INTRODUCTION

The native range of the American bullfrog (Lithobates catesbeianus) encompasses a large portion of the eastern United States and its non-native range is rapidly expanding, both in the United States and around the world (Casper & Hendricks, 2005). Within its range, American bullfrogs typically occur in a variety of permanent aquatic habitats (Casper & Hendricks, 2005). Given its large geographic range and its ability to use a variety of aquatic systems, American bullfrog tadpoles are likely to interact with a wide variety of species and occur in communities with varying species compositions.

In addition, American bullfrogs are common in permanent ponds across the agricultural regions of North America, including the Midwestern United States (Casper & Hendricks, 2005). American bullfrogs are thus likely to be exposed to a variety of agricultural chemicals that are regularly used in these areas (Puglis & Boone, 2007). Of particular concern are agricultural fertilizers, including both nitrogen and phosphorus based compounds. Agricultural runoff and atmospheric deposition of fertilizer compounds, especially nitrate, in ponds and lakes where amphibians breed have the potential to negatively affect amphibian populations by their direct toxic effect on individuals (e.g. Berger, 1989; Rouse et al., 1999). In addition to direct toxic effects, nutrient enrichment can change the composition of the algal community (e.g. Fairchild & Lowe, 1984; Fairchild et al., 1985; Jensen et al., 1994). In particular, Leibold (1999) found that less edible algae became more prevalent at high nutrient levels and more edible algae are more prevalent at low nutrient levels (see also Stevens & Steiner, 2006). Any changes in the quality or quantity of the algal resources has the potential to affect the performance of tadpoles. Such pollution of freshwater habitats is widespread (Naiman & Turner, 2000; Smil, 2000; Fenn et al., 2003; Carpenter & Bennett, 2011), and is likely to increase in the future (Tilman et al., 2001; Galloway et al., 2003; Canfield et al., 2010).

American bullfrog tadpoles therefore potentially face a range of background tadpole communities and a range of background abiotic environments across the species’ distribution. Each of these environmental contexts, biotic and abiotic, can have independent effects, but they may also have interactive effects on the tadpoles. For example, contaminants can change the impact or outcome of competition in tadpole communities (e.g. Warner et al., 1993; Smith et al., 2006; Boone et al., 2007; Distel & Boone, 2010). Anthropogenic pollution of aquatic ecosystems can therefore mediate community-level interactions among anuran tadpoles. We conducted an experiment in which we examined how American bullfrog tadpoles responded to manipulations of the background anuran tadpole community and the abiotic environment. In particular, we manipulated the presence and overall density of American toad (Anaxyrus americanus) and Gray treefrog (Hyla versicolor) tadpoles, and manipulated nutrients (nitrate and phosphate) to alter the abiotic environment, mimicking the effects of agricultural run-off. At low background tadpole densities, enrichment had a positive effect on American bullfrog tadpole mass when the background tadpole community consisted of Gray treefrog tadpoles only, but had a negative effect when the community contained American toad tadpoles (either alone or with Gray treefrogs). The situation was reversed at high background tadpole densities. Nutrient enrichment decreased survivorship in American bullfrog tadpoles. Our results suggest that the wide variety of environmental contexts in which American bullfrog tadpoles are found throughout their native and non-native ranges are likely to affect their success.

Key words: Anaxyrus americanus, Hyla versicolor, Lithobates catesbeianus, nitrate, phosphate
nutrients (nitrate and phosphate) to mimic the effects of agricultural run-off. We expected that the species composition and density of the background tadpole community would affect American bullfrog tadpoles via competition for their shared algal resource. Previous work has shown that Hyla spp. and Anaxyrus spp. can compete with Lithobates spp. (e.g. Hyla spp. and Lithobates spp.; Wilbur & Alford, 1985; Faragher & Jaeger, 1998; Smith et al., 2004; Purrenhage & Boone, 2009; Anaxyrus spp. and Lithobates spp.; Boone et al., 2007; Purrenhage & Boone, 2009, Distel & Boone, 2010). We expected that the outcome of these interactions might be influenced by the addition of the nutrients because of the potential impacts of the nutrients on the quantity or quality of tadpole resources (e.g. algae, periphyton; Leibold & Wilbur, 1992; Murphy et al., 2000; Kiffney & Richardson, 2001).

**MATERIALS AND METHODS**

We collected several egg masses representing clutches from multiple females (>3) of American toads and Gray treefrogs on 8 May 2003, and American bullfrogs on 27 May 2003 from a small pond on the Denison University Biological Reserve located in Licking Co., Ohio, USA (40°5’N, 82°31’W) and incubated them in aged tapwater at 17–19°C in the laboratory. After hatching, tadpoles were maintained in plastic containers (54 cm x 35 cm x 16 cm) and fed ground Purina Rabbit Chow *ad libitum* until they were transferred to mesocosms at Gosner stage 25 (Gosner, 1960).

We used 1135 L cattletanks (*n*=36) filled with 800 L (depth=44 cm) of well water (conductivity=453 mS, dissolved oxygen=9.56 mg L⁻¹, nitrate-N=2 ppm, phosphate-P<1 ppm, ammonium-N<0.1 ppm, hardness=180 ppm) to establish our experimental communities. Mesocosms were placed in an open field (sunlight throughout the experiment) of the mesocosm so all received the same potential amount of incident sunlight. Periphyton mass at the end of the experiment was estimated by removing the periphyton (mean mass=0.008+0.0003 g, *n*=10) and Gray treefrog (mean mass=0.010±0.001 g, *n*=10) tadpoles to the mesocosms on 23 May 2003, and 40 American bullfrog tadpoles (mean mass=0.007±0.0003 g, *n*=10) to each mesocosm on 6 June 2003. The timing of tadpole introductions reflected the natural phenology of these species in local ponds. For mesocosms containing the enrichment treatment we added nutrients (8 mg L⁻¹ NO₃ and 2 mg L⁻¹ PO₄) every 14 days starting on 2 June 2003 to simulate periodic run-off events. These concentrations are within the range of concentrations observed in ponds in agricultural regions of the USA, but above concentrations found in areas not impacted by nutrient run-off (e.g. Sims et al., 1998; Rouse et al., 1999). We did not measure or monitor nitrate or phosphate concentrations so these should be considered nominal concentrations. We ended the experiment on 14 July 2003 after several days of no metamorphs of American toad or gray treefrogs emerging from the mesocosms. At the end of the experiment, we removed all surviving bullfrog tadpoles, and counted and weighed them after blotting dry. Periphyton mass at the end of the experiment was estimated by removing the periphyton from a specified area (15.5 cm x 22.8 cm; all were from the south-facing interior wall of each mesocosm and thus all received the same potential amount of incident sunlight throughout the experiment) of the mesocosm wall, and weighing it after air drying.

**Table 1.** Results of analyses of variance tests examining the effects of background tadpole community density and composition and nutrient enrichment on American bullfrog (*Lithobates catesbeianus*) tadpole survivorship and mean tadpole mass.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>1</td>
<td>0.16</td>
<td>0.70</td>
<td>0.68</td>
<td>0.42</td>
</tr>
<tr>
<td>Composition</td>
<td>2</td>
<td>0.64</td>
<td>0.54</td>
<td>0.50</td>
<td>0.62</td>
</tr>
<tr>
<td>Enrichment</td>
<td>1</td>
<td>11.67</td>
<td>0.0024</td>
<td>1.27</td>
<td>0.27</td>
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<tr>
<td>Density x Composition</td>
<td>2</td>
<td>3.05</td>
<td>0.067</td>
<td>2.32</td>
<td>0.12</td>
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<tr>
<td>Density x Enrichment</td>
<td>1</td>
<td>0.0007</td>
<td>0.98</td>
<td>0.24</td>
<td>0.62</td>
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<tr>
<td>Composition x Enrichment</td>
<td>2</td>
<td>0.041</td>
<td>0.96</td>
<td>0.91</td>
<td>0.42</td>
</tr>
<tr>
<td>Density x Composition x Enrichment</td>
<td>2</td>
<td>3.06</td>
<td>0.067</td>
<td>5.83</td>
<td>0.0089</td>
</tr>
</tbody>
</table>

Error 23
To conform to the assumptions of parametric tests, we transformed tadpole survivorship (the proportion of tadpoles recovered at the end of the experiment) using an arcsine-square root transformation, and mean tadpole mass using a log transformation. We used ANOVAs to analyze treatment effects. For mean tadpole mass, we initially conducted an ANCOVA with the number of American bullfrog tadpoles surviving as the covariate; however, none of the terms in the ANCOVA involving the covariate were significant (all $P>0.13$) and had no effects on the significance or patterns of other terms in the model, thus we used ANOVA to analyze mean tadpole mass. All statistical analyses were conducted on mesocosm means. We removed one mesocosm (high density, American toad and Gray treefrog, enrichment) from our analyses because of a bloom of red algae early in the experiment.

### RESULTS

Survivorship of American bullfrog tadpoles was lower in enrichment mesocosms than in no enrichment mesocosms (enrichment: $0.67±0.08$, $n=17$; no enrichment: $0.94±0.02$, $n=18$; Table 1). No other terms were significant (all $P>0.05$).

There was a significant, complex three-way interaction for mean American bullfrog tadpole mass (Fig. 1; Table 1). In low background tadpole density mesocosms, nutrient enrichment lowered mean American bullfrog tadpole mass in mesocosms containing American toads only and a mix of American toads and Gray treefrogs, but enrichment increased mass in Gray treefrog only mesocosms. In high density mesocosms, enrichment increased mass of American bullfrog tadpoles with American toads only and American toad and Gray treefrog treatments, whereas enrichment decreased American bullfrog tadpole mass in Gray treefrog only treatments. No other terms were significant (Table 1).

High background tadpole density mesocosms had lower periphyton mass than low background tadpole density mesocosms (low density: $0.638±0.123$ g, $n=18$, high density: $0.354±0.096$ g, $n=17$; Table 2). Periphyton mass at the end of the experiment was lower in enrichment than in no enrichment mesocosms (no enrichment: $0.794±0.104$ g, $n=18$, enrichment: $0.189±0.068$ g, $n=17$; Table 2). No other treatment or interaction term was statistically significant (Table 2).

### DISCUSSION

The composition and density of the background tadpole community interacted with nutrient enrichment to affect the growth of American bullfrog tadpoles. At low densities, enrichment had a positive effect on American bullfrog tadpole mass when the background tadpole community consisted of Gray treefrog tadpoles only, but had a negative effect when the background tadpole community contained American toad tadpoles (either American toads only, or both Gray treefrogs and American toads). The situation was reversed at high densities, with enrichment having a negative effect on American bullfrog tadpole mass when the community consisted of Gray treefrog tadpoles only, but having a positive effect when the background tadpole community contained American toad tadpoles. We found no independent effects of background tadpole community composition or density on American bullfrog tadpoles. The patterns of the effects of background tadpole community density and composition and nutrient enrichment on periphyton mass.

Table 2. Results of analysis of variance tests examining the effects of background tadpole community density and composition and nutrient enrichment on periphyton mass.

<table>
<thead>
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<th></th>
<th>df</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>1</td>
<td>5.42</td>
<td>0.029</td>
</tr>
<tr>
<td>Composition</td>
<td>2</td>
<td>0.22</td>
<td>0.81</td>
</tr>
<tr>
<td>Enrichment</td>
<td>1</td>
<td>22.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Density x Composition</td>
<td>2</td>
<td>0.65</td>
<td>0.53</td>
</tr>
<tr>
<td>Density x Enrichment</td>
<td>1</td>
<td>0.63</td>
<td>0.43</td>
</tr>
<tr>
<td>Composition x Enrichment</td>
<td>2</td>
<td>0.058</td>
<td>0.94</td>
</tr>
<tr>
<td>Density x Composition x Enrichment</td>
<td>2</td>
<td>0.23</td>
<td>0.80</td>
</tr>
<tr>
<td>Error</td>
<td>23</td>
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</table>
tadpole density and nutrient enrichment, as well as the interactions between American toads and Gray treefrogs on the growth, survivorship and time to metamorphosis of the American toad and Gray treefrog tadpoles (see Table 3) may combine to be responsible for the patterns we observed in American bullfrog tadpole mass. For example competition between American bullfrogs and American toads is likely to be lowest in the high density, enrichment mesocosms since metamorphosis in American toads was accelerated at high density and in nutrient enrichment treatments, thus potentially explaining why American bullfrog masses tended to be highest in the high density, nutrient enrichment mesocosms with American toads. This effect, combined in complex ways with the other effects of the treatments on the American toads and Gray treefrogs appear likely to drive the observed pattern in the American bullfrogs; however, the linkages do not appear to be straightforward. Previous studies have suggested that bufonid and hylid tadpoles can have effects on ranid tadpoles, but the effects can be context dependent (e.g., Alford & Wilbur, 1985; Alford, 1989; Laurila, 2000). Thus, our results add to the evidence that the outcome of interactions among these groups of tadpoles are context-dependent.

How might nutrient enrichment have mediated the observed effects of the background tadpole community on American bullfrog tadpoles? One possible mechanism is the effect of nutrient enrichment on tadpole resources, namely phytoplankton and periphyton. Nutrient enrichment can change the make-up of the algal community with less edible algae becoming more abundant at high nutrient levels and more edible algae more abundant at low nutrient levels (Leibold, 1999; Stevens & Steiner, 2006). In our experiment, nutrient enrichment also decreased periphyton productivity at the end of the experiment, possibly due to the formation of filamentous algal mats that we observed forming late in the experiment. These mats may have shaded the periphyton. Indeed, increased phytoplankton productivity, including filamentous algae, can result from phosphorus over-enrichment, and can lower periphyton productivity (e.g., Vadeboncoeur et al., 2002; McCormick & Laing, 2003). Thus, nutrient enrichment likely altered the relative abundances of primary producers in our experimental communities, potentially mediating the interactions between the background tadpole community and the American bullfrog tadpoles, and within the background tadpole community. In addition, tadpole growth can be sensitive to the type of algae consumed (e.g., Kupferberg et al., 1994; Waringer-Löschekohl & Schagerl, 2001), and thus any changes in the algal community in response to nutrient enrichment could impact the interactions among the species in this community, especially if different species responded to changes in algal productivity in different ways.

In addition to changing the effects of the background tadpole community on American bullfrog tadpole growth, nutrient enrichment on its own decreased survivorship in American bullfrog tadpoles. The decrease in survivorship of American bullfrog tadpoles in our experiment is consistent with previous work on American bullfrogs and other ranids. The survival of American bullfrog tadpoles exposed to 5 mg L$^{-1}$ N-NO$_3$ as NaNO$_3$ for 6 weeks in mesocosms was reduced compared to control mesocosms (Smith et al., 2006). However, Puglis & Boone (2007) found that exposure to nitrate concentrations up to 10 mg L$^{-1}$ had no significant effect on American bullfrog tadpoles; however, they used ammonium nitrate as their source of nitrate. As far as we are aware, no studies have examined the toxicity of phosphate on American bullfrogs; however, Smith (2007) found no effect of potassium phosphate on the tadpoles of wood frogs (L. sylvaticus) at concentrations up to 20 mg L$^{-1}$. Thus, it may be that the nutrients we added, especially sodium nitrate, had toxic effects, either separately or interacting with each other. However, another possible, but not mutually exclusive, explanation for the negative effects of nutrient enrichment on American bullfrog tadpoles may have been the result of the increased production of large mats of filamentous algae that we observed forming on the surface of nutrient

### Table 3. Summary of the responses of the background tadpole community (Gray treefrogs and American toads) to the treatments used in this experiment (Smith & Burgett, in press).

<table>
<thead>
<tr>
<th>Species</th>
<th>Observed Effect</th>
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</table>
| **Gray treefrog (Hyla versicolor)** | 1) In enrichment mesocosms, Gray treefrogs survived better with American toads and Gray treefrogs than with Gray treefrogs alone, whereas the opposite was true in no enrichment mesocosms.  
2) No significant effects on mean proportion metamorphosing.  
3) At low density, mean number of days to metamorphosis tended to be higher in the American toad and Gray treefrog treatment than in the gray treefrog treatment, whereas at high density the opposite was true ($P=0.07$)  
4) No significant effects on mean metamorph mass. |
| **American toad (Anaxyrus americanus)** | 1) American Toad tadpoles tended to survive better in the American toad and Gray treefrog treatment than in the American toad only treatment ($P=0.064$).  
2) Mean metamorph mass was greater at high density than low density.  
3) American Toads metamorphosed earlier at high density than at low density.  
4) Enrichment accelerated metamorphosis in American toad tadpoles. |
enrichment mesocosms. These mats may have been a cause for mortality of the tadpoles. We observed partially decomposed American bullfrog tadpoles in these mats as we sampled the mesocosms at the end of the experiment. Thus, it appears nutrient enrichment in our experiment resulted in negative effects, likely through its effects on the composition of the algal community, primarily the shift to floating filamentous algae that could both shade out periphyton and serve as an entanglement threat.

In conclusion, nutrient enrichment altered the effects of the background tadpole community on American bullfrog tadpoles, and nutrient enrichment also negatively affected the survivorship of American bullfrog tadpoles. Our results suggest that the wide variety of environmental contexts in which American bullfrog tadpoles are found throughout their native and non-native ranges are likely to affect their success, suggesting that further studies exploring how environmental context affects American bullfrogs will prove useful for understanding their role in amphibian communities, both in their native and non-native ranges.

ACKNOWLEDGEMENTS

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two species of tadpoles mediated by nutrient enrichment. *Herpetologica.*


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