FINAL REPORT

Repatriation as a mechanism for restoring a pond-breeding amphibian community

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Repatriation as a mechanism for restoring a pond-breeding amphibian community.

Final Report on Project #T-22-P-1

This report details on-going work at Warbler Woods Nature Preserve (WWNP), Coles County, Illinois, an 81.5 ha piece of land owned by L. Barrie Hunt. The overall project concerns the monitoring of amphibian populations that utilize four breeding ponds in the southeast portion of the property. Predatory fish previously inhabited two of these ponds. The fish populations were removed by January 2003, and now the primary research objective at WWNP is to restore pre-settlement levels of amphibian diversity through the repatriation of tiger salamanders. This report details the continued recovery of amphibians using the breeding ponds at WWNP and the efforts to repatriate tiger salamanders at one of the ponds.

Study Site & Background

Four ponds in the southeast section of WWNP (Figure 1) are labeled from East to West: A, B, C, and D. Ponds A and B are separated by a 5 m ridge of secondary deciduous forest and understory vegetation. Ponds B and C are separated by 80 m of old field that has been planted with seedlings of deciduous hardwoods in accordance with an existing IDNR restoration objective. Ponds C and D are separated by 280 m of primarily old field that also has been planted with seedlings of deciduous hardwoods. A small access road leading to a barn and an extension of deciduous forest ravine bisecting the old field also separate the latter two ponds.

Prior to the fish removal in January 2003, Pond B contained a stable population of small *Ameiurus melas* (black bullhead catfish); Pond C contained a stable population of centrarchids

(*Lepomis macrochirus* [bluegill], and *Lepomis cyanellus* [green sunfish]). All ponds have stable populations of a variety of invertebrate species (e.g., snails, aquatic insects/larvae, *etc.*). All ponds permanently hold water except for Pond D that has gone dry in 5 of the past 7 years (usually on or before 1 August).

Activities at WWNP in 2006

From 23 February to 2 December, the drift fence-pitfall trap arrays around the four amphibian breeding ponds (constructed in May 2000) were monitored every other day. Specimens caught in the traps (Table 1) were measured (snout-vent length, tail length for metamorphs and salamanders) and sexed (where possible). Where possible, the gender of trapped individuals was also determined. All trapped specimens were given a pond-by-year series of toe clips for future identification. Occasional maintenance on the fence/trap array was also performed.

On 10 March 2006, approximately 2200 *Ambystoma tigrinum* eggs (in multiple masses) were collected from two sites within Stephen A. Forbes State Park (Marion County, Illinois). These egg masses were transported and immediately placed in enclosures (1 m^3) within Pond B at WWNP. From these egg masses, 293 larvae survived to hatching and were released into the pond. To date, one juvenile tiger salamander has been caught in the pitfall traps around that pond, although it was not certain if that individual was produced by the cohort of larvae released into the pond in 2006.

From mid-September to mid October, approximately 20 adult and 30 larval bullfrogs were removed from Pond B at WWNP and euthanized. Tissues from the muscle and gastrointestinal tract of adult specimens were preserved for future analysis of parasite content and levels of chemical pollutants. This action was sanctioned by the Illinois Nature Preserves Commission with the intent of reducing the potential for predation on juvenile tiger salamanders as they left the pond and entered the surrounding forest habitat. On 2 December, all traps sealed to prevent incidental capture during the Winter season of inactivity.

Activities at WWNP in 2007

From 25 February to 1 December, the drift fence and pit-fall trap array was monitored every other day. Specimens caught in the traps (Table 1) were measured (snout-vent length, tail length for metamorphs and salamanders) and sexed (where possible). Where possible, the gender of trapped individuals was also determined. All trapped specimens were given a pond-by-year series of toe clips for future identification. Occasional maintenance on the fence/trap array was also performed.

From mid-June to mid October, 31 adult and 19 juvenile bullfrogs were removed from Pond B at WWNP and euthanized. Tissues from the muscle and gastrointestinal tracts of adult specimens were preserved for future analysis of parasite content and levels of chemical pollutants. Throughout this same period, approximately 120 larvae were collected from Pond B using minnow traps; these specimens were also euthanized. On 1 December 2007, all pitfall traps were sealed to prevent incidental capture during the Winter season of inactivity.

Several attempts were made to collect either egg- or larval-stage tiger salamanders from multiple donor sites in Indiana. Because of erratic climate events in 2007 (late freeze, and a mid-Summer dry spell), however, I was not successful in obtaining tiger salamander egg masses (frozen) or larvae (dessicated). As such, no individuals from this species were repatriated to Pond B in 2007. Adults collected (n=20) from the donor site in early March 2007 have been maintained in an outdoor enclosure that simulates natural environmental conditions. I am hoping that these individuals will breed in captivity in 2008 so as to provide a source of specimens to be repatriated at Pond B.

Results & Projected Outcomes

The sizes of most of the amphibian populations of using the breeding ponds at WWNP have increased since this study began, especially in the ponds that previously held fish. The populations of each species have since fluctuated (Fig. 2), due to different levels of precipitation in each year and to adjustments in trophic dynamics of the larval community (in the absence of fish). Levels of species diversity, however, have increased in all ponds (Table 2), an effect that is especially apparent in ponds where fish have been removed (Fig. 3).

The initial effort of repatriating tiger salamanders was not successful, with a low survival rate to hatching and even lower survival rate to the conclusion of the larval period. I am not discouraged by this outcome for several reasons:

1. At this latitude, some tiger salamander populations are reported to require two activity seasons to complete larval period. As such, the larvae released into Pond B in Spring 2006 might not be detected until the 2008 activity season. Pond B is deep enough (up to 1.5 m at maximum depth) that it will not freeze solid so that larvae can survive periods of surface ice formation.
2. Bullfrog adults are generalist carnivores that attempt to eat much of what they can catch. The sanctioned removal of bullfrog larvae and adults should reduce the predator population over the course of the next few years. This will improve the chances of larval tiger salamanders surviving to metamorphosis and entering the forest habitat surrounding Pond B.

3. Erratic weather events in 2006 and 2007 that happened to coincide with peaks in either breeding activity or larval emergence of tiger salamanders made it very difficult to execute a successful repatriation. After they had been deposited in ponds at the donor sites, egg masses were either killed by severe freezes or low precipitation that caused subsequent dessication. Later in the same years, low precipitation resulted in the donor ponds drying down prior to larval emergence and the death of any tiger salamander larvae that had survived the earlier freezes.

To minimize the influence of anomalous weather events on future efforts to repatriate tiger salamanders at WWNP, I collected 20 adult specimens from a donor site in Indiana. These individuals have been housed in an outdoor enclosure (220 x 100 x 80 cm) under ambient conditions. A thick (75 cm) layer of leaf litter has been placed within the enclosure, thereby insulating the specimens from dramatic fluctuations in temperature. The intent is to have these specimens over-winter under ambient conditions such that they will then breed in captivity upon emergence. Once fertilized eggs are obtained they will be maintained in captivity until hatching, and the larvae subsequently released into Pond B at WWNP.

Tissue samples from the bullfrog larvae and adults removed from Pond B have been preserved. These samples represent material that can provide a preliminary data set for future research at WWNP concerning the impacts of chemical fertilizer and pesticide application on agricultural fields that are immediately adjacent to that area of the property.

Research Products

During the period covered by the grant award, the following presentations were made at scientific conferences based on research conducted at WWNP:

Posters

2006 Gross, L.M., and S.J. Mullin. An amphibian community after fish removal: A tale of four ponds. Midwest Partners for Amphibian & Reptile Conservation, Carbondale, IL.

2006 Walston, L.J., and S.J. Mullin. Population responses of wood frog (*Rana sylvatica*) tadpoles to overwintered bullfrog (*Rana catesbeiana*) tadpoles. Joint Meetings of Ichthyologists & Herpetologists, New Orleans, LA.

Papers

2007 Gross, L.M. and S.J. Mullin. A pond-breeding amphibian community after fish removal: A six-year study. Midwest Fish & Wildlife Conference, Madison, WI.

2007 Gross, L.M. and S.J. Mullin. Compositional changes in a pond-breeding amphibian community after fish removal: The saga continues. Midwest Partners for Amphibian & Reptile Conservation, Chesterton, IN.

2007 Gross, L.M. and S.J. Mullin. Compositional changes in a pond-breeding amphibian community after fish removal: The saga continues. Joint Meetings of Ichthyologists & Herpetologists, St. Louis, MO.

Furthermore, the following manuscripts have been published or accepted for publication based on work conducted at WWNP:

2007 Walston, L.J., and S.J. Mullin. Responses of a pond-breeding amphibian community to the experimental removal of predatory fish. Amer. Midl. Nat. 157:63-73.

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

2008 Walston, L.J., and S.J. Mullin. Variation in surrounding forest habitat influences the initial orientation of juvenile amphibians emigrating from breeding ponds. Can. J. Zool. 86:in press.

J. Brian Towey was the graduate student who helped initiate this project in 2000, and conducted his thesis research at this site from 2001-2003. After being away from campus, Brian returned and successfully defended his thesis in November 2007.

Table 1. Amphibian and reptile species documented in and around four ponds in the southeast portion of Warbler Woods Nature Preserve (Coles County, Illinois) during the 2006 and 2007 activity seasons. Amphibians were all caught in drift fence-pitfall trap arrays around the ponds, whereas reptiles were observed in the ponds or immediately adjacent habitat. Some amphibian specimens were captured on more than one occasion, but were counted only once in the tallies.

	Number Observed	
Taxon	**2006**	**2007**
Caudata		
Ambystoma texanum	794	489
Ambystoma tigrinum	1	
Eurycea cirrigera	2	2
Anura		
Bufo americanus	43	18
Acris crepitans blanchardi	4	3
Hyla versicolor (x *chrysoscelis*)	53	18
Pseudacris crucifer	25	33
Rana catesbeiana	148	192
Rana sylvatica	26	14
Rana utricularia	33	89

Table 1, cont'd.

Taxon	**Number Observed** **2006**	**2007**
Testudines		
Chelydra serpentina	3	1
Terrapene c. carolina	2	2
Chrysemys picta marginata	1	
Lacertilia		
Eumeces laticeps	2	2
Eumeces fasciatus		1
Serpentes		
Diadophis punctatus	1	1
Nerodia sipedon	1	2
Thamnophis sirtalis	2	1
Elaphe spiloides	1	

Table 2. Composition of an amphibian community (expressed as a percentage of individuals captured for each species) in four breeding ponds at Warbler Woods Nature Preserve (Coles County, Illinois) between 2001 and 2007. Reference ponds lacked any fish, whereas mitigated ponds contained populations of predatory fish that were extirpated by January 2003 (the delineation of the pre- and post-removal periods). Percentages do not sum to 100 % due to rounding errors. The pooled values for the Shannon-Wiener Index of species diversity are also shown.

	Pre-removal		Post-removal	
Species	reference pond	mitigated ponds	reference ponds	mitigated ponds
Ambystoma texanum	16.7	2.6	58.9	47.4
Bufo americanus	66.3	90.3	2.9	9.4
Hyla versicolor	0.6	—	1.2	8.1
Pseudacris crucifer	1.1	—	4.4	11.8
Rana catesbeiana	4.1	4.8	7.7	6.7
Rana sylvatica	7.7	1.0	14.5	2.5
Rana utricularia	3.0	0.7	10.3	13.6
Other species	0.5	0.6	0.3	0.6
Shannon-Wiener Index (H')	1.14	0.68	1.32	1.57

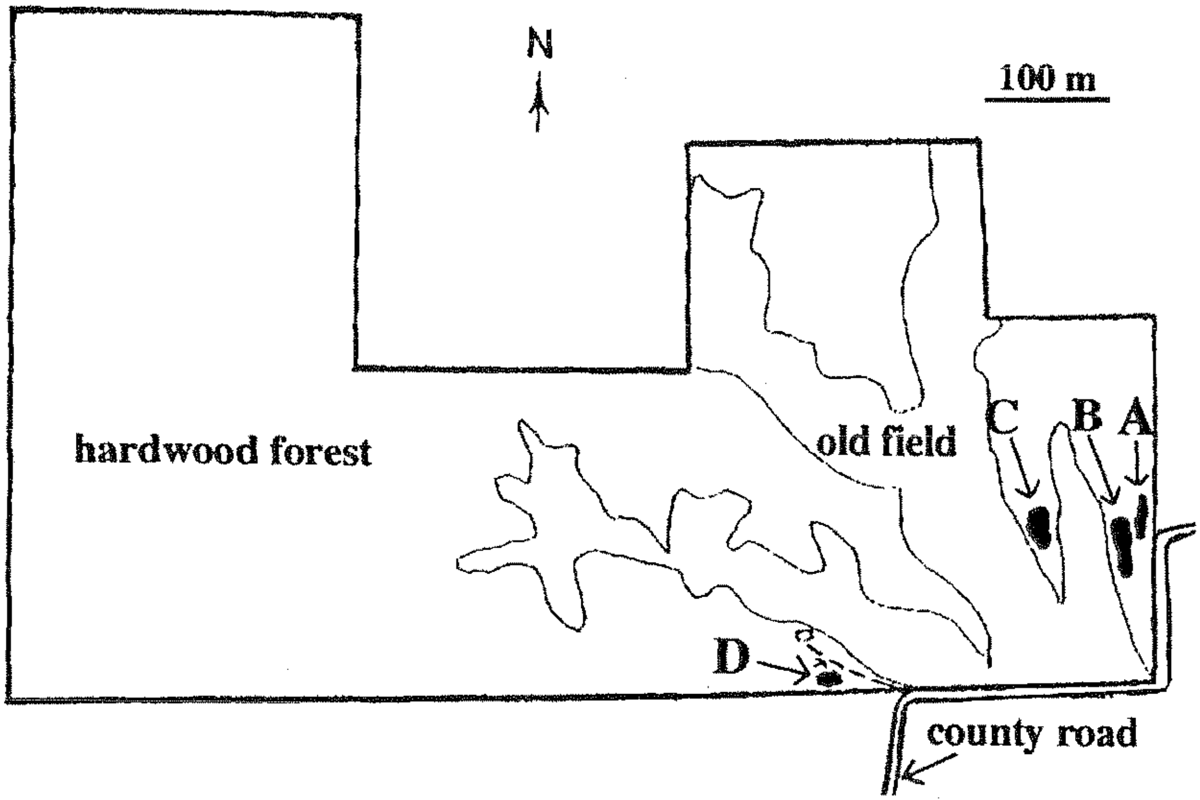

Figure 1. Schematic map of Warbler Woods Nature Preserve (81.5 ha) in Coles County, Illinois. Ponds A, B, and C are permanent, whereas Pond D typically dries down on or before 1 August of each year.

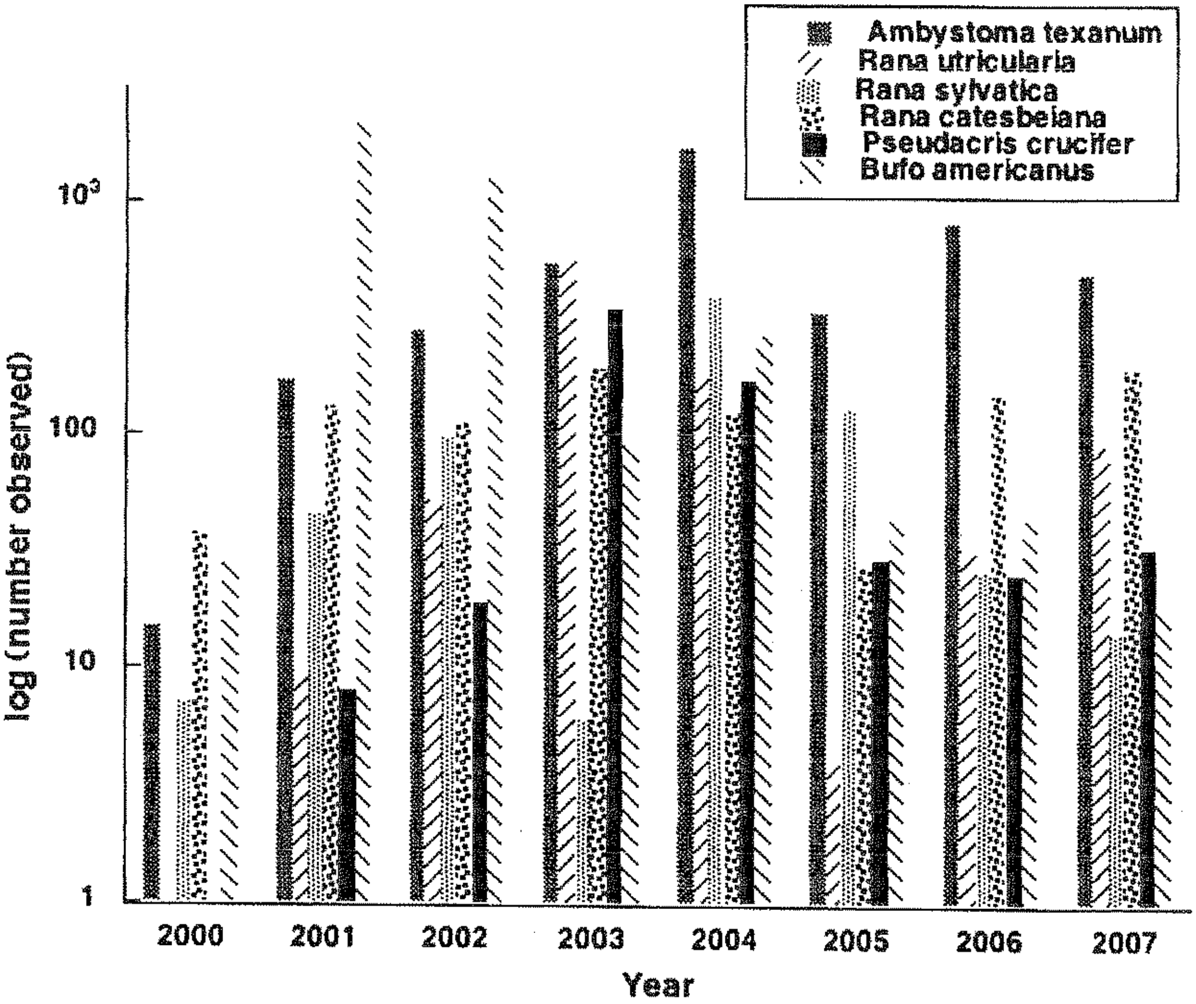

Figure 2. Numbers of individuals (shown in a logarithmic scale) for six species of amphibians trapped at four breeding ponds in Warbler Woods Nature Preserve (Coles County, Illinois) between 2000-2007. Predatory fish were extirpated from two of the ponds prior to the 2003 breeding season.

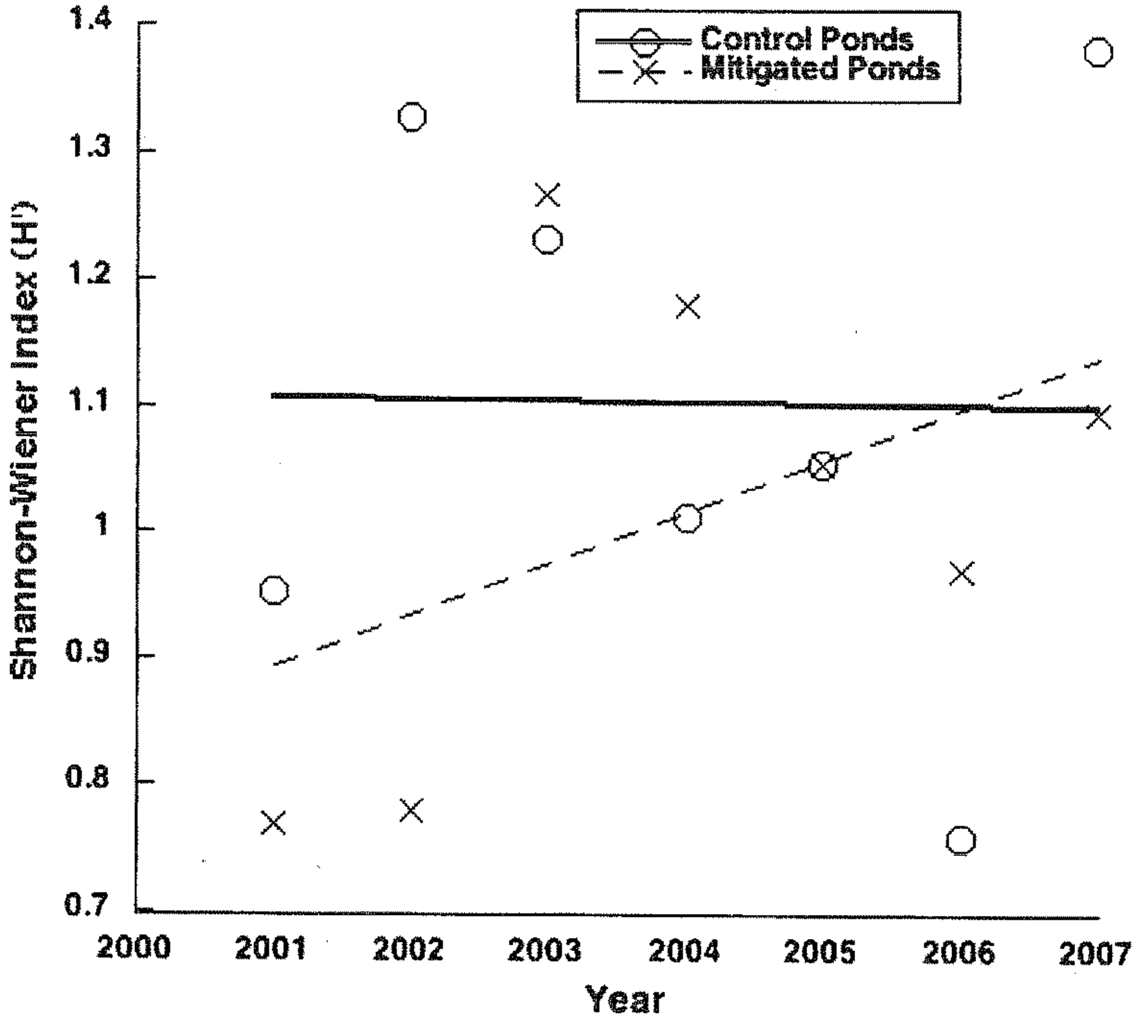

Figure 3. Variation in Shannon-Wiener Diversity Index values calculated from amphibians captured around ponds at Warbler Woods Nature Preserve, Coles County, Illinois. Predatory fish were extirpated from the mitigated ponds prior to the 2003 breeding season. Coefficients of determination (r^2-values) for control and mitigated ponds are 0.0002 and 0.21, respectively.

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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-

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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 depredate 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 naïve 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 22 March 2005, we returned to the two permanent ponds where the syntopic Wood Frogs were originally collected and seined 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 refugia, and the aluminum screening served as a partition between the two microhabitats (with refugia 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) perpendicular 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.

(A) MANOVA

Factor	Wilks λ	*F*	*P*
Population	0.38	12.17	< 0.001
Bullfrog	0.20	30.90	< 0.001
Population × Bullfrog	0.42	10.29	0.002

(B) ANOVA

Response	Population	Bullfrog	Population×Bullfrog
Growth	17.26 (< 0.001)	41.19 (< 0.001)	20.12 (< 0.001)
Survival	5.04 (0.04)	15.04 (0.001)	0.38 (0.55)

(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 ± 2.7%; syntopic, 94.0 ± 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 tad-

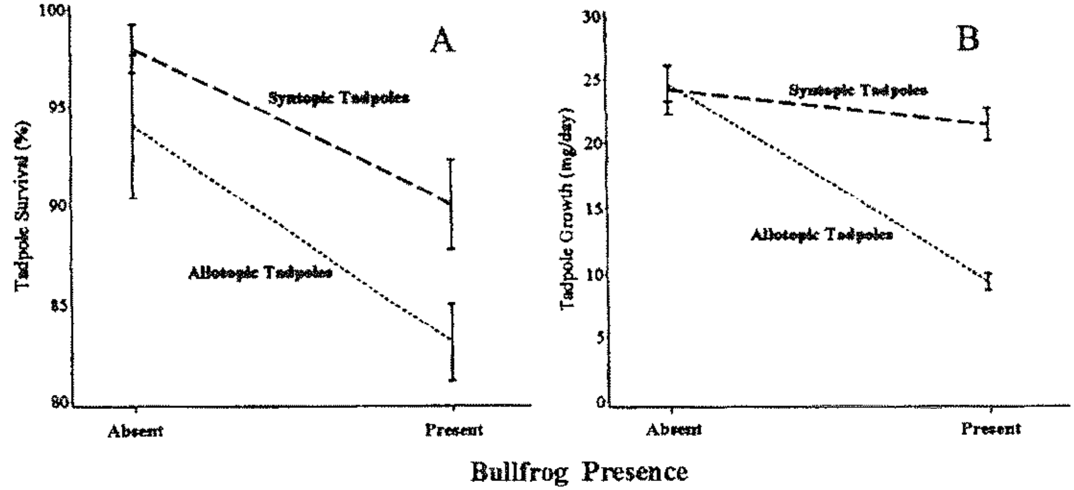

FIG. 1. Survival (A) and Growth (B) of Wood Frog (*Rana sylvatica*) tadpoles from syntopic or allotopic populations in response to the presence of a Bullfrog (*Rana catesbeiana*) tadpole. Data are represented as means ± 1 standard error.

TABLE 2. (A) MANOVA results for Experiment 2, which tested for the effects of Wood Frog (*Rana sylvatica*) population source (allotopic/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.

(A) MANOVA

Factor	Wilks λ	*F*	*P*
Population	0.42	10.52	0.001
Bullfrog	0.19	31.30	< 0.001
Population × Bullfrog	0.34	14.25	< 0.001

(B) ANOVA

Response	Population	Bullfrog	Population×Bullfrog
Activity	4.21 (0.06)	34.53 (< 0.001)	9.94 (0.006)
Refuge Use	19.68 (< 0.001)	38.18 (< 0.001)	22.91 (< 0.001)

poles declined by 61.9%, whereas the growth of syntopic Wood Frog tadpoles declined by 13.2% (Fig. 1B). The mean growth of all Bullfrog tadpoles was 17.6 ± 0.32 mg/day and did not differ between allotopic and syntopic Wood Frog populations ($t_8 = 0.41$; $P = 0.687$).

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 asymetrical effects of overwintered Bullfrog tadpoles on Wood Frog tadpoles from allotopic and syntopic populations. Although

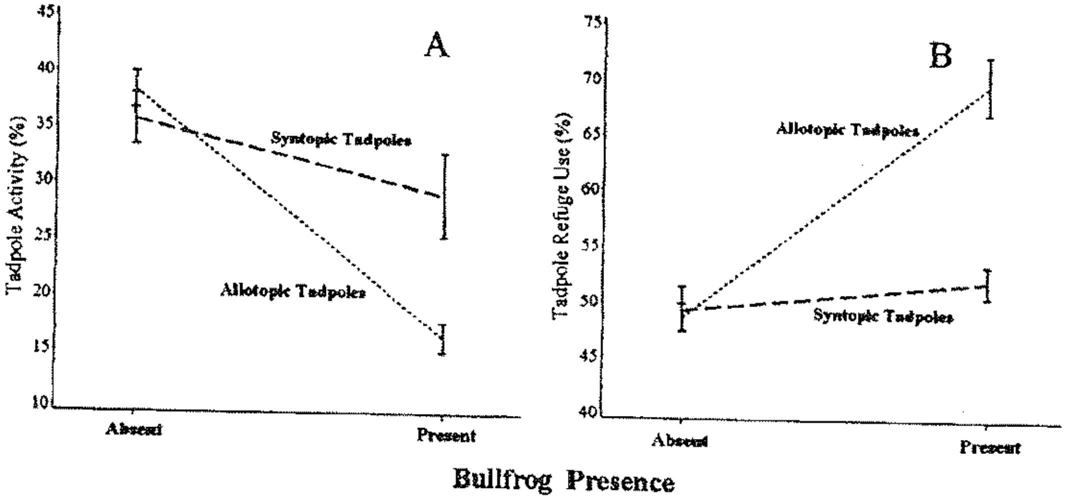

FIG. 2. Activity (A) and refuge use (B) of Wood Frog (*Rana sylvatica*) tadpoles from syntopic or allotopic populations in response to the presence of Bullfrog (*Rana catesbeiana*) tadpole visual and chemical cues. Data are represented as means ± 1 standard error.

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). Although antipredator responses were exhibited only by allotopic Wood Frogs, competition is the most plausible mechanism for our observations of Wood Frog growth and survival because survival rates of both Wood Frog populations to the presence of overwintered Bullfrog tadpoles were similar. We cannot rule out, however, the likelihood that predation occurred during this study.

Only allotopic Wood Frog tadpoles grew slower when in the presence of an overwintered Bullfrog tadpole. In a similar study using outdoor mesocosms, Boone et al. (2004) found that other allotopic larval amphibians (Spotted Salamanders, *Ambystoma maculatum* and Southern Leopard Frogs, *Rana sphenocephala*) grew more slowly when in the presence of sympatric overwintered Bullfrog tadpoles. Amphibian larval growth carries important population-level implications, as larger size at metamorphosis confers greater adult fitness in terms of survival and fecundity (Semlitsch, 1985; Berven, 1990; but see Boone, 2005). Two mechanisms may be responsible for the reduced growth of allotopic Wood Frog tadpoles in our study. Bullfrog tadpoles are superior competitors (Werner and Anholt, 1996), and researchers have observed declines in larval growth of other amphibians resulting from depleted food resources attributed to the presence of Bullfrog tadpoles (Lawler et al., 1999; Adams, 2000; Boone et al., 2004). Therefore, resource depletion resulting from interspecific competition might have resulted in decreased larval growth of allotopic Wood Frogs. This effect might also be a result of the antipredator behaviors, as larval growth is often associated with activity and refuge use (Petranka and Hayes, 1998; Relyea, 2002, 2004). Although these behavioral responses decrease the likelihood of being detected by predators (Werner and Anholt, 1996), this benefit often comes at a cost of slower growth (Relyea, 2002, 2004).

Organisms exhibiting phenotypic plasticity in variable environments are often at a selective advantage compared to organisms that do not exhibit plastic 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 Bullfrog 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 allotopic, fishless aquatic habitats that fail to dry between years may permit Bullfrogs to exploit these habitats where they may have adverse effects on allotopic 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.

Acknowledgments.—This study was conducted in accordance with Institutional Animal Care and Use Committee guidelines (protocol 04-008). We thank D. Mott and the Illinois Department of Natural Resources for permission to collect egg masses (IDNR Scientific Collection Permit NH04-0946). We are grateful to E. Bollinger, K. Kruse, M. Walston, and two anonymous reviewers for their constructive comments on previous drafts of this manuscript.

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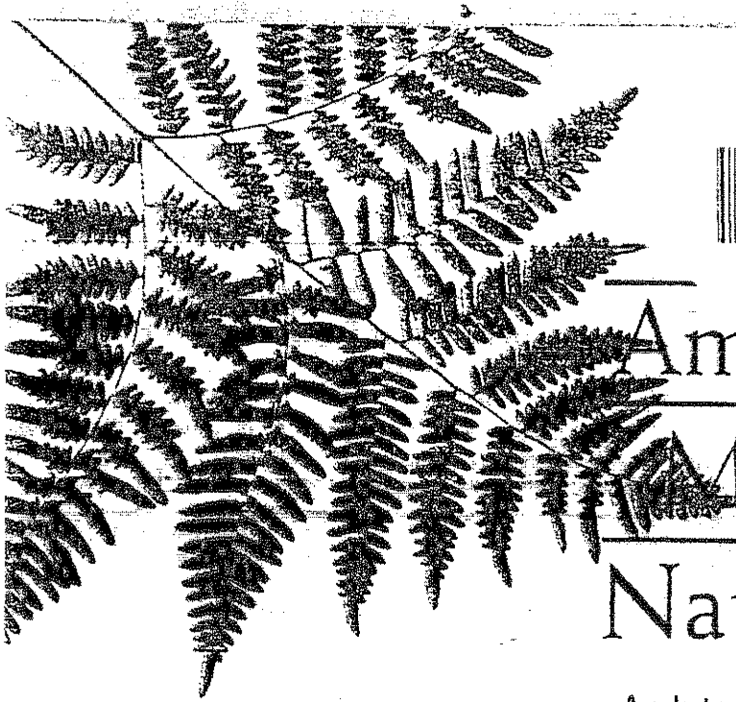

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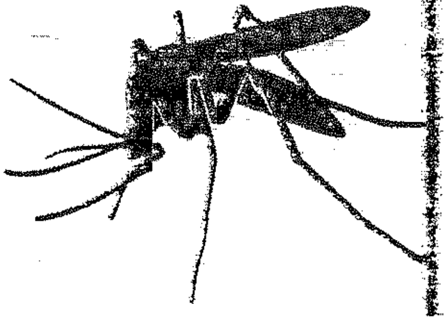

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Responses of a Pond-breeding Amphibian Community to the Experimental Removal of Predatory Fish

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ABSTRACT.—Introduced species have become one of the most important anthropogenic impacts in aquatic ecosystems and are implicated in the declines of many native amphibian populations. Despite the effects of introduced fish species on amphibian communities, there is little information concerning the responses of amphibians following the removal of introduced predatory fish. We present results of a 4-y field experiment designed to determine the amphibian community- and population-level responses to the removal of introduced predatory fish from two experimental ponds. We observed an increase in amphibian species diversity in experimental ponds following the removal of fish. We also observed temporal changes in smallmouth salamander (*Ambystoma texanum*) population dynamics. Although *A. texanum* size at metamorphosis was unaffected by introduced fish in experimental ponds, fish removal resulted in prolonged *A. texanum* larval period and improved reproductive success. We attribute these responses to increases in *A. texanum* larval densities, as we caught considerably more metamorphosed *A. texanum* juveniles from experimental ponds following the removal of fish. Collectively, these observations support previous studies indicating that introductions of predatory fish have adverse effects on amphibians at both the community and population levels. Furthermore, our results suggest that amphibian communities are capable of recovering from environmental perturbations.

INTRODUCTION

The effects of anthropogenic environmental perturbations on amphibian populations ive received considerable attention, particularly the effects of introduced species (Collins id Storfer, 2003). In many aquatic systems introduced species constitute the most iportant anthropogenic impact (U.S. Congress, 1993). Whether native or exotic, human-sisted introductions of predatory fish into previously fishless aquatic habitats have had lverse effects on amphibian populations (Kiesecker, 2003). Introduced fish may serve as mpetitors and predators to pond-breeding amphibians (Kiesecker and Blaustein, 1998; ler *et al.*, 1998; Knapp and Matthews, 2000) and may be responsible for the extirpation of tive amphibian populations (Bradford, 1989).

Predatory fish are important agents of amphibian community structure (*see* Hecnar and Closkey, 1997), capable of altering amphibian abundance and distribution via a number mechanisms. Many fish species are capable of consuming amphibian eggs and larvae yler *et al.*, 1998; Goodsell and Kats, 1999), directly influencing amphibian populations decreasing reproductive success and subsequent juvenile recruitment. Furthermore, males of many amphibian species avoid breeding in bodies of water containing fish (Kats id Sih, 1992; Hopey and Petranka, 1994), indicating that the presence of fish may directly effect amphibian populations by limiting the number of potential breeding sites. edatory fish also have adverse effects on amphibian larval behavior, growth, and survival etranka *et al.*, 1987; Kats *et al.*, 1988; Hoffman *et al.*, 2004), which may compromise the ness of surviving amphibian larvae and future amphibian population viability (Semlitsch *et* , 1988; Berven, 1990; *but see* Boone, 2005).

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The smallmouth salamander (*Ambystoma texanum*) is distributed throughout the midwestern United States and exhibits geographic variation in its mode of reproduction (Petranka, 1998). In some populations in Kentucky, Indiana and Ohio, *A. texanum* may breed in streams (Petranka, 1983), whereas most *A. texanum* populations in Illinois breed in woodland ponds (Phillips *et al.*, 1999). Larval *A. texanum* are important in structuring amphibian communities by depredating zooplankton and larval anurans (Wilbur, 1997). Because *A. texanum* larvae are susceptible to predation by native fish species (Kats, 1988), their distribution is often limited to fishless aquatic habitats (Petranka, 1983; Phillips *et al.*, 1999). Although the larval distributions of most Ambystomatid salamander populations are influenced by predatory fish, previous studies have shown that a small proportion of salamander larvae are capable of surviving to metamorphosis in the presence of fish (Petranka, 1983; Figiel and Semlitsch, 1990; Tyler *et al.*, 1998; Hoffman *et al.*, 2004). Therefore, low rates of juvenile recruitment might allow *A. texanum* populations exhibit a minimum level of viability in habitats occupied by fish. At the landscape level, these populations might also be augmented from the colonization of individuals from nearby source populations (Semlitsch, 2002).

Despite the numerous reports describing the negative effects of introduced fish on amphibian populations, there is a growing body of evidence indicating that these impacts can be reversed (Knapp *et al.*, 2001; Hoffman *et al.*, 2004; Vredenburg, 2004). Following the removal of predatory fish, researchers have observed increased rates of colonization by pond-breeding amphibian species to restored wetlands, as well as shifts in larval behavior that benefit amphibian larval growth and survival (Knapp *et al.*, 2001; Hoffman *et al.*, 2004; Vredenburg, 2004). However, no study has examined amphibian community-level responses following the removal of predatory fish. In this study, we elucidate the community- and population-level responses of amphibians following the removal of predatory fish from two permanent ponds in east-central Illinois by quantifying amphibian community and population dynamics during periods before and after the experimental removal of fish. We then compare these observations to those recorded at two nearby fishless ponds. The objectives of this study were to: (1) determine the effects of introduced fish on amphibian species diversity, and (2) describe the responses of *Ambystoma texanum* to the presence and absence of fish.

METHODS

Study site.—We conducted our study at Warbler Woods Nature Preserve (WWNP; 39°26′N; 88°8′W), an 81.5 ha parcel of land in Coles County, Illinois managed by the Illinois Nature Preserves Commission. The site is dominated by a mixture of oak-hickory hardwood forests and old-field upland habitat (primarily goldenrod *Solidago* spp.). Four woodland ponds, ranging between 0.05 and 0.10 ha in size, were created in the early 1980's (K. Kruse, pers. comm.). We labeled these ponds east to west alphabetically: A, B, C, and D. Vegetation in and immediately peripheral to all four ponds included green algae, common duckweed (*Lemna minor*), Dudley's rush (*Juncus dudleyi*), stinging nettle (*Urtica dioica*), as well as a few other aquatic macrophytes. All ponds were of permanent hydroperiod except for Pond D, which filled in late winter and dried before 2 Aug. of each year of this study.

Centrarchids (*Lepomis macrochirus* and *L. cyanellus*) were introduced into Pond C and black bullhead catfish (*Ameiurus melas*) were introduced into Pond B between 1985 and 1986 (K. Kruse, pers. comm.). Centrarchids and black bullhead catfish are capable of depredating amphibian larvae (Kruse and Francis, 1977; Figiel and Semlitsch, 1990; Adams, 2000), and laboratory trials have revealed that the centrarchids and black bullhead catfish captured from the ponds at WWNP depredate syntopic larval amphibians (S. Mullin, unpubl.). Therefore, we speculated that the introductions of these fish species into Ponds B and C at WWNP likely had adverse effects on amphibian community and population dynamics. Fish were observed in both ponds during intermittent field visits between the mid-1980's and late-1990's (K. Kruse, pers. comm.), and fish were still inhabiting the two ponds in the spring of 2000 when this study commenced.

Sampling.—Drift fence/pitfall trap arrays were constructed around each pond in May 2000 to census the amphibian community (Corn, 1994). The drift fence consisted of plastic-weave silt fence material, approximately 45 cm high with 10 cm buried beneath the soil surface. Pitfall traps (3-L plastic tubs, 20-cm deep) were placed on both sides of the fence at 7.5-m intervals. Due to inter-pond size variation, there were unequal numbers of pitfall traps among ponds. In total, there were 19, 23, 14 and 12 pairs of pitfall traps for Ponds A, B, C and D, respectively. Small (<2 mm diameter) holes were drilled in the bottom of the pitfalls to allow water to drain and prevent captured organisms from drowning. Pitfall traps were checked during the amphibian activity season (early Feb. through early Dec.) at least once every 48 h. We placed lids on the buckets during periods of inactivity to prevent mortality of non-target organisms such as small mammals. We were unable to obtain complete census data for all of 2000; therefore, only data collected from 2001 through 2004 were used in this study.

For all amphibians captured, we measured the snout-vent length (SVL; ±1 mm) and determined the sex of adult amphbibians captured during the breeding season by inspecting the swollen cloacae of male salamanders (*Ambystoma*) and the swollen nuptial pads on the fore-feet of male anurans. We identified juvenile amphibians as those that were captured the same year in which metamorphosis occurred. We marked all captured amphibians to a year-by-pond specific cohort using a pre-determined pattern of toe clipping (*from* Donnelly *et al.*, 1994). All recaptures were omitted from analyses.

In Dec. 2001, Rotenone, a plant-based isoflavonoid pesticide commonly used in fish management (McClay, 2000), was experimentally applied to Ponds B and C to eradicate all fish (*see* Mullin *et al.*, 2004). Although Rotenone can be toxic to non-target organisms including amphibians (Fontenot *et al.*, 1994), there is evidence that amphibians might be less sensitive to the pesticide than fish and are capable of recovering from exposure (Mullin *et al.*, 2004). Rotenone has a greater half-life in colder water (Gilderhus *et al.*, 1988) and is, therefore, most effective in lentic habitats when applied during the winter while most pond-breeding amphibians are dormant and utilizing the terrestrial habitats surrounding the ponds. We presumed that the poison was effective for less than 30 d (*from* Gilderhus *et al.*, 1988), becoming inert before amphibian activity resumed the following spring. Bullfrog (*Rana catesbeiana*) tadpoles were the only amphibian to overwinter in the WWNP ponds and were thus the only amphibian species exposed to Rotenone during application. However, *R. catesbeiana* were ubiquitous throughout WWNP and we assumed recolonization to the treated ponds was likely should the poison have adversely affected overwintering tadpoles. The application of Rotenone in December 2001 did not completely remove all black bullhead catfish from Pond B and a second dose was applied to only that pond in January 2003, approximately 40 d before amphibian breeding activity for the 2003 season commenced.

We grouped the four ponds into two categories: experimental ponds, ponds in which fish had been present and subsequently removed (Ponds B and C); and reference ponds, ponds in which fish had never been present (Ponds A and D). Temporal periods were determined with respect to Rotenone application (pre-application or post-application). The post-application period commenced after the 2001 season for Pond C and after the 2002 season for Pond B because these were the first years in which fish were completely removed from the respective experimental pond. The period between the 2002 and 2003 field season was used to distinguish temporal periods in reference ponds, as this was the final period in which Rotenone was applied to any of the experimental ponds.

Statistical analyses.—We determined the annual relative abundance of all amphibian species captured at each WWNP pond. To characterize temporal changes in amphibian community composition between pond types, we used the Shannon-Weiner Diversity Index ($H' = \Sigma p_i \log_{10} p_i$) to calculate amphibian species diversity within each pond for every year. We tested for the effects of temporal period and pond type on amphibian species diversity by performing a nonparametric Friedman two-way analysis of variance by ranks test (PROC GLM; SAS Institute, 1989).

We assessed *Ambystoma texanum* population-level responses to the presence and absence of fish by examining the temporal changes in larval period (amount of time for an individual larva to reach metamorphosis), size at metamorphosis, and juvenile recruitment. To calculate larval period, we used the Julian date at which metamorphosed juvenile *A. texanum* were captured. Size at metamorphosis was quantified as the SVL (±1 mm) of all juvenile *A. texanum* emigrating from each pond. Because *A. texanum* size and date at metamorphosis were not independent, we analyzed the effects of temporal period and pond type on *A. texanum* size and date at metamorphosis using multivariate analysis of variance (MANOVA; PROC GLM; SAS Institute, 1989) followed by univariate analyses of variance (ANOVA). We determined annual juvenile recruitment at each pond as the proportion of metamorphosing juveniles to the number of breeding female *A. texanum* that visited the pond during the breeding season and tested for the effects of fish on *A. texanum* juvenile recruitment by performing a nonparametric Friedman two-way analysis of variance by ranks test (PROC GLM; SAS Institute, 1989) between both pond types and temporal periods.

We followed all significant univariate interactions with a Tukey-Kramer Multiple Comparison Test. Due to unequal sample sizes among treatments, all analyses are reported using type III sums of squares. All analyses were performed using SAS 9.1 (SAS Institute, 1989).

RESULTS

Fish have not been documented in either of the two experimental ponds since the final application of Rotenone to Pond B in Jan. of 2003. We observed a total of 10 amphibian species among the four WWNP ponds during our study (Table 1). There was no effect of pond type ($F_{1,15} = 0.03$; $p = 0.86$) or temporal period ($F_{1,15} = 1.19$; $p = 0.30$) on amphibian species diversity; however, there was a significant pond type-by-temporal period interaction ($F_{1,15} = 11.42$; $p = 0.005$). Prior to the removal of fish from the experimental ponds, amphibian species diversity was 3.5 times greater in the reference ponds than in the experimental ponds (Table 1). American toads (*Bufo americanus*) were the most abundant amphibian species during this temporal period, accounting for 90.5% and 66.8% of the captures at experimental and reference ponds, respectively. Amphibian community composition changed for both pond types following the removal of fish from the experimental ponds. *Ambystoma texanum* became the most abundant species, accounting for 40.6% and 54.0% of all amphibians in experimental ponds and reference ponds, respectively. Results of the Tukey-Kramer Multiple Comparisons Test revealed that only the species diversity of the experimental ponds changed between temporal periods ($p = 0.01$). Following the removal of fish, amphibian species diversity within experimental ponds increased by 341%, whereas amphibian species diversity increased by 9.6% within reference ponds (Table 1).

TABLE 1.—Mean relative abundance (percent) of amphibians captured in experimental ($n = 2$) and reference ($n = 2$) ponds at Warbler Woods Nature Preserve, Coles County, Illinois, during each temporal period (pre-application or post-application). Mean annual Shannon-Weiner Diversity Index values (H': ±1 SE) are displayed for both pond types during each temporal period

Species	Pre-application		Post-application	
	Experimental ponds	Reference ponds	Experimental ponds	Reference ponds
Ambystoma texanum	2.55	16.82	40.60	53.96
Bufo americanus	90.50	66.78	12.03	2.32
Bufo fowleri	0.08	0.07	–	0.14
Acris crepitans	0.19	0.07	0.30	0.08
Hyla versicolor × *chrysoscelis*	–	0.34	3.31	1.11
Pseudacris crucifer	0.16	1.17	14.10	4.69
Pseudacris triseriata	–	–	0.24	0.05
Rana catesbeiana	4.84	4.14	6.81	7.43
Rana sylvatica	0.98	7.72	2.17	16.18
Rana utricularia	0.71	2.89	14.94	14.11
Mean H' (±1 SE)	0.15 (0.03)	0.52 (0.08)	0.66 (0.04)	0.57 (0.04)

We captured 1302 emerging juvenile *Ambystoma texanum* from both pond types during the study period. Twelve juveniles were captured at the experimental ponds during the years when fish were present, whereas 861 juveniles were captured during the years following fish removal. Twenty-nine juveniles were captured at the reference ponds prior to the removal of fish from the experimental ponds, whereas 400 juveniles were captured after fish removal. There was an effect of pond type, temporal period, and their interaction on *A. texanum* larval period and size at metamorphosis (Wilks' $\lambda > 0.98$; $F_{2,1297} > 4.38$; $p < 0.01$). Throughout the study, juvenile *A. texanum* from the experimental ponds metamorphosed earlier than juveniles from the reference ponds ($F_{1,1298} = 23.47$; $p < 0.001$) and there was a significant pond type-by-temporal period interaction on *A. texanum* larval period ($F_{1,1298} = 5.96$; $p = 0.015$; Fig. 1). Tukey-Kramer Multiple Comparisons Tests revealed that *A. texanum* larval period changed only in the experimental ponds following fish removal ($p = 0.025$). Following the removal of fish, *A. texanum* larval period from experimental ponds increased by 12.0%, whereas larval period length from reference ponds decreased by 6.6% (Fig. 1).

Throughout the study period, juvenile *Ambystoma texanum* emerging from experimental ponds were larger than juveniles emerging from reference ponds ($F_{1,1298} = 48.15$; $p < 0.001$; Fig. 1). The average size of juvenile *A. texanum* at metamorphosis was 36.3 ± 0.3 mm and 30.5 ± 0.1 mm for experimental and reference ponds, respectively. Overall, *A. texanum* size at metamorphosis decreased in the years following fish removal ($F_{1,1298} = 133.95$; $p < 0.001$; Fig. 1), decreasing by 19.3% and 18.7% for experimental and reference ponds, respectively. There was no pond type-by-temporal period interaction on *A. texanum* size at metamorphosis ($F_{1,1298} = 2.41$; $p = 0.12$).

There was no effect of pond type on *Ambystoma texanum* juvenile recruitment ($F_{1,12} = 2.21$; $p = 0.163$). However, juvenile recruitment was affected by temporal period ($F_{1,12} = 13.52$; $p = 0.003$), and there was a significant pond type-by-temporal period interaction ($F_{1,12} = 16.35$; $p = 0.002$; Fig. 2). Results of the Tukey-Kramer Multiple Comparisons Tests showed that *A. texanum* juvenile recruitment increased only for the experimental ponds following the removal of fish ($p = 0.017$). After the removal of fish, *A.*

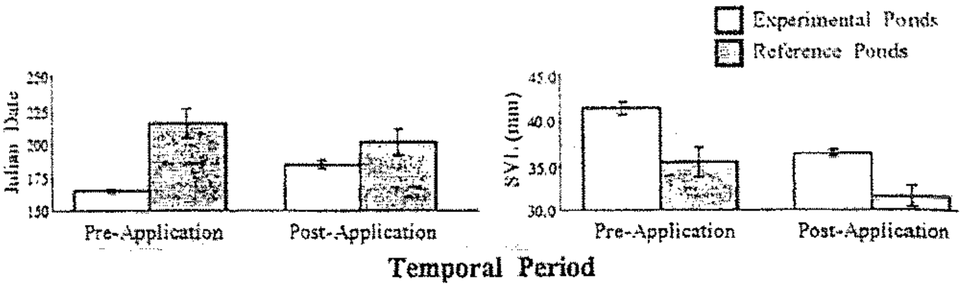

FIG. 1.—Changes in larval smallmouth salamander (*Ambystoma texanum*) larval period (date of metamorphosis) and size at metamorphosis between temporal periods and pond types at Warbler Woods Nature Preserve, Coles County, Illinois. All data are presented as means ± 1 SE

texanum juvenile recruitment increased 73-fold at the experimental ponds, whereas juvenile recruitment at the reference ponds increased 6-fold (Fig. 2). There was no temporal change in *A. texanum* juvenile recruitment at the reference ponds (p = 0.20)

DISCUSSION

This study demonstrates that the presence of introduced fish in the WWNP experimental ponds had adverse effects on native amphibians at the community and population levels. Differences in amphibian species diversity at WWNP were likely due to changes in species relative abundance rather than changes in species composition, as species richness within and between pond types did not vary considerably over the entire study period (Table 1). Our findings are consistent with previous studies describing the negative association between fish presence and amphibian community structure. Hecnar and M'Closkey (1997) discovered that amphibian species richness was lower in ponds containing predatory fish

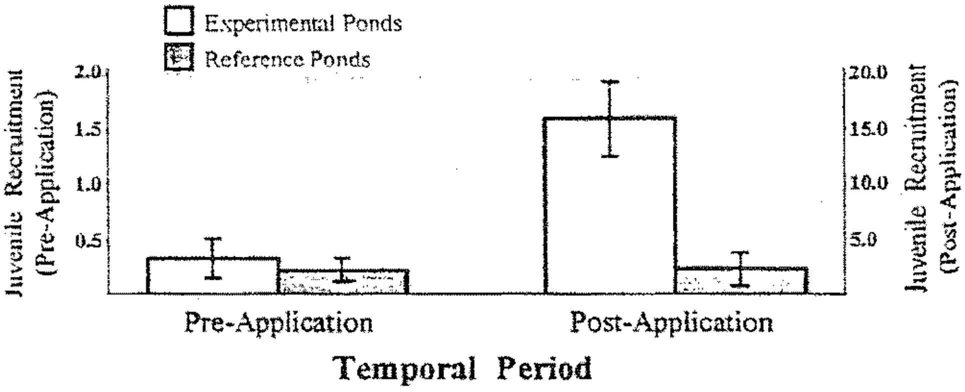

FIG. 2.—Changes in *Ambystoma texanum* juvenile recruitment (proportion of metamorphosing juveniles to number of breeding females) in experimental and reference ponds between temporal periods at WWNP. Note: the scale for post-application recruitment (right-axis) is ten times greater than the pre-application recruitment (left-axis). All data are presented as means ± 1 SE

than in ponds lacking predatory fish. Semlitsch (1988) also noted that the distributions of some *Ambystoma* communities are influenced by fish predation. During the years when fish were present at WWNP, the amphibian species diversity of reference ponds was (on average) 3.5 times greater than the diversity of experimental ponds. Following the removal of fish, amphibian species diversity improved only for experimental ponds, indicating that introduced fish were primarily responsible for the low amphibian species diversity at experimental ponds during the pre-application period. Thus, our before-and-after investigation provides causal evidence that introduced fish had negative effects on the amphibian community at the WWNP ponds, and that the removal of predatory fish improved amphibian species diversity.

The interaction between predation and interspecific competition is an important factor that regulates many amphibian communities (Wilbur, 1987) and is one plausible explanation for the temporal shifts in amphibian species diversity observed at the WWNP experimental ponds. During the years when fish were present, American toads (*Bufo americanus*) were the most abundant amphibian species at the experimental ponds, and metamorphosing juveniles accounted for 76.8% of all *B. americanus* captured during this study. Hence, the changes in *B. americanus* relative abundance likely occurred at the larval stage. Previous studies have demonstrated the relative unpalatability of *B. americanus* tadpoles compared to competing larval anurans (Kruse and Stone, 1984; Smith *et al.*, 1999). The presence of fish might have had positive indirect effects on *B. americanus* abundance by depredating and reducing the abundance of competing amphibians. However, *B. americanus* tadpoles are also inferior competitors (Wilbur, 1987). Therefore, the observed decline in *B. americanus* relative abundance after the removal of fish might be attributable to increased levels of interspecific competition following the release of predation pressure on other larval amphibians.

During the years when fish were present, juvenile *Ambystoma texanum* at the experimental ponds metamorphosed an average of 51 d earlier and were 17.5% larger than juveniles metamorphosing from fishless reference ponds. Following fish removal, juvenile *A. texanum* from the experimental ponds metamorphosed an average of 17 d earlier and were 15.5% larger than juveniles metamorphosing from reference ponds (Fig. 1). We found that the presence of fish in the experimental ponds reduced *A. texanum* larval period, as juvenile *A. texanum* at the experimental ponds metamorphosed earlier than juveniles from reference ponds during the years of fish presence. Others have discovered similar developmental responses of *Ambystoma* to predation risk. For example, larval Jefferson salamanders (*A. jeffersonianum*) increase development rate, metamorphosing earlier and at a larger body size when in the presence of aquatic predators (Cortwright, 1988; Wells and Harris, 2001). Reduced salamander larval period in the presence of predators may be due to decreased rates of intraspecific competition resulting from reduced larval density (Skelly, 1992; Wells and Harris, 2001). Increased amphibian larval densities often result in delayed metamorphosis (Scott, 1990), and may explain the prolonged larval period for *A. texanum* following the removal of fish from WWNP experimental ponds (Fig. 1). We captured considerably more juvenile *A. texanum* during the years following fish removal, and during this temporal period we captured over twice as many juvenile *A. texanum* at the experimental ponds (n = 861) than at reference ponds (n = 400). Although we were unable to directly quantify *A. texanum* larval densities in this study, we assumed that the increased number of juveniles captured following the removal of fish is attributable to increased larval densities.

Over the course of this study, juvenile *Ambystoma texanum* emerging from the experimental ponds were 15.7% larger than those emerging from the reference ponds. However, the presence of fish had no apparent effect on *A. texanum* growth, as the size of juveniles emerging from both pond types decreased following the removal of fish (Fig. 1). The decrease in *A. texanum* size at metamorphosis for juveniles at both pond types might be due to increased *A. texanum* larval densities. Among other abiotic and biotic factors, amphibian larval growth is density-dependent (Wilbur, 1987; Werner, 1986) and increased amphibian larval densities often result in slower growth (Scott, 1990). Therefore, the temporal variability in *A. texanum* larval densities may also explain the between pond-type similarity in *Ambystoma texanum* size at metamorphosis.

Following the removal of fish, *Ambystoma texanum* juvenile recruitment increased only within experimental ponds (Fig. 2). Field and laboratory experiments have demonstrated the adverse effects of introduced fish on the reproductive success of other native amphibian species (Goodsell and Kats, 1999; Smith *et al.*, 1999). Although the fish species introduced into the WWNP experimental ponds are capable of consuming amphibian egg masses and larvae (Kruse and Francis, 1977; Figiel and Semlitsch, 1990; Adams, 2000), we did not directly observe predation of *A. texanum* larvae by the introduced fish during this study. However, the increase in juvenile recruitment at the experimental ponds coincided with fish removal, indicating that fish presence likely suppressed *A. texanum* juvenile recruitment. These observations support those of previous studies that have documented rapid (*i.e.*, <5 y) improvement in amphibian reproductive success following the removal of introduced fish, providing further evidence that some amphibian populations are capable of recovery following the removal of predatory fish (Knapp *et al.*, 2001; Hoffman *et al.*, 2004; Vredenburg, 2004).

We observed significant changes in the amphibian community at WWNP in less than 2 y after the removal of fish from the experimental ponds. These observations indicate that the application of Rotenone might be an effective management strategy for future amphibian conservation efforts (*see* Mullin *et al.*, 2004). The quick recovery of amphibians at the experimental ponds was likely due to colonization of amphibians from the nearby reference ponds, despite low levels of juvenile recruitment for most amphibian species at the experimental ponds during the years when fish were present. Although improvements in amphibian species diversity and *Ambystoma texanum* larval dynamics at WWNP coincided with, and are most likely attributable to, the presence and absence of fish, some of our results might be explained by abiotic processes such as variation in precipitation, temperature, or pond hydroperiod (Semlitsch, 1985; Phillips *et al.*, 2002). However, analyses of daily climatic data obtained from Coles County Regional Airport, approximately 12 km from WWNP, revealed no differences in mean daily precipitation or temperature patterns between temporal periods (MANCOVA; $F_{2,1450} = 0.600$; $p = 0.549$). As such, our 4-y study provides evidence that amphibian communities are capable of recovering from environmental perturbations over an ecologically relevant time scale. Because amphibian population sizes and reproductive output naturally fluctuate among years (Pechmann and Wilbur, 1994), long-term studies will be vital for accurately determining the effects of anthropogenic perturbations on amphibian communities.

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1st YEAR REPORT

Repatriation as a mechanism for restoring a pond-breeding amphibian community

31 December 2007

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Project #T-22-P-1

Funded period: 1 January 2006 – 31 December 2007

Repatriation as a mechanism for restoring a pond-breeding amphibian community.

1st Year Report on Project #T-22-P-1

This report details on-going work at Warbler Woods Nature Preserve (WWNP), Coles County, Illinois, an 81.5 ha piece of land owned by L. Barrie Hunt. The overall project concerns the monitoring of amphibian populations that utilize four breeding ponds in the southeast portion of the property. Predatory fish previously inhabited two of these ponds. The fish populations were removed by January 2003, and now the primary research objective at WWNP is to restore pre-settlement levels of amphibian diversity through the repatriation of tiger salamanders. This report details the continued recovery of amphibians using the breeding ponds at WWNP and the efforts to repatriate tiger salamanders at one of the ponds.

Study Site & Background

Four ponds in the southeast section of WWNP (Figure 1) are labeled from East to West: A, B, C, and D. Ponds A and B are separated by a 5 m ridge of secondary deciduous forest and understory vegetation. Ponds B and C are separated by 80 m of old field that has been planted with seedlings of deciduous hardwoods in accordance with an existing IDNR restoration objective. Ponds C and D are separated by 280 m of primarily old field that also has been planted with seedlings of deciduous hardwoods. A small access road leading to a barn and an extension of deciduous forest ravine bisecting the old field also separate the latter two ponds.

Prior to the fish removal in January 2003, Pond B contained a stable population of small *Ameiurus melas* (black bullhead catfish); Pond C contained a stable population of centrarchids

(*Lepomis macrochirus* [bluegill], and *Lepomis cyanellus* [green sunfish]). All ponds have stable populations of a variety of invertebrate species (e.g., snails, aquatic insects/larvae, *etc.*). All ponds permanently hold water except for Pond D that has gone dry in 5 of the past 7 years (usually on or before 1 August).

Activities at WWNP in 2006

From 23 February to 2 December, the drift fence-pitfall trap arrays around the four amphibian breeding ponds (constructed in May 2000) were monitored every other day. Specimens caught in the traps (Table 1) were measured (snout-vent length, tail length for metamorphs and salamanders) and sexed (where possible). Where possible, the gender of trapped individuals was also determined. All trapped specimens were given a pond-by-year series of toe clips for future identification. Occasional maintenance on the fence/trap array was also performed.

On 10 March, approximately 2200 *Ambystoma tigrinum* eggs (in multiple masses) were collected from two sites within Stephen A. Forbes State Park (Marion County, Illinois). These egg masses were transported and immediately placed in enclosures (1 m^3) within Pond B at WWNP. From these egg masses, 293 larvae survived to hatching and were released into the pond. To date, one juvenile tiger salamander has been caught in the pitfall traps around that pond, although it was not certain if that individual was produced by the cohort of larvae released into the pond in 2006.

From mid-September to mid October, approximately 20 adult and 30 larval bullfrogs were removed from Pond B at WWNP and euthanized. Tissues from the muscle and gastrointestinal tract of adult specimens were preserved for future analysis of parasite content and levels of chemical pollutants. This action was sanctioned by the Illinois Nature Preserves Commission with the intent of reducing the potential for predation on juvenile tiger salamanders as they left the pond and entered the surrounding forest habitat. On 2 December, all traps sealed to prevent incidental capture during the Winter season of inactivity.

Results & Projected Outcome

The sizes of most of the amphibian populations of using the breeding ponds at WWNP have increased since this study began, especially in the ponds that previously held fish. The populations of each species have since fluctuated (Fig. 2), due to different levels of precipitation in each year and to adjustments in trophic dynamics of the larval community (in the absence of fish). Levels of species diversity, however, have increased in all ponds as a result of the removal of fish (Table 2).

The initial effort of repatriating tiger salamanders was not successful, with a low survival rate to hatching and even lower survival rate to the conclusion of the larval period. I am not discouraged by this outcome for several reasons:

1. At this latitude, some tiger salamander populations are reported to require two activity seasons to complete larval period. As such, the larvae released into Pond B in Spring 2006 should not be expected to show up in the pitfall trap array surrounding Pond B until the end of the 2007 activity season. Pond B is deep enough (up to 1.5 m at maximum depth) that it will not freeze solid so that larvae can survive periods of surface ice formation.
2. Bullfrog adults are generalist carnivores that attempt to eat much of what they can catch. The sanctioned removal of bullfrog larvae and adults should reduce the predator

population in the 2007 activity season as well as those in the next few years. This will improve the chances of larval tiger salamanders surviving to metamorphosis and entering the forest habitat surrounding Pond B.

3. One of the two donor sites for tiger salamanders (a privately-owned farm pond in Shepardsville, Vigo County, Indiana) experienced a mild drought during the early Spring of 2006. One result from this event is that the tiger salamander population known to breed in that pond either failed to reproduce that year, or experienced 100 % mortality among its egg masses before they could be collected (due to either desiccation or being frozen in shallow water during a subsequent freeze). Better monitoring of conditions at that site in 2007 should assure the collection of an adequate amount of eggs for repatriation to WWNP.

Another attempt at repatriating tiger salamanders through the release of larvae hatched from egg masses that are allowed to complete development in Pond B.

Research Products

During 2006, the following poster presentations based on research conducted at WWNP were made at scientific conferences:

Gross, L.M., and S.J. Mullin. 2006. An amphibian community after fish removal: A tale of four ponds. Midwest Partners for Amphibian & Reptile Conservation, Carbondale, IL.

Walston, L.J., and S.J. Mullin. 2006. Population responses of wood frog (*Rana sylvatica*) tadpoles to overwintered bullfrog (*Rana catesbeiana*) tadpoles. Joint Meetings of Ichthyologists & Herpetologists, New Orleans, LA.

Furthermore, the following manuscripts have been accepted for publication in 2007:

Walston, L.J., and S.J. Mullin. 2007. Responses of a pond-breeding amphibian community to the experimental removal of predatory fish. Amer. Midl. Nat. 154:in press.

Walston, L.J., and S.J. Mullin. 2007. Population responses of wood frog (*Rana sylvatica*) tadpoles to overwintered bullfrog (*Rana catesbeiana*) tadpoles. J. Herpetol. 41:23-30.

Tissue samples from the bullfrog larvae and adults removed from Pond B were obtained and preserved. These samples represent material that can provide a preliminary data set for future research at WWNP concerning the impacts of chemical fertilizer and pesticide application on agricultural fields that are immediately adjacent to that area of the property.

Table 1. Amphibian and reptile species documented in and around four ponds in the southeast portion of Warbler Woods Nature Preserve (Coles County, Illinois) during the 2006 activity season. Amphibians were all caught in drift fence-pitfall trap arrays around the ponds, whereas reptiles were observed in the ponds or immediately adjacent habitat. Some amphibian specimens were captured on more than one occasion, but were counted only once in the tallies.

Taxa Observed	**Number Observed**
Caudata	
Ambystoma texanum	794
Ambystoma tigrinum	1
Eurycea cirrigera	2
Anura	
Bufo americanus	43
Acris crepitans blanchardi	4
Pseudacris crucifer	25
Hyla versicolor (x *chrysoscelis*)	53
Rana catesbeiana	148
Rana sylvatica	26
Rana utricularia	33
Testudines	
Chelydra serpentina	3
Terrapene c. carolina	2
Chrysemys picta marginata	1
Lacertilia	
Eumeces laticeps	2
Serpentes	
Diadophis punctatus	1
Nerodia sipedon	1
Thamnophis sirtalis	2
Elaphe spiloides	1

Table 2. Composition of an amphibian community (expressed as a percentage of individuals captured for each species) in four breeding ponds at Warbler Woods Nature Preserve (Coles County, Illinois) between 2001 and 2006. Reference ponds lacked any fish, whereas mitigated ponds contained populations of predatory fish that were extirpated by January 2003 (the delineation of the pre- and post-removal periods). Percentages do not sum to 100 % due to rounding errors. The Shannon-Wiener Index of species diversity is also shown.

	Pre-removal		Post-removal	
Species	reference pond	mitigated ponds	reference ponds	mitigated ponds
Ambystoma texanum	16.7	2.6	53.8	40.5
Bufo americanus	66.3	90.3	4.6	12.0
Hyla versicolor	0.6	—	2.3	8.8
Pseudacris crucifer	1.1	—	7.3	14.2
Rana catesbeiana	4.1	4.8	14.1	6.8
Rana sylvatica	7.7	1.0	1.6	2.2
Rana utricularia	3.0	0.7	16.0	14.9
Other species	0.5	0.6	0.3	0.6
Shannon-Weiner Index (H')	0.48	0.20	0.61	0.74

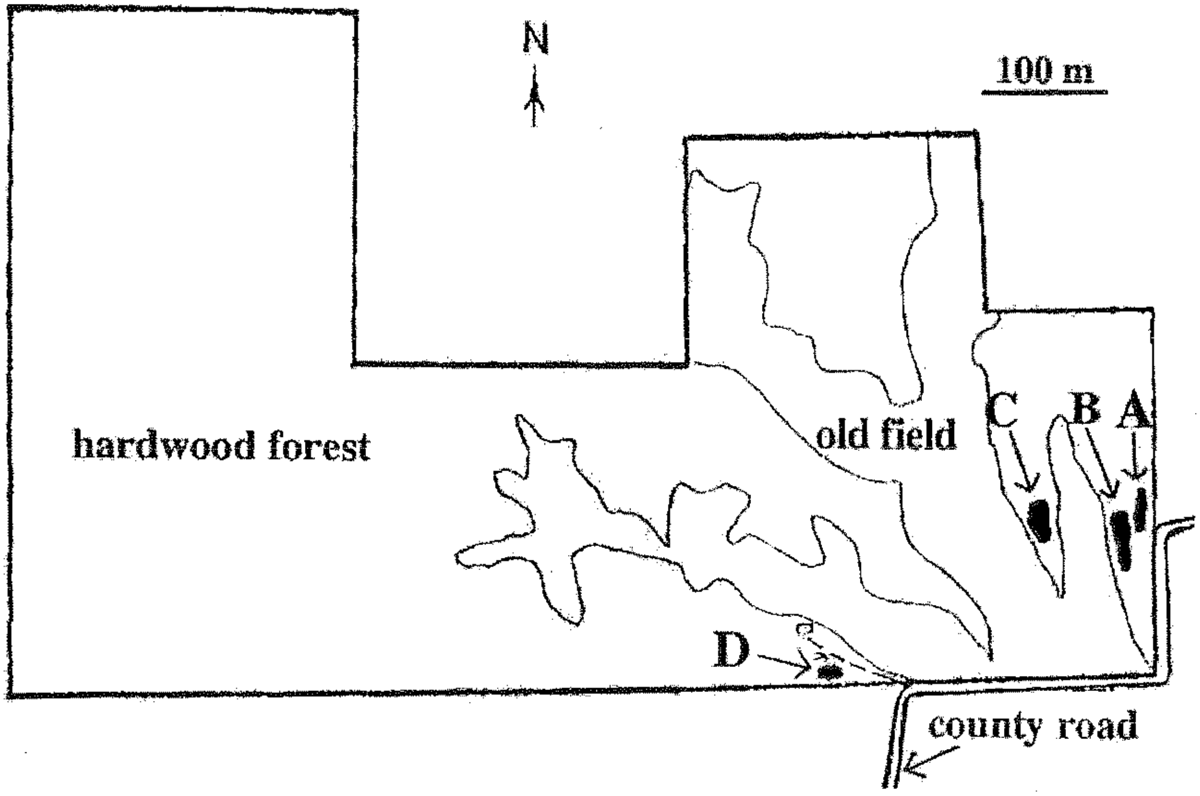

Figure 1. Schematic map of Warbler Woods Nature Preserve (81.5 ha) in Coles County, Illinois. Ponds A, B, and C are permanent, whereas Pond D typically dries down on or before 1 August of each year.

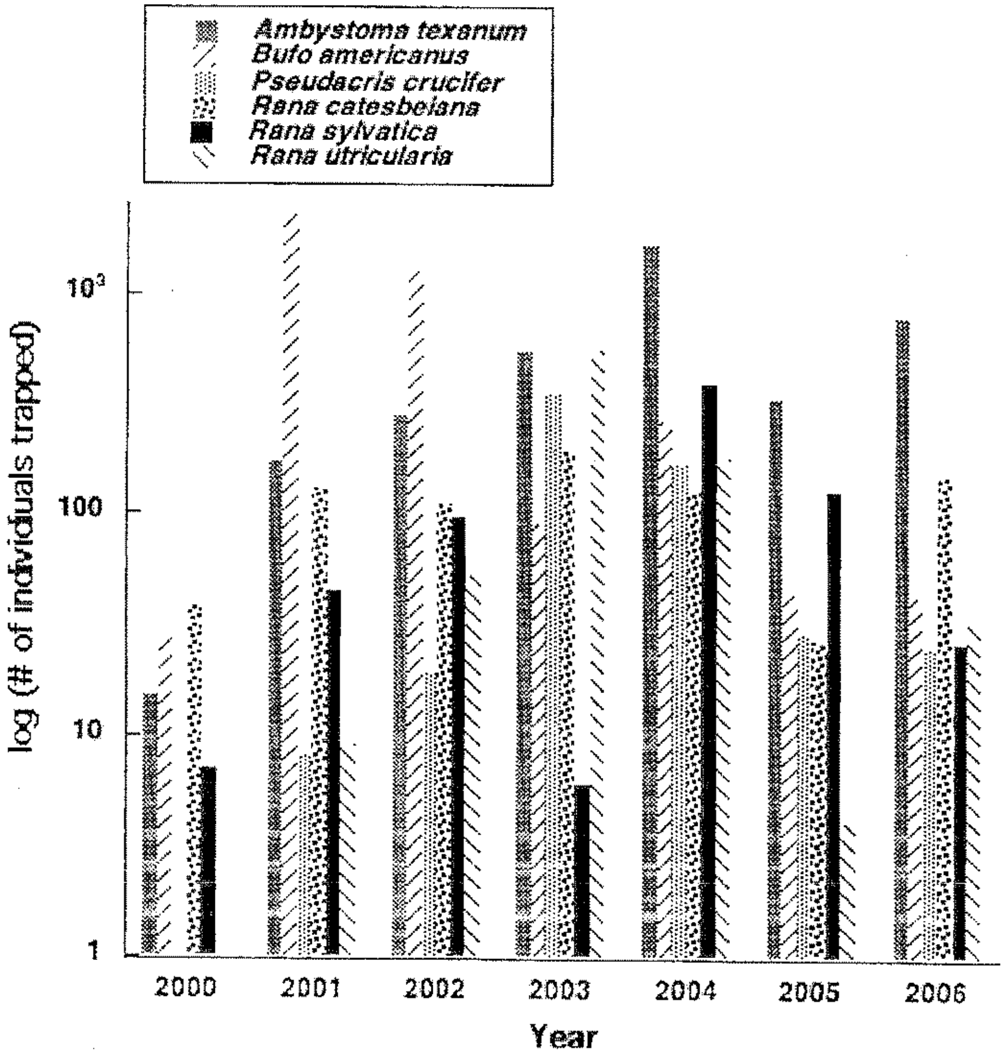

Figure 2. Numbers of individuals (shown in a logarithmic scale) for six species of amphibians trapped at four breeding ponds in Warbler Woods Nature Preserve (Coles County, Illinois) between 2000-2006. Predatory fish were extirpated from two of the ponds prior to the 2003 breeding season.