

**THE ECOLOGICAL ROLES OF AMPHIBIANS AND SUBSIDIES
IN PONDS**

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DEDICATION

I dedicate this dissertation to my family. My parents (Marcia Rowland, Gary Rowland, and Jennifer Clarke) and grandparents (Dick and Jean Clarke) encouraged me to follow my dreams. Most of all, my husband, Ryan Gauthier, has helped keep me afloat with his unwavering love and support.

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ABSTRACT

Freshwater ponds are often underappreciated landscape features, despite the fact they comprise a major portion of all freshwater ecosystems. Ponds support diverse food webs and species not found in other habitat types. Ponds ecosystems do not exist in isolation, and what happens in and around them influences their food webs and connections to nearby terrestrial ecosystems. Habitat alterations near ponds have strong potential to reduce animal diversity. In freshwater systems, loss of sensitive species, such as amphibians, is occurring rapidly and with large ecological consequences. Furthermore, although many amphibians are imperiled, there is a lack of data regarding their precise role in ecosystems. My research tries to fill this gap by adding crucial data to expand our knowledge of the ecological structure of pond food webs and the role of pond-breeding amphibians.

I used a combination of experiments, field observations, and statistical tools to address three main objectives: 1) what is the relative importance of top consumers vs. resources in aquatic food webs, 2) quantifying pond ecosystem response to variation in leaf inputs (i.e., such as would happen with land-use change), and 3) understanding how food webs and energy sources shift in ponds with varying leaf litter inputs. I used experimental ponds to establish a model food webs consisting of larval spotted salamanders (*Ambystoma maculatum*) and Southern leopard frog tadpoles (*Rana sphenoccephala*). Experimental ponds standardize pond features such as age, shape, and depth while retaining microbial and plankton communities similar to natural ponds. They have been used with high success in answering important basic and applied ecological questions.

Primary production can be controlled through bottom-up (e.g., resources) or top-down (e.g., predators) constraints. My experiment manipulated resources by altering light (low/high) and nutrient (low/high) availability; omnivores with the presence/absence of tadpoles; and predators with the presence/absence of spotted salamander larvae in an experiment conducted over 14 weeks. I observed that both bottom-up and top-down effects were important in predicting lower trophic level biomass. In particular, tadpoles were highly influential in the food web. We also found large temporal differences in food-web dynamics. These results underscore the need for more information into how ecosystem functioning could be altered by land use and amphibian species losses.

One central problem of amphibian recovery efforts is that we still do not really know why some ponds are better for amphibian growth and survival than others. The amount of tree cover over and around ponds can vary drastically, thus I hypothesized that these differences may impact ponds through leaf litter. I completed a second large experiment testing how ponds respond to gradients of leaf litter subsidies. I manipulated leaf litter inputs across seven levels (0.25, 0.5, 0.75, 1, 1.5, 2, 3 kg) in a replicated design using the model food web from the first experiment. I measured ecosystem responses such as dissolved oxygen, nutrients, algae, and amphibian responses. Data from this experiment indicate that nutrient concentrations increase with increasing leaf litter, but that frogs and salamanders respond differently to leaf litter than field observations would predict. Additionally, I observed a large proportion of the ecosystem responses were non-linear with increasing leaf litter. These data will be useful in developing models to inform the recovery of threatened amphibian species.

To round out my exploration of pond food webs, I conducted an experiment to understand shifts in pond food web structure as a function of leaf litter input (0.5, 1, 1.5, 2.5, 4 kg) the relative strength of brown (detrital) vs. green (algal) energy pathways in ponds. I used the same experimental pond set-up as in previous years, but also included a top predator in the form of larval dragonflies. This experiment took advantage of the relatively simple food webs found in ponds to better understand food-web dynamics. I used two stable isotopes to trace algal and leaf litter through the food web, contributing greatly to understanding of energy pathways in ponds. I found evidence that consumers shift their energy source and trophic level with increasing leaf litter. This research will identify how leaf litter affects pond food webs and their sources of energy.

All together, my research takes a novel approach integrating common scientific tools to bring together theory and conservation. The most important findings of my dissertation include: 1) amphibians can have strong and lasting effects on pond ecosystems, 2) leaf litter switches ponds from green to brown energy, and 3) consumer trophic position and energy sources can shift as a function of leaf litter, often in non-intuitive ways.

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INTRODUCTION

This dissertation was motivated by a simple question: what influences trophic interactions and food webs of small aquatic communities? Most of the previous work on aquatic food webs focuses on lakes, but ponds have several features that make them worthy of study. Freshwater ponds are common landscape features. In some regions like the glaciated northern U.S. and Canada they comprise a major portion of all freshwater ecosystems (e.g. vernal pools; Colburn 2004, Calhoun and DeMaynadier 2007). These ecosystems have a high degree of contact between benthic and pelagic food chains and support diverse food webs consisting of phytoplankton, periphyton, microbes, macrophytes, macroinvertebrates, and vertebrates. In fact, the cyclical nature of freshwater ponds creates habitat that supports species either not found in other habitat types or that attain their greatest population sizes in ponds (Williams 1997). Ponds can function as an abundant source of energy to terrestrial environments (Gibbons et al. 2006) or receive energy from terrestrial sources (Reger et al. 2006) thereby linking aquatic and terrestrial ecosystems. Habitat modifications near ponds (e.g. canopy loss) have strong potential to reduce vertebrate and invertebrate diversity (Findlay and Houlihan 1997, Colburn et al. 2008). In freshwater systems, loss of consumer diversity (especially sensitive species, such as dragonflies and amphibians) is occurring rapidly and with large ecological consequences (Calhoun and DeMaynadier 2007). Although ponds are common, many basic questions about pond ecosystem function remain unanswered.

Amphibian larvae are dominant consumers in freshwater ponds (Wilbur 1980), reach high densities (Gibbons et al. 2006), strongly influence ecosystem properties (Seale 1980, Iwai et al. 2012), serve as important prey for other species (Wells 2010), affect

species interactions and community structure (Morin 1983, Wilbur 1997), and can represent a substantial export of biomass to terrestrial systems (Gibbons et al. 2006). Yet despite these important ecosystem roles, we still have large gap in knowledge about the role of amphibians in ponds. Figuring this out may be particularly important because amphibians are declining more rapidly than other vertebrates, and many species are threatened with extinction (Stuart et al. 2004).

Canopy cover plays an important role in pond ecosystem functioning, affecting both light allowed in to the pond (that could support primary production) and inputs of leaf litter as a source of energy and nutrients. Alterations in forests affects canopy cover around and over ponds, and therefore inputs of light and leaf litter, which impact the base energy sources for pond food webs (Colburn et al. 2008). This has the strong potential to change trophic interactions and food web structure (Wilbur 1997, Schindler et al. 2001). Few previous studies separated canopy cover into its two main effects: light and nutrients (such as would be brought in via senesced leaf litter). In Chapter 1, I present the results from experiment testing whether light, nutrient availability, tadpole presence, or salamander presence were most important in shaping pond food webs.

A key step to understanding the functional relationship among community members is defining their trophic position and how energy sources affect the abundance and diversity of species (reviewed in Post 2002). Interest in energy pathways of food webs arose from the discovery that detritus supports larger predator biomass, diversity of species, and longer food chains than would be supported by autotrophs alone (Hairston and Hairston 1993). In fact, many fundamental questions in ecology including structure of food webs, length of food chains and trophic cascades are interpreted differently when

detritus is considered a central component of the ecosystem (Moore et al. 2004). Here we define detrital pathways (hereafter brown) as consisting of non-living organic matter including plant tissue, carrion, dead microbes, faeces, and secreted and excreted products from organisms (Moore et al. 2004) and photosynthetic pathways (hereafter green) as including plants, algae, and cyanobacteria. The strength of brown connections varies among ecosystems. Some—such as small, forested streams (Wallace et al. 1997)—are based entirely on detrital support, while in other systems such as some lakes, energy flow from detritus is relatively small (Cole et al. 2000). Despite the variation, brown support of food webs is common (Moore and Hunt 1998). In Chapter 2, I used experimental ponds to understand how small aquatic ecosystems responded to leaf litter subsidy size in terms of algal biomass, nutrient availability, and biomass export of amphibians.

There is still a general lack of consensus on how canopy cover affects carnivorous caudates (salamanders) compared to anurans (frogs). If anurans develop better in open canopy ponds because of increased algal energy, in theory, predators should be even more constrained by primary consumer energy because it has to travel through primary consumers before reaching them (Lindeman 1942). This supports the idea that salamanders benefit more from brown rather than green pathways in ponds. Still missing is a clear mechanistic understanding of how canopy cover influences food webs and the energy pathways of consumers.

A major limitation of previous work has been the assumption of simple trophic pathways. The trophic level concept is not able to capture species interactions and trophic omnivory (Vander Zanden and Rasmussen 1999). Stable isotope ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) are a powerful technique that can provide a continuous measure of

trophic position that integrates the assimilation of energy through all the different trophic pathways leading to an organism (Peterson and Fry 1987). Researchers have only a very vague idea of what amphibians actually eat (Altig et al. 2007), but it is becoming ever more apparent that anurans are not simply primary consumers (Schiesari et al. 2009, Whiles et al. 2010). In Chapter 3, I use stable isotopes to explore how leaf litter subsidy size can shift trophic positions and energy source in pond food webs.

Finally, I discuss what implications my dissertation might have for management in the conclusions. Amphibians are the most threatened vertebrate taxon on the planet (Stuart et al. 2004). Translating basic research into useful information for managers is key for management of amphibian populations in Missouri and elsewhere.

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CHAPTER 1: Joint effects of resources and amphibians on pond ecosystems

Abstract

Primary production can be controlled through bottom-up (e.g., resources) or top-down (e.g., predators) constraints. Two key bottom-up resources in small aquatic systems are light and nutrients, and forest canopy cover heavily influences these factors, whereas amphibian and invertebrate colonizers exert top-down pressure as grazers and predators. We designed our experiment to specifically manipulate two different top-down and bottom-up factors. We manipulated resources by altering light (low/high) and nutrient (low/high) availability; omnivores with the presence/absence of southern leopard frog tadpoles (*Lithobates sphenoccephalus*); and predators with the presence/absence of spotted salamander larvae (*Ambystoma maculatum*) in a full-factorial experiment conducted over 14 weeks. We observed that both bottom-up and top-down effects were important in predicting lower trophic level biomass. We found a significant top-down effect of salamanders on *Daphnia*, but tadpoles had the strongest overall effect on the food web, influencing phytoplankton (+), periphyton (-), *Daphnia* (-) and chironomids (-). None of our models were good predictors of phytoplankton biomass, but both shading and nutrient availability relatively equally boosted periphyton biomass. We also found large temporal differences in food-web dynamics. Our results underscore the need for more information into how ecosystem functioning could be altered by land use, amphibian

extirpation and climate change.

Keywords: food webs, salamanders, anurans, bottom-up, top-down

Introduction

Understanding how abiotic and biotic factors affect food webs is a major focus of ecology. Global changes such as increased nitrogen deposition (Elser et al. 2009), changes in forest cover (Hansen et al. 2013), and shifting leaf litter composition (Stoler and Relyea 2011) have altered many conditions in habitats. These interact with biotic effects, such as predation, that can change prey population dynamics through consumption (Werner and Peacor 2003) or through non-consumptive predator effects whereby a prey adopts costly defenses (Preisser et al. 2005). The magnitude of top-down predator effects, however, can also change with availability of resources (Preisser et al. 2009). Thus, disentangling top-down and bottom-up effects in food webs is important to understanding how ecosystems may respond to changes.

Pond food webs are constrained by dissolved nutrients, light and detritus at the base; and amphibians and other predators at the top. Alterations in forest canopy cover around and over ponds affects inputs of light and leaf litter, which form the base energy sources for pond food webs (Colburn et al. 2008). Changes in canopy cover, therefore, have the potential to shift trophic interactions and food web structure (Schiesari 2006; Schindler et al. 2001; Werner and Glennemeier 1999; Wilbur 1997). These effects are particularly striking in ponds (as opposed to lakes), because ponds have high edge-to-area ratios. Some of the most dominant consumers in ponds are amphibian larvae (Wilbur 1980). Amphibian larvae reach high densities (Gibbons et al. 2006), strongly influence ecosystem properties (Iwai et al. 2012; Seale 1980), serve as important prey for other species (Wells 2010), affect species interactions and community structure (Morin 1983;

Wilbur 1997), and can represent a substantial export of biomass to terrestrial systems (Gibbons et al. 2006).

Previous research has demonstrated that forest canopy is important in determining species distributions, community structure and ecosystem processes in aquatic habitats (Batzer et al. 2004). Canopy cover contributes to two important resources in pond ecosystems: negatively affecting light and positively affecting nutrient availability (via leaf litter). Both of these resources can be limiting for primary productivity in pond ecosystems (Mokany et al. 2008), and therefore impose bottom-up constraints on the food web. However, the effect of ‘canopy cover’ is rarely separated out into light and nutrients, and by testing them separately we can better understand pond dynamics.

From the top-down perspective, amphibians can exert strong forces on pond communities. Pond-breeding amphibians often form complex communities with high degrees of predation and competition (Morin 1983, Davenport and Chalcraft 2012). Salamanders can exert significant predatory control on herbivores, and thereby promote algal growth via trophic cascades (Morin 1995, Blaustein et al. 1996). Omnivorous anuran tadpoles can also be drivers of primary production and nutrient flux (Seale 1980). Most studies of top-down control with amphibians use larval salamanders as predators of tadpoles (Morin 1983), but comparing tadpole grazing and salamander predator effects on pond communities is rarely done within the same study.

Previous work comparing top-down and bottom-up effects in aquatic systems has found that predators indirectly boost primary production by consumptive effects on grazers (Rohr and Crumrine 2005; Wojdak 2005). Bottom-up and top-down effects can also interact. For example, the magnitude of snail growth reduction in the presence of

predatory crayfish depended on snail density and nutrient supply (Turner 2004). In another study, the positive effect of nutrient addition allowed snails to overcome negative non-consumptive effects of a predatory crayfish, even with the cost of added shell defense (Ruehl and Trexler 2013). Furthermore, a meta-analysis showed dynamic resource availability increased the strength of non-consumptive top-down effects (Preisser et al. 2009), and warming experimentally increased the strength of top-down effects of fish, even though nutrient addition boosted biomass of all trophic levels (Shurin et al. 2012).

Given that food-web structure can significantly alter how organisms respond to abiotic manipulations (Leibold and Wilbur 1992) and pesticides (Rohr and Crumrine 2005), it is important to tease apart the bottom-up and top-down effects on communities in small aquatic ecosystems. We sought to compare two different top-down and two bottom-up factors that affect food-web dynamics in ponds, using repeated samples and multi-trophic level approach. We hypothesized that the top-down effects of salamanders and tadpoles would have the strongest effect on ecosystems (Fig. 1a). We predicted salamanders would be important because of their role as predators of *Daphnia*, which could indirectly affect phytoplankton biomass as seen in previous work (Blaustein et al. 1996; Morin 1995). We predicted that tadpoles would have a strong effect on primary producers (Fig. 1a), directly through grazing on periphyton but also indirectly by boosting phytoplankton through nutrient flux (Seale 1980). Finally, we predicted that after salamander and tadpole presence, nutrients would be important as a stimulator of primary production (Fig. 1b), but light would be less important based on previous work in

a similar system (Rowland et al. 2016).

Materials and methods

Experimental design

To quantify top-down and bottom-up effects on pond communities we employed a 2 x 2 x 2 x 2 completely randomized factorial mesocosm experiment with two levels of light (low/high), two levels of nutrients (low/high), southern leopard frog tadpoles (*Lithobates sphenoccephalus*; presence/absence) and spotted salamander larvae (*Ambystoma maculatum*; presence/absence). We chose these two amphibian species, because they typically co-exist in ponds in the United States and they represent different feeding niches. Southern leopard frog tadpoles are mostly primary consumers in ponds (but see Altig et al. 2007; Schiesari et al. 2009), whereas spotted salamanders are gape-limited predators of zooplankton, macroinvertebrates, and any other prey small enough to consume. These niche differences allowed us to assess how habitat conditions and community complexity affect the same pond system. We conducted the experiment at a fenced outdoor research facility in Columbia, MO, U.S.A during the spring and summer of 2014 for *c.* 14 weeks. We included control treatments (without vertebrates) as a baseline to better assess how amphibian presence affected lower trophic levels. Control treatments were replicated three times and those with vertebrates four times, for a total of 60 experimental units. We randomly assigned treatments within two spatial blocks of thirty ponds each.

We created experimental ponds with polyethylene cattle watering tanks (1.52 m diameter, 50 cm water depth; hereafter ponds) that allowed us to control for pond features such as age, shape, substrate, photoperiod, temperature, hydroperiod and depth, while

retaining microbial, algal, and zooplankton communities similar to natural ponds (Semlitsch and Boone 2009). We filled the ponds with *c.* 1000 L of tap water each, and allowed the water to dechlorinate for 14 days. We collected litter from a deciduous forest dominated by oak (*Quercus* spp.) and maple (*Acer* spp.) in the Thomas S. Baskett Wildlife Research and Education Area (Ashland, MO, U.S.A.) in early April 2014 and added 0.5 kg to each pond on 09-Apr-2014 as a base nutrient load. This level of leaf litter is half of what is used many other studies (e.g., Davenport and Chalcraft 2012), and this functioned to limit nutrients in the low nutrient treatments while still providing habitat structure for hiding and foraging. We subsequently inoculated the ponds with phytoplankton, periphyton, and zooplankton from several natural ponds in the Baskett Research Area on 09-, 10-, 18-, and 23-Apr-2014. We inoculated with organisms from an experimental pond on 12-, 16-, and 26-Apr-2014 to ensure organisms adapted to experimental enclosures were included in the assemblage. On 27-Apr-2014, we suspended ceramic tiles (22.1 cm²) midway down the northeast side of each pond to monitor periphyton biomass. To assess temperature, we added iButtons to the bottom of seven low and seven high light ponds within each block on 15-May-2014. We programmed the iButtons to log temperature every hour for the duration of the experiment.

We manipulated light by covering ponds with high-density polyethylene PAK knit shade cloth (Hummert International, St. Louis, MO, U.S.A.) to achieve a 30% (low shade) and 70% (high shade) reduction in ambient light levels, starting on 11-Apr-2014. These covers also served as a way to prevent the colonization and oviposition of other anurans and insect predators. We allowed the pond communities to establish before

initiating high nutrient conditions. Beginning on 8-May-2014, high nutrient ponds received $5 \mu\text{g L}^{-1}$ of P as KH_2PO_4 and $50 \mu\text{g L}^{-1}$ of N as NH_4NO_3 three times a week, consistent with the concentrations used in previous aquatic studies (Dickman et al. 2008; Rowland et al. 2015). Nutrient addition continued for the duration of the experiment, and represented a total delivery of approximately 2 g N and 0.2 g P. Low nutrient treatments received no additional nutrient subsidies beyond the nutrients associated with the initial leaf litter addition. Water depth in the ponds was maintained at *c.* 50 cm through rain water or water amendments during dry periods.

In early April, we collected egg masses of both amphibian species from several ponds at Fort Leonard Wood, MO, U.S.A. We collected at least three egg masses from each species to add genetic diversity. Eggs were stored at 15°C until the ponds had developed well-established planktonic and microbial communities. On 01-May-2014, we warmed the southern leopard frog egg masses to room temperature and then allowed the hatchlings to develop to the free-swimming stage (Gosner 24-25; Gosner 1960). On 04-May-2014, we added 20 tadpoles to each frog treatment ($n = 32$ ponds) randomly in groups of 10, and then added four more to each pond on 05-May-2014 to achieve a density of 24 tadpoles pond^{-1} , or approximately 13 tadpoles m^{-2} , which is well within the natural range of densities for southern leopard frogs (F. Rowland, *personal observation*). Salamanders were brought to room temperature on 08-May-2014. We allowed hatchlings to develop until they had re-absorbed their egg yolks; then randomly added groups of six to salamander treatments ($n = 32$ ponds) on 19-May-2014 to achieve a density of 12 salamander hatchlings pond^{-1} , or approximately seven salamanders m^{-2} . This is within the range of natural densities for spotted salamanders (0.2–80.0 larvae m^{-2} , Figiel Jr and

Semlitsch 1990), and a moderate density for competition (Ousterhout and Semlitsch 2015). As we did not add invertebrate predators to our experiment, we expected high survival of amphibians. Thus, we added larvae at moderate but realistic densities to better reflect the population sizes found in ponds near the end of amphibian larval periods. We also reasoned that intense competition could restrict our ability to detect lower trophic level changes (e.g., if tadpole density was too high, periphyton biomass might be reduced below our detection limit). We delayed salamander development until the tadpoles were large enough to escape predation by salamanders, so any effect of salamanders on tadpoles was non-consumptive.

Sampling and analytical methods

We sampled all ponds at the beginning, middle, and near the end of the experiment to assess phytoplankton and periphyton biomass (29-May-2014, 25-Jun-2014, and 15-Jul-2014), *Daphnia* biomass (30-May-2014, 26-Jun-2014, and 16-Jul-2014), and chironomid density (02-Jun-2014, 26-Jun-2014, and 16-Jul-2014). To determine phytoplankton biomass, we pooled one 200-mL subsample of subsurface water from each cardinal direction of a pond, then filtered a known volume through a Pall A/E glass fiber filter (Pall A/E, 1 μm nominal pore size), and stored the filter at -20 °C until analyzed for chlorophyll *a* (chl *a*). To determine periphyton biomass, we collected one tile from the mesh and carefully scrubbed the entire area or a subsample of known area onto a Pall A/E glass fiber filter (Pall A/E, 1 μm nominal pore size), and stored filters at -20 °C until analyzed for chl *a*. Chl *a* filters samples were extracted for 20 minutes in a hot (70-75° C) 95% ethanol and assayed for phaeophyton-corrected chl *a* on a Turner TD-700 fluorometer (Turner Designs, Sunnydale, California USA) calibrated with commercial

standards dissolved in ethanol as in Jones and Bachmann (1974). All of the filters were unintentionally dried at 60°C for 24 h before freezing. To determine the effect of this drying on chl *a* measurement, we collected an additional round of phytoplankton and periphyton samples on 29-Jul-2014 that were then frozen, and compared them to 30 phytoplankton and 19 periphyton duplicates that were dried before freezing. We regressed the dry and frozen chl *a* measurements and found a highly significant, positive correlation: $\log(\text{frozen chl } a) = -1.04 + 1.08 * \log(\text{dry chl } a)$ ($n = 49$, $R^2 = 0.81$, $p < 0.001$). We then used this regression to estimate chl *a* concentrations from previously dried samples.

Zooplankton were collected using a PVC pipe (internal diameter: 6.1 cm) fitted with a plunger, and four integrated (i.e., surface to bottom) subsamples were pooled from each pond. We sieved one 1-L subsample from each cardinal direction through an 80 μm Wisconsin net, and then anesthetized the zooplankton using Alka-Seltzer before preserving them in 50% ethanol. The first 20 *Daphnia* encountered were measured using an ocular micrometer on a dissecting microscope. We then estimated *Daphnia* biomass following the same protocol as Gonzalez et al. (2010). We estimated chironomid density by counting the number of chironomid cases present within a 3.5 cm^2 area of one side and one bottom location in each pond. In rare instances (<3% of all observations for first two sampling dates, and <10% of all observations on last sampling date), periphyton growth was too thick to see the side or bottom of the pond. These observations were eliminated from analyses.

We ended the experiment on 11-Aug-2014, once at least three individuals from each pond had metamorphosed. When we drained the ponds, we thoroughly searched the

leaf litter for remaining individuals that did not yet make it to metamorphosis. These animals represented approximately 1.7% (± 1.3 SE) and 20.1% (± 3.0) of surviving frogs and salamanders, respectively.

Statistical analyses

We performed all statistical analyses in R. We analyzed temperature data from the iButtons using the ‘lme4’ package (Bates et al. 2014). To test how the light manipulations may have affected temperature, we fit a mixed model predicting log-transformed average daily temperature based on light, date and the interaction of light x date with pond as a random effect. We then used the ‘Anova’ function in the ‘car’ package (Fox and Weisberg 2011) to conduct Type II Wald χ^2 tests. An analysis of data from the iButtons found average daily temperature was strongly affected by light ($\chi^2 = 16.05$, $p < 0.001$), date ($\chi^2 = 12105.0$, $p < 0.001$), and a light x date interaction ($\chi^2 = 1258.0$, $p < 0.001$), suggesting that the cooling effect of low light compared to high light treatments was strongest early in the season and decreased as the summer progressed. Unfortunately, we did not have temperature data from every pond to include in our models, so we collapsed temperature and light into a common shading effect.

For variables measured at multiple time points—temperature, phytoplankton, periphyton, *Daphnia* biomass, and chironomids—we fit repeated-measures mixed models on log- (for algae) and log(x+1)-transformed data (for *Daphnia* and chironomids) using the ‘lmer’ function in the ‘lme4’ package (Bates et al. 2014). We first fit a full model with the main and all interactive effects of shading (low/high), nutrients (low/high), tadpoles (presence/absence), and salamanders (presence/absence). We also included block and time as main effects, and time within a pond as a random effect. We then used

the ‘Anova’ function in the ‘car’ package (Fox and Weisberg 2011) to identify significant interaction effects using Type II Wald χ^2 tests. To reduce the type I error rate associated with multiple comparisons, we used ‘p.adjust’ to yield Bonferroni-corrected p-values. Any interaction that was significant at $\alpha \leq 0.10$ was included in the reduced model. Reduced models included all main effects, time, and the random effect of time within a mesocosm, but block and interactions were only included if found to be marginally significant in the full model. We did this to avoid overfitting due to the complexity of the study and relatively low replication.

To estimate the relative strength of bottom-up and top-down effects as mediated through direct and indirect links in the food web, we utilized structural equation modeling (SEM) in the ‘lavaan’ package in R (Rosseel 2012). The goal of our causal networks was to assess the relative strength of top-down versus bottom-up control of primary producer biomass, *Daphnia* biomass and chironomid density. We used SEM to explore the relative importance of shade (coded as low shade = 0 and high shade = 1), nutrients (low = 0 and high = 1), tadpoles (presence/absence), and salamander larvae (presence/absence) in predicting phytoplankton, periphyton and *Daphnia* biomass, as well as chironomid density. All endogenous (response) variables were $\log(x+1)$ -transformed. We modeled our causal networks based on *a priori* hypothesized strength of interaction. For example, *Daphnia* are unlikely to be limited by phytoplankton in our system, but could exert strong control on phytoplankton biomass. Conversely, chironomids are unlikely to control periphyton biomass, but could reach higher densities with high periphyton biomass (bottom-up) or could be reduced through predation by tadpoles and salamanders (top-down; Fig. 1). We fit the same model to all three time periods and used a likelihood-ratio

chi-square statistic to test whether the covariance matrix generated by the model differed significantly from the data. A $p > 0.05$ would indicate that the observed and predicted models were not significantly different, and that the fit for our model was adequate (Shipley 2000). We also assessed model fit using the Comparative Fit Index (CFI), which is relatively insensitive to sample sizes (Fan et al. 1999).

Results

Algal and invertebrate responses

Phytoplankton biomass (as chl *a*) was positively influenced by tadpole presence, but biomass decreased over time (Table 1, Fig. 2a-c). Periphyton biomass (as chl *a*), was significantly and positively affected by shade and nutrients, but negatively affected by tadpole presence (Table 1, Fig. 2d-f).

Daphnia biomass ($\mu\text{g dry weight L}^{-1}$) was strongly affected by a shade x salamander interaction (Table 1, Fig. 2g-i). Salamander presence decreased *Daphnia* biomass, but in the absence of salamanders, high shade mesocosms had higher *Daphnia* biomass than low shade (Table 1, Fig. 2g-i). Chironomid density (individuals cm^{-2}) was negatively affected by tadpoles (Table 1, Fig. 2j-l), and chironomid density decreased over time (Fig. 2k-l). Chironomids were also significantly affected by block (Table 1).

Early food web model

Early in the experiment (late May/early June), the food web was affected by a mix of bottom-up and top-down effects (model $\chi^2 = 6.34$, CFI = 0.96, d.f. = 6, $n = 56$, $P = 0.109$; Fig. 3a; Online Resource Table A1). None of the variables were good predictors of phytoplankton biomass (Fig. 3a; Online Resource Table A1), but periphyton biomass was positively affected by shading ($P < 0.001$) and nutrients ($P < 0.001$), and negatively

influenced phytoplankton biomass ($P < 0.001$) and tadpole presence ($P < 0.001$). Chironomid density was directly, negatively affected by tadpole presence ($P < 0.001$), but also negatively affected by periphyton biomass ($P = 0.004$) and indirectly by all of the factors that affected periphyton growth including tadpole grazing, phytoplankton biomass, shading and nutrients (Online Resource Table A1). Neither salamander presence nor *Daphnia* biomass were significant predictors of chironomid density (Fig. 3a; Online Resource Table A1). Salamanders had a significant positive effect on *Daphnia* biomass ($P = 0.031$), but the positive effect of shading was 2x stronger ($P < 0.001$; Fig. 3a; Online Resource Table A1). Nutrients did not significantly predict *Daphnia* biomass, but tadpole presence had a marginally negative effect ($P = 0.058$; Online Resource Table A1).

Mid-experiment food web model

By the middle of the experiment (late June), the SEM ($\chi^2 = 10.18$, CFI = 0.97, d.f. = 6, $n = 59$, $P = 0.117$) suggested top-down effects may be more important than bottom-up (Fig. 3b, Table A2). Phytoplankton biomass was positively influenced by shading ($P = 0.039$), but tadpoles had a negative effect 2x stronger than the boost from shading ($P < 0.001$, Fig. 3b; Online Resource Table A2). Periphyton biomass was equally positively influenced by shading ($P = 0.007$) and nutrients ($P = 0.006$), but the negative effect of tadpoles on periphyton was twice as strong ($P < 0.001$; Fig. 3b, Online Resource Table A2). Phytoplankton biomass had a marginally significant ($P = 0.062$) negative effect on periphyton. Chironomid density was negatively influenced by *Daphnia* biomass ($P = 0.042$) and tadpole presence ($P < 0.001$). *Daphnia* biomass was strongly negatively influenced by salamander presence ($P < 0.001$), and positively affected by shading ($P < 0.001$). Neither tadpole presence nor nutrients were significant predictors of *Daphnia*

biomass.

Late experiment food web model

Near the end of the experiment (mid-July), most of the tadpoles and salamanders had metamorphosed. The SEM reflected this and had the lowest predictive power for all endogenous variables. Many of the previously strong top-down connections disappeared (model $\chi^2 = 4.78$, CFI = 1.00, d.f. = 6, $n = 55$, $P = 0.572$; Fig. 3c, Online Resource Table A3). None of the treatments significantly affected phytoplankton biomass (Fig. 3c).

Interestingly, although many of the tadpoles had already metamorphosed and left the system, they still had a strong negative effect on periphyton biomass ($P < 0.001$), roughly 1.5x stronger than the positive effect of nutrient addition ($P = 0.011$; Fig. 3c).

Chironomid density was positively affected by periphyton biomass ($P = 0.033$), but not by tadpoles or *Daphnia*. Salamanders were a strong negative predictor of *Daphnia* biomass ($P = 0.001$), roughly equivalent to the positive effects of shading ($P = 0.009$) and nutrients ($P = 0.001$) on *Daphnia*.

Discussion

We found that pond food webs were influenced by a complex mix of top-down (predator and consumer) and bottom-up (shading and nutrients) interactions. Our hypothesis that amphibians would have the strongest effects on ecosystems was partially supported.

Tadpoles were consistently strong regulators of periphyton, and sometimes chironomids, *Daphnia*, and phytoplankton. Salamanders, however, appeared to play a relatively minor role in the ecosystem. Salamander larvae significantly affected *Daphnia* biomass throughout the experiment, but we did not detect a trophic cascade resulting in increased phytoplankton biomass. In fact, none of our bottom-up or top-down manipulations had

very strong predictive power for phytoplankton biomass. Contrary to our prediction, shading tended to be more important than nutrients in predicting primary producer biomass. Shading significantly boosted *Daphnia* biomass during all sampling periods. Below we expand on the top-down and bottom-up effects we found, the temporal trends in ponds, and discuss the implications of our findings for our understanding of the effects of shading, nutrients, and consumers on food webs.

Top-down effects

The presence of tadpoles and larval salamanders exerted strong top-down effects on the food web throughout the experiment. Tadpoles had the biggest effect on the food web, causing a decrease in periphyton biomass, chironomid density, and *Daphnia* biomass, and an increase in phytoplankton biomass. Our results suggest that tadpoles either competed for periphyton with chironomids or consumed them directly, which would support recent work that tadpoles are omnivorous (Altig et al. 2007; Schiesari et al. 2009; Whiles et al. 2010). Our measurement of periphyton was restricted to biomass, but previous research suggests that tadpoles can change not only periphyton standing stock, but also algal species composition and inorganic sediment accumulation (Connelly et al. 2008). Furthermore, tadpoles can have a strong influence on nutrient flux and phytoplankton dynamics in systems (Seale 1980). Thus, the negative effect of tadpoles on *Daphnia* could be explained by shifts in phytoplankton species composition to unpalatable species. Alternatively, tadpole consumption of periphyton could have destroyed refuges where *Daphnia* hide from predators, or tadpoles could have consumed zooplankton directly (Schiesari et al. 2009). Further, tadpoles have been shown to positively influence detritivores through nutrient regeneration (Iwai and Kagaya 2007). Our results join a

growing body of literature showing that tadpoles alter many ecosystem properties, but more work is needed to elucidate their role in ecosystems.

We predicted that salamanders would have a strong top-down effect on our ponds. Previous research suggests that small larval salamanders rely mainly on crustacean zooplankton, but switch to consuming chironomids as they grow (Taylor et al. 1988). Although salamanders were a significant predictor of *Daphnia* biomass at all sampling points, we never detected any negative effect of salamanders on chironomid density. It may be that using chironomid casing density instead of actual biomass limited our ability to detect changes in chironomid populations. We also did not see a trophic cascade as expected based on previous work (Morin 1995); *Daphnia* biomass was not a significant predictor of phytoplankton biomass at any time point. *Daphnia* biomass was highest in the beginning of the experiment, and quickly declined in treatments with amphibians. Even though *Daphnia* populations were robust in all ponds before vertebrate additions, salamanders quickly reduced *Daphnia* populations and shifted zooplankton community composition (F. Rowland, *unpublished data*), and ponds without tadpoles were dominated by blooms of filamentous periphyton. Some research suggests that *Daphnia* can graze successfully on periphyton (Siehoff et al. 2009), and this may explain the negative effect of *Daphnia* on chironomids we saw in the middle of the experiment through competition.

Bottom-up effects

Both shading and nutrients were significant predictors of phytoplankton, periphyton, and *Daphnia* biomass, but in different ways. Phytoplankton was only significantly predicted by shading mid-experiment, but nutrients never significantly boosted biomass.

Periphyton biomass was predicted by shading and nutrients, and during the first sampling period, phytoplankton negatively affected periphyton biomass. This negative effect of phytoplankton on periphyton is likely due to a shading effect, as has been seen in eutrophication of lakes (Vadeboncoeur et al. 2003).

Shade manipulations increased periphyton biomass, which may be an artifact of using chl *a* as our metric. Under low light conditions, algae will typically develop more light harvesting machinery and therefore have higher chl *a* concentrations (Geider 1987). However, chl *a* and particulate organic carbon in phytoplankton and periphyton were significantly correlated under even larger light variations than those in this study (F. Rowland, *unpublished data*). Instead, we suggest that shading, nutrients and grazers likely induced algal community shifts. Previous work has found complex interactions between light, nutrients, and fish on phytoplankton community composition (Mette et al. 2011). Liess et al. (2009) altered light, nutrients, and snail grazing pressure and found increased periphytic diatom species richness was related to increased chl *a* and nutrient addition, as well as complex interactions between grazers, nutrients, and light. Light, in particular, seems to have a strong effect on algal community composition. More work is needed to improve our understanding of the mechanisms by which light, nutrients, tadpole grazers, and predators mediate algal composition.

We detected a positive influence of shading on *Daphnia* throughout the experiment, similar to what has been found in previous studies (Earl and Semlitsch 2013). This can likely be explained by algal food quality. Changes in light and nutrient supply altering the nutritional content of algae have been well-documented, especially with regards to carbon:nutrient stoichiometry (Sterner and Elser 2002). High shade and

high nutrient supply tends to result in algae with low carbon:nutrient ratios, and increased *Daphnia* growth on this high quality food (Hessen et al. 2002). Algal compositional and nutritional quality may be more important than predation and other grazers in determining *Daphnia* biomass, and this deserves further research. Moreover, *Daphnia* abundance can have other community effects such as reducing tadpole movement (Rohr and Crumrine 2005), highlighting the complexity of ecosystem responses to manipulations.

Unfortunately, we were unable to separate the effects of light and temperature. We found a significant difference in temperature between low and high light treatments, especially early in the season. Different trophic levels vary in their sensitivity to temperature changes. Small increases in water temperature can shift aquatic food webs toward greater heterotroph relative to autotroph biomass (O'Connor et al. 2009), increase top-down control of pond food webs by fish (Shurin et al. 2012), and increase ecosystem respiration (Yvon-Durocher et al. 2010). Future work should attempt to separate the effects of light and temperature to understand how shading over aquatic ecosystems will interact with predicted global temperature increases (IPCC 2014) to influence ecosystem properties.

Temporal trends and legacy effects

We observed high temporal variation in food web dynamics. For example, early in the experiment, periphyton biomass was relatively equally affected by treatment shading, nutrients, phytoplankton shading and tadpoles. By the middle, tadpole grazing was twice as strong of a predictor of periphyton biomass as either shading or nutrients. At the last sampling date, periphyton was only significantly predicted by tadpoles and nutrients, and

the effect of tadpoles was about 1.5x stronger. Initial *Daphnia* biomass was most strongly positively influenced by shading, and less so by salamanders. By the middle and end of the experiment, salamanders exerted a much stronger negative effect on *Daphnia* than the positive effect of shading. These shifting interaction strengths highlight the dynamism of small, aquatic ecosystems such as ponds where many of the residents metamorphose and leave.

Even after organisms leave, community responses can remain as a legacy for the next organisms to colonize. For example, Blaustein et al. (1996) found two-weeks post larval salamander removal that invertebrates had recovered completely, but zooplankton, bacteria, and algae remained significantly different than in control treatments. We found periphyton biomass remained lower in tadpole treatments than in control treatments weeks after the last frog metamorphosed. Additionally, *Daphnia* biomass was affected by salamander biomass throughout the experiment, even though on the last sampling day some ponds had no salamander larvae left. These legacy effects would change the biomass and community composition of primary producers and consumers for later breeding species, potentially facilitating colonization or growth (Connell and Slatyer 1977).

Conclusions

We found that the effects of bottom-up and top-down forces were important to understanding food web dynamics in ponds. Most studies tend to look at one top-down and one-bottom up effect, but our study separates out and simultaneously examines two top-down (predator and omnivore) and two bottom-up (shade and nutrient) effects. We found that resources such as light and nutrients interact with top-down effects in aquatic

ecosystems. Despite the fact that many amphibians are imperiled (Stuart et al. 2004), there is a paucity of data regarding their role in ecosystems. The results of our study are consistent with the limited existing data suggesting tadpoles can have strong effects on aquatic ecosystem properties (Connelly et al. 2008; Iwai et al. 2012; Seale 1980) and underscores the need for more information into how ecosystem functioning could be altered by land use, amphibian extirpation and climate change.

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Table 1 Results of mixed models for phytoplankton biomass (chl *a* $\mu\text{g L}^{-1}$), periphyton biomass (as chl *a* $\mu\text{g cm}^{-2}$), *Daphnia* biomass ($\mu\text{g dw L}^{-1}$) and chironomid density (cm^{-2}). All data were log- (algal parameters) or log($x + 1$)-transformed (*Daphnia* and chironomids) to meet assumptions of normality and homoscedasticity. Bolded values have a Bonferroni adjusted p-value of ≤ 0.05

Response	Predictors	χ^2	d.f.	adj-P
Phytoplankton	shade	1.56	1	1.000
	nutrients	2.62	1	0.527
	tadpoles	7.20	1	0.036
	salamanders	0.09	1	1.000
	time	30.91	1	<0.001
Periphyton	shade	21.95	1	<0.001
	nutrients	20.59	1	<0.001
	tadpoles	58.10	1	<0.001
	salamanders	0.08	1	0.778
	shade*tadpoles	2.53	1	0.111
	time	3.53	1	0.361
<i>Daphnia</i>	shade	17.50	1	0.002
	nutrients	5.66	1	0.104
	tadpoles	0.43	1	0.511
	salamanders	33.51	1	<0.001
	shade*salamanders	15.03	1	0.001
	time	0.00	1	1.000
Chironomids	shade	4.38	1	0.218
	nutrients	2.18	1	0.841
	tadpoles	70.68	1	<0.001
	salamanders	0.28	1	1.000
	block	12.24	1	0.003
	time	7.94	1	0.029

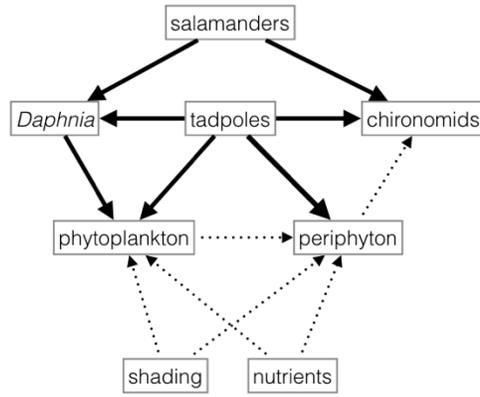
Figure Captions

Fig. 1 Conceptual diagram of a) top-down and b) bottom-up control of phytoplankton and periphyton biomass. Solid arrows represent significant pathways, and dotted lines non-significant pathways

Fig. 2 Biomass of phytoplankton (a-c), periphyton (d-f) and *Daphnia* (g-i); and chironomid density (j-l) at the beginning, middle, and end of the experiment. Each bar represents a mean \pm SE of three or four replicate ponds. No verts = no vertebrates added ($n = 3$ replicate ponds), frogs = presence of southern leopard frog tadpoles (*Lithobates sphenoccephalus*; $n = 4$ replicate ponds), sals = presence of spotted salamander larvae (*Ambystoma maculatum*; $n = 4$ replicate ponds), frogs+sals = both tadpoles and salamander larvae added ($n = 4$ replicate ponds). High shade treatments are hatched bars and low shade treatments are open bars. Low nutrient treatments are represented in white and high nutrients treatments in grey

Fig. 3 Causal diagrams of a) early (May, $n = 56$ ponds), b) middle (June, $n = 59$ ponds), and c) late (July, $n = 55$ ponds) experiment food web dynamics with light, nutrients, tadpoles and larval salamanders as exogenous predictors. Phytoplankton ($\text{chl } a \text{ } \mu\text{g L}^{-1}$), periphyton ($\text{chl } a \text{ } \mu\text{g cm}^{-2}$), *Daphnia* biomass ($\mu\text{g dw L}^{-1}$) and chironomid density (per cm^{-2}) data were $\log(x+1)$ transformed. Significant direct and indirect pathways ($P < 0.05$) are shown as solid arrows with widths proportional to the standardized path coefficient (shown on path). Non-significant paths are represented with dotted lines

a) Top-down



b) Bottom-up

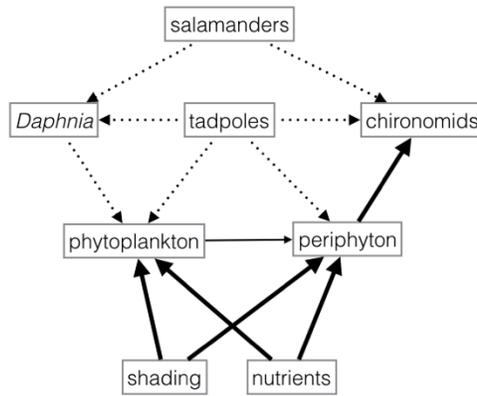


Fig. 1

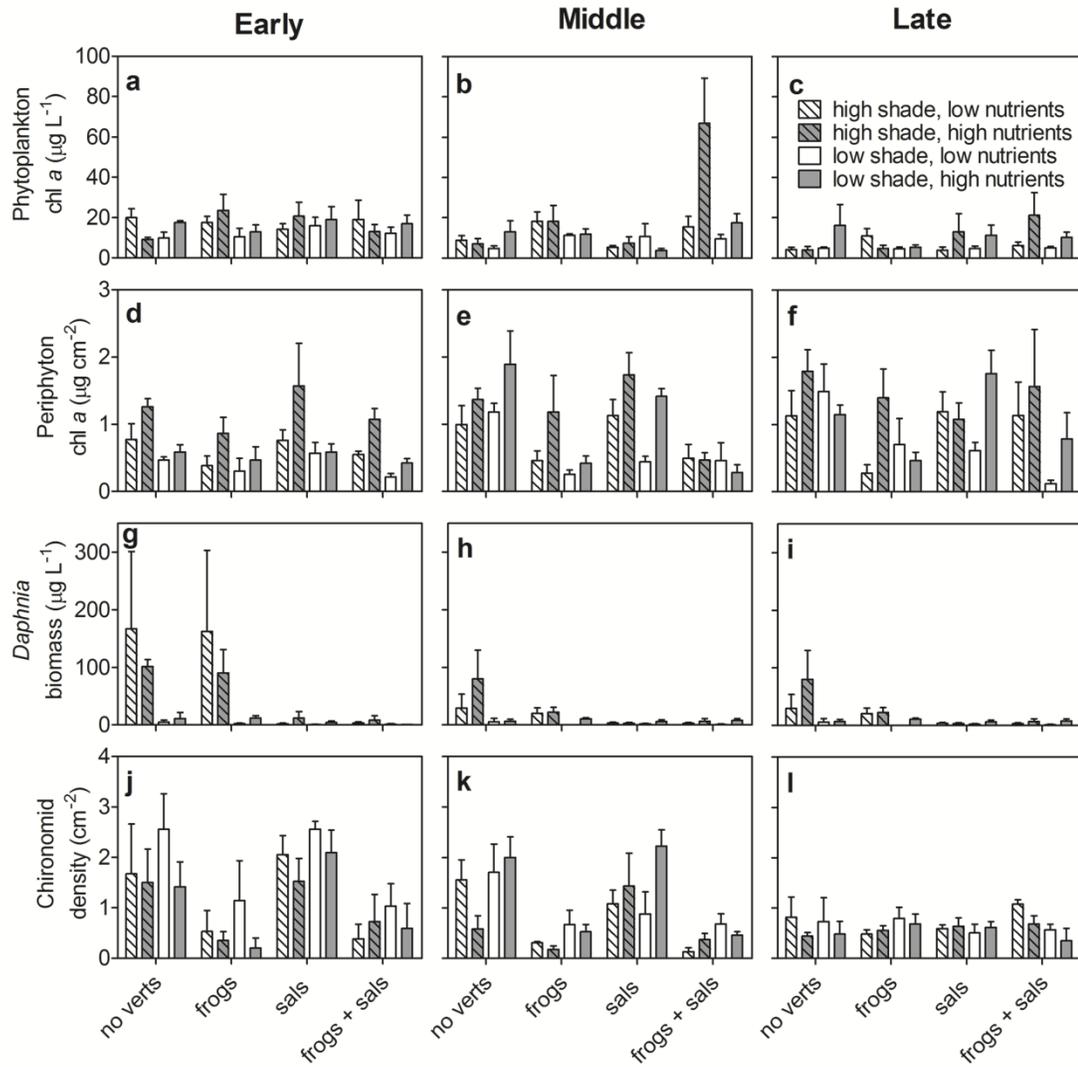


Fig. 2

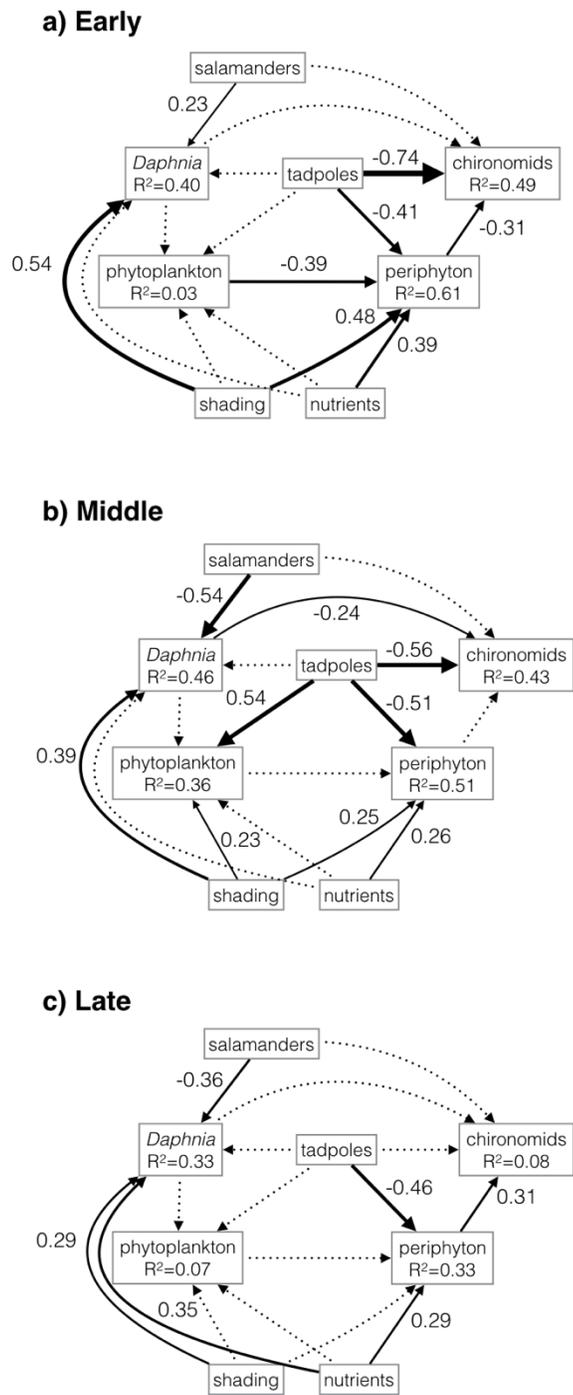


Fig. 2

CHAPTER 2: Nonlinear ecosystem responses to leaf litter subsidies in experimental ponds

Abstract

Spatial subsidies can strongly affect trophic interactions in food webs, yet most experiments use a presence/absence approach (i.e., subsidy vs. no subsidy). This emphasis on extremes assumes linear relationships and thus fails to capture potential nonlinear effects of gradients in subsidies seen in real systems and could overlook patterns in ecosystem responses. To test the effects of subsidies in aquatic systems, we manipulated leaf litter biomass across seven levels in 1000-L replicated experimental ponds. We used a simple pond food web that included southern leopard frog tadpoles (*Lithobates sphenoccephalus*) and spotted salamander larvae (*Ambystoma maculatum*) as the top consumers. We measured temperature, dissolved N and P; dissolved organic carbon (DOC); dissolved oxygen (DO); phytoplankton and periphyton biomass; and amphibian biomass export. We hypothesized that leaf litter would increase nutrient supply and DOC; decrease algal biomass; and that amphibian biomass export would closely track primary production. Our results suggest that leaf litter subsidies can affect abiotic and biotic components of ponds differently. Increased leaf litter linearly increased dissolved N, phytoplankton and periphyton biomass. DOC, periphyton ash-free dry mass, and DO were best described by nonlinear functions. Our results also suggest that frogs and salamanders have different responses to the size of leaf litter subsidies, and that the responses are nonlinear. Frog biomass export increased with the size of leaf litter subsidy, while salamander biomass export increased up to moderate subsidy levels, before decreasing to almost nothing at high subsidies, suggesting not only a nonlinear response, but a non-monotonic. In particular, these data show that a dose-

dependent approach is fundamental for understanding how the magnitude of the leaf litter subsidy affects ecosystems.

Key words: algae; canopy cover; dissolved nutrients; salamander; tadpole; wetland

Introduction

Ecosystems are often connected by the cross-boundary movement of resources and processes. The movement of nutrients, detritus, prey, and consumers can have important direct and indirect effects on population, community, and food web dynamics (Polis et al. 1997, Polis et al. 2004). Even systems originally thought of as isolated, such as lakes, are highly subsidized by exogenous carbon (Cole et al. 2000, Carpenter et al. 2005). In forested ecosystems, streams and ponds are strongly connected to the terrestrial landscape. Energy and nutrients enter through the soil, falling leaf litter, and deposition of terrestrial arthropods (Wallace et al. 1997, Wipfli 1997, Rubbo et al. 2006).

Our study focuses on freshwater ponds, which are common landscape features. In some regions like the glaciated northern U.S. and Canada they comprise a major portion of all freshwater ecosystems (e.g. vernal pools; Colburn 2004, Calhoun and DeMaynadier 2007). Temporary ponds can make an excellent system for studying relationships influence of subsidies because of their low levels of endogenous production and high respiration (Rubbo et al. 2006). Ponds can function as an abundant source (Gibbons et al. 2006) or sink (Regester et al. 2006) of energy for terrestrial environments, and thereby link aquatic and terrestrial ecosystems. Pond food webs are bookended by dissolved nutrients and detritus at the base, and amphibians and other predators at the top. Alterations in forests affect canopy cover around and over ponds, and therefore also affect inputs of light and leaf litter, which form the base energy sources for pond food webs (Colburn et al. 2008). These effects are particularly striking in ponds (as opposed to lakes) because ponds have a high edge-to-area ratio. Some of the most dominant consumers in ponds are amphibian larvae (Wilbur 1980). Amphibian larvae reach high densities (Gibbons et al. 2006), strongly influence ecosystem properties (Seale 1980, Iwai et al. 2012), serve as

important prey for other species (Wells 2010), and affect species interactions and community structure (Morin 1983, Wilbur 1997).

Despite numerous attempts to understand how leaf litter inputs influence ponds (Williams et al. 2008, Stoler and Relyea 2011, Cohen et al. 2012, Earl and Semlitsch 2013, Schriever and Williams 2013, Stephens et al. 2013, Stoler and Relyea 2013, Holgerson et al. 2016), emphasis on extreme input values (i.e., presence or absence of subsidy), plus focus on consumer responses only have failed to capture potential nonlinear effects of subsidy inputs. For example, although many experimental studies found addition (e.g., Richardson 1991) or exclusion (e.g., Wallace et al. 1999) of terrestrial leaf litter to streams to be important for consumers and overall productivity, almost all of these manipulations have been presence/absence-based (Richardson and Wipfli 2016). A gradient approach could offer important insights, as ecologists have long hypothesized the existence of a nonlinear “subsidy-stress gradient” and the effects of subsidies may be markedly different at population, community, and ecosystem levels (Odum et al. 1979). Thus there is a need to test not only subsidy gradients, but also to assess how the effects of subsidy may differ with scale.

We tested the relationships between terrestrial leaf litter subsidies and pond dissolved nutrients, algae, and amphibian larvae. We used a rigorous replicated design in experimental ponds to test for ecosystem response to a range of leaf litter subsidies, and hypothesized that leaf litter subsidies would nonlinearly increase nutrient supply and DOC; nonlinearly decrease algal biomass; and that amphibian biomass export would closely track primary production. Our approach tests ecosystem (e.g., nutrients) and population level responses (i.e., amphibians), both

of which are needed for evaluation of subsidy effects (Odum et al. 1979).

Materials and methods

Experimental design

We created experimental ponds in 1000-L mesocosms (hereafter ponds; 1.8 m² surface area) that allowed us to control for features such as age, shape, substrate, photoperiod, temperature, hydroperiod and depth, while retaining microbial, algal, and zooplankton communities similar to natural ponds (Semlitsch and Boone 2009). Previous work has found that processes observed in mesocosms are also important in natural ponds (Resetarits Jr and Fauth 1998, Van Buskirk and McCollum 1999, Van Buskirk 2009). To quantify the effects of leaf litter subsidies on pond ecosystems, we used a completely randomized design with seven subsidy levels: 0.25, 0.5, 0.75, 1.0, 1.5, 2, and 3 kg of leaf litter per pond. These values were within the range of values seen in an equivalent surface area in natural ponds (Earl 2012), but also represented extremes of very low and very high leaf litter input as well.

Each treatment was replicated six times within a 9 x 5 array for a total of 42 ponds, which were located in a fenced outdoor research facility owned by the University of Missouri, Columbia, MO. Each pond was filled with *c.* 1000 L of tap water on 14-Mar and 15-Mar-2015. After filling, we covered the ponds with high-density polyethylene PAK knit shade cloth (Hummert International, St. Louis, MO, U.S.A.) covers to prevent unwanted colonization of animals and escape of experimental animals. This resulted in a 30% reduction in ambient light levels. We allowed the water to dechlorinate for five days before adding leaf litter. We collected leaf litter from a deciduous forest dominated by oak (*Quercus* spp.) and maple (*Acer* spp.) in the Thomas S. Baskett Wildlife Research and Education Area (Ashland, MO, U.S.A.) on 16 March 2015, and added litter to the ponds on 17 March 2015. Aliquots of concentrated bacteria,

phytoplankton and zooplankton from local ponds were added on 19 March, 20 March, and 27 March 2015 as inocula to build the base of the pond food web. On 1 April 2015, we suspended ceramic tiles (22.1 cm²) midway down the water column along the wall of the northeast side of each pond to monitor periphyton biomass. Pond water depth was maintained at approximately 50 cm through rain water and water amendments during dry periods. To assess temperature, we sunk iButtons coated in Plasti Dip (Plasti Dip International, Blaine, MN USA) in the center of every pond on 12 May 2015. We programmed the iButtons to log temperature every four hours until 1 Aug 2016, the day before we ended the experiment.

Study system: top consumers

Southern leopard frogs (*Lithobates sphenoccephalus*) and spotted salamanders (*Ambystoma maculatum*) typically co-exist as larvae in ponds in the United States but represent different feeding niches. Southern leopard frogs are mostly primary consumers in ponds (but see Altig et al. (2007); Schiesari et al. 2009), whereas spotted salamanders are gape-limited predators of zooplankton, macroinvertebrates, and any other prey small enough to consume. We used these two species to build a model food web so we could examine how leaf litter subsidies would affect some of the most common and largest species occupying ponds on landscapes.

We collected four *L. sphenoccephalus* egg masses on 20 March 2015 and *A. maculatum* egg masses on 27 March 2015 from ponds at Fort Leonard Wood, Missouri, USA. We stored the egg masses in a protected area outside, and completed partial water changes every other day until hatching. We allowed tadpoles to develop to the free-swimming stage (Gosner 24–25; Gosner 1960). We randomly assigned tadpoles to treatments and added eight tadpoles to each pond on 8 April 2015, and an additional 16 on 10 April 2015 for a total density of 24 tadpoles pond⁻¹ or ~13 tadpoles m⁻², well within the natural density for *L. sphenoccephalus* (F. Rowland, *personal*

observation). We allowed salamander hatchlings to develop until they had re-absorbed their egg yolks before adding 12 larval salamanders to each treatment on 20 April 2015. Our salamander densities were within the range of natural densities for spotted salamanders (0.2–80.0 larvae m⁻², Figiel Jr and Semlitsch, 1990) and a moderate density for competition (Ousterhout and Semlitsch 2015). Thus, we added amphibian larvae at moderate but realistic densities to better reflect the population sizes found in ponds near the end of amphibian larval periods.

Sampling and analytical methods

We measured a number of chemical, physical, and biological response variables in the ponds approximately once per month. We sampled all ponds at the beginning (25 May 2015), middle (23 Jun 2015), and near the end of the experiment (20 July 2015) for both phytoplankton and periphyton algal biomass (as chlorophyll *a*), periphyton ash-free dry mass (to assess the algal, bacterial, and fungal biomass of periphyton biofilms [AFDM]), water column dissolved organic carbon (DOC), dissolved nitrogen (DN), and dissolved phosphorus (DP).

To determine phytoplankton biomass, we pooled one 200-mL subsample of subsurface water from each cardinal direction of a pond, and filtered a known volume through a Pall A/E glass fiber filter (Pall A/E, 1 µm nominal pore size). We stored filters at -20 °C until analyzed for chlorophyll *a* (chl *a*). To determine periphyton biomass, we collected one tile from the mesh and carefully scrubbed a known area onto a Pall A/E glass fiber filter and stored filters at -20 °C until analysis. Chl *a* samples were extracted for 20 minutes in a hot (78° C) 95% ethanol solution, then allowed to cool overnight in the dark before analysis (Sartory and Grobbelaar 1984). We measured pheophytin-corrected chl *a* on a Turner TD-700 fluorometer (Turner Designs, Sunnydale, California USA) calibrated with commercial standards dissolved in ethanol. We used

a flow-through method as described in Knowlton (1984).

Periphyton AFDM was estimated by Standard Methods 2540 D and E (American Public Health Association 1995). Briefly, we filtered a known area of each periphyton tile onto pre-ashed and weighed Whatman Grade 934-AH filters (1.5 μm nominal particle retention size). Filters were then dried at 105° C for an hour and re-weighed. We ashed the filters (30 minutes at 550° C) to remove organic matter, and used the difference between dry and ashed weight to estimate mass of periphyton.

DN, DP, and DOC were all measured on filtered pond water (1–1.5 μm retention), thus representing both dissolved materials and fine particles not retained by filters. All samples were acidified with sulfuric acid to a pH of approximately 2, and stored at 4° C until analysis. DN was analyzed using the second derivative method (Crumpton et al. 1992) after persulfate digestion. DP was analyzed using Standard Method 4500-P E, the ascorbic acid method following persulfate digestion (American Public Health Association 1995). Both DN and DP were run on a Spectronic Genesys 2 spectrophotometer (Thermo Fisher Scientific, Madison, WI USA). Water samples for DOC were filtered through membrane filters (0.45 μm pore size), and analyzed on a Shimadzu total organic carbon instrument (TOC 5000; Kyoto, Japan). All nutrient analyses were performed in duplicate.

We estimated dissolved oxygen (DO) using a handheld YSI temperature (to nearest 0.1 °C) and DO (to nearest 0.01 mg L^{-1}) meter during peak photosynthesis (11:00-14:00) and an hour before dawn as a proxy for minimum oxygen concentration. We did this on three occasions: 27/28 May, 27/28 June, and 30/31 July 2015. We only collected oxygen measurements when the previous 24 hours and time between peak and pre-dawn measurements had no precipitation,

minimal cloud cover, and low wind conditions with no rippling of water in ponds (< 8 mph).

We monitored the ponds daily for metamorphosed frogs (individuals with at least one forelimb) and salamanders (defined as the complete absorption of gills). We recorded date of metamorphosis, wet mass to the nearest 0.01 g, and body length as snout-vent length (SVL) to the nearest mm. Frog metamorphs were not weighed and measured until complete reabsorption of their tails. We ended the experiment on 4 August 2015 when at least three individuals of each species from each pond had metamorphosed. When we drained the ponds, we thoroughly searched the leaf litter for remaining individuals to estimate survival. Non-metamorphosing individuals were not included in any other analyses. These animals represented approximately 2.4% (± 1.0 SE) and 16.6% (± 3.2) of surviving frogs and salamanders, respectively.

Statistical analyses

We performed all statistical analyses in R (R Core Team 2017) on mean values of the three sampling periods. We used the *mle2* function within ‘*bbmle*’ package (Bolker and R Development Core Team 2017) to test for fit of various linear and nonlinear models using maximum likelihood. For the iButton temperature data we calculated daily means and the difference between minimum and maximum temperature within each day. Then we fit mixed models with litter treatment, date, and the interaction of litter x date as fixed effects, and pond as a random effect using the ‘*lme4*’ package (Bates et al. 2015). We then used the ‘*Anova*’ function in the *car* package (Fox and Weisberg 2011) to calculate χ^2 statistics and test for significance.

Results

Ecosystem responses to leaf litter

We found litter subsidy altered temperature dynamics (Fig. 1). Litter subsidy size ($\chi^2 = 96.05$, d.f. = 1, $p < 0.001$) and date ($\chi^2 = 4640.33$, d.f. = 1, $p < 0.001$) significantly affected mean daily temperature, but the interaction did not ($\chi^2 = 0.13$, d.f. = 1, $p = 0.715$; Fig. 1a). Higher litter subsidies resulted in lower mean daily temperatures. For example, across the entire season, the mean temperature was $25.2 \pm 1.6^\circ \text{C}$ (mean \pm S.E.) in the 0.25 kg subsidy treatment, $24.5 \pm 1.5^\circ \text{C}$ in the 1.5 kg subsidy, and $23.6 \pm 1.5^\circ \text{C}$ in the 3.0 kg subsidy. Unsurprisingly, ponds got warmer as the season progressed (Fig. 1a). The difference between the minimum and maximum daily temperature within each pond had a significant subsidy \times date interaction ($\chi^2 = 34.81$, d.f. = 1, $p < 0.001$; Fig. 1b). That is, the difference between daily maximum and minimum was highest in the low subsidy treatments, and increased as the weather got warmer. Higher subsidies appeared to buffer changes in temperature. For example, the 0.25 kg subsidy treatment had a mean daily difference of $3.3 \pm 0.6^\circ \text{C}$ (mean \pm S.E.), in the 1.5 kg subsidy it was $2.3 \pm 0.4^\circ \text{C}$, and in the 3.0 kg subsidy this difference was only $1.7 \pm 0.5^\circ \text{C}$. Essentially, ponds with the highest leaf litter subsidy experienced about half as much fluctuation in temperature as those with the smallest subsidy.

Our leaf litter subsidy gradient successfully created ecosystems varying from oligotrophic to eutrophic in nutrients and spanning a large range of DOC ($21\text{--}49 \text{ mg L}^{-1}$), and algal biomass responses indicative of oligotrophic to mesotrophic conditions (Table 1). Leaf litter subsidy size had strong effects on the chemical, physical, and biological properties of the pond ecosystems (Fig. 2). DN, phytoplankton chl *a*, and periphyton chl *a* all linearly increased with subsidy input (Fig. 2 b, d, and e; Table 2, Table 3), but DOC and DP increased nonlinearly (Fig. 2a, c; Table 2, Table 3). Periphyton AFDM exhibited a nonlinear fit, increasing up until 2 kg of leaf litter, and then decreasing at 3 kg (Fig. 2f). The only ecosystem property to show a decline with increased

leaf litter subsidy size was dissolved oxygen, which decreased nonlinearly with leaf litter (Fig. 2g, Table 2, Table 3). Dissolved oxygen in the surface water (first 10 cm of water column) varied widely. The 0.25 leaf litter subsidy had ~5x more oxygen than in the 3 kg subsidy treatment.

Population-level responses to subsidy size

Amphibians exhibited mostly nonlinear responses to leaf litter subsidy size (Fig. 3, Table 2). All responses were nonlinear, except for frog body condition (Fig. 3c), which had the highest support for a linear fit (Table 2, Table 3). Somewhat surprisingly, frogs and salamanders diverged in their response to subsidy size; frog biomass export and body condition continued to increase to the highest subsidy level, whereas salamander biomass export and body condition both peaked at a subsidy of 1.5 kg, and decreased at the highest levels (Fig. 3). This did not appear to be related to survival, as both species had high average survival rates (frogs $85.8 \pm 13\%$ SD; salamanders $83.5 \pm 18\%$) with similar responses to subsidy (Fig. 3e, f).

Discussion

We observed strong effects of leaf litter subsidy size on ponds. Leaf litter subsidies decreased temperature, and often increased nutrient concentrations, algal biomass, and frog body condition and export, but had a negative effect on dissolved oxygen and larval salamander growth and development (as evidenced by the reduced export and body condition). Our hypothesis that all of the relationships would be nonlinear was only partially supported. Nutrients and DOC did increase with leaf litter subsidy size, but dissolved nitrogen exhibited a linear relationship. We did not find support for a decrease in algal biomass with increasing subsidy, nor that amphibian biomass export tracked with algal biomass. Overall, DOC, DP, periphyton AFDM, and most of the amphibian responses were nonlinear, and similar to what Odum et al. (1979) proposed. That is, subsidies are only usable up until a certain point, and then beyond that

can stress ecosystems. Our results highlight that not all ecosystem properties and populations respond similarly to subsidy inputs, and that experimental approaches using a gradient of subsidy levels may offer key insights into how aquatic systems will change as subsidies increase or decrease.

Temperature and nutrient dynamics

Leaf litter subsidies induced several physical and chemical changes in our pond ecosystems worthy of note. As subsidy size increased, the mean daily temperature decreased. This was likely due to higher DOC with increased subsidy decreasing light penetration and thereby changing temperature dynamics. Temperature increases are suggested to affect ecosystem respiration more quickly than primary production (Yvon-Durocher et al. 2010), and thus the differences observed in our study could have implications for how well small aquatic ecosystems act as a source or a sink of carbon. Furthermore, we observed a temperature buffering capacity of high-subsidy ponds (Fig. 1b). Canopy cover over ponds influences buffers temperatures during leaf out, but the DOC within the pond could be especially important during times when the canopy is leafless in temperate areas. This could have large implications for ectothermic animals that occupy ponds for one or more of their life stages (which we discuss more below).

Dissolved nutrient concentrations were tightly linked to leaf litter subsidies in our experiment. Although all dissolved nutrient concentrations increased with subsidy size (Fig. 2), we found that DOC and dissolved P had positive, nonlinear relationships with increasing subsidy size, but dissolved N was positive and linear. Nutrient limitation is a fundamental control on primary production, algal biomass, and community composition (Tilman 1976). Thus, the relative balance of N and P could have large implications for the algal ‘food quality’ at the base

of the food web and the trophic transfer efficiency of energy (Dickman et al. 2008, Rowland et al. 2015).

Balance of autotrophy vs. heterotrophy

Although there is increasing appreciation of the importance of detritus to ecosystem processes and functioning (Moore et al. 2004), we still lack an understanding of the relative importance of detritus versus photosynthesis in small aquatic ecosystems. Some components of food webs may have key switching points where reliance on autotrophy vs. heterotrophy flips. The nonlinear response of periphyton AFDM in our experiment may underscore this; at low subsidy input algae, fungi, and microbes were all limited by resources, peaked in production at 2 kg, and by 3 kg of subsidy may have switched to a microbial-dominated system. We observed increases in phytoplankton biomass (as chl *a*) with increasing subsidy size, likely due to increases in dissolved nutrient supplies (Fig. 2). However, despite increasing nutrients, we observed larger variation in algal biomass with increasing subsidy, and a highly nonlinear response of periphyton AFDM to subsidies. At low subsidies, primary production appears to be limited by nutrients, but at high subsidies it may be limited by the DOC associated with leaf litter subsidies. Indeed, previous work has indicated that terrestrial DOC can depress primary production by chelating micronutrients (Jackson and Hecky 1980), diminishing enzymatic activity (Hättenschwiler and Vitousek 2000), and attenuating solar radiation (Thrane et al. 2014).

With increased DOC, phytoplankton biomass tends to decrease (Carpenter et al. 1998) and bacterial production increase (Hessen 1992). We did not observe decreased phytoplankton biomass, but although we were unable to quantify heterotrophic production, we observed a large gradient in DOC over the course of the entire experiment (15.8–64.5 mg L⁻¹), consistent with the range found in wetlands and bogs (Wetzel 2001). Recent work found that the relative

contribution of heterotrophic bacteria to overall ecosystem processes increases linearly with DOC (Jones and Lennon 2015). We observed a nonlinear relationship between subsidy size and DOC, an inverse relationship between DO and DOC, and increase in periphyton AFDM with DOC, suggesting that ponds with higher leaf litter subsidies shift to more heterotrophy. Although we did not alter light directly, increased dissolved organic carbon (DOC) with leaf litter input should strongly alter light availability (Thrane et al. 2014, Jones and Lennon 2015), and DOC increase resulted in visual water color changes within the experimental ponds. Thus, subsidies of leaf litter can shift relative autotrophy:heterotrophy in ponds, and reliance on heterotrophy in small aquatic systems may be even more pronounced than in lakes given the high values of DOC measured in small wetlands and marshes. Others have found forested ponds to be highly net heterotrophic (Rubbo et al. 2006, Holgerson 2015).

Consumer responses

Our hypothesis that consumer biomass export would be closely linked with algal biomass was only partially supported. Although frog body condition increased linearly with subsidy (similar to phytoplankton biomass), all of the other responses were nonlinear. Furthermore, our research suggests that consumer trophic level is an important determinant of the effects of subsidies. For most amphibian species, open canopy conditions consistent with 1 kg or less of leaf litter subsidy generally result in greater species diversity and survival (Skelly et al. 2002, Werner et al. 2007), presumably because higher light promotes higher algal biomass (Skelly et al. 2002). Southern leopard frogs are open-canopy specialists, thus we expected them to do poorly in the high subsidy treatment that is more typical of closed-canopy ponds. However, frog biomass export increased nonlinearly with subsidy, and their body condition was better with higher subsidy as well. We suggest frogs may opportunistically take advantage of whatever

resources are available. Frog tadpoles have previously been documented consuming detritus (Skelly and Golon 2003, Holgerson et al. 2016), and many species are much more flexible in their diet than previously considered (Schiesari et al. 2009, Whiles et al. 2010).

On the other hand, if anurans develop better in open canopy ponds because of increased green energy, in theory predators such as salamanders should be even more constrained by primary producer energy because it has to travel through primary consumers before reaching them (Lindeman 1942). Indeed, salamander larvae incorporate less detrital energy than frogs (Holgerson et al. 2016). This may suggest that organisms that are unable to consume biofilms may be more constrained under high subsidy conditions, and this deserves further research.

Interestingly, ponds surrounded by forest on a landscape not only receive higher leaf litter subsidies, but will also will dry out sooner (Skelly et al. 1999). High leaf litter subsidies reducing growth rates either directly through food limitation or indirectly through decreased temperatures can have strong effects on whether amphibians are successfully able to metamorphose before a pond dries. In addition to receiving leaf litter subsidies from the surrounding forest, whether species are able to metamorphose successfully will determine whether a pond functions as an abundant source of energy to terrestrial environments (Gibbons et al. 2006) or is a sink of energy from terrestrial sources (Regerter et al. 2006). Understanding the dose-dependent link leaf litter subsidies and flux of energy between aquatic and terrestrial ecosystems is vital to determining this relationship. Our research suggests that high subsidies will inhibit growth of consumers such that ponds will be an energy sink in terms of amphibian export.

Conclusions

Observational and theoretical modeling approaches need to be linked with experimental

approaches (Odum et al. 1979). The gradient approach we used can provide information to parametrize models and disentangle the complexities of ecosystems into real relationships that would be impossible under the usual presence/absence paradigm. Furthermore, we found ecosystem properties and populations to respond differently to leaf litter subsidies. Thus, global change altering landscapes and the movement of materials can have large effects on ecosystem processes at multiple scales.

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Table 1. Mean and standard error (SE) values across three sampling points for dissolved nitrogen (DN), dissolved phosphorus (DP), dissolved organic carbon (DOC), phytoplankton biomass (as chl *a*), and periphyton biomass (as chl *a* and ash-free dry mass [AFDM]) as a function of leaf litter subsidy size.

Litter (kg)	DN (mg/L)		DP (µg/L)		DOC (mg/L)		Phyto chl <i>a</i> (µg/L)		Peri chl <i>a</i> (µg/cm²)		Peri AFDM (mg/cm²)	
0.25	0.38	(0.02)	9.1	(1.08)	21.2	(0.72)	2.6	(0.47)	0.02	(0.002)	0.01	(0.002)
0.50	0.46	(0.01)	15.1	(0.36)	22.3	(0.83)	4.2	(0.59)	0.03	(0.007)	0.01	(0.005)
0.75	0.47	(0.01)	16.9	(0.70)	23.9	(1.13)	3.6	(0.51)	0.03	(0.005)	0.01	(0.001)
1.00	0.51	(0.02)	17.4	(1.14)	24.0	(0.70)	2.6	(0.54)	0.04	(0.007)	0.01	(0.003)
1.50	0.60	(0.02)	23.2	(1.71)	29.3	(1.52)	4.1	(0.70)	0.09	(0.018)	0.02	(0.001)
2.00	0.71	(0.03)	48.9	(4.65)	34.0	(0.61)	8.8	(0.79)	0.22	(0.157)	0.06	(0.009)
3.00	0.88	(0.02)	76.5	(4.15)	48.8	(1.21)	6.3	(1.01)	0.38	(0.139)	0.03	(0.007)

Table 2. Table of candidate models for the effect of leaf litter subsidy size on pond mesocosms abiotic and biotic variables. Models are ordered based on ΔAIC alongside their respective Akaike weights (w_i). Models with the most support (those $< 2 \Delta\text{AIC}$ from the model with the lowest AIC) are shown in bold.

Parameter	Model	ΔAIC_i	w_i
DOC	quadratic	0.0	1
	linear	20.2	<0.01
	Michaelis-Menton	75.4	<0.01
DN	linear	0.0	0.75
	quadratic	2.1	0.25
	Michaelis-Menton	54.4	<0.01
DP	quadratic	0.0	0.91
	Michaelis-Menton	4.5	0.09
	linear	13.0	<0.01
Phytoplankton	linear	0.0	0.46
	quadratic	1.0	0.28
	Michaelis-Menton	1.2	0.26
Periphyton	linear	0.0	0.75
	quadratic	2.2	0.25
Periphyton AFDM	third-order polynomial	0.0	0.99
	Michaelis-Menton	13.4	<0.01
	quadratic	14.1	<0.01
	linear	14.8	<0.01
Dissolved oxygen	quadratic	0.0	0.69
	linear	1.6	0.31
Frog export	quadratic	0.0	0.94
	linear	5.5	0.06
Frog body condition	linear	0.0	0.77
	quadratic	2.4	0.23
Frog survival	quadratic	0.0	0.98
	linear	8.5	0.02
Salamander export	quadratic	0.0	0.94
	linear	5.7	0.06
Salamander body condition	quadratic	0.0	0.84
	linear	3.3	0.16
Salamander survival	quadratic	0.0	0.80
	linear	2.8	0.20

Table 3. Top models describing leaf litter subsidy size (kg) effects on ecosystem properties.

Each equation was fit to seven levels of leaf litter times six replicates across 42 mesocosms.

Each data point used represented a mean value across three sampling periods. Key: phyto =

phytoplankton, peri = periphyton, sal = salamander

Variable	Equation	n	Fit	
			r ²	P value
DOC (mg L ⁻¹)	20.84 + 1.35 x litter + 2.66 x litter ²	42	0.94	<0.0001
DN (mg L ⁻¹)	0.35 + 0.18 x litter	42	0.93	<0.0001
DP (µg L ⁻¹)	8.69 + 4.49 x litter + 6.176 x litter ²	42	0.92	<0.0001
Phyto chl a (µg L ⁻¹)	2.44 + 1.68 x litter	42	0.34	<0.0001
Peri chl a (µg cm ⁻²)	-0.06 + 0.15 x litter	42	0.43	<0.0001
Peri AFDM (mg cm ⁻²)	0.03 – 0.09 x litter + 0.09 x litter ² – 0.02 x litter ³	42	0.57	<0.0001
Dissolved oxygen	8.09 - 3.15 x litter + 0.45 x litter ²	42	0.94	<0.0001
Frog export (g pond ⁻¹)	12.21 + 22.59 x litter – 3.85 x litter	42	0.70	<0.0001
Frog condition (g cm ⁻¹)	0.38 + 0.18 x litter	42	0.85	<0.0001
Frog prop. survival	19.57 + 4.21 x litter – 1.80 x litter ²	42	0.39	<0.0001
Sal export (g pond ⁻¹)	6.90 + 4.17 x litter – 1.98 x litter ²	42	0.38	<0.0001
Sal condition (g cm ⁻¹)	0.28 + 0.08 x litter – 0.03 x litter ²	42	0.16	0.0307
Sal prop. survival	10.73 + 0.89 x litter – 0.76 x litter ²	42	0.49	<0.0001

Figure Captions

Fig. 1 Temperature responses of experimental ponds to leaf litter subsidy. (a) Daily mean temperature and (b) daily temperature range ($^{\circ}$ C). Each line represents the mean value for a leaf litter subsidy (kg) treatment. $n = 5$ replicate ponds for 0.25, 1, and 3 kg subsidies (due to iButton failure or inability to find at end of experiment). $n = 6$ replicate ponds for 0.5, 0.75, 1.5, and 2 kg subsidies. Subsidy size (in kg pond^{-1}) is represented by gray shading.

Fig. 2 Ecosystem responses to leaf litter subsidy size: dissolved organic carbon (a), dissolved nitrogen (b), dissolved phosphorus (c), phytoplankton chl *a* (d), periphyton chl *a* (e), periphyton AFDM (f), and daytime dissolved oxygen (g). Lines represent best fit \pm 95% confidence intervals. $n = 6$ replicate ponds per treatment.

Fig. 3 Responses of frogs (left side) and salamanders (right side) to leaf litter subsidy size. Biomass export (a, b) is the sum of all individuals reaching metamorphosis. Body condition is mass divided by snout-vent length at metamorphosis (c, d). Proportional survival represents the fraction of original population at the end of the experiment (e, f). The solid line represents the top model and 95% confidence intervals are denoted with dashed lines. $n = 6$ replicate ponds per treatment.

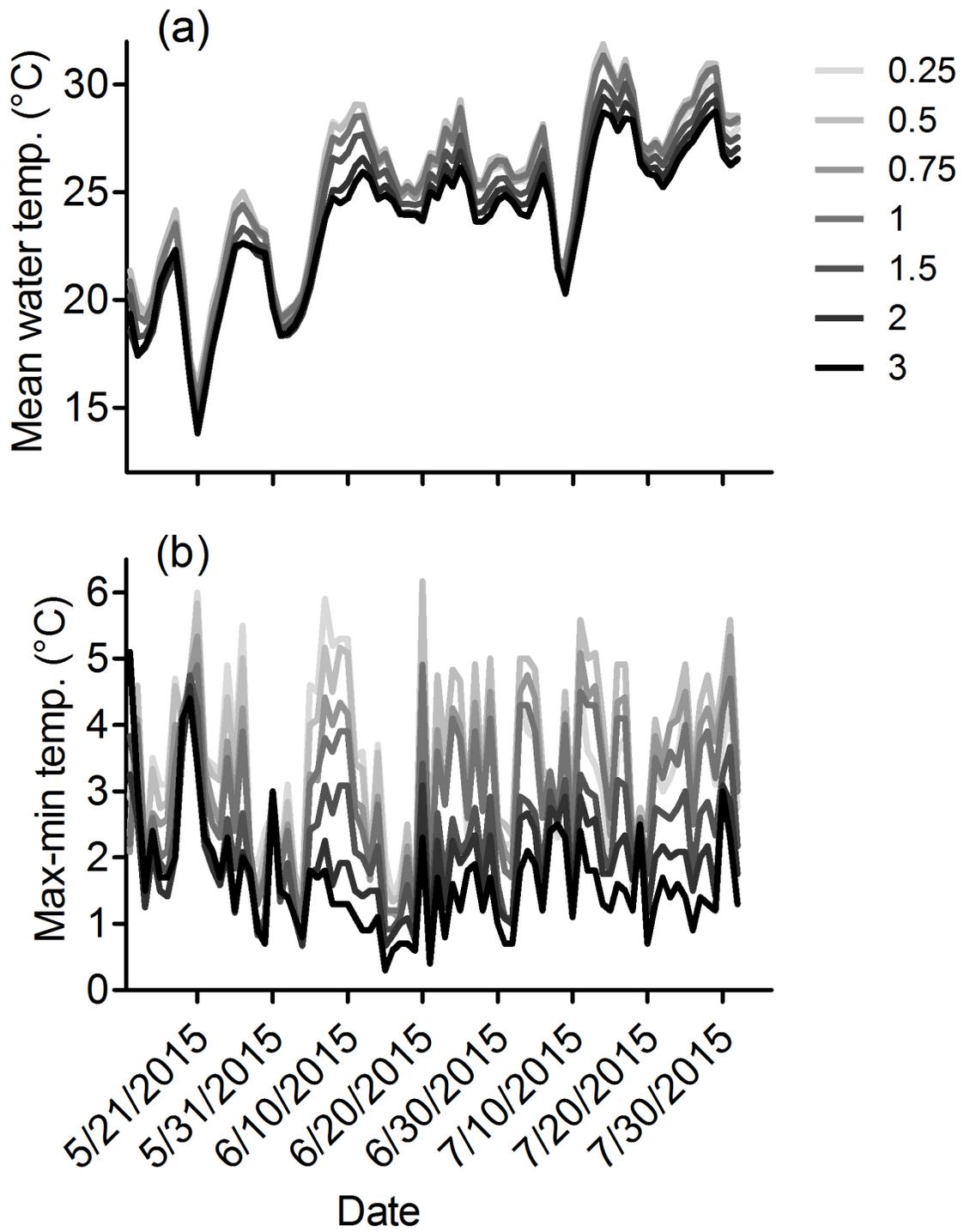


Fig. 1

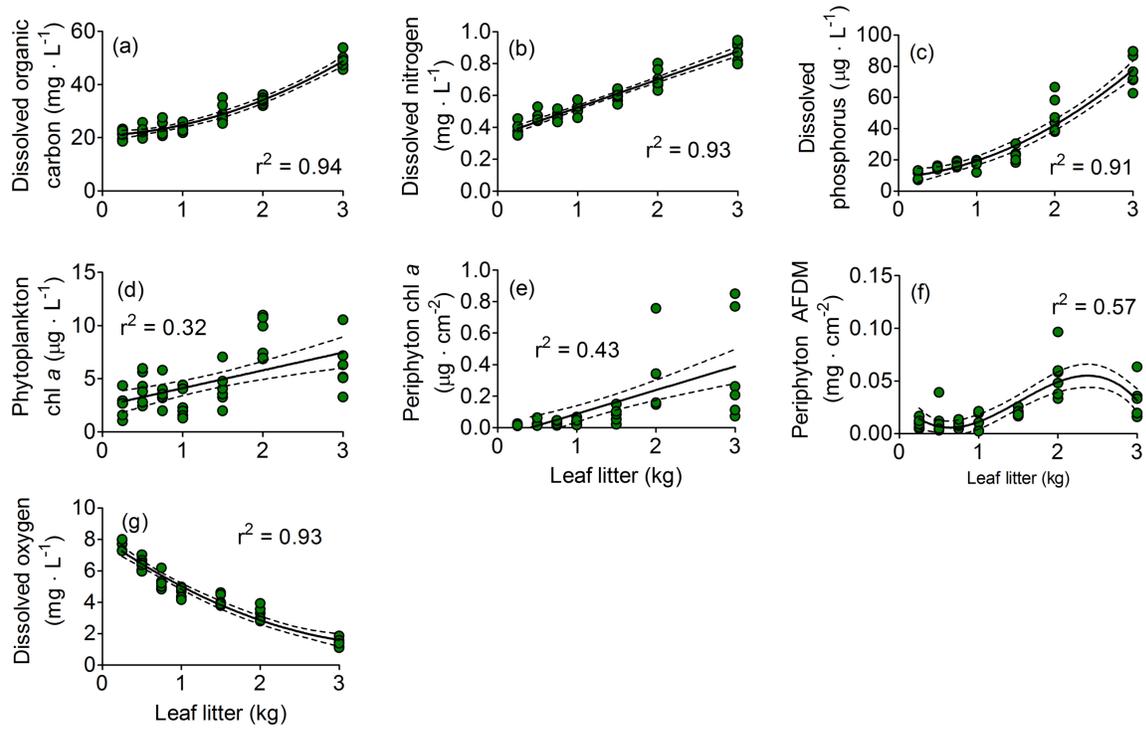


Fig. 2

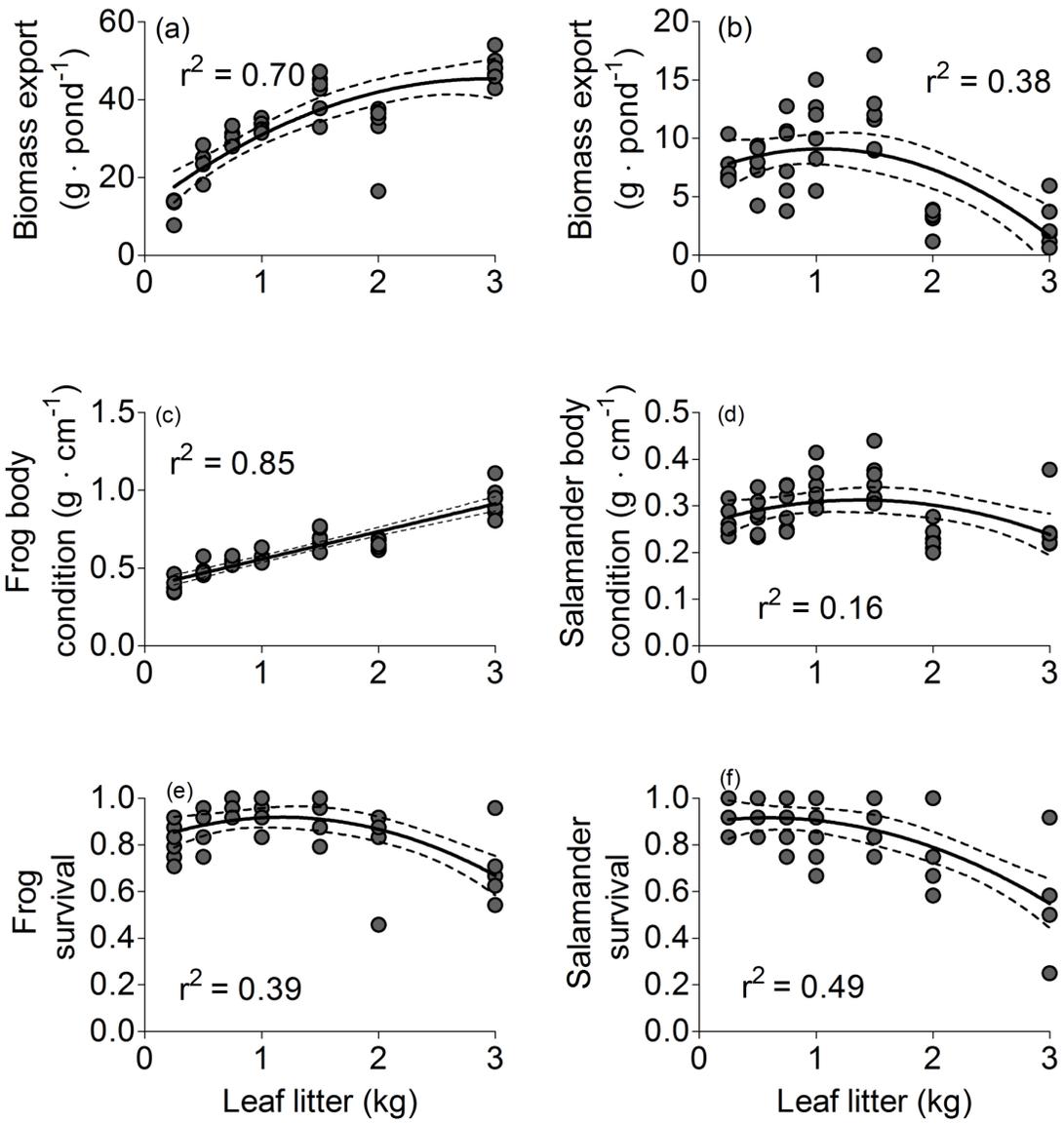


Fig. 3

CHAPTER 3: Leaf litter subsidies shift energy sources and trophic position for consumers

Abstract

Terrestrial leaf litter can heavily subsidize aquatic systems, yet the importance of these subsidies to aquatic food webs is still relatively unknown. Ponds present an excellent opportunity to explore questions related to subsidies, because their high edge:area ratio means they should be even more affected by subsidies than lakes, for example. We manipulated leaf litter subsidies in experimental ponds and used stable isotopes to assess shifts in food web position of consumers. We found strong evidence for shifts in all consumers to more detrital-derived energy as subsidy levels increased, but relatively few changes in trophic position. Surprisingly, the vertebrate, obligate predator in our system, *Ambystoma maculatum*, showed the strongest and most consistent changes with leaf litter subsidy. Overall, our study highlights that consumers are highly variable in their response to subsidies, and emphasizes the interconnectedness of the green and brown pathways in pond ecosystems.

Key words: canopy cover; salamander; stable isotopes; tadpole; wetland

Introduction

Understanding the structure and dynamics of food webs is a major focus of ecology. A key step to understanding the functional relationships among community members is defining their trophic position and how energy sources affect the abundance and diversity of species (Paine 1988; Persson 1999; Polis and Strong 1996; Post 2002; Vander Zanden and Rasmussen 1999). Interest in food web energy pathways arose from the discovery that detritus supports more predator biomass, higher species diversity, and longer food chains than would be supported by autotrophs alone (Hairston Jr and Hairston Sr 1993). In fact, many fundamental questions in ecology including those about the structure of food webs, length of food chains and trophic cascades are interpreted differently when detritus is considered a central component of the ecosystem (Moore et al. 2004). Although detritus (the ‘brown’ pathway hereafter) is important, we still lack an understanding of the relative importance of detritus versus photosynthesis (the ‘green’ pathway hereafter) in small aquatic ecosystems. Much of the detritus arrives as cross-ecosystem subsidies in the form of leaf litter. Such subsidies have been studied previously, but usually only at one level of input rather than integrated into the green vs. brown dichotomy and across the subsidy gradients that occur in real systems.

The strength of brown connections varies among ecosystems. Some—such as caves (Jesser 1998), small forested streams (Wallace et al. 1997), and below-ground food webs (Hunt et al. 1987)—are supported entirely by detritus. In contrast, in other systems (such as some lakes), energy flow from detritus is highly variable (Carpenter et al. 2005; Cole et al. 2000). Despite the variation, brown support of food webs is common. Moore and Hunt (1988) found that the majority (72.5%) of 40 community food webs they examined contained both brown and

green energy channels to consumers.

Freshwater ponds are common landscape features. In some regions like the glaciated northern U.S. and Canada they comprise a major portion of all freshwater ecosystems (e.g. vernal pools (Calhoun and DeMaynadier 2007; Colburn 2004). Ponds can function as an abundant energy source (Gibbons et al. 2006) or sink (Regeister et al. 2006) for or from terrestrial environments, thereby linking aquatic and terrestrial ecosystems. Habitat modifications near ponds (e.g. canopy loss) have strong potential to reduce vertebrate and invertebrate diversity (Colburn et al. 2008; Findlay and Houlihan 1997). In freshwater systems, loss of consumer diversity (especially sensitive species, such as dragonflies and amphibians) is occurring rapidly and with large ecological consequences (Calhoun and DeMaynadier 2007).

Alterations in forests affect canopy cover around and over ponds, and therefore also affect inputs of light and leaf litter, which form the base energy sources for pond food webs (Colburn et al. 2008). Changes in canopy cover therefore have the potential to shift trophic interactions and food web structure (Schindler et al. 2001; Wilbur 1997). These effects are particularly striking in ponds (as opposed to lakes) because ponds have a high edge-to-area ratio. Despite numerous attempts to understand pond food-webs, the role of amphibians, and the contribution of leaf litter input and light level (Cohen et al. 2012; Gillespie 2013; Holgerson et al. 2016; Schiesari et al. 2009; Schriever and Williams 2013; Stephens et al. 2013; Stoler and Relyea 2013; Whiles et al. 2010; Williams et al. 2008), it is still unknown how increasing leaf litter can shift diets of species; why some species like salamanders benefit from increasing canopy cover, especially given the fact that as predators they are constrained more by energy than a generalist would be; and how a balance of green versus brown energy affects energy flow through and out of ponds.

There is increasing evidence that open canopy ponds provide better support for most amphibian and invertebrate communities. For anuran primary consumers (frog and toad tadpoles), open canopies generally result in greater species diversity and survival (Skelly et al. 2002; Werner et al. 2007), presumably because higher light promotes the green pathway energy (Skelly et al. 2002). Indeed, mechanistic studies on tadpoles have suggested that decreased food quality and availability are major factors in reducing amphibian performance (Maerz et al. 2010; Skelly and Golon 2003). Food supplementation in closed canopy ponds has increased growth rates of tadpoles (Schiesari 2006; Skelly et al. 2002; Werner and Glennemeier 1999), and other studies have found a strong positive relationship between the amount of periphyton (a primary producer) and the performance of tadpoles (Hocking and Semlitsch 2008). Conversely, increasing canopy cover has been shown to reduce invertebrate richness (Batzer et al. 2004), abundance (Palik et al. 2001) and colonization (Binckley and Resetarits 2007). Midges in the family Chironomidae, however, tend to increase in biomass with increasing canopy cover and leaf litter input (Batzer et al. 2000; Mokany et al. 2008), although some studies show no effect of canopy (Batzer and Palik 2007). All of this implies that canopy openness can affect food web structure, but the mechanisms driving this process remain unknown.

There is still a general lack of consensus on how canopy cover affects carnivorous caudates (salamanders) compared to anurans (frogs). Two experimental studies have found negative impacts of closed canopy (Van Buskirk 2009; Van Buskirk 2011), and two have found positive impacts (Earl et al. 2011; Rowland et al. 2017) on salamanders. Observational studies sometimes find no effect (Werner et al. 2007), or find higher larval salamander abundance (Peterman et al. 2014) in closed-canopy ponds. If anurans develop better in open canopy ponds because of increased algal energy, in theory, predators should be even more constrained by

primary consumer energy because it has to travel through primary consumers before reaching them (Lindeman 1942). Still missing is a clear mechanistic understanding of how canopy cover influences food webs and the energy pathways of consumers.

The trophic level concept is not able to capture species interactions and trophic omnivory (Paine 1988; Persson 1999; Polis and Strong 1996; Vander Zanden and Rasmussen 1999), which are essential to understanding many basic questions in ecology. The use of stable isotope ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) represents a powerful technique that can provide a continuous measure of trophic position. Stable isotope values integrate the assimilation of energy through all the various trophic pathways leading to an organism (Cabana and Rasmussen 1996; Kling et al. 1992; Peterson and Fry 1987). In ponds, there are large gaps in knowledge about how important green vs. brown energy are for determining trophic position of consumers. Researchers have only a very vague idea of what amphibians actually eat (Altig et al. 2007), but it is becoming ever more apparent that anurans are not simply primary consumers (Holgerson et al. 2016; Schiesari et al. 2009; Whiles et al. 2010). Similarly, knowledge of larval salamander diets is primarily from stomach contents and observation, which does not reflect long-term consumption patterns. Stable isotopes are a tool for understanding amphibian diets and how much they rely on green and brown energy.

We manipulated leaf litter across five levels in experimental ponds to test the hypothesis that trophic relationships between consumers in the pond food web would shift as a function of leaf litter subsidy size. We had two predictions about energy sources: 1) in ponds with a lower level of leaf litter input, consumers will assimilate a higher proportion of pelagic species (more depleted in ^{13}C); and 2) under higher leaf litter input, consumers will assimilate more benthic species (heavier ^{13}C signature). Additionally, we predicted that obligate carnivores (i.e.,

dragonfly nymphs and salamander larvae) would increase their $\delta^{15}\text{N}$ with increasing leaf litter. Our rationale was that with increased dissolved organic carbon brought in with leaf litter subsidies, phytoplankton biomass tends to decrease (Carpenter et al. 1998) while bacterial production will increase (Hessen 1992; Jones and Lennon 2015). These changes will travel up the food web to top trophic levels by changing the biomass and species composition of available prey.

Materials and methods

Experimental design

We created experimental ponds in 1000-L mesocosms (hereafter ponds; 1.8 m² surface area) that allowed us to control for features such as age, shape, substrate, photoperiod, temperature, hydroperiod and depth, while retaining microbial, algal, and zooplankton communities similar to those of natural ponds (Semlitsch and Boone 2009). Most importantly, we were able to control subsidy input levels. Previous work has found that processes observed in mesocosms are also important in natural ponds (Resetarits Jr and Fauth 1998; Van Buskirk 2009; Van Buskirk and McCollum 1999). We used a completely randomized design with five subsidy levels: 0.5, 1.0, 1.5, 2.5, and 4 kg of leaf litter per pond.

Each treatment was replicated three times within a 6 x 5 array for a total of 15 ponds located in a fenced outdoor research facility owned by the University of Missouri, Columbia, MO (an additional 15 ponds were labeled with ¹³C and ¹⁵N, but are not presented here). Each pond was filled with *c.* 1000 L of tap water between 29-Feb and 1-Mar-2016. After filling, we covered the ponds with high-density polyethylene PAK knit shade cloth (Hummert International, St. Louis, MO, U.S.A.) covers to prevent unwanted colonization or escape of animals. This

resulted in a 30% reduction in ambient light levels. Dry leaf litter was collected from a deciduous forest dominated by oak (*Quercus* spp.) and maple (*Acer* spp.) in the Thomas S. Baskett Wildlife Research and Education Area (hereafter Baskett Wildlife Research Area; Ashland, MO, U.S.A.). We allowed the water to dechlorinate for five days before adding leaf litter collected the previous day on 6-Mar-2016. We inoculated experimental ponds with aliquots of concentrated bacteria, phytoplankton and zooplankton from ponds at Baskett on 10-Mar, 11-Mar, and 1-Apr. On 16-Apr-2016, we suspended ceramic tiles (22.1 cm²) midway down the water column along the wall of the northeast side of each pond to monitor periphyton biomass. Pond water depth was maintained at approximately 50 cm through rain water.

Study system

In addition to algae, bacteria, fungi, zooplankton, and naturally colonizing aquatic insects (i.e., chironomids and mosquito larvae), our simple food web included Southern leopard frogs (*Lithobates sphenoccephalus*) tadpoles, spotted salamander larvae (*Ambystoma maculatum*), and dragonfly nymphs (Family Libellulidae) as top predators.

All animals were collected at Basket Wildlife Research Area, the source of our leaf litter. We dipnetted dragonfly nymphs from a medium-sized, fishless permanent pond on 1-Apr, 2-Apr, and 5-Apr-2016. We visually size-matched nymphs and added four nymphs to each pond on 2-Apr, and an additional two nymphs on 5-Apr-2016 for a total of six dragonfly nymphs per pond. We collected four egg masses of *L. spehnocephalus* (on 14-Mar) and eight masses of *A. maculatum* eggs (on 10-Mar) masses from Baskett Wildlife Research Area. We stored the egg masses in a protected area outside, and completed partial water changes every other day until hatching. We allowed tadpoles to develop to the free-swimming stage (Gosner 24–25; Gosner 1960). We randomly assigned tadpoles to treatments and added 24 tadpoles to each pond on 7-

Apr and 8-Apr-2016. We allowed salamander hatchlings to develop until they had re-absorbed their egg yolks before adding 12 larval salamanders randomly to each pond on 17-Apr-2016.

Sampling

We sampled the experiment three times to understand how trophic structure and carbon source may shift with ontogeny. Thus, we sampled early (4-May to 5-May), in the middle of the experiment (11-May to 13-May); and just before the first animals started to metamorphose (24-May to 25-May).

We collected zooplankton using a 80- μ m mesh cup from a Wisconsin net. We collected integrated samples (surface to as close as we could get to the bottom without sampling leaf litter) until we had sufficient sample to clog the mesh. We then carefully rinsed the sample into a cup containing spring water. Tadpoles, salamander larvae, and dragonfly nymphs were dipnetted from experimental ponds. We aimed to obtain four tadpoles, two salamander larvae, and one nymph per sampling round because we were permanently removing the animals and wanted to keep the population sizes equal. We searched ponds until we found enough animals or for 4 h, whichever came first. All vertebrates were euthanized with MS-222, weighed, measured, and frozen until analysis.

Stable isotope analytical methods

We used stable isotope analysis to assess how trophic position and carbon source of consumers may shift with increasing leaf litter subsidy. We took measures to ensure any isotopic changes were representative of what was assimilated into body tissue instead of gut contents. Zooplankton and dragonfly nymphs were held in spring water overnight (~12 h) to clear their gut contents before freezing. For larger animals (tadpoles and salamanders), we euthanized the animals in MS-222 and removed guts and stomachs before drying. All samples were dried at 60°

C until constant dry mass and then homogenized with a mortar and pestle (animals) or coffee grinder (litter). We packed samples for animal tissue and litter into pressed tin capsules. For tadpoles and salamanders, we weighed out 0.9 ± 0.1 mg (mean \pm SD). For zooplankton with high enough biomass on the filter and large body sizes, we weighed out 0.9 ± 0.1 mg of dry sample. Some samples had low enough biomass that this was not feasible, in which case we wrapped the sample filter. Due to the high C and low N content of leaf litter, we ran these samples separately (1.8 ± 0.4 mg for C and 10.0 ± 0.4 mg for N).

We analyzed samples for percent C, percent N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ at the University of Missouri Stable Isotope Lab using a Carlo Erba NA 1500 Elemental Analyzer coupled to a Thermo Finnigan Delta Plus XL mass spectrometer via the ConFlo III interface. We present stable isotope ratio results using the standard delta (δ) notation, i.e., units per mil (‰) relative to international standards Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$ values) and atmospheric air ($\delta^{15}\text{N}$ values). Replicate analysis of an acetanilide standard yielded standard deviation estimates of 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$ estimates. Animal $\delta^{13}\text{C}$ was corrected for lipids based on Post et al. (2007)'s relationship between C:N and lipid content. In total, we analyzed 40 dragonfly nymphs, 45 zooplankton samples, 43 tadpoles, and 45 salamander larvae, for a total of 173 samples. We used linear models in R to assess how $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ changed for each consumer with leaf litter subsidy.

Results

All consumers changed their carbon source with increasing leaf litter for at least one of the sampled time periods. Dragonfly nymphs consumed items closer to the terrestrial leaf signature (higher $\delta^{13}\text{C}$) early on (Fig. 1a, Table 1), but this pattern disappeared as the experiment continued. Zooplankton $\delta^{13}\text{C}$ was significantly related to leaf litter in all time periods (Fig. 1b,

Table 1). As the leaf litter subsidy size increased, the zooplankton community assimilated heavier C (higher $\delta^{13}\text{C}$) into their body tissues. Early zooplankton samples appeared to rely on detrital energy sources more than late zooplankton samples (Fig. 1b). Tadpoles showed a similar pattern and their $\delta^{13}\text{C}$ shifted to heavier values as subsidy size increased. This pattern was significant in the early and middle sample periods (Fig. 1c, Table 1). There was also a positive relationship in all time periods between salamanders and $\delta^{13}\text{C}$. That is, salamanders relied more on detrital C as leaf litter subsidy increased (Fig. 1d, Table 1).

We observed relatively fewer changes in trophic level with increasing leaf litter. There was not a significant relationship between litter subsidy and $\delta^{15}\text{N}$ in any time period for dragonfly nymphs (Fig. 2a, Table 1). There was a positive relationship between zooplankton $\delta^{15}\text{N}$ and leaf litter subsidy for the early time period (Fig. 2b), but this relationship disappeared in the later time points (Table 1). Tadpoles did not show any relationship between $\delta^{15}\text{N}$ and leaf litter (Fig. 2c, Table 1), but salamanders significantly increased $\delta^{15}\text{N}$ with leaf litter in all time periods (Fig. 2d, Table 1).

Discussion

We found evidence that under low leaf litter conditions, all consumers were more depleted in $\delta^{13}\text{C}$, potentially suggesting they assimilated a higher proportion of pelagic species. Consumers also assimilated significantly more detrital energy (heavier $\delta^{13}\text{C}$ signature) as leaf litter subsidy increased. Our results agree with a growing body of research that detrital energy supports food webs (Carpenter et al. 2005; Holgerson et al. 2016; Moore et al. 2004). Increasing leaf litter subsidy greatly increases dissolved organic carbon (Chapter 2), which boosts bacterial production (Jones and Lennon 2015) and can suppress algal production (Thrane et al. 2014). The

flexibility to exploit both algal-derived energy and detrital energy is an important adaptation for organisms living in quickly changing environments such as ponds.

We had predicted that obligate carnivores (i.e., dragonfly nymphs and salamander larvae) would increase their $\delta^{15}\text{N}$ with increasing leaf litter, but this was only supported in salamanders. Dragonfly nymphs showed no evidence of eating higher on the food chain with increased leaf litter (e.g., they could have consumed amphibian larvae as zooplankton became more limited), even though they have no gape limitation. In contrast, salamanders showed the largest change across all time periods in their trophic position. Salamander larvae assimilated a higher proportion of $\delta^{15}\text{N}$ with increasing leaf litter across all time periods, which is unexpected given their gape limitation. This may suggest that salamander larvae are more flexible in diet than originally thought or hint at prey species composition changes.

Grazer responses

Although we were unable to separate out our zooplankton community to species due to biomass constraints, we found the entire zooplankton community shifted to consuming more detrital energy with increased subsidy. Previous work in ponds has suggested that zooplankton derive more than 80% of their biomass from algae (Holgerson et al. 2016), but work in lakes and bogs has indicated it can be as little as 25% of their energy in DOC-rich systems (Carpenter et al. 2005; Wilkinson et al. 2013). Our work shows that they are highly flexible. We cannot rule out that the shift in $\delta^{13}\text{C}$ may be due to species composition shifts. Indeed, *Daphnia*, the dominant grazer, tends to decrease in abundance later in the season in these systems (Rowland et al. 2017) and we have observed visual shifts from *Daphnia* to copepod and mosquito larvae dominated communities at high leaf litter (F. Rowland, *personal observation*). However, we did not see a change in $\delta^{15}\text{N}$ as expected if the zooplankton community shifted to more carnivorous species,

and supplementation of algal diets with humic substances similar to detrital-derived DOC have shown increased fitness in *Daphnia* (Lennon et al. 2013). It may be that because of their relatively flexible body nutrients (Sterner and Elser 2002), zooplankton are better able to adjust to shifting resources than larger invertebrates such as dragonfly nymphs or vertebrates.

The other grazers in our system, tadpoles, did increase detrital energy with increasing leaf litter subsidy. Most previous work could not quantify the importance of algal vs. detrital energy for tadpoles due to overlapping isotopic signatures (Schiesari et al. 2009; Schriever and Williams 2013). However, Holgerson et al. (2016) found relatively equal contribution of both of these sources to tadpole biomass. Both this finding and results from the current study support our hypothesis in Chapter 2 that tadpoles may be opportunistically feeding on whatever is available. The fact that they shifted their $\delta^{13}\text{C}$ but not $\delta^{15}\text{N}$ suggests they switched carbon source, likely from periphyton to biofilms. We found no evidence of the tadpoles occupying any other niche but primary consumer, and did not observe a switch to omnivory or carnivory as seen in previous studies (Schiesari et al. 2009; Whiles et al. 2010).

Carnivore responses

We saw differences between our two obligate carnivores. Dragonfly nymphs were only slightly affected by increasing leaf litter. Early on we found evidence of a shift from algae to more detrital energy sources, but this pattern disappeared as the experiment continued and there was no evidence of diet changes based on trophic level. Somewhat surprisingly, the other carnivore in our system, salamander larvae, changed their diet the most. In all time periods, salamander carbon source and trophic position were significantly related to leaf litter input. Salamander larvae are thought to primarily consume zooplankton, but also chironomids, isopods, and other larval amphibians (Branch and Altig 1981). However, whether salamander larvae rely

more on algal energy (Holgerson et al. 2016; Rubbo et al. 2008) or detrital energy (Earl et al. 2011; Werner et al. 2007) is still debated. Our results suggest that salamander larvae are flexible in both carbon source and somewhat in trophic position. This could be an important adaptation for a species such as our study species that prefers to breed in forested ponds (Peterman et al. 2014).

Conclusions

Our experiment provides evidence that consumers within pond food webs are highly variable in their response to leaf litter subsidies. Some, but not all species, showed shifts in food web node as subsidies increased. Depending on the quantity, leaf litter subsidies act as either a stimulator of primary production (Chapter 2), or increase bacterial production. Our data suggest that consumers are more likely to switch from algal to detrital-derived energy than change their trophic position. Ponds are highly reliant on leaf litter subsidies, and so as forest cover changes (Hansen et al. 2013), this could have strong effects not only on ecosystem properties (Earl and Semlitsch 2013), but also food web dynamics.

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Table 1. Fit statistics for linear regressions of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ vs. leaf litter. Significant ($P < 0.05$) results shown in bold.

Consumer		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		r^2	P	r^2	P
nymphs	early	0.45	0.0059	0.02	0.5778
	mid	0.23	0.0820	0.04	0.4800
	late	0.004	0.8717	0.04	0.3076
zooplankton	early	0.29	0.0472	0.34	0.0285
	mid	0.33	0.0254	0.21	0.0824
	late	0.37	0.0202	0.23	0.0866
tadpoles	early	0.55	0.0016	0.02	0.6495
	mid	0.48	0.0060	0.002	0.8807
	late	0.26	0.0610	0.22	0.0938
salamanders	early	0.61	0.0001	0.30	0.0441
	mid	0.73	<0.0001	0.35	0.0251
	late	0.60	0.0012	0.45	0.0009

Figure Captions

Fig. 1 $\delta^{13}\text{C}$ of a) dragonfly nymphs, b) zooplankton, c) tadpoles, and d) salamander larvae vs. leaf litter subsidy mass during the experiment. Each value represents the mean of three replicates. Linear regressions of the early, middle, and late sampling periods ($\pm 95\%$ C.I.) are shown only if the regression was significant.

Fig. 2 $\delta^{15}\text{N}$ of a) dragonfly nymphs, b) zooplankton, c) tadpoles, and d) salamander larvae vs. leaf litter subsidy mass. Each value represents the mean of three replicates. Linear regressions of the early, middle, and late sampling periods ($\pm 95\%$ C.I.) are shown only if regression was significant.

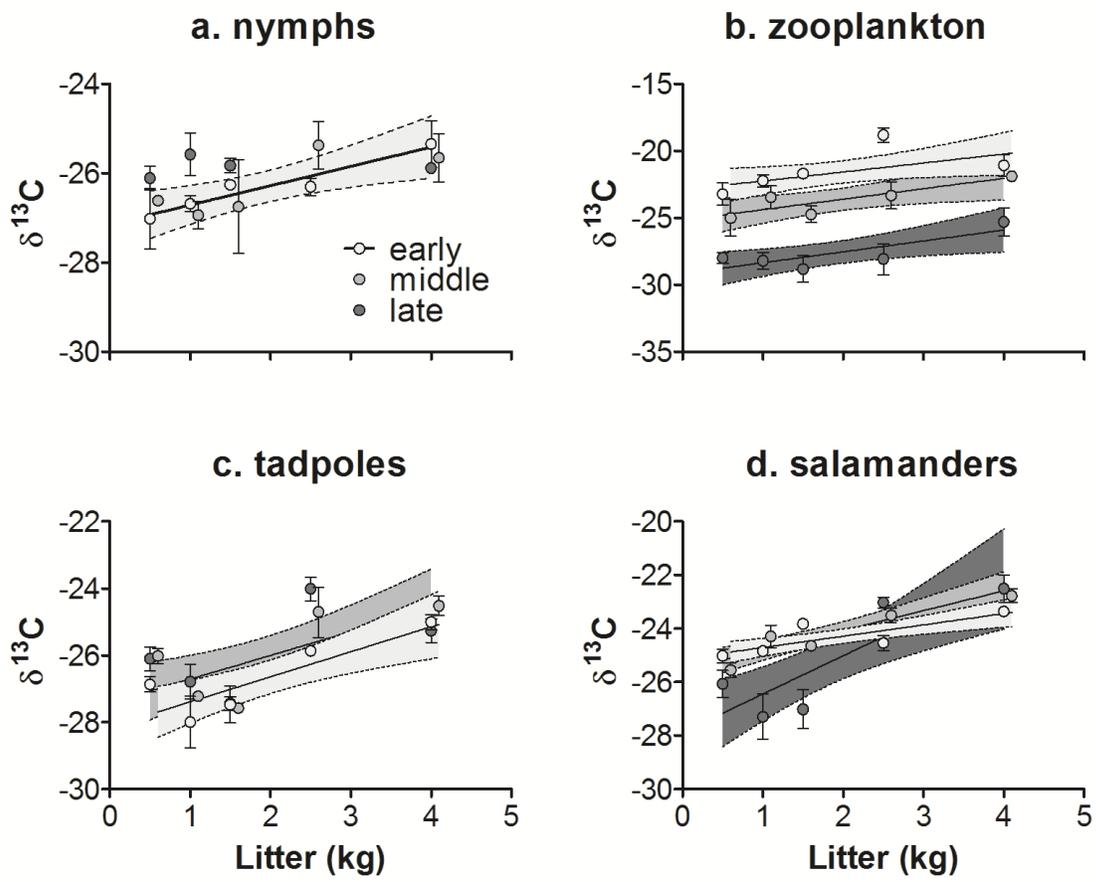


Fig. 1

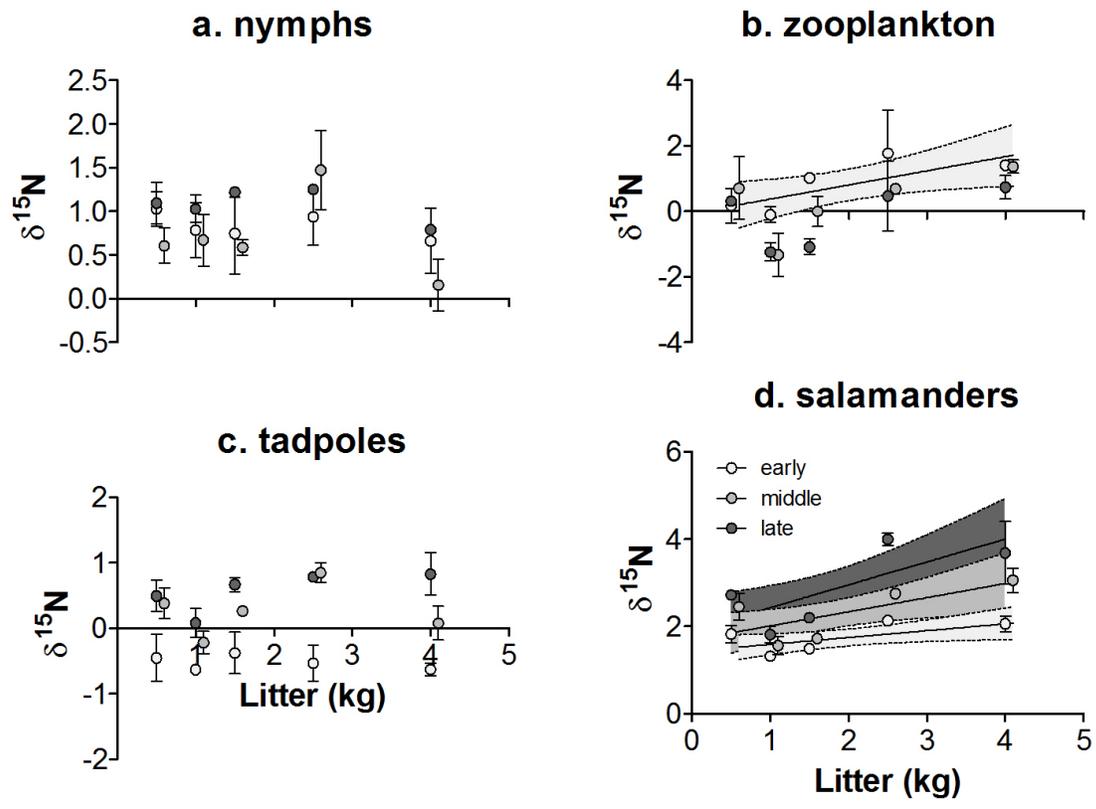


Fig. 2

SUMMARY AND CONSERVATION IMPLICATIONS

This dissertation adds to our understanding of the structure and functioning of pond ecosystems. In Chapter 1, I observed high temporal variation in food web dynamics. Whether shading, nutrients, tadpoles, or salamander larvae were most influential on the food web varied by sampling period and size of amphibian larvae. In general, canopy cover (as manipulated by shading and nutrients) had a stronger influence when amphibians were small, but I also found that tadpoles can strongly influence biomass of zooplankton, chironomids, and algae (three key components) in pond food webs. Perhaps even more importantly, I documented a legacy effect of amphibians on ponds. A month after most of the tadpoles and salamander larvae had metamorphosed, there was still a measurable effect of amphibian larvae on the food web. This means that even if there is no overlap within the pond, the next species to colonize will encounter a different environment than if the amphibian larvae had never been there. These legacy effects would change the biomass and community composition of primary producers and consumers for later breeding species, potentially facilitating colonization or growth (Connell and Slatyer 1977).

Chapters 2 and 3 highlight the role of leaf litter subsidy size on small aquatic ecosystems. Given the small size of ponds, leaf litter inputs can function as a major source of energy and nutrients. In Chapter 2, I found that leaf litter subsidy size strongly influenced dissolved nutrient concentrations, algal biomass, and the growth and survival of amphibians. Increasing leaf litter inputs increased nutrients and algal biomass, but not always in linear ways. Furthermore, amphibian larvae appear to be sensitive to high leaf litter loads in ponds, as evidenced by the decreased growth rates and number individuals metamorphosing in the highest litter treatment. Finally, in Chapter 3 I found that consumers within pond food webs are highly variable in their response to leaf litter input. Some (but not all) species showed shifts in trophic position and

energy source. My data suggest that consumers are more likely to switch from algal to detrital-derived energy than change their trophic position. Ponds are highly reliant on leaf litter subsidies, and so as forest cover changes (Hansen et al. 2013), this could have strong effects not only on ecosystem properties (Earl and Semlitsch 2013), but also food web dynamics.

These results show that forest cover around ponds is an important determinant of pond ecosystem function and biodiversity. Thus, forestry practices can have an impact on species occupying ponds for some or all of their life cycle. Partial forest cuts can be neutral or sometimes positive for amphibians (Semlitsch et al. 2009, Skelly et al. 2013), however clear-cutting would eliminate leaf litter as a resource in ponds and drastically change community dynamics. My research suggests that canopy removal could shift the ecosystem from a more detritus-based to an algal-based food web, change temperature dynamics, alter trophic positions, and even potentially decrease primary production through decreased nutrient input.

Generalizations are difficult due to the highly variable nature of ponds across time and space. However, my research indicates that tadpoles may be especially influential in shaping pond communities, and that it is important to consider canopy cover when designing or managing wildlife ponds. Canopy cover and amphibian community can dictate the energy brought out of the pond to the surrounding terrestrial environment at metamorphosis. There has been much debate on a federal level about whether small, isolated wetlands (such as those modeled in this dissertation) deserve protection. My research indicates that these systems are not isolated, but rather highly affected by and connected to the surrounding land. Additional research is needed to quantify this connectedness and determine the best management strategies for ponds and wetlands.

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VITA

Freya E. Rowland was born in Edina, MN on 30 Jan 1984. She spent her childhood in the suburbs of Minneapolis, MN, where she spent as much time as possible in or around lakes. Freya attended the University of Wisconsin for her undergraduate career, and can still remember the epiphany during a biology lecture discussing lake turnover when she realized she could make a career out of studying water. She was inspired by the excellent graduate students and faculty affiliated with the Center for Limnology and spent many happy days on lakes and enumerating zooplankton. After graduating in 2007, Freya started her first round of graduate work in the labs of Drs. Mike Vanni and María González at the Miami University, where she studied how light and nutrient variation affect food web efficiency. After receiving her MS in Aquatic Ecology in 2010, she spent several years working for local government monitoring stormwater, streams, and lakes in Minnesota before being inspired to come back to school to pursue her real interest: question-driven science. Freya will earn her PhD in Biology with an emphasis in Ecology in May 2018, and will begin a postdoctoral appointment at the Cooperative Institute for Great Lakes Research in Ann Arbor, MI in June 2018.