unknown. For all species n = 15.

Species	Age (d)	Gosner Stage	Body Length (mm)
Hyla squirella	<10	25	2.3 ± 0.03
Osteopilus septentrionalis	10	25-26	4.2 ± 0.12
Bufo marinus	8	26-29	4.0 ± 0.10

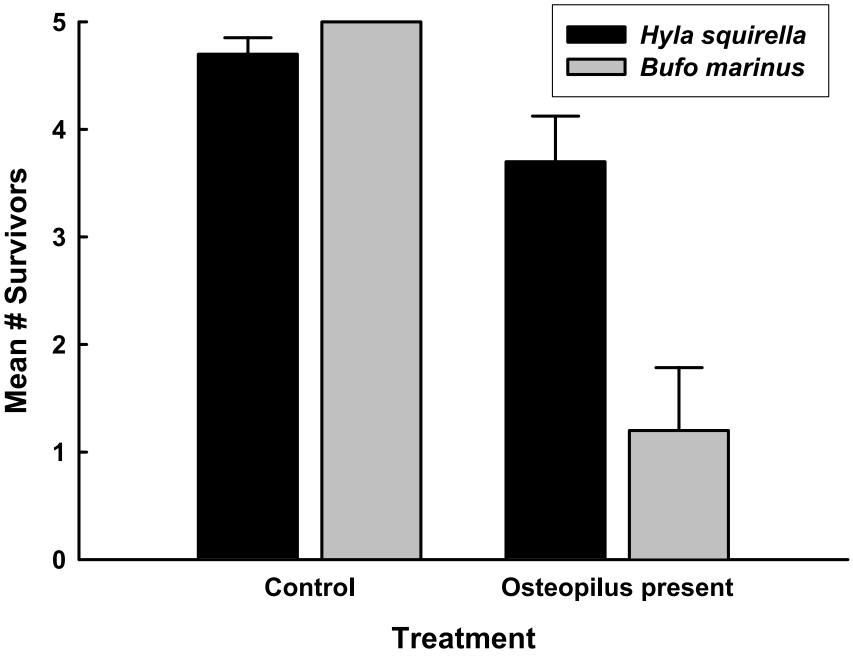

Fig. III-1. Effects of predation by larval *Osteopilus septentrionalis* on mean number of surviving tadpoles. Error bars show 1 SE.

PART IV

MECHANISM OF COMPETITIVE EFFECTS OF TADPOLES OF AN INVASIVE SPECIES: EXPLOITATION OR INTERFERENCE?

INTRODUCTION

The study of the impacts of invasive species has recently become a critical focus of conservation biology, in part because of the high ranking of invasive species among threats to native biodiversity (Simberloff 1997, Wilcove et al. 1998). For this reason, one of the most significant challenges facing ecologists and conservation biologists is the evaluation of the potential impacts of nonindigenous species (Mack et al. 2000). Ideally, such evaluations will yield results that contribute to the prediction, prevention, or mitigation of the adverse impacts of invasive species.

A significant obstacle to the successful evaluation of the potential impacts of nonindigenous species is the inherent contingency of ecological communities (e.g., Lawton 1999). Some of this contingency results from variations in membership of ecological communities and the presence of indirect, often unpredictable, interactions among community members. Consequently, although they are justifiable for practical reasons, ecological experiments using simplified communities can have results that may or may not apply to natural systems (Bender et al. 1984). However, studies specifically designed to identify the mechanisms of competitive interactions can potentially overcome this obstacle, allowing for the formulation of specific predictions regarding the outcome of competition within unstudied or novel species assemblages (Tilman 1987). For this reason, mechanistic studies of competition can be useful tools in the evaluation of the potential impacts of invasive species.

Within this framework, I conducted a study to evaluate the impacts of the larvae of an invasive anuran in Florida, USA. The state of Florida is one of the most highly invaded regions of the United States (Mooney and Drake 1986, Simberloff et al. 1997), and presently includes at least 40 species of established nonindigenous amphibians and reptiles (Meshaka et al. 2004). Few of these species have been studied with regard to their impacts on native taxa and almost none have been studied thoroughly. Among those that have been studied minimally is the Cuban treefrog (*Osteopilus septentrionalis*), which occurs widely throughout peninsular Florida since its original introduction to mainland south Florida over 50 years ago (Schwartz 1952, Meshaka 2001, Meshaka et al. 2004). Laboratory and quasi-natural, phenomenological studies have shown that the presence of larval *O. septentrionalis* results in significantly delayed metamorphosis, smaller size, and reduced survivorship of the larvae of several native species (Smith 2005 and Part V). However, as these studies were not designed to determine the specific mechanisms responsible for the observed results, the extending these results to natural systems is problematic.

To understand better and predict the potential impacts of larval *O. septentrionalis* in natural aquatic amphibian communities, I designed a two-part laboratory study to identify the mechanisms responsible for the previously documented competitive effects of *O. septentrionalis* larvae on the tadpoles of a common native species in Florida, the Southern Toad, *Bufo terrestris*. The first experiment implemented a factorial design to determine the significance of exploitation and interference competition to the competitive impacts of *O. septentrionalis* in *B. terrestris*. I designed the second experiment as an exclusion experiment to determine further if chemical or allelopathic interference competition contributes to the competitive effects of *O. septentrionalis*.

MATERIALS AND METHODS

I designed both experiments in this study as controlled, laboratory microcosm experiments, which allowed for precise manipulations of resource levels (artificial food, a 3 : 1 ratio of ground alfalfa pellets : ground Tetrafin™ fish food) and tadpole densities. Both experiments were conducted in the North Wet Lab of the U.S. Geological Survey Florida Integrated Science Center (FISC), Gainesville, FL in 10 gal (37.85 L; 25.5 cm X 50 cm) glass aquaria. Aquaria in both experiments were fitted with screen-covered standpipe drains to maintain the water volume (well water) at ca. 18 L (14.5 cm depth) and were arranged on a pallet rack shelving system under 12 h light : 12 h dark intervals. In Experiment 1 a 20 cm X 10 cm knotted piece of fiberglass insect screen was added to each aquarium to provide inedible spatial complexity and microhabitat. In Experiment 2 I added 10 g of oven-dried oak leaves to each aquarium for spatial complexity. I conducted statistical analyses for both experiments at $\alpha = 0.05$ using NCSS and JMP 5.1 statistical software (Hintze 2001, SAS Institute Inc 2004). All means are presented in the text ± 1 S.D..

Experiment 1: interference vs. exploitation competition

The first experiment used a two-factor ANOVA design to determine the significance of exploitation and interference competition to the overall competitive effects of larval *O. septentrionalis* on *B. terrestris* tadpoles. I manipulated two factors in Experiment 1; the first factor, *O. septentrionalis* density, had three levels (density levels 1, 2, and 4, with 10, 20, or 40 *O. septentrionalis* per tank, respectively). The second factor, feeding level, had three levels of food supply (feeding levels 1, 2, and 4, or 0.05, 0.10, or 0.20 g per tank / 2 d, increased to 0.10, 0.20, or 0.30 g per tank / 2 d after 12 d). I crossed the two factors completely, resulting in a total of nine treatment combinations. I replicated each treatment eight times for a total of 72 aquaria, which were divided into two trials due to space limitations. The first trial began May 5 and the other June 22 and each trial was composed of four complete replicates of each treatment.

To eliminate the conflation of the effects of intra- and interspecific competition, I included only one individual of *B. terrestris* in each treatment in combination with the prescribed number of *O. septentrionalis*. Responses of *B. terrestris* were therefore related solely to the effects of interspecific competition with *O. septentrionalis*.

I collected clutches of eggs of both species immediately after deposition on April 30-31 from Lee Co., FL (May 5 trial) and on June 16 from Alachua Co., FL (June 22 trial). I transported all clutches to FISC, where they were held in separate containers until larvae hatched and reached the free-swimming stage, about Gosner stage 25 (Gosner 1960). Elapsed time from oviposition to hatching was ca. 48 h for *B. terrestris* and ca. 36 h for *O. septentrionalis*. At this time, larvae from multiple clutches were mixed by species to minimize possible genetic effects on competition during the experiment (Travis 1980). At the start of each trial, each *B. terrestris* tadpole (72 total) and a sample of 30 *O. septentrionalis* were identified to Gosner stage and weighed (wet mass) to the nearest 0.1 mg. Tadpoles of both species were randomly assigned and added to aquaria as prescribed by the experimental design. At the onset of the experiments, differences in mean tadpole mass between *B. terrestris* and *O. septentrionalis* were not statistically significant (unequal variance $t = 0.856$, $P = 0.397$). In each trial all *B. terrestris* tadpoles were weighed again after 10 d and a final time at metamorphosis (Gosner stage 42).

The response variable in this study was k, a growth rate constant based on the exponential growth equation (as suggested for growth in tadpoles, Alford and Jackson 1993) calculated for each individual *B. terrestris* from differences in the initial and day 10 mass measurements:

$$k = [\log_e(\text{mass}_{\text{final}}) - \log_e(\text{mass}_{\text{initial}})] / \text{age}$$

where $\text{mass}_{\text{final}}$ is the mass of *B. terrestris* on day 10, $\text{mass}_{\text{initial}}$ is the mass of *B. terrestris* on day 0, and "age" is 10 d. I conducted two separate statistical analyses to assess the effect of exploitation and interference competition on the growth rate of *B. terrestris* larvae. First, I conducted a two-factor randomized block ANOVA (with each trial coded as a block) to test the effects of competitor density level, feeding level, and their interaction on k. Because feeding level in this analysis represented food availability per aquarium rather than per capita, I also conducted a second analysis in which I converted feeding level to "relative feeding level" (RFL), by dividing feeding level (1, 2, or 4) by competitor density level (1, 2, or 4). This conversion yielded five unique values of RFL (0.25, 0.5, 1, 2, and 4) that represent supply : demand ratio based on the density of competing *O. septentrionalis*. These levels were crossed incompletely with the three density levels, resulting in an unbalanced design. I analyzed both trials together as a randomized block, two-factor ANOVA with RFL and density as the manipulated factors. This analysis statistically separates the effects of RFL from the effects of density, which represent exploitation and interference competition, respectively (see also Wilbur 1977, Alford 1994).

Experiment 2: behavioral vs. allelopathic interference

The second experiment was designed to determine if allelopathy contributes to the overall competitive effects of *O. septentrionalis* on *B. terrestris*. This study implemented a substitutive design to control for the effects of overall tadpole density. The four treatments in this study were: 36 *B. terrestris* tadpoles reared alone ("control"); 18 *B. terrestris* larvae reared in combination with 18 *O. septentrionalis* ("mixed"); 18 *B. terrestris* larvae separated from 18 *O. septentrionalis* larvae by fiberglass insect screen ("allelopathy"); and two groups of 18 *B. terrestris* tadpoles separated from each other by fiberglass insect screen ("screen control"). The four treatments were each included in one of four spatial blocks in a randomized block design. The comparison of life history characters of *B. terrestris* across the four treatments in this study allows for the assessment of the presence of allelopathic interference competition as a contributor to the overall competitive effects of *O. septentrionalis*. In particular, the design of the allelopathy treatment is such that direct physical interactions between *B. terrestris* and *O. septentrionalis* larvae were prevented, while the screen barrier allowed for the flow of water and allelopathic compounds between the two sides of the aquaria. Competitive interactions between the two species were therefore limited to water-borne allelopathic interactions, as both exploitation competition and behavioral interference were precluded. The screen control treatment was designed to control for any effects of screen presence on the larval development of *B. terrestris*.

Eggs of both species were collected and cared for as in Experiment 1. I collected eggs from Alachua Co., FL on June 9-10 and tadpoles were randomly assigned to aquaria on June 14. Throughout the experimental period artificial food was supplied at a ration of 0.8 g per aquarium every other day. When screen barriers were present (allelopathy and screen control treatments), the food ration was divided into two 0.4 g allotments added to each side of the barrier. I flushed all tanks with fresh well water for 15 min once weekly, which replaced approximately one-half of the water volume in each tank.

I checked all aquaria daily for transforming *B. terrestris* (Gosner stage 42). Transforming tadpoles were removed from the aquaria and weighed to the nearest 0.1 mg. I used aquarium means for all analyses. I first used a randomized block MANOVA to simultaneously test for the effects of the four treatments on *B. terrestris* mass at metamorphosis, time to metamorphosis, and proportion surviving to metamorphosis. The use of MANOVA prior to ANOVA reduces the likelihood of committing a Type I error when conducting multiple tests of the same hypothesis (Moran 2003). If the MANOVA results indicated significant treatment effects I then used individual univariate ANOVAs to determine the response variables within which significant differences existed among treatments. Finally, I used Tukey-Kramer multiple comparisons to determine the treatment groups among which significant differences occurred. I log-transformed mass at metamorphosis and time to metamorphosis and arcsine-square-root transformed proportional survival to meet ANOVA assumptions.

RESULTS

Experiment 1: interference vs. exploitation competition

Across both trials, the proportion of *B. terrestris* tadpoles surviving to day 10 was 0.972. Competitor density and feeding level significantly and independently influenced the growth rate, *k*, of *B. terrestris* larvae surviving to day 10 (Table IV-1[4]). Increases in the density of *O. septentrionalis* resulted in significant decreases in the growth rate of *B. terrestris* larvae, while increases in feeding level resulted in significant increases in the growth rate *B. terrestris* larvae (Fig. IV-1A).

When feeding levels were standardized to per capita relative feeding levels (RFL), the growth of *B. terrestris* larvae was significantly affected by variation in RFL, but not by variation in density of *O. septentrionalis* (Table IV-2). The growth rate, *k*, of *B. terrestris* larvae increased significantly with increases in RFL, but did not change across density levels (Fig. IV-1B). The qualitative similarity of the slopes of the three density-specific curves strongly suggests that RFL and density did not interact significantly to affect the growth rate of *B. terrestris*.

Experiment 2: behavioral vs. allelopathic interference

Overall, there was a significant effect of the four treatments on life history characteristics of larval *B. terrestris* (MANOVA, Treatment: $F_{9,17} = 9.77$, $P < 0.0001$; Block: $F_{9,17} = 0.66$, $P = 0.731$). Survival of *B. terrestris* was greater in the screen control group than in the control or mixed groups (ANOVA $F_{3,9} = 6.40$, $P = 0.013$ and Tukey-

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[4] All tables and figured appear in Appendix IV-A

Kramer; Fig. IV-2A). Across all treatments, the mean proportion of *B. terrestris* surviving to metamorphosis was 0.868 ± 0.139.

Bufo terrestris tadpoles reared in combination with *O. septentrionalis* transformed significantly later as compared to any other group, transforming after 34.4 ± 0.81 d (ANOVA, Treatment: $F_{3,9} = 56.25$, $P < 0.00001$; Block: $F_{3,9} = 0.85$, $P = 0.502$ and Tukey-Kramer at $\alpha = 0.05$). There were not significant differences among the other three groups in mean time to metamorphosis of *B. terrestris* (Fig. IV-2B). The mean value among these groups was 20.5 ± 2.01 d.

There was not a significant treatment effect on mass at metamorphosis of *B. terrestris* $\alpha = 0.05$, although differences among treatments in this variable approached significance (ANOVA, $F_{3,9} = 3.30$, $P = 0.072$; Block: $F_{3,9} = 0.61$, $P = 0.626$). Consequently, a weak nonsignificant trend of greater body mass in control group *B. terrestris* tadpoles as compared to individuals in the mixed treatment was evident (Fig. IV-2C).

DISCUSSION

Larval anuran ecology

Overall, the results presented here are consistent with the hypothesis that exploitation competition is primarily responsible for the competitive effects of *O. septentrionalis* on *B. terrestris*. Although growth rates of *B. terrestris* were significantly affected by both food level and density of *O. septentrionalis*, standardizing food level (as RFL) eliminated the influence of competitor density on *B. terrestris* growth. For example, within RFL 1, increasing the number of *O. septentrionalis* competitors from 10 to 40 (density level 1 to 4) resulted in a decrease in the exponential growth rate, *k*, of *B. terrestris* of only 3.5%. In contrast, when the density of competing *O. septentrionalis* was constant at 20 (density level 2), the reduction of RFL from 2 to 0.5 resulted in a 22.0% decrease in *k* (Fig. IV-1B). The results of the two analyses in Experiment 1 together suggest that the effect of *O. septentrionalis* density in this experiment was mediated strictly through the consumption of food resources. Consequently, the competitive effects of larval *O. septentrionalis* on *B. terrestris* in this experiment are most attributable to exploitation competition, with no evidence for a significant role of interference competition.

Given that interference competition was not a significant component of the effects of *O. septentrionalis* larvae on the growth of *B. terrestris* larvae in Experiment 1, the results of Experiment 2 are not surprising in that they confirm that allelopathy, one form of interference competition, was not significant to the competitive effects of *O. septentrionalis*. Significant differences in growth of *B. terrestris* larvae occurred only when *O. septentrionalis* larvae were physically present in the mixed treatment, in which *B. terrestris* larvae experienced delayed metamorphosis and grew to qualitatively smaller body mass (Fig. IV-2B, C). In contrast, the presence of water conditioned by *O. septentrionalis* had no effect on life history characteristics of *B. terrestris* larvae, as evidenced by the similarity of growth responses in the allelopathy and screen control groups.

Survival of *B. terrestris* larvae was greater in the screen control group than in either the control or mixed treatment groups (Fig. IV-2A). The cause of this result is unknown, but is possibly some positive effect of the screen divider, such as the presence of an additional foraging substrate. This result should not have obscured evidence of allelopathic interference by *O. septentrionalis* since any positive effect of the screen in the allelopathy treatment was controlled for by the screen control treatment.

It is somewhat surprising that other forms of interference competition, especially behavioral interference, were not an important mechanism of competition by *O. septentrionalis* in this study. In this study, *O. septentrionalis* larvae rapidly became larger than *B. terrestris* as a consequence of higher growth rates (Smith 2005 and Part V) and were highly active and frequently harassed and collided with other tadpoles. That the effects of this harassment by the larger-bodied *O. septentrionalis* were not density-dependent and did not result in interference competition is unexpected, especially since similar behavior was observed in a study of competition between *Rana sphenocephala* and *Hyla cinerea*, contributing to reduced growth in the latter (Faragher and Jaeger 1998). In contrast, competition between the large tadpoles of introduced bullfrogs (*Rana catesbeiana*) and tadpoles of two native species was strictly the result of exploitation competition (Kupferberg 1997).

Steinwascher (1978) suggested that interference mechanisms may be more important at low food levels, so it may be that food levels in these experiments were too high for interference competition to become evident relative to exploitation competition. Alternatively, because *O. septentrionalis* is apparently a strong competitor, the effects of interference competition may plateau at relatively low densities of *O. septentrionalis* (e.g., at < 10 tadpoles per aquarium), leading to an apparent lack of density-dependent interference effects. Alford (1999) described one example of such asymptotic effects of intraspecific interference competition in Australian *Limnodynastes ornatus*. Although these alternative explanations are possible, it seems unlikely that they would undermine the conclusion that competition by *O. septentrionalis* is primarily exploitative in nature since the effects of exploitation competition in this study were so strong. Furthermore, empirical evidence of food limitation in tadpole communities in natural habitats suggests that exploitation is the most important form of competition in tadpoles assemblages (Steinwascher 1978, Banks and Beebee 1988, Alford 1999).

Implications to ecological impacts of *Osteopilus septentrionalis*

Despite the frequency with which interspecific competition has been studied in larval anurans (reviewed in Alford 1999), few studies have specifically determined the mechanisms contributing to observed competitive effects (Griffiths 1995). This fact may be responsible, in part, for disagreement regarding the importance of various competitive mechanisms in aquatic amphibian communities (Petranka 1989, e.g., Beebee 1995, Griffiths 1995, Petranka 1995). Such disagreements notwithstanding, the results of this research are unambiguous and generally indicate that the competitive effects of larval *O. septentrionalis* on a native tadpole, *B. terrestris*, are the result of exploitation competition.

This conclusion leads to several implications regarding the competitive interactions of larval *O. septentrionalis* with native anuran larvae. First, this result suggests that because *O. septentrionalis* did not have an allelopathic effect on *B. terrestris*, negative water-conditioning by *O. septentrionalis* is unlikely to occur in natural ponds. Empirical evidence from a study of 17 North American tadpole species indicates that allelopathic growth inhibition caused by crowding is not species-specific (Licht 1967), suggesting that the results of the present study apply to species other than the focal species, *B. terrestris*. Additionally, despite evidence of allelopathic growth inhibition from a number of laboratory studies, Petranka (1989) found that allelopathy is relatively rare in natural habitats. For these reasons it is unlikely that nonindigenous *O. septentrionalis* larvae will adversely impact native anuran larvae via allelopathic interference competition.

In contrast to the absence of interference competition, the strong effect of exploitation competition in this study suggests that larval *O. septentrionalis* may compete significantly with native tadpoles in the field. This prediction is supported by past studies demonstrating the importance of interspecific competition, particularly exploitation competition, in natural or field-manipulated tadpole communities (Seale 1980, Banks and Beebee 1988, Kupferberg 1997, Alford 1999). Furthermore, the tadpoles of *B. terrestris* are superior competitors in some native tadpole communities (e.g., Morin 1983). The competitive superiority of *O. septentrionalis* relative to *B. terrestris* in this and other studies (Smith 2005 and Part V) therefore suggests that *O. septentrionalis* may replace *B. terrestris* as the competitively dominant species in some Floridian tadpole communities, potentially resulting in adverse impacts to *B. terrestris* and other competitively inferior species. This possibility is supported by additional experimental evidence showing that *O. septentrionalis* outcompetes most other North American tadpole species against which it has been tested (e.g., *H. cinerea*, *Hyla squirella*) (Smith 2005 and Part V)). Evidence from other systems also suggests that competition with the larvae of introduced species can adversely impact native amphibians (Kupferberg 1997, Kiesecker and Blaustein 1998).

The results of this and other studies predict that larval *O. septentrionalis* compete significantly with native anuran larvae in Florida, with deleterious results to survival, time to metamorphosis, and size at metamorphosis of native anurans (Smith 2005 and Part V). Although the ultimate effect on native anuran populations of such competition with *O. septentrionalis* is unknown, studies on anuran life history indicate that increases in larval period and decreases in metamorphic body size can result in reduced fitness of adult anurans, as a result of either decreased survival to reproduction, delayed first reproduction, or reduced fecundity (Howard 1978, Berven 1981, Smith 1987, Newman 1988). Furthermore, because *O. septentrionalis* occur syntopically and breed synchronously with several native anurans in Florida (Meshaka 2001 and personal observations), several native species are exposed to the competitive impacts of *O. septentrionalis*.

It is presently unknown if *O. septentrionalis* larvae have deleterious impacts on native amphibian populations in nature, and as substantial debate surrounds the relevance of microcosm and mesocosm studies to natural systems (Morin 1989, Wilbur 1989, Skelly and Kiesecker 2001, e.g., Chalcraft et al. 2005, Skelly 2005), the results presented here cannot alone be taken as evidence of the adverse impacts of *O. septentrionalis* in Florida or elsewhere. What these results do suggest, however, is that additional investigations of this topic are needed, especially studies in which the predictions provided by this research are explicitly tested under natural conditions. Such ecological pluralism (e.g., Bender et al. 1984, Resetarits and Bernardo 1998) is likely to provide realistic and useful approximations of the impacts of *O. septentrionalis* on native amphibians in Florida, and perhaps elsewhere.

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APPENDIX IV-A

TABLES AND FIGURES

Table IV-1. Results of an analysis of variance assessing the influence of density of larval *Osteopilus septentrionalis* (Density) and food availability (Food) on the exponential growth rate, *k*, of *Bufo terrestris* tadpoles.

Factor	**df**	**MS**	***F***	***P***
Density	2	0.0051	11.36	0.0046
Food	2	0.0042	9.35	0.0081
Density X Food	4	0.0059	0.13	0.9668
Block (Date)	1	0.0462	102.04	< 0.0001
Error	8	0.0005		

Table IV-2. Results of an analysis of variance assessing the influence of relative feeding level (RFL) and density of *Osteopilus septentrionalis* on the exponential growth rate, *k*, of *Bufo terrestris* tadpoles.

Factor	**df**	**MS**	***F***	***P***
RFL	4	0.0022	5.92	0.0104
Density	2	0.0000	0.06	0.9413
Block (Date)	1	0.0462	126.35	< 0.0001
Error	10	0.0004		

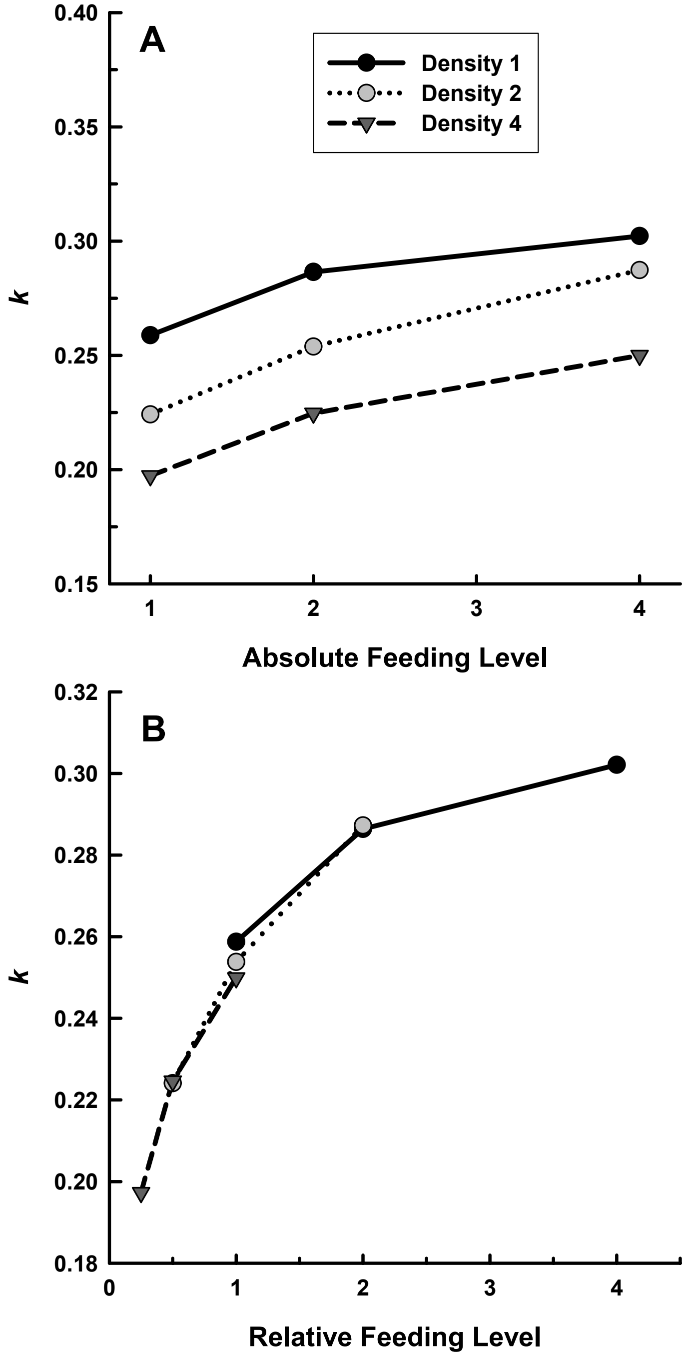
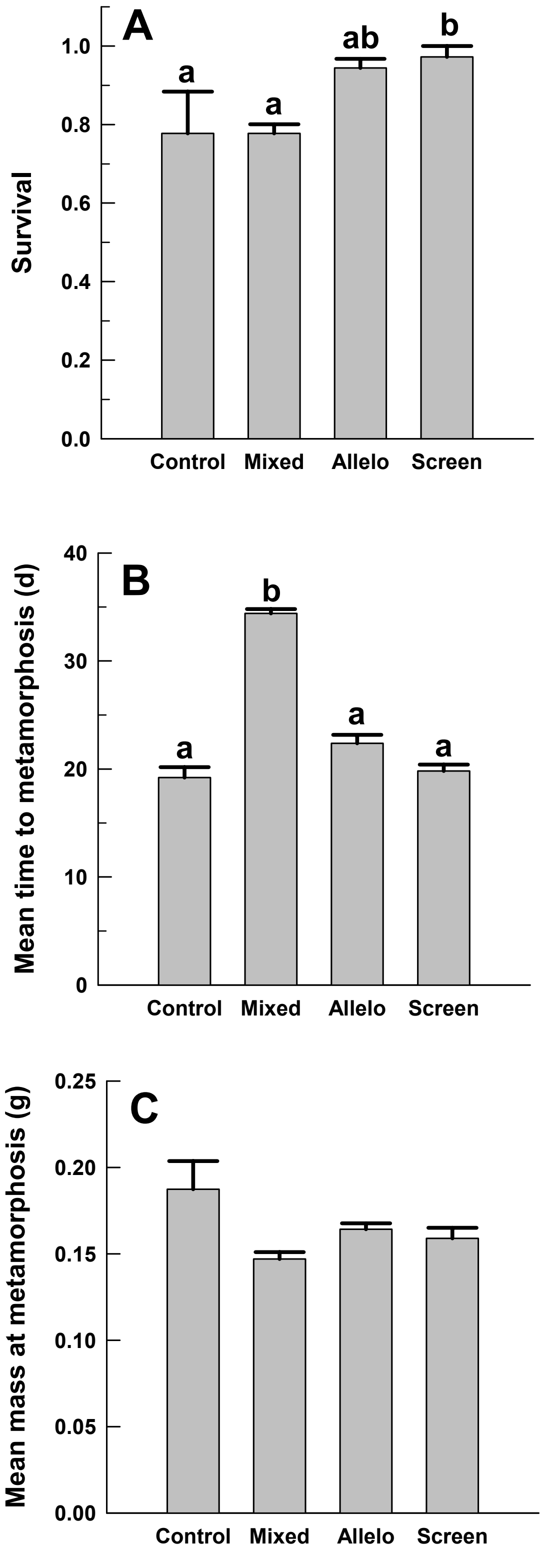


Fig. IV-1. Effects of *Osteopilus septentrionalis* tadpole density and absolute (A) and relative feeding levels (B) on exponential growth rate (k) of *Bufo terrestris* tadpoles after 10 d (Experiment 1; see text for description of density and feeding levels). Standard error bars are omitted for clarity.

Fig. IV-2. Proportional survival (A), mean time to metamorphosis (B), and mean mass at metamorphosis (C) of *Bufo terrestris* tadpoles compared across the four treatments in Experiment 2 (see text for description of treatments). Bars with dissimilar letters are significantly different (Tukey-Kramer α = 0.05).


A
Survival
1.0
0.8
0.6
0.4
0.2
0.0
a
a
ab
b
Control
Mixed
Allelo
Screen
B
Mean time to metamorphosis (d)
40
30
20
10
0
a
b
a
a
Control
Mixed
Allelo
Screen
C
Mean mass at metamorphosis (g)
0.25
0.20
0.15
0.10
0.05
0.00
Control
Mixed
Allelo
Screen

PART V

REDUCTION OF IMPACTS OF AN AQUATIC INVASIVE SPECIES BY A KEYSTONE PREDATOR (EASTERN NEWT, *NOTOPHTHALMUS VIRIDESCENS*)

INTRODUCTION

Keystone species are important components of many of the ecosystems in which they have been studied (Menge et al. 1994). In the most traditional sense, keystone species are top predators that maintain community diversity by preying selectively on competitively superior prey taxa, thereby preventing the exclusion of relatively weak competitors (Paine 1966, 1969, Morin 1983, Mills et al. 1993, Schmitz 2003). Despite some criticism questioning the utility of the keystone species concept (Mills et al. 1993), evidence that some species are disproportionately important in their communities suggests a number of important implications for the conservation of biodiversity (Simberloff 1998, Davic 2003).

Surprisingly, it is unknown if keystone predators play a similarly important role in invaded communities. This is an important omission in applied ecology, as invasive species are a widespread threat to native biodiversity (Wilcove et al. 1998). Furthermore, although relatively few studies have conclusively demonstrated competitive effects of invasive species, it is widely assumed that invasive species may compete with native and nonindigenous taxa for resources (Simberloff 1997, Mack et al. 2000). In those cases where such competition does exist, keystone predators may contribute to the maintenance of species diversity in invaded communities as they do in native communities. Given the global penetration of nonindigenous species into native biotas, keystone predators may be expected to play an invaluable role in reducing or mitigating the impacts of some invasive species. If this is the case, the importance of keystone species to biological conservation may be undervalued (cf. Mills et al. 1993).

A candidate species for the evaluation of the relationship between keystone predators and invasive species is the eastern newt, *Notophthalmus viridescens*, of the United States. Empirical evidence suggests that *N. viridescens* are keystone predators in larval anuran communities and that their presence is an important factor in the structuring of these communities across a gradient of prey densities and richnesses and in the presence of other aquatic predators (Morin 1981, 1983, Wilbur 1987, Fauth and Resetarits 1991). Mechanistic evidence from these studies suggests that newts feed selectively on competitively superior tadpoles, thereby contributing to coexistence among community members. Keystone status in this species appears to be robust given that a competitive hierarchy exists within the tadpole community (but see Fauth 1999). If the larvae of successful invasive anurans exhibit competitive dominance in native communities, it may be predicted that newts will prey selectively on the invasive tadpoles, thereby reducing their competitive impacts.

In peninsular Florida, USA, two nonindigenous anurans (cane toad, *Bufo marinus*, and Cuban treefrog, *Osteopilus septentrionalis*) are established and relatively widespread (Meshaka 2001, Meshaka et al. 2004). Both species occur in sympatry with a number of native amphibians (personal observations and Bartlett and Bartlett 1999) and an experimental study suggests that *O. septentrionalis* is competitively superior to the larvae of at least two native anurans (Smith 2005a). In contrast, *B. marinus* tadpoles are not known to have strong competitive effects, but they are toxic to a number of vertebrate and invertebrate predators (Crossland and Alford 1998, Crossland and Azevedo-Ramos 1999, Smith 2005a) and may therefore modify the role played by *N. viridescens* as a predator. In this study, I use replicated, experimentally invaded larval anuran communities a) to establish that *O. septentrionalis* has significant, negative competitive effects on a native tadpole community, b) to test if the presence of a keystone predator, *N. viridescens*, significantly reduces the impacts of *O. septentrionalis*, and c) to determine if the presence of *B. marinus* inhibits keystone predation by *N. viridescens*.

MATERIALS AND METHODS

Mesocosms and experimental design

I used outdoor aquatic mesocosms as experimental ponds in this study, following the general methods of previous studies that resulted in the establishment of diverse, naturalistic aquatic communities (e.g., Morin 1983, Wilbur 1987, Fauth and Resetarits 1991). The mesocosms used in this study were 150 gal (ca. 568 L) black vinyl tanks, filled to a depth of 33 cm with ca. 285 L of well water on May 10. Dry, grassy pond litter was also added in 150 g allotments to provide a natural substrate and to inoculate the mesocosms with resting stages of plankton and microinvertebrates. Tanks were securely covered with fiberglass mesh screen to prevent egg deposition by endemic anurans and to retain metamorphic anurans until they could be collected. On May 17, 10 stems each of live *Hydrocotyle sp.* and *Hydrilla sp.* were added to each tank to provide additional spatial complexity. At this time I also added 10 g of alfalfa pellets to each experimental tank to act as a resource base for the aquatic communities. Twenty snails (Gastropoda: Lymnaeidae, Physidae, and Planorbidae) were also added to each tank. On June 7, visual inspection showed that diverse planktonic and microinvertebrate communities had become established in the tanks. On this day I also collected and mixed a 1 L volume of water from each tank and redistributed this water among the 40 tanks to help ensure relative homogeneity among the mesocosms. To each mesocosm I also added a 1 L volume of water from a non-experimental tank that contained a concentrated assemblage of aquatic organisms filtered from local pond water.

I designed this study as a complete, randomized-block experiment with two treatment factors to assess the influence of newt predation on the competitive effects of the nonindigenous tadpoles. The first factor was a community factor, with four levels representing four substitutive combinations of native and nonindigenous larval anurans, each with an overall larval density of 240 larvae per tank (Table V-1[5]). This factor represents the competitive effects imposed by nonindigenous species in a native tadpole community. Although this design results in varying levels of intraspecific competition among treatments, the substitutive design provides a conservative test of the effects of the presence of nonindigenous tadpoles independent of overall tadpole density given a limited number of mesocosms. I crossed each level of the community factor with two levels of a second factor, presence or absence of a single adult male *N. viridescens*. This resulted in a total of eight treatments which I replicated across five spatial blocks. Each block was comprised of eight tanks, with each of the eight treatments randomly assigned to a tank within each block. The five blocks were arranged on a concrete substrate to the north of the U.S. Geological Survey Florida Integrated Science Center North Wet Lab, Gainesville, FL, and the entire array of tanks was shaded with shadecloth on an aluminum frame ca. 2.5 m high to reduce the thermal effects of the substrate.

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[5] All tables and figures appear in Appendix V-A

The experimental communities included a four-species native tadpole community, which included equal numbers of *Bufo terrestris*, (southern toad), *Gastrophryne carolinensis* (eastern narrow-mouthed toad), *Hyla squirella* (squirrel treefrog), and *Rana sphenocephala* (southern leopard frog). In Florida, these species can occur syntopically with both *B. marinus* and *O. septentrionalis*. I collected the eggs of all species between June 2 and 8 during a period of frequent rain in peninsular Florida. Tadpoles of all species were from clutches collected in Alachua and Hillsborough Counties or from wild-caught amplectant pairs that oviposited in captivity. Tadpoles of all species were from multiple clutches to minimize the influence of genetic effects on the results of this experiment (e.g., Travis 1980). I collected 20 adult male *N. viridescens* from Putnam County, Florida between March and May 2004.

On June 9 I weighed and measured (snout-vent length, SVL) each newt to the nearest 0.1 mg and 0.1 cm, respectively, and randomly assigned them to 20 of the 40 mesocosms. On June 10 I collected a sample of 10 individuals from each anuran species to confirm that the larvae of all six species had reached the free-swimming stage (approximately Gosner stage 25, Gosner 1960) and randomly allocated larval anurans to each of the 40 mesocosms as prescribed by the substitutive design (Table V-1).

Beginning on June 11, tanks were searched daily for transforming tadpoles and general observations (e.g., predation events, behavior, activity) were recorded. Transforming tadpoles were identified by the presence of both forelimbs (Gosner Stage 42) and were removed from each tank and immediately identified, weighed, and preserved. Response data for each tadpole included mass at metamorphosis and time to metamorphosis, two important predictive measures of adult anuran fitness that reflect competitive conditions during larval development (Wilbur 1980, Berven and Gill 1983, Smith 1987). The experiment was allowed to continue until September 10-11, when all tanks were drained and surveyed completely for remaining tadpoles. Because of their comparatively long larval period, nonmetamorphic individuals of *R. sphenocephala* removed at the end of the experimental period were included in the analyses, whereas the few remaining tadpoles of other species were excluded from analyses.

Data and statistical analyses

I used several measures at two ecological scales of measurement (single-species and community) to test for the effects of tadpole community, newt presence, and their interaction on native tadpole performance. For all analyses I used treatment means as response variables. Single-species response variables were survival rate, mean growth rate (mg / d), mean mass and time to metamorphosis. Because species-specific analyses of variables describing growth were limited by low survival of some species, I used *Bufo terrestris* as a focal species. Among the four native species, data for *B. terrestris* were the most complete and previous experimental research has shown that larval *B. terrestris* are significantly impacted by the presence of *O. septentrionalis* (Smith 2005a). I used overall survival of native tadpoles and mean deviation from expected relative abundance of tadpoles as community response variables. The latter variable was calculated as the difference between the relative abundance of surviving individuals of a given species and the expected relative abundance of that species based on the initial number of tadpoles. I used this variable as a surrogate variable for proportional abundance, which is confounded by varying species-specific abundances across treatments. I analyzed each of these response categories separately for native and nonindigenous tadpoles.

To minimize type I error rate inflation when multiple tests addressed the same hypothesis, I first analyzed responses with multivariate analyses of variance (MANOVA). In the analysis of deviation from expected relative abundance within the native community I eliminated *G. carolinensis* to prevent linear dependence in this variable. If MANOVAs detected significant effects I then used univariate analyses of variance (ANOVAs) to identify significant response variables and Tukey-Kramer multiple comparison tests to determine the groups among which the differences occurred. I arcsine-square-root-transformed proportional survival and $\log_e$-transformed growth rate, mass, and time to metamorphosis to meet the assumptions of ANOVA (Sokal and Rohlf 1995). All statistical analyses were conducted with NCSS and JMP software at $\alpha = 0.05$ (Hintze 2001, SAS 2004). All mean values presented in the text are ± 1 SD.

RESULTS

Native anuran responses

Survival

Tadpole community and newt presence interacted to affect significantly the overall proportion of native tadpoles that survived to metamorphosis (ANOVA interaction: $F_{3,28} = 4.02$, $P = 0.017$), although main effects were not significant (ANOVA community: $F_{3,28} = 2.50$, $P = 0.080$; newt presence: $F_{1,28} = 0.11$, $P = 0.738$). When newts were absent, the proportion of native tadpoles reaching metamorphosis was reduced by the presence of *O. septentrionalis* tadpoles (ANOVA $F_{3,12} = 5.01$, $P = 0.018$ and Tukey-Kramer), while the presence of newts virtually eliminated this negative effect of *O. septentrionalis* on total native community survival (Fig. V-1A).

The survival of individual native species reflected overall survival of the native community and the interaction of tadpole community and newt presence significantly affected species-specific survival rates of native tadpoles (MANOVA on four native species, interaction: $F_{12,66} = 2.44$, $P = 0.011$; community: $F_{12,66} = 1.11$, $P = 0.369$; newt presence: $F_{4,25} = 0.72$, $P = 0.584$). More specifically, when newts were not present, tadpole community significantly affected the survivorship of native tadpoles (MANOVA community: $F_{12,35} = 2.71$, $P = 0.011$). *Bufo terrestris* tadpoles experienced reduced survival when reared in the presence of *O. septentrionalis* when newts were absent (ANOVA: $F_{3,12} = 11.85$, $P < 0.001$ and Tukey-Kramer; Fig. V-1B). Mean proportional survival for *B. terrestris* reared in the native community was 0.707, as compared to 0.371 in the *O. septentrionalis* treatment.

In contrast, when newts were present the effect of tadpole community on *B. terrestris* survival was no longer significant (ANOVA community: $F_{3,12} = 0.83$, $P = 0.500$; community-newt interaction: $F_{3,28} = 9.97$, $P < 0.001$; Fig. V-1B). This effect appears to be mediated through the modification of the negative effects of the presence *O. septentrionalis* on *B. terrestris*, as survival of *B. terrestris* reared in the presence of *O. septentrionalis* larvae increased from 0.371 to 0.683 as a result of newt presence (Fig. V-1B).

The effect of tadpole community on survivorship of the other native species was not significant ($P > 0.05$), although there was evidence of a trend toward reduced survival in *H. squirella* due to the presence of *O. septentrionalis* (ANOVA: $F_{3,12} = 2.76$, $P = 0.088$).

Growth and development

The growth and development of *B. terrestris* over the course of the larval period was independently affected by tadpole community and newt presence (MANOVA on growth rate, mass at metamorphosis, and larval period, community: $F_{9,63} = 2.86$, $P = 0.007$; newt presence: $F_{3,26} = 8.86$, $P < 0.001$; interaction: $F_{9,63} = 1.42$, $P < 0.198$). The growth rate of *B. terrestris* larvae in the natives-only treatment was significantly greater than that of any other treatment group (Table V-2) and the presence of newts also increased growth significantly overall (Fig. V-1C). These variations in growth rate in turn affected the length of the larval period for *B. terrestris*, as well. Regardless of newt presence, the presence of *O. septentrionalis* in the larval community significantly increased the length of the larval period of *B. terrestris*, resulting in a 7.4 d delay in metamorphosis compared to the natives-only community. Tadpole community had only a marginal influence on the mass of *B. terrestris* at metamorphosis (Table V-2).

Community

The structure of the natives-only community treatment at metamorphosis varied substantially from the expected (i.e., initial) community structure. The expected mean relative abundance for each of the four native species in the natives-only treatment was 0.25, whereas by metamorphosis, species-specific mortality shifted the relative community composition in favor of *B. terrestris* (mean relative abundance ± SD = 0.6442

± 0.1325). The remainder of the native community composition at metamorphosis was dominated by *R. sphenocephala* (0.2738 ± 0.1359), while *G. carolinensis* and *H. squirella* comprised 0.0469 ± 0.1406 and 0.0397 ± 0.0362 of the community, respectively.

Deviations from expected mean relative abundance were not consistent among treatments, however, and tadpole community and newt presence interacted to significantly alter native community dynamics (Table V-3). For *B. terrestris*, this effect corresponded with the presence of *O. septentrionalis* (Tukey-Kramer). Although *B. terrestris* was always relatively more abundant than expected with a positive mean deviation from expected relative abundance, the presence of *O. septentrionalis* reduced the relative dominance of *B. terrestris* significantly (Table V-3, Figs. V-1D and V-2). The newt X community interaction was not significant at $\alpha = 0.05$ for *B. terrestris*. However, predation by newts apparently modified the negative effect of *O. septentrionalis* on *B. terrestris*, as the presence of newts resulted in an elimination of the negative effects of *O. septentrionalis* on *B. terrestris* relative dominance (Fig. V-1D and 2). Deviations from expected relative abundance were not affected by tadpole community or newt presence for *R. sphenocephala*, but tadpole community influenced the relative abundance of *H. squirella* (Table V-3).

Nonindigenous anuran responses

Survival

Tadpole community and newt presence acted independently to significantly affect survival of *O. septentrionalis* (ANOVA community: $F_{1,12} = 8.07$, $P = 0.015$; newt presence: $F_{1,12} = 23.72$, $P < 0.001$; interaction $F_{1,12} = 0.12$, $P = 0.736$). *O. septentrionalis* survival was greater in the *B. marinus* + *O. septentrionalis* community, an effect that is most likely related to decreased intraspecific competition due to the substitutive design. Newts preyed heavily on *O. septentrionalis*, reducing species-specific survival rate by 50 % over both treatments in which *O. septentrionalis* was present (Fig. V-2). In contrast, there were no statistically significant effects of either community or newt presence on survival of *Bufo marinus* (ANOVA community: $F_{1,12} = 1.08$, $P = 0.319$; newt presence: $F_{1,12} = 1.51$, $P = 0.243$), although newt presence and tadpole community interacted nonsignificantly to increase survival of *B. marinus* larvae reared in the presence of both newts and *O. septentrionalis* tadpoles (interaction: $F_{1,12} = 4.01$, $P = 0.068$).

Growth and development

Only newt presence significantly affected the growth rates of *O. septentrionalis* larvae (ANOVA community: $F_{1,12} = 0.99$, $P = 0.339$; newt presence: $F_{1,12} = 32.57$, $P < 0.001$; interaction $F_{1,12} = 2.13$, $P = 0.170$). On average, *O. septentrionalis* reared in the presence of newts grew at approximately double the rate of tadpoles reared in the absence of newts (newts present: 11.5 mg/d ± 3.1; newts absent: 5.8 mg/d ± 1.1). Consequently, *O. septentrionalis* tadpoles transformed earlier (ANOVA $F_{1,12} = 18.96$, $P = 0.001$) and at larger mass (ANOVA $F_{1,12} = 68.95$, $P < 0.001$) when newts were present.

In contrast to the significant effects of newt presence on growth of *O. septentrionalis*, growth rates of *B. marinus* tadpoles did not vary significantly among treatments (ANOVA community: $F_{1,9} = 0.17$, $P = 0.692$; newt presence: $F_{1,9} = 2.72$, $P = 0.133$; interaction $F_{1,9} = 0.09$, $P = 0.775$).

Community

Deviation from expected relative abundance was large and positive for *O. septentrionalis*, suggesting competitive dominance of this species. This dominance was not affected by tadpole community (ANOVA $F_{1,12} = 1.41$, $P = 0.259$), but was reduced by the presence of newts (ANOVA $F_{1,12} = 22.49$, $P < 0.001$; Fig. V-2). When newts were absent, *O. septentrionalis* were much more abundant than expected (mean deviation from expected relative abundance = 0.334 ± 0.116), but were only slightly more abundant than expected when newts were present (mean deviation = 0.067 ± 0.118). In contrast, the relative abundance of *B. marinus* larvae was not affected by either tadpole community or newt presence and were generally as abundant as expected (mean deviation = - 0.015 ± 0.117).

Notophthalmus responses

All newts survived the entire experimental period and mean growth rates of newts did not vary among tadpole community treatments (ANOVA $F_{3,11} = 1.18$, $P = 0.362$). Newts gained a mean of 779 mg (± 53) body mass during the study period, with a mean growth rate of 14 mg / d (± 9).

DISCUSSION

Collectively, the results presented here are consistent with two broad patterns. First, *O. septentrionalis* tadpoles compete significantly with native tadpoles, resulting in deleterious effects to the native larval anuran community. The second broad pattern is that the evidence from this study suggests that newts maintain their role as an important predator in the invaded tadpole community, reducing the negative impacts of the competitively-dominant invader, *O. septentrionalis*. Here I discuss the evidence for and possible mechanisms contributing to these patterns.

Competitive effects of *Osteopilus septentrionalis*

The presence of *O. septentrionalis* larvae consistently resulted in adverse impacts to the native tadpole community. In particular, survival of native species was drastically reduced when *O. septentrionalis* were present. Although laboratory observations have shown that larval *O. septentrionalis* are cannibalistic and predatory under certain circumstances (Crump 1986, Smith 2005b), a microcosm study suggested that predation by *O. septentrionalis* on heterospecific tadpoles is insignificant when alternative food is present (Smith 2005a). The cause of increased mortality in this study is therefore more probably attributable to the general competitive superiority of *O. septentrionalis* larvae, which exhibited higher growth rates than any other species in this study, including the larger *R. sphenocephala*. A high relative growth rate in anuran larvae is sometimes associated with competitive superiority (e.g., Morin 1983, Wilbur 1987) and natural selection is expected to maximize growth rates of larval anurans in temporary aquatic environments (Wilbur 1980), such as those favored by *O. septentrionalis* (Meshaka 2001). Although resource levels in the mesocosms were not monitored in this study, increased mortality of native species most likely resulted from exploitation of limited food resources by *O. septentrionalis*. The results of a separate laboratory study support this conclusion, as they suggest that the primary mechanism of competitive effects of *O. septentrionalis* on *Bufo terrestris* is the exploitation of limited food resources (Part IV).

In addition to increased mortality of native tadpoles, the presence of *O. septentrionalis* also resulted in sublethal impacts to *B. terrestris*, including reduced growth rate, longer larval period, and smaller size at metamorphosis. Each of these effects is of ecological significance; delayed metamorphosis may expose anuran larvae to higher mortality rates as a consequence of predation or desiccation (Wilbur 1980), while small metamorphic body size is correlated with small adult body size (Smith 1987) which relates with reproductive success and adult fitness (Berven 1981, Woolbright 1983). The impacts of competition with larval *O. septentrionalis* may therefore transcend the larval period of native anurans and indirectly affect the population dynamics of subsequent generations.

Another, more subtle, effect of *O. septentrionalis* in this study was the alteration of native community dynamics resulting from its presence. Although the implications of the alteration of community structure by invasive species are not well known, it may be important if invasive species become the most significant determinant of the structure of communities which originally assembled in their absence. In fact, examples of "community disassembly" by invasive species suggest that community-level effects beyond reductions in richness or diversity may be ecologically significant (e.g., Sanders et al. 2003).

Collectively, these results contribute to a growing body of evidence that larval *O. septentrionalis* compete with native anuran larvae in Florida. Although there is presently no significant evidence of amphibian declines in the southeastern United States

(Pechmann et al. 1991), the presence of nonindigenous aquatic species has contributed to declines of amphibians elsewhere (Blaustein and Kiesecker 2002). Consequently the results of this study contribute to the early detection and assessment of potential threats to native biodiversity, an essential component of biodiversity conservation.

Keystone effects of *Notophthalmus viridescens*

Newts were important predators in the invaded communities containing competitively-dominant *O. septentrionalis* larvae in this experiment. The ecological effects of newt predation frequently interacted significantly with the effects of the tadpole community. This interaction generally resulted in reduced impacts of *O. septentrionalis* on the native community, as evidenced in part by improved survival and growth of native tadpoles. Additional evidence for a modifying effect of newt predation is the observation that newts preyed selectively on *O. septentrionalis* tadpoles. In the 6-species *B. marinus* + *O. septentrionalis* treatment, only *O. septentrionalis* experienced a decrease in survival rate as a result of newt presence (Table V-4). As the putative mechanism of keystone predation is selective consumption of competitively superior species (Paine 1966, 1969, Morin 1983), this result supports the role of newts as keystone predators in the *O. septentrionalis*-invaded community.

Higher mortality of *O. septentrionalis* through predation by newts is somewhat surprising, given the high growth rate of *O. septentrionalis* tadpoles in this study and in the wild (Meshaka 2001). Because newts (and several other tadpole predators) are gape-limited, high growth rates generally benefit anuran larvae by reducing their exposure to predation (Wilbur and Fauth 1990). However, as high larval growth rates and competitive superiority are thought to result from high activity levels in some species (Werner 1992), predation on early larval stages of rapidly-growing species may be disproportionately high, as activity may aid the detection of prey by predators. Observations made during this study suggest that *O. septentrionalis* are highly active, as they frequently were seen swimming openly in the pelagic zone of the tanks, behavior that is typical of *O. septentrionalis* larvae in natural water bodies (personal observation and Meshaka 2001). This contrasts sharply with the behavior of the other species in this study, which were less active and generally absent from the open water column, perhaps in part as a response to the presence of newts (e.g., Nicieza 2000). These observations suggest that the behavior of *O. septentrionalis* may expose them to greater predation pressure from newts, which rely in part on visual cues while foraging (Attar and Maly 1980). Similar preferential predation by newts occurred in a previous study in which competitively-dominant, fast-growing, active larvae of *Scaphiopus holbrookii* were selectively preyed upon to the benefit of other members of the tadpole community (Morin 1983).

Although newt presence and tadpole community interacted in several cases, the interaction of predation and competition was not universal in this study. Newts modified and reduced the negative effects of *O. septentrionalis* on survival and native community structure, while the growth rates of native tadpoles were independently affected by tadpole community and newt presence. These discordant results might suggest that different mechanisms affect the survival and growth of native tadpoles, since the interaction of predation and competition can result from several mechanisms (Chase et al. 2002). One plausible explanation is that differences in survival among community treatments in this study were primarily caused by interspecific competition with *O. septentrionalis*, while differences in growth were in part caused by either intraspecific competition or competition among the native species, factors which may have been affected by newt predation to lesser degrees.

Interestingly, the presence of *Bufo marinus* larvae did not significantly influence predation by newts, despite their toxicity to several vertebrate and invertebrate predators (Crossland and Alford 1998, Crossland and Azevedo-Ramos 1999). This suggests that newts avoided *B. marinus* larvae or are tolerant of *B. marinus* toxins. Evidence from laboratory feeding trials (unpublished data) suggests that both mechanisms are responsible. In these trials, newts consumed live *B. marinus* larvae without experiencing mortality. However, when alternative prey was available (e.g., *B. terrestris* larvae) newts consumed fewer *B. marinus*. These observations are also consistent with the absence of evidence of significant predator-associated mortality of *B. marinus* in this study (Table V-4; Fig. V-2).

CONCLUSIONS AND IMPLICATIONS

This study is the first to demonstrate keystone effects of predation by a native species on an invasive species. Assuming that the ecological relationship between *N. viridescens* and *O. septentrionalis* in this experiment is not unique, these results suggest that keystone species may be important factors in the maintenance of diversity in invaded communities in addition to being important factors in native systems. Although this assumption has been validated by past studies on the ecological role played by *N.*

viridescens in various aquatic communities (Morin 1981, 1983, Fauth and Resetarits 1991) the ecological properties of individual species that contribute to their keystone status may be idiosyncratic. Consequently, several factors may constrain whether other keystone species have similar moderating effects on the impacts of invasive species. First, competitive asymmetry must exist among the group of potential prey species (Fauth and Resetarits 1991). This constraint suggests that only invasive species whose impacts arise through interspecific competition may be susceptible to selective predation by keystone species. As even the general impacts of most nonindigenous species are unknown, this study highlights the importance of assessing the ecological impacts of invasive species and, especially, the parsing of such impacts into specific mechanisms.

An additional constraint is that the invasive competitor must also be accessible to the predator. In this study, the ecological similarity of *O. septentrionalis* to competitively dominant native anuran larvae (*S. holbrookii* (Morin 1981, 1983)) may have contributed to its suppression by newts. In contrast, a competitively superior, but toxic or cryptic, invasive species may be unavailable to a potential keystone predator. The results of this study notwithstanding, keystone status is contingent and should not be considered an irrevocable trait of *N. viridescens* or any other species (Power et al. 1996, Fauth 1999, Kotliar 2000).

The results of this study also bear on the frequently invoked but rarely supported notion of biotic resistance (Elton 1958, Simberloff 1995, D'Antonio and Levine 1999), wherein it is hypothesized that diverse natural communities possess some inherent resistance to invasive species or, as in this study, some resistance to the impacts of invasive species. Although ecologists have yet to reach a consensus on the existence of a relationship between native diversity and invasiveness, this study agrees with the suggestion by Simberloff (1995, 1997) that biotic resistance may not be the result of diversity *per se*, but rather of the presence of particular species, in this case a keystone predator. Alternatively, because keystone predation effects can be the result of the additive diffuse predation from a large number of species (Menge et al. 1994), the control of invasive species may yet be a property of intact native communities. Additional experimental studies exploring this possibility could provide evidence of the importance of intact native communities to invasion resistance and may explain why diversity appears to contribute to invasion resistance in some communities, but not others (D'Antonio and Levine 1999). Further assessments such as these will help elucidate the importance of keystone interactions to the maintenance of biotic diversity, especially in highly invaded, highly endangered aquatic ecosystems (Ricciardi and Rasmussen 1999, Sala et al. 2000).

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APPENDIX V-A

TABLES AND FIGURES

Table V-1. Substitutive combination of anuran larvae in the four levels of community in this study. "*N*" is the number of larvae of each species introduced at establishment. Total tadpole density was 240 per tank for each levels. Each community level was crossed with two levels of newt presence (a single adult newt present or absent) for a total of eight treatment combinations.

Community Level	***N***	**Species**
Native Community	60	*Bufo terrestris, Gastrophryne carolinensis, Hyla squirella, Rana sphenocephala*
Bufo marinus (Bma) Treatment	48	*B. terrestris, G. carolinensis, H. squirella, R. sphenocephala, B. marinus*
Osteopilus septentrionalis (Ose) Treatment	48	*B. terrestris, G. carolinensis, H. squirella, R. sphenocephala, Osteopilus septentrionalis*
B. marinus + *O. septentrionalis* (Bma + Ose) Treatment	40	*B. terrestris, G. carolinensis, H. squirella, R. sphenocephala, B. marinus, O. septentrionalis*

Table V-2. Summary of ANOVA testing for significant effects of tadpole community and newt presence on larval performance of *Bufo terrestris*.

		ANOVA: growth rate	
Source	**df**	***F***	***P***
Community (C)	3,28	5.02	0.007
Newt presence (N)	1,28	12.63	0.001
C X N interaction	3,28	1.44	0.251
		ANOVA: mass at metamorphosis	
Source	**df**	***F***	***P***
Community (C)	3,28	2.86	0.054
Newt presence (N)	1,28	16.49	< 0.001
C X N interaction	3,28	2.76	0.061
		ANOVA: larval period	
Source	**df**	***F***	***P***
Community (C)	3,28	3.83	0.020
Newt presence (N)	1,28	4.96	0.034
C X N interaction	3,28	0.37	0.774

Table V-3. Summary of MANOVA testing for overall effects of community and newt presence on native tadpole community structure and individual ANOVAs on each native species.

	MANOVA: Deviation from expected relative abundance* of *B. terrestris*, *H. squirella*, and *R. sphenocephala*		
Source	df	*F*	*P*
Community (C)	9,73	3.76	< 0.001
Newt presence (N)	3,30	3.08	0.042
C X N interaction	9,73	2.16	0.035

	ANOVA: Deviation from expected relative abundance*		
Source	df	*F*	*P*
B. terrestris			
Community (C)	3,32	4.28	0.012
Newt presence (N)	1,32	3.36	0.076
C X N interaction	3,32	2.75	0.059
H. squirella			
Community (C)	3,32	6.90	0.001
Newt presence (N)	1,32	1.65	0.208
C X N interaction	3,32	1.65	0.198
R. sphenocephala			
Community (C)	3,32	1.07	0.375
Newt presence (N)	1,32	2.33	0.136
C X N interaction	3,32	0.93	0.439

*See Methods section for description of deviation from expected relative abundance.

Table V-4. Mean proportion (± 1 SD) of surviving tadpoles for each species in the 6-species *Bufo marinus* + *Osteopilus septentrionalis* treatment.

	Mean Proportional Survival		
	Newt Absent	**Newt Present**	**Difference**
B. marinus	0.090 ± 0.134	0.380 ± 0.134	0.290 ± 0.252
B. terrestris	0.520 ± 0.147	0.665 ± 0.147	0.145 ± 0.233
G. carolinensis	0.010 ± 0.014	0.055 ± 0.014	0.045 ± 0.085
H. squirella	0.005 ± 0.011	0.015 ± 0.011	0.010 ± 0.025
O. septentrionalis	0.800 ± 0.175	0.460 ± 0.175	- 0.340 ± 0.275
R. sphenocephala	0.170 ± 0.315	0.220 ± 0.315	0.050 ± 0.364

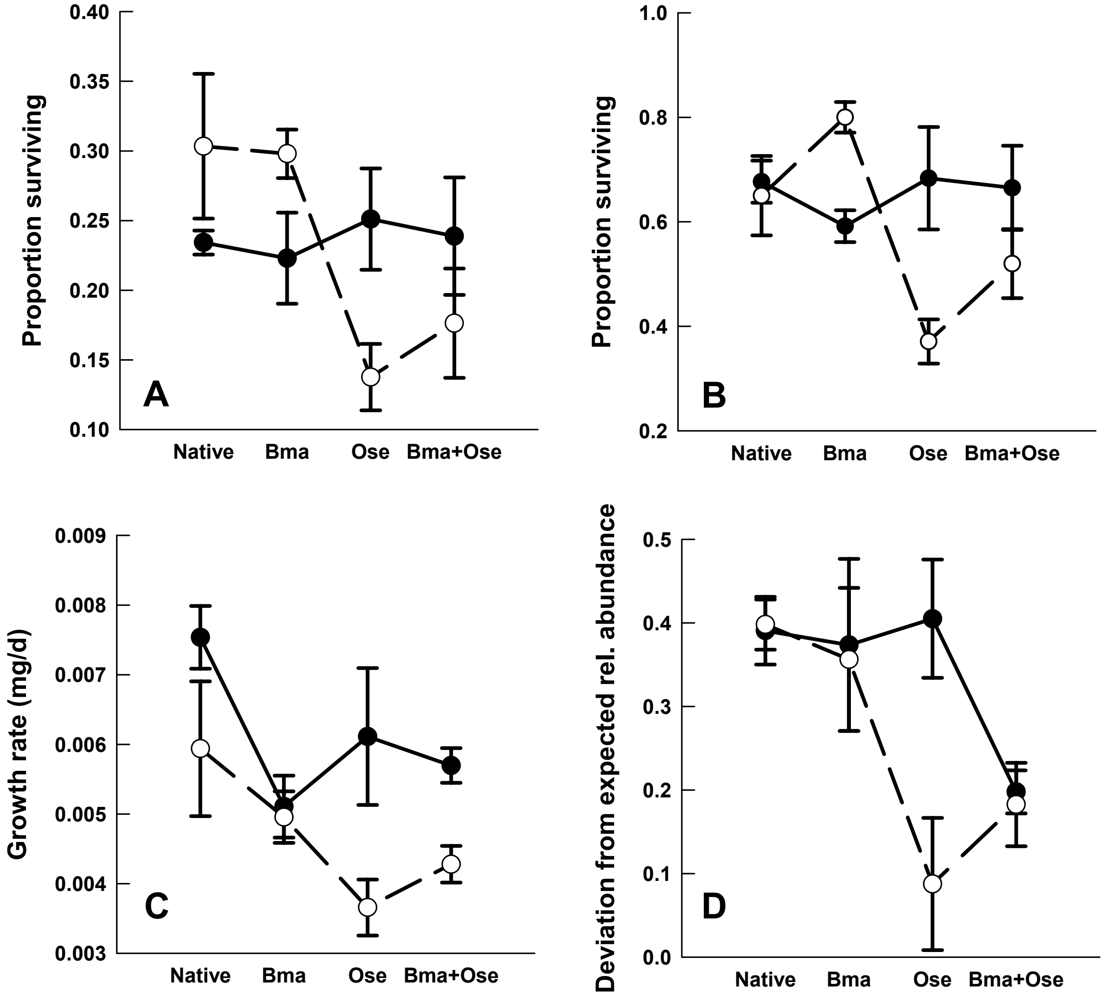

Fig. V-1. The effects of tadpole community and newt presence (closed symbols) or absence (open symbols) on (A) proportion of native tadpoles surviving, (B) proportion of *Bufo terrestris* surviving, (C) growth rate of surviving *B. terrestris*, and (D) deviation from expected relative abundance of *B. terrestris*. Symbols are mean values ± 1 SE. See Table V-1 for community descriptions.

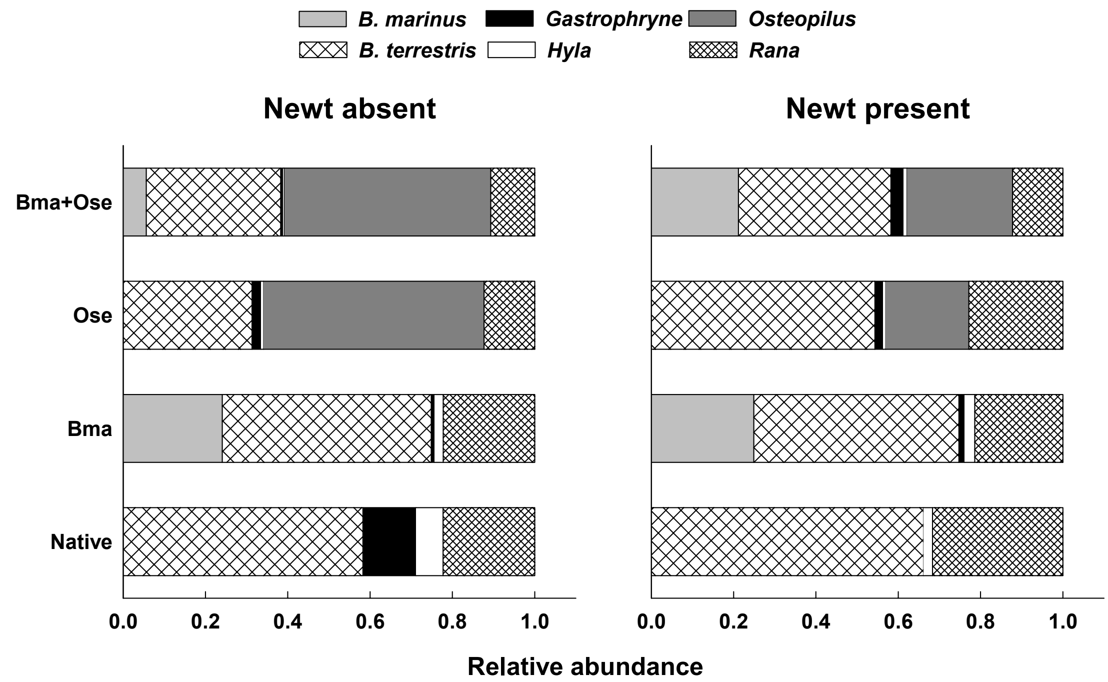

Fig. V-2. Composition of the larval anuran communities compared across all factor levels. Values are mean relative abundances of larvae surviving until metamorphosis, or until the end of the experimental period for *R. sphenocephala*.

CONCLUSIONS

In this section I summarize the primary conclusions reached through the research described in this dissertation and briefly describe the implications of these conclusions.

Part I: The pattern of establishment of nonindigenous amphibians and reptiles in Florida reinforces the important role played by human activities in the establishment of nonindigenous species. Several studies have shown a strong relationship between human population and nonindigenous species richness across taxa and scale, and this research also confirms this trend. In contrast, the strong negative influence of latitude on the richness of nonindigenous amphibians and reptiles in Florida contradicts general relationships between diversity and latitude. This trend is probably particular to Florida, however. The positive relationship between university presence and nonindigenous herpetofaunal richness in this study is most likely related to survey effort, and confirms that unequal effort can influence apparent patterns of species diversity at the spatial scale of counties. Despite the presence of at least 40 established nonindigenous amphibians and reptiles in Florida, there is presently no significant evidence of biotic homogenization. The spread of presently established species, the widespread introduction of new invasive species, and the extirpation of relatively rare native species may all contribute to future homogenization, however.

Parts II and III: The results of the experimental case studies indicate that larvae of one of the two invasive amphibians may have significant ecological impacts to native amphibians communities. Competition with larval Cuban treefrogs, *Osteopilus septentrionalis*, resulted in reduced growth, smaller size at metamorphosis, and longer larval periods in the tadpoles of several native anurans. Although not explicitly investigated in this study, if similar impacts occur in natural tadpole communities in

Florida, the presence of *O. septentrionalis* mar result in adverse impacts to the fitness of adult native anuran populations. In contrast, predation on heterospecific anuran larvae by *O. septentrionalis* tadpoles was only documented under relatively extreme conditions, and may not be a significant ecological effect in natural tadpole communities.

In contrast to the ecological effects of *O. septentrionalis*, in no case did the presence of *B. marinus* tadpoles result in adverse impacts to native tadpoles. Adverse impacts to native anurans as a result of competition with larval *B. marinus* therefore seem unlikely.

Part IV: The competitive effects of *O. septentrionalis* on native tadpoles were the result of exploitation competition for food resources. Because natural tadpole communities are frequently resource-limited, this result suggests that *O. septentrionalis* may also compete significantly with native tadpoles in natural habitats, resulting in adverse impacts to growth, survivorship, and eventually recruitment of the native species. This prediction should be explicitly tested in natural tadpole communities in Florida.

Part V: The addition of a keystone predator of native tadpole communities, eastern newts, *Notophthalmus viridescens*, resulted in a reduction of the impacts of *O. septentrionalis* on the native tadpole community. Survival of native tadpoles was higher in the presence of *N. viridescens*, and growth rates and mass at metamorphosis were higher in one species, *Bufo terrestris*. Reduced survival of *O. septentrionalis* in the presence of *N. viridescens* suggests that newts preyed selectively on tadpoles of the competitively-superior invasive species. This results supports the role of *N. viridescens* as a keystone predator in this invaded tadpole community. If these results are general, keystone predators may play important roles in the maintenance of diversity in invaded communities by reducing the impacts of invasive species.

These results present a number of testable predictions that can be assessed with field studies to better determine the impacts of *O. septentrionalis* on native amphibians communities in Florida. Such additional studies will contribute not only to the understanding of the impacts of *O. septentrionalis*, but will also be important tests of the ability of simplified experimental communities to predict the impacts of invasive species. Furthermore, the descriptive geographic analysis presented in Part I of this dissertation defines some patterns that may be used to focus efforts on the prevention of future introductions of nonindigenous amphibians and reptiles and perhaps other taxa, as well. As the rate of introductions of these taxa is apparently accelerating with human population growth in Florida, such studies are increasingly essential components of the conservation of Florida's unique and threatened biodiversity and that of other regions.

VITA

Kevin G. Smith was born in California in 1974. After a very short stay in that state, he moved with his family to Florida and then New York before settling for his formative years in North Andover, Massachusetts, where he eventually attended Brooks School for his secondary education. After graduating from high school in 1992, Kevin chose to stay in New England for his Bachelor's degree, attending Colby College in Waterville, Maine while spending his summers in Naknek, Alaska. He received a B.A. in Biology with a Concentration in Environmental Science from Colby College in 1996. In the fall of 1996, Kevin entered graduate school in the Department of Zoology and Wildlife Studies at Auburn University in Auburn, Alabama. He graduated with a Master of Zoological Studies degree in 1998.

After working for a few years as a wildlife biologist in Great Smoky Mountains National Park and Okefenokee National Wildlife Refuge for the United States Geological Survey, Kevin began his PhD program in 2001 with Sandy Echternacht in the Department of Ecology and Evolutionary Biology at the University of Tennessee, Knoxville. After receiving his PhD in the summer of 2005, Kevin plans to move to Potchefstroom, South Africa to begin a post-doctoral position at the Potchefstroom campus of North-West University conducting research related to amphibian conservation in southern Africa.