

Leaf litter resource quality induces morphological changes in wood frog (*Lithobates sylvaticus*) metamorphs

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Abstract For organisms that exhibit complex life cycles, resource conditions experienced by individuals before metamorphosis can strongly affect phenotypes later in life. Such resource-induced effects are known to arise from variation in resource quantity, yet little is known regarding effects stemming from variation in resource quality (e.g., chemistry). For larval anurans, we hypothesized that variation in resource quality will induce a gradient of effects on metamorph morphology. We conducted an outdoor mesocosm experiment in which we manipulated resource quality by rearing larval wood frogs (*Lithobates sylvaticus*) under 11 leaf litter treatments. The litter species represented plant species found in open- and closed-canopy wetlands and included many plant species of current conservation concern (e.g., green ash, common reed). Consistent with our hypothesis, we found a gradient of responses for nearly all mass-adjusted morphological dimensions. Hindlimb dimensions and gut mass were positively associated with litter nutrient content and decomposition rate. In contrast,

forelimb length and head width were positively associated with concentrations of phenolic acids and dissolved organic carbon. Limb lengths and widths were positively related with the duration of larval period, and we discuss possible hormonal mechanisms underlying this relationship. There were very few, broad differences in morphological traits of metamorphs between open- and closed-canopy litter species or between litter and no-litter treatments. This suggests that the effects of litter on metamorph morphology are litter species-specific, indicating that the effects of changing plant community structure in and around wetlands will largely depend on plant species composition.

Keywords Amphibian · Limb length · Intestine · Snout-vent length · Temperate wetland

Introduction

The effects of resource availability are commonly expressed over the lifespan of an organism. Changes in resource conditions early in ontogeny can alter later life-history traits (Pechenik 2006) for many types of organisms, such as insects, plants, mollusks, reptiles, and birds (e.g., Bernays 1986; Merilä and Svensson 1997; Pechenik et al. 1998; Shine and Downes 1999; Grant et al. 2001). Such effects are particularly important for organisms with complex life cycles in which the progression of growth is marked by distinct changes in life form, habitat, and resource niche (i.e., metamorphosis; Pechenik 2006). Indeed, resource conditions experienced during early life stages can strongly influence post-metamorphic mass and fitness (Pechenik et al. 1998). Although most studies have focused only on demonstrating the existence or absence of such connections between life stages, there is increasing

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evidence that variation in resources can induce highly detailed phenotypic changes across multiple life stages (Tejedo et al. 2010).

Variation in later-stage phenotypes might arise from changes in both larval resource quantity and quality. For example, competition (i.e., competitor-mediated declines in resource quantity) during larval stages is often inversely related to offspring number and growth after metamorphosis, and can induce several types of post-metamorphic morphological variants (Brockelman 1975; Alford and Harris 1988; Tejedo et al. 2010). Similarly, variation in larval resource quality has demonstrable effects on post-metamorphic mass, survival, and phenotype (Bernays 1986; Nicleza et al. 2006). However, most manipulations of resource quality are performed with simple manipulations of nutrient ratios in resources [e.g., carbon:nitrogen (C:N), carbon:phosphorus (C:P)]. In contrast, natural variation in resource quality can be far more complex and multifaceted than variation in quantity, since multiple variables (e.g., variables related to nutrient content and availability) often independently and simultaneously determine quality (Marcarelli et al. 2011). For this reason, it is likely that our current understanding of resource-induced morphological connections between early and later life stages is severely limited relative to the range of natural phenomena.

A common source of natural variation in resource quality arises from the chemistry of resource subsidies. For example, leaf litter serves as an abundant and qualitatively variable resource in a variety of systems. This is particularly true in temperate forests, where up to 90 % of all foliar material senesces during the fall (Facelli and Pickett 1991). A substantial amount of this litter falls into ponds and streams, where microbes and numerous species of macro-consumers use the litter as a source of energy and nutrients (Tiegs et al. 2009). Plant species can exhibit tremendous inter-specific variation in the concentration of nutrients, and of other compounds that reduce nutrient availability (e.g., Ostrofsky 1997). Generally, species that have high nutrient concentrations, rapid decay rates, and low phenolic concentrations are thought to offer a higher quality resource to consumers (Webster and Benfield 1986).

In temperate ecosystems, larval anurans are an important and abundant grazer of plant litter resources (Schiessari et al. 2009), and there has been much recent interest in understanding how litter quality influences anuran phenotypes. Indeed, several studies have demonstrated that leaf litter quality influences larval anuran survival (Maerz et al. 2010; Earl et al. 2014), and can alter body mass and size, tail and body shape, and gut length (Stoler and Relyea 2011, 2013; Stephens et al. 2013). Although effects of litter resource quality on metamorph phenotypes have not been researched, Nicleza et al. (2006) offers some predictions by demonstrating that increased energy content of artificial

larval diets can increase metamorph head width and length. It is also reasonable to hypothesize that decreases in litter quality may mimic effects of higher larval competition (i.e., low resource quantity), which can cause metamorphs to exhibit shorter and narrower bodies (Relyea 2001; Relyea and Hoverman 2003). Studies have also observed a positive correlation between limb length and duration of larval period, which may be driven by a combination of low resource availability and hormonal mechanisms (Emerson 1986; Relyea 2001). Because such morphological changes may have fitness consequences (Tejedo et al. 2010), identifying the effects associated with litter resources might have important implications for amphibian conservation and forest ecology.

Despite the potential complexity and interspecific diversity of leaf litter quality, the effects of litter chemistry may be comparable among plants with similar growth forms. Due to trade-offs along the leaf economics spectrum (Wright et al. 2004), plant species with similar resource niches are likely to have more similar chemistry than plants in different habitats and varying niches (Cornelissen 1996). For example, water bodies with reduced overhead canopy typically contain small shrubs and emergent herbaceous species (e.g., grasses, sedges, rushes) that exhibit rapid growth and higher foliar nutrient content (Evans 1989). In contrast, water bodies with overhead canopy typically contain slower-growing trees with less foliar nitrogen (Evans 1989; Skelly et al. 2002). The nutritional differences between tree and smaller emergent plant litter may be important for aquatic consumers. For example, Williams et al. (2008) found that tadpoles growing in ponds receiving deciduous tree leaf litter grew and developed slower relative to tadpoles in ponds with grass litter. Given broad differences in foliar chemistry among plant types, it may be possible to generalize the effects of litter quality according to these types.

In a previous study, Stephens et al. (2013) exposed wood frog (*Lithobates sylvaticus*) tadpoles to multiple species of tree, grass, and shrub leaf litter. This species actively grazes the litter and associated microflora, and increased litter quality led to increases in both growth and development rates. Here, we extend the results of Stephens et al. (2013) and examine the morphology of metamorphs from that study. We hypothesized that decreases in litter nutritional quality will induce changes in metamorph morphology that are similar to morphological responses to increased competition, including decreases in relative snout–vent length and head width, and increases in limb length, limb width, body mass, and gut mass (Relyea 2001; Relyea and Auld 2004). We predicted that larval exposure to different litter species would produce a gradient of morphological responses among metamorphs that corresponds to gradients in litter chemistry among plant species. Second, we predicted

that the absence of litter resources would produce phenotypes expected of the lowest quality litter (i.e., smaller bodies with increased limb lengths and widths, and larger gut mass). Owing to broad differences in litter chemistry among plant types, we further explored whether effects of litter quality were litter species-specific, or if effects would be specific to two broad plant groups including trees versus emergent shrubs and herbs. Finally, based on observations by Relyea (2001), we predicted that the duration of larval period would positively correlate with individual mass, gut mass, and limb lengths, and negatively correlate with body size. Together, the results of this study offer insights into how larval resource quality and ongoing changes in plant species composition (e.g., Abrams 1998) can have lasting effects on a common consumer phenotype.

Materials and methods

Wood frog metamorphs were collected during a study conducted by Stephens et al. (2013) in accordance with the guidelines of Oakland University's Institutional Animal Care and Use Committee. This study, conducted at the Oakland University Biological Preserve (MI, USA), assessed the influence of ten litter species belonging to two litter types (five broadleaf tree litter species and five smaller shrub and herbaceous plant litter species) on larval wood frog growth and development. All species can be present in and around wetlands containing moderate to high canopy cover where wood frogs breed (Werner et al. 2007). Several of the plant species are of major conservation concern (e.g., green ash), and several are widespread invasives (e.g., common reed, glossy buckthorn). A complete list of the common and scientific names of litter species used in this study can be found in Table 1, including the abbreviations used for each species herein.

The experiment was a completely randomized design with 10 litter species treatments and 1 no-litter control. The 11 treatments were replicated five times for a total of 55 experimental units. Experimental units were 110-L plastic mesocosms filled with tap water in the autumn prior to the experiment. After allowing tap water to age for 7 days, we inoculated each mesocosm with equal aliquots of pond water from Saginaw Pond (MI, USA; 42°40'N, 83°13'W) which contained bacteria, fungi, algae, and zooplankton. At this time, we also added 150 g of recently senesced and dried leaves to all mesocosms except the no-litter control. This biomass of litter is within the range observed in natural wetlands (Rubbo et al. 2008). The mesocosms overwintered until the start of the experiment in the spring. We set aside a subset of dried litter for chemical analysis of carbon, nitrogen, and phosphorus content. Details on litter collection and chemical analyses are in Supplementary Appendix A.

At the same time that we introduced leaf litter into the mesocosms in the autumn, we placed a single ceramic tile into each mesocosm as a standardized substrate to measure periphyton biomass. In addition, we placed a coarse mesh bag (10 mm pore size) containing ~5 g of dried and pre-weighed leaf litter in each mesocosm to measure litter decomposition. The mesh bag allowed tadpoles to graze litter, so our measure of leaf decomposition includes grazing activity.

We introduced tadpoles into the mesocosms in the spring of 2010. We reared tadpoles from eggs produced by 10 pairs of adults collected in Saginaw Pond (Sec. 26 T25 R5E, Washtenaw County, MI; 42°40'N, 83°13'W). Adults were collected individually and paired in the laboratory, in separate containers, which resulted in 10 egg masses. Five days after hatching, we mixed larvae from all egg masses and haphazardly selected 20 individuals for each mesocosm (see Stephens et al. 2013 for further details). The day

Table 1 List of litter species used in the study, including the abbreviation used in this article, vegetation type, family and species names, and results from nutrient analysis

Treatment	Abbreviation	Vegetation type	Family	Species	% C	% N	% P
Green ash	FRPE	Tree	Oleaceae	<i>Fraxinus pennsylvanica</i>	43.18	2.20	0.20
Red maple	ACRU	Tree	Aceraceae	<i>Acer rubrum</i>	44.26	0.59	0.06
Eastern cottonwood	PODE	Tree	Salicaceae	<i>Populus deltoides</i>	43.30	1.03	0.09
Black willow	SANI	Tree	Salicaceae	<i>Salix nigra</i>	43.11	1.30	0.08
White oak	QUAL	Tree	Fagaceae	<i>Quercus alba</i>	45.11	1.45	0.16
Glossy buckthorn	RHFR	Emergent	Rhamnaceae	<i>Rhamnus frangula</i>	40.80	1.96	0.18
Common reed	PHAU	Emergent	Poaceae	<i>Phragmites australis</i>	42.16	1.55	0.12
Reed canary grass	PHAR	Emergent	Poaceae	<i>Phalaris arundinacea</i>	41.37	2.00	0.30
Tussock sedge	CAST	Emergent	Cyperaceae	<i>Carex stricta</i>	45.29	0.99	0.06
Cattail	TYLA	Emergent	Typhaceae	<i>Typha latifolia</i>	43.98	0.56	0.04

of tadpole introduction to the mesocosms is defined as day 0 of the experiment. We set aside 20 individuals to assess 24-h mortality due to handling effects, which was 0 %.

Before the tadpoles began metamorphosing, we measured several factors in the mesocosms that can affect amphibian performance in wetlands: the rate of litter decomposition, the concentration of dissolved organic carbon, the concentration of phenolics, periphyton biomass, dissolved oxygen, and pH (Maerz et al. 2010; Stoler and Relyea 2011; Stephens et al. 2013). We conducted the measurements 30 days after the tadpoles were added to the mesocosms. Measurement procedures are detailed in Stephens et al. (2013).

The first tadpole metamorphosed on day 45. After this date, we checked mesocosms daily and any individuals with emergent forelimbs were collected and housed in the laboratory using plastic bins containing 1 cm of water. We considered metamorphosis complete when a metamorph's tail was completely resorbed. At this time, we recorded the time to metamorphosis and the individual was euthanized in MS-222, and preserved in 4 % formalin for further morphometric analysis.

Morphological measurements

We measured the morphology of individuals that remained from each mesocosm after using five preserved individuals for nutrient analysis (reported in Stephens et al. 2013). For measurement of gut mass, we used two to three individuals from each mesocosm. We quantified dry gut mass using a procedure described by Castañeda et al. (2006) with modification. Gut mass included food contents remaining in the alimentary canal, although this was minimal for most organisms. For details on gut mass measurement, see Supplementary Appendix A.

After removing individuals for gut mass measurement, we measured the external morphology of all remaining frogs. Because of differences in survival and the need to remove individuals for other measurements, the number of individuals used to assess external morphology differed among mesocosms. We measured between one and seven individuals from each mesocosm, but were able to measure at least three individuals from most mesocosms. On average, we measured three more individuals among PHAR replicates than among ACUR replicates.

We quantified external morphology after imaging the ventral side of all individuals using a dissecting scope attached to a digital camera (Olympus SZX16, Japan). We standardized the focal distance of the camera by mounting the lens at the same distance from the stage for all images, and pinning the forelimbs and hindlimb to a clay surface. After images were taken for an individual, we blotted it dry and then weighed it. For each individual, we measured

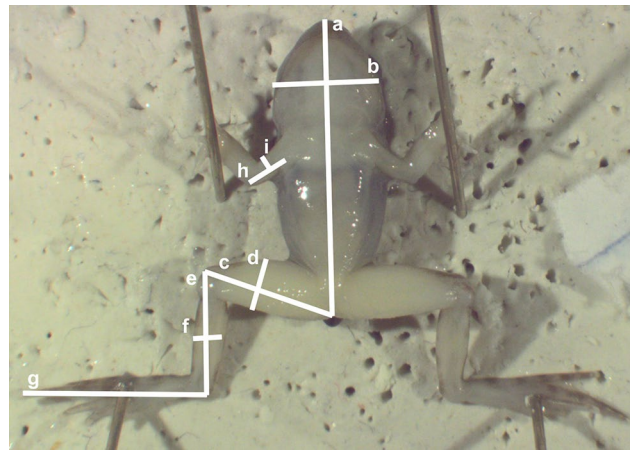


Fig. 1 Morphological dimensions measured in the study, including snout–vent length (*a*), head width (*b*), upper-hindlimb length (*c*), upper-hindlimb width (*d*), lower-hindlimb length (*e*), lower-hindlimb width (*f*), foot length (*g*), forelimb length (*h*), and forelimb width (*i*)

nine morphological features (Fig. 1), including head width, snout–vent length (SVL), upper-hindlimb length and width, lower-hindlimb length and width, foot length, forelimb length, and forelimb width.

Statistical analysis

Effects of litter on metamorph morphology

We log-transformed mass and all morphological measurements. Due to a processing error, there were no preserved metamorphs available for gut or external morphological measurements in one no-litter replicate. An additional processing error caused the loss of gut data for one red maple replicate; all external morphological measurements for this replicate were retained in the analysis. Since morphological features are often correlated with individual mass, we mass-adjusted all morphological responses (Supplementary Appendix B). Adjusting morphological variables by SVL revealed similar results.

To assess the effect of litter treatment on responses, we conducted a separate ANOVA for each response. We adjusted the results to correct for an increase in type I error that results from conducting multiple tests (Benjamini and Hochberg 1995). We excluded the no-litter treatment from these analyses, since we later compare this treatment to the individual litter species treatments using planned comparisons. Response variables included gut mass, metamorph mass, SVL, head width, forelimb width and length, and all hindlimb dimensions. The five hindlimb dimensions were combined into a composite variable (hereafter, “hindlimb dimensions”) using principal components analysis (PCA), such that an increase in this component describes longer and

wider legs (see Supplementary Appendix B for details on the PCA, loading scores, and attempts to combine other variables). Note that the effect of litter on metamorph mass was previously assessed by Stephens et al. (2013); we included the mass data in the present paper to explore the effects of litter type, litter chemistry, and aquatic environmental attributes on mass which were not examined previously. Correlations among response variables are reported in Table B2.

Effect of litter chemistry and aquatic environmental conditions on morphology

We then explored how litter chemistry and aquatic environmental conditions were associated with morphological dimensions by using a redundancy analysis (RDA). RDA is a constrained, linear, multivariate analysis that combines regression and ordination to explore how variation in the structure of an independent dataset (i.e., litter chemistry and aquatic environmental conditions) explains variation of a dependent dataset (i.e., morphological dimensions). Canonical axes (i.e., ecological gradients) for each dataset are derived such that the first ecological gradient derived from the independent dataset explains the maximum variation within the dependent dataset. In our analysis, the independent dataset included the eight litter and aquatic environment variables: litter C:N, percent litter decomposition, concentration of DOC, concentration of phenolics, periphyton biomass, dissolved oxygen, conductivity, and pH. The dependent dataset included all morphological dimensions. All individual hindlimb dimensions were used for this analysis. We excluded data from the no-litter treatment since it had no associated litter chemistry variables. Further details regarding RDA data preparation and interpretation can be found in Supplementary Appendix B.

Effect of litter absence and canopy litter types on morphology

For all morphological responses that exhibited significant differences among litter species (i.e., significant univariate effects), we conducted two planned comparisons on each response using 2-tailed *t* tests. The first comparison contrasted the responses of all litter treatments with the no-litter control to test the prediction that the absence of litter would produce phenotypes similar to those generated by low quality litter species. The second comparison contrasted the responses of all tree litter treatments with shrub and herbaceous litter treatments. This comparison tested the prediction that tree litter species would be associated with morphological changes associated with low resource quantity, whereas shrub and herbaceous species treatments would be associated with opposite responses. The no-litter treatment was excluded from the latter planned comparison.

Effect of larval period on morphology

When we detected a significant effect of litter on a morphological trait, we used linear regression to test the prediction that the duration of larval period would positively affect mass, gut mass, and limb lengths, and negatively affect body width and SVL. We adjusted significance values for an increase in type I error due to multiple testing (Benjamini and Hochberg 1995).

We conducted mass-adjustments, PCA, ANOVAs, planned comparisons, and regression analyses using SPSS (v.19). We conducted RDA using the package *vegan* in R (v.3.1.2).

Results

Effect of leaf litter on metamorph morphology

Due to the immense number of treatment differences found in our analysis, we only present major differences among treatments. A detailed list of all pairwise comparisons can be found in Supplementary Appendix C.

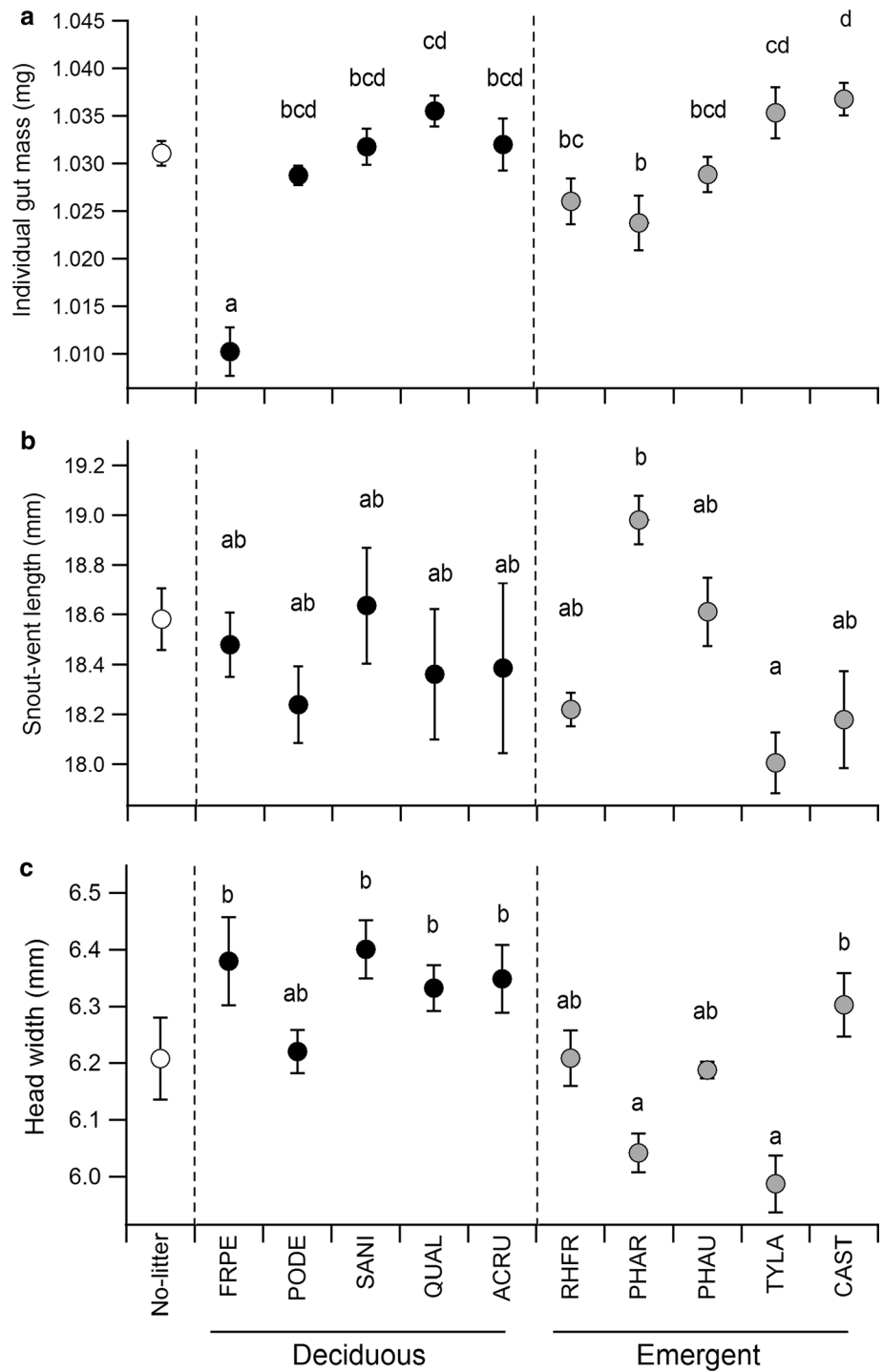
Treatment differences in metamorph mass are reported in Stephens et al. (2013) and repeated in Supplementary Appendix C. Planned comparisons found no difference in mass between tree species versus shrub and herb species ($P = 0.421$) and no difference between the no-litter and 10 litter treatments ($P = 0.098$).

We found a univariate effect of litter species on relative gut mass ($F_{9,39} = 12.973$, $P < 0.001$; Fig. 2a). After conducting mean comparisons, we found a gradient of differences among treatments. The most substantial difference was that individuals had 1–3 % smaller guts in the green ash treatment relative to all other treatments ($P \leq 0.002$). Planned comparisons did not reveal a difference in gut mass between the two litter types ($P \geq 0.285$) and no difference between litter and no-litter treatments ($P \geq 0.610$).

SVL was also affected by litter treatment ($F_{9,40} = 2.128$, $P = 0.050$; Fig. 2b). When conducting mean comparisons, the only difference we found was that metamorphs from the cattail treatment were 5 % shorter than metamorphs in the reed canary grass treatment ($P = 0.027$). Planned comparisons revealed no difference in SVL between the two litter types ($P = 0.884$) and no difference between no-litter and the 10 litter treatments ($P = 0.471$).

Head width was affected by litter species ($F_{9,40} = 8.198$, $P < 0.001$; Fig. 2c). When conducting mean comparisons, we found several differences among treatments; the largest difference was that metamorphs in the cattail treatment had 4–6 % narrower heads relative to individuals in all other treatments except reed canary grass, common reed, and buckthorn ($P \leq 0.041$). Planned contrasts revealed that

Fig. 2 Mass-adjusted gut mass (a) snout–vent length (b) and head width (c) of metamorphs in 11 different leaf litter treatments ($n = 5$), including closed- and open-canopy litter species, and a no-litter control (white circle). Within canopy categories, litter species are arranged according to associated larval period. All data were mass-adjusted (see Supplementary Appendix B). Litter treatment abbreviations are found in Table 1. Letters above points indicate significant differences among individual litter species. Data are means of mesocosm averages ± 1 SE



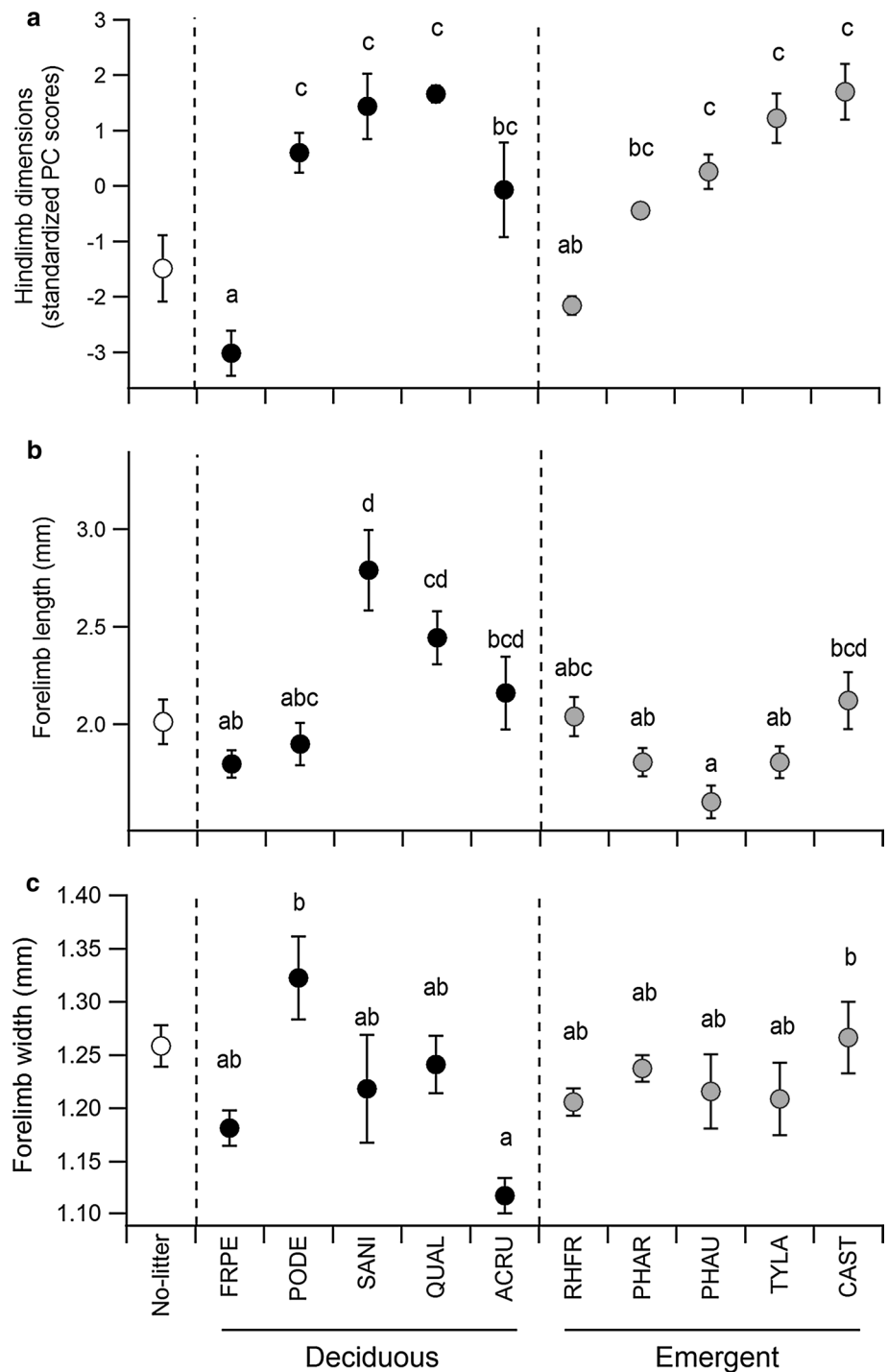
metamorph head width in shrub and herbaceous litter treatments averaged 2 % narrower than in tree litter treatments ($t_{48} = 4.873$, $P < 0.001$), but no difference between no-litter and litter treatments ($P = 0.663$).

Hindlimb dimensions (i.e., a composite response resulting from a PCA) were affected by litter species ($F_{9,40} = 13.201$, $P < 0.001$; Fig. 3a). After conducting mean comparisons, we found a gradient of differences in

hindlimb dimensions among treatments; the largest difference consisted of shorter and narrower hindlimbs in the green ash treatment relative to all treatments except glossy buckthorn ($P \leq 0.007$). Planned contrasts did not reveal any difference between the two litter types ($P = 0.987$) or between no-litter and litter treatments ($P = 0.087$).

Both forelimb length and width were affected by litter species treatment (forelimb length: $F_{9,40} = 7.773$,

Fig. 3 Mass-adjusted hindlimb dimensions (a), forelimb length (b) and width (c) of metamorphs in 11 different leaf litter treatments ($n = 5$), including closed- and open-canopy litter species, and a no-litter control (white circle). Within canopy categories, litter species are arranged according to associated larval period. All data were mass-adjusted (see Supplementary Appendix B). Litter treatment abbreviations are found in Table 1. Letters above points indicate significant differences among individual litter species. Data are means of mesocosm averages ± 1 SE



$P < 0.001$; forelimb width: $F_{9,40} = 3.056$, $P = 0.007$; Fig. 3b, c). After conducting mean comparisons, we found a gradient of differences in forelimb length among litter treatments; the largest difference consisted of 25–43 % shorter forelimbs in the common reed treatment relative to tussock sedge, red maple, white oak, and black willow treatments ($P \leq 0.048$). Likewise, we found a gradient of

differences in forelimb width; the largest difference consisted of 1–11 % narrower forelimbs in red maple relative to tussock sedge and cottonwood treatments, respectively ($P \leq 0.033$). Planned comparisons revealed 2 % shorter forelimbs among shrub and herbaceous litter treatments relative to tree litter treatments ($t_{48} = 3.075$, $P = 0.003$), but no difference in forelimb width ($P = 0.576$). There

were no differences between litter and no-litter treatments ($P \leq 0.351$).

Effect of litter chemistry and aquatic environmental conditions on morphology

We tested how litter chemistry and aquatic environment attributes were associated with morphological dimensions using RDA. Collectively, litter chemistry and aquatic environmental attributes explained a significant amount of variation in morphological dimensions (permutation test: $F = 4.567$, $P = 0.005$; Fig. 4). The first two ecological gradients explained 83 % of the variation in the dependent dataset, so we only explored the relationship of these two gradients. For the first ecological gradient (RDA1), periphyton, percent litter decomposition, DOC concentration, and dissolved oxygen loaded positively, whereas litter C:N loaded negatively. For the second ecological gradient (RDA2), DOC concentration and percent phenolics both loaded negatively whereas periphyton, dissolved oxygen, and pH all loaded negatively.

When we examined how the relative morphological dimensions of wood frogs correlated with these two RDA gradients, we found that metamorph mass was positively associated with RDA1 whereas gut mass and hindlimb dimensions were negatively associated with RDA1. In addition, head width and forelimb length were negatively associated with RDA2.

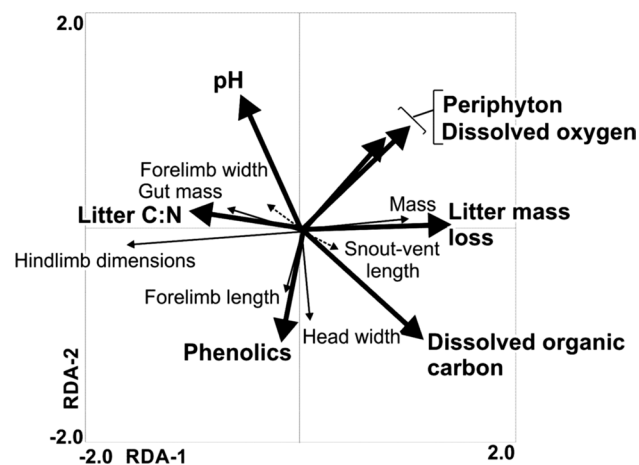


Fig. 4 Biplot of redundancy analysis for mass-adjusted morphological responses. Independent values (*thick arrows*) are litter attributes and aquatic environmental attributes. Dependent values (*thin arrows*) are morphological responses among the 10 litter species treatments (not including the no-litter treatment). The angle of the arrows with respect to the axes indicate the magnitude of correlation between a variable and a canonical variate. *Solid arrows* indicate variables that exhibit significant correlations with one or more canonical variates (see Supplementary Appendix B for details). Note that the length of the *arrows* only approximates the strength of this correlation

Table 2 Adjusted r^2 , correlation coefficients, degrees of freedom (regression and residual) and regression statistics of linear regression models of morphological responses as predicted by duration of larval period (i.e., time to metamorphosis)

Response	Adjusted r^2	β	t	df	P^a
Metamorph mass	0.404	-0.644	-6.072	1,52	0.002
SVL	-0.019	-0.026	0.187	1,52	0.852
Head width	0.083	0.316	2.403	1,52	0.028
Hindlimb dimensions	0.236	0.501	4.169	1,52	0.002
Forelimb length	0.142	0.398	3.124	1,52	0.005
Forelimb width	-0.018	-0.032	-0.228	1,52	0.852
Gut mass	0.248	0.513	4.265	1,51	0.002

Bold type indicates significant responses ($P \leq 0.05$)

^a Adjusted using the method of Benjamini and Hochberg (1995)

Effect of larval period on morphology

To test our predictions regarding the effect of larval period on morphological traits (i.e., positive effects on individual mass, gut mass and limb dimensions, and negative effects on body width and length), we conducted linear regression analyses on all responses for which there was a significant effect of litter treatments (Table 2). Head width, hindlimb dimensions, forelimb length and gut mass were all positively affected by larval period. Metamorph mass was negatively affected by larval period.

Discussion

Our study provides novel insights into the variation of metamorph morphology that may result from variation in resource quality experienced early in ontogeny. Importantly, we examined detailed patterns in metamorph morphology that may have fitness consequences (Nicieza et al. 2006, discussed below). In general, we found numerous treatment effects on metamorph morphology that were not readily explained by litter chemistry, but were explained by the effects of litter treatments on water quality during the larval period and by treatment effects on larval period. Our study adds to a growing body of evidence that indicates amphibians are sensitive to the quality of allochthonous resources in their larval environment (e.g., Maerz et al. 2010).

As we predicted, variation in litter quality and aquatic environmental factors generated gradients of morphological responses. The first ecological gradient in the RDA (RDA1) was primarily associated with factors related to the quantity of nutrients available to consumers, including litter C:N, litter mass loss, and periphyton biomass. As litter C:N decreases and litter palatability increases,

periphyton growth on the litter surface increases and there is an increase in litter mass loss. Increased rates of litter mass loss are also associated with greater leaching rates and elevated levels of DOC (Wetzel 2001). Additionally, increased periphyton growth can lead to elevated dissolved oxygen, as a result of increased photosynthesis. In response to these changes, we found that decreases in litter C:N were associated with smaller hindlimb dimensions and gut mass, and increased metamorph mass. These trends are consistent with expectations from previous experiments. For example, Relyea and Hoverman (2003) found that hindlimb length decreased as tadpole resources increased. Likewise, Stoler and Relyea (2013) demonstrated that tadpole gut length decreases with increased litter nitrogen content, which may result in shorter and possibly smaller metamorph guts.

The second ecological gradient in the RDA (RDA2) was primarily associated with secondary compounds in the litter that can inhibit tadpole growth, particularly litter phenolics. As the concentration of phenolics in the water column increased, there was an associated decrease in pH. There was also an increase in DOC, likely because phenolic acids often comprise a major part of total soluble carbon in litter (Wetzel 2001). In response to an increase in phenolics, we found an associated increase in head width and forelimb length. Physiological mechanisms associated with this phenomenon are unclear. Increasing phenolic concentrations may serve to reduce per-capita resource availability, which is associated with increased limb lengths (Relyea and Hoverman 2003). The effect of phenolic acids may also involve negative physiological effects on development, as suggested by Maerz et al. (2005). Given the abundance and diversity of phenolic acids in plant species across the globe, elucidating these physiological mechanisms should be a goal of future research.

The effects of litter type and litter absence

There was substantial variation in chemistry among all litter species which prevented generalizations of chemical attributes among litter types. Not surprisingly, there were few differences in metamorph morphology between tree species and herbaceous and shrub litter species treatments. Indeed, the only significant differences included 2 % narrower head and 2 % shorter forelimbs in herbaceous and shrub litter treatments relative to tree litter treatments. It is worth noting that nutrient content was generally lower on among tree litter species (Table 1), yet ash tree litter had more nitrogen than any of the shrub or herbaceous species. Such lack of generalization corroborates prior results indicating that litter species often have species-specific results that cannot be categorized by litter growth form (e.g., coniferous vs. deciduous; Stoler and Relyea 2011; Stephens et al. 2013).

The lack of any nutrient subsidies, as represented by our no-litter treatment, did not generate many extreme effects on morphology. This refuted our prediction that morphological responses in the absence of litter would represent the extremes of morphological gradients. However, it is worth noting that no-litter replicates were not devoid of larval resources; a thin, but visible layer of periphyton had grown in these mesocosms. In addition, survival was relatively low in this treatment (see Stephens et al. 2013), so it is likely that deceased tadpoles contributed to the resource pool for surviving tadpoles. This finding corroborates the growing realization that both quantity and quality are critical factors to examine when investigating ecosystem function (Marcarelli et al. 2011).

Effect of larval period

In support of our prediction, we found that larval period (measured as time to metamorphosis) positively correlated with hindlimb dimensions and forelimb length. Others have found similar trends with wood frogs and other anuran species (Relyea 2001; Gomez-Mestre et al. 2010). In addition, Martof and Humphries (1959) found that wood frogs at northern latitudes, which experience short growing seasons, have small leg length to body length ratios relative to individuals at southern latitudes. The hormonal mechanism behind this phenomenon is suggested to be lengthened exposure to thyroid hormone during limb development (Emerson 1986). In turn, the anuran larval period appears to be strongly tied with the nutritional quality of litter resources (Stephens et al. 2013). However, it is worth noting that the effects of larval period on morphological traits may be species-specific: Relyea and Hoverman (2003) found that competition increased larval period of gray treefrogs (*Hyla versicolor*) but decreased relative limb lengths. Further work should explore the phylogenetic patterns of resource-induced metamorph morphology and link those patterns to physiological mechanisms (e.g., hormones).

Adaptive nature of responses

It is unknown whether variation in limb size is adaptive, or merely a by-product of prolonged development. In larval environments that are predictive of adult environments, there could be an adaptive link between larval and adult traits (Relyea 2001), such as a positive correlation between limb length and hopping performance. However, neither Niecieza et al. (2006) nor Van Buskirk (2011) found differences in hopping distance between metamorphs emerging from environments with qualitatively different resources, despite significant differences in limb morphology. Similarly, Relyea and Hoverman (2003) found that

morphological effects resulting from low and high larval competition treatments were unlikely to alter juvenile growth or survival. Nevertheless, there is a correlation between limb length and hopping performance of adults (Zug 1978). If subtle changes in limb length are magnified through juvenile and adult development, then changes in limb length observed in our study may still be adaptive in the long term (i.e., throughout juvenile and adult development). Alternatively, morphological effects in our study may simply result from the redistribution or alteration of energy and nutrient allocation in the developing tadpole. Indeed, recent studies have demonstrated that aquatic stressors can alter whole-body stoichiometry (Costello and Michel 2013). Future studies should address whether the effects of larval resources on metamorph morphology are a byproduct of developmental trends or if they have long-term adaptive significance.

Likewise, changes in gut mass might also have adaptive significance or might be a byproduct of tadpole development. In our study, we found that metamorph gut mass increased with litter C:N (i.e., increased with decreasing litter nutritional quality). This result may simply be due to changes in the amount of food left in the gut following metamorphosis. However, tadpoles cease feeding once metamorphosis begins, and their guts are generally empty once metamorphosis is complete. An alternative, adaptive explanation is that heavier guts may be a result of longer gut lengths, which can increase the amount of nutrients absorbed from a low resource environment (Sibly 1981). Indeed, previous studies have found that tadpoles developed longer guts in response to fewer per capita resources or lower quality resources (Relyea and Auld 2004; Stoler and Relyea 2013). Although gut size during a non-feeding period of anuran development is not directly indicative of adaptation, metamorph gut size is likely to be predictive of future gut length. Such an adaptive significance would necessitate a link between aquatic and terrestrial resource abundance or quality. To our knowledge, such a link has not been investigated but could offer tremendous insight for aquatic-terrestrial linkages.

Implications for changing landscapes

Our results suggest that ongoing changes in the vegetative species composition in freshwater ecosystems and surrounding forests have the potential to promote substantial changes in metamorph morphology. In our study, we used litter species that are commonly found in or around temperate forest wetlands, and are also the subject of conservation concerns. For example, red maple is rapidly expanding its range through temperate North American forests following the decline of other species (e.g., oaks) due to disturbances such as fire suppression and

over-browsing by deer (Abrams 1998). Several species of ash are disappearing throughout North America owing to the invasive emerald ash borer. Similarly, invasive strains of phragmites are dominating wetlands and replacing species such as native cattail (Keller 2000). Moreover, an increasing number of wetlands are being converted to open-canopy systems, which will change the dominant plant litter type from woody to herb or grass species. Our results suggest that such large-scale shifts in plant species assemblage will be accompanied by changes in consumer growth, development, and morphology. Our results suggest that these changes can be predicted from litter chemistry, but may not be predictable from broad shifts in vegetation type. Further work must be conducted to determine if these shifts are adaptive, and will permit consumers to acclimate to novel habitat.

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Author contribution statement JPS, KAB, and SDT conceived and designed the experiment. JPS conducted the experiment. ABS and JPS equally contributed to the analysis of data and the writing of the manuscript. RAR provided the assistants and facilities to collect the morphological data, contributed to the analysis of data, and the writing of the manuscript.

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