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Leaf litter quality induces morphological and developmental changes in larval amphibians

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Abstract

Aquatic consumers exhibit many types of inducible phenotypic responses to variation in resource quantity and quality. Leaf litter constitutes a primary resource in freshwater systems and variation in litter quality can alter the growth and development of aquatic consumers. It is therefore reasonable to hypothesize that variation in litter quality might also induce phenotypic changes in consumers. To test this hypothesis, we exposed two densities of wood frog (*Lithobates sylvaticus* [*Rana sylvatica*]) tadpoles to six chemically distinct species of leaf litter from temperate broadleaf and coniferous trees. After several weeks, we quantified development rate, growth rate, intestinal length, size of the oral disc, and five external dimensions of the tadpoles. In addition to substantial changes in growth and development rates, we found striking changes in all morphological responses among different leaf litter environments, including up to 14% longer intestines, 11% deeper tails, and 6% deeper tail muscles. In addition, we found strong relationships of total nitrogen content with all morphological features except growth rate. Our results indicate that differences in resource quality can induce phenotypic changes that are as large as or larger than changes induced by resource quantity. Our study also has substantial implications for the future of aquatic consumers living in forested wetlands given that these forests are currently experiencing widespread changes in tree composition.

Keywords: aquatic subsidies, decomposition, gut length, lignin, mouthpart size, phenolics, resource-induced plasticity, temperate forests, wetlands

Introduction

Variation in environmental resources can have profound effects on the fitness of an individual. Resource limitation can promote competition while hindering development, growth, and other physiological processes (Price 1992). As a means of improving fitness, organisms frequently exhibit resource-induced phenotypic changes (i.e. phenotypic plasticity; Agrawal 2001, Weiner et al. 2004, Pigliucci 2005). For example, to improve resource use efficiency, many plant species growing in resource-limited environments alter growth rates and resource allocation strategies, including changes in allocation to root versus shoot growth (Weiner 2004). Similarly, many animal species can alter behavior, morphology, development, and life history traits; examples include insects (Bernays 1986, Greene 1989, Thompson 1992, Reiskind et al. 2009), fish (Day et al. 1994), and amphibians (Walls et al. 1993, Relyea 2002). These phenotypic changes are likely adaptive responses that improve individual performance, affect ecological interactions, and may lead to species diversification (Agrawal 2001, Miner et al. 2005).

Phenotypic responses to resource fluctuations are often studied in the context of variation in resource quantity (i.e. changes in competition), but resource fluctuations can occur due to changes in resource quality (Thompson 1992, Marcarelli et al. 2011). In many systems, resources are derived from both inorganic and organic sources whose quality is a function of their chemical composition. *In situ* changes in production or changes in resource inputs from surrounding ecosystems (i.e. resource subsidies; Polis et al. 1997) can lead to both quantitative and qualitative resource variation. Resource chemistry is determined by numerous factors, including biological causes (e.g., changes in resource stoichiometry) and abiotic causes (e.g., rainfall, temperature) and it can change independently of resource quantity (Marcarelli et al. 2011). Several studies have found that the effects of different resource chemistry on individual phenotypes can be

substantial, particularly for morphological traits (Greene 1989, Thompson 1992, Day et al. 1994), and may have significant implications for ecological interactions (Greene 1989). Hence, discerning how chemical variation in resources alters phenotypes can greatly improve our understanding of how organisms respond to environmental variation.

Plant litter represents a resource in terrestrial and aquatic ecosystems that can vary in both quantity and quality. Whereas litter quantity is simply a function of how much litter is produced, litter quality varies due to interspecific and intraspecific variation in tissue chemistry that remains after senescence (Ostrofsky 1993, Webster & Benfield 1986). Such variation can have important effects on litter-based food webs, which often contain diverse communities of microbes and larger consumers that mineralize and process the nutrients of litter (Facelli and Pickett 1991). For example, elevated nutrient content in litter can promote microbial growth, whereas increased concentrations of structural (e.g., lignin, cellulose) or toxic compounds (e.g., phenolics) can slow or inhibit such growth. Although the effects of litter quality on ecosystem-level processes (e.g., decomposition, nutrient cycling) are well studied (Marcarelli et al. 2011), less attention has been given to the effect on individuals within such food webs.

Moreover, when the effects of litter quality on individuals are considered, the focus is commonly on the survival and growth of individuals. However, changes in litter quality might also alter many other traits of consumers—such as morphological traits—and do so in ways that could represent adaptive responses, similar to how changes in living plant chemistry are known to influence herbivore morphology (Bernays 1986). Despite the potential importance of such changes, there appears to have only been one study that has ever examined how senesced leaf litter alters the morphological traits of consumers. In that study, Reiskind et al. (2009) found that adult mosquitoes developed different wingspans when larvae were fed different litter types.

Recently, there has been growing interest in examining how differences in leaf litter species and chemistry affect the survival and growth of wetland organisms. Much interest has surrounded larval amphibians, which feed off microbial and algal communities growing on litter surfaces (i.e. periphyton; Altig et al. 2007, Schiesari 2006). To date, the focus of this work has been on the survival and growth of consumers in the system (Rubbo and Kiesecker 2004, Maerz et al. 2005, Williams et al. 2008, Stoler and Relyea 2011, Cohen et al. 2012). For example, Cohen et al. (2012) found that tadpole growth was positively related to litter nitrogen (N) content whereas Maerz et al. (2005) found that increased concentrations of polyphenols in litter can have severely adverse effects on tadpole survival. Such effects may be due to changes in the nutritional quality of litter resources (Cohen et al. 2012), or more direct effects of changing aquatic chemistry (e.g., from leached soluble carbon and phenolics; Horne & Dunson 1995, Maerz et al. 2005). However, there has never been an investigation of whether manipulations of litter species or chemistry can induce morphological changes in tadpoles.

Although there has been no examination of litter-induced changes in tadpole morphology, there has been a great deal of work examining how tadpole morphology changes in response to resource quantity, predation risk, and pesticides (Relyea 2000, 2002, Relyea and Auld 2004, 2005, Relyea 2012). Wood frog tadpoles (*Lithobates sylvaticus* [*Rana sylvatica*]) are particularly well studied for their response to reductions in per-capita resource quantity; lower resources induce slower growth and development, and higher foraging activity. Morphologically, lower resource quantity induces relatively smaller tails, larger bodies, longer intestines, and wider mouths, although the magnitude of response depends on the presence of predation risk (Relyea 2002, Relyea and Auld 2004, 2005). These morphological changes appear adaptive, as they improve the growth performance of tadpoles (Relyea 2002) likely due to increased assimilation

and growth efficiency (Sibly 1981, Wassersug and Yamashita 2001). Given the variety of morphological responses to variation in resource quantity, it is reasonable to ask if tadpoles also have the ability to alter their morphology in response to variation in resource quality.

In this study, we investigated whether tadpole consumers can respond to changes in leaf litter quality by altering their internal and external morphology. Using six litter species that varied in nutrient content, recalcitrance, and toxin content, we analyzed the species-specific effects of each litter species and the effects of individual litter chemical components. To investigate how responses to litter chemistry interact with resource quantity, we also manipulated litter species at two densities of tadpoles. We predicted that tadpoles given litter with high N will exhibit morphological responses similar to tadpoles experiencing low competition (e.g., shorter intestines, smaller bodies, and larger tails). In contrast, we predicted that tadpoles given litter with elevated phenolic content or lignin (i.e. structural compounds) will exhibit morphological responses similar to tadpoles experiencing high competition. Regarding effects of density, we predicted that decreasing per-capita resource supply would increase the magnitude of phenotypic responses to litter species.

Methods

Our experiment was conducted at the Pymatuning Laboratory of Ecology in northwest Pennsylvania. The experiment used a completely randomized design with six leaf litter species treatments crossed with two tadpole densities. To increase the applicability of our work with regard to realistic changes in resource chemistry, we used litter species that are dominant in eastern North America and common to areas where wood frogs breed in northeastern temperate forests: American sycamore (SYC), bigtooth aspen (ASP), black willow (BW), sugar maple (SM), red pine (RP), and white pine (WP; Table 1). All species vary substantially in multiple

aspects of litter chemistry, including total N, total phenolic content, and total lignin, thereby allowing us to determine the specific components of litter chemistry that are responsible for morphological changes. Each of the 12 treatment combinations was replicated four times, for a total of 48 experimental units.

The experimental units were 100-L outdoor, plastic mesocosms covered by a 60% shade mesh cloth to simulate a moderate amount of canopy cover and prevent entrance of unwanted organisms. Mesocosms were filled with well water on 6 May. We then introduced microbes, algae, and zooplankton to each mesocosm by providing an aliquot of water taken from five nearby wetlands. A small amount of rabbit chow was provided to each mesocosm as a form of nutrients to accelerate growth of microfauna.

We added leaf litter to the mesocosms on 7 May. We collected litter immediately after senescence during the autumn prior to the experiment and allowed it to dry indoors during the winter in an unheated facility. We placed 100 g of litter into each mesocosm. This provided a litter density within the natural range for the northeastern United States (Rubbo et al. 2008) and a similar density relative to previous experiments (Stoler and Relyea 2011). After adding litter, we allowed periphyton, phytoplankton, and algae to develop for 2 wks before tadpoles were added.

We collected the wood frogs as 10 egg masses from a local wetland and placed all masses in wading pools containing aged well water where they hatched and were then fed rabbit chow *ad libitum*. After reaching stage 25 (Gosner 1960) and a safe handling mass (66.8 mg; 1 SE = ±3.4), we added tadpoles to mesocosms on 23 May (hereafter, day 0). We mixed tadpoles from all egg masses and placed 20 and 40 individuals in low and high density treatments, respectively. This established natural densities of tadpoles and replicated the two lower experimental densities of Relyea and Auld (2004, 2005). Twenty additional tadpoles were selected haphazardly to

assess 24-hr survival, which was 100%.

Tadpoles developed in mesocosms until day 23, at which time we collected and euthanized all surviving individuals and preserved them in 10% formalin. We stopped development of tadpoles at this time because several individuals were at Gosner stage 41. At this stage, tadpole body mass reaches a peak and is soon followed by metamorphosis.

We digitally imaged all preserved tadpoles from the low-density treatments, and 20 randomly selected individuals from the high-density treatments. Because survival was high across all treatments, we were able to image at least 15 individuals per mesocosm. We took separate pictures of the right lateral side, oral disc, and uncurled intestines. For images of the lateral side, we ensured that the tail was on the same focal plane as the body in the image by propping the tail on top of a glass slide to so that the center line of the individual was parallel with the focal plane of the camera.

From these images, we made morphological measurements using ImageJ (Version 1.6.0_20, NIH). We chose to conduct linear measurements instead of landmark-based geometric measurements (e.g., Van Buskirk 2011) because linear dimensions are often easier to visually interpret and both methods often provide the same general illustration of body shape. We began by measuring several dimensions on the right side of the body. We made five measurements identical to those made in Relyea (2001): body length, body depth, tail length, tail depth, and tail muscle depth. Next, we measured several dimensions of the oral disc. We imaged the oral disc after forcing the mouth open by pinning down the lower labium. For mouthparts, we traced the length of each denticle row excluding any gaps in keratinization and denticle structure. As is common for wood frogs, particularly among individuals under high competition (Relyea & Auld 2004), the fourth denticle row was frequently missing or lacked keratinization. When this

occurred, the length of this denticle row was given a measurement of zero. The total keratinized length for each denticle row was summed into a single measure. We also measured the width of the beak and traced the length of the lower beak edge. Finally, we dissected the intestines, and measured intestine length by tracing the entire length of the intestines from the end of the lower stomach to the beginning of the colon.

Litter chemistry analysis

To elucidate potential chemical mechanisms underlying changes in tadpole growth, development, and morphology, we assessed three key components of litter chemistry: total N, percentage of total phenolics, and percentage of total lignin. We also analyzed total phosphorous, but this was highly correlated with total N, so we dropped it from our analysis. Details regarding the chemical analyses can be found in Appendix A.

Statistical analysis

Mass and all morphological dimensions were log-transformed to fit a normal distribution prior to all analyses, and morphological dimensions were mass-adjusted (see Appendix B). During digital analysis, all images from one high-density replicate of red pine were lost, and it was not possible to re-image them because the tadpoles had already been dissected. This replicate was removed from all analyses. Preliminary analysis revealed no significant differences in survival among the 12 treatment means, which ranged from 92 to 100%.

Prior work has demonstrated that the numerous dimensions of the oral disc are typically correlated and can therefore be simplified with ordination analysis without significant loss of information (Relyea and Auld 2005). Following mass-adjustments, we included all mouth dimensions in a principal components analysis (PCA). The first axis explained 71% of the variation, so we used the scores associated with axis as a single response variable (hereafter,

"mouth size") in place of all mouthpart dimensions.

As a result of these analyses, our dataset included individual mass, developmental stage, and seven mass-adjusted morphological measurements: intestine length, mouth size, body length, body depth, tail length, tail depth, and muscle width. We also attempted to reduce external body dimensions using PCA, but the resulting axes did not produce interpretable gradients. Consequently, we retained all external body dimensions as separate response variables in our analysis. In all cases, we used the mean responses from a mesocosm as our response variables. Preliminary analyses revealed that mass-adjustment of linear dimensions also removed any correlations between developmental stage and linear dimensions, and that adding development stage as a covariate in our analyses did not change the interpretation of our results.

We analyzed the effects of density and litter species on the nine response variables using a multivariate analysis of variance (MANOVA) with litter species and density as fixed effects in a full-factorial model. Upon finding a significant multivariate effect, we conducted univariate analyses. For significant univariate effects of litter, we conducted Tukey's post-hoc pairwise comparisons to determine treatment differences.

To assess the effect of litter chemistry on growth, development, and morphological dimensions at different density levels, we conducted a multivariate multiple regression analysis on mesocosm means of phenotypic responses. Preliminary analysis revealed that all regressions were best fit by a linear model. Thus, we employed the general linear model (GLM) procedure in SPSS, using a model that included density as a fixed factor, total N, total lignin, and total phenolics as covariates, and the nine response variables as dependent variables. The model included all main effects and all three possible interactions of density with the covariates. Upon finding a significant multivariate effect, we conducted separate univariate Pearson correlation

analyses to determine correlation coefficients.

Results:

Effects of litter species and tadpole density on tadpole morphology

We found a significant multivariate effect of litter species, tadpole density, and their interaction on mass, development, and relative morphology of tadpoles (Table 2). As a result, we conducted univariate ANOVAs on each response. When we detected a litter species-by-density interaction, we conducted separate univariate ANOVAs within each density treatment.

The mass of individual tadpoles was marginally affected by litter species, and significantly affected by density, and their interaction (Table 2, Fig. 1A). At low density, litter species affected mass ($F_{5,18} = 5.442$, $P = 0.003$). Mean comparisons indicated that tadpoles raised with SYC had 19 to 25% more mass than any other treatment ($P \leq 0.042$). At high density, litter species had a marginal effect on mass ($F_{5,17} = 2.722$, $P = 0.055$); mass in BW tended to be greater than in RP, yet there were no significant differences among the pairwise comparisons ($P \geq 0.068$). Relative to low density treatments, individuals at high density were an average of 30% less massive across all litter treatments.

The developmental stage of the tadpoles was affected by litter species, density, and their interaction (Table 2; Fig. 1B). Litter species affected developmental stage at low density ($F_{5,18} = 6.585$, $P = 0.001$), but not at high density ($F_{5,17} = 1.865$, $P = 0.154$). At low density, tadpoles in WP were one to two developmental stages behind individuals in SM, BW, and SYC ($P \leq 0.022$). Additionally, tadpoles in RP were about one stage behind individuals in SYC ($P = 0.017$). Relative to low density treatments, developmental stage decreased at high densities among SM, ASP, and SYC treatments (1.1 to 3.5%), whereas stage increased slightly (1.3%) in WP.

Tadpole mouth size was affected by litter species and density but not their interaction

(Table 2; Fig. 2A). Averaged across both density treatments, tadpoles in the SYC treatment developed larger mouths than individuals in all other treatments ($P \leq 0.048$). In addition, tadpoles in the BW treatment developed larger mouths than in the WP treatment ($P = 0.003$). Averaged across all litter treatments, mouth size was larger at high density than at low density.

Intestine length was affected by litter species, density, and their interaction (Table 1; Fig. 2B). At low density, litter species affected intestine length ($F_{5,18} = 3.686$, $P = 0.018$); tadpole intestines in the BW treatment were 13 to 14% shorter than in the RP and WP treatments, respectively ($P \leq 0.038$) and 12% shorter than in the SYC treatment ($P = 0.068$). At high density, litter species had a marginal effect ($F_{5,17} = 2.754$, $P = 0.053$); intestines were 12% shorter in the ASP treatment than in the SYC treatment ($P = 0.038$). Relative to low density treatments, intestines increased in length among all treatments, yet this increase was subtle (≤ 3.5%) among ASP, SP, and WP treatments while intestinal length increased by 12, 13, and 20% among SYC, SM, and BW treatments, respectively.

Body length and depth were affected by litter species and density, but not their interaction (Table 2; Fig. 3A,B). Averaged across the density treatments, tadpole bodies in the SYC treatment were 2.7 to 2.9% longer than in the RP or SM treatments ($P \leq 0.054$). In the WP, RP, and SYC treatments, individuals had 3.4 to 5.3% deeper bodies than in the ASP or BW treatments ($P \leq 0.026$). Additionally, bodies in SM were 3.9% deeper than in BW treatments ($P = 0.002$). Averaged across all litter treatments, bodies were 4.2% longer and 2.2% deeper at high density than at low density.

Tail length, tail depth, and tail muscle width were affected by litter species and density, and there was a marginal litter-by-density interaction on tail length (Table 2; Fig. 3C-E). Regarding tail length, litter species had an effect at both densities (low density: $F_{5,18} = 10.039$, P

< 0.001; high density: $F_{5,17} = 2.876$, $P = 0.046$); At low density, tails in the BW treatment were 8.0 to 10.9% longer than in the WP and RP treatments ($P \leq 0.004$). Tails in ASP were 8.6% longer than in RP ($P = 0.002$) and 5.7% longer than in WP ($P = 0.059$). At high density, mean comparisons failed to reveal any significant differences among treatments ($P \geq 0.086$). Relative to low density treatments, tail length of individuals decreased 1.2 to 4.2% among SM, ASP, SYC, and BW treatments while tail length increased 2.9% with RP.

Regarding tail depth, tadpoles in the SM, ASP, and BW treatments had 4.3 to 6.3% deeper tails than in RP and WP ($P \leq 0.040$) when averaged across both density treatments. Averaged across all litter treatments, tails were 2.2% deeper at low density than at high density.

Regarding tail muscle depth, tail muscles were 5.6% wider in the BW treatment than in the SYC treatment ($P = 0.046$) and slightly deeper than in the SM treatment ($P = 0.073$) when averaged across both density treatments. Averaged across all litter treatments, tail muscles were 5.0% deeper at low density than at high density.

Relationships between tadpole phenotypes and litter chemistry

When we tested for relationships between tadpole phenotypes and the chemical traits of the six litter species, we found significant multivariate effects of density ($F_{9,31} = 11.601$, $P < 0.001$), total N ($F_{9,31} = 12.892$, $P < 0.001$), lignin ($F_{9,31} = 2.699$, $P < 0.019$), a marginally significant effect of phenolics ($F_{9,31} = 2.087$, $P = 0.062$), and a significant density-by-N interaction ($F_{9,31} = 2.268$, $P = 0.044$). We did not find significant density-by-lignin or density-by-phenolic interactions ($P \geq 0.717$).

We then examined the univariate regression coefficients (Table 3). Because of the density-by-N interaction, we conducted univariate regression analyses on the effects of N within each density level. At low density, there were significant negative relationships of N with

intestine length and body depth; there were significant positive relationships of N with development stage, mouth size, tail depth, tail length, body length, and tail muscle depth. At high density, N was positively related to mouth size, tail length, and tail depth, and negatively related to body depth. For the percentage of total lignin, there were no significant univariate relationships with any response variable. For the percentage of total phenolics, there was a negative relationship with tail depth across both densities.

Discussion

While previous studies have demonstrated the effects of resource quantity on tadpole morphology (Relyea 2000, 2002, Relyea and Auld 2004, 2005), our study is the first to demonstrate that variation in resource quality can induce dramatic effects on tadpole phenotypes. All measured developmental and morphological responses exhibited at least marginally significant changes in response to the leaf litter treatments. In many cases, the magnitudes of changes caused by resource quality were equal to or greater than those induced by changes in resource quantity (i.e. competition).

Effects of litter quality on phenotypes

The primary question posed by this study is how litter quality influences tadpole phenotypes. Many responses could be generalized through correlations with litter chemistry, and particularly nutrient content. Litter species with greater N content (e.g. sycamore, black willow), which was positively correlated with litter P content, were associated with shorter intestines, larger mouths, longer and shallower bodies, longer and deeper tails, and deeper tail muscles. These correlations indicate wood frogs are capable of ingesting the nutrients in litter, either by direct litter consumption or grazing of microbial communities. Since the litter was generally un-fragmented by the end of the study, it is also likely that the majority of resources were microbial-

derived. Interestingly, there was a positive correlation of litter nutrients with development rate, yet no correlation of mass with nutrients. This suggests that wood frog tadpoles use nutrients towards development instead of growth. Schiesari (2006) also found evidence of this trend, noting that leopard frogs (*L.* [*R.*] *pipiens*) gained more mass than wood frogs when provided high N resources, while wood frogs developed faster than leopard frogs in the same conditions. Similarly strong effects of litter nutrients have also been noted in mosquitoes; Walker et al. (1997) noted that mosquito larvae (*Aedes triseriatus*) increased both development rate and body size with increasing litter N content.

Surprisingly, there were few correlations of total lignin or total phenolics with tadpole responses. This is interesting because past studies have demonstrated strong negative association between lignin and litter decomposition rate, which is largely regulated by the grazing of consumers (e.g., tadpoles) on the litter surface (Melillo et al. 1982, Aerts 1997, Swan & Palmer 2006). Moreover, studies have revealed negative effects of phenolic leachates on tadpole survival (Maerz et al. 2005). There are at least three potential explanations for non-significant effects of phenolics and lignins on tadpole phenotypes. First, wood frogs may be adapted to moderate amounts of phenolic leachates and generally poor-quality substrate; they are one of the few anuran species that consistently inhabits closed-canopy wetlands, which have high inputs of leaf litter and low primary production due to a high amount of shading from the overhead canopy (Werner et al. 2007). This hypothesis appears unlikely, as wood frogs are negatively impacted by dissolved organic carbon and low pH (Horne and Dunson 1995), which are both associated with high phenolic leachates. An alternative explanation is that lignin and phenolic content are inversely related to each other and subsequently counterbalanced their effects. However, there is no evidence for such a relationship in our study and such a relationship has not been reported in

the literature. A more likely explanation is that the concentration of secondary compounds in the litter was not sufficiently high to elicit a response from the tadpoles. Previous studies demonstrating an effect of litter phenolic chemistry on tadpoles used litter of an invasive species (*Lythrum salicaria*) with a dry weight consisting of over 20% phenolic content (Maerz et al. 2005, Brown et al. 2006). In contrast, the highest concentration of phenolic content among our native litter species was 2.1%. Given that litter phenolic content of most native, temperate deciduous tree species is generally between 0-2% (Ostrofsky 1993), our results suggest that the effects of phenolics in native litter species may be largely overshadowed by nutrient content.

Interaction of litter quality and density

Another central question of this study is how the effects of litter chemistry on tadpole phenotypes compare to the effects of per-capita resource quantity. One prediction is that the effects of increasing N content, decreasing lignin content, or decreasing phenolic content would parallel the effects of decreasing density on phenotypes. Although we found no correlation of phenotypic traits with lignin or phenolics, correlations with N provided mixed support for this prediction. For several phenotypic traits in our study, including developmental stage, intestinal length, tail depth, tail length, body depth, and tail muscle depth, responses to increasing litter N were in the same direction as decreasing density. For other phenotypic traits, including mouth size and body length, responses to increasing litter N were in the opposite direction as decreasing density. Moreover, most phenotypic responses exhibited a weaker response to litter N at high density. In addition, increasing density decreased tadpole mass while increasing litter N had no significant effect on tadpole mass at either density level. These results indicate that increased litter nutrient content generate many of the same phenotypic responses as decreasing density, however the relationship is not perfect. Reasons for this are unclear and warrant further research,

such as an investigation of how tadpoles allocate nutrients at different densities.

It is worth noting that the interaction effects observed for several phenotypic responses were not merely due to changes in response magnitude, but also to changes in response direction. This suggests that tadpole development strategies depend on relative litter nutrient content and competitor density, in addition to the unique chemical composition of each litter species. For example, intestinal length and mouth size generally increased at higher densities, yet this was not the case for individuals in bigtooth aspen treatments. One explanation may be the relatively low phenolic and high N content of aspen leaves, which likely promoted microbial growth and efficient tadpole grazing, even at high tadpole densities. In contrast, the relative lack and nutrients and high recalcitrance of the two conifer litters (i.e. red and white pine) may explain the consistently long intestinal length, large body size, and short tail lengths in these treatments. As another example, the relatively high tadpole mass in sycamore may have been generated by distinctively large surface area of the litter species. Large surface area, combined with high N content, can promote microbial growth (Gunnarsson et al. 1988), may have reduced the energetic demands of tadpole foraging, and allowed energy to be used in other aspects of the phenotype.

Implications of results for changes in forest composition

Our study suggests that changes or heterogeneity in forest composition will have cascading effects on consumer phenotypes and potentially on consumer fitness. This is important, considering the numerous impacts that humans are currently exerting on forest structure and function. For example, sugar maple is undergoing a dramatic decline in abundance due to climate change, deer browsing, and other factors (Lovett and Mitchell 2004). Multiple species are likely to replace this, including red maple (*A. rubrum*), which differs substantially in chemistry and may induce changes in wetland food webs (Stoler and Relyea *in review*). Natural

succession in forests may also shift tree composition, through the replacement of fast growing tree species (e.g., pines and poplars) with more shade tolerant and slow growing species (e.g., maples, oaks; Abrams 1998). Our results indicate that wood frogs may cope with such changes through phenotypic plasticity, yet future research should elucidate whether such plasticity will influence ecosystem processes within wetlands (e.g., rate of litter decomposition) and across aquatic-terrestrial boundaries (e.g., organic subsidies to land). Such effects may provide a novel link between forest diversity and ecological function.

The importance of phenotypic changes will also depend on whether they are adaptive within and among ecological contexts. Although Relyea (2002) suggests that the phenotypic changes observed in our study may be adaptive, explicit tests of this with regard to litter-induced changes should be considered in the future. Moreover, many phenotypic changes were in the opposite direction to changes that wood frogs exhibit when challenged with predators (Relyea 2002), indicating potential maladaptation in the context of predator presence. Additionally, litter-induced phenotypic changes may not occur among amphibian populations or species less adapted to the litter-based conditions of closed-canopy wetlands. Further studies on the combined effects of litter chemistry and predation for wood frogs and other amphibian species should be conducted to fully elucidate the effects of changing forest composition on amphibian fitness.

Conclusions

Discussions of resource subsidies in ecosystems have focused on either quality or quantity, but rarely consider the impacts of both simultaneously (Marcarelli et al. 2011). This disconnect has resulted in the use of separate analyses to uncover the effects of resource chemistry and quantity, and has led to little comparison of their effects. This is particularly the case with leaf litter; except for a few notable studies (e.g., Maerz et al. 2005) the majority of

community-level studies have ignored the impacts of litter species variation even though ecosystem ecologists continually stress the importance of this variation for whole-ecosystem function (Scott and Binkley 1997, Aerts 1997). Our study is among the first to examine how litter quality alters consumer morphology, and the first study to examine the effects of litter quality on tadpole morphology. In doing so, we have shown that variation in litter chemistry can have an equal, if not greater, impact on individual-level processes than resource quantity. Future work should escalate this research to the community level, and attempt to understand how resource variation impacts food web structure and function.

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Supplemental Material

Appendix A

Details on litter chemical analyses.

Appendix B

Details on mass-adjustment methodology.

Table 1: The leaf litter species used in the experiment, including common names, abbreviations, and family. Values for total lignin, total phenolics, and total nitrogen are mean values based on analyses that were performed in triplicate.

Treatment	Abbreviation	Family	Species	Lignin (%)	Phenolics (%)	Nitrogen (%)
American sycamore	SYC	Platanaceae	*Platanus occidentalis*	24.0	0.5	1.0
Bigtooth aspen	ASP	Salicaceae	*Populus grandidentata*	23.9	0.2	0.9
Black willow	BW	Salicaceae	*Salix nigra*	14.9	1.0	1.0
Red pine	RP	Pinaceae	*Pinus resinosa*	7.7	1.0	0.4
Sugar maple	SM	Aceraceae	*Acer saccharum*	7.3	2.1	0.7
White pine	WP	Pinaceae	*Pinus strobus*	20.5	0.2	0.6

Table 2: Results of a MANOVA and subsequent ANOVAs on mass, development stage, and seven mass-adjusted morphological dimensions of wood frog tadpoles. All measurements were performed on preserved tadpoles that were raised in mesocosms for 23 days. The term "mouth size" represents the first axis of a PCA conducted on 10 dimensions of the oral disc.

	Litter species			Density			Litter species x Density		
	F	df	P	F	df	P	F	df	P
MANOVA	5.221	45,124	<0.001	15.903	9,27	<0.001	1.963	45,124	0.002
Univariate effects									
Mass	2.364	5,35	0.060	1.499	1,35	<0.001	5.421	5,35	0.001
Development stage	5.412	5,35	0.001	4.155	1,35	0.049	2.220	5,35	0.074
Mouth size	11.876	5,35	<0.001	42.195	1,35	<0.001	1.255	5,35	0.305
Intestines	3.564	5,35	0.010	26.190	1,35	<0.001	2.917	5,35	0.026
Body length	2.543	5,35	0.046	61.029	1,35	<0.001	1.033	5,35	0.414
Body depth	11.307	5,35	<0.001	16.612	1,35	<0.001	0.893	5,35	0.496
Tail length	11.515	5,35	<0.001	4.791	1,35	<0.001	2.301	5,35	0.066
Tail depth	8.029	5,35	<0.001	8.277	1,35	0.007	0.339	5,35	0.886
Tail muscle depth	2.813	5,35	0.031	19.782	1,35	<0.001	0.865	5,35	0.514

Table 3: Univariate regression coefficients of the correlation between three litter chemical components (total nitrogen, total lignin, total phenolics) with nine developmental and morphological responses of wood frog tadpoles. Because there was a significant interaction of density with nitrogen, coefficients at both density levels are provided.

	Nitrogen			
Measurement	Low Density	High Density	Lignin	Phenolics
Mass	0.378	-0.288	0.047	0.037
Development stage	**0.664**	0.368	0.037	0.216
Mouth size	**0.531**	**0.590**	-0.114	-0.122
Intestines	**-0.487**	0.144	0.045	-0.025
Tail depth	**0.592**	**0.483**	0.155	**0.290**
Tail length	**0.815**	**0.515**	-0.037	0.139
Body depth	**-0.608**	**-0.492**	0.226	-0.048
Body length	**0.453**	0.048	0.009	-0.151
Tail muscle depth	**0.448**	0.030	-0.007	0.176

Note: Coefficients in boldface are significant ($P < 0.05$).

Figure legends:

Figure 1. Individual mass (a) and Gosner stage (b) of wood frog tadpoles in six different litter treatments at two density levels. Responses were measured on tadpoles preserved on day 23 of the experiment. Litter treatments and abbreviations are found in Table 1. Data are means ± 1 SE.

Figure 2. Mass-independent mouth size (a) and intestine length (b) of wood frog tadpoles in six different litter treatments at two density levels. Responses were measured on tadpoles preserved on day 23 of the experiment. Mouth size data represent principal component scores of a single axis that explain the majority of variation among 10 mass-independent measurements of the oral disc. Litter treatment abbreviations are found in Table 1. Data are back-transformed means ± 1 SE.

Figure 3. Mass-independent body length (a), body depth (b), tail length (c), tail depth (d), and tail muscle width of wood frog tadpoles in six different litter treatments at two density levels. Responses were measured on day 23 of the experiment. Litter treatment abbreviations are found in Table 1. Data are back-transformed means ± 1 SE.

Figure 1

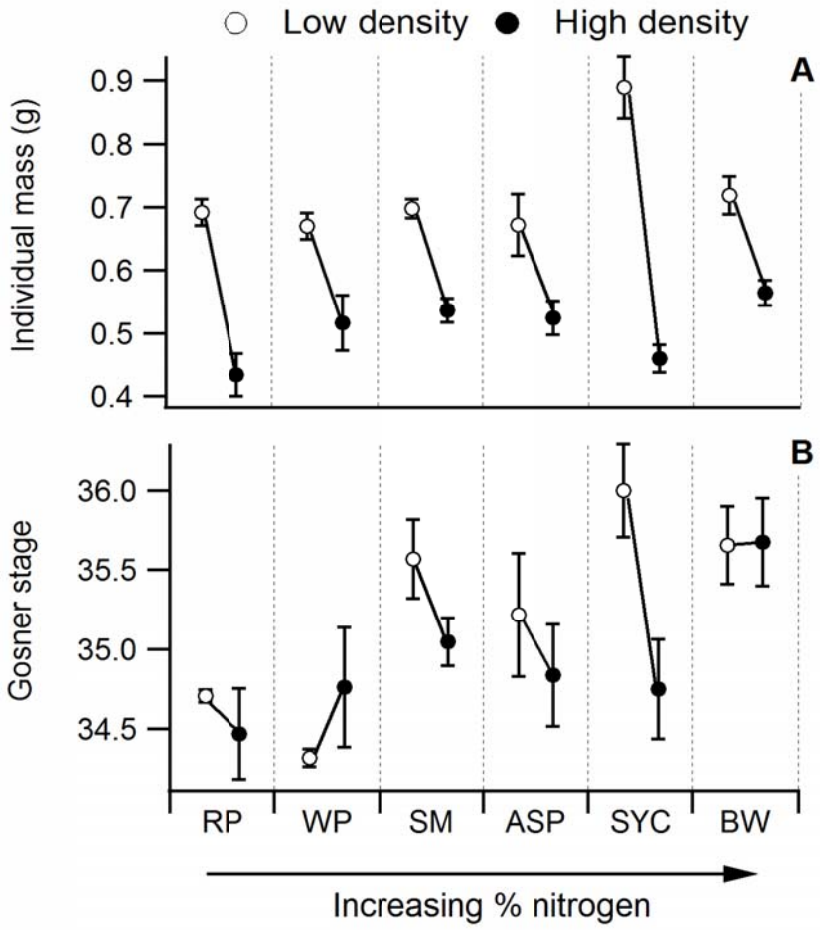


Figure 2

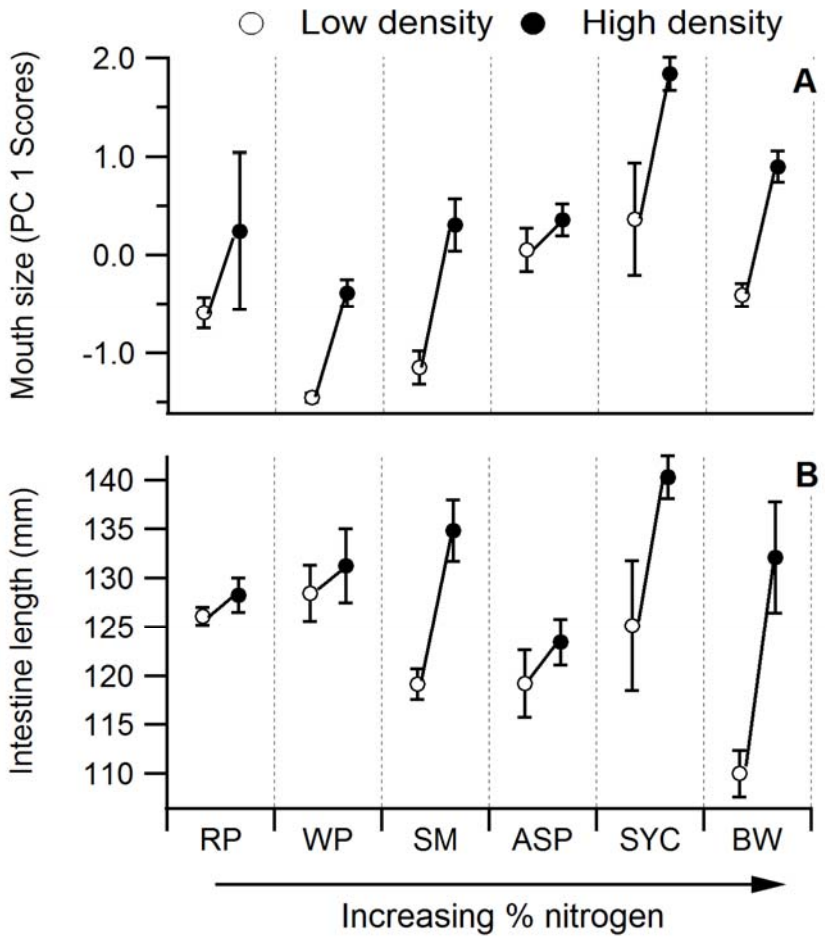
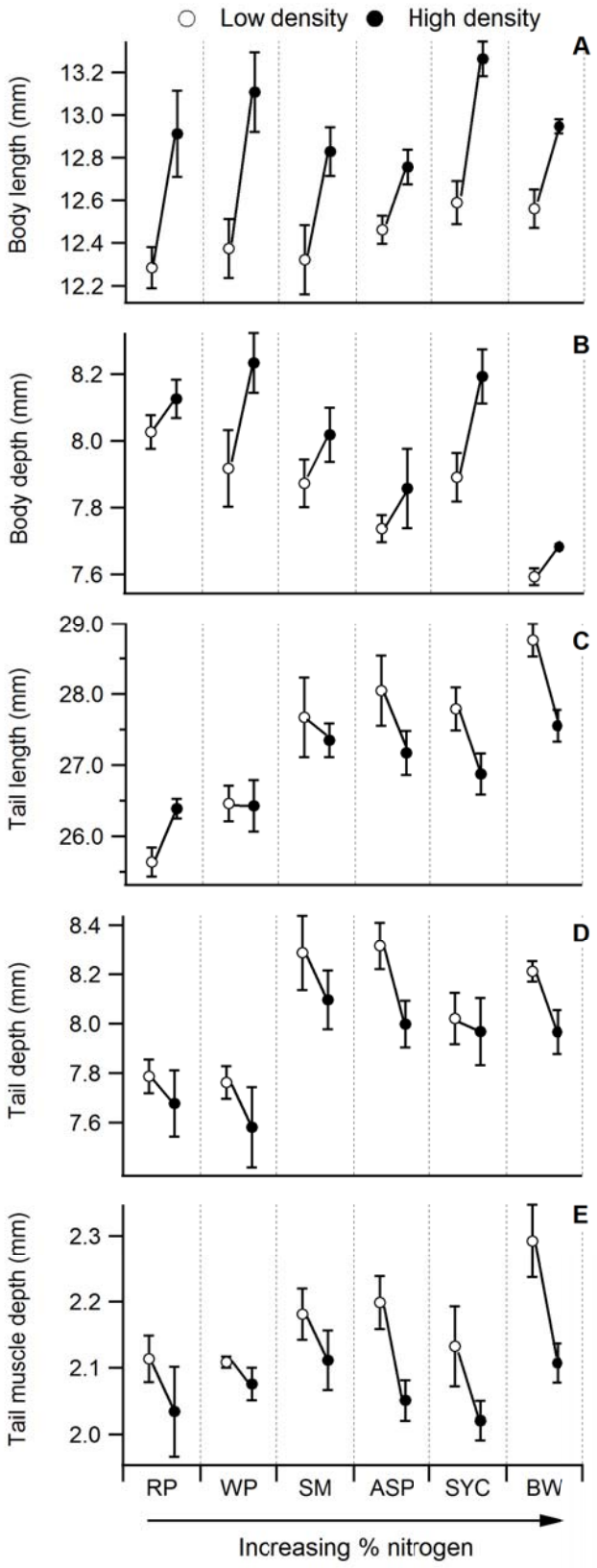


Figure 3


Low density
High density
A
Body length (mm)
13.2
13.0
12.8
12.6
12.4
12.2
B
Body depth (mm)
8.2
8.0
7.8
7.6
C
Tail length (mm)
29.0
28.0
27.0
26.0
D
Tail depth (mm)
8.4
8.2
8.0
7.8
7.6
E
Tail muscle depth (mm)
2.3
2.2
2.1
2.0
RP
WP
SM
ASP
SYC
BW
Increasing % nitrogen