USING PLANT TRAITS TO UNDERSTAND THE IMPACTS OF PLANT INVASIONS ON LARVAL ANURANS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Jillian Standish Cohen

May 2009

ABSTRACT

Plant detritus is the primary source of energy in freshwater benthic food webs, and the flow of energy from detritus to consumers is largely a function of detritus quality. Theory suggests that invasions by nonnative plants can impact consumers by changing the quality of the detrital pool, but empirical evidence for this is limited. Larval anurans, an abundant constituent of wetlands, may be particularly responsive to shifts in litter quality, as they feed largely on detrital biofilms. I conducted two experiments to evaluate the impacts of nonnative plant litter on larval anurans. For each experiment, I hypothesized that litter quality, including C:N:P, tannin content and lignin content, would determine the number of tadpoles that completed metamorphosis, as well as the productivity, or aggregate mass, of those metamorphs. First, I conducted a field experiment involving four native and five nonnative plant species at five different wetland complexes in central New York. I installed field cages in sites dominated by a single species of each plant and added three tadpole species according to their natural phenology. I monitored cages regularly, collecting and weighing all metamorphosed individuals as they became available. I observed unique interactions between plant and amphibian species, largely driven by the response of larval anurans to plant traits (e.g. sensitivity to plant phenolics). Importantly, my data shows that tadpole performance did not differ in habitats dominated by native or nonnative plants, largely because there are no consistent differences in native and nonnative litter quality. However, my findings do show that plant traits, irrespective of plant origin, do affect tadpole performance. For my second experiment, I raised tadpoles in experimental mesocosms containing an algal slurry and litter from 15 populations of a single nonnative species, *Phalaris arundinacea* L. (reed canarygrass), that varies widely in litter quality. I observed significant differences in tadpole performance among *P. arundinacea* populations, and found that

litter traits explain a significant portion of the observed variation in tadpole productivity. Increases in *P. arundinacea* C:P had a negative impact on tadpole performance, while increases in plant phenolics had a positive effect. Overall, my work shows that variation in litter quality, both between and within species, influences secondary productivity in these experimental communities. This suggests that functional traits, irrespective of species origin or identity, can have important consequences for ecosystem function.

BIOGRAPHICAL SKETCH

Jill Cohen was born in Washington, D.C. in May 1984. She decided to become an environmental scientist in the fourth grade, when each student in her class raised a guppy in old 2-L soda bottles. Jill was immensely proud when her guppy, Tina, survived months longer than all the other students' guppies. During high school, she gained a foothold in both biology and French. Loving both subjects equally, she decided to attend college at McGill University in Montreal, Quebec, where she could major in environmental science and hear French spoken constantly. At McGill she got a job feeding guppies in Dr. Andrew Hendry's lab. This led to a summer internship with Dr. Tony Ricciardi studying herbivore communities on aquatic plants, where she fell in love with fieldwork. After graduating from McGill in 2006, she found a home for herself in Bernd Blossey's lab in the Department of Natural Resources at Cornell. She looks forward to furthering her research on the impacts of plant invasions on aquatic ecosystems by pursuing a Ph.D. under the guidance of Dr. Blossey.

To Nedda and Igou,

for reminding me why I love to learn

ACKNOWLEDGMENTS

I would first like to thank my major advisor, Dr. Bernd Blossey, for his guidance and support. Bernd has always shown a genuine interest in my opinion, and his faith in me has helped me find my voice as a scientist and a person. He motivates me through his example, as he constantly explores new areas of thought and research. Even more, he has reinforced my resolve to conduct research that will help people make informed decisions about environmental management.

I would also like to thank Dr. John Maerz for laying the groundwork for the research presented in this thesis, and for continually trusting me, even when I haven't trusted myself. John has a way of talking that continually excites and engages those around him. Whenever we spend time together, I pay close attention not just to what he says, but also how he says it. I would also like to thank him for his efforts to make me feel like part of the Georgia family. I've truly enjoyed spending time with him and his students.

Dr. Lars Rudstam provided advice and encouragement throughout my degree. My conversations with Lars allowed me to gain a fresh perspective on my research, and he was particularly helpful during my statistical analysis. Most of all, he has always been friendly and supportive, inviting me to his home to meet his family and reminding me that I am always welcome at CBFS.

I would like to thank the following organizations who provided financial support for this research: the National Science Foundation, the Environmental Protection Agency, the New York Biodiversity Research Institute, the Doris Duke Conservation Foundation, and the Kieckhefer Adirondack Fellowship.

There are many individuals to whom I am grateful for their help throughout:

- The Bernd Blossey Band: Charlotte Acharya, Amy Blair, Jeremy Dietrich, Brian Green, Victoria Nuzzo, Laura Martin, Holly Menninger, and Sarah Reilly.

- A special thanks to B and L for being great co-workers and fantastic friends.
- The many undergraduate assistants who have helped me over the years, including Tami Wynn, Matt Vallente, Bjorn Whitmore, Ben Scott-Killian, and Stacey[2]. They made working at the REM a pleasure and never complained, even when they had plenty of reason to.
- The F-house crew, past and present. They make work fun and have provided invaluable advice.
- Dr. Ed Mills and Dr. Rich Stedman, for supporting me on a whim and showing tremendous enthusiasm for communicating science to the public.
- To Jim Tantillo, for teaching me how to teach.
- My family, for all their love and support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

PLANT TRAITS, NOT ORIGIN, PREDICT IMPACTS OF PLANTS ON LARVAL AMPHIBIANS

Abstract

Plant detritus is the dominant basal resource in freshwater benthic food webs. The rate of energy flow and nutrient release to consumers is largely a function of detritus quality. It is hypothesized that one way plant invasions could alter native food webs is by changing the compositional quality of the detrital pool; however, empirical evidence to support this hypothesis is limited and strictly experimental. Here, I report the results of a field study of relationships between nine emergent wetland plant species and the performance of a mixed community of three amphibian species. I examined how litter quality (%N, C:N, and phenolic content) of native and nonnative plants was related to tadpole performance. I installed field cages in sites dominated by a single species of each plant and added tadpoles of three common species, then monitored cages for environmental parameters (temperature, dissolved oxygen, and pH) and recorded the number of days that a cage held at least 2 cm of water (hydroperiod). I collected and weighed all metamorphosed individuals as they became available. I used linear and logistical regression analyses to test for the effects of plant litter quality and environmental parameters on larval performance, then used Aikaike's information criterion (AIC) to select the top models. I also used analyses of variance to compare larval performance in sites dominated by native and nonnative plants.

I found that hydroperiod, C:N, phenolic content, and temperature were significant in many of our top models for larval performance. I observed unique

interactions between species, largely driven by the response of larval anurans to plant traits (e.g. sensitivity to plant phenolics). Importantly, my data shows that tadpole performance did not differ in habitats dominated by native or nonnative plants. Rather, my findings suggest that plant traits, irrespective of plant origin, determine larval amphibian performance.

Introduction

Senesced plant matter is the primary source of energy supporting food webs in freshwater ecosystems (Wetzel 1995, Wallace et al. 1999). Detrital subsidies can vary greatly in both quality and quantity, influencing the release of energy and nutrients and ultimately affecting the performance of aquatic heterotrophs (Cebrian and Lartigue 2004, Moore et al. 2004). Plant invasions have the potential to alter native community composition and affect ecosystem processes. Few studies have identified the mechanisms underlying impacts of plant invasions (Levine et al. 2003), particularly impacts at higher trophic levels (Gerber et al. 2008). If plant invasions change characteristics of detritus inputs, they may support different heterotroph communities (Rubbo and Kiesecker 2004, Levin et al. 2006, Maerz et al., in review) in freshwater ecosystems. Plant traits, including percent nitrogen, C:N ratio, and phenolic concentrations predict rates of plant decomposition across a variety of taxa (Webster and Benfield 1986, Ostrofsky 1997). If nonnative plants possess different traits than native species they replace, and hence exhibit different rates of decomposition, we would expect bottom-up changes to the flow of energy to aquatic consumers.

Larval amphibians are a dominant group of aquatic consumers in many freshwater ecosystems (Alford 1999). The larvae of anurans in northeastern North America are generally opportunistic benthic omnivores, preferentially grazing on

detrital biofilms that include algae, fungi, and bacteria. They will also consume detritus itself or filter suspended phytoplankton (Alford 1999). Until recently, little attention was paid to the role of shifts in plant detritus in tadpole development and community dynamics (Maerz et al. in review). Several mesocosm studies show that larval amphibian performance is strongly influenced by plant detritus (Schiesari 2006, Rubbo et al. 2008, Williams et al. 2008). In particular, litter species composition (Williams et al. 2008) and biomass (Rubbo et al. 2008) determine the strength of bottom-up effects on amphibian performance. The rate of plant decomposition influences the flow of energy to the biofilms that tadpoles graze, which in turn affects tadpole development rate and mass at metamorphosis (Williams et al. 2008).

Experimental evidence indicates that specific plant invasions can negatively impact tadpoles. *Anaxyrus americanus* Holbrook (American toad, formerly *Bufo americanus*) tadpoles raised in mesocosms with nonnative *Lythrum salicaria* L. (purple loosestrife) extract showed reduced survivorship and developmental rates compared to conspecifics raised in native *Typha latifolia* L. (broadleaf cattail) extract (Maerz et al. 2005, Brown et al. 2006). The authors suggest that the high phenolic content in *L. salicaria* leachate damaged tadpole gills. Maerz et al. (in review) raised five species of tadpoles in mesocosms containing litter from three native and three nonnative wetland plant species. They found that total metamorph productivity (biomass) and richness were negatively correlated with litter C:N. Further, they observed higher C:N in nonnative plants, suggesting that nonnative plants generally have lower litter quality compared to native species. Each of these experiments provides evidence that plant invasions negatively affect tadpoles by altering litter quality (phenolics and C:N); however, each was conducted under highly controlled conditions. A field experiment that exposes larval anurans to native and nonnative plant species across many sites would allow me to test the hypothesis that plant origin

affects habitat quality for these animals. Specifically, if nonnative plant litter differs in phenolic content and C:N compared to native species, I would expect to see changes in tadpole development.

Here, I studied larval performance of three amphibian species in habitats dominated by native or nonnative plants at five wetland complexes in central New York. I explored potential mechanisms by which plant invasions impact aquatic communities by examining differences between native and nonnative plant chemistry and how that relates to water chemistry, algal productivity, and larval amphibian performance. I hypothesized that nonnative plant species produce lower quality litter compared to native plant species, and would therefore reduce tadpole performance. My specific hypotheses were:

1. As litter %N increases, amphibian performance will increase.
2. As litter C:N and phenolic content increase, amphibian performance will decrease.

Methods

I established experimental sites in central and western New York at the Montezuma, Oak Orchard, and Tonawanda Wildlife Management Areas, the Iroquois National Wildlife Refuge, and the Cornell University Experimental Pond facility. Each site was dominated by either a single native or nonnative emergent plant. I included the nonnative *L. salicaria* and the native *Decodon verticallatus* L. (swamp loosestrife), two members of the *Lythraceae*. I also located sites in native *T. latifolia*, nonnative *T. angustifolia* L. (narrowleaf cattail), and their hybrid, *T. X glauca.* I also included the nonnative *Phalaris arundinacea* L. (reed canary grass) and *Phragmites australis* Type M (common reed), and the native *Sparganium eurycarpum* Engelm.

(broadfruit bur-reed) and *Schoenoplectus taebernaemontani* Gmel. (soft-stem bulrush) in my experiment.

On 2 May 2007 I collected four *Lithobates sylvaticus* LeConte (wood frog, formerly *Rana sylvatica*) egg masses at Montezuma. I placed eggs in plastic bags with water, then into coolers, and transported them back to the Resource Ecology and Management (REM) Facility at Cornell University. I placed egg clutches in individual 10 L plastic containers with coarsely filtered pond water, covered them with mesh, and then floated them in a small outdoor pond. I exchanged water in the containers every other day until eggs began to hatch approximately one week later. Eggs and tadpoles remained in floating containers until tadpoles from all clutches reached the free-swimming stage (stage 25, Gosner 1960). To homogenize genetic influences, I placed eight tadpoles from four different clutches (n = 32) into 250 mL plastic containers containing fresh pond water. I then placed containers in a cooler to be transported to the field sites. From 7-10 May 2007 I collected seven egg clutches of *L. palustris* LeConte (pickerel frog, formerly *Rana palustris*) eggs at the Arnot Teaching and Research Forest in Van Etten, NY and five clutches of *A. americanus* eggs in Richford, NY. I maintained them as described for the *L. sylvaticus* tadpoles.

I installed field cages (Reptarium™ 65 gallon [41 x 75 x 70 cm], Dallas MFG Co. Dallas, Texas) in wetland sites between 19 and 20 May 2007. Cages were made of nylon mesh (2 mm) to allow free flow of water, algae, and zooplankton. I placed cages in areas of dense monospecific vegetation in at least 35 cm deep water. Occasionally, I manually cleared plants to allow room for cage placement. I added dry senescent vegetation of the focal species (volume filling a 4 L plastic bag) to each cage. I recorded water temperature, dissolved oxygen, and pH using a YSI 556 MPS (YSI Environmental, Yellow Springs, OH) before adding all *L. sylvaticus* tadpoles

from a single container to each cage. Between 5 June and 13 June 2007, I added 32 *L. palustris* and 64 *A. americanus* tadpoles at Gosner stage 25 to each cage.

I monitored cages every other week following installation. At each sampling date I measured temperature, conductivity, dissolved oxygen, pH, and salinity. I also measured water depth from the bottom of the cage to the water line at each corner of the cage. I used 10 standardized sweep net samples (using a 15 cm fish net) to tally the number of surviving tadpoles of each species. Water samples (1 L) were taken from each cage from 19-23 June and 26-28 August to obtain measurements of algal abundance and aqueous phenolics. Each water sample was immediately passed through a 53 μm mesh filter in the field. The filtrate was preserved on ice, then taken back to the lab and passed through a 0.7 μm Whatman GF/F filter to collect phytoplankton. Each filter was placed in a small desiccator and kept frozen for analysis of chlorophyll-α (algal abundance). I also froze 12.5 mL of the filtrate from this process to analyze for phenolic concentration. In December 2007 I extracted chlorophyll-α from the filters following Wetzel and Likens (2000). At this time I also performed a colorimeteric analysis of phenolics on the filtrate by adding a Folin phenol reagent (Sigma-Aldrich, St Louis, MO, USA) to reduce active phenolics, then adding a pre-made Folin-Ciocalteu solution to determine sample concentration compared to a phenol standard (Clesceri and Eaton 1998).

I observed metamorphic tadpoles (legs and arms erupted, stage 42, Gosner 1960) of *A. americanus* and *L. sylvaticus* beginning on 17 June 2007, while *L. palustris* metamorphs first appeared on 25 June. From this time on I visited each cage on a three day rotation and collected all tadpoles Gosner stage 42 and higher and transported them back to Cornell University to be weighed. I placed metamorphs in 250 mL plastic containers outfitted with several air holes and a moist paper towel. I held metamorphs in these containers until they fully absorbed their tales (1-4 days), at

which time I weighed and measured their snout-vent length (SVL). Roughly 85% of metamorphs survived this process. Those that did not were preserved in ethanol in 50 mL plastic tubes and their SVL measured. I used the SVL of each preserved metamorph to estimate mass at metamorphosis by taking the mean mass of all live conspecifics with the same SVL. These estimates were then factored into our comparisons of total metamorph productivity per cage and proportion surviving to metamorphosis in each cage. I calculated metamorph productivity for each cage by summing the mass of all metamorphs produced by that cage.

From 20 October through 11 November 2007 I returned to cage locations and randomly sampled all emergent biomass within three replicate 30 x 30 cm quadrats. By this date all plants at my sites had senesced. I then stored the material in paper bags in a greenhouse for several weeks to dry. Some samples were lost during a flood of our storage facility; however, the loss was random across plant species and sites, so it did not significantly impact my analyses. In December 2007 I created aqueous extracts, or 'tea' from senescent leaves from each sample to measure leaf phenolics. To make tea, I submerged 2 g of leaf litter in 1 L of distilled water for 48 hours, stirring occasionally to ensure that leaf material remained submerged. I then removed leaf material and measured aqueous phenolics as outlined above. The remaining leaf litter was ground to less than 1mm thickness using a ball mill in December 2007. I then weighed out 3 mg of material for each sample and submitted it to the University of Georgia Institute of Ecology Soil, Water, and Plant Stable Isotope Facility for analysis of total C, total N, and C:N.

To test the hypothesis that cages in wetlands dominated by nonnative plants supported less amphibian metamorph productivity and a lower percentage of larvae reaching metamorphosis, I used nested analyses of variance (ANOVA) with plant species nested within plant origin (native or nonnative). To understand the

environmental parameters impacting the performance of each amphibian species I used a two stage analysis. I used an information theoretic approach to determine (1) which logistic regression models best explained whether an anuran species could metamorphose within a cage, (2) which linear regression models best explained the percentage of larvae that metamorphosed at sites where species successfully metamorphosed, and (3) which linear regression models best explained the productivity of anuran metamorphs produced at a site. I used Akaike's Information Criterion (AIC) to compare potential models. I used mean substitution to handle missing data, and data were log transformed when necessary to meet the assumption of normality.

Variables of interest included environmental parameters known to impact larval amphibian performance, including hydroperiod (number of days after cage installation a site held water), mean June water temperature, mean July water temperature, mean June dissolved oxygen (%) and mean July dissolved oxygen (%). I also included variables suspected to influence performance, such as reactive phenolic concentration in June water samples, chlorophyll-α content in June water samples, plant biomass (detrital inputs g/m^2), senescent leaf C:N, % senescent leaf phenolics (log transformed), N inputs from plant litter (g/m^2, log transformed), and soluble reactive phenolic inputs from plant litter (g/m^2, log transformed).

Results

There was no measurable difference in metamorph productivity (total g metamorphs) between habitats dominated by native or nonnative plant species (Origin: $F_{1, 52}= 0.173$, $P = 0.679$), nor was there significant variation in metamorph productivity among native or nonnative plant species (plant species [nested within origin]: $F_{7, 52}= 0.902$, $P = 0.512$). There were 17 linear regression models predicting

metamorph productivity within 2 AIC that warranted consideration as the possible best model (Burnham and Anderson 2002). All 17 top models included hydroperiod and mean July dissolved oxygen level as factors. Sixteen models also included mean June water temperature. Twelve top models included the amount of plant biomass, eight included detrital phenolic inputs, seven included plant phenolic concentrations, six included plant C:N, and six included detrital N inputs as variables. Eleven top models included phenolic concentrations in June water samples, and four included chlorophyll-α concentration in June water samples as variables. The number of days with water, leaf phenolic concentrations, mean June water temperature, and mean July dissolved oxygen level were significant variables, based on Wald's statistic ($\alpha = 0.05$). Plant C:N and detrital phenolic inputs were also marginally significant based on Wald's statistic, ($\alpha = 0.1$).

The top model for predicting total metamorph productivity included hydroperiod, phenolic concentration in June water sample, plant detrital biomass, mean June water temperature, and mean July dissolved oxygen level. This model explained ~47% of the variation in total metamorph productivity (multiple regression: adjusted $r^2 = 0.472$, SE of estimate = 0.939). Only hydroperiod, June water temperature and July dissolved oxygen levels were significantly correlated with anuran metamorph productivity. Hydroperiod was positively correlated with metamorph productivity, as was June water temperature. July dissolved oxygen levels were negatively correlated with metamorph productivity. Hydroperiod was significant in all top linear regression models of metamorph productivity.

There was a significant difference in the composition of metamorph communities between habitats dominated by native and nonnative plants (Origin, Table 1-1, $P = 0.002$). The effect of plant origin was mostly driven by the

Table 1-1. Wilk's tests for the effect of origin (native or nonnative) on amphibian larval communities in habitats dominated by either nonnative or native plants.

	Value	*F*	Effect df	Error df	*P*
Intercept	0.289	40.950	3.000	50.000	0.000
Plant (Origin)	0.590	1.387	21.000	144.123	0.134
Origin	0.750	5.565	3.000	50.000	0.002

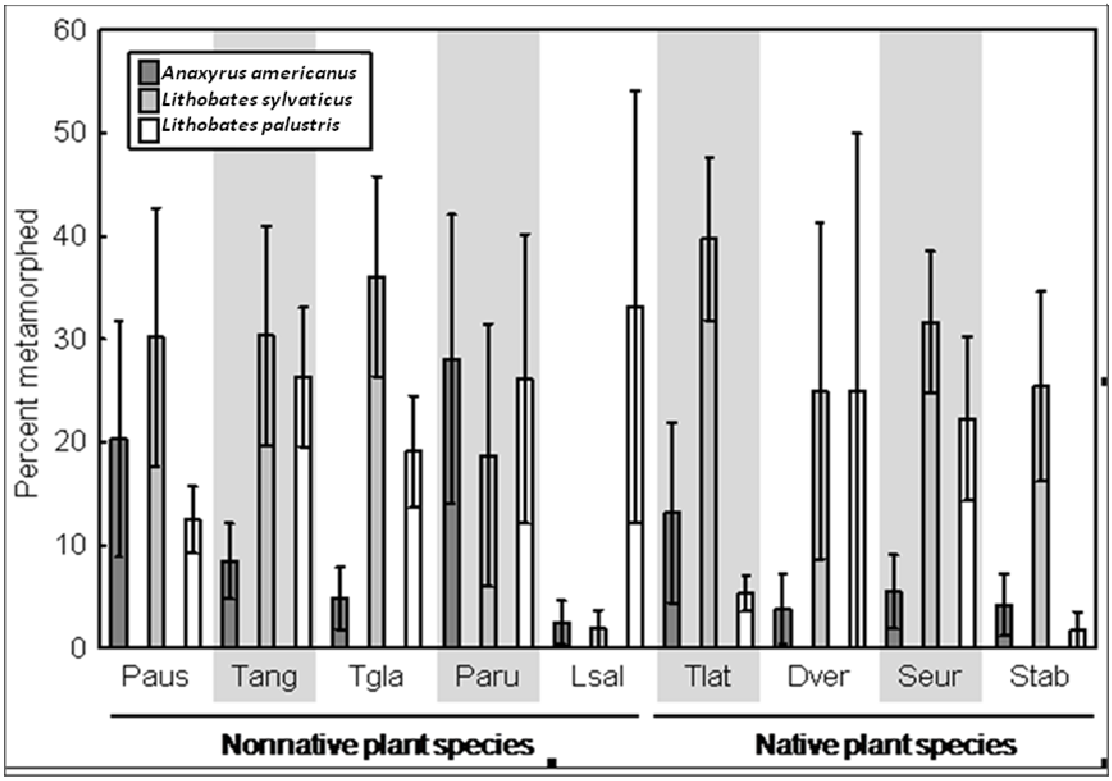

Figure. 1-1. The percentage of larval amphibians of each species that survived to metamorphosis in cages in wetlands dominated by five nonnative and four native plant species. Plant codes: Paus=*Phragmites australis* (M); Tang=*Typha angustifolia*; Tgla=*Typha glauca*; Paru=*Phalaris arundinacea*; Lsal=*Lythrum salicaria*; Tlat=*Typha latifolia*; Dver=*Decodon verticillatus*; Seur=*Sparganium eurycarpum*; Stab=*Schoenoplectus tabernaemontani*.

number of metamorphosing *L. palustris* in habitats dominated by nonnative plants. Otherwise, there was no measurable difference in the percent metamorphosed of any species among native or nonnative plant species (plant species term nested within origin, Table 1, $P = 0.134$, Figure. 1-1).

The differences in percentage of metamorphs produced for each species indicated that there were two questions that needed to be addressed: (1) what factors predict whether a species can metamorphose in a particular habitat, and (2) among sites where metamorphosis was possible, what factors explain the variation in percent metamorphosed? There were 17 models within two AIC for predicting whether *A. americanus* metamorphosed at a site. All top models included the concentration of phenolics in June water samples, concentration of soluble phenolics in plant leaves, and mean July water temperature as variables. Ten models included June dissolved oxygen as a variable, and 12 and six models also included detrital inputs of N and phenolics as variables, respectively. Few other variables were regular constituents of top models. The top model for *A. americanus*, which included June aqueous phenolics, plant litter phenolic concentrations, plant litter N inputs, mean July water temperature and mean June dissolved oxygen levels, explained 28% of the variation in whether *A. americanus* metamorphosed. June water phenolics and mean July water temperature were significant variables in the model, and were positively correlated with the probability that *A. americanus* would metamorphose. Leaf phenolic concentration was also a significant variable and negatively correlated with whether *A. americanus* would metamorphose (Figure. 1-2).

There were 26 and 9 models within two AIC for predicting whether a habitat produced a *L. sylvaticus* or *L. palustris* metamorph, respectively. For both species, all top models included hydroperiod as a variable. Using Wald statistics I determined that the number of days with water was the only variable significantly, independently

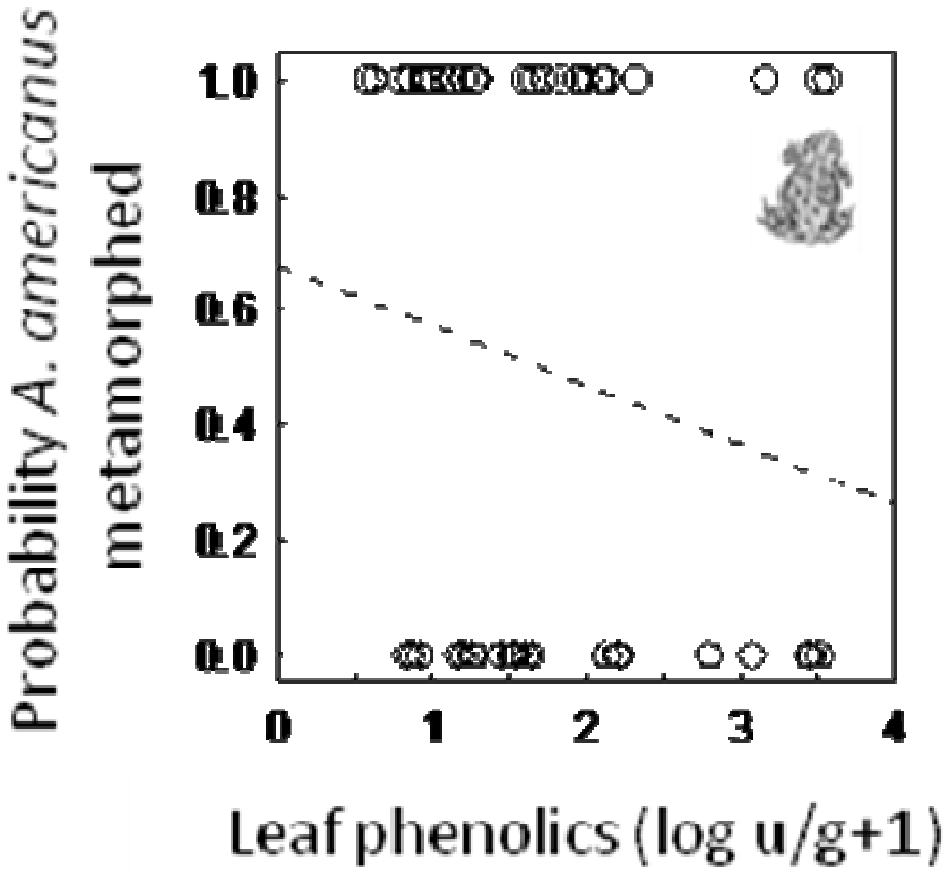

Figure. 1-2. The probability that a cage produced at least one *A. americanus* metamorph as a function of leaf phenolics in the plant surrounding the cage.

correlated with the probability that *L. sylvaticus* or *L. palustris* would metamorphose at a site. For both species, hydroperiod explained ~80% of the variation whether the species metamorphosed at a site. For *L. sylvaticus* the effect of hydroperiod was more graded (Figure. 1-3). That is, *L. sylvaticus* tadpoles required ~ 40 days of water minimum to metamorphose, but beyond 40 days, there was still variation in whether the site would support metamorphosis even if the site never dried. This suggests other factors also affect whether *L. sylvaticus* tadpoles metamorphosed at a site. In contrast, *L. palustris* tadpoles did not metamorphose at any site that held water for less than 50 days; however, nearly all sites that held water longer than 50 days produced *L. palustris* metamorphs. For both species, all top models also included leaf phenolic concentrations as a variable. For *L. sylvaticus* 25 of 26 top models also included plant C:N, 18 included mean July water temperature, 10 included detrital phenolic inputs, six included June chlorophyll-α concentration, five included water phenolic concentration in June, three included mean July dissolved oxygen, two included plant litter biomass, two included June dissolved oxygen, and one included mean June water temperature. For *L. palustris*, nine of nine top models included phenolic concentrations in June water samples, plant litter biomass, and mean July dissolved oxygen level. Eight models included June chlorophyll-α concentration and mean June water temperature. Few other variables were regular constituents of top models.

Logistic regression showed that the top model for predicting whether *L. sylvaticus* metamorphosed, which included number of days with water, litter C:N and phenolic concentrations, and July water temperature, explained 48 % of the variation. Two variables, days with water and plant C:N, were significant in that model. Beta (β) values for the two variables show that days with water was positively correlated with the probability of producing a metamorph, and plant C:N was negatively correlated. The top model for *L. palustris*, which included number of days with water, phenolic

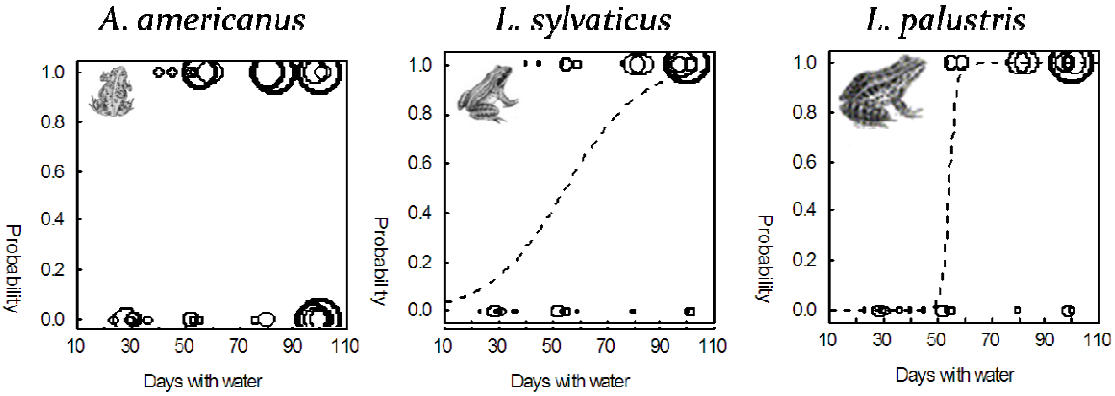

Figure. 1-3. The probability that a cage produced at least one *A. americanus, L. sylvaticus*, and *L. palustris* metamorph as a function of hydroperiod. The size of the circle represents the variance around the estimate.

and chlorophyll-α concentrations in June water samples, plant litter biomass, mean July water temperature and mean June dissolved oxygen levels, explained 57% of the variation in whether the species metamorphosed at a site. Days with water, June water phenolics, and plant litter biomass were all significant variables. Days with water and plant litter biomass were positively correlated with the probability that *L. palustris* would metamorphose, and June water phenolics were negatively correlated.

For *A. americanus*, there were five linear regression models for predicting the percentage of larvae that metamorphosed within 2 AIC, all of which included June chlorophyll-α levels, plant litter phenolic concentration, plant litter C:N, plant litter biomass, N and phenolic inputs from litter, and mean June and July water temperatures as variables. The top model explained 48% of the variation in percent of *A. americanus* larvae that metamorphosed. June chlorophyll-α, plant litter C:N, plant litter biomass, nitrogen inputs from litter, and mean June and July water temperatures were all significant variables in that model. Plant litter biomass and mean June temperature were positively correlated with percentage of *A. americanus* tadpoles that metamorphosed, while plant litter C:N, detrital nitrogen inputs, and June chlorophyll-α levels were negatively correlated with the percentage of *A. americanus* tadpoles that metamorphosed. Leaf phenolic concentrations were not significantly correlated with the percentage of *A. americanus* tadpoles that metamorphosed, but this variable was positively correlated with N inputs. The negative relationship between *A. americanus* survival and N inputs may have reflected the negative effects of phenolics.

For *L. sylvaticus* there were 17 models within 2 AIC, all of which included June chlorophyll-α levels and mean July water temperature as variables. Thirteen of the top models also included plant litter C:N, 12 models included N, and four included phenolic inputs from litter as variables. A few other variables appeared in one of the top models. The top model, which included June chlorophyll-α , plant C:N, estimated

N input from plant litter, and mean July temperature as variables, explained only 16% of the variation in percent of *L. sylvaticus* larvae that metamorphosed. Plant litter C:N and mean July water temperatures were the only significant variables in that model, and both were negatively correlated with the percentage of *L. sylvaticus* tadpoles that metamorphosed.

For *L. palustris* there were 27 models within 2 AIC. Twenty-three models included mean July water temperature as a variable, and 22 models included the amount of plant litter biomass as a variable, or the amount of phenolic inputs from plant litter as variables. Several other variables included mean July dissolved oxygen level, June chlorophyll-α levels, days with water, and June water phenolic levels all frequently ($x < 25\%$) appeared in the top models. The top model, which included June chlorophyll-α, plant litter biomass, estimated phenolic inputs from litter, and mean July water temperature, explained only 25% of the variation in percent of *L. palustris* larvae that metamorphosed. Plant litter and estimated phenolic inputs from plant litter were the only two significant variables in that model. Plant litter biomass was negatively correlated and phenolic inputs from litter were positively correlated with the percent of *L. palustris* tadpoles that metamorphosed.

Discussion

My results indicate that plant traits, rather than plant origin, are useful for predicting larval amphibian survival and development in freshwater habitats. I predicted that nonnative plants would show reduced litter quality (higher C:N and phenolic content) and would support reduced algal productivity, with associated decreases in larval amphibian performance. There is no measurable difference in plant chemistry or tadpole performance in habitats dominated by native versus nonnative plants; however, variation in plant traits, including biomass production, C:N, and

phenolics, are associated with altered tadpole performance. For example, increased plant biomass tends to improve larval performance for *A. americanus* and *L. sylvaticus*, while increased C:N and phenolic concentrations reduced larval performance for those species.

Hydrology was the most significant predictor of whether *L. sylvaticus* and *L. palustris* would successfully metamorphose at a site; however, hydrology was not generally important for determining *A. americanus* success. *Anaxyrus americanus* tadpoles are known for their rapid development and exploitation of ephemeral breeding sites (Alford 1999). *Lithobates sylvaticus* take longer to develop than *A. americanus*, but exhibit more rapid development that *L. palustris*. Therefore *L. sylvaticus* were affected by hydroperiod, but less so than *L. palustris*. The effects of hydroperiod were not even across plant species. For example, 75% of *P. arundinacea* cages dried out, while none of the *T. angustifolia* cages were lost. Our experimental design does not allow us to evaluate whether plant species cause water levels to recede (e.g. by taking water up into plant tissues) or whether those species simply occupy areas where water recedes rapidly.

Several results from this study are consistent with previous research. The percent N and C:N of litter influenced the performance of *A. americanus* and *L. sylvaticus* tadpoles. Generally, as percent N increased, or C:N decreased, larval amphibian performance increased. This supports my hypothesis that as litter quality increased larval amphibian production and survival would improve. It also agrees with work by Schiesari (2006) and Maerz et al. (in review), who demonstrated that food nutritional quality (C:N) substantially increased growth in tadpoles. Similar to Maerz et al (2005) and Brown et al (2006), I observed that *A. americanus* performance in nonnative *L. salicaria* was very poor (mean % metamorphosed < 5%). It is important to note that *A. americanus* also performed very poorly in habitat dominated

by the native *D. verticillatus*. Both *L. salicaria* and *D. verticillatus* were very high in soluble phenolics, and my results show that leaf phenolic concentration was a significant predictor of whether *A. americanus* larvae could survive to metamorphosis. My results demonstrate that the case study with *L. salicaria* was not generally indicative of all nonnative plants. Rather, it was a result of choosing a plant species rich in phenolics, which have a demonstrated negative effect on *A. americanus* performance. I observed the same result with a native loosestrife with similar traits. Our results underscore the importance of understanding the mechanisms behind the ecological impacts of nonnative species.

Understanding the ecological impacts of plant traits, not just plant species, will improve our ability to predict community responses to invasions and can better inform management decisions. Managers concerned with preserving amphibian habitat could use the information from this study to make decisions about where to allocate resources for managing plant invasions. Consider the current scenario at Montezuma, where populations of nonnative *T. angustifolia* are invading stands of native *T. latifolia* (B. Blossey, pers. obs.). My research suggests that these species are equivalent habitat for amphibian larvae, so we would not expect changes to amphibian populations. In other areas of the refuge, nonnative *P. australis* Type M is replacing *T. angustifolia* (B. Blossey, pers. obs). Here we would anticipate reduced performance of *A. americanus* and *L. sylvatica*, since *P. australis* Type M litter is off lesser quality than *T. angustifolia*. If managers relied on origin to predict which invasion was harmful to amphibians, they would devote resources to the first case, where a nonnative plant is replacing a native species, rather than the second case, where a nonnative plant is replacing another nonnative species. However, based on my trait data, I would recommend that managers focus their attention on the second case and prevent the spread of *P. australis* Type M. This trait-based framework can

help managers reconcile the desire to control nonnative plants with other goals, such as protecting wildlife habitat.

I observed unique species-specific interactions between amphibians and plants, suggesting that wetlands characterized by a 'mosaic' of plant species with different traits will host the greatest amphibian diversity and productivity. Plant traits created habitats that favored certain species over others. For example, *L. palustris* showed high survival in habitats dominated by phenolic-rich species, such as the native and nonnative loosestrife, while *A. americanus* and *L. sylvaticus* had much lower survival at those sites. Conversely, both *A. americanus* and *L. sylvaticus* fared better than *L. palustris* in species with low phenolic content, such as *S. taebernaemontani* and *T. latifolia*. While the origin of a plant matters less than the traits it exhibits, plant invasions still have the potential to impact amphibian populations. A plant invasion could replace the desired mosaic pattern of plant species with a monoculture, reducing not just species diversity, but more importantly, reducing 'trait' diversity.

REFERENCES

1. Alford, R. A. 1999. Ecology: resource use, competition and predation. Pages 240-278 *in* R. W. McDiarmid and R. Altig, editors. Tadpoles: the biology of anuran larvae. The University of Chicago Press, Chicago, IL.
2. Brown, C. J., B. Blossey, J. C. Maerz, and S. J. Joule. 2006. Invasive plant and experimental venue affect tadpole performance. Biological Invasions 8:327-338.
3. Burnham, K. P. and D. R. Anderson. 2002. Model Selection and Multimodel Inference: a Practical Information-theoretic Approach. Springer-Verlag, New York, NY.
4. Clesceri, L. S. and A. D. Eaton, editors. 1998. Standard Methods for Examination of Water and Wastewater, 20th edition. American Public Health Association, Washington, D. C., USA.
5. Gerber, E., C. Krebs, C. Murrell, M. Moretti, R. Rocklin, and U. Schaffner. 2008. Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. Biological Conservation 141:646-654.
6. Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183-190.
7. Levin, L. A., C. Neira, and E. D. Grosholz. 2006. Invasive cordgrass modifies wetland trophic function. Ecology 87:419-432.

8. Levine, J. M., M. Vila, C. M. D'Antonio, J. S. Dukes, K. Grigulis, and S. Lavorel. 2003. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society B 270:775-781.
9. Maerz, J. C., C. J. Brown, C. T. Chapin, and B. Blossey. 2005. Can secondary compounds of an invasive plant affect larval amphibians? Functional Ecology 19:970-975.
10. Maerz, J. C., J. S. Cohen, and B. Blossey. In review. Can detritus quality be used to predict the impacts of nonnative plant invasions on larval amphibians? Freshwater Biology.
11. Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. deRuiter, Q. Dong, A. Hastings, N. C. Johnson, et al. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters 7:584-600.
12. Ostrofsky, M. L. 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. Journal of the North American Benthological Society 16:750-759.
13. Rubbo, M. J. and J. M. Kiesecker. 2004. Leaf litter composition and community structure: translating regional species changes into local dynamics. Ecology 85:2519-2525.
14. Rubbo, M. J., L. K. Belden, and J. M. Kiesecker. 2008. Differential responses of aquatic consumers to variations in leaf-litter inputs. Hydrobiologia 605:37-44.
15. Schiesari, L. 2006. Pond canopy cover: a resource gradient for anuran larvae. Freshwater Biology 51:412-423.

16. Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. Effects of resource limitation on a detrital-based ecosystem. Ecological Monographs 69:409-442.
17. Webster, J. R. and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Annual Reviews in Ecology and Systematics 17:567-594.
18. Wetzel, R. G. 1995. Death, detritus, and energy flow in aquatic ecosystems. Freshwater Biology 33:83-89.
19. Wetzel, R. G. and G. E. Likens. 2000. Limnological Analyses, 3rd edition. Springer-Verlag, New York, USA.
20. Williams, B. K., T. A. G. Rittenhouse. 2008. Leaf litter input mediates tadpole performance across forest canopy treatments. Oecologia 155:377-384.

CHAPTER 2

INTRASPECIFIC VARIATION IN PLANT LITTER CHEMISTRY DRIVES AMPHIBIAN PRODUCTIVITY IN EXPERIMENTAL MESOCOSMS

Abstract

Functional traits link individuals to ecosystem processes. Functional traits of live plants influence direct plant-animal interactions, particularly the productivity of insect herbivores. However, many plant-animal interactions are indirect, as when senesced plant material supports animal productivity by sustaining the primary producers that animals feed on. Experimental evidence for a link between functional traits of senesced plants and animal productivity is lacking. I raised tadpoles in experimental mesocosms containing an algal slurry and litter from 15 populations of a single species, *Phalaris arundinacea* L. (reed canarygrass), that varied widely in litter chemistry. Tadpoles are dominant consumers in many freshwater ecosystems, feeding largely on detritus, detrital biofilms, and suspended algae. I hypothesized that litter functional traits, including C:N:P, tannin, and lignin content, would determine the flow of energy to, and hence productivity of, tadpoles in this simplified community.

I find that litter traits, including C:N:P and tannin content, explain a significant portion of the variation I observed in tadpole productivity. Increases in tannin content and N:P were associated with improved larval performance, while increases in C:P had negative impacts. I observed no association between litter lignin content or phytoplankton abundance and tadpole productivity. This study demonstrates the influence of litter functional traits on secondary productivity in detritus-based food webs. My work adds to a growing body of evidence that intraspecific variation in functional traits can have important consequences for ecosystem function, and that

functional traits, rather than species *per se*, are useful for understanding ecosystem processes.

Introduction

Plants display immense genetic variability and phenotypic plasticity in their elemental composition (Aerts & Chapin 2000) and defense chemistry (Poelman, van Loon & Dicke 2008), resulting in dynamic interactions of plants with their herbivores and higher trophic levels (Kessler & Baldwin 2001; Halitschke *et al.* 2008). The nutritional quality of plants is generally expressed as the ratio of C:N:P in plant tissue (Sterner & Elser 2002), with lower C:N:P tissues considered higher quality resources for consumers. While increases in N and P in plant tissues may positively impact plant function, it also increases their attractiveness to herbivores, resulting in the development of mechanical and chemical defense mechanisms (Herms & Mattson 1992; Stamp 2003; Stamp 2004). Concentrations of nutrients (Thompson *et al.* 1997; Aerts & Chapin 2000) and secondary compounds (Castells *et al.* 2002; Kraus, Zasoski & Dahlgren 2004; Nyman *et al.* 2005; Kaplan *et al.* 2008; Lacerf & Chauvet 2008) can vary greatly within a species. This variation may be driven by abiotic factors, such as soil pH (Kraus, Zasoski & Dahlgren 2004), or biotic factors, such as the intensity of insect herbivory (Kaplan *et al.* 2008).

The same traits that determine palatability to herbivores are also recognized as the principal determinant of how quickly decomposer organisms consume senesced plant tissues (Webster & Benfield 1986; Enriquez *et al.* 1993; Ostrofsky 1997; Cornwell *et al.* 2008). Not surprisingly, correlations have been observed between leaf palatability and decomposition rate in a variety of ecosystems (Schadler *et al.* 2003; Chapman *et al*. 2003, Cornelissen *et al.* 2004; Kurokawa &Nakashizuka 2008; Palkova & Leps 2008). Further, experimental evidence shows that intraspecific

variation in C:N:P, tannins, and lignin causes variation in decomposition rate comparable to the variation observed between species (Epps *et al.* 2007; Meir & Bowman 2008). Thus intraspecific variation in plant chemistry directly affects organisms that consume plant tissue, regardless of whether tissues are green or senesced.

Effects of intraspecific variation in litter chemistry have received relatively little attention (Moore *et al.* 2004), despite their major importance for energy flows, particularly in freshwater benthic communities (Hairston & Hairston 1993; Wetzel 1995; Wallace *et al.* 1999). Given the strong relationship between senesced leaf chemistry and decomposition rate, variations in litter chemistry are expected to cascade through detritus-based food webs. For example, decreases in litter tannin concentrations are expected to increase the rate of litter decomposition, thereby increasing decomposer productivity, resulting in increased food availability for species feeding on decomposers. Despite strong theoretical evidence for such multitrophic impacts of litter chemistry, experimental evidence for these effects is lacking (Moore *et al.* 2004).

Benthic tadpoles, as generalist 'biofilm grazers' that consume fungi, bacteria and algae that colonize plant litter, the plant litter itself, as well as phytoplankton in the water column (Alford 1999), are ideally suited to assess the role of litter chemistry in structuring aquatic communities. Laboratory and field experiments to assess the influence of different native and introduced plant species on tadpole performance (Cohen *Chapter 1*, Maerz *et al. in review*), demonstrated that specific plant qualities were important factors determining tadpole performance in aquatic habitats. In addition to the differences among plant species, our experiments revealed large intraspecific variation in litter chemistry of *Phalaris arundinacea* L. (reed canarygrass), which resulted in variable tadpole performance (Cohen *Chapter 1*).

Tadpole performance was positively correlated with N, and negatively correlated with tannins and lignin concentrations of *P. arundinacea*.

In the experiment described below, I further examined potential bottom-up effects of litter chemistry on detrital food webs by assembling experimental outdoor mesocosms using 15 *P. arundinacea* populations that varied in litter chemistry. While site history, predation, hydroperiod, landscape context, and other factors are of importance in determining local tadpole performance, creating simplified pond communities containing litter, pond slurry, and tadpoles allowed me to focus on the effects of litter chemistry. My work was guided by the following hypotheses:

Hypothesis 1: *P. arundinacea* populations differ in litter chemistry.

Hypothesis 2: Tadpole performance will be affected by litter from different *P. arundinacea* populations.

Hypothesis 3: Metamorph abundance, individual mass at metamorphosis (g), and total amphibian productivity (g) will increase as C:N:P, tannin, and lignin concentrations in *P. arundinacea* litter decrease.

Hypothesis 4: Length of larval period will decrease as C:N:P, tannin, and lignin concentrations in *P. arundinacea* litter decrease.

Methods

In early May 2008, I established a 'common garden' consisting of 150 mesocosms (100L tree pots, BFG supply, Lancaster NY) at the Cornell Resource Ecology and Management (REM) Facility in Ithaca, NY. I evenly spaced mesocosms in four rows on an open, level area that was covered with wood chips to reduce weed growth. I placed two screen-covered overflow pvc pipes (2·5 cm diameter) at the 80 L level in each mesocosm to allow excess rainwater to drain. I labeled each mesocosm

and covered it with a screen (5 mm mesh) to prevent colonization by other aquatic organisms. I filled each pot with 80 L of tap water on 13 May 2008 and allowed water to age for 3 days before adding *P. arundinacea* litter.

I collected senesced *P. arundinacea* litter in February 2008 at 15 different sites in New York State (Table 2-1) by clipping emergent material (leaves and stems) from several monospecific patches at each site (total approx 5 m^2). I kept litter from different sites in separate mesh bags and air-dried the material in a greenhouse under ambient temperature (15-30 °C). For each collection location, I prepared 10 bags of litter, each with 65 g (representing a typical amount of *P. arundinacea* litter produced in 0·25 m^2, the surface area of our mesocosms, in the field. J. Cohen, unpubl data). I randomly assigned each bag to one of the 150 different pots. I also prepared four random samples of 3 g of ground material (< 1 mm) from each site. I submitted three samples per population to the Cornell Nutrient Analysis Laboratory for C and N analysis, and one to Dairy One, Ithaca, NY for P and lignin analysis.

I added litter to mesocosms on 13 May 2008. On 14 May 2008 I collected 100 L of pond slurry from a shallow pond containing a well-established *P. arundinacea* stand at the Cornell Experimental Pond Facility in Ithaca, NY. I filtered the pond water through 80 μm mesh, removing all organisms except for algae and microbes. I continuously homogenized the resulting slurry and added 0·5 L of the mixture to each mesocosm.

On 9 May 2008, I collected eight *Lithobates palustris* LeConte (formerly *Rana palustris*, pickerel frog) egg masses (approx. 500-1,000 eggs per mass) from a pond largely surrounded by *P. arundinacea* at the Arnot Teaching and Research Forest in Van Etten, NY. I maintained individual egg masses in pond water in 10 L plastic containers covered with mesh floating in a small, shaded, outdoor pond. I changed pond water in the containers every other day until eggs began to hatch on 18 May

Table 2-1. *Phalaris arundinacea* collection sites in New York

Population Code	Site of collection
AU	42° 00' N, 79° 06' W
BE	43° 11' N, 73° 24' W
DA	44° 08' N, 75° 20' W
EX	42° 30' N, 76° 27' W
FI	43° 30' N, 76° 02' W
FR	44° 24' N, 74° 16' W
HR	42° 04' N, 79° 05' W
HS	42° 04' N, 78° 14' W
HF	44° 11' N, 74° 04' W
MN	43° 05' N, 76° 42' W
O1	43° 07' N, 78° 18' W
O2	43° 07' N, 78° 18' W
TN	43° 06' N, 78° 29' W
VF	44° 58' N, 73° 31' W
WG	42° 20' N, 76° 50' W

2008. I provided dry fish flakes *ad libitum* until tadpoles from all clutches reached the free-swimming stage (stage 25, Gosner 1960). On 1 June 2008 I placed 40 tadpoles (five each from eight different clutches to homogenize potential genetic influences) into 250 mL plastic containers containing aged tap water. I then added the contents of one container to each mesocosm, resulting in a density of 0·5 tadpoles L^{-1}, a density equivalent to previous work with *L. palustris* (Wilbur & Fauth 1990).

I checked mesocosms every few days until I noticed metamorphs on 21 July 2008, and from then on I checked mesocosms daily. I removed only individuals who had fully metamorphosed (Gosner stage 46). I occasionally observed drowned metamorphs (Gosner stage 42 or higher), and in such cases I used mean substitution from the individual's mesocosm to estimate mass at metamorphosis. I collected the final metamorph on 1 October 2008 and terminated the experiment before the first killing frost on 6 October 2008. At this time, I euthanized all remaining tadpoles (Gosner stage 40 and below) in a bath of 2% buffered MS-222 (tricaine methane sulfonate) and disassembled the mesocosms.

Beginning 29 May 2008, I measured temperature (°C), dissolved oxygen (mg L^{-1}), and pH in the middle water column of each mesocosm every two weeks using a YSI 556 MPS (YSI Environmental, Yellow Springs, OH). On 9 June and 10 Aug 2008 I took 1 L water samples from the middle water column of each mesocosm for measurements of algal abundance and aqueous tannins. I immediately passed each water sample through a 53 μm mesh filter. I preserved the filtrate on ice, then took it back to the lab and passed it through a 0·7 μm Whatman GF/F filter to collect phytoplankton. I placed each filter in a small dessicator and kept these frozen for later analysis of chlorophyll-α (algal abundance). I also froze 12·5 mL of the filtrate for tannin analysis. In December 2008 I measured chlorophyll-α on filters using a fluorometer (Wetzel & Likens 2000). At this time, I also performed a colorimeteric

analysis of tannins on the filtrate by adding a Folin phenol reagent (Sigma-Aldrich, St Louis, MO, USA) to reduce active tannins, then adding a pre-made Folin-Ciocalteu solution to determine sample concentration compared to a phenol standard (Clesceri & Eaton 1998).

All statistical analyses were performed using R (version 2.7.2, R Development Core Team 2008). I examined residuals to verify that assumptions of normality and homogeneity of variance were not violated. For each statistical test I assumed a significance level of 0·05. To test for differences in litter chemistry between populations (hypothesis 1) I conducted two sets of one-way ANOVAS with population as a factor and either C:N or aqueous tannin content as the variable. I conducted similar ANOVAS to test for differences in tadpole performance (hypothesis 2), with either metamorph mass or length of larval period as the variables. I also used Pearson product-moment correlations (r^2) to examine relationships between litter C and lignin content, as well as litter N and P.

To address hypotheses 3 and 4, I used a three step approach involving logistic and linear regressions. I used an information theoretic approach, Aikaike's Information Criterion (AIC), at each step to determine which model best predicted our measures of amphibian performance. I also considered models within 2 AIC of the top model (Burnham & Anderson 2002). First, I performed logistic regressions to determine what conditions best explained whether a pot produced at least one metamorph. I used metamorph production as a binomial response variable, where 0 corresponded to pots where no metamorph was produced, and 1 corresponded to pots where at least one was metamorph produced. Predictor variables included dissolved oxygen, temperature, pH, algal abundance, aqueous tannin concentration, and population (factor).

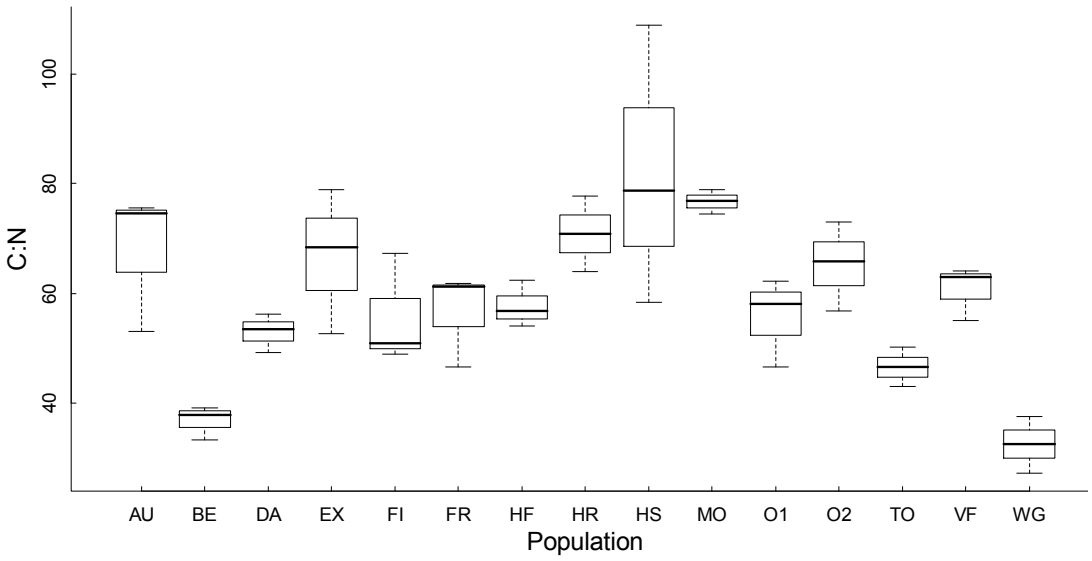

Figure. 2-1. Box-and-whisker plot of C:N for different populations (n = 3). Each box contains data within the 50% quartiles, while lines extend to the maximum and minimum value. The median is represented by a dark line inside each box.

Second, using data only from those pots that produced at least one metamorph, I conducted multiple linear regressions to evaluate which variables best explained the performance of those individuals. I conducted separate analyses for abundance of metamorphs, mean mass at metamorphosis, total biomass of metamorphs, and length of larval period, with individual pots as the unit of analysis. I used the same response variables as in the logistic regressions. In my final step, I pooled data on metamorph abundance and biomass from each pot into a population-level mean. Using these pooled values as our response variables, I conducted multiple linear regressions, where our predictor variables included the population-level characteristics C:N, C:P, N:P, lignin, and mean tannin concentration.

Results

Across my experiment, the length of larval period ranged from 51 to 127 days (mean 90·3), and metamorph mass ranged from 0·328 to 1·812 g (mean 0·7036). Overall, only approximately 3% of tadpoles reached metamorphosis by 6 October 2008. Litter C:N differed significantly among *P. arundinacea* populations (ANOVA $P < 0{\cdot}0001$, Figure. 1, Table 2-2), as did aqueous tannin concentration (ANOVA $P < 0{\cdot}0001$, Table 2-2). Similarly, both the mass of metamorphs (ANOVA $P < 0{\cdot}01$, Table 2-2) and length of larval period (ANOVA $P < 0{\cdot}0001$, Table 2-2), were significantly different among *P. arundinacea* populations. The Pearson's product moment correlation was significant for C and lignin content ($r^2 = 0{\cdot}579$, $P < 0{\cdot}001$), but not significant for N and P ($r^2 = 0{\cdot}113$, $P = 0{\cdot}169$).

Differences in tadpole survival to metamorphosis were best explained (lowest AIC, Table 2-3) by a model that contained tannin content, dissolved oxygen levels, and temperature. All three variables were significant. An additional model containing

Table 2-2: One-way ANOVAs examining variation in RCG population means for metamorph mass (g), length of larval period (days), C:N, and aqueous tannins (mg L^{-1})

Source	*df*	*SS*	*MS*	*F*	*P*
Metamorph mass	14,163	2·0246	0·1446	2·3829	0·00481**
Length of larval period	14,163	14999	1071	3·8146	$P < 0·001$***
C:N	14,30	7745·6	553·3	5·7274	$P < 0·001$***
Aqueous tannins	14,135	3·2233	0·2302	5·3575	$P < 0·001$***

Table 2-3. Top logistical regression model on metamorph survival, *P<0·05, ** P<0·01, ***P<0·001

Predictor	Coefficient	SE Coefficient	z	P
Intercept	-38·9	19·5	-1·99	0·0461*
Tannins	2·21	0·791	2·80	0·00513**
DO	-1·01	0·434	-2·34	0·0194*
pH	5·55	2·76	2·01	0·0442*

Table 2-4. Top multiple regression models for four measures of amphibian performance for pots that produced at least one metamorph, *P<0·05, ** P<0·01, ***P<0·001, †denotes variables coded as factors. Pop = *P. arundinacea* Population, DO = dissolved oxygen, Tan = tannins

	β_0	β_1	β_2	β_3	*df*	*F*	*adj* r^2	*P*
Abun-dance	-0.597	Pop† *	pH 0·091	--	15,68	2·21	0·182	0·0147 *
Mean mass	-5·17*	Tan 0·231*	pH 0·081*	DO 0·973	3,78	3·67	0·090	0·0157 *
Bio-mass	-30·8**	Pop† ***	pH 4·42**	Tan 0·948	16,65	2·87	0·270	0·0014 **
Larval period	319*	Tan -13.0	pH 28·1	--	2,79	3·63	0·061	0·0309 *

temperature was within 2 AIC of this top model (Appendix). A total of 82 out of 150 pots, or 55%, produced at least one metamorph; these were included in the second step of our regression analyses. The top model for metamorph abundance included pH and population, and explained 18·2% of the observed variation (Table 2-4). Only population was significant in this model. Two models that included temperature and one that included tannin content were also within 2 AIC of the top model for metamorph abundance (Appendix). Mean mass at metamorphosis was best explained by a model containing tannins, pH, and dissolved oxygen (0·9% of the observed variation). An additional model that included temperature was within 2 AIC of the top model (Appendix). Finally, population, pH, and tannins together explained 27·0% of the variation in metamorph biomass, both population and pH were significant in the model. Two additional models that included temperature and one containing chlorophyll-α were within 2 AIC of the top model for metamorph biomass (Appendix). Tannin levels and pH together explained 6·1% of the variation in the length of larval period, however neither was significant. Two additional models containing dissolved oxygen and one containing temperature were within 2 AIC of the top model (Appendix). For the analysis of mean metamorph abundance and biomass grouped by *P. arundincacea* population, both top models included C:P and N:P (Table 2-5). In each of these models C:P was the only significant predictor, and for each a second model that included C:N was within 2 AIC. The top models for metamorph abundance and biomass explained 30.0% and 38·4% of the observed variation, respectively. The relationship between litter C:P and population biomass is presented in Figure. 2-2.

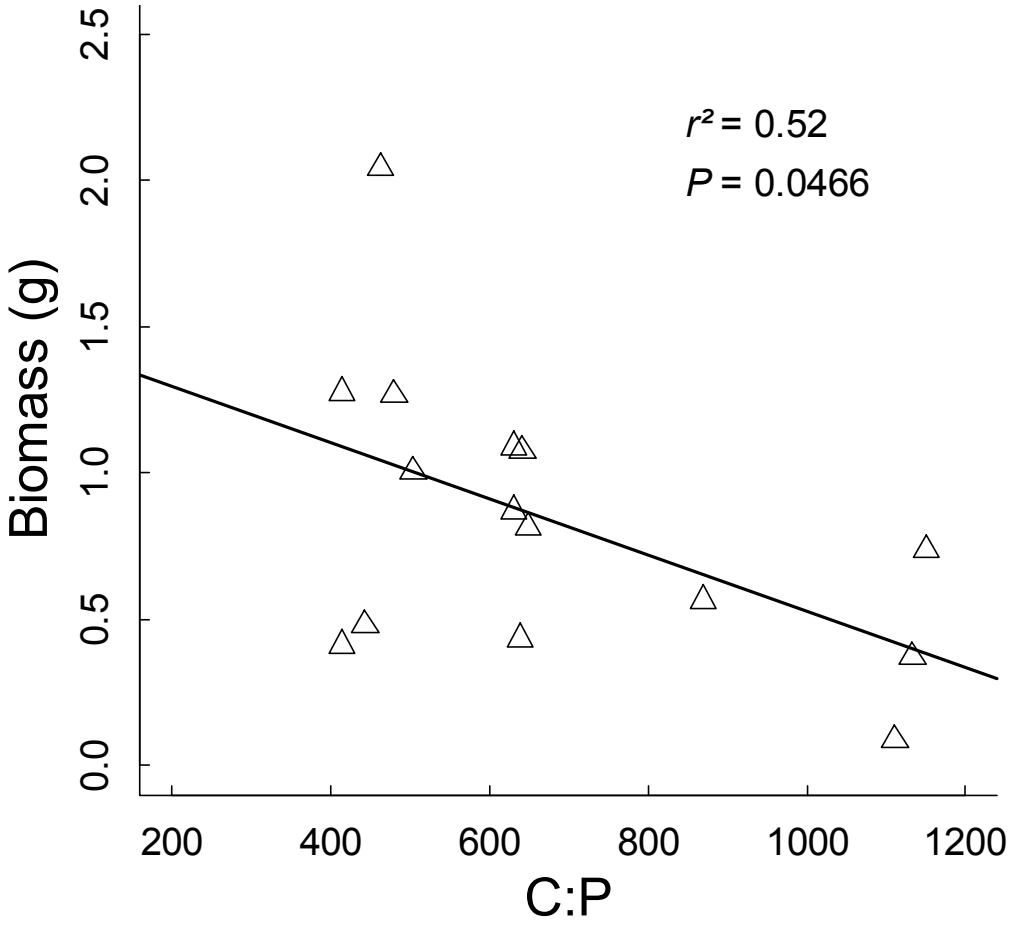

Figure. 2-2. Relationship between the mean biomass of metamorphs and C:P for each plant population. The Pearson correlation (r^2) and associated P-value are also shown.

Table 2-5. Top multiple regression models for amphibian performance on grouped means per population, *P<0·05, ** P<0·01, ***P<0·001

	β_0	β_1	β_2	*df*	*F*	*adj* r^2	*P*
Metamorph abundance	19·7*	C:P -0·0319*	N:P 1·12	2,12	5·94	0·300	0·0467*
Metamorph biomass	14·4***	C:P -0·0234**	N:P 0·802	2,12	5·37	0·384	0·0216*

Discussion

This study shows that natural within-species variation in litter chemistry can alter the strength of bottom-up effects on larval amphibians. I find that litter chemistry, including C:N:P and the concentrations of tannins, varies between populations of *P. arundinacea*, and that this variation impacts larval amphibian performance. All top models in each step of my analysis, except for one (larval period, Table 2-4), contained either population, tannin concentration, or litter C:P as a significant predictor of amphibian performance. Therefore, capturing intraspecific variation in *P. arundinacea* significantly improved our ability to predict amphibian productivity in this simplified food web.

My results agree with previous studies in showing that plant litter is a key component of larval amphibian habitat (Skelly *et al.* 2002, Maerz *et al.* 2005, Brown *et al.* 2006, Schiesari 2006, Rubbo & Kiesecker 2008, Williams *et al.* 2008, Maerz *et al. in review*). Schiesari (2006), Williams *et al.* (2008), and Maerz *et al.* (in review) suggest that litter C:N influences tadpole development. My study expands on this body of work by examining litter quality not only in terms of C:N, but also C:P and N:P. It is noteworthy that in my study system, C:P and N:P had greater explanatory power than C:N alone. Like others, I find that including P in stoichiometric analyses improves our understanding of how energy and nutrients cycle through benthic communities (Frost, Cross & Benstead 2005, Cross *et al.* 2005, Cross, Wallace & Rosemond 2007).

Interestingly, lignin concentration and phytoplankton abundance did not appear in any of our top models. Since lignin was correlated to C concentration, it is likely that C:P and C:N better explained variation in tadpole performance than lignin concentration alone. Algal abundance was distributed randomly across populations and was not significantly related to any of our amphibian performance metrics.

However, my methods measured suspended phytoplankton, which is not a preferred food for *L. palustris*. I did not measure algal abundance or microbial biomass in biofilms, each of which is more likely to influence pickerel frog development. Future experiments could investigate the impact of litter chemistry on detrital biofilms and explore whether that affects tadpole performance.

Contrary to my expectation, tannin concentration had a positive impact on the probability that a pot would produce an *L. palustris* metamorph, as well as a positive effect on mean mass at metamorphosis. In addition, increased tannin content was associated with a shorter larval period, which is also a positive impact on amphibian performance. Several authors have observed that tannins retard development in certain tadpole species, potentially by impairing gills (Maerz *et al*. 2005, Leonard 2008). *Lithobates palustris* develop lungs early, and I observed tadpoles gulping air from the surface throughout the experiment. This behavior explains why *L. palustris* escaped negative impacts of tannins, but why they would actually *benefit* from increased tannin levels remains unclear. One possible explanation is that increased tannins may have favored algal species that are more nutritious for tadpoles, as studies have observed differences in algal communities depending on tannin levels (Pillinger et al. 1994). This represents an important area of future inquiry.

Plant functional traits explain a range of ecosystem properties, from soil carbon sequestration (DeDyne *et al.* 2008), to patterns of succession (Garnier *et al.* 2004) and rates of decomposition (Cornwell *et al.* 2008). To date, however, most work on the relationship between plant functional traits and ecosystem function has operated at the species-level, emphasizing interspecific differences in traits and explicitly ignoring variation within species (e.g. Wright *et al.* 2004; Shipley, Vile & Garnier 2006; Cornwell *et al.* 2008; Hattenschwiler, Tiunov & Scheu 2008). Intraspecific variation is considered to be much smaller than variation among species,

making it possible to assign 'species-specific attributes' for each trait (e.g. Shipley, Vile & Garnier 2006).

This study contributes to a growing body of evidence that intraspecific variation in plant traits can significantly affect ecosystem function. Other workers have demonstrated that within-species variation in green tissue chemistry impacts herbivore productivity and diversity (e.g. Johnson and Agrawal 2005). My work builds on this by demonstrating the significance of intraspecific variation in litter chemistry to ecosystem function; specifically, its effect on secondary productivity in detritus-based food webs. Further, given that increases in nitrogen deposition and atmospheric CO_2 are expected to increase chemical variation within species (McDonald, Agrell & Lindroth 1999; Henry *et al.* 2005; Kasurinen *et al.* 2007; Knops, Naeem & Wright 2007; Xia & Wan 2008), studies such as this, which examine how plant chemistry impacts ecosystem processes, will enhance our ability to predict ecosystem responses to global change. My work shows that intraspecific variation in litter chemistry is already great, and that changes in litter chemistry can impact plant-animal interactions in detritus-based communities.

REFERENCES

1. Alford, R.A. (1999) Ecology: resource use, competition and predation. *Tadpoles: The Biology of Anuran Larvae* (eds. R.W. McDiarmid & R. Altig), pp 240-278. The University of Chicago Press, Chicago, IL.
2. Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference: a Practical Information-theoretic Approach*. Springer-Verlag, New York, NY.
3. Castells, E., Roumet, C, Penuelas, J. & Roy, J. (2002) Intraspecific variability of phenolic concentrations and their responses to elevated CO_2 in two Mediterranean perennial grasses. *Environmental and Experimental Botany* **47**, 205-216.
4. Chapman, S.K., Hart, S.C., Cobb, N.S., Whitham, T.G., & Koch, G.W. (2003) Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. *Ecology* **84**, 2867-2876.
5. Clesceri, L.S., & Eaton, A.D. eds. (1998) *Standard Methods for Examination of Water and Wastewater*, 20th edn. American Public Health Association, Washington, D. C., USA.
6. Cornelissen, J.H.C., Quested, H.M., Gwynn-Jones, D., Van Logtestijn, R.S.P, De Beus, M.A.H, Kondratchuk A., Callaghan, T.V. & Aerts, R. (2004) Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. *Functional Ecology* **18**, 779-786.

7. Cornwell, W.K., Cornelissen, J.H.C., Amatangelo., K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N.P., Quested, H.M., Santiago, L.S., Wardle, D.A., Wright, I.J., Aerts, R., Allison, S.D., van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T.V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N.A., Vaieretti, M.V. & Westoby, M. (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* **11**, 1065-1071.
8. Cross, W.F., Benstead, J.P., Frost, P.C. & Thomas, S.A. (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology* **50**, 1895-1912.
9. Cross, W.F., Wallace, J.B. & Rosemond, A.D. (2007) Nutrient enrichment reduces constraints on material flows in a detritus-based food web. *Ecology* **88**, 2563-2575.
10. De Dyne, G.B., Raaijmakers, C.E., van Ruijven, J., Berendse, F. & van der Putten, W.H. (2004) Plant species identity and diversity on different trophic levels of nematodes in the soil food web. *Oikos* **106**, 576-586.
11. Epps, K.Y, Comerford, N.B., Reeves, J.B., Cropper, W.P. & Araujo, Q.R. (2007) Chemical diversity- highlighting a species richness and ecosystem function disconnect. *Oikos* **116**, 1831-1840.
12. Frost, P.C., Cross, W.F. & Benstead, J.P. (2005) Ecological stoichiometry in freshwater benthic ecosystems: an introduction. *Freshwater Biology* **50**, 1781-1785.

13. Gerber, E., Krebs, C., Murrell, C., Moretti, M., Rocklin, R. & Schaffner, U. (2008) Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. *Biological Conservation* **141**, 646-654.

14. Garnier, E., Cortez, J., Billes, G., Navas, M.L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C. & Toussaint, J.P. (2004) Plant functional markers capture ecosystem properties during secondary succession. *Ecology* **85**, 2630-2637.

15. Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.

16. Halitschke, R., Stenberg, J. A., Kessler, D., Kessler, A. & Baldwin, I. T. (2008) Shared signals - 'alarm calls' from plants increase apparency to herbivores and their enemies in nature. *Ecology Letters,* **11,** 24-34.

17. Hättenschwiler S., Tiunov, A.V. & Scheu, S. (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology Evolution and Systematics* **36**, 191-218.

18. Henry, H.A.L., Cleland, E.E., Field, C.B. & Vitousek, P.M. (2005) Interactive effects of elevated CO_2, N deposition and climate change on plant litter quality in a California annual grassland. *Oecologia* **142**: 465-473.

19. Herms, D. A. & Mattson, W. J. (1992) The dilemma of plants: to grow or defend. *The Quarterly Review of Biology,* **67,** 283-335.

20. Johnson, M.T.J. & Agrawal, A. (2005) Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* **86**, 877-885.

21. Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, S. & Denno, R. F. (2008) Constitutive and induced defenses to herbivory in above- and belowground plant tissues. *Ecology* **89,** 392-406.

22. Kasurinen, A., Peltonen, P.A., Julkunen-Tiitto, R., Vapaavuori, E., Nuutinen, V., Holopainen, T. & Holopainen, J.K. (2007) Effects of elevated CO_2 and O_3 on leaf litter phenolics and subsequent performance of litter-feeding soil macrofauna. *Plant and Soil* **292**, 25-43.

23. Kessler, A. & Baldwin, I. T. (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science* **291,** 2141-2144.

24. Knops, J.M.H., Naeem, S. & Reich, P.B. (2007) The impact of elevated CO_2, increased nitrogen availability and biodiversity on plant tissue quality and decomposition. *Global Change Biology* **13**, 1960-1971.

25. Kraus, T.E.C., Zasoski, R.J., & Dahlgren, R.A. (2004) Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. *Plant and Soil* **262**, 95-109.

26. Kurokawa, H. & Nakashizuka, T. (2008) Leaf herbivory and decomposability in a Malaysian tropical rain forest. *Ecology* **89**, 2645-2656.

27. Lacerf, A. & Chauvet, E. (2008) Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. *Basic and Applied Ecology* **9**, 598-605.

28. Leonard, N. E. (2008) The effect of the invasive exotic Chinese tallow tree (*Triadica sebifera*) on amphibians and aquatic invertebrates. Dissertation. University of New Orleans, New Orleans, LA.

29. Maerz, J. C., J. S. Cohen, and B. Blossey. In review. Can detritus quality be used to predict the impacts of nonnative plant invasions on larval amphibians? Freshwater Biology.

30. McDonald, E.P., Agrell, J. & Lindroth, R.L. (1999) CO_2 and light effects on deciduous trees: growth, foliar chemistry, and insect performance. *Oecologia* **119**, 389-399.

31. Meir, C.L. & Bowman, W.D. (2008) Links between plant litter chemistry, species diversity, and below-ground ecosystem function. Proceedings of the National Academy of Sciences of the United States of America. **105**:19780-19785.

32. Moore, J.C., Berlow, E. L., Coleman, D. C., deRuiter, P. C., Dong, Q., Hastings, A., Johnson, N.C., McCann, K.S., Melville, K., Morin, P.J., Nadelhoffer, K., Rosemond, A.D., Post, D.M., Sabo, J.L., Scow, K.M., Vanni, M.J., Wall, D.H. (2004) Detritus, trophic dynamics and biodiversity. *Ecology Letters* **7,** 584-600.

33. Nyman, T. & Julkunen-Tiitto, R. (2005) Chemical variation within and among six northern willow species. *Phytochemistry* 66, 2836-2843.

34. Ostrofsky, M.L. (1997) Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *Journal of the North American Benthological Society* **16**, 750-759.

35. Palkova, K. & Leps, J. (2008) Positive relationship between plant palatability and litter decomposition in meadow plants. *Community Ecology* **9**, 17-27.

36. Pillinger, J.M., Cooper, J.A. & Ridge, I. (1994) Role of phenolic-compounds in the anti-algal activity of barley straw. *Journal of Chemical Ecology* **20**, 1157-1569.

37. R Development Core Team. (2008) R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org)

38. Rubbo, M.J. & Kiesecker, J.M. (2004) Leaf litter composition and community structure: translating regional species changes into local dynamics. *Ecology* **85**, 2519-2525.

39. Shipley, B., Vile, D. & Garnier, E. (2006) From plant traits to plant communities: A statistical mechanistic approach to biodiversity. *Science* **314**, 812-814.

40. Stamp, N. (2003) Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology,* **78,** 23-55.

41. Stamp, N. E. (2004) Can the growth-differentiation balance hypothesis be tested rigorously? *Oikos,* **107,** 439-448.

42. Sterner, R.W. & Elser, J.J. (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere.* Princeton University Press, Princeton, NJ.

43. Thompson, K., Parkinson, J.A., Band, S.R., & Spencer, R.E. (1997) A comparative study of leaf nutrient concentrations in a regional herbaceous flora. *New Phytologist* **136**, 679-689.

44. Wallace, J.B., Eggert, S.L., Meyer, J.L., & Webster, J.R. (1999) Effects of resource limitation on a detrital-based ecosystem. *Ecological Monographs* **69**, 409-442.

45. Wetzel, R.G. (1995) Death, detritus, and energy flow in aquatic ecosystems. *Freshwater Biology* **33**, 83-89.

46. Wetzel, R.G. & Likens, G.E. (2000) *Limnological Analyses*, 3rd edn. Springer-Verlag, New York, USA.

47. Wilbur, H.M. & Fauth, J.E. (1990) Experimental aquatic food webs: interactions between two predators and two prey. *The American Naturalist* **135**, 176-204.

48. Wright, I.J, Reich, P.B, Westoby, M., Ackerly, D.D, Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C, Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. (2004) The worldwide leaf economics spectrum. *Nature* **428**, 821-827.

49. Xia, J.Y. & Wan, S.Q. (2008) Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist* **179**, 428-439.

APPENDIX

AIC VALUES FOR LINEAR ANALYSES IN CHAPTER TWO

Response variable: Probability that a cage produced at least one metamorph

Predictor variables: Tannins + Population (factor) + DO + pH + Temperature + Chla

Logistic Model	**AIC**
Tannins + Population(factor) + DO + pH + Temperature + Chla	210.49
Tannins + DO + pH + Temperature + Chla	201.07
Tannins + DO + pH + Temperature	199.26
Tannins + DO + pH	198.96

Response variable: Abundance of metamorphs for pots that produced at least one metamorph

Predictor variables: Tannins + Population (factor) + DO + pH + Temperature + Chla

Linear Model	**AIC**
Tannins + Population(factor) + DO + pH + Temperature + Chla	69.82
Tannins + Population(factor) + pH + Temperature + Chla	67.88
Tannins + Population(factor) + pH + Temperature	65.96
Population(factor) + pH + Temperature	65.05
Population(factor) + pH	64.79

Response variable: Mean mass of metamorphs for pots that produced at least one metamorph

Predictor variables: Tannins + Population (factor) + DO + pH + Temperature

Linear Model	**AIC**
Tannins + Population(factor) + DO + pH + Temperature + Chla	-215.98
Tannins + DO + pH + Temperature + Chla	-225.34
Tannins + DO + pH + Temperature	-227.01

Tannins + DO + pH	-228.31

Response variable: Biomass of metamorphs for pots that produced at least one metamorph

Predictor variables: Tannins + Population (factor) + DO + pH + Temperature

Linear Model	**AIC**
Tannins + Population(factor) + DO + pH + Temperature + Chla	17.11
Tannins + Population(factor) + pH + Temperature + Chla	15.45
Tannins + Population(factor) + pH + Temperature	14.34
Tannins + Population(factor) + pH	13.81

Response variable: Length of larval period of metamorphs for pots that produced at least one metamorph

Predictor variables: Tannins + Population (factor) + DO + pH + Temperature

Linear Model	**AIC**
Tannins + Population(factor) + DO + pH + Temperature + Chla	456.87
Tannins + DO + pH + Temperature + Chla	452.91

Tannins + DO + pH + Temperature	451.01
Tannins + DO + pH	449.32
Tannins + pH	449.15

Response variable: Biomass of metamorphs (population means)

Predictor variables: C:N, C:P, N:P, Tannins

Model	**AIC**
C:N + C:P + N:P + Tannins	46.78
C:N + C:P + N:P	44.79
C:N + C:P	42.82

Response variable: Abundance of metamorphs (population means)

Predictor variables: C:N, C:P, N:P, Tannins

Model	**AIC**
C:N + C:P + N:P + Tannins	59.95
C:N + C:P + N:P	57.97
C:N + C:P	56.1