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Influence Of Fish Presence And Removal On Woodland Pond Breeding Amphibians

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Influence of Fish Presence and Removal on Woodland Pond Breeding Amphibians in Central Illinois

By

J. Brian Towey

THESIS

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recorded at four ponds in 2001 and 2002 at Warbler Woods Nature Preserve, Coles County, Illinois. Ponds A and D have no historical presence of fish. In December 2001, *Ameiurus melas* were removed from pond B and *Lepomis cyanellus* and *L. machrochirus* were removed from pond C. Data are standardized using pond circumference. Asterisks (*) indicate differences in recruitment values between years at $\alpha = 0.05$.

Abstract

Predatory fish can have devastating effects on populations of amphibians, many of which are already declining around the world. Land managers seeking to restore disturbed woodland ponds to more historically-accurate conditions may consider removing fish, a potentially difficult or costly process. I report on the effects of introduced sunfish (*Lepomis* spp.) and black bullhead catfish (*Ameiurus melas*), and their removal, on the reproductive efforts of three species of amphibians: smallmouth salamanders (*Ambystoma texanum*), American toads (*Bufo americanus*) and wood frogs (*Rana sylvatica*). Using drift fences and pitfall traps around four woodland ponds, amphibians were monitored for one year prior to and one year following removal of fish from two ponds (two control ponds never contained fish). Prior to treatment, few adult amphibians entered the pond with sunfish, while more American toads were found in association with the pond with bullhead than at other ponds. Numbers of adult amphibians using ponds did not increase as a result of fish removal. Sunfish decreased amphibian recruitment more severely than bullhead catfish. American toads bred successfully with bullheads, although recruitment of toads increased after removal of the fish. In the year following treatment, recruitment estimates increased in treated ponds for all species examined, but were unchanged in control ponds. Conservationists should consider *Lepomis* a threat to many native amphibian populations and *Ameiurus* a threat to some. Removal of predatory fish can be an effective method of improving conditions for some pond-breeding amphibians.

Introduction

For nearly two decades, a considerable amount of research has focused on amphibian declines and malformations, and factors influencing these events. Amphibians with aquatic larval stages and land-dwelling adult stages are limited to areas containing suitable terrestrial and aquatic habitat. In Illinois, about 90% of pre-settlement wetlands have been lost due to human modification, mostly through conversion for agricultural use (Suloway and Hubbell 1994). Gibbs (1998) reported that the physiological constraints of amphibians may make them less tolerant than other taxa of environmental changes such as loss and fragmentation of habitat. Additionally, populations of amphibians are highly susceptible to extinction-recolonization events (Wilbur 1984). Recolonization, however, hinges on the existence of local populations that serve as a source of dispersing individuals. Fragmentation and loss of habitat can eliminate immigration of amphibians to a declining or extirpated population. Reduced immigration increases the importance of recruitment in maintaining viable populations at isolated wetlands. Therefore, when managing land having amphibian populations that may be subject to isolation, it is important to consider aquatic factors which may pose a threat to amphibian recruitment.

Movement and dispersal patterns of amphibians affect the colonization of breeding habitat. Gibbs (1998) reported that neither forest borders nor streambeds influenced movements of *Rana sylvatica*, but that the relative permeability of road-forest edges was much less than forest interior or forest-open land borders. Rothermel and Semlitsch (2002) reported that the juvenile dispersal patterns of *Bufo americanus* may not follow patterns of adult habitat use such as occupying open canopy habitats. They found that juvenile toads avoided fields in favor of forest habitat. Smallmouth salamanders also tended to avoid field habitat (Rothermel and Semlitsch 2002).

Levels of amphibian dispersal between breeding ponds are variable. Newman and Squire (2001) found a higher than expected degree of genetic relatedness between populations of *R. sylvatica* when they sampled populations breeding at ponds on a local landscape. This relatedness indicates high gene flow and dispersal between these populations. Genetic differences can exist between populations separated by as little as 1070 m, but most populations had similar allele frequencies, even if separated by kilometers (Squire and Newman 2002).

Fish presence impacts local distribution and abundance of amphibians. Many fish depredate species of aquatic-breeding amphibians (Sexton et al. 1994). Some amphibians are limited to breeding in habitats without fish, but other amphibians can coexist with fish (Manteifel 1995). Fish are likely the sole vertebrate predator able to eliminate larval amphibians in ponds (Voris and Bacon 1966; Bronmark and Edenhamn 1994), thus restricting the reproductive success of some species to temporary ponds. Ireland (1989) found roughly the same hatching rate at two Virginia ponds, one with and one without fish. The pond without fish successfully produced metamorphs; but, there was no successful recruitment in the pond with fish for any of the four years of the study. In Missouri, Sexton and Phillips (1986) observed a reduction in the number of reproducing amphibian species after the introduction of fish. They cited the green sunfish (*Lepomis cyanellus*) as the species most responsible for this decline.

Varying levels of predation have been observed as a function of toxicity of different amphibian life history stages. For instance, Petranka et al. (1994) showed that

B. americanus eggs and larvae are palatable to larvae of *R. sylvatica*, but are toxic to other invertebrate and vertebrate predators (Voris and Bacon 1966; Crossland 1998). The larvae of this species are unpalatable in at least some stages to many species. Predators known to refuse either eggs or larvae of *B. americanus* include: fish (largemouth bass [*Micropterus salmoides*], central mudminnow [*Umbra limi*], bluegill sunfish [*L. macrochirus*], green sunfish [*L. cyanellus*], black bullhead catfish [*A. melas*]), newts (*Notophthalmus viridescens*), shrews (*Blarina* sp.), and invertebrates such as dragonfly naiads (*Anax* sp.), giant water bugs [*Belostoma* sp.], crayfish [*Procambrus* sp.], and leeches (*Batrachobella* sp.) among other species (Kruse and Francis 1977; Formanowicz and Brodie 1982; Kruse and Stone 1984; Brodie and Formanowicz 1987; Kats et al. 1988).

Habitat mitigation for aquatic-breeding amphibian populations can include the creation or protection of appropriate terrestrial habitat, creation of new wetlands, and improvement of existing wetlands through removal of predaceous fish. Unless wetlands can be drained of water, removal of all fish may be very difficult by mechanical means. A common method of fish removal is piscicide application (*e.g.*, Mullin et al. 2004), the most common of which is Rotenone™. The present study demonstrates the effectiveness of fish removal using Rotenone™, with amphibian conservation as the goal. The null hypotheses for this study are that (a) all ponds at Warbler Woods Nature Preserve (WWNP) are equally productive for breeding amphibians before fish removal; and, (b) fish removal will have no effect on numbers of adult amphibians, emerging metamorphs, or overall recruitment of any of the surveyed amphibian species.

Natural History

Ambystoma texanum – The smallmouth salamander is a common and widely distributed member of the "mole salamander" clade, Ambystomatidae. Smallmouth salamanders breed in late winter and early spring in central Illinois, moving to breeding ponds during heavy rain events. Females lay several hundred eggs attached to submerged plants and branches. Young transform from May through July (Conant and Collins 1998; Phillips et al. 1999). Kats et al. (1988) lists *A. texanum* larvae as palatable to green sunfish but also as a species that rarely encounters fish due to breeding habitat demographics. Adult *A. texanum* can migrate a mean distance of 52.4 m from breeding sites, with a range from 0 to 125 m (Williams 1973). This estimate is lower than the mean calculated by Semlitsch (1998) for 6 species of Ambystomatid salamanders.

Bufo americanus – The American toad is common and widely distributed in Illinois. In central Illinois, the species breeds from mid-April to early May in many types of aquatic habitat. Females lay membranous strings of several thousand eggs which hatch in approximately two weeks. Larvae transform within 40 days of hatching (Phillips et al. 1999). Newly hatched and metamorphic stages are unpalatable to most vertebrate and invertebrate predators, but the intermediate stages are palatable to those same predators (Brodie and Formanowicz 1987; Kats et al. 1988). Petranka et al. (1994) suggested that larval *Rana sylvatica* could depredate egg and larval stages of *B. americanus* in some populations.

Rana sylvatica – In Illinois, wood frogs exist in spotty populations typically in moist, mature forests. Wood frogs have been declining over much of their previous range in Illinois likely due to forest reduction (Thurow 1994). In central Illinois, the wood frog breeds in mid-February through March following warm rains in forest ponds and temporary pools. Females deposit between 300 and 900 eggs, which hatch in two weeks or less (Phillips et al. 1999). Larval wood frogs metamorphose after approximately 2 months. Formanowicz and Brodie (1982) showed that wood frogs, while palatable to fish throughout most of their larval stage, become unpalatable with the advent of metamorphosis, which is otherwise the most vulnerable larval stage of frogs (due to decreased mobility). Adult wood frogs show a high degree of fidelity to their first breeding pond, and 18% of juveniles disperse to breed in a pond other than their natal pond (Berven and Grudzien 1990).

Rana catesbeiana- In Illinois, the bullfrog is a common resident of permanent bodies of water. Breeding occurs from April to August in central Illinois, with each female laying thousands of eggs over the season (Phillips et al. 1999). Larvae spend their winter in aquatic habitat and metamorphose the following year (Phillips et al. 1999). The large size of *R. catesbeiana* larvae may make them unavailable to invertebrate or fish predators that cannot ingest large prey (Formanowicz and Brodie 1982). Kats et al. (1988), list the bullfrog as an unpalatable species to some fish species (*L. cyanellus* and *L. macrochirus*). Their unpalatability may be due to a combination of large larval size and some degree of toxicity (Kruse and Francis 1977).

Other frogs (*Rana utricularia, R. blairi, B. fowleri, Acris crepitans, Hyla versicolor, Pseudacris triseriata*, and *Pseudacris crucifer*) use ponds at WWNP either in low numbers or are not as easily trapped by the methods used in this study.

Materials and Methods

Study site

Warbler Woods Nature Preserve, located in Hutton Township, Coles County, Illinois, was established as an Illinois Nature Preserve in 1999. The preserve covers 81 ha and consists of about 66.5 ha of deciduous forest and 14.5 ha of old field, the latter were previously used for livestock and hay agriculture. This portion was planted with seedlings of native hardwoods shortly before this study commenced. There are four man-made ponds on the site (Table 1, Fig. 1), created by previous landowners decades before this study (K. Kruse, pers. comm.). The ponds were established by damming the flow of drainage gullies with clay dikes. I assigned letters (A-D) to specific ponds for the purposes of this study. Pond A is 5 m east of Pond B. Pond C is 74 m west of B, with 28 m of forest adjacent to the ponds and 46 m of old field separating the two. Pond D is approximately 290 m southwest of Pond C, separated by 50 m of forest adjacent to the ponds and 240 m of mostly old field with some deciduous forest. All ponds are surrounded on all sides by a minimum of 3 m of forest habitat.

Ponds A and D have never contained fish, whereas ponds B and C contained fish species prior to this study, introduced in 1986 (K. Kruse, pers. comm.). Pond B contained black bullhead catfish (*A. melas*). Pond C contained both bluegill (*L. macrochirus*) and green sunfish (*L. cyanellus*). Ponds A and B had a few rooted aquatic plants (*e.g., Scirpus*, *Juncus*, *Phalaris*). Pond C had virtually no rooted aquatic plants but contained a substantial cover of duckweed (*Lemna minor*); at times nearly 90% of the pond's surface area was covered. Pond D periodically dried completely and had no rooted aquatic plants, although falling summer water levels did allow some terrestrial plant growth. None of the other ponds have a record of drying. In the latter half of year 1 of this study, I detected laundry effluent from a neighboring property reaching pond D. This issue was addressed and the effluent line was re-directed to a septic system prior to amphibians breeding in year 2. The potential impact of this effluent was not addressed in the design of the study and might have impacted my ability to consider amphibian use at pond D as a control group.

Monitoring amphibian populations

I monitored amphibian populations at the four WWNP ponds using drift fences and pitfall traps. Fences were constructed of 50-cm aluminum screening supported by wooden stakes with pitfall traps spaced 7.5 m apart on both sides of the fence. Due to dense tree and briar thickets, steep slopes, and periodic flooding, complete enclosure of ponds was not logistically feasible. Pond A was 86% enclosed, pond B was 89% enclosed, pond C was 44% enclosed, pond D was 91% enclosed. Ponds were monitored throughout the activity season.

I marked individuals by removing a toe to designate cohort groups by year of capture; only one toe was removed per individual. Toe clipping is a commonly used method of marking amphibians and is not known to influence subsequent behavior, mobility, or return rates when only one toe is removed (Ott and Scott 1999; McCarthy and Parris 2004). I only marked adult individuals in this study. After capture and marking amphibians, I released them on the opposite side of the fence, allowing them to continue moving in the direction they were assumed to be traveling at the time of capture. Marking individuals prevents a recapture from being counted as another breeding individual at the pond and allows determination of multiple entries into the same or other ponds.

Clipping toes is effective because regeneration (even when possible) would not occur in the time frame required to breed at any pond (> 35 weeks in *Plethodon*; Davis and Ovaska 2001). Limb and/or digit regeneration can occur in some anuran species and is common in salamanders. Bufonids have not been reported to have regenerative capabilities (Scadding 1983), and Berven (1990) reports that *R. sylvatica* does not regenerate digits.

Fish Removal

In December of 2001, personnel from the Illinois Department of Natural Resources (IDNR) removed fish from ponds B and C by applying Rotenone™ using a combination of a motorized sprayer and hand-pumped applicators. The Rotenone™ solution was sprayed from several points along the banks of each pond to ensure complete coverage. Pond B was treated at 7 ppm Rotenone™ and pond C at 3.5 ppm due to susceptibility differences between *Ameiurus* and *Lepomis*. The lethal effects of Rotenone™, a naturally occurring piscicide derived from the roots of plants (*Lonchocarpus* or *Derris* sp.), are manifest by inhibiting oxygen uptake at the cellular level (Singer and Ramsay 1994; Fajt and Grizzle 1998). Ponds B and C were treated in mid-winter to avoid chemical contact with most species of amphibian larvae.

We applied Rotenone™ a second time to Pond B in January 2003 due to an incomplete kill of black bullhead in the previous year. Black bullhead are known to be among the fish species most resistant to Rotenone poisoning (Marking and Bills 1976). In the 2003 application, IDNR personnel pumped Rotenone™ into the pond through numerous holes drilled in the ice thereby circulating it throughout the water column (Mullin et al. 2004). I sampled with wire minnow traps, D-frame nets, and visual investigations to verify that all fish had been killed following treatment.

Winter treatments exposed bullfrog larvae and adults overwintering in ponds to Rotenone™. We did not predict the effect of Rotenone™ on bullfrogs (described below), and assumed that there would be some larval bullfrog mortality associated with its application. Because bullfrogs are common across Illinois and at other ponds at WWNP, we deemed any loss acceptable from a management perspective.

Statistical analyses

In addition to capture records, I calculated recruitment for each species as the number of emerging metamorphs captured at a pond divided by the number of adults captured entering that pond during regular breeding periods. I included males in this calculation because it was not always possible to determine sex of individuals. I did not capture any known female *R. sylvatica* at ponds B or C in 2000, or female *B. americanus* at pond C in 2001. I caught males, however, of both of these species. This method underestimates recruitment, but provides quantitative values that were compared across years and ponds. The two-year larval period of *R. catesbeiana* prevents my calculation of recruitment for that species.

To account for differences in pond size, I standardized capture data and recruitment values across all ponds using measures of pond circumference. I used multivariate analyses of variance (MANOVA) to compare the response variables (numbers of adults and metamorphs, and recruitment) for *A. texanum*, *B. americanus* and *R. sylvatica* between ponds and years (SPSS v 15.0, 2006). I used an inverse transformation of the data (1 / [y-1]) to eliminate zero values (Sabin and Stafford 1990). The number of bullheads had been drastically reduced following the first application of Rotenone™ as evidenced by the low number of killed fish observed following the second treatment. Therefore, I considered pond B in analyses as having been successfully treated after the 2001 application.

I compared data across treatments (fish present [B, C] vs. absent [A, D]) and years (2001 vs. 2002), as well as any interaction between these variables. I also compared pond A to pond D to detect any differences between the two "control" ponds. Lastly, I compared all ponds without distinguishing between treatments (A vs. B vs. C vs. D) to assess differences in response variables that were unique to each pond.

To determine if there were differing effects of the presence or removal of fish on different amphibian species, I performed Chi-square tests on breeding season adult captures, emerging metamorph captures, and recruitment figures specific to each amphibian species (*A. texanum*, *B. americanus* and *R. sylvatica*) at each pond. I also performed Chi-square tests on the number of emerging *R. catesbeiana* metamorphs from each pond to assess effect of Rotenone™ application on survival of overwintering larvae. I calculated expected frequencies of capture per year (2001 and 2002) as half of the captures for the two years combined because this would assume no difference between years. In cases in which an expected frequency was less than 5, I used Yates' correction for continuity. Using this method, I subtracted 0.5 from the difference between each observed value and its expected value in 2×2 contingency tables. While this method decreases the possibility of detecting significance by increasing the P-values obtained, it prevents overestimation of statistical significance for small datasets (Yates 1934).

Results

There were no differences between the numbers of adult amphibians captured at ponds, although fewer adult amphibians tended to utilize pond C as compared to other ponds ($F = 2.70$, $P = 0.07$; Fig. 2). The number of emerging metamorphs trended lower at pond C in 2001 than at pond B in 2001 and ponds B or C in 2002 ($F = 2.92$, $P = 0.059$). Considering values pooled between ponds A and D, neither the number of amphibians trapped nor the larval recruitment differed between years. Ponds A and D did not differ from each other in numbers of metamorphs or adults captured, but recruitment was higher at pond A than at pond D ($F = 6.63$, $P = 0.028$). Recruitment was lower at pond D than at any other pond ($F = 3.75$, $P = 0.03$). Recruitment was greater at ponds B and C in 2002 than at ponds A and D ($F = 3.48$, $P = 0.05$). Recruitment was similar between years at both ponds B and C though figures trended higher in 2002 ($F = 3.45$, $P = 0.09$).

A. texanum – Numbers of emerging *A. texanum* metamorphs were greater at treated ponds and those without fish (A; D; B, 2002; C, 2002) than at untreated ponds with fish (B and C, 2001; $F = 7.21$, $P = 0.036$). After removing fish from ponds B and C, the number of emergent *A. texanum* metamorphs increased ($F = 2363.4$, $P < 0.0001$; Fig. 3). More adult *A. texanum* entered pond B in 2002 ($\chi^2 = 26.43$, $P < 0.001$) and fewer entered pond D in 2002 ($\chi^2 = 3.88$, $P = 0.05$; Fig. 2). More *A. texanum* metamorphs emerged from pond B in 2002 than in 2001 ($\chi^2 = 22.2$, $P < 0.0001$) and from pond C in 2002 than in 2001 ($\chi^2 = 202.7$, $P < 0.0001$). Recruitment of *A. texanum* was higher at pond C in 2002 than in 2001 ($\chi^2 = 8.6$, $P = 0.003$; Fig. 4).

B. americanus – Numbers of emerging *B. americanus* metamorphs were similar between treated and fishless ponds (A; D; B, 2002; C, 2002) and untreated ponds with fish (B and

C, 2001; $F = 4.96$, $P = 0.067$). Recruitment of *B. americanus* was lower at pond D than other ponds ($F = 36.23$, $P = 0.002$). Fewer adult *B. americanus* entered ponds B ($\chi^2 = 13.32$, $P < 0.001$) and C ($\chi^2 = 4.35$, $P = 0.037$) in 2002 than in 2001 (Fig. 2). Fewer *B. americanus* metamorphs emerged from pond B in 2002 than in 2001 ($\chi^2 = 78.55$, $P < 0.0001$). More *B. americanus* metamorphs emerged from pond C in 2002 than in 2001 ($\chi^2 = 81.1$, $P < 0.0001$; Fig. 3). Recruitment of *B. americanus* was lower at pond A in 2002 than in 2001 ($\chi^2 = 7.44$, $P = 0.006$). Recruitment of *B. americanus* ($\chi^2 = 13.98$, $P = 0.0002$) was higher at pond C in 2002 than in 2001 (Fig. 4).

R. sylvatica – Recruitment of *R. sylvatica* was higher at ponds after treatment with Rotenone™ than at either untreated ponds with fish, or fishless ponds (A and D; $F = 32.86$, $P = 0.001$). Recruitment increased at ponds B and C after treatment with Rotenone™ ($F = 46.32$, $P = 0.02$). Adult captures of *R. sylvatica* were higher at pond D than at ponds B or C ($F = 2216.77$, $P < 0.001$). More adult *R. sylvatica* entered pond D in 2002 than in 2001 (Fig. 2). More *R. sylvatica* metamorphs emerged from pond B in 2002 than in 2001 ($\chi^2 = 10.07$, $P = 0.002$). More *R. sylvatica* metamorphs emerged from pond D in 2002 than in 2001 ($\chi^2 = 21.0$, $P < 0.0001$; Fig. 3).

R. catesbeiana – More *R. catesbeiana* metamorphs emerged from ponds B and C than A or D ($F = 2127.32$, $P < 0.0001$). There were no differences between numbers of *R. catesbeiana* metamorphs between years although larger numbers of individuals were captured emerging from ponds B and C in 2002 than in 2001, and fewer were captured emerging from pond A in 2002 than in 2001 (Fig. 3).

Discussion

Treating fish-inhabited ponds with Rotenone™ facilitated greater amphibian reproductive success at WWNP. Increases in recruitment (Fig. 3) and numbers of metamorphs (Fig. 4) at ponds B and C after treatment demonstrate that increased breeding potential is possible in the first year after fish removal. There were no such differences between years in ponds without fish. Increases in recruitment following fish removal for all three amphibian species (each in a different Genus) illustrate the potential of this technique to be implemented for the purposes of conservation of a variety of pond-breeding amphibians.

Recruitment in all three species was greater at Ponds B and C following fish removal, than at ponds with no history of fish (Fig. 4). This result is likely due to a low level of inter- and intra-specific competition in treated ponds, in addition to the lack of predation by fish. Vredenburg (2004) described a "rapid recovery" of *Rana mucosa* after removing trout from lakes in the Sierra Nevada. Bronmark and Edenhamn (1994) reported on the abrupt end to breeding and chorusing of *Hyla arborea* after the introduction of fish to a pond, and the resumption of successful breeding after removing the fish with Rotenone™. Fish can reduce populations of other species that compete against or depredate amphibian larvae. In the absence of these species immediately following removal of fish, amphibian breeding can be more successful than several years after the removal of fish. Future monitoring of amphibians using the WWNP ponds might reveal the duration of this period of increased breeding potential.

The most dramatic increase in amphibian recruitment was observed at Pond C after removal of *Lepomis* spp. In 2001, no recruitment occurred for *A. texanum*, *B.*

americanus or *R. sylvatica*. After removal of sunfish, these species produced recruitment values of 10.5, 1.0, and 14.3, respectively. Of all *A. texanum* metamorphs captured at WWNP in 2002, 85.4 % were from pond C (Table 2). Without further disturbance at this pond, the number of captures for each amphibian species should continue to increase to carrying capacity assuming some degree of adult fidelity to natal ponds (Sinsch 1997).

Prior to removal of black bullheads from pond B, *B. americanus* exhibited a fairly high rate of recruitment (16.8 metamorphs per adult) in spite of the presence of the fish. Metamorphs leaving pond B comprised 72.8% of all emerging *B. americanus* captures at WWNP in 2001 (Table 3). After removal of bullheads, recruitment of this species increased to 21.0 (Fig. 4) and 83.9% of *B. americanus* metamorphs were captured at Pond B. This species seems to have benefited from removal of bullheads in the short (1 yr) term. Further study should determine the longer term effect of bullhead removal as populations of competitors and invertebrate predators respond. Bullheads are capable of depredating larval *B. americanus* without apparent ill effect to the fish (Appendix I). Given the success of *Bufo* in pond B prior to removal of bullheads, however, it is apparent that *B. americanus* larvae are able to avoid some degree of bullhead predation.

The redirecting of laundry effluent drainage away from pond D appears to have benefited the *A. texanum* and *R. sylvatica* populations breeding in that pond, both of which experienced increases in numbers of metamorph captures in 2002 (Fig. 3). Metamorphs of *A. texanum* and *R. sylvatica* leaving pond D in 2002 comprised 2.1% and 55.7%, respectively, of all metamorph captures of those species at WWNP after pond D had not produced any metamorphs in 2001 (Table 2). Water chemistry analyses in 2002 and 2003 and subsequent Chi-square tests indicate that pond D was, during that time, similar to ponds A-C in temperature, pH, dissolved oxygen, chlorophyll A, turbidity, total volatile solids, and dissolved volatile solids, while it demonstrated higher conductivity, contained more total solids, and less suspended organic solids than the other ponds (Appendix II). The observation of higher conductivity and total solids may indicate that the pond had not fully recovered from its history of effluent runoff or that additional sources of runoff persisted. Additional research on the effects of laundry effluent pollution would be valuable to land managers and lawmakers.

Pond A was the only pond not manipulated in this study between 2001 and 2002. Amphibians breeding in that pond experienced no increases in metamorph emergence (Fig. 3) or recruitment (Fig. 4). In fact, decreases in metamorph captures were observed for two species (*A. texanum* and *B. americanus*) in 2002, and no metamorphs of the third (*R. sylvatica*) were captured there in either year. These results strengthen my conclusions that fish removal and removal of laundry effluent caused increases in recruitment for these species of amphibians rather than other influences.

Bullfrog larvae overwintering in ponds B and C at the time of Rotenone™ application metamorphosed and emigrated from those ponds in 2002. Bullfrog metamorphs from Ponds B and C were more numerous (Fig. 3), and comprised a greater percent of total captures in 2002 than in the previous year (Table 2). This indicates that the application of Rotenone™ did not have negative effects on bullfrog larval survival. Bullfrogs are increasingly common within and outside their historic range and are often considered a threat to native species of conservation concern (Schwalbe and Rosen 1988). Additional study of Rotenone™ use on overwintering larvae of *R. catesbeiana* and other species, such as *R. clamitans*, could provide useful information to land managers who may or may not be inclined to accept losses to populations of those species.

My conclusion that fish presence can have negative effects on the breeding success of amphibians is consistent with other studies (Werschkul and Christensen 1977; Ireland 1989; Sih et al. 2003; Egan and Paton 2004). There are fewer studies comparing the degree of impact that different species of fish have on amphibian breeding. Sexton and Phillips (1986) found that green sunfish (*L. cyanellus*) have greater negative effects on amphibians than 5 other fish species; my data generally support this finding. Pond C contained only *Lepomis* sp., and it was the least utilized and least productive pond for amphibians prior to fish removal (Table 3, Fig. 4). Black bullhead catfish at Pond B apparently had less of an impact on amphibians, especially *B. americanus* (Fig. 4). Laboratory studies have demonstrated that *L. machrochirus*, a less aggressive predator of amphibians than *L. cyanellus*, readily feeds on two species of ranid larvae, and prefers their larvae over eggs (Werschkul and Christensen 1977).

Adult choice of oviposition sites is a means by which amphibians can avoid breeding in ponds that are population sinks for reasons such as presence of predaceous fish (Kats and Sih 1992). Choosing an appropriate oviposition site is a direct, time-specific assessment of the potential of a breeding pond than pond fidelity; however, the large number of factors that influence egg and larval survival may be difficult for adult amphibians to assess. Choice of oviposition site by adult *Hyla* sp. can play a role in structuring larval amphibian communities in ponds (Resetarits and Wilbur 1989; Binkley and Resetarits 2002). Laurila and Aho (1997) found no evidence, however, that common frogs (*R. esculenta*) selectively avoid pools after the experimental introduction of predatory fish. At WWNP, few adult *A. texanum* and *R. sylvatica* entered ponds containing fish (Table 3, Fig. 2) in spite of their close proximity to fishless ponds that also were used by these species. The tendency to enter only fishless ponds indicates that adults were not entering ponds at random and selectively ovipositing depending on conditions perceived in ponds through chemosensory reception, direct interaction with fish, or other factors. Ponds without fish produced far more adult captures than ponds with fish, except in the case of *B. americanus* at Pond B (Fig. 2), where toads were captured in abundance. American toads demonstrated the ability to breed successfully with *A. melas*, but not *Lepomis* spp. Unless adult amphibians possess mechanisms by which they can assess potential predators in a pond without entering it, mechanisms other than selective oviposition contribute more to the composition of amphibian communities at WWNP. Fidelity to natal ponds (Sinsch 1997) may be such a process because it can result in low numbers of adult entries into historically low quality breeding ponds, as I observed at WWNP.

My conclusions that fish had a negative effect on amphibian reproduction and that fish removal increased amphibian recruitment at WWNP are consistent with previous studies (Bronmark and Edenhamn 1994; Vredenburg 2004). Taken together, these studies indicate that fish removal can be a valuable tool for the conservation of amphibians. Based on the lack of any metamorph captures of *A. texanum*, *B. americanus*, or *R. sylvatica* from pond C prior to treatment and the fact that metamorphs of *B. americanus* and *R. sylvatica* were captured from pond B in that time, *Lepomis* spp. posed a greater threat to more species of amphibians in Pond C than did *A. melas* in Pond B. Although many species of amphibians might benefit from the removal of many species of fish, it is important for land managers to consider the particular interspecific relationships involved. Conservation strategies for amphibians should consider introduced populations of *Lepomis* spp. as a significant threat to amphibian reproduction. Longer term studies assessing the benefit to these and other amphibians of removal of other types of fish should be conducted to facilitate better decision making and better understanding of aquatic communities.

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Table 1. Approximate dimensions and characteristics of four ponds at Warbler Woods Nature Preserve, Coles County, Illinois.

Pond	Maximum length (m)	Maximum width (m)	Area (m^2)	Circumference (m)	Maximum depth (m)	Fish species present in 2001	Hydroperiod
A	53	13	610	111	0.7	No fish	Permanent
B	60	18	770	135	1.2	*Ameiurus melas*	Permanent
C	51	26	1070	141	1.5	*Lepomis machrochirus* and *L. cyanellus*	Permanent
D	49	16	130	73	1	No fish	Ephemeral

Table 2. Percent of emergent metamorphs for each of four amphibian species captured leaving four ponds in 2001 and 2002 at Warbler Woods Nature Preserve, Coles County, Illinois.

	2001				2002			
Species	Pond A	Pond B	Pond C	Pond D	Pond A	Pond B	Pond C	Pond D
Ambystoma. texanum	100.00%	0.00%	0.00%	0.00%	3.20%	9.35%	85.35%	2.11%
Bufo americanus	27.10%	72.80%	0.00%	0.10%	11.87%	83.90%	3.98%	0.25%
Rana sylvatica	0.00%	100.00%	0.00%	0.00%	0.00%	39.23%	5.12%	55.66%
Rana catesbeiana	14.99%	38.51%	46.50%	0.00%	8.55%	46.99%	43.52%	0.94%

Table 3. Percent of adults of three amphibian species captured entering four ponds during their breeding seasons in 2001 and 2002 at Warbler Woods Nature Preserve, Coles County, Illinois.

	2001				2002			
Species	Pond A	Pond B	Pond C	Pond D	Pond A	Pond B	Pond C	Pond D
Ambystoma. texanum	44.13%	4.77%	14.97%	36.13%	45.80%	23.90%	10.39%	19.91%
Bufo americanus	17.11%	72.48%	8.26%	2.15%	20.51%	68.69%	4.89%	5.91%

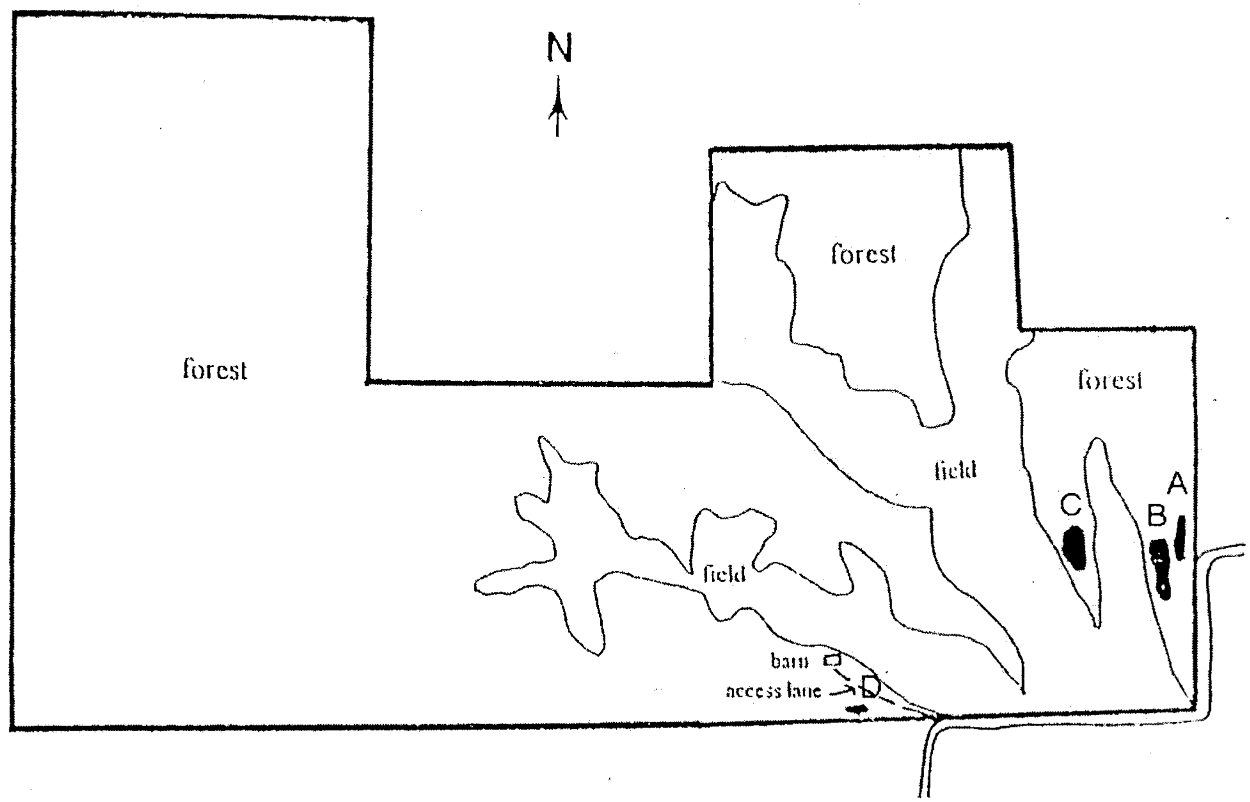


Fig. 1. Map of ponds, and habitat type at Warbler Woods Nature Preserve, Hutton Township, Coles County, Illinois. Ponds (dark-shaded areas) are labeled as (A-D) from east to west.

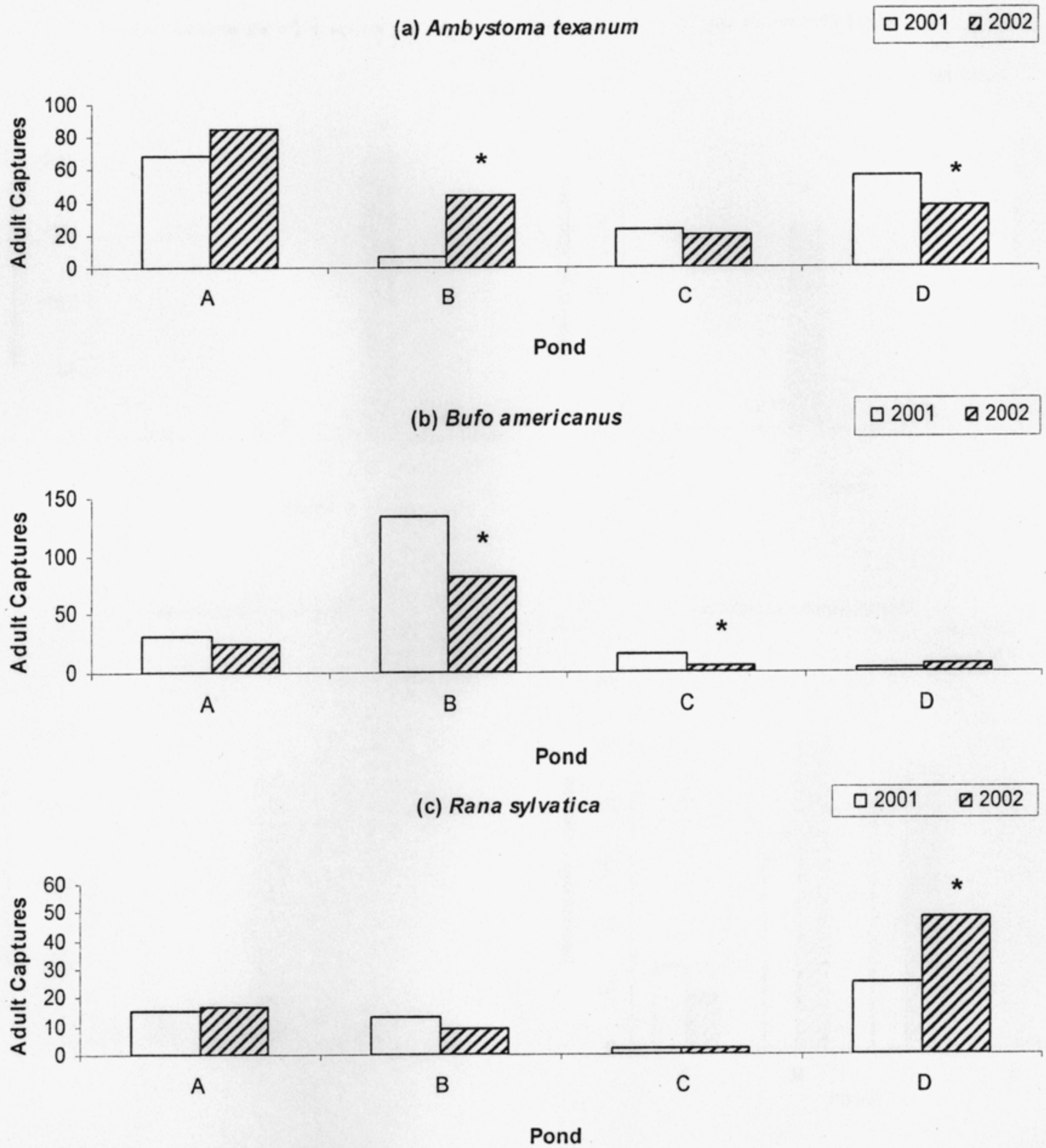


Fig. 2. Numbers of adult (a) *Ambystoma texanum,* (b) *Bufo americanus*, and (c) *Rana sylvatica* captured in spring entering four ponds in 2001 and 2002 at Warbler Woods Nature Preserve, Coles County, Illinois. Ponds A and D have no historical presence of fish. In December 2001, *Ameiurus melas* were removed from pond B and *Lepomis cyanellus* and *L. machrochirus* were removed from pond C. Data are standardized using pond circumference. Asterisks (*) indicate differences in individual abundance between years at $\alpha = 0.05$.

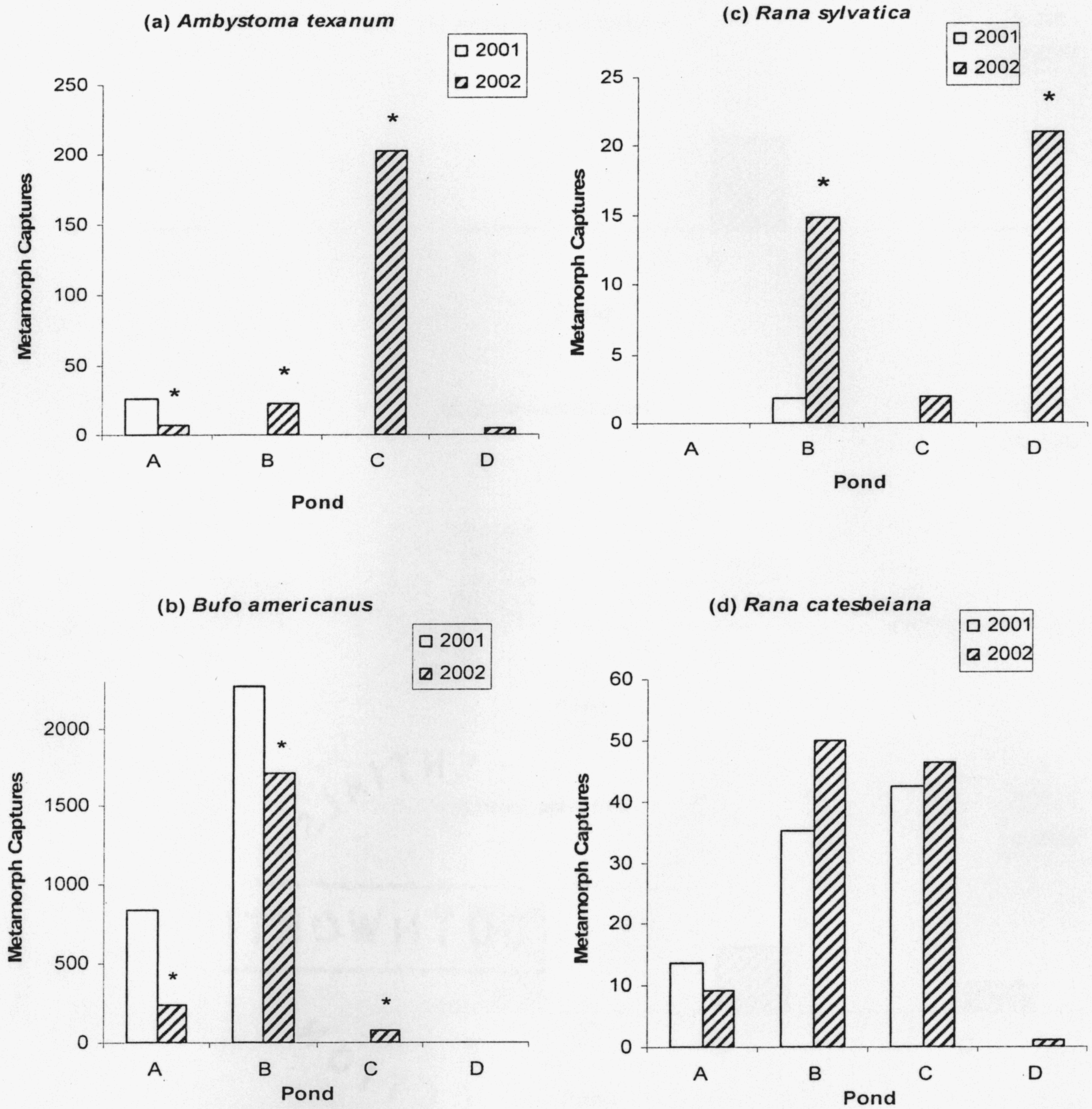
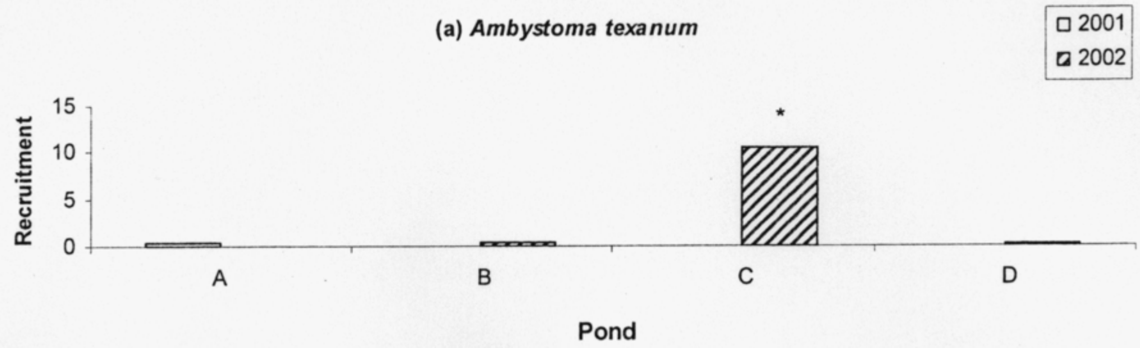
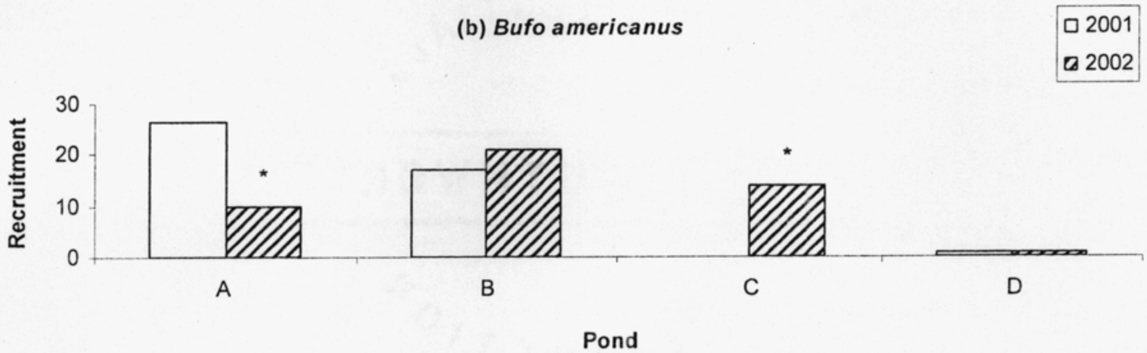


Fig. 3. Metamorphs of (a) *Ambystoma texanum,* (b) *Bufo americanus*, (c) *Rana sylvatica,* and (d) *R. catesbeiana* captured leaving four ponds in 2001 and 2002 at Warbler Woods Nature Preserve, Coles County, IL. Ponds A and D have no historical presence of fish. In December 2001, *Ameiurus melas* were removed from pond B and *Lepomis cyanellus* and *L. machrochirus* were removed from pond C. Data are standardized using pond circumference. Asterisks (*) indicate differences in individual abundance between years at $\alpha = 0.05$.





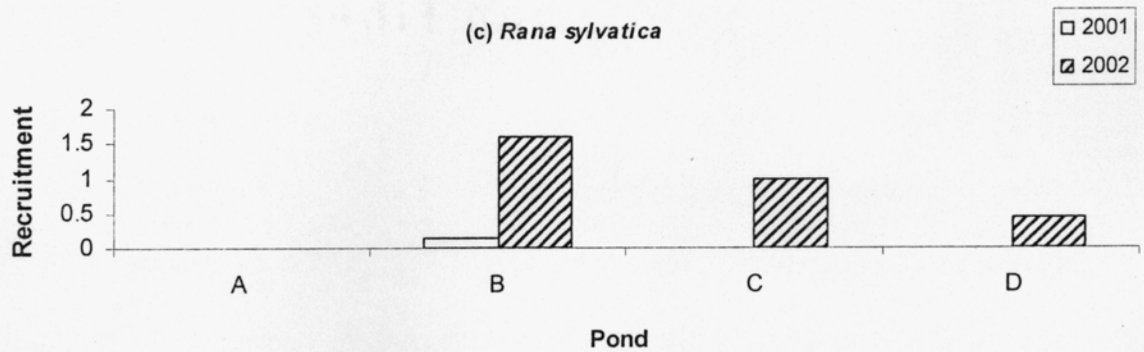


Fig. 4. Recruitment (number of captured emerging metamorphs / number of adults entering ponds) of (a) *Ambystoma texanum,* (b) *Bufo americanus*, and (c) *Rana sylvatica* recorded at four ponds in 2001 and 2002 at Warbler Woods Nature Preserve, Coles County, Illinois. Ponds A and D have no historical presence of fish. In December 2001, *Ameiurus melas* were removed from pond B and *Lepomis cyanellus* and *L. machrochirus* were removed from pond C. Data are standardized using pond circumference. Asterisks (*) indicate differences in recruitment values between years at $\alpha = 0.05$.

APPENDIX I

Black Bullhead Catfish (*Ameirus melas*) Predation on Amphibian Larvae.

Introduction

Fish are likely the sole aquatic predators able to eliminate larval amphibians in ponds (Voris and Bacon 1966; Bronmark and Edinhamn 1994). To effectively manage amphibian populations in aquatic habitats, it is important to determine which fish species threaten successful amphibian reproduction. Predation of larval amphibians by some species of fish has been examined (Kruse and Francis 1977; Kruse and Stone 1984), but the role of catfish as predators of amphibian larvae is relatively unknown. Kruse and Francis (1977) reported that black bullhead catfish (*Ameiurus melas*) readily ate larvae of *Pseudacris triseriata*, *Rana blairi*, *R. pipiens*, and *Scaphiopus bombiferons*, but refused *R. catesbeiana* larvae. Black bullheads are omnivorous. Much of their diet can be composed of aquatic insect larvae, especially those of midges and mayflies. Other prey items include small arthropods and mollusks, oligochaete worms, and a wide variety of animal and plant matter, as well as minnows and the eggs of other fishes (Mayhew 1987). The black bullhead is a predatory fish and often exists in breeding ponds of amphibians. Rosen et al. (1995) credit black bullhead and other introduced predatory fish species with partial responsibility for the decline of *Rana chiricahuensis* in Arizona.

The relatively high predation rates experienced by many amphibian larvae are not necessarily matched in other life history stages, or in other species. For example, three black bullheads were offered eggs of *Bufo valliceps* and other food types soaked in crushed ovarian eggs (Licht 1968). One of the bullheads mouthed but refused to eat eggs or egg parts, one swallowed and later regurgitated a bit of jelly coating, and one ate a worm soaked in crushed eggs. The third fish was found dead the following morning.

Other aquatic predators (e.g., larvae of the diving beetle, *Dyctiscus verticalis*) also exhibit differential ability as amphibian predators depending on the life history stage consumed (Formanowicz and Brodie 1982). I determined the willingness of *A. melas* to feed on amphibian larvae that have unpalatable life history stages (Kruse and Francis 1977). I offered larvae of the wood frog (*R. sylvatica)* and American toads (*B. americanus)* to black bullheads collected from east-central Illinois to determine if the fish was a willing predator of these amphibians.

Methods

From August to September 2002, I captured juvenile black bullhead from one pond at Warbler Woods Nature Preserve (WWNP), in Hutton Township, Coles County, Illinois. The collected individuals were kept in 37-l aquaria and fed commercially available shrimp pellets and aquatic turtle diet until larval amphibians were collected in Spring 2003. Both fish and larvae were collected from shore with a dip net. Larvae of *B. americanus* and *R. sylvatica* were kept in 37-l aquaria prior to feeding trials, and fed shrimp pellets and aquatic turtle diet.

From March through May of 2003, I conducted 30 feeding trials using *R. sylvatica* larvae and 26 trials with *B. americanus* larvae. I placed one bullhead and five amphibian larvae in a 9.5-l opaque aquarium and recorded the number of larvae consumed, killed, and dead after 24 h. I distinguished between larvae that were "killed" rather than simply "dead" when there was visible damage to their bodies. I used five amphibian larvae because of the relatively small size of the bullheads used (50 - 100 mm total length). In spite of this consideration, some of the smaller bullheads may have been satiated by fewer than five larvae. Two bullheads were used in a trial for *B. americanus* larvae after having been used in a trial for *R. sylvatica* several weeks prior but this was generally avoided to preserve a level of inexperience in the bullheads. I used Chi-square tests to detect differences in the survival rates for both amphibian species, as well as differences in the fates of the larvae (dead vs. killed vs. consumed).

Results

Rana sylvatica – Of the 30 trials conducted, 17 (57 %) resulted in all *R. sylvatica* larvae being consumed, and 3 (10 %) resulted in all larvae being killed, with some being consumed and some killed but not eaten or regurgitated. Those larvae found dead after 24 h all had some degree of injury. Of the 150 *R. sylvatica* larvae used in these trials, only 18% survived the 24 h exposure to bullheads. All five larvae were killed and/or consumed in more trials than any other result (df = 5, $\chi^2 = 56.4$, $P < 0.001$). Across trials, more larvae were killed and/or consumed than survived (df = 1, $\chi^2 = 59.55$, $P < 0.001$).

Bufo americanus – Of the 26 trials conducted, 20 (77%) resulted in all 5 *B. americanus* larvae being consumed by bullheads and 3 more resulted in all 5 *B. americanus* being killed and/or consumed by bullheads. Of the 130 *B. americanus* larvae used in these trials, 96% were killed and/or eaten, 94% were consumed, 2% were killed and not eaten, 4% survived the 24-h exposure to bullheads, and none were found dead without evidence of injury. All five larvae were killed and/or consumed in more trials than any other result (df = 5, $\chi^2 = 97.23$, $P < 0.001$). Across trials, more larvae were killed and/or consumed than survived (df = 1, $\chi^2 = 81.64$, $P < 0.001$). The two occasions in which bullheads had previously been used in a feeding trial with *R. sylvatica* resulted in all five *B. americanus* larvae being eaten. If these trials are removed from comparisons of numbers of trial results or numbers killed or survived, P-values remain below 0.001. No bullheads exhibited any ill symptoms or death as a result of eating larvae of either amphibian species.

Discussion

Bullhead catfish are an important predator of amphibian larvae. The fact that bullheads routinely feed on fathead minnows (Mayhew 1987) illustrates their capability to consume swimming vertebrates. Given the ability of *A. melas* to use a variety of water depths and vegetative covers (Cucherousset et al. 2006) and the demonstrated ability of *Ameiurus* to capture relatively agile, vertebrate prey (Dahle and Hatch 2002), it is likely that the bullhead is capable of capturing the larvae of *B. americanus* and *R. sylvatica*.

Larval *B. americanus* are unpalatable when newly hatched, but intermediate stages are reported to be palatable (Brodie and Formanowicz 1987). My results show a willingness on the part of bullheads to prey on *Bufo*. If black bullheads acquire an avoidance of *Bufo* larvae resulting from experiences with unpalatable, newly-hatched larvae, that avoidance may allow for *Bufo* to persist successfully in the presence of the fish without suffering intense predation even during palatable periods of development. If acquired avoidance is not a significant factor in the ability of *Bufo* to thrive with *Ameiurus*, other antipredatory defenses of *Bufo*, such as behavioral adaptations, crypsis, or habitat use could explain their persistence with bullheads. It should be noted that in my experimental design, I did not attempt to replicate a natural foraging location or cover area for the subjects and therefore antipredatory adaptations of *Bufo* relating to those factors would have been confounded in my trials. Manipulative experiments in field enclosures would be useful to study predator-prey interactions between these species.

The results of my laboratory experiments show that *A. melas* from WWNP consume both *Rana* and *Bufo* larvae in captivity and suffer no severe physiological consequences such as noticeably impaired or unusual movement or death. These findings are consistent with Formanowicz and Brodie (1982) which demonstrated periods of development of the larvae of these species during which they are palatable to predators.

Bufo americanus were consumed by bullheads at a greater rate than *R. sylvatica* (96% compared to 82% consumed respectively). While I do not have conclusive evidence to address this difference, two factors of my experimental design may have influenced these results. I conducted trials in the spring as amphibian specimens became available in the field. Because *R. sylvatica* bred and hatched earlier than *B. americanus*, trials for *R. sylvatica* were conducted about 3-4 weeks in advance of the latter. It may be that the temporal difference influenced behavior and appetite of bullheads. An additional possibility is that *Bufo* larvae were slightly smaller than *Rana* larvae. The larger size of *Rana* larvae may have allowed for some bullheads to reach a point of satiation which they would not have reached with the smaller larvae of *Bufo*. An additional study that eliminated temporal variation and accounted for differences in mass of prey could also help to explain the differences noted in my study. Behavioral differences between *B. americanus* and *R. sylvatica* could also have influenced the numbers consumed by bullheads.

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APPENDIX II

Appendix II. Means (±1 standard deviation) of water chemistry parameters of four ponds at Warbler Woods Nature Preserve, Coles County, Illinois. Samples were taken in April, May, June, August, and September of 2002, and March and April of 2003.

Parameter	Pond A	Pond B	Pond C	Pond D
Water Temperature (°C)	19.6 ± 8.7	18.3 ± 9.1	19.1 ± 8.4	13.5 ± 8.6
Water pH	8.1 ± 0.8	7.8 ± 0.6	7.7 ± 0.5	7.9 ± 0.4
Dissolved O_2 (mg/L)	4.5 ± 1.6	3.1 ± 1.8	2.4 ± 1.4	2.8 ± 1.5
Chlorophyll A (mg/L)	2.3 ± 0.9	7.4 ± 10.9	1.9 ± 0.9	2.5 ± 1.0
Conductivity	194.4 ± 40.6	296.3 ± 78.6	202.2 ± 50.7	556 ± 104.2
Turbidity (NTU)	15.7 ± 13.2	28.2 ± 34.1	19.3 ± 19.9	19.7 ± 18.7
Total Solids (mg/L)	174.3 ± 88.6	248.3 ± 58.9	196.1 ± 50.2	349.1 ± 73.6
Total Volatile Solids (mg/L)	118.1 ± 42.9	127.4 ± 35.0	127.2 ± 75.4	155.8 ± 55.3
Suspended Volatile Solids (mg/L)	9.4 ± 2.4	30.0 ± 44.1	12.1 ± 10.0	5.0 ± 2.7
Dissolved Volatile Solids (mg/L)	110.2 ± 42.5	112.9 ± 34.7	115.9 ± 78.6	137.1 ± 36.9