Biological Invasions (2006) 8: 327–338
DOI 10.1007/s10530-004-8244-x

Invasive plant and experimental venue affect tadpole performance

Carrie J. Brown[1], Bernd Blossey[1,*], John C. Maerz[1] & Steve J. Joule [2,3]
[1]*Department of Natural Resources, Fernow Hall, Cornell University, Ithaca, NY 14853, USA;* [2]*Ft. Drum Military Installation, 85 First Street West, Ft. Drum, NY 13602, USA;* [3]*Present address: New York Department of Environmental Conservation, Bureau of Wildlife, 21 South Putt Corners Road, New Paltz, NY 12561, USA; *Author for correspondence (e-mail: bb22@cornell.edu; fax: +1-607-255-0349)*

Received 30 May 2004; accepted in revised form 28 December 2004

Key words: amphibian declines, food web effects, freshwater algae, invasion, mesocosms

Abstract

Introductions of non-native predators and competitors appear to contribute to worldwide amphibian declines; however, potential negative impacts of invasive plants on habitat quality and amphibian populations have not been examined. Loss of diversity and alterations in ecosystem function associated with plant invasions may disrupt food webs, potentially leading to further declines of already threatened amphibian populations. We used a combination of small bins, mesocosms, and field experiments to examine the impacts of Eurasian purple loosestrife (*Lythrum salicaria*) replacing native cattails (*Typha latifolia*) in North American freshwater wetlands on survival, developmental rate, and diet (freshwater algae) of American toad (*Bufo americanus*) tadpoles. Tadpoles developed slower in *L. salicaria* compared to tadpoles developing in *T. latifolia*. This effect was consistent across experimental venues, although mesocosms showed this effect only in the second year of our study. Survival and development rates were always more variable in purple loosestrife than in cattail. In bins, tadpoles showed significantly reduced survival when raised in purple loosestrife extract and addition of leaf litter exacerbated this negative effect. Tadpole survival rates in mesocosms and field cages were not significantly different between plant species, most likely an effect of high variability among replicates. We suspect a combination of direct toxicity of high tannin concentrations in *L. salicaria* leaves and their indirect negative impacts on aquatic food webs are responsible for these results. Tadpole gut analyses revealed differences in algal communities among venues and between *L. salicaria* and *T. latifolia* suggesting that alterations in tadpole food quality and quantity contribute to the observed reduced tadpole performance. The replacement of native wetland plant species by *L. salicaria* does not represent a simple exchange of ecological equivalents and the function of invaded habitats for native species has clearly changed. While we were investigating only a single amphibian species, our results suggest that the impact of *L. salicaria* on ecosystem processes and aquatic food webs may be more general and likely to negatively affect other wetland species. The threats non-indigenous plants represent for amphibian populations and food webs may be underestimated, and warrant further investigation.

Introduction

Amphibian populations are declining worldwide, in part due to introduction of invasive species (Alford and Richards 1999; Houlahan et al. 2000; Blaustein and Kiesecker 2002; Stuart et al. 2004). Although introduction of non-indigenous predators, diseases, and competitors has received considerable attention, effects of replacement of native by non-native plant species in aquatic

breeding and terrestrial feeding sites of amphibians have not been studied. Changes in plant species composition may alter chemical and physical habitat features, influence decomposition and nutrient dynamics, and alter the trophic structure of invaded ecosystems (Wedin and Tilman 1990; Chapin et al. 1997; Knops et al. 2002). Coupled with other stressors, plant invasions could exacerbate the current precarious status of many amphibian populations.

The purpose of our study was to examine impacts of purple loosestrife (*Lythrum salicaria* L.) on American toads (*Bufo americanus* Holbrook). The Eurasian *L. salicaria* invades habitats important for breeding, tadpole development, and summer foraging of adults and juveniles of many amphibian species throughout northeastern North America (Pope et al. 2000). Any decline in habitat quality as a result of *L. salicaria* invasion may add additional stress to already fragmented and isolated amphibian populations.

Investigations of invasive plant impacts need to carefully consider appropriate venues (Oksanen 2001; Petersen and Hastings 2001; Skelly and Kiesecker 2001; Skelly 2002). Field abundance counts can be unreliable (van Horne 1983; Schmidt and Whelan 1999; Bock and Jones 2004); more appropriate measures sensitive to subtle changes in habitat quality include demographic parameters such as fecundity or recruitment (Doak 1994; Byers and Goldwasser 2001). Experimental mini-ecosystems (mesocosms) allow easy replication, but they are usually small and operated for short periods of time with reduced physical and biological complexity (Petersen and Hastings 2001). Additionally, short-term experiments can not capture distinct temporal and spatial components of invasions (Blossey 1999). Conducting field investigations under relevant spatial and temporal scales while achieving appropriate replication can also present formidable challenges (Oksanen 2001). We chose to investigate impacts of *L. salicaria* on *B. americanus* in three different venues; small bins, large mesocosms, and field enclosures. If similar results are obtained in different venues, resulting conclusions are less likely to represent artifacts due to methodology (Skelly and Kiesecker 2001).

To assess habitat quality, we compared developmental rates and survival of *B. americanus* tadpoles in wetlands dominated by *L. salicaria* or cattails (*Typha* spp.). Under optimal conditions, tadpoles both grow and develop quickly resulting in high survival rates, larger size, and earlier timing of metamorphosis (Berven 1990; Kupferberg et al. 1994); all factors likely to increase post-larval fitness (Smith 1987; Semlitsch et al. 1988; Berven 1990; Scott 1994). We assessed variation in quality and quantity of tadpole diet by comparing algal communities in mesocosms and field enclosures through tadpole gut analysis. This approach allowed us to assess how different experimental venues influenced availability and composition of tadpole food which may have important impacts on size and development time (Steinwascher and Travis 1983; Ahlgren and Bowen 1991; Kupferberg et al. 1994; Kupferberg 1997). Based on other (Wilbur 1977; Alford and Harris 1988; Bardsley and Beebee 2000) and our own pilot studies (Maerz et al. unpublished data) that show negative effects of reduced resource availability on *Bufo* survival and development, we expected that any reduction in habitat quality as a result of *L. salicaria* invasion would slow development rates or increase mortality. Purple loosestrife leaves contain high concentrations of potentially toxic secondary compounds (Rauha et al. 2001), therefore exposing tadpoles to *L. salicaria* extract and litter in small bins also allowed us to explore the possibility of direct toxic effects.

Materials and methods

Experimental organisms

Lythrum salicaria is an erect herbaceous perennial plant of Eurasian origin now widespread in North American freshwater wetlands. Plants can grow across a wide moisture regime from permanently flooded (up to 1 m deep) to uplands, but are most common in periodically flooded areas (after snow melt in spring or after heavy rains). The same range of habitats is occupied by the emergent, clonal, rhizomatous *T. latifolia*, although the species is able to thrive in deeper water than *L. salicaria*. These littoral zones constitute prime breeding habitat for many amphibian species across temperate North America.

Leaves of *L. salicaria* contain significantly more tannins (24% of dry weight, stems contain 6%), and have twice the phosphorus concentration of *Typha* spp. leaves (Emery and Perry 1996; Rauha et al. 2001). Leaves of *L. salicaria* drop off the plant in the fall and decompose quickly, while leaves of *Typha* spp. and other native species such as *Carex* spp. decompose slowly (Bärlocher and Biddiscombe 1996; Emery and Perry 1996; Grout et al. 1997; Templer et al. 1998). In contrast, the woody stems of *L. salicaria* break down very slowly (3–5 years) and significant amounts of standing litter can accumulate.

American toads (*Bufo americanus*) are common throughout eastern North America in open canopy ponds that are under threat of *L. salicaria* invasion. American toads are one of six anuran species (other species typical for the region are woodfrog, *Rana sylvatica*; greenfrog, *R. clamitans*; pickerel frog, *R. palustris*; spring peeper, *Pseudacris crucifer*; and gray treefrog, *Hyla versicolor*). In the absence of fish, two salamander species, adult red-spotted newt, *Notophthalmus viridescens*, and larval yellow spotted salamander, *Ambystoma maculatum*, are typical keystone vertebrate predators in these ponds. In New York, toads breed in late April–early May in water bodies ranging from puddles to large shallow marshes (Wright and Wright 1949; Conant and Collins 1998). Toad tadpoles are microphagous filter feeders of detritus, periphytic and free-floating algae, zooplankton, and microorganisms (Holomuzki 1997) and their development is highly synchronized. Tadpoles reach metamorphosis in 5–12 weeks after hatching. We observed breeding activity in both *Typha* spp. and *L. salicaria* dominated stands although we noticed reduced male calling activity at the latter invaded sites.

Experiment 1: plant and venue effects on toad performance

We studied the effect of *L. salicaria* replacement of *T. latifolia* on larval toad survival and development in two venues: field cages and mesocosms. In October 1999, we established 10 outdoor mesocosms (two rows of five each) on the Cornell University campus in Ithaca, NY using untreated wooden frames ($1.8 \times 1.8 \times 0.5$ m) lined with plastic (20 mil PVC). Mesocosms were two-third filled with wetland soils (high at the edges and deeper in the center) excavated from a *Lythrum*/*Typha* invasion front at the Northern Montezuma Wetlands Complex (Savannah, NY) but stored dry for 2 months to eliminate potential predators. We assigned mesocosms to treatments (*L. salicaria* or *T. latifolia*) at random, and established treatments by planting rhizomes or rootstocks along the perimeter. We established potential maximum water levels by creating overflow outlets at the same height and then mesocosms were allowed to fill with rainwater over the winter. In early spring 2000, mesocosms were enclosed with nets (Lumite® screening, 20 by 20 mesh size, shade 15%, porosity 1629CFM, Synthetic Industries, Gainesville, Georgia) placed over a galvanized steel pipe frame ($2 \times 2 \times 1.8$ m). Caging prevented potential colonization by predatory invertebrates (dragonflies, etc.) and deposition of eggs by other amphibians. Water levels were allowed to fluctuate naturally, but mesocosms never fell dry. Plants were allowed to grow, with the exception of emergent volunteer plants, which we removed as needed to maintain single species treatments. Nets were removed from mesocosms in the fall to prevent snow damage and were replaced early each spring.

In early spring 2000, we established 60 tadpole enclosures along West Creek on Fort Drum Military Reservation (44°2′ N 75°49′ W) in northern New York State. West Creek is a low gradient stream with an unconsolidated silt or organic substrate. The water is slow-moving to stagnant and runs through a series of beaver dams, resulting in the formation of an emergent marsh complex throughout the course of the creek. Two of these marshes, approximately 3 km apart, were selected based on similar hydrology, but with different dominant vegetation; one dominated by *Typha* spp. and the other by *L. salicaria*. Enclosures (50 cm diameter, 90 cm height) were constructed of Lumite mesh (20×20 mesh size) attached to four 90 cm tall wooden stakes. To maintain a circular shape, we attached plastic rings (10 cm high) to the top and bottom of each enclosure. Two types of enclosures (20 bottomless and 10 with a Lumite screen bottom for a total of 30 per site) were established at similar water depths (*Typha* spp.: mean 25.9 ± 0.8 cm;

L. salicaria: mean 25.7 ± 0.9 cm) and water levels were allowed to fluctuate naturally until termination of the experiment. The bottom plastic ring of bottomed enclosures was secured into the marsh sediment preventing escape of tadpoles, as well as access by aquatic predators, but restricted tadpole access to natural marsh substrates. We added plant material (500 g) from the vicinity of the enclosure to each bottomed cage. Bottomless cages allowed contact with natural marsh substrates, but we had to search for and remove predatory insect larvae throughout the experiment.

We collected egg masses in the field and obtained additional eggs by breeding toads in the lab. We combined tadpoles from five egg masses hatching within 24 h of one another and fed them lettuce for 1 week in the laboratory. On 12 May 2000, we stocked each enclosure with 20 randomly selected, 1-week-old tadpoles, stage 27–28, (Gosner 1960). On 13 May 2000 we stocked each mesocosm with 125 randomly selected 1-week-old tadpoles, stage 27–28. Average initial tadpole densities in cages and mesocosms were 0.13 and 0.40 tadpoles/l of water respectively; both moderate to low initial densities (Morin 1983); these stocking rates avoided potential confounding density effects. To assess algal communities and tadpole diet in the different treatments, we randomly collected one tadpole from each field enclosure and two from each mesocosm on 15/16 June 2000 and preserved them in 10% formalin. We monitored cages and mesocosms several times per week and took weekly water temperature measurements using a Corning Checkmate Deluxe Field System (Corning, Elmira, NY, USA). We monitored tadpole development until we discovered the first metamorphosing individuals (after 38 day in cages and 42 day in mesocosms). All surviving tadpoles and metamorphs were harvested the following day using standardized timed searches (15 min) and dip nets. Tadpoles were killed in a Chloretone solution and preserved in 10% formalin immediately following collection.

We repeated our mesocosm experiment in 2001, an unusually dry season with substantially reduced toad breeding activity. All tadpoles originated from a single egg mass. We did not harvest tadpoles for diet analysis; temperatures were measured at bi-weekly intervals, and all tadpoles were removed after 45 days when we noticed the first metamorphs.

For each tadpole we determined stage (Gosner 1960) and wet mass. For tadpoles harvested for diet analysis, we randomly selected 20 tadpoles from bottomless (10 per treatment), 10 from bottom enclosures (5 per treatment), and one from each mesocosm for dissection. We removed the entire gut from each tadpole, and preserved the contents from the first two centimeters in 5 ml of 0.25% glutaraldehyde solution. From each sample, three slides were prepared and 400 natural units from each slide were identified (to genus level for live algal cells) and measured to determine biovolume. Natural units were classified as either live cells or detritus, which included dead plant material, dead algal cells, and any other amorphous material. Algae were identified and their biovolume determined by PhycoTech Inc., St. Joseph, MI, USA.

For all field enclosures and mesocosms, we calculated tadpole survival (%), mean stage, and mean larval mass. We used two factor ANOVAs to compare the effects of plant type and venue on toad survival and mean Gosner stage. In addition, we conducted ANOVAs on field cage data to determine the effects of cage design (bottomed v. bottomless), and we conducted a repeated measures ANOVA of mesocosm data to compare the effects of plant type across years 1 and 2.

We used two canonical analyses to compare the effects of plant type, experimental venue, and field enclosure design on tadpole diets. The first analysis used the relative biovolumes of seven functional food groups: detritus, chlorophytes, cyanophytes, chrysophytes, diatoms, euglenophytes, and a miscellaneous category which included microflagellates, unidentifiable cysts, and *Gonyostomum* semen. For the second analysis, we used the relative biovolumes of detritus, miscellaneous material, and 32 genera of algae, diatoms, and euglenophytes found in tadpole diets. All analyses were performed using Statistica 6.0 (© 2002, StatSoft, Tulsa, OK, USA).

Experiment 2: direct and indirect effects on toad performance

We harvested *L. salicaria* and *T. latifolia* leaves at different field sites in central New York in fall

2002. Plants were taken to the lab, dried immediately, and stored in paper bags. On 10 May 2003 we leached dry leaves of each plant in reverse osmosis water at a ratio of 3.33 g/l for 48 h. Then we removed and froze the leached litter, and transferred extracts to a 25-l opaque plastic container stored in a dark cooler at 4 °C. In spring 2003, we measured benthic phenolic concentrations in a natural cattail wetland and the cattail extract. We used Folin phenol reagent to reduce the active phenolics (Clesceri and Eaton 1998) and a pre-made Folin–Ciocalteu reagent (Sigma) to determine sample concentration against a phenol standard (expressed as concentrations of reactive phenolics in extracts and water as mg/l). Based on the measurements in the cattail wetland, we determined that a 10% dilution of stock extracts would emulate natural concentrations of plant compounds in our experiments (Maerz et al. 2004).

We set up 40, 10-l plastic bins for our experiment each containing 0.5 l of small and 0.5 l of large crushed stones (purchased from a garden center and rinsed thoroughly before use) and 6.3 l of filtered pond water. We added 0.7 l of stock extracts to each of 20 bins (20 replicates × 2 plant species). To half of the replicates per plant species, we added 1 g of frozen leached plant material. All bins were covered with screen lids, and placed into one of six outdoor ponds (water depth 5–10 cm) to regulate temperature.

On 2 May 2003, we collected four toad egg masses from a local wetland near Ithaca, NY. We placed egg masses in a 30-l plastic bin containing 15 l of filtered pond water and an aerator, and housed it near a window in our lab to provide hatching tadpoles with natural light and photoperiod. On 16 May, we placed five 1-week-old toad larvae (stage 25) into each bin. As in experiment 1, this stocking rate (0.6 tadpoles/l) represents a low (but similar) density, thus avoiding potential confounding density effects. We also collected benthic periphyton, flocculent algae, and floating bloom algae from artificial ponds, homogenized them in a plastic tub, strained out the pond water, and added 30 ml to each bin.

To ensure that all treatments were replicated equally among ponds, we used a stratified random design to assign bins to ponds and locations within ponds. Ponds were covered with Lumite® screening (20 × 20 mesh size, shade 15%, porosity 1629CFM, Synthetic Industries, Gainesville, GA, USA) placed over a 3.6 × 3.6 × 1.8 m galvanized steel pipe frame. We monitored food abundance every other day, and we added an additional 30 ml of algae to all bins on days 10 and 30 of the experiment. After 40 days, we harvested all remaining larvae from bins, killed them in MS-222, and preserved them in 10% buffered formalin.

For each bin, we determined the number of larvae that survived and we measured mean development stage (Gosner 1960). We used a two factor ANOVA to analyze the effects of the two plant extracts and the addition of plant litter on toad survival and development.

Results

Experiment 1: plant and venue effects on toad performance

We did not include tadpole mass at metamorphosis in our analyses because in all *L. salicaria* field cages and many mesocosms toads failed to advance to the metamorph stage. There was no overlap in mean Gosner stage of tadpoles from *T. latifolia* (range = 38.5–42.5) and *L. salicaria* (range = 29–38) field cages, and the relationship between mass and development (Gosner stage) was non-linear across the range of development stages observed. Mass increased with Gosner stage from stage 29 through 38, then declined from stage 38 through 42; therefore, we could not compare stage specific mass among our treatments.

There were large temperature differences between *L. salicaria* field cages and mesocosms and *T. latifolia* field cages, and temperature had a significant positive effect on tadpole survival ($r = 0.457$, $P < 0.001$) and development ($r = 0.740$, $P < 0.001$; Figure 1). Therefore, we included temperature as a covariate in our subsequent analyses. Analysis of 2000 field cage and mesocosm data showed that temperature had a marginal effect on survival and a highly significant effect on mean Gosner stage (Table 1). Corrected for temperature effects, there was a significant interaction between plant type and

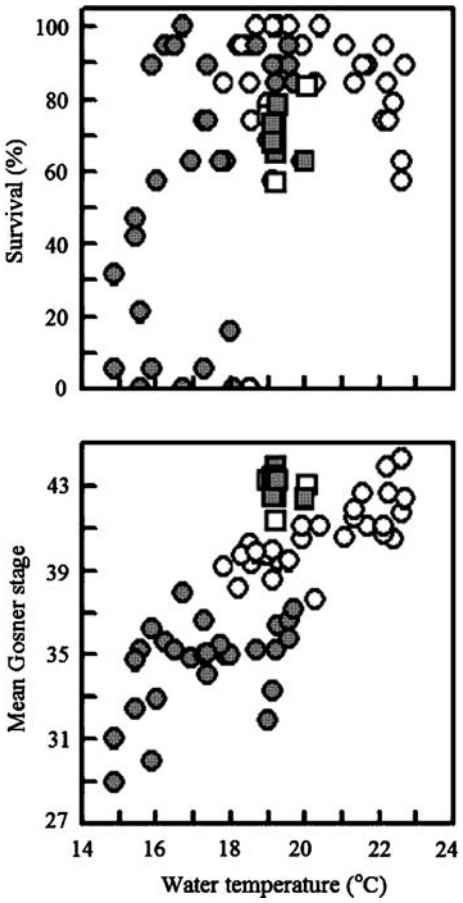

Figure 1. Relationships between mean water temperature through the experimental period and percent survival and mean Gosner (1960) stage across all 2000 venues, cage designs, and plant treatments.

venue on mean development stage but not survival (Table 1, Figure 2).

We probably failed to find a significant effect of plant type or interaction between plant type and venue because variance of tadpole survival in *L. salicaria* field cages was significantly larger than in other treatments (Levene's Test for Homogeneity of Variance: $MS_{\text{effect}} = 1527$, $MS_{\text{error}} = 154$, $F_{3,62} = 9.941$, $P < 0.001$). This variance (adjusted for temperature) was explained, in part, by an interaction of plant type and cage design within the field cage experiment ($MS = 3239.79$, $F_{(1,51)} = 6.539$, $P = 0.013$). Tadpole survival was not significantly different between *L. salicaria* and *T. latifolia* in bottomed cages, but was significantly different between plant species in bottomless cages (Figure 2).

Cage design had no effect on tadpole development in either marsh (Plant type × Cage design interaction: $MS = 2.125$, $F_{(1,51)} = 0.901$, $P = 0.347$). Mean tadpole development stage in *L. salicaria* was significantly lower than in *T. latifolia* (Plant type main effect: $MS = 109.86$, $F_{(1,51)} = 46.583$, $P < 0.001$; Figure 2).

Tadpole survival in mesocosms differed between years and between plant types across the years. Tadpole survival declined significantly between 2000 and 2001 in all mesocosms, but declined more in *L. salicaria* than in *T. latifolia* mesocosms (Plant type × Year interaction: $MS = 115.94$, $F_{(1,8)} = 6.278$, $P = 0.037$; Figure 2). In 2000, there was no difference in survival between mesocosm types ($MS = 18.5$, $F_{(1,8)} = 0.265$, $P = 0.620$), but by 2001 survival in

Table 1. Results of plant type, venue and temperature (covariate) effects on toad tadpole survival and mean Gosner stage.

Variable Source of variation	*MS*	df	F	P
Percent survival				
Intercept	5	1	0.010	0.919
Temperature (covariate)	1806	1	3.786	0.056
Plant type	285	1	0.598	0.442
Venue	239	1	0.501	0.482
Plant × Venue	90	1	0.189	0.666
Error	477	61		
Mean Gosner stage				
Intercept	276	1	130.556	< 0.001
Temperature (covariate)	65	1	30.473	< 0.001
Plant type	25	1	12.014	0.001
Venue	211	1	99.535	< 0.001
Plant × Venue	33	1	15.620	< 0.001
Error	2.676	61		

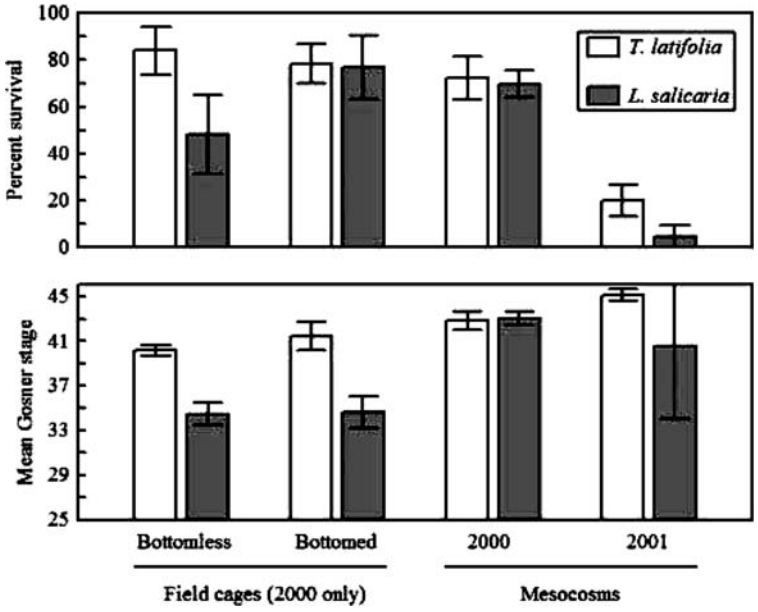

Figure 2. Mean (±2 SE) percent survival and Gosner (1960) developmental stage of *Bufo americanus* tadpoles reared in native *T. latifolia* or *L. salicaria* field cages (bottomed or bottomless; 2000 only) and mesocosms (2000 and 2001).

L. salicaria mesocosms was significantly lower than in *T. latifolia* mesocosms ($MS = 577.6$, $F_{(1,8)} = 13.127$, $P = 0.007$; Figure 2).

Tadpole guts from *L. salicaria* mesocosms contained 45.3% detritus (mean biovolume) and guts from *T. latifolia*-reared tadpoles contained 59.7% detritus. In bottomless enclosures in the natural marshes, detritus accounted for an average of 64.7 and 78.3% of tadpole gut biovolume contents in *L. salicaria* and *Typha* spp., respectively. In bottomed enclosures, 61.8% of the gut contents of *L. salicaria*-reared tadpoles were detritus compared to 84.9% in *Typha* spp.-reared tadpoles. The remaining gut contents consisted mainly of algae (chlorophytes, diatoms, chrysophytes, and cyanophytes; Table 2). There was a large overlap in algal communities (at the genus level) in all the experimental venues. Divisions of algae found in the field enclosures were the same as those in the mesocosms, but represented in different proportions (Table 2). Based on tadpole gut analyses, mesocosms showed depauperate algal taxonomic richness compared to field enclosures (Table 2). Among field cages, tadpoles in bottomless enclosures had the highest diversity of algae in their diets (excluding genera represented in trace amounts).

Canonical analysis using functional groups yielded one significant canonical root that explained 77% of the variation in tadpole diets and clearly distinguished between mesocosm and field enclosure tadpole diets, but showed no diet pattern associated with plant type (Eigenvalue = 4.008, $\chi^2 = 82.746$ df = 35, $P < 0.001$). Tadpoles in mesocosms had a much higher biovolume of cyanophytes in their diets than did field cage tadpoles, while diets in field enclosures contained a greater biovolume of detritus. The second canonical analysis using prey genera identified two significant canonical roots that explained 99.7% of the variation in tadpole diets and clearly separated tadpole diets according to venue, cage design, and dominant plant. The second canonical root shows a common effect of plant type regardless of experimental venue or cage design on tadpole diets (Figure 4A) (Eigenvalue = 23.430, $\chi^2 = 174.664$ df = 128, $P = 0.004$). A significantly greater biovolume of the filamentous chlorophyte *Spirogyra* in *T. latifolia* mesocosms and field cages and relatively (within venue) lower biovolumes of filamentous blue-green algae was the largest contributor to tadpole diet differences related to plant type.

Table 2. Mean percent biovolume of algal genera found in tadpole guts.

Genus	AD[a]	Mesocosms		Field cages			
				Bottomless		Bottomed	
		Typha	Lyth	Typha	Lyth	Typha	Lyth
Spirogyra sp.	C	16.88	0.74	–	2.75	17.18	–
Mougeotia sp.	C	0.14	0.13	0.33	4.09	–	2.22
Oedogonium sp.	C	0.57	–	5.09	0.83	5.49	–
Microspora sp.	C	9.90	16.47	–	–	–	–
Bulbochaete sp.	C	–	–	–	3.04	–	–
Scenedesmus sp.	C	0.01	–	0.03	0.05	0.06	0.07
Chlorococcum sp.	C	1.01	0.38	0.69	0.23	1.63	0.68
Ulothrix sp.	C	3.74	5.01	0.61	–	0.12	–
Amphipleura sp.	D	–	–	0.13	–	–	–
Meridion sp.	D	–	–	0.08	0.02	–	0.02
Cymbella sp.	D	0.02	–	0.40	0.86	0.13	1.24
Amphora sp.	D	–	–	0.03	1.03	0.19	1.49
Eunotia sp.	D	–	–	0.62	1.95	0.33	0.72
Cyclotella sp.	D	0.04	0.04	0.14	1.77	0.28	8.19
Fragilaria sp.	D	0.17	0.01	1.89	2.86	0.38	5.60
Navicula sp.	D	1.44	1.20	1.37	2.34	0.86	6.05
Surirella sp.	D	–	0.21	0.13	–	–	–
Diploneis sp.	D	–	–	0.09	–	–	–
Synedra sp.	D	0.15	0.01	0.09	0.09	0.06	2.27
Gomphonema sp.	D	–	–	0.15	2.39	0.24	2.31
Achnanthes sp.	D	0.01	0.01	0.41	1.22	0.71	1.39
Nitzschia sp.	D	0.08	0.61	0.22	0.04	0.04	0.08
Diatoma sp.	D	–	–	0.06	0.64	0.07	0.99
Denticula sp.	D	–	–	–	0.44	–	0.37
Non-motile blue-greens (>1 UM)	CY	0.04	4.98	–	0.01	–	–
Pseudanabaena sp.	CY	–	0.03	–	–	–	–
Oscillatoria sp.	CY	2.30	0.05	0.07	0.72	0.01	0.17
Tolypothrix sp.	CY	0.22	6.45	–	–	–	–
Cyst	CH	0.07	0.03	–	0.01	–	–
Blue-green Akinete	M	1.24	5.47	–	–	–	–
Misc. microflagellate	M	–	–	–	0.03	–	0.01

– Genera present in trace amounts not included in table.
[a] Alagal divisisions: C = chlorophyte; D = diatom; CH = chrysophyte; CY = cyanophyte; M = miscellaneous.

Experiment 2: direct and indirect effects on toad performance

Toad survival was significantly lower in *L. salicaria* treatments than in *T. latifolia* treatments, and there was a significant extract by plant litter interaction (Table 3, Figure 4). Previous experiments showed that toad survival and development were not significantly different between a water control and a treatment using *T. latifolia* extract (Maerz et al. 2005). Compared to *T. latifolia* treatments, exposure to *L. salicaria* extracts alone reduced toad survival by 36%. The addition of leached *L. salicaria* litter reduced toad survival by 89% compared to *L. salicaria* extracts alone and by 93% compared to *T. latifolia* treatments. The low survival of toads in *L. salicaria* treatments with litter added limited analysis of effects on tadpole development. Comparison of mean development stages from treatments without litter showed no significant difference in mean development stage between *L. salicaria* and *T. latifolia* treatments ($t = -0.461$, $df = 18$, $P = 0.650$).

Table 3. ANOVA plant extract and litter additions on *B. americanus* tadpole survival.

Source of variation	df	MS	F	P
Plant species	1	72.9	66.949	< 0.001
Litter treatment	1	16.9	15.520	< 0.001
Plant × Litter treatment	1	12.1	11.112	0.002
Error	36	1.1		

Discussion

To the best of our knowledge, this is the first study to investigate impacts of a non-indigenous plant on larval amphibian survival and development. Our designs used low (but similar) stocking rates to avoid potential confounding density effects and the results support our main hypotheses that *B. americanus* tadpole performance is negatively affected by *L. salicaria* and we suspect a combination of indirect mechanisms (change in quality and quantity of algal food) and direct toxicity (high tannin concentrations) to be responsible for this effect. While we conducted fieldwork only at two marshes, our results are robust because negative impacts were confirmed in additional experiments. Our experiments may be the first comparison of food resources for tadpoles in mesocosms versus field enclosures and show that algal community differences may contribute to venue effects (Skelly and Kiesecker 2001; Skelly 2002).

The effect of experimental venue

Bins and mesocosms are popular in ecological research, however, their realism due to reduced physical and biological complexity has been questioned (Carpenter 1996; Skelly and Kiesecker 2001; Skelly 2002). Our results show that venue was a significant effect in our experiments, but we reproduced similar results across a range of biological complexity. Subtle design changes can have substantial effects: adding a bottom screen affected tadpole survival (Figure 2); adding litter dramatically reduced tadpole survival (Figure 4). Access to decomposing *L. salicaria* litter has major negative implications for tadpoles and we can only speculate that this may be a direct toxic effect of non-water soluble compounds, present in or on detritus, that are released in tadpole guts. In isolation, any of our experiments could have produced misleading results about the presence or magnitude of an effect; but in combination they provide evidence for the negative impact of *L. salicaria* on *B. americanus*.

Freshwater algae (Table 2) and detritus are the most important food items of *B. americanus* tadpoles in our experiments and tadpole gut contents should accurately represent algal communities present in their habitats (Farlowe 1928; Johnson 1991). This allows us to make inferences about algal communities in our mesocosms and the marshes, which were drastically different (Figure 3). The dominant algal genera

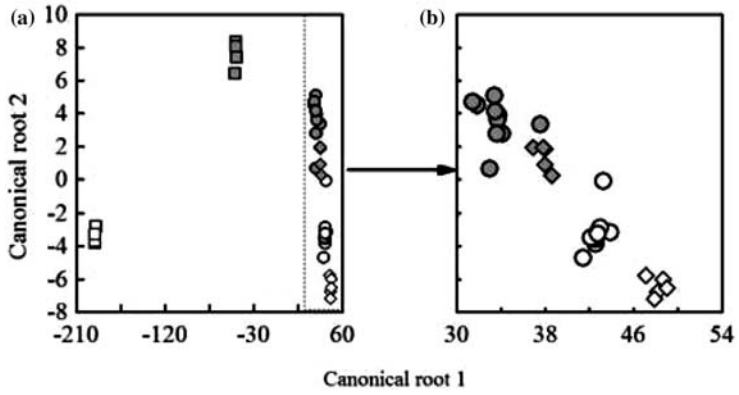

Figure 3. Scatterplot of root 1 and 2 scores from canonical analysis of percent biovolumes of algal genera in tadpole diets from *L. salicaria* (grey symbols) and *T. latifolia* (white symbols) treatments. Circles represent bottomless enclosures, diamonds represent bottomed enclosures, and squares represent mesocosms. (b) Enlargement of area encompassed by the dashed line in (a).

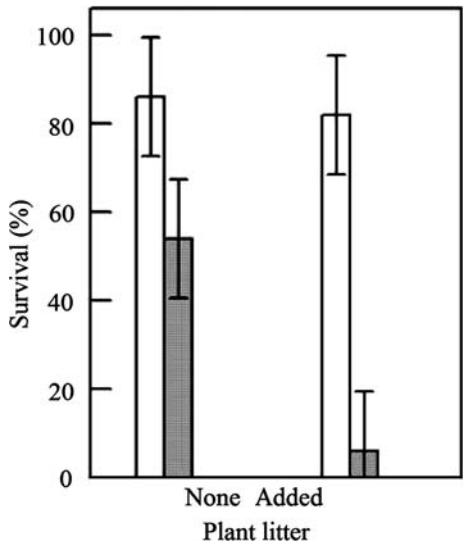

Figure 4. Mean (±2 SE) percent survival of *Bufo americanus* tadpoles reared in native *T. latifolia* (white bars) or *L. salicaria* (grey bars) extracts with or without plant litter added.

and functional group were different between venues, and mesocosms showed reduced algal richness. We introduced dormant stages of many organisms with natural wetland soils to our mesocosms but they still produced species poor algal communities (Table 2) that undoubtedly were linked to differences in tadpole performance across venues. Unexpectedly, algal communities and tadpole diet were also clearly different between bottomed and bottomless enclosures (Figure 3). We attribute the decline in tadpole survival from first to second year in all *T. latifolia* mesocosms (Figure 2) to increase in emergent plant biomass reducing nutrient pools availability for algal growth.

The effect of dominant plant species

Emergent plants have important impacts on structure and nutrient availability of aquatic habitats. Differences in secondary compounds, nutrient content, and leaf toughness among wetland plants create species-specific effects on mineralization and nutrient availability (Irons et al. 1988; Driebe and Whitham 2000; Miki and Kondoh 2002) during decomposition with major implications for aquatic food webs (Naeem et al. 2000). Surprisingly, mesocosm studies of larval amphibians seldom include living plants in their design, which may greatly change the outcome of ecological experiments. The replacement of native wetland plant species by *L. salicaria* does not represent a simple exchange of ecological equivalents. This invasion introduces leaves with high tannin contents to an aquatic food web which may reduce microbial conditioning of leaf litter with important consequences for decomposition rates and detritivore communities (Findlay and Arsuffi 1989; Driebe and Whitham 2000). Tannins with their antibacterial and antimicrobial properties also function as digestive inhibitors and concentrations of as little as 2% dry weight can deter herbivore feeding (Rosenthal and Janzen 1979). Moreover, tannins bind with proteins (Suberkropp et al. 1976) rendering even an abundant food supply inaccessible for tadpoles (Britson and Kissell 1996).

In our experiments, tadpole survival in *L. salicaria* was significantly reduced and more variable; most likely the result of a combination of direct toxic and indirect food web effects. These effects of invasive species may take years to develop; if we had restricted our investigations to mesocosms and a single season, we would have, erroneously, concluded that there was no indication for a negative impact of *L. salicaria*. In addition to changes in quantity and quality of the food supply, the predictability of habitat quality for tadpoles has decreased. While *T. latifolia* habitat appeared exceptionally stable, we documented an increased variability of survival rates, developmental rates, and tadpole diets in *L. salicaria* regardless of venue. Such uncertainty of habitat quality will have important consequences for organisms, which, over evolutionary time, have adjusted to prevailing conditions.

While we were investigating only a single amphibian species, it is likely that the impact on ecosystem processes and aquatic food webs is more general and *L. salicaria* is negatively affecting other wetland species. Many breeding ponds and wetlands used for foraging (Semlitsch 1998) in the Northeast and Midwest now have extensive *L. salicaria* populations (Thompson et al. 1987; Pope et al. 2000). A reduction in wetland quality for amphibians through *L. salicaria* invasion would likely reduce survival and fitness of

juveniles and adults and could contribute to population declines (Berven 1990; Morey and Reznik 2001; Álvarez and Nicieza 2002; Relyea and Hoverman 2003). We need additional studies to assess whether negative impacts of non-indigenous plants invading amphibian habitat are commonplace or whether effects documented here for *L. salicaria* are a special case. Amphibians are already threatened by multiple factors (Alford and Richards 1999; Houlahan et al. 2000; Kiesecker et al. 2001), and additional stress may further compromise currently healthy populations. We conclude by suggesting that threats non-indigenous plants represent to amphibians and food webs may be underestimated, and warrant further investigation.

Acknowledgements

We thank K. Zamudio, S. Morreale, V. Nuzzo, and members of the Blossey lab at Cornell University for contributions throughout the study and A. Silvano, A. Tompkins, and K. Ellis for field assistance. The Environmental Division on Fort Drum Military Installation provided access, financial, and logistical support. Additional funding was provided by the Postgraduate Environmental Management Participation Program at the U.S. Army Environmental Center administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and USAEC and a Kieckhefer Adirondack Fellowship awarded to CJB. We also acknowledge helpful comments by anonymous reviewers.

References

Ahlgren MO and Bowen SH (1991) Growth and survival of tadpoles (*Bufo americanus*) fed amorphous detritus derived from natural waters. Hydrobiologia 218: 49–51

Alford RA and Harris R (1988) Effects of larval growth history on anuran metamorphosis. The Amercian Naturalist 131: 91–106

Alford RA and Richards SJ (1999) Global amphibian declines: a problem in applied ecology. Annual Review of Ecology and Systematics 30: 133–165

Álvarez D and Nicieza AG (2002) Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecologia 131: 186–195

Bardsley L and Beebee TJC (2000) Competition between *Bufo* larvae in a eutrophic pond. Oecologia 124: 33–39

Bärlocher F and Biddiscombe NR (1996) Geratology and decomposition of *Typha latifolia* and *Lythrum salicaria* in a freshwater marsh. Archiv für Hydrobiologie 136: 309–325

Berven KA (1990) Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71: 1599–1608

Blaustein AR and Kiesecker JM (2002) Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters 5: 597–608

Blossey B (1999) Before, during, and after: the need for long- term monitoring in invasive plant species management. Biological Invasions 1: 301–311

Bock CE and Jones ZF (2004) Avian habitat evaluation: should counting birds count? Frontiers in Ecology and the Environment 2: 403–410

Britson CA and Kissell REJ (1996) Effects of food type on developmental characteristics of an ephemeral pond-breeding anuran, *Pseudacris triseriata feriarum*. Herpetologica 52: 374–382

Byers JE and Goldwasser L (2001) Exposing the mechanism and timing of impact of nonindigenous species on native species. Ecology 82: 1330–1343

Carpenter SR (1996) Microcosm experiments have limited relevance for community and ecosystem ecology. Ecology 77: 677–680

Chapin FS, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE and Tilman D (1997) Biotic control over the functioning of ecosystems. Science 277: 500–504

Clesceri LS and Eaton AD (eds) (1998) Standard Methods for Examination of Water and Wastewater, Vol, pp 5–51, American Public Health Association, Method #5550

Conant R and Collins JT (1998) A Field Guide to Reptiles and Amphibians of Eastern and Central North America, Vol. Houghton Mifflin Company, Boston

Doak DF (1994) Source-sink models and the problem of habitat degradation: general models and applications to the Yellowstone grizzly. Conservation Biology 9: 1370–1379

Driebe E and Whitham TG (2000) Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. Oecologia 123: 99–107

Emery SL and Perry JA (1996) Decomposition rates and phosphorus concentrations of purple loosestrife (*Lythrum salicaria*) and cattail (*Typha* spp.) in fourteen Minnesota wetlands. Hydrobiologia 323: 129–138

Farlowe V (1928) Algae of ponds as determined by an examination of the intestinal contents of tadpoles. Biological Bulletin 55: 443–448

Findlay SEG and Arsuffi TL (1989) Microbial growth and detritus transformations during decomposition of leaf litter in a stream. Freshwater Biology 21: 261–269

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183–190

Grout JA, Levings CD and Richardson JS (1997) Decomposition rates of purple loosestrife (*Lythrum salicaria*) and Lyngbyei's sedge (*Carex lyngbyei*) in the Fraser River estuary. Estuaries 20: 96–102

Holomuzki JR (1997) Habitat-specific life-histories and foraging by stream-dwelling American toads. Herpetologica 53: 445–453

Houlahan JE, Findlay CS, Schmidt BR, Meyer AH and Kuzmin SL (2000) Quantitative evidence for global amphibian population declines. Nature 404: 752–755

Irons JGI, Oswood MW and Bryant JP (1988) Consumption of leaf detritus by a stream shredder: influence of tree species and nutrient status. Hydrobiologia 160: 53–61

Johnson LM (1991) Growth and development of larval northern cricket frogs (*Acris crepitans*) in relation to phytoplankton abundance. Freshwater Biology 25: 51–59

Knops JMH, Bradley KL and Wedin DA (2002) Mechanisms of plant species impacts on ecosystem nitrogen cycling. Ecology Letters 5: 454–466

Kupferberg SJ (1997) The role of larval diet in anuran metamorphosis. American Zoologist 37: 146–159

Kupferberg SJ, Marks JC and Power ME (1994) Effects of variation in natural algal and detrital diets on larval anuran (*Hyla regilla*) life-history traits. Copeia 1994: 446–457

Maerz JC, Brown CJ, Chapin CT and Blossey B (2005) Plant secondary metabolites affect larval amphibian performance. Functional Ecology (submitted)

Miki T and Kondoh M (2002) Feedbacks between nutrient cycling and vegetation predict plant species coexistence and invasion. Ecology Letters 5: 624–633

Morey S and Reznik D (2001) Effects of larval density on postmetamorphic spadefoot toads (*Spea hammondii*). Ecology 82: 510–522

Morin PJ (1983) Predation, competition, and the composition of larval anuran guilds. Ecological Monographs 53: 119–138

Naeem S, Hahn DR and Schuurman G (2000) Producer-decomposer co-dependency influences biodiversity effects. Nature 403: 762–764

Oksanen L (2001) Logic of experiments in ecology: is pseudoreplication a pseudoissue? Oikos 94: 27–38

Petersen JE and Hastings A (2001) Dimensional approaches to scaling experimental ecosystems: designing mousetraps to catch elephants. American Naturalist 157: 324–333

Pope SE, Fahrig L and Merriam HG (2000) Landscape complementation and metapopulation effects on Leopard frog populations. Ecology 81: 2498–2508

Rauha JP, Wolfender JL, Salminen JP, Pihlaja K, Hostettmann K and Vuorela H (2001) Characterization of the polyphenolic composition of purple loosestrife (*Lythrum salicaria*). Zeitschrift für Naturforschung 56: 13–20

Relyea RA and Hoverman JT (2003) The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. Oecologia 134: 596–604

Rosenthal GA and Janzen DH (eds) (1979) Herbivores: Their Interaction with Secondary Plant Metabolites, Vol. Academic Press, New York

Schmidt KA and Whelan CJ (1999) Effects of exotic *Lonicera* and *Rhamnus* on songbird nest predation. Conservation Biology 13: 1502–1506

Scott DE (1994) The effect of larval density on adult demographic traits in amphibians in *Ambystoma opacum*. Ecology 75: 1383–1396

Semlitsch RD (1998) Biological delineation of terrestrial buffer zones for pond-breeding salamanders. Conservation Biology 12: 1113–1119

Semlitsch RD, Scott DE and Pechmann JHK (1988) Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69: 184–192

Skelly DK (2002) Experimental venue and estimation of interaction strength. Ecology 83: 2097–2101

Skelly DK and Kiesecker JM (2001) Venue and outcome in ecological experiments: manipulations of larval anurans. Oikos 94: 198–208

Smith DC (1987) Adult recruitment in chorus frogs – effects of size and date at metamorphosis. Ecology 68: 344–350

Steinwascher K and Travis J (1983) Influence of food quality and quantity on early larval growth of two anurans. Copeia 1983: 238–242

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL and Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306: 1783–1786

Suberkropp K, Godshalk GL and Klug MJ (1976) Changes in the chemical composition of leaves during processing in a woodland stream. Ecology 57: 720–727

Templer P, Findlay S and Wigand C (1998) Sediment chemistry associated with native and non-native emergent macrophytes of a Hudson River marsh ecosystem. Wetlands 18: 70–78

Thompson DQ, Stuckey RL and Thompson EB (1987) Spread, Impact, and Control of Purple Loosestrife (*Lythrum salicaria*) in North American Wetlands. US Fish and Wildlife Service, Washington, DC, USA

van Horne B (1983) Density as a misleading indicator of habitat quality. Journal of Wildlife Management 47: 893–901

Wedin DA and Tilman D (1990) Species effect on nitrogen cycle: a test with perennial grasses. Oecologia 84: 433–3441

Wilbur HM (1977) Density-dependent aspects of growth and metamorphosis in *Bufo americanus*. Ecology 58: 196–200

Wright AH and Wright AA (1949) Handbook of Frogs and Toads of the United States and Canada. Comstock Publishing Company, Ithaca, New York