Oikos 121: 259–267, 2012
doi: 10.1111/j.1600-0706.2011.19221.x

Subject Editor: Richard Stevens. Accepted 29 April 2011

Synergistic effects of multiple mechanisms drive priority effects within a tadpole assemblage

Jason P. Hernandez and David R. Chalcraft

J. P. Hernandez and D. R. Chalcraft (chalcraftd@ecu.edu), Dept of Biology and the North Carolina Center for Biodiversity, East Carolina Univ., Greenville, NC, 27858, USA.

Mounting evidence suggests that the history of species arrival to a locality can have important effects on species performance but the mechanism(s) through which priority effects are produced is not always clear. Differences in the developmental time of frog tadpoles provide an opportunity to examine mechanisms through which priority effects may influence fitness components of a late arriving taxon. Specifically, tadpoles of the southern leopard frog (*Rana*) can often require more than a year to complete metamorphosis so they overwinter in a pond and compete with newly deposited tadpoles in the spring. We conducted an experiment in artificial ponds to evaluate mechanisms through which overwintering *Rana* tadpoles influence fitness components of southern toad (*Bufo*) tadpoles deposited into ponds during the spring. We found that overwintered *Rana* reduced *Bufo* performance while newly hatched *Rana* that enter a pond simultaneously with *Bufo* did not. The production of this priority effect was primarily the result of *Rana* depleting algal resources in a pond during the winter prior to *Bufo* arrival. The performance of *Bufo* did not correspond with variation in the abundance of algae present in a pond during the spring and we found evidence to indicate that, in the absence of resource exploitation during the winter, overwintered *Rana* do not compete strongly with *Bufo* during the spring when both species co-occur. When *Rana* deplete algal resources during the winter, however, interactions between *Rana* and *Bufo* during the spring became much more important as both the development rate and survival of *Bufo* was reduced to a greater extent than what would have been predicted by resource exploitation alone. Our results demonstrate that priority effects can result from early colonists depleting resource availability prior to the arrival of other species which can intensify behavioral/physical interactions between species when they co-occur.

A vast amount of research has demonstrated that both biotic and abiotic processes can play an important role in controlling biodiversity (reviewed by Dunson and Travis 1991, Wellborn et al. 1996, Chase 2003), but a growing amount of work has demonstrated that a system's history can also be important (Chase 2003, 2010, Fukami 2004, Louette and De Meester 2007). For example, the order in which species colonize can have a profound impact on the diversity and relative abundances of taxa within a system and on fitness components of constituent species (Alford and Wilbur 1985, Wilbur and Alford 1985, Robinson and Dickerson 1987, Almany 2003, Chase 2010). The dependency of a system's (or species) properties on the order of species arrival to a particular site is called a priority effect (Alford and Wilbur 1985). Variation in the order in which species arrive to a particular locality could result from stochastic variation in arrival time, or interspecific differences in dispersal rates or breeding phenology (Benke and Benke 1975, McCauley et al. 2008, 2010). Although a number of studies have demonstrated the occurrence of priority effects in a number of different ecological systems (Shorrocks and Bingley 1994, D'Antonio et al. 2001, Almany 2003, Stoks and McPeek 2003, Farris-Lopez et al. 2004), the mechanisms through which these effects are produced are seldom evaluated. An investigation of the mechanisms through which historical phenomena affect ecological systems is imperative if we are to better predict how a system's history will affect its properties. Some possible mechanisms through which priority effects can be produced include: 1) size-based competition, in which early arriving taxa grow to a larger size that give them competitive superiority over late arriving taxa; 2) habitat manipulation, in which early arriving taxa alter the habitat in a way that makes it less suitable for late arriving taxa (e.g. depleting the availability of resources); and 3) density-dependent effects, in which late arriving taxa are forced to share resources with more individuals.

We investigated the potential for priority effects and the mechanisms through which they could be produced in a model system involving larval anurans. Priority effects involving larval anurans have been reported in a number of studies (Alford and Wilbur 1985, Lawler and Morin 1993), but very few have involved overwintered tadpoles (i.e. tadpoles that complete metamorphosis after spending the winter in a pond) or directly evaluate mechanisms associated with the production of priority effects. We focused our study on potential priority effects produced by overwintered southern leopard frog *Rana sphenocephala* tadpoles entering ponds before tadpoles of southern toad *Bufo terrestris*. Eggs of these two species can often be oviposited into ponds at the same time (early spring to early fall) and the two species co-occur (Morin 1983, Lanoo 2005) but the larval period of these two species is very different. *Bufo* completes metamorphosis within 1 or 2 months and never overwinters while *Rana* requires >2 months to complete metamorphosis (Martof et al. 1980, Lannoo 2005) and often needs to overwinter in a pond prior to completing metamorphosis (Caldwell 1986, Gibbons and Semlitsch 1991). Consequently, *Bufo* may be oviposited into ponds during the spring that 1) contain no *Rana* tadpoles, 2) contain newly deposited *Rana* tadpoles, 3) contain overwintered *Rana* tadpoles, or 4) contained overwintered *Rana* during the winter but the *Rana* completed metamorphosis prior to the arrival of *Bufo* in the spring. Thus, this study system provides an excellent opportunity to examine mechanisms through which the historical presence of a species in a community affects species that arrive later in assembly process.

We believe that the ability of *Rana sphenocephala* to overwinter will exert a more negative effect on *Bufo* tadpoles than *R. sphenocephala* tadpoles hatched at the same time as *Bufo* because studies with other overwintered *Rana* species (*Rana catesbeiana* and *Rana clamitans*) have demonstrated the occurrence of a priority effect by overwintered *Rana* on spring tadpole cohorts (Kupferberg 1997, Boone et al. 2004, Walston and Mullin 2007, Mackey and Boone 2009). Although these studies were very useful in documenting the occurrence of a priority effect, their experimental design did not allow for an evaluation of various mechanisms that could produce priority effects. Studies that manipulate the particular time that overwintered *Rana* are present in ponds, the size of *Rana* tadpoles present, and the density of tadpoles in a pond are necessary to evaluate mechanisms that could cause priority effects. Our goal was to conduct an experiment that included these manipulations in order to better understand mechanisms associated with the production of priority effects.

We expect that there are at least two reasons why the particular time during community assembly that *Rana* enters a pond will affect *Bufo* performance. First, we expect that an overwintered cohort of *Rana* could exert a greater negative effect on spring oviposited *Bufo* than spring oviposited *Rana* because overwintered cohorts of *Rana* are composed of dramatically larger individuals (often >1 g) than individuals within a cohort of newly hatched *Rana* (often <0.01 g). Werner (1994) demonstrated that size advantage played an important role in identifying which of two *Rana* species will be competitively dominant over the other. Second, we could also expect newly hatched *Rana* to have a different impact on *Bufo* than overwintered *Rana* because newly hatched *Rana* are more abundant than overwintered *Rana*. The importance of population density on the intensity of competitive interactions involving larval anurans is well documented (reviewed by Alford 1999). Scaling relationships between metabolic rate and body size and between body size and population abundance suggest that populations composed of many smaller individuals can have impacts as large as or even greater than populations composed of larger but fewer individuals (Chalcraft and Resetarits 2004).

Competition is an important mechanism through which larval anurans interact and any priority effect caused by variation in the time at which *Rana* enters a pond during the community assembly process would be the result of variation in the intensity of competitive interactions. Resource exploitation is often thought to be the most important mechanism through which competition occurs between larval anurans but there is evidence to also indicate that larval anurans compete via interference competition (reviewed Alford 1999, Wells 2007). For example, larval anurans can suppress growth and enhance mortality of other larval anurans by intimidating them and reducing their foraging success or by producing growth inhibitors that suppress the growth of other larval anurans (Faragher and Jaeger 1998). Consequently, variation in the time at which *Rana* enters a pond can be very important as overwintered *Rana* have a longer time to suppress algal resources and they are more likely to directly interact with *Bufo* in a manner that suppresses *Bufo* performance than newly hatched *Rana* that enter the pond contemporaneously with *Bufo*.

Material and methods

To examine the influence through which overwintered *Rana* affects *Bufo* tadpoles, we conducted an experiment in 30 artificial ponds (modified 1100 liter cattle watering tanks) at East Carolina University's West Research Campus in Greenville, NC, USA. The use of artificial ponds such as these have been useful in advancing our understanding of the ecology of larval anurans (reviewed by Wilbur 1997, Alford 1999) and processes that have been found to be important in artificial ponds have also been found to be important in natural ponds (Scott 1990, Resetarits and Fauth 1998, Loman 2004, Rubbo et al. 2006).

We evaluated the effects of *Rana* on 250 spring oviposited *Bufo* by manipulating the time, size, and density at which *Rana* enter artificial ponds. Our experimental design included 6 treatments that are summarized in Fig. 1. Four treatments contained 250 spring oviposited *Bufo* and differed in the particular time at which 20 overwintered *Rana* were present (not present, present during the winter but not spring, present during the spring but not winter, and present during both winter and spring). Although natural ponds never have overwintered *Rana* present in the spring that were not present during the winter, the inclusion of this treatment as part of the factorial design facilitates the evaluation of the total effect of overwintered *Rana* on *Bufo*, the effect of overwintered *Rana* on *Bufo* that is the result of overwintered *Rana* changing the quality of the environment prior to *Bufo* arrival, and the effect of overwintered *Rana* on *Bufo* that is due to contemporary interactions between larger overwintered *Rana* and spring hatched *Bufo*. To determine if overwintered *Rana* have a different impact on *Bufo* than newly hatched *Rana* that enter ponds at the same time as *Bufo*, we included a treatment where 250 newly-hatched *Rana* and 250 newly-hatched *Bufo* were added to a pond at the same time during the spring. Although this treatment increased the number of *Rana* present in a mesocosm, the higher abundance of newly hatched than overwintered *Rana* reflects the fact that newly hatched *Rana* are both more abundant and smaller than overwintered *Rana* in natural ponds. We also included a sixth treatment with 500 *Bufo* added to a mesocosm in the spring. The purpose of this treatment was to evaluate whether the addition of *Rana* had an impact on

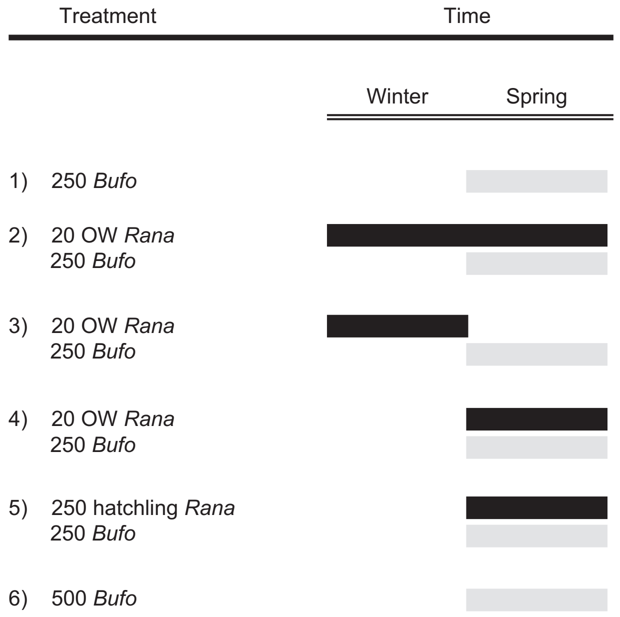

Figure 1. Experimental treatments, showing numbers and type of tadpoles added to mesocosms, and time of introduction to mesocosms. Black bars indicate presence of overwintered (OW) or hatchling (new) *Rana*, grey bars indicate presence of *Bufo*.

Bufo that was different from merely increasing the density of tadpoles present. All tadpole numbers added to mesocosms fall within the range of densities found in nature (0–7659 tadpoles m^{-2}; Morin 1983, Alford 1999).

We used a blocked design, in which a block consisted of six mesocosms spatially clustered together, and each treatment was randomly assigned to one mesocosm within each block. There were five blocks for a total of 30 experimental units. All methodological procedures applied to mesocosms were always conducted on a block by block basis. This approach was done to minimize random variation within blocks (Chalcraft and Resetarits 2003).

Mesocosms were filled with water to a depth of 50 cm on 22 November 2008 and covered with screening to prevent colonization by unwanted organisms and escape of study organisms. Two days later, we added 1 kg raked loblolly pine straw (*Pinus taeda*) to each mesocosm to provide a natural substrate and source of nutrients representative of the loblolly pine forests characterizing much of the southeastern Coastal Plain. On 3 December 2008 all mesocosms were inoculated with a constant volume of concentrated algae and zooplankton collected from ponds in the Croatan National Forest, NC. Twelve days later, a second inoculation was made to ensure adequate establishment.

Overwintering *Rana sphenocephala* tadpoles were collected from flooded tire ruts in the Croatan National Forest, NC and added to mesocosms designated to contain overwintered *Rana* during the winter (two treatments) on 9 December 2008. Removal of all *Rana* from mesocosms designated to contain *Rana* during the winter but not during the spring was accomplished by intensive minnow-trapping from 29 March to 9 April 2009. Overwintered *Rana* tadpoles were again collected from the same tire ruts in the Croatan National Forest on 10 April 2009 and added to mesocosms designated to contain overwintered *Rana* during the spring only.

Newly deposited eggs of *Rana sphenocephala* and *Bufo terrestris* were collected from the same stormwater detention pond on 29 March 2009 and hatched in the laboratory. Tadpoles were pooled from six *Bufo* clutches and three *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 mesocosm designated as the hatchling *Rana* treatment; 500 *Bufo* to each mesocosm designated to be the high-density treatment that contains no *Rana*; and 250 *Bufo* to each mesocosm for all other treatments.

Mesocosms were checked daily and each mesocosm was provided with a floating 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. After being counted and weighed, all metamorphosed animals were released at their location of initial capture. We destructively sampled each mesocosm on 22 June 2009 because it appeared that all surviving *Bufo* had completed metamorphosis. All remaining tadpoles were identified, counted and weighed and then released at the location from which they were collected.

To assess the amount of algal resources present, we placed two weighted white strips of flagging tape (approx. 3 × 12 cm) on the bottom southeast corner of each mesocosm to serve as a substrate for algal growth. One strip of flagging was removed on 5 April 2009, just prior to adding *Bufo*, and the second was removed on 20 May 2009, just after peak metamorphosis had passed but before algal stocks could recover from the exit of tadpoles. Algae was scraped from the surface of one side of the flagging (surface area of scraping was measured) and the resulting slurry was filtered for pigment analysis. Periphyton samples were frozen for later chlorophyll extraction using the spectrophotometric methods for chlorophyll described by Clesceri et al. (1998). Resource availability was represented by the sum of the chlorophyll a and phaeophytin content as tadpoles graze both living and decomposing algal matter (reviewed by Alford 1999).

Statistical analyses

Mass at metamorphosis, larval period, and survival of *Bufo* were the primary response variables because these are important fitness components in anurans (Wilbur 1972, Smith-Gill and Gill 1978, Smith 1987). Analyses on mass at metamorphosis and larval period were performed on average values for a mesocosm because different individuals within a mesocosm are not independent data points. Geometric mean was used for mass as the mass of individuals within a mesocosm are lognormally distributed. Arithmetic mean was used for larval period. Survival in a mesocosm is the proportion of *Bufo* added to a mesocosm that metamorphosed. Residuals for all analyses involving these response variables did not differ from a normal distribution and variances were homogeneous so no additional transformations (beyond the derivation of the geometric mean for mass at metamorphosis) were necessary.

We conducted four analyses to evaluate four families of hypotheses pertaining to *Bufo* performance. Each hypothesis family focused on a different conceptual question and included responses from different sets of treatments. As such, each hypothesis family was analyzed separately as recommended by Hochbeg and Tamhane (1987) and Quinn and Keough (2002). We maintained the family-wise error rate within each hypothesis family at 5% and adjusted the per comparison error rate within each hypothesis family using the REGWQ pairwise comparison procedure if necessary (e.g. the overall hypothesis of no difference among treatments within a particular hypothesis family is rejected).

The first hypothesis family (HF1) focused on whether variation in the natural timing of *Rana* arrival into a pond (overwintered *Rana* present during the winter and spring, overwintered *Rana* present during the winter but leaves before the spring, or newly hatched *Rana* that arrive in the spring) produces a priority effect on *Bufo* performance. We tested this hypothesis with ANOVA and used Dunnett's procedure to determine whether *Bufo* performance differed in ponds with newly hatched *Rana* from ponds in which overwintered *Rana* were present during the winter or in which overwintered *Rana* were present during the winter and spring.

The second hypothesis family (HF2) pertained to the independent and interactive effects on *Bufo* performance that were associated with variation in the particular time of overwintered *Rana* presence. We examined these hypotheses with a factorial ANOVA that compared responses in all treatments with overwintered *Rana* and the treatment containing a low abundance of *Bufo*. The factorial ANOVA specified the main and interactive effects involving the factors 1) overwintered *Rana* presence/absence during the winter and 2) overwintered *Rana* presence/absence during the spring. The factorial ANOVA allows us to identify the critical time at which the presence of overwintered *Rana* exerts its effect on *Bufo* and it also evaluates whether the continued presence of overwintered *Rana* through the winter and spring exerts an effect on *Bufo* that is greater or less than that expected by the sum of the winter effect and the spring effect.

The third hypothesis family (HF3) addressed whether overwintered *Rana* exert a stronger effect on *Bufo* performance than newly hatched *Rana* independent of historical effects produced by overwintered *Rana*. We used a t-test to evaluate this hypothesis by comparing *Bufo* responses in the treatment where overwintered *Rana* were only present during the spring to the treatment where newly hatched spring *Rana* were present. A statistically significant t-test here would indicate that differences in performance are due to either variation in *Rana* size (with overwintered *Rana* being larger) or abundance (with newly hatched *Rana* being more abundant).

The fourth hypothesis family (HF4) pertained to whether *Bufo* performance was altered by intraspecific and interspecific variation in the abundance of tadpoles present. To test these hypotheses, we conducted a single factor ANOVA comparing *Bufo* responses in treatments with only 250 *Bufo*, 250 *Bufo* + 250 newly hatched *Rana*, and 500 *Bufo*. We would have conducted pairwise comparisons of means among these treatments with REGWQ if the F-test of the overall ANOVA were statistically significant.

To determine whether overwintered *Rana* altered the abundance of algal resources prior to the addition of *Bufo* we used a t-test to compare algal resource levels measured in April. The t-test compared algal levels in the mesocosms containing overwintered *Rana* during the winter (two treatments) to mesocosms with no tadpoles present during the winter (four treatments). We employed this t-test comparing two groups of mesocosms during the winter rather than an ANOVA that considers each of the six treatments as six different groups of mesocosms because the only difference among all experimental units during the winter was whether or not overwintered *Rana* were present or absent. Due to the fact that mesocosms differed in two ways as the result of experimental manipulations during the winter but six ways during the spring, it was not possible to perform a repeated measures ANOVA on algal data because the repeated measures approach enforces the spring treatment labels on mesocosms during the winter even though these labels have no meaning during the winter. Enforcing the spring treatment structure on the analysis of data collected during the winter reduces the statistical power associated with actual treatment effects during the winter which is our most crucial hypothesis test to determine if overwintered *Rana* reduce algal resources during the winter.

We compared algal abundances measured during the spring by employing the same family of hypotheses and analyses as used for assessing *Bufo* performance. We employed the same family of hypotheses on algal abundances measured during the spring as used for assessing *Bufo* performance because 1) all six treatments were in place during the spring and 2) we were interested in examining variation in the abundance of algal resources within the context of how treatments produce variation in *Bufo* performance. One periphyton sample from a mesocosm containing only 250 *Bufo* was lost from the late sampling date. Algal data were log transformed to homogenize variances.

We present all results with no block effects included in the statistical analyses because block effects never explained a substantial amount of variation and their inclusion did not alter the interpretation of our results.

Results

Rana survival was relatively high in treatments containing overwintered *Rana* (range from 70% – 100%) and in the treatment with newly hatched *Rana* (56%). A very small number (<1 individual/mesocosm on average) of overwintered *Rana* metamorphosed prior to the time of peak *Bufo* metamorphosis and the sampling of algae in the spring (May 20) and the first newly hatched *Rana* did not metamorphose until June 6. Consequently, the majority of *Bufo* interacted with *Rana* during their entire time in mesocosms. All surviving overwintered *Rana* completed metamorphosis by the end of the experiment except one individual but only 4% of newly hatched *Rana* metamorphosed by the end of the experiment.

Variation in the natural timing at which *Rana* enters a pond affected *Bufo* performance (mass: $F_{2,14} = 7.53$, $p = 0.008$; larval period: $F_{2,14} = 10.57$, $p = 0.002$; survival: $F_{2,12} = 78.64$, $p < 0.001$). Dunnet's procedure indicates that the early arrival of *Rana* during the winter produced a priority effect because the presence of overwintered *Rana* in a pond during the winter and spring caused *Bufo* to have lower survival, and metamorphose later and at a smaller size than would be observed in ponds where *Rana* enter in the spring as newly hatched larvae (all adjusted $p < 0.005$; Fig. 2, treatments associated with HF1). When overwintered *Rana* were present in a pond during the winter and left before *Bufo* arrived in a pond, however, the priority effect was weaker as Dunnet's procedure indicates that *Bufo* survival was comparable to that observed in ponds with newly hatched spring *Rana* (adjusted $p > 0.99$; Fig. 2b, treatments associated with HF1). Nonetheless, *Bufo* had a tendency to metamorphose later (adjusted $p = 0.06$) and metamorphose at a smaller size (adjusted $p = 0.03$) in ponds where overwintered *Rana* were present during the winter but left before the spring than ponds containing newly hatched *Rana* in the spring (Fig. 2a, c; treatments associated with HF1).

The effect of overwintered *Rana* on *Bufo* performance depended on the particular time(s) that overwintered *Rana* were present in a pond. The presence of overwintered *Rana* during the winter suppressed *Bufo* mass at metamorphosis ($F_{1,16} = 12.34$, $p = 0.003$) but their presence during the spring did not ($F_{1,16} = 0.42$, $p = 0.525$) and the effect of the presence of overwintered *Rana* during the winter was not altered by the presence of overwintered *Rana* during the spring ($F_{1,16} = 0.02$, $p = 0.898$; Fig. 2a, treatments associated with HF2). Furthermore, the presence of overwintered *Rana* during the winter prolonged the larval period of *Bufo* ($F_{1,16} = 24.26$, $p < 0.001$) and this effect was enhanced further when overwintered *Rana* was also present during the spring ($F_{1,16} = 4.57$, $p = 0.048$; Fig. 2c, treatments associated with HF2). The occurrence of overwintered *Rana* during the spring by itself, however, had no effect on the larval period of *Bufo* ($F_{1,16} = 1.57$, $p = 0.228$). Although *Bufo* survival varied in a statistically meaningful way in response to either the presence of overwintered *Rana* during the winter ($F_{1,16} = 34.72$, $p < 0 .001$) or during the spring ($F_{1,16} = 44.12$, $p < 0.001$), the observed responses (Fig. 2b; treatments associated with HF2) and the highly significant interaction between the winter and spring effect ($F_{1,16} = 33.06$, $p < 0.001$) indicates that it is the continued presence of overwintered *Rana* during the winter and spring that causes a biologically meaningful reduction in *Bufo* survival.

The effect of overwintered *Rana* did not appear to be driven by the fact that overwintered *Rana* are larger than newly hatched *Rana* (HF3) because none of the *Bufo* performance measures differed between the treatment with overwintered *Rana* present during the spring only and the treatment containing smaller, newly (spring) hatched *Rana* (mass: $t_8 = 0.280$, $p = 0.79$; survival: $t_8 = 0.14$, $p = 0.89$; larval period: $t_8 = 0.28$, $p = 0.783$; Fig. 2). Furthermore, the effect of *Rana* on *Bufo* does not appear to merely be the result of *Bufo* competing with more tadpoles (HF4) because the performance of *Bufo* did not vary among treatments differing in the abundance of newly hatched (*Rana* or *Bufo*) tadpoles present during the spring (mass: $F_{2,16} = 0.28$, $p = 0.76$; larval period: $F_{2,16} = 0.37$, $p = 0.70$; survival: $F_{2,16} = 0.33$, $p = 0.72$; Fig. 2).

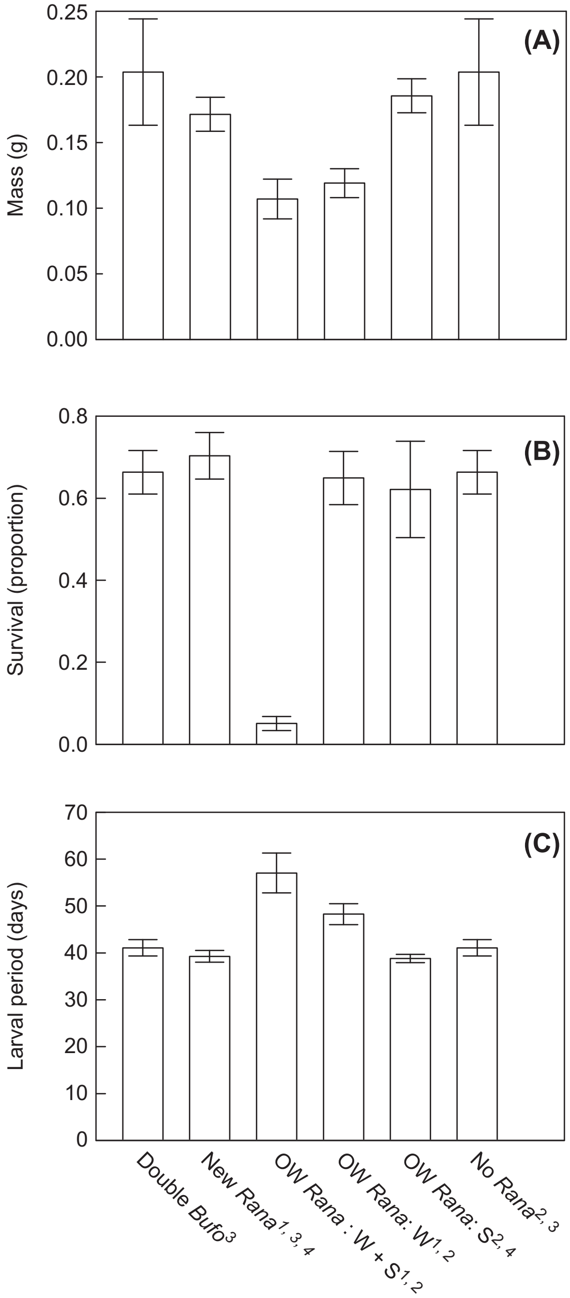

Figure 2. Mean (±SE) (A) mass at metamorphosis, (B) survival, and (C) larval period of *Bufo* represented by the six treatment conditions included in the experiment. Numbered superscripts following the treatment name corresponds to 1 of 4 hypothesis families outlined in the methods section. Comparisons among treatments within the same hypothesis family are the most relevant comparisons as each hypothesis family focuses on a different conceptual question pertaining to the occurrence of priority effects. Statistical results for each family of hypotheses are presented in the results section.

The presence of overwintered *Rana* during the winter tended to reduce the amount of periphyton ($t_{28} = 1.82$, $p = 0.08$) present in a pond by the time *Bufo* entered a pond (Fig. 3a). Variation in the natural phenology of *Rana*, however, did not account for a statistically significant amount of variation in the amount of periphyton present in May

($F_{2,14} = 2.12$, $p = 0.16$) but there was a tendency for ponds with overwintered *Rana* present during the winter to have less algae than ponds with newly hatched *Rana* (Fig. 3b, treatments associated with HF1). The effect of overwintered *Rana* on the amount of periphyton present during May, however, depended on the time periods in which overwintered *Rana* were present (winter effect: $F_{1,15} = 1.82$, $p = 0.19$; spring effect: $F_{1,15} = 2.10$, $p = 0.17$; winter × spring interaction: $F_{1,15} = 4.46$, $p = 0.05$). Specifically, spring algal abundance was enhanced by overwintered *Rana* present during the spring when overwintered *Rana* were absent during the winter but not when overwintered *Rana* were present during the winter (Fig. 3b, treatments associated with HF2).

Variation in *Rana* size does not appear to be an important contributor to the amount of periphyton present in May because treatments with overwintered *Rana* present during the spring only had a similar amount of periphyton present in May as the treatment with newly hatched *Rana* ($t_{28} = 1.07$, $p = 0.31$; Fig. 3b, treatments associated with HF3). Furthermore, variation in the abundance of newly hatched tadpoles did not account for a significant amount of variation in the amount of periphyton present ($F_{2,11} = 2.39$, $p = 0.14$; Fig. 3b, treatments associated with HF4).

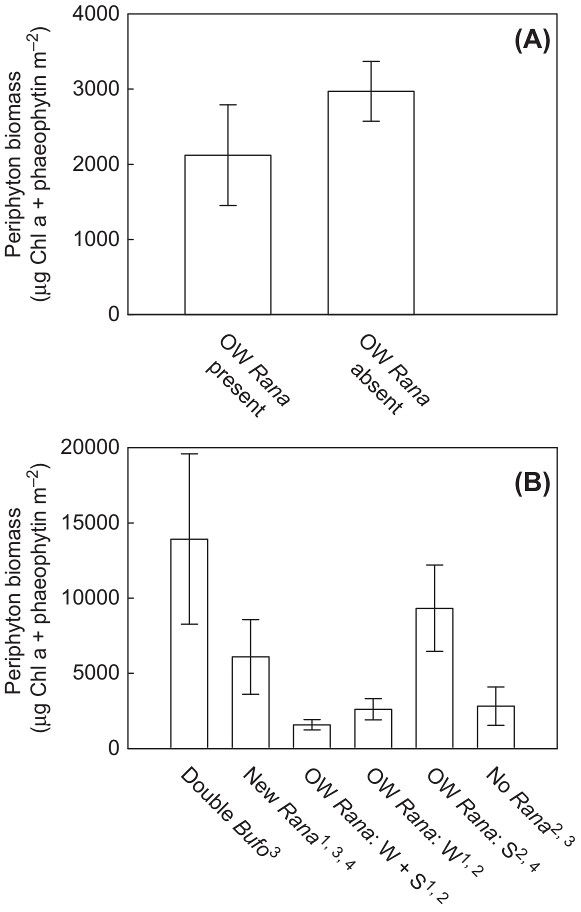

Figure 3. Mean (± SE) concentration of periphyton (µg Chl a + Phaeophytin m^{-2}) at (A) the end of the winter and (B) during the spring at the time of peak *Bufo* metamorphosis. At the end of the winter there were effectively only two treatments: overwintered *Rana* present or absent. During the spring, all six treatments applied in the experiment were in effect. Numbered superscripts following the treatment name corresponds to 1 of 4 hypothesis families outlined in the methods section. Comparisons among treatments within the same hypothesis family are the most relevant comparisons as each hypothesis family focuses on a different conceptual question pertaining to how overwintered *Rana* may cause priority effects in *Bufo* performance by altering the abundance of algae present. Statistical results for each family of hypotheses are presented in the results section.

Discussion

In nature, local communities differ in their assemblage histories as both deterministic and stochastic processes influence the relative timing at which species arrive to a community. Important and current issues in ecology include understanding the extent to which differences in a communities' assembly history affect community structure and the performance of taxa (Chase 2003, 2010). We found that the history of assembly affects how well species perform in a community. In our case, *Bufo* performance was lower when *Rana* entered a pond during the year prior to *Bufo* arrival (i.e. overwintered *Rana* are present) than when *Rana* entered a pond contemporaneously with *Bufo* (i.e. newly hatched *Rana*). Although our study is not the first to demonstrate the occurrence of priority effects (Alford and Wilbur 1985, Wilbur and Alford 1985, Morin 1987, Lawler and Morin 1993), our study provides novel insight into the actual mechanisms that produce priority effects. Specifically, we demonstrate that multiple kinds of interactions between taxa are involved in the production of priority effects, these mechanisms operate at different times during the community assembly process, and these mechanisms interact in a synergistic manner that enhances the overall effect of early arriving taxa on late arriving taxa.

Resource exploitation by early arriving taxa is a commonly proposed mechanism to explain the production of priority effects; this is especially true of studies involving larval anurans even though resource levels were not quantified in these studies (Alford and Wilbur 1985, Wilbur and Alford 1985, Morin 1987, Lawler and Morin 1993). Our study confirms the important role of resource exploitation as a mechanism contributing to the production of a priority effect because overwintered *Rana* depleted the availability of algal resources in a pond prior to the arrival of *Bufo* which resulted in *Bufo* metamorphosing at a smaller size. Nonetheless, resource exploitation that occurs during the time in which early and late arriving taxa co-occur does not appear to be important. Despite tremendous differences in *Bufo* performance between the treatment with overwintered *Rana* present during the winter and spring and the treatment with a low abundance of *Bufo* (Fig. 2), algal resource levels at the time of peak *Bufo* metamorphosis was comparable between these treatments (Fig. 3). Consequently, resource exploitation prior to the arrival of *Bufo*, and not during the time where *Bufo* and *Rana* co-occur, is the primary mechanism through which a priority effect was produced.

One of the most interesting findings of our work is that the effect of resource exploitation prior to the colonization of late arriving species augmented the impact of direct interactions that operate between early and late arriving taxa when both species are present in the community together. We observed that *Bufo* survival and larval period was reduced to a greater extent when overwintered *Rana* was present in a pond during both the winter and spring than when overwintered *Rana* was only present in a pond during the winter (Fig. 2). If no processes were operating during the spring the performance of *Bufo* should have been the same in the treatment with overwintered *Rana* present during the winter and spring and the treatment where overwintered *Rana* was only present during the winter. The absence of an effect of overwintered *Rana* on *Bufo* performance when overwintered *Rana* are only present during the spring suggests that mechanisms operating during the spring are not sufficiently strong to affect *Bufo* performance in the absence of mechanisms (resource exploitation) imposed by overwintered *Rana* during the winter.

Potential mechanisms through which overwintered *Rana* could directly interact with *Bufo* during the spring include interference competition (via the production of growth inhibitors or aggressive behavior) and predation. Faragher and Jaeger (1998) conducted short term studies in the lab and found that *Rana sphenocephala* influence newly hatched *Hyla cinerea* tadpoles (a summer breeding species) via predation, aggressive behavior and production of growth inhibitors. We do not believe that predation by overwintered *Rana* augmented the overall effect of overwintered *Rana* on *Bufo* in our study. Predation generally reduces survival and accelerates the larval period of anurans (Wilbur 1987). If predation is an important mechanism operating during the spring we would have expected the larval period of *Bufo* to be shorter when overwintered *Rana* were present but we observed that overwintered *Rana* tended to increase the larval period of *Bufo*. Reductions in survivorship and larval development rate are more consistent with competition so we believe that overwintered *Rana* compete via interference competition during the spring. Unfortunately, we were not able to identify the particular manner in which interference competition occurred because the depth and murkiness of the water in our experimental units made it very difficult to make behavioral observations and we did not quantify the production of growth inhibitors, in part, because it is not clear what the actual nature of the growth inhibitor is. Proposed inhibitors include metabolic waste products, some unknown chemical compound, and different kinds of parasites (e.g. particular species of yeast and algae, reviewed by Alford 1999).

Laboratory work supports our suggestion that the early arrival of *Rana* entering a pond can enhance the intensity of interference competition by depleting the availability of resources prior to the arrival of *Bufo*. Both Steinwascher (1978) and Beebee and Wong (1992) reported that the production of growth inhibitors is greater when the amount of food resources supplied to larval anurans is reduced. Consequently, depression of algae by overwintered *Rana* during the winter may have stimulated the production of growth inhibitors by overwintered *Rana* during the spring. Although Petranka's (1989) survey for larval anuran growth inhibitors in 11 natural ponds concluded that the production of larval anuran growth inhibitors was uncommon outside of lab conditions, he did find evidence that growth inhibitors were important in a pond which contained overwintered *Rana sphenocephala* but not in ponds with younger *Rana sphenocephala*. These observations support our contention that the effect of growth inhibitors produced by *Rana* is greatest when larval *Rana* are present in a pond during the winter. We do not know of any evidence to indicate that larval anurans increase their aggressive behavior when food resources are lower but studies of other organisms have found an increase in aggression with a reduction in food availability in some cases (Feinsinger 1976, Ducey and Heuer 1991, Hodge et al. 2009) but not others (Ovaska 1993, Rychlik and Zwolak 2006).

The ability to grow to a larger size by arriving early did not appear to allow overwintered *Rana* to have a competitive advantage over *Bufo* during the time in which *Rana* and *Bufo* co-occur. *Bufo* performance was comparable when they interacted with overwintered *Rana* that were only present during the spring to when they interacted with newly hatched *Rana* during the spring. We maintained abundances of each size class of *Rana* at levels that *Bufo* would likely encounter in nature but we do not believe that the lack of a size effect was due to the fact that newly hatched *Rana* were more abundant than overwintered *Rana* because 250 newly hatched *Rana* had no impact on *Bufo* performance in comparison to the treatment where *Bufo* occurred alone. Furthermore, the absence of a change in *Bufo* performance when *Bufo* abundance is increased by 250 individuals and when *Bufo* competed with 250 newly hatched *Rana* suggests that overwintered *Rana* will not likely reduce *Bufo* performance due to density-dependent processes. Despite the absence of size and density-dependent effects in our study, we believe that variation in the size and density of overwintered *Rana* that are present during the winter (two factors that we did not manipulate) could be important factors in determining the extent of resource exploitation during the winter and could regulate the intensity of interference mechanisms that operate during the spring.

Our work sheds light on mechanisms that may generally produce priority effects involving organisms other than frogs. Many studies of priority effects either lack sufficient information to describe the mechanism(s) producing priority effects or they present a singular mechanism through which a priority effect is produced. Our work demonstrates that priority effects can be the product of multiple mechanisms that operate at different times in the community assembly process and that these mechanisms can operate in a synergistic manner. Resource exploitation by early arriving taxa is clearly an important contributor to the production of a priority effect but the stressful conditions imposed by low resource levels can enhance the magnitude of processes that may otherwise have unimportant effects had resource exploitation not occurred. We expect that such a synergy may be important in the production of priority effects for a diverse array of taxa because resource exploitation is a commonly reported mechanism of interspecific competition for a diverse array of taxa in a number of different ecological systems (Schoener 1983) and species rarely all arrive at a site simultaneously.

The importance of historical context in biology is very clear in evolution and there is an increasing amount of work to demonstrate that the history of community assembly has important effects in ecological time as well (Robinson and Dickerson 1987, Chase 2003, 2010, Fukami 2004, Fukami et al. 2010). We believe that an increased attention toward synergistic interactions of mechanisms that operate between taxa at different times in the colonization process will better allow us to understand how historical contingencies have such important effects on ecological systems. For example, such detailed focus on mechanisms may better allow us to manage landscapes where the early arrival of invasive taxa may convey a competitive advantage over native taxa. Reducing the abundance of early arriving taxa early in the assembly process may be especially critical as resource exploitation during this time may increase the sensitivity of late arriving taxa to other stressors. Although the vagaries of species arrival order does introduce stochasticity into the trajectory of future community development, a focus on understanding the mechanisms through which priority effects occur will better allow us to predict community responses to stochastic events when they do arise.

Acknowledgements – We thank H. Vance-Chalcraft, C. Goodwillie and M. O'Driscoll for comments and critiques of JH's thesis which served as the foundation for this paper and to J. Loman for his review of this paper. We thank J. Davenport, N. Amoroso, L. Connell, R. Cooper, R. Deans, M. Albecker, C. Ruehl, L. McCarthy and C. Williams, for assistance in the field and lab. This project was funded in part by a National Science Foundation grant (DEB-0716558) to DRC.

References

Alford, R. A. 1999. Ecology: resource use, competition, and predation. – In: McDiarmid, R. W. and Altig, R. (eds), Tadpoles: the biology of anuran larvae. – Univ. of Chicago Press,, pp. 240–278.

Alford, R. A., and Wilbur, H. M. 1985. Priority effects in experimental pond communities: competition between *Bufo* and *Rana*. – Ecology 66: 1097–105.

Almany, G. R. 2003. Priority effects in coral reef fish communities. – Ecology 84: 1920–1935.

Beebe, T. J. C. and Wong, A. L.-C. 1992. Prototheca-mediated interference competition between anuran larvae operates by resource diversion. – Physiol. Zool. 65: 815–823.

Benke, A. C. and Benke, S. S. 1975. Comparative dynamics and life histories of coexisting dragonfly populations. – Ecology 56: 302–317.

Boone, M. D. et al. 2004. Overwintered bullfrog tadpoles negatively affect salamanders and anurans in native amphibian communities. – Copeia 2004: 683–690.

Caldwell, J. P. 1986. Selection of egg deposition sites: a seasonal shift in the southern leopard frog, *Rana sphenocephala*. – Copeia 1986: 249–253.

Chalcraft, D. R. and Resetarits Jr., W. J. 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? – Ecology 84: 2407–2418.

Chalcraft, D. R. and Resetarits Jr., W. J. 2004. Metabolic rate models and the substitutability of predator populations. – J. Anim. Ecol. 73: 323–232.

Chase, J. M. 2003. Community assembly: when should history matter? – Oecologia 136: 489–498.

Chase, J. M. 2010. Stochastic community assembly causes higher biodiversity in more productive environments. – Science 328: 1388–1391.

Clesceri, L. S. et al. 1998. Section 1020H. Chlorophyll. – Am. Public Health Ass.

D'Antonio, C. M. et al. 2001. Factors influencing dynamics of two invasive C-4 grasses in seasonally dry Hawaiian woodlands. – Ecology 82: 89–104.

Ducey, P. K. and Heuer. J. 1991. Effects of food availability on intraspecific aggression in salamanders of the genus *Ambystoma*. – Can. J. Zool. 69: 288–290.

Dunson W. A. and Travis, J. 1991. The role of abiotic factors in community organization. – Am. Nat. 138: 1067–1091.

Faragher, S. G. and Jaeger, R. G. 1998. Tadpole bullies: examining mechanisms of competition in a community of larval anurans. – Can. J. Zool. 76: 144–153.

Farris-Lopez, K. et al. 2004. Influence of a common palm, *Oenocarpus mapora*, on seedling establishment in a tropical moist forest in Panama. – J. Trop. Ecol. 20: 429–438.

Feinsinger, P. 1976. Organization of a tropical guild of nectarivorous birds. – Ecol. Monogr. 46: 257–291.

Fukami, T. 2004. Assembly history interacts with ecosystem size to influence species diversity. – Ecology 85: 3234–3242.

Fukami, T. et al. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. – Ecol. Lett. 13: 675–684.

Gibbons, J. W. and Semlitsch, R. D. 1991. Guide to the reptiles and amphibians of the Savannah River Site. – Univ. Georgia Press.

Hochberg, Y. and Tamhane, A. C. 1987. Multiple comparison procedures. – Wiley.

Hodge, S. J. et al. 2009. Food limitation increases aggression in juvenile meerkats. – Behav. Ecol. 20: 930–935.

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

Lannoo, M. 2005. Amphibian declines. – Univ. California Press.

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

Louette, G. and De Meester, L. 2006. Predation and priority effects in experimental zooplankton communities. – Oikos 116: 419–426.

Loman, J. 2004. Density regulation in tadpoles of *Rana temporaria*: a full pond experiment. – Ecology 85: 1611–1618.

Mackey, M. J. and Boone, M. D. 2004. Single and interactive effects of malathion, overwintered green frog tadpoles, and cyanobacteria on gray treefrog tadpoles. – Environ. Toxicol. Chem. 28: 637–643.

Martof, B. S. et al. 1980. Amphibians and reptiles of the Carolinas and Virginia. – Univ. North Carolina Press.

McCauley, S. J. et al. 2008. Metacommunity patterns in larval odonates. – Oecologia 158: 329–342.

McCauley, S. J. et al. 2010. Foraging rates of larval dragonfly colonists are positively related to habitat isolation: results from a landscape-level experiment. – Am. Nat. 175: 66–73.

Morin, P. J. 1983. Predation, competition, and the composition of larval anuran guilds. – Ecol. Monogr. 53: 120–138.

Morin, P. J. 1987. Predation, breeding synchrony, and the outcome of competition among treefrog tadpoles. – Ecology 68: 675–683.

Ovaska, K. 1993. Aggression and territoriality among sympatric western plethodontid salamanders. – Can. J. Zool. 71: 901–907.

Petranka, J. W. 1989. Chemical interference competition in tadpoles: does it occur outside laboratory aquaria? – Copeia 1989: 921–930.

Quinn, G. P. and Keough, M. J. 2002. Experimental design and data analysis for biologists. – Cambridge Univ. Press.

Resetarits, Jr., W. J. and Fauth, J. E. 1998. From cattle tanks to Carolina Bays: the utility of model systems for understanding natural communities. – In*:* Resetarits Jr., W. J. and Bernardo, J. (eds), Experimental ecology: issues and perspectives. Oxford Univ. Press, pp. 133–151.

Robinson, J. F. and Dickerson, J. E. 1987. Does invasion sequence affect community structure? – Ecology 68: 587–595.

Rubbo, M. J. et al. 2006. Evaluting a predator–prey interaction in the field: the interaction between beetle larvae (predator) and tadpoles (prey). – J. Zool. 269: 1–5.

Rychlik, L. and Zwolak, R. 2006. Interspecific aggression and behavioral dominance among four sympatric species of shrews. – Can. J. Zool. 84: 434–448.

Schoener, T. W. 1983. Field experiments on interspecific competition. – Am. Nat. 122: 240–285.

Scott, D. E. 1990. Effects of larval density in *Ambystoma opacum*: an experiment in large-scale field enclosures. – Ecology 71: 296–306.

Shorrocks, B. and Bingley, M. 1994. Priority effects and species coexistence: experiments with fungal-breeding *Drosophila*. – J. Anim. Ecol. 63: 799–806.

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

Smith-Gill, S. J. and Gill, D. E. 1978. Curvilinearities in the competition equations: an experiment with ranid tadpoles. – Am. Nat. 112: 557–570.

Steinwascher, K. 1978. Interference and exploitation competition among tadpoles of *Rana utricularia*. – Ecology 59: 1039–1046.

Stoks, R. and McPeek, M. A. 2003. Predators and life histories shape *Lestes* damselfly assemblages along a freshwater habitat gradient. – Ecology 84: 1576–1587.

Walston, L. J. and Mullin, S. J. 2007. Population responses of wood frog (*Rana sylvatica*) tadpoles to overwintered bullfrog (*Rana catesbeiana*) tadpoles. – J. Herpetol. 41: 24–31.

Wellborn, G. A. et al. 1996. Mechanisms creating community structure across a freshwater habitat gradient. – Annu. Rev. Ecol. Syst. 27: 337–363.

Wells, K. D. 2007. The ecology and behavior of amphibians. – Univ. of Chicago Press.

Werner, E. E. 1994. Ontogenetic scaling of competitive relations: size-dependent effects and responses in two anuran larvae. – Ecology 75: 197–213.

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

Wilbur, H. M. 1987. Regulation of structure in complex systems: experimental temporary pond communities. – Ecology 68: 1437–1452.

Wilbur, H. M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. – Ecology 78: 2279–2302.

Wilbur, H. M. and Alford, R. A. 1985. Priority effects in experimental pond communities: responses of *Hyla* to *Bufo* and *Rana*. – Ecology 66: 1106–1114.