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ABSTRACT

IMPORTANCE OF HABITAT STRUCTURE FOR POND-BREEDING AMPHIBIANS IN MULTIPLE LIFE STAGES

Jennifer L. Purrenhage

Habitat loss and alteration is a major cause of population declines; thus, habitat conservation is essential for preventing species extinctions. Habitat conservation, however, can be hindered by an inadequate understanding of species' habitat requirements. Pond-breeding amphibians have a biphasic life history, which requires both aquatic and terrestrial habitats; thus, pond-breeding amphibians are particularly vulnerable to habitat alteration because they experience its effects in multiple habitats during successive life stages. The goal of this dissertation was to examine the importance of habitat structure (i.e., vegetation structure, canopy cover) for larval and juvenile pond-breeding amphibians. In the first and second chapters, I assessed how aquatic vegetation structure mediates biotic interactions (competition and predation) for larval amphibians. In the second, third, and fourth chapters, I established that aquatic and terrestrial canopy cover can strongly influence growth and survival of amphibian larvae, as well as locomotor performance, growth, and survival of terrestrial juveniles. Taken together, the findings of my research suggest that, although habitat structure influences growth and survival of larvae, some pond-breeding amphibians appear to be more sensitive to the suitability of their terrestrial habitat; thus, successful conservation of pond-breeding amphibians must involve the preservation of high-quality (i.e., closed canopy) terrestrial habitat and the maintenance of corridors that facilitate juvenile dispersal from natal wetlands to suitable terrestrial habitat.

IMPORTANCE OF HABITAT STRUCTURE FOR POND-BREEDING AMPHIBIANS IN MULTIPLE LIFE STAGES

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Figure 1. This dissertation aimed to address how individual performance, species interactions, and population parameters vary across multiple environmental gradients (aquatic vegetation structure, canopy cover, predators), and specifically explored these questions: Can aquatic vegetation structure influence competitive and predator-prey interactions and play a role in community regulation? (Chapters 1 & 2); Can canopy cover influence fitness-correlated traits of individuals and mediate biotic interactions? (Chapter 2); & Can canopy cover in one life stage have consequences for individual performance in subsequent life stages, and are there tradeoffs in performance in different habitat types? (Chapters 3 & 4).

GENERAL INTRODUCTION

A common goal of population, community, and conservation biologists is to identify and understand the factors that regulate individual performance, population dynamics, species interactions, and community composition. Competition and predation are important regulating factors for many species, but habitat quality can also be an important determinant of individual performance, population persistence, and community composition (Morin 1999). In this dissertation, I will explore how habitat quality, competition, and predation can independently and interactively influence pond-breeding amphibians at the individual, population, and community levels.

Pond-Breeding Amphibians

Aquatic and terrestrial habitats are critical for the persistence of amphibian populations, yet both habitats are being lost or altered at an alarming rate (Dodd and Smith 2003). Patterns of habitat alteration on the landscape level (e.g., changes in forest cover) are reflected by altered habitat structure on the local level (e.g., vegetation structure, canopy cover), and can have serious impacts on local populations and communities. Organisms with complex life cycles, such as pond-breeding amphibians, are particularly vulnerable to habitat alteration because they experience its effects in multiple habitats during successive life stages (Semlitsch 2003; Cushman 2006). In fact, the current worldwide decline of amphibian populations has been attributed, in great part, to habitat loss and alteration (Alford and Richards 1999; Dodd and Smith 2003; Stuart et al. 2004; Halliday 2008).

Because pond-breeding amphibians require both aquatic and terrestrial habitats to complete their biphasic life cycle, it is critical to consider species' habitat requirements during multiple life stages when setting conservation priorities and planning habitat management, restoration, and mitigation (Semlitsch 2002; Houlahan and Findlay 2003). Historically, much attention has been given to the study of pond hydroperiod, larval competition, and larval predation as factors that regulate amphibian populations and structure amphibian communities (reviewed in Wilbur 1987, Wellborn et al. 1996). Although the importance of these factors is well-documented, other habitat features (e.g., vegetation structure and canopy cover) have more recently emerged as potential environmental gradients (Werner et al. 2007) for amphibians. Furthermore, a shift in the research focus of amphibian biologists away from habitat-related questions and towards the study of 'novel stressors' (e.g., chytridiomycosis caused by

Batrachochytrium dendrobatidis), despite the consensus agreement that habitat alteration is a leading stressor for amphibian populations, has prompted a call for more habitat-focused research (Halliday 2008); a similarly strong need to examine amphibian life stages beyond metamorphosis has also been identified (Biek et al. 2002; Boone 2005; Rothermel and Semlitsch 2006). The overarching goal of my dissertation research was to explore the importance of vegetation structure and canopy cover for larval and juvenile pond-breeding amphibians.

Vegetation Structure and Canopy Cover

Variation in emergent vegetation structure and canopy cover has been shown to influence animals. Despite the demonstrated effects of vegetation structure on growth, survival, and reproduction in a variety of taxa (Werner et al. 1983; Bell et al. 1991; Diehl and Eklov 1995; Persson and Eklov 1995; Grenouillet et al. 2002; Langellotto and Denno 2004), the effects of vegetation structure on larval amphibians have been largely overlooked (Alford 1999). However, the available data suggest that structural variation within and among ponds, often by providing refugia and reducing predation, can have important consequences for growth, metamorphosis, and survival of larval amphibians (Alford 1986; Sredl and Collins 1992; Warkentin 1992; Babbitt and Jordan 1996; Babbitt and Tanner 1998; Tarr and Babbitt 2002; Baber and Babbitt 2004; Smith and Doupnik 2005). As has been documented in fish and invertebrate communities (e.g., Grenouillet et al. 2002; Langellotto and Denno 2004), variation in aquatic and terrestrial habitat structure may also modify competitive and predator-prey interactions in amphibians, thus influencing growth and survival during multiple life stages. The majority of vegetation-structure research in freshwater systems, however, has focused on the importance of vegetation structure in mediating fish predation (e.g., Werner et al. 1983; Diehl and Eklov 1995; Persson and Eklov 1995; Grenouillet et al. 2002; Hartel et al. 2007). Although fish are important predators in many freshwater environments, they are generally excluded from the temporary wetlands where many amphibians complete the larval life stage. In fishless ponds, however, the mediation of competitive interactions by vegetation structure may have important consequences for growth, survival, and the structure of communities.

Aquatic and terrestrial canopy cover can influence growth, survival, and the structure of amphibian communities (e.g., Rothermel and Semlitsch 2006; Werner et al. 2007). Pond canopy cover has recently emerged as an environmental gradient along which individual performance and community composition appear to vary for pond-breeding amphibians (Skelly et al. 1999;

Skelly et al. 2002; Schiesari 2006; Werner et al. 2007). For most species studied in the existing literature, increased pond canopy cover has been shown to negatively affect larval amphibians. Specifically, increased canopy cover can be associated with longer developmental time, smaller size at metamorphosis, and lower probability of surviving to metamorphosis (Werner and Glennemeier 1999; Skelly et al. 2002; Schiesari 2006), as well as decreased species richness with increasing canopy cover over time (Skelly et al. 1999). Conversely, although far less is known about the terrestrial canopy gradient, most amphibians studied to date tend to perform better in forested rather than unforested terrestrial habitats (deMaynadier and Hunter 1999; Rothermel and Semlitsch 2002, 2006). Most amphibians inhabit heterogeneous and changing landscapes. Individuals may reside in open-canopy habitat during one life stage and in closed-canopy habitat during another, and each of these habitats presents unique challenges for growth, dispersal, and survival. Thus, more detailed information is needed about the importance of aquatic and terrestrial habitat variation along the canopy gradient for amphibians with biphasic life cycles.

Multiple Life Stages

The typical pond-breeding amphibian has a complex life cycle that involves an aquatic larval stage, metamorphosis from aquatic larvae to terrestrial juveniles, and a terrestrial adult stage, during which breeding adults migrate to ponds seasonally (Wells 2007). Ecological sensitivity analyses for pond-breeding amphibians have shown that vital rates of post-metamorphic life stages (e.g., terrestrial juveniles) can more strongly influence population growth than those of pre-metamorphic stages (e.g., aquatic larvae; Biek et al. 2002). Overwhelmingly, the best-studied life stage of pond-breeding amphibians is the aquatic larval stage (McDiarmid and Altig 1999; Wells 2007); however, it is equally if not more important to establish cause-and-effect relationships for terrestrial life stages (Pechmann 1995; Parris 2001; Biek et al. 2002; Boone 2005). Studies that follow individuals through multiple life stages are rare, but are particularly important for a comprehensive understanding of the factors affecting individual performance and population dynamics. Moreover, because pond-breeding amphibians require two different habitats during their biphasic life cycle, and thus, those habitats cannot be managed separately (Semlitsch 2003), it is important to study the effects of various aquatic-terrestrial habitat associations on individuals in multiple life stages.

Dissertation Overview

In this dissertation, I address critical questions about the impacts of aquatic and terrestrial habitat (emergent vegetation and canopy cover) on pivotal points in the development of amphibians with a complex life cycle: (1) Can aquatic vegetation structure influence competitive and predator-prey interactions and play a role in community regulation? ; (2) Can canopy cover influence fitness-correlated traits of individuals and mediate biotic interactions? ; and (3) Can canopy cover in one life stage have consequences for individual performance in subsequent life stages, and are there tradeoffs in performance in different habitat types? (Fig. 1). These questions have implications for understanding factors that influence community composition and that are critical to maintenance of species with complex life histories.

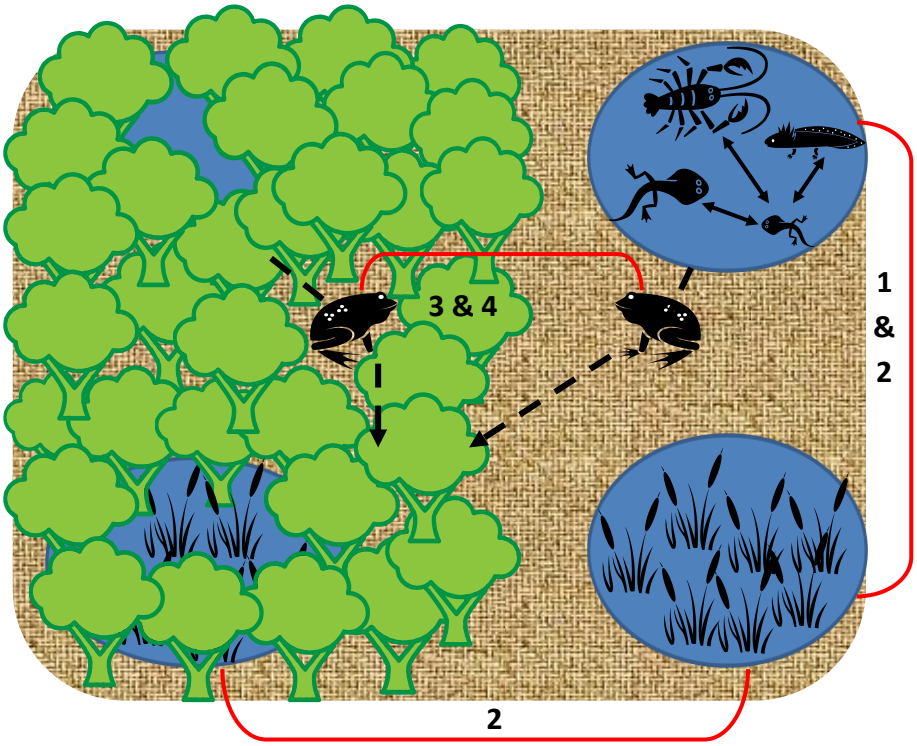

Fig. 1 – This dissertation explores three overarching questions: Can aquatic vegetation structure influence competitive and predator-prey interactions and play a role in community regulation? (Chapters 1 & 2); Can canopy cover influence fitness-correlated traits of individuals and mediate biotic interactions? (Chapter 2); & Can canopy cover in one life stage have consequences for individual performance in subsequent life stages, and are there tradeoffs in performance in different habitat types? (Chapters 3 & 4).

In Chapter 1, I describe the response of larval amphibian communities in experimental ponds to variation in emergent vegetation structure and competitor density. In Chapter 2, I describe a series of pond mesocosm experiments used to examine how vegetation influences predator-prey relationships and interactions with heterospecifics. In Chapter 3, I describe a study in which we reared American toad (*Bufo americanus*) larvae in open- and closed-canopy pond mesocosms and subsequently conducted locomotor performance trials to test the effects of pond canopy cover on post-metamorphic speed and endurance abilities, which can have important implications for dispersal potential of juveniles. In Chapter 4, I describe complementary pond mesocosm and terrestrial enclosure experiments designed to test the effects of aquatic and terrestrial canopy cover on growth and survival of American toad larvae and juveniles. Together these studies suggest that vegetation within and surrounding a wetland can influence the quality and quantity of individuals that reach metamorphosis, and the performance of those individuals in the terrestrial environment. My research represents the first to examine cause-and-effect relationships between key habitat features in the aquatic and terrestrial life stages and offers important insights for habitat restoration and conservation.

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CHAPTER 1:

Amphibian Community Response to Variation in Habitat Structure and Competitor Density

Abstract – Habitat destruction and alteration is a major cause of population declines; thus, habitat restoration is essential for preventing species extinctions. Habitat restoration, however, may be hindered by an inadequate understanding of species' habitat requirements. Habitat structure may play a major role in influencing the biotic interactions among species. Pond-breeding amphibians are one group that has experienced loss and alteration of critical habitats. Vegetation structure in wetland environments may be important to amphibian larvae if its presence mediates biotic interactions, such as competition, which can affect growth and survival to metamorphosis. Despite the potential importance of habitat structure and competition, few studies have examined these factors simultaneously. In a full-factorial pond mesocosm experiment, we tested for the effects of variation in habitat structure and density of a larger tadpole competitor (*Rana pipiens*, northern leopard frog) on development and survival of three species of larval amphibians: *Bufo americanus* (American toad), *Hyla versicolor* (gray treefrog), and *Ambystoma maculatum* (spotted salamander). Survival of *Bufo* was greater in mesocosms with live or artificial cattails than in those lacking vegetation. Although response varied among species, our data suggest that time to metamorphosis, survival, and species evenness can be influenced by both aquatic habitat structure and competition, whereas size at metamorphosis is affected primarily by competition. Our study is the first to demonstrate that vegetation structure alone can affect the expression of metamorphic traits for some anuran species.

Introduction

Amphibians are currently experiencing worldwide population declines due, in part, to habitat loss and alteration (Alford and Richards, 1999; Collins and Storfer, 2003; Dodd and Smith, 2003; Kiesecker et al., 2001; Semlitsch, 2003; Stuart et al., 2004). Wetland habitats have been especially vulnerable to loss and alteration through human-mediated changes such as physical disturbance, alteration of hydroperiod, and biological invasions (Mitsch and Gosselink,

2007; Whigham, 1999; Zedler and Kercher, 2004). Such habitat modifications can result in altered wetland plant communities, which can in turn affect the chemical, biological, and structural quality of the aquatic environment. For instance, secondary compounds leached from invasive plants (e.g., purple loosestrife, *Lythrum salicaria*) have been shown to slow larval amphibian development (Maerz et al., 2005). Biological attributes of the environment, such as algal community composition, may also vary among different plant communities and can affect size of metamorphs and length of the larval period in some amphibians that depend on periphyton as a primary food resource (Brown et al., 2006; Skelly et al., 2002). Finally, habitat structure varies among and within wetland sites (Colburn, 2004), and differences in habitat structure may alter behavior and the outcome of biotic interactions. Each of these changes can affect amphibian population dynamics and transform local community structure, potentially influencing species diversity at the landscape level (Gray et al., 2004; Knutson et al., 1999; Schiesari, 2006; Semlitsch, 2003; Skelly et al., 1999; Van Buskirk, 2005; Wellborn et al., 1996).

Although loss of suitable wetland habitat presents a general threat to amphibians (Dodd and Smith, 2003), many species are known to readily use newly constructed wetlands (Lehtinen and Galatowitsch, 2001; Pechmann et al., 2001; Petranka et al., 2003), which suggests that habitat restoration or construction of new wetlands could help offset past or present effects of habitat destruction. However, effective restoration, construction, and protection of wetland habitats require an understanding of which habitat features most strongly influence wetland fauna. Pond hydroperiod is known to be a strong predictor of the aquatic community that assembles at a site (Wellborn et al., 1996), and changes in pond canopy cover through time have been correlated with changes in amphibian community structure (Skelly et al., 1999). However, the effects of other physical attributes of ponds, including vegetation structure, are unclear and could play an important role in determining community dynamics. Habitat structure has emerged as a key determinant of habitat suitability for many taxa, including fishes (Diehl and Eklov, 1995; Eklov, 1997; Grenouillet et al., 2002; Persson and Eklov, 1995; Werner et al., 1983), invertebrates (Langellotto and Denno, 2004), reptiles (e.g., Pounds, 1988), mammals (e.g., Wilder and Meikle, 2005), and birds (e.g., Whittingham and Evans, 2004). The effects of habitat structure on amphibians have been largely overlooked (McDiarmid and Altig, 1999), but the limited available data suggest that habitat variation within ponds can have important consequences for growth and survival of larval amphibians (Alford, 1986; Babbitt and Jordan,

1996; Babbitt and Tanner, 1997, 1998; Baber and Babbitt, 2004; Smith and Doupnik, 2005; Sredl and Collins, 1992; Tarr and Babbitt, 2002; Warkentin, 1992).

Variability in the distribution and complexity of habitat structure can result in differing availability of microhabitat and food resources, which may alter competitive and trophic interactions (Bell et al., 1991). For instance, past studies have shown that larger ranids can affect metamorphic traits of smaller heterospecific amphibians (Boone et al., 2004*a*; Kupferberg, 1997; Lawler et al., 1999); larger ranids can also affect larval traits of smaller heterospecifics, consequently altering interactions between the smaller species (Peacor and Werner, 1997). The presence of habitat structure may mediate these competitive and trait-mediated indirect interactions via habitat partitioning and food supplementation. Alteration of biotic interactions may be especially important for species with complex life cycles that are restricted to specific habitats during distinct life stages, such as larvae of pond-breeding amphibians that must complete metamorphosis prior to pond drying (Wilbur, 1980). Information about the consequences of variation in aquatic habitat structure on amphibians is valuable for managers interested in taking data-driven approaches to habitat conservation and restoration. In the absence of comprehensive and accurate data, we risk protecting and restoring habitat that is insufficient to support many pond-dependent species.

We conducted an outdoor, pond mesocosm experiment to examine the effects of habitat structure and density of a larger tadpole (*Rana pipiens*) in larval amphibian communities composed of two anurans (*Bufo americanus*, *Hyla versicolor*) and one salamander (*Ambystoma maculatum*). We made the following predictions for our experimental communities: (1) the presence of aquatic habitat structure positively affects amphibian metamorphosis by increasing survival to and size at metamorphosis and decreasing length of the larval period; (2) the presence of physical structure alone, rather than the increased living plant matter, positively affects amphibian metamorphosis; (3) increasing *Rana* density negatively affects amphibian metamorphosis by decreasing survival to and size at metamorphosis and increasing length of the larval period; and (4) the presence of habitat structure mediates the effect of increased *Rana* density on growth and survival to metamorphosis.

Materials and Methods

Study System

The amphibian species used in our mesocosm experiment occur as larvae in temporary and permanent ponds, their breeding seasons overlap, and in some areas they breed in the same wetlands (Babbitt et al., 2003; Trenham et al., 2003; J. Purrenhage, personal observation). Therefore, these communities have the spatial and temporal potential to occur in natural ponds, and have been used here to represent an assemblage of species employing a range of larval period lengths and trophic positions. Larval densities vary widely in nature, and the densities used in our experiment fall well within the observed range (14-4238 per 1000 L; e.g., Morin, 1983; Petranka, 1989). Initial stocking densities for the three focal species (45 *Bufo*, 45 *Hyla*, and 10 *Ambystoma* per pond mesocosm) reflect the relative availability of each species' eggs at the collection sites, and thus, approximate their natural larval densities.

Field Collection and Rearing

We collected eggs from *Bufo americanus* (American toad) and *Hyla versicolor* (gray treefrog) from a fishless, temporary pond at Rush Run Wildlife Area (Preble County, Ohio) on 17 May 2005, and *Ambystoma maculatum* (spotted salamander) eggs from a fishless, temporary pond at Indian Creek Preserve MetroPark (Butler County, Ohio) on 25 April 2005. Anuran eggs were maintained in the lab at 22-24 C until hatching. Upon hatching, tadpoles were reared in plastic bins filled with dechlorinated (aged 48 h) tap water. Water was changed daily and tadpoles were given food (TetraMin® Tropical Flakes) *ad libitum*. *Bufo* and *Hyla* tadpoles were added to the experiment at Gosner stage 25 (Gosner, 1960). Salamander eggs were maintained in a cattle tank (containing water, leaf litter, and plankton) outdoors at the Miami University Ecology Research Center (ERC), Oxford, Ohio, until hatching. Larvae were raised from multiple egg masses (7 *Bufo* egg strings, 15 *Ambystoma* egg masses, and *Hyla* eggs were collected from multiple sites within a pond where many males were calling) and mixed prior to addition to pond mesocosms to incorporate genetic diversity from the respective species' populations. Using dipnets, we collected *Rana pipiens* (northern leopard frog) larvae for *Rana* density treatments from Rush Run Wildlife Area on 1 June 2005. *Rana* larvae had recently developed hind limbs when they were added to the experimental ponds (Gosner stage 33-36, Gosner, 1960). Northern leopard frogs are explosive breeders that often precede breeding of American toads or gray treefrogs. Northern leopard frog larvae grow fast and reach a large size by the time the other

anurans hatch, thereby representing a natural competitor of toads and treefrogs; we therefore refer at times to northern leopard frog (*Rana*) larvae as competitors. Although *Rana* larvae do not compete directly with the zooplanktivorous salamander larvae in our experiment, they could indirectly affect salamanders via the food web (i.e., changes in algal abundance can affect abundance of zooplankton food resources).

Experimental Design

A mesocosm experiment was conducted outdoors at the Miami University ERC from May through July 2005. To test the effects of habitat structure and density of a larger heterospecific anuran (*Rana pipiens*), we used a full-factorial design and manipulated three levels of habitat structure (none, live cattail structure, and artificial cattail structure) and four levels of *Rana* density (0, 6, 12, or 24 *Rana* larvae) in cattle-tank pond mesocosms (hereafter referred to as mesocosms). We included four replicates of each treatment, and treatments were randomly assigned to a grid of 48 mesocosms (1.85-m diameter, 1480-L capacity), each containing 1000 L water, 1 kg leaf litter from a mixed deciduous forest (primarily *Acer*), and inoculations of algae and zooplankton from natural ponds. Mesocosms were covered with fiberglass mesh lids to inhibit colonization of predators and escape of metamorphs.

Fourteen days prior to the addition of amphibian larvae, one of three habitat-structure treatments was randomly assigned to each mesocosm: controls (no structure) contained five 20-cm diameter plastic pots with soil and pea gravel; live-cattail treatments contained five pots with soil, pea gravel, and three individual *Typha laxmannii* (graceful cattail) plants per pot (total of 15 plants per mesocosm); and artificial-cattail treatments contained five pots with soil, pea gravel, and three individual artificial plants per pot (total of 15 plants per mesocosm). We used cattails (*Typha*) in our habitat structure treatments because *Typha* species are common in the majority of wetlands in our area, and because they provide a relatively simple, uniform structure similar to that of other common wetland species (e.g., *Phragmites*, *Iris*). To mimic common cattails, each artificial cattail was constructed of one 61-cm long, 2-cm diameter PVC stem and two green, artificial leaf blades attached with two transparent plastic cable ties. Graceful cattails were expected to have a similar structure to common cattails (and our artificial cattails), but the stems were more slender and the plants were more structurally complex than common cattails. For all treatments, pots were arranged in a tight cluster in one quadrant of the mesocosm; the location of

the structure quadrant was randomized such that the four replicates of each treatment represented structure treatments in each of the four cardinal directions (i.e., N, S, E, W structure quadrants).

As we mentioned previously, we manipulated *Rana pipiens* density as an experimental treatment in our pond mesocosms because of the expectation that this species may exert strong competitive effects on anuran heterospecifics due to its large size; in nature, there may be priority effects because this species is generally the earliest breeder in the community (Alford and Wilbur, 1985). The treatment levels of *Rana* density (0, 6, 12, or 24 *Rana* larvae per 1000 L) were based on expected field densities with high early larval mortality and are comparable to the densities used in previous studies of large ranid competitors (Boone et al., 2004*a*; Kupferberg, 1997; Lawler et al., 1999). *Rana* larvae were introduced to mesocosms in the specified treatment densities on 2 June. On 3 June, each mesocosm was stocked with 100 focal larvae: 45 *Hyla*, 45 *Bufo*, and 10 *Ambystoma* (3 June represented day zero for all measurements of time to metamorphosis). Mesocosms were checked daily for metamorphs, and anurans with at least one front limb emerged (Gosner stage 42, Gosner, 1960) or salamanders with resorbed gills were collected and transported to the laboratory at Miami University for processing. Anurans were held in the lab until tail resorption (≤ 3 days), at which point we recorded time to metamorphosis and size at metamorphosis. All metamorphs were released at the egg-collection sites within 48 h of metamorphosis.

We measured response for all species as survival to metamorphosis, mass at metamorphosis (g), time to metamorphosis. Time to metamorphosis represents the number of days from the start of the experiment until full resorption of the tail for anurans (Gosner stage 46, Gosner, 1960) or the gills for salamanders. We calculated proportion survived for each species in each treatment as the number of individuals that successfully metamorphosed by the end of the experiment (29 July) divided by stocking density for that species. We calculated mesocosm means for mass and time to metamorphosis, as mesocosms were the experimental units in all analyses.

We scraped uniform samples of periphyton (3 cm below the water surface x 1.7 cm width of scraped area) from both the mesocosm edge and the vegetation structure, and collected zooplankton samples, from each mesocosm three times during the experiment to determine if habitat structure and *Rana* density treatments affected availability of food resources for anuran and salamander larvae, respectively. Measurements of chlorophyll a concentration (µg/L),

determined via fluorometry of periphyton samples, were used as a proxy for relative abundance of algal resources. Periphyton samples were collected from the mesocosms on 9 June, 22 June, and 13 July. Zooplankton samples were collected from the mesocosms on 8 June, 29 June, and 28 July. We also measured pH, water temperature (C), and dissolved oxygen (DO) of each mesocosm weekly to determine any treatment effects on these water characteristics. Finally, we monitored whether the behavior of amphibian larvae differed among treatments by recording the number and location of amphibian larvae visible in each mesocosm on four separate occasions (6, 10, 11, and 14 June) before larvae began to metamorphose. *Bufo* and *Hyla* larvae were grouped in these counts because the two species could not be reliably distinguished during all observations. Number of larvae was then divided by the initial stocking density to calculate the proportion of larvae visible and active above the leaf litter in each mesocosm (only larvae that were either hidden in the leaf litter or resting on the mesocosm bottom were not counted as active); the proportion active was used as the response variable in statistical analyses.

Data Analyses

Using multivariate analyses of covariance (MANCOVA) and univariate analyses of covariance (ANCOVA), we tested for main and interaction effects of habitat structure and *Rana* density on mass and time to metamorphosis of the focal amphibians (*Bufo*, *Hyla*, *Ambystoma*) and the *Rana* larvae used in competitor-density treatments. We used survival to metamorphosis as the covariate in these analyses so that treatment differences were not confounded by differential survival among treatments and because it explained a significant amount of the variation in the data. We used ANOVA to test for treatment effects on survival to metamorphosis of each species. Differences in periphyton abundance, zooplankton abundance, and water characteristics (pH, temperature, DO) among treatments were analyzed separately using repeated measures ANOVA. We tested for treatment effects on the proportion of larvae that were active and visible during each of the four observations using repeated measures ANOVA. To identify potential relationships in survival of focal amphibian species, we ran correlation analysis on percent survival for all pairs of species; we were particularly interested in the potential role of *Ambystoma* larvae as predators of anuran larvae. The correlations were all positive and were not significant ($0.0166 < r < 0.2596$, $P > 0.07$), indicating that *Ambystoma* was unlikely acting as a predator of anurans in this experiment. We evaluated the community-level response (species evenness) to experimental treatments by calculating Simpson's Diversity Index

for each mesocosm based on final survival of the focal amphibian species. Simpson's Diversity Index (D) equals $1/\Sigma$ (proportion of species i)^2. We tested for treatment effects on values of D using ANOVA. For all analyses, data were transformed as necessary to meet the assumption of normality. Data expressed as proportions (i.e., survival, activity) were angularly transformed; mass and time data were log-transformed. Statistical analyses were performed using SAS version 9.1.

Results

Community Response

Species evenness in our experimental pond communities was significantly lower in mesocosms with a high density of *Rana pipiens* tadpoles (Fig. 1A; $F_{3,36} = 5.68$, $P < 0.01$) and in mesocosms lacking habitat structure (Fig. 1B; $F_{2,36} = 6.44$, $P < 0.01$). In mesocosms with decreased evenness, the skew was due to an increase in the ratio of *Hyla versicolor* relative to *Bufo americanus*. There was approximately a 10 percent change in the relative abundances of *Hyla* and *Bufo* between *Rana* controls (*Hyla*, 0.574 ± 0.026; *Bufo*, 0.348 ± 0.026) and high *Rana*-density treatments (*Hyla*, 0.679 ± 0.019; *Bufo*, 0.242 ± 0.021). There was a similar shift in relative abundances of *Hyla* and *Bufo* between mesocosms with either live vegetation (*Hyla*, 0.580 ± 0.026; *Bufo*, 0.353 ± 0.026) or artificial vegetation (*Hyla*, 0.568 ± 0.022; *Bufo*, 0.352 ± 0.022) and mesocosms lacking habitat structure (*Hyla*, 0.664 ± 0.018; *Bufo*, 0.257 ± 0.019).

Effects of Habitat Structure and Competitor Density on Bufo

Habitat structure significantly influenced the multivariate response (i.e., mass and time to metamorphosis) of *Bufo* (Wilks' lambda = 0.6797, $F_{4,68} = 3.62$, $P < 0.01$), which was mainly attributable to effects on time to metamorphosis (Fig. 2A). *Bufo* in artificial structure treatments had a longer larval period (time to metamorphosis) than individuals in live structure and control (no structure) treatments (Fig. 2A; $F_{2,35} = 7.60$, $P < 0.01$), and individuals in artificial structure treatments tended to be smaller at metamorphosis relative to those in live cattail and control treatments (Fig. 2A; $F_{2,35} = 1.53$, $P = 0.23$). Density of *Rana* competitors significantly influenced the multivariate response of *Bufo* (Wilks' lambda = 0.6347, $F_{6,68} = 2.89$, $P = 0.01$). Univariate analyses revealed that *Bufo* in lower competitor-density treatments were generally larger at metamorphosis than those in higher competitor-density treatments ($F_{3,35} = 4.42$, $P < 0.01$), regardless of time to metamorphosis ($F_{3,35} = 0.81$, $P = 0.50$) (Fig. 3A).

Habitat structure had a significant effect on survival to metamorphosis ($F_{2,36} = 7.63$, $P < 0.01$); *Bufo* survivorship was approximately 20% higher in mesocosms with artificial and live cattail structure than in controls (Fig. 4). Increased competitor density had a negative effect on survival to metamorphosis (Fig. 4; $F_{3,36} = 7.70$, $P < 0.01$). Moreover, there was a significant interaction effect of habitat structure and competitor density on survival to metamorphosis for *Bufo* (Fig. 4; $F_{6,36} = 2.35$, $P = 0.05$). *Bufo* exhibited increased survival when either live or artificial cattail structure was present, particularly at low *Rana* density, except in mesocosms with live cattails and no *Rana* competitors (Fig. 4).

***Effects of Habitat Structure and Competitor Density on* Hyla**

There was no significant multivariate response (Wilks' lambda = 0.9158, $F_{4,68} = 0.76$, $P = 0.55$) and no significant univariate effects of habitat structure treatments on any of the measured response variables for *Hyla* (time, $F_{2,35} = 1.31$, $P = 0.28$; mass, $F_{2,35} = 0.72$, $P = 0.49$; survival, $F_{2,36} = 0.03$, $P = 0.97$) (Fig. 2B). However, density of *Rana* competitors significantly influenced the multivariate response (Wilks' lambda = 0.4345, $F_{6,68} = 5.86$, $P < 0.001$). Univariate analyses revealed that as competitor density increased, time to metamorphosis increased ($F_{3,35} = 11.70$, $P < 0.001$) and mass at metamorphosis decreased ($F_{3,35} = 7.23$, $P < 0.001$) (Fig. 3B). There was not a significant effect of competitor density on *Hyla* survival ($F_{3,36} = 2.58$, $P = 0.07$; LS mean percent survival ± SE: 0 *Rana*, 0.846 ± 0.03; 6 *Rana*, 0.778 ± 0.03; 12 *Rana*, 0.767 ± 0.03; 24 *Rana*, 0.854 ± 0.03). There were no significant treatment interaction effects on any of the response variables measured for *Hyla* (Wilks' lambda = 0.6695, $F_{12,68} = 1.26$, $P = 0.26$; time, $F_{6,35} = 0.34$, $P = 0.91$; mass, $F_{6,35} = 1.73$, $P = 0.14$; survival, $F_{6,36} = 0.67$, $P = 0.67$).

***Effects of Habitat Structure and* Rana *Density on* Ambystoma**

Habitat structure (Wilks' lambda = 0.9914, $F_{4,68} = 0.07$, $P = 0.99$) and *Rana* density (Wilks' lambda = 0.9235, $F_{6,68} = 0.46$, $P = 0.84$) did not influence the multivariate response of *Ambystoma*. Univariate analyses similarly showed no significant effects of habitat structure (mass: $F_{2,35} = 0.12$, $P = 0.89$; time: $F_{2,35} = 0.03$, $P = 0.97$; survival: $F_{2,35} = 1.32$, $P = 0.28$) or *Rana* density (mass: $F_{3,35} = 0.81$, $P = 0.50$; time: $F_{3,35} = 0.09$, $P = 0.96$; survival: $F_{3,35} = 1.06$, $P = 0.38$) on any response variables measured.

Effects of Habitat Structure and Population Density on Rana

Habitat structure did not significantly influence the multivariate response of *Rana* (Wilks' lambda = 0.7520, $F_{4,50} = 1.91$, $P = 0.12$). However, univariate analyses indicated a

significant effect of habitat structure on time to metamorphosis in *Rana* (Fig. 2C). *Rana* in both live and artificial structure treatments had a longer larval period than individuals in the control treatment (Fig. 2C; $F_{2,26} = 4.03$, $P = 0.03$), but there was no difference in mass at metamorphosis (Fig. 2C; $F_{2,26} = 0.17$, $P = 0.84$) or survival ($F_{2,27} = 0.86$, $P = 0.44$) among treatments.

Intraspecific density significantly influenced the multivariate response of *Rana* (Wilks' lambda = 0.2490, $F_{4,50} = 12.55$, $P < 0.001$). Univariate analyses revealed that as intraspecific density increased, *Rana* exhibited a dramatic decrease in mass at metamorphosis ($F_{2,26} = 29.9$, $P < 0.001$), and there was a trend suggesting that time to metamorphosis increased ($F_{2,26} = 2.77$, $P = 0.08$) in the low-density treatment (Fig. 3C). There was not a significant effect of intraspecific density on *Rana* survival ($F_{2,27} = 0.64$, $P = 0.53$). There were no significant effects of treatment interactions on any of the response variables measured for *Rana*.

Behavioral Observations

During behavioral observations, the proportion of combined *Bufo* and *Hyla* larvae that were visible and active (above leaf-litter substrate) varied among sampling dates ($F_{3,108} = 70.0$, $P < 0.001$). Where significantly different, the proportion of active *Bufo* and *Hyla* larvae was greater in low (0 or 6 *Rana*/mesocosm) relative to high (12 or 24 *Rana*/mesocosm) competitor-density treatments. These differences were significant during the first ($F_{3,36} = 3.73$, $P = 0.02$; LS means ± SE: 0 *Rana*, 47.7 ± 2.48; 6 *Rana*, 43.1 ± 2.48; 12 *Rana*, 36.0 ± 2.48; 24 *Rana*, 37.6 ± 2.48 individuals/mesocosm) and last ($F_{3,36} = 3.49$, $P = 0.03$; LS means ± SE: 0 *Rana*, 22.7 ± 1.73; 6 *Rana*, 26.2 ± 1.73; 12 *Rana*, 22.7 ± 1.73; 24 *Rana*, 17.6 ± 1.73 individuals/mesocosm) of the four behavioral observations. The proportion of *Rana* visible and active also varied across sampling dates ($F_{3,81} = 7.64$, $P < 0.001$); however, there were no significant treatment effects on activity of *Rana* larvae.

Periphyton and Zooplankton Resources

Repeated-measures ANOVA revealed that the abundance of periphyton from the mesocosm edge varied greatly through time ($F_{2,72} = 34.77$, $P < 0.001$). Periphyton abundance decreased initially during the peak growth period for anuran larvae (between the first two sampling dates, on days 6 and 19 of the experiment) and then increased dramatically once most of the anuran larvae had metamorphosed (between the second and final sampling dates, days 19 and 40), except in the high *Rana* density (24 *Rana*/mesocosm) treatment where algal abundance remained suppressed (Fig. 5A). There were significant time x *Rana* density (Fig. 5A; $F_{6,72} =$

3.91, $P < 0.01$) and time x habitat structure ($F_{4,72} = 2.58$, $P = 0.04$) interaction effects on periphyton abundance on the mesocosm edge. Neither interaction revealed a clear pattern.

Repeated-measures ANOVA also revealed that the abundance of periphyton growing on the vegetation differed between the live and artificial cattail structure treatments (Fig. 5B; $F_{1,24} = 7.28$, $P = 0.01$). Although we were unable to quantify the total additional periphyton available in vegetation structure treatments, the chlorophyll data indicate that the presence of structure augmented total periphyton; this effect was approximately 0.89 µg/L, 2.34 µg/L, and 12.78 µg/L greater per unit area in artificial than in live cattail treatments at each of the three sampling dates (days 12, 26, and 56), respectively (Fig. 5B). Overall, abundance of periphyton on vegetation structure increased over time (Fig. 5B; $F_{2,48} = 14.68$, $P < 0.001$), and there was a trend of decreased abundance of periphyton on vegetation structure with increased *Rana* density ($F_{3,24} = 2.47$, $P = 0.09$).

Repeated measures ANOVA revealed a significant time effect on total zooplankton abundance ($F_{2,72} = 44.48$, $P < 0.001$), which represented a decrease in abundance throughout the experiment. The decrease in zooplankton abundance was observed across vegetation structure and *Rana* density treatments.

Water Characteristics

The pH of the water in pond mesocosms generally declined over time ($F_{7,252} = 92.33$, $P < 0.001$), but the difference between mesocosms was never more than 0.5 units. Similarly, pH was generally lower in live cattail structure treatments ($F_{2,36} = 6.36$, $P < 0.01$), but the difference between treatments was never more than 0.1 units. Water temperature fluctuated among sampling dates, ranging from 26.8 to 30.8 C ($F_{7,252} = 4187.2$, $P < 0.001$). Repeated-measures ANOVA revealed a significant interaction between *Rana* density and habitat structure on water temperature ($F_{6,36} = 2.38$, $P = 0.05$). The interaction effect seems to reflect the pattern that, in the absence of *Rana* (0 *Rana* treatments), temperature was greater in habitat structure controls (mesocosms lacking vegetation) than in live or artificial structure treatments; whereas, in the presence of *Rana* (6, 12, and 24 *Rana* treatments), temperature did not appear to differ with varying habitat structure. However, temperature differences among treatments were generally small (< 0.5 C). Dissolved oxygen (DO) concentration fluctuated through time ($F_{7,252} = 20.67$, $P < 0.001$). There was also a significant interaction of *Rana* density and time on DO ($F_{21,252} = 3.50$, $P < 0.001$), which seems to reflect generally higher DO in *Rana* controls (0

Rana/mesocosm) relative to moderate and high (12 or 24 *Rana*/mesocosm) *Rana*-density treatments. Actual significant differences in DO concentration between *Rana* density treatments ranged from a 0.30-mg/L to a 2.08-mg/L difference. We observed significant differences in DO between habitat-structure treatments on several mid-season sampling dates: 30 June (day 27; $F_{2,36} = 3.20$, $P = 0.05$); 6 July (day 33; $F_{2,36} = 4.09$, $P = 0.03$); and 13 July (day 40; $F_{2,36} = 5.93$, $P < 0.01$). On all three dates, DO was lower in mesocosms with live cattails than in mesocosms with no vegetation structure or artificial cattails; however, the greatest difference in average DO between structure treatments was 0.5 mg/L.

Discussion

Ours is the first study to demonstrate that the presence of vegetation structure alone can affect the expression of metamorphic traits for some anuran species. The presence of artificial or live structure increased *Bufo* survivorship in mesocosms where the density of *Rana* competitors was low. That this effect was not observed for other amphibian species may be a reflection of the different life-history strategies employed by the species in our experimental community. Werner (1986) suggested that amphibian life histories differ based on two main factors: the opportunity for growth and the risk of mortality in the aquatic and terrestrial phases. In general, bufonids have shorter larval periods and metamorphose smaller relative to their adult size than hylids and ranids; therefore, bufonids may be more sensitive than species with longer larval periods to changes in food resources. The addition of vegetation structure appears to be associated with increased periphyton food resources, and toads may experience such benefits of structure when the competitive environment is more favorable (i.e., at low competitor density). It is interesting that we observed a significant effect of vegetation structure on survival in *Bufo americanus*, the species that spends the least amount of time in the pond environment. However, the short larval period of *Bufo* also reflects the strong influence that the aquatic environment can have on this species in a short timeframe. A closely related species, *Bufo woodhousii*, has been shown to respond to environmental changes even when other species in the community showed little to no response (e.g., insecticide exposure; Boone et al., 2004*b*). Similarly, we found in this study that *Bufo americanus* responded to variation in habitat structure despite brief exposure during their relatively short larval stage.

In many studies, the advantages of habitat structure are attributed to a mediation of predator-prey dynamics. Prey detection by foraging predators (e.g., many fish predators) is often compromised in structurally complex environments, which confers clear benefits to survivorship and condition of prey (Babbitt and Tanner, 1997, 1998; Crowder and Cooper, 1982). In this experiment, however, we observed a positive effect of habitat structure on larval survival and community evenness in the absence of predators, which suggests that the advantages of habitat structure are not limited to the availability of refugia for predator evasion. Other possible explanations of the benefits of habitat structure include increased foraging behavior due to the perception of safety in the presence of vegetation structure and increased food resources provided by periphyton growing on vegetation. Although we cannot empirically evaluate differences in the perception of predation risk among habitat structure treatments based on our dataset, others have documented this behavior in a range of terrestrial, freshwater, and marine systems (Horat and Semlitsch, 1994; Lima, 1990; Nicieza, 2000; Sih, 1986; Werner and Hall, 1988; Wirsing et al., 2007). We did not directly observe differences in behavior among habitat-structure treatments; however, increased foraging due to perception of safety cannot be ruled out as a possible mechanism for the observed differences in *Bufo* survivorship. Alternatively, benefits to *Bufo* larvae could be explained by an alleviation of exploitative competition with *Hyla* and *Rana* due to supplemental food resources provided by periphyton growing on vegetation structure. For instance, the community shift toward *Hyla* dominance in mesocosms lacking vegetation structure could be due to *Hyla*'s superior exploitation of shared resources in structurally simple habitats. Chlorophyll analysis of periphyton samples collected from the vegetation in our mesocosms confirmed additional periphyton growth in mesocosms with both live and artificial cattails, although periphyton was more abundant on artificial cattails. Thus, the availability of supplemental periphyton growing on vegetation may contribute to increased survival in some cases. A third possible explanation is that the presence of habitat structure may reduce interference competition between species. Behavioral observations of *Bufo americanus* and *Hyla versicolor* larvae did not reveal changes in activity or avoidance behavior when they were observed in the presence of *Rana pipiens* larvae, which suggests that interference competition is not important between these species and further supports the former hypotheses about the mechanisms of habitat structure effects.

Our results also demonstrate that habitat structure can affect the length of the larval period for some anurans. Larvae of pond-breeding amphibians have been shown to make trade-offs between the length of the larval period and body size at the time of metamorphosis in response to changes in environmental attributes (e.g., Boone et al., 2004*b*; Crump, 1989; Laurila and Kujasalo, 1999). Different species employ different life history strategies; *Bufo* generally represent the strategy of metamorphosing earlier and smaller relative to their adult size, *Rana* and *Ambystoma* remain in the aquatic environment much longer and metamorphose at a larger size relative to their adult size, and *Hyla* tend to fall somewhere in the middle of this continuum. Shortening the larval period reduces exposure to aquatic predators and minimizes the risk of desiccation, both of which increase the likelihood of surviving to metamorphosis. However, larvae that leave the pond environment early often do so at a smaller size, and size at metamorphosis is considered an indicator of future fitness for many species (e.g., Semlitsch and Pechmann, 1988). Thus, factors that influence plasticity in these traits can have potentially serious effects on populations.

We had expected to see larger metamorph size and a shorter larval period in mesocosms with habitat structure because of the supplemental periphyton resource available on the vegetation. In contrast, we observed an increase in the length of the larval period of *Bufo* in mesocosms with artificial cattails and of *Rana* in mesocosms with artificial or live cattails. For both species, the larval periods between mesocosms with and without habitat structure differed by approximately three days. It is interesting that variation in habitat structure can significantly influence the timing of metamorphosis, but it remains unclear why this difference occurred and whether there are biologically significant consequences of a three-day difference in larval period. If, for instance, the presence of habitat structure induced a trade-off between time spent in the larval stage and size at metamorphosis, we would have expected that metamorphs emerging later would be larger in size; however, our data do not support such a trade-off.

The observed difference in *Bufo* response to artificial versus live cattails could be attributed to either biological or structural differences between the two vegetation treatments. A biological explanation could be that artificial and live cattails differ in their capacities to support growth of microorganisms and algae that benefit amphibian larvae. Live cattails appeared to be associated with a biofilm at the water surface and larvae were observed feeding in these areas (J. Purrenhage, personal observations). If live cattails did support microorganisms or algae that

enhanced the food resources for *Bufo*, this may help to explain the apparent size and developmental advantage conferred to larvae in mesocosms with live cattail structure. Alternatively, the relevant difference between live and artificial cattails could have been structural. The live cattails in our mesocosms grew throughout the experiment and provided a more structurally complex habitat than did the artificial cattails, which more closely approximated the structure of most *Typha* species commonly found in Midwestern ponds. Consequently, *Bufo* may have responded to levels of structural complexity rather than to some biological factor associated with live, but not artificial, cattails. However, we cannot definitively say whether one or both of these mechanisms drove the observed effect.

As predicted, and consistent with previous findings (Werner, 1986; Wilbur, 1977; Wilbur and Collins, 1973), the anurans in our experiment exhibited smaller sizes at metamorphosis in high-competition environments (higher *Rana*-density treatments), although trade-offs between size at metamorphosis and length of the larval period differed among species. On average, *Bufo* metamorphosed at a smaller size with increasing *Rana* density, but showed no significant alteration in the length of the larval period. This pattern is consistent with a strategy that minimizes the time spent in the aquatic, larval stage, regardless of the resultant size at metamorphosis (Werner, 1986), and provides further evidence for metamorphic synchrony in *Bufo* (Breden and Kelly, 1982; DeVito, 2003), even in the absence of predation or changing water levels. In contrast, *Hyla* exhibited both a graded decrease in size at metamorphosis and a graded increase in the length of the larval period with increasing *Rana* density. This pattern suggests that increased competition with *Rana* inhibits growth of *Hyla* larvae, such that they must delay metamorphosis until they reach a minimum size required by this species for metamorphosis. Competition among larval amphibians may occur via both interference and exploitation. However, as has been seen with other ranids (Boone et al., 2007; Kupferberg, 1997), the observed suppression of periphyton abundance in mesocosms with high *Rana* density suggests that the competitive effect of the larger *Rana pipiens* larvae on smaller heterospecifics may be exerted mainly via exploitation of the shared algal food resource. As previously mentioned, behavioral observations suggest that interference by *Rana* is not the mechanism of competition between these species, which further supports the alternative hypothesis of exploitative competition.

The nature of the response by *Rana* to habitat structure and larval density differed from the responses of *Bufo* and *Hyla*. Whereas *Bufo* metamorphosed earlier in mesocosms with live cattails than in those with artificial cattails, *Rana* larvae metamorphosed earlier in mesocosms lacking vegetation than in those with either artificial or live cattails. This difference suggests that the biological or structural aspect of live cattail structure that benefited *Bufo* does not confer a similar advantage to *Rana*. If *Bufo* and *Rana* are competing for the same food resource, then we might expect to see this differential response since a condition that favors one species should have the opposite effect on its competitor. *Rana* also differed in their response to population density. Whereas *Hyla* exhibited both a smaller size at metamorphosis and a longer larval period with increased density of *Rana* competitors, *Rana* exhibited a different pattern in these traits. *Rana* did demonstrate a strong density-dependent response of greatly reduced size at metamorphosis in moderate- and high-density treatments, but the density of *Rana* competitors did not significantly influence the larval periods of either *Rana* or *Bufo*. However, unlike *Bufo*, *Rana* did exhibit a trend of a longer larval period in low-density treatments, which may indicate a trade-off between size at metamorphosis and time spent in the larval stage. In accordance with the general life-history strategy of ranids, *Rana* larvae in our experiment may have weighed the cost of a long larval period against the benefit of a large size at metamorphosis (Werner, 1986; Wilbur and Collins, 1973). Alternatively, the different response by *Rana* could be a consequence of the environmental conditions they were exposed to during the early larval stage in the natural pond from which they were collected. However, even if exposure to unknown factors in the natural environment did influence *Rana* response, they nevertheless showed response to experimental treatments after shorter-term exposure than the other species, relative to their own developmental period.

In summary, variation in habitat structure and density of *Rana* larvae (competitor density) significantly influenced length of the larval period, mass at metamorphosis, and survival to metamorphosis of larval anurans, as well as species evenness of the experimental communities. Our prediction that habitat structure increases survival to and mass at metamorphosis and decreases length of the larval period was partially supported for *Bufo*, which exhibited increased survival in the presence of vegetation, but not for the other species in our experimental community. Our prediction that density of *Rana pipiens*, a heterospecific competitor, decreases survival to and mass at metamorphosis and increases length of the larval period was supported

for *Bufo* and *Hyla*. Finally, our prediction that habitat structure mediates the negative effect of competitor density was partially supported for *Bufo*, which exhibited increased survival at low densities of the *Rana* competitor and showed a similar trend in high *Rana*-density treatments. Habitat structure has been shown to affect the outcome of biotic interactions for a range of taxa, but has been largely overlooked in studies of larval amphibians (McDiarmid and Altig, 1999; Bell et al., 1991). Our study is the first to show that for larval amphibians in structurally complex habitats there may be delayed time to metamorphosis and increased probability of survival, even in the absence of predation. Moreover, the presence of habitat structure increased species evenness and may increase the likelihood for diverse communities over time.

Understanding the role of habitat structure for pond-breeding amphibians is critical for successful restoration and management practices. We have demonstrated that aquatic vegetation has the potential to affect survival and length of the larval period for some anuran larvae, but that species may differ in their habitat needs and that habitat structure may be important for mediating different biotic interactions (e.g., predation) and structuring communities (e.g., species evenness). While it may be premature to label vegetation structure, with hydroperiod and predator community composition (Wellborn et al., 1996), as an important factor affecting the structure and dynamics of larval amphibian communities, aquatic vegetation has been shown to be important for courtship and oviposition in pond-breeding amphibians (Egan and Paton, 2004; Howard, 1978). Thus, we should continue to explore the role of vegetation structure for amphibians in multiple life stages (e.g., metamorphs, breeding adults). Moreover, our finding that habitat structure can enhance survival to metamorphosis of anurans even in the absence of predators, justifies further examination of the effects of habitat structure on amphibians in the aquatic stage.

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Fig. 1.—Simpson's Diversity Index (D) for experimental larval amphibian communities at each level of *Rana pipiens* density (1A) and at each level of habitat structure (1B). Simpson's Diversity Index incorporates final survival of the three focal amphibians: *Ambystoma maculatum*, *Bufo americanus*, and *Hyla versicolor*. Based on initial stocking densities, the maximum possible value of D is 2.41, and is represented by the thick, horizontal line. Different letters indicate significant differences in D between treatments based on Scheffe's post hoc test.

Fig. 2.—Effects of habitat structure treatments on *time* (days) to metamorphosis and *mass* at metamorphosis of (A) *Bufo americanus*; (B) *Hyla versicolor*; and (C) *Rana pipiens*. Error bars represent ± 1 SE for least-square means. Different letters indicate significant differences in time to metamorphosis between treatments based on Scheffe's post hoc test. Neither mass nor time to metamorphosis was significant for *Hyla*.

Fig. 3.—Effects of competitor density treatments on *time* (days) to metamorphosis and *mass* at metamorphosis for (A) *Bufo americanus*; (B) *Hyla versicolor*; and (C) *Rana pipiens*. Error bars represent ± 1 SE for least-square means. Different letters indicate significant differences in time to (capital letters) and mass at (lowercase letters) metamorphosis between treatments based on Scheffe's post hoc test.

Fig. 4.—Significant interaction of habitat structure and competitor density treatments for percent survival of *Bufo americanus*. Error bars represent ± 1 SE for least-square means.

Fig. 5.—Relative abundance of periphyton (represented by chlorophyll a concentration) in samples taken from (A) the pond edge in ponds with different *Rana*-competitor densities and (B) the vegetation structure in ponds with artificial and live cattail structure. Error bars represent ± 1 SE for least-square means.

Fig. 1

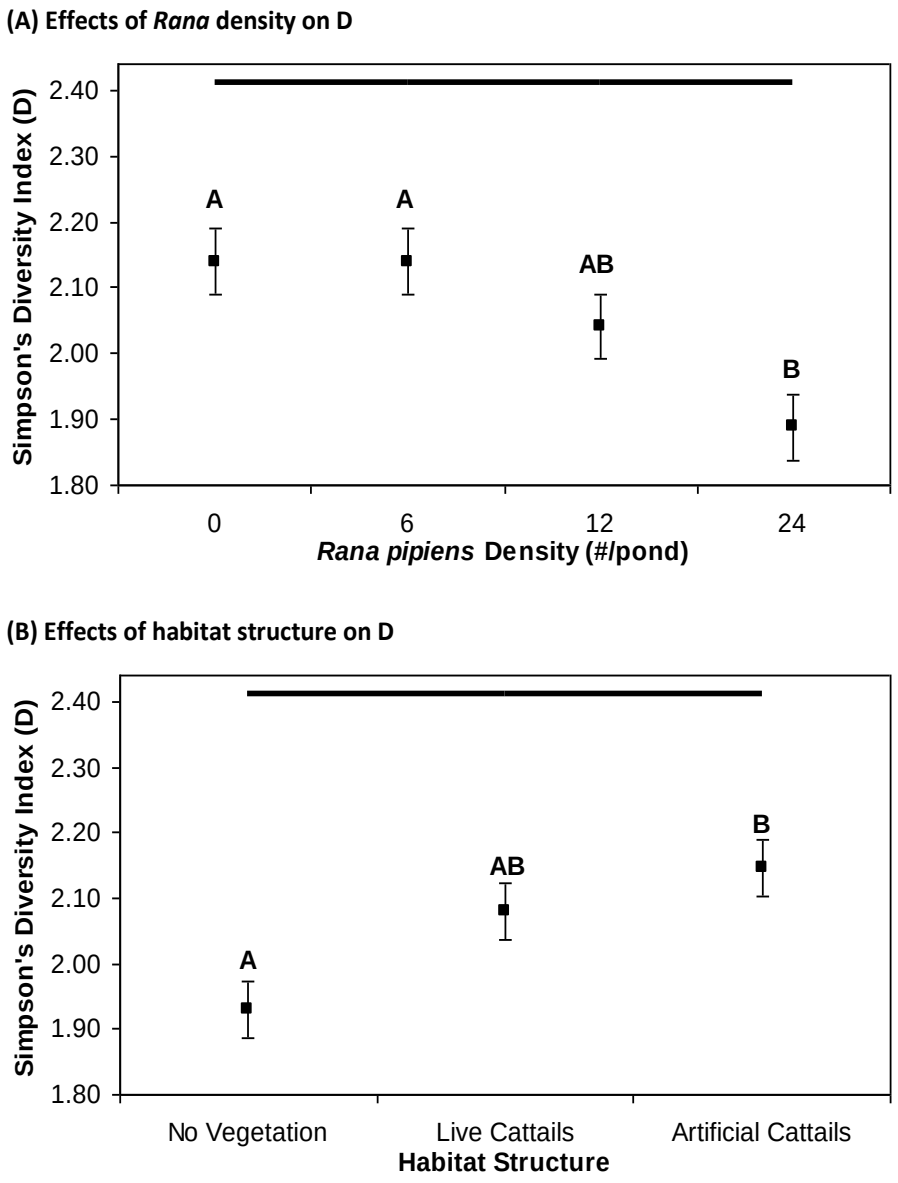

(A) Effects of *Rana* density on D
Simpson's Diversity Index (D)
2.40
2.30
2.20
2.10
2.00
1.90
1.80
A
A
AB
B
0
6
12
24
Rana pipiens Density (#/pond)
(B) Effects of habitat structure on D
Simpson's Diversity Index (D)
2.40
2.30
2.20
2.10
2.00
1.90
1.80
A
AB
B
No Vegetation
Live Cattails
Artificial Cattails
Habitat Structure

Fig. 2

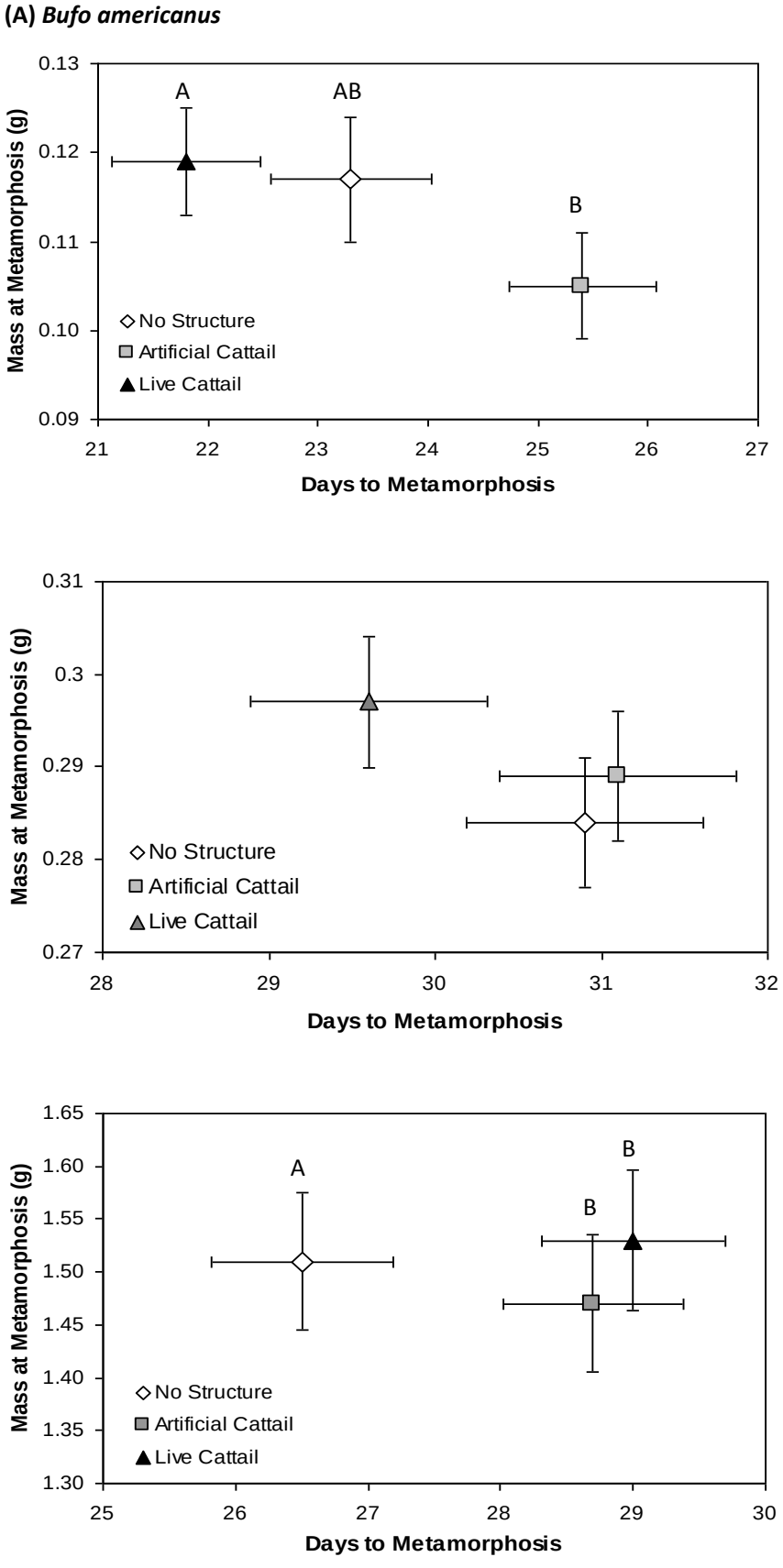

(A) Bufo americanus
Mass at Metamorphosis (g)
0.13
0.12
0.11
0.10
0.09
A
AB
B
No Structure
Artificial Cattail
Live Cattail
21
22
23
24
25
26
27
Days to Metamorphosis
Mass at Metamorphosis (g)
0.31
0.3
0.29
0.28
0.27
No Structure
Artificial Cattail
Live Cattail
28
29
30
31
32
Days to Metamorphosis
Mass at Metamorphosis (g)
1.65
1.60
1.55
1.50
1.45
1.40
1.35
1.30
A
B
B
No Structure
Artificial Cattail
Live Cattail
25
26
27
28
29
30
Days to Metamorphosis

Fig. 3

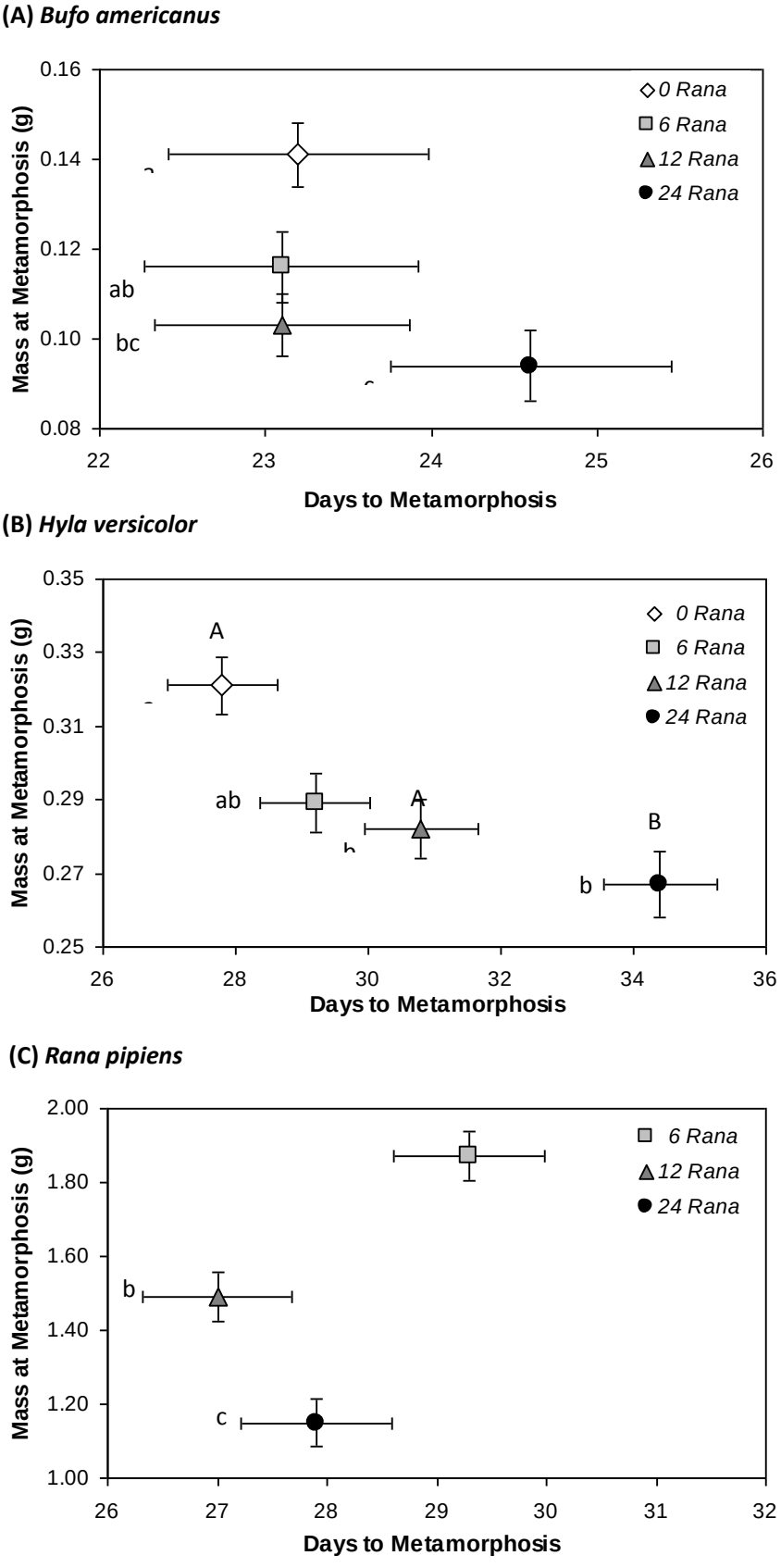

(A) Bufo americanus
Mass at Metamorphosis (g)
Days to Metamorphosis
0 Rana
6 Rana
12 Rana
24 Rana
ab
bc
(B) Hyla versicolor
A
ab
A
B
b
(C) Rana pipiens
b
c

Fig. 4

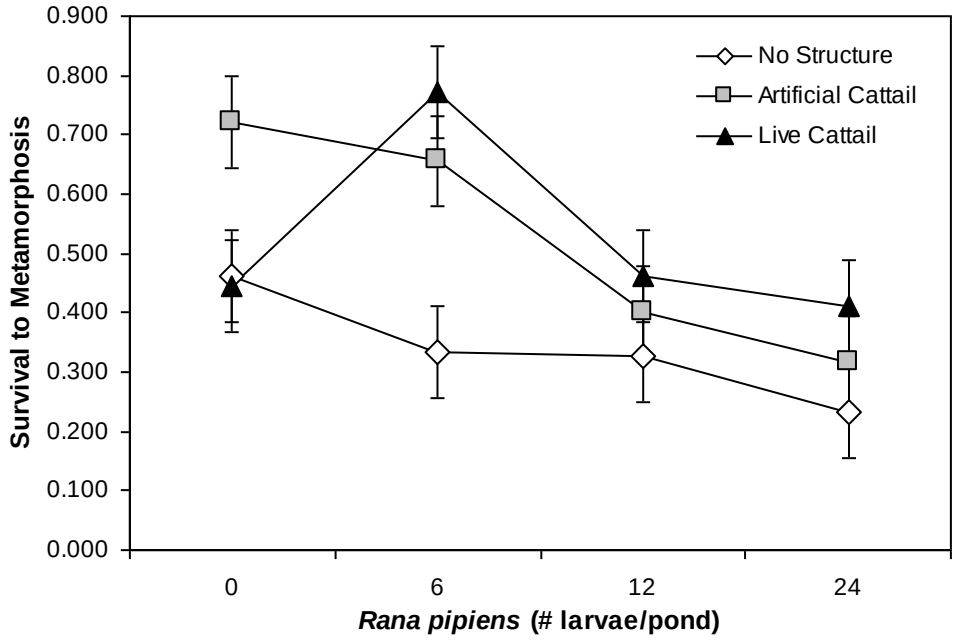
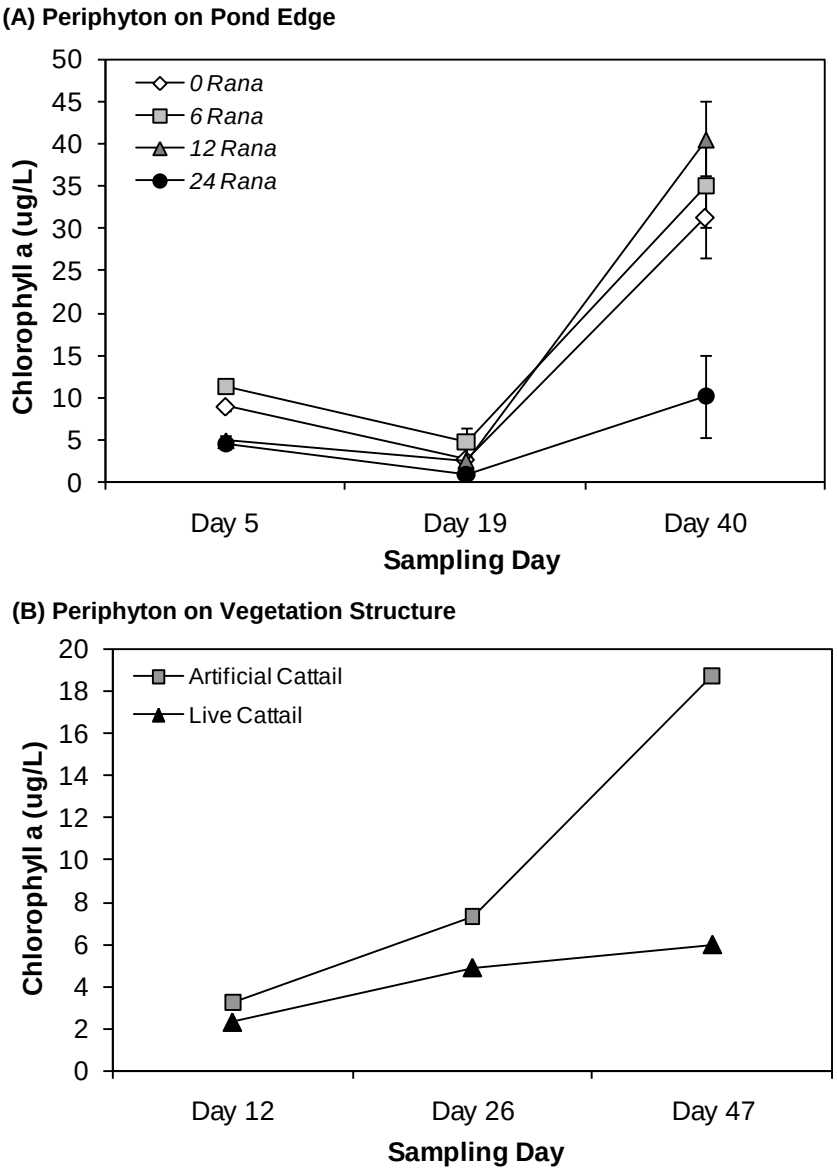

0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Survival to Metamorphosis
No Structure
Artificial Cattail
Live Cattail
0
6
12
24
Rana pipiens (# larvae/pond)

Fig. 5


(A) Periphyton on Pond Edge
Chlorophyll a (ug/L)
0 Rana
6 Rana
12 Rana
24 Rana
Day 5
Day 19
Day 40
Sampling Day
(B) Periphyton on Vegetation Structure
Chlorophyll a (ug/L)
Artificial Cattail
Live Cattail
Day 12
Day 26
Day 47
Sampling Day

CHAPTER 2:

Habitat Structure Mediates Biotic Interactions in Pond-Breeding Amphibians

Abstract. – Many taxa are experiencing population declines and loss of biodiversity, and habitat alteration has been overwhelmingly cited as a major threat to these species. Amphibians are one group that has suffered worldwide population declines due in great part to habitat loss and alteration. Our objective was to examine cause-and-effect relationships between several key habitat attributes and the response of amphibians during a critical life stage. We conducted three simultaneous experiments in outdoor pond mesocosms to test the separate and combined effects of pond canopy cover, emergent vegetation, crayfish predators, and presence/absence of larval salamanders on fitness-correlated metamorphic traits of American toads (*Bufo americanus*). Canopy cover was consistently important for toad metamorphosis: in general, toads reared in closed-canopy ponds were larger at metamorphosis and had a greater probability of surviving to metamorphosis than toads reared in open-canopy ponds. Moreover, emergent vegetation had a positive influence on mass at metamorphosis and survival to metamorphosis of toads in the absence of crayfish predators, but had the opposite effect when crayfish were present. Thus, although we observed strong effects of canopy cover alone, we also found that variation in habitat structure (i.e., canopy cover and emergent vegetation) can affect amphibian metamorphosis via mediation of biotic interactions with crayfish predators and larval salamanders.

Introduction

Habitat alteration is a major threat to the persistence of populations and the maintenance of biodiversity (Allan and Flecker 1993; Wilcove et al. 1998; Cushman 2006). During the past century, the landscape of the eastern United States has undergone dramatic changes in land use as the burden of agricultural production has shifted from the northeastern region to the midwestern states (Manspeizer 2006). Consequently, some regions have become reforested after longtime agricultural use, while in other regions forests have been cleared to make the land suitable for agriculture. Moreover, such large-scale habitat alteration across the landscape (e.g., changes in forest cover) is often accompanied by small-scale alteration of local habitat

characteristics (e.g., changes in habitat complexity). All these changes alter wildlife habitats in ways that may lead to declining populations and shrinking species' ranges (Skelly et al. 1999; Werner et al. 2007).

One group that has been severely impacted by habitat loss and alteration is amphibians (Lehtinen et al. 1999; Dodd and Smith 2003; Cushman 2006). Pond-breeding amphibians are particularly vulnerable because their biphasic life cycle requires the use of two distinct habitats – aquatic and terrestrial – both of which can be compromised by anthropogenic changes to the landscape (Semlitsch 2003). Because pond-breeding amphibians have complex habitat requirements and are experiencing population declines that have been in part attributed to habitat loss and alteration, they make good model species for examining the impacts of land-use changes.

Pond-breeding amphibians utilize a range of aquatic habitats that vary in their key habitat attributes including pond canopy cover and vegetation structure (Wellborn et al. 1996; Semlitsch 2003; Colburn 2004). For amphibians, pond canopy cover has been shown to influence both individual performance and species' distributions (Skelly et al. 1999; Werner and Glennemeier 1999; Skelly et al. 2002; Skelly et al. 2005; Schiesiari 2006; Binckley and Resetarits 2007; Werner et al. 2007). Based on their distributions in different habitat types, some species have been characterized as either open-canopy specialists or as canopy generalists (Skelly et al. 2005). However, regardless of these habitat affiliations, most species appear to exhibit lower larval performance (i.e., growth, survival) in closed-canopy environments (e.g., Werner and Glennemeier 1999; Schiesiari 2006).

Independent of an association with variation in pond canopy cover, differences in vegetation structure within and among ponds can influence survival and growth of aquatic organisms (Heck and Crowder 1991). The influence of vegetation structure on fish and aquatic invertebrates has been reviewed in Heck and Crowder (1991), but less is known about how vegetation structure affects amphibians in aquatic environments. The available data do, however, suggest a potentially important role of vegetation structure in mediating larval amphibans' interactions with competitors and predators. The presence of vegetation structure can reduce the influence of interspecific competition on mass at metamorphosis (Sredl and Collins 1992) and survival to metamorphosis (Purrenhage and Boone 2009) of some amphibian larvae. Interestingly, vegetation structure may also influence predation of amphibian larvae, but the outcome for prey

may depend on the identity of the predator. In environments with vegetation structure, amphibian larvae experienced reduced predation by fish and aquatic insects (Tarr and Babbitt 2002; Baber and Babbitt 2004; Kopp et al. 2006; Hartel et al. 2007), but increased predation by crayfish (J.P. and M.B., unpublished manuscript).

Although the influences of canopy cover, vegetation structure, and biotic interactions have been examined separately to some extent, none of these factors exists in isolation. Natural habitats are complex, and understanding the influences of each component requires an approach that evaluates this complexity. We conducted three concurrent pond mesocosm experiments to test the effects of pond canopy cover, aquatic vegetation structure, and biotic interactions (i.e., interactions with crayfish or salamander cohabitants) on fitness-correlated metamorphic traits of the pond-breeding American toad (*Bufo americanus*). Based on published studies and previous work of our own, we predicted the following: (1) increased canopy cover will negatively affect mass at metamorphosis, larval period, and survival to metamorphosis of toads (e.g., Skelly et al. 2002; Schesiari 2006); (2) vegetation structure will negatively affect larval period (i.e., a longer larval period), but positively affect mass and survival of toads (Purrenhage and Boone 2009, Chapter 1); (3) presence of crayfish predators will negatively affect mass and survival (J.P. and M.B., unpublished manuscript), and may accelerate the timing of metamorphosis of toads (Benard 2004); and (4) presence of *Ambystoma* salamanders will have indirect positive effects on mass, larval period, and survival of toads (Purrenhage and Boone 2009) by reducing zooplankton and leading to increased algal food resources. Moreover, we expected that these factors would have complex interactions that affect toad metamorphosis, such that variation in physical habitat characteristics (i.e., canopy cover and vegetation structure) may alter the outcomes of biotic interactions.

Methods

Field Collection and Rearing

We collected American toad (*Bufo americanus*) larvae from six larval aggregations located throughout a pond at Woodland Trails Wildlife Area, Preble County, Ohio, USA, on 19 April 2007. Toad larvae had very recently hatched and were presumably still in sibling groups when collected, as they were densely schooled in distinct aggregations in shallow vegetation near the remnant egg strings. We collected five egg masses of spotted salamanders (*Ambystoma*

maculatum) from a temporary pond at Indian Creek Preserve MetroPark, Butler County, Ohio, USA, on 10 April 2007. Eggs hatched and larvae were maintained in separate cattle tanks outdoors at the Miami University Ecology Resarch Center (ERC) until they were added to experimental ponds. To reduce clutch-related biases within species, larvae from different aggregations (toads) and egg masses (salamanders) were mixed prior to being added to experimental ponds. We used red swamp crayfish (*Procambarus clarkii*) as predators in Experiment 2; crayfish were obtained from Carolina Biological Supply Company, maintained in a cattle tank until the start of the experiment, and sacrificed at the end of the experiment.

During May – July 2007, we conducted three simultaneous experiments in outdoor pond mesocosms at the Miami University ERC, Oxford, Ohio, USA. Collectively, the three experiments were designed to examine the influence of vegetation structure and canopy cover on biotic interactions and metamorphic traits of American toad tadpoles; each experiment focused on a different aspect of the overarching goal (Table 1). For all experiments, we used a full-factorial design with four replicates per treatment, and treatments were randomly assigned to the mesocosm grid.

Each mesocosm was filled with 1000 L water, 1 kg dry leaf litter from a nearby mixed deciduous (primarily *Acer*) forest, and inoculations of algae and zooplankton from a fishless pond on the ERC property. We filled the mesocosms with water on 15 April 2007, allowed the water to age 48 h prior to adding leaf litter and vegetation structure, and added experimental animals on 5 May 2007. We stocked all mesocosms at the same initial density of larval toads, 70 per mesocosm, which falls within the wide range of larval densities that has been observed in natural ponds (14 - 4238 per 1000 L; e.g., Morin, 1983; Petranka, 1989). Toads were added to experimental mesocosms at Gosner stage 25 (Gosner 1960). We checked mesocosms daily for metamorphs; anurans with at least one front limb emerged (Gosner stage 42, Gosner 1960) or salamanders with resorbed gills were collected and transported to the laboratory at Miami University for processing. Anurans were held in the laboratory until tail resorption was complete (≤ 3 days to reach Gosner stage 46, Gosner 1960), at which point we recorded time to metamorphosis (larval period) and mass at metamorphosis. Metamorphs were released at the egg-collection sites after being processed. We ended the experiments on 27 July 2007, once all mesocosms had not produced metamorphs for a minimum of 7 consecutive days.

Experiment 1: Vegetation Structure and Canopy Cover

To test the effects of vegetation structure and canopy cover on toad metamorphosis, we manipulated three levels of vegetation structure (none, live cattail vegetation, and artificial cattail vegetation) and two levels of canopy cover (open canopy, closed canopy) with four replicates in 24 mesocosms (Table 1). Each mesocosm was randomly assigned one of three vegetation structure treatments: no-vegetation treatments (control) contained a 39 x 56 x 13.5-cm (27-L capacity) clear Rubbermaid container with soil and pea gravel; live vegetation treatments contained a Rubbermaid container with soil, pea gravel, and 15 individual cattail (*Typha latifolia*) plants; and artificial vegetation treatments contained a Rubbermaid container with soil, pea gravel, and 15 individual artificial cattail plants per pot. We used cattails (*Typha*) in our vegetation structure treatments because *Typha* species are common in the majority of wetlands in our area, and because they provide a relatively simple, uniform structure similar to that of other common wetland species (e.g., *Phragmites*, *Iris*). Artificial cattails were designed to mimic *T. latifolia*; each artificial cattail plant was constructed of one 61-cm long, 2-cm diameter PVC stem and two green, artificial leaf blades attached with two transparent plastic cable ties. Open- and closed-canopy treatments, high and low light environments respectively, were simulated using different materials for experimental mesocosm lids. Open-canopy lids were constructed of a single layer of 1.9-cm black plastic netting; closed-canopy lids were constructed of two overlapping layers of 0.2-cm black fiberglass mesh. We used a Biospherical Instruments QSL spherical PAR detector to test the percent transmission of visible light (PAR) through each mesocosm lid type. These measurements were carried out in a darkened room with no light other than our intended light source, a white compact fluorescent lamp (GE Helical 26W) mounted inside a 30.5-cm photoreflector; the probe was mounted perpendicular to the light source, 60 cm from the front of the reflector. Each mesocosm lid material was placed in front of the detector (60 cm from the probe); three readings were taken and the mean percent transmission was calculated for each material. Open-canopy lids blocked ~30% overhead light (mean % transmission = 69.5%), whereas closed-canopy lids blocked ~65% (mean % transmission = 34.5%). Lids also served to deter immigration of predators and emigration of metamorphs from mesocosms.

Experiment 2: Vegetation Structure, Canopy Cover, and Crayfish Predators

To test the effects of vegetation structure, canopy cover, and crayfish predators on toad metamorphosis, we manipulated two levels of vegetation structure (no vegetation, live cattail vegetation), two levels of canopy cover (open canopy, closed canopy), and two levels of predator exposure (crayfish absent, crayfish present) with four replicates in 32 mesocosms (Table 1). Live cattail vegetation treatments, vegetation structure controls, and canopy cover treatments were the same as those described for Experiment 1. Mesocosms assigned to the crayfish predator treatment received two red swamp crayfish, *Procambarus clarkii*, (9-12 cm in length). Crayfish were added to predator treatments on 5 May 2007, ~ 8 h prior to adding amphibian larvae, so that chemical cues would be present in the water when larvae were added. *Procambarus clarkii* is non-native to Ohio, but was introduced to the state during the twentieth century (Thoma and Jezerinac 2000). In addition to being highly invasive and an efficient predator, *P. clarkii* can greatly reduce vegetation biomass through grazing and non-consumptive plant clipping (Gamradt et al. 1997; Cruz and Rebelo 2005; Gherardi and Acquistapace 2007).

Experiment 3: Pond Habitat Type and Presence/Absence of Larval Salamanders

To test the combined effects of vegetation and canopy cover (i.e., pond habitat type) and the presence of larval salamanders, *Ambystoma maculatum*, in the pond community on toad metamorphosis, we simulated two pond habitat types (open-canopy and closed-canopy habitat) and two pond communities (70 *Bufo*; 70 *Bufo* + 14 *Ambystoma*) with four replicates in 16 mesocosms (Table 1). Open-canopy habitat treatments included an open-canopy mesocosm lid and live cattail vegetation. Closed-canopy habitat treatments included a closed-canopy mesocosm lid and no vegetation. These pond habitat treatments represent the vegetation and light availability of natural ponds in that open-canopy ponds often contain emergent vegetation, whereas closed-canopy ponds are less likely to support emergent vegetation (Colburn 2004). *Ambystoma* presence/absence treatments allowed comparisons of *Bufo* response to habitat treatments when reared with conspecifics alone versus with conspecifics and heterospecifics. *Ambystoma* larvae could influence toad metamorphosis directly (via predation or competition) or indirectly (via trophic effects); however, previous work shows no evidence of a predator-prey relationship between these species (Purrenhage and Boone 2009). Initial stocking densities of *Bufo* (70 larvae per 1000 L) and *Ambystoma* (14 larvae per 1000 L) reflect the relative

availability of each species at the collection sites, and thus, approximate realistic larval densities in natural ponds.

Food Resources and Water Characteristics

To assess the availability of possible food resources for larval toads (periphyton, phytoplankton) and salamanders (zooplankton) in our experimental treatments, we scraped uniform samples of periphyton from the mesocosm edge, filtered phytoplankton (filtered 1 L water from 4-L composite samples), and collected zooplankton samples from each mesocosm. Periphyton samples were taken by scraping 3 cm x 1.7 cm patch off the mesocosm wall just below the water surface; periphyton was then transferred to a filter. Filter papers containing periphyton and phytoplankton were submerged in 15 mL neutralized 90% acetone in the dark at 4°C for 24 h prior to being analyzed by fluorometry (Greenberg et al. 1992). We sampled zooplankton by collecting composite samples from each mesocosm; 1 L of each composite sample was filtered through 80-µm mesh, and zooplankton were stored in 80% ethanol. Using a dissecting microscope, we counted all zooplankton in four categories (cladocerans, copepods, ostracods, other) for each sample. Zooplankton data presented here represent total zooplankton counts (all four categories combined) because the majority of zooplankton in the samples were cladocerans and there were no significant differences in the other categories of zooplankton between treatments. During Experiments 1-3, we sampled periphyton and phytoplankton on 9 May, 23 May, 6 June, and 20 June; during Experiment 3, we collected a fifth sample on 4 July. Measurements of chlorophyll a concentration (µg/L) in periphyton and phytoplankton samples, determined via fluorometry, were used as a proxy for relative abundance of these algal resources. During Experiments 1-3, we collected zooplankton samples on 11 May and 25 June; during Experiment 3, we collected a third zooplankton sample on 20 July. We measured pH, water temperature (C), and dissolved oxygen (DO) of each mesocosm on the same dates that periphyton and phytoplankton were sampled to determine any differences in these water characteristics between experimental treatments.

Response Variables and Data Analyses

We measured treatment effects on the following metamorphic traits for *Bufo*: length of the larval period (time to metamorphosis), mass at metamorphosis (g), and survival to metamorphosis. We calculated percent survival in each treatment as the number of individuals that successfully metamorphosed by the end of the experiment (27 July) divided by stocking density for each

species. For larval period and mass at metamorphosis variables, we calculated means for each experimental mesocosm and treated mesocosms as the experimental units in all analyses. We tested for main effects and interaction effects of vegetation structure (Experiments 1 and 2), canopy cover (Experiments 1 and 2), crayfish predators (Experiment 2), pond habitat type (Experiment 3), and larval salamanders (Experiment 3) on larval period, mass at metamorphosis, and survival to metamorphosis of *Bufo* (and *Ambystoma* in Experiment 3) using multivariate analyses of variance (MANOVA) and univariate analyses of variance (ANOVA). In preliminary analyses of larval period and mass response, we used survival to metamorphosis as a covariate to account for differences in response that were linked to differential survival between treatments; however, reduced survival was associated with reduced mass, which resulted in the survival covariate compensating for differences between treatments in a way contrary to the typical density-dependent manner. For this reason we did not include a covariate in our final analyses. We used repeated measures ANOVA to test for differences in food resources (periphyton, phytoplankton, zooplankton) and water characteristics (pH, temperature, DO) among treatments. All data were transformed as necessary to meet the assumption of normality. Survival data were angularly transformed; mass and larval period data were log-transformed. Statistical analyses were performed using SAS version 9.1.

Results

Experiment 1: Vegetation Structure and Canopy Cover

Vegetation structure treatments influenced the length of the larval period of American toads, such that the mean larval period of toads in artificial vegetation treatments was shorter than that of toads in either live vegetation or no vegetation treatments (Table 2). Vegetation structure treatments did not significantly influence either mass at metamorphosis or survival to metamorphosis of toads (Table 2). Canopy cover influenced mass at metamorphosis and the length of the larval period, but not survival to metamorphosis (Table 2). Toads from closed-canopy ponds metamorphosed on average at a larger size and remained in the ponds slightly longer than those from open-canopy ponds (Fig. 1A).

Toads in closed-canopy ponds had a significantly longer larval period relative to those in open-canopy ponds when the pond environments lacked vegetation structure; there was, however, little to no difference in toad larval period between canopy cover treatments in the

presence of live or artificial vegetation (Fig. 1B). The interaction of vegetation structure and canopy cover treatments influenced the overall multivariate response of toads (Wilks' lambda = 0.3915, $F_{6,32} = 3.19$, P = 0.01). There was a significant univariate interaction of vegetation and canopy cover on larval period (Fig. 1B; $F_{2,18} = 5.26$, P = 0.01), but not on mass at metamorphosis ($F_{2,18} = 1.00$, P = 0.38) or survival to metamorphosis ($F_{2,18} = 0.38$, p = 0.68).

Experiment 2: Vegetation Structure, Canopy Cover, and Crayfish Predators

Vegetation structure treatments did not significantly influence mass at metamorphosis, larval period, or survival to metamorphosis (Table 3). However, canopy cover influenced mass at metamorphosis and the length of the larval period, but not survival to metamorphosis (Table 3). As in Experiment 1, toads from closed-canopy ponds metamorphosed on average at a larger size and remained in the ponds slightly longer than those from open-canopy ponds (Table 3). Finally, crayfish predators influenced survival to metamorphosis and mass at metamorphosis, but not larval period (Table 3). Survival to metamorphosis was lower in ponds with crayfish predators, and toads from ponds with crayfish predators were smaller on average than those from ponds without predators (Table 3).

There were several interactions of vegetation structure, canopy cover, and predator treatments on metamorphic traits of toads. The interaction of vegetation structure and predator treatments did not influence the overall multivariate response of toads (Wilks' lambda = 0.8011, $F_{3,22} = 1.82$, P = 0.17). In the univariate analysis, though, the interaction of vegetation and predators influenced mass at metamorphosis (Fig. 2A; $F_{1,24} = 5.69$, P = 0.02). In ponds lacking vegetation structure, mass at metamorphosis did not differ between predator treatments; however, in ponds with live cattail vegetation, toads metamorphosed larger in the absence of crayfish predators. There was a non-significant trend that the interaction of vegetation and predators similarly influenced toad survival (Fig. 2B; $F_{1,24} = 3.18$, P = 0.08). In ponds lacking vegetation structure, there was no difference in survival between predator treatments; however, in ponds with vegetation structure, toad survival tended to be lower in the presence of crayfish predators (Fig. 2B).

The interaction of vegetation and canopy cover treatments did not significantly influence the multivariate response (Wilks' lambda = 0.7667, $F_{3,22} = 2.23$, P = 0.11). The interaction of vegetation and canopy cover treatments did influence the univariate response of toad larval period ($F_{1,24} = 7.25$, P = 0.01; means $\pm$ 1 SE: no vegetation/open canopy, 29.3 $\pm$ 0.3; no

vegetation/closed canopy, 31.3 ± 0.3; live vegetation/open canopy, 30.4 ± 0.3; live vegetation/closed canopy, 30.8 ± 0.3), but not mass at metamorphosis ($F_{1,24} = 0.00$, $P = 0.99$). In ponds lacking vegetation, larval period was shorter in open-canopy than in closed-canopy treatments; in ponds with vegetation, there was no difference in length of the larval period between canopy cover treatments. Finally, the interaction of vegetation structure, canopy cover, and predator treatments influenced the multivariate response of toads (Wilks' lambda = 0.6871, $F_{3,22} = 3.34$, $P = 0.03$). However, the interaction of these treatments did not significantly influence the univariate responses of mass at metamorphosis ($F_{1,24} = 1.22$, $P = 0.28$), larval period ($F_{1,24} = 0.00$, $P = 0.98$), or survival to metamorphosis ($F_{1,24} = 2.53$, $P = 0.12$).

Experiment 3: Pond Habitat Type and Presence/Absence of Larval Salamanders

Pond habitat (closed-canopy vs. open-canopy pond habitat) influenced mass at metamorphosis, such that toads from closed-canopy habitats were larger than those from open-canopy habitats (Table 4). As we observed for closed-canopy treatments in Experiments 1 and 2, toads in Experiment 3 also metamorphosed at a larger size from closed-canopy habitats, which had greater survival to metamorphosis. The effects of pond habitat on survival to metamorphosis and larval period were marginally significant, and reflected trends of greater survival and a longer larval period of toads in closed-canopy habitats (Table 4).

The presence of *Ambystoma* salamanders alone did not significantly influence mass at metamorphosis, larval period, or survival to metamorphosis (Table 4). However, the interaction of pond habitat and *Ambystoma* treatments did affect survival to metamorphosis of toads (Fig. 3; $F_{1,12} = 6.44$, $P = 0.02$). The interaction appeared to be driven by a significant increase in toad survival in closed-canopy ponds with *Ambystoma* larvae present. There were no significant treatment interactions on either mass at metamorphosis ($F_{1,12} = 0.97$, $P = 0.34$) or larval period ($F_{1,12} = 0.32$, $P = 0.57$).

Pond habitat influenced the multivariate response of salamanders used in *Ambystoma* presence/absence treatments (Wilks' lambda = 0.0739, $F_{4,3} = 9.39$, $P = 0.04$). Univariate analyses revealed a significant effect of pond habitat on mass at metamorphosis ($F_{1,6} = 8.79$, $P = 0.02$; means ± SE: open canopy, 1.38 ± 0.05 g; closed canopy, 1.12 ± 0.05 g), a marginally significant effect on larval period ($F_{1,6} = 5.50$, $P = 0.06$; means ± SE: open canopy, 54.9 ± 1.6 d; closed canopy, 59.7 ± 1.6 d), and no effect on survival to metamorphosis ($F_{1,6} = 0.37$, $P = 0.56$; means ± SE: open canopy, 0.73 ± 0.05; closed canopy, 0.77 ± 0.05) for salamanders.

Food Resources and Water Characteristics

With the exception of water temperature, none of the food resources or water characteristics measured in Experiments 1-3 varied in statistically or biologically significant ways between canopy treatments. Means (± 1 SE) and statistical significances for all food and water variables are provided in Appendix A. In all three experiments, water temperature was consistently higher in open-canopy relative to closed-canopy mesocosms. Water temperature varied over time ($P < 0.01$ in all experiments) and by canopy-cover treatment over time ($P < 0.01$ in all experiments; Appendix A).

Discussion

For aquatic species, pond canopy cover (e.g., Skelly et al. 2002), vegetation structure (e.g., Sredl and Collins 1992; Purrenhage and Boone 2009) and biotic interactions (e.g., Benard 2004; Relyea 2001) each have been shown to influence plasticity of fitness-correlated traits. These factors, however, do not exist in isolation from one another. Thus, to explore the more realistic interactive effects of these factors on amphibian metamorphosis, we conducted several concurrent pond mesocosm experiments with American toads. Four key findings about the influences of habitat structure and community composition on amphibian metamorphosis emerged from this study, each of which is discussed further below: (1) A closed-canopy environment can be advantageous for anuran metamorphosis; (2) In closed-canopy environments, community composition (i.e., presence of larval salamanders) can influence survival to metamorphosis; (3) The interaction between vegetation and predators is important for metamorphosis; and (4) The mechanism of emergent vegetation effects on larval amphibians is not merely structural.

A closed-canopy environment can be advantageous for metamorphosis. – Our results indicate that canopy cover is a major factor influencing metamorphosis in American toads. However, contrary to our prediction that increased canopy cover negatively affects metamorphic traits, we found that, in general, toads responded favorably to high canopy cover. In all three experiments, toads from closed-canopy environments were larger at metamorphosis than those from open-canopy environments; and in experiment three, we observed a trend of higher survival to metamorphosis in the closed-canopy habitat treatments. Although toads metamorphosed approximately two days later from closed-canopy ponds, this slightly longer larval period was

traded off for a size advantage; toad metamorphs from closed-canopy ponds were approximately 17% larger than those from open-canopy ponds.

This finding of positive canopy cover effects was initially surprising, as it seems to contradict much of what existed in the literature about canopy cover effects on larval amphibians (e.g., Skelly et al. 2002; Schiesiari 2006). However, the incongruity may be explained by species-specific responses or by differences in environmental variables associated with canopy treatments in the different studies. We used American toads (*Bufo americanus*) as our focal species, whereas most canopy cover studies have focused on *Rana sylvatica*, *R. pipiens*, and *Pseudacris crucifer* (Werner and Glennemeier 1999; Skelly et al. 2002; Halverson et al. 2003; Skelly et al. 2005; Schiesiari 2006), all of which typically have longer larval periods than toads. Werner and Glennemeier (1999) did include *B. americanus* in their study of canopy-cover effects in Michigan, but in that region toads were not found to breed in closed-canopy ponds; conversely, toads in southern Ohio, where our study was conducted, will breed in both open- and closed-canopy ponds (J.P., personal observation). It is possible that the differences in response observed between studies may be in part due to differences between species or regions, or both. Schiesiari (2006) observed differences in response to canopy cover between northern leopard frogs (*R. pipiens*) and wood frogs (*R. sylvatica*): wood frogs, the canopy generalists, were less impacted by increased canopy cover than were leopard frogs, the open-canopy specialists. That toads in our study region may be considered canopy generalists, could explain their tolerance of closed-canopy conditions.

The availability and quality of food resources along the canopy gradient may be a primary mechanism by which differences in metamorphic response arise. In our study, during the period before most toads reached metamorphosis, periphyton was either equally abundant in both habitats or was more abundant in closed-canopy environments, whereas others have found more abundant and diverse periphyton communities in open-canopy environments (Skelly et al. 2002). In a transplant experiment lasting for approximately two weeks of the larval period, Schiesiari (2006) tested the effects of canopy cover on growth and development of northern leopard frog and wood frog larvae, analyzed C:N ratio of foregut contents, and analyzed C:N ratio of major food resources in each habitat. Schiesiari (2006) demonstrated that food quality (C:N) can vary along the canopy gradient, such that overall C:N ratio of food resources (periphyton, phytoplankton, and detritus combined) was lower (i.e., higher quality) in open-canopy

environments. However, when each food resource was examined separately, Schiesiari (2006) found that C:N ratio of periphyton and phytoplankton did not differ between open- and closed-canopy ponds, whereas closed-canopy detritus had 30% higher C:N ratio (lower quality) than open-canopy detritus. In our experiments, detritus (i.e., benthic leaf litter substrate) persisted in closed-canopy ponds and biodegraded in open-canopy ponds throughout the larval period; moreover, toad larvae were almost always observed scraping periphyton from the pond edge (J.P., personal observation). Thus, in our experimental ponds, the apparent primary food resource for toads (i.e., periphyton rather than detritus) was either equally or more abundant in closed-canopy environments, which may contribute to larger average mass at metamorphosis for toads in closed- versus open-canopy ponds. Furthermore, like wood frogs (Schiesiari 2006), canopy-generalist toads may be able to ingest higher quality food in closed-canopy environments.

Alternatively, some closed-canopy environments may simply provide higher-quality food than open-canopy environments. Dickman et al. (2008) showed that food chain efficiency in aquatic systems can be constrained by algal food quality, and that quality was higher (i.e., lower C:nutrient ratio) in low-light/high-nutrient environments than in high-light environments. Because algal food quality can depend on both light and nutrient availability (Dickman et al. 2008), and because ponds in nature vary in their autochthanous and allochthanous nutrient supplies (Polis et al. 1997), differences in nutrients along a canopy gradient (or among experimental venues) may also explain some of the apparent incongruities between our findings and those of others (e.g., Schiesieari 2006).

Finally, differences in temperature and dissolved oxygen (DO) have been pointed to as possible contributing factors to differential species responses along the canopy gradient. Not surprisingly, temperature is 1-5°C higher in open-canopy ponds; however, the range over which temperature varies can differ between studies conducted in different regions and different venues (e.g., Werner and Glennemeier 1999; Skelly et al. 2002; Schiesiari 2006; this study). By facilitating metabolism and foraging in ectotherms, a two- to five-degree temperature difference between open and closed canopy may have a strong impact on growth when the entire range of observed temperatures is low (see Skelly et al. 2002, ~14-19°C), but a similar increase in temperature may not be as important when overall temperatures are higher (this study, 18.9-25.9°C). Differences in DO concentration, which are associated with variation in algal abundance, may also explain differential growth in open- and closed-canopy environments.

Werner and Glennemeier (1999) and Skelly et al. (2002) described that DO tended to be higher in open-canopy relative to closed-canopy ponds, and both suggested that low DO in closed-canopy environments may slow larval growth (e.g., via a reallocation of energy from foraging and metabolism to surface bobbing for respiration). As specified by Werner and Glennemeier (1999), toads may be particularly sensitive to low DO because they do not develop their lungs until late in the larval stage. In our study, DO concentration was higher in closed-canopy relative to open-canopy treatments in five of six statistically significant samples; thus, DO may also help explain our finding of better toad performance in closed-canopy environments.

In closed-canopy environments, community composition can influence survival to metamorphosis. – As potential predators of smaller amphibian larvae, *Ambystoma* larvae might be expected to exert negative direct and trait-mediated effects on larval toads (Resetarits and Wilbur 1989). However, we found no evidence of negative effects in a previous study (Purrenhage and Boone 2009); rather we observed a positive trend of higher survival of larval anurans in the presence of larval salamanders (*Ambystoma maculatum*). In this study, we introduced *Ambystoma* larvae into some of our experimental ponds (Experiment 3); we predicted that, perhaps via an indirect food-web mediated effect, *Ambystoma* presence could have a positive effect on toad metamorphic traits. We found that survival to metamorphosis was highest in closed-canopy ponds when *Ambystoma* larvae were present relative to both open- or closed-canopy ponds without *Ambystoma* and open-canopy ponds with *Ambystoma* present. Initially we suspected that this effect was driven by increased algal abundance due to greater consumption of zooplankton by *Ambystoma* larvae, a response similar to food supplementation effects observed in closed-canopy environments by Werner and Glennemeier (1999). However, our zooplankton and algal abundance data do not support this explanation; when statistically significant, zooplankton abundance was highest in closed-canopy habitats with *Ambystoma*, and phytoplankton abundance was lower in closed-canopy habitats. Due to competition for resources, we might expect periphyton to be abundant when phytoplankton was not; however, differences in periphyton abundance among treatments were not statistically significant in Experiment 3.

The interaction between vegetation and predators is important for metamorphosis. – As predicted, we found that the presence of crayfish predators resulted in lower survival to metamorphosis for toads, and we observed interesting effects of the interaction of vegetation and predators on mass at metamorphosis of toads. In the absence of predators, cattail vegetation was

beneficial for mass at metamorphosis. Conversely, in the presence of crayfish predators, cattail vegetation was detrimental for mass at metamorphosis. As mentioned previously, there was a trend of a vegetation-by-predators interaction effect on survival to metamorphosis that mirrored the mass response. These results are consistent with the findings from a previous study of the role of vegetation structure in mediating predator-prey interactions between a crayfish predator (*Orconectes rusticus*) and green frog tadpoles (*Rana clamitans*) (J.P. and M.B., unpublished manuscript). A scenario in which the presence of vegetation apparently benefits crayfish predators rather than providing a refuge for the amphibian prey (this study; J.P. and M.B., unpublished manuscript) is quite different than the typical scenario in which habitat complexity leads to reduced predation of amphibian larvae (Sredl and Collins 1992; Tarr and Babbitt 2002; Kopp et al. 2006). However, the fact that we observed the same pattern of response to cattail vegetation and crayfish predators in two separate systems that differed both in the identities of the study species and in experimental venue, suggests that this effect may be generalizable to crayfish-tadpole interactions.

The mechanism of emergent vegetation effects on larval amphibians is not merely structural. – In Experiment 1, we included artificial structure treatments in addition to live cattail treatments to determine whether the influence of vegetation was structural or biological in nature. The observed differential effects of artificial and live vegetation on timing of metamorphosis may indicate a biological mechanism rather than a structural mechanism. As was observed in two previous studies (Purrenhage and Boone 2009, Chapter 1; J.P. and M.B., unpublished manuscript), a biofilm was observable on the water surface in association with live cattail vegetation. Moreover, tadpoles were observed feeding at these water surface – vegetation interfaces, which may harbor a nutritious supplemental food source (Altig et al. 2007). Although the role of vegetation has traditionally been considered structural in nature (Heck and Crowder 1991), various non-structural benefits of aquatic vegetation have been described as well (e.g., Taylor and Crowder 1983; Gifford 2002; Lajmanovich et al. 2003). Thus, while toads sometimes metamorphosed later in the presence of live vegetation, those individuals may have done so to take advantage of some benefit of the vegetation, which resulted in observed increases in mass.

Conclusions

Identifying priorities for conservation is a necessary goal of wildlife and habitat managers. Whether the goal is to protect a particular species or a diversity of species, it is essential to begin with the most complete understanding possible of the factors that influence individual fitness, population persistence, and community structure. In controlled environments, we examined the effects of canopy cover, emergent vegetation, and biotic interactions on fitness-correlated traits of American toads. Two aspects of our findings are particularly relevant for managers and applied ecologists. Firstly, canopy cover has emerged as one of several environmental gradients influencing pond-breeding amphibians (Wellborn et al. 1996; Schiesiari 2006; Werner et al. 2007). The findings from our study, however, indicate that we may need to rethink our current understanding of *how* the canopy cover gradient influences amphibians. Specifically, certain biotic (i.e., quantity and quality of food resources) and abiotic (e.g., temperature, DO) attributes of open- and closed-canopy ponds appear to be associated with larval amphibian performance along an environmental gradient, but variation of these attributes along the canopy gradient may differ with other habitat characteristics, such as nutrients. Differences in nutrients and other habitat characteristics along the canopy gradient may be in part an artifact of experimental venue in this study (i.e., higher nutrients in experimental ponds); however, similar differences can occur in nature due to the landscape context of ponds (Polis et al. 1997; e.g., higher allochthanous nutrients in agricultural regions), which may contribute to apparent canopy-cover differences within and between regions.

Secondly, our study confirmed an important interaction between emergent vegetation and crayfish predators on larval amphibians. In a previous laboratory experiment (J.P. and M.B., unpublished manuscript), we found that in the absence of a crayfish predator (*O. rusticus*), cattail vegetation was beneficial for green frog tadpoles (increased growth in *R. clamitans*); however, vegetation was shown to facilitate predation of tadpoles by crayfish and resulted in decreased growth of survivors. In this study, we observed the same response by toad tadpoles (*B. americanus*) in experimental ponds with cattail vegetation and a different crayfish predator (*P. clarkii*). Concordant responses by two different amphibians to two different crayfish species, and in two different experimental venues (10-L laboratory microcosms, 1000-L outdoor mesocosms), may indicate that this effect is representative of predator-prey interactions between crayfish and tadpoles. As much of the existing literature about vegetation structure and predator-prey

interactions indicates that vegetation reduces predation of tadpoles (e.g., Tarr and Babbitt 2002; Kopp et al. 2006; Hartel et al. 2007), this opposing finding for a widespread predator (i.e., crayfish) is important for understanding the impact that planting and removal of vegetation during habitat restoration and management can have on larval amphibian communities.

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Table 1. – Summary of experimental designs for three mesocosm experiments testing the effects of vegetation, canopy cover, and biotic interactions on metamorphic traits of American toads (*Bufo americanus*).

	Experimental Treatments	Treatment Levels
Experiment 1	Vegetation Structure	1. No Vegetation 2. Live Cattails 3. Artificial Cattails
	Canopy Cover	1. Open Canopy (30% cover) 2. Closed Canopy (65% cover)
Experiment 2	Vegetation Structure	1. No Vegetation 2. Live Cattails
	Canopy Cover	1. Open Canopy (30% cover) 2. Closed Canopy (65% cover)
	Crayfish Predators	1. Absent (0 crayfish/pond) 2. Present (2 crayfish/pond)
Experiment 3	Pond Habitat	1. Open Canopy + Live Cattails 2. Closed Canopy + No Vegetation
	Larval Salamanders	1. Absent (0 *Ambystoma* /pond) 2. Present (14 *Ambystoma* /pond)

Table 2. – Summary of univariate analyses of variance (ANOVA) of survival to metamorphosis, larval period (time), and mass at metamorphosis for American toads (*Bufo americanus*) in Experiment 1; pond means are presented ± 1 SE.

Response Variable	Source of Variation	df	*F*	*P*	No Vegetation	Artificial Cattails	Live Cattails
Survival	Vegetation	2	1.62	0.23	0.74 ± 0.06	0.66 ± 0.06	0.79 ± 0.06
	Error	18					
Time (d)	Vegetation	2	6.70	< 0.01	30.3 ± 0.3	29.2 ± 0.3	30.5 ± 0.3
	Error	18					
Mass (g)	Vegetation	2	1.43	0.27	0.195 ± 0.011	0.177 ± 0.011	0.198 ± 0.011
	Error	18					
					Open Canopy	**Closed Canopy**	
Survival	Canopy	1	0.72	0.40	0.70 ± 0.05	0.76 ± 0.05	
	Error	18					
Time (d)	Canopy	1	7.24	0.01	29.6 ± 0.2	30.4 ± 0.2	
	Error	18					
Mass (g)	Canopy	1	6.86	0.02	0.173 ± 0.009	0.206 ± 0.009	
	Error	18					

Table 3. – Summary of univariate analyses of variance (ANOVA) of survival to metamorphosis, larval period (time), and mass at metamorphosis for American toads (*Bufo americanus*) in Experiment 2; pond means are presented ± 1 SE.

Response Variable	Source of Variation	df	*F*	*P*	No Vegetation	Live Cattails
Survival	Vegetation	1	0.11	0.74	0.78 ± 0.04	0.79 ± 0.04
	Error	24				
Time (d)	Vegetation	1	0.75	0.39	30.3 ± 0.2	30.6 ± 0.2
	Error	24				
Mass (g)	Vegetation	1	0.10	0.75	0.200 ± 0.007	0.205 ± 0.007
	Error	24				
					No Predators	**Crayfish Predators**
Survival	Predators	1	13.49	< 0.01	0.87 ± 0.04	0.70 ± 0.04
	Error	24				
Time (d)	Predators	1	0.08	0.77	30.5 ± 0.2	30.4 ± 0.2
	Error	24				
Mass (g)	Predators	1	11.71	< 0.01	0.219 ± 0.007	0.186 ± 0.007
	Error	24				
					Open Canopy	**Closed Canopy**
Survival	Canopy	1	1.62	0.21	0.75 ± 0.04	0.82 ± 0.04
	Error	24				
Time (d)	Canopy	1	14.90	< 0.01	29.8 ± 0.2	31.0 ± 0.2
	Error	24				
Mass (g)	Canopy	1	20.23	< 0.01	0.180 ± 0.007	0.225 ± 0.007
	Error	24				

Table 4. – Summary of univariate analyses of variance (ANOVA) of survival to metamorphosis, larval period (time), and mass at metamorphosis for American toads (*Bufo americanus*) in Experiment 3; pond means are presented ± 1 SE.

Response Variable	Source of Variation	df	*F*	*P*	Open Canopy	Closed Canopy
Survival	Canopy	1	3.45	0.08	0.82 ± 0.04	0.90 ± 0.04
	Error	12				
Time (d)	Canopy	1	4.03	0.06	30.4 ± 0.4	31.3 ± 0.4
	Error	12				
Mass (g)	Canopy	1	5.32	0.03	0.202 ± 0.009	0.235 ± 0.009
	Error	12				
					Salamanders Absent	**Salamanders Present**
Survival	Salamanders	1	1.04	0.32	0.84 ± 0.04	0.88 ± 0.04
	Error	12				
Time (d)	Salamanders	1	0.02	0.89	30.9 ± 0.3	30.8 ± 0.3
	Error	12				
Mass (g)	Salamanders	1	1.72	0.41	0.226 ± 0.009	0.211 ± 0.009
	Error	12				

Fig. 1. – For American toads (*Bufo americanus*) in Experiment 1: (A) The trade-off between larval period (days to metamorphosis) and mass at metamorphosis for toads reared in open-canopy and closed-canopy experimental ponds and (B) The interaction of canopy cover and vegetation treatments on toad larval period (days to metamorphosis). Error bars represent ± 1 SE.

Fig. 2. – Influence of vegetation and predators on mass at metamorphosis (A) and survival to metamorphosis (B) for American toads (*Bufo americanus*) in Experiment 2. Error bars represent ± 1 SE.

Fig. 3. – Influence of pond habitat type and *Ambystoma* presence/absence on survival to metamorphosis for American toads (*Bufo americanus*) in Experiment 3. Error bars represent ± 1 SE.

Fig. 1A

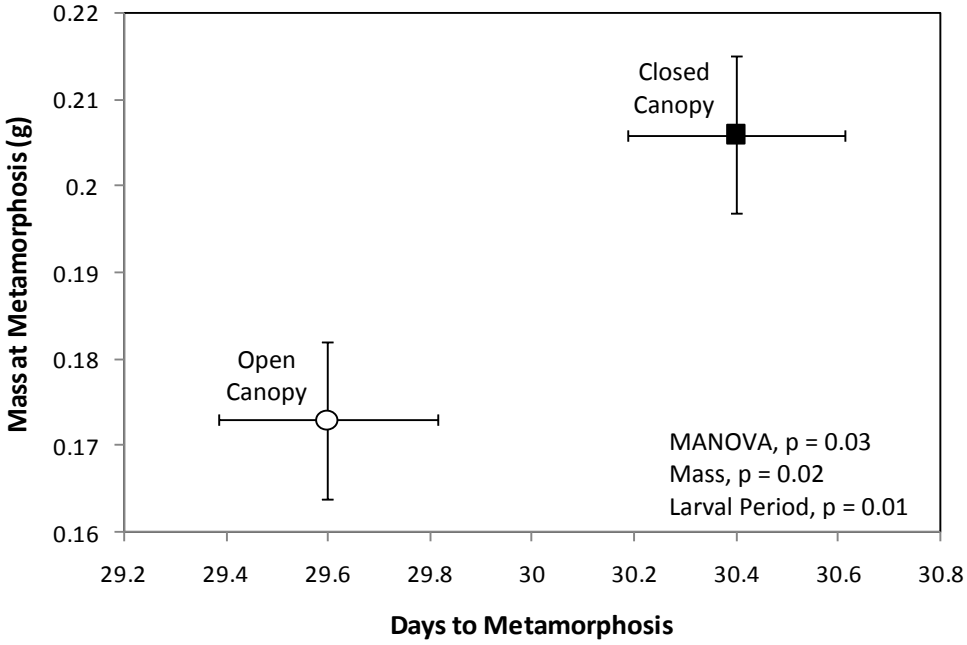

0.22
0.21
0.2
0.19
0.18
0.17
0.16
Mass at Metamorphosis (g)
Closed Canopy
Open Canopy
MANOVA, p = 0.03
Mass, p = 0.02
Larval Period, p = 0.01
29.2
29.4
29.6
29.8
30
30.2
30.4
30.6
30.8
Days to Metamorphosis

Fig. 1B

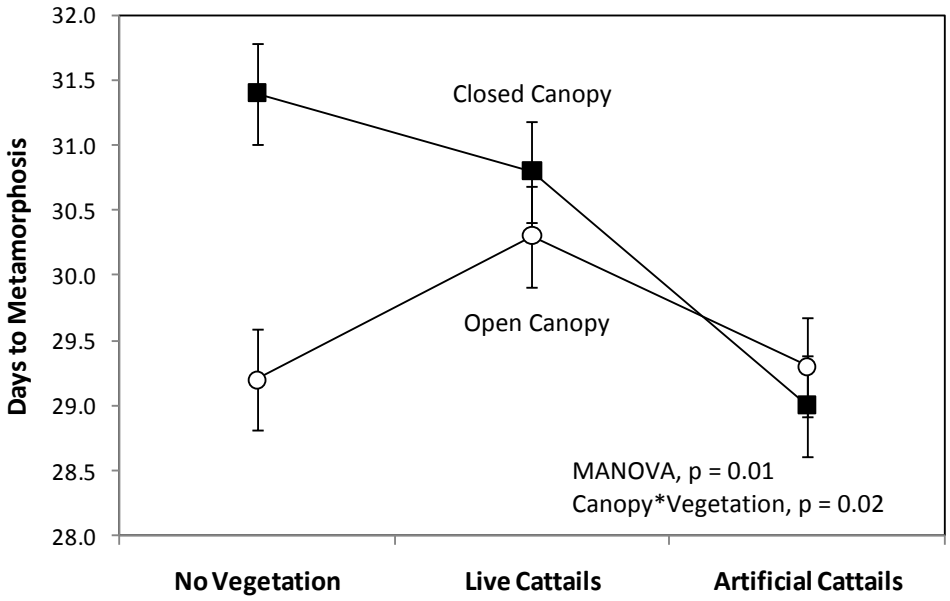

32.0
31.5
31.0
30.5
30.0
29.5
29.0
28.5
28.0
Days to Metamorphosis
Closed Canopy
Open Canopy
MANOVA, p = 0.01
Canopy*Vegetation, p = 0.02
No Vegetation
Live Cattails
Artificial Cattails

Fig. 2A

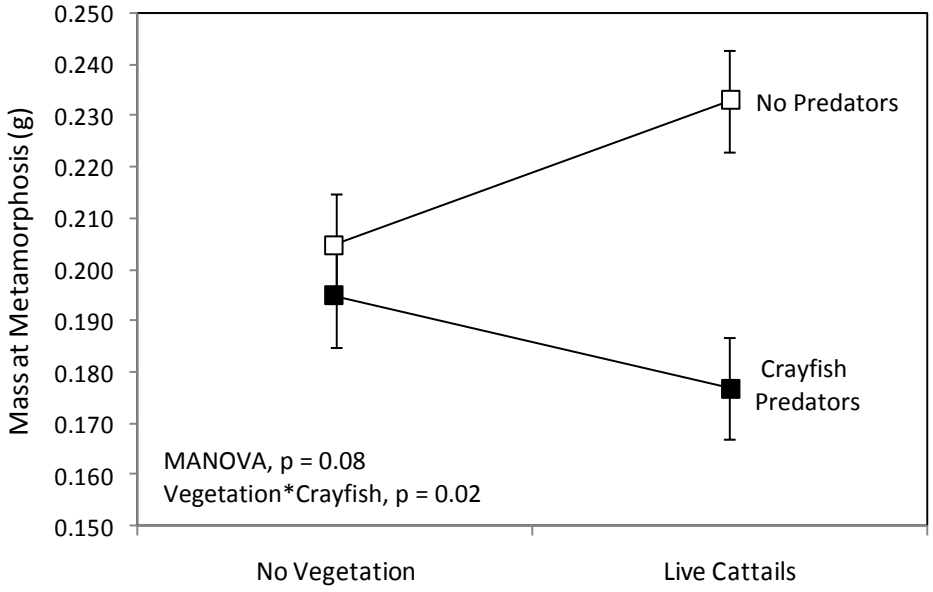

0.250
0.240
0.230
0.220
0.210
0.200
0.190
0.180
0.170
0.160
0.150
Mass at Metamorphosis (g)
No Predators
Crayfish Predators
MANOVA, p = 0.08
Vegetation*Crayfish, p = 0.02
No Vegetation
Live Cattails

Fig. 2B

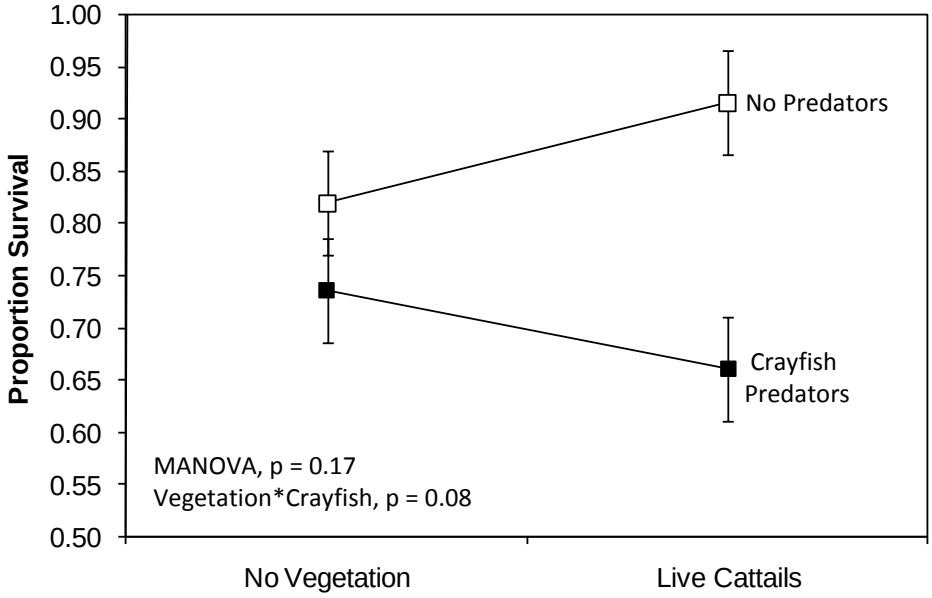
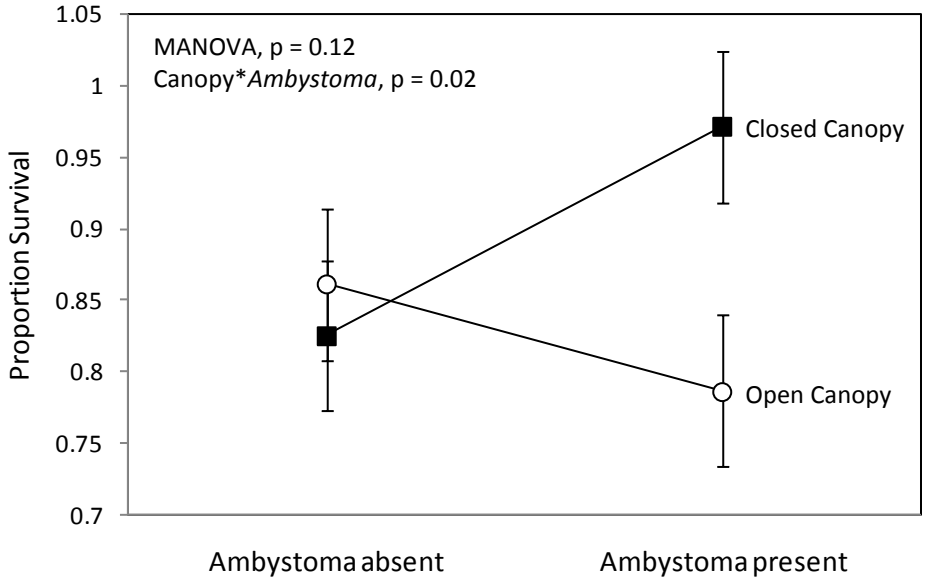

1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
Proportion Survival
No Predators
Crayfish Predators
MANOVA, p = 0.17
Vegetation*Crayfish, p = 0.08
No Vegetation
Live Cattails

Fig. 3


MANOVA, p = 0.12
Canopy*Ambystoma, p = 0.02
Closed Canopy
Open Canopy
Proportion Survival
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
Ambystoma absent
Ambystoma present

Appendix A – Periphyton, phytoplankton, zooplankton, pH, dissolved oxygen, and water temperature (means ± 1 SE) by date for Experiments 1-3. Asterisks (*) indicate significant differences ($P < 0.05$) between canopy treatments.

		Periphyton (µg/L)		Phytoplankton (µg/L)			Zooplankton Abundance	
	Date	Open Canopy	Closed Canopy	Open Canopy	Closed Canopy	**Date**	Open Canopy	Closed Canopy
Experiment 1	May 9	27.6 ± 25.0*	112.3 ± 25.0*	7.2 ± 2.5	8.9 ± 2.5	May 11	20.8 ± 4.3	31.3 ± 4.2
	May 23	85.5 ± 67.7	210.1 ± 67.7	3.4 ± 10.1	16.9 ± 10.1	June 25	646.2 ± 86.5*	192.7 ± 86.5*
	June 6	98.9 ± 31.2	158.5 ± 31.2	135.2 ± 80.9	4.8 ± 80.9			
	June 20	182.2 ± 56.0	223.4 ± 56.0	52.9 ± 16.7	20.4 ± 16.7			
Experiment 2	May 9	47.7 ± 22.9*	135.9 ± 22.9*	13.9 ± 3.1	8.7 ± 3.1	May 11	30.8 ± 7.0	30.7 ± 7.1
	May 23	100.0 ± 24.9	119.1 ± 24.9	3.4 ± 7.6	12.9 ± 7.6	June 25	554.4 ± 82.0*	212.3 ± 82.0*
	June 6	209.1 ± 38.2	131.1 ± 38.2	99.5 ± 60.6	4.2 ± 60.6			
	June 20	302.2 ± 115.3	265.3 ± 115.3	47.9 ± 21.1	56.3 ± 21.1			
Experiment 3	May 9	98.8 ± 29.5	96.8 ± 29.5	16.7 ± 5.0	5.36 ± 5.0	May 11	22.4 ± 7.1	25.4 ± 7.0
	May 23	87.3 ± 30.5	62.7 ± 30.5	2.60 ± 0.4	1.81 ± 0.4	June 25	606.0 ± 146.7	321.6 ± 146.6
	June 6	259.6 ± 72.7	81.7 ± 72.7	18.9 ± 3.4*	4.7 ± 3.4*	July 20	411.4 ± 134.2	553.8 ± 134.1
	June 20	427.7 ± 208.7	146.0 ± 208.7	23.9 ± 24.8	40.0 ± 24.8			
	July 4	455.3 ± 175.4	395.8 ± 175.4	31.7 ± 29.6	44.8 ± 29.6			

		pH		Dissolved Oxygen (mg/L)		Temperature (°C)	
	Date	Open Canopy	Closed Canopy	Open Canopy	Closed Canopy	Open Canopy	Closed Canopy
Experiment 1	May 9	7.95 ± 0.03	7.94 ± 0.03	5.89 ± 0.34	6.00 ± 0.34	20.9 ± 0.07*	19.3 ± 0.07*
	May 23	7.94 ± 0.04*	8.13 ± 0.04*	2.74 ± 0.26*	4.90 ± 0.26*	22.5 ± 0.16	22.3 ± 0.16
	June 6	7.95 ± 0.06*	8.14 ± 0.06*	8.85 ± 0.92	9.50 ± 0.92	25.5 ± 0.07*	23.5 ± 0.07*
	June 20	8.00 ± 0.07	8.05 ± 0.07	4.55 ± 0.30	4.65 ± 0.30	19.6 ± 0.07*	21.0 ± 0.07*
Experiment 2	May 9	7.96 ± 0.03	7.94 ± 0.03	6.51 ± 0.21	6.49 ± 0.21	20.1 ± 0.07*	19.3 ± 0.07*
	May 23	7.89 ± 0.04*	8.09 ± 0.04*	2.66 ± 0.21*	4.82 ± 0.21*	22.7 ± 0.14*	22.0 ± 0.14*
	June 6	7.94 ± 0.06*	8.14 ± 0.06*	8.89 ± 0.80	9.58 ± 0.80	25.5 ± 0.12*	23.5 ± 0.12*
	June 20	7.98 ± 0.07	8.05 ± 0.07	3.83 ± 0.26*	4.74 ± 0.26*	19.8 ± 0.08*	21.1 ± 0.08*
Experiment 3	May 9	8.03 ± 0.02*	7.93 ± 0.02*	7.34 ± 0.25*	6.18 ± 0.25*	20.4 ± 0.08*	19.3 ± 0.08*
	May 23	7.86 ± 0.05*	8.08 ± 0.05*	2.68 ± 0.33*	4.95 ± 0.33*	23.0 ± 0.23*	21.9 ± 0.23*
	June 6	7.94 ± 0.07	8.09 ± 0.07	8.68 ± 0.77	8.48 ± 0.77	25.6 ± 0.12*	23.5 ± 0.12*
	June 20	8.00 ± 0.09	8.13 ± 0.09	4.01 ± 0.46	5.31 ± 0.46	20.0 ± 0.15*	20.9 ± 0.15*
	July 4	7.85 ± 0.13	7.79 ± 0.13	4.88 ± 0.56	5.51 ± 0.56	20.9 ± 0.07	20.8 ± 0.07

CHAPTER 3:

Habitat-Induced Plasticity in Metamorphic and Post-Metamorphic Traits of Pond-Breeding Amphibians

Abstract – Phenotypic plasticity has been observed in a range of taxa, including amphibians which have been shown to exhibit widespread plasticity in metamorphic traits. In addition to substantial evidence of plastic metamorphic responses to biotic factors, other studies have demonstrated similar responses to changes in abiotic factors (e.g., pond hydroperiod, canopy cover). These physical attributes of the larval environment may also have latent effects on plastic phenotypes in subsequent life stages. For juvenile pond-breeding amphibians, terrestrial locomotor ability is an important trait because juveniles must typically disperse overland from their natal pond to suitable terrestrial habitat. Differences in the larval environment could alter the developing physiological capabilities that underlie locomotor ability, which can impact individual fitness and population persistence. We raised toad (*Bufo americanus*) and salamander (*Ambystoma maculatum*) larvae in outdoor pond mesocosms designed to simulate open- and closed-canopy pond habitats, and examined both metamorphic traits (i.e., size at metamorphosis, larval period, and survival) and post-metamorphic performance (i.e., speed and endurance abilities). Toads raised in closed-canopy ponds were larger at metamorphosis and had a greater probability of survival to metamorphosis than those raised in open-canopy ponds. Toads exhibited a size-mediated trade-off between speed and endurance, such that small toads were slower but had better endurance relative to large toads. Moreover, after correcting for the influence of body size, toads from open-canopy ponds still exhibited better endurance ability than those from closed-canopy ponds. Conversely, salamanders from open-canopy habitats were larger than those from closed-canopy habitats; post-metamorphic salamanders did not exhibit a trade-off between speed and endurance abilities. Our results suggest that pond habitat type can affect not only fitness-related metamorphic traits of pond-breeding amphibians, but also the potential ability of juveniles to disperse to suitable terrestrial habitat.

Introduction

The environment can play a significant role in determining the expression of various traits throughout ontogeny (Wilbur et al. 1974). Phenotypic changes, particularly those induced early in development, can have negative impacts (e.g., Henry and Ulijaszek 1996); however, many plastic phenotypic responses to changing environmental conditions have been shown to be adaptive (Dewitt and Scheiner 2004). For instance, predators can induce phenotypic plasticity in their prey, such as the growth of defensive structures by zooplankton (Hammill et al. 2008), different shell morphologies for predator resistance by freshwater snails (DeWitt et al. 2000), and morphological structures that facilitate escape by fish (Domenici et al. 2008). Thus, the maintenance of plastic traits is advantageous because it gives animals a better opportunity for survival in environments that are suboptimal or unpredictable.

Amphibians are one group that shows widespread plasticity in metamorphic traits, such as timing of metamorphosis and size at metamorphosis, in response to variation in the larval environment. Much of the existing literature on phenotypic plasticity in amphibians has focused on plastic metamorphic responses to predator cues in the larval environment (Relyea and Werner 2000; Van Buskirk and Schmidt 2000; Relyea 2001a, 2001b); however, other studies have shown similarly strong metamorphic responses to changes in abiotic factors such as pond drying (Crump 1989; Newman 1992; Merila et al. 2000; Relyea 2001a) and canopy cover (Werner and Glennemeier 1999; Skelly et al. 2002; Schiesari 2006).

Pond-breeding amphibians are currently threatened by habitat loss and alteration (Lehtinen et al. 1999; Dodd and Smith 2003; Cushman 2006). Alterations to larval aquatic habitats, such as changes in hydroperiod and habitat structure, can have dramatic effects on amphibians by altering traits such as size at and timing of metamorphosis, which are correlates of future fitness (e.g., Skelly et al. 2002; Wells 2007; Purrenhage and Boone 2009). Therefore, persistence of amphibian populations in regions affected by habitat loss and alteration depend in part on species' capacities to exploit alternative phenotypes in a changing environment (Newman 1992; Wells 2007). Together, the pervasiveness of habitat alteration and evidence that amphibians can respond to abiotic factors during the larval phase suggest that variation in the physical environment should be further explored as a major factor influencing phenotypic plasticity in amphibians.

One aspect of plasticity that remains understudied in developing amphibians, as well as other taxa (reviewed in Pechenik 2006), is the potential for latent effects of the larval environment on subsequent life stages; most of the existing literature for amphibians describes delayed effects of chemical exposure (e.g., Rehage et al. 2002; Boone 2005; Griffis-Kyle 2005; Rohr et al. 2006) and larval density (Goater 1994; Morey and Reznick 2001; Chelgren et al. 2006) on post-metamorphic individuals. However, variation in other characteristics of the larval environment (e.g., habitat structure) may similarly influence developing physiological and biochemical mechanisms that underlie performance in the terrestrial stage. Altered terrestrial performance resulting from certain attributes of the larval environment could have an enormous impact on fitness of terrestrial juveniles via potential effects on dispersal abilities, anti-predator behaviors, and foraging efficiency (Irschick and Garland 2001; Kingsolver et al. 2001; Irschick 2003).

For pond-breeding amphibians, locomotor ability is, arguably, the most important performance trait of individuals in the terrestrial juvenile stage. Post-metamorphic pond-breeding amphibians must disperse overland, often long distances relative to their small body size, from their natal pond to suitable terrestrial habitat, where they forage and locate appropriate overwintering sites (Breden 1987; Rothermel and Semlitsch 2002; Semlitsch 2003). Plasticity resulting from variation in larval habitats can impact size at and timing of metamorphosis, but it is not known whether habitat variation can also cause changes in morphology and physiology that affect dispersal abilities of terrestrial juveniles. If so, these latent effects on locomotor ability may have significant effects on individual fitness and population persistence.

Sprinting ability (speed) is generally advantageous for successful foraging, predator avoidance, mate location, and dispersal (Arnold 1983; Bennett 1989; Jayne and Bennett 1990). However, depending on the location of an amphibian's natal pond in the landscape (i.e., proximity to suitable terrestrial habitat), a higher endurance capacity may be more important than speed for successful post-metamorphic dispersal. It has been suggested that there is a trade-off between sprint speed and endurance within vertebrates, since maximizing these two activities requires different proportions of muscle fiber types. Most of the cited evidence for this trade-off, though, stems from observations that human world-class sprinters possess a higher proportion of fast-twitch muscle fibers, whereas marathoners have more fatigue-resistant slow-twitch muscle fibers (Komi 1984; Heinrich 1985; Esbjornsson et al. 1993). However, there is little evidence

supporting the conflict between speed and endurance in other animals; most studies have shown little or no correlation between these two performance measures at the whole-organism level (e.g. Huey et al. 1990; Sorci et al. 1995; Vanhooydonck et al. 2001) despite a trade-off being observed between maximum power output and fatigue resistance at the muscle level (Wilson et al. 2002). If differences in the environment of developing larval amphibians can shape the morphological and physiological traits of post-metamorphic individuals, then selection might be expected to favor plastic traits that permit individuals to optimize performance in different landscapes. For example, individuals that develop in ponds located far from optimal terrestrial habitats might possess adaptive plastic traits that promote an increase in endurance capacity over those that develop in ponds closer to or within favorable terrestrial habitats. Consequently, if there is a trade-off between speed and endurance in recently metamorphosed amphibians, it is reasonable to expect the direction of the trade-off (i.e., endurance favored over speed or vice versa) to be driven by the characteristics of the larval habitats.

We raised toad and salamander larvae in outdoor pond mesocosms designed to simulate open- and closed-canopy pond habitats. We assessed typical metamorphic traits (i.e., size at metamorphosis, larval period, and survival) and post-metamorphic dispersal abilities of toads and salamanders. We predicted the following results for both species: (1) toads and salamanders from open-canopy habitats will be larger at metamorphosis, have shorter larval periods, and have a greater probability of surviving to metamorphosis than those from closed-canopy habitats; (2) because of the different locomotor challenges presented to metamorphs from open- and closed-canopy pond habitats, toads and salamanders from open-canopy habitats will optimize endurance ability to facilitate dispersal to closed-canopy terrestrial habitat, whereas individuals from closed-canopy pond habitats will optimize sprinting ability to enhance foraging and predator avoidance in the absence of a predominating need to disperse; and (3) the influence of pond habitat type on metamorphic traits and on post-metamorphic locomotor ability may differ between toads and salamanders due to differences in these species' life history characteristics.

Methods

Study System

American toads (*Bufo americanus*) and spotted salamanders (*Ambystoma maculatum*) are typical pond-breeding amphibians; both species spend the majority of their lives in the terrestrial

environment. This biphasic lifestyle requires multiple migrations between their wetland and upland habitats. Toads and salamanders employ very different strategies during the larval phase: American toads have a characteristically short larval period and are primarily herbivorous (McDiarmid and Altig 1999), whereas spotted salamanders have a longer larval period and are primarily zooplanktivorous (Petranka 1998). Both species breed in a range of pond types that vary in hydroperiod, canopy cover, and vegetation structure, among other habitat attributes, and in some areas they coexist during the larval stage (Babbitt et al. 2003; J.P., personal observation). Larval amphibian densities vary widely in nature (14-4238 per 1000 L; e.g., Morin 1983, Petranka 1989). We reared toads (70 per 1000 L) and salamanders (14 per 1000 L) at initial densities that reflected the relative availability of each species' eggs at the collection sites, and thus, experimental densities approximated natural larval densities in southeastern Ohio, USA.

On 19 April 2007, we collected American toad larvae from six larval aggregations located throughout a permanent, open-canopy pond at Woodland Trails Wildlife Area (Preble County, Ohio, USA); the larvae were densely schooled in distinct aggregations in shallow vegetation near the remnant egg strings, and were presumably still in sibling groups when collected. On 10 April 2007, we collected spotted salamander eggs from five distinct egg masses from a temporary, partial-canopy pond at Indian Creek Preserve MetroPark (Butler County, Ohio, USA). Toad and salamander larvae were reared, segregated by species, in outdoor cattle tanks (with 1000 L water, 1 kg leaf litter, and plankton from a natural pond) at the Miami University Ecology Research Center (ERC) until they were added to the experimental ponds; for both species, larvae from different aggregations (toads) or egg masses (salamanders) were mixed prior to being added to experimental ponds in order to minimize potential clutch-related biases.

Pond Mesocosms

We created larval amphibian communities composed of toads and salamanders in pond mesocosms to test the effects of pond habitat type (open- vs. closed-canopy pond habitats) on metamorphic traits, as well as the latent effects of pond habitat type on terrestrial locomotor ability of post-metamorphic toads and salamanders. The pond mesocosm experiment was conducted outdoors in eight 1000-L cattle tanks at the Miami University ERC from 5 May through 27 July 2007. Each pond was stocked with both species: 70 *Bufo americanus* larvae and 14 *Ambystoma maculatum* larvae. We used two habitat treatments (open- and closed-canopy habitats) with four replicates of each treatment, and treatments were randomly assigned to the

grid of ponds. Each pond was filled with 1000 L water, 1 kg dry leaf litter from a nearby mixed deciduous forest (primarily *Acer*), and inoculations of algae and zooplankton from a fishless pond located at the Miami University ERC. Open-canopy habitat was simulated using: (1) a mesocosm cover that blocked 30% of incoming light and (2) 15 cattail (*Typha latifolia*) plants that occupied approximately one-third of the pond area to further simulate a typical open-canopy pond. Closed-canopy habitat was simulated using a mesocosm cover that blocked 65% of incoming light; there was no vegetation added to closed-canopy mesocosms because in nature these ponds typically lack understory pond vegetation. Open-canopy mesocosm covers were constructed of a single layer of 1.9-cm black plastic netting; closed-canopy covers were constructed of two overlapping layers of 0.2-cm black fiberglass mesh. We used a Biospherical Instruments QSL spherical PAR detector to test the percent transmission of visible light (PAR) through each mesocosm cover. These measurements were carried out in a darkened room with no light other than our intended light source, a white compact fluorescent lamp (GE Helical 26W) mounted inside a 30.5-cm photoreflector; the probe was mounted perpendicular to the light source, 60 cm from the front of the reflector. Samples of each mesocosm cover were placed in front of the detector (60 cm from the probe), three readings were taken, and the mean percent transmission was calculated for each material. Open-canopy covers blocked ~30% overhead light (mean % transmission = 69.5%), whereas closed-canopy covers blocked ~65% (mean % transmission = 34.5%). Cattails used in open-canopy treatments were collected from a pond at the ERC and transplanted into 39 x 56 x 13.5-cm (27-L capacity) clear Rubbermaid® containers filled with soil and pea gravel; closed-canopy treatments had no cattails, but did contain a Rubbermaid® container with soil and pea gravel.

We checked the ponds daily for metamorphs; toads with at least one front limb emerged (Gosner stage 42, Gosner 1960) or salamanders with resorbed gills were collected and transported to the laboratory at Miami University for processing. We measured size at metamorphosis (mass and snout-vent length) of toads once tail resorption was complete (Gosner stage 46, Gosner 1960); however, we only recorded snout-vent length of toads used in locomotor performance trials. We measured size at metamorphosis (mass and snout-vent length) of salamanders once gill resorption was complete. We also calculated percent survival for each species in each pond (i.e., the number of individuals that successfully metamorphosed by the end

of the experiment, 27 July, divided by the stocking density for that species). We calculated pond means for mass and survival, and used ponds as the experimental units in all analyses.

To assess differences in water chemistry and food resources between pond habitat treatments, we measured water pH, dissolved oxygen concentration (DO), water temperature, periphyton abundance, and zooplankton abundance throughout the experiment. We measured pH, DO, and water temperature (^{o}C) for each mesocosm on 9 May, 23 May, 6 June, 20 June, and 4 July. Following the procedure described in chapter 2, we collected uniform periphyton samples from the walls of each mesocosm on the same dates that we measured water characteristics. We obtained measurements of chlorophyll a concentration (µg/L) via fluorometry of periphyton samples (as described in chapter 2), and used chlorophyll a concentration as a proxy for relative abundance of algal resources. We collected zooplankton samples (as described in chapter 2) from each pond on 11 May, 25 June, and 20 July.

Locomotor Performance

We retained 80 metamorphs of each species (40 from open-canopy ponds and 40 from closed-canopy ponds) for use in locomotor performance trials. Following the collection of morphological data, these toad and salamander metamorphs were placed individually in 18 x 13 x 8-cm (1-L capacity) plastic containers lined with a moistened paper towel. Locomotor performance trials were conducted beginning one day following the acquisition of morphological data (i.e., one day after completion of metamorphosis). For both species, maximum sprint speed and endurance were measured one day apart and the order of performance trials for each individual was randomly determined. For half of the toads and salamanders used in this study, sprint speed was measured one day after metamorphosis, and endurance was measured on the following day; the other half was measured in reverse (i.e., first endurance, then sprint speed). All performance trials were conducted at room temperature (24 ± 1 °C). If an individual failed to cooperate during either its speed or endurance trial, we repeated that performance trial on the following day; if the second attempt was unsuccessful, then that individual was excluded from the appropriate analysis (speed or endurance).

Maximum Sprint Speed – The dorsal view of each toad and salamander was videotaped with a Sony DCR-TRV460 Digital8 (New York, NY, USA) at 30 frames/s while being stimulated to move at maximal speed down a 1.0 m- long track. Each individual was stimulated to move down the track by lightly tapping its foot, backside, or tail with a spatula for three

consecutive trials. Maximum speed, and maximum hop length and hop frequency for toads, was later determined from video analyses. For each trial, we estimated maximum sprint speed as the fastest speed over three or more consecutive movements. For each individual, the maximum speed was the fastest of the three trials. We obtained maximum speed data for 80 toads (100%) and 69 salamanders (38 of 40 from closed-canopy ponds; 31 of 40 from open-canopy ponds).

Endurance – Endurance trials were conducted on a custom-built treadmill (25 x 10 cm) connected to a rheostat to control speed. The treadmill contained a burlap tread and was set to a constant predetermined speed (0.02 m/s) that represented sub-maximum speed for both toads and salamanders. Oftentimes, the animals would exhibit a short burst of speed during the first few seconds of the trial before slowing their pace to closely match that of the treadmill with minimal stimulation (using a spatula or finger). Animals were considered exhausted when they no longer moved ahead of the posterior barrier (piece of styrofoam) of the treadmill following three stimulation attempts. The time until exhaustion was used as a measure of endurance capacity. We obtained endurance data from 80 toads (100%) and 72 salamanders (37 of 40 from closed-canopy ponds; 35 of 40 from open-canopy ponds).

Data Analyses

For both species, we tested for effects of pond habitat type (open-canopy, closed-canopy) on metamorphic traits (i.e., survival, size at metamorphosis, larval period) for two datasets: the complete dataset (all individuals that metamorphosed from our experimental ponds) and a reduced dataset (only those individuals included in the locomotor performance trials). We tested for effects of pond habitat type on larval period, mass at metamorphosis, and survival to metamorphosis of toads and salamanders using analysis of variance (ANOVA), and we tested the multivariate metamorphic response using multivariate analyses of variance (MANOVA). In preliminary analyses of larval period and mass response, we used survival to metamorphosis as a covariate to account for the influence of differential survival between treatments; however, reduced survival was associated with reduced mass. Because the survival covariate compensated for differences between treatments in a way contrary to the typical density-dependent manner, we did not include a covariate in our final analyses. We tested for latent effects of pond habitat type on subsequent locomotor performance (maximum sprint speed and endurance) of toads and salamanders using analyses of covariance (ANCOVA). Because body size has been shown to have a strong influence on locomotor ability (Alexander 2003; Jones et al. 2007), we used

metamorph body mass and snout-vent length (SVL) as covariates in separate analyses testing for treatment effects on speed and endurance performance. We tested for differences in water characteristics (pH, temperature, DO), periphyton, and zooplankton between pond habitat treatments using repeated-measures ANOVA. All data were transformed as necessary to meet the assumption of normality. Survival data were expressed as proportions and were angularly transformed, whereas mass and snout-vent length data were log-transformed. Statistical analyses were performed using SAS version 9.1.

Results

Effects of Larval Pond Habitat on Metamorphic Traits

For both species, the response of the reduced dataset was slightly stronger than that of the complete dataset in some instances, but reflected the same pattern of response. Pond habitat type influenced the multivariate response of toads (complete dataset: Wilks' Lambda = 0.2451, $F_{3,4}$ = 4.10, P = 0.10; reduced dataset: Wilks' Lambda = 0.0413, $F_{4,3}$ = 17.39, P = 0.02), but the multivariate response of salamanders was not significant (complete dataset: Wilks' Lambda = 0.1863, $F_{4,3}$ = 3.27, P = 0.17; reduced dataset: Wilks' Lambda = 0.1195, $F_{4,3}$ = 5.53, P = 0.09). Univariate analyses revealed that survival to metamorphosis was greater in closed-canopy habitats than in open-canopy habitats for toads (Fig. 1A; Table 1); survival of salamanders did not differ significantly between habitats (Fig. 1A; Table 1). Pond habitat type influenced mass at metamorphosis and larval period for toads (Fig. 1B; Table 1). Toads from closed-canopy habitats had a slightly longer larval period, but were larger at metamorphosis despite higher survival in closed-canopy treatments (Fig. 1B). Salamanders also had a longer larval period in closed-canopy habitats, but they metamorphosed at a larger size from open-canopy habitats (Fig. 1C; Table 1). The responses of snout-vent length at metamorphosis for toads and salamanders mirrored their respective mass-at-metamorphosis responses to pond habitat treatments (Table 1).

Water Characteristics and Food Resources in Open-Canopy and Closed-Canopy Habitats

Water pH did not vary significantly over time (Wilks' Lambda = 0.6890, $F_{4,11}$ = 1.24, P = 0.35), but did vary between pond habitat treatments over time (Wilks' Lambda = 0.3667, $F_{4,11}$ = 4.75, P = 0.02); pH was significantly different ($P \leq 0.03$) on two of five sample dates. However, there was no consistent pattern and differences in pH were likely not biologically relevant; pH ranged from 7.9 to 8.1 across treatments (Table 2). Dissolved oxygen (DO) varied over time

(Wilks' Lambda = 0.0818, $F_{4,11}$ = 30.87, P < 0.01) and between pond habitat treatments over time (Wilks' Lambda = 0.1975, $F_{4,11}$ = 11.17, P < 0.01). DO was significantly different (P ≤ 0.05) on three of four sample dates; DO was slightly greater in closed-canopy treatments in two of those samples (Table 2). Water temperature also varied over time (Wilks' Lambda = 0.0023, $F_{4,11}$ = 1187.6, P < 0.01) and between pond habitat treatments over time (Wilks' Lambda = 0.0269, $F_{4,11}$ = 99.37, P < 0.01). Temperature was significantly different (P < 0.01) between pond habitats on four of five sample dates; open-canopy treatments were typically 1-2° warmer than closed-canopy treatments (Table 2). Periphyton abundance (µg/L chlorophyll a) varied over time (Wilks' Lambda = 0.0658, $F_{4,3}$ = 10.64, P = 0.04), but did not vary between pond habitat treatments over time (Wilks' Lambda = 0.0658, $F_{4,3}$ = 0.34, P = 0.84); there was a trend ($F_{1,6}$ = 4.88, P = 0.07) of greater periphyton abundance in open-canopy habitats on one of five sample dates (Table 2). Zooplankton abundance varied over time (Wilks' Lambda = 0.1674, $F_{2,5}$ = 12.43, P = 0.01), but did not vary between pond habitat treatments over time (Wilks' Lambda = 0.7910, $F_{2,5}$ = 0.66, P = 0.56); there was a trend ($F_{1,6}$ = 4.28, P = 0.08) of greater zooplankton abundance in closed-canopy habitats on one of five sample dates (Table 2).

Effects of Larval Pond Habitat on Post-Metamorphic Performance

Toads reared in closed-canopy habitats were faster than those reared in open-canopy habitats (Fig. 2A); however, after accounting for the influence of size differences with the analyses of covariance, maximum sprint speed of toads did not differ between pond habitats (Fig. 2B; Table 3). The disappearance of a significant difference in speed when a body-size covariate was included indicates that toads from closed-canopy ponds were faster because they metamorphosed at a larger size. In contrast, toads that were reared in open-canopy ponds displayed better endurance relative to body size than those from closed-canopy ponds (Figs. 2A and 2B; Table 3). Although in part due to habitat-mediated differences in size at metamorphosis, toads exhibited a trade-off between speed and endurance abilities at the whole-organism level (Figs. 2A and 2B). We observed no differences in speed or endurance abilities of salamanders reared in open-canopy and closed-canopy habitat treatments either with or without body size covariates (Fig. 2C; Table 3).

Discussion

Our study demonstrated that habitat characteristics of the larval pond environment not only induce phenotypic plasticity in metamorphic traits of pond-breeding amphibians, but can also influence the plasticity of vital post-metamorphic traits of terrestrial juveniles. We observed habitat-induced phenotypic plasticity in American toads (*B. americanus*) and spotted salamanders (*A. maculatum*) reared in open- and closed-canopy experimental habitats, as well as differential responses between species. Both species breed in a range of pond habitats, and several key habitat gradients, including pond hydroperiod and canopy cover, have been identified as determinants of individual performance (Denver et al. 1998; Skelly et al. 2002; Schiesari 2006) and community composition (Wellborn et al. 1996; Skelly et al. 1999; Werner et al. 2007) for amphibians. However, a pond's suitability may extend beyond the influence of the pond environment on aquatic larvae to the implications of the pond's location in the landscape for terrestrial juveniles and adults. For instance, an open-canopy pond environment may facilitate faster growth in some species (e.g., this study; Werner and Glennemeier 1999; Skelly et al. 2002), but the pond's distance from suitable upland habitat may inhibit terrestrial dispersal by juveniles (Rothermel and Semlitsch 2002, 2006). Superiority in fitness-correlated metamorphic traits (e.g., greater size at metamorphosis) in certain environments may become relatively inconsequential if performance of terrestrial juveniles from those same environments is compromised. Ultimately, recruitment into the adult population, and persistence of that population, depends on successful post-metamorphic dispersal and survival of juveniles.

We found that toad and salamander larvae exhibited differential metamorphic responses to open- and closed-canopy habitat treatments. Toads had a greater probability of survival to metamorphosis in closed-canopy habitats, whereas salamanders showed no difference in probability of survival between habitats. Toads metamorphosed later from closed-canopy habitats, but traded off a slightly longer larval period for a significant increase in size at metamorphosis. Conversely, salamanders also metamorphosed later from closed-canopy ponds, but without the growth benefit that toads exhibited; salamanders metamorphosed larger from open-canopy habitats. Although in nature both species utilize a range of pond habitats for breeding and are primarily associated with forested upland habitats, toad and salamander larvae occupy different trophic positions in the aquatic environment, which could explain their differential metamorphic responses to environmental gradients. Toad larvae are herbivorous

grazers and salamander larvae are zooplanktivorous predators (Wells 2007); this has implications for the relative importance of food quality and energy expenditure for each species. In accordance with stoichiometry theory, algae have been shown to respond strongly to variation in light availability, such that the C:nutrient (e.g., C:N and C:P) ratio of algae decreases (i.e., higher food quality for toads) in low-light environments (Dickman et al. 2008). Conversely, stoichiometry theory suggests that the C:nutrient ratio of zooplankton remains much more stable across environments (Sterner and Elser 2002). Therefore, the quality of algal resources likely differed between open- and closed-canopy treatments, whereas the quality of zooplankton resources likely did not. Thus, habitat effects on food quality due to variation in light availability may explain the greater mass at metamorphosis of toads from closed-canopy treatments, because algae are more stoichiometrically responsive to environmental variation along the canopy gradient. The explanation for differences in mass at metamorphosis for salamanders (i.e., larger salamanders from open-canopy ponds) is more likely related to temperature effects on energy expenditure of these active predators than it is to variation in food quality. Salamander larvae actively pursue their mobile prey, and thus, must expend more energy to forage compared to grazers. Furthermore, because they are ectothermic, ambient temperature can present strong constraints on this energy expenditure (Bennett 1990). Consequently, it is likely that even small differences in temperature experienced by salamanders throughout the larval period would result in significantly decreased growth rates; hence, smaller metamorphs from closed-canopy habitats.

As we just discussed, the maintenance of plasticity in larval and metamorphic traits of pond-breeding amphibians is important for tolerating the range of local pond characteristics that exists in nature. However, ponds also differ in their locations on the landscape, and so it is equally essential to maintain plasticity in post-metamorphic traits that allow individuals to move between ponds and suitable terrestrial habitats. By definition, open-canopy ponds are often surrounded by unforested terrestrial habitat (e.g., fields, agriculture), whereas closed-canopy ponds are more likely to be surrounded by forested terrestrial habitat. For species that breed in both open- and closed-canopy ponds, but are more dependent on forested habitat during the terrestrial phase, locomotor ability is critical for utilizing open-canopy breeding ponds. Because breeding in open- versus closed-canopy ponds presumably presents different challenges for terrestrial dispersal by juveniles, we expected to observe plasticity in post-metamorphic locomotor abilities of canopy-generalist species. We did not observe habitat-related differences

in locomotor abilities of salamanders; however, toads exhibited plasticity in terrestrial locomotor traits in a manner that suggests adaptive plasticity.

Toads that metamorphosed from open-canopy ponds had better endurance ability than those from closed-canopy ponds. Interestingly, open-canopy toads were on average smaller at metamorphosis. The pattern of smaller toads having better endurance is counterintuitive; typically, locomotor ability is positively correlated with body size (Arnold 1983; Carrier 1996). After the influence of body size was accounted for, there remained a significant effect of pond habitat on endurance ability of toads; this indicates that the influence of pond habitat on endurance ability was not due to size-mediated effects. The multifaceted mechanisms of pond-habitat effects on endurance emphasize that plasticity in locomotor ability is habitat induced. Though the specific mechanism triggering plasticity in endurance ability is unclear, it is likely related to either differences in muscle fiber composition of the leg musculature (i.e., slow-twitch oxidative vs. fast-twitch glycolytic fibers) or differences in metabolic capacity. Most importantly, greater endurance ability of juveniles from open-canopy ponds could facilitate the longer terrestrial dispersal events required by those animals in nature.

Conversely, toads from closed-canopy ponds were faster (i.e., greater maximum sprint speed) than those from open-canopy ponds. Maximizing speed is advantageous for many life activities, including predator avoidance and foraging (Arnold 1983; Bennett 1989). However, it is generally believed that an individual cannot simultaneously maximize speed and endurance abilities because these activities require different muscle fiber types (Huey et al. 1984; Komi 1984). A trade-off between speed and endurance capabilities at the muscle level has been demonstrated repeatedly in vertebrates (Wilson et al. 2002), but a performance trade-off at the whole-organism level is not as commonly observed (Wilson et al. 2002) presumably because other physiological processes can mediate the translation of the muscle-level trade-off to individual performance. We did observe a trade-off between terrestrial speed and endurance abilities in toads. Moreover, the nature of the trade-off differed between open- and closed-canopy habitats, such that toads from open-canopy ponds exhibited better endurance at the cost of reduced speed, and vice versa for closed-canopy toads. The speed portion of the trade-off was explained by a body size-mediated mechanism: closed-canopy toads were larger at metamorphosis and were faster in terrestrial speed trials, whereas open-canopy toads were smaller and slower. The endurance portion of the trade-off, however, could not be explained by

body size differences between habitats; this suggests that some other aspect of toad development or physiology was influenced by larval habitat type and affected subsequent endurance ability of terrestrial juveniles. Further study is needed to identify the specific habitat-induced mechanisms of differential endurance abilities along the canopy gradient. This plasticity in locomotor abilities exhibited by toads is assumed to be adaptive since the direction of the trade-off in each habitat (especially open-canopy ponds) would give toads the best chance of survival.

Conclusions

Most pond-breeding amphibians are cryptic in the terrestrial phase, and studies of the effects of the larval environment on amphibians often end at metamorphosis. In this study, if only metamorphic traits had been considered, closed-canopy environments would appear to recruit superior juveniles: closed-canopy toads were larger at metamorphosis and had a greater probability of surviving to metamorphosis. However, successful recruitment into the adult population requires successful juvenile dispersal following metamorphosis. Assuming the capacity for compensatory growth once individuals reach suitable terrestrial habitat (Boone 2005), our data suggest that locomotor performance trade-offs in toads may act as a potential equalizer of differential larval habitat quality along the canopy gradient. Specifically, although toad metamorphs from open-canopy ponds were smaller, they could probably compensate for their small size at metamorphosis with increased growth rates during the pre-winter period of terrestrial growth (Chapter 4), once they reached suitable terrestrial habitat. Open-canopy toads in our study showed greater endurance ability, which may be an adaptive plastic response that would allow them to overcome the challenge of dispersing through a poor-quality terrestrial environment to locate high-quality terrestrial habitat for foraging and overwintering. By maintaining plasticity in metamorphic traits (Wells 2007; this study), post-metamorphic locomotor traits (Beck and Congdon 2000; this study), and terrestrial juvenile growth rate (Boone 2005; Chapter 4), amphibians, and other taxa with complex life cycles, may cope with changes to their aquatic and terrestrial habitats by equalizing the advantages and disadvantages of each via multi-stage phenotypic plasticity.

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Table 1. – Summary of univariate analyses of variance (ANOVA) of survival to metamorphosis, larval period (time), mass at metamorphosis, and snout-vent length (SVL) at metamorphosis for American toads (*Bufo americanus*) and spotted salamanders (*Ambystoma maculatum*). Results for the complete dataset (i.e., all individuals that metamorphosed from experimental ponds) are shown in parentheses following the results for the reduced dataset (i.e., only individuals included in the locomotor performance study); SVL data were not available (N.A.) for the complete toad dataset.

Response Variable	Source of Variation	df	*F*	*P*	Open-Canopy Habitat means ± 1 SE	Closed-Canopy Habitat means ± 1 SE
B. americanus						
Survival	Habitat	1	12.55	0.01	0.78 ± 0.05	0.97 ± 0.05
	Error	6				
Time (d)	Habitat	1	12.26 (2.79)	0.01 (0.15)	47.9 ± 0.3 (30.2 ± 0.5)	49.2 ± 0.3 (31.4 ± 0.5)
	Error	6				
Mass (g)	Habitat	1	10.30 (4.28)	0.02 (0.08)	0.195 ± 0.010 (0.191 ± 0.014)	0.245 ± 0.010 (0.235 ± 0.014)
	Error	6				
SVL (mm)	Habitat	1	9.28 (N.A.)	0.02 (N.A.)	11.40 ± 0.20 (N.A.)	12.28 ± 0.20 (N.A.)
	Error	6				
A. maculatum						
Survival	Habitat	1	0.37	0.56	0.73 ± 0.05	0.77 ± 0.05
	Error	6				
Time (d)	Habitat	1	4.15 (5.50)	0.09 (0.06)	55.2 ± 1.4 (54.9 ± 1.6)	58.7 ± 1.4 (59.7 ± 1.6)
	Error	6				
Mass (g)	Habitat	1	10.02 (8.79)	0.02 (0.03)	1.39 ± 0.05 (1.38 ± 0.05)	1.12 ± 0.05 (1.12 ± 0.05)
	Error	6				
SVL (mm)	Habitat	1	5.38 (4.12)	0.06 (0.09)	34.9 ± 0.48 (34.9 ± 0.49)	33.2 ± 0.48 (33.3 ± 0.49)
	Error	6				

Table 2. – Water pH, dissolved oxygen concentration, temperature, periphyton abundance, and zooplankton abundance (means ± 1 SE) by date. Asterisks (*) indicate significant differences ($P < 0.05$) and double asterisks (**) indicate marginally significant differences ($P \leq 0.08$) between open-canopy and closed-canopy habitat treatments.

	pH		Dissolved Oxygen (mg/L)		Temperature (°C)	
Date	Open Canopy	Closed Canopy	Open Canopy	Closed Canopy	Open Canopy	Closed Canopy
May 9	8.0 ± 0.03*	7.9 ± 0.03*	7.3 ± 0.2*	6.2 ± 0.2*	20.4 ± 0.1*	19.3 ± 0.1*
May 23	7.9 ± 0.05*	8.1 ± 0.05*	2.7 ± 0.3*	5.0 ± 0.3*	23.0 ± 0.2*	21.9 ± 0.2*
June 6	7.9 ± 0.07	8.1 ± 0.07	8.7 ± 0.7	8.5 ± 0.7	25.6 ± 0.1*	23.5 ± 0.1*
June 20	8.0 ± 0.09	8.1 ± 0.09	4.0 ± 0.4*	5.3 ± 0.4*	20.0 ± 0.1*	20.9 ± 0.1*
July 4	7.9 ± 0.12	7.8 ± 0.12	4.9 ± 0.5	5.5 ± 0.5	20.9 ± 0.1	20.8 ± 0.1

	Periphyton (µg/L)			Zooplankton Abundance	
Date	Open Canopy	Closed Canopy	**Date**	Open Canopy	Closed Canopy
May 9	118.1 ± 45.9	98.3 ± 45.9	**May 11**	15.3 ± 3.8	26.3 ± 3.8
May 23	92.7 ± 22.0**	24.0 ± 22.0**	**June 25**	592.5 ± 180.2	518.3 ± 180.2
June 6	136.1 ± 31.1	66.1 ± 31.1	**July 20**	472.0 ± 243.2**	887.5 ± 243.2**
June 20	207.0 ± 76.9	116.9 ± 76.9			
July 4	695.3 ± 325.6	443.3 ± 325.6			

Table 3. – Summary of analyses of variance (ANOVA) and analyses of covariance (ANCOVA) of larval habitat effects on subsequent terrestrial locomotor performance (maximum speed and endurance) of post-metamorphic American toads (*Bufo americanus*) and spotted salamanders (*Ambystoma maculatum*). Significant *P*-values ($P \leq 0.05$) and the associated means are bolded.

Response Variable	Source of Variation	df	*F*	*P*	Open-Canopy Habitat means ± 1 SE	Closed-Canopy Habitat means ± 1 SE
B. americanus						
Max. Speed	Habitat, no covariate	1	3.81	**0.05**	**0.063 ± 0.005**	**0.077 ± 0.005**
	Error	78				
	Habitat, mass covariate**	1	1.39	0.24	0.065 ± 0.006	0.075 ± 0.006
	Error	77				
	Habitat, svl covariate*	1	1.30	0.26	0.065 ± 0.006	0.075 ± 0.006
	Error	77				
Endurance	Habitat, no covariate	1	4.31	**0.04**	**186.30 ± 14.27**	**144.35 ± 14.28**
	Error	78				
	Habitat, mass covariate	1	8.50	**< 0.01**	**198.09 ± 14.95**	**132.56 ± 14.95**
	Error	77				
	Habitat, svl covariate	1	9.81	**< 0.01**	**199.57 ± 14.64**	**131.08 ± 14.64**
	Error	77				
A. maculatum						
Max. Speed	Habitat, no covariate	1	0.02	0.89	0.147 ± 0.016	0.150 ± 0.017
	Error	67				
	Habitat, mass covariate	1	0.06	0.81	0.151 ± 0.016	0.145 ± 0.018
	Error	66				
	Habitat, svl covariate	1	0.00	0.98	0.149 ± 0.018	0.148 ± 0.016
	Error	66				
Endurance	Habitat, no covariate	1	0.11	0.74	415.51 ± 60.52	443.62 ± 58.86
	Error	70				
	Habitat, mass covariate	1	0.45	0.51	398.55 ± 63.17	459.67 ± 61.31
	Error	69				
	Habitat, svl covariate**	1	0.85	0.36	389.26 ± 60.34	468.46 ± 58.62
	Error	69				

Asterisks (*) indicate that the covariate was significant (Type I, P < 0.05)
Double asterisks (**) indicate that the covariate was marginally significant (Type I, P ≤ 0.06)

Fig. 1. – (A) Differences in survival to metamorphosis between pond habitat treatments for American toads (*B. americanus*) and spotted salamanders (*A. maculatum*); different letters indicate significant differences between habitat treatments within species. Trade-off between mass at metamorphosis and larval period (days to metamorphosis) for *B. americanus* (B) and *A. maculatum* (C). Mass and larval period means represent the reduced datasets for both species (i.e., only individuals used in locomotor performance trials); error bars represent means ± 1 SE.

Fig. 2. – Trade-offs between maximum sprint speed and endurance for American toads (*B. americanus*) using means without a body-size covariate (A) and with snout-vent length (svl) as a covariate (B), and for spotted salamanders (*A. maculatum*) using means without a body-size covariate (C). Error bars represent means ± 1 SE.

Fig. 1

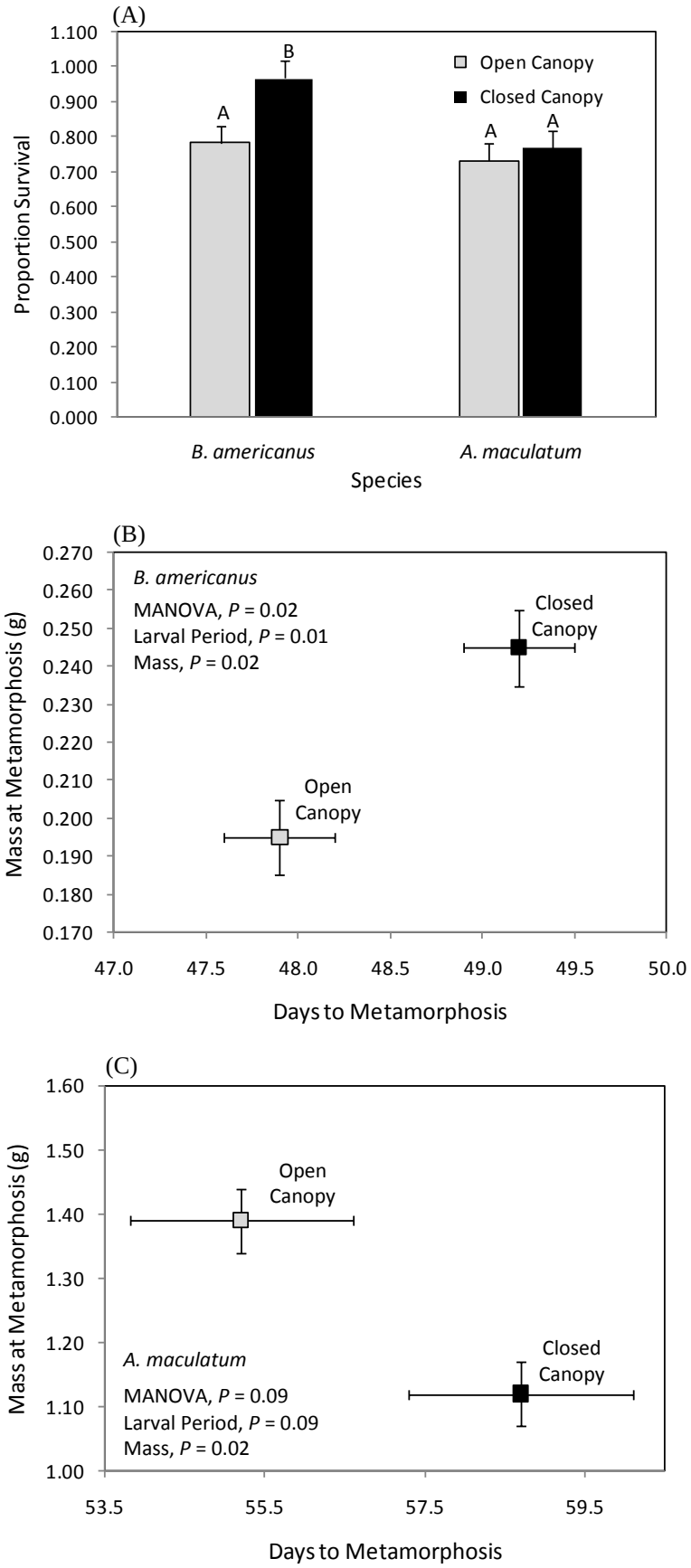
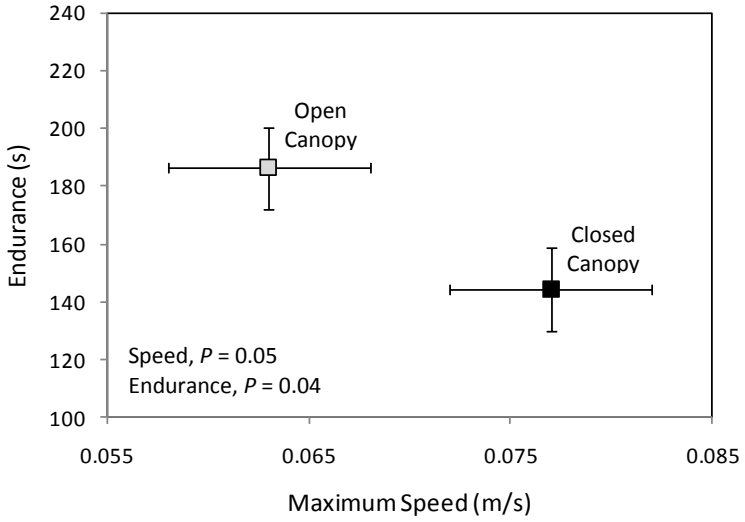
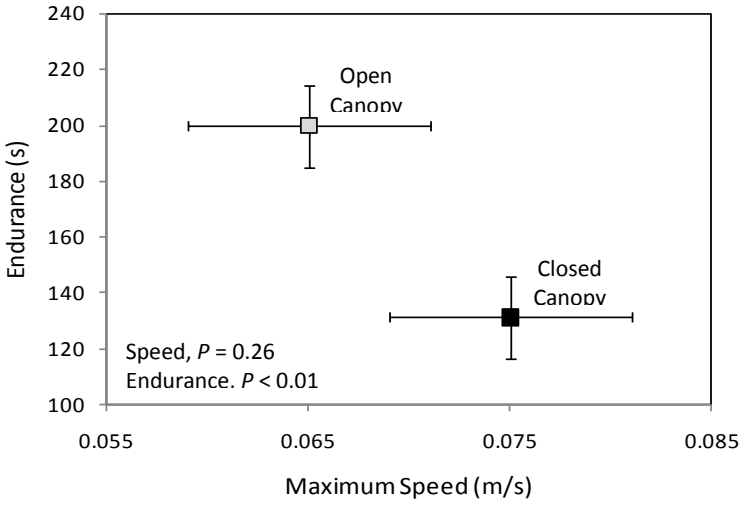

(A)
Proportion Survival
1.100
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
A
B
A
A
Open Canopy
Closed Canopy
B. americanus
A. maculatum
Species
(B)
B. americanus
MANOVA, P = 0.02
Larval Period, P = 0.01
Mass, P = 0.02
Closed Canopy
Open Canopy
Mass at Metamorphosis (g)
0.270
0.260
0.250
0.240
0.230
0.220
0.210
0.200
0.190
0.180
0.170
47.0
47.5
48.0
48.5
49.0
49.5
50.0
Days to Metamorphosis
(C)
Mass at Metamorphosis (g)
1.60
1.50
1.40
1.30
1.20
1.10
1.00
Open Canopy
Closed Canopy
A. maculatum
MANOVA, P = 0.09
Larval Period, P = 0.09
Mass, P = 0.02
53.5
55.5
57.5
59.5
Days to Metamorphosis

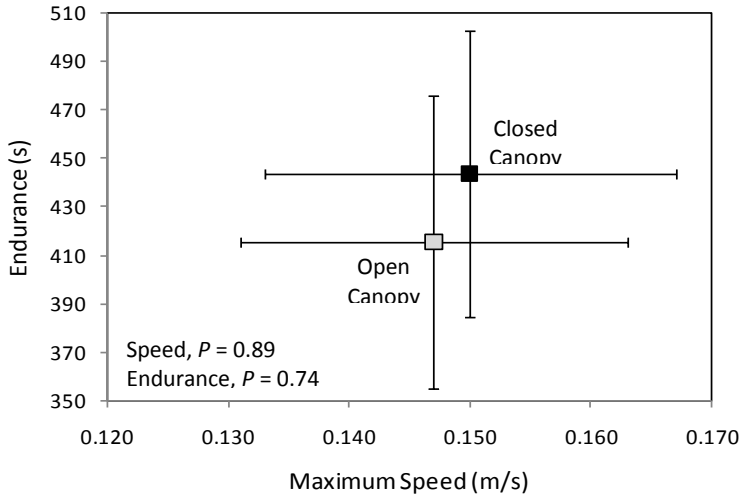
Fig. 2 (A) *B. americanus*, no covariate



(B) *B. americanus*, svl covariate



(C) *A. maculatum*, no covariate



CHAPTER 4:

Impacts of Canopy Cover on American Toads (*Bufo americanus*) in Multiple Life Stages

Abstract – A major goal of community ecology is to identify factors that regulate species abundance and community composition, both of which are known to transition across environmental gradients (e.g., hydroperiod, canopy cover). For species with complex life histories, such as pond-breeding amphibians, predicting species abundance, population persistence, and community structure also requires an understanding of how habitat changes may independently or simultaneously affect individuals in multiple life stages. We conducted complementary aquatic and terrestrial field experiments to evaluate the impacts of open-canopy and closed-canopy aquatic and terrestrial habitats on larval and juvenile American toads (*Bufo americanus*). Toads from closed-canopy ponds exhibited greater survival to metamorphosis and were on average larger at metamorphosis than toads from open-canopy ponds. However, although toads from open-canopy ponds were smaller at metamorphosis, they exhibited compensatory growth as terrestrial juveniles in forested habitats. Toads from both open- and closed-canopy ponds had very low survival as juveniles in unforested (i.e., open field) terrestrial habitats. Both aquatic and terrestrial canopy cover had strong impacts on growth and survival of American toad larvae and juveniles; however, our data suggest that the availability of suitable terrestrial habitat may ultimately determine the likelihood of population persistence for this species.

Introduction

A major goal of community ecology is to identify factors that regulate species abundance and community composition, both of which are known to transition across environmental gradients such as hydroperiod (Wellborn et al. 1996) and canopy cover (Werner et al. 2007). However, for species with complex life histories, predicting species abundance, population persistence, and community structure requires a consideration of how habitat changes may independently or simultaneously affect multiple life stages (Wilbur 1980; Pechenik 2006). Certain life stages may be more vulnerable than others, and identifying such vulnerabilities can be critical to the longevity of a population (e.g., Crowder et al. 1994; Biek et al. 2002). Conversely, focusing

conservation attention on less-critical life stages may result in a waste of resources and a failure to maintain population viability.

One group for which it is crucial to examine multiple life stages is pond-breeding amphibians. A greater percentage of described amphibian species are threatened worldwide than is true for other vertebrate taxa, including mammals and birds (IUCN 2008). Amphibians can constitute a significant proportion of the vertebrate biomass in aquatic and terrestrial ecosystems, and their loss may have serious ecological ramifications (Burton and Likens 1975; Beard et al. 2003; Halliday 2008). Habitat loss and alteration is a leading cause of amphibian population declines, and pond-breeding amphibians are particularly vulnerable because their biphasic life cycle depends on two distinct habitats, as well as multiple movements between those habitats (Semlitsch 2003). Interestingly, regeneration of previously cleared forests can also cause shifts in communities that can lead to local extinctions and species turnover (e.g., Skelly et al. 1999). Identifying priorities for habitat-based conservation is essential (Semlitsch 2002; Dodd and Smith 2003), and for species with a biphasic life history, it is important to consider the respective impacts of the aquatic and terrestrial habitats when setting conservation priorities. However, we should extend our examination of habitat effects, beyond the immediate influences of the aquatic habitat on larvae, to also consider the potential carryover and latent effects of the larval environment on later life stages, such as terrestrial juveniles (Pechenik 2006).

Biek et al. (2002) constructed stage-structured population matrices and conducted ecological sensitivity analyses for three pond-breeding anurans, and demonstrated that vital rates (i.e., survival) of post-metamorphic life stages more strongly influenced population growth than did vital rates of pre-metamorphic stages. Overwhelmingly, however, the best-studied life stage of pond-breeding amphibians is the larval stage (McDiarmid and Altig 1999; Wells 2007). Many species are cryptic in the terrestrial environment, making experimental studies of terrestrial juveniles and adults more difficult to execute and, consequently, much less common. Studies that follow individuals through multiple life stages are similarly scarce, but are particularly important for understanding the proximate, carryover, and latent effects of larval environmental conditions. The majority of multi-stage experiments have tested the effects of population density, predator exposure, or chemical exposure during the larval stage on metamorphic and post-metamorphic traits (e.g., Berven 1990; Goater 1994; Scott 1994; Boone 2005; Vonesh 2005; Chelgren et al. 2006). Carryover effects from larval environmental conditions, such as density or exposure to a

chemical, have been demonstrated, as has subsequent compensation for initial negative effects of larval environmental conditions (e.g., Goater 1994; Boone 2005; Chelgren et al. 2006). It is important to understand how other environmental factors affect recruitment at various ontogenetic transitions, and this is especially true for post-metamorphic transitions (Biek et al. 2002).

Given that habitat alteration is known to affect amphibian populations in general (Cushman 2006), and specifically that larval habitat characteristics (e.g., canopy cover) have been shown to influence metamorphosis in some amphibians (e.g., Skelly et al. 2002; Schiesari 2006; Chapters 2 and 3), it is critical to establish how different larval habitats can exert carryover and latent effects on subsequent life stages, as has been demonstrated in response to other larval environmental conditions (e.g., density, predators, chemical exposure). Two recent commentaries on amphibian conservation have noted that there has been an inappropriate shift away from habitat studies toward studies of 'more novel' stressors (Gardner et al. 2007) and that detailed studies of habitat alteration on amphibian populations are seriously lacking (Halliday 2008). It is especially true that there is a paucity of data about how terrestrial juveniles perform in different habitats (but see Pechmann 1995; Chazal and Niewiarowski 1998; Rothermel and Semlitsch 2002, 2006). Moreover, pond-breeding amphibians live in heterogenous landscapes, and individuals may find themselves using one of many possible combinations of aquatic and terrestrial habitats. Thus, studies that follow individuals from different larval (aquatic) habitats into different juvenile (terrestrial) habitats are necessary to understand the implications of landscape composition on amphibians during multiple ontogenetic transitions and to determine the underlying mechanisms that influence community composition.

The objective of our study was to examine how aquatic and terrestrial canopy cover influences growth and survival of larval and juvenile American toads (*Bufo americanus*). The American toad is an ideal species for which to explore the effects of extreme variation in larval and juvenile habitats because it is a pond-breeding, habitat generalist that could experience in nature a range of aquatic and terrestrial habitats, as simulated in this study. We reared toad larvae in open- and closed-canopy pond mesocosms, translocated metamorphs from both pond-canopy treatments into terrestrial enclosures located in forest and field habitats, and monitored growth and survival of terrestrial juveniles. We predicted the following results for our habitat crossover experiment: (1) toads reared in closed-canopy ponds will be larger at metamorphosis, have a

longer larval period, and have a greater probability of surviving to metamorphosis than toads reared in open-canopy ponds (Chapters 2 and 3); (2) toads reared in forested terrestrial habitat will exhibit greater growth and survival to overwintering than toads reared in field habitat (e.g., Rothermel and Semlitsch 2006); and (3) differences in pond canopy cover during the larval stage will have carryover effects on growth and survival during the post-metamorphic juvenile stage (e.g., Beck and Congdon 2000; Boone 2005).

Methods

Pond Mesocosms

On 19 April 2008, we collected five American toad (*Bufo americanus*) egg strings from a pond on the property of the Miami University Ecology Research Center (ERC), Oxford, Ohio, U.S.A. The eggs hatched and larvae were maintained together in a cattle tank outdoors at the ERC until they were added to experimental mesocosms; clutch-related biases were reduced by mixing larvae from different egg strings prior to the experiment. To test the effects of pond canopy cover on metamorphic traits and subsequent terrestrial growth and survival of post-metamorphic juveniles, we conducted a pond mesocosm experiment from 1 May through 23 June 2008. In ten outdoor mesocosms at the ERC, we simulated two canopy-cover treatments (open- and closed-canopy) with five replicates of each treatment; treatments were randomly assigned to the grid of ponds. Prior to the addition of toad larvae, each pond was filled with 1000 L water, 1 kg dry leaf litter from a nearby mixed deciduous forest (primarily *Acer*), and inoculations of algae and zooplankton from a fishless pond located at the ERC. On 1 May, each pond was stocked with 60 toad larvae (Gosner stage 25, Gosner 1960). Ponds were checked daily for toads with at least one front limb emerged (Gosner stage 42, Gosner 1960), at which point toads were collected and transported to the laboratory at Miami University for processing. We measured size at metamorphosis (mass and snout-vent length) of toads once tail resorption was complete (Gosner stage 46, Gosner 1960). We measured mass at metamorphosis for all toad metamorphs, and we measured both mass and snout-vent length for the subset of metamorphs used in the terrestrial enclosure experiment. We also calculated proportion survival in each pond (i.e., the number of individuals that successfully metamorphosed by the end of the experiment, 23 June, divided by 60, the stocking density). We calculated pond means for mass, snout-vent

length, larval period (days to metamorphosis), and survival, and used ponds as the experimental units in all analyses.

Open- and closed-canopy treatments were simulated using mesocosm lids that blocked 30% and 65%, respectively, of incoming light. Open-canopy lids were constructed of a single layer of 1.9-cm black plastic netting; closed-canopy lids were constructed of two overlapping layers of 0.2-cm black fiberglass mesh. We used a Biospherical Instruments QSL spherical PAR detector to test the percent transmission of visible light (PAR) through each type of lid material. These measurements were carried out in a darkened room with no light other than our intended light source, a white compact fluorescent lamp (GE Helical 26W) mounted inside a 30.5-cm photoreflector; the probe was mounted perpendicular to the light source, 60 cm from the front of the reflector. Samples of each material were placed in front of the detector (60 cm from the probe), three readings were taken, and the mean percent transmission was calculated for each material. Open-canopy lid material blocked ~30% overhead light (mean % transmission = 69.5%), whereas closed-canopy lid material blocked ~65% (mean % transmission = 34.5%).

To assess differences in water chemistry and food resources between canopy-cover treatments, we measured water pH, dissolved oxygen concentration (DO), water temperature, periphyton abundance, and phytoplankton abundance throughout the experiment. We measured pH, DO, and water temperature (^{o}C) for each mesocosm on 10 May, 16 May, 23 May, 28 May, and 12 June. We scraped uniform samples of periphyton from the walls of each mesocosm on 7 May, 14 May, 21 May, 28 May, and 12 June. We filtered phytoplankton (filtered 1 L water from 4-L composite samples) from each mesocosm on 7 May, 21 May, and 12 June. We obtained measurements of chlorophyll a concentration (µg/L) via fluorometry of periphyton and phytoplankton samples, and used chlorophyll a concentration as a proxy for relative abundance of algal resources. Detailed methods for collection of periphyton and phytoplankton data are included in chapter 2.

Terrestrial Enclosures

To test the effects of aquatic and terrestrial habitats on juvenile toads, we added toads from open- and closed-canopy pond mesocosms to sixteen terrestrial enclosures located in forest and field habitats using a full-factorial habitat crossover design (two aquatic habitats X two terrestrial habitats X four replicates). Terrestrial enclosures were located in four spatial blocks (replicates) at the Miami University ERC (Fig.1); each spatial block was made up of two sub-

blocks (a set of forest enclosures and field enclosures). Each sub-block was composed of four enclosures, which shared common inner walls (Fig.1). Enclosures were constructed of aluminum flashing; each enclosure measured 3 x 3m with a height of 0.63m, and buried 0.25m in the ground. To prevent escape of toads, window screen was attached perpendicular to the top edges of the enclosures. We dug a pit (48cm deep, 33cm diameter) in the center of each enclosure. The pits, intended to serve as overwintering sites, were filled with 0.5kg dry leaf litter and covered with a rectangular pine board (39cm x 54cm). Vegetation within the enclosures was left undisturbed, with the exception of a 25-cm wide strip along the interior border of each enclosure that was trimmed to prevent escape by toads.

Between 12 and 14 June, we added a total of eight toads to each of the sixteen enclosures, giving a stocking density of 0.9 animals/m^2 (Chazal and Niewiarowski 1998), within the range of natural field densities (Pechmann 1995). In an effort to sample more broadly across the population and to use a representative sample in the terrestrial experiment, every other toad metamorph from open- and closed-canopy mesocosms was marked with a unique toe clip and translocated to a terrestrial enclosure located in either forest or field habitat. Because toads metamorphose synchronously, the individuals used in the terrestrial experiment all metamorphosed within a few days of one another (between 10 and 13 June). On seven separate occasions during the summer and early fall (18 July, 1 Aug, 8 Aug, 21 Aug, 2 Sept, 18 Sept, and 28 Sept), we searched each enclosure for juvenile toads. We recorded the unique toe clip and snout-vent length for each recaptured toad and immediately released each individual where it was located. On the final sample date before winter (28 Sept), we transported all recaptured toads to the laboratory at Miami University to record each individual's unique toe clip, snout-vent length, and body mass; these toads were returned to the appropriate enclosures later the same day, and were left to overwinter in the terrestrial enclosures.

Data Analyses

Using analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA), we tested for effects of pond canopy cover on metamorphic traits (i.e., survival, size at metamorphosis, larval period) for two toad datasets: the complete dataset (all toads that metamorphosed from our experimental ponds) and a reduced dataset (only those individuals translocated to terrestrial enclosures). In preliminary analyses of larval period and mass response, we used survival to metamorphosis as a covariate to account for the influence of differential

survival between treatments; however, reduced survival was associated with reduced mass. Because the survival covariate compensated for differences between treatments in a way contrary to the typical density-dependent manner, we did not include a covariate in our final analysis. We tested for effects of terrestrial habitat structure (forest or field) and spatial block, as well as carryover effects of pond canopy cover, on subsequent terrestrial survival of juvenile toads using analyses of variance (ANOVA). Because of low toad survival in field enclosures, we only tested for effects of aquatic canopy cover and spatial block on juvenile growth in forest habitat using repeated-measures ANOVA. Survival to pre-winter in the terrestrial experiment was calculated by pooling the data from the final three observations (2, 18, and 28 Sept). We tested for the effects of aquatic canopy cover, terrestrial habitat type, and spatial block on survival of juvenile toads using ANOVA with a split-plot design. For toads that were recaptured during the final pre-winter sample (28 Sept), we calculated initial (at metamorphosis) and final (on 28 Sept) body condition (body mass in grams / snout-vent length in millimeters); we tested for effects of terrestrial habitat, as well as carryover effects of pond canopy cover, on initial and final body condition using ANOVA. In all analyses, block was included if it was significant; however, if the spatial block was non-significant, it was removed from the model to conserve degrees of freedom.

We tested for differences in water characteristics (pH, temperature, DO) and algal food resources (periphyton, phytoplankton) between pond canopy-cover treatments using repeated-measures ANOVA, and for differences in soil moisture between forest and field terrestrial enclosures using ANOVA. Pond mesocosm and terrestrial enclosure means were used in analyses of variance testing for habitat treatment effects on mass, snout-vent length, time to metamorphosis (larval period), and survival. For terrestrial analyses, in the event that a single individual in a given enclosure was observed, that individual's mass or snout-vent length was used as the enclosure mean. All data were transformed as necessary to meet the assumption of normality. Survival data were expressed as proportions and so were angularly transformed, whereas mass and snout-vent length data were log-transformed. Statistical analyses were performed using SAS version 9.1.

Juvenile-recruitment biomass for each of the four aquatic-terrestrial habitat pairings (e.g., closed-canopy pond to forest habitat) was calculated with the following algorithm, in which N_m is the mean number of metamorphs that survived from open- or closed-canopy ponds, G_m is the

mean mass at metamorphosis (grams) from open- or closed-canopy ponds, S_J is the mean proportion survival to September for juveniles in forest or field terrestrial habitats, and G_J/G_m (mass on 28 Sept / mass at metamorphosis) is the mean proportion of mass gained in forest or field terrestrial habitats:

Juvenile-recruitment biomass = $N_m * G_m * S_J * G_J/G_m$

Mean values (e.g., survival, mass) for biomass estimates were generated by first calculating means for each pond mesocosm or terrestrial enclosure, and from those, calculating overall means for the respective groups (e.g., closed-canopy ponds, forest habitat).

Results

Effects of Larval Pond Habitat on Metamorphic Traits

Pond canopy cover influenced the multivariate metamorphic response of toads (complete dataset: Wilks' Lambda = 0.2674, $F_{3,6} = 5.48$, P = 0.04; reduced dataset: Wilks' Lambda = 0.2523, $F_{3,6} = 5.93$, P = 0.03). The univariate analysis revealed that toad survival to metamorphosis was greater in closed-canopy than in open-canopy pond mesocosms (Fig. 2; $F_{1,8} = 13.63$, P < 0.01). Toads also reached a greater mass at metamorphosis in closed-canopy than in open-canopy mesocosms (complete dataset: Fig. 3A, $F_{1,8} = 7.54$, P = 0.03; reduced dataset: Fig. 3B, $F_{1,8} = 6.08$, P = 0.04). The response of snout-vent length at metamorphosis for toads in the reduced dataset mirrored the response of mass-at-metamorphosis to pond canopy cover ($F_{1,8} = 6.41$, P = 0.03; means ± 1 SE: open canopy, 11.3 ± 0.42; closed canopy, 12.8 ± 0.42). Toads from closed-canopy mesocosms had a slightly longer larval period than those from open-canopy mesocosms (complete dataset: Fig. 3A, $F_{1,8} = 18.72$, P < 0.01; reduced dataset: Fig. 3B, $F_{1,8} = 1.29$, P = 0.29). The response of the reduced dataset (terrestrial experiment animals only) mirrored the response of the complete dataset (all toads that metamorphosed from the experimental ponds) with one exception: the animals used in the terrestrial experiment (reduced dataset) all metamorphosed within a few days of one another, whereas the complete dataset included the individuals that metamorphosed earlier and later than those in the main emergence period (88% of toads metamorphosed between 10 and 13 June).

Water Characteristics and Food Resources in Open-Canopy and Closed-Canopy Habitats

Water temperature and DO were the only water-characteristic or food-resource variables measured in this study that differed significantly between pond canopy cover treatments. Water

temperature varied over time (Wilks' Lambda = 0.0003, $F_{4,5}$ = 3918.3, P < 0.01) and between canopy cover treatments over time (Wilks' Lambda = 0.0322, $F_{4,5}$ = 37.5, P < 0.01). Temperature was significantly greater in open-canopy mesocosms than in closed-canopy mesocosms on all five sample dates (P < 0.01 in all cases). Throughout the experiment, water temperature ranged from 16.3°C to 25.7°C in open-canopy mesocosms, and from 15.9°C to 24.8°C in closed-canopy mesocosms; open-canopy treatments were 0.5-2° warmer than closed-canopy treatments. Water pH varied significantly over time (Wilks' Lambda = 0.1742, $F_{4,5}$ = 5.92, P = 0.04), but did not vary between canopy cover treatments over time (Wilks' Lambda = 0.4569, $F_{4,5}$ = 1.49, P = 0.33); pH measurements varied little throughout the experiment, ranging from 7.8 to 8.1. Dissolved oxygen (DO) varied significantly over time (Wilks' Lambda = 0.0133, $F_{4,5}$ = 92.3, P < 0.01) and between pond habitat treatments over time (Wilks' Lambda = 0.1386, $F_{4,5}$ = 7.77, P = 0.02); however, DO did not vary significantly on any of the sample dates (0.23 > P < 0.62). DO varied little throughout the experiment, ranging from 4.4 mg/L and 6.6 mg/L, and the difference between open- and closed-canopy treatments on any given sample date was never greater than 0.7 mg/L. Periphyton abundance (µg/L chlorophyll a) varied over time (Wilks' Lambda = 0.0312, $F_{4,5}$ = 38.8, P < 0.01), but did not vary between canopy cover treatments over time (Wilks' Lambda = 0.2190, $F_{4,5}$ = 4.46, P = 0.07). Similarly, phytoplankton abundance (µg/L chlorophyll a) varied over time (Wilks' Lambda = 0.3816, $F_{2,7}$ = 5.67, P = 0.03), but did not vary between pond habitat treatments over time (Wilks' Lambda = 0.6815, $F_{2,7}$ = 1.64, P = 0.26).

Effects of Larval Pond Habitat and Terrestrial Habitat on Post-metamorphic Traits

Survival of terrestrial juveniles did not differ between toads from open- and closed-canopy pond mesocosms ($F_{1,6}$ = 1.03, P = 0.35), but did differ between toads living in field and forest terrestrial habitats ($F_{1,6}$ = 6.05, P = 0.05). Juvenile toads had a much greater probability of survival in forested terrestrial habitats than in field habitats (Fig. 2). The interaction of pond canopy cover and terrestrial habitat treatments was not significant ($F_{1,6}$ = 0.14, P = 0.72).

Body size (snout-vent length) of juvenile toads living in forested terrestrial habitat increased over time (Fig. 4A; $F_{7,7}$ = 287.44, P < 0.01) and also differed significantly between toads from open- and closed-canopy larval environments for 3 of 8 observations (Fig. 4A). As was previously mentioned, toad body size differed between pond canopy cover treatments at the time of metamorphosis; this was reflected in an analysis of body size for the subset of individuals that were recaptured throughout the summer and fall in forest terrestrial enclosures (Fig. 4A; $F_{1,1}$

= 137.8, P = 0.05). Body size differed significantly between toads from open- and closed-canopy larval environments on two recapture dates: 1 Aug (Fig. 4A; $F_{1,1}$ = 271.7, P = 0.04) and 2 Sept (Fig. 4A; $F_{1,1}$ = 460.2, P = 0.03); there was a significant influence of spatial block on terrestrial body size on both recapture dates (1 Aug: $F_{2,1}$ = 13220.2, P = 0.01; 2 Sept: $F_{2,1}$ = 1653.6, P = 0.02).

Pond canopy cover influenced the multivariate response of body condition at metamorphosis and body condition of pre-winter juvenile toads (Wilks' Lambda = 0.0301, $F_{2,2}$ = 32.2, P = 0.03). In the univariate analyses, pond canopy cover had a statistically significant effect on body condition at metamorphosis (Fig. 4B; $F_{1,3}$ = 10.42, P = 0.04) and a marginally significant effect on body condition of pre-winter juveniles (Fig. 4B; $F_{1,3}$ = 5.91, P = 0.09). Toads from closed-canopy pond environments had greater body condition (g/mm) at metamorphosis, whereas toads from open-canopy pond environments had greater body condition as pre-winter juveniles (on 28 Sept) (Fig. 4B). Terrestrial habitat did not significantly affect body condition of juvenile toads ($F_{1,3}$ = 1.29, P = 0.33).

Our biomass estimates indicated that juvenile-recruitment biomass (total grams of juveniles that survived to overwinter) would be highest for hypothetical toad populations that metamorphosed from closed-canopy ponds and utilized forested terrestrial habitat (Fig. 4C). We estimated moderate juvenile-recruitment biomass for hypothetical populations utilizing open-canopy ponds and forested terrestrial habitat (62% lower biomass than for closed-canopy to forest), whereas juvenile-recruitment biomass would be extremely low for hypothetical populations that utilized field habitats, regardless of their larval habitat type (open- or closed-canopy ponds) (Fig. 4C).

Discussion

We implemented complementary aquatic and terrestrial field experiments to evaluate the impacts of open-canopy and closed-canopy aquatic and terrestrial habitats on larval and juvenile American toads (*B. americanus*). Our study generated several key findings about the importance of larval and juvenile habitats for this pond-breeding species: (1) survival rate of larvae to metamorphosis in closed-canopy aquatic habitats was nearly double the survival rate in open-canopy aquatic habitats, 64% and 38% respectively; (2) apparent survival of terrestrial juveniles from metamorphosis to pre-winter (over approximately three and a half months) was much

greater in forest habitats than in field habitats, 36% and 3% respectively; and (3) larval growth differed between open- and closed-canopy aquatic habitats, resulting in smaller metamorphs from open-canopy habitats (approximately 35% smaller) relative to closed-canopy habitats; however, size differences eventually disappeared for juveniles that were translocated to forested terrestrial habitats. We elaborate on these findings below, and discuss their collective implications for a species with a biphasic life history that exists in heterogenous landscapes.

Toads from open-canopy ponds, which entered the terrestrial environment at a smaller average size relative to toads from closed-canopy ponds, were able to compensate for smaller size at metamorphosis with accelerated growth in the terrestrial juvenile stage; however, due to extremely low apparent survival in field habitats, this was only testable for toads translocated to forest habitats. It appeared that during the first 2.5 months following metamorphosis, open-canopy toads remained smaller on average relative to toads from closed-canopy ponds, and that toads from both pond habitats followed similar growth trajectories. However, around the 2.5-month mark (2 Sept), open-canopy toads were suddenly slightly larger on average than closed-canopy toads. By the final pre-winter observation (28 Sept), though, snout-vent length (SVL) of open- and closed-canopy toads did not differ significantly; this could reflect compensatory growth by open-canopy toads. Although field data (Semlitsch et al. 1988; Scott 1994) and theory (Werner 1986) suggest that relative differences in size at metamorphosis may be fixed for the rest of the life of an individual, some species of amphibians may be able to overcome consequences of small size at metamorphosis. Toads have relatively short-larval periods (2-6 weeks) and reach metamorphosis at small sizes, a strategy that may optimize the potential for terrestrial growth. Compensatory growth has been documented in a closely related species (*Bufo woodhousii*) that exhibited reduced size at metamorphosis in response to larval density and larval exposure to the insecticide carbaryl (Boone 2005). Interestingly, although pre-winter (28 Sept) SVL of toads in our study did not differ significantly between juveniles from open- and closed-canopy larval habitats, we observed a non-significant trend that body condition (grams of body mass per millimeter of SVL) of open-canopy juveniles was greater than body condition of closed-canopy juveniles. Greater body condition may indicate greater energy stores in juvenile toads from open-canopy larval habitats. Though not specifically tested in this study, greater energy stores theoretically may be explained by greater food intake (food quantity), greater intake of high-quality prey items (food quality), or greater assimilation efficiency of food items.

For instance, toads may overcompensate behaviorally for small size at metamorphosis through increased foraging or by choosing more nutritious prey items, or they may do both. Alternatively, the lower absolute metabolic rate of smaller metamorphs (e.g., Beck and Congdon 2000) can result in increased gut passage time of prey items, which may facilitate greater assimilation of nutrients (McConnachie and Alexander 2004). Regardless of the mechanism, achieving greater body condition prior to overwintering could confer a greater probability of surviving the winter.

The previous finding that open-canopy toads exhibited compensatory growth as terrestrial juveniles, and may also have achieved greater body condition than closed-canopy toads prior to overwintering, may indicate that the complementation of an open-canopy aquatic (larval) habitat and a forested terrestrial (juvenile) habitat would be more suitable for toads than would a closed-canopy aquatic habitat and a forested terrestrial habitat. However, despite the suspected relative importance of post-metamorphic vital rates to population growth (Biek et al. 2002), the dramatic differences in both body size (mass) and survival to metamorphosis that we observed in this study between toads from open- and closed-canopy larval environments could have important consequences for recruitment in natural populations. To explore the implications of this scenario, we calculated juvenile-recruitment biomass for the four combinations of aquatic and terrestrial habitats simulated in our habitat crossover design. We recognize that there are inherent complications of pooling data from individuals across spatial blocks for these calculations; however, the biomass calculations demonstrate potential differences in biomass that could occur with different aquatic and terrestrial habitat combinations. We estimated that relative juvenile biomass would be: high for toad populations in which larvae metamorphosed from closed-canopy ponds and dispersed to forest habitat; intermediate for populations utilizing the combination of open-canopy ponds and forest habitat; and very low for populations utilizing either open-canopy ponds or closed-canopy ponds if juveniles are forced into unsuitable field habitats. Therefore, our biomass estimates partially support our previous conclusions that: (1) forest habitat is far superior to field habitat for survival of juvenile toads and (2) if forest habitat is available, smaller toads from open-canopy larval habitat can catch up with larger toads from closed-canopy larval habitat (via compensatory post-metamorphic growth). Further, our biomass estimates, which incorporated the vital rates of both metamorphic and post-metamorphic life stages, suggested that relative to all other habitat combinations closed-canopy aquatic habitat

combined with forested terrestrial habitat can by far generate the greatest biomass of juvenile recruitment for American toads. However, it is important to note that incorporating the vital rates of overwintered juvenile toads into our biomass estimates could dramatically alter our understanding of the importance of aquatic and terrestrial habitats along the canopy gradient for recruitment to adult breeding populations.

For many pond-breeding amphibians, fitness potential is often inferred from certain metamorphic traits, such as size at metamorphosis (Werner 1986; Goater et al. 1994; Semlitsch et al. 1988); however, realized fitness of individuals, and consequent growth and persistence of populations, necessarily depends on successful recruitment into the adult breeding population. Metamorphic traits that appear to be correlated with fitness in some species (e.g., *Ambystoma talpoideum*, Semlitsch et al. 1988), seem to be less important for fitness of toads than other factors, such as the capacity for compensatory growth of juveniles in the terrestrial environment (e.g., Werner 1986; Boone 2005; this study). Our findings confirm the assertion that growth and survival in bufonids, and possibly other species with relatively short larval periods, may be tied more closely to terrestrial growth rather than to growth during the aquatic larval stage (Werner 1986). More generally, our study underscores the importance of following individuals throughout multiple life stages, and supports the emerging contention that, for species with complex life histories, evaluations of the impacts of environmental conditions during early life stages should include considerations of both immediate and carryover effects.

We have shown that aquatic and terrestrial canopy cover can have strong impacts on growth and survival of larval and juvenile American toads (*B. americanus*), and that suitable terrestrial habitat ultimately determines the likelihood of population persistence. Closed-canopy ponds produced more metamorphs, and larger metamorphs on average, than open-canopy ponds. Although toads from open-canopy ponds were smaller at metamorphosis, they exhibited compensatory growth as terrestrial juveniles in forested habitats. After 2.5 months in forested terrestrial habitats, toads from open- and closed-canopy ponds did not differ in body size; however, toads from both pond canopy treatments had extremely low juvenile survival in field habitats. Together these findings may suggest that, as long as suitable terrestrial habitat (i.e., forested, closed-canopy) is accessible by juveniles, toads and other species with similar life histories (i.e., short larval period, emphasis on terrestrial growth) can successfully utilize ponds along the canopy gradient for breeding. Our findings thus corroborate previous studies that

demonstrated avoidance of open terrestrial habitats (i.e., fields, clearcuts, powerlines) by *Bufo americanus*, *Rana sylvatica*, and *Ambystoma maculatum* during post-metamorphic dispersal (deMaynadier and Hunter 1999; Rothermel and Semlitsch 2002), and greatly reduced growth and survival of *Ambystoma maculatum* and *Ambystoma opacum* in old-field habitat relative to forest habitat (Rothermel and Semlitsch 2006). Given the heterogeneity of natural landscapes, and the frequency of land-use changes, it is critical to establish that some pond-breeding amphibians appear to be far more sensitive to the alteration of terrestrial habitat than aquatic habitat. This information may be particularly useful when resources are limited and priorities must be set for habitat conservation.

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Fig. 1. – Schematic of terrestrial enclosure locations and design at the Miami University Ecology Research Center (ERC), Oxford, Ohio, U.S.A.

Fig. 2. – Proportion survival to metamorphosis in open- and closed-canopy pond mesocosms ('metamorphs') and proportion survival to overwintering in field and forest terrestrial enclosures ('juveniles') for American toads (*Bufo americanus*). Error bars represent ± 1 SE.

Fig. 3. – Trade-off between larval period (days to metamorphosis) and mass at metamorphosis for American toads (*Bufo americanus*) in open- and closed-canopy pond mesocosms: (A) complete dataset and (B) reduced dataset, only individuals used in the terrestrial experiment. Error bars represent ± 1 SE.

Fig. 4. – (A) Growth (snout-vent length over time) of juvenile American toads (*Bufo americanus*) from open- and closed-canopy ponds in forest terrestrial enclosures. Asterisks indicate statistical significance ($P \leq 0.05$); (B) Body condition (grams of body mass / millimeters of snout-vent length) of *B. americanus* at metamorphosis ('metamorphs') and on 28 Sept ('juveniles'); (C) Comparisons of juvenile-recruitment biomass of *B. americanus* for each of the four aquatic-terrestrial habitat pairings (i.e., open- and closed-canopy ponds to field and forest terrestrial habitats). Biomass estimates are based on the number of metamorphs that survived to metamorphosis and their mean mass at metamorphosis, as well as juvenile survival and mass gained in the terrestrial environment. Error bars represent ± 1 SE.

Fig. 1 –

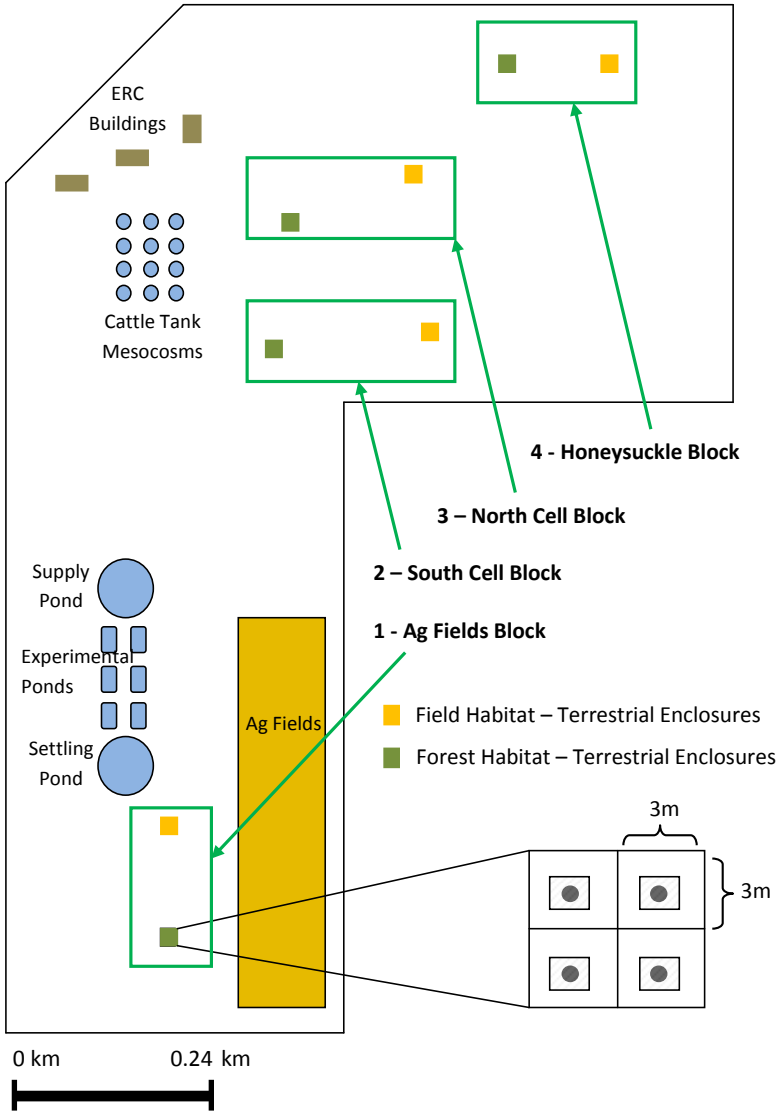

ERC
Buildings
Cattle Tank
Mesocosms
4 - Honeysuckle Block
3 – North Cell Block
2 – South Cell Block
1 - Ag Fields Block
Supply
Pond
Experimental
Ponds
Settling
Pond
Ag Fields
Field Habitat – Terrestrial Enclosures
Forest Habitat – Terrestrial Enclosures
3m
3m
0 km
0.24 km

Fig. 2 –

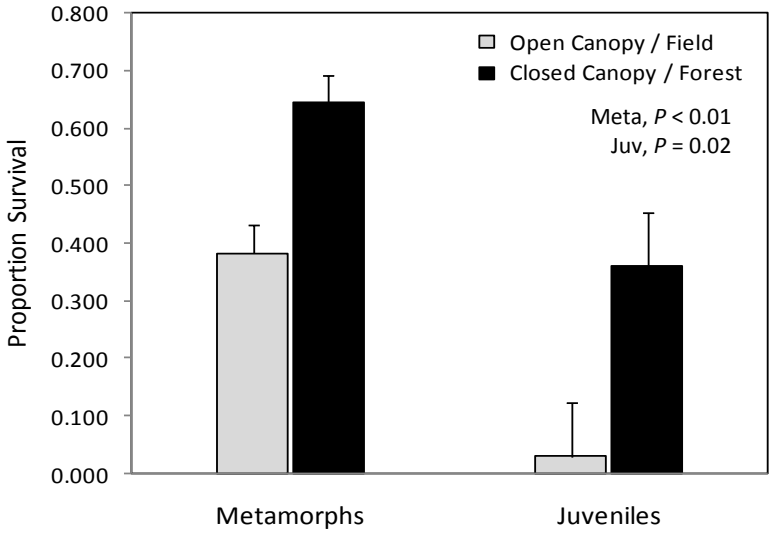

0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Proportion Survival
Open Canopy / Field
Closed Canopy / Forest
Meta, P < 0.01
Juv, P = 0.02
Metamorphs
Juveniles

Fig. 3 –

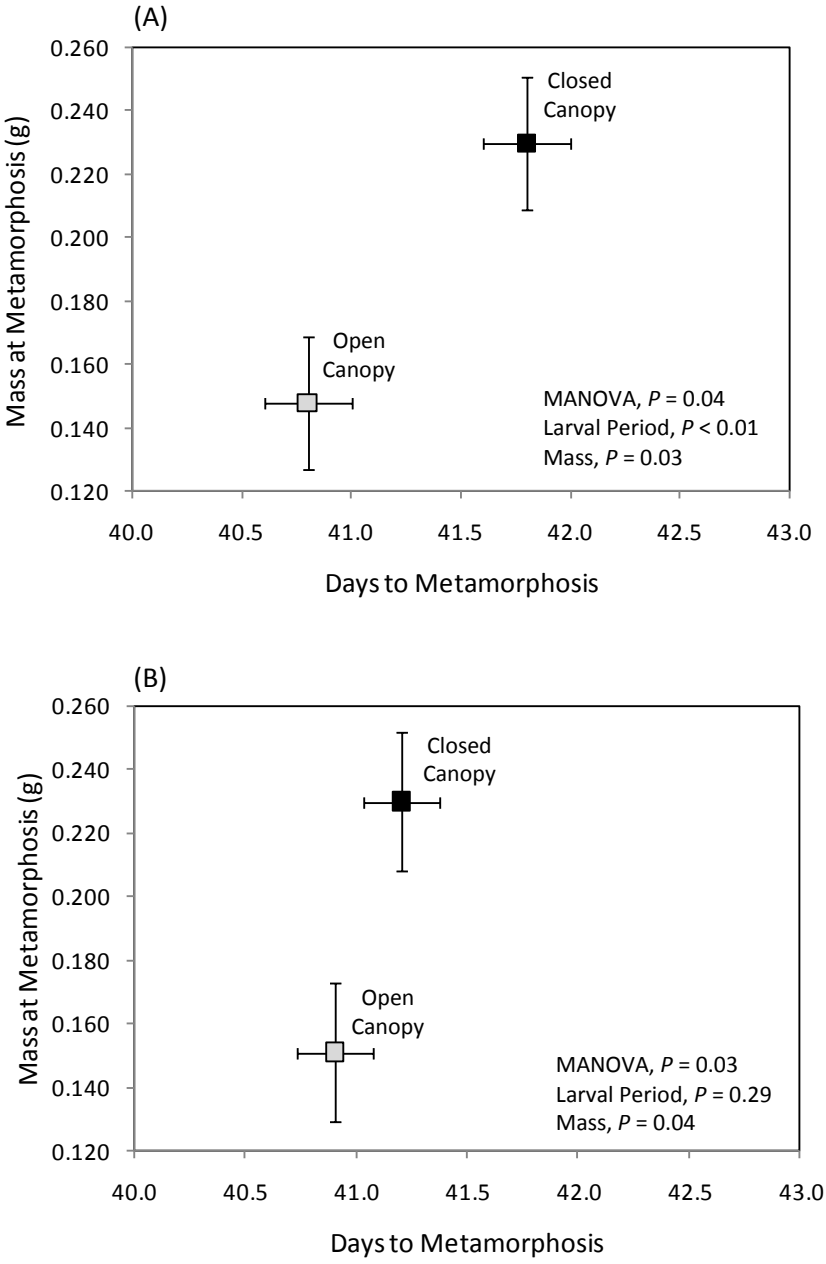
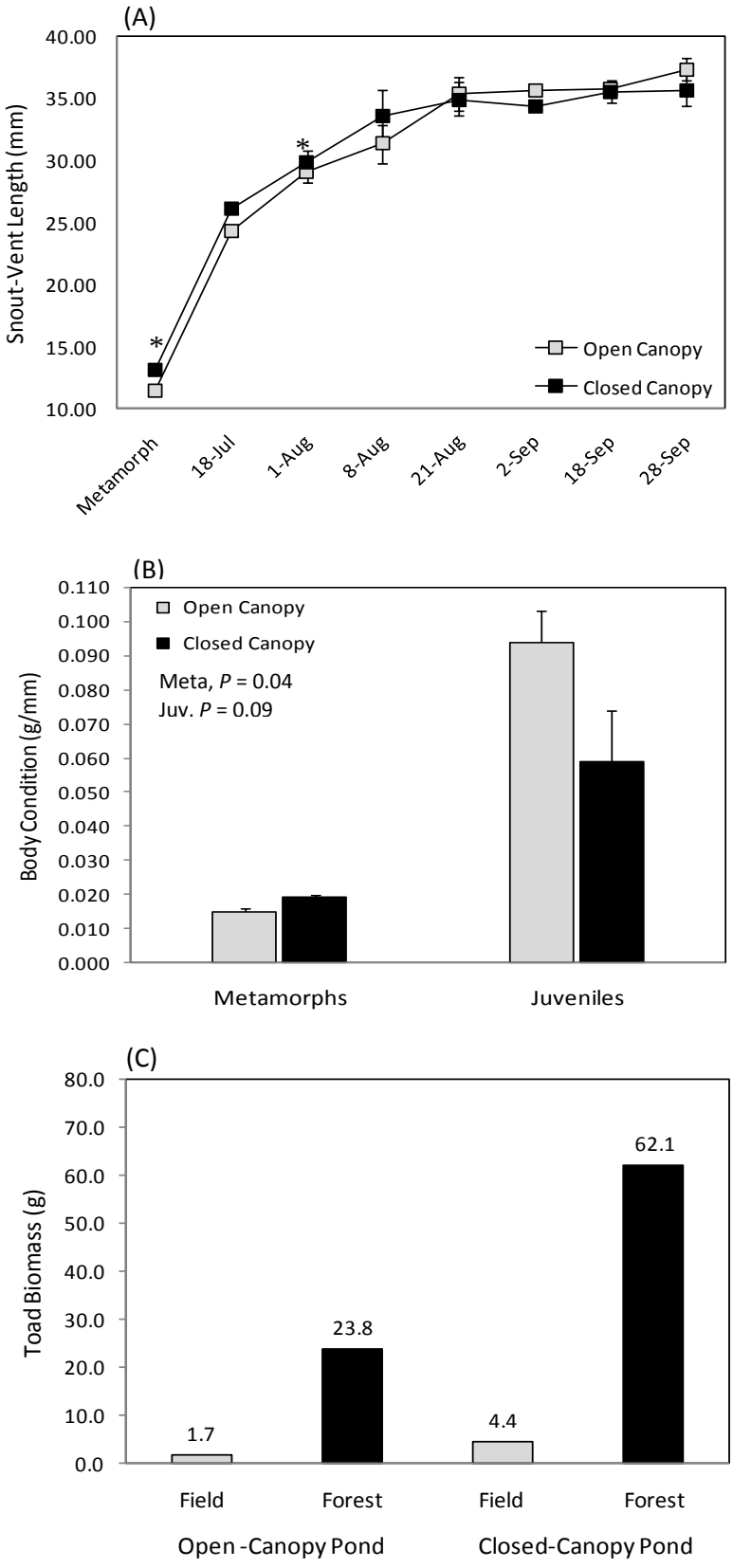

(A)
Mass at Metamorphosis (g)
0.260
0.240
0.220
0.200
0.180
0.160
0.140
0.120
Closed Canopy
Open Canopy
MANOVA, P = 0.04
Larval Period, P < 0.01
Mass, P = 0.03
40.0
40.5
41.0
41.5
42.0
42.5
43.0
Days to Metamorphosis
(B)
Mass at Metamorphosis (g)
0.260
0.240
0.220
0.200
0.180
0.160
0.140
0.120
Closed Canopy
Open Canopy
MANOVA, P = 0.03
Larval Period, P = 0.29
Mass, P = 0.04
40.0
40.5
41.0
41.5
42.0
42.5
43.0
Days to Metamorphosis

Fig. 4 –


(A)
Snout-Vent Length (mm)
40.00
35.00
30.00
25.00
20.00
15.00
10.00
*
*
Open Canopy
Closed Canopy
Metamorph
18-Jul
1-Aug
8-Aug
21-Aug
2-Sep
18-Sep
28-Sep
(B)
Body Condition (g/mm)
0.110
0.100
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
Open Canopy
Closed Canopy
Meta, P = 0.04
Juv. P = 0.09
Metamorphs
Juveniles
(C)
Toad Biomass (g)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1.7
23.8
4.4
62.1
Field
Forest
Field
Forest
Open -Canopy Pond
Closed-Canopy Pond

GENERAL CONCLUSIONS

My dissertation research aimed to address how individual performance, population parameters, and species interactions vary across multiple environmental gradients: aquatic vegetation structure, canopy cover, and predators. Specifically, in this dissertation, I described a series of field experiments and behavioral trials that examined the importance of aquatic and terrestrial habitat characteristics (emergent vegetation and canopy cover) for mediating biotic interactions and performance of larval and juvenile pond-breeding amphibians (Fig. 1).

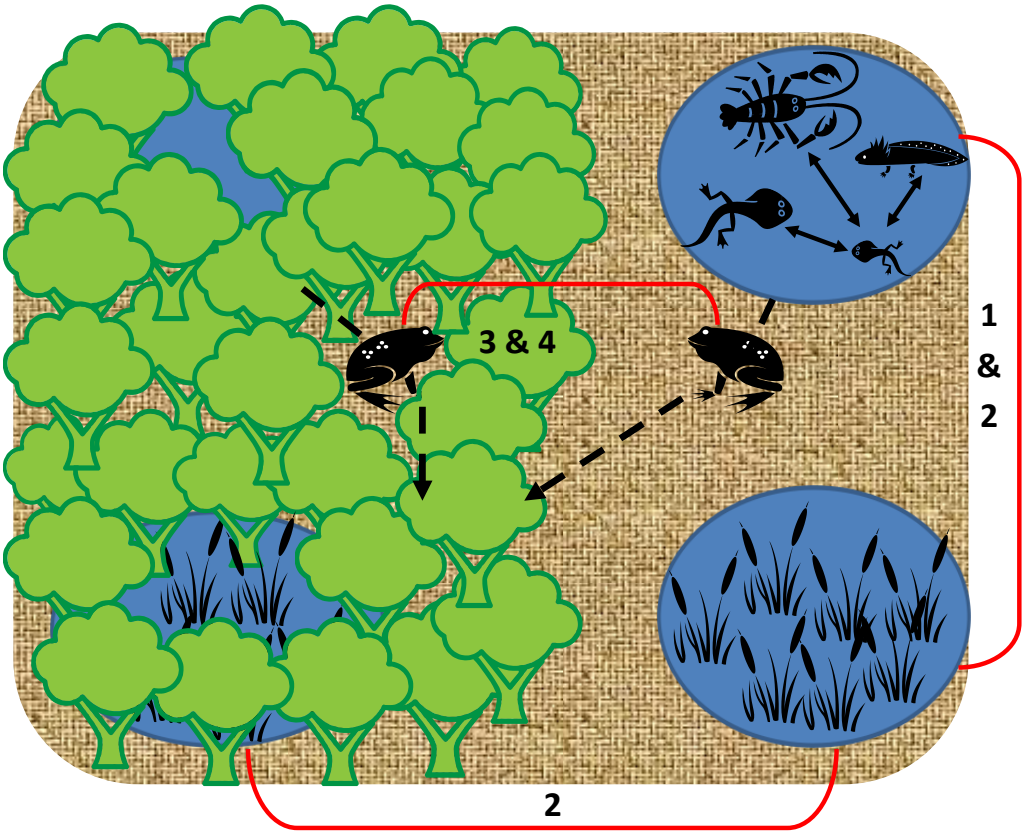


Fig. 1 – This dissertation aimed to address how individual performance, species interactions, and population parameters vary across multiple environmental gradients (aquatic vegetation structure, canopy cover, predators), and specifically explored these questions: Can aquatic vegetation structure influence competitive and predator-prey interactions and play a role in community regulation? (Chapters 1 & 2); Can canopy cover influence fitness-correlated traits of individuals and mediate biotic interactions? (Chapter 2); & Can canopy cover in one life stage have consequences for individual performance in subsequent life stages, and are there tradeoffs in performance in different habitat types? (Chapters 3 & 4).

In Chapter 1, I showed that, in the absence of predators, aquatic vegetation structure can influence both the length of the larval period and survival to metamorphosis for some pond-breeding species, and can mediate larval competition at low densities. Ours was the first study to show that vegetation structure alone, rather than by reducing predation rates, can increase the probability of survival to metamorphosis for amphibian larvae. In Chapter 2, I described several pond mesocosm experiments, in which we examined the effects of canopy cover and aquatic vegetation structure on metamorphosis and biotic interactions of American toads (*B. americanus*). Two novel and important findings emerged from the experiments in Chapter 2: (1) contrary to what has been shown for other pond-breeding species (e.g., Skelly et al. 2002; Schiesari 2006), pond canopy cover can positively impact growth and survival to metamorphosis in American toads; and (2) contrary to most of the existing literature that describes reduced predation in structurally complex environments (e.g., Sredl and Collins 1992; Baber and Babbitt 2004), vegetation structure can facilitate predation of larval toads by crayfish, resulting in lower toad survival in ponds with vegetation structure. In Chapter 3, I again demonstrated that closed-canopy ponds can produce more, and larger, toad metamorphs than open-canopy ponds. However, I also described our finding that post-metamorphic locomotor abilities of toads differed between metamorphs from open- and closed-canopy ponds in ways that may facilitate successful post-metamorphic dispersal and survival in the terrestrial environment. Specifically, toads from closed-canopy ponds exhibited greater speed, whereas toads from open-canopy ponds exhibited greater endurance capacity; greater endurance capacity may be especially advantageous to toads from open-canopy ponds because they generally have to disperse greater distances to reach suitable terrestrial habitat. Finally, in Chapter 4, I showed that, although toads metamorphosed larger, and had greater survival, from closed-canopy ponds, juveniles from open-canopy ponds were able to catch up with those from closed-canopy ponds via compensatory growth in forested terrestrial habitat. However, toads that metamorphosed from both pond canopy treatments had extremely low apparent survival when they were restricted to unforested (i.e., open field) terrestrial habitat during the post-metamorphic juvenile stage, which substantiates the findings of others that post-metamorphic amphibians tend to avoid open terrestrial habitats during dispersal (deMaynadier and Hunter 1999; Rothermel and Semlitsch 2002).

Together the findings presented in this dissertation offer new insights about the importance of emergent vegetation structure and canopy cover for pond-breeding amphibians. These findings are relevant to conservation biologists interested in habitat restoration and setting habitat conservation priorities, to population biologists interested in how habitat factors can affect population viability, and to community ecologists interested in how species assemblages may transition along habitat gradients and how habitat structure can mediate biotic interactions in unexpected ways:

1. Emergent vegetation in wetlands can influence timing of metamorphosis (larval period) and survival to metamorphosis, even in the absence of predators.
2. The role of vegetation structure in mediating predator-prey relationships may depend on the identity and behavior of the predator. Specifically, the efficiency of crayfish as predators of amphibian larvae can increase in structurally complex habitats.
3. Pond canopy cover can be advantageous for amphibian metamorphosis in some habitat-generalist species (e.g., American toads, *Bufo americanus*).
4. Post-metamorphic locomotor abilities can differ between individuals from open- and closed-canopy ponds; however, these differences may be adaptive given the post-metamorphic locomotor requirements generally associated with each pond habitat.
5. Small size at metamorphosis can be overcome via compensatory growth in the juvenile stage; however, this is apparently contingent on the availability of suitable terrestrial habitat (i.e., forested habitat).
6. Open-canopy terrestrial habitats (e.g., open fields) can be associated with extremely low survival of juvenile pond-breeding amphibians.

The dependence of pond-breeding amphibians on two distinct habitats makes them particularly vulnerable to anthropogenic changes to the landscape, and complicates conservation efforts (Semlitsch 2003). Understanding the relative importance and key habitat features of aquatic and terrestrial environments will allow us to both manage and restore habitats in an effort to reverse the extinction tide. The findings from this research indicate that habitat managers interested in conserving amphibians should consider that, in addition to hydroperiod and predators (Wellborn et al. 1996), the vegetation within and surrounding a wetland can influence the quality and quantity of individuals that reach metamorphosis, and may also influence

community composition. Managers should also keep in mind that: (1) predicting predation risk in habitats that vary in structural complexity can depend on the types of predators in the system and (2) although open-canopy wetlands have been shown to facilitate faster tadpole growth and greater survival for some species (e.g., Werner and Glennemeier 1999; Skelly et al. 2002; Schiesiari 2006), my research demonstrated that not all pond-breeding species are alike; canopy-generalist American toads repeatedly showed greater growth and survival to metamorphosis in closed-canopy wetland habitats. Moreover, aquatic and terrestrial canopy cover can strongly influence individual performance of juvenile pond-breeding amphibians, and may have serious implications for juvenile recruitment into the adult population. My research demonstrated that survival of juvenile anurans can be greatly reduced when they are restricted to unsuitable terrestrial habitats (e.g., open fields); this has been shown for juvenile pond-breeding salamanders as well (Rothermel and Semlitsch 2006). Suitable terrestrial habitat is critical for juvenile survival, and post-metamorphic life stages may more strongly influence population growth than pre-metamorphic life stages (Biek et al. 2002); thus, successful conservation of pond-breeding amphibians must involve the preservation of high-quality (i.e., closed canopy) terrestrial habitat and the maintenance of corridors that facilitate juvenile dispersal from natal wetlands to suitable terrestrial habitat.

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