PREDATION AND COMPETITION AMONG LARVAL SALAMANDERS: THE

INFLUENCE OF DENSITY DEPENDENCE, PHENOLOGY, FOOD WEB STRUCTURE

AND HABITAT COMPLEXITY

A Dissertation Presented to the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

THOMAS LEE ANDERSON

Dr. Raymond D. Semlitsch, Dissertation Advisor

MAY 2016

The undersigned, appointed by the dean of the Graduate School, have examine the

dissertation entitled:

PREDATION AND COMPETITION AMONG LARVAL SALAMANDERS: THE
INFLUENCE OF DENSITY DEPENDENCE, PHENOLOGY, FOOD WEB
STRUCTURE AND HABITAT COMPLEXITY

Presented by Thomas L. Anderson,

a candidate for the degree of doctor of philosophy,

and hereby certify that, in their opinion, it is worth of acceptance

---
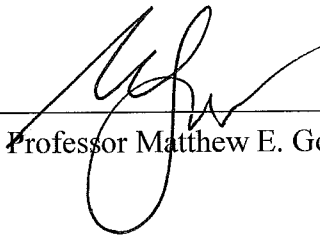Professor Raymond D. Semlitsch

---
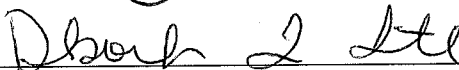Professor Ricardo M. Holdo

---
Professor Reginald B. Cocroft

Professor Matthew E. Gompper

Professor Deborah L. Finke

Dedicated to Raymond D. Semlitsch and Rosemary S. Anderson

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

Chapter

1. ECOLOGICAL CONTEXT IN PREDATOR-PREY INTERACTIONS

2. HIGH INTRAGUILD PREDATOR DENSITY INDUCES THINNING EFFECTS ON
AND INCREASES TEMPORAL OVERLAP WITH PREY POPULATIONS

5. SUMMARY

LIST OF TABLES

Chapter 3.

Chapter 4.

LIST OF FIGURES

ABSTRACT

The influence of biotic and abiotic factors on species interactions and overall community structure has long interested ecologists. Despite a legacy of interest, there is still ambiguity into the role of biotic and abiotic factors due to highly nuanced, complex networks of interactions that are difficult to comprehend. Yet, understanding how such nuances is an essential goal to determine how they affect population and community structure. Thus, the goal of my dissertation was to understand how multiple biotic and abiotic mechanisms alter interactions among larval stages of two pond-breeding salamanders. Larval stages of pond-breeding salamanders represent an excellent system for understanding how species interactions vary along abiotic and biotic gradients. Intra- and interspecific interactions are frequently determined by size differences among individuals, where larger larvae are predators of smaller larvae and can out-compete them for shared resources. However, when size differences are minimized, only competition occurs. Such conjoined competition and predation is termed intraguild predation, and is a common interaction in many taxa. The factors that determine size differences among individuals (both within and between species) are critical towards to determining both the type of interaction, as well as the strength of such interactions. The focal species I used were the ringed salamander (*Ambystoma annulatum*) and spotted salamander (*A. maculatum*). The former breeds earlier than the latter, creating a larval size advantage which results in predation as the dominant interaction between species. However, factors that influence growth rates of ringed salamanders could result in minimized size differences, resulting in a change to the strength or type of interaction that occurs. For my dissertation, I experimentally investigated three different processes that were expected to

ix

affect the relative importance of predation and competition: density dependence, food web structure, and phenological shifts. In my first chapter, I tested whether the density of ringed salamanders influenced their growth rates to such a degree that the interaction type with spotted salamanders would switch from predation to competition. I found that increased intraspecific competition in ringed salamanders reduced their body size and increased their larval period length. However, intraspecific competition did not reduce their size to such a degree that predation on spotted salamanders was precluded. Spotted salamanders showed decreased survival and increased size at higher predator densities, indicative of thinning effects. The period of overlap in ponds also increased at higher predator densities, resulting in a larger temporal window for interactions to occur. In my second chapter, I tested how six different top predator food webs would influence intraguild predation between ringed and spotted salamanders. I also tested whether food web configuration would be simultaneously impacted by increased habitat complexity. I found that ringed salamander body size and survival were unaffected by habitat complexity, and that only certain combinations of predators affected these demographic rates. Spotted salamander body size and survival showed positive and negative relationships with ringed salamander survival, but the strength of these relationships varied depending on the predator and habitat complexity treatment. Thus, pairwise interactions may not exemplify typical interactions when embedded in more complex food webs with other predators. For my third chapter, I investigated whether phenological shifts in both the ringed and spotted salamanders, simultaneous to density dependence in the ringed salamander would influence the type and strength of their interactions. I found ringed salamander survival varied with phenological shifts but only when at high

intraspecific densities. Spotted salamanders were relatively unaffected by phenological

shifts, and that their interactions were, similar to the previous chapters, influenced

primarily by survival of ringed salamanders. As phenological shifts are predicted for

many species with climate change, this study highlights that not all species interactions

will be subsequently affected, and that other underlying factors (e.g. density dependence)

may be more important. Thus, the most important findings of my dissertation include 1)

predator density can be a dominant factor in species interactions, 2) pairwise interactions

may change when embedded in different habitats or food webs in non-intuitive ways, and

3) simultaneously testing multiple mechanisms can elicit a greater understanding of the

relative importance of different ecological processes.

Chapter 1

ECOLOGICAL CONTEXT IN PREDATOR-PREY INTERACTIONS

Thomas L. Anderson

Introduction

Ecologists have historically been interested in the effects of species interactions on both population demography and community structure. The initial perception of species interactions was that it was the dominant mechanism regulating population growth. This view was epitomized by the debate in the early to mid-twentieth century over the relative role of density-dependent and density-independent factors in regulating population growth rates (Turchin 1995, Hixon et al. 2002). In recent decades it has been increasingly recognized that other factors are more important than the effects of species interactions for structuring populations and communities (Hixon et al. 2002). As a result of the integration of experimental, field and theoretical approaches, the contemporary viewpoint is that multiple mechanisms structure populations and communities, such as biotic and abiotic effects, ontogenetic niche shifts or stage-structure (Werner and Gilliam 1984, Hixon et al. 2002, Miller and Rudolf 2011, Morin 2011). Furthermore, there is increasing recognition that subtle variation in environmental context or traits of individual species are critical for understanding the mechanisms by which species-specific responses emerge from species interactions (Agrawal et al. 2007).

Several biotic and abiotic mechanisms exist that generate variation in predator-prey interactions (e.g., predator consumption and prey survival rates). Predators are frequently larger than their prey (Cohen et al. 1993, Woodward et al. 2005), but the magnitude of the size difference can be critical, as many predators are gape-limited, which reduces their ability to consume prey of similar size (Persson et al. 1996, Urban 2007a). The habitat type or physical environment also influences predator-prey interactions, as different structural complexities can simultaneously be regarded as prey refuges, hunting perches and/or offer concealment to predators or prey (Finke and Denno 2002, Janssen et al. 2007, Orrock et al. 2013). Also, predator-prey interactions do not occur in isolation with food webs, and are often affected by which other prey or predators are present (Holt and Huxel 2007, Schmitz 2007). In sum, predator-prey interactions can be affected by a variety of factors, all of which ultimately can alter ecological or evolutionary trajectories (Werner and Gilliam 1984), population cycles (Krebs et al. 2001, De Roos et al. 2003, Wissinger et al. 2010), or community structure (Hairston et al. 1960, Paine 1966, Persson et al. 2003). However, because multiple, synergistic mechanisms are simultaneously acting on predator-prey interactions, developing general principles can be difficult. Therefore, a greater assessment of the various mechanisms that simultaneously affect predatory-prey interactions is critical across several levels of biological organization (e.g., populations or communities).

My dissertation utilized larval stages of pond-breeding salamanders as a model system for testing multiple mechanisms that promote, inhibit or otherwise modulate the effects of interspecific size variation, the critical factor in determining the balance between predation and competition as the dominant type of interaction among individuals

of different species. Specifically, I focused on interactions between larval ringed (*Ambystoma annulatum*) and spotted salamanders (*A. maculatum*), which form an intraguild predation module (Polis et al. 1989). Larvae of both species share basal prey resources (e.g., aquatic invertebrates) but can also prey upon one another (Anderson et al. 2016). Ringed salamanders breed from September-November, and larvae overwinter in ponds before undergoing metamorphosis from April-June (Semlitsch et al. 2014). Spotted salamanders breed from February-April and undergo metamorphosis in June-October (Semlitsch and Anderson *In Press*). Thus, overwintering ringed salamanders reach greater sizes by the time spotted salamander larvae appear in the spring, creating large size disparities between species based on breeding phenology. Such size differences ultimately promote strong asymmetries in larval interactions. Factors that affect growth rates and survival of ringed salamanders therefore would have a large impact on spotted salamanders by shifting density or size differences between species, but have not been tested for these species, and only infrequently tested among other species pairs with similar life histories (Stenhouse 1985, Urban 2007b).

I used a series of experiments to test the role of different biotic and abiotic mechanisms that I hypothesized would impact larval salamander interactions. In Chapter 2, I tested whether increased density dependence in the predatory ringed salamander would decrease predator growth rates such that a switch in interaction type from predation to competition with spotted salamanders would occur at high densities (Anderson and Semlitsch 2014). In Chapter 3, I investigated whether top predator food web diversity and habitat heterogeneity mediates the effects of larval ringed on spotted salamanders (Anderson and Semlitsch 2016). In Chapter 4, I tested whether the joint

effects of phenological shifts and density dependence in both ringed and spotted salamanders would influence the relative size differences among species, and again shift the interaction type and strength (Anderson and Semlitsch, in review). I also tested whether resource depletion or predation by ringed salamanders was a more likely mechanism that resulted in changes to their intraguild prey, larval spotted salamanders.

Overall, my dissertation unravels complexities of synergistic relationships of biotic and abiotic factors on species interactions. My research suggests accounting for ecological context in species interactions can a powerful aspect of community ecology, yet difficult to disentangle. Further tests of combinatorial processes in ecological research will therefore lead to greater insight into how causal mechanisms vary and whether the strength of mechanisms change when placed into different contexts.

References:

Agrawal, A. A., D. D. Ackerly, F. Adler, A. E. Arnold, C. Cáceres, D. F. Doak, E. Post, P. J. Hudson, J. Maron, and K. A. Mooney. 2007. Filling key gaps in population and community ecology. Frontiers in Ecology and the Environment **5**:145-152.

Anderson, T. L., C. Linares, K. Dodson, and R. D. Semlitsch. 2016. Variability in functional response curves among larval salamanders: comparisons across species and size classes. Canadian Journal of Zoology **94**:23-30.

Anderson, T. L., and R. D. Semlitsch. 2014. High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations. Population Ecology **56**:265-273.

Anderson, T. L., and R. D. Semlitsch. 2016. Top predators and habitat complexity alter
    an intraguild predation module in pond communities. Journal of Animal Ecology.

Cohen, J. E., S. L. Pimm, P. Yodzis, and J. Saldaña. 1993. Body sizes of animal predators
    and animal prey in food webs. Journal of Animal Ecology:67-78.

De Roos, A. M., L. Persson, and E. McCauley. 2003. The influence of size-dependent
    life-history traits on the structure and dynamics of populations and communities.
    Ecology Letters **6**:473-487.

Finke, D. L., and R. F. Denno. 2002. Intraguild predation diminished in complex-
    structured vegetation: implications for prey suppression. Ecology **83**:643-652.

Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure,
    population control, and competition. American Naturalist:421-425.

Hixon, M. A., S. W. Pacala, and S. A. Sandin. 2002. Population regulation: historical
    context and contemporary challenges of open vs. closed systems. Ecology
    83:1490-1508.

Holt, R. D., and G. R. Huxel. 2007. Alternative prey and the dynamics of intraguild
    predation: theoretical perspectives. Ecology **88**:2706-2712.

Janssen, A., M. W. Sabelis, S. Magalhães, M. Montserrat, and T. van der Hammen. 2007.
    Habitat structure affects intraguild predation. Ecology **88**:2713-2719.

Krebs, C. J., R. Boonstra, S. Boutin, and A. R. E. Sinclair. 2001. What drives the 10-year
    cycle of snowshoe hares? Bioscience **51**:25-35.

Miller, T. E. X., and V. H. W. Rudolf. 2011. Thinking inside the box: community-level
    consequences of stage-structured populations. Trends in Ecology & Evolution
    26:457-466.

Morin, P. J. 2011. Community ecology. 2 edition. John Wiley & Sons, Oxford, UK.

Orrock, J. L., E. L. Preisser, J. H. Grabowski, and G. C. Trussell. 2013. The cost of
safety: Refuges increase the impact of predation risk in aquatic systems. Ecology
94:573-579.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist
100:65-75.

Persson, L., J. Andersson, E. Wahlström, and P. Eklöv. 1996. Size-specific interactions in
lake systems: predator gape limitation and prey growth rate and mortality.
Ecology:900-911.

Persson, L., A. M. De Roos, D. Claessen, P. Bystrom, J. Lovgren, S. Sjogren, R.
Svanback, E. Wahlstrom, and E. Westman. 2003. Gigantic cannibals driving a
whole-lake trophic cascade. Proc Natl Acad Sci U S A **100**:4035-4039.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild
predation: potential competitors that eat each other. Annual Review of Ecology
and Systematics **20**:297-330.

Schmitz, O. J. 2007. Predator diversity and trophic interactions. Ecology **88**:2415-2426.

Semlitsch, R. D., and T. L. Anderson. *In Press*. Structure and dynamics of Spotted
Salamander (*Ambystoma maculatum*) populations in Missouri. Herpetologica **72**.

Semlitsch, R. D., T. L. Anderson, M. S. Osbourn, and B. H. Ousterhout. 2014. Structure
and dynamics of ringed salamander (*Ambystoma annulatum*) populations in
Missouri. Herpetologica **70**:14-22.

Stenhouse, S. L. 1985. Interdemic variation in predation on salamander larvae. Ecology
66:1706-1717.

Turchin, P. 1995. Population regulation: old arguments and a new synthesis. Pages 19-40 *in* N. Cappuccino and P. W. Price, editors. Population dynamics: new approaches and synthesis Academic Press, San Diego, California, USA.

Urban, M. C. 2007a. The growth-predation risk trade-off under a growing gape-limited predation threat. Ecology **88**:2587-2597.

Urban, M. C. 2007b. Predator size and phenology shape prey survival in temporary ponds. Oecologia **154**:571-580.

Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics **15**:393-425.

Wissinger, S., H. H. Whiteman, M. Denoel, M. L. Mumford, and C. B. Aubee. 2010. Consumptive and nonconsumptive effects of cannibalism in fluctuating age-structured populations. Ecology **91**:549-559.

Woodward, G., B. Ebenman, M. Emmerson, J. M. Montoya, J. M. Olesen, A. Valido, and P. H. Warren. 2005. Body size in ecological networks. Trends in Ecology & Evolution **20**:402-409.

Chapter 2

HIGH INTRAGUILD PREDATOR DENSITY INDUCES THINNING EFFECTS ON

AND INCREASES TEMPORAL OVERLAP WITH PREY POPULATIONS

Thomas L. Anderson and Raymond D. Semlitsch

Abstract

Intraguild (IG) predator density can alter its effects on intraguild prey populations through several mechanisms, including density-dependent processes that affect IG predator traits such as size or growth that enhance or limit its predatory abilities. We examined whether intraspecific density-dependence altered IG predator traits, as well as the subsequent interspecific effects among its intraguild prey within a larval salamander guild. Four densities of ringed salamanders (*Ambystoma annulatum*), the IG predator, were combined with the presence/absence of spotted salamanders (*A. maculatum*), the IG prey, within experimental mesocosms. We modeled the effects of *A. annulatum* density on both conspecific and heterospecific responses that would be indicative of density-dependent competition and predation, respectively. We also modeled the reciprocal interspecific effects of *A. maculatum* on *A. annulatum*. We found that increasing intraspecific density negatively affected morphological traits but not survival of *A. annulatum*. No interspecific effects of *A. maculatum* on *A. annulatum* were observed. Alternatively, traits of *A. maculatum* showed nonlinear relationships with increasing *A. annulatum* density. Thinning effects of *A. annulatum* on *A. maculatum* were observed, as survival was positively and size negatively related for *A. maculatum* with IG predator density. The temporal overlap of the IG predator and prey also increased nonlinearly with IG predator density, intensifying the potential encounter rate of the two species. Overall, this study shows that density-dependent processes in IG predators can significantly affect traits of both themselves, as well as IG prey, which could ultimately change whether competition or predation occurs between the two groups.

Keywords: *Ambystoma* · Competition · Priority effects · Predation · Salamander

Introduction

Intraguild predation (IGP), where two species simultaneously compete for resources and prey upon one another, is an important ecological process across a wide diversity of organisms that has a strong theoretical basis (Polis et al. 1989, Holt and Polis 1997, Arim and Marquet 2004). Many factors influence the outcome of IGP, such as basal resource levels, the presence of alternative prey, size- or stage-structure within a population, habitat structure and the densities of the organisms involved (Mylius et al. 2001, Borer et al. 2003, Holt and Huxel 2007, Janssen et al. 2007). Variation in these factors can lead to alternative outcomes, including species coexistence, exclusion, as well as fluctuating and stochastic dynamics of the IG predator and prey.

The effects of intraguild (IG) predator density can vary in its importance depending on how and when density-dependence occurs during ontogeny. Predator aggregation (i.e., numerical responses) that occurs through different mechanisms such as changes in basal or prey resources results in greater predator densities (Mylius et al. 2001, Borer et al. 2003). These changes in density can increase predator-predator interactions (i.e., higher levels of interference competition) that limit their effectiveness in consuming IG prey (Sih et al. 1998). Alternatively, exclusion of IG prey can also occur through increased per capita predation rates at higher IG predator densities (Vance-Chalcraft et al. 2007). Examinations of the numerical responses often focus on fully-developed predators (Borer et al. 2003, Stier et al. 2013), or do not follow predator

growth rates and/or trait changes that result from density-dependence. Similarly, experimental approaches which mimic a numerical response often use a limited range of predator densities (Wissinger 1989, Boone et al. 2002, Balfour et al. 2003), and such experiments infrequently track predator growth rates that limits the inference on how factors such as density-dependent growth can alter predators' ecological role.

In IGP systems, density-dependent processes could ultimately determine relationships between the IG predator and prey by affecting traits of each group, such as growth rates or size. For example, if the average size of the IG predators at high densities is reduced to such a degree that their ability to consume the IG prey is limited, a shift in the nature of their interactions with the putative IG prey towards a solely competitive relationship can occur. Alternatively, reduced densities of the IG predator may allow for higher growth rates, leading to larger predator sizes, and elimination of the IG prey due to enhanced foraging abilities. Direct manipulation of density-dependence, or combined manipulations of density and size that elucidate similar mechanisms, have been infrequently examined, especially in IG predators (Stenhouse et al. 1983, Burley et al. 2006). Furthermore, examinations of growing predators and prey often are focused only on examining prey growth, such as strategies that allow them to coexist with different predators, rather than the mechanisms that induce differences between traits such as gape size of the predator themselves (Urban 2007a). Understanding processes that influence predator growth patterns would thus be critical to determine patterns of coexistence and/or exclusion of interacting species (Holt and Polis 1997).

To investigate the effects of density on an IG predator, and subsequently on its IG prey, we experimentally manipulated larval densities of ringed salamanders (*Ambystoma*

annulatum) in the presence/absence of an IG prey, the spotted salamander (*A. maculatum*). Larval stages of both species occur in pond habitats where IGP is a well-established (e.g., Wissinger and McGrady 1993, Davenport and Chalcraft 2012). Many organisms in these communities have immature stages that compete and/or prey upon one another due to variation in breeding phenology and the occurrence of overlapping generations; both of these processes result in size-structured populations that promote IGP (Polis et al. 1989, Wissinger 1989, Yurewicz 2004). Many of these organisms also show density-dependent growth rates during the larval stage (Semlitsch 1987, Wissinger 1989, Wilbur 1997). For the two focal species, breeding phenology differences (fall versus spring breeding for *A. annulatum* and *A. maculatum*, respectively) influences their relative sizes when they co-occur in ponds, and both exhibit density-dependent growth (Semlitsch and Walls 1993; this study). Thus, the ability of *A. annulatum* to act as an IG predator may depend upon initial densities within a pond if it alters their growth rates and size, and ultimately determines their relationship with *A. maculatum*.

Our primary goal was to examine how density-dependent intraspecific competition in *A. annulatum* would affect conspecific traits (e.g., size at metamorphosis) and survival, as well as the same variables for sympatric *A. maculatum*. We expected larvae of *A. annulatum* reared at low initial densities to have higher growth rates, and thus greater size at metamorphosis as a result of reduced intraspecific competition. This was expected to translate into strong negative effects on *A. maculatum* traits when the two species were jointly reared. Specifically, we hypothesized reduced survival but greater growth rates and larger size at metamorphosis of *A. maculatum* due to thinning effects from the fewer, larger *A. annulatum* (Van Buskirk and Yurewicz 1998). In

contrast, high initial densities of *A. annulatum* larvae were predicted to result in smaller size due to higher levels of intraspecific competition. Survival of *A. maculatum* would increase with these smaller larvae due to reduced size differences between IG predator and prey that would limit predation. Decreased growth and size of *A. maculatum* would occur, however, due to greater intra- and interspecific competition. Reciprocal negative effects from *A. maculatum* on *A. annulatum* were not expected due to the large size differences hypothesized to be present when the species were combined. Facilitation was also an alternative outcome, whereby predation on *A. maculatum* would minimize the effects of intraspecific competition within *A. annulatum* at higher densities, leading to convergent size at metamorphosis in lower density allopatric and higher density sympatric populations. We also examined whether density of the IG predator affected the degree of temporal overlap within the aquatic habitat between the two species, which can have a strong effect on species interactions (Lawler and Morin 1993).

Methods

Study system

Ambystoma annulatum is a pond-breeding salamander endemic to the Ozark Plateau in Missouri, Arkansas and Oklahoma (Petranka 1998). Adults migrate in September through November to breed in ephemeral and permanent ponds. Larvae overwinter in ponds and undergo metamorphosis in the late spring to early summer (Hocking et al. 2008). *Ambystoma maculatum* are ubiquitous across the southeastern US, and a common prey species in studies on IGP (Stenhouse 1985, Brodman 2004, Urban 2007b). Adults of this species often breed in the late winter and early spring in ponds containing *A. annulatum*,

and eggs hatch approximately one month before most *A. annulatum* metamorphose (T.L. Anderson, unpublished data). The larval period for *A. maculatum* lasts approximately 8-10 weeks, leading to metamorphosis that occurs from early summer into the fall. These species use similar breeding pond habitat throughout much of the range of *A. annulatum*, and often occur in sympatry (Hocking et al. 2008; T.L. Anderson, unpublished data). Thus, temporal overlap within the pond environment between larvae of these species can range from approximately a few weeks to two months (T.L. Anderson, unpublished data).

Mesocosm experiment

Experimental pond mesocosms (1000 L, 1.52 m diameter; hereafter, cattle tanks), were set up in September 2011 in a circular array at a fenced outdoor research complex at the University of Missouri in Columbia, MO. Tanks were filled with tap water and allowed to de-chlorinate for 5 days. Approximately 3 kg dry weight of leaves collected from Baskett Wildlife Research Area (BWRA) near Ashland, MO were added to each tank (primarily *Quercus* spp., *Carya* spp. and *Acer* spp.) as a nutrient base and to provide refuge. Tanks were inoculated with concentrated zooplankton, a primary food source of larval salamanders (Taylor et al. 1988) every other day for approximately two weeks following collection from ponds at BWRA (approximately 3 L total volume over two weeks). Tanks were left open to allow for natural colonization by flying insects (e.g., chironomids and other dipterans) as an additional source of food for salamander larvae. Some potentially predaceous insects (dytiscid beetles) also colonized the open tanks but were removed as observed. Holes were drilled into the lip of each tank to permit water drainage, and water was added as needed to maintain constant water depth (~ 50 cm).

Late-stage embryos of *A. annulatum* were collected from Daniel Boone

Conservation Area (DBCA) near Hermann, MO in late September 2011 and transported

to environmental chambers (held at 10 ° C) at the University of Missouri. Cattle tank

water was added every three days to the containers to acclimate the embryos/hatchlings

to mesocosm conditions. Upon hatching, individuals were transferred into plastic cups

filled with tank water for overnight observation to ensure viability of the hatchlings; non-

viable individuals were replaced prior to addition. Hatchlings were then added on

October 13 after a 1 h acclimation period of floating the cups on the water surface within

the cattle tanks. There were four experimental density treatments (8, 16, 24, and 32

hatchings per tank) for *A. annulatum*, with six replicates of each tank. Tanks were

randomly assigned both a density treatment, as well a presence/absence treatment of *A.*

maculatum (see below).

Egg masses of *A. maculatum* were collected during the first week of April 2012

from the same ponds at DBCA where *A. annulatum* collections occurred the previous

fall. Egg masses were allowed to hatch, after which 24 hatchlings were added in a similar

manner to *A. annulatum* on April 10, resulting in prior residence time of 181 days by *A.*

annulatum. We introduced *A. maculatum* to half of the six replicates of the *A. annulatum*

density treatments. The other three replicates of the *A. annulatum* density treatments

served as control treatments for the tanks containing *A. maculatum*. Four control tanks

with no *A. annulatum* and 24 hatchlings of *A. maculatum* were also set up at this same

time but occurred over a period of five days due to slight hatching asynchrony. Due to

several tank failures (i.e., 100 % mortality), however, replication of the *A. maculatum*

addition was not equal across all *A. annulatum* densities. The resulting treatments (with

replicates of *A. maculatum* presence/absence in parentheses) were: four low-density tanks (two with *A. maculatum*, two without), six medium-density (three, three), six medium-high density (three, three), and three high-density (two, one). Weekly nighttime behavioral observations that were conducted on all tanks (T.L. Anderson, unpublished data) indicated that hatchlings added to failed tanks died shortly after addition; no salamanders were ever observed in three of the failed tanks, and one tank exhibited signs of a bacterial infection that resulted in 100% mortality (i.e., dead larvae were observed). No diseased animals were observed in any other tanks, leading us to believe the tank failures do not imply confounding mortality which affected our results. Furthermore, growth rates/sizes of *A. annulatum* match other studies, with similar model selection results (B.H. Ousterhout and R.D. Semlitsch, unpublished data). Thus, despite the loss of some experimental tanks, we feel our results accurately portray interactions between these species.

Both species were captured and measured to assess larval growth at one time point, which occurred at approximately 75 % of aquatic ontogeny (Day 154 for *A. annulatum*, Day 50 for *A. maculatum*); this measurement also provided an estimate of the size of larval *A. annulatum* at the time of *A. maculatum* addition. Captures were performed using aquarium nets at night for *A. annulatum*, and a combination of nets and mesh funnel traps for *A. maculatum*, as visibility was obscured by algae. While the goal was to capture at least three larvae in order to estimate a mean size, three tanks for *A. annulatum* resulted in two, two, and one larvae, respectively. Captures for *A. maculatum* were less successful, as survival was very low in most tanks, often resulting in only one

larval capture per tank. Measurements taken on the larvae included snout-vent length (nearest mm), total length, and mass (nearest 0.01 g).

Beginning in April 2012, tanks were checked every other night for metamorphosing individuals, and continued until the end of the experiment in July, at which point 99% of both species had completed metamorphosis. Individuals were considered metamorphs if gills were < 1 mm in length, in combination with behavior indicative of leaving the tank (e.g., crawling out of the water onto the tank lip). Upon removal, metamorphs were photographed, measured for SVL, TL and mass, and euthanized.

We analyzed population (i.e., cattle tank) mean values for larval SVL (mm), overall growth rate (mm $\times$ day^{-1}), SVL and mass (g) at metamorphosis, days to metamorphosis and survival for each focal species. All data were tested for normality and homogeneity of variances using Shapiro and Levine's tests, respectively; no violations were found and the raw data was used thereafter. Preliminary analyses followed a similar format for each species, where we compared different mechanistic growth models using an AIC model selection approach in the R statistical program version 3.0.0 (R Development Core Team 2013). Maximum likelihood estimates of parameters for each model were calculated using the 'nls' function in R except survival. Survival of both species was modeled with a binomial error structure, and parameter estimates were calculated using the 'mle2' function from the 'bbmle' package (Bolker 2012) in R.

The results of the model selection showed that several models were equally supported for both species (see Appendix A). For *A. annulatum*, the nonlinear models

were also not clearly different from a linear model; thus, we report the results of the linear model for response variables of *A. annulatum*. For *A. maculatum*, the results showed that several nonlinear models were better supported than the linear model. We report the results of the best model for each response (see Appendix A). We used an ANCOVA to test for the effects of conspecific density and the presence/absence of *A. maculatum*. As survival was variable in *A. annulatum*, we used the number of *A. annulatum* metamorphs as the density in analyses of *A. maculatum*. This value likely represents the biologically-relevant predator density that *A. maculatum* experienced rather than the initial densities of *A. annulatum*. Using either density provided qualitatively similar outcomes.

We examined how the final density of *A. annulatum* affected temporal overlap of the two species by comparing linear, three-parameter Michaelis-Menten, negative exponential, Shepherd and power law models (Bolker 2008; Appendix A). We calculated the minimum and maximum days of overlap which were measured as the first and last days that metamorphs of *A. annulatum* were captured, respectively, from a tank. Again, nonlinear models were clearly supported for both temporal overlap metrics, and we report the results of the top model (monomolecular function $[y = a(1-e^{-bX})]$). As nonlinear regression does not produce equivalent test statistics to linear regression, we report the R^2 of the top model as an estimate of the effects of density of *A. annulatum* on *A. maculatum*, and for the relationship of temporal overlap and density (Crawley 2012).

Results

For *A. annulatum*, the linear model and nonlinear models were equally supported, indicating that the pattern of intraspecific density-dependence was slightly nonlinear for most response variables (Fig. 1). Larval SVL for *A. annulatum* was significantly influenced by conspecific density ($R^2 = 0.30$, $t_{1,17} = -2.70$, $P = 0.015$). Larvae of this species from the lowest density tanks were approximately 20% larger in SVL than larvae from the three higher densities. This pattern also existed for *A. annulatum* metamorphs, where individuals from the lowest density were approximately 10% larger in mean SVL, 26% greater mass and had 15% higher mean growth rates than metamorphs from the other three densities (SVL: $R^2 = 0.19$, $t_{1,17} = -2.26$, $P = 0.04$; mass: $R^2 = 0.14$, $t_{1,17} = -2.00$, $P = 0.06$; growth: $R^2 = 0.19$, $t_{1,17} = -2.30$, $P = 0.03$). Individuals from the lowest density treatment completed metamorphosis on average five days earlier than the tanks with 16 and 24 initial densities, and 10 days earlier than the tanks with 32 individuals ($R^2 = 0.17$, $t_{1,17} = 2.17$, $P = 0.04$). Survival of *A. annulatum* in tanks ranged from 13-100% and was greater than 60% for most tanks; no relationship with density was apparent, however (residual deviance = 95.8, $t_{1,17} = -1.01$, $P = 0.3$). Linear ANCOVA models found no significant effects of *A. maculatum* presence for any response variables for *A. annulatum* (all $P > 0.15$); a generalized linear model using a binomial error structure also found no effect of *A. maculatum* on survival of *A. annulatum*.

Nearly all of the most supported models for *A. maculatum* showed a saturating curve where most size and growth metrics of both larvae and metamorphs increased nonlinearly with *A. annulatum* density (Appendix A; Fig. 2). In the control tanks with no *A. annulatum,* larval and metamorphic *A. maculatum* showed nonlinear patterns, and were approximately 15% smaller in SVL ($R^2 = 0.86$ and 0.74, respectively), and had 18%

slower growth rates ($R^2 = 0.74$) compared with high density tanks of *A. annulatum*. The linear model was best supported for metamorph mass, and were mean values were 35% smaller in the control tanks compared to higher density tanks ($R^2 = 0.63$). Survival showed an inverse relationship, however, where survival in control tanks was 86% higher than high density tanks. Even when *A. maculatum* were combined with the lowest density of *A. annulatum*, a strong pattern existed, where survival was three times less and size metrics were greater than 10% compared with control tanks. Days to metamorphosis for *A. maculatum* showed no strong pattern ($R^2 = -0.02$).

Both the maximum and minimum days of overlap within tanks had a strong saturating relationship with the density of *A. annulatum* (maximum days: $R^2 = 0.92$; minimum days: $R^2 = 0.81$, Fig. 3). The two responses approached asymptotes at approximately 20 and 40 for minimum and maximum days of overlap, respectively (Table 2, Fig. 3), indicating a window of approximately three weeks that the larvae overlapped before metamorphosis started to occur for *A. annulatum*. Another three week window of overlap existed during which the density of *A. annulatum* was decreasing due to metamorphosis and extended to just prior to when *A. maculatum* started metamorphosis.

Discussion

Density is an important aspect of predator-prey and IGP interactions that can both limit and enhance the abilities of a predator to consume prey. We found that the density of an IG predator altered its growth rate and size but not its survival, whereas the same density-dependent processes affected both size and survival of its IG prey. These findings support

the conclusion that environments that are poor in quality for an IG predator (i.e., higher intraspecific competition) may be significantly worse for its IG prey. In this system, the more and longer IG predators (larval *A. annulatum*) remained in the aquatic environment, the greater the effect on size and survival of its IG prey. Furthermore, many of the observed patterns also showed asymptotic relationships for the IG prey, whereby density thresholds occurred for interspecific interactions. This pattern was also reversed for the IG predator and prey, such that the IG predator, *A. annulatum*, showed a negative pattern of intraspecific density-dependence and *A. maculatum*, the IG prey, a positive saturating function with interspecific density (Figs. 1, 2). No reciprocal effects from the IG prey on the IG predator were observed, indicating the direct interactions between these species may be highly asymmetric and unidirectional. The hypothesis that consumption of *A. maculatum* would reduce the negative effects of increased density for *A. annulatum* (i.e., convergence on similar size at metamorphosis between low and high densities when sympatric) was also not supported here.

Inverse relationships of survival and size with density were apparent (i.e., thinning effects), where both the control and the lowest density of the IG predator resulted in higher survival but a reduced size of its IG prey. Higher densities of *A. annulatum* nearly eliminated the IG prey, but the survivors were substantially larger (Fig. 2). These thinning effects are a common occurrence in intraguild and predator-prey interactions, especially among aquatic invertebrates and amphibians (Van Buskirk and Yurewicz 1998, Brodin and Johansson 2002). The resulting larger size of *A. maculatum* metamorphs from tanks that experienced thinning indicates the potential for higher juvenile survival (Scott 1990, 1994, Rothermel and Semlitsch 2006), leading us to

hypothesize a potential feedback loop between fall and spring breeding salamanders: predation by larvae of fall breeding species may reduce overall densities (i.e., reduced competition) of larvae of spring breeders that facilitates higher juvenile survival due to larger size at metamorphosis. While we cannot conclusively say that predation was the cause of the effects on *A. maculatum* (resource depletion by *A. annulatum* is also a potential mechanism), zooplankton were still visible in most tanks, indicating resources were not exhausted (T.L. Anderson, personal observation). Hatchlings of *A. maculatum* are palatable to *A. annulatum* larvae (T.L. Anderson, unpublished data), but their importance to, or prevalence in, the diet of *A. annulatum* is unknown. The densities that we manipulated may not have been strong enough to eliminate the potential for predation, which would be needed to examine the sole effects of competition. However, the experimental densities and sizes did match field observations of larval size distributions for the focal species (Shoop 1974, Peterson et al. 1991, Petranka 1998).

Sympatric populations of the focal species exist throughout much of the range of *A. annulatum*, but an understanding of the mechanisms that permit coexistence is lacking. Field studies of other fall -breeding salamanders (e.g., *A. opacum*) suggest that high larval densities of these IG predators affect patterns of abundance of *A. maculatum*, but their interactions alone do not exclude them from ponds (Stenhouse 1987, Urban 2007b). Intraguild predation theory predicts that the IG prey should be superior in resource exploitation to persist with an IG predator (Holt and Polis 1997). This hypothesis has some empirical support (Morin 1999, Borer et al. 2003) but has not been tested for many systems. Larval *A. maculatum* are superior exploitative foragers as larvae over congeneric competitors (Walls 1996), but comparisons of foraging efficiency between

fall and spring-breeding salamanders (i.e., IG predators and prey) have not been performed. IGP theory also predicts that basal resources must be sufficiently high for the IG predator to persist (Holt and Polis 1997, Mylius et al. 2001); in our system, either this was the case, or larval *A. annulatum* were more efficient than *A. maculatum* at consuming basal food resources. Alternatively, because the window of larval interactions is short for these two species compared to their larval period lengths, the relative consumptive abilities may be inconsequential to the outcome of their interactions; *A. maculatum* just has to survive past when metamorphosis occurs for *A. annulatum* to allow for their own metamorphosis.

Other factors may also influence their coexistence. In particular, predation on larval *A. annulatum* may reduce their densities to such a degree that the relative impacts of the few remaining larvae do not result in exclusion of *A. maculatum*. Predictions from IGP models also suggest that abundant alternative prey or increased habitat complexity may promote coexistence (Holt and Huxel 2007; Janssen et al. 2007). In ponds where high densities of *A. annulatum* occur, both of these factors may promote their coexistence with *A. maculatum*, despite density-dependent growth that would prolong their temporal overlap. Other prey sources, such as eggs and larvae of other spring-breeding amphibians, as well increased densities of invertebrate prey as the season progresses due to warmer water temperatures, provides additional food sources for *A. annulatum* which may reduce predation on *A. maculatum*. Spatial segregation within a pond by other ambystomatids suggests some partitioning of the aquatic microhabitat occurs in this guild, including the use of vegetated areas or locations of increased habitat complexity by IG prey species that would reduce encounter rates with IG predators (Brodman and

Krause 2007). The effects of density on behavioral strategies such as spatial segregation are unknown, however. Other empirical studies of IGP systems have also found increased habitat complexity can reduce the incidence of IGP (Finke and Denno 2006).

An important implication from this study is when density-dependent processes occur in an IG predator, a potential switch in the type of interactions between two species (i.e., from predation to competition) can follow (Yang and Rudolf 2010). This effect may be particularly relevant in systems where priority effects occur. Density-dependent inhibition of growth in the early-arriving species (a presumed IG predator) may negate their temporal advantage by reducing size asymmetries between species, thereby shifting the relationship from predator-prey to competition. In our study system, *A. annulatum* breeds in the fall and their larvae overwinter in ponds, providing them with a significant size advantage over hatchlings of *A. maculatum* that appear in the spring. Yet, high densities of larval *A. annulatum* may inhibit their own growth rates to such a degree that they are equally sized with their IG prey, resulting in increased competition and reduced predation effects. The intensity of species interactions may also scale to the temporal duration over which they occur, such that the influence of priority effects may matter more when the degree of overlap is greater (Yang and Rudolf 2010). When resources are exploited in temporal pulses, such as when breeding occurs in ephemeral bodies of water by invertebrates or amphibians, the degree of overlap plays an important role in the outcome of interactions (Alford 1989, Lawler and Morin 1993). Minimal overlap due to phenological mismatches or temporal segregation would reduce the effect of competition or predation and result in potentially negligible effects on the inferior/prey species (Alford 1989, Yang and Rudolf 2010), whereas longer overlap increases encounter rates,

amplifying the effects of each interaction. In our study, temporal overlap ranged from 14 to 51 days, which is within the range of observed from field observations of these two species but only a portion of the potential duration as *A. annulatum* metamorphs have been observed exiting ponds as late as July (Anderson et al. 2015). The density of the IG predator increased this overlap at higher densities due to slower growth rates, but was shown to saturate at a density threshold (Fig. 3). When temporal overlap is greater due to higher density-dependence, increased competition between individuals of the IG prey that survive initial predation may occur.

Overall, the results of our study show that when traits such as size are affected by density, the resulting differences between individuals can determine the type and strength of species interactions that occurs. This effect is important because the cascading effects of competition and predation/IGP can result in different outcomes for both the individual species, as well as have community-level consequences (Chase et al. 2002). For species that undergo ontogenetic transitions between life stages, such as metamorphosis in amphibians and insects, this study indicates varying densities can also alter the temporal overlap of several species, which can significantly affect the outcome of their interactions (Lawler and Morin 1993). Further manipulations that vary both temporal overlap and density would reveal the relative strengths of these two mechanisms, and simultaneous manipulations of IG predator and prey densities would be useful to understand how density-dependent processes in each species contributes to the outcome of their interactions.

Acknowledgements

References

Alford, R. A. 1989. Variation in predator phenology affects predator performance and prey community composition. Ecology **70**:206-219.

Anderson, T. L., D. J. Hocking, C. A. Connor, J. E. Earl, E. B. Harper, M. S. Osbourn, W. E. Peterman, T. A. Rittenhouse, and R. D. Semlitsch. 2015. Abundance and phenology patterns of two pond-breeding salamanders determine species interactions in natural populations Oecologia **177**:761-773.

Arim, M., and P. A. Marquet. 2004. Intraguild predation: a widespread interaction related to species biology. Ecology Letters **7**:557-564.

Balfour, R. A., C. M. Buddle, A. L. Rypstra, S. E. Walker, and S. D. Marshall. 2003. Ontogenetic shifts in competitive interactions and intra-guild predation between two wolf spider species. Ecological Entomology **28**:25-30.

Bolker, B. M. 2008. Ecological models and data in R. Princeton University Press, Princeton, NJ.

Bolker, B. M. 2012. bbmle: Tools for general maximum likelihood estimation R package

    version 1.0.5.2 http://CRAN.R-project.org/package=bbmle

Boone, M. D., D. E. Scott, and P. H. Niewiarowski. 2002. Effects of hatching time for

    larval ambystomatid salamanders. Copeia **2**:511-517.

Borer, E. T., C. J. Briggs, W. W. Murdoch, and S. L. Swarbrick. 2003. Testing intraguild

    predation theory in a field system: does numerical dominance shift along a

    gradient of productivity? Ecology Letters **6**:929-935.

Brodin, T., and F. Johansson. 2002. Effects of predator-induced thinning and activity

    changes on life history in a damselfly. Oecologia **132**:316-322.

Brodman, R. 2004. Intraguild predation on congeners affects size, aggression, and

    survival among *Ambystoma* salamander larvae. Journal of Herpetology **38**:21-26.

Brodman, R., and H. D. Krause. 2007. How blue-spotted and small-mouthed salamander

    larvae coexist with their unisexual counterparts. Herpetologica **63**:135-143.

Burley, L. A., A. T. Moyer, and J. W. Petranka. 2006. Density of an intraguild predator

    mediates feeding group size, intraguild egg predation, and intra- and interspecific

    competition. Oecologia **148**:641-649.

Chase, J. M., P. A. Abrams, J. P. Grover, S. Diehl, P. Chesson, R. D. Holt, S. A.

    Richards, R. M. Nisbet, and T. J. Case. 2002. The interaction between predation

    and competition: a review and synthesis. Ecology Letters **5**:302-315.

Crawley, M. J. 2012. The R book. Wiley, West Sussex, England.

Davenport, J. M., and D. R. Chalcraft. 2012. Evaluating the effects of trophic complexity

    on a keystone predator by disassembling a partial intraguild predation food web.

    Journal of Animal Ecology **81**:242-250.

Finke, D. L., and R. F. Denno. 2006. Spatial refuge from intraguild predation: implications for prey suppression and trophic cascades. Oecologia **149**:265-275.

Hocking, D. J., T. A. Rittenhouse, B. B. Rothermel, J. R. Johnson, C. A. Connor, E. B. Harper, and R. D. Semlitsch. 2008. Breeding and recruitment phenology of amphibians in Missouri oak-hickory forests. American Midland Naturalist **160**:41-60.

Holt, R. D., and G. R. Huxel. 2007. Alternative prey and the dynamics of intraguild predation: theoretical perspectives. Ecology **88**:2706-2712.

Holt, R. D., and G. A. Polis. 1997. A theoretical framework for intraguild predation. American Naturalist **149**:745-764.

Janssen, A., M. W. Sabelis, S. Magalhães, M. Montserrat, and T. van der Hammen. 2007. Habitat structure affects intraguild predation. Ecology **88**:2713-2719.

Lawler, S. P., and P. J. Morin. 1993. Temporal overlap, competition, and priority effects in larval anurans. Ecology **74**:174-182.

Morin, P. 1999. Productivity, intraguild predation, and population dynamics in experimental food webs. Ecology **80**:752-760.

Mylius, S. D., K. Klumpers, A. W. de Roos, and L. Persson. 2001. Impact of intraguild predation and stage structure on simple communities along a productivity gradient. American Naturalist **158**:259-276.

Peterson, C. L., R. F. Wilkinson, D. Moll, and T. Holder. 1991. Premetamorphic survival of *Ambystoma annulatum*. Herpetologica **47**:96-100.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington [D.C.].

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics **20**:297-330.

R Development Core Team. 2013. R: A Language and Environment for Statistical Computing 3.0.0 http://www.R-project.org/

Rothermel, B., and R. Semlitsch. 2006. Consequences of forest fragmentation for juvenile survival in spotted (*Ambystoma maculatum*) and marbled (*Ambystoma opacum*) salamanders. Canadian Journal of Zoology **84**:797-807.

Scott, D. E. 1990. Effects of larval density in *Ambystoma opacum*: An experiment in large-scale field enclosures. Ecology **71**:296-306.

Scott, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology **75**:1383-1396.

Semlitsch, R. D. 1987. Density-dependent growth and fecundity in the paedomorphic salamander *Ambystoma talpoideum*. Ecology **68**:1003-1008.

Semlitsch, R. D., and S. C. Walls. 1993. Competition in two species of larval salamanders: a test of geographic variation in competitive ability. Copeia **1993**:587-595.

Shoop, C. R. 1974. Yearly variation in larval survival of *Ambystoma maculatum*. Ecology **55**:440-444.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends Ecol Evol **13**:350-355.

Stenhouse, S. L. 1985. Interdemic variation in predation on salamander larvae. Ecology **66**:1706-1717.

Stenhouse, S. L. 1987. Embryo mortality and recruitment of juveniles of *Ambystoma maculatum* and *Ambystoma opacum* in North Carolina. Herpetologica **43**:496-501.

Stenhouse, S. L., N. G. Hairston, and A. E. Cobey. 1983. Predation and competition in *Ambystoma* larvae: field and laboratory experiments. Journal of Herpetology **17**:210-220.

Stier, A. C., S. W. Geange, and B. M. Bolker. 2013. Predator density and competition modify the benefits of group formation in a shoaling reef fish. Oikos **122**:171-178.

Taylor, B. E., R. A. Estes, J. H. K. Pechmann, and R. D. Semlitsch. 1988. Trophic relations in a temporary pond: larval salamanders and their microinvertebrate prey. Canadian Journal of Zoology **66**:2191-2198.

Urban, M. C. 2007a. The growth-predation risk trade-off under a growing gape-limited predation threat. Ecology **88**:2587-2597.

Urban, M. C. 2007b. Predator size and phenology shape prey survival in temporary ponds. Oecologia **154**:571-580.

Van Buskirk, J., and K. L. Yurewicz. 1998. Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. Oikos **82**:20-28.

Vance-Chalcraft, H. D., J. A. Rosenheim, J. R. Vonesh, C. W. Osenberg, and A. Sih. 2007. The influence of intraguild predation on prey suppression and prey release: a meta-analysis. Ecology **88**:2689-2696.

Walls, S. C. 1996. Differences in foraging behaviour explain interspecific growth inhibition in competing salamanders. Animal Behaviour **52**:1157-1162.

Wilbur, H. M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology **78**:2279-2302.

Wissinger, S., and J. McGrady. 1993. Intraguild predation and competition between larval dragonflies: direct and indirect effects on shared prey. Ecology **74**:207-218.

Wissinger, S. A. 1989. Seasonal variation in the intensity of competition and predation among dragonfly larvae. Ecology **70**:1017-1027.

Yang, L. H., and V. H. Rudolf. 2010. Phenology, ontogeny and the effects of climate change on the timing of species interactions. Ecology Letters **13**:1-10.

Yurewicz, K. L. 2004. A growth/mortality trade-off in larval salamanders and the coexistence of intraguild predators and prey. Oecologia **138**:102-111.

Figure Legends

Fig. 1: Response variables for ringed salamanders (*Ambystoma annulatum*): larval snout-vent length (SVL) (a), metamorph SVL (b), mass (c), growth rate (d), days to metamorphosis (e), and survival (f). All points represent mean values from each cattle tank. The *x*-axis is the initial number of *A. annulatum*. The lines represent the predicted values from the top model of each response.

Fig. 2: Response variables for spotted salamanders (*Ambystoma maculatum*): larval snout-vent length (SVL) (a), metamorph SVL (b), mass (c), growth rate (d), days to metamorphosis (e), and survival (f). All points represent mean values from each cattle tank. The *x*-axis is the number of surviving ringed salamanders (*A. annulatum*), which better represents the predator density experienced by *A. maculatum*. The zero density of *A. annulatum* is the control tanks for *A. maculatum*. The lines represent the predicted values from the top model of each response.

Fig. 3: The relationship between final ringed salamander (*Ambystoma annulatum*) density and days of temporal overlap with spotted salamanders (*A. maculatum*). Maximum days of overlap (solid line, filled circles) was calculated as the last day an *A. annulatum* metamorphosed from a tank with *A. maculatum*. Minimum days of overlap (dashed line, open circles) represents when the first *A. annulatum* metamorphosed from a tank. The lines represent the predicted values from the top model of each response. The zero density of *A. annulatum* is the control tanks for *A. maculatum*.
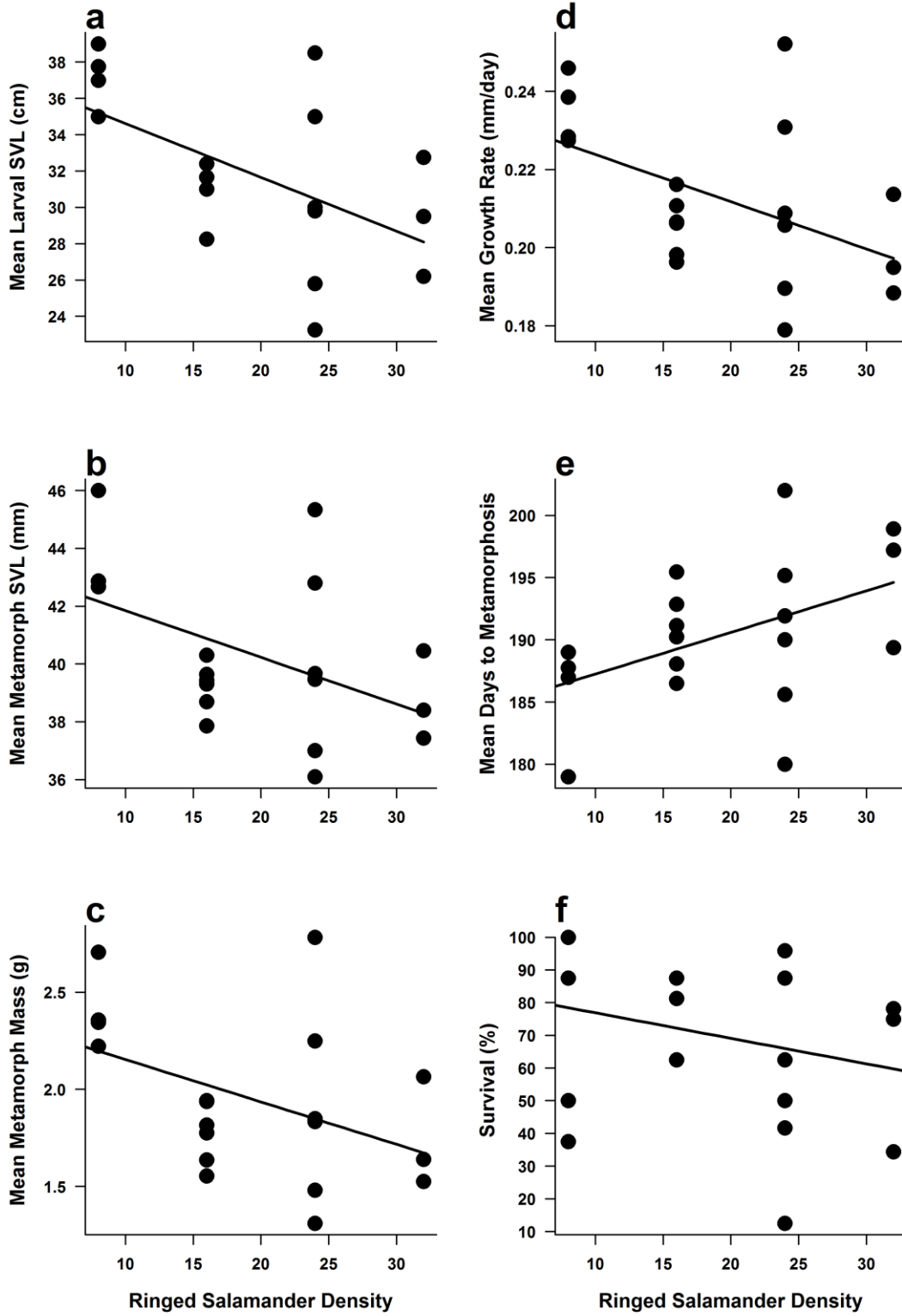
Figure 1
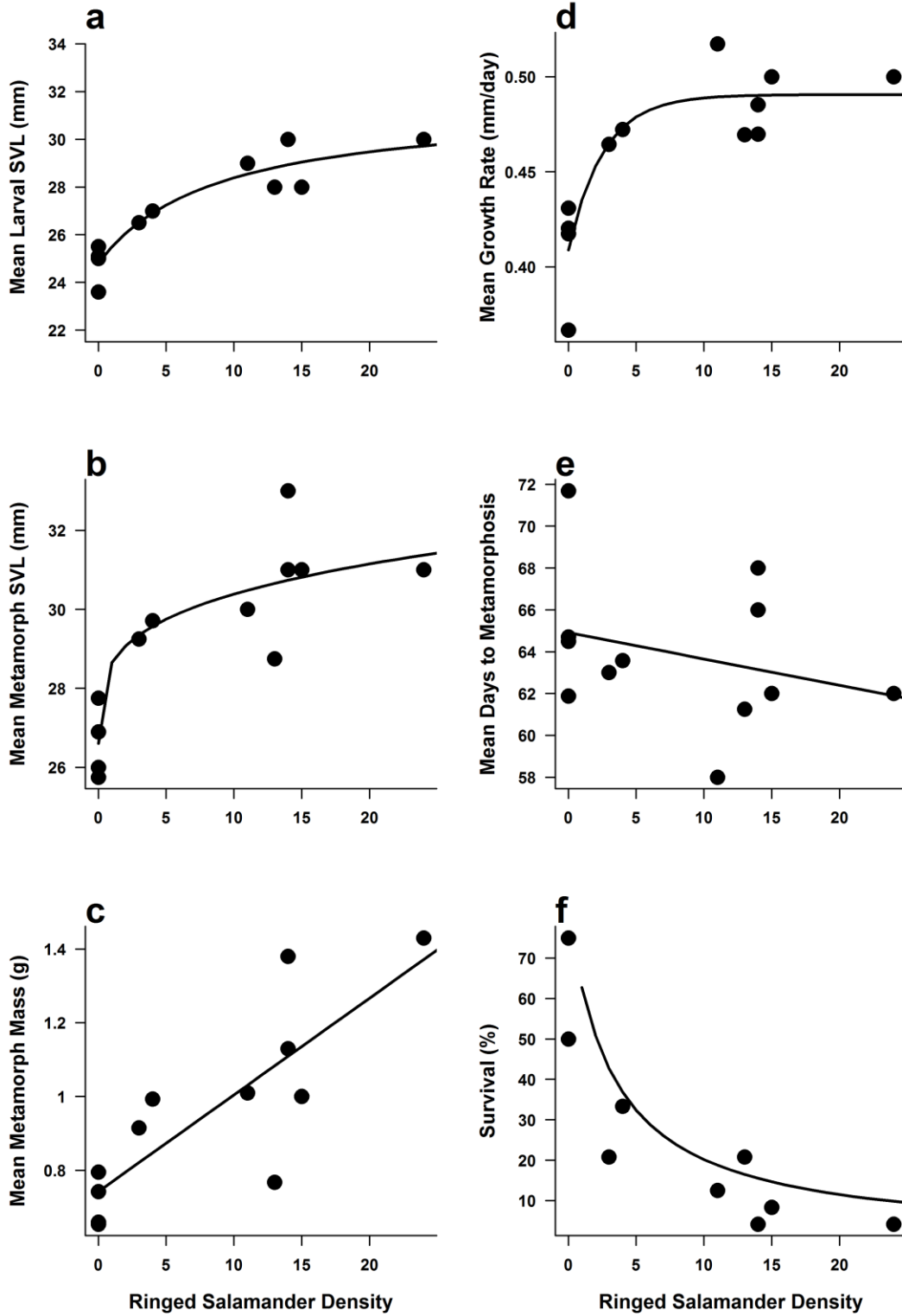
Figure 2
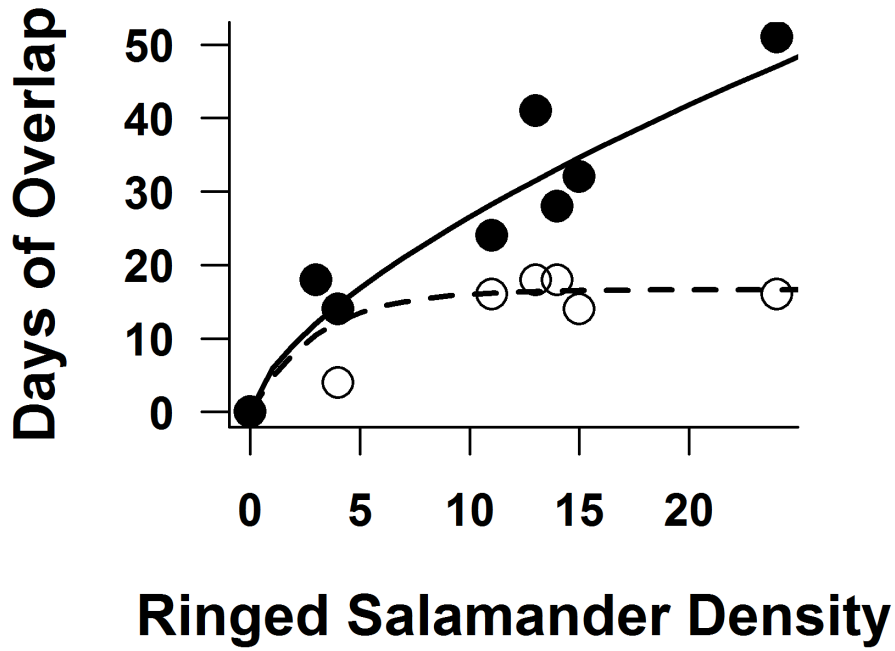
Figure 3

Chapter 3

TOP PREDATORS AND HABITAT COMPLEXITY ALTER AN INTRAGUILD

PREDATION MODULE IN POND COMMUNITIES

Thomas L. Anderson and Raymond D. Semlitsch

Abstract

Predator diversity and habitat complexity frequently influence species interactions at lower trophic levels, yet their joint investigation has been performed infrequently despite the demonstrated importance of each individual factor. We investigated how different top predators and varying habitat complexity influence the function of an intraguild predation module consisting of two larval salamanders, intraguild predator *Ambystoma annulatum* and intraguild prey *A. maculatum*. We manipulated predator food webs and habitat complexity in outdoor mesocosms. Top predators significantly influenced body condition and survival of *A. annulatum*, but habitat complexity had minimal effects on either response. A three-way interaction among the covariates top predator identity, habitat complexity and *A. annulatum* survival influenced body condition and survival of *A. maculatum* via a density mediated indirect effect. Different top predator combinations had variable effects in different habitat complexity treatments on intraguild predator (*A. annulatum*) survival that subsequently influenced intraguild prey (*A. maculatum*) body condition and survival. Future work should consider how different top predators influence other food web components, which should vary due to predator attributes and the physical environments in which they co-occur.

Keywords: ambystomatid, amphibian, competition, density-mediated effect, mosquitofish, salamander

Introduction

Community structure is simultaneously determined by both biotic and abiotic factors (Morin 2011). Food web composition or diversity, traits of each organism, and their relative abundances can all alter species interactions, which altogether are simultaneously influenced by habitat heterogeneity or complexity. These synergistic components of communities result in highly complex systems that are often difficult to interpret, as they contain direct and indirect effects, feedback loops, and other context-dependent associations (Werner and Peacor 2003, Agrawal et al. 2007). Yet, teasing apart the complexity of such systems is a priority, as nuanced and synergistic processes concurrently affect population dynamics, species interactions and community structure. Here, we test how predator food web complexity and habitat heterogeneity simultaneously influence intraguild predation.

Intraguild predation (IGP) is a widespread interaction that occurs in many ecosystems (Arim and Marquet 2004) that can strongly influence population dynamics and community structure (Polis et al. 1989, Polis and Holt 1992, Holt and Polis 1997). IGP is most commonly depicted as a simplified food web (i.e. module) composed of three nodes: an intraguild (IG) predator, an IG prey, and their shared basal resource. In addition to competing for resources, the IG predator and IG prey also can prey upon one another (Holt and Polis 1997). Examinations of simplified food webs such as IGP modules have been useful to understand the importance of species interactions. However, reducing complex ecological communities to two or three-way interactions may not capture important underlying processes that alter their outcomes. These underlying factors can stem from within an IGP module, such as the amount of basal resources (Borer et al.

2003), or from size and age structure of the predator and/or prey populations (Mylius et al. 2001). IGP modules can also be affected by external factors, such as habitat or food web complexity (e.g. Finke and Denno 2002, Finke and Denno 2004). When food webs consist of multiple predator species, simultaneously competing with and preying upon one another (i.e. multispecies IGP; Holt and Huxel 2007), further complications can arise in attempting to understand predator population dynamics, as different species can have both direct and indirect effects on each other as well their shared prey. Although examination of diverse predator communities is more difficult due to the myriad of connections among species, understanding of these systems is critical given the importance of predator identity and diversity to overall trophic dynamics (McPeek 1998, Finke and Denno 2004, Schmitz 2007).

In addition to biotic factors, the physical landscape can further alter the functioning and outcome of IGP modules. In particular, increased habitat complexity can mediate the effect of predators by limiting visibility of prey to predators, altering attack rates of predators, and providing refuge that predators cannot navigate into (Swisher et al. 1998, Finke and Denno 2002, Warfe and Barmuta 2006, Hossie and Murray 2010). Alternatively, when different predators exhibit complementary hunting strategies, prey can be driven from refuges (or habitat types), increasing their susceptibility to other predators in a different habitat (Huang and Sih 1990, Soluk 1993, Losey and Denno 1998, Carey and Wahl 2010). The effect of habitat complexity also varies in its impacts on the outcome of IGP, and depends on traits of both the predators and prey. Because of this, predicting the impact of habitat complexity on IGP is difficult. Furthermore, few studies have evaluated the overall importance of habitat complexity, predator food web

complexity, and their synergistic effects (e.g. Finke and Denno 2006, Grabowski et al. 2008). Such studies are needed to synthesize the effects of habitat complexity on IGP and overall community structure.

IGP in pond communities is largely driven by size-mediated priority effects, whereby early-arriving species attain larger sizes over later-arriving species, which provides them predatory and competitive advantages (Rasmussen et al. 2014). This type of priority effect occurs for many organisms that utilize pond habitats, including larval amphibians (e.g. ambystomatid salamander larvae) and holometabolous insects (e.g. dragonfly naiads) (Wissinger 1989, Padeffke and Suhling 2003, Segev and Blaustein 2007, Urban 2007, Anderson and Semlitsch 2014). Initial ontogenetic stages of both early-arriving (i.e. the IG predator) and later-arriving (the IG prey) species are subject to predation by a wide diversity (and in some cases extremely high densities) of vertebrate and invertebrate predators (Urban 2007). Individuals of the early-arriving species that escape predation transition into size classes impervious to gape-limited predators, and can exist as IG predators (or top predators) and superior competitors to later-arriving species due to size advantages gained during ontogeny (Mylius et al. 2001, Miller and Rudolf 2011, Rasmussen et al. 2014). Therefore, the factors that influence the number of IG predators that survive, and the size of each individual relative to their IG prey, are critical to determining the outcome of IGP. Furthermore, the relative size of the IG predator to IG prey influences the type of interaction that occurs, where smaller size classes of IG predators may primarily compete with their IG prey and larger size classes primarily interact with smaller size classes as predator and prey (Hin et al. 2011). As predation limits survival and often alters growth rates of prey, top predator identity, diversity and

abundance would be expected to influence this link between IG predator and prey, but has infrequently been investigated (Wissinger et al. 1999). Habitat features also frequently influence predation in pond communities by altering attack rates, as well as providing refuges for prey species (Babbitt and Tanner 1998, Baber and Babbitt 2004, Hossie and Murray 2010). The simultaneous effects of predator food web composition and habitat complexity on IGP modules have only been infrequently investigated in pond systems.

We tested whether the presence of different top predators and increasing habitat complexity influenced the outcome of an intraguild predation module within experimental pond food webs (Figure 1). Specifically, we tested whether different combinations of three top predators in pond food webs that varied in life history traits influenced an IGP module among larval stages of two species of salamanders. We expected survival and body size of each salamander species to vary by predator combination due to gape limitations, hunting strategies, and the presence/absence of habitat complexity.

Methods

Study system

Our experimental food webs consisted of up to five species that commonly co-occur in ponds in Missouri, USA (Shulse et al. 2010, Peterman et al. 2014) : ringed salamanders (*Ambystoma annulatum*) and spotted salamanders (*A. maculatum*), the central newt (*Notophthalmus viridescens louisianensis*), aeshnid dragonfly nymphs (*Anax* spp.), and adult mosquitofish (*Gambusia holbrooki*; Figure 1). Larval stages of the two salamanders

form an IGP module because of differences in breeding phenology (*A. annulatum* = fall-breeder and IG predator; *A. maculatum* = spring-breeder and IG prey) that lead to larval size asymmetries that permits predation (Anderson and Semlitsch 2014). High larval densities of *A. annulatum* positively affect body size and negatively affect survival of *A. maculatum* (Anderson and Semlitsch 2014), but individual *A. annulatum* are limited in their attack rates if size disparities with *A. maculatum* are reduced (Thomas L. Anderson, unpubl. data), creating the expectation that the IGP module would be altered if top predators influenced either growth or survival of *A. annulatum*.

Because the top predators exhibit variable hunting strategies and have different gape limitations, alteration to IGP between salamanders should vary based on the top predator present. Newts are visually oriented, active foragers that search through dense vegetation and leaf litter for prey items (Petranka 1998). Mosquitofish are an introduced species of top minnow that actively forages in open water as well as in dense vegetation (Baber and Babbitt 2004), and often have substantial effects on amphibian recruitment (Segev et al. 2009, Shulse et al. 2012, Shulse and Semlitsch 2013). In contrast, aeshnid dragonfly naiads are voracious predators of larval amphibians that use ambush tactics (i.e. sit and pursue; Preisser et al. 2007). All three top predators can consume hatchlings of both species (Walters 1975, Drake et al. 2014). However, mosquitofish and newts are both gape-limited, and salamander larvae quickly grow into size classes invulnerable to consumption (Urban 2007, Shulse and Semlitsch 2013). Dragonfly nymphs are less gape-limited and can consume large amphibian larvae, meaning ambystomatid larvae are susceptible throughout their aquatic ontogeny (Caldwell et al. 1980).

Based on these life history traits, we expected the gape-unlimited dragonflies would have the greatest impact on both salamanders, followed by the gape-limited mosquitofish and adult newts (Preisser et al. 2007, Davenport et al. 2014). We also expected predation by larval dragonflies, a sit-and-pursue predator, would be heightened in more structurally complex habitats than the active predators (mosquitofish and newts) (Preisser et al. 2007, Orrock et al. 2013). If the top predators influenced the IG predator (*A. annulatum*), we expected cascading effects would ensue for the IG prey (*A. maculatum*).When all three predators were present, we expected the greatest overall negative effects as all microhabitats (cover and open water) would be occupied by the different predators. Finally, we only focus on the direct consumptive effects of top predators on the larval salamanders, though we recognize interspecific competition for zooplankton or other invertebrates, as well as non-consumptive effects (i.e. behavioral shifts), likely occur among predators and with both salamander species (Davenport et al. 2014).

Experimental set-up

We conducted the experiment in 1000 L outdoor mesocosms (hereafter, tanks) from October 2012 to July 2013. Tanks were arranged in a rectangular array within a fenced enclosure at the University of Missouri, Columbia, MO, USA. We completely filled tanks with tap water on 6 September 2012, and allowed them to de-chlorinate for approximately two wks. We added 2.5 kg of dry leaves to each tank that were collected from Baskett Wildlife Area near Ashland, MO on 21 September. Concentrated zooplankton additions collected from nearby wildlife ponds began on 25 September and continued until the start of the experiment, totaling approximately 3 L in volume per tank.

We initially covered tanks with window screen covers to prevent colonization by predaceous insects, but were removed at the initiation of the experiment as many of these predators were entering dormancy. Libellulid dragonflies did colonize tanks in the last few weeks of the experiment, but none reached a size that would allow them to prey upon the remaining larval salamanders.

We collected eggs of *A. annulatum* from two pond basins at Fort Leonard Wood (FLW), MO in late October 2012, and brought them back to finish development in indoor environmental chambers near the mesocosm array. We added hatchlings ($n = 32$ individuals) to each tank on 4 November. We collected eggs of *A. maculatum* in early April 2013 from the same locations at FLW where *A. annulatum* collections had occurred. We added 24 hatchlings of *A. maculatum* split across two separate addition dates (12 hatchlings on 24 and 29 April) due to hatching asynchronies. Densities of both species matched those found in natural populations (Thomas L. Anderson, unpubl. data).

We collected predators from several ponds at FLW on 3 November 2012. They were transported back to the University of Missouri and stored in a controlled temperature room at approximately 10 degrees C prior to adding to tanks on 10 November. We collected predators of approximately equal size within each group. Mean total length of mosquitofish was 38.9 ± 5.18 SD mm. For newts, we tried to collect only immature or male adult newts (mean ± SD snout-vent length [SVL] = 36.5 ± 2.4 mm; mean ± SD head width (HW) = 7.2 ± 0.4 mm). We did not identify aeshnids below family, assuming functional equivalency between species. Because dragonflies were of approximately equal size (ca. 5th instar), we expected behavioral interference among the naiads, but did not expect predation between dragonflies; we also did not expect them to

physically interact with newts or mosquitofish. Due to the duration of the experiment, reproduction by newts and mosquitofish occurred in some tanks during the last few weeks of the experiment. Nearly all of *A. annulatum*, and the majority of *A. maculatum* had metamorphosed by this point, however, suggesting these additional animals likely had minimal effects on the outcome of the experiment.

We manipulated habitat complexity within tanks by randomly assigning vertical structure treatments to half of all predator treatment replicates on 1 November (Figure A1 in Appendix A). Structure consisted of strips of 70% shade cloth (ca. 50 x 5 cm, PAK Unlimited, Cornelia, GA) that were glued along the entire length of five separate pieces of polyvinyl chloride (PVC) pipe (lengths of 35, 41, 49, 55 and 58 cm). We added the five pieces of PVC parallel to each other to the south side of each tank, spaced about 20 cm apart, with the longest piece spanning the center of the tank. The shade cloth was sufficiently buoyant to remain vertical (perpendicular to the water surface) throughout the course of the experiment, and would be analogous to cattails in natural ponds in both structure and density. Thus, our habitat complexity treatments were absent (leaves only) and present (leaves + vertical cover).

Our design included twelve total experimental treatments with four replicates per treatment (Figure 1). These treatments included six food webs that were then crossed with the presence/absence of vertical cover. The six food webs included a simple IGP module consisting of only larval salamanders and no top predators, the IGP module + mosquitofish only ($n = 3$ individuals), the IGP module + newts only ($n = 2$ individuals), the IGP module + aeshnids only ($n = 4$ individuals), the IGP module + aeshnids + newts, and the IGP module + all three predators (Figure 1). We did not have all possible

45

combinations of predators because of the logistical difficulties in setting up that many mesocosms; therefore, our results are somewhat limited on how multiple top predators affect IGP. The densities of predators we used are low relative to some natural populations, but given that we were interested in the effects of lethal predation, the low densities were expected to permit survival of at least some ambystomatids. Our predator treatments were additive in nature for the combinations that involved more than one species (i.e. overall density of predators was not held constant).

If dead and/or sick looking predators were observed, we replaced them with similarly-sized individuals collected from a nearby pond to maintain a constant density until metamorphosis had begun for *A. annulatum* (20 May 2013). The number of predators replaced was relatively low ($n = 6$ newts, $n = 3$ aeshnids), and primarily occurred due to overwintering mortality. While replacement of predators stopped once *A. annulatum* began to undergo metamorphosis, the presence of exuvia and/or dead nymphs that unsuccessfully metamorphosed was recorded to estimate the length of time that aeshnids overlapped with both salamander species. While this would impose a different predation regime on *A. maculatum* (i.e. a shorter length of time with aeshnids), it matches the natural phenology of pond food webs. All mosquitofish and newts were recovered from tanks at the end of the experiment.

We monitored larval *A. annulatum* growth by capturing larvae at night at two time points during the spring (ca. 6 April and 22 April). We captured up to five larvae per tank, and salamanders were dorsally photographed in a plastic tray filled with water over a ruler. We then measured approximate snout-vent length (SVL) by measuring to the distal junction of the hind limbs to the body using ImageJ (Rasband 1997).

Beginning in May 2013, we checked tanks for metamorphosing individuals at least every other night. We removed salamanders that had completely reabsorbed gills from tanks, and recorded SVL, total length and mass (in grams) measurements, as well as the date. Size at metamorphosis is correlated to adult fecundity, fitness and survival in amphibians (Semlitsch et al. 1988, Scott 1994), and thus is important to assess as a proxy for predation effects on population dynamics. We scored metamorphs for injury in four categories: (1) missing tail tip, (2) missing limb, (3) missing majority of tail, and (4) missing multiple body parts (Semlitsch and Reichling 1989). We terminated the experiment the second week of July after > 90% of salamanders had completed metamorphosis, drained the tanks and carefully searched the leaf litter to recover any remaining salamanders and predators.

Analysis

We analyzed body condition at metamorphosis and larval period length for both *A. annulatum* and *A. maculatum* using linear mixed models in the *lme4* package within R (Bates et al. 2015, R Development Core Team 2015). Body condition was calculated by dividing mass by SVL ($g*m^{-1}$). Individual salamanders were used as data points with tank as a random effect in all mixed models to account for non-independence of the response variables. We analyzed survival of both species with generalized linear models using a quasibinomial error structure to account for overdispersion. We calculated survival as the number of metamorphs and larvae successfully recovered from tanks when the experiment was terminated. The majority of *A. annulatum* had completed metamorphosis (99%) whereas a greater number of *A. maculatum* remained larval (75% had undergone metamorphosis). Therefore, for *A. maculatum* we separately analyzed overall survival

(larvae + metamorphs), the number that underwent metamorphosis, and the number that remained larval in each treatment. We analyzed metamorph injury for *A. annulatum* using a two-column matrix response variable (number injured, number uninjured) with a generalized linear model and a quasibinomial error distribution; predator treatment, cover and their interaction were predictive factors. Few injuries were observed on metamorphs of *A. maculatum*, leading us to not statistically analyze these individuals.

In all models for both species, we tested for the main effects of predator treatment, cover and their interaction. For *A. maculatum*, we also added the survival of *A. annulatum* as an additional covariate, including all two- and three-way interaction terms. We retained interaction terms at marginally significant p-values ($P < 0.1$) as they contributed meaningful variation to the outcome, but dropped the three-way interaction if $P > 0.1$. We always included the habitat and predator treatment interaction term, as that was our original experimental design. Tukey's HSD post-hoc tests were performed to examine treatment differences using the "*lsmeans*" package in R (Hothorn et al. 2008). The survival of *A. annulatum* was also included to understand if the responses of *A. maculatum* were the result of indirect effects from top predators influencing *A. annulatum*. If the three-way interaction was significant, this would indicate that predators directly affected *A. annulatum*, and this effect varied by habitat treatment, which resulted in differential effects on *A. maculatum*.

Finally, we tested whether predators and cover influenced the growth rate of larval *A. annulatum* to understand if the predators altered the size ratio between larval *A. annulatum* and hatchling *A. maculatum*. We used larval SVL from 22 April as our response variable, as this was the approximate date of *A. maculatum* addition, with

predator treatment and cover as fixed effects and tank as a random effect. Using SVL

from 6 April or a calculated growth curve through both larval measurements and

metamorph size did not alter these results.

Results

IG predator (*Ambystoma annulatum*)

Predator treatment significantly affected metamorph body condition of *A. annulatum*, but

cover and the interaction of cover and predator treatment were not significant (Table 1).

Post-hoc tests showed that mosquitofish only was the only treatment that differed

significantly from the IGP-only treatment; metamorphs were smaller in body condition

with fish compared to aeshnid, newt, aeshnid+newt, and all predators (Figure 2a). Larval

period length was not affected by either predator or cover treatments, or their interaction

(Table 1; Figure 2b). Larval size of *A. annulatum* at the time of *A. maculatum* addition

(ca. April 22) did not vary significantly by cover or predator treatments (Figure A2 in

Appendix A).

We recovered 637 metamorphs and 6 larvae of *A. annulatum* at the end of the

experiment. Survival of *A. annulatum* was significantly affected by predator treatment,

but cover and their interaction were both not significant (Table 1). Survival was highest

in the IGP-only and mosquitofish treatments (83% and 75%, respectively), both of which

had significantly higher survival rates than tanks with aeshnids, aeshnids + newts, and all

three predators combined (Figure 2c). The lowest average survival occurred with all three

predators (17%), but post-hoc tests showed this treatment was not significantly different

from the aeshnids only (42%) or aeshnids + newts (26%) treatments. Overall, 14% of

metamorphs emerged with at least one injury. The prevalence of injuries on metamorphs was significantly influenced by predator treatment, but cover and the predator-cover interaction term were not significant ($\chi^2 = 35.98$, $df = 5$, $P < 0.001$). IGP only and newts only treatments had the lowest number of injuries (Figure 2d). The highest percentage of injuries were in in the mosquitofish only treatments (32% of individuals), and the overwhelming majority of injuries (93%) were of low severity, e.g. missing tail tips (Figure 2d; Figure A3 in Appendix A). The most severe injuries occurred more commonly in the tanks with aeshnids, aeshnids + newts, and all three predators. In each case, 33-64% of the observed injuries included missing the entire tail, a limb or both (Figure A3 in Appendix A).

IG prey (*Ambystoma maculatum*)

We saw no significant effects of predator treatment, cover treatment or their interaction on metamorph body condition, larval period length, or survival for *A. maculatum* (Table 1; Figure 2d-f). However, when we accounted for *A. annulatum* survival, we observed significant differences among treatments for all response variables (Tables A1-A3 in Appendix A). Metamorph body condition of *A. maculatum* was significantly affected by a three-way interaction of top predator, cover, and *A. annulatum* survival (Table 1). On average, metamorphs of *A. maculatum* had higher body condition when more *A. annulatum* survived (Figure 3a-f). However, this pattern was reversed with mosquitofish; body condition of *A. maculatum* decreased when more *A. annulatum* survived in tanks without vertical cover (Figure 3b; Table A1, Figure A4 in Appendix A). A similar negative pattern was observed with aeshnids+newts in the presence of cover,

though the low overall variability in *A. annulatum* survival likely influenced this result (Figure 3e).

The interaction of predator treatment with the survival of *A. annulatum* significantly affected the larval period length for *A. maculatum*, but no other higher order interactions were significant (Table 1). Larval period length of *A. maculatum* had a positive relationship with *A. annulatum* survival in IGP only treatment and in aeshnids only treatments (Figure 3g,i; Table A2, Figure A5 in Appendix A). This relationship switched to negative in tanks with all three predators (Figure 3l), and showed no relationship with newts, mosquitofish, or aeshnids+newts (Figure 3h,j,k; Table A2, Figure A5 in Appendix A).

At the end of the experiment, we recovered 177 metamorphic and 92 larval *A. maculatum*. A three-way interaction of predators, cover, and survival of *A. annulatum* significantly affected the number of metamorphs and overall survival of *A. maculatum* (Table 1). Overall survival showed a negative relationship with *A. annulatum* survival, but the slope of that relationship varied by predator and cover treatment (Figure 3m-r). With aeshnids, the absence of cover resulted in a switch to a positive relationship of *A. annulatum* and *A. maculatum* survival compared to a negative relationship in the presence of cover (Figure 3o; Table A3, Figure A6 in Appendix A). A similar pattern existed with newts, where the co-variance of survival among the two focal salamanders was only significant in the cover treatment (Figure 3p; Table A3, Figure A6 in Appendix A). With all three predators, survival was extremely low for *A. maculatum* (<10%). The number of *A. maculatum* that remained larval was affected by predator treatment and *A. annulatum* survival but no interaction terms were significant ($\chi^2 = 17.79$, $df = 1$, $P <$

0.001). The percent of individuals that remained larval was on average highest in tanks with mosquitofish (21%), newts (24%) and in the IGP only treatment (14%).

Discussion

Both top predators and habitat complexity can affect IGP by influencing the IG predator, the IG prey or both. Furthermore, while synergistic effects of these two factors has been documented for some taxa (Finke and Denno 2006, Grabowski et al. 2008), their combined influence on IGP is largely untested and unknown for many taxa. Our study shows that 1) IG predators (*A. annulatum*) were affected by top predators but not habitat complexity, 2) IGP was modified by the presence of top predators through their species-specific impact on the survival of the IG predator (*A. annulatum*), and 3) habitat complexity altered IGP with some top predators but not others.

Influence of Top Predators

Top predators often influence species interactions at lower trophic levels (e.g Paine 1966, Dodson 1970, Morin 1983b), including IGP (e.g. McPeek 1998, Wissinger et al. 1999, Finke and Denno 2005). In particular, IGP is altered when top predators negatively impact the IG predator (Hall 2011). In our study, as the top predators reduced the survival of the IG predator, survival of the IG prey (*A. maculatum*) increased, typical of a density-mediated indirect effect (Abrams et al. 1996; Trussell 2006). No top predators limited the growth of the IG predator such that they were too small to consume the IG prey, potentially because when mortality of the IG predator occurred, the survivors were released from intraspecific competition. Interestingly, larval size of *A. annulatum* was not different among treatments six weeks prior to metamorphosis, indicating that

growth rates were homogenized despite variable survival- potential evidence for a behavioral shift. Wissinger *et al.* (1999) and Yurewicz (2004) both showed that IG predators experienced heightened mortality due to their higher activity rates compared with their IG prey. Predation also may not have occurred until late in the larval period of *A. annulatum*. Alternatively, density-dependence may not have been strong enough to minimize predator-prey size disparities to the point where predation would be precluded (Rasmussen et al. 2014). Body condition of the IG prey survivors increased in tanks with high predation rates, however, also likely due to reduced intraspecific competition. As size at metamorphosis in amphibians is strongly correlated with adult fitness (Semlitsch et al. 1988, Scott 1994), this release from competition via predation could benefit IG prey.

While we did not observe a statistically significant increase in mortality with our additive design, the variance in survival for each salamander was much reduced with all three predators, indicating some additivity among predators, similar to other studies with multiple lethal predators (Relyea and Yurewicz 2002). Yet, not all predators had equivalent effects, supporting the hypothesis that different predator species can have dissimilar effects on prey populations and community structure (Relyea 2001, Chalcraft and Resetarits 2003). The presence of aeshnids resulted in high mortality regardless of predator combination, potentially from their sit-and-pursue hunting tactics and reduced gape limitations that often results high prey mortality (Preisser et al. 2007, Davenport et al. 2014). This supports the idea of a sampling effect, where one predator contributes disproportionately to prey mortality compared with the other species are present (Ives et al. 2005). Our study also did not include all possible combinations of top predators,

which we recognize limits our understanding of how multiple top predators influence IGP.

Mosquitofish are often a devastating invasive species that strongly affects aquatic food webs (Segev et al. 2009, Shulse and Semlitsch 2013). However, we saw nearly equivalent levels of survival in the IGP only treatment compared with mosquitofish as top predators, indicating their deleterious effects on amphibian recruitment may be density-dependent. Body condition at metamorphosis for *A. annulatum* was the smallest in tanks with mosquitofish and metamorphs had substantially more injuries, despite equivalent survival to the IGP-only treatments, indicating indirect and/or sublethal costs to co-existing with mosquitofish. Interestingly, *A. maculatum* and *A. annulatum* metamorphosed at the same size from tanks with mosquitofish but at disparate survival rates (higher for *A. annulatum*). The highest proportion of individuals remaining as larvae for *A. maculatum* also occurred in the mosquitofish treatment, indicating that the predominant effects on *A. maculatum* were from *A. annulatum*, but that mosquitofish continued to affect *A. maculatum*'s ability to metamorphose.

In contrast to mosquitofish, aeshnids had the greatest negative impact on larval survival. Yet, we did not see the extreme mortality observed in other studies, which is surprising given our experimental duration was 10 months rather than days or weeks (e.g. Relyea and Yurewicz 2002, Yurewicz 2004, Hossie and Murray 2010). Predation by aeshnids may have been minimized due to our use of multiple dragonfly naiads, as other studies have shown that dragonfly predation rates saturate as their density increases due to behavioral interference (Ramos and Van Buskirk 2011). We observed some cannibalism among naiads, but the extent to which is somewhat unknown; thus, naiad

density may confound our results and explain the large variance in response variables within dragonfly treatments.

Finally, newts had the weakest lethal and sublethal effects on both salamanders, which was not surprising given that they have the narrowest gape, resulting in a limited temporal period where consumption can occur before larvae grow into an invulnerable size class. Newts often forage on amphibian egg masses, and can substantially reduce embryonic survival (Morin 1983a, Drake et al. 2014), and thus our use of free-swimming hatchlings may have reduced their impact.

Influence of Habitat Complexity

Habitat complexity can have contrasting effects on predation rates. For some predators, cover reduces consumption by providing refuges from predators (e.g. Finke and Denno 2002, Warfe and Barmuta 2004), though substantial nonconsumptive effects can subsequently occur (Orrock et al. 2013). Alternatively, cover has been shown to have minimal effects on some predators, primarily because of their hunting strategy or body size (e.g. Babbitt and Tanner 1998, Carey and Wahl 2010, Toscano and Griffen 2013). Our study supports this latter point, as cover did not have strong effects on metamorph traits, injury prevalence and survival for *A. annulatum*, and limited effects on *A. maculatum* that acted only in concert with specific predators. Although our cover matches cattails in form, one possible explanation for the reduced habitat effects is that it was still structurally simple; other studies have shown that structurally simple cover does not influence predators compared to more complex cover (Warfe and Barmuta 2004). All three predators and both salamanders were observed to use the vertical cover more often

than the open side of the tank (mean ± SE number of larvae observed on open versus cover sides: 1.21 ± 0.04 vs 1.93 ± 0.05, Thomas L. Anderson, unpubl. data), but it may not have been dense enough to restrict predator foraging behavior.

Interaction of Top Predators and Habitat Complexity

We observed that certain predators in complex habitats had disproportionate effects on the IGP module, similar to Finke and Denno (2006) and Grabowski et al. (2008) who each found that different predator species had variable effects on interactions at lower trophic levels that depended upon habitat type. In our study, increased habitat complexity reversed the relationship of *A. annulatum* and *A. maculatum* survival (no effect to negative with newts or aeshnids). In the absence of cover, the positive relationship between *A. maculatum* and *A. annulatum* survival with aeshnids could suggest a behavioral response: *A. annulatum* may have foraged less on *A. maculatum* when there was no vertical cover to conceal the aeshnids, resulting in increased survival. The presence of cover for the mosquitofish treatment reversed the relationship of body condition of *A. maculatum* and *A. annulatum* survival from positive to negative, which could suggest that the few survivors did not benefit from thinning effects (e.g. Anderson and Semlitsch 2014) because zooplankton levels were potentially cropped back by mosquitofish. When cover was present, zooplankton may have had more refuge, though we did not collect quantitative data on this aspect of the food web. Further investigation of behavioral observations (Thomas L. Anderson, unpubl. data) will potentially elucidate the mechanisms of such survival relationships among IG predator and prey with the different top predators in varying habitats.

Implications for Natural Systems

While predator food web complexity and IGP have strong experimental and theoretical underpinnings, observations from natural systems are needed to verify such findings for many taxa. For larval salamanders, experimental studies have shown larval IGP can be pervasive (Stenhouse 1985, Cortwright and Nelson 1990, Walls and Williams 2001, Anderson and Semlitsch 2014), yet inferences on long-term coexistence in natural populations in light of such interactions have been infrequently investigated. Many ambystomatids co-occur in ponds with no direct evidence of exclusion either by predation or competition (e.g. Ousterhout et al. 2015). We hypothesize that top-down predation may be mechanism that permits coexistence. As permanent hydroperiod ponds typically contain high densities of top predators (Wellborn et al. 1996, Semlitsch et al. 2015), larval densities may be reduced such that their pairwise interactions are minimized, similar to our treatment with all three top predators, though *A. annulatum* still negatively affected recruitment of *A. maculatum* in some natural populations (Anderson et al. 2015). In ephemeral ponds, we hypothesize IGP would be amplified, as pond drying would reduce invertebrate predator density, leaving larval ambystomatids as top predators. Other prey species may provide alternative food sources that mediates both IGP and predation by top predators, though Davenport & Chalcraft (2012) found that alternative prey did not reduce top predators. Additional investigations of habitat complexity and predator food webs are needed for natural populations (e.g. Hartel et al. 2007), as documenting such context-dependent interactions are critical to elucidating the mechanisms that promote coexistence in natural populations, for both this and other systems (Agrawal et al. 2007).

Conclusions

Top predators and habitat complexity frequently alter the strength or outcome of pairwise species interactions. Yet, experiments that investigate such factors often simplify their various complexities in order to test for the influence of specific mechanisms. All but the simplest of communities have many organisms that co-occur and interact with one another, and thus interactions among two links of a food web would be inextricably linked to what community members are also present, in addition to the habitat in which such interactions occur. Identifying these critical biotic or abiotic components will continue to help elucidate the mechanisms that determine the function and structure of food webs.

Acknowledgements

Data Accessibility

All data are available in the Dryad Digital Repository http://doi.org/10.5061/dryad.66j20 (Anderson 2015)

References

Agrawal, A. A., D. D. Ackerly, F. Adler, A. E. Arnold, C. Cáceres, D. F. Doak, E. Post, P. J. Hudson, J. Maron, and K. A. Mooney. 2007. Filling key gaps in population and community ecology. Frontiers in Ecology and the Environment **5**:145-152.

Anderson, T. L. 2015. Data from: Top predators and habitat complexity alter an intraguild predation module in pond communities. Journal of Animal Ecology.

Anderson, T. L., D. J. Hocking, C. A. Connor, J. E. Earl, E. B. Harper, M. S. Osbourn, W. E. Peterman, T. A. Rittenhouse, and R. D. Semlitsch. 2015. Abundance and phenology patterns of two pond-breeding salamanders determine species interactions in natural populations Oecologia **177**:761-773.

Anderson, T. L., and R. D. Semlitsch. 2014. High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations. Population Ecology **56**:265-273.

Arim, M., and P. A. Marquet. 2004. Intraguild predation: a widespread interaction related to species biology. Ecology Letters **7**:557-564.

Babbitt, K. J., and G. W. Tanner. 1998. Effects of cover and predator size on survival and development of *Rana utricularia* tadpoles. Oecologia **114**:258-262.

Baber, M. J., and K. J. Babbitt. 2004. Influence of habitat complexity on predator–prey interactions between the fish (*Gambusia holbrooki*) and tadpoles of *Hyla squirella* and *Gastrophryne carolinensis*. Copeia **2004**:173-177.

Bates, D., M. Maechler, B. M. Bolker, and S. Walker. 2015. lme4: Linear mixed-effects models using S4 classes R package version 1.1-9 http://CRAN.R-project.org/package=lme4

Borer, E. T., C. J. Briggs, W. W. Murdoch, and S. L. Swarbrick. 2003. Testing intraguild predation theory in a field system: does numerical dominance shift along a gradient of productivity? Ecology Letters **6**:929-935.

Caldwell, J. P., J. H. Thorp, and T. O. Jervey. 1980. Predator-prey relationships among larval dragonflies, salamanders and frogs. Oecologia **46**:285-289.

Carey, M. P., and D. H. Wahl. 2010. Interactions of multiple predators with different foraging modes in an aquatic food web. Oecologia **162**:443-452.

Chalcraft, D. R., and W. J. Resetarits, Jr. 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? Ecology **84**:2407-2418.

Cortwright, S. A., and C. E. Nelson. 1990. An examination of multiple factors affecting community structure in an amphibian aquatic community. Oecologia **83**:123-131.

Davenport, J. M., B. R. Hossack, and W. H. Lowe. 2014. Partitioning the non-consumptive effects of predators on prey with complex life histories. Oecologia **176**:149-155.

Dodson, S. I. 1970. Complementary feeding niches sustained by size-selective predation. Limnology and Oceanography **15**:131-137.

Drake, D. L., T. L. Anderson, L. M. Smith, K. L. Lohraff, and R. D. Semlitsch. 2014. Predation of eggs and hatchlings of Ringed Salamanders (*Ambystoma annulatum*) by native and non-native predators. Herpetologica **70**:378-387.

Finke, D. L., and R. F. Denno. 2002. Intraguild predation diminished in complex-structured vegetation: implications for prey suppression. Ecology **83**:643-652.

Finke, D. L., and R. F. Denno. 2004. Predator diversity dampens trophic cascades. Nature **429**:407-410.

Finke, D. L., and R. F. Denno. 2005. Predator diversity and the functioning of
    ecosystems: the role of intraguild predation in dampening trophic cascades.
    Ecology Letters **8**:1299-1306.

Finke, D. L., and R. F. Denno. 2006. Spatial refuge from intraguild predation:
    implications for prey suppression and trophic cascades. Oecologia **149**:265-275.

Grabowski, J. H., A. R. Hughes, and D. L. Kimbro. 2008. Habitat complexity influences
    cascading effects of multiple predators. Ecology **89**:3413-3422.

Hall, R. J. 2011. Intraguild predation in the presence of a shared natural enemy. Ecology
    92:352-361.

Hartel, T., S. Nemes, D. Cogălniceanu, K. Öllerer, O. Schweiger, C. I. Moga, and L.
    Demeter. 2007. The effect of fish and aquatic habitat complexity on amphibians.
    Hydrobiologia **583**:173-182.

Hin, V., T. Schellekens, L. Persson, and A. M. d. Roos. 2011. Coexistence of predator
    and prey in intraguild predation systems with ontogenetic niche shifts. The
    American Naturalist **178**:701-714.

Holt, R. D., and G. R. Huxel. 2007. Alternative prey and the dynamics of intraguild
    predation: theoretical perspectives. Ecology **88**:2706-2712.

Holt, R. D., and G. A. Polis. 1997. A theoretical framework for intraguild predation.
    American Naturalist **149**:745-764.

Hossie, T. J., and D. L. Murray. 2010. You can't run but you can hide: refuge use in frog
    tadpoles elicits density-dependent predation by dragonfly larvae. Oecologia
    163:395-404.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general
parametric models. Biometrical Journal **50**:346-363.

Huang, C., and A. Sih. 1990. Experimental studies on behaviorally mediated, indirect
interactions through a shared predator. Ecology **71**:1515-1522.

Ives, A. R., B. J. Cardinale, and W. E. Snyder. 2005. A synthesis of subdisciplines:
predator-prey interactions, and biodiversity and ecosystem functioning. Ecology
Letters **8**:102-116.

Losey, J. E., and R. F. Denno. 1998. Positive predator-predator interactions: enhanced
predation rates and synergistic suppression of aphid populations. Ecology
79:2143-2152.

McPeek, M. A. 1998. The consequences of changing the top predator in a food web: a
comparative experimental approach. Ecological Monographs **68**:1-23.

Miller, T. E. X., and V. H. W. Rudolf. 2011. Thinking inside the box: community-level
consequences of stage-structured populations. Trends in Ecology & Evolution
26:457-466.

Morin, P. J. 1983a. Competitive and predatory interactions in natural and experimental
populations of *Notophthalmus viridescens dorsalis* and *Ambystoma tigrinum*.
Copeia **1983**:628-639.

Morin, P. J. 1983b. Predation, competition, and the composition of larval anuran guilds.
Ecological Monographs **53**:120-138.

Morin, P. J. 2011. Community ecology. 2 edition. John Wiley & Sons, Oxford, UK.

Mylius, S. D., K. Klumpers, A. W. de Roos, and L. Persson. 2001. Impact of intraguild predation and stage structure on simple communities along a productivity gradient. American Naturalist **158**:259-276.

Orrock, J. L., E. L. Preisser, J. H. Grabowski, and G. C. Trussell. 2013. The cost of safety: Refuges increase the impact of predation risk in aquatic systems. Ecology **94**:573-579.

Ousterhout, B. H., T. L. Anderson, D. L. Drake, W. E. Peterman, and R. D. Semlitsch. 2015. Habitat traits and species interactions differentially affect abundance and body size in pond-breeding amphibians. Journal of Animal Ecology **84**:914-924.

Padeffke, T., and F. Suhling. 2003. Temporal priority and intra-guild predation in temporary waters: an experimental study using Namibian desert dragonflies. Ecological Entomology **28**:340-347.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist **100**:65-75.

Peterman, W. E., T. L. Anderson, D. L. Drake, B. H. Ousterhout, and R. D. Semlitsch. 2014. Maximizing pond biodiversity across the landscape: a case study of larval ambystomatid salamanders. Animal Conservation **17**:275-285.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington [D.C.].

Polis, G. A., and R. D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends in Ecology & Evolution **7**:151-154.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics **20**:297-330.

Preisser, E. L., J. L. Orrock, and O. J. Schmitz. 2007. Predator hunting mode and habitat domain alter nonconsumptive effects in predator-prey interactions. Ecology **88**:2744-2751.

R Development Core Team. 2015. R: A Language and Environment for Statistical Computing 3.2.2 http://www.R-project.org/

Ramos, O., and J. Van Buskirk. 2011. Non-interactive multiple predator effects on tadpole survival. Oecologia **169**:535-539.

Rasband, W. S. 1997. ImageJ, US National Institutes of Health, Bethesda, Maryland, USA.

Rasmussen, N. L., B. G. Van Allen, and V. H. W. Rudolf. 2014. Linking phenological shifts to species interactions through size-mediated priority effects. Journal of Animal Ecology **83**:1206-1215.

Relyea, R. A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology **82**:523-540.

Relyea, R. A., and K. L. Yurewicz. 2002. Predicting community outcomes from pairwise interactions: integrating density-and trait-mediated effects. Oecologia **131**:569-579.

Schmitz, O. J. 2007. Predator diversity and trophic interactions. Ecology **88**:2415-2426.

Scott, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology **75**:1383-1396.

Segev, O., and L. Blaustein. 2007. Priority effects of the early breeding fire salamander on the late breeding banded newt. Hydrobiologia **583**:275-283.

Segev, O., M. Mangel, and L. Blaustein. 2009. Deleterious effects by mosquitofish (*Gambusia affinis*) on the endangered fire salamander (*Salamandra infraimmaculata*). Animal Conservation **12**:29-37.

Semlitsch, R. D., W. E. Peterman, T. L. Anderson, D. L. Drake, and B. H. Ousterhout. 2015. Intermediate pond sizes contain the highestdensity, richness, and diversity ofpond-breeding amphibians. PLoS ONE **10**:e0123055.

Semlitsch, R. D., and S. B. Reichling. 1989. Density-dependent injury in larval salamanders. Oecologia **81**:100-103.

Semlitsch, R. D., D. E. Scott, and J. H. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology **69**:184-192.

Shulse, C. D., and R. D. Semlitsch. 2013. Western mosquitofish (*Gambusia affinis*) bolster the prevalence and severity of tadpole tail injuries in experimental wetlands. Hydrobiologia **723**:131-144.

Shulse, C. D., R. D. Semlitsch, K. M. Trauth, and J. E. Gardner. 2012. Testing wetland features to increase amphibian reproductive success and species richness for mitigation and restoration. Ecological Applications **22**:1675-1688.

Shulse, C. D., R. D. Semlitsch, K. M. Trauth, and A. D. Williams. 2010. Influences of design and landscape placement parameters on amphibian abundance in constructed wetlands. Wetlands **30**:915-928.

Soluk, D. A. 1993. Multiple predator effects: predicting combined functional response of stream fish and invertebrate predators. Ecology **74**:219-225.

Stenhouse, S. L. 1985. Interdemic variation in predation on salamander larvae. Ecology **66**:1706-1717.

Swisher, B. J., D. A. Soluk, and D. H. Wahl. 1998. Non-additive predation in littoral habitats: influences of habitat complexity. Oikos **81**:30-37.

Toscano, B. J., and B. D. Griffen. 2013. Predator size interacts with habitat structure to determine the allometric scaling of the functional response. Oikos **122**:454-462.

Urban, M. C. 2007. Predator size and phenology shape prey survival in temporary ponds. Oecologia **154**:571-580.

Walls, S. C., and M. G. Williams. 2001. The effect of community composition on persistence of prey with their predators in an assemblage of pond-breeding amphibians. Oecologia **128**:134-141.

Walters, B. 1975. Studies of interspecific predation within an amphibian community. Journal of Herpetology **9**:267-279.

Warfe, D. M., and L. A. Barmuta. 2004. Habitat structural complexity mediates the foraging success of multiple predator species. Oecologia **141**:171-178.

Warfe, D. M., and L. A. Barmuta. 2006. Habitat structural complexity mediates food web dynamics in a freshwater macrophyte community. Oecologia **150**:141-154.

Wellborn, G. A., D. K. Skelly, and E. E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics **27**:337-363.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology **84**:1083-1100.

Wissinger, S. A. 1989. Seasonal variation in the intensity of competition and predation among dragonfly larvae. Ecology **70**:1017-1027.

Wissinger, S. A., H. H. Whiteman, G. B. Sparks, G. L. Rouse, and W. S. Brown. 1999. Foraging trade-offs along a predator-permanence gradient in subalpine wetlands. Ecology **80**:2102-2116.

Yurewicz, K. L. 2004. A growth/mortality trade-off in larval salamanders and the coexistence of intraguild predators and prey. Oecologia **138**:102-111.

Table 1: Test statistics and p-values for body condition, larval period length and survival of ringed salamanders (*Ambystoma annulatum*) and spotted salamanders (*A. maculatum*) without and with accounting for the survival of *A. annulatum* survival. Values for body condition and larval period length are Wald's chi square statistic and survival is likelihood ratio statistic, with p-values in parentheses. Pred= predator treatment, Hab= habitat treatment, and AA = survival of *A. annulatum*. Degrees of freedom for each term are: Predator = 5, Habitat =1, Pred-Hab = 5, Pred:AA=5,Hab-AA= 5, Hab:Pred:AA=5.

Ambystoma annulatum		
Covariate	**Body condition**	**Larval period length**	**Survival**
Predator	**11.44 (P = 0.04)**	1.79 (P = 0.88)	**17.81 (P =0.003)**
Habitat	0.01 (P = 0.93)	0.12 (P = 0.89)	0.30 (P = 0.58)
Pred-Hab	4.78 (P = 0.44)	1.95 (P = 0.86)	1.67 (P = 0.89)
Ambystoma maculatum (without IG predator effects)		
Covariate	**Body condition**	**Larval period length**	**Survival**
Predator	4.93 (P = 0.42)	3.12 (P = 0.68)	6.49 (P = 0.26)
Habitat	1.30 (P = 0.25)	0.72 (P = 0.40)	0.01 (P = 0.94)
Pred-Hab	4.11 (P = 0.53)	1.16 (P = 0.95)	4.62 (P = 0.46)
Ambystoma maculatum (with IG predator effects)		
Covariate	**Body condition**	**Larval Period Length**	**Survival**
Predator	5.99 (P = 0.31)	21.27 (P = 0.001)	15.40 (P = 0.01)
Habitat	0.00 (P = 0.99)	0.37 (P = 0.54)	1.31 (P = 0.25)

AA	4.79 (P = 0.03)	7.75 (P = 0.005)	9.80 (P = 0.002)
Pred-Hab	11.17 (P = 0.05)	1.42 (P = 0.92)	14.91 (P = 0.01)
Pred-AA	9.87 (P = 0.08)	**14.73 (P = 0.01)**	10.31 (P = 0.07)
Hab-AA	0.25 (P = 0.62)	0.03 (P = 0.87)	0.98 (P = 0.32)
Pred-Hab-AA	**12.46 (P = 0.03)**	NA	**17.84 (P = 0.003)**

Figure Legends

Figure 1: Diagram of basic IGP module of two larval salamanders (1) combined with different top predator treatments (2-6). All food webs were crossed with the presence (filled circle) or absence (filled circle with crossbar) of habitat complexity. Open circles represent ringed salamanders, *Ambystoma annulatum* (AA), and spotted salamanders, *A. maculatum* (AM), late instar dragonfly nymphs, Aeshnidae (AE), adult Central newts, *Notophthalmus v. louisianensis* (NT), mosquitofish, *Gambusia holbrooki* (MF). The basal resource (R) was not manipulated. Text under treatment names indicate predator gape type and hunting strategy. Thickness of arrows indicates the predicted relative effects of each predator.

Figure 2: Mean (± SE) body condition, larval period length, and survival of ringed salamanders (*Ambystoma annulatum*; panels a–c) and spotted salamanders (*A. maculatum,* panels e–g) among the different predator and cover treatments. Panel d shows the mean (± SE) percent of injured metamorphic ringed salamanders (*Ambystoma annulatum*) within predator food web and cover treatments. "IGP" refers to no top predators present, "MF" refers to mosquitofish only, "AE" refers to aeshnids only, "NT" refers to newts only, "AE-NT" refers to aeshnid+newts, and "ALL" refers to all three predators present. Sample sizes for the total number of metamorphs in each of the predator treatments (cover/no-cover) are: IGP: 102/91; MF: 20/15, AE: 46/24, NT: 77/85, AE-NT: 60/67, and ALL: 34/22.

Figure 3: Three-way interaction of predator treatment, habitat complexity and *Ambystoma annulatum* survival on metamorph body condition (a-f), larval period length

(g-l) and survival (m-r) of *A. maculatum*. Bold names above each column of panels

indicate the predator treatment. Gold circles/lines indicate the absence and black

circles/solid lines indicate presence of habitat complexity. Each dot is one individual

salamander for body condition and larval period panels, and each dot is one tank in the

survival panels.
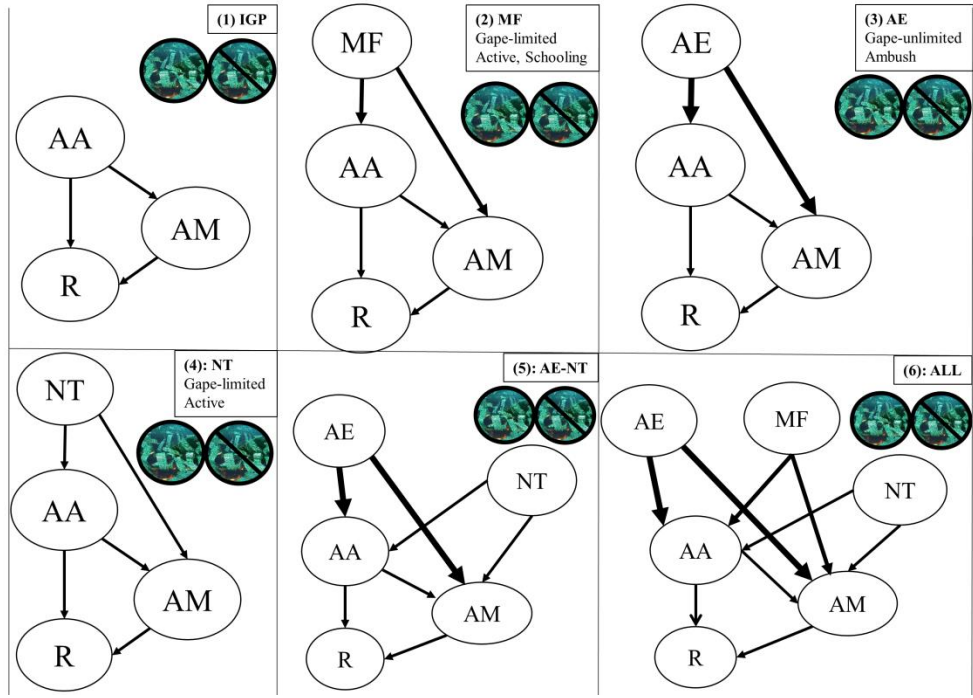
Figure 1
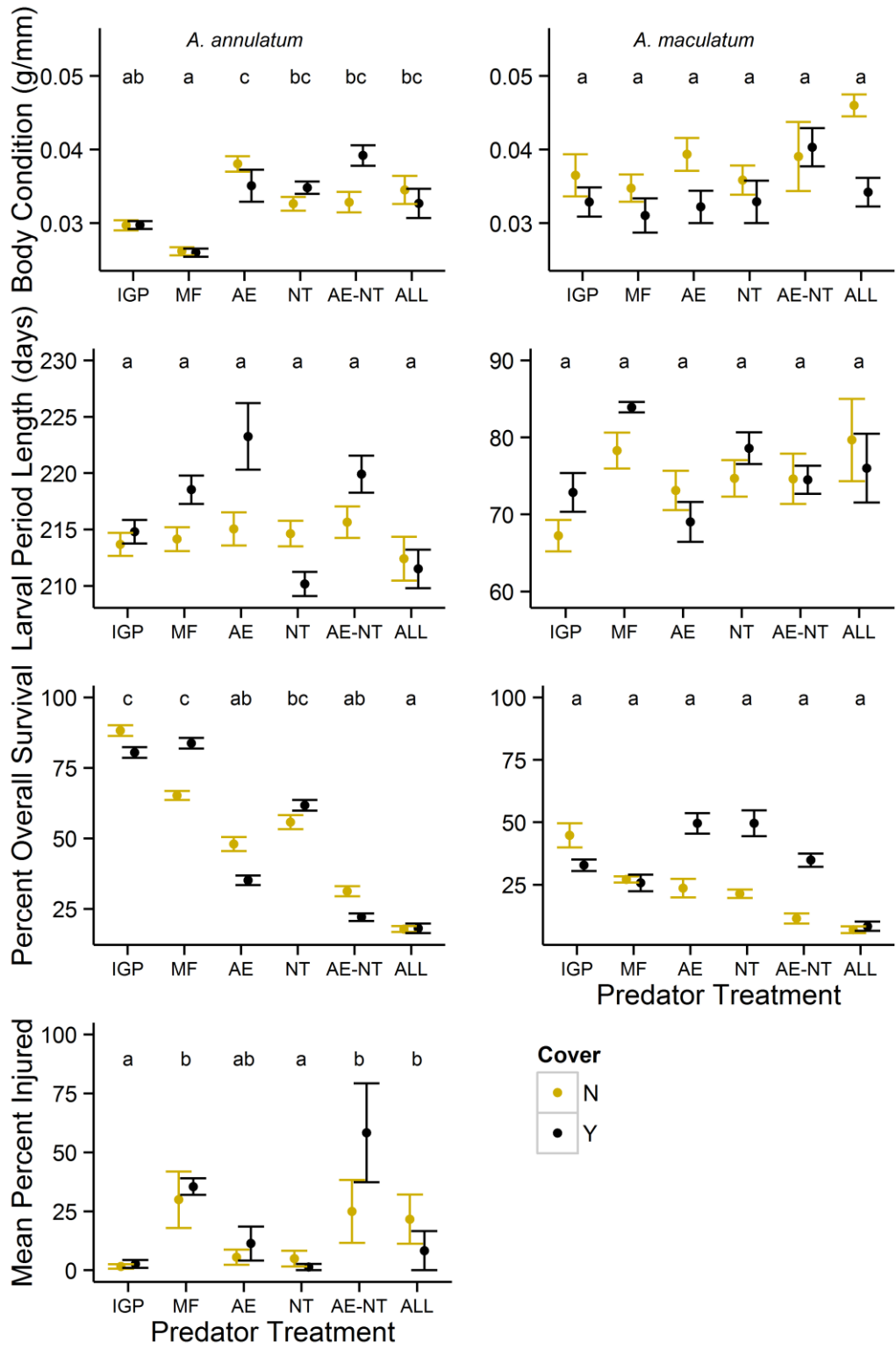
Figure 2
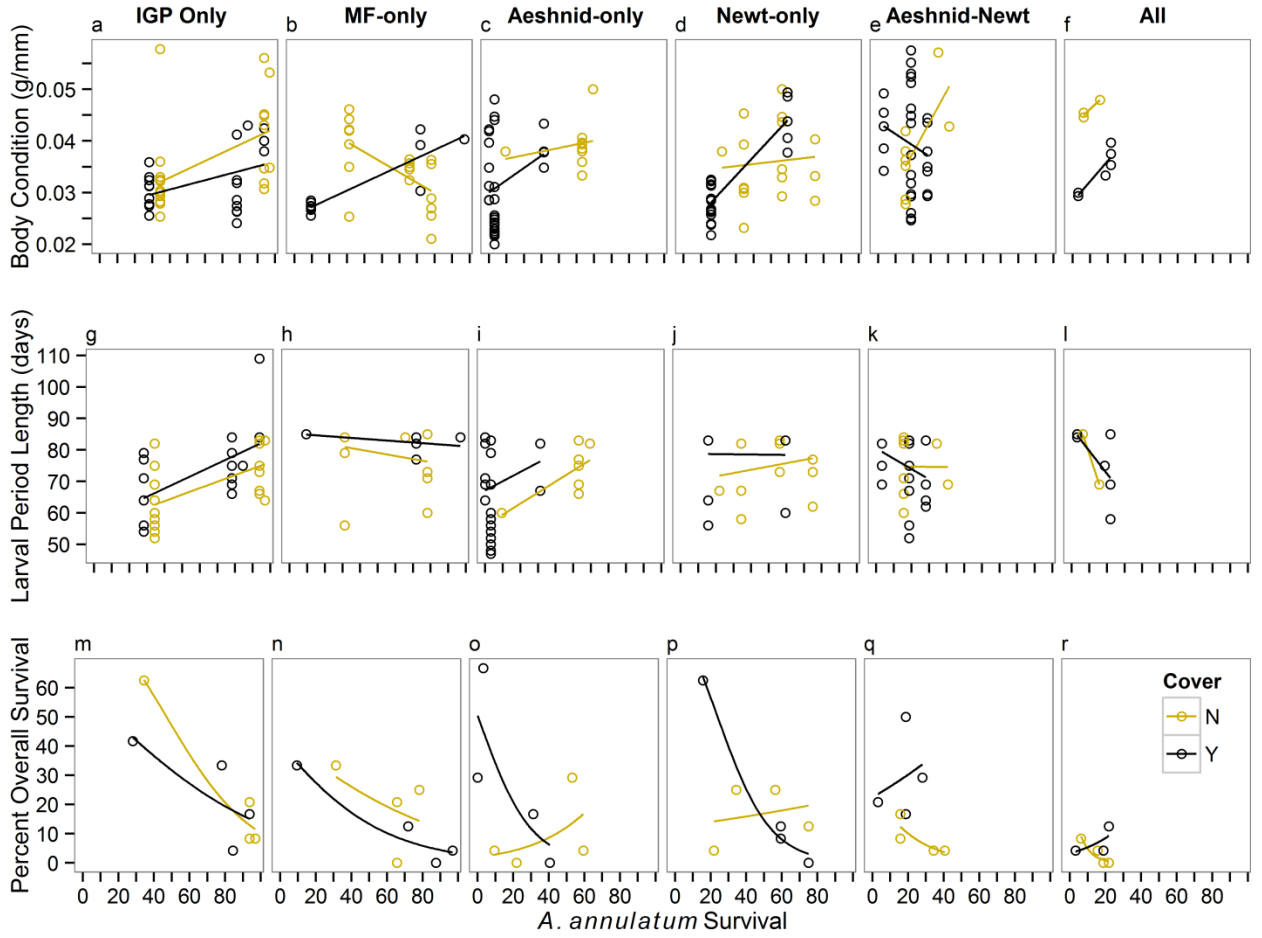
Figure 3

Chapter 4

ASYMMETRIC EFFECTS OF PHENOLOGICAL SHIFTS AND LARVAL DENSITY

INFLUENCE INTRAGUILD PREDATION BETWEEN SALAMANDERS

Thomas L. Anderson and Raymond D. Semlitsch

Abstract: Phenological shifts are occurring for many species, which may have strong consequences on species interactions and community structure. These altered interactions can be further compounded by other processes, such as density-dependent competition within or across species. Yet, the outcome of simultaneous shifts in phenology and density are primarily untested. We tested whether the outcome of intraguild predation between two pond-breeding salamanders varied in response to altered phenology and intraguild (IG) predator density. In outdoor mesocosm ponds, we experimentally manipulated phenology by adding larvae of each species at one of two time points in their normal breeding phenology (early or late), and the density of the IG predator. Additionally, we examined potential priority effects mechanisms by which IG predators affected IG prey. Body size of the IG predator was only affected by intraspecific density, whereas larval period length and survival were dictated by both density and phenology treatments. For IG prey, body size, larval period length and survival were all related to the density of IG predators, with very limited effects of phenology treatments. We also found support for predation as the likely mechanism by which the IG predator limits the IG prey. Overall, density-dependence had a substantially greater influence on the outcome of intraguild predation compared with phenological shifts. Identifying such context dependencies will lead to greater insight into if and when shifts in phenology will substantially alter species interactions and community structure.

Keywords: *Ambystoma*, breeding migration, compensatory growth, competition, phenology, priority effects, species interactions

Introduction

Climate-induced shifts in phenology have been documented in a growing number of taxa (Parmesan and Yohe 2003, Visser and Both 2005, Parmesan 2006). Furthermore, it is increasingly recognized that not all species will respond similarly to changes in environmental cues, resulting in the potential for decoupling of species interactions, match-mismatch scenarios, and the potential development of novel communities (Urban et al. 2012, Revilla et al. 2014). However, the resulting consequences of such variability in phenological shifts on population demography and species interactions have only recently been advocated as an essential component missing from community ecology (Miller-Rushing et al. 2010, Nakazawa and Doi 2012). Filling these gaps in knowledge is especially critical to predict how phenological shifts will alter species interactions and community structure under future climate scenarios (Yang and Rudolf 2010, Zarnetske et al. 2012, Blois et al. 2013).

While documenting shifts in phenology among natural populations is a first step in understanding their importance to species interactions, the underlying mechanisms that determine how these shifts affect such interactions are not always clear (Rafferty et al. 2013). Early-arriving species often dominate over later-arriving species (i.e. priority effects) via several mechanisms, including resource pre-emption, habitat modification, or direct interference (Connell and Slatyer 1977). Such priority effects are a general phenomenon and have been observed in many taxa (e.g. Shorrocks and Bingley 1994, Almany 2003, Louette and De Meester 2007, Hernandez and Chalcraft 2012, Kardol et al. 2013); however, the most important underlying mechanism prevails (if there is one) is

unknown. Therefore, if shifts in phenology occur (either within a species or in arrival order among species), a greater understanding of which process (e.g. physical interference or resource pre-emption) determines the outcome of interactions may facilitate a robust understanding of how species interactions will change.

The effects of phenological shifts on species interactions do not occur independently of other biotic or abiotic factors, and these other effects may either suppress or magnify effects of phenological shifts. In particular, advancements or delays in the timing of breeding may be offset or augmented by differential growth rates (either for individuals or populations) resulting from variable population densities, which can result in density-dependent growth (Reed et al. 2015). Such synergistic interactions between density dependence and phenological shifts may be especially relevant for predator-prey interactions whose outcome is determined by body size (i.e. gape limitations), as intra- and interspecific density dependence and interspecific differences in phenology can both dictate body size differences. Shifts in interaction type or strength may therefore ensue through either density-dependent or phenology- dependent processes (Yang and Rudolf 2010) yet simultaneous tests of these effects on species interactions are rare (but see Hunter and Elkinton 2001, Durant et al. 2005, Reed et al. 2015).

Studies of phenological variation have an established history with pond-breeding amphibians, due to adult reliance on environmental cues (rainfall and temperature) to stimulate breeding migrations in these systems, and thus the order of arrival into these communities. Because of inter-annual variation in rainfall and temperature patterns, the timing of breeding migrations can vary within and among species from several weeks to months (Petranka 1998). Asynchrony in arrival times of adults creates size asymmetries

among resulting offspring, which can impact the type of interactions and the amount of temporal overlap among species within aquatic habitats. Thus, because environmental variation can influence changes in timing of breeding and larval development, shifts in phenology already plays a critical role in amphibian population and community dynamics, and indeed numerous experimental studies have documented that such variation strongly influences both competition and predation (Alford and Wilbur 1985, Wilbur and Alford 1985, Morin 1987, Alford 1989, Lawler and Morin 1993, Boone et al. 2002, Orizaola et al. 2010, Rasmussen and Rudolf 2015). Demographic rates and species interactions in natural populations have also been found to be influenced, at least in part, by phenological variation (Anderson et al. 2015, Benard 2015). Additionally, breeding patterns of some amphibians have shifted with climate change (Todd et al. 2011), such that migrations of some fall-breeding species are occurring later whereas some spring-breeding species are breeding earlier, exactly the scenario that might induce shifts in the type of interaction due to changes in relative size differences. The underlying mechanism afforded by size advantages attained by early arriving species, which can result in superior exploitative, interference or predatory abilities, has also rarely been demonstrated (Hernandez and Chalcraft 2012). Density-dependent interactions are also common among larval amphibians, such that high densities result in slower individual growth rates, increased larval period lengths, and decreased size at metamorphosis (Van Buskirk and Smith 1991, Scott 1994, Anderson and Whiteman 2015). Yet, the joint effects of density dependence and phenological shifts have rarely been investigated for amphibians (Ryan and Plague 2004, Rasmussen and Rudolf 2015).

We conducted a mesocosm experiment to test how shifts in phenology and larval densities influence intraguild predation (IGP) between the ringed (*Ambystoma annulatum*) and spotted salamander (*A. maculatum*). Intraguild predation involves a three-species food web module, where two species compete for a shared prey resource while also preying upon one another (Polis et al. 1989). The focal species in our study breed at different times of the year (fall and winter/spring for ringed and spotted salamanders, respectively), making the former the intraguild (IG) predator and latter the IG prey. We experimentally manipulated phenology by introducing larvae to outdoor mesocosms at differing times (early/late in the fall or spring) while simultaneously altering IG predator density. We also investigated a potential underlying mechanism of the priority effects (resource pre-emption or predation), and expected that the latter process would be the dominant interaction. We expected phenological shifts to influence interactions through one of several potential pathways: 1) phenological shifts that increase size differences (early breeding of ringed salamanders in the fall and late breeding of spotted salamanders in the spring) would result strong interspecific effects via increased predation relative to competition (Figure 1), 2) shifts that change the in relative timing of breeding and development in the same direction (i.e. both species breeding early or late) may result in no change of interspecific effects, because both species breeding are moving synchronously, and 3) shifts that decrease size differences (ringed salamanders breeding later in the fall and spotted salamanders breeding earlier in the spring) will shift interactions from IGP to solely competition (Figure 1). In all cases, we predicted that higher density dependence in ringed salamanders would result in both

reduced intraspecific growth rates and size disparities, and further compound phenology effects (Figure 1).

Methods

Study system

Ringed salamanders are endemic to the Ozark and Ouachita Mountains in Missouri, Arkansas and Oklahoma, USA. Adults migrate to ponds in September through November to breed, larvae overwinter in ponds and undergo metamorphosis from April–June (Figure 1; Semlitsch et al. 2014). Spotted salamanders breed in late February to April and undergo metamorphosis from June–October (Semlitsch and Anderson *In Press*). Thus, larvae of these two species co-occur in ponds anywhere from several weeks to months (Anderson et al. 2015), resulting in variable size structure patterns within and across species. Larger ringed salamander larvae are also more voracious predators than smaller on spotted salamander hatchlings (Anderson et al. 2016), indicating that phenology and density (both determinants of larval size) could have strong implications for survival of spotted salamanders.

Experimental Set-Up

We conducted the experiment in 1000 l outdoor mesocosms (hereafter, tanks) arranged in a rectangular array on the University of Missouri campus. We filled tanks with water on 13 September 2013, and let them sit for 10 days to dechlorinate. We added 1.25 kg of dry leaves (primarily *Quercus spp.*, *Carya spp.*, and *Acer spp.*) that had been collected from Baskett Wildlife Research Area near Ashland, MO. We initially covered tanks with mesh lids to prevent colonization by insects, but we removed them at the start of the experiment. We added pond water from nearby natural ponds every three to five days

until the start of the experiment, totaling approximately 4 L per tank. We sieved water samples through an aquarium net to remove any large predaceous invertebrates prior to addition.

We collected late-stage egg masses of ringed salamanders on 27 September 2013 from a permanent pond at Fort Leonard Wood, MO, and transported them back to a greenhouse near the tank array. To minimize genetic bias of collection dates on the outcomes of the experiment, we cut each egg mass approximately in half with scissors, and placed one half in a cold storage room (ca. 8 degrees C) and one half in a greenhouse (ca. 22 degrees C). Eggs in the greenhouse hatched within 48 hours, whereas those in the cold room began hatching on 19 October and completed hatching within 12 d.

We collected eggs of spotted salamanders during the first week of April 2014 also from ponds at Fort Leonard Wood. Egg masses were cut in half and placed in the same rooms where ringed salamander eggs had been stored (8 degrees C for cold and 19 degrees C for warm). However, the eggs experienced some mortality in the greenhouse, and had to be supplemented with further eggs that were not cut in half. Thus, our ringed salamander treatments had equivalent genetic contributions mixed across phenology treatments, but spotted salamander treatments was slightly confounded in genetic contribution.

Phenology-Density Experiment

Our phenology treatments included an "early" and "late" addition for each species to represent early- or late- hatching larvae, resulting in four possible combinations of the ambystomatid community (read as Ringed salamander-Spotted salamander): early-early, early-late, late-early and late-late. We consider the early-early and late-late treatments

"synchronous" as both species are changing phenology in the same direction, and the early-late and late-early as "asynchronous" shifts because species are changing in dissimilar directions. Each community contained 24 spotted salamanders, and one of two densities of ringed salamanders (low: N = 8 individuals, high: N = 24 individuals). We added ringed salamander hatchlings to tanks assigned the "early" phenology for both density treatments on 3 October 2013, and the "late" treatment 31 d later on 3 November. We added spotted salamander hatchlings to tanks assigned the "early" treatment on 18 April 2014, and the "late" treatment 21 d later on 9 May. Tanks assigned to the low density, late-early combination erroneously received a high density of ringed salamanders, thus eliminating the full-factorial nature of the design; we discuss how we dealt with this error below. Beginning in late April, we began checking all tanks for metamorphs (individuals that had reabsorbed their gills) at least every other night. We removed metamorphs, and recorded snout-vent length (SVL), mass and determined the larval period length, the number of elapsed days from the date of introduction. All tanks were drained in early July 2014 and the leaf litter thoroughly searched for surviving individuals.

We tested for the effects of phenology and surviving density for each species using linear mixed effects models with tank as a random effect to account for non-independence of metamorphs from the same tank. For survival of ringed salamanders, we used generalized linear models with a binomial error distribution, and tested whether phenology and initial density influenced survival. For spotted salamanders, we fit similar models (linear mixed effects models for SVL and larval period length, and generalized linear model with binomial errors for survival) with the covariates phenology treatment

and the number of surviving ringed salamanders, and tank as a random effect. Mass and SVL were highly correlated ($r = 0.89$), and thus we only report results on SVL (see Appendix A for mass). Because initial density influenced survival of ringed salamander (see *Results*) and was mistakenly added in the wrong amount initially, we used the number of surviving individuals rather than initial density for analyses of SVL and larval period length, as it is likely a more accurate representation of functional density.

Priority Effects Experiment

In a second experiment, we tested the mechanism by which the prior establishment of ringed salamanders affect spotted salamanders: resource pre-emption or interference/predation. We set up all tanks for this experiment in an identical manner as above and interspersed them within the *Phenology-Density* experimental array. We used a 4 x 2 design, with four priority effects treatments (Sequential, Synchronous, Low IG Prey Density Control, and High IG Prey Density Control, described below) and two IG predator density treatments (Low: $N = 8$; High: $N = 24$) with three replicates of each treatment. In the Sequential treatment, ringed salamander larvae were added on 3 October 2013 at either low or high density, and then removed prior to the addition of spotted salamanders ($N = 24$) on 27 April 2014; this treatment represents communities where ringed salamanders metamorphose prior to hatching of spotted salamanders in the spring. The Synchronous treatment, where both species were added at the same time on 27 April 2014 to tanks that had been set up the previous fall but left devoid of salamanders, isolates direct effects of species interactions (interference and predation) from indirect effects because larval ringed salamanders did not have time to deplete resources prior to adding spotted salamanders (i.e. the Sequential treatment). We did measure zooplankton

resource levels, and observed reductions in *Daphnia* biomass in the Sequential treatment compared with the other three treatments, though overall zooplankton biomass was not significantly reduced (TLA, unpublished data).

On 21 April 2014, we began capturing ringed salamander larvae from the Sequential treatment tanks with aquarium nets and collapsible mesh minnow traps. This process continued for 6 d, at which point we did not observe or capture any more ringed salamander larvae. No remaining ringed salamanders were captured at the end of the experiment in the Sequential treatments, indicating all larvae had been successfully removed. Sequential treatment tanks had been assigned a matching tank within the Synchronous treatment: ringed salamander larvae removed from the former were transferred to the latter after capture. Prior to this move, larvae were dorsally photographed over a ruler to obtain SVL using ImageJ. For the Low and High Density Control treatments, we added either 24 or 48 hatchling spotted salamanders on 27 April, which served as controls against the combined larval density in a tank (up to 24 ringed salamanders + 24 spotted salamanders).

For the *Priority Effects* experiment, we analyzed identical metrics of metamorphs (SVL, larval period length, and survival) for both species using similar mixed effects models and error distributions as the *Phenology-Density* experiment. For ringed salamanders, the only predictor variable was larval density. We also analyzed larval SVL of ringed salamanders at the time they were moved in relation to larval density. For spotted salamanders, we tested for differences in each response across the four treatments (High and Low Controls, Sequential and Synchronous treatments). We also examined whether the surviving number of ringed salamanders influenced metrics of spotted

salamanders in the Synchronous treatment, the only treatment in this subset where the salamanders co-occurred.

Mixed effects models from both *Phenology-Density* and *Priority Effects* experiments were fit in a Bayesian framework in R through the jagsUI package (Plummer 2003, Kellner 2015). We ran models for 50,000 simulations on three separate chains, a burn-in period of 10,000 iterations and a thinning rate of 1:20. Posterior predictive checks showed all models converged (Gelmin-Rubin statistic < 1.1). We also calculated Bayesian p-values for each model, and all showed satisfactory fit (range: 0.45–55). To assess all treatment differences, we calculated the difference in slope and/or intercept values as derived quantities for all combinations of treatments. If the 95% credible intervals of these derived quantities did not overlap zero, we considered the treatments different. We also calculated an overall slope value to test whether for relationships between surviving ringed salamander density and each individual metric across all treatments in both experiments. We also discuss posterior distributions of contrasts that had a distribution where > 90% shared the same sign as the posterior mean.

Results

Phenology-Density

Overall, SVL of ringed salamander metamorphs showed a negative relationship with the number of surviving ringed salamanders, and on average synchronous (i.e. Early-Early and Early-Late) phenology treatments resulted in larger metamorphs than asynchronous shifts (Early-Late and Late-Early; Figure 2a). All contrasts among phenology treatments for intercepts and slopes overlapped zero, though several posterior distributions exhibited marginal effects (Table 1). The Early-Early treatment had a 94% probability that the

intercept was greater than that of the Early-Late treatment. There were also 97% and 91% probabilities that the slope was steeper in the Early-Early treatment compared to the Early-Late and Late-Early treatments, respectively. The Early-Early and Early-Late treatments had slopes with 95% CRI that did not overlap zero, whereas both the Late-Early and Late-Late treatments had less than a 90% probability of being different from zero (Table A1, Appendix A).

Larval period length were approximately 30 d longer in the Early ringed salamander phenology treatments (Early-Early and Early-Late) compared with the Late ringed salamander phenology treatments (Late-Early and Late-Late; Figure 2c), and both showed positive relationships with the number of survivors. Contrasts among intercepts confirmed this result, as the Early-Early and Early-Late had greater intercepts than all other treatments, and were not different from each other (Table 1). The Late-Early and Late-Late were not different from each other. All contrasts among treatments for slopes included zero, indicating few treatment effects.

Across all phenology treatments, survival of ringed salamanders was higher in the low-density treatment than the high-density treatment (Figure 2e), and the posterior distribution of the contrast between low and high-density treatments did not overlap zero (Table 1). At low initial density, the Early-Early and Early-Late treatments had higher survival than the Late-Late treatment. At high initial density, the asynchronous (Early-Late and Late-Early) treatments had higher survival than the synchronous treatments, and the Early-Early had higher survival than the Late-Late treatment (Table 1).

The overall relationship of metamorph SVL of spotted salamanders with ringed salamander survival was positive (Figure 2b). However, all contrasts among phenology

treatments for slope and intercept values overlapped zero, indicating no strong treatment differences. The intercept of the Early-Early treatment had a moderate support (92% probability) that it was greater than Late-Late treatment (Table 2).

Larval period length of spotted salamanders exhibited a positive relationship with ringed salamander survival (Figure 2d). The intercept for larval period length was greater for the Early-Early compared with the Early-Late treatment (Table 2). There was moderate support (96% probability) that the intercept was greater for the Early-Early treatment than the Late-Early treatment (Table 2). Only the asynchronous treatments (Early-Late and Late-Early) had slopes different from zero (Table A2, Appendix A). All contrasts among slope values included zero, though there was a 93% probability the Late-Early treatment had a more positive slope than the Early-Early treatments (Table 2).

Survival of spotted salamanders was negatively related to ringed salamander survival across all phenology treatments (Figure 2f). All contrasts among slopes and intercepts overlapped zero, though several showed marginal support for differences among treatments (Table 2). The intercept of the Early-Early treatment had a 95% probability of being greater than the Early-Late treatment. The Early-Late intercept also had a 96% probability of being lower than each of the Late-Early and Late-Late treatments. The slope of the Early-Early treatment had a 96% probability of being steeper than the Early-Late treatment. There was a 97% probability that the Early-Late was less steep than each of the Late-Early and Late-Late treatments (Table 2).

Priority Effects

Larval ringed salamanders averaged 30 mm SVL (range: 23–42 mm) in the Sequential treatments at the time they were removed, and showed a negative relationship between

SVL and the number of larvae transferred to the Synchronous treatment. This pattern in SVL continued for these individuals as metamorphs, where tanks with lower survival resulted in larger metamorphs compared with tanks that had higher survival (Figure A2, Appendix A). Metamorphic ringed salamanders also had longer larval periods when survival was higher (Figure A2, Appendix A). Survival of ringed salamanders was not different in the low versus high initial density treatments within the Sequential treatment, and was relatively high for both (mean $\pm$ SD: $84 \pm 24\%$ vs. $72 \pm 23\%$; Figure A2, Appendix A).

SVL of metamorphic spotted salamanders varied among Priority Effects treatments. Individuals from the Synchronous treatment were largest, followed by the Low Control, the High Control, and then the Sequential treatment (Figure 3a). The contrasts between the Synchronous-High Control, the Synchronous-Low Control, the Synchronous-Sequential, and the High-Low Controls treatments all did not include zero. There were also 97% and 92% probabilities that metamorphs in the High Control were smaller than individuals from the Low Control and Sequential treatments, respectively. For larval period length, the contrast was between the Synchronous and High Control was the only one that did not overlap zero (Figure 3b; Table A3, Appendix A). There was also moderate support that larval period length was longer in the Sequential compared to the Synchronous (97%) treatments, and in the High Control compared with the Low Control (96%). Survival of spotted salamanders was higher in the Sequential compared with both controls (Table A3, Appendix A), though the raw mean values were not substantially different among these three treatments ($82 \pm 0.8\%$, $81 \pm 0.07\%$ and $85 \pm 18\%$, respectively). The Synchronous had lower survival than the Sequential treatment, but a

95% probability of being higher than the Low Control. There was also a 96% probability that survival was higher in the Low Control compared with the High Control. Average survival was $61 \pm 29\%$ in the Synchronous treatment whereas the other three treatments were. The survival of ringed salamanders within the Synchronous treatment explained some of the high variance in spotted salamander survival, which showed a negative relationship between species (β = -0.218; 95% CI = -0.259, -0.179; Figure 3c).

Discussion

Phenological shifts due to climate change are increasingly being documented among numerous taxa (Parmesan 2006), but mechanistic investigations into the consequences of such shifts on community ecology have received relatively little attention (Yang and Rudolf 2010, Rafferty et al. 2013). Furthermore, synergistic effects of shifts in phenology with other background processes, such as density dependence, are likely to be important in structuring both populations and communities but have been rarely investigated (Hunter and Elkinton 2001, Ryan and Plague 2004). We found highly asymmetric effects of these factors within an IGP module, where density dependence negatively affected size and survival of both the IG predator and IG prey substantially more than did altering shifts in either species' phenology. We also found support for density-dependent predation as the dominant mechanism by which the IG predator in our system limits its IG prey, as opposed to resource pre-emption. Overall, our study provides critical insight into the relative magnitude of phenological shifts needed to alter species interactions, and highlights that considering alternative mechanisms (e.g. density dependence) will help identify the relative importance of shifts in phenology on species interactions.

Density Dependence

We found that intraspecific density-dependent competition negatively affected body size and positively affected larval period length of ringed salamanders. Similar patterns of density dependence have been observed in numerous taxa, especially amphibians (Van Buskirk and Smith 1991, Scott 1994, Anderson and Whiteman 2015). As body size at metamorphosis is a strong predictor of adult fitness and survival in amphibians (Semlitsch et al. 1988, Altwegg and Reyer 2003, Earl and Whiteman 2015), understanding the mechanisms that influence growth rates and body size are critical to understanding selective pressures on individuals. Furthermore, natural populations that vary in densities would result in juveniles of variable sizes (Van Buskirk and Smith 1991), which can translate into differential contributions toward metapopulation structure (W.E. Peterman, unpublished data). We also observed strong compensatory growth in the IG predator, where the late additions for ringed salamanders overcame a 30 day deficit in breeding phenology to metamorphose at the same time and at the same size as the early additions. Compensatory growth has been documented in numerous taxa (Metcalfe and Monaghan 2001), but especially in organisms that inhabit ephemeral aquatic ecosystems, as such plasticity is likely critical to advance development time and complete metamorphosis under deteriorating conditions (Altwegg 2002, Stoks et al. 2006, Orizaola et al. 2010, 2014). Such compensatory growth in ringed salamanders is surprising, however, because it has a larval period nearly double that of any other known amphibian to have exhibited compensatory growth and thus requires more permanent ponds, which would suggest the selection from pond drying on growth rates would likely be reduced for this species. In contrast, we did not observe compensatory growth responses in the IG prey, spotted salamanders. These differences could result from the high quality food

source added to tanks for ringed salamander (i.e. spotted salamander hatchlings) which promoted growth late in the larval period, a process that has been shown to influence metamorphic rates in other salamanders (Ryan and Semlitsch 2003).

We observed density-dependent mortality in ringed salamanders, which is contrary to other experimental studies on this species (Anderson and Semlitsch 2014, Ousterhout and Semlitsch 2015). The overwintering portion of their larval period during the study coincided with an extremely cold winter that resulted in tanks freezing nearly solid. However, at least one salamander survived in all tanks, despite a small volume of liquid water for a long portion of the winter (T.L. Anderson, personal observation). Larval mortality due to pond freezing has been reported in other studies (Herstoff and Urban 2014), though our study shows that this effect can be density-dependent. The underlying mechanism behind this pattern is not explicitly known from the current study, but could stem from competition for limited food resources, oxygen depletion or multiple synergistic factors.

Survival of the IG prey in this study showed a negative relationship with IG predator survival. Similar to other studies, we observed thinning effects, where body size and mortality rates of prey were positively correlated (Van Buskirk and Yurewicz 1998, Anderson and Semlitsch 2014). However, we also observed greater variation and a reduced strength in this effect compared with previous studies, which could stem from different winter conditions among these studies that limited basal prey levels to such a degree that there was reduced prey available to the few survivors. Identifying such variability in ecological processes such as thinning effects is critical, as it will lead to a better understanding of spatiotemporal variation in species interactions. One predicted

consequence of phenological shifts for gape-limited predators such as ringed salamanders would be a switch in interaction type from predation to competition as size asymmetries were minimized (Figure 1; Yang and Rudolf 2010), which in this system could also result from density-dependent processes. Our experiment indicates that only extreme density-dependence within the IG predator would induce reductions in size asymmetries, or other processes that similarly limit their growth rates, resulting in a switch of interaction type.

Phenology Shifts

Phenological shifts in both the IG predator and prey had relatively little influence on either species' individual traits or survival. This finding highlights a context-dependence to when shifts in phenology may be important to species interactions, as not all species are changing in a similar manner and thus only certain interactions may be influenced (Rafferty and Ives 2011, CaraDonna et al. 2014). Specifically, species interactions determined by phenology but spaced out over greater temporal separation may not be as influenced by phenological shifts as those more synchronous in time. In our case, phenology shifts of 30 days represent ca. 15% of the total larval period length for ringed salamanders, which may not be enough of a shift to influence interspecific size differences. Todd et al. (2011) reported a 15 d shift in median breeding phenology of another fall breeding ambystomatid (marbled salamanders, *A. opacum*), which though it represented a statistically significant phenological shift, such a difference may not unduly alter interspecific interactions with spring-breeding species. However, Todd et al. (2011) also reported three species all converged on a similar breeding date (two species earlier and one later) that spanned 60-75 d shifts. Such extreme shifts are therefore possible, and represent scenarios where species interactions are more likely to be altered.

We compared phenological shifts in breeding between two dates for each species, analogous to a shift in the mean breeding date. However, mean shifts in phenology may underestimate how species' phenological patterns are shifting, and rather changes in phenological variance, skewness, or earliest/last dates may also occur and be more critical to species interactions (CaraDonna et al. 2014, Rasmussen and Rudolf 2015). For salamanders, we expect that altered variance in breeding would be particularly important in the development of larval size-structure: a greater number of breeding pulses spread out across a longer time period may increase size variance both within and between species, especially if cannibalism occurs among cohorts. Alternatively, decreased phenological variance to where more individuals are synchronized (either con- or heterospecifics) may reduce size structure but increase density-dependence, which we found to be a much stronger predictor of both IG predator and prey responses. Further tests are therefore needed to determine what temporal component of phenological variation, if any, is most critical towards explaining shifts in species interactions.

Priority Effects

The mechanism by which early colonizing individuals either enhance or limit subsequent arrival patterns has been a long-standing area of investigation in ecology, and likely will have important consequences for interactions altered by phenology. However, tests of which mechanism is pervasive are rarer. We found that prior occupancy of tanks by ringed salamanders did not influence body size or survival of spotted salamanders, and resulted in similar patterns to control treatments without ringed salamanders. However, when the two co-occurred without resource depletion present, spotted salamander survival showed a density-dependent response to ringed salamanders, indicating that the

relative impacts of resource depletion on spring breeding species are substantially less than the direct effects of predation. We cannot entirely disentangle the exact process (avoidance mechanisms of the IG prey, interference competition, or predation by the IG predator), but we speculate predation as the likely culprit due to the foraging rates observed in laboratory studies (Anderson et al. 2016). Behavioral avoidance seems unlikely to have occurred as spotted salamanders do not always respond to predatory threats by congeners (Walls 1995). Overall, identifying priority effects mechanisms is critical, as it shows that breeding phenology shifts resulting in reduced temporal overlap or minimized size differences would reduce predation rates and ultimately the importance of their interaction.

Conclusions

Experimental investigations of shifts in phenology can set a baseline for understanding how current variation in phenology determine interspecific interactions, as well as simulate scenarios of how future phenological shifts will alter community structure under climate change (Visser and Both 2005, Rafferty et al. 2013, Forrest 2015). Such investigations, coupled with natural observations, would be exceedingly powerful to unravel the mechanistic pathways in species interactions associated with phenology shifts. It also important to not discount background processes, such as density-dependence, that may be equally or more important for determining the outcome of species interactions. Assessments of these asymmetric or synergistic effects on species interactions is an important next step in understanding how climates shifts will alter species interactions and community structure.

Acknowledgements

References

Alford, R. A. 1989. Variation in predator phenology affects predator performance and prey community composition. Ecology **70**:206-219.

Alford, R. A., and H. M. Wilbur. 1985. Priority effects in experimental pond communities: competition between *Bufo* and *Rana*. Ecology **66**:1097-1105.

Almany, G. R. 2003. Priority effects in coral reef fish communities. Ecology **84**:1920-1935.

Altwegg, R. 2002. Predator-induced life-history plasticity under time constraints in pool frogs. Ecology **83**:2542-2551.

Altwegg, R., and H. U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution **57**:872-882.

Anderson, T. L., D. J. Hocking, C. A. Connor, J. E. Earl, E. B. Harper, M. S. Osbourn, W. E. Peterman, T. A. Rittenhouse, and R. D. Semlitsch. 2015. Abundance and phenology patterns of two pond-breeding salamanders determine species interactions in natural populations Oecologia **177**:761-773.

Anderson, T. L., C. Linares, K. Dodson, and R. D. Semlitsch. 2016. Variability in

    functional response curves among larval salamanders: comparisons across species

    and size classes. Canadian Journal of Zoology **94**:23-30.

Anderson, T. L., and R. D. Semlitsch. 2014. High intraguild predator density induces

    thinning effects on and increases temporal overlap with prey populations.

    Population Ecology **56**:265-273.

Anderson, T. L., and H. H. Whiteman. 2015. Asymmeric effects of intra- and

    interspecific competition on a pond-breeding salamander. Ecology **96**:1681-1690.

Benard, M. F. 2015. Warmer winters reduce frog fecundity and shift breeding phenology,

    which consequently alters larval development and metamorphic timing. Global

    Change Biology **21**:1058-1065.

Blois, J. L., P. L. Zarnetske, M. C. Fitzpatrick, and S. Finnegan. 2013. Climate change

    and the past, present, and future of biotic interactions. Science **341**:499-504.

Boone, M. D., D. E. Scott, and P. H. Niewiarowski. 2002. Effects of hatching time for

    larval ambystomatid salamanders. Copeia **2002**:511-517.

CaraDonna, P. J., A. M. Iler, and D. W. Inouye. 2014. Shifts in flowering phenology

    reshape a subalpine plant community. Proceedings of the National Academy of

    Sciences **111**:4916-4921.

Connell, J. H., and R. O. Slatyer. 1977. Mechanisms of succession in natural

    communities and their role in community stability and organization. American

    Naturalist **111**:1119-1144.

Durant, J. M., D. O. Hjermann, T. Anker-Nilssen, G. Beaugrand, A. Mysterud, N.

    Pettorelli, and N. C. Stenseth. 2005. Timing and abundance as key mechanisms

affecting trophic interactions in variable environments. Ecology Letters **8**:952-958.

Earl, J. E., and H. H. Whiteman. 2015. Are commonly used fitness predictors accurate? A meta-analysis of amphibian size and age at metamorphosis. Copeia **103**:297-309.

Forrest, J. R. K. 2015. Plant-pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations? Oikos **124**:4-13.

Hernandez, J. P., and D. R. Chalcraft. 2012. Synergistic effects of multiple mechanisms drive priority effects within a tadpole assemblage. Oikos **121**:259-267.

Herstoff, E., and M. C. Urban. 2014. Will pre-adaptation buffer the impacts of climate change on novel species interactions? Ecography **37**:111-119.

Hunter, A. F., and J. S. Elkinton. 2001. Interaction between phenology and density effects on mortality from natural enemies. Journal of Animal Ecology **68**:1093-1100.

Kardol, P., L. Souza, and A. T. Classen. 2013. Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. Oikos **122**:84-94.

Kellner, K. 2015. jagsUI: Run JAGS from R (an alternative user interface for rjags) 1.3.1 http://CRAN.R-project.org/package=jagsUI

Lawler, S. P., and P. J. Morin. 1993. Temporal overlap, competition, and priority effects in larval anurans. Ecology **74**:174-182.

Louette, G., and L. De Meester. 2007. Predation and priority effects in experimental zooplankton communities. Oikos **116**:419-426.

Metcalfe, N. B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends in Ecology & Evolution **16**:254-260.

Miller-Rushing, A. J., T. T. Hoye, D. W. Inouye, and E. Post. 2010. The effects of phenological mismatches on demography. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences **365**:3177-3186.

Morin, P. J. 1987. Predation, breeding asynchrony, and the outcome of competition among treefrog tadpoles. Ecology **68**:675-683.

Nakazawa, T., and H. Doi. 2012. A perspective on match/mismatch of phenology in community contexts. Oikos **121**:489-495.

Orizaola, G., E. Dahl, and A. Laurila. 2010. Compensating for delayed hatching across consecutive life-history stages in an amphibian. Oikos **119**:980-987.

Orizaola, G., E. Dahl, and A. Laurila. 2014. Compensatory growth strategies are affected by the strength of environmental time constraints in anuran larvae. Oecologia **174**:131-137.

Ousterhout, B. H., and R. D. Semlitsch. 2015. Non-additive response of larval ringed salamanders to intraspecific density. Oecologia.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics **37**:637-669.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature **421**:37-42.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian

    Institution Press, Washington [D.C.].

Plummer, M. 2003. JAGS: A program for analysis of Bayesian graphical models using

    Gibbs sampling. Page 125 *in* Proceedings of the 3rd international workshop on

    distributed statistical computing. Technische Universit at Wien.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild

    predation: potential competitors that eat each other. Annual Review of Ecology

    and Systematics **20**:297-330.

Rafferty, N. E., P. J. Caradonna, L. A. Burkle, A. M. Iler, and J. L. Bronstein. 2013.

    Phenological overlap of interacting species in a changing climate: an assessment

    of available approaches. Ecology and Evolution **3**:3183-3193.

Rafferty, N. E., and A. R. Ives. 2011. Effects of experimental shifts in flowering

    phenology on plant-pollinator interactions. Ecology Letters **14**:69-74.

Rasmussen, N. L., and V. H. W. Rudolf. 2015. Phenological synchronization drives

    demographic rates of populations. Ecology **96**:1754-1760.

Reed, T. E., P. Gienapp, and M. E. Visser. 2015. Density dependence and microevolution

    interactively determine effects of phenology mismatch on population dynamics.

    Oikos **124**:81-91.

Revilla, T., F. Encinas-Viso, and M. Loreau. 2014. (A bit) Earlier or later is always

    better: Phenological shifts in consumer–resource interactions. Theoretical

    Ecology **7**:149-162.

Ryan, T. J., and G. R. Plague. 2004. Hatching asynchrony, survival, and the fitness of

    alternative adult morphs in *Ambystoma talpoideum*. Oecologia **140**:46-51.

Ryan, T. J., and R. D. Semlitsch. 2003. Growth and the expression of alternative life

    cycles in the salamander *Ambystoma talpoideum* (Caudata: Ambystomatidae).

    Biological Journal of the Linnean Society **80**:639-646.

Scott, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma*

    opacum. Ecology **75**:1383-1396.

Semlitsch, R. D., and T. L. Anderson. *In Press*. Structure and dynamics of Spotted

    Salamander (*Ambystoma maculatum*) populations in Missouri. Herpetologica **72**.

Semlitsch, R. D., T. L. Anderson, M. S. Osbourn, and B. H. Ousterhout. 2014. Structure

    and dynamics of ringed salamander (*Ambystoma annulatum*) populations in

    Missouri. Herpetologica **70**:14-22.

Semlitsch, R. D., D. E. Scott, and J. H. Pechmann. 1988. Time and size at metamorphosis

    related to adult fitness in *Ambystoma talpoideum*. Ecology **69**:184-192.

Shorrocks, B., and M. Bingley. 1994. Priority effects and species coexistence:

    experiments with fungal-breeding *Drosophila*. Journal of Animal Ecology

    63:799-806.

Stoks, R., M. D. Block, and M. A. McPeek. 2006. Physiological costs of compensatory

    growth in a damselfly. Ecology **87**:1566-1574.

Todd, B. D., D. E. Scott, J. H. Pechmann, and J. W. Gibbons. 2011. Climate change

    correlates with rapid delays and advancements in reproductive timing in an

    amphibian community. Proceedings of the Royal Society B: Biological Sciences

    278:2191-2197.

Urban, M. C., J. J. Tewksbury, and K. S. Sheldon. 2012. On a collision course: competition and dispersal differences create no-analogue communities and cause extinctions during climate change. Proc Biol Sci **279**:272-280.

Van Buskirk, J., and D. C. Smith. 1991. Density-dependent population regulation in a salamander. Ecology **72**:1747-1756.

Van Buskirk, J., and K. L. Yurewicz. 1998. Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. Oikos **82**:20-28.

Visser, M. E., and C. Both. 2005. Shifts in phenology due to climate change: the need for a yardstick. Proceedings of the Royal Society of London, Series B: Biological Sciences **272**:2561-2569.

Walls, S. C. 1995. Differential vulnerability to predation and refuge use in competing larval salamanders. Oecologia **101**:86-93.

Wilbur, H. M., and R. A. Alford. 1985. Priority effects in experimental pond communities: responses of *Hyla* to *Bufo* and *Rana*. Ecology **66**:1106-1114.

Yang, L. H., and V. H. Rudolf. 2010. Phenology, ontogeny and the effects of climate change on the timing of species interactions. Ecology Letters **13**:1-10.

Zarnetske, P. L., D. K. Skelly, and M. C. Urban. 2012. Ecology. Biotic multipliers of climate change. Science **336**:1516-1518.

Table 1: Summary of posterior distribution of contrasts among the phenology and density treatments for ringed salamanders.

Treatment Contrast		Estimate	Snout-vent Length				Larval Period Length				Survival*			
			mean	2.50	97.50	f	mean	2.50	97.50	f	mean	2.50	97.50	f
early-early	early-late	intercept	2.82	-0.93	6.59	0.94	-2.26	-9.56	5.16	0.73	-0.14	-0.67	0.39	0.30
early-early	late-early		4.65	-5.57	15.14	0.82	25.99	7.62	44.58	1.00	NA	NA	NA	NA
early-early	late-late		2.24	-4.07	8.45	0.77	22.91	10.45	35.46	1.00	0.82	0.31	1.33	1.00
early-late	late-early		1.83	-8.52	12.25	0.64	28.25	9.80	46.75	1.00	NA	NA	NA	NA
early-late	late-late		-0.59	-6.56	5.38	0.58	25.17	12.92	37.08	1.00	0.96	0.46	1.45	0.53
late-early	late-late		-2.41	-14.19	8.98	0.66	-3.08	-23.73	17.69	0.62	NA	NA	NA	NA
early-early	early-late	slope	-0.38	-0.81	0.04	0.97	0.50	-0.34	1.32	0.88	-1.40	-1.60	-1.19	1.00
early-early	late-early		-0.44	-1.11	0.21	0.91	0.57	-0.65	1.77	0.82	-1.66	-1.84	-1.48	1.00
early-early	late-late		-0.46	-1.56	0.65	0.80	0.88	-1.27	3.09	0.79	0.66	0.41	0.92	1.00
early-late	late-early		-0.06	-0.64	0.53	0.58	0.07	-0.96	1.12	0.55	-0.26	-0.40	-0.12	1.00
early-late	late-late		-0.08	-1.12	0.98	0.55	0.38	-1.68	2.45	0.64	2.06	1.84	2.30	1.00
late-early	late-late		-0.02	-1.16	1.16	0.51	0.31	-1.92	2.55	0.61	2.32	2.12	2.54	1.00

*Columns show: mean = posterior distribution mean; 2.5/97.5% = 95% credible interval, f = the proportion of the posterior distribution that shares the same sign as the mean. Survival contrasts show low (light gray shading) and high (dark gray shading) initial density treatments.

Table 2: Summary of posterior distribution of contrasts among the phenology treatments for spotted salamanders.

Treatment Contrast		Parameter	Snout-vent Length				Larval Period Length				Survival			
			mean	2.50	97.50	f	mean	2.50	97.50	f	mean	2.50	97.50	f
early-early	early-late	intercept	1.08	-1.22	3.43	0.82	0.58	-10.64	11.29	0.54	1.30	-0.27	2.86	0.95
early-early	late-early		1.78	-5.35	8.75	0.70	-11.45	-20.61	-2.34	0.99	-2.87	-7.55	2.00	0.89
early-early	late-late		2.59	-1.19	6.26	0.92	2.76	-6.34	11.76	0.73	-0.79	-3.28	1.78	0.74
early-late	late-early		0.70	-6.63	7.60	0.58	-12.03	-22.87	-1.28	0.99	-4.17	-8.89	0.66	0.96
early-late	late-late		1.51	-2.21	5.02	0.81	2.18	-8.22	12.48	0.66	-2.09	-4.43	0.32	0.96
late-early	late-late		0.81	-6.66	8.69	0.59	14.21	5.02	23.20	1.00	2.09	-3.03	7.13	0.80
early-early	early-late	slope	0.24	-0.13	0.63	0.89	0.70	-0.26	1.69	0.92	-0.24	-0.50	0.02	0.96
early-early	late-early		0.12	-0.41	0.65	0.66	0.86	-0.31	2.03	0.92	0.03	-0.34	0.37	0.57
early-early	late-late		-0.16	-0.84	0.57	0.69	0.16	-0.46	0.80	0.69	0.14	-0.33	0.61	0.73
early-late	late-early		-0.12	-0.53	0.31	0.72	0.15	-1.24	1.55	0.58	0.27	-0.02	0.54	0.97
early-late	late-late		-0.40	-1.02	0.27	0.89	-0.54	-1.50	0.42	0.87	0.38	-0.04	0.79	0.97
late-early	late-late		-0.27	-1.00	0.46	0.78	-0.69	-1.88	0.51	0.88	0.12	-0.35	0.60	0.69

*Columns show: mean = posterior distribution mean; 2.5/97.5% = 95% credible interval, f = the proportion of the posterior distribution that shares the same sign as the mean.

Figure Legends

Figure 1: Life history diagram and theoretical predictions on the shift in importance of competition vs. predation between ringed salamanders (intraguild predator, RS) and spotted salamanders (intraguild prey, SS). Life history timing represents patterns observed in Missouri (Anderson et al. 2015a). * indicate when our Phenology treatments occurred.

Figure 2: Snout-vent length (A, B), larval period length (C, D), and survival (E, F) of the intraguild predator (ringed salamander) and intraguild prey (spotted salamander) among phenology treatments, and initial density treatments (E). Each point represents one individual, and are slightly adjusted horizontally to avoid overlap.

Figure 3: Snout-vent length (A), larval period length (B), and survival (C) of intraugild prey (spotted salamander) in Priority Effects treatments ('Low Cont' = Low Density Control, 'High Cont' = High Density Control, 'Synchr' = Synchronous, 'Sequen' = Sequential). In Panel C, filled circles and solid line represent the Synchronous treatment, open circles and dashed line the Sequential treatment, open squares the High Control, and open triangles the Low Control.
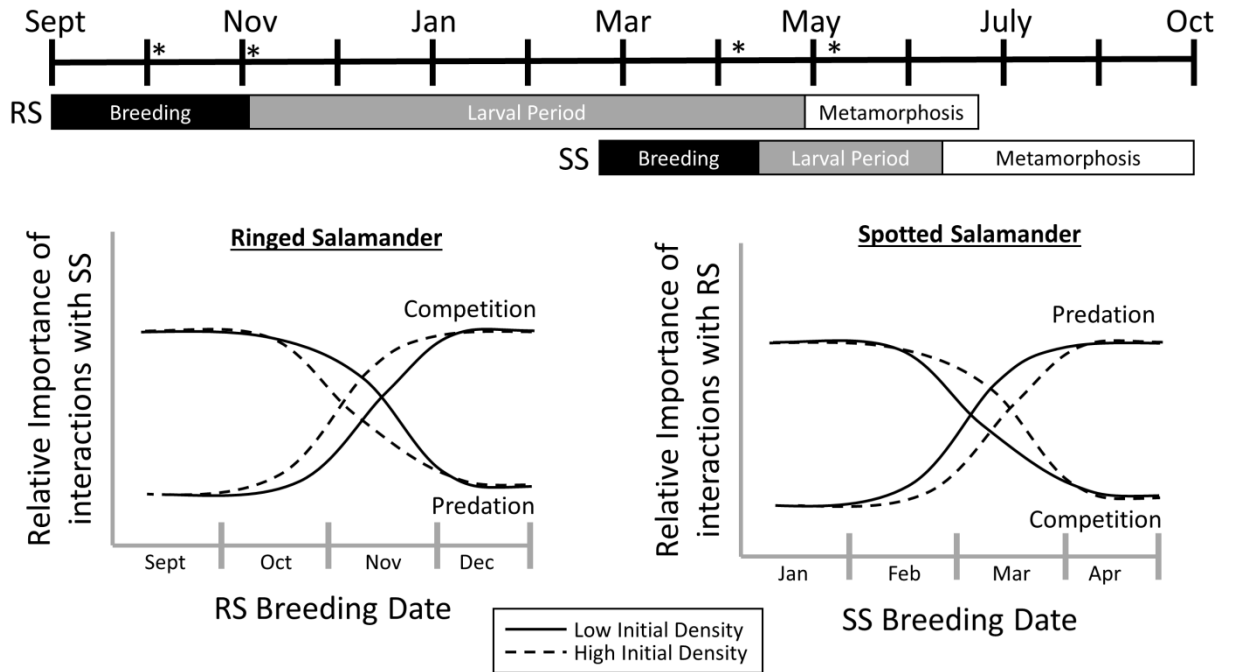
Figure 1
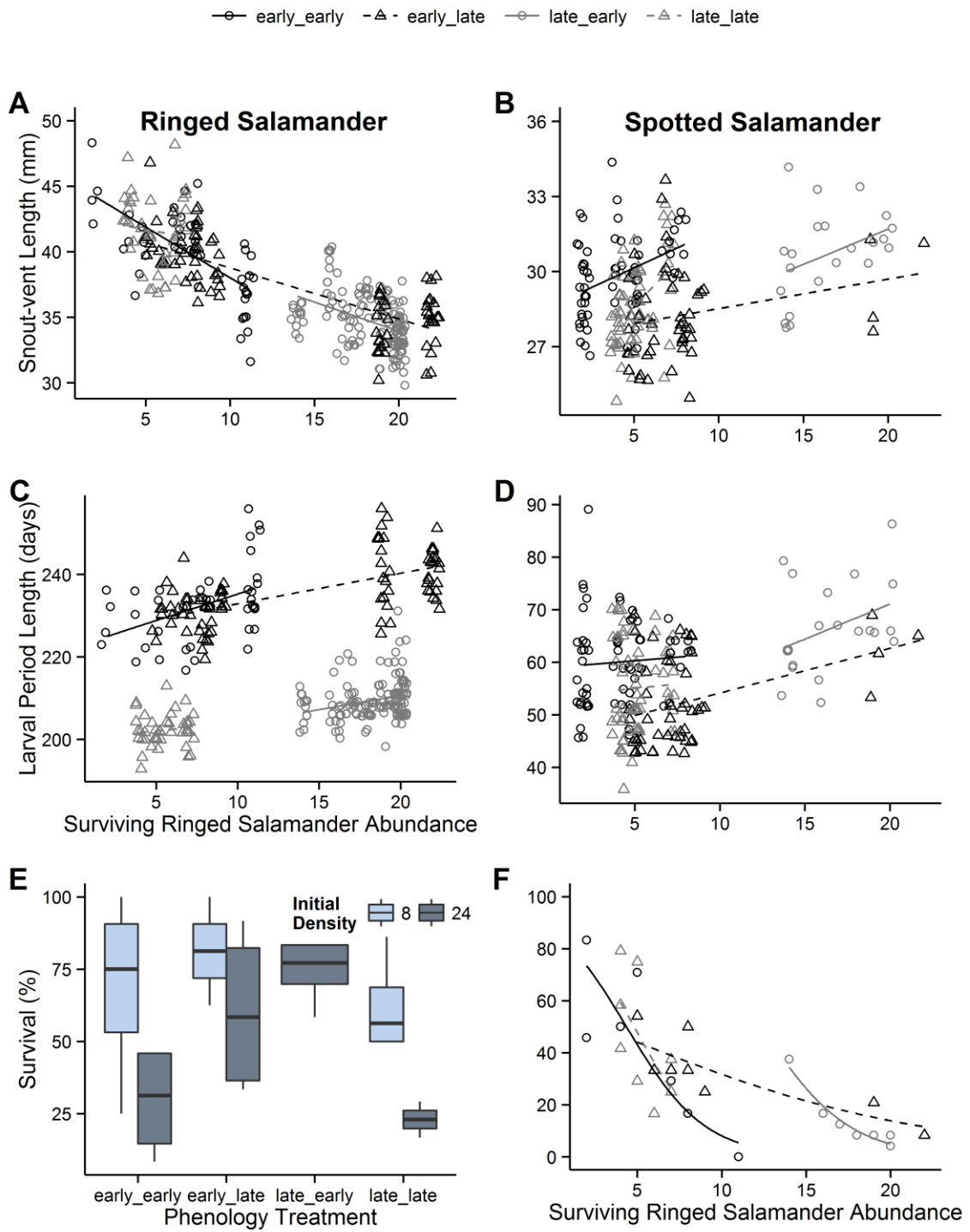
Figure 2
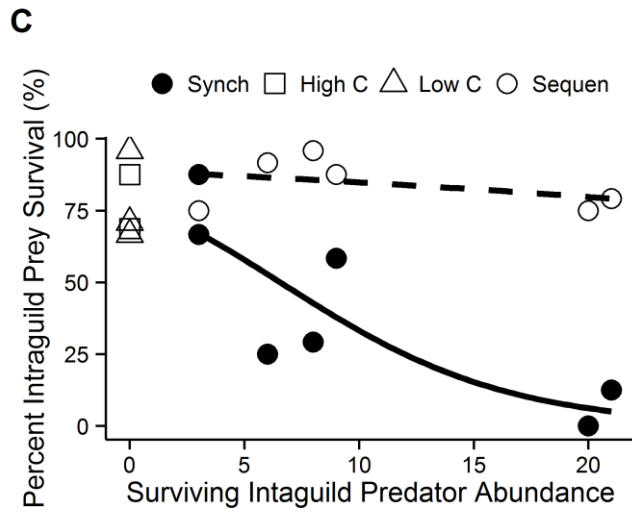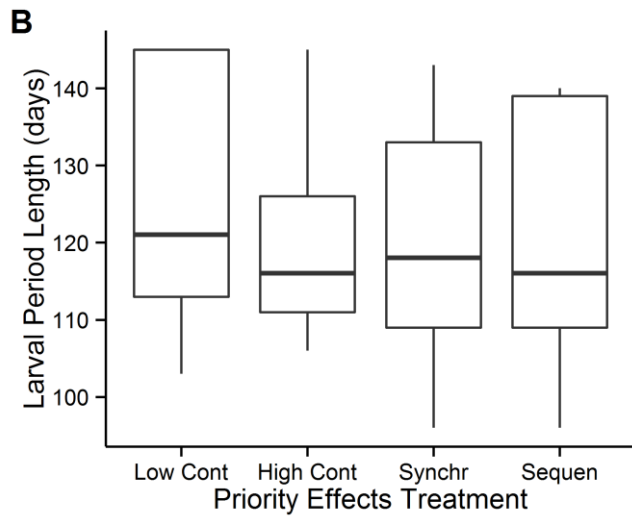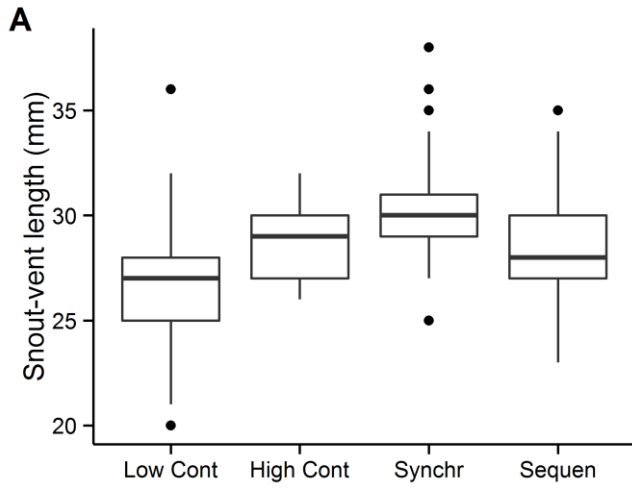
Figure 3

Chapter 5

SUMMARY

Thomas L. Anderson

Predator-prey interactions are a key process in ecological communities that can have implications for the predator, prey or the entire community in which they are embedded. Many biotic and abiotic factors can influence survival rates of the prey, capturing abilities of the predator, or other aspects of their interactions. How synergies between these biotic and abiotic factors influence predation is unknown for many systems, yet are likely critical towards developing a deep understanding of the complexities of such processes. This dissertation develops a comprehensive understanding of the myriad factors that affect intraguild interactions between the ringed and spotted salamander. The summary below highlights the contributions and key findings of each dissertation chapter:

Chapter 2. – High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations

- High predator densities results in reduced prey survival but increased prey body size via thinning effects.
- Increased predator densities lengthened their larval period, increasing their temporal overlap with prey.

Chapter 3. – Top predators and habitat complexity alter an intraguild predation module in pond communities

- Intraguild predator body size and survival was negatively affected different combinations of top predators, primarily driven by whether aeshnids were present as a predator. Habitat complexity had limited effects.
- Intraguild prey body size and survival showed complex patterns with different top predators and habitat complexity that was mediated by how many intraguild predators survived.

Chapter 4. – Asymmetric effects of phenological shifts and larval density influence intraguild predation between salamanders

- Density dependence negatively affected body size of intraguild predators, but phenology did not have significant effects. Survival was negatively affected by both density dependence and phenology.
- Intraguild prey body size and survival were negatively affected the density of intraguild predators, but not phenology treatments.
- The mechanism by which intraguild predators limit intraguild prey is predation rather than resource pre-emption.

Appendix A

NONLINEAR MODEL SELECTION RESULTS

We compared six nonlinear growth models for larval SVL of *A. annulatum* (i.e.,
as this was prior to *A. maculatum* addition). Models that were compared included a
linear, Ricker, negative exponential, power law, Shepard and hyperbolic curves (Bolker
2008, Table A1). We tested the same candidate set for intraspecific effects on metamorph
response variables (snout-vent length (SVL), mass, growth rate and days to
metamorphosis). Days to metamorphosis showed opposite relationships with density
compared to the other metamorph response variables, and appropriate functional models
were tested for that response (Table A1). Due to small sample sizes, nonlinear models
would not converge to compare intra- and interspecific effects on the metamorph
response variables for *A. annulatum*. However, the results from the model comparison
showed that the linear model was often close (< 2.0 ΔAIC_c) to the nonlinear models
(Table A2); therefore, we used an ANCOVA to test for the effects of conspecific density
and the presence/absence of *A. maculatum*, under the assumption the results of this
analysis would be analogous to the nonlinear models. We examined how the final density
(i.e. number of surviving metamorphs) of *A. annulatum* affected *A. maculatum* response
variables by comparing linear, three-parameter Michaelis-Menten, negative exponential,
Shepherd and power law models (Table S1). All models in this set included estimation of
an intercept term. These same five models were also tested for both maximum and
minimum days of temporal overlap to examine how the final density of *A. annulatum*
affected the time of co-occurrence between the two species. Minimum and maximum

days were calculated by the first and last days that metamorphs of *A. annulatum* were captured, respectively, in a tank.

Nearly all models for the response variables of *A. annulatum* provided similar fits, except for the Ricker model (Table A2). The power law model was the highest supported model (AIC_c weight range: 0.33-0.40), but all four nonlinear models, as well as the linear model, were less than two ΔAIC_c for most response variables. This indicates that the pattern of intraspecific density-dependence was slightly nonlinear for most response variables, and that most of the models were similar in predicting how each response varied with conspecific density.

For *A. maculatum*, the top model for each response varied, but similar to *A. annulatum*, several models were nearly identical in terms of support (Table A2). However, the nonlinear models were more highly supported for this species compared to the linear model for most responses; the linear model was supported for larval SVL and overall growth rate. The Ricker, negative exponential, power and Michaelis-Menten models were all within one ΔAIC_c value of the top model for larval and metamorph SVL, metamorph mass, and overall growth rate. The Shepherd model was consistently not supported for any response variables (Table A2). The variance (R^2) explained by the fitted models for each response variable was much higher for *A. maculatum* compared to *A. annulatum*, except for days to metamorphosis.

Table A1: Nonlinear regression models tested for *Ambystoma annulatum* and *A. maculatum*. For all models, *X* represents the abundance of *A. annulatum*, *e* is Napier's constant, and letters *a-c* are estimated parameters. No intraspecific effects were modeled for *A. maculatum* because their density was not manipulated beyond presence/absence.

Response Variables	Model Name	Formula
▪ Mean SVL and mass at metamorphosis ▪ Mean growth rate ▪ Mean days to metamorphosis[1] ▪ Survival[1,2]	Hyperbolic (Hyper)	$a/(b+X)$
	Negative Exponential (NegExp)	ae^{-bX}
	Power	aX^b
	Shepherd	$aX/(b+X^c)$
	Ricker	$aXe^{(-bX)}$
	Linear	$a+bX$
	Michaelis-Menten (M-M)	$aX/(1+b*X)$
	Monomolecular (Mono)	$a(1-e^{-bX})$

[1] The power law and Shepard models would not converge for *A. maculatum*, and were not compared for this response variable.

[2] Only the linear, hyperbolic and negative exponential models were converged for this response in *A. maculatum*, and were not compared for this response variable.

Table A2: Model selection results for *Ambystoma annulatum* and *A. maculatum*. *K* is the number of parameters in the model and *ω* is the model weight. See Table S1 for model formulas.

Response	*Ambystoma annulatum*				*Ambystoma maculatum*			
	Model	**K**	**ΔAIC$_c$**	**ω**	**Model**	**K**	**ΔAIC$_c$**	**ω**
Larval SVL (mm)	Power	2	0.0	0.391	M-M	3	0.0	0.240
	Hyper	2	1.6	0.178	Power	3	0.0	0.237
	Shepherd	3	1.8	0.156	Mono	3	0.3	0.204
	NegExp	2	1.9	0.149	Ricker	3	0.8	0.160
	Linear	2	2.3	0.126	Linear	2	1.0	0.148
	Ricker	2	19.2	<0.001	Shepherd	4	6.2	0.011
Metamorph SVL (mm)	Power	2	0.0	0.404	Power	3	0.0	0.264
	Shepherd	3	0.9	0.258	M-M	3	0.0	0.258
	Hyper	2	1.6	0.179	Mono	3	0.3	0.226
	Linear	2	1.9	0.160	Ricker	3	1.3	0.137
	NegExp	2	17.7	<0.001	Linear	2	1.9	0.103

	Ricker	2	33.4	<0.001	Shepherd	4	6.3	0.012
Metamorph mass (g)	Power	2	0.0	0.333	Linear	2	0.0	0.767
	Shepherd	3	1.1	0.195	M-M	3	4.6	0.077
	Hyper	2	1.2	0.181	Ricker	3	4.6	0.076
	NegExp	2	1.5	0.155	Power	3	4.7	0.074
	Linear	2	1.8	0.135	Shepherd	4	9.8	0.005
	Ricker	2	11.9	<0.001				
Growth rate (mm/day)	Power	2	0.0	0.342	Mono	3	0.0	0.285
	Hyper	2	1.2	0.188	M-M	3	0.0	0.281
	NegExp	2	1.3	0.176	Power	3	0.1	0.271
	Linear	2	1.5	0.166	Ricker	3	2.2	0.100
	Shepherd	3	2.0	0.128	Linear	2	3.3	0.055
	Ricker	2	24.1	<0.001	Shepherd	4	6.3	0.012
Days to metamorphosis	Power	2	0.0	0.327	M-M	3	0.0	0.469
	Linear	2	0.1	0.307	Linear	2	0.0	0.466

	M-M	2	0.2	0.302	Ricker	3	4.0	0.065
	Shepherd	3	3.3	0.064	Power	3	66.4	<0.001
	Ricker	2	50.1	<0.001				
Survival	Linear	3	0.0	0.197	Hyper	3	0.0	0.748
	NegExp	2	28.9	0.191	NegExp	3	2.2	0.252
	Hyper	2	96.4	0.179				
	Ricker	2	96.6	0.149				

Appendix B

PARAMETER ESTIMATES AND ADDITIONAL FIGURES FOR RINGED SALAMANDERS (*AMBYSTOMA ANNULATUM*)

AND SPOTTED SALAMANDERS (*A. MACULATUM*)

Table B1: Parameter estimates of the linear mixed effects ANCOVA model for *A. maculatum* metamorph body condition. Lower and upper are 95% confidence intervals. Bold values represent whether the intercept and slope are different from zero, and an approximate test of significance among treatments is:

$t = (estimate_1-estimate_2)/sqrt((SE_1^2+SE_2^2)/2)$, with $t > 2$ indicates a significant difference.

	No Cover					Cover				
Predator	**Estimate**	**SE**	**t-value**	**Lower**	**Upper**	**Estimate**	**SE**	**t-value**	**Lower**	**Upper**
Intercept										
IGP	**0.026**	**0.006**	**4.269**	**0.014**	**0.038**	**0.026**	**0.006**	**4.392**	**0.014**	**0.038**
MF	**0.046**	**0.008**	**6.095**	**0.031**	**0.061**	**0.026**	**0.005**	**5.623**	**0.017**	**0.035**
AE	**0.036**	**0.009**	**3.957**	**0.018**	**0.054**	**0.031**	**0.003**	**10.598**	**0.025**	**0.037**
NT	**0.035**	**0.007**	**4.881**	**0.021**	**0.050**	**0.022**	**0.005**	**4.259**	**0.012**	**0.033**

AE-NT	**0.027**	**0.007**	**3.656**	**0.012**	**0.041**	**0.044**	**0.005**	**9.125**	**0.034**	**0.053**
ALL	**0.043**	**0.011**	**3.945**	**0.022**	**0.064**	**0.028**	**0.006**	**4.512**	**0.016**	**0.041**
Slope										
IGP	**0.017**	**0.008**	**2.188**	**0.002**	**0.033**	0.012	0.008	1.391	-0.005	0.028
MF	-0.020	0.012	-1.601	-0.044	0.004	**0.016**	**0.008**	**1.919**	**0.000**	**0.032**
AE	0.010	0.018	0.521	-0.026	0.046	0.022	0.018	1.168	-0.015	0.058
NT	0.001	0.014	0.072	-0.026	0.028	**0.037**	**0.012**	**2.999**	**0.013**	**0.061**
AE-NT	**0.059**	**0.029**	**2.029**	**0.002**	**0.116**	-0.021	0.024	-0.884	-0.069	0.026
ALL	0.031	0.101	0.309	-0.167	0.230	0.038	0.038	1.012	-0.036	0.113

Table B2: Parameter estimates of the linear mixed effects ANCOVA model for *A. maculatum* larval period length. Lower and upper are 95% confidence intervals. Bold values represent whether the intercept and slope are different from zero, and an approximate test of significance among treatments is:

$t = (\text{estimate}_1 - \text{estimate}_2)/\text{sqrt}((SE_1^2 + SE_2^2)/2)$, with $t > 2$ indicates a significant difference.

Predator	No Cover					Cover				
	Estimate	**SE**	**t-value**	**Lower**	**Upper**	**Estimate**	**SE**	**t-value**	**Lower**	**Upper**
Intercept										
IGP	**55.49**	**8.15**	**6.81**	**39.52**	**71.46**	**56.64**	**7.94**	**7.14**	**41.09**	**72.20**
MF	**84.38**	**9.85**	**8.56**	**65.06**	**103.69**	**85.06**	**6.13**	**13.88**	**73.05**	**97.08**
AE	**55.95**	**12.26**	**4.57**	**31.93**	**79.98**	**69.18**	**3.93**	**17.60**	**61.48**	**76.89**
NT	**69.52**	**9.76**	**7.12**	**50.38**	**88.66**	**78.67**	**7.00**	**11.24**	**64.95**	**92.39**
AE-NT	**75.01**	**9.78**	**7.67**	**55.83**	**94.18**	**80.68**	**6.42**	**12.57**	**68.09**	**93.26**
ALL	**95.67**	**14.73**	**6.49**	**66.79**	**124.55**	**86.86**	**8.48**	**10.24**	**70.23**	**103.49**

Slope										
IGP	20.82	10.67	1.95	-0.09	41.74	**27.49**	**11.23**	**2.45**	**5.47**	**49.51**
MF	-10.04	16.26	-0.62	-41.91	21.83	-3.54	10.94	-0.32	-24.98	17.89
AE	36.32	24.81	1.46	-12.30	84.94	25.50	24.78	1.03	-23.07	74.06
NT	9.07	18.47	0.49	-27.13	45.27	0.00	16.41	0.00	-32.15	32.16
AE-NT	-1.15	39.35	-0.03	-78.28	75.98	-30.67	32.54	-0.94	-94.46	33.11
ALL	-170.67	137.22	-1.24	-439.60	98.27	-71.37	51.29	-1.39	-171.90	29.16

Table B3: Parameter estimates of the generalized linear mixed effects ANCOVA model for *A. maculatum* survival. Estimates are on the logit scale. Lower and upper are 95% confidence intervals. Bold values represent whether the intercept and slope are different from zero, and an approximate test of significance among treatments is:

$t = (estimate_1 - estimate_2)/sqrt((SE_1^2 + SE_2^2)/2)$, with $t > 2$ indicates a significant difference.

	No Cover						Cover					
Predator	Estimate	SE	t-value	p-value	Lower	Upper	Estimate	SE	t-value	p-value	Lower	Upper
Intercept												
IGP	1.92	1.04	1.85	0.08	-0.12	3.97	0.33	0.94	0.35	0.73	-1.52	2.18
MF	-0.27	1.25	-0.21	0.83	-2.72	2.18	-0.37	0.73	-0.50	0.62	-1.80	1.06
AE	**-3.92**	**1.64**	**-2.38**	**0.03**	**-7.14**	**-0.70**	0.02	0.46	0.03	0.97	-0.88	0.91
NT	-1.96	1.07	-1.83	0.08	-4.06	0.14	1.61	0.88	1.84	0.08	-0.11	3.34
AE-NT	-1.12	1.37	-0.82	0.42	-3.80	1.56	-1.24	0.77	-1.61	0.12	-2.75	0.27
ALL	-1.18	1.85	-0.64	0.53	-4.80	2.45	**-3.38**	**1.33**	**-2.54**	**0.02**	**-5.98**	**-0.77**

Slope												
IGP	**-4.09**	**1.38**	**-2.97**	**0.01**	**-6.79**	**-1.39**	-2.20	1.35	-1.63	0.12	-4.84	0.44
MF	-1.94	2.10	-0.93	0.36	-6.06	2.17	**-3.01**	**1.38**	**-2.18**	**0.04**	**-5.72**	**-0.30**
AE	3.89	3.23	1.21	0.24	-2.44	10.23	**-6.77**	**2.65**	**-2.55**	**0.02**	**-11.97**	**-1.57**
NT	0.73	2.02	0.36	0.72	-3.23	4.69	**-6.76**	**2.05**	**-3.30**	**0.00**	**-10.77**	**-2.74**
AE-NT	-5.35	5.81	-0.92	0.37	-16.74	6.05	2.00	3.87	0.52	0.61	-5.58	9.59
ALL	-18.05	16.88	-1.07	0.30	-51.15	15.04	5.01	7.82	0.64	0.53	-10.32	20.33

Figure B1: Habitat complexity used in the experiment. Panel A shows a recently added ringed salamander (*Ambystoma annulatum*) larva. Panel B shows cover orientation in tanks. Panel C shows an underwater view of cover. Panel D shows the density of cover from above.

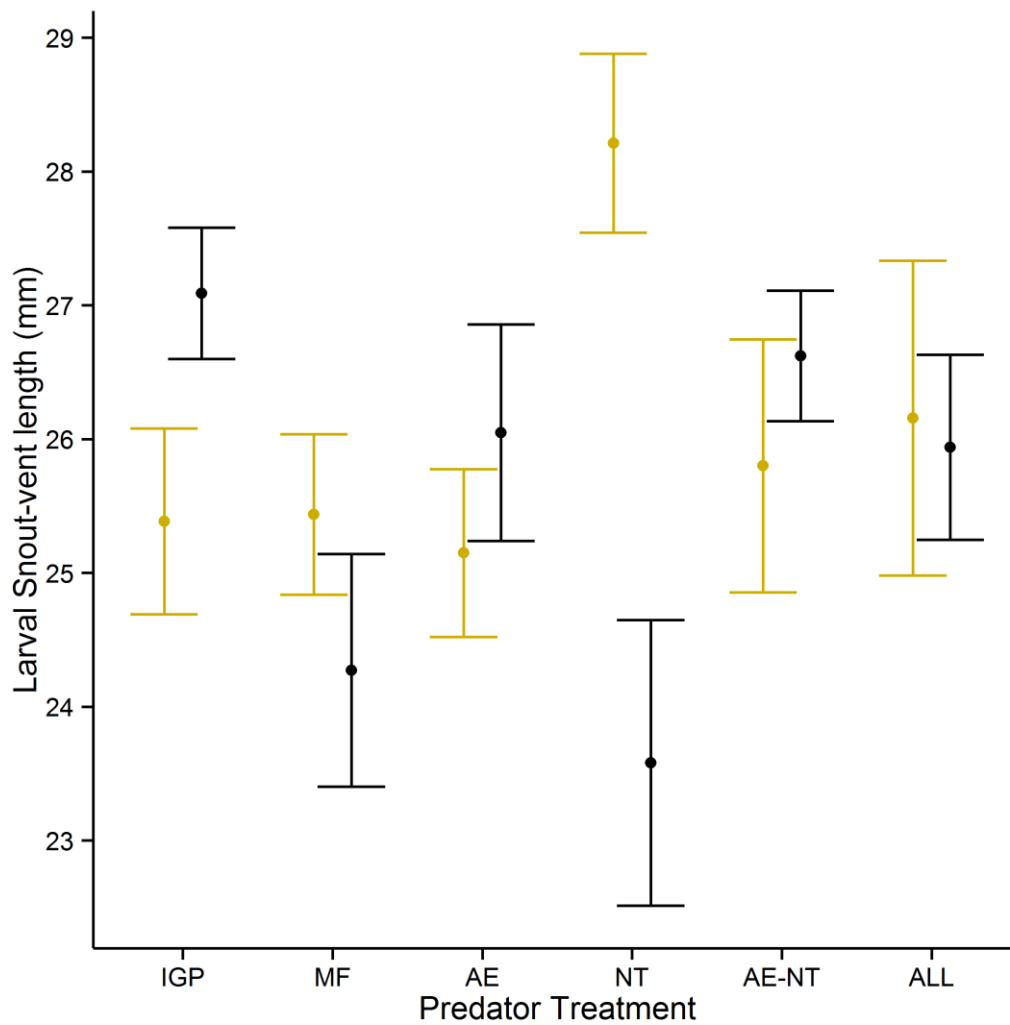Figure B2: Mean SVL (± SE) of larval ringed salamanders (*Ambystoma annulatum*) on 22 April in different predator and cover treatments. Gold dots and bars indicate the absence of habitat complexity and black indicate the presence of habitat complexity.
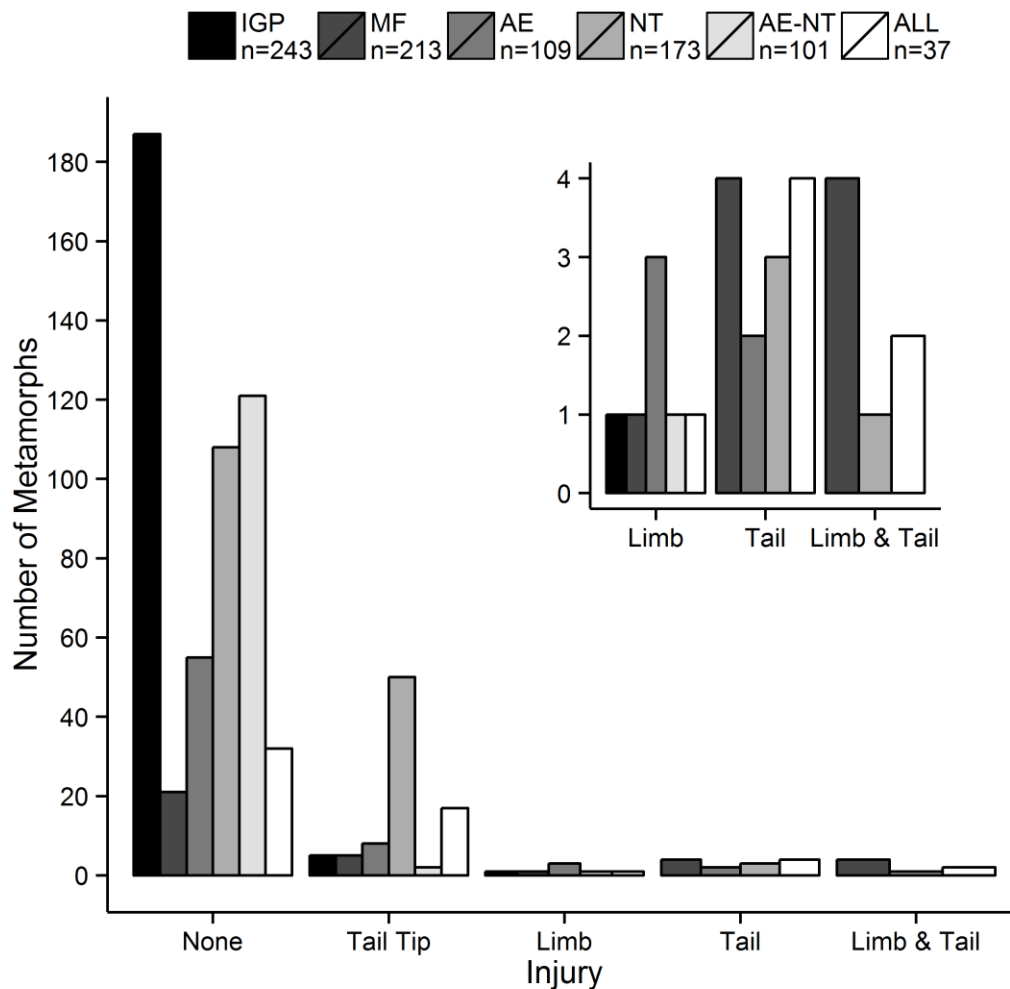
Figure B3: Distribution of injuries for metamorphic ringed salamanders (*Ambystoma annulatum*) within six predator food web treatments. The x-axis indicates the type of injury, and the y-axis the number of individuals, and the different colors indicate predator treatment. The "None" injury indicates metamorphs that had no observable injuries. The inset graph shows an expanded y-axis for the values for the Limb, Tail and Limb & Tail categories. Total sample sizes for the number of metamorphs from each treatment are next to the figure legend.

Figure B4: Model coefficients for body condition of *A. maculatum*. The thicker line indicates 1 SD and the lighter line indicates 2 SD of each estimate. Above the solid black line are intercept values and below the line are slope values.

Figure B5: Model coefficients for larval period length of *A. maculatum*. The thicker line indicates 1 SD and the lighter line indicates 2 SD of each estimate. Above the solid black line are intercept values and below the line are slope values.
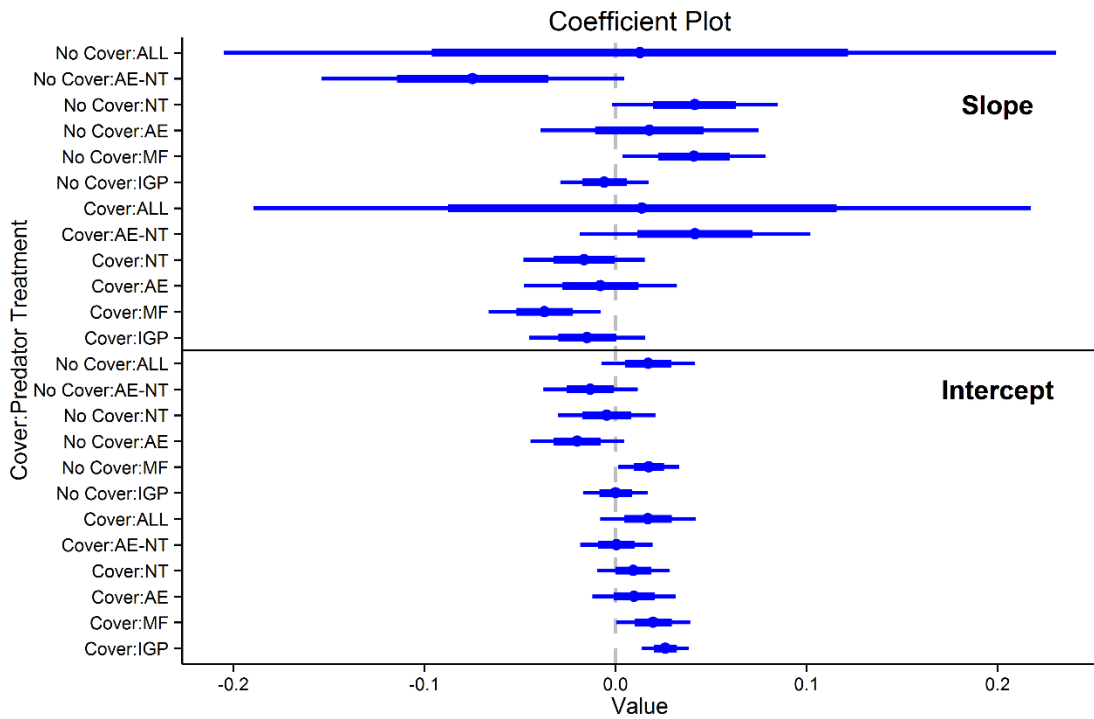
Figure B6: Model coefficients for survival of *A. maculatum*. The thicker line indicates 1 SD and the lighter line indicates 2 SD of each estimate. Above the solid black line are intercept values and below the line are slope values.
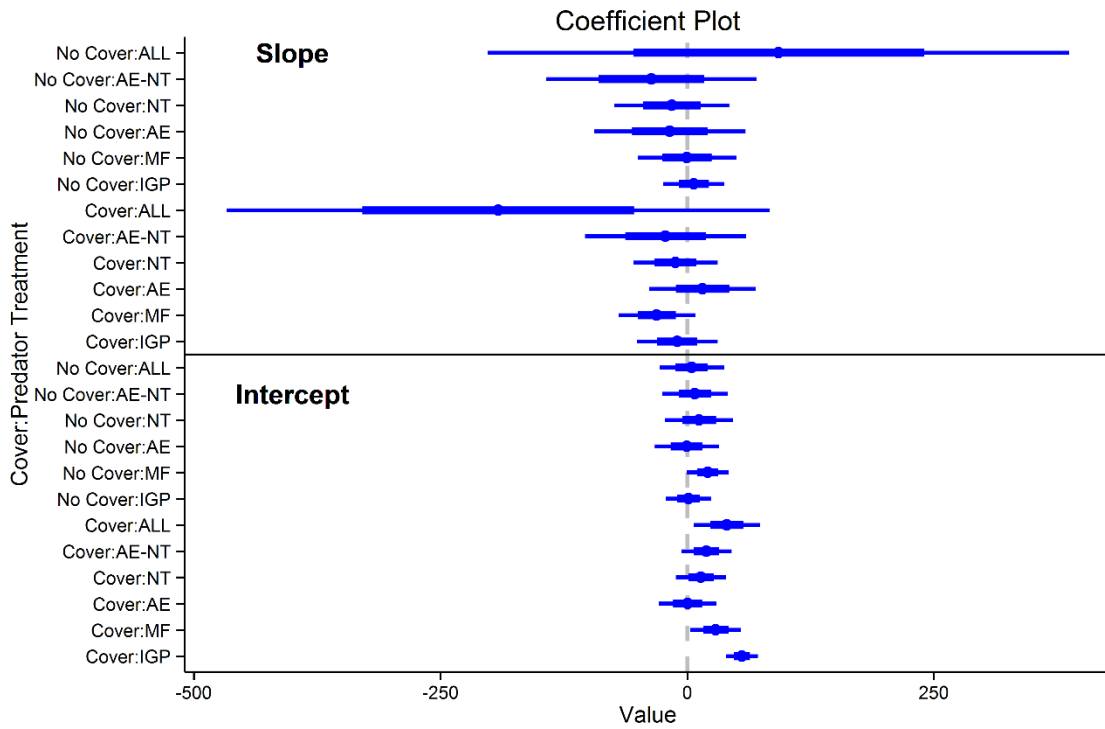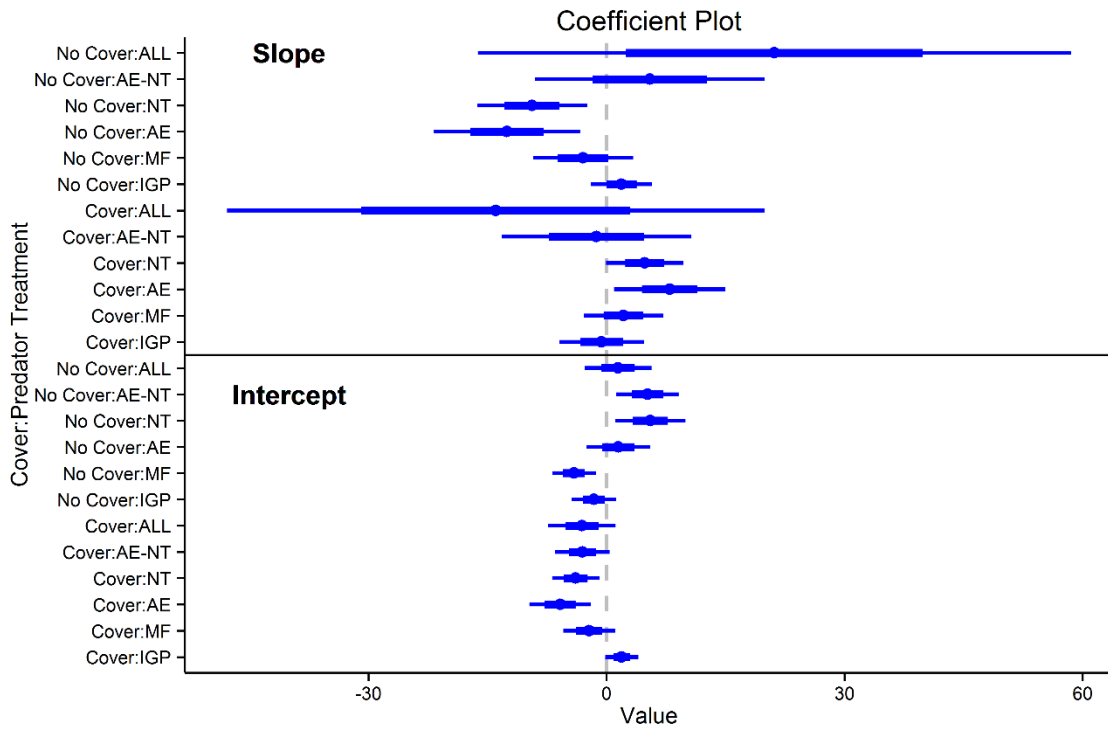
Appendix C

ADDITIONAL TABLES, FIGURES AND STATISTICAL ANALYSES OF

PHENOLOGY-DENSITY AND PRIORITY EFFECTS EXPERIMENTS

Results: Mass at metamorphosis in ringed salamanders followed similar patterns to SVL, where it exhibited a negative relationship with survival and was greater in the synchronous (Early-Early and Late-Late) versus asynchronous (Early-Late and Late-Early) treatments. All contrasts among intercepts and slopes overlapped zero, though there was a 94% probability that the intercept of the Early-Early treatment was greater than the Early-Late treatment. There was also a 96% and 91% probability that the early-early treatment had a stronger negative relationship with ringed salamander survival compared to the Early-Late and Late-Early treatments. Additionally, only the Early-Early and Early-Late treatments had slopes whose 95% CRI did not overlap zero.

The general relationship of Ringed salamander survival with metamorph mass of spotted salamanders was positive, though the 95% CRI overlapped zero (88% probability the effect was positive). All contrasts among intercept and slope values had 95% CRI that overlapped zero, and no slope values were significantly different from zero. The slope of the Early-Late treatment was marginally greater than zero (86% probability).
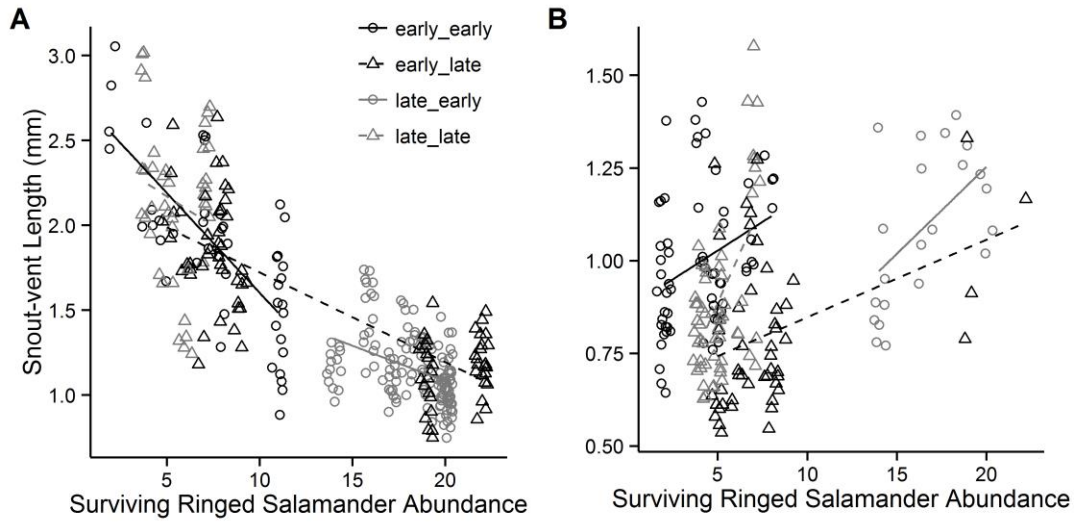
Figure C1: Mass at metamorphosis for ringed (A) and spotted salamanders (B) in the

Phenology-Density experiments

Table C1: Posterior distribution estimates for ringed salamanders. Phenology = phenology treatment, Parameter = whether it was the slope or intercept term from the ANCOVA models, mean = posterior distribution mean, SD = standard deviation of posterior mean, 2.5/97.5% = 95% credible interval (CI), overlap = whether 95% CI overlapped 0, f = the proportion of the distribution that shares the same sign as the mean.

Treatment	Parameter	Snout-vent Length					
		mean	sd	2.50%	97.50%	overlap0	f
early-early	slope	-0.777	0.192	-1.15	-0.398	FALSE	1
early-late		-0.399	0.097	-0.591	-0.206	FALSE	1
late-early		-0.342	0.28	-0.874	0.232	TRUE	0.892
late-late		-0.313	0.521	-1.344	0.704	TRUE	0.732
early-early	intercept	45.554	1.419	42.742	48.311	FALSE	1
early-late		42.791	1.205	40.344	45.137	FALSE	1
late-early		40.941	5.06	30.557	50.591	FALSE	1
late-late		43.305	2.828	37.82	48.824	FALSE	1
		Mass					
early-early	slope	-0.13	0.037	-0.203	-0.058	FALSE	1
early-late		-0.055	0.021	-0.097	-0.014	FALSE	0.996
late-early		-0.036	0.06	-0.152	0.081	TRUE	0.725
late-late		-0.101	0.105	-0.312	0.106	TRUE	0.832
early-early	intercept	2.849	0.268	2.323	3.359	FALSE	1
early-late		2.281	0.255	1.776	2.785	FALSE	1
late-early		1.804	1.085	-0.299	3.947	TRUE	0.953

late-late		2.69	0.57	1.571	3.827	FALSE	1
		Larval Period Length					
early-early	slope	1.238	0.385	0.454	1.986	FALSE	1
early-late		0.744	0.186	0.366	1.101	FALSE	1
late-early		0.685	0.512	-0.3	1.714	TRUE	0.917
late-late		0.444	1.005	-1.543	2.491	TRUE	0.669
early-early	intercept	223.221	3.003	217.396	229.176	FALSE	1
early-late		225.418	2.38	220.704	230.17	FALSE	1
late-early		196.937	9.269	178.611	214.898	FALSE	1
late-late		199.808	5.522	188.746	210.761	FALSE	1
	Density	Survival					
early-early	High	-0.497	0.086	-0.662	-0.327	FALSE	1
early-late		0.902	0.058	0.789	1.016	FALSE	1
late-early		0.892	0.056	0.783	1.003	FALSE	1
late-late		-1.165	0.102	-1.367	-0.968	FALSE	1
early-early	Low	1.496	0.198	1.129	1.901	FALSE	1
early-late		1.643	0.189	1.287	2.025	FALSE	1
late-early		NA	NA	NA	NA	NA	NA
late-late		0.68	0.166	0.366	1.017	FALSE	1

Table C2: Posterior distribution estimates for spotted salamanders. Phenology =

phenology treatment, Parameter = whether it was the slope or intercept term from the

ANCOVA models, mean = posterior distribution mean, SD = standard deviation of

posterior mean, 2.5/97.5% = 95% credible interval (CI), overlap = whether 95% CI

overlapped 0, f = the proportion of the distribution that shares the same sign as the mean.

		Snout-vent Length					
Treatment	**Parameter**	**mean**	**sd**	**2.50%**	**97.50%**	**overlap0**	**f**
early-early	slope	0.359	0.183	0.002	0.719	FALSE	0.976
early-late		0.123	0.078	-0.028	0.277	TRUE	0.946
late-early		0.243	0.2	-0.163	0.64	TRUE	0.888
late-late		0.51	0.312	-0.121	1.121	TRUE	0.949
early-early	intercept	28.451	0.908	26.642	30.222	FALSE	1
early-late		27.377	0.806	25.783	28.978	FALSE	1
late-early		26.705	3.493	19.768	33.685	FALSE	1
late-late		25.911	1.645	22.664	29.199	FALSE	1
		Mass					
early-early	slope	0.036	0.06	-0.08	0.156	TRUE	0.724
early-late		0.022	0.021	-0.02	0.063	TRUE	0.856
late-early		0.03	0.059	-0.088	0.144	TRUE	0.7
late-late		0.069	0.1	-0.127	0.269	TRUE	0.759
early-early	intercept	0.863	0.311	0.228	1.466	FALSE	0.997
early-late		0.64	0.248	0.146	1.132	FALSE	0.994
late-early		0.603	1.073	-1.493	2.745	TRUE	0.711

late-late		0.521	0.54	-0.549	1.576	TRUE	0.836
		Larval Period Length					
early-early	slope	0.349	0.564	-0.758	1.433	TRUE	0.732
early-late		0.885	0.322	0.261	1.51	FALSE	0.996
late-early		1.699	0.709	0.349	3.116	FALSE	0.992
late-late		0.581	0.995	-1.329	2.531	TRUE	0.719
early-early	intercept	58.552	2.462	53.699	63.367	FALSE	1
early-late		45.255	2.829	39.671	50.849	FALSE	1
late-early		38.228	11.768	14.897	60.59	FALSE	1
late-late		52.073	5.084	42.095	61.876	FALSE	1
		Survival					
early-early	slope	-0.269	0.028	-0.323	-0.214	FALSE	1
early-late		-0.108	0.021	-0.15	-0.068	FALSE	1
late-early		-0.41	0.061	-0.533	-0.293	FALSE	1
late-late		-0.419	0.051	-0.521	-0.32	FALSE	1
early-early	intercept	1.416	0.125	1.173	1.663	FALSE	1
early-late		0.393	0.166	0.084	0.721	FALSE	0.993
late-early		5.167	0.941	3.37	7.028	FALSE	1
late-late		2.282	0.261	1.768	2.796	FALSE	1

Table C3: Summary of posterior distribution of contrasts among treatments in the Priority Effects experiment for spotted salamanders. The Treatment Contrast columns show the treatments being compared for differences in intercepts. Other columns show: mean = posterior distribution mean; 2.5/97.5% = 95% credible interval, f = the proportion of the posterior distribution that shares the same sign as the mean.

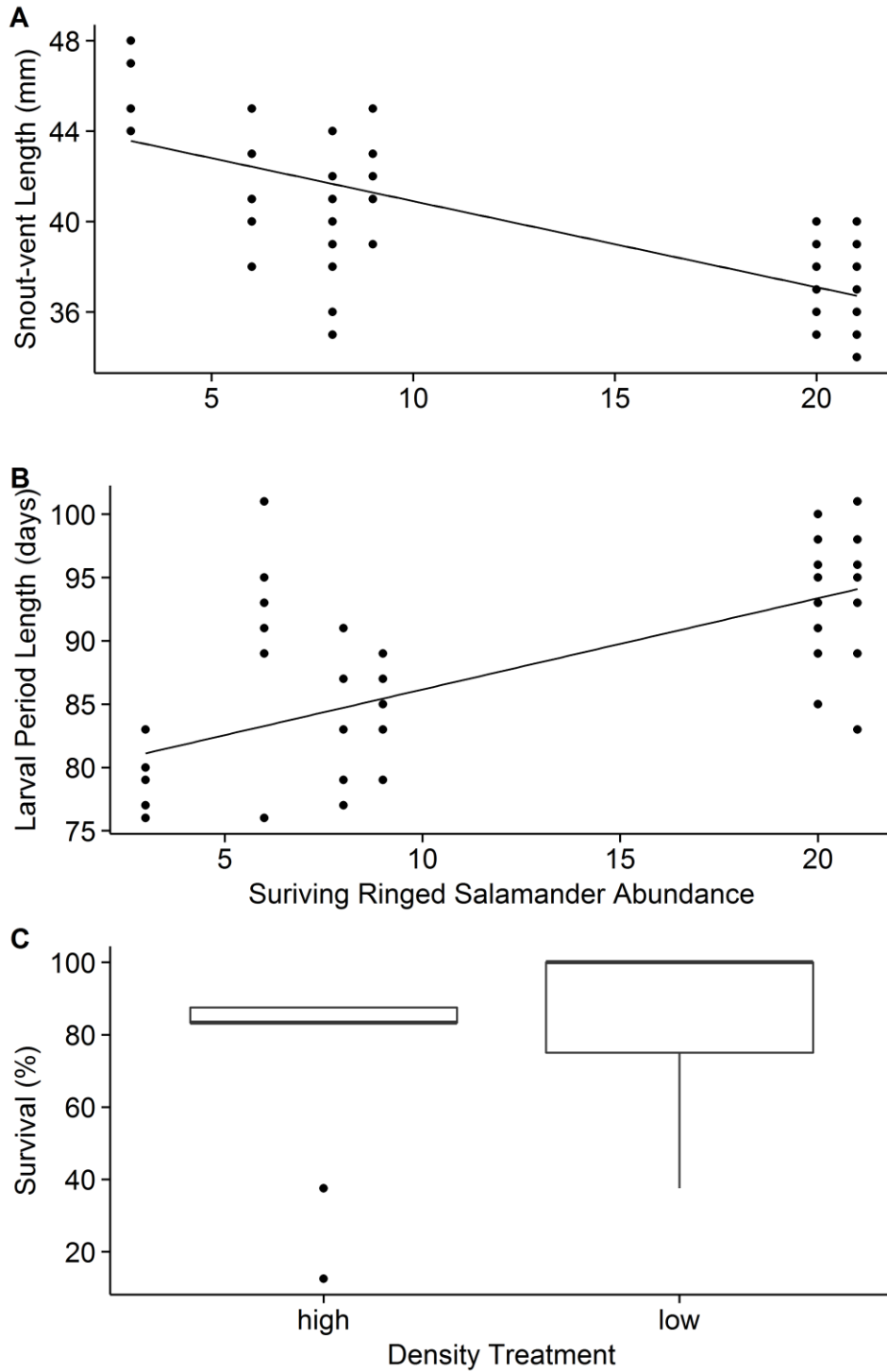| Treatment Contrast		Snout-vent Length				Larval Period Length				Survival			
		mean	2.50	97.50	f	mean	2.50	97.50	f	mean	2.50	97.50	f
Synchronous	High Control	2.72	1.59	3.92	1.00	-6.02	-10.36	-1.66	1.00	0.09	-0.17	0.36	0.75
Synchronous	Low Control	1.53	0.29	2.76	0.99	-2.36	-7.39	2.56	0.83	0.25	-0.05	0.56	0.95
Synchronous	Sequential	2.01	1.05	3.00	1.00	-3.78	-7.78	0.15	0.97	-0.38	-0.70	-0.06	0.99
High Control	Low Control	-1.19	-2.44	0.14	0.97	3.65	-0.58	7.96	0.96	0.16	-0.03	0.34	0.96
High Control	Sequential	-0.71	-1.80	0.31	0.92	2.24	-2.08	6.49	0.85	-0.48	-0.70	-0.25	1.00
Low Control	Sequential	0.48	-0.67	1.61	0.81	-1.42	-6.23	3.53	0.72	-0.63	-0.90	-0.37	1.00

Figure C2: Snout-vent length (SVL) at metamorphosis (A), larval period length (B) and survival (C) of ringed salamanders from the Synchronous treatments.

VITA

Thomas Lee Anderson was born on April 20, 1983 in Dubuque, IA in time for his mother, Rosemary, to still catch Guiding Light, a requirement of hers. He grew up in Platteville, WI, attending St. Mary's School from grades K-8 and graduating from Platteville High School in 2001. Tom earned a Bachelor of Science from the University of Wisconsin-Eau Claire in 2006. During his last summer as an undergraduate, he underwent a life-changing and career-altering experience at the Rocky Mountain Biological Laboratory, which ultimately led to his desire to conduct ecological research. After a two-year post-baccalaureate hiatus from school that involved field technician jobs working on sapsuckers, ground squirrels, black ducks and spotted owls, Tom returned to school in 2008 to work on a Master of Science at Murray State University under the supervision of Howard Whiteman. Thus began his journey into the world of amphibian and experimental ecology, with his thesis research focusing on how intra- and interspecific competition of larval salamanders influenced growth, phenotypic expression, and survival in both natural and experimental conditions. Tom completed his MS degree in Water Science in 2011. He fortuitously talked to Julia Earl during his last semester, who placed him in contact with Ray Semlitsch at the University of Missouri. He graciously accepted an offer to work on his PhD at Missouri with Ray, where he began in 2011. His research focused on how biotic and abiotic factors affected species interactions among larval salamanders, again using both experimental and natural pond communities. Tom completed his PhD in May 2016. He will be starting a postdoctoral research position with Daniel Reuman at the University of Kansas in May 2016.