PRIORITY EFFECTS OF OVERWINTERED *RANA* TADPOLES ON LARVAL

SOUTHERN TOAD (*BUFO TERRESTRIS* BONNATERRE)

by

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In natural ecosystems, the order of species arrival can impact the development of the community. In the Atlantic Coastal Plain of the Carolinas, wetland ponds exhibit a wide range of hydroperiods, ranging from ponds that dry up in late summer, to those that persist through fall and winter into spring. Ponds that persist through the fall and winter can be colonized by late-summer breeding southern leopard frogs, whose tadpoles must remain in the ponds until the following spring. Tadpoles of spring-breeding anurans, including Southern toads, are adversely affected by the presence of large tadpoles from the prior summer. I examined several mechanisms potentially responsible for this effect: overwintering tadpoles changing the environment during the winter; large tadpoles outcompeting small hatchling tadpoles; interspecific differences in competitive ability between leopard frog and Southern toad; and density-dependent effects. Leopard frog tadpoles had their primary adverse effect on Southern toads through processes occurring during the winter, prior to the arrival of Southern toads. The other mechanisms tested were not significant. The algal resource on which both species depend also showed a response to the presence of overwintered leopard frog tadpoles, being reduced where tadpoles had been present through winter and spring, and increased where overwintered tadpoles were present only in spring. This response did not become apparent until late in the

spring, suggesting that resource depletion per se is not the mechanism at work, but that some other process occurring in winter leads to adverse conditions for spring-hatched tadpoles where overwintered tadpoles are present through winter. Because isolated wetlands often do not receive the same level of protection as wetlands connected to navigable waters, important amphibian habitat is often altered by humans in ways that change its habitat value. In managing for optimum biodiversity, it is important to consider the often conflicting needs of different species, and to conserve a range of different pond types of varying hydroperiods and degrees of connectivity.

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CHAPTER 1: BACKGROUND

As the importance of functioning ecosystems to life on earth becomes increasingly better understood, the need to understand how these complex systems work becomes more and more necessary. Often, biodiversity itself is an important component in maintaining ecosystem function (Naeem 2002). Biodiversity may be measured in two ways: evenness, that is, the relative abundance of each species within a community; or species richness, that is, the total number of species present. The composition of ecological communities is affected by many interacting factors, such as predation, competition, and abiotic conditions like temperature regimes and rainfall patterns. Two of these factors -- competition and predation -- are direct interactions between species. Indeed, the term "community" in ecology has been aptly defined as "the set of organisms that occur together, and that significantly affect each other's abundance" (Connell and Slatyer 1977). This study focused on competition, which has been shown to occur widely in natural ecosystems, both within populations of a species (intraspecific competition), and between two or more interacting species (interspecific competition) (reviewed by Connell 1983; Schoener 1983). Because competition is affected by a number of different factors, we must understand the biology of the particular suite of species involved, and the factors influencing competition.

One such factor is known as the priority effect. Simply defined, a priority effect is caused when the order of arrival of species into a community affects the outcome of interactions between species in that community. Much of the early work with priority effects focused on their role in plant succession. Because of this focus, successional theory has tended to focus strongly on competition among species for such resources as space, sunlight, and soil nutrients. This led to three putative models of succession (Connell and Slatyer 1977). The first is facilitation, in

which the early-successional species alter the environment in a way that favors the establishment of later-successional species. Tolerance is the model in which the first species to colonize has no effect on the success of subsequent arrivals. Finally, in the inhibition model, the first species to arrive alters the environment to favor its own persistence, and reduces the success of later-successional species. The tolerance model is based on the absence of priority effects, whereas the other two both depend on priority effects in the broad sense. In the narrower sense, however, the term priority effect tends to apply mainly to the third, or inhibition, model, and it is used in that sense in this study.

Sometimes, priority effects can counteract intrinsic competitive ability, as D'Antonio *et al.* (2001) found among grasses: an inferior competitor uses a priority effect – it colonizes first – to succeed over a superior competitor. Where the inferior competitor fails to colonize first, the superior competitor will dominate. Although plant succession was the focus of early work in priority effects, they have been found in animal succession also, both in competition and predation. In some cases, priority effects can determine the direction of trophic interactions (Blaustein and Margalit 1996). Even where trophic interactions are not affected, priority effects can still determine the strength of competition among member species of a guild, that is, a group of species filling similar ecological niches.

The role of priority effects in competition has been especially well studied in larval anuran assemblages. This is likely because of the distinctive approaches used by the two stages of amphibians: whereas adults of different species tend to partition habitat space, i.e., occupy different microhabitats, larvae tend to partition seasonal time, i.e., hatch and develop at different times during the year (Gillespie *et al.* 2004). This partitioning of seasonal time is more difficult in regions with short breeding seasons. Many of these studies have investigated interactions

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among larvae of different genera in seasonal wetlands on the Southeastern Coastal Plain of North America. Alford and Wilbur (1985) found that the natural breeding phenology, with *Bufo americanus* breeding first, *Rana sphenocephala* second, optimized performance in both species relative to other phenologies tested. *Bufo* did better when it preceded *Rana*, and, perhaps unexpectedly, *Rana* did better when it came *after Bufo*. This was so even though either species alone did best when introduced late. Likewise, *Pseudacris crucifer* performed best in ponds where neither *Bufo* nor *Rana* had been (Wilbur and Alford 1985). Similar effects were seen between *P. crucifer* and *B. woodhousii*: the natural phenology, in which *Pseudacris* breeds first, enables it to avoid adverse effects of competition with *Bufo* (Lawler and Morin 1993). This may explain the temporal partitioning of breeding sites by anuran species in high diversity tropical ecosystems (Crump 1974; Bowker and Bowker 1979). Priority effects have also been found in intraspecific competition, when the first cohort to arrive adversely affects later conspecific cohorts. (Chen *et al.* 2001).

So far, the majority of studies of priority effects have focused on within-year order of arrival, that is, the order in which breeding anurans colonize an unoccupied pond or vernal pool in the spring. In North America, a few species of anurans overwinter as larvae. On the Southeastern Coastal Plain, these are all Ranid frogs: the bullfrog, *Rana catesbeiana*; the green frog, *R. clamitans*; and the southern leopard frog, *R. sphenocephala*. All three of these have long spawning seasons, lasting from the beginning of spring into late summer (Mount 1975), so that there are several age cohorts developing at the same time. Some montane populations of *Rana clamitans* require two or three years to complete larval development due to short growing seasons (Berven *et al.* 1979). In lowland populations, where the majority of individuals complete larval development within a single year, there is conflicting data on the reason why

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some overwinter: Morin (1983) found it to be triggered by density-dependent effects of competition, whereas Berven *et al.* (1979) found it to be the result of time of hatching and temperature. In both these cases, the overwintering tadpoles face the trade-off between risk of desiccation and advantage of larger size.

This trade-off results in part from the fact that larval anuran habitat occupies a spectrum of wetland hydroperiods, from temporary (submerged only for brief periods), through seasonal (submerged early in the growing season, but water absent by the end of the growing season in most years), to semipermanent (submerged throughout growing season in most years) and permanent (always submerged) (Cowardin *et al.* 1979). Because rainfall patterns vary from year to year, a given pond may have a longer or shorter hydroperiod in wetter or drier years, respectively. There is a linear relationship between increased rainfall and increased hydroperiod (O'Driscoll and Parizek 2008). Hydroperiod is also affected by geological substrate, sandy pools having a longer hydroperiod than clay pools (O'Driscoll and Parizek 2008); and by anthropogenic activities, as both overgrazing (Munger *et al.* 1998) and deforestation (Neckel-Oliveira and Lannoo 2007) can shorten the hydroperiod. Conversely, in regions which have, or formerly had, populations of native grazing animals, grazing can increase hydroperiod in some years (Marty 2005). Hydroperiod is an important factor in anuran reproductive success. Overwintering tadpoles obviously require a semipermanent to permanent pond, which will retain water from late summer through to spring; conversely, such species as narrowmouth toads (*Gastrophryne caroliniana*) and pine woods treefrogs (*Hyla femoralis*) require a pond that dries up completely between breeding seasons (Semlitsch 2000).

Jones and Gresham (1985) developed a regional classification scheme for the Southeastern Coastal Plain, based on whether a wetland was alluvial or not, and on

geomorphology and water table fluctuation. In their scheme, non-alluvial wetlands fall into three categories: *Pine Savanna*, with water present mainly in winter and spring; *Pocosin*, with water table at or above the surface most of the year; and *Bay*, with a higher water table than pocosin. Their *Bay* is different from the geological Carolina Bay, as Carolina bays may contain either bay or pocosin wetland types as they define these. Sharitz (2003) listed 11 different kinds of Carolina bays, based on hydroperiod, soils, and vegetation assemblages; hydroperiod ranged from permanent lakes to seasonal vernal pools. Alluvial wetland types in the Southeastern Coastal Plain also fall into three geomorphological categories, but only two hydrological types: *Sandy Alluvial Swamp* and *Red River Swamp* both show extreme water table fluctuations, whereas *Low Wet Alluvial Swamp* shows persistently high water table (Jones and Gresham 1985). Among permanent wetlands, some contain fish, which are important predators of amphibian larvae (Chalcraft and Resetarits 2003), whereas others do not. Because of these differences in water table fluctuation, hydroperiod, and presence or absence of fish, we would expect to see different species assemblages within these different wetland types.

A pond retaining water through the winter may or may not contain overwintering tadpoles, especially if it persists only in some years. Colonization of a given pond depends in part on whether breeding adults find it. Due to the generally short distances traveled by most amphibian species, and the high levels of site fidelity shown by many (Blaustein *et al.* 1994), many ponds persisting through winter may nevertheless fail to be colonized by overwintering tadpoles. Thus, anurans using such a pond in the spring may find it either occupied or not by overwintered tadpoles, and may or may not face the competitive pressures of priority effects.

In this study, I examined the different outcomes of possible combinations of pond colonization by anuran larvae in the prior summer and current spring, and the mechanisms

involved in these different outcomes. I attempted to separate out the different mechanisms

which may be driving the effects of overwintered tadpoles on spring-hatched tadpoles, in order

better to understand how priority effects occur in this system. Understanding these mechanisms

can help elucidate some of the ecological processes that drive aquatic biodiversity.

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CHAPTER 2: TESTING PRIORITY EFFECTS OF OVERWINTERED RANID LARVAE ON

Bufo terrestris TADPOLES

Introduction

Among the factors influencing biodiversity is the competitive interactions between
species. The principle of competitive exclusion predicts that where two species both use the
same resource in the same way, one will tend to exclude the other over time (Armstrong and
McGehee 1980). Competition can sometimes determine the structure of the resulting community
through the effect of early arrivals on the availability of resources for later arrivals (Connell and
Slatyer 1977; Robinson and Dickerson 1987), or the relative efficiencies of the interacting
species in using the available resources (Hall 2004). Conversely, Armstrong and McGeehe
(1980) showed that competing species need not exclude each other, and in some systems, two
species dependent on the same limiting resource may coexist indefinitely. In such cases,
competition can nevertheless influence relative abundances of species, since competitively
dominant species are often more abundant relative to poorer competitors (Levine and Rees
2002), or fitness components of the interacting species, as the dominant competitor may be better
able to maximize growth and development at the expense of the poorer competitor (Wilbur and
Alford 1985).

One possible outcome of species interactions is the priority effect, that is, the effect the
first species to arrive in an ecosystem or habitat patch has on later arrivals. This may relate
either to competition or predation. Priority effects have been investigated in a wide variety of
taxa, including grasses (D'Antonio *et al.* 2001), rain forest understory plants (Farris-Lopez *et al.*
2004), coral reef fishes (Almany 2003), fungus-breeding drosophilids (Shorrocks and Bingley

1994) and larval damselflies (Stoks and McPeek 2003). The first species to arrive in a habitat patch may appropriate resources to such a degree that not enough remain for later arrivals (Hall 2004), or may attain a larger size before the arrival of other species, allowing it to escape predation (Blaustein and Margalit 1996), prey on smaller competitors (Stoks and McPeek 2003), or outcompete newly-arriving competitors (Shorrocks and Bingley 1994).

Although many studies have investigated priority effects among different anuran larvae oviposited during the same season, (e.g., Alford and Wilbur 1985, Lawler and Morin 1993), there have been few studies of the effects of overwintering anuran larvae on spring cohorts. These few have generally focused on specific scenarios, e.g., invasive populations of bullfrogs in California (Kupferberg 1997a), or the anthropogenic conversion of temporary or seasonal ponds into semipermanent ones, allowing bullfrogs to become established in new sites (Walston and Mullin 2007). In both these cases, the overwintered tadpoles had adverse effects on spring cohorts. The more general adverse effect of overwintered bullfrog tadpoles on several other larval amphibian species has also been investigated (Boone *et al.* 2004), but no studies have yet examined in detail the mechanisms underlying these effects. Some possible mechanisms include: 1) size-based competition, in which larger tadpoles are superior competitors to smaller ones; 2) changes in habitat quality, as overwintering tadpoles feed on algae through the winter, potentially leading to a lowered food supply in spring relative to ponds without overwintering tadpoles; or 3) density-dependent effects, as ponds with two or more cohorts may have higher total tadpole density than ponds with just one.

Competition has been demonstrated in a number of studies of anuran larvae. *Pseudacris crucifer* is a poor competitor relative to more active larvae of *Bufo woodhousii* and *Rana sphenocephala* (Wilbur and Alford 1985). Also, *Pseudacris* larvae grew larger when predatory

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newts reduced competing populations of spadefoot and southern toad larvae (Morin 1983). Some species may minimize the effects of competition by spatially dividing the resources (Seale and Beckvar 1980). Other species may compensate by other behavioral changes (Richter-Boix *et al.* 2007), which allow them to metamorphose and leave the pond sooner, but at a smaller size. Longer larval periods allow a larger size at metamorphosis (Kehr and Marangoni 1999), which in turn results in improved adult performance (Beck and Congdon 2000); so where competition forces larvae to focus on development at the expense of growth, adult fitness may be reduced. Related species can be differentially affected by competition: *Bufo bufo* did equally well in single-species or mixed groups, but *B. viridis* showed larger mass at metamorphosis in single-species and shorter larval period in mixed groups (Katzmann *et al.* 2003).

A pond persisting through fall and winter in some years can potentially be occupied during those years by the offspring of late-summer breeding amphibians, depending on its proximity to source populations. In ponds where *Rana* tadpoles hatch in late summer and overwinter as larvae, they have attained a much larger size by the following spring than the newly-hatched tadpoles of that spring. The possibility that priority effects related to size advantage are important to competitive interactions is suggested by the size-dependent competition effects found in *Rana clamitans* and *R. catesbeiana*, the nature of which differed according to which species was the larger (Werner 1994). These are sister species, so it might be expected that they would respond in similar ways. The fact that they showed different responses suggests the difficulty of generalizing from studies of one species to another. Although that study did not specifically address between-year priority effects, it does illustrate the importance of relative sizes of competing tadpoles.

Filter-feeding tadpoles significantly reduce primary production by phytoplankton, feeding on all algal species and size classes equally (Seale 1980). Grazing tadpoles, by contrast, feed preferentially on some algal species over others, affecting the composition of the algal species assemblage (Dickman 1968; Kupferberg 1997b). This in turn can affect the performance of tadpoles in a number of ways. Many taxa show a prolongation of the juvenile or larval period in situations of resource scarcity (Alford 1999). Conversely, Skelly (1995) suggested that smaller larvae, because of their lower food requirements, may be better able to cope with resource depletion than larger larvae, creating the possibility of a reversed size advantage, in which a large cohort of smaller individuals can in some species outcompete a small cohort of larger individuals (Claessen *et al.* 2000). In some cases, populations with smaller, more abundant individuals can have effects at least as large as those with fewer, larger individuals (Chalcraft and Resetarits 2004).

There is debate as to whether resources in a temporary pool are highest in a flush when the pond first fills, or in the drying stage (Harris 1999). Nitrogen is input into wetlands through wet deposition, i.e., dissolved nitrogen in rainfall, and dry deposition, from the settling of particles and gases onto the water surface (Paerl *et al.* 2002). These two forms of deposition are variable – wet deposition would be increased in wetter years and seasons, and decreased in drier periods. Likewise, atmospheric deposition increases in regions of increased intensive livestock production. The timing of maximum resources would affect the performance of tadpoles showing different reproductive phenology: if resources are highest when a pond first fills, tadpoles colonizing new ponds would have the advantage. This would be reversed if resources are highest in the drying stage, which would favor tadpoles colonizing old ponds.

Density-dependent effects may account for a portion of the perceived priority effects. As later cohorts are added to the existing population, the total density of larvae increases. In some cases, one species shows a priority effect in which older cohorts suppress their younger conspecifics, whereas another shows only density-dependent, intra-cohort competition among the oldest cohort (Lehinten 2004). Density-dependent effects have also been seen in cases where priority effects were not demonstrated (Alford 1999).

Research Question

This project examined the potential occurrence of resource-limitation, size-based competition, and density-dependent effects in contributing to the overall priority effect of overwintered tadpoles on spring-hatched tadpoles. The null hypothesis is that the timing at which *Rana* is present in the ponds has no effect on *Bufo*. The alternate hypothesis is that *Bufo* are adversely affected by the presence of overwintered *Rana* from the prior year. I investigated several mechanisms of competition to determine which are important in causing the adverse effects. Mechanism 1 is that overwintered *Rana* affect *Bufo* by depleting algal resources through the winter. Mechanism 2 is that overwintered *Rana* affect *Bufo* by competitive advantage due to larger size. Mechanism 3 is that overwintered or newly hatched *Rana* affect *Bufo* regardless of size due to interspecific differences in competitive ability. Mechanism 4 is that the presence of *Rana* affects *Bufo* due to greater total population density relative to a pond without *Rana*.

Study System

Bufo terrestris Bonnaterre, the Southern toad, is the largest and most common of three species of *Bufo* inhabiting the Southeastern Coastal Plain in North Carolina. *Bufo terrestris* begins breeding earlier than the other two, and is thus the most likely to encounter a pond either unoccupied, or with only overwintered *Rana* tadpoles. It breeds in a variety of natural wetland

habitats, as well as ditches along roads, stormwater detention ponds, and flooded agricultural fields. The southern leopard frog, *Rana sphenocephala* Cope, breeds throughout most of the growing season, from early spring into late summer, producing several age cohorts per year. The late summer cohorts are unable to complete development before the onset of winter, and thus must spend the winter in the ponds as larvae.

Materials and Methods

To examine the influence of priority effects associated with competition between Rana and Bufo tadpoles, I conducted an experiment in artificial ponds (mesocosms) at the East Carolina University West Research Campus, Pitt County, North Carolina The mesocosms used in this study are made from modified cattle watering tanks (Rubbermaid model 4247, Newell Rubbermaid, Atlanta, Georgia), designed to mimic seasonal or semipermanent ponds (Cowardin *et al.*1979) of a type commonly encountered on the Southeastern Coastal Plain. Natural ponds vary widely in size, but the mesocosms fall within this range of size variation. Although mesocosms have been criticized as being potentially less realistic than natural ponds (Skelly 2002, 2005), they possess certain advantages: they can be standardized to eliminate natural between-pond variation that reduces statistical power, and the ability to reduce confounding variables, and are independent of each other, unlike enclosures within a single pond (Chalcraft *et al.* 2005). Also, the use of mesocosms reduces historical effects, which cannot always be fully assessed in a natural pond (Morin 1983). Mesocosm studies offer a greater level of realism than is available in the laboratory (Odum 1984) and provide an opportunity for greater statistical power for complex questions than does a field study in a natural pond.

The experiment focused on the responses of *Bufo* to *Rana*. To evaluate the effects of *Rana* on *Bufo,* I manipulated the time at which *Rana* entered the ponds. In order to separate fully

the several mechanisms being tested, I had to include some treatments that might not occur in nature. The treatments were as follows (Table 1): in the treatment designated Winter + Spring, 20 *R. sphenocephala* overwintered in the ponds, to which were added 250 newly hatched *Bufo terrestris* at the onset of spring while the *R. sphenocephala* remained present, reflecting the combined effects to be tested individually in other treatments. In the treatment called Winter only, 20 *R. sphenocephala* overwintered, then were removed at the onset of spring, and 250 newly hatched *B. terrestris* were added at the onset of spring, to test for the effect of resource depletion apart from other factors. In the Spring only treatment, 20 overwintered *R. sphenocephala* tadpoles raised elsewhere were added at the onset of spring concurrently with 250 newly-hatched *B. terrestris*, to test for the effects of contemporaneous competition apart from other factors. The hatchling *Rana* ponds had 250 newly-hatched *R. sphenocephala* and 250 newly-hatched *B. terrestris*, to see if *R. sphenocephala* has an interspecies effect on *B. terrestris* apart from any size-based effects. In the high density *Bufo* treatment were 500 newly hatched *B. terrestris*, to test for density-dependent effects apart from other factors. The final treatment, low density *Bufo*, had 250 newly hatched *B. terrestris*, representing the condition with none of the factors being tested. All tadpole numbers reflect natural densities found in the wild (Morin 1983).

I used a blocked design, in which a block consisted of six mesocosms spatially clustered together, and one treatment randomly assigned to each. This was done to minimize random variation within blocks, and within-treatment variances (Chalcraft and Resetarits 2003). There were five blocks, each consisting of one replicate of each treatment, for a total of 30 experimental units. After initial filling, the mesocosms received only precipitation inputs and evaporation losses, consistent with the hydrological regime of many Carolina bays and similar

wetland depressions with perched water tables and lacking inlet and outlet streams (Sharitz 2003).

On 22 November 2008, the mesocosms were filled block by block with well water to a depth of 50 cm, for a volume of ~1000 liters of water, and covered with insect screening to prevent colonization by unwanted organisms, and escape of study organisms. Two days later, each mesocosm was supplied block by block with 1 kg raked loblolly pine straw (*Pinus taeda* L.) from Otter Creek Natural Area, Pitt County, North Carolina, to provide a natural substrate representative of the loblolly pine forests characterizing much of the Southeastern Coastal Plain. Pine straw lots were randomized for each tank, on a block by block basis, and contained small amounts of broadleaf and herbaceous material, reflecting the vegetation actually occurring on the collection site. On 3 December 2008 all mesocosms were inoculated with naturally occurring phytoplankton and zooplankton of the region, collected from ponds in Croatan National Forest, Craven County, North Carolina (Figure 1). Twelve days later, a second inoculation of plankton was made to ensure adequate establishment.

Overwintering *Rana sphenocephala* tadpoles were collected from the Croatan National Forest, Craven County, North Carolina, from flooded ruts made by tracked logging equipment in a loblolly pine thinning area along County Line Road, and added to the Winter + Spring and Winter only treatments on 9 December 2008. All overwintering *Rana* tadpoles were weighed and randomized before being placed in the mesocosms. Randomization was accomplished by assigning each tadpole a number, and using a random number generator to assign numbered tadpoles to lots in which the range of masses was fairly even across lots. Removal of *Rana* from the Winter only treatment was accomplished by intensive minnow-trapping from 29 March to 9 April, 2009. All individuals were recovered, weighed, and released at the location of initial

Table 1. Experimental treatments, showing numbers and type of tadpoles added to mesocosms, and time of introduction to mesocosms. Black lines indicate presence of overwintered (OW) or hatchling (new) *Rana*, gray lines indicate presence of *Bufo*.

| Treatment	Time	
	Winter	Spring
1) 20 OW *Rana* 250 *Bufo*	▬▬▬▬▬▬▬▬▬▬▬▬	
2) 20 OW *Rana* 250 *Bufo*	▬▬▬▬	▬▬▬▬
3) 20 OW *Rana* 250 *Bufo*		▬▬▬▬ ▬▬▬▬
4) 250 new *Rana* 250 *Bufo*		▬▬▬▬ ▬▬▬▬
5) 500 *Bufo*		▬▬▬▬
6) 250 *Bufo*		▬▬▬▬
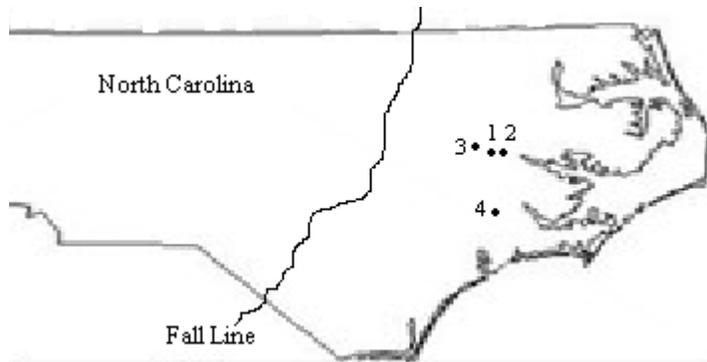
Figure 1. Study location and collection sites. 1) East Carolina University West Research Campus. 2) City of Greenville. 3) Otter Creek Natural Area. 4) Croatan National Forest. The Fall Line is the transition from Coastal Plain to Piedmont.

capture. Overwintered *Rana* tadpoles were again collected from the same site in Croatan on 10 April 2009, randomized in the same way, and added to the Spring only treatment.

On 29 March, 2009, *R. sphenocephala* and *Bufo terrestris* eggs were collected from a stormwater detention pond within the City of Greenville, Pitt County, North Carolina (Figure 1), and hatched in the laboratory. Tadpoles were pooled from 6 *Bufo* clutches and 3 *Rana* clutches in such a way that the proportion of individuals from each clutch was uniform across mesocosms, in order to equalize genetic diversity as much as possible. Tadpoles were assigned to lots of 250 each. Tadpole lots were then randomized, and added to the mesocosms on 5 April 2009 as follows: 250 hatchling *Rana* and 250 *Bufo* to each tank in the hatchling *Rana* treatment; 500 *Bufo* to each tank in the high-density treatment; and 250 *Bufo* to each tank in the low density treatment.

While tadpoles were present, all mesocosms were checked daily. Before metamorphosis, each mesocosm was provided with a ramp and platform to allow emerging metamorphs to leave the water.

All metamorphs, defined as the emergence of at least one forelimb, were removed from a mesocosm and held in the lab until tail resorption was completed, then weighed (Travis 1980). Towel-dried wet weights were used throughout the experiment (Morin 1983). On 22 June 2009, mesocosms were destructively sampled to account for any surviving, non-metamorphosed individuals. *Rana* metamorphs were collected and weighed in the same way. After being counted and weighed, all metamorphosed animals were returned to the location of initial capture and released into the wild.

As a further test of the resource-depletion mechanism, algal production was also tested. On 7 February 2009, two periphytometers were installed in each mesocosm, to measure

periphyton growth through the winter in the presence or absence of *Rana* tadpoles. Each

periphytometer consisted of a length of plastic ribbon serving as a substrate for algal growth,

weighted with a ring of PVC pipe to anchor it in place. All periphytometers were placed in the

southeast corner of their respective tanks, to eliminate variations in sun exposure. One

periphytometer from each tank was removed on 6 April 2009, just prior to adding *Bufo*, to test

for differences in the initial algal stocks available to hatchling *Bufo*. The second periphytometer

from each tank was removed on 20 May 2009, after peak metamorphosis had passed but before

algal stocks could recover from the exit of tadpoles. Phytoplankton samples were collected on

the same dates, to see whether tadpole feeding differentially affects periphyton and

phytoplankton. Each sample was filtered through fiberglass filters (Fisher Scientific, Pittsburgh,

PA) and stored frozen until analysis. Analysis was conducted using spectrophotometry test for

chlorophyll *a* using a Milton Roy Spectronic 1201 (Milton Roy, Ivyland, PA), for samples

collected just prior to adding *Bufo*, to assess initial algal stocks available to hatchling *Bufo*, and

after peak metamorphosis had passed, to assess final stocks remaining. For periphyton, the area

of periphytometer scraped was measured, to quantify algal production in ug m^{-2}; for

phytoplankton, a known volume was filtered, to quantify production in ug l^{-1}. The change in

chlorophyll *a* from early to late sampling dates was also calculated.

Statistical Analyses

Mass at metamorphosis, larval period, and survival were the primary measures, because

these are important fitness components in anurans (Alford and Wilbur 1985; Wilbur and Alford

1985). Mass and larval period were averaged by mesocosm, since tadpoles within a single

mesocosm affect each other and cannot be considered independent data points (Hurlbert 1984).

Geometric mean was used for mass, to normalize the distribution. Arithmetic mean was used for

larval period. Because the sample sizes were equal (n=5 for each treatment, that is, one mean value per mesocosm), there was no need to test variances for homogeneity; unequal variances will have little effect in this case (McGuinness 2002).

Comparisons are summarized in Table 2. Analyses were conducted as follows: To test for the effects of resource depletion through the winter, ANOVA was used to compare the four overwintering treatments: *Rana* present in winter only, overwintered *Rana* present in spring only, *Rana* present in winter and spring, and *Rana* never present. Ryan's multiple range test (REGW) was used to make pairwise comparisons.

To test for effect of larger size of overwintered *Rana*, two-sample t-tests were conducted for each of the following combinations: overwintered *Rana* × new *Rana*; overwintered *Rana* present only in spring × new *Rana*; and overwintered *Rana* × high-density *Bufo*.

To compare the interspecific effect of hatchling *Rana* with effects of high conspecific density, paired t-test used the Population Impact ratio (Chalcraft and Resetarits 2004):

$$\ln\left(\frac{\text{Experimental}}{\text{Control}}\right)$$

for the hatchling *Rana* treatment as the numerator and *Bufo* at low density as the denominator vs. the same ratio for high density *Bufo* as the numerator. Two-sample t-test was used to test for density-dependent effects: *Bufo* at high density × *Bufo* at low density, and *Bufo* with hatchling *Rana* × *Bufo* alone at low density.

Contrasts in the algal resource were made by treatment as follows: for initial algal stocks, overwintered *Rana* present vs. absent, as this was the only treatment applied by that date. For final algal stocks, factorial ANOVA using Block, Winter, and Spring. The factor "Winter" was

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Table 2. Statistical comparisons, by treatment, for variables: mass at metamorphosis (Mass), length of larval period (Larvl per), and percent survival (Surv.).

Mechanism Tested	Comparison	Statistical Test
Priority effect of OW *Rana*	OW *Rana* x Hatchling *Rana*; OW *Rana* x *Bufo* High Density	Mass: $t_8=3.42$, P=0.0091; $t_8=3.57$, P=0.0078 Larvl per: $t_8=3.766$, P=0.005; $t_8=4.024$, P=0.003 Surv.: $t_8=9.536$, P<0.0001 $t_8=11.0$, P<0.0001
Size-based effects of large *Rana*	OW *Rana* Present in Spring x Hatchling *Rana*	Mass: $t_8=0.28$, P=0.79 Larvl per: $t_8=0.284$, P=0.783 Surv.: $t_8=0.137$, P=0.89
Timing of effect of OW *Rana*	OW *Rana* present in Winter, Spring, Both, and Neither	Mass: $F_{5,24}=3.36$, P=0.02 Larvl per: $F_{5,24}=9.94$, P<0.0001 Surv.: $F_{5,24}=13.28$, P<0.0001
Interspecific effect of *Rana*, independent of size and priority	Effect Size Hatchling *Rana* x Effect Size *Bufo* High Density	Mass: $t_8=0.74$, P=0.48 Larvl per: $t_8=0.84$, P=0.43 Surv.: $t_8=0.52$, P=0.62
Density-dependent effects	*Bufo* High Density x Low Density	Mass: $t_8=0.16$, P=0.88 Larvl per: $t_8=0.65$, P=0.55 Surv.: $t_8=0.3$, P=0.77

both the treatment with *Rana* present in both winter and spring, and the treatment with *Rana* present only in winter. Likewise the "Spring" factor used the treatment with *Rana* present in both winter and spring, and that with overwintered *Rana* present only in spring. REGW was conducted comparing treatments with overwintered *Rana* present in winter, spring, both, and neither; and t-tests contrasting treatments with high-density *Bufo*, low-density *Bufo*, and *Bufo* together with new *Rana*. Repeated measures ANOVA was used to compare initial and final algal stocks where overwintered *Rana* were present in winter, spring, both, and neither, to determine whether there was an effect of time on the algal response to different treatments.

Results

Rana.

Survival of overwintered *Rana* tadpoles was 100% in the treatment where *Rana* were removed at the onset of spring. At metamorphosis, survival of overwintered *Rana* was 64% for those that spent all winter and spring in the ponds, and 78% for those that spent only spring in the ponds. All surviving overwintered *Rana* completed metamorphosis prior to the end of the experiment except one individual. Current year *Rana* began emergence on 6 June. Survival was relatively high (56%), but only 4% reached metamorphosis prior to termination of the experiment.

Average mass of fall tadpoles when introduced to the ponds was 0.496g. Although the average mass of overwintered tadpoles removed in spring was 2.272g, those introduced in spring were significantly smaller at an average of 1.58g (t_8=7.64, P<0.0001). This difference was the result of my inability to find larger overwintered *Rana* in the wild at the onset of spring. The same ponds were revisited at the spring collection date; however, the surviving tadpoles found were on average smaller than those collected from those ponds in fall. Nevertheless,

overwintered *Rana* present only in spring metamorphosed at 2.46g, significantly larger than those present through both winter and spring, which averaged 1.66g (t_8=4.35, P=0.003) at metamorphosis.

Bufo.

The first metamorph emerged on 7 May, and peak metamorphosis was on 18 May. At termination of the experiment, ten unmetamorphosed tadpoles were found, of which five came from one pond. Survival overall was near the upper end of the range observed in other mesocosm experiments (e.g., Alford and Wilbur 1985), except that in the treatment where *Rana* were present in both winter and spring, which showed lower survival.

Priority effects were demonstrated to occur in that there was a significant reduction in *Bufo* mass and survival, and significant lengthening of larval period, where overwintered *Rana* were present relative to where hatchling *Rana* were present (mass: t_8=3.42, P=0.0091; larval period: t_8=3.766, P=0.0055; survival: t_8=9.536, P<0.0001) (Figure 2). Likewise, presence of overwintered *Rana* also showed a significant adverse effect on all variables compared with increased density of *Bufo* (mass: t_8=3.527, P=0.0078; larval period: t_8=4.024, P=0.0038; survival: t_8=11.0, P<0.0001). Conversely, overwintered *Rana* presence only in spring showed no significant difference in any variable compared with presence of hatchling *Rana* (mass: t_8=0.28, P=0.79; larval period: t_8=0.284, P=0.783; survival: t_8=0.137, P=0.89) (Figure 3).

Bufo survival differed among the three treatments containing overwintered *Rana* and the treatment containing only low-density *Bufo* (mass $F_{5,24}$=3.36, P=0.02; larval period, $F_{5,24}$=9.94, P<0.0001; survival $F_{5,24}$=13.28, P<0.0001) (Figure 4). Survival was significantly lower where overwintered *Rana* were present in both winter and spring relative to the other three conditions, which did not significantly differ from each other. Mass was significantly lower where

overwintered *Rana* were present in winter and in both winter and spring, relative to when they

were absent and present only in spring. Larval period showed a more complex pattern: *Bufo*

took about 18 days longer to metamorphose where *Rana* were present in both winter and spring

relative to where overwintered *Rana* were present only in spring. *Bufo* larval period was

intermediate in length where *Rana* were present only in winter, compared to where overwintered

Rana were present in both winter and spring, and where they were present only in spring. Where

Rana were never present, *Bufo* larval period did not significantly differ from the treatment in

which overwintered *Rana* were present only in spring.

Where overwintered *Rana* were never present, neither species identity nor increased

density of tadpoles affected fitness components of *Bufo*. There was no significant difference

(paired t-test) between the effect size of increased density of *Bufo* vs. presence of hatchling *Rana*

(mass: t_8=1.704, P=0.16; larval period: t_8=0.047, P=0.96; survival: t_8=0.611, P=0.57). (Figure 5).

Likewise, for density, there were no significant differences (two-sample t-test) between *Bufo* at

high vs. normal density (mass: t_8=0.74, P=0.48; larval period: t_8=0.84, P=0.43; survival: t_8=0.52,

P=0.62), nor between *Bufo* with vs. without hatchling *Rana* (mass: t_8=0.16, P=0.88; larval

period: t_8=0.63, P=0.55; survival: t_8=0.3, P=0.77) (Figure 6).

Algae.

One phytoplankton sample from a pond where *Rana* were present in both winter and

spring and one periphyton sample from the control were lost, both from the late sampling date.

For periphyton chlorophyll *a* repeated measures ANOVA showed that chlorophyll *a*

increased in all tanks between the early and late sampling dates ($F_{3,11}$=4.4, P=0.028), and that

there was a trend toward treatment differences emerging late rather than early ($F_{3,11}$=3.23,

P=0.065). No significant differences were found among treatments with overwintered *Rana*

compared to *Bufo* alone at normal density in the samples collected early ($F_{5,24}=0.67$, P=0.65), but significant differences were found at the late sampling date ($F_{3,15}=3.97$, P=0.03). Mesocosms with *Rana* present in both winter and spring had significantly lower algal stocks than those with *Rana* present only in spring, but neither significantly differed from those with presence of *Rana* only in winter, or with *Rana* never present (Figure 7).

For phytoplankton, repeated measures ANOVA showed an increase in phytoplankton from the early to late sampling dates ($F_{1,19}=12.4$, P=0.002), but no significant emergence of treatment effects through time ($F_{5,19}=0.3$, P=0.9). ANOVA showed no significant differences among treatments with overwintered *Rana* compared to *Bufo* alone at normal density at either sampling date (early $F_{3,16}=1.37$, P=0.287; late $F_{15,3}=1.98$, P=0.316) (Figure 7).

Discussion

From these analyses, it appears that overwintering *Rana* have important priority effects on *Bufo* through processes that occur during the winter, before *Bufo* are present, as indicated by the significant effect of *Rana* presence in winter on larval period and mass of *Bufo* whether or not *Rana* continued into spring. The overwintered *Rana* present only in spring did not affect the larval period or mass of emerging *Bufo*. Thus, larger size *per se* did not confer an adverse effect on these performance components when overwintered tadpoles did not have priority in these ponds. Larval period of *Bufo* was adversely affected by *Rana*'s presence in winter, and by the interaction of *Rana*'s presence in winter combined with its presence in spring, but not by *Rana*'s presence in spring without the effects of presence in winter. There was a significant effect on *Bufo* survival only when overwintered *Rana* were present in both winter and spring. When overwintered *Rana* were removed prior to introduction of *Bufo*, they nevertheless had an adverse
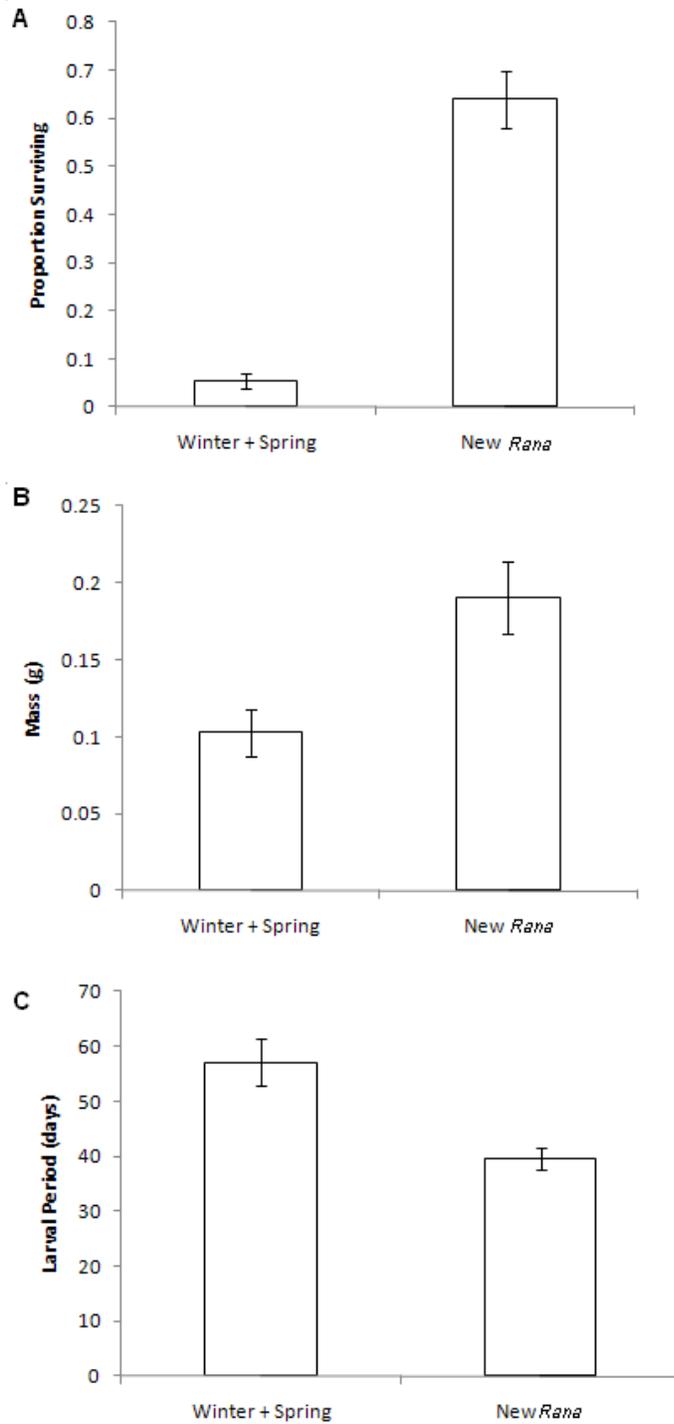
Figure 2. Mean (± SE) A) survival, B) mass at metamorphosis and C) larval period of *Bufo* in tanks where i) overwintered *Rana* are present in both winter and spring and ii) hatchling *Rana* are present.
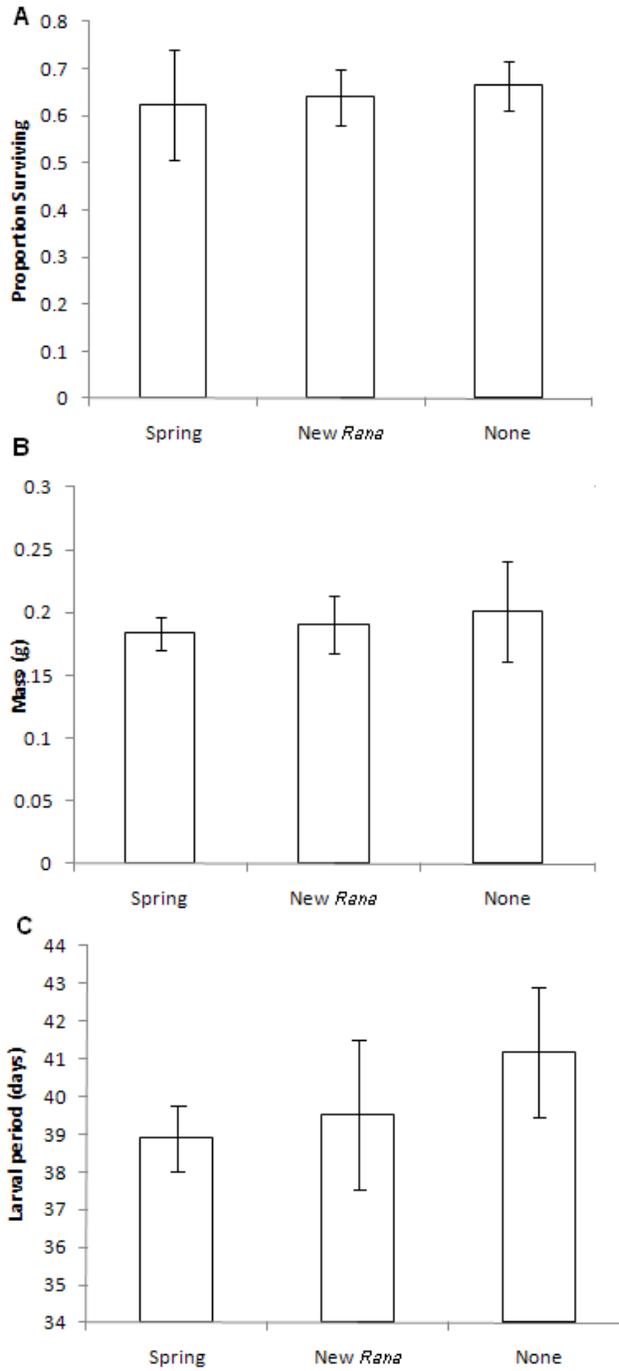
Figure 3. Mean (± SE) A) survival, B) mass at metamorphosis, and C) larval period of *Bufo* where i) overwintered *Rana* present only in spring, ii) hatchling *Rana* present, and iii) absence of *Rana*.
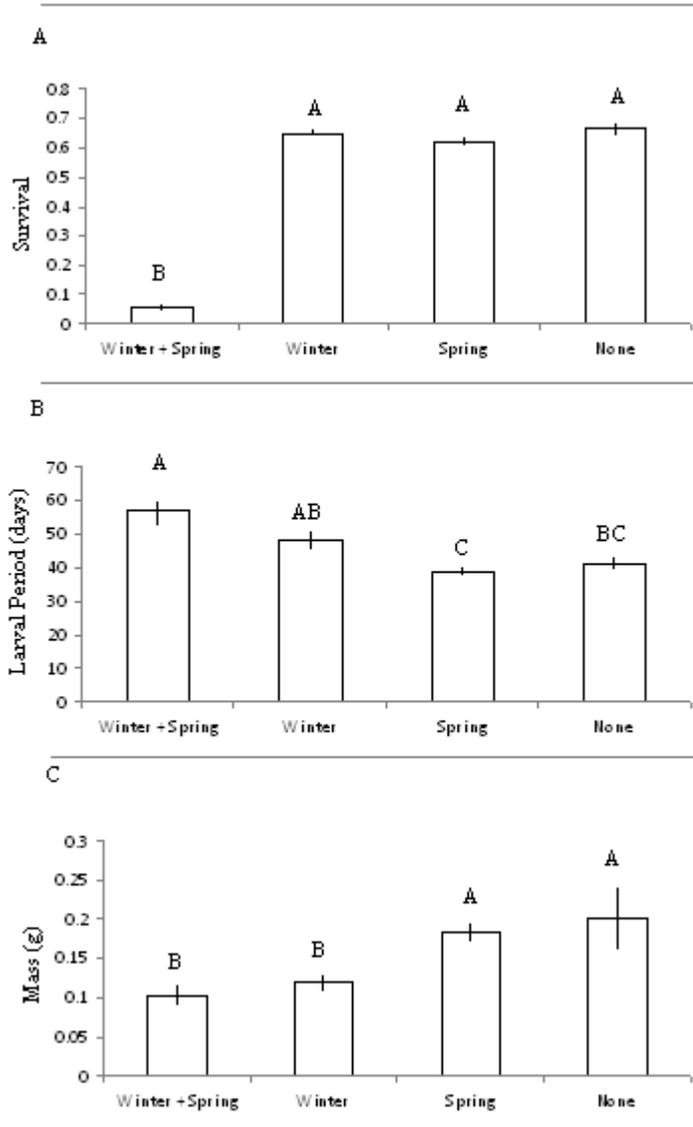
Figure 4. Mean (± SE) A) survival, B) Larval period, and C) mass of *Bufo* where i) *Rana* present in winter and spring, ii) *Rana* present only in winter, iii) overwintered *Rana* present only in spring, and iv) *Rana* never present. Columns within a graph with the same capital letter do not significantly differ from each other (REGW).
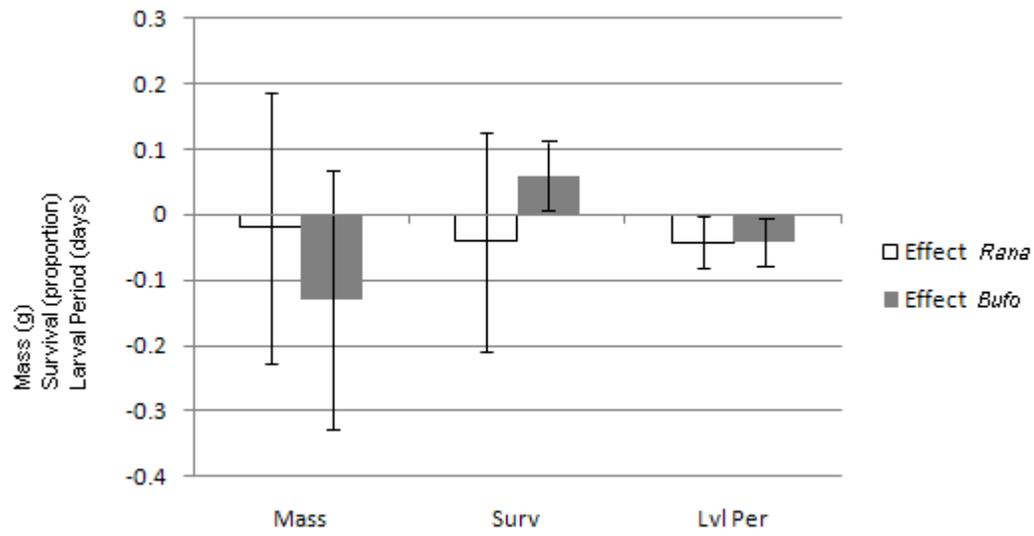
Figure 5. Effect on *Bufo* performance of hatchling *Rana* and high density *Bufo*, relative to low density *Bufo*. Error bars are one SE. Zero line is *Bufo* performance at low density.
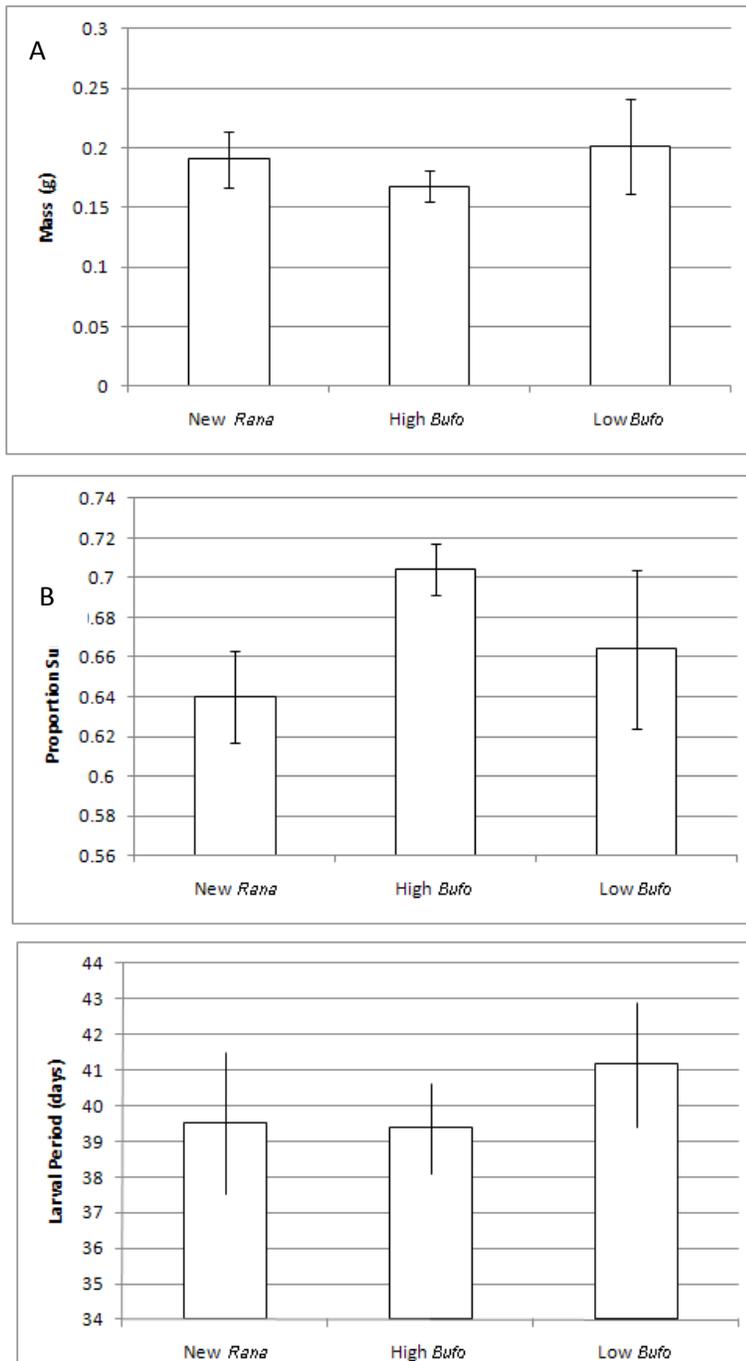
Figure 6. Mean (± SE) A) survival and mass at metamorphosis, and B) larval period of *Bufo* where i) hatchling *Rana* present, ii) *Bufo* at high density, and iii) *Bufo* at low density.
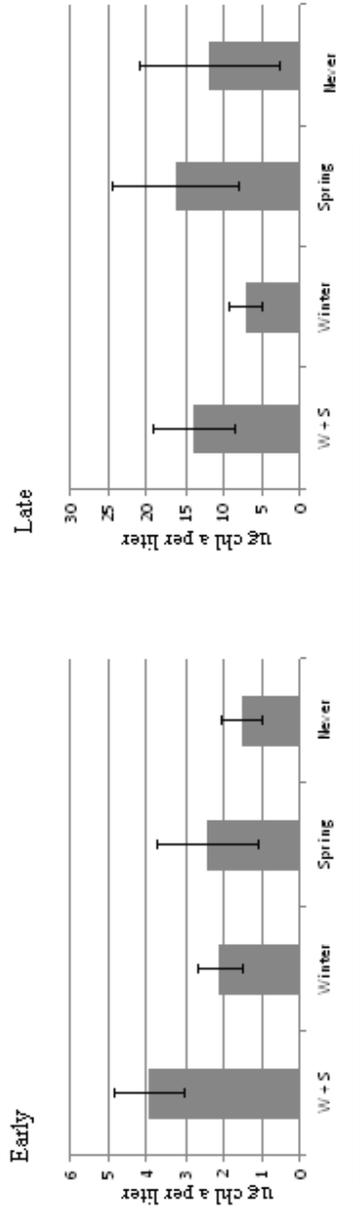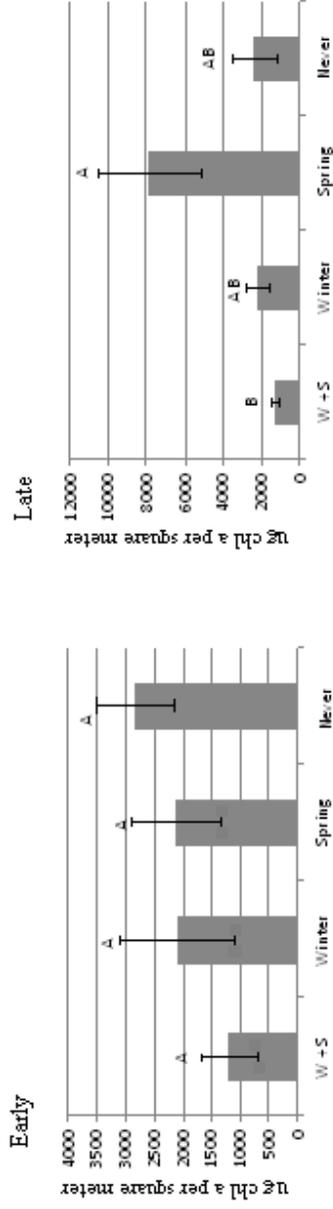
Figure 7. Average chlorophyll a on early (just prior to adding Bufo) and late (after peak Bufo metamorphosis) sampling dates. No significant differences found in phytoplankton. Within periphyton graphs, columns with the same letter do not significantly differ from each other. (REGW) Error bars are one SE.

effect on larval period and mass of *Bufo*, suggesting a legacy effect of resource competition, i.e. that the effect of overwintering tadpoles persists in time. This has been previously documented in other anuran systems for priority effects among anurans breeding within the same year (Wilbur and Alford 1985). It appears presence of *Rana* through the winter affects both larval period and mass of *Bufo*. Larval period was lengthened both by presence of *Rana* in winter and by continued competition with larger tadpoles in spring, but not by such competition where *Rana* had not been present in winter.

Mass at metamorphosis appears to be the least sensitive fitness component, as mass of survivors was reduced only by *Rana* presence in winter. It is unknown whether mass of those which did not survive was affected. It is possible that mortality was caused in part by failure to attain sufficient mass. Nevertheless, the fact that survival and mass responded differently suggests that there are effects on each apart from effects on the other.

Survival was significantly reduced by the combination of the two factors, but not by either factor alone. It is interesting to compare this with Wilbur and Fauth (1990), which examined *Bufo* survival in the presence of predation. In that study, a single predator species reduced *Bufo* survival to levels intermediate between that observed in this study in the treatment where *Rana* were present in both winter and spring, and those in which *Rana* were present in only one or neither season. Two predator species reduced *Bufo* survival to levels comparable to that seen in this study in the treatment where *Rana* were present in both winter and spring. This suggests that competition and predation can change in relative importance depending on which is more intense in a given habitat patch.

Conversely, the lack of significant differences among all treatments without overwintered *Rana* suggests that density-dependent effects and interspecific differences are not of importance

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in competitive interactions between same-aged *Rana* and *Bufo*. If density-dependence was operating, we would expect to see reduced *Bufo* performance at high density relative to low density. Likewise, if *Rana* had an effect on *Bufo* due to interspecific differences in competitive ability, we would expect to see reduced *Bufo* performance where hatchling *Rana* were present relative to where no *Rana* were present.

The effects on the algae were more complex. Periphyton, in particular, behaved unexpectedly in light of the *Bufo* results. Overwintered *Rana* present only in spring resulted in higher periphyton stocks at the end of spring than when no overwintered *Rana* had been present. Yet, if the presence of *Rana* in spring was having a beneficial effect on periphyton, we would expect to see similarly high levels of chlorophyll *a* where *Rana* were present in both winter and spring, which instead had the lowest periphyton production. Likewise, if periphyton stocks are depressed by the presence of *Rana* in winter, we would expect to see high levels of chlorophyll *a* where overwintered *Rana* were never present, which instead was the same at the end of spring as where *Rana* were present only in winter.

The answer to the algal response may lie in nutrient availability. Nutrient levels were not measured during this study, but it is possible that tadpole activity affected availability. Amphibian larvae are important in nutrient cycling in aquatic ecosystems. Tadpole feces can become a solid layer by the end of summer in ponds where tadpoles are in high abundance, creating a nitrogen-rich substrate, and this in turn may affect the composition of the aquatic plant community (Alford 1999). In some studies, tadpole presence had a favorable effect on the algae they grazed, due to their enhancement of nutrient cycling: tadpole excretions facilitate leaf litter microbes and detritivores, which in turn release nutrients locked in the leaf litter (Iwai and Kagaya 2007). Conversely, other research has found that by feeding on planktonic algae,

tadpoles may reduce eutrophication (Seale 1980), and that algal standing stocks can drop to 1-2% of previous levels when tadpoles are introduced (Dickman 1968). Thus there may be two different mechanisms involved in these mesocosms, which change in relative importance with the seasons: in the winter, when algal growth is slow, presence of *Rana* may prevent buildup of initial algal stocks by their maintenance feeding, while ponds without *Rana* can build up greater initial stocks in the absence of tadpole grazing. In spring, when increased temperatures speed algal growth, the presence of large overwintered *Rana* may then become beneficial to algae as these large larvae effectively facilitate the release of nutrients by leaf litter microbes and detritivores. However, this effect is only seen where *Rana* had not been present in winter, suggesting that depleted algal stocks, or those experiencing continued heavy grazing pressure after depletion, are unable to make effective use of the released nutrients.

This grazer-enhanced productivity has been found to depend on grazer species identity. In part, this appears to be mediated by selective grazing by tadpoles: by removing epiphytic diatoms and adhering silt and detritus, large *Rana* tadpoles have been shown to increase the biomass of filamentous green algae, whereas *Pseudacris* in the same system do not (Kupferberg 1997b). Early in the growing season, the presence of key consumers has been observed to exert a controlling effect on primary productivity (Mokany *et al.* 2008).

If indeed overwintering *Rana* are adversely affecting *Bufo* by their presence through the winter, as suggested by their adverse effect on *Bufo* mass and larval period even when removed in spring, why do we see no significant difference in algal biomass at the early sampling date, when *Rana* have been present in some treatments all winter? This study quantified total algal biomass, but did not sort out algal species composition. Different algal species are of different nutritional quality for tadpole growth and development (Richter-Boix *et al.* 2007). It may be that

overwintering *Rana* change the algal species composition during the winter (Dickman 1968), resulting in a lower quality food resource in spring in those tanks where they were present in the winter, relative to those where they were not. Dickman's result suggests the importance of quality, in addition to quantity, of a food resource. Other possible effects worth investigating might be changes in water chemistry in winter when *Rana* are present, such as dissolved oxygen levels, or the presence of inhibitory metabolic compounds.

If seasonal wetlands reliably filled in late winter and dried in late summer, there would be no need to study overwintering tadpoles; but as rainfall patterns, groundwater inputs, and hurricane landfalls differ from year to year (Sharitz 2003), in some years a seasonal pond may persist through the fall and winter. In these years, seasonal-pond breeding amphibians using the ponds the following spring will face different competitive pressures in a pond with overwintered tadpoles than in similar but unoccupied ponds, or in the same pond in years when it did not persist through the winter. Many factors influence whether a given pond will persist in any given year, including geological substrate (O'Driscoll and Parizek 2008), and such large-scale mechanisms as regional water table fluctuations and connectivity to river systems (Jones and Gresham 1985). Anthropogenic changes such as ditching can also change the hydrological regime by lowering the soil surface relative to the seasonal water table. The overwintered *Rana* collection site in this study was an example of this: tracked logging equipment created ruts which functioned as small depressional ponds in what was otherwise a site without standing water. These ponds persisted through the fall and winter, allowing overwintered *Rana* tadpoles to be collected from them in April.

Likewise, even when a pond persists through the winter, the presence or absence of overwintering tadpoles is influenced by patterns of colonization, that is, whether or not late-

breeding *Rana* selected that pond the previous August. Due to the ovipostion site fidelity and limited dispersal distances exhibited by many anurans (Blaustein *et al.* 1994), proximity of a pond to a source population probably influences the likelihood of that pond being colonized by *Rana* tadpoles in those years when it persists through the winter.

Amphibians are in decline throughout the world, for reasons that still remain unclear. In some cases, amphibian species have disappeared from seemingly pristine habitat, for example, the golden toad (*Bufo periglenes* Savage) of Costa Rica (Crump *et al.* 1992). In order to develop effective conservation strategies for amphibians, it is important to understand the factors affecting fitness of different species, under conditions of annual variability. Priority effects do not always allow one species to predominate in all localities (Shorrocks and Bingley 1994); where habitat is patchy, different species may arrive first in different patches, and thus coexist in complex ways over the landscape. As the complexity of natural systems becomes better understood, it is clear that what is advantageous to one species is deleterious to another, often even within the same guild. If we want to optimize biodiversity, conservation strategies will need to take these differences into account. By understanding how competing species affect each other, more effective conservation decisions can be made.

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CHAPTER 3: PUTTING IT IN CONTEXT

The Atlantic Coastal plain in the Carolinas ranges in elevation from sea level to ~91m (Dahl 1999), and contains approximately 16,548 ha of freshwater wetlands in North Carolina (calculated from Cashin *et al.* 1992), and 889,069 ha in South Carolina (calculated from Dahl 1999), including thousands of Carolina bays as well as numerous small ponds and seasonal wetlands <0.4 ha and thus too small to map (Sharitz 2003), with small wetlands far outnumbering large (Semlitsch and Bodie 1998). The Coastal Plain is an area of high amphibian diversity and endemism, with the Fall Line at its inland boundary serving as a barrier to dispersal of many species (Mount 1975). About 50% of the original wetlands extent has been converted to other land uses, primarily agriculture and silviculture (Cashin *et al.* 1992). Managed loblolly pine (*Pinus taeda* L.) is the predominant species in Southeastern silvicultural lands (Baker and Langdon 1990). Pine forests have more diverse herpetofauna than mixed forests, and density of leaf pack is negatively correlated with amphibian species richness (Russell 2000). The Coastal Plain receives ~1,335 mm of precipitation year^{-1}, of which the greatest amount occurs in summer, and a moderate amount in winter and early spring (Sharitz 2003); severe regional droughts occur approximately every 15 years, with localized droughts every 7 or 8 years (Jones and Gresham 1985). Hydroperiod is affected by composition of the substrate, with sandy pools persisting longer than clay pools (O'Driscoll and Parizek 2008). Although wetland size can influence hydroperiod, it does not always predict hydroperiod (Snodgrass *et al.* 2000b).

The 2001 Supreme Court decision, *Solid Waste Agency of Northern Cook County v. U.S. Army Corps of Engineers,* 531 U.S. 314 (SWANCC), removed isolated wetlands from Clean Water Act (CWA) protections, based on the premise that the CWA was meant to apply to navigable waterways, and to those wetlands sufficiently connected as to affect said navigable waterways. In 2006, *Rapanos v. United States,* 547 U.S. 715, similarly excluded isolated

wetlands from CWA protection. Because most Carolina bays and similar depressional wetlands are considered isolated as defined in those cases (Sharitz 2003), any protection of these must be achieved at the state and local level. In North Carolina, the Temporary Isolated Wetland/Waters Permitting Rules, 15A NCAC 2H.1300, explicitly included isolated wetlands as waters of the state after the SWANCC decision, and South Carolina has similar legislation (Christie and Hausman 2003), but uncertainty over the limits of federal jurisdiction has proven challenging to state management efforts.

Anthropogenic alterations can change the hydrological regime of isolated wetlands, sometimes in contrasting ways. At the overwintered tadpole collection site, for example, ruts created by tracked logging equipment, and ditches along the access road, served as permanent ponds. In other cases, ditching for drainage can shorten hydroperiod, or even eliminate a wetland altogether. Where ditches connect an isolated wetland to a permanent water body, this can allow fish to colonize the wetland, potentially changing species composition (Snodgrass *et al.* 2000a), amphibian abundance (Sharitz 2003),and the competitive interactions among anuran larvae (Wilbur and Fauth 1990).

Where overwintered *Rana* are not present, *Bufo* may face either high or low conspecific density, and may occur alone or with other species of the same age. The results of this experiment reveal no significant differences in *Bufo* performance under any of these conditions, suggesting that, in the absence of predators, *Bufo* should have about equal chances of success in ponds lacking overwintered Ranids, so long as the hydroperiod is sufficiently long (~ 40 days). This is consistent with prior research (Rogers and Chalcraft 2008) which found that density-dependent effects on *B. terrestris* operated only in short-hydroperiod ponds, not in long-hydroperiod ponds.

Gascon and Travis (1992) investigated effects of pond size, and found that larval period was longer in deeper pools, possibly due to water temperature, and that interference competition was stronger in smaller-scale systems. In the present study, *Bufo* survival in the presence of overwintered *Rana*, was affected by prior presence in the pond and contemporaneous competition acting in concert, but not either factor in isolation; whereas *Bufo* mass was affected only by prior presence in the pond, and larval period, by prior presence in the pond alone or in tandem with contemporaneous competition.

In ponds persisting through winter, i.e. permanent ponds, and semipermanent ponds in wet years, the potential presence of overwintered Ranids may be a significant factor affecting the performance of *Bufo*. This appears to be due primarily to resource competition, since treatments with *Rana* present in winter and spring showed reduced *Bufo* survival and mass, and increased *Bufo* larval period, relative to those without, and this effect persisted for mass and larval period even where overwintered *Rana* were removed prior to arrival of *Bufo*. This agrees with Kupferberg (1997a), who found that a small impact of an introduced Ranid on algae resulted in a large indirect effect on a native Ranid.

It might be argued that the treatment where overwintered *Rana*, not present in winter, were added in spring, does not reflect natural conditions. However, this treatment was necessary in order to separate out the mechanism of winter depletion of resources from that of size-based competitive advantage. In addition, there may be situations in which this could occur in nature, e.g. a spring rain event causes a pond containing overwintered *Rana* to overflow into one that did not.

Among ponds persisting through the winter, only a subset is likely actually to contain overwintering tadpoles, due to the limited dispersal distances of adult amphibians, and the high site fidelity shown by many species (Blaustein *et al.* 1994). Those ponds persisting only in some

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years, and/or those at greater distances from established Ranid populations, are less likely to be colonized by late-breeding Ranids in any given year. A female Southern toad, therefore, ovipositing in persisting ponds, faces stochastic variation in her larvae's chances of optimal performance. Many female anurans can detect the presence of predators (Reiger *et al.* 2004) and competitors (Resetarits and Wilbur 1989, 1991) and preferentially oviposit in ponds lacking these. At least some species also select oviposition site based on assessment of pond hydroperiod (Spieler and Linsenmair 1997), and make different site selections based on the relative importance at a given site of different variables (Crump 1991). In order for an ovipositing female to make effective decisions, future conditions must be sufficiently predictable based on present conditions, i.e. a species of predator likely to colonize a pond after oviposition tends not to elicit the avoidance response even when already present (Resetarits and Wilbur 1989).

Given the results of this study, there should be selective pressure against *Bufo terrestris* ovipositing in ponds containing overwintered Ranids. Relatively few studies of oviposition site selection have been made (Resetarits 1996), and no such studies were found for *Bufo* oviposition in the presence of competitors. *Hyla chrysoscelis* avoided ovipositing in ponds containing conspecific tadpoles, but did not avoid *R. catesbeiana* tadpoles (Resetarits and Wilbur 1989, 1991). If *B. terrestris* shows the same lack of discrimination against ponds containing overwintered Ranid larvae, this would result in reduced fitness. Where a competitor is evolutionarily unfamiliar, such maladaptive decisions can constrain a species' ability to use potential habitat effectively (Twomey 2008).

Given that, with the exception of certain species specialized for non-persisting ponds (Semlitsch 2000), anuran species diversity generally increases with increasing pond hydroperiod up to a hydroperiod of 8-10 months, then declines somewhat at longer hydroperiods (Snodgrass

et al. 2000a), it would seem that optimal biodiversity would likely be achieved with a mix of pond types, weighted somewhat toward the longer-hydroperiod types. Conversely, there is some data to suggest that small, isolated wetlands have higher amphibian diversity than large wetland areas, due to such factors as the lack of fish in smaller wetlands (Semlitsch and Bodie 1998), whereas other data suggests no relationship between wetland size and amphibian species richness (Snodgrass *et al.* 2000b). Because overwintered tadpoles have adverse effects on spring cohorts, it is also important that not every pond persisting through winter be colonized by late-breeding Ranids.

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December 11, 2008

David Chalcraft, Ph.D
Department of Biology
Howell Science Complex
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Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "Effects of Overwintered Ranid Tadpoles on the Survival and Development of Spring-Hatched Anuran Tadpoles," (AUP #D228) was reviewed by this Institution's Animal Care and Use Committee on 12/11/08. The following action was taken by the Committee.

   "Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure