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Population Responses of Wood Frog (Rana sylvatica) Tadpoles to Overwintered Bullfrog (Rana catesbeiana) Tadpoles

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ABSTRACT.—A fundamental goal in ecology is to understand how environmental variation influences the distribution of individuals within a population. In this study, we used laboratory experiments to examine the population responses of sympatric Wood Frog (Rana sylvatica) tadpoles to native overwintered Bullfrog (Rana catesbeiana) tadpoles. For periods of up to two weeks, we measured growth, activity, and refuge use of Wood Frog tadpoles in small mesocosms with and without an overwintered Bullfrog tadpole present. Bullfrog tadpoles had a negative effect on the growth of Wood Frog tadpoles allotopic (naïve) to Bullfrogs, whereas the presence of Bullfrogs had no effect on growth of syntopic (experienced) Wood Frog tadpoles. There were also differential behavioral responses of the Wood Frog populations to overwintered Bullfrog tadpole visual and chemical cues. Only allotopic Wood Frog tadpoles decreased activity levels and increased use of refugia in the presence of overwintered Bullfrog tadpoles. These observations indicate overwintered Bullfrog tadpoles might exert a selective pressure on sympatric Wood Frog tadpoles, and that experience might allow for the development of strategies to maximize performance for species coexisting with overwintered Bullfrog tadpoles.

A fundamental goal in ecology is to understand how organisms respond to their environment and how environmental variation influences the distribution of individuals within a population. Amphibian larvae developing in ephemeral aquatic habitats often experience a different suite of competitors and predators than those larvae developing in permanent aquatic habitats (Semlitsch, 1988; Werner and McPeek, 1994). To persist in these environments, amphibians must have adaptations to tolerate not only variation in pond hydroperiod but also to tolerate different suites of competitors and predators. One possible adaptation to environmental variation is for a species to be phenotypically plastic (Van Buskirk and Relyea, 1998). In response to competitors and predators, many larval anurans exhibit competitor- and predator-induced plasticity in behavior, growth rate, and morphology (Van Buskirk and Relyea, 1998; Relyea, 2002, 2004).

Throughout their native range, Bullfrogs are considered important agents of amphibian community structure (Werner et al., 1995; Hecnar and M’Closkey, 1997; Boone et al., 2004). Laboratory experiments have demonstrated that Bullfrogs are competitively superior to other amphibian species (Werner and Anholt, 1996), although the outcome of competition in the field may be influenced by the presence of predators (Werner, 1991). The effects of Bullfrogs on sympatric amphibians are often mediated by pond hydroperiod. In the Midwest, Bullfrogs typically require two years for tadpoles to complete metamorphosis (Phillips et al., 1999) and are, thus, restricted to permanent wetlands where fish may also occur. Native fish species are predators of most amphibians (Semlitsch, 1988; Werner and McPeek, 1994), capable of eliminating many amphibian populations from permanent ponds. Bullfrog tadpoles, however, are usually unpalatable to fish (Kruse and Francis, 1977; Werner and McPeek, 1994) and are capable of persisting in these environments. Although ephemeral ponds do not typically support Bullfrog tadpole populations in the Midwest, ephemeral ponds may become semipermanent after receiving high levels of precipitation or after human landscape alterations. In these situations, Bullfrog tadpoles may be capable of surviving for over one year, allowing overwintered Bullfrog tadpoles to interact with other sympatric amphibian larvae. Boone et al. (2004) observed that, within its native range, the presence of overwintered Bullfrog tadpoles resulted in decreased growth and survival of larval amphibians allotopic (naïve) to Bullfrogs, presumably via interspecific competition. Thus, the exploitation of historically ephemeral aquatic habitats by Bullfrogs might present a novel competition threat to resident amphibian species.

Wood Frog (Rana sylvatica) tadpoles commonly exhibit behavioral and morphological plas-
ticity in response to predators and competitors (Van Buskirk and Relyea, 1998; Relyea, 2002, 2004). Wood Frogs and Bullfrogs are sympatric throughout much of the United States and Canada. Although their distributions are usually allotopic with respect to pond hydroperiod (Phillips et al., 1999; Paton and Crouch, 2002), tadpoles of the two species may be able to coexist in permanent aquatic environments that lack fish. Overwintered Bullfrog tadpoles possess a size advantage over Wood Frog tadpoles and have been observed to deplete tadpoles of other ranids (Ehrlich, 1979; Kiesecker and Blaustein, 1997). Therefore, overwintered Bullfrog tadpoles might have adverse effects on Wood Frog populations through an interaction of interspecific competition and predation. There is additional evidence suggesting that larval amphibians with prior experience to competitors and predators exhibit contrasting responses to the presence of the competitor or predator than inexperienced larval anurans (Kiesecker and Blaustein, 1997; Van Buskirk and Relyea, 1998; Gomez-Mestre and Tejedo, 2002). Thus, we believe that syntopic (experienced) Wood Frog tadpoles from semipermanent fishless ponds will respond differently to the presence of overwintered Bullfrog tadpoles than allotopic Wood Frog tadpoles from ephemeral ponds.

Herein, we present results of laboratory experiments addressing the responses of Wood Frog populations to native overwintered Bullfrog tadpoles. We used natural populations of Wood Frogs and Bullfrogs to test two hypotheses: (1) overwintered Bullfrog tadpoles induce changes in behavior, growth, and survival of sympatric Wood Frog tadpoles; and (2) Wood Frog tadpoles from populations syntopic to Bullfrogs respond differently than allotopic populations when in the presence of overwintered Bullfrog tadpoles.

**Materials and Methods**

**Study Organisms.**—We collected eight Wood Frog egg masses on 7 March 2005 from four ponds in Coles County, Illinois. Four egg masses each were collected from populations syntopic and allotopic to Bullfrogs. Syntopic Wood Frog egg masses were collected from two permanent fishless ponds that also contained a breeding population of Bullfrogs. Successful recruitment of Bullfrog and Wood Frog juveniles has been observed at both ponds in each of the previous six years (S. Mullin, unpubl. data). Syntopic Wood Frog tadpoles, therefore, were considered experienced to overwintered Bullfrog tadpoles. Allotopic Wood Frog egg masses were collected from two ephemeral ponds; each pond completely dried before 1 August in each of the previous six years (S. Mullin, pers. obs.) such that larval Bullfrogs could not complete development. Although adult Bullfrogs have been observed along the banks of these ephemeral ponds during the summer months, we have not observed any Bullfrog reproductive activity at these ponds in any of the previous six years. Thus, allotopic Wood Frog tadpoles were considered naive to overwintered Bullfrog tadpoles. All Wood Frog embryos collected were of similar developmental stage (Gosner stage 10; Gosner, 1960).

Upon collection, each egg mass was brought back to the laboratory and incubated in isolated glass aquaria in 25 liters of natal pond water. All tadpoles hatched within seven days of collection. Upon hatching, tadpoles were transferred to population-specific aquaria (allotopic or syntopic) filled with 25 liters of aged tap water, where they were raised until the commencement of this study. Water was changed every 5–7 days and tadpoles were fed powdered rodent chow ad libitum. On 23 March 2005, we returned to the two permanent ponds where the syntopic Wood Frogs were originally collected and seiined each pond for Bullfrog tadpoles. These tadpoles were 7.32 ± 1.84 g (mean ± 1 SE), too large to have hatched in 2005 (Gosner stage 32–36; Gosner, 1960) and were considered to have overwintered from the previous year. Those Bullfrog tadpoles not immediately added to experimental aquaria were kept in a separate aquarium filled with aged tap water. Bullfrog tadpoles were fed and their water was changed in the same manner as the Wood Frog tadpoles. All aquaria were maintained on a 12:12 L:D photoperiod in a temperature-controlled environment (20°C).

**Experiment 1: Wood Frog Growth and Survival.**—We used a randomized block design to test for the effects of overwintered Bullfrog tadpoles on the growth and survival of Wood Frog tadpoles. The two independent variables each had two levels—the Wood Frog population type was allotopic or syntopic, and Bullfrog tadpoles were present or absent. We randomly assigned glass aquaria (38-liter volume) to the four combinations of these variables, each of which was replicated five times.

On 23 March 2005, we filled each aquarium with 25 liters of aged tap water. We then added 2 g of leaf litter and 1 g of ground rodent chow to each enclosure to serve as a refuge and food resource. For the Bullfrog treatments, we added a single Bullfrog tadpole to an aquarium containing either 20 allotopic or syntopic Wood Frog tadpoles. In treatments in which Bullfrog tadpoles were absent, we added 20 Wood Frog tadpoles from either population. We placed
brown construction paper between each aquarium to prevent the potentially confounding effects of Bullfrog visual stimuli on Wood Frogs in adjacent aquaria. We measured the mass (± 1.0 mg) of all tadpoles before adding them to all aquaria. The mean (±1 SE) mass of all Wood Frog tadpoles added to the aquaria was 35.40 ± 0.51 mg and there was no difference in mass between populations (t 18 = 0.39; P = 0.70). All Wood Frog tadpoles were of similar developmental stage (Gosner stage 24–27; Gosner, 1960), and all Bullfrog tadpoles used in this experiment were within the same size distribution (7.27 ± 2.05 g; Kolmogorov-Smirnov test; P = 0.20).

On 5 April, we terminated the experiment and measured Wood Frog tadpole growth and survival. To quantify growth, we measured the mass (± 1.0 mg) of each Wood Frog tadpole and calculated the mean for each aquarium. We then subtracted the mean initial mass from the mean final mass to determine Wood Frog growth within each aquarium. Bullfrog tadpole growth was calculated in a similar manner. We quantified survival in each aquarium as the proportion of Wood Frog tadpoles that were alive to the total number initially stocked (N = 20).

Experiment 2: Wood Frog Activity and Refuge Use.—We tested for chemically and visually mediated avoidance of Bullfrogs by Wood Frog tadpoles through activity and use of microhabitat refugia. For this experiment, we used a 2 × 2 factorial design using 38-liter glass aquaria, different from those used in the previous experiment. We partitioned each aquarium into equal lengthwise sections by attaching a 1-cm high aluminum screen to the bottom of the aquarium with silicone sealant. We then placed 2 g (2–3 layers) of leaf litter (primarily oak, Quercus spp.) on one side of each aquarium. The leaf litter served as refuge, and the aluminum screening served as a partition between the two microhabitats (with refuge and without). Additionally, the 1-cm high screen partitioning was low enough to allow tadpoles to swim freely between both sides.

To determine the effects of Bullfrog chemical and visual cues on Wood Frog activity and refuge use, we conducted trials once per day, beginning on 24 March 2005. Prior to each trial we added 20 liters of aged tap water to all aquaria. Bullfrog cages, made of perforated clear 20-cm tall plastic cups, were submerged 12 cm beneath the water surface such that the opening of the cup was above the water surface, preventing Bullfrog tadpoles from escaping. We suspended one Bullfrog cage (using monofilament) perpendicularly to the 1-cm high screen partitioning. Bullfrog tadpoles placed into these cages were visible to Wood Frog tadpoles and chemical cues could disperse into the water column occupied by the Wood Frog tadpoles. For the Bullfrog treatments, we randomly selected one Bullfrog tadpole from a stock and placed it in the cage. To mimic the same amount of disturbance that the Bullfrog cages create, empty Bullfrog cages were suspended in all control aquaria. For all treatments, 20 randomly selected Wood Frog tadpoles were added from either an allotopic or syntopic stock population. The order in which trials occurred was randomly determined.

After a 12-h habituation period, we quantified tadpole activity and microhabitat use. To reduce bias, only one of us (LJW) quantified tadpole activity, using a method of scan sampling (from Altmann, 1974) by cautiously approaching each aquarium and counting the number of Wood Frog tadpoles actively moving during a 30–60-sec period. We divided this number by the number of total tadpoles present to provide an estimate of tadpole activity. So as not to disturb the leaf-litter substrate and any tadpoles that might be using it, we quantified refuge use by counting the number of tadpoles present in the side of the aquarium that lacked the leaf-litter refuge. Subtracting the number observed in this side from the number of tadpoles present provided the number of tadpoles located on the side containing the leaf-litter refuge. We divided this number by the total number of tadpoles present to provide an estimate of refuge use. We assumed that tadpoles on the side of the aquarium containing the leaf-litter were using the refuge, regardless of the tadpole’s position in the water column. For each replicate, we recorded activity and refuge use three times, each measurement separated by 1–2 h, and we calculated the mean of the three observations as the metric of tadpole activity and refuge use. After the final measurement was recorded for each replicate, aquaria were drained and tadpoles were placed in separate containers. Tadpoles were never used in more than one treatment. We terminated all experiments on 6 April, after five replicates had been completed for each treatment.

Statistical Analyses.—We tested for the effects of population source, Bullfrog presence, and their interaction on the growth and survival of Wood Frogs using a multivariate analysis of variance (MANOVA), followed by univariate analyses of variance (ANOVA). Spatial blocks were not significant and were pooled with the error term to increase the power of the test. We performed a multivariate analysis of variance (MANOVA) to test for the effects of population source, Bullfrog presence, and their interaction on activity and refuge use of Wood Frogs, followed by univariate analyses of variance
Table 1. (A) MANOVA results for Experiment 1, which tested for the effects of Wood Frog (Rana sylvatica) population source (allotopic/syntopic), presence of Bullfrog (Rana catesbeiana) tadpoles, and their interaction on Wood Frog growth and survival. Degrees of freedom equal 2,15 in all cases. (B) ANOVA results for Wood Frog growth and survival. The $F$-statistic is reported with the associated $P$-value in parentheses. Degrees of freedom equal 1,16 in all cases.

<table>
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<th>Factor</th>
<th>Wilks $\lambda$</th>
<th>$F$</th>
<th>$P$</th>
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</thead>
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<tr>
<td>Population</td>
<td>0.38</td>
<td>12.17</td>
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<tr>
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<td>&lt; 0.001</td>
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<td>10.29</td>
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<th>Bullfrog</th>
<th>Population $\times$ Bullfrog</th>
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<td>Growth</td>
<td>17.26 (&lt; 0.001)</td>
<td>41.19 (&lt; 0.001)</td>
<td>20.12 (&lt; 0.001)</td>
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<tr>
<td>Survival</td>
<td>5.04 (0.04)</td>
<td>15.04 (0.001)</td>
<td>0.38 (0.55)</td>
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(ANOVA). If the univariate analyses revealed a significant interaction, we followed this analysis with Tukey-Kramer multiple comparison tests. Preliminary analyses revealed that the response variables conformed to all assumptions of parametric statistics; thus, no transformations were necessary. All analyses were performed using SAS 9.1 (SAS Institute, Cary, NC) using a significance level of $\alpha = 0.05$.

**RESULTS**

*Tadpole Growth and Survival.*—Wood Frog tadpole growth and survival were influenced by population source and the presence of an overwintered Bullfrog tadpole (Table 1). The presence of an overwintered Bullfrog tadpole similarly affected the survival of tadpoles from both Wood Frog populations (i.e., no population-by-Bullfrog interaction, Table 1B; Fig. 1A). Overall, there was a difference in survival between the two Wood Frog populations (percent survival: allotopic, $88.5 \pm 2.7\%$; syntopic, $94.0 \pm 1.8\%$; Table 1B). In the presence of Bullfrogs, survival of allotopic and syntopic larvae declined by $11.7\%$ and $8.2\%$, respectively (Fig. 1A). For all treatments, Bullfrog survival was $100\%$. There was a population-by-Bullfrog interaction on Wood Frog tadpole growth (Table 1B). Only the growth of allotopic Wood Frog tadpoles was reduced in the presence of an overwintered Bullfrog tadpole (Tukey-Kramer Test; $P < 0.001$). In the presence of the Bullfrog tadpole, growth of allotopic Wood Frog tadpoles declined significantly compared to the absence of the Bullfrog tadpole (Tukey-Kramer Test; $P < 0.001$).

**Fig. 1.** Survival (A) and Growth (B) of Wood Frog (Rana sylvatica) tadpoles from syntopic or allotopic populations in response to the presence of a Bullfrog (Rana catesbeiana) tadpole. Data are represented as means ± 1 standard error.
Table 2. (A) MANOVA results for Experiment 2, which tested for the effects of Wood Frog (Rana sylvatica) population source (alloptic/syntopic), presence of Bullfrog (Rana catesbeiana) tadpoles, and their interaction on Wood Frog activity and refuge use. Degrees of freedom equal 2,15 in all cases. (B) ANOVA results for Wood Frog activity and refuge use. The F-statistic is reported with the associated P-value in parentheses. Degrees of freedom equal 1,16 in all cases.

<table>
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<th>(A) MANOVA</th>
<th>Wilks λ</th>
<th>F</th>
<th>P</th>
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<tr>
<td>Population</td>
<td>0.42</td>
<td>10.52</td>
<td>0.001</td>
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<tr>
<td>Bullfrog</td>
<td>0.19</td>
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<td>Population × Bullfrog</td>
<td>0.34</td>
<td>14.25</td>
<td>&lt; 0.001</td>
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<table>
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<th>(B) ANOVA</th>
<th>Population</th>
<th>Bullfrog</th>
<th>Population × Bullfrog</th>
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<tr>
<td>Activity</td>
<td>4.21 (0.06)</td>
<td>34.53 (&lt; 0.001)</td>
<td>9.94 (0.006)</td>
</tr>
<tr>
<td>Refuge Use</td>
<td>19.68 (&lt; 0.001)</td>
<td>38.18 (&lt; 0.001)</td>
<td>22.91 (&lt; 0.001)</td>
</tr>
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</table>

Tadpole Activity and Refuge Use.—Wood Frog tadpole activity and refuge use were affected by a population-by-Bullfrog interaction (Table 2). Only allotopic Wood Frog tadpoles reduced their activity in the presence of a Bullfrog (Tukey-Kramer Test; P < 0.001). In response to Bullfrog chemical and visual cues, allotopic Wood Frogs reduced their activity by 57.5%, whereas syntopic Wood Frogs reduced their activity by 18.8%. Only allotopic Wood Frog tadpoles altered their microhabitat use in the presence of Bullfrog tadpole visual and chemical cues (Tukey-Kramer Test; P < 0.001). In the presence of Bullfrogs, allotopic Wood Frog tadpoles increased their use of leaf-litter refugia by 43.1%, whereas syntopic Wood Frog tadpoles exhibited a 5.4% increase in refuge use (Fig. 2B).

Discussion
To our knowledge, this study is the first to compare the population-level responses of a sympatric anuran species to native overwintered Bullfrog tadpoles. For the most part, we observed asymmetrical effects of overwintered Bullfrog tadpoles on Wood Frog tadpoles from allotopic and syntopic populations. Although...
both Wood Frog populations exhibited similar survival rates, only the growth and behavior of allotopic Wood Frog tadpoles was affected by the presence of a Bullfrog tadpole. The experiments in this study examined two different paradigms of species interaction. In Experiment 1, changes in Wood Frog growth and survival in response to the physical presence of Bullfrog tadpoles might be the result of exploitative or interference interspecific competition or predation. In Experiment 2, however, we observed changes in Wood Frog activity and refuge use in response to chemical and visual cues from caged Bullfrog tadpoles. These responses are the result of interference mechanisms only.

Overall, Wood Frog tadpole survival declined by 9.9% when an overwintered Bullfrog tadpole was present. Although Bullfrog tadpoles are capable of consuming congeneric tadpoles (Ehrlich, 1979; Kiesecker and Blaustein, 1997), we did not directly quantify predation in this study. Overwintered Bullfrog tadpoles often reduce the survival of other larval anurans through exploitative competition (Kupferberg, 1997; Adams, 2000; Boone et al., 2004), and the relatively large size of overwintered Bullfrog tadpoles suggests that interference competition might also occur (Wilbur, 1984). In response to overwintered Bullfrog chemical and visual cues, the decrease in activity and increase in refuge use exhibited only by allotopic Wood Frog tadpoles are characteristic of predator-induced responses (Van Buskirk and Relyea, 1998; Relyea, 2002, 2004). For instance, Kiesecker and Blaustein (1997) reported that, in response to nonnative overwintered Bullfrog tadpoles, only syntopic Red-Legged Frogs (Rana aurora) exhibited behavioral plasticity in activity and refuge use. As a result, syntopic Red-Legged Frogs experienced higher survival rates in the presence of Bullfrog tadpoles than allotopic Red-Legged Frogs (which did not exhibit behavioral plasticity). Our results are interesting in that we discovered that only allotopic Wood Frog tadpoles exhibited behavioral plasticity in the presence of overwintered Bul- lfrog tadpole chemical- and visual cues. Thus, we were surprised to find that the performance of allotopic Wood Frog tadpoles did not improve as a result of the expression of these behaviors. In fact, allotopic Wood Frog tadpoles suffered from slower growth in the presence of overwintered Bullfrog tadpoles. These observations contrast previous studies that have found that, when in the presence of a competitor or predator, larval anurans that exhibiting phenotypic plasticity experience greater fitness (Kiesecker and Blaustein, 1997; Van Buskirk and Relyea, 1998).

Our results indicate that the syntopic Wood Frog tadpoles in our study do not perceive overwintered Bullfrog tadpoles as a predation threat. Consequently, syntopic Wood Frog tadpoles might use food resources more efficiently than allotopic Wood Frog tadpoles. Experience is the most plausible explanation for the performance of Wood Frog tadpoles. Related studies have shown that the performance of organisms experienced to a competitor or predator is greater than the performance of those with no prior experience (Chivers and Smith, 1994; Kiesecker and Blaustein, 1997; Van...
Buskirk and Relyea, 1998; Gomez-Mestre and Tejedo, 2002). Therefore, prolonged exposure to Bullfrog tadpoles may condition sympatric Wood Frogs and allow them to recognize Bullfrog tadpoles as less of a potential competition or predation threat. Our results suggest that there is a genetic basis for the development of this strategy because we observed the differential population responses of Wood Frogs to overwintered Bullfrog tadpoles without any conditioning of Wood Frog tadpoles to Bullfrog tadpoles.

Although the distributions of native Bullfrogs and Wood Frogs are usually alloptic, fishless aquatic habitats that fail to dry between years may permit Bullfrogs to exploit these habitats where they may have adverse effects on alloptic amphibian populations. Evolutionary experience might play an important role in the adaptation of strategies that promote coexistence with Bullfrog tadpoles. Therefore, in areas where Bullfrogs have been introduced, native larval amphibians might perceive overwintered Bullfrogs differently than amphibians that have evolved with Bullfrogs.

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