

Bottom-up meets top-down: leaf litter inputs influence predator–prey interactions in wetlands

Aaron B. Stoler · Rick A. Relyea

Received: 14 June 2012 / Accepted: 11 January 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract While the common conceptual role of resource subsidies is one of bottom-up nutrient and energy supply, inputs can also alter the structural complexity of environments. This can further impact resource flow by providing refuge for prey and decreasing predation rates. However, the direct influence of different organic subsidies on predator–prey dynamics is rarely examined. In forested wetlands, leaf litter inputs are a dominant energy and nutrient resource and they can also increase benthic surface cover and decrease water clarity, which may provide refugia for prey and subsequently reduce predation rates. In outdoor mesocosms, we investigated how inputs of leaf litter that alter benthic surface cover and water clarity influence the mortality and growth of gray treefrog tadpoles (*Hyla versicolor*) in the presence of free-swimming adult newts (*Notophthalmus viridescens*), which are visual predators. To manipulate surface cover, we added either oak (*Quercus* spp.) or red pine (*Pinus resinosa*) litter and crossed these treatments with three levels of red maple (*Acer rubrum*) litter leachate to manipulate water clarity. In contrast to our predictions, benthic surface cover had no effect on tadpole survival while darkening the water caused lower survival. In addition, individual tadpole mass was lowest in the high maple leachate treatments, suggesting an interaction between bottom-up effects of leaf litter and top-down effects of predation risk that altered mortality and growth of tadpoles. Our results indicate that realistic

changes in forest tree composition, which cause concomitant changes in litter inputs to wetlands, can substantially alter community interactions.

Keywords Aquatic refuge · Benthic structure · Water clarity · *Hyla versicolor* · *Notophthalmus viridescens*

Introduction

Ecological function is reliant on the flow of resource subsidies between food webs and on the biological processes that assimilate and process these inputs (Polis et al. 1997; Marcarelli et al. 2011). In food webs, the most common conceptual role of subsidies is the bottom-up supply of energy and nutrients that permit greater in situ production than internal resources would allow (Polis et al. 1997). However, resource subsidies also alter the structural complexity of an environment by generating microhabitat and altering other abiotic factors (e.g., water chemistry; Dobson et al. 1992; Richardson 1992; Moore et al. 2004). Both bottom-up forces and environmental changes can alter food web dynamics, particularly predator–prey interactions (Crowder and Cooper 1982). For example, the response of prey to the threat of predators is often mediated by bottom-up energy availability, and prey may be able to utilize changes to the environment as chemical or physical refugia (Flecker and Allan 1984; Carpenter et al. 2010; Evans et al. 2011). Consideration of subsidies as mediating predator–prey dynamics is rare, yet is critical to assessing the full impact of subsidies on ecological function.

Senescent plant tissue (i.e., litter) is one of the largest sources of coarse particulate subsidies (Polis et al. 1997) and ecological function is often reliant on the decomposition of this resource pool. This is particularly true in

Communicated by Ross Alford.

A. B. Stoler (✉) · R. A. Relyea
Department of Biological Sciences, University of Pittsburgh,
4249 Fifth Avenue, 101 Clapp Hall, Pittsburgh,
PA 15260, USA
e-mail: abs45@pitt.edu

temperate forests where 70–90 % of all terrestrial leaf tissue and substantial amounts of woody debris fall to the ground each year (Facelli and Pickett 1991). Much of this litter gravitates towards streams and wetlands containing heterotrophic food webs that rapidly decompose the litter (Marecchelli et al. 2011). These inputs, which frequently exhibit interspecific variation in both chemical and physical structure (Webster and Benfield 1986), can have positive bottom-up effects on prey growth through the provision of nutrients and energy (Wallace et al. 1997; Motomori et al. 2001; Leroy and Marks 2006; Stoler and Relyea 2011). In contrast, leachate from litter can contain detrimental levels of dissolved organic carbon (DOC) and other compounds such as phenolic acids that interfere with growth and development of prey and their predators (Horne and Dunson 1995; Maerz et al. 2005; Canhoto and Laranjeira 2007). Changes in growth can have substantial consequences for predator–prey dynamics. Higher growth can result in prey reaching size-refugia from gape-limited predators and possessing greater evasion speeds (Wilbur et al. 1983), whereas lower growth can make prey easier to catch.

Leaf litter inputs can also alter the physical and chemical environment in multiple ways that may directly interfere with predator–prey dynamics (Richardson 1992; Yee and Juliano 2006). First, litter inputs to wetlands persist as relatively stationary sources of microhabitat that can provide shelter and visual protection from predators (Richardson 1992; Dudgeon and Wu 1999). Indeed, in artificial wetland mesocosms a greater percentage of prey are often found hiding in litter when predators are present (e.g., Hoverman and Relyea 2008). Second, leachate from litter inputs can darken the water (Karlsson et al. 2009), which may increase prey survival by making it harder for visual predators to find and catch prey. Acid leachates from litter (e.g., phenolics) may interfere with prey responses to predators by reducing pH, which may reduce the effectiveness of chemical cues emitted by predators (i.e., kairomones) that prey use to detect and respond to predators (Brown et al. 2002; Leduc et al. 2004). Finally, changes in predation rates resulting from such direct effects of litter may have further effects on prey by changing per capita resource availability for prey. Although these effects may not be of great importance in lotic (i.e., flowing) systems such as streams and rivers where litter and leachates rapidly flow downstream (Dobson et al. 1992; Richardson 1992; Dudgeon and Wu 1999), they are likely important in lentic (i.e., non-flowing) systems where material is retained for much longer periods of time.

Our goal was to investigate how predator–prey interactions respond to changes in benthic surface cover and water clarity generated by inputs of leaf litter of equal biomass. We made three predictions: increased benthic surface cover would increase prey survival due to increased refuge

availability, decreased water clarity from litter leachate would increase prey survival due to reduced visual detection by predators, and the combination of increased benthic surface cover and decreased water clarity would increase prey survival more than either factor alone. These predictions assume that the influence of litter on pH or prey growth rate is negligible. If increased structure or decreased water clarity cause decreases in prey growth, which would make the prey more susceptible to gape-limited predators, then the predicted increases in prey survival could be weakened or even reversed.

To test these predictions, we altered surface cover and water clarity in the benthos of outdoor, artificial wetland mesocosms by manipulating the species of litter inputs. Using wetland mesocosms, we examined how these manipulations affected the growth and survival of gray tree frog tadpoles (*Hyla versicolor*) when in the presence of adult eastern red-spotted newts (*Notophthalmus viridescens*) as predators. Although these predictions could be tested using artificial structure and coloring agents, this would preclude any bottom-up effects of litter chemistry on prey.

System background

Our experiment was conducted at the University of Pittsburgh's Pymatuning Laboratory of Ecology in northwest Pennsylvania. Senesced tree litter constitutes a dominant source of nutrient and energy subsidies to the ponds that these newts and tadpoles cohabit. In the region where this study was conducted and where organisms were collected, red maple (*Acer rubrum*), red pine (*Pinus resinosa*), and oak (*Quercus* spp.) constitute three of the dominant tree species. In addition, these trees are associated with contemporary changes in forest diversity. Currently, maples are increasing in abundance throughout eastern temperate forests through such forces as selective browsing by mammals (e.g., white tailed deer, *Odocoileus virginianus*) and human-driven fire suppression (Abrams 1998, 2003). At the same time, the ranges of many maple species are predicted to shift northwards following current models of climate change (Hansen et al. 2001; Iverson and Prasad 2001).

The ranges of gray tree frogs and newts overlap in most areas where these tree species are dominant. Both species can be found in a wide variety of habitats, from large lakes to small wetlands, open- to moderate-canopy systems, and from deciduous to coniferous forests (Lannoo 2005; Werner et al. 2007). Many of these habitats receive substantial inputs of litter from surrounding trees, either from overhead litterfall or wind-blown inputs. Often, these inputs are concentrated in shallow littoral zones where amphibians spend much of their time foraging and seeking refuge (Porej and Hetherington 2005). Gray tree frogs are a

summer-breeding species that typically lay their eggs during June and July (Kiesecker and Skelly 2000). They are active foragers, typically metamorphosing in 3–6 weeks (Werner et al. 2007). In many systems, they constitute an important prey item for eastern red-spotted newts, which are predominantly visual predators that forage during both day and night (Martin et al. 1973) and are often keystone predators in vernal ponds (Wilbur et al. 1983). Gray tree frogs and other prey items of eastern red-spotted newts, including larval newt conspecifics, are alerted to the threat of predation through chemical cues (i.e., kairomones; Dodson et al. 1994; Relyea 2001; Mathis 2003). Upon eating and digesting tadpole prey, newts release kairomones that induce phenotypic responses in gray tree frog tadpoles that make the tadpoles less susceptible to predation (Lawler 1989; Relyea 2001). These include relatively immediate reductions in movement and activity to diminish visual detection, and more gradual changes in body shape that increase chances of escape.

Materials and methods

The experiment used a completely randomized design with six treatments in which we crossed two benthic surface cover treatments (oak vs. pine litter) and three water clarity treatments. Low, medium, and high water clarity treatments were generated using variable amounts of red maple litter that was removed prior to the experiment to avoid altering the total biomass of litter in treatment, which might confound results. Due to expected variability in predator feeding behavior, we replicated each treatment eight times, resulting in 48 experimental units. Although it was unlikely that litter inputs would have any effect on tadpole survival or mass over the short duration of our study (Stoler and Relyea 2011), we assessed these direct, bottom-up effects by including two control replicates for each of the six treatments that included a caged predator. This design produced a total of 60 experimental units.

Our experimental units were 100-L plastic wading pools. The pools were 1 m in diameter and approximately 0.2 m in height. Each pool was covered with a 60 % shade-cloth lid to prevent escape or entry of any organisms and to simulate a medium level of canopy cover relative to the range of canopy cover in ephemeral wetlands (Werner and Glennemeier 1999).

Pine and oak litter were placed into mesocosms on 8 June 2011. Litter used for this experiment was collected immediately after senescence during the autumn prior to the experiment. While the chemistry of this litter is substantially different from that of older litter to which summer-breeding amphibians would be naturally exposed, prior work has demonstrated that the physical structure of

oak and pine does not deteriorate much from the time of senescence and the time of the experiment. Furthermore, stained water due to red maple leachate remains dark throughout the spring and summer in many ponds (A. Stoler, unpublished data). To manipulate low and high benthic surface cover, we added 100 g of red pine needles or 100 g of oak leaves to the mesocosms, respectively. This biomass is within the range of observed litter inputs to forest wetlands (Rubbo et al. 2008) and is similar to the biomass of inputs used in past experiments (e.g., Stoler and Relyea 2011). These two species were used to manipulate structure due to their common co-occurrence, conservation concern, and similarity in lignin content and breakdown rate (Webster and Benfield 1986), which indicate a similarity in physical rigidity. Biomass was used to standardize inputs in accordance with the methods of nearly all other litter manipulation studies and mesocosm experiments (e.g., Rubbo et al. 2008).

Maple litter was added to mesocosms on 10 June 2011. To generate high, medium, or low water clarity, we added 15, 50, or 85 g of maple litter to the mesocosms. These amounts span the range of observed red maple inputs to forest wetlands as observed in field surveys (A. Stoler, unpublished data); the highest biomass leached sufficient DOC into the water so that the benthos was no longer visible. Because we wanted an equal biomass of benthic leaf litter in all treatments, we placed the maple litter into 5-mm-mesh bags that were later removed. Soluble carbon began leaching from leaves almost immediately and clarity ceased to change after 2 days. Bags were left in mesocosms for 9 days and were removed prior to tadpole introduction, while oak and pine litter were kept in the mesocosms for the duration of the study. Although red maple served as the primary source of leachate, both oak and pine do leach some carbon into the water. However, this amount is nominal relative to the leachate of maple, primarily due to the slow decomposition rate of oak and pine species (Webster and Benfield 1986).

Two days after maple litter introduction, in accordance with common protocol for setting up mesocosms, we collected and mixed water from six nearby ponds to serve as a source of periphyton, phytoplankton, zooplankton, bacteria and fungi. We inoculated each mesocosm by placing 1.5-L aliquots of the water into all mesocosms. We chose ponds for water collection based on their proximity to tree species whose litter was represented in this experiment. We allowed the mesocosms to sit for 7 days prior to the introduction of tadpoles. Given this short time period, there was no substantial increase in zooplankton that could serve as an alternative food source for the newts. Hence, we made no attempt to quantify zooplankton. Growth of periphyton biomass was quantified in a previous study (Stoler and Relyea 2011) that found greater growth among

conifer litter treatments relative to broadleaf litter treatments, and relatively low growth of periphyton with red maple litter relative to oak litter. Hence, we did not quantify periphyton biomass in the current study.

In accordance with accepted Institutional Animal Care and Use Committee protocol, the gray tree frogs were collected as 24 amplexing pairs that were allowed to oviposit into laboratory containers. After oviposition, we transferred eggs to outdoor wading pools. Tadpoles were fed rabbit chow ad libitum until introduced into the experiment when they reached a safe handling mass (initial mean mass \pm 1 SE = 25 ± 18 mg). On 19 June 2011 (defined as day 0 of the experiment), individuals from all 24 clutches were mixed and 30 tadpoles were placed into each mesocosm. This resulted in a density of 38 tadpoles m^{-2} , which is well within the natural range of densities for *H. versicolor* (Relyea and Hoverman 2003). Thirty additional tadpoles were chosen at random to assess 24 h survival post-handling, which was 100 %.

We collected the newts from a local wetland and held them in laboratory tubs containing filtered water and refugia for 7 days. While in the laboratory, we kept four newts in each container and fed them with 15–20 gray tree frog tadpoles daily (at a size that was similar to the tadpoles they would experience during the experiment). To ensure that the newts used in the experiment had similar propensities to consume tadpoles, we attempted to feed all individuals two tadpoles prior to their introduction into mesocosms. We only used individuals that readily ate both tadpoles.

On day 1 of the experiment (20 June 2011), one newt was introduced into each mesocosm. This resulted in a density of approximately 1 individual m^{-2} , which is comparable to densities observe in natural ponds (Gill 1978). Since predator–prey interactions can be altered by phenotypic changes that tadpoles undergo when sensing predatory risk, we caged all newts for the first 2 days to provide tadpoles with predator cues. Cages were made of corrugated drain pipe, capped on both sides by 1-mm mesh and held in place along the edge of each mesocosm with binder clips. Immediately after placing newts in cages, each newt was fed 300 mg of gray tree frogs to cause the production of kairomones by the newts. This biomass of prey is sufficient to elicit a response by tadpoles (Schoepner and Relyea 2005). The newts were not fed for the next 2 days, which is a sufficient time to ensure they are hungry when released (Lefcort and Blaustein 1995). On day 3, all newts in the uncaged treatments were released from their cages; all cages were left in the mesocosms. Newts in mesocosms assigned to the caged-predator treatment were not released. Instead, they were fed 300 mg of tadpoles on day 1, 3, and 5 so that the tadpoles were continually exposed to the kairomones. To equalize disturbance caused by feeding the caged newts, all empty

cages were also lifted out of the water and placed back after each feeding.

To monitor tadpole survival over time, we randomly selected a single replicate from each treatment on each morning of the experiment, removed all litter, and netted and counted all tadpoles. We stirred the litter in all other mesocosms to equalize the disturbance generated by this activity. Mortality was ≤ 30 % by day 3, so we attempted to increase the rate of predation by increasing visibility in the water. To do this, we replaced the 60 % shade cloth lids with 10 % shade cloth lids (made of nylon window screen).

The experiment ended on day 7. By that time, newts had foraged for 4 days. In addition, the tadpoles in some treatments had grown nearly tenfold, indicating a potential size refuge from newts. Upon termination, we collected all newts and placed them into individual containers. Because tadpole survival may be influenced by the body size of a predator, we measured the snout–vent length (SVL) of each newt using digital calipers. While treatments may have influenced newt mass, SVL was unlikely to change significantly over the short duration of the experiment (average growth rate of adult newts is ~ 5 mm SVL $year^{-1}$; Caetano and Leclair 1996).

After measuring the newts, all tadpoles were removed from each mesocosm and counted to determine percent mortality. The tadpoles from each mesocosm were weighed and we used the mean individual mass as our response variable. To verify that leaf litter did not exert a bottom-up influence on tadpole over the short duration of our study, tadpoles in caged-predator replicates were also weighed. We did not attempt to assess tadpole behavior among treatments, as the dark water of high-leachate treatments made it difficult to see individuals and posed a sampling bias.

Water chemistry

Using the method of Collier (1987), we quantified the concentration of DOC in the water column via spectrophotometric absorbance, which has been shown to be accurate across large ranges and types of DOC. We took samples on day 3, after the newts were released, and kept samples at 4 °C for 2 days until they were processed. We filtered samples through a 0.42- μ m cellulose membrane and allowed samples to reach room temperature before assaying in a spectrophotometer (Perkin Elmer UV/Vis Lambda 20 Spectrophotometer). Absorbance values were transformed to $g\ m^{-3}$ of DOC via the equation:

$$DOC\ (g\ m^{-3}) = 59.6a + 1.9$$

where a is equal to the absorbance of the sample at 360 nm with a path length of 1 cm in acrylic cuvettes. We also quantified pH in all mesocosms on day 7 with a handheld

meter (P4 Multiline meter; WTW Instruments). Sub-sampling of treatments for dissolved oxygen and temperature revealed no difference among treatments, which was expected due to the high surface area to volume ratio of our mesocosms that allowed rapid surface air and heat exchange.

Statistical analysis

We used multivariate ANOVA to test for effects of surface cover and leachate on tadpole mortality, tadpole mass, pH, and the mass of DOC in the water column. This effectively controlled for type I error when conducting subsequent univariate analyses. We employed type III sums of squares based on unweighted marginal means to account for our unbalanced experimental design due to missing replicates. We used a full-factorial model including benthic surface cover treatments and red maple-leachate treatments as independent, fixed factors. Preliminary tests revealed that inclusion of newt SVL as a covariate in the model had no effect on the biological interpretation of results, so this covariate was dropped from the multivariate model. For leachate treatments, we used Tukey's test to conduct mean comparisons between treatments after finding significant univariate effects. Caged-predator treatments were not included in this analysis as they were used only to confirm a lack of any direct, bottom-up effect of litter; however, values and ranges of these treatments are reported in Table 1. All variables were assessed for normality using probability plots. Percent mortality was log-transformed to fit a normal distribution. One newt escaped from an uncaged-predator replicate containing oak and a low maple leachate, so we discarded all data from this replicate. Using Dixon's *Q*-test (confidence level = 95 %; Sokal and Rohlf 1995) we detected one outlier among mortality responses in the high maple leachate and oak litter treatment, and so discarded all data from this replicate.

Results

Caged-predator treatments confirmed that tadpole mortality and individual mass did not differ among caged-predator controls, thus indicating no bottom-up effect of litter over

the short duration of our study. Across all caged-predator treatments, mortality was never higher than 6 %. Means and ranges of tadpole mortality and individual mass are provided in Table 1. For all uncaged-predator treatments, our analysis revealed significant multivariate effects of benthic surface cover species and maple leachate level. There was no interaction between surface cover species and red maple leachate (Table 2).

Effects of surface cover species

We did not detect any univariate effects of benthic surface cover species on tadpole mortality, but there was a marginal effect on individual tadpole mass (Fig. 1; Table 2). Tadpoles in oak litter treatments were approximately 10 % smaller relative to individuals in pine litter treatments.

We detected a significant effect of benthic surface cover species on DOC concentration in the water column, as measured by absorbance, and on pH (Table 2; Fig. 2). DOC concentration was approximately 21 % higher in oak litter treatments relative to pine litter treatments. Mean comparisons revealed that pH was approximately 0.3 pH units less in oak litter treatments relative to pine litter treatments.

Effects of red maple leachate

Red maple leachate affected tadpole mortality (Table 2; Fig. 1a). Mean comparisons found that mortality in high- and medium-leachate treatments was at least 13 % greater than in low-leachate treatments ($P \leq 0.007$). Mortality among high- and medium-leachate treatments did not differ ($P = 0.78$).

Red maple leachate also affected individual tadpole mass (Table 2; Fig. 1b). Tadpoles in the high-leachate treatment were 50 mg (26 %) smaller than tadpoles in the medium-leachate treatment ($P < 0.001$), and tadpoles in the medium-leachate treatment were 36 mg (16 %) smaller than tadpoles in low-leachate treatments ($P = 0.011$).

The concentration of DOC in the water column was also affected by maple leachate (Table 2; Fig. 2a). Mean comparisons revealed that the concentration of DOC was significantly different between all three levels of maple

Table 1 Means \pm 1 SE of tadpole mortality and individual mass among caged-predator treatments

	Mortality (%)			Individual mass (mg)		
	Low	Medium	High	Low	Medium	High
Pine litter	1.5 \pm 1.1	3.1 \pm 2.2	0.0 \pm 0.0	231.9 \pm 21.4	275.3 \pm 11.8	219.9 \pm 43.2
Oak litter	1.5 \pm 1.1	0.0 \pm 0.0	1.5 \pm 1.1	263.3 \pm 4.5	206.5 \pm 28.6	198.9 \pm 11.4

Values are divided among the three levels of maple leachate treatments (low, medium, and high) within the two treatments of benthic surface cover (pine and oak)

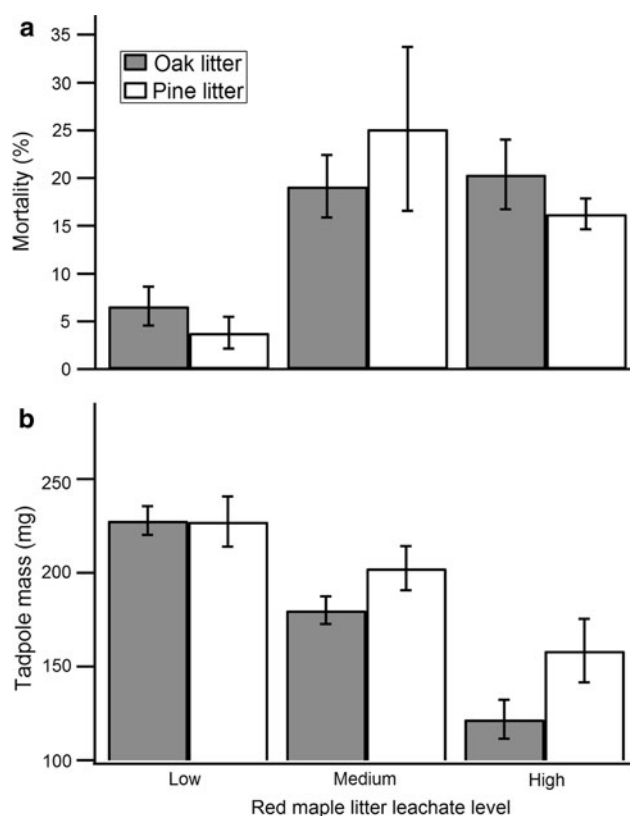


Fig. 1 Effect of leachate level from red maple litter and benthic surface cover (oak vs. pine litter) on individual tadpole **a** mortality and **b** mass (means \pm 1 SE). Results displayed are for uncaged-predator treatments only

leachate ($P < 0.001$). The concentration of DOC was 30 % greater in medium-leachate treatments relative to low-leachate treatments, and 48 % higher in high-leachate treatments relative to medium-leachate treatments.

Red maple leachate also influenced pH (Table 2; Fig. 2b). Mean comparisons revealed that pH differed between all leachate levels ($P < 0.001$). Water in low-leachate treatments was 0.4 pH units greater than in

medium-leachate treatments, which were approximately 0.5 pH units greater than high-leachate treatments.

Discussion

Our study suggests that leaf litter inputs alter the physical and chemical environment of wetlands in a manner that influences prey growth and subsequent interactions between predators and prey. While tadpole mortality was relatively low (0–6 %) when newts were caged, tadpole mortality ranged from 5 % to more than 20 % with lethal predators as the amount of red maple leachate increased. However, mortality was unaffected by our manipulation of benthic surface cover using oak or pine litter. In addition, tadpole mass increased as maple leachate decreased and individuals reached a potential size refuge from predation by the conclusion of the study.

These results refuted our three predictions, which were based on the assumption that visibility was the dominant factor influencing newt-tadpole interactions (Martin et al. 1973). It is possible that this assumption was incorrect; indeed, studies examining other newt-prey and newt-predator interactions indicate that newts are responsive to chemical cues (Dodson et al. 1994; Mathis 2003). However, there is no strong indication that newts use chemical cues when detecting heterospecific prey items (Martin et al. 1973). Moreover, this suggests that the increased mortality of tadpoles recorded in the high-leachate treatments of our study was due to increased perception of tadpole cues in these treatments. This is unlikely, as experimental manipulations of prey cues in increasingly high- and low-light environments registered no change in newt predation activity (Martin et al. 1973). Hence, it is unlikely that changes in predator perception of prey chemical cues were a major mechanism underlying our results.

One possible explanation for our results is that treatments with elevated DOC (i.e., high maple leachate)

Table 2 Multivariate and univariate results of the multivariate ANOVA for benthic surface cover and leachate treatments on mass of dissolved organic carbon (DOC), mortality, tadpole mass, and pH in mesocosms containing uncaged predators

Multivariate	<i>F</i>		<i>P</i>					
Benthic surface cover	12.4 _{4,37}		<0.001					
Maple leachate	49.2 _{8,74}		<0.001					
Benthic cover \times maple leachate	1.4 _{8,74}		0.22					
Univariate	Tadpole mortality		Tadpole mass		DOC		pH	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Benthic surface cover	0.1 _{1,40}	0.76	3.9 _{1,40}	0.055	26.3 _{1,40}	<0.001	49.9 _{1,40}	<0.001
Maple leachate	8.8 _{2,40}	0.001	26.0 _{2,40}	<0.001	567.5 _{2,40}	<0.001	145.3 _{2,40}	<0.001

Univariate results for the interaction term are not provided, as the multivariate effect was not significant. *df* are given as subscripts with *F*-values

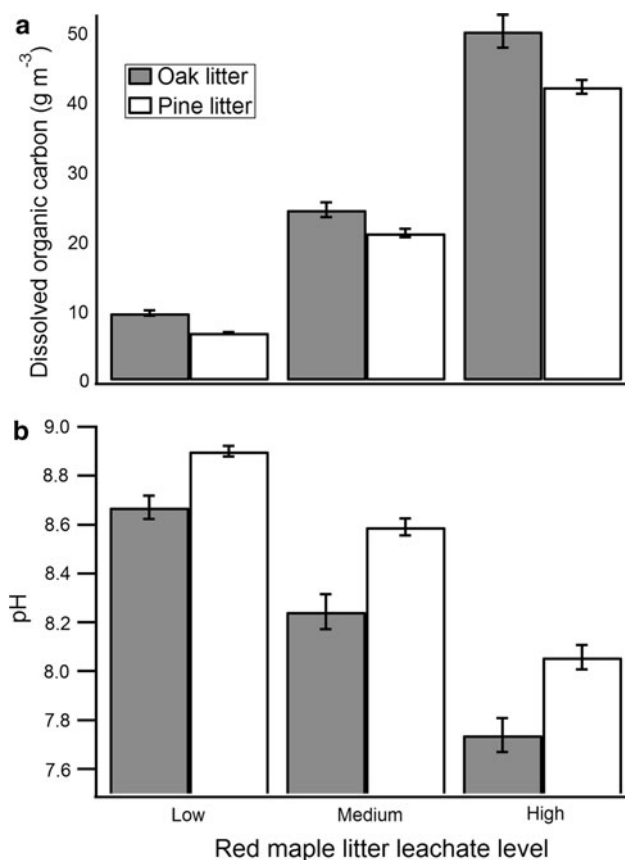


Fig. 2 Effect of leachate level from red maple litter and benthic surface cover (oak vs. pine litter) on **a** mass of dissolved organic carbon and **b** pH (means \pm 1 SE). Results displayed are for uncaged-predator treatments only

decreased light availability in the visible spectrum more for the tadpoles than the newts, thereby affording newts a visual advantage. Such differences in spectral sensitivities have been documented for other aquatic organisms and their predators, particularly among fish (Endler 1992). While newts are visual predators, their spectral sensitivity is unknown, so we cannot determine if elevated DOC altered their visual acuity in our study. However, many species of aquatic prey, including tadpoles, use chemical cues to detect their predators (Dodson et al. 1994; Brönmark and Hansson 2000), so it is unlikely that differences in visual acuity had a strong influence on tadpole predation. It is more likely that high amounts of maple leachate interfered with the chemical cues of newts or reduced prey size, thereby making it easier for the newts to consume more tadpoles.

Chemical cues such as kairomones are common in pond environments (Dodson et al. 1994), and previous work demonstrates that gray tree frogs reduce their movement when kairomones are present (Schoeppner and Relyea 2005). However, the effectiveness of chemical alarm cues can be pH dependent. In streams, Brown et al. (2002) found

that a reduction of pH by one unit (i.e., from 7 to 6) reduced predator avoidance behavior of a minnow and dace species, likely due to a permanent deformation of the alarm cue's molecular structure. Such changes in pH can be caused by litter inputs, particularly when litter species rich in phenolic acids (e.g., red maple) are introduced into the system. Although our observed pH values (pH 7.8–8.9) were not below neutral and were not within a range that would directly harm tadpoles (Grant and Licht 1993), the decline in pH of 0.9 units with increased maple leachate may have been sufficient to alter the detection of kairomones by the prey. In turn, this would have prevented tadpoles from activating their normal suite of anti-predator strategies, such as hiding or reducing movement, which would result in increased predation rates and decreased tadpole survival. This hypothesized mechanism certainly requires further investigation.

Differences in predation may have also been affected by differences in tadpole size. Body size is a critical factor in determining prey survival, particularly when the predator is gape-limited (Wilbur et al. 1983). Unlike many predators that pierce or chew their prey (e.g., dragonfly larvae), newts consume tadpoles by engulfing the body (Wilbur and Fauth 1990). Larger prey are both faster and more difficult to engulf, making successful predation attempts harder (Relyea 2004). In our study, tadpole mass increased as leachate decreased; by the end of the experiment tadpoles in low-leachate treatments were nearly twice the mass of tadpoles in high-leachate treatments and were likely closer to a size refuge from newt predation. Hence, larger body size likely contributed to increased tadpole survival under low-leachate treatments.

Differences in body size among treatments may have been caused by variation in litter inputs that differed in the quality and availability of resources (Brinson et al. 1981; Webster and Benfield 1986; Marcarelli et al. 2011). Low tadpole survival has been associated with red maple litter in wetland mesocosms, likely due to large inputs of DOC and phenolic acids that can inhibit periphyton production through shading and chemical inhibition, and can also interfere with gill functioning (Rubbo and Kiesecker 2004; Maerz et al. 2005). High levels of red maple may increase aerobic microbial respiration, leading to reduced dissolved oxygen and suffocation of tadpoles (Wassersug and Feder 1983; A. Stoler, unpublished data). In addition, DOC leached from maple litter darkens the water column and reduces algal growth, which is a nutrient-rich food source for gray tree frog tadpoles (Kupferberg 1997). In contrast, pine litter possesses relatively little soluble carbon (Berg and McLaugherty 2008), resulting in clearer water that promotes greater algal productivity (Karlsson et al. 2009). Indeed, in mesocosms of similar size and with similar litter species to those used in this experiment, Stoler and Relyea

(2011) found greater biomass of algal-dominated periphyton in litter treatments with relatively clear water.

Given the nominal mortality among caged-predator treatments, tadpole mortality was not likely a direct result of litter chemistry or leachates in our study. Moreover, we did not find any dead tadpoles and did not note any individuals that appeared sickly or weak in either caged- or uncaged-predator treatments. Considering the detrimental effects of leachates when tadpoles are exposed for longer durations, it is possible that more time is needed for leachates to have pronounced bottom-up effects on tadpole fitness (Rubbo and Kiesecker 2004; Stoler and Relyea 2011). Yet even over short durations, the presence of sublethal stressors can have important consequences on tadpole fitness when combined with other stressors (Relyea 2003), such as elevated kairomone levels and the sight of a free-swimming predator. Hence, an important implication of our study that deserves further investigation is that bottom-up stresses caused by the effects litter inputs on the chemical and physical environment may exacerbate the effects of stress from top-down forces.

Further work should aim to understand how increasing environmental and ecological complexity mediate the effect of litter inputs on predator–prey dynamics. It is worth noting that natural water chemistry may substantially differ from that of our mesocosms and will depend on many environmental variables (e.g., timing of litterfall, hydroperiod, soil composition, temperature). Understanding how such climactic factors influence the effects of leaf litter and predators on prey fitness is necessary to fully elucidate how our experimental results translate to natural phenomena. Further work should also aim to understand how increasing food web complexity mediates these effects. For example, the presence of litter grazers with functionally different feeding habits can facilitate consumer growth (Iwai and Kagaya 2007), which may have further impacts on predation rates. Incorporation of such complexity may offer detailed and important insight into the effects of litter in natural food webs.

Implications for future shifts in forest composition

By manipulating leaf litter species, the results of our study suggest that predicted changes in eastern temperate forests of the United States have the potential to dramatically change the dynamics of forested wetlands. While red maple naturally colonizes forests through succession—replacing trees such as pines and poplars (*Populus* spp.)—it is also rapidly increasing in abundance throughout the northeastern United States due to fire suppression and selective mammalian browsing (Abrams 1998, 2003). At a local scale, many forests are becoming near-monocultures of red maple. Our study indicates that such shifts in forest tree

composition will influence predator–prey dynamics in wetlands. This is important for both wetlands and the surrounding forest since many predator and prey species, particularly amphibians, significantly contribute to nutrient cycling in large regions surrounding wetlands (Beard et al. 2002). Thus, our study suggests a biological consequence of changing forest composition that should be considered to fully estimate future changes in the ecological functioning of forests.

Acknowledgments Our thanks to R. J. Bendis, Rickey Cothran, John Hammond, Chris Hensley, Marco Katzenberger, and Zach Zbinden for assisting with the experiment. Comments from Rickey Cothran, John Hammond, and two anonymous reviewers greatly improved this manuscript. The experiments described comply with current US law. This research was funded by a Leasure K. Darbaker Prize in Botany awarded to A. B. S. and supported by NSF funding to R. A. R. This is PLE research paper no. 310.

References

- Abrams MD (1998) The red maple paradox. *Bioscience* 48:355–364
- Abrams MD (2003) Where has all the white oak gone? *Bioscience* 53:927–939
- Beard KH, Vogt KA, Kulmatiski A (2002) Top-down effects of a terrestrial frog on forest nutrient dynamics. *Oecologia* 133: 583–593
- Berg B, McLaugherty C (2008) Plant litter: decomposition, humus formation, carbon sequestration. Springer, Berlin
- Brinson MM, Lugo AE, Brown S (1981) Primary productivity, decomposition and consumer activity in freshwater wetlands. *Annu Rev Ecol Syst* 12:123–161
- Brönmark C, Hansson L (2000) Chemical communication in aquatic systems: an introduction. *Oikos* 88:103–109
- Brown GE, Adrian JC Jr, Lewis MG, Tower JM (2002) The effects of reduced pH on chemical alarm signaling in ostariophysan fishes. *Can J Fish Aquat Sci* 59:1331–1338
- Caetano MH, Leclair R Jr (1996) Growth and population structure of red-spotted newts *Notophthalmus viridescens* in permanent lakes of the Laurentian Shield, Quebec. *Copeia* 1996:866–874
- Canhoto C, Laranjeira C (2007) Leachates of *Eucalyptus globulus* in intermittent streams affect water parameters and invertebrates. *Int Rev Hydrobiol* 92:173–182
- Carpenter SR, Cole JJ, Kitchell JF, Pace ML (2010) Trophic cascades in lakes: lessons and prospects. In: Terborgh J, Estes JA (eds) *Trophic cascades: predators, prey, and the changing dynamics of nature*. Island Press, Washington, pp 55–70
- Collier KJ (1987) Spectrophotometric determination of dissolved organic carbon in some South Island streams and rivers (Note). *N Z J Mar Freshwater Res* 21:349–351
- Crowder LB, Cooper WE (1982) Habitat structural complexity and the interaction between bluegills and their prey. *Ecology* 63: 1802–1813
- Dobson M, Hildrew AG, Ibbotson A, Garthwaite J (1992) Enhancing litter retention in streams: do altered hydraulics and habitat area confound field experiments? *Freshwater Biol* 28:71–79
- Dodson SI, Crowl TA, Peckarsky BL, Kats LB, Covich AP, Culp JM (1994) Non-visual communication in freshwater benthos: an overview. *J N Am Benthol Soc* 13:268–282
- Dudgeon D, Wu KKY (1999) Leaf litter in a tropical stream: food or substrate for macroinvertebrates? *Arch Hydrobiol* 146:65–82

- Endler JA (1992) Signal conditions, and the direction of evolution. *Am Nat* 139:S125–S153
- Evans DM, Dolloff A, Aust WM, Villamagna AM (2011) Effects of eastern hemlock decline on large woody loads in streams of the Appalachian mountains. *J Am Water Resour*
- Facelli JM, Pickett STA (1991) Plant litter: its dynamics and effects on plant community structure. *Bot Rev* 57:1–32
- Flecker AS, Allan JD (1984) The importance of predation, substrate and spatial refugia in determining lotic insect distributions. *Oecologia* 64:306–313
- Gill DE (1978) The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). *Ecol Monogr* 48:145–166
- Grant KP, Licht LE (1993) Acid tolerance of anuran embryos and larvae from central Ohio. *J Herpetol* 27:1–6
- Hansen AJ, Neilson RP, Dale VH, Flather CH, Iverson LR, Currie DJ, Shafer S, Cook R, Bartlein PJ (2001) Global change in forests: responses of species, communities, and biomes. *Bioscience* 51:765–779
- Horne MT, Dunson WA (1995) The interactive effects of low pH, toxic metals, and DOC on a simulated temporary pond community. *Environ Pollut* 89:155–161
- Hoverman JT, Relyea RA (2008) Temporal environmental variation and phenotypic plasticity: a mechanism underlying priority effects. *Oikos* 117:23–32
- Iverson LR, Prasad AM (2001) Potential changes in tree species richness and forest community types following climate change. *Ecosystems* 4:186–199
- Iwai N, Kagaya T (2007) Positive indirect effects of tadpoles on a detritivore through nutrient regeneration. *Oecologia* 152:685–694
- Karlsson J, Byström P, Ask J, Ask P, Persson L, Jansson M (2009) Light limitation of nutrient-poor lake ecosystems. *Nature* 460:506–510
- Kiesecker JM, Skelly DK (2000) Choice of oviposition site by gray treefrogs: the role of potential parasitic infection. *Ecology* 81:2939–2943
- Kupferberg S (1997) Facilitation of periphyton production by tadpole grazing: functional differences between species. *Freshwater Biol* 37:427–439
- Lannoo M (2005) Amphibian declines: the conservation status of United States species. University of California Press, London
- Lawler SP (1989) Behavioural responses to predators and predation risk in four species of larval anurans. *Anim Behav* 38:1039–1047
- Leduc AOHC, Ferrari MCO, Kelly JM, Brown GE (2004) Learning to recognize novel predators under weakly acidic conditions: the effects of reduced pH on acquired predator recognition by juvenile rainbow trout. *Chemoecology* 14:107–112
- Lefcort H, Blaustein AR (1995) Disease, predator avoidance, and vulnerability to predation in tadpoles. *Oikos* 74:469–474
- Leroy CJ, Marks JC (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. *Freshwater Biol* 51:605–617
- Maerz JC, Brown CJ, Chapin CT, Blossey B (2005) Can secondary compounds of an invasive plant affect larval amphibians? *Funct Ecol* 19:970–975
- Marcarelli AM, Baxter CV, Mineau MM, Hall RO Jr (2011) Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology* 92:1215–1225
- Martin JB, Witherspoon NB, Keenleyside MHA (1973) Analysis of feeding behavior in the newt *Notophthalmus viridescens*. *Can J Zool* 52:277–281
- Mathis A (2003) Use of chemical cues in detection of conspecific predators and prey by newts, *Notophthalmus viridescens*. *Chemoecology* 13:193–197
- Moore JC, Berlow EL, Coleman DC, de Ruiter PC, Dong Q, Hastings A, Johnson NC, McCann KS, Melville K, Morin PJ, Nadelhoffer K, Rosemond AD, Post DM, Sabo JL, Scow KM, Vanni MJ, Wall DH (2004) Detritus, trophic dynamics and biodiversity. *Ecol Lett* 7:584–600
- Motomori K, Mitsuhashi H, Nakano S (2001) Influence of leaf litter quality on the colonization and consumption of stream invertebrate shredders. *Ecol Res* 16:173–182
- Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annu Rev Ecol Syst* 28:289–316
- Porej D, Hetherington TE (2005) Designing wetlands for amphibians: the importance of predatory fish and shallow littoral zones in structuring of amphibian communities. *Wetl Ecol Manage* 13:445–455
- Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* 82:523–540
- Relyea RA (2003) Predator cues and pesticides: a double dose of danger for amphibians. *Ecol Appl* 13:1515–1521
- Relyea RA (2004) Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* 85:172–179
- Relyea RA, Hoverman JT (2003) The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. *Oecologia* 134:596–604
- Richardson JS (1992) Food, microhabitat, or both? Macroinvertebrate use of leaf accumulations in a montane stream. *Freshwater Biol* 27:169–176
- Rubbo MJ, Kiesecker JM (2004) Leaf litter composition and community structure: translating regional species changes into local dynamics. *Ecology* 85:2519–2525
- Rubbo MJ, Belden LK, Kiesecker JM (2008) Differential responses of aquatic consumers to variations in leaf-litter inputs. *Hydrobiologia* 605:37–44
- Schoeppner NM, Relyea RA (2005) Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defenses. *Ecol Lett* 8:505–512
- Sokal RR, Rohlf FJ (1995) Biometry: the principles and practices of statistics in biological research. Freeman, New York
- Stoler AB, Relyea RA (2011) Living in the litter: the influence of tree species on wetland communities. *Oikos* 120:862–872
- Wallace JB, Eggert SL, Meyer JL, Webster JR (1997) Multiple levels of a forest stream linked to terrestrial litter inputs. *Science* 277:102–104
- Wassersug RJ, Feder ME (1983) The effects of aquatic oxygen concentration, body size and respiratory behavior on the stamina of obligate aquatic (*Bufo americanus*) and facultative air breathing (*Xenopus laevis* and *Rana berlandieri*) anuran larvae. *J Exp Biol* 105:173–190
- Webster JR, Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. *Annu Rev Ecol Syst* 17:567–594
- Werner EE, Glennemeier KS (1999) Influence of forest canopy cover on the breeding pond distributions of several amphibian species. *Copeia* 1999:1–12
- Werner EE, Skelly DK, Relyea RA, Yurewicz KL (2007) Amphibian species richness across environmental gradients. *Oikos* 116:1697–1712
- Wilbur HM, Fauth JE (1990) Experimental aquatic food webs: interactions between two predators and two prey. *Am Nat* 135:176–204
- Wilbur HM, Morin PJ, Harris RN (1983) Salamander predation and the structure of experimental communities: anuran responses. *Ecology* 64:1423–1429
- Yee DA, Juliano SA (2006) Consequences of detritus type in an aquatic microsystem: effects on water quality, micro-organisms and performance of the dominant consumer. *Freshwater Biol* 51:448–459