

EFFECTS OF SPATIAL SUBSIDIES AND CANOPY COVER ON POND
COMMUNITIES AND MULTIPLE LIFE STAGES IN AMPHIBIANS

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COMMUNITIES AND MULTIPLE LIFE STAGES IN AMPHIBIANS

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DEDICATION

I dedicate my dissertation to my wonderful husband, my parents, and my grandfather. My husband, Dylan J. Allen, has kept my head above water with love, humor, and understanding. My parents, Alice and Steven Earl, have always supported me, no matter what and I love them dearly. My grandfather, Howard Lindsey Wilson, loved and cared for me as a child and showed me many wonders in this world before he passed on 13 June 1994. I wish he were here to see how far I've come.

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ABSTRACT

Spatial subsidies are resources that move from one ecosystem to another. In aquatic systems, canopy cover often determines both light availability (a major determinant of system productivity) and subsidy input in the form of senescing leaves and terrestrial invertebrates. This creates a system where subsidies are more prevalent in areas with low primary productivity and less prevalent in areas with high primary productivity. This phenomenon has been well studied in streams, and general patterns of ecosystem production, community structure, and the reciprocal export of animals with complex life histories have been discovered. I was interested in whether these patterns also occurred in ponds. I examined these patterns using experimental pond mesocosms and supported the results on community structure using an observational study of natural ponds. For the pond mesocosm experiment, I placed mesocosms along a canopy cover gradient and manipulated spatial subsidy input. For subsidy input treatments, I had mesocosms with subsidy input (leaves), a within-system resource (an aquatic grass), and no input. I examined the ratio of primary production to community respiration (GPP/CR_{24}) and community structure seasonally over two years and the reciprocal export of frog metamorph biomass over three years. I found that canopy cover, but not subsidy input, affected GPP/CR_{24} , indicating a shift from net heterotrophy in closed canopy mesocosms to a balance between heterotrophy and autotrophy in open canopy mesocosms. This is concordant with that found in streams, indicating that this shift in trophic state with changes in light availability may be common for aquatic ecosystems. The

macroinvertebrate community structure responded to both canopy cover and subsidy input in mesocosms. The biomass of collectors (detritivores) was highest in mesocosms with litter input and increased with canopy cover. I found a similar trend in natural ponds with collectors, but herbivores decreased with increases in litter input and canopy cover. Herbivores were rare in mesocosms and did not respond to treatments. Unlike stream systems, shredders were rare in both natural ponds and pond mesocosms. Finally, I found that litter input (both grass and leaves) increased the reciprocal export of amphibian biomass compared to no input. Amphibian biomass also decreased with increases in primary productivity, but this trend may be due changes in temperature or in the algal community from the differences in light availability. This research highlights the importance of spatial subsidies that connect different ecosystem types. I found that many of the responses that stream ecosystems have to spatial subsidies are similar to that in ponds. Conserving these ecosystem connections will help maintain biodiversity and ecosystem function.

CHAPTER 1

Introduction

Cross-ecosystem transfer (spatial subsidies) of energy, materials, and various chemical compounds can have profound effects on recipient ecosystems (Polis, Anderson & Holt, 1997; Marczak, Thompson & Richardson, 2007). These fluxes are the major determinants of many economically important ecosystem services, including crop pollination (Morandin & Winston, 2006; Kremen *et al.*, 2007) and pest control (Lundberg & Moberg, 2003). For example, ocean-derived nutrients in salmon fertilize inland forests in the Pacific northwest at a similar level to silvicultural application (Quinn *et al.*, 2009). Alternately, these chemical transfers can also result in major environmental degradation, such as the Gulf of Mexico dead zone (Rabalais, Turner & Wiseman, 2002) and the destruction of tundra from agriculturally subsidized waterfowl (Jefferies, Henry & Abraham, 2004). Recent evidence has shown that spatial subsidies can also move contaminants from impacted sites to un-impacted sites, creating potential problems for the management of waste disposal (Rasmussen & Zanden, 2004; Walters, Fritz & Otter, 2008). With both positive and negative consequences of cross-ecosystem transfers, it is valuable to be able to predict effects of subsidies.

In aquatic systems, canopy cover often determines both light availability and subsidy input. Subsidy input consists mainly of senescing leaves, terrestrial invertebrates, and amphibian egg masses (Nakano, Miyasaka & Kuhara, 1999; Batzer,

Palik & Buech, 2004; Kraus, Pletcher & Vonesh, 2011). In ponds, many studies have shown that this gradient drives many biotic and abiotic changes. There is a general consensus that closed canopy ponds (low light and high subsidy input) have lower dissolved oxygen, temperature, and pH than open canopy ponds (Table 1). The decrease in light from canopy cover generally translates in to lower biomass of primary producers (Table 2) and lower overall primary production (Sand-Jensen & Staehr, 2007; Sand-Jensen & Staehr, 2009). Because of the low light availability in closed canopy ponds, the ecosystem tends to be dominated by community metabolism driven by decomposition (Rubbo, Cole & Kiesecker, 2006; Sand-Jensen & Staehr, 2007; Sand-Jensen & Staehr, 2009; Staehr *et al.*, 2011). Most of this evidence presents patterns in natural systems. Thus, the dual effects of light and subsidy input from canopy cover cannot be separated. In Chapter 2, I present the results of an experimental manipulation using artificial ponds (cattle tanks) to determine how light and subsidy input affect primary production, community respiration, and trophic state.

In ecology, it is well known that changes in ecosystem processes and the overall base of the food web can produce dramatic changes in the communities of animals that depend upon them. Previous work on ponds shows similar results. Increases in canopy cover greatly impact pond invertebrate and amphibian communities by decreasing abundances and species richness (Table 3). However, changes in the base of the food web suggests that different groups of invertebrates might be affected differently in response to canopy cover. Due to lower primary production, grazers likely would have slower growth and developmental rates and lower survival in closed canopy than open canopy ponds. On the other hand, detritivores would likely perform better in closed

canopy ponds than open canopy ponds. These differences in performance should lead to changes in community composition and structure, as seen in stream ecosystems under the river continuum concept (Vannote *et al.*, 1980). The one study that has investigated a change in community structure with canopy cover in ponds found mixed results (Batzer, George & Braccia, 2005). To provide further data on this topic, I examined these principals in ponds using an experimental manipulation (Chapter 2) and an observational study on natural ponds (Chapter 3). In both studies, I was interested in how the two components of canopy cover affected trophic community structure, which I examined using biomass in different functional feeding groups.

There are two main proximate factors that determine community composition and community structure: habitat choice and performance within the habitat. Habitat choice occurs when organisms directly choose their own habitat (adult aquatic beetles) or when organisms choose habitat for their larvae through oviposition sites (insects and amphibians). The organism's performance determines whether it will persist in a given habitat after the choice has been made. Binckley and Resetarits (2007) examined insect and amphibian habitat choice in relation to canopy cover and found that beetles and amphibians overwhelmingly chose open canopy ponds over closed canopy ponds. However, Hocking and Semlitsch (2007) found that treefrogs preferred open canopy sites near forest edges. Despite these preferences, there are also clear differences in amphibian performance between open and closed canopy ponds (Table 4). These performance metrics are very important in the population dynamics of these species, but also determine the amount of amphibian biomass exported from ponds to the surrounding terrestrial landscape (McCoy, Barfield & Holt, 2009; Earl *et al.*, 2011), a spatial subsidy.

Ponds can export large amounts of amphibian biomass (Gibbons *et al.*, 2006). This can be on the same order of magnitude as aquatic insects (Table 5), which are known to alter community dynamics in the riparian areas surrounding streams (Baxter *et al.*, 2004; Baxter, Fausch & Saunders, 2005; Fausch, Baxter & Murakami, 2010; Marcarelli *et al.*, 2011). In Chapter 4, I examined the effects of canopy cover on amphibian biomass export in ponds, specifically investigating the input of leaf litter and primary productivity.

Finally, for pond canopy to cause changes in amphibian population dynamics, canopy must affect pond vital rates in the tadpole stage or cause carryover effects. Carryover effects occur when conditions early in life affect subsequent performance (Pechenik, Wendt & Jarrett, 1998; Pechenik, 2006). Amphibians experience carryover effects from a variety of factors, including competition and predation (Relyea & Hoverman, 2003) and pesticides (James & Semlitsch, 2011). In Chapter 5, I examine the effects of pond canopy cover on post-metamorphic (i.e. after emerging from the pond) survival in different environments using three different species of anurans. Finally, in Chapter 6, I bring many of these ideas together and discuss management implications.

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Table 1. Effects of canopy cover on abiotic factors in ponds and artificial ponds (buckets and cattle tanks). DO indicates dissolved oxygen. ↓ forest indicates that the parameter is lower in the forest ponds or under closed canopy conditions. n/a indicates not applicable, and No diff. indicates that there was not a statistically significant effect. Most of the studies listed below use a binary categorization of canopy.

Reference	Habitat	DO	Temperature	pH
Halverson <i>et al.</i> 2003	Ponds	n/a	↓ forest	n/a
Mokany <i>et al.</i> 2008	Buckets	n/a	↓ forest	No diff.
Scheisari 2006	Ponds	↓ forest	↓ forest	n/a
Skelly <i>et al.</i> 2002	Ponds	↓ forest	↓ forest	n/a
Werner and Glennemeir 1999	Ponds	↓ forest ¹	↓ forest	↓ forest ¹
Williams <i>et al.</i> 2008	Cattle Tanks	↓ forest	↓ forest	↓ forest

¹Indicates marginal significance (p = 0.06).

Table 2. Effects of canopy cover on primary producers in ponds and artificial ponds (buckets and cattle tanks). ↓ forest indicates that the parameter is lower in the forest ponds or under closed canopy conditions. n/a indicates not applicable, and No diff. indicates that there was not a statistically significant effect. Most of the studies listed below use a binary categorization of canopy.

Reference	Habitat	Periphyton	Phytoplankton	Macrophytes
Mokany <i>et al.</i> 2008	Buckets	↓ forest	↓ forest	n/a
Palik <i>et al.</i> 2001	Ponds	n/a	n/a	No diff.
Sand-Jensen and Staehr 2009	Small Lakes	n/a	↓ forest	n/a
Skelly <i>et al.</i> 2002	Ponds	↓ forest	n/a	n/a
Williams <i>et al.</i> 2008	Cattle Tanks	↑ forest	↓ forest	n/a

Table 3. Effects of canopy cover on invertebrate and amphibian communities in ponds and artificial ponds (buckets and cattle tanks). ↓ forest indicates that the parameter is lower in the forest ponds or under closed canopy conditions. n/a indicates not applicable, and No diff. indicates that there was not a statistically significant effect. Most of the studies listed below use a binary categorization of canopy. Amphibian data is from both tadpole surveys and egg mass counts.

Reference	Habitat	Invert. Richness	Invert. Abundance	Amphibian Richness	Amphibian Abundance
Batzer <i>et al.</i> 2004	Ponds	↓ forest	n/a	n/a	n/a
Binckley and Resetarits 2007	Cattle Tanks	↓ forest	↓ forest	n/a	↓ forest
Egan and Patton 2004	Ponds	n/a	n/a	n/a	↑ forest ¹ , No. diff. ²
Halverson <i>et al.</i> 2003	Ponds	n/a	n/a	n/a	↓ forest ³ , No. diff. ²
Hocking and Semlitsch 2007	Cattle Tanks	n/a	n/a	n/a	↓ forest ⁴
Mokany <i>et al.</i> 2008	Buckets	No diff.	No diff.	n/a	n/a
Palik <i>et al.</i> 2001	Ponds	n/a	↓ forest	n/a	No diff.
Skelly <i>et al.</i>	Ponds	n/a	n/a	↓ forest	n/a

2005

Werner *et al.* Ponds n/a n/a ↓ forest n/a

2007

¹For a salamander species, *Ambystoma maculatum*.

²For a frog species, *Rana sylvatica*.

³For a frog species, *Pseudacris crucifer*.

⁴For a frog species, *Hyla versicolor*.

Table 4. Effects of canopy cover on amphibian performance in ponds and artificial ponds (buckets and cattle tanks). All studies refer to frogs, except Earl *et al.* 2011, which examines two ambystomatid salamander species. ↓ forest indicates that the parameter is lower in the forest ponds or under closed canopy conditions. n/a indicates not applicable, and No diff. indicates that there was not a statistically significant effect. Most of the studies listed below use a binary categorization of canopy.

Reference	Habitat	Growth Rate	Developmental Rate	Survival
Earl <i>et al.</i> 2011	Cattle tanks	↑ forest	No diff.	↑ forest
Halverson <i>et al.</i> 2003	Ponds	↓ forest ¹ , No diff. ²	↓ forest	n/a
Hocking and Semlitsch 2008	Cattle tanks	↑ forest	↓ forest	↓ forest
Scheisari 2006	Ponds	↓ forest	↓ forest ³ , No diff. ²	n/a
Skelly <i>et al.</i> 2002	Ponds	↓ forest	n/a	↓ forest ² , No diff. ¹
Skelly <i>et al.</i> 2002	Aquarium	↑ forest ² , ↓ forest ¹	n/a	n/a
Skelly <i>et al.</i> 2005	Ponds	n/a	↓ forest	n/a
Werner and Glennemeir 1999	Ponds	↓ forest	n/a	↓ forest ^{3,4} , No diff. ²
Williams <i>et al.</i>	Cattle tanks	n/a	↓ forest ^{1,5} , No diff. ⁶	↑ forest ⁶ , ↓

¹For frog species, *Pseudacris crucifer*.

²For frog species, *Rana sylvatica*.

³For frog species, *Rana pipiens*.

⁴For the toad species, *Bufo americanus*.

⁵For the frog species, *Rana sphenoccephala*.

⁶For the frog species, *Hyla versicolor*.

Table 5. Amphibian and insect emergence estimates in biomass for ponds and wetlands in central and southern USA.

Taxa	Location	Biomass (mg DM/m ² /y)	Reference
Amphibians	Semi-permanent pond, SC	3,180*	Gibbons et al. 2006
Amphibians	Permanent ponds, MO	117 - 4,220*	Earl et al., unpublished data
Chironomids	Temporary pond, SC	516 - 1,599	Leeper and Taylor 1998
Chironomids	Wetland, AL	463 - 2,366	Stagliano et al. 1998
All Insects	Wetland, IL (various hydroperiods)	260 – 5,100	Whiles and Goldowitz 2001

*This estimate was originally made for wet weight. We adjusted it by assuming amphibians are 80% water.

CHAPTER 2

SPATIAL SUBSIDIES, TROPHIC STATE, AND COMMUNITY STRUCTURE: EXTRAPOLATING THE RIVER CONTINUUM CONCEPT TO PONDS

ABSTRACT

In aquatic systems, light and subsidy input often co-vary along a canopy cover gradient. The River Continuum Concept (RCC) predicts how this light/subsidy gradient affects ecosystem and community attributes in lotic systems. We examined the effects of light and subsidy input on ponds, using the RCC as a foundation for predictions. We placed pond mesocosms along a canopy gradient and manipulated subsidy input. We then sampled mesocosms for ecosystem and community parameters for two years during spring, summer and fall. Neither canopy cover nor litter input tended to dominate effects overall, highlighting the importance of the light-subsidy gradient combination that is frequently encountered in temperate aquatic systems. Ponds shifted from autotrophy or an autotrophy/heterotrophy balance with low canopy cover to net heterotrophy under high canopy cover in concordance with the RCC. Although litter input affected dissolved oxygen, we did not detect an effect of litter on trophic state, primary production or community respiration. We additionally found effects of both canopy cover and litter input on community composition, but very differently than that found in streams. In general, the grazer and shredder macroinvertebrate functional feeding groups were rare in

the pond mesocosms, and thus did not respond to treatments. The collector proportion of the community, mainly chironomids, increased with canopy cover and was higher in mesocosms with litter input than those without. This study along with many others highlights the importance of preserving intact terrestrial ecosystems surrounding ponds to maintain aquatic-terrestrial linkages for overall ecosystem function and community composition.

Spatial subsidies are resources (prey, detritus, nutrients) that move from a donor habitat to a recipient habitat (Polis and others 1997). This resource movement provides linkages among ecosystems and results in the open nature of most systems (Loreau and Holt 2004). These linkages help maintain ecosystem function and, in many cases, operate as ecosystem services (Tylianakis and others 2004). Theory predicts that the subsidy's trophic level and the food web structure determine how the subsidy cascades through the system. When subsidies are nutrients or detritus, bottom-up effects tend to occur due to the increase in nutrient availability to primary producers (Polis and others 1997). Studies have shown that subsidy amount and type influence community attributes and the degree to which resources cascade through a community and affect certain trophic levels (Rubbo and Kiesecker 2004; Nowlin and others 2007; Nowlin and others 2008; Rubbo and others 2008).

The importance of bottom-up effects and low trophic level subsidies has been predicted to vary in relation to the productivity of the recipient system (Persson and others 1996; Polis and others 1997). Marczak et al. (2007) performed a meta-analysis and found that consumer response was affected by the ratio of subsidy input to equivalent resources, but not by recipient habitat productivity. We might expect low trophic level subsidies to be especially important in supporting systems where primary productivity is severely limited due to low nutrient availability or, in the case of detritus, in providing an alternative resource for primary consumers.

In aquatic systems, canopy cover often determines both light availability and subsidy input in the form of senescing leaves and terrestrial invertebrates (Nakano and others 1999). This creates systems where subsidies are more prevalent in areas with low

primary productivity and less prevalent in areas with high primary productivity. The River Continuum Concept (RCC, Vannote and others 1980) is a framework that predicts the effects of the light/subsidy gradient as related to changes in stream order on the available organic matter types, the trophic status of the ecosystem, and the proportions of organisms from different functional feeding groups. However, the light/subsidy gradient also corresponds with other stream changes that relate to stream order, such as flow rate and water depth, among others (Vannote and others 1980). These corresponding gradients make it difficult to separate the effects the light/subsidy gradient and other characteristic changes associated with stream order.

We used the RCC as a framework to examine the applicability of its light/subsidy gradient predictions to the overall effects of subsidies on recipient ecosystems. We chose to examine the effects of subsidies and light availability on pond ecosystems because of their location along a similar light/subsidy gradient but with key differences in terms of their lentic nature. Thus, if the light/subsidy gradient is the key factor in the RCC, we would expect that closed canopy ponds follow the predictions for low-order streams with low light levels and high subsidy input, while open canopy ponds should follow the predictions of mid-order streams with high light levels and low subsidy input. In this study, we manipulated subsidy input (leaf litter) in *in situ* pond mesocosms placed along a canopy gradient to tease apart the two separate effects of canopy cover (light availability and subsidy input) and to examine the following tenets of the RCC on pond ecosystems:

1. An increase in light availability (decrease in canopy cover) increases the ratio of gross primary productivity (GPP) to community respiration (CR_{24}) indicating a shift from net heterotrophy to net autotrophy.
2. Changes in light availability and subsidy input change the proportions of biomass of functional feeding groups. Higher light availability will lead to higher proportions of grazers, while higher allochthonous input will lead to higher proportions of shredders.
3. A lack of coarse particulate organic matter will result in a shift from dominance by macroinvertebrates to dominance by zooplankton.

We additionally examined the effects of light availability and subsidy input on other important ecosystem parameters and the effects of a within system resource (a senesced aquatic grass) as another common pond detrital resource to better describe changes in ponds, including the following: nutrient concentrations, dissolved oxygen, pH, and litter decomposition rate. While other studies have examined many of these ideas (e.g. Batzer and others 2000; Sand-Jensen and Staehr 2009), our study offers a controlled experiment that eliminates much of the potentially confounding variation caused by pond size and hydroperiod.

METHODS

Experimental Design

In order to examine the effects of spatial subsidies across a light gradient, we used *in situ* mesocosms instead of natural ponds. Mesocosms allowed us to focus on our manipulated variables while standardizing many other variables known to influence pond communities

including pond size (surface area and depth), shape, age, and hydroperiod (Wellborn and others 1996; Gee and others 1997; Søndergaard and others 2005). Mesocosms were placed within experimental forestry plots (Hocking and Semlitsch 2007; Semlitsch and others 2008; Semlitsch and others 2009) in Oak-Hickory forest at Daniel Boone Conservation Area, Warren County, Missouri in early 2006 for another experiment (Hocking and Semlitsch 2008). Twelve mesocosms (1000L, 1.5 m diameter cattle watering tanks) were placed along a canopy cover gradient created by clearcutting at three sites (total N = 36 tanks) in winter 2004 to spring 2005. Each site contained mesocosms located within approximately 0-95% tree canopy cover, though the presence of shade cloth covers for most of the year (see below) made the shading gradient approximately 30-100%. Mesocosms were filled with water and 1 kg of leaves in February 2006, inoculated with zooplankton in April and May of 2006, and were used for a tadpole experiment until August (Hocking and Semlitsch 2008), after which tanks were left uncovered and allowed to develop communities through colonization and oviposition.

In fall of 2007 (28 and 29 November), litter manipulations were applied to tanks to examine the effects of spatial subsidies on pond ecosystems. Our three treatments consisted of spatial subsidies added (1 kg deciduous leaf litter added), a within system resource (1 kg of aquatic grass), and no input. Preliminary data in our high canopy cover areas (\approx 95% canopy cover) indicated that leaf litter deposition averaged 1.01 kg tree leaves per cattle tank surface area. Additionally, this is consistent with many other mesocosm studies (Boone and others 2007; Williams and others 2008; Earl and others 2011). For the leaf litter input, we used the natural mixture of deciduous leaves found in our area, consisting mostly of white oak (*Quercus alba*) and northern red oak (*Quercus*

rubra) with some hickory (*Carya* spp.) and sugar maple (*Acer saccharum*). For the within system input, we used prairie cordgrass (*Spartina pectinata*), a wetland obligate species found across much of the United States (USDA and NRCS 2010). This grass dies in the fall around the same time as leaf fall, acting as detrital storage. For the no input treatment and the within system input prior to the grass addition, we removed leaf litter present from the cattle tanks using a net with 2.5 cm gauge mesh. This method removed whole leaves and large leaf fragments, while retaining small leaf fragments and fine particulate organic matter. Subsidy treatments were renewed on 9 November 2008 by adding an additional 1kg of grass, leaves, or no litter to appropriate tanks. Leaves and grass were all collected after natural senescence from within the same conservation area where the study took place a few days before addition to tanks. In our experiment, grass was higher in both nitrogen (grass: 0.96%, deciduous leaves: 0.76%) and phosphorus (grass: 0.073%, deciduous leaves: 0.045%; Earl and Semlitsch, unpublished data) than leaves. Grass also had a lower C:N ratio than leaves (grass: 43.7, deciduous leaves: 60.5; Earl and Semlitsch, unpublished data).

After implementing subsidy treatments, tanks were covered with 27% high-density polyethylene PAK knit shade cloth with 2 mm sized openings (Hummert International, St. Louis, MO, USA) to prevent further introduction of leaf material and the escape of metamorphosing tadpoles as part of another study (40 wood frog hatchlings were introduced to each tank in spring of 2008 and 72 American toad hatchlings were introduced to each tank in spring of 2009 [Earl and Semlitsch, in press]). Tank covers were pushed into the water to facilitate oviposition by invertebrates and anurans. Tank covers were removed for at least 24 hours every 4-6 weeks to further facilitate

oviposition and colonization by aquatic animals, except during winter. Tanks were additionally left uncovered after the completion of the tadpole experiment from mid-August to late September to further allow oviposition and colonization.

Tanks were sampled in spring (mid-April), summer (mid-July), and fall (early November) in 2008 and 2009 for ecosystem parameters (GPP and CR_{24}), water quality (dissolved oxygen, pH, and nutrient concentrations), macroinvertebrates, and zooplankton. All samples for each sampling period were taken within a five-day period. Macroinvertebrate samples were always taken last, because the sampling technique suspended benthic material, likely altering the ecosystem and water quality measurements. Canopy cover was estimated each year in late spring or early summer using a convex spherical densiometer by taking four measurements from each tank (Ben Meadows, Janesville, WI, USA).

Ecosystem and Water Chemistry Sampling

To estimate GPP and CR_{24} , we utilized the diel oxygen method (Wetzel and Likens 2000) taking dissolved oxygen (to 0.01 mg L^{-1}) and temperature (to 0.1°C) at three consecutive sunrises and sunsets with a YSI 55 handheld meter (Yellow Springs Instruments, Yellow Springs, OH, USA). All measurements were taken within 1hr of sunrise or sunset (Fontaine and Ewel 1981), and diel oxygen measurements were only taken when the time between the first and last measurements contained no precipitation, low clouds during daylight hours, and low wind conditions which resulted in no rippling of water in tanks ($< 8 \text{ mph}$), which allowed us to use a 0.05 diffusion coefficient for all calculations (Wetzel and Likens 2000). Many studies use the daily peak in dissolved oxygen for their measurements instead of sunset. In a similar study using cattle tanks, the oxygen

concentrations an hour before sunset were not statistically different from any afternoon measurement of dissolved oxygen (Williams et al., unpubl. data; Williams and others 2008), indicating that our measurements were similar to the peak concentration. We measured pH to 0.01 units in the morning between 1-4hrs after sunrise using a pH testr3 (Oakton Instruments, Vernon Hills, IL, USA).

We additionally measured nutrient concentrations (total and dissolved nitrate and soluble reactive phosphorus [SRP]) in water by taking two 60mL water samples (one raw sample and one filtered) per tank during each sampling period, freezing them until analysis, and analyzing them using a Technicon Flow Injection Auto-Analyzer II (Technicon Systems, Oakland, CA, USA). Filtered samples were filtered through glass fiber filters with a 0.7 μm pore size (AP40 filters, Millipore, Billerica, MA, USA) for measurement of dissolved nitrate and SRP. Raw water samples were digested using the sulfuric acid-nitric acid digestion (Clesceri and others 1989) for measurement of total nitrate and total SRP. Nitrate was analyzed to 0.01 mg L^{-1} using the cadmium reduction method, and SRP was analyzed to 1 $\mu\text{g L}^{-1}$ using the molybdenum blue method (Clesceri and others 1989). Filtered water samples were also analyzed for total tannin concentration using the Tyrosine method. We used the purchased Tanniver® 3 tannin/lignin reagent (Hach, Loveland, CO, USA) and a DR/2400 portable spectrophotometer (Hach, Loveland, CO, USA) to determine sample tannin concentration in relation to tannic acid standards (Clesceri and others 1989).

Macroinvertebrate and Zooplankton Sampling

We took macroinvertebrate samples by plunging a 5.2 cm diameter PVC into the cattle tank approximately 10cm from the edge of the tank and covering the bottom of the pipe

with our hand, capturing the entire water column. We repeated this method at three different locations in each tank that were combined into one sample. We ran the samples through a sieve with 590 μm mesh size (Fisher Scientific, Pittsburgh, PA, USA) and preserved the whole sample in 95% ethanol until sorted in the laboratory, when we transferred macroinvertebrates into 70% ethanol. All macroinvertebrates were identified to genus (except chironomids, which were identified to tribe and snails and worms were identified to family) and assigned a functional feeding group (shredder, grazer, collector or predator) using Merritt and Cummins (1996) and Thorp and Covich (2009). We measured the length of invertebrates to 0.01 mm using a stage micrometer with a dissecting microscope (Nikon type 102, Tokyo, Japan) for a subset of each taxa and calculated the biomass and proportion of biomass in each feeding group by tank using published length/mass relationships (Myer 1989; Nyström and Pérez 1998; Benke and others 1999; Edwards and others 2009). Because of the high levels of worm fragmentation, we separated worms from other invertebrates, dried them to a constant weight at 60°C, and weighed them on a Mettler AT261 balance (Mettler-Toledo International, Columbus, OH, USA) to obtain biomass estimates.

We sampled zooplankton using an integrated pipe sampler, consisting of a 6.5 cm diameter PVC pipe fitted with a rubber stopper to capture a portion of the water column. 1 L subsamples were taken from three locations in each tank. When water levels were too low, two 0.5 L samples were taken from the same location and combined for each 1L subsample. Water was then filtered through a 0.10 mm plankton net and preserved in 70% ethanol for later examination in the laboratory. We counted and classified zooplankton into four categories: ostraods, cladocera, calanoid copepods, and cyclopoid

copepods using a dissecting microscope (Nikon type 102, Tokyo, Japan). Cladocera were further identified to genus for biomass estimates. Plankton were measured to 0.01 mm using a stage micrometer and calculated the biomass and proportion of biomass in each feeding group by tank using published length/mass relationships (McCauley 1984; Anderson and others 1998). Cladocera and ostracods were classified as filterer/herbivores and copepods were classified as omnivores (Thorp and Covich 2009).

Decomposition Study

Leaf packs were used to examine differences in decomposition rates between leaves and grass along the canopy gradient within tanks containing that litter type. We did not assess decomposition in no input tanks to maintain the no litter input treatments in those tanks. Leaf packs were made out of 27% high-density polyethylene PAK knit shade cloth with 2 mm sized openings (Hummert International, St. Louis, MO, USA). Litter material was collected 24 March 2008 from Daniel Boone Conservation Area. Five grams of the appropriate dry litter was weighed out on a Mettler BD Balance (Mettler-Toledo International, Columbus, OH, USA) to 0.01 g and gathered in mesh, which we secured with a zip tie. Twelve leaf packs were placed around the edge of each tank by tethering them with fishing line that was taped to the rim of the tank, so that the leaf packs would sit in the litter at the bottom equally spaced apart. Leaf packs were placed in tanks on 13 April 2008, at which time an extra leaf pack for each tank was transported and dunked in the water to estimate mass loss due to handling. Two leaf packs were removed from each tank after 3, 13, 28, 61, 123, and 183 days. After collection, we sorted leaf packs to remove macroinvertebrates, and we cleaned leaves by hand to remove biofilms. We then allowed leaves to dry in paper bags at room temperature for at

least a month until constant weight. We stored leaf packs in the refrigerator until processing, and we processed all leaf packs within 48 hr of collection.

Statistical Analysis

We used SAS/STAT for all analyses (SAS 2004). For all response variables except decomposition, we first averaged each variable in each season across years. This reduced the number of analyses and allowed us to focus on only the most robust patterns. We separated variables into five categories: ecosystem (GPP, CR₂₄, GPP/CR₂₄), water chemistry (minimum dissolved oxygen, pH, tannin concentration), nutrients (total and dissolved nitrogen and SRP, N/P ratio), macroinvertebrates (biomass and proportion of functional feeding groups), and zooplankton (biomass and proportion of functional feeding groups). Within each variable category, we examined Pearson's correlations and removed any variables that were highly correlated ($-0.70 > r > 0.70$). We then performed a MANOVA using Wilk's Lambda for each category to look for effects of litter treatment, canopy cover (as a continuous variable) and the interaction between litter treatment and canopy cover with site as a blocking variable. For categories with significant factors, we then used a repeated-measures ANCOVA to examine seasonal differences in the effects of litter treatment, canopy cover (as a continuous variable), the interaction of the two variables, and site as a blocking variable for each variable within that category. We square-root transformed all zooplankton and macroinvertebrate variables. All other variables met the assumption of homoscedasticity. All tests were performed with $\alpha = 0.05$.

For the decomposition study, we calculated the decomposition coefficient (k) for each tank and ran an ANCOVA to examine the effects of subsidy input treatments, canopy

cover (as a continuous variable), the interaction of the two variables, and used site as a blocking variable (Bärlocher 2005). When the overall model was found to be nonsignificant, we ran a t-test to determine whether grass and leaves decomposed at different rates in our study as found elsewhere (Brinson and others 1981).

RESULTS

Ecosystem Parameters

GPP and CR₂₄ were highly correlated in each season ($r = 0.94 - 0.99$; Table 1), so we did not include CR₂₄ in the MANOVA. The ecosystem MANOVA indicated a strong effect of canopy cover ($F_{6,17} = 14.61$, $p < 0.0001$), but no effect of litter ($F_{12,34} = 1.33$, $p = 0.25$) or the interaction between litter and canopy cover ($F_{12,34} = 1.19$, $p = 0.33$). The ratio GPP/CR₂₄ was affected by the interaction between season and canopy cover ($F_{2,52} = 4.01$, $p = 0.02$, Table 2). The ratio decreased with increasing canopy cover in summer and fall but not in spring (Figure 1a). GPP was also affected by the interaction between season and canopy cover ($F_{2,62} = 76.33$, $p < 0.0001$). GPP decreased with increases in canopy cover in summer and fall, but not spring. The slope was steeper in summer than fall (Figure 1b).

Community Structure

Zooplankton communities consisted of cyclopoid copepods, *Daphnia*, *Ceriodaphnia*, *Bosmina*, *Scaphlopheberis*, and ostracods. All taxa were detected in all sampling periods, except *Daphnia* and *Scaphlopheberis* in spring 2008. Total zooplankton biomass was highly correlated with herbivore biomass (all $r > 0.96$; Table 3) due to high proportions of cladocera in samples. The proportion of omnivores was highly negatively correlated

with the herbivore proportion (all $r < -0.87$; Table 3). Thus, we removed the omnivore proportion from our overall zooplankton community composition MANOVA. We found no effect of canopy cover, litter or the interaction between litter and canopy cover (all $p > 0.11$, Table 2) on zooplankton community composition. However, there was a marginally significant positive relationship between total zooplankton biomass and canopy cover ($F_{1,27} = 3.69$, $p = 0.07$). Total zooplankton biomass was not affected by litter, season or any interactions (all $p > 0.33$).

Macroinvertebrate communities were dominated by chironomids, *Chaoborus* (phantom midges), and naidid worms. We also frequently detected lymnaeid snails, culicids (mosquitoes), and *Pachydiplax longipennis* (blue dasher dragonfly). Because collectors dominated communities, total invertebrate biomass and collector biomass were highly correlated (all $r > 0.94$; Table 4). The biomass and proportions of grazers and shredders were highly correlated, as were the proportion of collectors and proportion of predators (all $r > 0.86$; Table 4). Due to the aims of the study, we chose to eliminate the variables grazer biomass, shredder biomass, and the proportion of predators in the MANOVA on macroinvertebrate community composition. The MANOVA indicated a marginal effect of litter treatment ($F_{30,22} = 1.83$, $p = 0.07$) and canopy cover ($F_{15,11} = 2.48$, $p = 0.07$) but no effect of their interaction ($p = 0.20$) on the macroinvertebrate community composition.

The collector proportion was significantly affected by both canopy cover ($F_{1,27} = 6.84$, $p = 0.01$) and litter ($F_{2,27} = 4.55$, $p = 0.02$, Table 2) across seasons, but not affected by season or interactions with season (all $p > 0.25$). The collector proportion increased with canopy cover and was higher in grass and leaf litter treatments than no litter (Figure

2). Collector biomass was affected by an interaction between season and canopy cover ($F_{2,58} = 5.52$, $p = 0.006$) and litter across seasons ($F_{2,29} = 24.64$, $p < 0.0001$). Collector biomass increased with canopy cover in spring and summer, but not fall, and was higher in mesocosms containing grass and leaves than those with no litter. Predator biomass was significantly affected by the interaction between season and canopy cover ($F_{2,58} = 8.19$, $p = 0.001$) and season and litter treatment ($F_{4,58} = 5.09$, $p = 0.002$). Predator biomass increased with canopy cover in spring, decreased in fall and was not affected in summer. In spring, there was higher predator biomass in the leaf litter treatment than the grass or no litter treatments. There was no effect of canopy cover, litter, season, or any interaction of grazer proportion (all $p > 0.11$) or shredder proportion (all $p > 0.46$).

Water Chemistry

Dissolved oxygen and pH were often correlated but not consistently enough to be removed from the model (Table 5). The water chemistry MANOVA showed a significant interaction between litter and canopy cover ($F_{18,28} = 3.42$, $p = 0.0007$).

Dissolved oxygen was affected by the interaction between season and canopy cover ($F_{2,54} = 30.75$, $p < 0.0001$) and season and litter treatment ($F_{4,54} = 5.08$, $p = 0.002$), but not the three-way interaction ($F_{4,54} = 2.01$, $p = 0.11$). Dissolved oxygen decreased with canopy cover in all seasons, but the slope was much steeper in fall than spring and summer was intermediate. In summer, dissolved oxygen was lower in mesocosms with grass litter than with no litter and leaf litter was intermediate. Litter did not affect dissolved oxygen in spring or fall.

pH had a significant interaction between season, litter, and canopy cover ($F_{4,54} = 2.53$, $p = 0.05$, Table 2). In spring, there was no effect of litter or canopy cover on pH,

while in summer, there was a negative relationship between pH and canopy cover in mesocosms with grass and no litter, but no relationship in mesocosms with leaf litter. The slope was steeper in mesocosms with no litter than with grass litter. In fall, there was a negative relationship with canopy cover, but no effect of litter input. Tannin concentrations had a significant interaction between season and canopy cover ($F_{2,54}=4.53$, $p = 0.02$) and season and litter input ($F_{4,54}= 3.21$, $p = 0.02$). Tannin concentration increased with canopy cover in all seasons, but the slope was much steeper in fall. In spring, tannin concentrations were much higher in mesocosms with leaf litter, but in summer and fall, concentrations were highest in grass litter mesocosms.

Several nutrient variables were correlated, but not highly or consistently enough to be removed from the model (Table 6). The nutrient MANOVA indicated that the interaction between litter input and canopy cover significantly affected nutrient concentrations ($F_{30,26}= 2.85$, $p = 0.004$). Several of the nutrient measures indicated that nutrient concentrations increased with canopy cover in the no litter treatment, but not with grass litter or leaf litter input (Table 2). However, these trends varied by nutrient and season. Dissolved SRP, dissolved nitrate, and total SRP were significantly affected by the three-way interaction among season, litter treatment and canopy cover (all $p < 0.003$). In spring, dissolved SRP increased with canopy cover in all litter treatments similarly. In summer, dissolved SRP increased with canopy cover in no litter and grass treatments, but did not in the leaf litter treatment, while in fall, dissolved SRP increased with canopy cover in no litter treatments only. In summer and fall, dissolved nitrate increased with canopy cover in the no litter treatment, but not in the grass or leaf

treatments or in spring. Total SRP increased with canopy cover in no litter mesocosms in fall, but not spring or summer.

Total nitrate was affected by season ($F_{2,54} = 19.67$, $p < 0.0001$), but not by the interaction between season and canopy cover or litter input (all $p > 0.16$, Table 2). Total nitrate was higher in summer than spring or fall. Total nitrate decreased with canopy cover across all seasons ($F_{1,27} = 10.13$, $p = 0.004$). For the ratio of total nitrate to total SRP (TN/TP), there was a significant interaction between season and litter ($F_{4,54} = 4.62$, $p = 0.003$) and an overall negative relationship with canopy cover across all seasons ($F_{1,27} = 16.39$, $p = 0.0004$). In fall, mesocosms with grass litter had a higher TN/TP ratio than those with leaves or no litter.

Decomposition Study

The grass litter decomposed faster than leaf litter ($p = 0.02$; grass $k = 0.0057 \pm 0.0004$, leaf $k = 0.0043 \pm 0.0004$). Canopy cover and the interaction between canopy cover and leaf litter did not affect decomposition rates (all $p > 0.09$).

DISCUSSION

Our results conformed to some of the predictions in the RCC. This indicates that the combination of spatial subsidies and light availability drive various outcomes in a predictable way across different aquatic habitats, despite differences in their lentic or lotic nature. The most important aspect was the shift in trophic states (Dodds and Cole 2007) in relation to canopy cover, a central tenet of the RCC. However, we did find some trends that differed from lotic systems, particularly in relation to effects on functional feeding groups. These dissimilarities may be due mainly to differences in

community characteristics between the two systems. Additionally, our study found strong influences of both canopy cover (light availability) and litter input (spatial subsidies) on ponds, though few interactions between the two factors. Neither canopy cover nor litter input tended to dominate effects overall, highlighting the importance of the light-subsidy gradient combination that is frequently encountered in temperate aquatic systems.

As predicted, we found that GPP/CR_{24} decreased with increases in canopy cover indicating a shift from net autotrophy or an auto/heterotrophy balance in high light environments to net heterotrophy in low light environments (Dodds and Cole 2007). This relationship occurred only in summer and fall. Our spring sampling period occurred prior to leaf out, which indicates that light availability is a driving factor in the relationship between canopy cover and pond trophic status, as would be expected. We found the same general effects of canopy cover and season on GPP. As further corroboration of the shift from autotrophy in open canopy ponds to heterotrophy in closed canopy ponds, dissolved oxygen and pH also decreased with increasing canopy cover due to the consumption of oxygen during respiration and association of pH with dissolved carbon dioxide (Sand-Jensen and Staehr 2007). Other studies have also found that small lakes change in trophic status with relation to canopy cover (Sand-Jensen and Staehr 2007, 2009; Staehr and others 2011).

Contrary to previous work, our ecosystem variables did not respond to the manipulation of litter input. Rubbo and others (2006) found that the input of plant material in temporary ponds strongly influences net ecosystem production, a similar measure to GPP/CR_{24} . In their study and ours, whole leaves and large leaf fragments

were removed, but fine particulate organic matter remained. This source of organic matter likely continues to provide substrates for microbial decomposition, which may result in delayed effects on ecosystem parameters. Rubbo and others (2006) detected an effect in the first year, but their use of temporary ponds may have resulted in a larger effect, because the oxidation of dry litter increases nutrient leaching and decomposition in broadleaf litter (Battle and Golladay 2001). Interestingly, GPP and CR₂₄ were not correlated in Rubbo and others (2006) but were highly correlated in our study. Our high correlation is similar to a study on small Danish Lakes (Sand-Jensen and Staehr 2007), in which the correlation resulted from phytoplankton dominating respiration as supported by chlorophyll data. We unfortunately did not measure chlorophyll and cannot address this mechanism.

Rubbo and others (2006) also found that the removal of leaf litter was related to an increase in dissolved oxygen due to lowered community respiration. Dissolved oxygen frequently decreases with increased leaf litter quantity (Magnusson and Williams 2006; Rubbo and others 2008; Cohen and others 2012b). Our data displayed a similar trend with lower dissolved oxygen in mesocosms with plant litter than no litter. Furthermore, the dissolved oxygen was lower with grass litter than leaf litter, which is likely related to the faster decomposition rates of grass than leaves. This suggests that respiration would be higher in grass mesocosms, but there was no evidence for this. It is possible that we were simply unable to detect a difference due to the lower accuracy of membrane oxygen meters than newer optical technology and the use of point measurements instead of continuous logging meters.

We also found that the community composition responded to our treatments, but these did not support our predictions. Our major predictions related to macroinvertebrate grazers and shredders. We found that the two groups were relatively rare within our experimental mesocosms, and we were unable to detect any differences with regard to canopy cover or litter input. Although shredders are very important to litter decomposition in stream systems (Graça 2001), shredders tend to occur in low abundances in ponds (Batzler and Wissinger 1996; Palik and others 2001; Batzler and Palik 2007). In fact, few pond invertebrates even fill the shredder niche, primarily pyralid moth larvae, limnephilid caddisflies (Smyers and others 2011), and haliplid beetles, which were very rare or not present in our mesocosms. They also may be just as likely to shred dead macrophytes as leaf litter input (Taylor and Batzler 2010), which may obscure any relationship to canopy cover in natural ponds. Alternatively, shredders may actually respond positively to the increase in macrophyte biomass associated with open canopy ponds, as seen for haliplids in temporary ponds in Minnesota (Palik and others 2001).

The proportion of collectors did respond to our treatments, perhaps in part because they dominated communities. The proportion of macroinvertebrate collectors increased with canopy cover and was higher in mesocosms with plant litter than without. This functional feeding group consisted mostly of chironomini chironomids, which are partially characterized by their red color from the presence of hemoglobin that helps them survive in low oxygen conditions. Low oxygen conditions were present in the closed canopy mesocosms containing plant litter in the present study. Interestingly, in Minnesota, chironomid abundance declines with canopy cover in temporary ponds

(Hanson and others 2009). Recent evidence suggests that chironomids are generalists, consuming both algae and detritus (Taylor and Batzer 2010), though preferences may change depending on species and geographic locale.

Other studies have found very mixed results with regard to functional feeding groups in ponds and litter manipulations, finding different trends in different years (Batzer and Palik 2007) or weak effects (Batzer and others 2004; Hanson and others 2009). Many of these studies have looked at ponds that vary in canopy cover as well as hydroperiod and the presence of fish. Hydroperiod in particular is a very strong driver of pond community composition (Wellborn and others 1996). It may be that the relationship of canopy cover to community composition varies with hydroperiod. These different gradients may act as different sized filters for the community (Poff 1997). We may have been able to detect an effect of canopy cover on macroinvertebrates while others have not, because we focused on one end of the hydroperiod gradient: permanent ponds. Further examination of the effects of canopy on pond communities may benefit from limiting variability in hydroperiod.

Zooplankton also did not respond to treatments as predicted. We expected zooplankton to dominate communities in mesocosms without litter, though zooplankton did not respond to the litter treatment at all. Rubbo et al. (2008) also found no response of zooplankton to litter input. We did find that total zooplankton biomass increased with canopy cover. In Minnesota, copepods and cladocera abundances show a positive relationship with canopy cover (Hanson and others 2009). This may be due to changes in food sources such as suspended organic matter, which were not measured in this study.

When we did find effects of plant litter, the types of plants tended to act similarly but different from no input, despite differences in quality between our two litter types. There is a great deal of evidence that litter quality can have wide ranging consequences in both streams (reviewed in Graça 2001) and ponds (Palik and others 2006; Stoler and Relyea 2011; Cohen and others 2012a). Grass was higher in quality for all measures (nitrogen, phosphorus, and carbon to nitrogen ratio) and also decomposed faster than leaves, likely the cause of differences in dissolved oxygen. We found differences in tannin concentration among litter types, but they varied with season. Williams et al. (2008) used a very similar experimental design and found that litter type affected phytoplankton and periphyton levels. Although we did not measure these two parameters, they would have likely been reflected in our GPP measure. The litter quality differences in our study simply may not be great enough to affect many community and ecosystem variables, as indicated by our data.

Our study demonstrates the importance of both shading and litter input to pond ecosystems. We found that changes in trophic state due to canopy cover occur in ponds in concordance with the RCC. However, our pond mesocosms appear to have fundamental differences from streams in community composition, mainly the rarity of shredders, which appears to affect how functional feeding groups respond to canopy cover and litter input, significantly diverging from the RCC. Overall, the input of spatial subsidies (plant litter) appears to be an important factor for community composition and water chemistry. Further research on pond organism diet and mechanistic pathways may further elucidate the importance of leaf litter and canopy cover in pond ecosystems. This study along with many others highlights the importance of preserving intact terrestrial

ecosystems surrounding ponds to maintain aquatic-terrestrial linkages for overall ecosystem function and community composition.

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Table 1. Pearson's correlations among ecosystem variables. All data points contained an average of values from 2008 and 2009 for each season. Correlations in bold indicate significance at an alpha of 0.05. Sample sizes are reported below each correlation.

		CR ₂₄	GPP/CR ₂₄
GPP	Spring	0.94	-0.16
		n = 35	n = 35
	Summer	0.99	0.08
		n = 35	n = 34
	Fall	0.95	0.26
		n = 35	n = 30
CR ₂₄	Spring		-0.25
			n = 35
	Summer		0.02
			n = 34
	Fall		0.10
			n = 30

Table 2. Summary of results from repeated measures ANOVAs. CC = canopy cover, NE = no effect. - = a negative relationship or lower, + = a positive relationship or higher. SP = spring, SU = summer, F = fall. L = leaves, G = grass, NL = no litter.

	CC ¹	Litter	Season	Season* CC	Season* Litter	Season* Litter * CC
Ecosystem						
GPP	NE	NE	NE	- SU, F; NE SP	NE	NE
GPP/CR ₂₄	NE	NE	NE	- SU, F; NE SP	NE	NE
Phys./Chem.						
pH	-	NE	NE	NE	NE	NE
Dissolved oxygen	NE	NE	NE	always -, SP > Su > F	SU: NL > L > G	NE
Tannins	+	NE	NE	always +, more + F	SP: L > G, NL; Su, F: G > L, NL	NE
Nutrients						
Total Nitrate	-	NE	SU > SP, F	NE	NE	NE

Dissolved Nitrate	NE	NE	NE	NE	NE	SU, F: + NL
Total SRP	NE	NE	NE	NE	NE	F: + NL
Dissolved SRP	NE	NE	NE	NE	NE	SP: +; SU: + G, NL; F: + NL
Total N/P	-	NE	NE	NE	F: G > L, NL	NE
Macroinvertebrates						
Collector biomass	NE	L, G > NL	NE	+ SP, F	NE	NE
Collector proportion	+	L, G > NL	NE	NE	NE	NE
Grazer proportion	NE	NE	NE	NE	NE	NE
Predator biomass	NE	NE	NE	+ SP, - F, NE SU	SP: L > G, NL	NE
Shredder proportion	NE	NE	NE	NE	NE	NE
Zooplankton						

Total Biomass	+	NE	NE	NE	NE	NE
Herbivore biomass	NE	NE	NE	NE	NE	NE
Herbivore proportion	NE	NE	NE	NE	NE	NE
Omnivore biomass	NE	NE	NE	NE	NE	NE

¹Canopy cover is a continuous variable.

Table 3. Pearson's correlations among zooplankton community variables. All data points contained an average of values from 2008 and 2009 for each season. Correlations in bold indicate significance at an alpha of 0.05. Sample sizes are reported below each correlation. All variables have been square root transformed.

		Omnivore Biomass	Herbivore Biomass	Omnivore Proportion	Herbivore Proportion
Total	Spring	0.32	0.96	-0.33	0.27
Biomass		n = 35	n = 35	n = 34	n = 34
	Summer	0.36	0.99	-0.28	0.28
		n = 35	n = 35	n = 35	n = 35
	Fall	-0.17	1.00	-0.37	0.22
		n = 35	n = 35	n = 35	n = 35
Omnivore	Spring		0.06	0.63	-0.47
Biomass			n = 35	n = 34	n = 34
	Summer		0.22	0.66	-0.47
			n = 35	n = 35	n = 35
	Fall		-0.21	0.36	-0.28
			n = 35	n = 35	n = 35
Herbivore	Spring			-0.56	0.49
Biomass				n = 34	n = 34
	Summer			-0.41	0.42
				n = 35	n = 35

	Fall	-0.42	0.26
		n = 35	n = 35
Omnivore	Spring		-0.90
Proportion			n = 34
	Summer		-0.87
			n = 35
	Fall		-0.91
			n = 35

Table 4. Pearson’s correlations among macroinvertebrate community variables. All data points contained an average of values from 2008 and 2009 for each season. Correlations in bold indicate significance at an alpha of 0.05. Sample sizes are reported below each correlation. All variables have been square root transformed.

		Collector	Predator	Grazer	Shredder	Collector	Predator	Grazer	Shredder
		Biomass	Biomass	Biomass	Biomass	Proportion	Proportion	Proportion	Proportion
Total	Spring	0.94	0.58	0.21	0.23	0.12	-0.04	0.09	0.23
Biomass		n = 35	n = 35	n = 35	n = 35	n = 33	n = 33	n = 33	n = 33
	Summer	0.99	0.08	-0.03	-0.09	0.33	-0.34	-0.06	-0.01
		n = 35	n = 35	n = 35	n = 35	n = 34	n = 34	n = 34	n = 35
	Fall	0.99	0.15	0.26	0.13	0.43	-0.50	0.10	0.13
		n = 35	n = 35	n = 35	n = 35	n = 35	n = 35	n = 35	n = 35
Collector	Spring		0.29	0.09	0.15	0.37	-0.32	0.02	0.14
Biomass			n = 35	n = 35	n = 35	n = 33	n = 33	n = 33	n = 33
	Summer		-0.02	-0.12	0.08	0.44	-0.42	-0.15	-0.03

		n = 35	n = 35	n = 35	n = 34	n = 34	n = 34	n = 34
	Fall	0.06	0.21	0.14	0.48	-0.56	0.04	0.14
		n = 35	n = 35	n = 35	n = 35	n = 35	n = 35	n = 35
Predator	Spring		0.27	0.37	-0.43	0.64	0.09	0.37
Biomass			n = 35	n = 35	n = 33	n = 33	n = 33	n = 33
	Summer		0.33	0.03	-0.69	0.77	0.26	0.11
			n = 35	n = 35	n = 34	n = 34	n = 34	n = 34
	Fall		0.25	-0.05	-0.30	0.55	0.20	-0.05
			n = 35	n = 35	n = 35	n = 35	n = 35	n = 35
Grazer	Spring			-0.06	-0.11	0.05	0.94	-0.06
Biomass				n = 35	n = 33	n = 33	n = 33	n = 33
	Summer			-0.11	-0.48	0.13	0.98	-0.09
				n = 35	n = 34	n = 34	n = 34	n = 34
	Fall			-0.07	-0.09	-0.04	0.87	-0.07
				n = 35	n = 35	n = 35	n = 35	n = 35

Shredder	Spring	-0.09	0.20	-0.06	1.00
Biomass		n = 33	n = 33	n = 33	n = 33
	Summer	-0.07	0.00	-0.12	0.87
		n = 34	n = 34	n = 34	n = 34
	Fall	0.10	-0.12	-0.07	1.00
		n = 35	n = 35	n = 35	n = 35
Collector	Spring		-0.88	-0.06	-0.09
Proportion			n = 33	n = 33	n = 33
	Summer		-0.87	-0.45	-0.18
			n = 34	n = 34	n = 34
	Fall		-0.86	-0.16	0.10
			n = 35	n = 35	n = 35
Predator	Spring			-0.04	0.20
Proportion				n = 33	n = 33
	Summer			0.09	0.10

			n = 34	n = 34
	Fall		-0.02	-0.12
			n = 35	n = 35
Grazer	Spring			-0.06
Proportion				n = 33
	Summer			-0.10
				n = 34
	Fall			-0.07
				n = 35

Table 5. Pearson’s correlations among water chemistry variables. All data points contained an average of values from 2008 and 2009 for each season. Correlations in bold indicate significance at an alpha of 0.05. Sample size is 35 for each correlation.

		pH	Tannin
DO	Spring	0.78	-0.62
	Summer	0.63	-0.65
	Fall	0.86	-0.83
pH	Spring		-0.47
	Summer		-0.61
	Fall		-0.72

Table 6. Pearson's correlations among nutrient concentration variables. All data points contained an average of values from 2008 and 2009 for each season. Correlations in bold indicate significance at an alpha of 0.05. Sample size is 35 for each correlation.

		TP	TN/TP	DN	DP
TN	Spring	0.78	-0.25	0.02	0.19
	Summer	0.65	-0.16	0.00	-0.02
	Fall	0.22	-0.06	-0.05	-0.10
TP	Spring		-0.46	-0.10	0.44
	Summer		-0.73	0.45	0.60
	Fall		-0.74	0.43	0.78
TN/TP	Spring			0.62	-0.51
	Summer			-0.45	-0.68
	Fall			-0.42	-0.64
DN	Spring				-0.14
	Summer				0.76
	Fall				0.61

Figure 1. Relationship of GPP/CR₂₄ (a) and GPP (b) with canopy cover in pond mesocosms during different seasons. Each data point represents a single mesocosm in a single season with data averaged over two years.

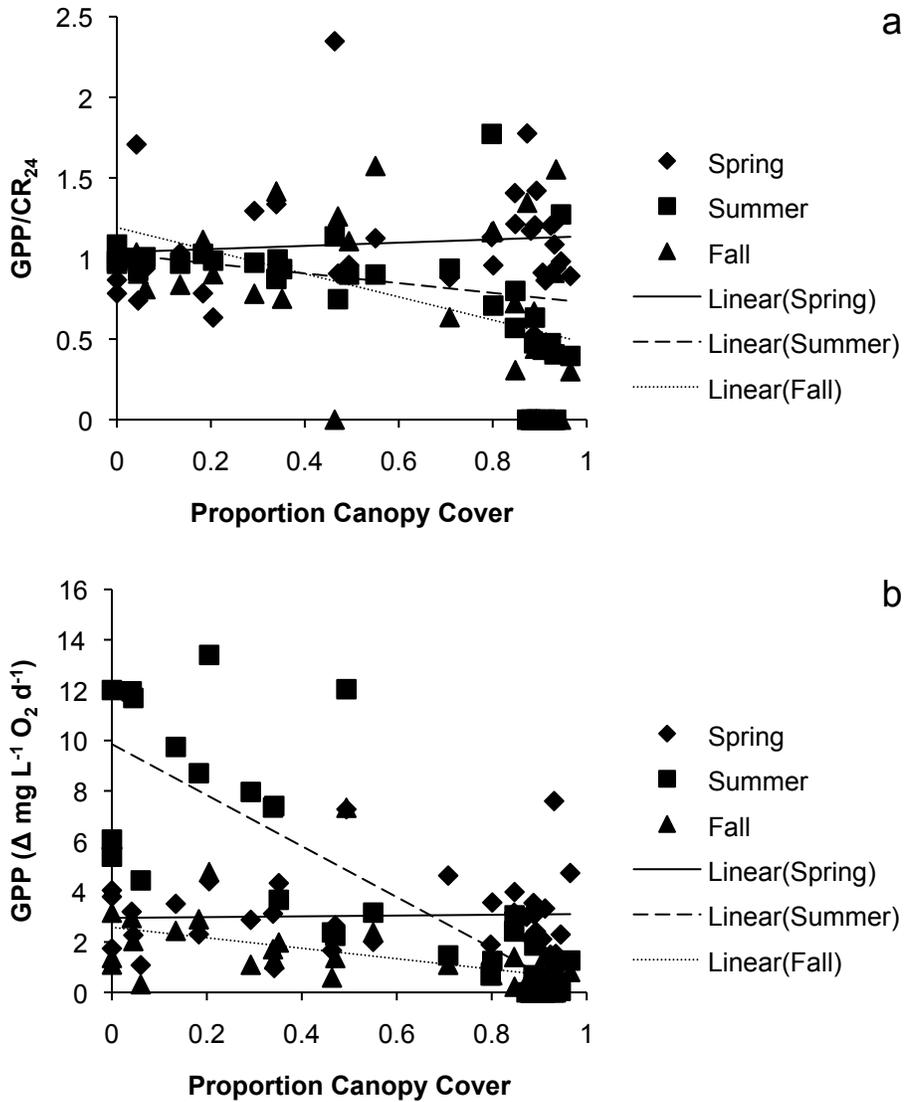
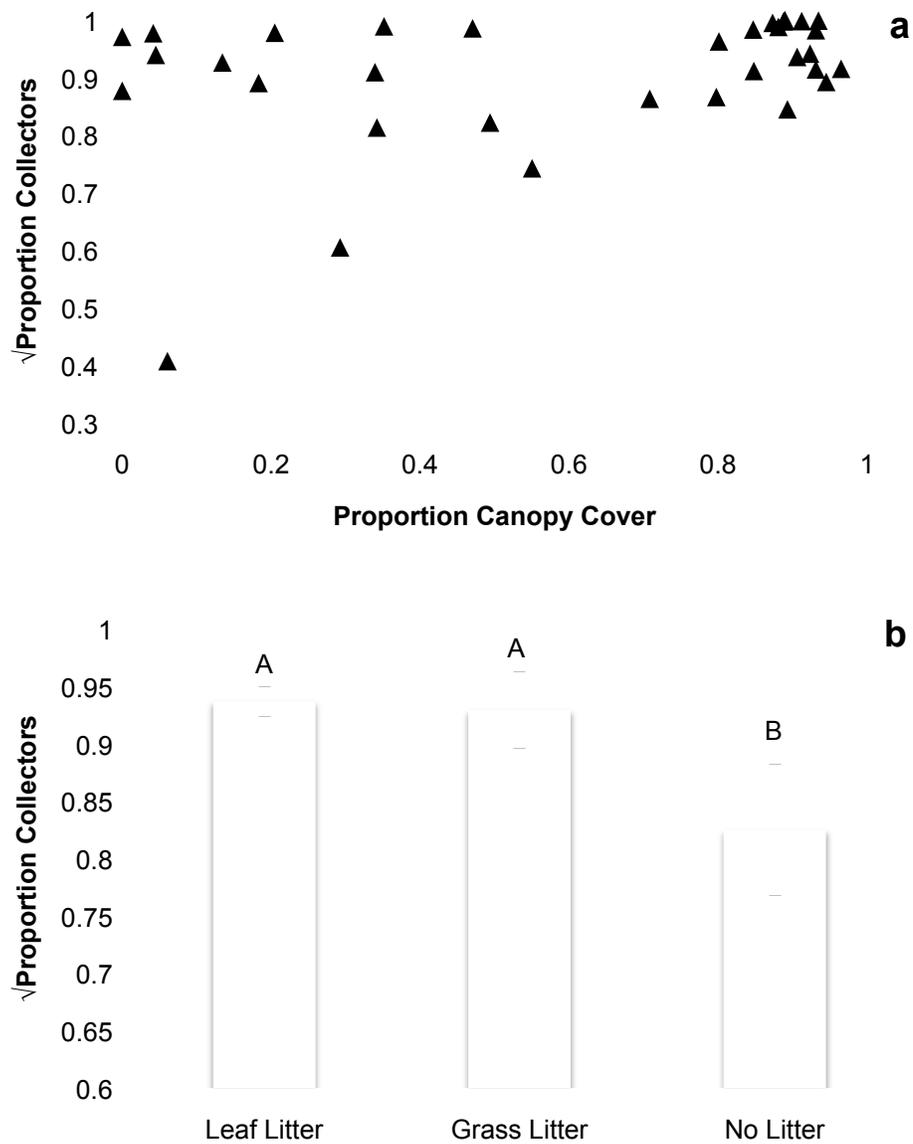


Figure 2. Relationship of macroinvertebrate collector proportion with canopy cover in pond mesocosms under different litter treatments. Each data point represents a single mesocosm with data averaged across three seasons and two years and is square root transformed.



CHAPTER 3

EFFECTS OF CANOPY ON POND COMMUNITY STRUCTURE IN PERMANENT, FISHLESS PONDS IN MISSOURI, USA

ABSTRACT

Hydroperiod and the presence of fish are thought to be two important structuring gradients in pond habitats. However, other gradients may be secondarily important, such as canopy cover. Previous studies have shown weak or conflicting patterns of changes in community structure with canopy cover, but many of these studies have simultaneously examined canopy and hydroperiod. Hydroperiod is such a strong gradient that it may mask effects of other variables. We examined the effects of canopy cover on macroinvertebrate community structure in twelve fishless, permanent ponds in central Missouri, USA. We chose only fishless, permanent ponds to eliminate variation due to hydroperiod and the presence of fish. Ponds were sampled in June 2009 and 2010 and biomass (mg/m^2) of macroinvertebrates was estimated and classified in terms of functional feeding group, habit (microhabitat use), and taxonomic class. We performed non-metric multidimensional scaling to examine relationships between community structure and canopy cover, leaf litter input, pond surface area, and pond depth. Leaf

litter input affected functional feeding groups with marginal significance in both years, and leaf litter and canopy cover affected habit significantly in 2010. The biomass of collectors increased and herbivores decreased with increasing leaf litter. Similarly, burrowers increased and plant-associated macroinvertebrates decreased with increasing leaf litter and canopy cover. We found no associations with taxonomic structure and no effect of surface area or water depth. Our study suggests that canopy cover is a structuring gradient for macroinvertebrate communities in fishless, permanent ponds. Further research on this topic will further characterize this relationship and determine whether the effects of canopy cover change with hydroperiod. This study and many others highlight the need to consider the terrestrial habitat surrounding ponds when managing them for aquatic biodiversity and ecosystem function.

Environmental gradients are thought to act as “filters” in determining community composition. Different species can “pass through” a filter if they possess traits suitable for surviving and reproducing under the conditions of that filter (Poff, 1997). In pond habitats, two environmental gradients have been identified as very strong site-level filters: hydroperiod and the presence of fish (Batzer & Wissinger, 1996; Wellborn, Skelly & Werner, 1996). However, other gradients likely play a large role as well. One such gradient is canopy cover. Canopy cover alters light availability necessary for primary production and subsidy input of leaf litter that provides an important source of energy and nutrients. These factors are extremely important in many ecosystems, as primary production and detritus are both basal trophic levels (Moore *et al.*, 2004).

In streams, this light/subsidy gradient is well known to alter community structure by altering proportions of different functional feeding groups (Vannote *et al.*, 1980). In low-order streams with high canopy cover, communities have high proportions of shredders, which mechanically break down leaves and subsist on the biofilms growing on the leaves. In mid-order streams with open canopies, communities have higher proportions of grazers or scrapers that consume algae growing on the surfaces of rocks and other substrates (Vannote *et al.*, 1980). In ponds, the effect of canopy on community structure is much less clear. Previous examinations have found ambiguous results with trends that are counterintuitive or reverse in different years for functional feeding groups (Batzer & Palik, 2007) and weak or nonexistent effects for general community structure (Batzer, Palik & Buech, 2004; Hanson *et al.*, 2009). However, studies do show lower overall macroinvertebrate abundance (Palik *et al.*, 2001; but see Chapter 2) and species

richness in closed canopy ponds than open canopy ponds, likely due in part from lower colonization rates (Binckley & Resetarits, 2007).

The lack of a pond community response to canopy cover may result from two factors: the use of abundance instead of biomass and the simultaneous study of multiple environmental gradients. Abundance can be an excellent measure for communities, particularly when species tend to be similar in body size (Lampert & Sommer, 1997). Unfortunately, few systems contain organisms whose sizes are all on the same order of magnitude. Although time-consuming, biomass is thought to be a much better measure for examining community structure because it allows researchers to examine the amount of biological tissue in different trophic guilds and ecological habits (Lampert & Sommer, 1997). Additionally, many studies examine gradients of hydroperiod and canopy cover simultaneously. Hydroperiod is such a strong structuring gradient that it may mask the effects of other more subtle gradients. Thus, studying canopy cover within a hydroperiod category may better illuminate functional differences.

A recent manipulation of light availability and subsidy input in permanent, fishless pond mesocosms in Missouri, USA showed that light and leaf litter input affects macroinvertebrate functional feeding groups consistently across three seasons in two years (Chapter 2). This study found very low abundance of grazers and shredders, but the proportion of collectors (dominated by chironomids) increased with canopy cover and with the input of plant litter. We were particularly interested in whether this pattern occurred in natural ponds in the same geographical area. In order to assess the effects of canopy cover on macroinvertebrate community structure, we sampled permanent, fishless ponds in early June of two years and examined functional feeding groups, biological

habit (i.e. mode of existence, Merritt & Cummins, 1996), family richness and diversity using biomass estimates.

METHODS

We conducted our study on ponds at Little Dixie Lake Conservation Area, Callaway County in central Missouri, USA. Approximately 30 ponds are scattered around Little Dixie Lake, which is a small reservoir (~83 ha). The surrounding terrestrial environment is a mostly oak-hickory forest with some additional restored prairie and old fields. Twelve permanent, fishless ponds were chosen across a gradient of canopy cover. Ponds were sampled in early June in 2009 and 2010. 2009 sampling occurred between 8 June and 10 June, and 2010 sampling occurred on 2 June and 3 June.

Our sampling protocol consisted of taking three pipe samples to estimate a density of macro-invertebrates and 15 dipnet sweeps to detect any rare species that might be able to escape the pipe sampler. The three pipe-sample locations were haphazardly chosen, evenly spaced around the pond, and were taken approximately 1.5m from the water's edge. Pipe samples were taken by plunging a large trashcan (46 cm diameter or ~0.17m² benthic area) with the bottom cut off into the water. Then, all invertebrates and small debris were removed from the water column and benthos within the pipe sampler with a small aquarium net (1.6 mm mesh size) and placed in white sorting tubs. All crayfish and amphibians were counted and then released. Large debris (sticks and whole leaves) was removed from the tub, and the remaining contents were run through a 0.59 mm sieve and preserved in 95% ethanol for later sorting in the laboratory. All macroinvertebrates (> 1 mm) were separated from debris and identified to the lowest taxonomic classification

possible: genus for most taxa and family for larval beetles, snails, and worms (Merritt & Cummins, 1996; Thorp & Covich, 2009). Each taxa was separated into size classes ranging 2-3 mm. Ten individuals for each size class were measured to the nearest 0.5mm and biomass was estimated using published length-mass regressions (Eckblad, 1971; Myer, 1989; Nyström & Pérez, 1998; Benke *et al.*, 1999; Edwards *et al.*, 2009). Each taxa was grouped by functional feeding groups, habit (general microhabitat use), and taxonomic class (Merritt & Cummins, 1996; Thorp & Covich, 2009).

We measured some aspects of the habitat. Most importantly, we estimated canopy cover and leaf litter input. Canopy cover was estimated by taking densiometer readings using a convex spherical densiometer (Ben Meadows, Janesville, WI, USA) in the four cardinal directions at the two ends, two sides and approximate center of each pond in mid to late August 2009, resulting in 20 measurements per pond, which were averaged. We measured water depth to 0.5 cm at each of the five canopy locations. Leaf litter input was estimated by placing three floating, partially submersible baskets (54cm x 37cm or ~0.2 m² per trap; Williams, 2008) evenly spaced around the pond. Baskets were tethered to the shore but were able to drift somewhat. Baskets were left in the ponds from 6 October 2009 until 23 January 2010. Leaf litter was then taken back to the lab, separated from non-leaf litter, gently cleaned and allowed to air dry in paper bags at room temperature for at least three months until constant weight. Leaf litter was then weighed to 0.01g on a Mettler BD Balance (Mettler-Toledo International, Columbus, OH, USA). We measured pond size in the summer of 2008 for each pond, when we used a laser range finder (Yardage Pro® Sport 450, Bushnell, Overland Park, KS, USA) to estimate

the length and width of each pond. Pond surface area was estimated using the formula for an ellipse.

Statistical analyses of pond characteristics were performed using SAS/STAT (SAS, 2004). We examined Pearson's correlations between canopy cover, leaf litter input, pond surface area and water depth to determine relationships between these major variables. We calculated family richness, which included the number of species detected in the pipe samples and dipnet samples, for each year. We also calculated family diversity with the Shannon Diversity Index (Meffe, Carroll & Contributors, 1997) using family biomass instead of abundance from the pipe samples for each year. We performed repeated-measure ANOVAs (Proc GLM) on family richness, diversity and total macroinvertebrate biomass using year as a repeated sample and canopy cover, litter input and pond surface area as independent variables. We chose not to include water depth, because of the high correlation between pond surface area and depth. We used box-cox transformations to determine the most suitable transformation for our data (Proc TRANSREG). Thus, we log transformed biomass and richness. After transformation, all variables met the assumptions of normality and homogeneity of variances.

Community structure was analyzed using Non-metric Multidimensional Scaling (NMDS) using the vegan package in R (Oksanen, 2011). We determined that NMDS was an appropriate ordination technique, because it does not assume normality or linear relationships with explanatory variables and preserves distance properties among samples (Zuur, Ieno & Smith, 2007). We performed ordination on community biomass classified using functional feeding group, habit (Merritt & Cummins, 1996), and taxonomic class for each year using the Bray-Curtis distance metric. We limited the number of groups for

each classification because of small samples sizes. For functional feeding groups, we had four groups: collectors (including both collector-gatherers and collector filterers), herbivores (including both scrapers and piercers), predators (including both piercers and engulfers), and shredders. For habit, we had six groups: burrowers, surface active (including both surface swimmers and skaters), swimmers, planktonic, sprawlers, and plant-associated (clingers and climbers). For each ordination, we calculated Kruskal's stress and regressed the distance in the original data (using Bray-Curtis distance measure) with the ordination distance to determine how well the ordination represented the data (Oksanen, 2011). Kruskal's stress indicates whether the number of dimensions used in the ordination sufficiently represents the variation in the data, where values less than 0.1 are considered good and values greater than 0.2 unacceptable (Zuur *et al.*, 2007). We associated each ordination with four environmental variables (canopy cover, leaf litter, pond surface area, and pond depth) and tested for associations with ordination axes by calculating the squared correlation coefficient and testing for significant correlations using 1000 random permutations of the data (Oksanen, 2011). We used an alpha of 0.05 for all tests. However, because of our small sample size and the exploratory nature of the analyses, we reported marginally significant results ($0.10 < p < 0.05$). We additionally performed power analyses to determine the sample size needed for significance.

RESULTS

Ponds varied in size from approximately 100 to 900m² surface area. Average water depths varied from about 20 to 80 cm deep. Surface area was correlated with water depth ($r = 0.85$). The ponds varied from approximately 30-90% canopy cover, which resulted

in a range of approximately 0.15 to 1.00 kg/m² of leaf litter input. Leaf litter input was positively correlated with canopy cover ($r = 0.75$), and pond surface area was negatively correlated with canopy cover ($r = -0.74$) but not litter input ($r = -0.35$).

Communities were dominated by planorbidae snails, libellulidae dragonfly larvae, chironomidae fly larvae, sphaeriidae clams, and *Chaoborus* fly larvae. These five taxa had the highest biomass in both years, but the relative dominance of each varied by year. We detected 35 macroinvertebrate families overall during the study, and family richness varied from 5-20 families per pond per sampling period. Neither canopy cover, litter input, pond surface area nor year affected total macroinvertebrate biomass (all $p > 0.27$) or diversity (all $p > 0.18$). For family richness, we found a significant interaction between year and pond surface area ($F_{1,8} = 7.91$, $p = 0.02$) and no effect of litter or canopy cover (all $p > 0.14$). Family richness increased with pond surface area in 2010, but not 2009 (Figure 1).

For pond community structure, Kruskal's stress was low (< 0.1), indicating that the number of NMDS dimensions was sufficient for the data, and the relationship between distance in the data and in the ordination was very strong (Table 1). Communities were affected by litter input and canopy cover but not pond surface area or water depth. These effects were most evident when ordination was conducted on functional feeding groups in which the effect of leaf litter was marginally significant in both years (2009: $p = 0.08$; 2010: $p = 0.06$; Table 2). Power analysis indicated that five additional ponds were needed for statistical significance in 2009 and four additional ponds were needed for significance in 2010. The input of leaf litter increased the biomass of collectors and decreased the biomass of herbivores (Table 3; Figure 2).

Shredders were rare, only occurring in two ponds in 2009 and three ponds in 2010 with biomass estimates two to three orders of magnitude lower than the other functional feeding groups. Thus, their placement on the ordination plot cannot be readily interpreted.

We found that canopy cover and leaf litter affected macroinvertebrate habit in 2010 (canopy: $p = 0.03$; leaf litter: $p = 0.05$; Table 2), but not 2009 (all $p > 0.21$). Canopy cover and leaf litter increased the biomass of burrowers and decreased the biomass of plant-associated macroinvertebrates (Table 3; Figure 3). This trend was consistent in both years, although not statistically significant in 2009. None of our environmental variables affected community structure in terms of taxa in either year (all $p > 0.27$; Table 2; Table 3; Figure 4).

DISCUSSION

We found that canopy cover was a structuring gradient for macroinvertebrates in fishless, permanent ponds when using biomass estimates. We found these effects in functional feeding groups with marginal significance in both years and significantly in habit in 2010, but never in taxonomic structure. Previous studies have shown weak patterns or conflicting patterns of community structure and community composition in relation to canopy cover (Batzer *et al.*, 2004; Batzer & Palik, 2007; Hanson *et al.*, 2009). While our results may be considered weak, the power analysis indicated that only 4-5 additional ponds were needed for significance. Additionally, we found effects that were consistent across years despite our small sample size. Other studies have focused on seasonal ponds. Hydroperiod is known to have a strong influence on pond ecosystems (Wellborn

et al., 1996). Small changes in hydroperiod may mask the effects of canopy cover. Alternatively, the effects of canopy cover may change with hydroperiod. Our study shows that canopy cover provides some community structure in fishless, permanent ponds, but trends may be similar in temporary ponds in wet years. Future work is needed to determine whether canopy cover alters community structure at other points along the hydroperiod gradient and in the presence of fish.

Leaf litter input was most often a marginally significant variable, but the correlation between leaf litter and canopy cover means that these two variables cannot really be separated. In most aquatic systems, tree canopy simultaneously alters both light and leaf litter input. Previous studies indicate that both of these variables are likely important to macroinvertebrate communities. Studies on microhabitat use by macroinvertebrates show that community composition varies with the type of detritus (deciduous leaves or macrophytes) and light availability (Spyra, 2011), but this data has not been summarized by functional feeding group. Additionally, a recent mesocosm study shows that the biomass of collectors are affected by both variables as well, but not their interaction (Chapter 2).

Overall the input of leaf litter promoted higher biomass of collectors and lower biomass of herbivores. This pattern is very similar to a recent study in pond mesocosms (Chapter 2), but somewhat different from typical patterns found in streams. In streams, shredders and herbivores are the primary functional feeding groups that respond to changes in canopy cover (Vannote *et al.*, 1980). While we did find decreased herbivore biomass (dominated by planorbidae snails) with increasing canopy cover as in streams, shredders were very rare in our samples. Shredders are very important for leaf litter

decomposition in streams (Graça, 2001). Interestingly, shredders appear to be rare or low in biomass in pond ecosystems altogether (reviewed in Batzer & Wissinger, 1996). Crayfish are known to be very important for leaf litter breakdown in streams (Momot, 1995) but are rarely included in pond macroinvertebrate studies despite their occurrence. While crayfish were present in our study ponds, we did not estimate their biomass and, thus, did not include them in our analysis as well. Including crayfish in future studies may change the patterns of functional feeding group structure in relation to leaf litter input. Recent evidence shows that snails may also contribute to leaf litter breakdown (Brady & Turner, 2010), though they are typically included in the grazer/herbivore category. However, the mechanism for the snail contribution to litter breakdown is still unclear.

Collectors (dominated by sphaeriidae clams and chironomidae fly larvae in our samples) are detritivores, feeding primarily on fine particulate organic matter and suspended solids (Merritt & Cummins, 1996). In streams, collectors do not really respond to changes in canopy cover (Vannote *et al.*, 1980), but they responded in our ponds. There is no real reason to suspect that the amount of fine particulate matter differed among our study ponds, so the amount of food for collectors may not have changed. However, a recent study in streams demonstrated that the quality of biofilms on leaves was higher in shaded areas than those in direct sunlight (Albariño, Villanueva & Canhoto, 2008). This pattern may occur in pond fine particulate organic matter as well, potentially increasing food quality for collectors in ponds with high shade and leaf litter input. Alternatively, the dominant collector taxa (Sphaeriidae clams and chironomidae fly larvae) are also tolerant of low dissolved oxygen and may actually prefer low-oxygen

conditions (Merritt & Cummins, 1996; Joyner-Matos *et al.*, 2011). Increases in canopy cover and leaf litter input result in decreased dissolved oxygen, sometimes to very low levels (Skelly, Freidenburg & Kiesecker, 2002; Schiesari, 2006). Thus, collectors may dominate in high canopy ponds due to higher tolerance of low oxygen than other taxa.

We additionally found that burrowers increased and plant-associated (climbers and clingers) macroinvertebrates decreased with increasing leaf litter and canopy cover in 2010, but not 2009. This is not surprising, given the trends with collectors, because the dominant burrowing taxa (sphaeriidae and chironomidae) were also collectors.

Additionally, although we did not measure macrophyte density in our ponds, macrophyte density did appear to decrease somewhat with increasing canopy cover (Earl, personal observation), which likely explains the effect on plant-associated macroinvertebrates.

Taxonomic community structure did not respond to any of our environmental gradients. Because we used class as our taxonomic group, this may have been too broad to really capture the variation in invertebrate taxa. However, family richness did increase with pond surface area in 2010, similar to the species area relationship (Connor & McCoy, 1979). Other studies have found a decrease in richness with canopy cover (Batzer *et al.*, 2004; Binckley & Resetarits, 2007), which may be related to somewhat harsher conditions with increased canopy cover, such as very low dissolved oxygen (Skelly *et al.*, 2002; Schiesari, 2006) and increased phenolic concentrations (Earl and Semlitsch, unpublished data). However, we did not find this effect in our study ponds.

We found that canopy cover can structure macroinvertebrate communities in fishless, permanent ponds. Our study supports the idea that canopy cover may be an important community structuring gradient, though likely secondary to hydroperiod and

the presence of fish (Wellborn *et al.*, 1996). Further research on this topic will help determine if the patterns seen in our study occur in other geographic localities and whether the effects of canopy cover change with hydroperiod. The importance of canopy cover highlights the need to consider the terrestrial environment surrounding ponds when managing them for biodiversity and aquatic ecosystem function.

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Table 1. Kruskal's stress and the correlation between distance in the data and the distance in ordination space for each ordination in each year.

Ordination	Stress	R ²
Feeding Group 2009	0.004	1.00
Feeding Group 2010	0.06	0.99
Habit 2009	0.06	0.99
Habit 2010	0.06	0.98
Taxa 2009	0.08	0.97
Taxa 2010	0.07	0.97

Table 2. Correlation (R^2) and p-values (in parentheses) of relationship between environmental variables and the NMDS ordination of macroinvertebrate biomass for 2009 and 2010.

Analysis	Canopy Cover	Leaf Litter	Area	Depth
Functional Feeding Group:				
2009	0.28	0.40	0.03	0.11
	(0.23)	(0.08)	(0.83)	(0.57)
2010	0.28	0.43	0.03	0.11
	(0.18)	(0.06)	(0.84)	(0.55)
Habit:				
2009	0.28	0.29	0.16	0.26
	(0.23)	(0.21)	(0.39)	(0.27)
2010	0.56	0.47	0.13	0.15
	(0.03)	(0.05)	(0.48)	(0.43)
Taxa:				
2009	0.10	0.16	0.14	0.26
	(0.64)	(0.47)	(0.49)	(0.27)
2010	0.07	0.18	0.02	0.01
	(0.68)	(0.42)	(0.91)	(0.97)

Table 3. Pearson's correlation between invertebrate groups (log transformed) and NMDS axes for each ordination in each year. Swimmers were not detected in 2009, and surface active invertebrates and amphipoda were not detected in 2010.

Ordination	Macroinvertebrate Group	NMDS1	NMDS2
Feeding Group 2009	Collector	0.42	-0.77
	Herbivore	-0.74	-0.52
	Predator	-0.73	0.43
	Shredder	-0.73	-0.61
Feeding Group 2010	Collector	0.66	-0.12
	Herbivore	-0.13	0.61
	Predator	-0.48	0.56
	Shredder	0.47	0.64
Habit 2009	Burrower	0.64	-0.64
	Plant-associated (clinger, climber)	-0.51	-0.77
	Planktonic	-0.63	0.56
	Surface active (skater, surface swimmer)	0.14	-0.47
	Sprawler	-0.86	-0.34
Habit 2010	Burrower	0.79	-0.14
	Plant-associated (clinger, climber)	0.19	0.82
	Planktonic	-0.67	-0.04
	Sprawler	0.63	0.50
	Swimmer	-0.48	0.21

Taxa 2009	Amphipoda	0.47	0.66
	Annelida	0.38	-0.35
	Bivalvia	-0.83	0.19
	Insecta	0.22	-0.44
	Gastropoda	-0.64	-0.46
Taxa 2010	Amphipoda	-0.07	-0.23
	Annelida	0.48	-0.45
	Bivalvia	0.55	0.20
	Insecta	0.58	-0.44
	Gastropoda	0.73	0.26

Figure 1. The relationship between species richness and pond surface area in 2009 and 2010. Each data point represents a single pond. The regression is statistically significant in 2010 but not 2009.

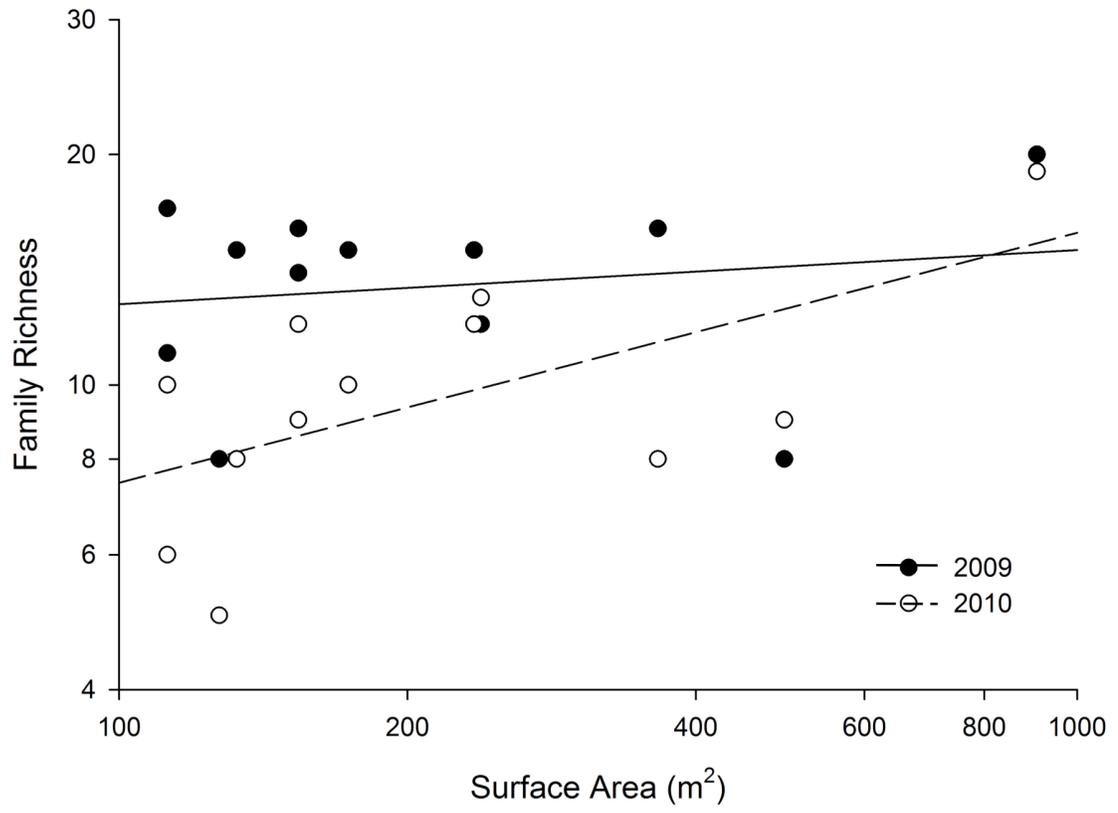
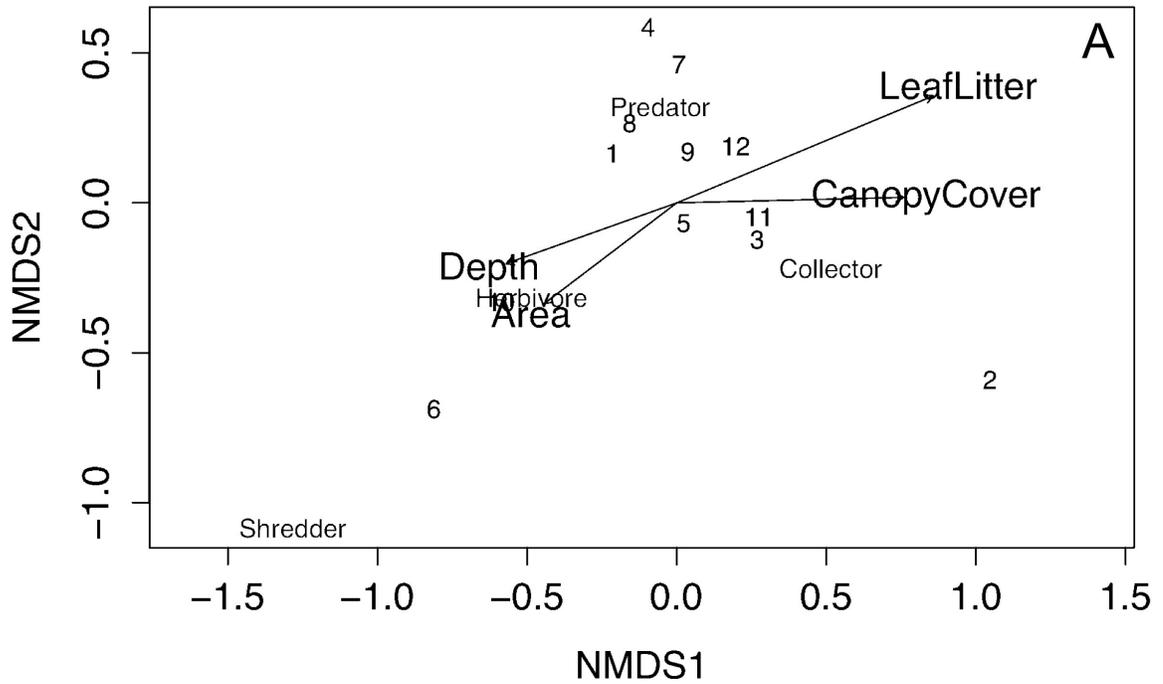


Figure 2. Non-metric Multidimensional Scaling plot of community structure by functional feeding groups in relation to environmental vectors in 2009 (A) and 2010 (B). Length of vectors represents the strength of relationship between environmental variables and ordination axes (NMDS1 and 2). Numbers 1-12 represent ponds.



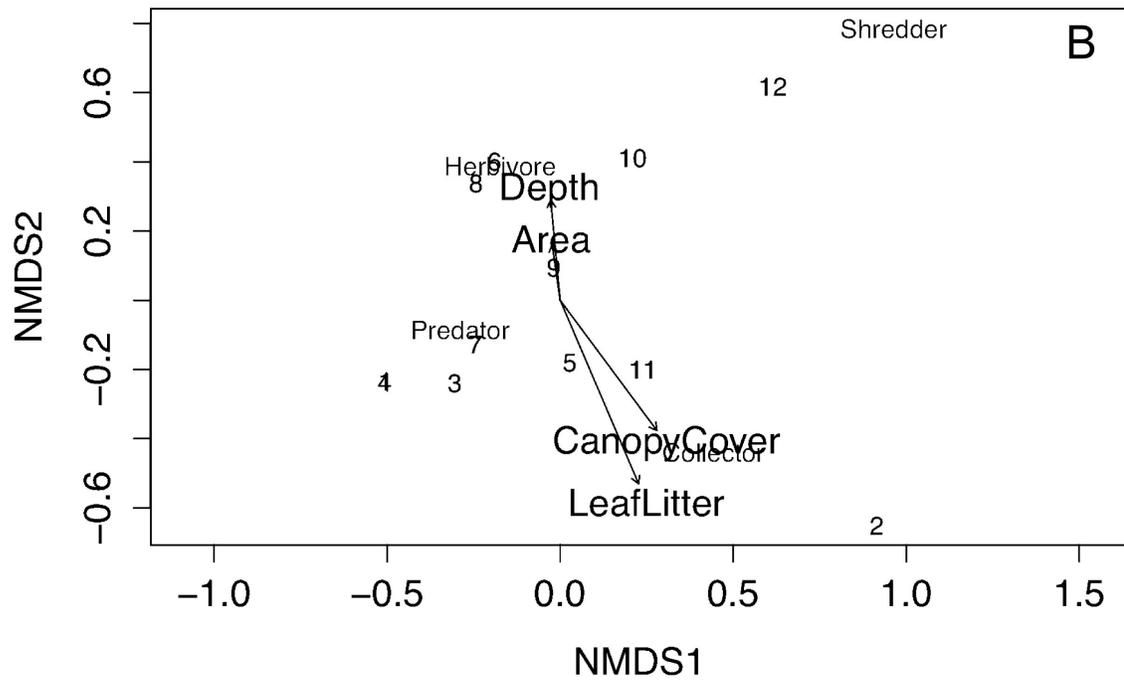
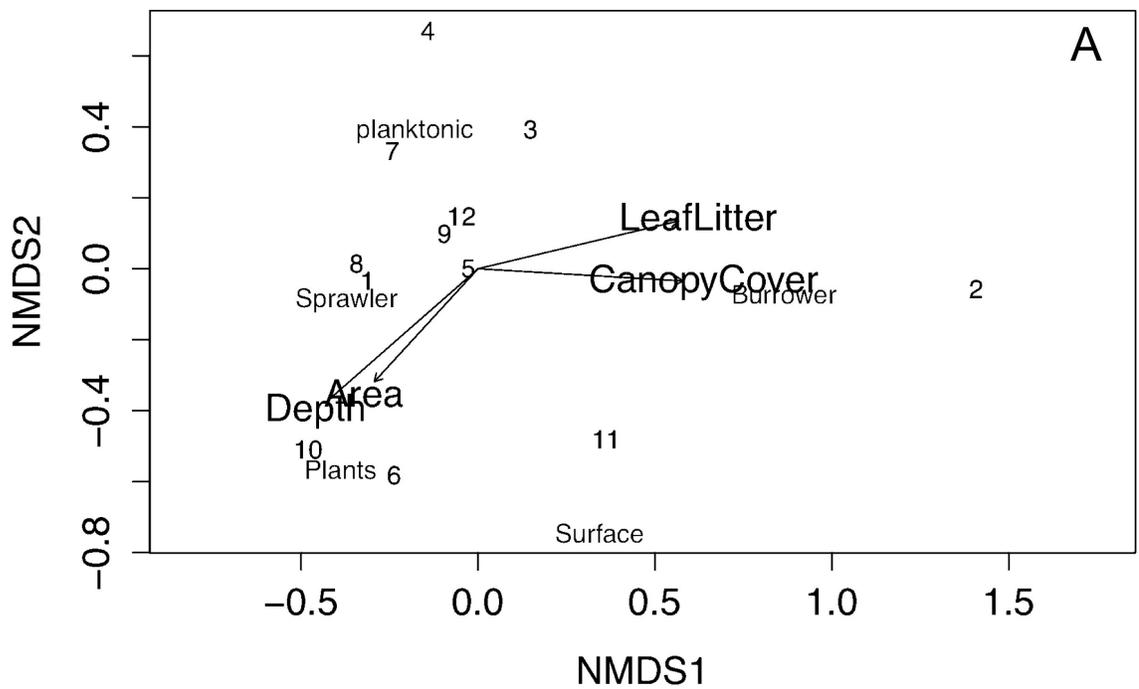


Figure 3. Non-metric Multidimensional Scaling plot of community structure by habit in relation to environmental vectors in 2009 (A) and 2010 (B). Length of vectors represents the strength of relationship between environmental variables and ordination axes (NMDS1 and 2). Numbers 1-12 represent ponds. Plant-associated macroinvertebrates included clingers and climbers. Surface macroinvertebrates (skaters and surface swimmers) were not detected in 2010 samples.



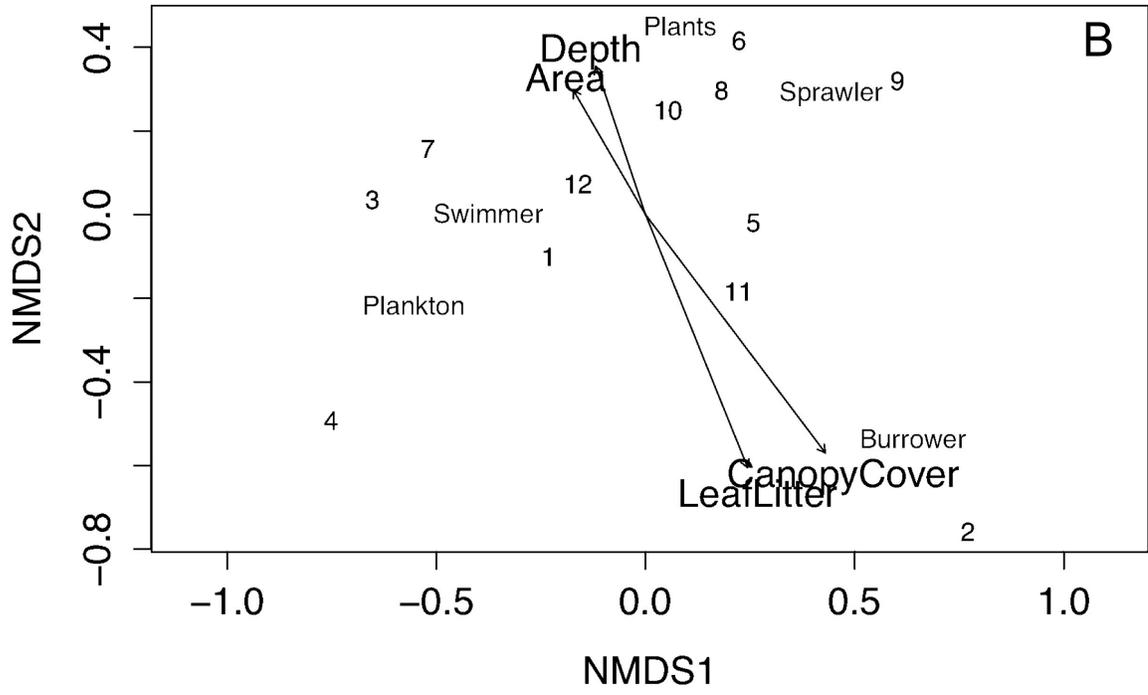
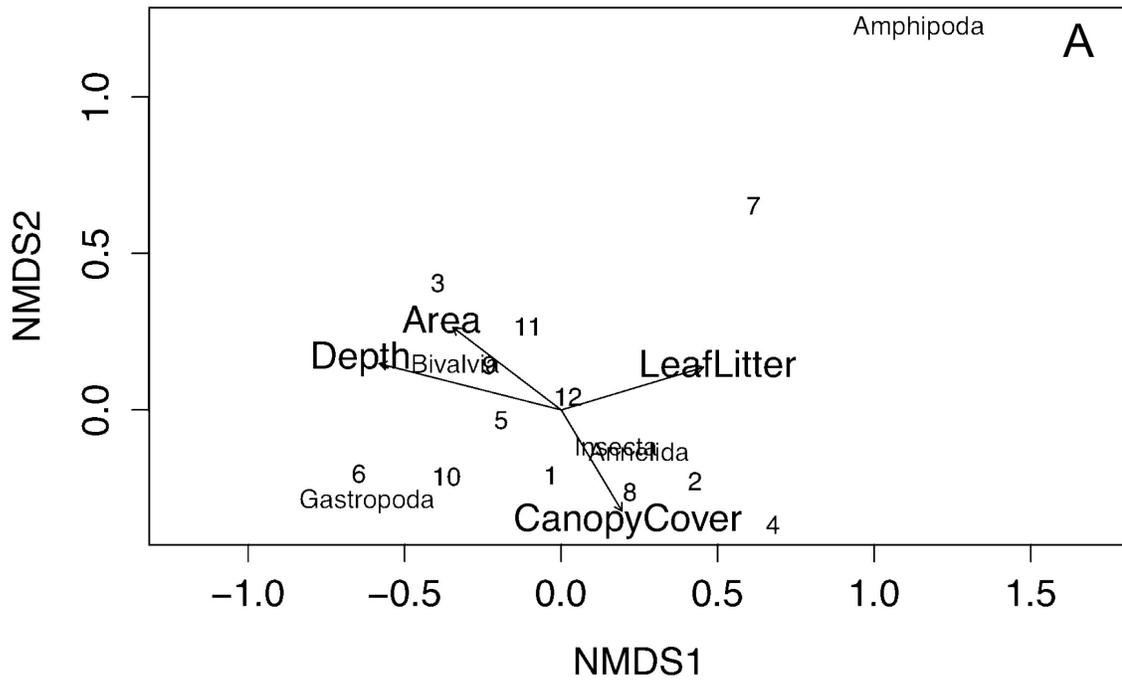
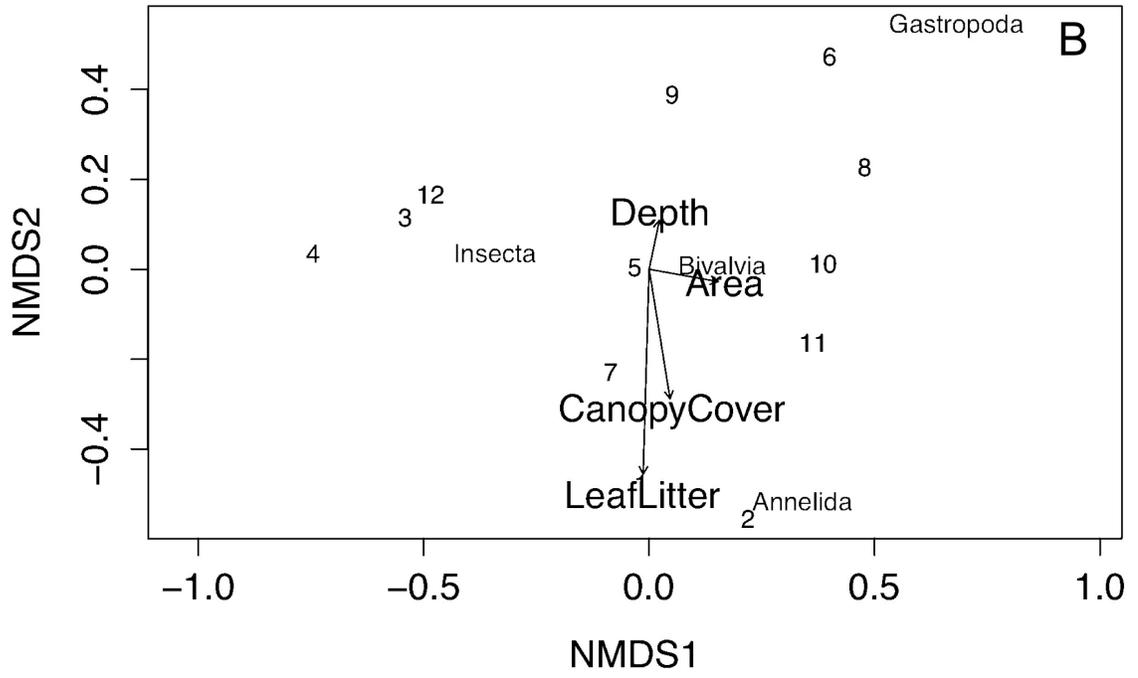


Figure 4. Non-metric Multidimensional Scaling plot of community structure by taxa in relation to environmental vectors in 2009 (A) and 2010 (B). Length of vectors represents the strength of relationship between environmental variables and ordination axes (NMDS1 and 2). Numbers 1-12 represent ponds. Amphipods were not detected in 2010 samples.





CHAPTER 4

RECIPROCAL SUBSIDIES IN PONDS: DOES LEAF INPUT INCREASE

FROG BIOMASS EXPORT?

ABSTRACT

Reciprocal subsidies occur when ecosystems are paired, both importing and exporting resources to each other. The input of subsidies increases reciprocal subsidy export, but it is unclear how this changes with other important factors, such as ambient resources. We provide a conceptual framework for reciprocal subsidies and empirical data testing this framework using a pond-forest system in Missouri, USA. Our experiment used *in situ* pond mesocosms and three species of anurans: wood frogs, American toads, and southern leopard frogs. We predicted that increases in ambient resources (primary productivity) and detrital subsidy input (deciduous tree leaves) into pond mesocosms would increase reciprocal exports (frog biomass) to the surrounding terrestrial ecosystem. In contrast, we found that increases in primary productivity consistently decreased frog biomass except with leaf litter inputs. With leaf inputs, primary productivity did not affect the export of frogs, indicating that leaf detritus and associated microbial communities may be more important than algae for frog production. We found subsidy inputs tended to increase reciprocal exports and, thus, partial concordance with our conceptual framework.

Spatial subsidies are resources that flow between ecosystems (Polis et al. 1997). These fluxes provide important linkages among ecosystems. Subsidies influence processes and species interactions in the recipient system, which has been shown by empirical data (e.g. Murakami and Nakano 2002; Janetski et al. 2009) and simulation models (e.g. Leroux and Loreau 2008). For example, subsidies can increase consumer production when ambient resources are low (reviewed in Marczak et al. 2007). Sometimes, two ecosystems become paired, both donating and receiving subsidies from each other. This pairing is referred to as reciprocal subsidies, and this scenario may be more widespread than currently recognized (Nakano and Murakami 2001). When reciprocal subsidies occur, this mutual exchange may increase the secondary production of both systems when compared to similar closed systems (Nakano and Murakami 2001). Additionally, the input of a subsidy might increase the export of another subsidy to the partner ecosystem (Baxter et al. 2005), assuming a trophic link between the two subsidies.

Experimental manipulations in stream ecosystems have shown this to be the case in two different ways. Leaf litter input from forests to streams provides basal food resources to support the export of adult aquatic insects from streams to forests (Wallace et al. 1997). Also, terrestrial insect input from forests to streams provides an alternative food resource for fish, releasing larval aquatic insects from predation pressures, which increases the export of adult aquatic insects to forests (Baxter et al. 2004; Baxter et al. 2005). However, in other systems, subsidy input can increase predation (or herbivore) pressures on *in situ* resources (e.g. Murakami and Nakano 2002). It is currently unclear whether subsidy input increases reciprocal exports in systems other than streams.

In addition to the knowledge gap for non-stream systems, the role of many factors in reciprocal subsidy dynamics have been mostly disregarded. Many factors likely affect the feedback loop between inputs and exports, such as ambient resources. Ambient resources are important for predicting consumer responses to inputs within recipient systems (Marczak et al. 2007), but have not been examined in systems with reciprocal subsidies. Here, we present a conceptual framework to predict reciprocal export in relation to subsidy input and ambient resource gradients (Fig. 1). Our framework is based on previous knowledge of the effects of spatial subsidies and ambient resources on consumer responses (Marczak et al. 2007), which we have applied to reciprocal subsidies. We then test this framework through experimental manipulations, described below.

In systems with reciprocal subsidies, we might expect the input of a basal resource (e.g. detritus) to be more important for reciprocal export under low ambient resources than high ambient resources (Fig. 1). Thus, with no input, subsidy export would increase greatly with increases in ambient resources (Persson et al. 1996). In systems with the input of a subsidy food source, reciprocal export may also increase with ambient resources, but we predict this would occur with a much shallower slope and higher intercept. The shallower slope results from inputs buffering the effects of low productivity on consumer production. This slope may decrease and intercept increase with rises in detrital subsidy quantity and quality, because subsidies of higher quality and/or quantity would provide greater support of consumers under low ambient resources (Marcarelli et al. 2011). Thus, the difference in exports between systems with and

without subsidy input would be greatest with low ambient resources and large, nutrient-rich subsidy inputs.

In order to test this conceptual framework, we used an experiment to examine reciprocal subsidies in the pond-forest system. In this system, the forest inputs to ponds primarily consist of tree leaf litter (Kraus et al. 2011), and pond exports to forests consist of metamorphosing amphibians and aquatic insects (Whiles and Goldowitz 2001; Gibbons et al. 2006; T. Schriever and D.D. Williams, unpublished data). In our study, we focused on a portion of this exchange, examining how a subsidy input (tree leaves) from forests to ponds influences one taxa of reciprocal exports (metamorphosing frog biomass) to forests across a gradient of ambient resources (primary productivity, created by differences in light availability) in the pond ecosystem. Although more research has focused on insect export from aquatic ecosystems in temperate North American ponds, amphibian biomass export can be on the same order of magnitude (Earl et al., unpublished data; Leeper and Taylor 1998; Whiles and Goldowitz 2001; Gibbons et al. 2006; but see Regester et al. 2006) or substantially greater (T. Schriever and D.D. Williams, unpublished data) than whole insect community biomass export.

We expected leaf litter to increase frog biomass due to tadpoles' common consumption of biofilms and algae that would be bolstered by the addition of plant material and associated nutrients (Altig et al. 2007; Schiesari et al. 2009; Whiles et al. 2010). We expected leaf litter to increase frog biomass export to a greater degree in low productivity systems than high productivity systems (Fig. 1). We also compared leaf litter to a within-system resource (detrital storage), a senesced aquatic grass from the previous year. The within-system resource (grass) was of higher quality than the subsidy

(tree leaves), which led us to predict higher frog biomass export in systems containing the within-system resource than the subsidy input. To better explain the processes creating these patterns, we additionally examined the effects of leaf litter, aquatic grass litter, and no input on the importance of different underlying community and environmental mechanisms for altering frog biomass export.

METHODS

Experimental Design

To examine the effects of leaf litter on frog biomass export, we used *in situ* mesocosms. Mesocosms allowed us to focus on our manipulated variables while standardizing many other variables known to influence pond communities including pond size (surface area and depth), shape, age, and hydroperiod (Wellborn et al. 1996; Gee et al. 1997; Søndergaard et al. 2005). Thirty-six mesocosms (1000L cattle watering tanks, 1.5m in diameter) were placed at three sites within experimental forestry plots (Hocking and Semlitsch 2007; Semlitsch et al. 2008; Semlitsch et al. 2009) in oak-hickory forest at Daniel Boone Conservation Area, Warren County, Missouri in early 2006 for another experiment (Hocking and Semlitsch 2008). Nutrient input for that past experiment was equal among mesocosms. We initiated two treatments: the placement of mesocosms under different levels of canopy cover to create a primary productivity gradient and litter input. We used this experimental design to investigate the direct and interactive effects of litter input and primary productivity (measured during the study) on the production of a reciprocal subsidy, emigrating metamorphosed frogs.

We examined three species of frogs in different years: wood frogs (*Rana sylvatica*) in 2008, American toads (*Bufo americanus*) in 2009, and southern leopard frogs (*Rana sphenoccephala*) in 2010. We chose three different species to determine if there was generality in our findings across species. The examination of different species in different years was confounded but necessary, due to the logistics of performing the experiment *in situ*. These three species are native to the eastern and central United States with overlapping ranges. Juveniles of each species emigrate away from their natal pond after metamorphosis and reside in the terrestrial habitat, though leopard frogs sometimes seek wetter areas like temporary ponds and ditches (Lanoo 2005). All three species have high mortality immediately after emigrating from their pond (Bridges 2000; Harper and Semlitsch 2007), resulting in the export of energy and nutrients from the pond to terrestrial predators and detritivores (Gibbons et al. 2006).

Twelve mesocosms were placed along a canopy cover gradient created by clearcutting at three sites (total N = 36 tanks) in winter 2004 to spring 2005. Each site contained mesocosms ranging from approximately 0-95% tree canopy cover (Online Appendix 1), though the presence of shade cloth covers made the shading approximately 30-100%. All mesocosms were filled with water, received equal amounts of leaves (1kg) in February 2006, and were inoculated with zooplankton in April and May of 2006 as part of another experiment examining the effects of canopy cover on tadpoles (Hocking and Semlitsch 2008). All tanks were treated the same except for their placement along a canopy cover gradient during this experiment. Tanks were left uncovered and allowed to develop communities through colonization and oviposition of invertebrates and treefrogs from autumn 2006 through autumn 2007. While the use of previously used mesocosms

with natural colonization is not a standard practice (Semlitsch and Boone 2009), it has been successfully used for a number of experiments (Lay et al. 1993; Schulman and Chase 2007). This method has been criticized, because established communities and colonization increase variation among replicates. However, this variation makes our experiment more similar to natural systems. Our experimental setup likely has an intermediate amount of environmental variation between more controlled mesocosms at research facilities and natural ponds (Semlitsch and Boone 2009).

In autumn of 2007, litter manipulations were applied to mesocosms to examine the effects of leaf litter input on frog biomass export (Table 1). The three treatments consisted of 1kg deciduous leaf litter, a within-system resource (1 kg of prairie cordgrass), and no input (12 mesocosms/litter treatment). Preliminary data in our high canopy cover areas ($\approx 95\%$ canopy cover) indicated that leaf litter deposition averaged 1.01kg tree leaves per cattle tank. Additionally, this is consistent with many other mesocosm studies (Boone et al. 2007; Hocking and Semlitsch 2008; Williams et al. 2008). For the leaf litter input, we used the natural mixture of deciduous leaves found in our area, consisting mostly of white oak (*Quercus alba*) and northern red oak (*Quercus rubra*) with some hickory (*Carya* spp.) and sugar maple (*Acer saccharum*). For the within-system resource, we used prairie cordgrass (*Spartina pectinata*), a wetland obligate species found across much of the United States (USDA and NRCS 2010). Leaves and grass were all collected after natural senescence from within Daniel Boone Conservation Area a few days before addition to mesocosms. For the no input treatment and the within-system resource prior to the grass addition, we removed leaf litter already present from the mesocosms using a net with 2.5 cm gauge mesh. This method removed

whole leaves and large leaf fragments, while retaining small leaf fragments and fine particulate organic matter that had accumulated previously. We replicated this disturbance in the leaf litter treatment by stirring the tanks for about the same amount of time it took to remove leaves in the other treatments. All subsidy treatments were renewed in autumn of 2008 and 2009 (Table 1) by adding an additional 1kg of grass, leaves, or no litter to designated mesocosms to maintain subsidy treatments for each anuran species (i.e. mesocosms retained the same treatment for all three years). In our experiment, grass was higher in both nitrogen (grass: 0.96%, deciduous leaves: 0.76%) and phosphorus (grass: 0.073%, deciduous leaves: 0.045%; Earl and Semlitsch, unpublished data) than leaves. Grass also had a lower C:N ratio than leaves (grass: 43.7, deciduous leaves: 60.5; Earl and Semlitsch, unpublished data).

After implementing litter treatments, mesocosms were covered with high-density polyethylene PAK knit 27% shade cloth with 3mm sized openings (Hummert International, St. Louis, MO, USA) to prevent further introduction of leaf material and the escape of metamorphosing tadpoles. Tank covers were pushed into the water to facilitate oviposition by invertebrates and anurans. Tank covers were removed for at least 24 hours every 4-6 weeks to further facilitate oviposition and colonization by aquatic animals, except during winter (from mid-November to mid-March). Mesocosms were also left uncovered after the completion of the tadpole experiment from mid-August to late September to further allow oviposition and colonization to retain natural communities for the next season.

Reciprocal Subsidy Patterns

To examine the effects of leaf litter input on frog biomass export, we stocked mesocosms with hatchling frog tadpoles in three different years. This allowed us to standardize the initial density of hatchlings and measure the reciprocal export of one frog species across subsidy and canopy treatments in each year. We stocked hatchlings at densities that resulted in metamorphs of comparable size to natural populations (Harper and Semlitsch 2007). Thus, we stocked wood frogs and leopard frogs at 40 hatchlings/mesocosm and American toads at 72 hatchlings/mesocosm (Table 1). All hatchlings were at Gosner stages 25-26 at stocking, the free-swimming stage where tadpoles are absorbing their external gills, generally 2-3 days after hatching (Gosner 1960).

We monitored mesocosms for metamorph emergence every 2-3 days starting at the known minimum time to metamorphosis and then daily from the detection of the first metamorph until one week after the last metamorph and tadpole was seen. Metamorphs were defined as Gosner stage 42, characterized by the emergence of at least one forelimb and both hind limbs (Gosner 1960). Metamorphs were held in the laboratory until tail absorption was complete, when we weighed them to the nearest 0.01g. This method allowed us to remove metamorphs from tanks at the earliest possible stage to prevent drowning and give us a reliable estimate of mass at metamorphosis (Travis 1980).

Reciprocal Subsidy Mechanisms

We examined four possible mechanisms driving differences in frog biomass export under different litter input treatments. The four possible mechanisms included water quality (dissolved oxygen), food availability (net primary productivity, NPP), predators (dragonflies), and competitors (treefrogs). These possible mechanisms are all known to

affect tadpole performance (Alford 1999; Schiesari 2006; Hocking and Semlitsch 2008). Mesocosms were sampled in spring and summer in 2008-10 (Table 1) for NPP and water quality (dissolved oxygen). We additionally measured temperature and pH to better characterize the environment experienced by tadpoles. All samples for each sampling period were taken within the same day. Dissolved oxygen and temperature were measured as part of the NPP estimation. We measured pH to 0.01 units in the morning between 1-4hrs after sunrise using a pH probe (testr3, Oakton Instruments, Vernon Hills, IL, USA). Canopy cover was estimated each year in late spring or early summer by taking four canopy estimates per tank using a convex spherical densiometer (Ben Meadows, Janesville, WI, USA).

To estimate NPP, we measured the diel flux in dissolved oxygen (Wetzel and Likens 2000). This method approximates NPP from the increase in dissolved oxygen in water during daylight hours when oxygen is produced through photosynthesis. We measured dissolved oxygen (to 0.01mg/L) and temperature (to 0.1°C) at consecutive sunrises and sunsets with a YSI 55 handheld meter (Yellow Springs Instruments, Yellow Springs, OH, USA). All measurements were taken within 1hr of sunrise or sunset (Fontaine and Ewel 1981), and diel oxygen measurements were only taken when the time between the first and last measurements contained no precipitation, low clouds during daylight hours, and low wind conditions which resulted in no rippling of water in mesocosms (< 8 mph). These conditions allowed us to use a 0.05 diffusion coefficient (k) for all calculations (Wetzel and Likens 2000). We used the following equation to estimate NPP:

$$\text{NPP} = \text{O}_{2(\text{sunset})} - \text{O}_{2(\text{sunrise})} - k \cdot (\text{O}_{2(\text{average})} - \text{O}_{2(\text{average saturation})})$$

Many studies use the daily peak in dissolved oxygen for their measurements instead of sunset. In a similar study using cattle tanks, the oxygen concentrations an hour before sunset were not different from any afternoon measurement of dissolved oxygen (Williams et al., unpubl. data; Williams et al. 2008), indicating that our measurements were close the peak concentration. In our mesocosms, NPP is highly correlated with gross primary productivity in both spring and summer (data over 2 years: $N = 35$, all $R^2 = 0.84-0.98$; Earl and Semlitsch, unpublished data).

To estimate relative abundance of predators and competitors, we monitored the number of adult dragonfly predators (almost exclusively the blue dasher, *Pachydiplax longipennis*) and treefrog competitors (spring peepers, *Pseudacris crucifer*, and gray treefrogs, *Hyla versicolor*) emerging from each tank each year. We identified, counted, and then released predator and competitor metamorphs emerging from each tank daily during the metamorphosis period of the focal amphibian species. We additionally monitored mesocosms for predator and competitor emergence weekly for the month prior to the metamorphosis of the focal amphibian and for 1-2 weeks following the metamorphosis of the last focal frog individual. Our predator and competitor abundance estimates are likely very accurate during the time monitored, because, similar to our focal species, adult dragonflies and treefrog metamorphs were contained within mesocosms by shade cloth covers until we counted and removed them.

Statistical Analyses

For all analyses, a single mesocosm was used as the unit of replication (i.e. each data point represented one mesocosm/tank). All statistical analyses were performed in SAS

(SAS 2004). We first performed repeated-measures ANOVAs on pH and dissolved oxygen across the three years with litter treatment as an added factor. This allows us to report differences in the water chemistry experienced by the different species and examine whether there was an interaction between year and litter treatment. For all other analyses, we analyzed each year separately to look for overall patterns of frog biomass export and mechanisms determining that export. Frog metamorph biomass was used as a measure of reciprocal subsidy export. We calculated biomass by summing the mass at metamorphosis for all individual metamorphs of the focal species produced from each mesocosm in each year. The NPP for spring and summer were averaged to assess NPP experienced by tadpoles during the entire larval period.

In some amphibian studies, greater tadpole survival can deplete algal resources via top down pressures, resulting in a negative correlation between algal production (such as NPP) and tadpole biomass (Loman 2001). We used partial correlation coefficients (Proc CORR) to determine whether amphibian biomass or canopy cover was explaining more of the variation in NPP. In cases where amphibian biomass was likely not controlling NPP, we performed mixed effects models (Proc GLM) to examine the patterns of reciprocal subsidy. Mixed effects models contained frog biomass as the dependent variable, and the independent variables included litter input (categorical variable), NPP (continuous variable), and the interaction between litter input and NPP with site as a random blocking variable (Potvin 2001). In the wood frog model, we log transformed NPP, and in the toad model, we square-root transformed biomass to meet the assumption of homogeneity of variance.

To examine the mechanisms for frog biomass export, we used path analysis to determine differences in importance of four possible mechanisms: water quality (dissolved oxygen), food availability (NPP), predator abundance, and competitor abundance. Although temperature was another possible mechanism, it was not included in these models because of the high correlations between temperature and NPP and between temperature and dissolved oxygen. When we attempted to include temperature in the model, the VIF values indicated problems with collinearity. We used path analysis as a qualitative assessment of the importance of different mechanisms and associated indirect effects (including the effects of canopy cover on our proposed mechanisms). We only assessed relative differences (using standard deviations) in the strength of standardized path coefficients rather than performing statistical tests because of our low sample sizes ($N = 9-12$ per litter treatment per frog species). This allowed us to identify the most important mechanism(s) for each path diagram. We additionally report the R^2 value for each path analysis to show the amount of variation of all four mechanisms in explaining frog biomass. We performed a separate path analysis for each subsidy input treatment and each focal frog species to compare patterns, resulting in nine total analyses. Each path analysis had identical structure and was created by performing a series of regressions (Proc REG) and calculating path coefficients (otherwise known as standardized regression coefficients), indicating the relationship between the dependent and a given independent variable while holding all other independent variables constant (Mitchell 2001). Spring and summer values were averaged for dissolved oxygen and NPP. Predator and competitor abundance were summed for each mesocosm for each year. In all analyses, variables were normal and displayed homoscedasticity, except for

NPP and competitor abundances in wood frogs and toads and biomass in toads. We log transformed NPP and square-root transformed competitor abundance and toad biomass to meet the assumptions of the analysis. We additionally report the means and range of values for NPP, dissolved oxygen, predator abundance, competitor abundance, pH, temperature, and canopy cover (Table 2) and Pearson's correlations between these parameters (Table 3). We also report the correlations between frog biomass, frog survival rate, and frog average mass per mesocosm to help translate our findings to other studies that do not use frog biomass.

RESULTS

Reciprocal Subsidy Patterns

Our sample sizes were lower than planned due to two factors: mesocosms leaking after the freeze/thaw of winter and oviposition by green frogs (*Rana clamitans*). We did not use mesocosms containing green frog larvae, because they were found in only a few replicates and no metamorph of our three focal species ever emerged from mesocosms containing them. This resulted in differential sample sizes of mesocosms for each species (wood frog: N = 34; American toad: N = 33; leopard frog: N = 31). We found differences among years in water chemistry (all $p < 0.0001$), but there was no significant interaction between year and litter treatment (all $p > 0.49$). pH was higher in 2008 than 2009 or 2010, and dissolved oxygen was higher in 2009 than 2008 or 2010 (Table 2). Canopy cover appeared to be driving variation in NPP, not grazing pressures from frog biomass. In all three species, the partial correlation coefficient between NPP and canopy cover (wood frogs: $R = -0.60$; American toads: $R = -0.71$; leopard frogs: $R = -0.78$) was

much higher than the coefficient between NPP and frog biomass (wood frogs: $R = -0.05$; American toads: $R = -0.21$; leopard frogs: $R = 0.05$). Thus, we were able to examine reciprocal subsidy patterns in all three species.

We consistently found that frog biomass export declined with increases in NPP (Fig. 2). Wood frog biomass export was affected by litter input and the interaction between litter input and NPP (Table 4). Wood frog biomass export decreased with increases in NPP with no input and grass, but there was no effect of NPP with leaf litter (Fig. 2a). For American toads, biomass export was not affected by either litter treatment or NPP alone (Table 2), but there was a marginally significant interaction between subsidy input and NPP (Table 4, Fig. 2b). Similar to wood frogs, toad biomass decreased with increasing NPP with grass litter and no input but was not affected by NPP with leaf litter. Leopard frog biomass production was significantly affected by litter treatment, but not NPP or the interaction between the two (Table 4). Tukey tests did not reveal significant differences in litter treatments, but there was a trend for higher frog biomass production with leaf litter input than either no input or grass litter (Fig. 2c). We also found total frog biomass to be highly positively correlated with both frog survival (wood frog: $R = 0.91$, American toad: $R = 0.96$, leopard frog: $R = 0.77$) and frog average individual mass per mesocosm (wood frog: $R = 0.57$, American toad: $R = 0.70$, leopard frog: $R = 0.71$).

Reciprocal Subsidy Mechanisms

The four proposed mechanisms explained between 18 and 89 percent of the variation in frog biomass export (Fig. 3). R^2 values were not consistently higher or lower for one

amphibian species or litter treatment. Primary productivity was the most important mechanism or one of the most important mechanisms in most of the path diagrams (6 out of 9; Fig. 3). In all of these cases but one, the relationship between primary productivity and frog biomass export was negative, similar to the data on reciprocal subsidy patterns. With leaf litter, primary productivity was not important in two of three path diagrams and community interactions (competition and predation) became the determinants of biomass export. All of the proposed mechanisms were strongly affected by shading (canopy cover) but not in all path diagrams. Some diagrams additionally showed important indirect effects, but these did not appear to be consistent across data sets.

DISCUSSION

We experimentally examined the relationship between primary productivity and frog biomass export with subsidy input (leaves), a within-system resource (grass), and no input to test the conceptual framework presented in Figure 1. Although our results were not always as predicted, we found that subsidy input did tend to increase reciprocal exports as predicted. However, reciprocal exports did not always increase with subsidy quality and actually declined with increases in ambient resources, though the latter may have resulted from correlations with other variables.

While not conforming to all our predictions, our results do show some consistent trends. With regard to primary productivity, we found that frog biomass export always decreased or was not affected by primary productivity, but never increased. We also consistently found that, in the leaf litter treatment, primary productivity did not alter the export of frogs. It would be reasonable to expect that higher biomass of tadpole grazers

depleted algal resources. Subsequently, grazing pressures would create a negative correlation between primary productivity and frog biomass export, as seen in other studies (Loman 2001). However, our partial correlation analysis showed that canopy cover drove the primary productivity gradient not frog biomass. This was consistent in all three years. Thus, the negative relationship between frog biomass and primary productivity may indicate that algae are not as important as tadpole food resources as generally thought (Schiesari et al. 2009; Whiles et al. 2010). Recent studies have shown that tadpoles assimilate more detritus and associated microbial biofilms than any other food type, though the importance of algae increases with increased canopy openness (Schiesari et al. 2009; Whiles et al. 2010). Hence, tadpoles may actually prefer the subsidized resource (leaves) to the ambient one (algae), which has been found for consumers in other systems including streams and desert islands (Huxel et al. 2004; Quinn et al. 2009).

Alternately, the NPP gradient likely represents more complex interactions than were measured in our mesocosms. The NPP gradient in our experiment was created by placing mesocosms along a canopy cover gradient, resulting in a range of light levels. This change in light likely altered a number of interacting physical, chemical, and biological factors. Some of these were measured and accounted for in the path analysis. However, temperature was too highly correlated with NPP and dissolved oxygen to be used in the analyses, and thus, we cannot separate effects from NPP and those from temperature. Previous studies have shown that higher temperatures actually result in metamorphs with lower body masses (Smith-Gill and Berven 1979; Harkey and Semlitsch 1988; Alvarez and Nicieza 2002), which would be consistent with trends in the

present study. Temperature can also alter survival rates with intermediate temperatures resulting in higher survival (Harkey and Semlitsch 1988). However, a recent study shows no change in frog export from ponds with experimental warming (Greig et al. 2012). Additionally, there is likely a distinct shift in types of algae available under different light levels. There tends to be more phytoplankton and filamentous algae (some of which is toxic to tadpoles) under high light conditions and more periphyton (better tadpole food; Altig et al. 2007) under low light in mesocosms (Williams et al. 2008), similar to that seen in streams (Biggs 1996). However, studies in natural ponds and smaller mesocosms show both reduced phytoplankton and reduced periphyton under low light (Skelly et al. 2002; Mokany et al. 2008).

Our results showed variation in species/years with regard to subsidy input and subsidy quality effects of frog biomass export. The predictions for subsidy quantity and quality were based on the idea that more nutrients would result in higher reciprocal export. In most cases, the input of litter did increase frog biomass export, but litter quality showed mixed results. Similar to our input versus no input comparison, Rubbo et al. (2008) examined different levels of leaf input, finding that higher quantities of leaf litter input resulted in higher survival and body mass of amphibians, which conforms to our prediction. With regard to input quality, another study showed higher leopard frog and spring peeper biomass export with grass than leaves (Earl et al. 2011), similar to the wood frogs in our study. However, Rubbo and Kiesecker (2004) found that higher quality red maple litter produced lower amphibian biomass than the lower quality oak litter (Ostrofsky 1997), similar to leopard frogs in our study. The maple litter supported higher levels of microbial activity, while oak litter promoted higher algal growth (Rubbo

and Kiesecker 2004). While we did not measure microbial activity, there may have been a similar shift in our mesocosms. Leopard frogs are known to eat more algae than other species (Schiesari et al. 2009) and may have benefitted from this shift. Additionally, grass is known to decompose faster than leaves (Ostrofsky 1997). The slower nutrient release from leaves may also benefit leopard frogs, which have longer larval periods than the other species in our study.

Our study also examined whether the important mechanisms in determining frog biomass export changed under different subsidy input treatments. We predicted that primary productivity would be an important mechanism with no input, which we found. Primary productivity was actually an important mechanism in determining frog biomass export under most scenarios (6 out of 9 path diagrams, 67%). However, similar to our analysis of reciprocal subsidy patterns, this relationship was almost always negative (5 of the 6 relationships, 83%). This effect could be due to other correlated variables, particularly temperature. Interestingly, though, primary productivity was not important with leaf litter for wood frogs and American toads, indicating that leaves or leaf-associated biofilms may be a more important resource than algae for tadpoles of these species or during this year of investigation. Detritus is a large proportion of the diet of toads (Diaz-Paniagua 1985; Diaz-Paniagua 1989) and wood frogs, whereas leopard frogs eat more algae (Schiesari et al. 2009). However, we know very little about how these species alter their diets in relation to available resources.

Other mechanisms, particularly competition and predation were also important in some path diagrams, but these were not consistent across treatments. Additionally, sometimes these mechanisms had unexpected relationships, such as positive effects of

predators and competitors on frog biomass. These relationships may simply indicate that the environments that were favorable to our focal species were also favorable to competitors and predators, as found in a natural community (Semlitsch et al. 1996). However, it is important to note that these are proposed mechanisms, that path analysis assumes causation, and that the causation assumption may not be met. Important variables could be highly correlated to other variables that were not measured or, in the case of temperature that was too correlated to be included in analyses, possibly masking the actual mechanism responsible. Manipulative experiments separating effects of primary productivity, food quality, and temperature, as well as detailed diet studies, will help provide more substantial evidence on mechanisms determining reciprocal biomass export.

It is possible that our results did not consistently match our predictions, because we examined primary productivity at a whole system level, but only analyzed a portion of the subsidy inputs and exports. Other amphibians, such as spring peepers and gray treefrogs, and various insects also emerged from the mesocosms, representing additional reciprocal subsidy exports. Although we counted all emerging frogs of these other species and dragonflies, we did not record mass and, thus, cannot test our predictions with a broader portion of the reciprocal subsidies. However, other data from the mesocosms in this study show that chironomid biomass also declines with increasing primary productivity and is higher in tanks with litter input than no input, suggesting that these patterns may translate into similar differences in chironomid biomass export from these mesocosms. Chironomids represent the majority of aquatic insect biomass in our mesocosms (Earl and Semlitsch, unpublished data).

Our data do indicate that, when examining a portion of the reciprocal subsidies, species- or year-specific trends occur, likely due to complex inter-specific interactions. Other studies have investigated all emerging aquatic insects from streams, which may represent the majority of the reciprocal subsidies from streams to forests in those systems (Wallace et al. 1997; Baxter et al. 2004; Baxter et al. 2005). Stream salamanders may also be a significant export (Peterman et al. 2008), but have not been addressed. These studies have shown that subsidy input as leaves (Wallace et al. 1997) and terrestrial insects (Baxter et al. 2004; Baxter et al. 2005) to streams increases the reciprocal export of aquatic insects to forest (Baxter et al. 2005), supporting our second prediction. There is also broad evidence that subsidies increase consumer biomass (reviewed in Marczak et al. 2007), which applies to reciprocal subsidy export if consumers are themselves subsidies. Further tests of conceptual framework in various systems using a whole system approach will provide additional insight into broad patterns related to reciprocal subsidies.

We provided a conceptual framework (Fig. 1) to begin examining the feedback loop between ecosystems with reciprocal subsidies. We tested these ideas using pond-forest linkages, finding some expected and unexpected results but differences in species/years. These data are the first to examine reciprocal subsidies experimentally along an ambient resource gradient, finding that reciprocal export declined with increases in primary productivity. Spatial subsidies are thought to be pervasive across the landscape (Polis and Strong 1996; Loreau and Holt 2004) and are often important as ecosystem services (Tylianakis et al. 2004; Janetski et al. 2009). Further studies, such as

ours, will advance our knowledge of how subsidies function and allow us to better preserve these flows between ecosystems to maintain their services.

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Table 1. Important dates for reciprocal subsidy experiments.

	Wood Frogs: 2008	American Toads: 2009	Leopard Frogs: 2010
Litter additions	28,29-Nov ^a	09-Nov ^a	04-Nov ^a
Hatchling additions	09-Apr	09-May	12,13-Apr
Spring NPP Sampling	19-Apr	16-Apr	13-Apr
1 st Metamorph Emergence	30-May	11-Jun	09-Jun
Last Metamorph Emergence	06-Aug	27-Jul	17-Sep
Summer NPP Sampling	09-Jul	26-Jul	28-Jul

^a Litter additions were performed in the previous year.

Table 2. Means \pm standard error for abiotic and biotic variables for each litter treatment and each frog species. Ranges are in parentheses. Canopy cover refers to tree overstory canopy. Because of 30% shade cloth covers, actual shading percentages were higher. Temperature, oxygen and pH values are the average of the spring and summer sampling periods. Pred. refers to the number of predators (dragonfly adults) emerging from a mesocosm, and comp. refers to the number of competitors (treefrog metamorphs) emerging from a mesocosm.

	Canopy Cover	Temperature	Oxygen	pH	Pred.	Comp.
	(%)	(°C)	(mg/L)		(#)	(#)
Wood Frogs: 2008						
Leaf Litter	43.8 \pm 10.2 (0.0–87.5)	14.1 \pm 0.5 (12.0–17.1)	2.5 \pm 0.5 (0.5–6.1)	7.7 \pm 0.1 (7.2–7.9)	2.8 \pm 1.6 (0–17)	104 \pm 41.9 (0–157)
Grass Litter	46.4 \pm 10.9 (0.0–93.1)	14.7 \pm 0.4 (12.9–17.0)	3.1 \pm 0.5 (0.9–8.1)	7.8 \pm 0.1 (7.3–9.1)	7.2 \pm 3.9 (0–39)	40.0 \pm 13.9 (0–393)
No Input	48.9 \pm 12.0 (0.0–89.6)	14.7 \pm 0.5 (11.4–17.7)	5.7 \pm 0.8 (0.81–9.8)	7.8 \pm 0.2 (7.2–9.2)	6.5 \pm 3.0 (0–24)	12.2 \pm 9.7 (0–125)

 American Toads:

2009

Leaf Litter	60.4 ± 11.7	23.3 ± 0.6	3.9 ± 0.7	7.0 ± 0.2	5.6 ± 5.3	38.5 ± 9.6
	(0.0–100.0)	(20.7–27.3)	(1.9–8.5)	(6.5–8.1)	(0–59)	(5–115)
Grass Litter	66.6 ± 11.5	22.2 ± 0.4	5.5 ± 0.4	7.0 ± 0.1	33.4 ± 27	70.1 ± 25.4
	(0.0–99.5)	(20.3–25.7)	(3.6–7.9)	(6.6–7.5)	(0–323)	(1–313)
No Input	73.0 ± 11.0	22.0 ± 0.5	8.1 ± 0.4	7.1 ± 0.1	10.6 ± 6.4	24.9 ± 7.7
	(0.0–99.5)	(18.8–25.7)	(6.3–10.8)	(6.7–8.5)	(0–29)	(0–73)

 Leopard Frogs: 2010

Leaf Litter	65.0 ± 10.6	25.5 ± 0.7	2.0 ± 0.5	7.0 ± 0.1	11.8 ± 7.3	48.2 ± 22.9
	(0.1–100.0)	(23.5–29.)	(0.9–5.5)	(6.7–7.5)	(0–55)	(0–185)
Grass Litter	71.0 ± 10.7	24.9 ± 0.4	3.5 ± 0.3	6.9 ± 0.1	7.0 ± 4.3	32.5 ± 16.1
	(0.0–99.5)	(23.6–27.9)	(2.4–5.5)	(6.7–7.3)	(0–45)	(0–186)
No Input	75.1 ± 11.0	25.2 ± 0.6	6.4 ± 0.4	7.2 ± 0.2	0 ± 0	10.6 ± 6.0
	(0.0–99.5)	(23.5–28.8)	(4.8–8.1)	(6.9–8.5)	(0–0)	(0–56)

Table 3. Pearson's correlation coefficients (r) between biotic and abiotic variables for each subsidy treatment for each frog species. Top number is for leaf litter (L), middle is for grass litter (G), and bottom number is for no input (N). Pred. is predator (adult dragonfly) abundance, and Comp. is competitor (treefrog) abundance. Bolded coefficients are statistically significant.

		NPP (mg/L)	Oxygen (mg/L)	Pred. (#)	Comp. (#)	pH	Temperature (°C)
Wood Frogs							
Canopy	L (n=12)	-0.56	-0.44	-0.40	-0.55	-0.57	-0.78
	G (n=12)	-0.74	-0.21	-0.48	-0.44	-0.63	-0.94
	N (n=11)	-0.88	-0.88	-0.43	0.38	-0.86	-0.92
NPP			-0.02	0.70	0.81	0.54	0.72
			-0.01	0.78	0.09	0.87	0.75
			0.63	0.51	0.59	0.75	0.74
Oxygen				-0.06	-0.10	0.57	0.61
				0.32	-0.17	-0.23	0.12
				0.39	0.24	0.69	0.91
Pred.					0.67	0.25	0.49
					-0.38	0.70	0.51
					0.55	0.35	0.31
Comp.						0.50	0.57
						0.20	0.48

					0.37	0.39
pH					0.79	
					0.77	
					0.83	
American Toads						
Canopy	L (n=11)	-0.90	-0.85	-0.03	0.35	-0.49 -0.80
	G (n=12)	-0.72	-0.55	0.09	-0.28	-0.12 -0.85
	N (n=11)	-0.85	-0.22	-0.47	-0.52	-0.09 -0.63
NPP		0.81		-0.31	-0.18	0.40 0.86
		0.85		0.49	-0.09	0.67 0.61
		0.48		0.31	0.43	0.26 0.91
Oxygen				-0.21	-0.46	0.69 0.65
				0.34	-0.05	0.68 0.53
				-0.00	-0.08	0.72 0.58
Pred.				0.03		-0.08 -0.24
				-0.29		0.72 -0.08
				0.71		0.56 0.20
Comp.						-0.46 -0.19
						-0.06 -0.00
						0.22 0.33
pH						0.11
						-0.05
						0.39

Leopard Frogs

Canopy	L (n=9)	-0.91	-0.79	-0.29	-0.54	-0.76	-0.97
	G (n=11)	-0.93	-0.88	-0.47	-0.27	-0.67	-0.88
	N (n=9)	-0.66	-0.50	n/a	-0.81	-0.31	-0.85
NPP		0.82		0.32	0.21	0.78	0.96
		0.80		0.52	-0.21	0.77	0.96
		0.57		n/a	0.17	0.59	0.93
Oxygen				-0.17	0.10	0.96	0.80
				0.51	-0.08	0.74	0.76
				n/a	0.37	0.87	0.69
Pred.				0.28		-0.06	0.35
				-0.27		0.62	0.42
				n/a		n/a	n/a
Comp.						0.04	0.41
						0.29	-0.03
						0.03	0.51
pH							0.79
							0.78
							0.59

Table 4. Statistical results for reciprocal subsidy patterns.

	df	F	p
<hr/> Wood Frogs			
Block	2	0.87	0.43
Litter	2	5.71	0.009
NPP	1	2.43	0.13
NPP*Subsidy	2	5.66	0.009
Error	26		
<hr/> American Toads			
Block	2	0.11	0.90
Litter	2	2.32	0.12
NPP	1	1.34	0.26
NPP*Subsidy	2	2.71	0.08
Error	25		
<hr/> Leopard Frogs			
Block	2	1.82	0.19
Litter	2	6.43	0.007
NPP	1	0.22	0.65
NPP*Subsidy	2	3.24	0.06
Error	20		

Figure 1. Conceptual relationship between ambient resources (primary productivity) and subsidy export comparing systems with no subsidy (dotted line) to systems with subsidy input (leaf litter; dashed line) and within system input (grass litter; solid line). For the experimental system in this study, this relationship illustrates our predictions for frog biomass export when comparing prairie cordgrass (detritus produced within the system retained from a previous year) to leaf litter. The grass has higher nutrient content than the leaves, which is predicted to result in higher subsidy export.

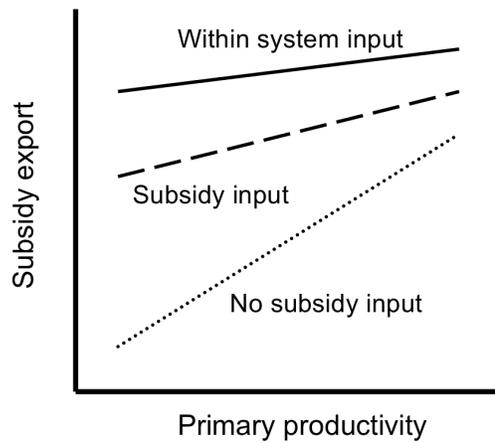


Figure 2. Relationship between frog biomass export, net primary productivity (NPP) and litter input treatment for a) wood frogs in 2008, b) American toads in 2009, and c) southern leopard frogs in 2010 (raw data shown). Leaf litter regression slopes are not significantly different from zero; grass and no input slopes are negative. All regression slopes for leopard frogs are marginally significant ($p = 0.06$, Table 2). Models were an analysis of covariance that also included site as a random blocking variable.

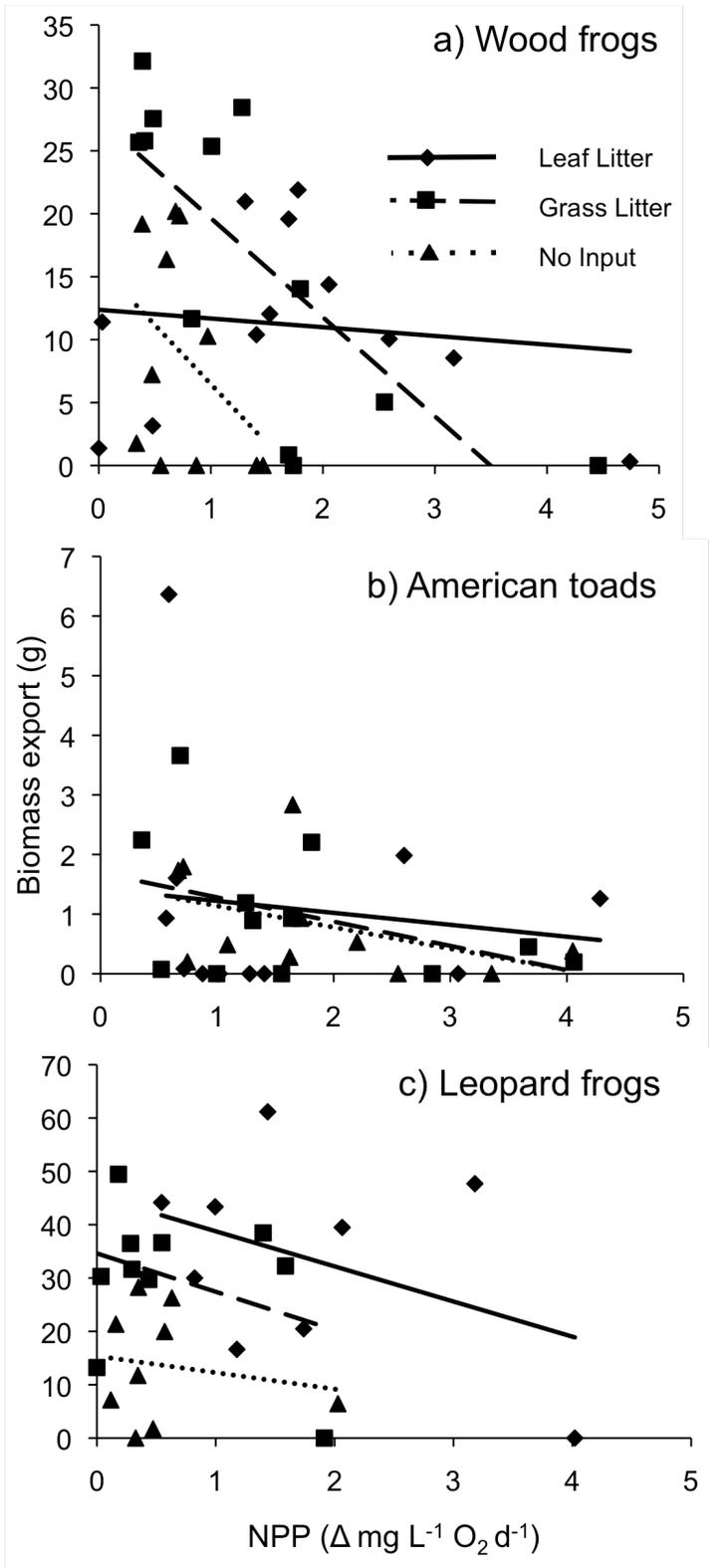
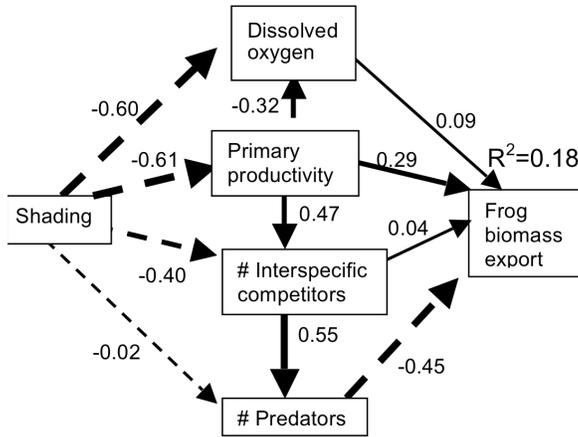


Figure 3. Path analysis diagrams examining the importance of different possible mechanisms in determining frog biomass export under spatial subsidy input (leaf litter; left column), within system input (grass litter; center column), or no input (right column) for (a,b,c) wood frogs in 2008, (d,e,f) American toads in 2009, and (g,h,i) southern leopard frogs in 2010. Solid lines are positive relationships, and dashed lines are negative relationships. Line thickness denotes the magnitude of the path coefficient. Numbers indicate the path coefficient (standardized regression coefficient). N refers to sample size. R^2 is the squared Pearson's correlation coefficient for the model explaining frog biomass export. There is no line connecting number of predators to frog biomass mass export for i) Leopard frogs in 2010 with no input, because no predators were found emerging from any mesocosm from that treatment.

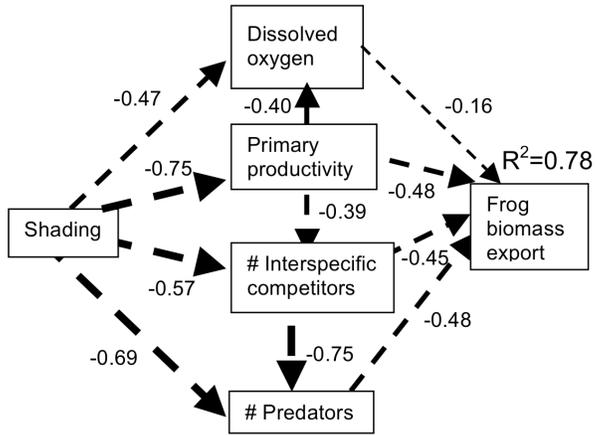
Spatial subsidy input:
Leaf litter

a) Wood frogs: 2008 (n = 12)

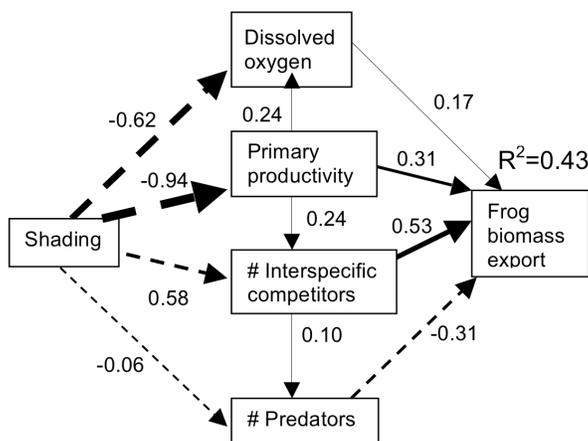


Within system input:
Grass litter

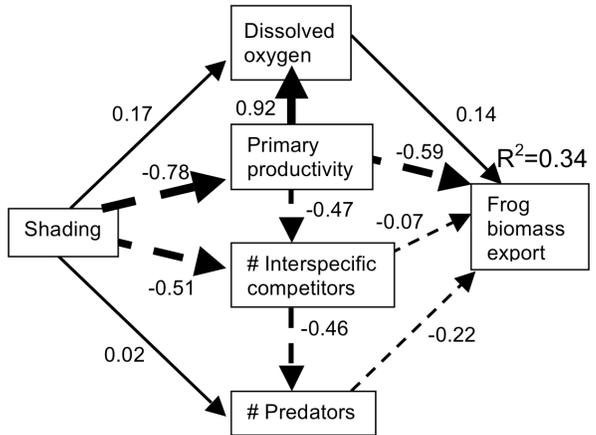
b) Wood frogs: 2008 (n = 12)



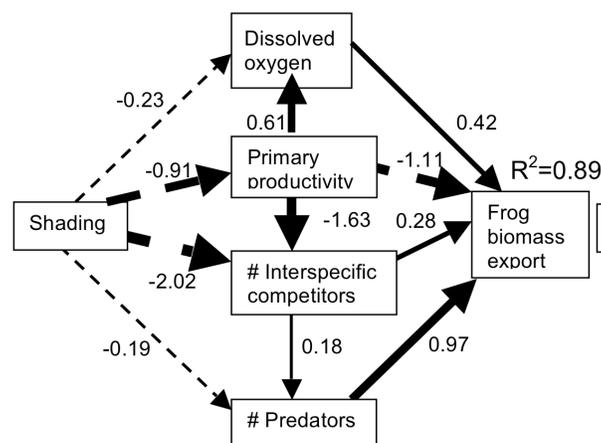
c) American toads: 2009 (n=11)



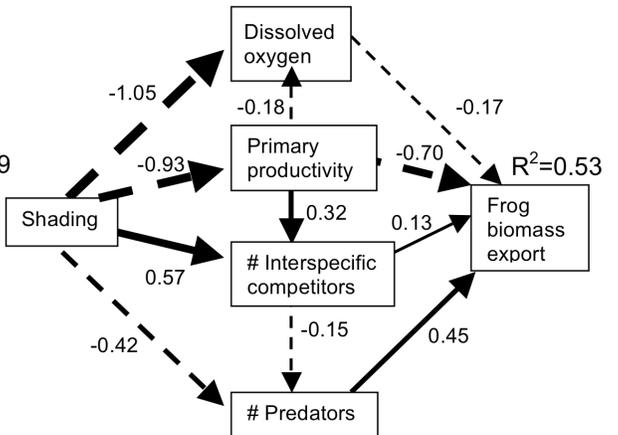
d) American toads: 2009 (n=12)



e) Southern leopard frogs: 2010 (n = 9)

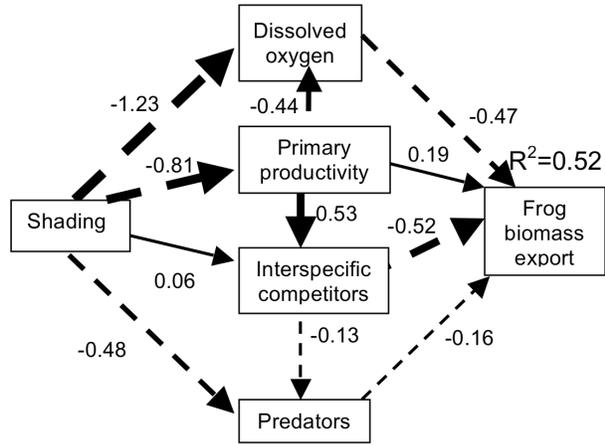


f) Southern leopard frogs: 2010 (n = 11)

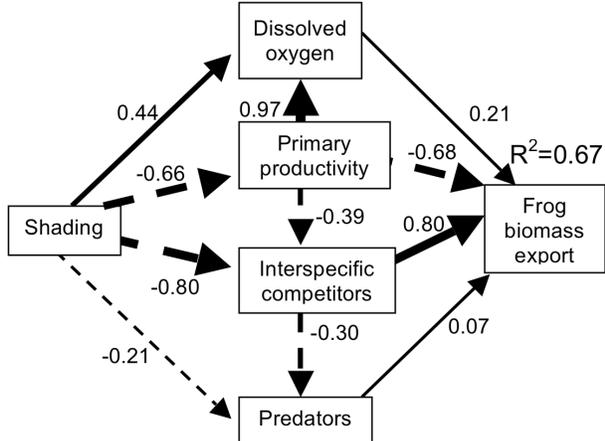


No Input

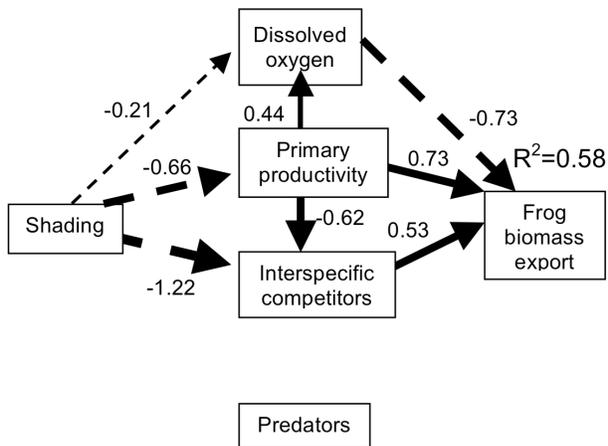
c) Wood frogs: 2008 (n = 11)



f) American toads: 2009 (n = 11)



i) Southern leopard frogs: 2010 (n = 9)



CHAPTER 5

CARRYOVER EFFECTS IN AMPHIBIANS: HOW MUCH COMPLEXITY IS NEEDED TO PREDICT SURVIVAL?

ABSTRACT

Carryover effects occur when experiences early in life affect an individual's performance at a later stage and are important in many socially and economically critical research areas, including human health, food production, and conservation. Recent studies have shown that experiences in early stages can, but not always, interact with later stages to create unintuitive patterns. These studies with different outcomes vary in taxa and types of environmental variation. We were interested in whether similar patterns would emerge under the same experimental design with similar taxa. To examine this, we implemented a four-way factorial experimental design with different forestry practices on three species of anurans (each examined in different years) in the aquatic larval stage and terrestrial juvenile stage. We investigated whether one environment, both environments, or the interaction between the environments best predicted survival. We found that the most complex model (the four-way interaction) was never the best model. However, in American Toads (*Bufo americanus*; 2009/2010) and southern leopard frogs (*Rana sphenoccephala*; 2010/2011), the best predictors of juvenile survival included factors from both the terrestrial and aquatic environments. In wood frogs (*Rana sylvatica*; 2008/2009), factors from only the terrestrial environment best predicted survival, but low

survival may have limited our ability to detect other differences. Both life history parameters (size and time to metamorphosis) and aquatic habitat variables indicated carryover effects, and these effects were found after both two and ten months. Our study and others show that environmental factors from different habitats have the potential to affect a single demographic parameter. Future work recognizing the potential importance of multiple life stages simultaneously will better assess their impacts on population dynamics.

Carryover effects (also known as latent effects) occur when an individual's history affects their subsequent performance (Pechenik et al. 1998). Carryover effect research has demonstrated that an early life experience can have lasting effects for months to decades and can be major factors in a variety of socially and economically important research areas. In humans, such effects have found that pre-natal and toddler environments can affect educational outcomes (Anderson et al. 2003) and the prevalence of disease in adults (Desai and Hales 1997). For example, evidence suggests that reduced fetal nutrition can lead to obesity and heart disease in adulthood by raising blood pressure and altering hormone and metabolic responses (Ravelli et al. 1976, Barker 1995). In ecology, recent studies have found that carryover effects from the larval stage of marine invertebrates can alter survival and reproductive output (Dias and Marshall 2010, Crean et al. 2011). Changes in demographic parameters (i.e. survival, fecundity) have the ability to alter population dynamics and evolutionary change (Pechenik 2006, Crean et al. 2011).

Early work focused on documenting the existence of carryover effects. More recent research has expanded this effort, finding that experiences early in life can, but not always, interact with later experiences to determine an individual's performance. For example, James and Semlitsch (2011) found that frogs with a cadmium exposure history had lower survival than unexposed individuals in forest habitats, but this pattern was reversed in field habitats. Factorial experiments, such as this, have not been frequently used to investigate carryover effects. However, two additional examples show interactions between environmental factors in different life stages affect individual performance (Vonesh 2005, Smith and Shima 2011), while two others do not (Relyea and

Hoverman 2003, Webber et al. 2010). If these types of non-intuitive patterns are common, they could prove quite problematic for solving social, economic, and ecological problems related to carryover effects, but clearly there is no current understanding of when these occur. These studies with different outcomes vary in taxa and types of environmental variation. One way to proceed is to test the same types of environmental variation with similar taxa to see if similar patterns emerge, which is the approach we took in this study.

To look for similar patterns, we implemented a four-way factorial experimental design with different forestry practices on three species of anurans in the aquatic larval stage and terrestrial juvenile stage. We investigated whether one environment, both environments, or the interactions between the environments best predicted survival. We chose this study system to investigate carryover effects for two main reasons. First, forestry practices are widespread forms of land use that have the potential to cause declines in amphibian species (i.e. Patrick et al. 2006, Peterman and Semlitsch 2009, Popescu and Hunter 2011). Second, recent evidence has shown that tree canopy removal can be positive for the growth and survival of larval anurans in the aquatic habitat but is overwhelmingly negative for the growth and survival of juveniles in the terrestrial habitat (reviewed in Semlitsch et al. 2009). These different effects in different life stages have the potential to result in important carryover effects that may relate to forest management and land use decisions in general.

We used model-ranking with an information-theoretic approach to determine the best predictors of juvenile survival (at two and ten months) among models containing factors from the larval aquatic and/or juvenile terrestrial stage. We were particularly

interested in the juvenile terrestrial stage, because previous studies have shown it to be the population regulating stage for pond-breeding amphibians, including the two genera examined in our study (Biek et al. 2002, Vonesh and De la Cruz 2002). We predicted that carryover effects would be important for juveniles after two months, but the effects of the larval aquatic environment would diminish after ten months in the juvenile terrestrial environment due to the equalizing effects of compensatory growth (Bernardo 1996, Heath and Blouw 1998, Boone 2005). We additionally included a model containing metamorphic life history parameters to determine whether carryover effects were best reflected by individual characteristics or habitat.

METHODS

Study System

Our study took place at Daniel Boone Conservation Area, Warren County in central Missouri, USA. The conservation area consists of continuous oak-hickory forest with many small permanent constructed wildlife ponds that support a diverse community of amphibians. It is also the site of ongoing research on the effects of forestry practices on amphibians (Semlitsch et al. 2009) for which experimental forestry plots were created in late 2004 and early 2005 (Semlitsch et al. 2009). In brief, plots (4 in total) were circular (164m radius) with a small constructed pond in the center. Plots were divided into quadrants and each quadrant received one of four forestry treatments: control forest, partial cut forest (thinned to 60% stocking level by removing or girdling low quality trees), clearcut with downed wood removed (trees greater than 25cm in diameter were

removed and trees under 25cm were girdled and left standing), and clearcut with downed wood retained (as in removed treatment except trees under 25cm were felled). The two clearcuts were opposite each other in all plots (Semlitsch et al. 2009). Our study took place during the 4-6 years after timber harvest and contained very thick shrubby vegetation. Thus, we will refer to these clearcuts as early successional forest (ESF).

For our study, we used three species of anurans: wood frogs (*Lithobates sylvaticus*), American toads (*Anaxyrus americanus*), and southern leopard frogs (*L. sphenoccephalus*). In Missouri, all three anuran species breed in ponds during the spring, though wood frogs tend to breed approximately one month earlier than American toads and southern leopard frogs. The three species have larval periods that last a few months, after which they metamorphose and emigrate away from their natal ponds into the surrounding terrestrial habitat during mid- to late summer (Lanoo 2005).

Experimental Design

To examine the importance of forestry practices in the aquatic and terrestrial stages on juvenile performance, we used *in situ* aquatic and terrestrial mesocosms within experimental forestry plots at Daniel Boone Conservation Area. Aquatic mesocosms (1000L cattle water tanks) allowed us to focus on our manipulated variables while standardizing many other variables known to influence pond communities including pond size (surface area and depth), shape, age, and hydroperiod (Wellborn et al. 1996, Gee et al. 1997, Søndergaard et al. 2005). Terrestrial mesocosms (3m x 3m enclosures made from hardware cloth) allowed us to restrict the movement of juvenile frogs in order to increase our detection probability and manipulate variables of interest (Harper et al.

2009). Mesocosms were placed within the forestry quadrants (Hocking and Semlitsch 2007, Semlitsch et al. 2008, Semlitsch et al. 2009) in early 2006 for a different aquatic experiment (Hocking and Semlitsch 2008) and in 2004 for a different terrestrial experiment (Harper 2007).

We created two treatments in the aquatic environment: litter input (1kg leaves, 1kg grass, or none) and mesocosm placement (edge or center) within control and ESF quadrants. We also had two treatments in the terrestrial environment: density (high or low) and forestry treatment (control forest, partial cut forest, ESF with downed wood retained, and ESF with downed wood removed). To perform the experiment, hatchlings were added to aquatic mesocosms and collected at metamorphosis. Metamorphs were then placed into terrestrial mesocosms such that one (low density) or two (high density) metamorphs from each aquatic treatment were present in each terrestrial mesocosm. This was the target scenario, but low survival for some aquatic treatments limited our ability to compare all possible treatment combinations. We examined three species of frogs in different years: wood frogs in 2008/2009, American toads in 2009/2010, and southern leopard frogs in 2010/2011 (dates of hatchling and metamorph additions for each species are in Table 1). We chose to use these species to help us probe for common outcomes of carryover effects. The examination of different species in different years was confounded but logistically necessary.

Aquatic Mesocosms

Three pond mesocosms (1000L cattle watering tanks) were placed in each of four positions: early successional forest (ESF) at 10m (ESF edge) and 50m (ESF center) from

the nearest forest edge and in the forest 10m (forest edge) and 50m (forest center) from the same edge, which resulted in a canopy (and primary productivity) gradient . Twelve mesocosms were placed in this manner at three replicate forestry plots (total N = 36) where clearcutting took place in winter 2004 to spring 2005. All mesocosms were filled with water, received equal amounts of leaves (1kg) in February 2006, and were inoculated with zooplankton in April and May of 2006 as part of another experiment examining the effects of canopy cover on tadpoles (Hocking and Semlitsch 2008). All tanks were treated the same except for their placement within forestry plots during this experiment. Tanks were left uncovered and allowed to develop communities through colonization and oviposition of invertebrates and treefrogs from autumn 2006 through autumn 2007.

In autumn of 2007, litter manipulations were applied to mesocosms (Table 1). The three treatments consisted of 1kg deciduous leaf litter, 1 kg of grass litter, and no input. Preliminary data in our control forest plots (\approx 95% canopy cover) indicated that leaf litter deposition averaged 1kg tree leaves per cattle tank. Additionally, this is consistent with many other mesocosm studies (Boone et al. 2007, Hocking and Semlitsch 2008, Williams et al. 2008). For the leaf litter, we used the natural mixture of deciduous leaves found in our area, consisting mostly of white oak (*Quercus alba*) and northern red oak (*Quercus rubra*) with some hickory (*Carya* spp.) and sugar maple (*Acer saccharum*). For the grass litter, we used prairie cordgrass (*Spartina pectinata*), a wetland obligate species found across much of the United States (USDA and NRCS 2010). Leaves and grass were all collected after natural senescence from within Daniel Boone Conservation Area a few days before addition to mesocosms. For the no input and grass litter

treatments prior to the grass addition, we removed leaf litter already present from the mesocosms using a net with 2.5 cm gauge mesh. This method removed whole leaves and large leaf fragments, while retaining small leaf fragments and fine particulate organic matter. We replicated this disturbance in the leaf litter treatment by stirring the tanks for about the same amount of time it took to remove leaves in the other treatments. All litter treatments were renewed in autumn of 2008 and 2009 (Table 2) by adding an additional 1kg of grass, leaves, or no litter to designated mesocosms to maintain subsidy treatments for each anuran species.

After implementing litter treatments, aquatic mesocosms were covered with high-density polyethylene PAK knit 27% shade cloth with 3mm sized openings (Hummert International, St. Louis, MO, USA) to prevent further introduction of leaf material and the escape of metamorphosing tadpoles. Tank covers were pushed into the water to facilitate oviposition by invertebrates and anurans. Tank covers were removed for at least 24 hours every 4-6 weeks to further facilitate oviposition and colonization by aquatic animals, except during winter (from mid-November to mid-March) when there is little to no insect colonization and oviposition in ponds (Merritt and Cummins 1996). Mesocosms were additionally left uncovered after the completion of the tadpole experiment from mid-August to late September to further allow oviposition and colonization.

We stocked aquatic mesocosms with hatchling frog tadpoles in three different years. We collected 3-6 egg masses from Daniel Boone Conservation Area and allowed them to hatch out in the laboratory. All hatchlings were at Gosner stages 25-26 at stocking, the free-swimming stage where tadpoles are absorbing their external gills, 2-4

days after hatching (Gosner 1960). We stocked hatchlings at densities that resulted in metamorphs of comparable size to natural populations (Harper and Semlitsch 2007). Thus, we stocked wood frogs and leopard frogs at 40 hatchlings/mesocosm and American toads at 72 hatchlings/mesocosm (Table 1). We monitored mesocosms for metamorph emergence every 2-3 days starting at the known minimum time to metamorphosis and then daily from the detection of the first metamorph until one week after the last metamorph and tadpole was seen. Metamorphs were defined as Gosner stage 42, characterized by the emergence of at least one forelimb and both hind limbs (Gosner 1960). Metamorphs were held in the laboratory until tail absorption was complete. At this time, we measured mass to 0.01g, snout to vent length (SVL) to 0.1mm (toads were measured to 0.01mm using Mitutoyo CD-6”P Calipers, Japan), and recorded time to metamorphosis in days. This method allowed us to remove metamorphs from tanks at the earliest possible stage to prevent drowning and give us a reliable estimate of mass at metamorphosis (Travis 1980).

Terrestrial Mesocosms

Two terrestrial mesocosms were placed in pairs in each forestry treatment of the four experimental forestry plots. Each pair of mesocosms was placed approximately 100m from the plot’s central pond in fall of 2004 using the methods described in Harper (2007). Briefly, mesocosms were 3 x 3m enclosures made from galvanized steel hardware cloth with 0.32cm mesh to allow small invertebrate prey to move through. Hardware cloth was buried 30cm below ground and extended 80 cm above ground with a 10 cm lip around the top. The amount of downed wood in each mesocosm was standardized by forestry

treatment, based on data from Shifley and Brookshire (2000). We removed all downed wood from ESF mesocosms with downed wood removed and standardized downed wood in all other treatments. We added 2m of 15cm diameter downed wood of decay class 3 to pens in the control, partial cut and downed wood retained pens. Class 3 downed wood as defined by Maser et al. (1979) includes logs that are still round, but are faded, lack twigs, and have only a trace of bark remaining. This type of downed wood was likely present prior to the application of the forestry treatments. Downed wood resulting from treatment application is considered decay class 1, which includes round logs with intact twigs and bark (Maser et al. 1979). We added 3m class 1 downed wood to mesocosms in the partial cut and 9m of class 1 downed wood to mesocosms in the downed wood retained treatment. In spring 2005, leaf litter depth was standardized by treatment to a depth of 2cm in the two ESF treatments and 4 cm in the partial cut and control. These depths were based on the averages measurements taken throughout the forestry treatment quadrants (Rittenhouse, Harper, Hocking, Conner and Semlitsch, unpublished data). After 2005, leaves were allowed to accumulate and logs were allowed to decay naturally in the mesocosms.

We assigned individual metamorphs to terrestrial mesocosms and individually marked them by clipping three toes each. To assign metamorphs to terrestrial mesocosms, we randomly chose a treatment in each forestry plot to start and then systematically added individuals to the low and high density of each forestry treatment going clockwise around the plot. Once one individual from an aquatic treatment was present in each mesocosm, we then continued clockwise to add the second metamorph to each high-density mesocosm.

We searched terrestrial mesocosms for juvenile frogs in two censuses for each species (Table 1). The first census occurred in late September, when the temperatures start to decline, to assess survival and growth over the summer, or after approximately two months. The second census occurred the following May to assess survival and growth over the winter, or after approximately ten months. The September censuses consisted of two searches of each pen performed on different days. May censuses consisted of at least two searches and additional searches were added until it appeared we detected all but 2 or fewer individuals. For each search, 2-3 people surveyed the entire area of the pen by disturbing leaf litter, turning cover objects, and investigating large holes. Harper and Semlitsch (2007) report that one application of this method results in capture probabilities of approximately 92% for juvenile wood frogs and 79% for juvenile toads. After the September census, all frogs were returned to their mesocosm of origin. After the May census, all frogs were sacrificed using a lethal dose of MS-222 and preserved.

Statistical Analysis

We analyzed metamorphic characteristics for differences among terrestrial treatments to determine whether there was any initial bias. We performed analyses on the following metamorphic traits for each species: SVL, body condition (mass/SVL), and time to metamorphosis. We chose not to examine mass, because it was highly correlated to SVL and body condition in all three species (Table 3). Independent variables consisted of the forestry treatment, the density treatment, and the interaction between the two. We included time to metamorphosis in the SVL and body condition analyses. The

unit of replication was the terrestrial mesocosm, and thus, each data point represented an mesocosm average. We Bonferroni corrected our alpha value for each species, because of the number of similar analyses we performed (Hochberg 1988). We performed three analyses per species changing our target alpha = 0.05 to the corrected value of 0.02. To determine the importance of the carryover effects on juvenile survival, we used an information theoretic approach to identify the best-fit model using logistic regression (Burnham and Anderson 2002). We examined survival for the September census, the May census, and the stage-specific survival between September and May for each species. We used each individual as a replicate in each analysis, because each experienced a unique combination of aquatic and terrestrial mesocosms and had a unique time, size, and body condition at metamorphosis. Eight models were ranked using Akaike's Information Criterion (AIC), where the lowest value indicates the best supported model, and any model within two units is considered a competing model (Burnham and Anderson 2002). Additionally, Akaike weights were computed to examine the relative support for each model (Burnham and Anderson 2002). Models consisted of a null model (intercept only), a metamorphosis model (time, SVL and body condition at metamorphosis), and different combinations of the aquatic and terrestrial treatments (Table 2). We considered all models containing aquatic treatments to indicate carryover effects. We chose SVL as our measure of size at metamorphosis, because mass and body condition were highly correlated (Appendix 3). When model-ranking revealed multiple competing models, we used model-averaging to estimate parameter values and standard errors for all parameters included in competing models. All models (proc logistic) were performed using SAS (Cary, North Carolina, USA).

RESULTS

Terrestrial Mesocosms

We placed 300 or more metamorphs into mesocosms for each species/year. The target numbers were 432 for wood frogs and 576 for leopard frogs and American toads.

Because of differential survival in some aquatic treatments, not all aquatic treatments were equally represented in terrestrial mesocosms (Table 4). There were no wood frogs placed in terrestrial mesocosms from the aquatic mesocosms in the ESF center with no litter. Also, there were no toads placed in terrestrial mesocosms from the aquatic mesocosms in the forest edge with leaf litter or in the ESF edge with grass litter. For leopard frogs, individuals from all aquatic treatments were represented in terrestrial mesocosms. Wood frog high-density (target density: 24 frogs/mesocosm) and low-density (target: 12 frogs/mesocosm) terrestrial mesocosms were close to target densities ($p < 0.0001$; high: 22.00 ± 0.69 frogs/mesocosm; low: 11.08 ± 0.29), as were leopard frogs ($p < 0.0001$; high: 19.27 ± 0.41 [mean \pm standard error] frogs; low: 9.93 ± 0.23). American toads had lower densities overall than planned due to low aquatic survival ($p < 0.0001$; high: 12.13 ± 0.53 frogs; low: 6.63 ± 0.45). We found no initial bias among individuals placed in terrestrial treatments, as indicated by no statistical difference in metamorphic SVL, body condition or length of larval period among terrestrial treatments for wood frogs (all $p > 0.13$), toads (all $p > 0.05$) or leopard frogs (all $p > 0.20$). For toads, there was a slight trend that individuals placed in the high-density mesocosms had a longer time to metamorphosis (1.5 ± 0.5 days longer) than individuals placed in low-density mesocosms ($p = 0.05$ with bonferroni-corrected $\alpha = 0.02$).

Carryover Effects

Thirty wood frogs were detected in September 2008 and 12 in May 2009. Three of those detected in May had not been found in the September census. We estimate that overall 10.7% of wood frogs survived to September and 4.3% to May. For American toads, 51 individuals were found in September 2009 and 26 in May 2010. Seven of those found in May were not detected in September. We estimated 19.3% survival to September and 8.7% to May. 145 leopard frogs were found in the September 2010 and 33 in May 2011. Ten individuals found in May had not been detected in September. Survival was approximately 36.3% to September and 7.8% to May.

Because of our experimental design, we are not attempting to compare these three species and attribute differences to characteristics of these species, because differences could easily be due to temporal variation or some interaction between species and temporal variation. Below, to emphasize this point, we use species with the years in parentheses at the first mention of each species in each paragraph.

Carryover effects influenced survival of American toads (2009/2010) and leopard frogs (2010/2011) to both September and May census dates. Conversely, carryover effects were not significant for wood frog (2008/2009) survival. Instead, for wood frogs, the terrestrial main effects model ranked highest for survival to September and survival from September to May (with one competing model, i.e. within two AIC units), while the null model best-fit survival from metamorphosis to May (with one competing model; Table 5). For wood frog survival to September, only parameters relating to forestry practices (not density) had confidence intervals that did not include zero (had significant effects), and these indicated lower survival in the ESF with downed wood removed than

the other three treatments (Table 6). For wood frog survival from September to May, no parameters had confidence intervals that did not include zero, all of which were estimated using model averaging.

American toad (2009/2010) survival to September was best predicted by the aquatic main effects model (Table 5) with the aquatic/terrestrial: main effects model and metamorphosis model as competing models (within two AIC units). Parameter estimates (Table 7) indicated that the probability of surviving to September increased with SVL at metamorphosis. Other parameters had confidence intervals that included zero. American toad survival to May was best predicted with the aquatic/terrestrial: interactions model, but the aquatic: interactions model, terrestrial: interactions model, and null model were competing models. After model-averaging, one aquatic parameter and one terrestrial parameter from these models had confidence intervals that did not include zero.

Juveniles that spent their larval period in forest center mesocosms with grass and resided in terrestrial mesocosms in the ESF with downed wood removed under high density had the highest survival (Table 7). Survival from September to May was best predicted by the null model (Table 5).

Leopard frog (2010/2011) survival to September was best predicted with the metamorphosis model (Table 5), but only SVL at metamorphosis had a confidence interval that did not include zero, indicating that larger metamorphs were more likely to survive (Table 8). Leopard frog survival to May was best predicted by the terrestrial main effects model with the aquatic/terrestrial: main effects model competing (Table 5). Model-averaging indicated that survival was highest in the ESF with downed wood removed terrestrial treatment and in the ESF center aquatic treatment, but lower in the

partial cut terrestrial treatment (Table 8). Survival from September to May had six competing models, including the null model (Table 5). Model-averaging determined three significant variables (Table 8). Leopard frogs were more likely to survive in the ESF with downed wood removed terrestrial treatment and that same treatment under high density and were less likely to survive with a longer time to metamorphosis.

DISCUSSION

We found that carryover effects were important for two of the species/years, but not for the third. In all cases, the most complicated model was never the best predictor of juvenile anuran survival. Some previous studies have found non-intuitive patterns between factors in different life stages (Vonesh 2005, James and Semlitsch 2011, Smith and Shima 2011), while others have not (Relyea and Hoverman 2003, Webber et al. 2010). Our study found a consistent lack of these non-intuitive, interactive patterns under the same experimental design for three similar taxa. Although model-ranking did not always result in a clear best model, the examination of parameter confidence intervals showed that both habitats were important for juvenile survival for American toads (2009/2010) and leopard frogs (2010/2011). For wood frogs (2008/2009), survival was best predicted by the terrestrial environment only. However, our power to detect differences among models for wood frogs may have been limited by the low number of juveniles surviving to the final census (only 12).

We predicted that carryover effects would be more important in the short-term than the long-term (Bernardo 1996, Heath and Blouw 1998). Other studies have found consistent evidence of this. For instance, southern toads have a strong correlation

between size at metamorphosis and growth after ten days, but this effect disappears given an additional two months post-metamorphosis (Beck and Congdon 1999). Similarly, others have found strong maternal effects in the short-term that slowly diminish as individuals reach adulthood (Lindholm et al. 2006). However, we found that carryover effects were important during all time periods for two species/years, despite the relatively long duration of our study. Interestingly, the significant carryover parameters were different factors in different time periods. Thus, we were unable to examine changes in parameter values over time that might have demonstrated diminished effects.

Evidence for carryover effects was shown both through significant larval habitat variables and metamorphic life history parameters. In both American toads (2009/2010) and leopard frogs (2010/2011), larger sizes at metamorphosis increased juvenile survival to September (2 months), but carryover effects were indicated by aquatic habitat variables for juvenile survival to May. Additionally, for leopard frogs, longer times to metamorphosis decreased the probability of survival between September and May. Similarly, many studies have shown that larger sizes and shorter times to metamorphosis positively affect fitness correlates in amphibians (i.e. Martof 1956, Semlitsch et al. 1988, Chelgren et al. 2006). Some studies have found effects of environmental factors but not life history parameters (Pahkala et al. 2001), while others have found effects of life history parameters but not environmental factors (Distel and Boone 2010). These differences may arise through different mechanisms.

As reviewed in Pechenik (2006), many authors have attributed carryover effects to differences in energy stores, but other hypotheses include alteration of metabolism, transcriptional and translational processes, and the damage of DNA or enzymes. We also

know that some early experiences alter morphology (Relyea and Hoverman 2003), chemical defenses (Hagman et al. 2009), and brain development (Trokovic et al. 2011), all of which could easily change how a carryover effect is revealed in later stages.

Clearly, the mechanisms of carryover effects are beyond the scope of this study, but further research into this area could help elucidate when carryover effects will manifest from environmental factors or life history parameters, as well as improve predictions of carryover effects in general.

Because we chose an important demographic parameter (Biek et al. 2002, Vonesh and De la Cruz 2002), our results have potential implications for population dynamics and thus amphibian conservation. We often found that different variables were significant for different species/year combinations and at different census times. Despite our inability to separate these factors, some information for management is present within our results. Additionally, we cannot separate species and year effects, but some of our results are consistent with previous research on the species we studied. Many studies find that wood frogs prefer and perform better in forested habitat when compared to clearcuts (Patrick et al. 2006, Patrick et al. 2008, Rittenhouse and Semlitsch 2009). Similarly, our parameter estimates indicate that partial cut or thinned forest increased juvenile survival probability, which recommends this practice as positive for wood frogs (Semlitsch et al. 2009).

Also, many studies have found that clearcutting is negative for the terrestrial stage of amphibians (Semlitsch et al. 2009). This is generally examined in the first few years post-harvest. Our study took place from four to six years post-harvest, during which the habitat contained thick, shrubby vegetation, and was better described as early

successional forest (ESF). We found that American toads (2009/2010) had higher survival probabilities when their terrestrial stage was spent in ESF when compared to control forest, and leopard frogs (2010/2011) had higher survival probabilities when both their aquatic and terrestrial stages were spent in ESF. During forest succession, changes in microclimate, habitat structure and/or prey availability likely provide favorable conditions for some amphibian species, particularly when compared to very recent clearcuts.

Our study found no complex, non-intuitive interactions between factors in different life stages for anurans. Although we implemented the same experimental design for similar taxa, we still found different results in different species/years. However, for two species/years, we did find factors from two different life stages were necessary to best predict survival. Few studies, thus far, have investigated the potential for environmental variation in multiple habitats to affect a single demographic parameter. Future work recognizing the potential importance of multiple life stages simultaneously will better assess the impacts of different environmental factors on population dynamics.

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Table 1. Important dates for carryover effect experiments.

	Wood Frogs: 2008	American Toads: 2009	Leopard Frogs: 2010
Litter additions*	28, 29 November	9 November	4 November
Hatchling additions	9 April	9 May	12, 13 April
1 st Metamorph Emergence	30 May	11 June	9 June
Last Metamorph Emergence	6 August	27 July	17 September
September Census	26, 27 September	12, 13 September	18, 19, 21 September
May Census†	20, 28 May, 4 June	23, 24 May	18, 19, 27, 31 May, 11 June

*Leaf additions were performed in the previous year.

†May censuses occurred the following year.

Table 2. Model set used to determine the importance of carryover effects to juvenile anuran survival. The metamorphosis model and models containing aquatic treatments indicate carryover effects. The number of levels in each treatment are indicated in parentheses at their first mention with “c” for continuous variables. K indicates the number of parameters and trt is an abbreviation for treatment.

Model	K	Parameters (besides intercept and variance)
Null model (intercept only)	1	none
Aquatic: Main Effects	6	Aquatic litter (3), aquatic forestry trt (4).
Aquatic: Interaction	7	Aquatic litter x aquatic forestry trt.
Terrestrial: Main Effects	4	Terrestrial density (2), terrestrial forestry trt (4).
Terrestrial: Interaction	4	Terrestrial density x terrestrial forestry trt.
Aquatic/Terrestrial: Main Effects	10	Aquatic litter, aquatic forestry trt., terrestrial density, terrestrial forestry trt.
Aquatic/Terrestrial: Interactions	11	Aquatic litter x aquatic forestry trt., terrestrial density x terrestrial forestry trt.
Aquatic x Terrestrial Interaction	19	Aquatic litter x aquatic forestry trt. x terrestrial density x terrestrial forestry trt.
Metamorphosis	4	Size at metamorphosis (c), time to metamorphosis (c), body condition at metamorphosis (c).

Table 3. Pearson's product moment correlation coefficients between metamorphic parameters for each species.

	Wood frog	American toad	Leopard frog
Mass-SVL	0.864	0.882	0.911
Mass-BC	0.988	0.980	0.984
SVL-BC	0.793	0.788	0.844
Mass-Time to Metamorphosis	-0.467	-0.389	-0.347
SVL-Time to Metamorphosis	-0.408	-0.497	-0.345
BC-Time to Metamorphosis	-0.476	-0.349	-0.363

Table 4. Number of individuals placed in terrestrial mesocosms from each aquatic treatment. Total number for each species is in parentheses. Target number per treatment was 36 for wood frogs and 48 for toads and leopard frogs. Some treatments exceed that to reach target densities, while other are lower due to low survival in the aquatic stage.

Treatments	Forest Center	Forest Edge	ESF Edge	ESF Center
Wood Frogs: (334)				
Leaf Litter	17	22	48	47
Grass Litter	36	50	36	22
No Litter	21	23	12	0
American Toads: (300)				
Leaf Litter	5	0	50	30
Grass Litter	58	50	0	13
No Litter	23	48	11	12
Leopard Frogs: (437)				
Leaf Litter	45	41	21	43
Grass Litter	44	45	46	24
No Litter	23	47	10	48

Table 5. Best models ($\Delta AIC < 2$) predicting juvenile survival as indicated by model ranking using an information theoretic approach. Complete AIC tables are in tables 9-11.

	N	Best Model	ω_i
Wood Frog:			
September 2008	334	Terrestrial: Main Effects	0.98
May 2009	334	Null Model	0.33
		Aquatic: Main Effects	0.22
September to May	34	Terrestrial: Main Effects	0.39
		Aquatic/Terrestrial: Main Effects	0.31
American Toad:			
September 2009	300	Aquatic: Main Effects	0.32
		Aquatic/Terrestrial: Main Effects	0.27
		Metamorphosis	0.20
May 2010	300	Aquatic/Terrestrial: Interactions	0.30
		Aquatic: Interaction	0.29
		Terrestrial: Interaction	0.18
		Null Model	0.15
September to May	51	Null Model	0.56
Leopard Frog:			

September 2010	437	Metamorphosis	0.95
May 2011 ^a	417	Terrestrial: Main Effects	0.58
		Aquatic/Terrestrial: Main Effects	0.36
September to May	137	Aquatic/Terrestrial: Main Effects	0.24
		Terrestrial: Main Effects	0.22
		Terrestrial: Interaction	0.18
		Metamorphosis	0.15
		Aquatic: Main Effects	0.11
		Null Model	0.10

^a The sample size for May for leopard frogs was lower than September due the deaths of some individuals during transport.

Table 6. Parameter estimates for parameters from wood frog models predicting juvenile survival with high support ($\Delta AIC < 3$). Model-averaging was used to determine estimates for May and September to May. ESF removed = early successional forest with downed wood removed, HD = high density. * indicates variables with confidence intervals that do not include zero.

	Environment	Variable	Estimate	SE
September 2008		Intercept*	-2.634	0.306
	Terrestrial	Control	0.190	0.404
	Terrestrial	Partial Cut*	0.732	0.3651
	Terrestrial	ESF Removed*	-1.915	0.769
	Terrestrial	HD	0.232	0.207
May 2009		Intercept	-7.655	152.0
	Aquatic	Grass	4.912	152.0
	Aquatic	Leaves	3.743	152.0
	Aquatic	Forest Edge	-0.571	0.606
	Aquatic	Forest Center	0.190	0.534
	Aquatic	ESF Edge	0.504	0.460
September to May		Intercept	0.041	54.281
	Terrestrial	Control	-1.037	45.108
	Terrestrial	Partial Cut	-3.412	45.116
	Terrestrial	ESF Removed	6.655	135.295
	Terrestrial	HD	-1.448	0.828

Aquatic	Grass	4.725	41.098
Aquatic	Leaves	2.952	41.012
Aquatic	Forest Edge	0.007	1.134
Aquatic	Forest Center	0.293	1.107
Aquatic	ESF Edge	0.382	0.881

Table 7. Parameter estimates for parameters from American toad models predicting juvenile survival with high support ($\Delta AIC < 3$). Model averaging was used for all parameter estimates. ESF removed = early successional forest with downed wood removed, HD = high density. * indicates variables with confidence intervals that do not include zero.

	Environment	Variable	Estimate	SE
September 2009		Intercept*	-2.040	0.944
	Terrestrial	Control	-0.088	0.274
	Terrestrial	Partial Cut	0.605	0.357
	Terrestrial	ESF Removed	0.060	0.265
	Terrestrial	HD	-0.015	0.161
	Aquatic	Grass	0.097	0.274
	Aquatic	Leaves	-0.484	0.376
	Aquatic	ESF Edge	-0.269	0.526
	Aquatic	ESF Center	-0.183	0.513
	Aquatic	Forest Center	0.584	0.436
	Aquatic	Body condition	-92.927	97.065
	Aquatic	SVL*	0.581	0.248
	Aquatic	Time to Metamorphosis	0.045	0.026
May 2010		Intercept*	-2.713	0.500
	Aquatic	ESF Center, Grass	-1.205	1.194

Aquatic	ESF Edge, Grass	1.972	1.213
Aquatic	Forest Center, Grass*	1.162	0.591
Aquatic	ESF Center, Leaves	0.481	0.775
Aquatic	ESF Edge, Leaves	0.187	0.808
Aquatic	Forest Center, Leaves	-1.854	1.057
Terrestrial	Control, HD	-0.194	0.367
Terrestrial	Partial Cut, HD	-0.797	0.436
Terrestrial	ESF Removed, HD*	0.798	0.367

Table 8. Parameter estimates for parameters from southern Leopard Frog models predicting juvenile survival with high support ($\Delta AIC < 3$). Model averaging was used to determine parameter estimates for May and May to September. ESF removed = early successional forest with downed wood removed, HD = high density. * indicates variables with confidence intervals that do not include zero.

	Environment	Variable	Estimate	SE
September 2010		Intercept*	-4.230	1.388
	Aquatic	Body condition	-1.343	1.479
	Aquatic	SVL*	1.864	0.645
	Aquatic	Time to metamorphosis	0.004	0.008
May 2011		Intercept	-2.801	0.255
	Terrestrial	Control	-0.383	0.394
	Terrestrial	Partial Cut*	-0.961	0.470
	Terrestrial	ESF Removed*	1.016	0.288
	Terrestrial	HD	0.220	0.207
	Aquatic	Grass	-0.449	0.311
	Aquatic	Leaves	0.398	0.258
	Aquatic	ESF Center*	0.629	0.298
	Aquatic	ESF Edge	-0.137	0.436
	Aquatic	Forest Center	-0.292	0.370
Sept. to May		Intercept	-0.479	1.684
	Terrestrial	Control	-0.488	0.431

Terrestrial	Partial Cut	-0.776	0.504
Terrestrial	ESF Removed*	0.757	0.333
Terrestrial	HD	0.195	0.236
Aquatic	Grass	-0.649	0.351
Aquatic	Leaves	0.207	0.302
Aquatic	ESF Edge	0.607	0.357
Aquatic	ESF Center	-0.069	0.492
Aquatic	Forest Center	-0.355	0.403
Terrestrial	Control, HD	0.375	0.361
Terrestrial	Partial Cut, HD	-0.773	0.420
Terrestrial	ESF Removed, HD*	0.633	0.312
Aquatic	Body condition	-3.167	3.668
Aquatic	SVL	0.2702	1.397
Aquatic	Time to metamorphosis*	-0.052	0.023

Table 9. Full AIC tables for wood frog survival.

Model	k	-2 Log Likelihood	AIC	ΔAIC	ω_i
Survival to September					
Terrestrial: Main Effects	4	200.97	210.97	0.00	0.98
Aquatic/Terrestrial: Main Effects	10	199.53	219.53	8.56	0.01
Null Model	1	219.78	221.78	10.81	0.00
Terrestrial: Interaction	4	214.91	222.91	11.94	0.00
Metamorphosis	4	215.59	223.59	12.62	0.00
Aquatic: Interaction	7	210.28	224.28	13.31	0.00
Aquatic/Terrestrial: Interactions	11	204.60	224.60	13.63	0.00
Aquatic: Main Effects	6	218.07	230.07	19.10	0.00
Aquatic x Terrestrial	19	201.60	239.60	28.63	0.00
Survival from September to May					
Terrestrial: Main Effects	4	33.41	43.41	0	0.39
Aquatic/Terrestrial: Main Effects	10	23.89	43.89	0.47	0.31
Null Model	1	44.15	46.15	2.74	0.10
Aquatic: Main Effects	6	34.44	46.44	3.03	0.09
Terrestrial: Interaction	4	38.72	46.72	3.30	0.07
Aquatic: Interaction	7	33.63	47.63	4.22	0.05
Aquatic x Terrestrial	19	12.14	48.14	4.73	0.04
Aquatic/Terrestrial: Interactions	11	28.64	48.64	5.23	0.03
Metamorphosis	4	41.27	49.27	5.86	0.02

Survival to May

Null Model	1	103.39	105.39	0	0.33
Aquatic: Main Effects	6	94.22	106.22	0.83	0.22
Terrestrial: Interaction	4	99.72	107.72	2.33	0.10
Aquatic: Interaction	7	93.83	107.83	2.44	0.10
Terrestrial: Main Effects	4	97.98	107.98	2.59	0.09
Aquatic/Terrestrial: Main Effects	10	88.04	108.04	2.65	0.09
Metamorphosis	4	102.06	110.06	4.67	0.03
Aquatic/Terrestrial: Interactions	11	90.13	110.13	4.74	0.03
Aquatic x Terrestrial	19	86.08	124.08	18.68	0.00

Table 10. Full AIC tables for American toad survival.

Model	k	-2 Log Likelihood	AIC	ΔAIC	ω_i
Survival to September					
Aquatic: Main Effects	6	279.14	291.14	0	0.32
Aquatic/Terrestrial: Main Effects	10	271.50	291.50	0.36	0.27
Metamorphosis	4	284.06	292.06	0.91	0.20
Null Model	1	291.73	293.73	2.60	0.09
Terrestrial: Main Effects	4	284.51	294.51	3.37	0.06
Aquatic: Interaction	7	282.05	296.05	4.91	0.03
Terrestrial: Interaction	4	288.32	296.32	5.19	0.02
Aquatic/Terrestrial: Interactions	11	278.50	298.50	7.36	0.01
Aquatic x Terrestrial	19	270.77	308.77	17.63	0.00
Survival from September to May					
Null Model	1	70.68	72.68	0	0.56
Terrestrial: Main Effects	4	65.13	75.13	2.45	0.16
Terrestrial: Interaction	4	68.18	76.18	3.50	0.10
Metamorphosis	4	69.22	77.22	4.54	0.06
Aquatic: Interaction	7	64.30	78.30	5.62	0.03
Aquatic: Main Effects	6	66.39	78.39	5.71	0.03
Aquatic/Terrestrial: Interactions	11	58.96	78.96	6.28	0.02
Aquatic x Terrestrial	19	43.44	79.44	6.75	0.02
Aquatic/Terrestrial: Main Effects	10	60.22	80.22	7.54	0.01

Survival to May

Aquatic/Terrestrial: Interactions	11	153.10	173.10	0	0.33
Aquatic: Interaction	7	159.65	173.65	0.56	0.25
Terrestrial: Interaction	4	166.38	174.38	1.29	0.18
Null Model	1	172.70	174.70	1.611	0.15
Aquatic: Main Effects	6	165.67	177.67	4.58	0.03
Metamorphosis	4	169.69	177.69	4.60	0.03
Terrestrial: Main Effects	4	169.20	179.20	6.10	0.02
Aquatic/Terrestrial: Main Effects	10	161.60	181.60	8.50	0.00
Aquatic x Terrestrial	19	152.51	190.51	17.42	0.00

Table 11. Full AIC tables for southern leopard frog survival.

Model	k	-2 Log Likelihood	AIC	ΔAIC	ω_i
Survival to September					
Metamorphosis	4	549.76	557.76	0	0.95
Aquatic/Terrestrial: Main Effects	10	544.75	564.75	6.99	0.03
Terrestrial: Main Effects	4	555.85	565.85	8.09	0.02
Aquatic: Main Effects	6	559.15	571.15	13.39	0.00
Null Model	1	569.55	571.55	13.79	0.00
Terrestrial: Interaction	4	566.76	574.76	17.00	0.00
Aquatic: Interaction	7	562.17	576.17	18.41	0.00
Aquatic/Terrestrial: Interactions	11	559.13	579.13	21.37	0.00
Aquatic x Terrestrial	19	550.71	588.71	30.95	0.00
Survival from September to May					
Aquatic/Terrestrial: Main Effects	10	131.491	151.49	0	0.24
Terrestrial: Main Effects	4	141.74	151.74	0.25	0.22
Terrestrial: Interaction	4	144.12	152.12	0.63	0.18
Metamorphosis	4	144.43	152.43	0.94	0.15
Aquatic: Main Effects	6	141.11	153.11	1.62	0.11
Null Model	1	151.27	153.27	1.78	0.10
Aquatic/Terrestrial: Interactions	11	143.19	163.19	11.70	0.00
Aquatic: Interaction	7	150.10	164.10	12.61	0.00

Aquatic x Terrestrial	19	131.72	169.72	18.23	0.00
Survival to May					
Terrestrial: Main Effects	4	213.88	223.88	0	0.58
Aquatic/Terrestrial: Main Effects	10	204.81	224.81	0.93	0.36
Terrestrial: Interaction	4	221.94	229.94	6.06	0.03
Metamorphosis	4	222.88	230.88	7.00	0.02
Null Model	1	230.73	232.73	8.85	0.01
Aquatic: Main Effects	6	222.03	234.03	10.15	0.00
Aquatic/Terrestrial: Interactions	11	218.04	238.04	14.16	0.00
Aquatic: Interaction	7	227.12	241.12	17.24	0.00
Aquatic x Terrestrial	19	214.73	252.73	28.85	0.00

CHAPTER 6

SUMMARY AND CONSERVATION IMPLICATIONS

This dissertation adds to the body of research showing that spatial subsidies can have large impacts on recipient systems. In Chapter 2, I found that the input of leaf litter to ponds changes basic ecosystem processes like primary productivity and community respiration. Chapters 2 and 3 highlight that the input of leaf litter profoundly changes pond community structure. This change is different than that seen in stream systems (Vannote *et al.*, 1980). In Chapter 4, I found that the input of leaves increases the reciprocal export of amphibian biomass. This indicates that ponds and forests may have a positive feedback loop of subsidy exchange. Finally, in Chapter 5, I show that subsidies can affect amphibian population dynamics through carryover effects from one life stage to the next.

The results and others indicate the importance of maintaining connections between different ecosystem types. The work in this dissertation shows that maintaining some forest cover around ponds is important for maintaining pond ecosystem function and biodiversity. For pond-breeding amphibians in particular, adequate buffers around ponds are necessary to assure population persistence, because they provide habitat for juveniles and adults (Semlitsch & Bodie, 2003). Chapter 4 suggests that buffers may also be important by providing resources in the tadpole stage.

Besides maintaining connections between ecosystems, habitat or ecosystem quality is likely also important. Because of the emphasis of my work on canopy cover, there is direct application to forestry practices. Small changes in canopy likely will not have large impacts on pond ecosystems. Several studies have indicated that partial forest cuts (sometimes referred to as uneven-aged stand management) are neutral or sometimes positive for amphibians (Chapter 5, Semlitsch *et al.*, 2009). However, larger changes in canopy, such as clearcutting (also called even-aged stand management), will have profound impacts on ponds. My research suggests that canopy removal would likely cause a shift in ecosystem function from decomposition to primary production, and subsequently cause shifts in the invertebrate and amphibian communities.

Despite all of this, habitat destruction and alteration is largely a matter of scale. When forest cutting is in small, isolated areas that conserve much of the natural habitat, the natural metapopulation dynamics will likely maintain diversity in the area by retaining refuges for species that cannot tolerate the disturbance (Adler & Neurnberger, 1994; Conradt *et al.*, 2003; Loreau, Mouquet & Holt, 2003). This type of cutting may mimic natural disturbances, such as forest gap creation through natural events (Phillips & Shure, 1990). However, forest cutting on a large scale would have much greater impacts. By restricting or eliminating the ability of organisms to disperse to new subpopulations, there would likely be population declines or extirpation of species that are forest dependent. In management, it is also important to consider what will occur after forest cutting takes place and what type of forest is being cut. If the forest grows quickly and succession is allowed to take place, many populations may be able to rebound to sustainable levels or recolonize from nearby areas (Phillips & Shure, 1990). However, if

forests grow very slowly or the habitat type is altered, those populations may be lost permanently. Thus, it is very important to consider various aspects of context when making decisions about forestry practices. Making generalizations is quite difficult, but additional research will help determine optimal management strategies.

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VITA

Julia E. Earl was born in Berkeley, CA on 19 July 1982. She spent her childhood in Charlotte, NC, where she developed a love for the natural world during trips to the Blue Ridge Mountains and the Carolina coast. Julia does not remember when she first fell in love with frogs, but she walked around her high school with a stuffed red-eyed tree frog pinned on the shoulder of her backpack and was summoned to rescue coquis out of toilets during a high school mission trip to Puerto Rico. Julia graduated from Myers Park High School in May 2000. She then attended college at Emory University, where she was active in a number of environmental and social justice organizations, including a part-time job at the on-campus recycling center. She originally intended to go into environmental policy, but discovered she could not bear to be stuck in an office. She was inspired by Dr. John Wegner, who was appointed as Campus Environmental Officer and taught her favorite class, Ecosystems of the Southeast. Julia earned her B.S. in Environmental Studies in December 2003. For the next year, she held temporary jobs. First, she worked in eastern North Carolina at Pocosin Lakes National Wildlife Refuge, where she caught tundra swans and pintail ducks with rocket nets. Then, she moved to Gainesville, FL, where she worked for the Amphibian Research and Monitoring Initiative through USGS. During this position, Julia learned to be herpetologist under the direction of Dr. Ken Dodd. In January 2005, Julia moved to Kentucky to start graduate school at Murray State University. There, she studied the effects of phosphate and nitrate pollution on frog fluctuating asymmetry under Dr. Howard H. Whiteman. She received her M.S. in Water Science in May 2007. Julia earned her Ph.D. in Biological Sciences with an

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