Biomass export of salamanders and anurans from ponds is affected differentially by changes in canopy cover

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SUMMARY

1. Previous research shows that canopy-associated shifts from an algal to a detritus-based food web can affect anuran tadpoles negatively. This may not be true of salamander larvae, however, because they are predators.

2. To investigate the influence of canopy cover on the survival and growth of salamanders, and on the subsequent export of biomass from ponds, we conducted a mesocosm experiment examining effects of shading (high or low) and litter (leaves or grass) on *Ambystoma maculatum* (a forest specialist) and *A. texanum* (a habitat generalist). Additionally, we reanalysed data from Williams, Rittenhouse & Semlitsch (2008) to examine the effects of shading and litter on biomass export of three anurans: *Rana sphenocephala*, *Pseudacris crucifer* and *Hyla versicolor*.

3. In contrast to previous studies, we found that salamanders performed better in mesocosms with the characteristics of closed canopy ponds (high shade and leaf litter), which resulted in a greater export of biomass. Salamanders grew larger under closed canopy conditions, probably because of differences in prey abundance among treatments. Anurans responded differently to canopy cover than caudates. The biomass export of *R. sphenocephala* and *P. crucifer* was reduced under closed canopy conditions (although differently affected by litter and shading), while the biomass of *H. versicolor* was not affected.

4. This and other studies suggest that changes in canopy cover may induce a shift in the amphibians emerging from ponds, from primarily anurans in open canopy ponds to primarily salamanders in closed canopy ponds. Additional multispecies studies will determine whether these trends hold true for more diverse amphibian assemblages. Further investigation into the effects of canopy cover on salamanders will be important for understanding aquatic–terrestrial linkages.

[1] *Keywords*: allochthonous input, *Ambystoma*, anurans, biomass, detritus, mesocosm, shading

Introduction

Forest canopy cover is a major factor in determining species distributions, community structure and ecosystem processes in aquatic habitats (Vannote *et al.*, 1980; Batzer, Palik & Buech, 2004). Canopy cover alters two main attributes: light availability and leaf litter input. Both of these factors can result in bottom-up effects, greatly altering primary productivity and decomposition (Vannote *et al.*, 1980; Mokany, Wood & Cunningham, 2008). Such changes can affect the subsequent export of biomass of some species from these habitats and their role in ecosystem processes.

Previous studies on the effects of canopy cover on amphibian assemblages in ponds have focussed almost exclusively on anuran larvae. There is a general consensus that increasing canopy cover

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1

decreases amphibian abundance (Halverson *et al.*, 2003; Binckley & Resetarits, 2007), species richness (Skelly *et al.*, 2005; Werner *et al.*, 2007), survival (Werner & Glennemeier, 1999; Thurgate & Pechmann, 2007), growth (Skelly, Freidenburg & Kiesecker, 2002; Schiesari, 2006) and development (Halverson *et al.*, 2003; Williams *et al.*, 2008). Studies have also demonstrated similar trends for overall invertebrate richness (Batzer *et al.*, 2004), abundance (Palik *et al.*, 2001) and colonisation (Nilsson & Svensson, 1995; Binckley & Resetarits, 2007). For tadpoles, mechanistic studies have demonstrated that decreased food quality and availability are major factors in reducing amphibian performance (including survival, growth and development; Skelly & Golon, 2003; Maerz, Cohen & Blossey, 2010). In support of these findings, food supplementation in closed canopy ponds increased the growth rate of tadpoles (Werner & Glennemeier, 1999; Skelly *et al.*, 2002; Schiesari, 2006). Other studies have found strong relationships between the amount of periphyton, an important food resource in open canopy ponds, and the performance of tadpoles (Hocking & Semlitsch, 2008).

Caudates (salamanders) and anurans differ in many ways, but particularly in larval diet. Salamander larvae are predatory, in contrast to anuran tadpoles, which are omnivorous (Altig, Whiles & Taylor, 2007; Schiesari, Werner & Kling, 2009). Studies have shown that zooplankton biomass and macroinvertebrate density decrease with increasing canopy cover (Palik *et al.*, 2001; Binckley & Resetarits, 2007; Hall *et al.*, 2007). However, this overall reduction does not translate into a reduced abundance of the preferred prey of salamanders (McWilliams & Bachmann, 1989; Regester, Whiles & Lips, 2008), such as midge (Chironomidae) larvae (Palik *et al.*, 2001; Batzer *et al.*, 2004; Mokany *et al.*, 2008). The density of midge larvae can be higher in closed canopy ponds (Batzer, Jackson & Mosner, 2000; Mokany *et al.*, 2008) as a result of leaf litter input (Batzer & Palik, 2007); though, some studies show similar midge densities in open and closed canopy ponds (Palik *et al.*, 2001; Batzer *et al.*, 2004; Batzer, George & Braccia, 2005). These responses of the prey of salamanders to canopy cover suggest that salamander larvae could perform better in closed canopy ponds or, alternatively, not respond to canopy differences. Thus, we predicted that salamanders would not display reduced performance in closed canopy ponds, as do many anurans. Only two studies have investigated the relationship between canopy cover and the performance of salamander larvae. In contrast to our predictions, two studies have shown decreased growth and developmental rates of European newts (*Triturus alpestris* Laurenti and *T. vulgaris* Linneaus) in closed canopy ponds (Van Buskirk, 2009, 2011).

In addition to a lack of studies on the effect of canopy cover on salamanders, most previous research has examined the effects of canopy cover on individual or population-level parameters. Amphibians breeding in water provide important linkages between aquatic and terrestrial systems (Regester, Lips & Whiles, 2006; McCoy, Barfield & Holt, 2009) and also make up a large proportion of vertebrate biomass in some habitats (Gibbons *et al.*, 2006; Peterman, Crawford & Semlitsch, 2008). The export of amphibian biomass from lentic systems (159 kg ha^{-1} year^{-1}; Gibbons *et al.*, 2006) can exceed that of various invertebrate taxa by orders of magnitude (1.3–17.9 kg ha^{-1} year^{-1}; White, 1985) and can approach that of entire invertebrate communities in certain years (Leeper & Taylor, 1998). We were particularly interested in how changes in canopy cover might alter the flux of amphibian biomass from aquatic to terrestrial ecosystems. To do so, it is important to use biomass as a community-level measure (Begon, Townsend & Harper, 2006), which would also allow better comparisons of amphibian data to that of other taxa than survival or growth.

To investigate further the effects of canopy cover on salamander larvae, and on the export of amphibian biomass from ponds, we performed an aquatic mesocosm experiment investigating the effects of canopy cover on the survival, growth and development of two species of ambystomatid salamanders. Because canopy cover alters both shading and litter input, we manipulated these factors separately. Additionally, to examine the effects of canopy cover on the export of a wider range of pond-breeding amphibians, we also investigated the effects of shading and litter on salamander biomass production and reanalysed data on anuran biomass production from Williams *et al.* (2008), who used a similar experimental design.

Methods

We tested the effects of shading and litter type on two species of salamander: *Ambystoma maculatum* Shaw (spotted salamander) and *A. texanum* Matthes (smallmouthed salamander). These two species are distantly

related within the genus (Shaffer, Clark & Kraus, 1991) and have widespread, overlapping distributions in the central United States with *A. maculatum* extending across the eastern states (Savage & Zamudio, 2005; Trauth, 2005). We chose to compare these two species, because adult *A. maculatum* is a forest specialist, whereas *A. texanum* is a habitat generalist (Petranka, 1998; Guerry & Hunter, 2002; Rothermel & Semlitsch, 2002). We predicted that these habitat preferences might translate into differences in the aquatic habitat as well, with *A. maculatum* performing better under a closed canopy and *A. texanum* performing better in open ponds.

To test the effects of shading and litter type on *A. maculatum* and *A. texanum*, we conducted a pond mesocosm experiment at a fenced outdoor research facility at the University of Missouri in Columbia, MO, U.S.A. Cattle tank mesocosms standardise some features of ponds, such as age, size and shape, while retaining microbial, phytoplankton, zooplankton and periphyton communities similar to those in natural ponds (Semlitsch & Boone, 2009). We used a full factorial design of three factors: shading (high or low), litter type (grass or leaves) and salamander species (*A. maculatum* or *A. texanum*). Each treatment combination was replicated three times for *A. maculatum*. Low hatching success in *A. texanum* resulted in three replicates for high-shade treatment combinations and two replicates for all other treatment combinations resulting in 22 total cattle tanks.

High (77%) and low (27%) shading was obtained by covering the tanks with high-density polyethylene PAK knit shade cloth of the appropriate level with 1 mm (high shade) or 2 mm (low shade) gauge mesh (Hummert International, St. Louis, MO, U.S.A.). These covers additionally prevented unwanted colonisation and oviposition by tree frogs and insect predators. We had two litter treatments: grass and tree leaf litter are common in open canopy and closed canopy ponds, respectively. We collected litter from the Thomas S. Baskett Wildlife Research and Education Area, Boone County, Missouri (hereafter, Baskett Wildlife Area). Freshly fallen leaves were collected from deciduous forest dominated by oak (*Quercus* spp.) and hickory (*Carya* spp.). Dead grass litter was raked and cut from around a partially dried pond in March and consisted of a mix of grasses and sedges. Both litter types were air-dried for at least 1 week prior to addition to tanks.

We used polyethylene cattle watering tanks (1.52 m diameter) as pond mesocosms. We filled tanks with *c.* 800 L of tap water each, allowed the water to stand for 5 days and added the randomly assigned litter type (1 kg per tank) on 10 April 2007. One litre of concentrated aliquots of plankton from several ponds at the Baskett Wildlife Area was added to tanks on 10, 12 and 16 April to allow the establishment of natural planktonic, microbial and other pelagic communities. **2**

On 10 April, *c.* 20 egg masses of each salamander species were collected from the same pond at the Baskett Wildlife Area. Eggs were hatched in plastic wading pools. We haphazardly counted out salamander larvae into groups of 12 that were randomly assigned to tanks, resulting in densities of *c.* 7 m^{-2}, which is within the range of natural densities for *A. maculatum* (0.2–80.0 larvae m^{-2}, Figiel & Semlitsch, 1990). We added larvae to tanks on 17 April, hereafter referred to as day 0 of the experiment. Water depth was measured to the nearest 0.5 cm in each tank on day 68, and water temperature was measured on day 78 to the nearest 0.1 °C.

Salamanders were recaptured at metamorphosis by searching tanks for metamorphs at night with a light at least every 2 days. At metamorphosis, we recorded the date of metamorphosis and wet mass to the nearest 0.0001 g with a Mettler AT-100 electronic balance (Mettler Toledo, Columbus, OH, U.S.A.). The first salamander metamorphosed on 9 June (day 53). We ended the experiment on 29 August (day 134), when we drained the tanks and searched the litter thoroughly for remaining salamanders to estimate survival. Non-metamorphosing salamanders were not included in other analyses, but these individuals represented <3% of surviving individuals.

All statistical analyses were performed using SAS/-STAT (SAS, 2004). Univariate three-way ANOVAS (Proc GLM) were used to examine the effects of shading, litter type and salamander species on survival, time to metamorphosis, mass at metamorphosis and salamander biomass export. Survival was used as a covariate in the time to metamorphosis analysis, and survival and time to metamorphosis were used as covariates in the analysis of salamander mass. Salamander biomass was calculated by summing the mass of all metamorphs found in each tank. All analyses were based on tank averages or proportions. Because none of the three-way interactions were significant, we removed them from analyses to increase power.

We additionally performed two-way ANOVAS (Proc GLM) on water temperature and water depth to determine differences among shading and litter treatments. All variables met the assumption of normality and homoscedasticity and, thus, were not transformed.

We also examined the effects of shading and litter type on anuran biomass using data from Williams *et al.* (2008), whose study is described briefly later for comparison with the salamander experiment. The treatments for the anuran experiment included three shading treatments (high, medium and low) and two litter types (grass and leaves). The high- and low-shade treatments were identical to those in the salamander study, while the medium-shade treatment was made from shade cloth resulting in 52% shade. These treatments were used to examine the effects of shading and litter on three species of anurans: *Pseudacris crucifer* Weid-Neuwied (spring peeper), *Rana sphenocephala* Cope (southern leopard frog) and *Hyla versicolor* LeConte (eastern grey tree frog). Tanks were set up similar to the salamander study, with several days allowed for water dechlorination prior to litter additions, three plankton inoculations and shade cloth used as cover to exclude the colonisation and oviposition of insect predators and competitors. Litter, plankton and amphibian egg masses were collected from the same site as the salamander study (Baskett Wildlife Area). All three anuran studies were performed at the same research facility with the same type of cattle tanks as in the salamander study. The spring peeper and leopard frog studies were conducted simultaneously from 21 April to 17 July 2003, and the grey tree frog study was conducted from 23 July to 4 September 2003. These studies all began when 40 hatchlings (Gosner stage 25; Gosner, 1960) of the appropriate species were added to each tank and ended when no tadpoles or metamorphs had been detected for several days. We summed the mass of all metamorphs found in each tank for each of the three species. We then performed a two-way ANOVA for each species to determine the effects of shading and litter on anuran biomass export to make qualitative comparisons with results for salamanders.

Results

Overall, salamanders performed better under closed canopy conditions (high shade and/or leaf litter).

Ambystoma maculatum had higher survival than *A. texanum* ($F_{1,15} = 11.58$, $P = 0.004$; Fig. 1a). The interaction between shading and litter on survival was marginally significant. Salamanders from high-shade tanks with grass litter had higher survival than low-shade tanks or high-shade tanks with leaves ($F_{1,15} = 4.00$, $P = 0.06$; Fig. 1b). Salamanders from high-shade tanks took longer time to metamorphose than those from low-shade tanks; though, the difference was marginal ($F_{1,15} = 3.75$, $P = 0.07$), but there was no effect of litter, species or any interactions on time to metamorphosis. Salamanders from the high-shade treatment were heavier than those from the low-shade treatment ($F_{1,15} = 47.50$, $P < 0.0001$; Fig. 2a). *Ambystoma texanum* from tanks with leaves were larger than those from tanks with grass ($F_{1,15} = 9.95$, $P = 0.008$; Fig. 2b), but the interaction between litter and shading was not significant. Survival and time to metamorphosis were not significant predictors of mass at metamorphosis (all $F < 2.67$, all $P > 0.12$).

Salamanders and frogs responded differently to canopy and litter treatments in terms of biomass
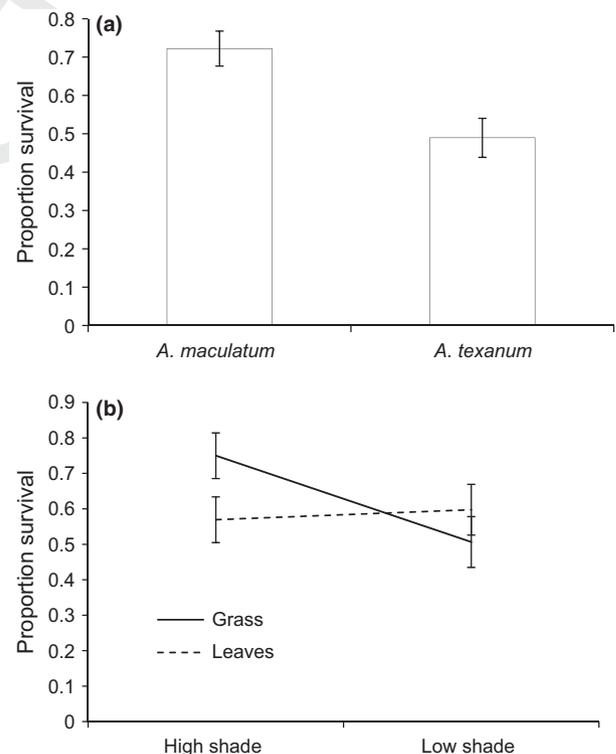
Fig. 1 Differences in salamander larvae survival between species (a) and shading and litter treatments (b). Data are presented as least square means (±SE) that adjust for other factors in the model.
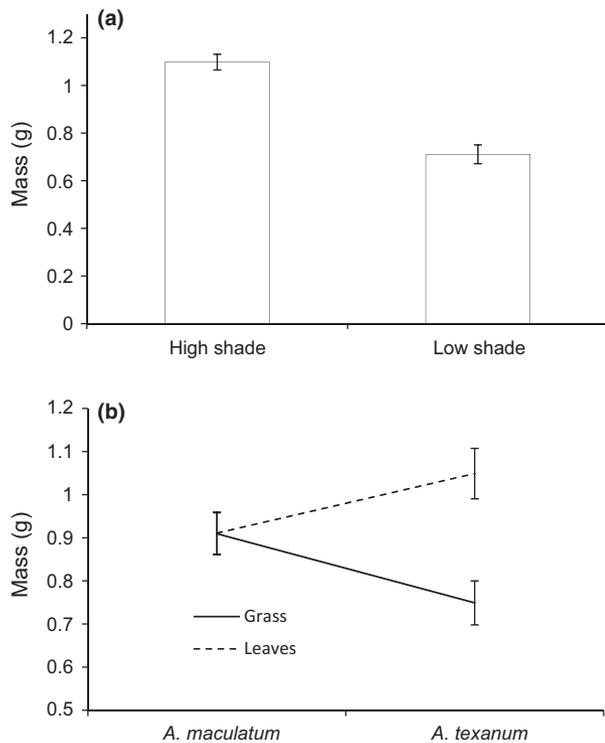
Fig. 2 Differences in salamander mass at metamorphosis among shading treatments (a) and species and litter treatments (b). Data are presented as least square means (±SE) that adjust for other factors in the model.

export. Salamander biomass was higher in the high-shade than in the low-shade treatment ($F_{1,15} = 26.53$, $P = 0.0001$; Fig. 3a) and was higher for *A. maculatum* than *A. texanum* ($F_{1,15} = 5.98$, $P = 0.03$). *Rana spheno-cephala* had higher biomass production in tanks with grass litter than leaves ($F_{1,12} = 258.35$, $P < 0.0001$; Fig. 3b) and in medium shade than high or low shade ($F_{2,12} = 7.24$, $P = 0.009$). *Pseudacris crucifer* had higher biomass in grass tanks but only in medium- and low-shade treatments ($F_{2,12} = 6.00$, $P = 0.02$; Fig. 3c). Neither litter nor shading affected *H. versicolor* biomass ($F_{5,11} = 0.19$, $P = 0.96$; Fig. 3d).

For the salamander experiments, water depth was affected by shading treatment. High-shade tanks were an average of 7.7 cm deeper than low-shade tanks ($F_{1,15} = 156.66$, $P < 0.0001$). Water temperatures were on average higher in low-shade tanks ($\bar{X} = 30.3 \pm 0.4$ °C) than high-shade tanks ($\bar{X} = 28.9 \pm 0.3$°C; $F_{1,15} = 8.08$, $P = 0.01$). This pattern of slightly higher temperatures in low-shade tanks was also found in the anuran experiment (Williams *et al.*, 2008). While pH was not measured in the salamander study,

Williams *et al.* (2008) found slightly higher pH in low-shade than high-shade tanks. Overall, weather patterns were similar for the salamander and anuran studies in terms of temperature and wind speed. There was more precipitation in 2003 during the anuran experiments than in 2007 during the salamander experiment (Appendix S1).

Discussion

We found that *Ambystoma* larvae of both species performed better overall in tanks with characteristics of closed canopy ponds (high shade and leaf litter) than open canopy ponds (low shade and grass litter), which translated into greater overall biomass in the closed canopy pond environment. These results for ambystomatids contrast with salamandrids, *Triturus alpestris* and *T. vulgaris*, which show reduced growth and developmental rates in closed canopy ponds and mesocosms with high shade and leaf litter (Van Buskirk, 2009, 2011). Our results also contrast with many studies on anuran larvae, which often display lower survival and slower growth and development in closed canopy ponds or under closed canopy pond conditions (e.g. Werner & Glennemeier, 1999; Schiesari, 2006; Williams *et al.*, 2008). Additionally, on further examination of the data from Williams *et al.* (2008), we found species differences in response to litter type and shading. However, we found lower frog biomass emerging from closed canopy environments (high shade and/or leaf litter) than open canopy environments (low shade and/or grass litter) for *Rana sphenocephala* and *Pseudacris crucifer*, but not *Hyla versicolor*.

Amphibian biomass production represents an export of nutrients (such as nitrogen and phosphorus; Regester *et al.*, 2006; Regester & Whiles, 2006), prey (for large spiders and mesopredators) and predators (on terrestrial invertebrates; McCoy *et al.*, 2009) from the aquatic to the terrestrial environment. The differences in biomass in *Amybystoma* salamanders, *Psuedacris crucifer* and *Rana sphenocephala* resulted when canopy cover reduced both survival and mass. *Hyla versicolor*, in contrast, had lower body mass but higher survival under closed canopy conditions than under open canopy conditions, which led to no difference among treatments in overall biomass (Williams *et al.*, 2008). Other studies on anurans have shown both reduced size or growth rates and survival in closed
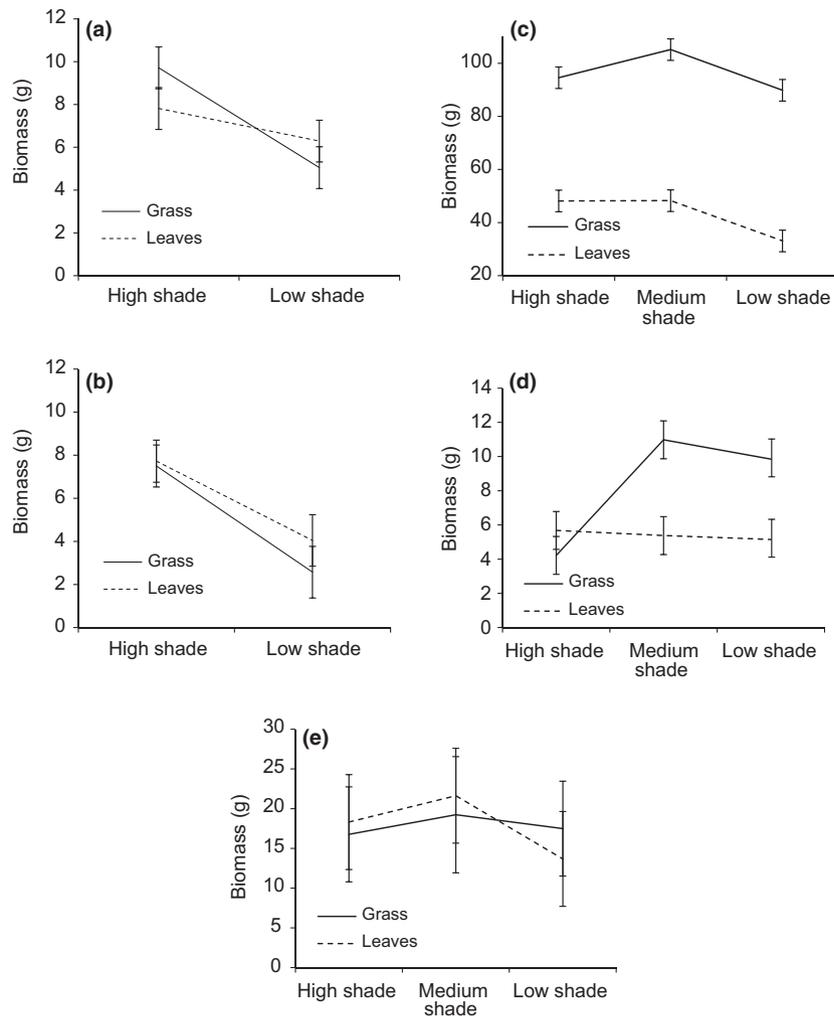
Fig. 3 Differences in biomass export among shading and litter treatments for *Ambystoma maculatum* (a) and *Ambystoma texanum* (b) in our study and *Rana sphenocephala* (c), *Pseudacris crucifer* (d), and *Hyla versicolor* (e) from Williams *et al.* (2008). Data are presented as least square means (±SE) that adjust for other factors in the model. Note differences in shading treatments between salamander and anuran graphs.

canopy ponds (Werner & Glennemeier, 1999; Skelly *et al.*, 2002), which would presumably lead to lower biomass. Although current data are limited, the differences between salamanders and frogs may result in a shift in the export of biomass from mainly anurans in open canopy ponds to a greater proportion of salamanders in closed canopy ponds (at least in areas where ambystomatids are the most common pond-breeding salamander). This shift could alter prey availability and nutrient deposition in terrestrial ecosystems. Gibbons *et al.* (2006) found that anurans made up *c.* 95% of amphibian biomass exported from an open canopy pond in South Carolina, U.S.A. In ponds with intermediate to closed canopies in Mis-

souri, U.S.A., amphibian biomass averages $64 \pm 5\%$ anuran (mean ± SE for five ponds over 4 years; Earl *et al.*, unpubl. data). Although not directly compara- **3** ble, these data suggest that further studies, particularly those on more complex assemblages, are warranted to examine the effects of canopy cover on amphibian biomass production and the relative amounts of salamander and anuran biomass.

The underlying cause of differences in biomass export in our experiment is differential survival and growth. These differences may also exist in natural ponds; though, reproductive input (initial recruitment) is also a major factor in ponds (Loman, 2002; Karraker & Gibbs, 2009). Anuran larvae tend to have

lower performance in closed canopy ponds than open canopy ponds, primarily because of lower primary production and food availability and quality (Werner & Glennemeier, 1999; Skelly *et al.*, 2002; Schiesari, 2006). Because salamander larvae are predators, increased canopy cover may affect salamander larvae in different ways than omnivorous anuran larvae. Invertebrate predators (primarily dytiscid beetles) tend to be more common in open canopy ponds (Nilsson & Svensson, 1995; Batzer *et al.*, 2004; Tuno *et al.*, 2005; Binckley & Resetarits, 2007); though, some studies showed inconsistent (Batzer & Palik, 2007) or no differences (Fairchild *et al.*, 2003; Mokany *et al.*, 2008). Nilsson & Svensson (1995) showed that dytiscids and their prey (mosquito larvae) both decreased in abundance with increasing canopy cover, hypothesising that the reduced prey availability drove the pattern seen. For salamanders, however, changes in canopy cover often do not alter the overall abundance of preferred prey (such as chironomids) in natural ponds (Palik *et al.*, 2001; Batzer *et al.*, 2004). Midge larvae can even be more numerous in high-shade than low-shade tanks in other similar studies (in July, closed canopy: 1.8 ± 0.3 chironomids cm^{-2}, open canopy: 0.6 ± 0.3 chironomids cm^{-2}; Earl & Semlitsch, unpubl. data). This may have driven the higher production of *Ambystoma* biomass in our study. Although *Ambystoma* and *Triturus* have similar diets (Bell, 1975; Taylor *et al.*, 1988; Kuzmin, 1991; Regester *et al.*, 2008), future studies in mesocosms that sample available prey (e.g. zooplankton and fly larvae) and analyse stomach contents may explain the observed differences in response to canopy cover (Van Buskirk, 2009, 2011).

Our study was designed to examine the two main effects of canopy cover, shading and leaf litter input, separately. These factors affected amphibian species biomass in different ways. *Ambystoma* biomass was doubled with higher shading, and *R. sphenocephala* biomass was twice as high with grass than leaves. *Pseudacris crucifer* biomass was two times higher in low and medium shade and grass than other treatment combinations, while *H. versicolor* biomass was affected by neither shading nor litter. Differences in canopy cover among ponds affect a number of different community, ecosystem and water quality parameters (Skelly *et al.*, 2002; e.g. Batzer *et al.*, 2004; Egan & Paton, 2004; Schiesari, 2006), which may be driven by shading or litter, or by the interaction

between them. These changes subsequently affect different amphibian species in different ways. Williams *et al.* (2008) found that shading was the most important driver of water temperature, pH and dissolved oxygen, but that shading and litter were both important influences on phytoplankton and periphyton. Leaf litter also affects the concentration of tannins, which is known to affect some tadpole species negatively (Maerz *et al.*, 2005; Brown *et al.*, 2006). Additionally, low dissolved oxygen (Schiesari, 2006) and high tannin concentrations (Maerz *et al.*, 2005) are known to have species-specific effects for amphibians. More mechanistic studies may help explain some of the species-specific differences between open canopy and closed canopy ponds on amphibians.

Contrary to our predictions, *A. maculatum* and *A. texanum* showed similar responses to litter and shading treatments. Because *A. maculatum* is a forest specialist (Guerry & Hunter, 2002; Savage & Zamudio, 2005) and *A. texanum* is a habitat generalist (Trauth, 2005), we expected *A. maculatum* to perform better under closed canopy conditions and *A. texanum* to perform equally well under open and closed canopy conditions. Other studies have shown canopy generalists to perform better in closed canopy ponds than open canopy specialists (Werner & Glennemeier, 1999; Schiesari, 2006). We found that species responded to the treatments similarly, except that *A. texanum* had greater mass at metamorphosis in tanks with leaves than those with grass. This effect may be due to differences in invertebrate abundances in leaf and grass tanks, as found in another study (in July, leaf tanks: 2.1 ± 0.4 chironomids cm^{-2}, grass tanks: 1.3 ± 0.4 chironomids cm^{-2}; Earl & Semlitsch, unpubl. data) and differences in diet preferences or foraging behaviour between *A. maculatum* and *A. texanum* (McWilliams & Bachmann, 1989; Regester *et al.*, 2008).

Given the results of this and other studies, anurans may represent the majority of amphibian biomass exported from open canopy ponds (Gibbons *et al.*, 2006), and salamanders have the potential to comprise the majority of biomass exported from closed canopy ponds in areas dominated by ambystomatids. Amphibians and aquatic insects represent an important link between aquatic and terrestrial ecosystems (Polis, Anderson & Holt, 1997; Regester *et al.*, 2006). Knowledge of trophic interactions in aquatic habitats is important for examining nutrient and energy

transfers between aquatic and terrestrial environments and for understanding subsequent trophic dynamics in the terrestrial habitat.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Weather summary by month for anuran (2003) and salamander (2007) experiments, listed as 2003, 2007.

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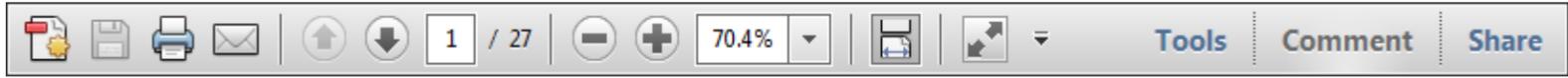
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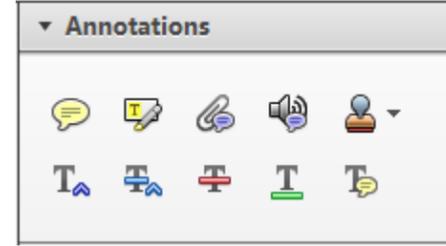
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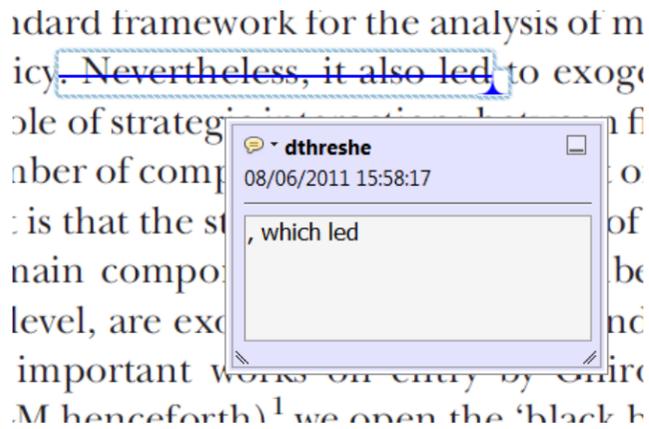
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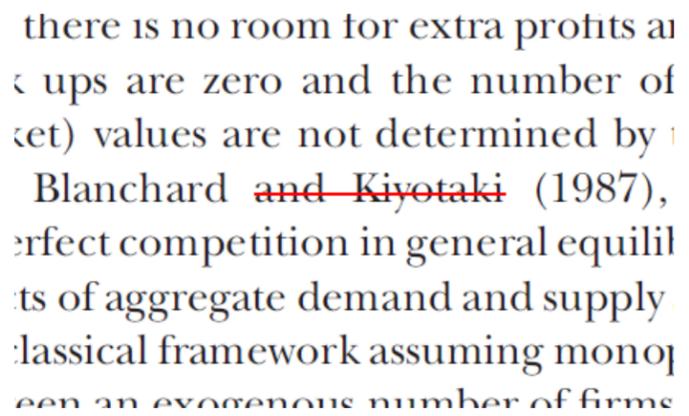
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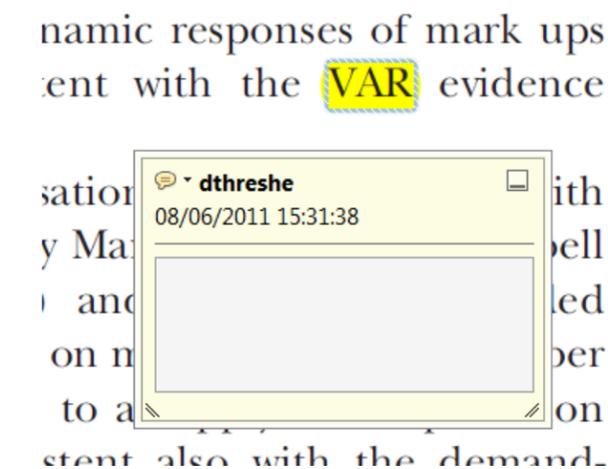
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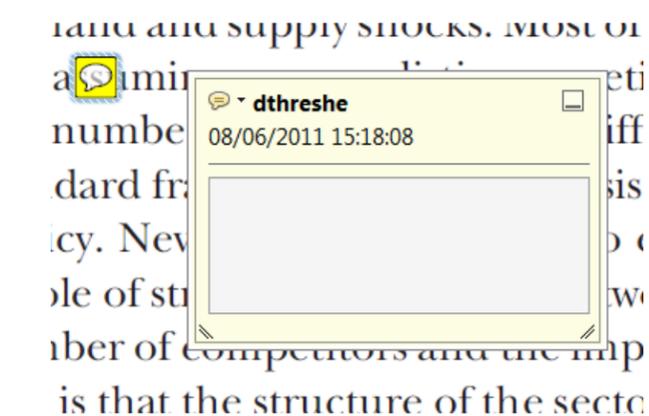
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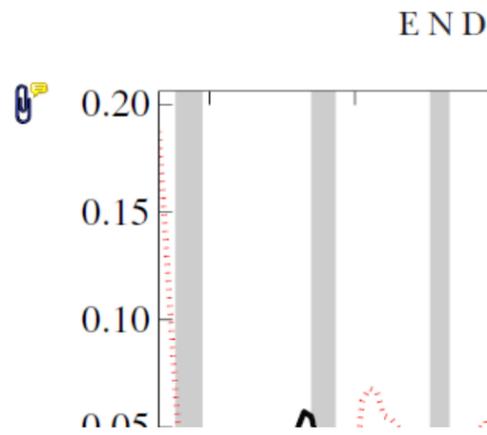
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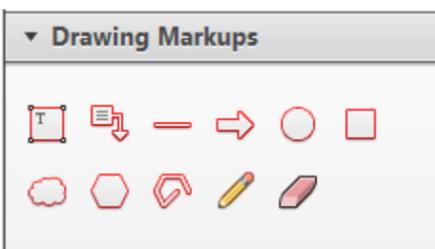
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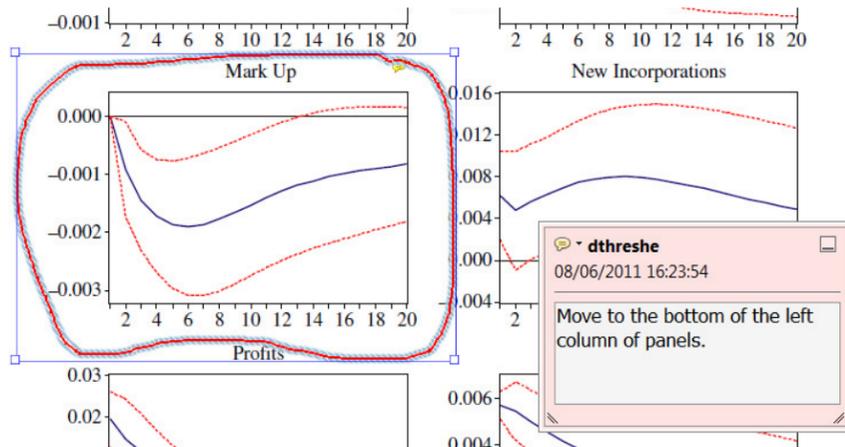
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