Spring 3-25-2022

Size Structure and Intraguild Interactions between Two Ambystoma Species

Gwyneth Daunton

COLUMBUS STATE UNIVERSITY

Size Structure and Intraguild Interactions between Two *Ambystoma* Species

A THESIS SUBMITTED TO

THE COLLEGE OF LETTERS AND SCIENCE

IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF BIOLOGY

BY

GWYNETH M. DAUNTON

COLUMBUS, GEORGIA

2022

Size Structure and Intraguild Interactions between Two *Ambystoma* Species

By

Gwyneth M. Daunton

Committee Chair:

Dr. Clifton B. Ruehl

Committee Members:

Dr. Daniel Holt
Dr. Jon Davenport
Dr. Chester Figiel

Columbus State University
February 2022

Abstract

Intraguild interactions affect population and community structure through a combination of competition and predation. Changes in size structure influence intraguild interactions by affecting the strength and direction of these species' interactions. I tested for size-structured intraguild interactions in temporary pond ecosystems between marbled salamanders (*Ambystoma opacum*) and tiger salamanders *(Ambystoma tigrinum*) using an outdoor mesocosm experiment. I hypothesized that 1) *A. opacum* survivorship and growth would be negatively impacted when both species start at a similar size because tiger salamanders grow faster and are more aggressive 2) *A. tigrinum* will grow slower and have lower survivorship when they occur with large *A. opacum* because *A. opacum* will outcompete *A. tigrinum* and prey upon them. There was no support for the second hypothesis, *A. tigrinum* grew and survived the same regardless of *A. opacum* size. However, I found support for the first hypothesis. Small *A. opacum* mortality was greatest when they occurred with small *A. tigrinum* suggesting changes in size structure resulting in these two species occurring together at similar sizes will negatively impact *A. opacum* populations through intraguild predation. Climate change will likely affect the distribution and abundance of species in temporary pond ecosystems and influence size-structured interactions that impact population and community structure.

Index words: amphibian larvae survivorship, mesocosm, intraguild predation, size-structure

DEDICATION

It has been said that it takes a village to raise a child, I have found that it takes a small army to raise approximately 200 larval salamanders. I dedicate this research to the small army of friends, family, and professors who have helped me persevere through my academic career. I thank my parents Clive and Dianna Daunton for always encouraging me to do what I love even though reptiles and amphibians aren't quite their cup of tea. They have helped me become the "salamander expert" that I am today. I thank my undergraduate advisors Dr. Gregory McGee and Dr. James Gibbs for encouraging me to further my education against all odds. I thank my graduate advisor, Dr. Clifton Ruehl, for always assuring me that perfection isn't achievable when working with wild animals and never giving up on me or my research. Through this research, my understanding of experimental design and statistics has grown exponentially which is mainly due to his guidance. I would also like to thank my committee members Dr. Daniel Holt, Dr. Jon Davenport, and Dr. Chester Figiel for their help and support. I would especially like to thank Dr. David Scott and the Savannah River Ecology lab at the University of Georgia for providing me with salamanders, I could not have done my research without your assistance. I thank my friends at Columbus State University both within the natural science department and outside, especially Kari Goodwin, Dominika Sherwood, Billy Daffin, Spencer Cruz, Mary-Clair Halbrook, Esteban Pinto, Melanie Flood, and Samuel Dixon. They have helped me during research, sometimes in the rain, and kept me sane in my attempt to keep hundreds of salamanders alive. Lastly, I would like to thank Mary Anning, Jane Goodall, and Beatrix Potter for inspiring me and paving a way for women to have a place in science even though some of them were deprived of that luxury.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

Communities are structured by a variety of abiotic and biotic factors. These factors interact in complex ways to influence population growth and community composition (Wilber, 1987, Wellborn et al. 1996, Peacor & Werner, 2000, Zalfman et al., 2017). Among biotic factors, intraguild interactions affect community composition through competition for shared resources and predation between and within species (Holt & Polis 1997, Polis et al. 1998, Should 1997 citation go before 1998 citation?). For example, in a simple intraguild food web, an intermediate predator competes with a top predator for a shared resource, but the intermediate predator also gets eaten by the top predator. Models predict that the outcome of these interactions can depend on which species is a better competitor (Polis et al. 1998). The two predators are predicted to coexist when the intermediate predator is a better competitor than the top predator for the shared resource; otherwise, the top predator will exclude the intermediate predator (Polis & Holt 1992, Vance-Chalcraft et al. 2007) Coexistence could also occur if there is an abundance of resources or the top predator was not effective at consuming the intermediate predator, assuming the intermediate predator remains the superior competitor.

Size structure of the top and intermediate predators influences the outcome of intraguild interactions (Wissinger 1992, Boone et al., 2002, Crumrine 2005, Sours & Petranka 2007, Rudolf & Armstrong 2008). Different size classes (or developmental stages) of top predators have different effects on intermediate predators (Yurewicz, 2004, Hawley, 2009, Anderson et al. 2013, Carter et al. 2018). For example, large top predators tend to eat more intermediate predators than small top predators (Crumrine 2005). Size-structured populations of top predators might mitigate predation on intermediate predators if top predators are large enough to eat smaller conspecifics (i.e., cannibalism; Rudolf & Armstrong 2008). Top predators also have the opportunity to consume more intermediate predators than the shared prey species (Yurewicz, 2004). The different outcomes reveal the importance of size in affecting community composition (Yurewicz, 2004, Crumrine,2005, Rudolf & Armstrong 2008).

Abiotic parameters like temperature, water permanence, and seasonal predictability can also affect intraguild interactions (Todd et al., 2011, Lund et al., 2016, Zalfman et al., 2017). For example, variation in temperature shifts communities to different latitudes and elevations in search of conditions that meet their physiological needs (Kelly & Goulden, 2008, Zellweger et al. 2017). These range shifts lead to novel species interactions as species assimilate into existing communities at higher latitudes or altitudes (Lurgi et al. 2012, Barley et al. 2019, Shepard et al. 2021). In some cases, species shift to habitats with historically longer hydroperiods because of changes in rainfall patterns that produce novel intraguild interactions that negatively affect resident species (Shepard et al., 2021). Temperature variation can induce changes in life-history switch points like breeding time (Beebee, 1995, Gibbs & Breisch, 2001, Todd et al.,2011). For example, in response to warmer temperatures the southern chorus frog (*Pseudacris nigrita*) breeds later in the winter leading to an overlap in pond use with the spring-breeding western chorus frog (*P. triseriata*) that negatively affects the southern chorus frog because of the change in size structure (Rudolf & Singh, 2013).

Temporary ponds are a model system to test for the effects of size structure on intraguild interactions. The communities that develop in these systems are often size-structured and intraguild predation is an important component structuring communities (Sours & Petranka, 2007, Hawley, 2009, Anderson et al., 2013). Many species that occupy temporary ponds use temperature and rainfall as cues to change development (Petranka, 1998, Shepard et al. 2021). For example, warmer temperatures lead to amphibians that breed earlier in the spring (Beebee 1995, Gibbs & Breisch 2001, Todd et al. 2011) and fall-breeding amphibians that breed later in the fall (Todd et al. 2011, Rudolf & Singh, 2013). These shifts in breeding time have the potential to affect predator-prey interactions and competition for resources because the size structure of larval populations within these systems changes.

In this study, I considered the consequences of changing size structure on the intraguild interactions between the fall-breeding marbled salamander (*Ambystoma opacum)* and the spring-breeding tiger salamander (*Ambystoma tigrinum)*. Both of these species shift their breeding time response to temperature such that *A. opacum* breeds later in the fall and *A. tigrinum* breeds earlier in the spring (Todd et al., 2011). I conducted a mesocosm experiment that manipulated the size structure of these two species to better understand how breeding time might affect species interactions between these two species I hypothesized that 1) *A. opacum* survivorship and growth would be negatively impacted by a shift in size structure that resulted in small individuals of both species occupying the pond at the same time and 2) *A. tigrinum* should grow slower and have lower survivorship when large *A. opacum* coexist with small *A. tigrinum*.

METHODS

Study System

The range of *A. opacum* and *A. tigrinum* overlap in temporary ponds of the coastal plain in the U.S.A. (Gibbons & Semlitsch,1991, Whiteman et.al., 1995, Petranka, 1998, Todd, 2011). Currently, *A. opacum* is more common in Georgia, and the range of *A. tigrinum* is much smaller (Jensen, 2008). Both species are voracious predators as larvae and therefore compete for resources (Petranka,1998). *Ambystoma opacum* lays eggs in the fall in dry areas of temporary ponds (Petranka, 1990). The eggs hatch as the pond fills, enabling them to be among the first temporary pond residents. They encounter few competitors or predators and few resources and consequently grow slowly (3–6-month larval period). If ponds do not fill or the mother leaves, eggs risk desiccation, freezing, and fungal or algal infections (Petranka, 1990). Conversely, *A. tigrinum* lays eggs in the spring after ponds fill and the eggs hatch in an environment with more competitors and predators, but also many resources enabling them to grow quickly with a range of development from 2-4 months (Petranka,1998; Jensen, 2008). Therefore, historically *A. opacum* has a head start on *A. tigrinum* enabling them to have a size advantage in size-structured species interactions.

Experimental design

I used a substitutive design of five treatments arranged in four replicate spatial blocks to test for the effects of size structure on intraguild interactions. Treatments were randomized within each block and included eight small *A. opacum* alone, eight large *A. opacum* alone, eight small *A. tigrinum* alone, four small *A. opacum* with four small *A. tigrinum* together, and four large *A. opacum* with four small *A. tigrinum* together. The treatment with large *A. opacum* and small *A. tigrinum* represented the size structure of these two species when the two species have greater differences in their breeding times, while the treatment with *A. opacum* and *A. tigrinum* of the same size represent size structure when the two species breed closer to the same time (Fig.1).

Salamander capture and rearing

Salamander larvae were collected from the Savanah River Ecology Lab in Aiken, South Carolina where *A. opacum* and *A. tigrinum* co-occur in the same pond ecosystems (Gibbons & Semlitsch,1991, Todd, 2011). Small *A. opacum* were hatched into plastic containers in the lab from 5 clutches by flooding the clutches on 9 February 2021 with pond water. The clutches were originally split at the beginning to control for genetic diversity, but due to widespread mortality, the clutches were haphazardly combined to have enough individuals for the experiment. Small *A. opacum* (1.72 ± 0.025 cm, mean ± SE) were maintained in the lab in 76-l aerated aquaria with brine shrimp and weekly water changes. Large *A. opacum* (3.44 ± 0.079 cm, mean ±SE) were collected from temporary ponds on 12 March, transported to the lab, and kept as above but were fed mosquito larvae instead. *Ambystoma tigrinum* eggs were collected from temporary ponds on 10 March and then hatched in large mesocosms during the next week at the Savannah River Ecology site before being transported to Columbus State University on 12 March where they were maintained as above (1.72 ± 0.029 cm, mean ±SE). There were no size differences in larval salamanders within the same size treatment and species at the start of the experiment: small *A. opacum* ($F_{11,52}$= 0.297; P = 0.984), large *A. opacum* ($F_{10, 49}$= 0.687; P = 0.731), and small *A. tigrinum* ($F_{8,39}$= 0.037; P = 0.517). All of the salamander larvae were randomly assigned to a treatment and added to experimental tanks on 5 April 2021(Day 1).

Mesocosm Set up

Mesocosms were filled with 375-l of well water, 100 grams of oak leaf litter, 4-g of rabbit chow, and 6-l of zooplankton from the Savannah River Ecology Lab (the amount of rabbit chow was based on Wilber, 1987). The zooplankton water was filtered through two screens, this prevented large carnivorous water beetles or dragonfly larvae from entering into the mesocosms. Mesocosms were covered with screens on both the top and the standpipe to prevent colonization by other organisms. Once salamanders were added, 6-l of zooplankton from a nearby wetland was added to each mesocosm every week.

I instituted a drying regime of 3-cm a week starting on 24 May (Day 49) and ending on 23 June (Day 79). This drying regime was based on temporary pond ecosystems at the Savanah River site where salamanders were collected. Many of these ponds, especially those with open canopies dry quickly and are typically dry by the end of June. (Gibbons & Semlitsch,1991, Scott, unpublished). The water level started at 28 cm and ended at 13 cm

Salamander measuring

Initial salamander length was measured by putting individuals into a clear plastic box with dividers that held a single salamander. The box was placed on a copy stand equipped with a Canon EOS REBEL T3i and 50mm F-3.5 prime lens that was used to capture images of each salamander that were processed using Image J (Schneider et al., 2012). Each salamander was measured from the tip of its tail to the tip of the snout three times and then the average was taken to the nearest tenth of a centimeter. Instead of snout-to-vent length, total length was used because it was less invasive and had less risk of mortality when measuring small larval salamanders with delicate gills.

I caught metamorphosed salamanders with a net before 7 May and used minnow traps thereafter that were placed halfway underwater to prevent drowning. Minnow traps were checked daily to prevent mortality. Metamorphosed salamanders were captured and taken back to the lab where the total length (cm) and mass (g) were taken. The length was recorded as above and mass was taken by blotting the salamander dry on a paper towel before recording the mass using a semi-micro balance (Sartorius CPA225D). Metamorphosed salamanders were maintained on crickets and termites in an aquarium.

Halfway through the experiment on 12 May (Day 37), larval salamanders were measured again as described above. Tanks were swept with large aquarium nets for 15 minutes in an attempt to capture salamanders. Although this may have disrupted intraguild interactions in the tanks for a short period, getting data on growth was critical for understanding salamander development. Since few salamanders were found on 12 May, especially in the treatment with small *A. tigrinum* and small *A. opacum*, the experiment was ended. On 23 June, all remaining larval salamanders, a total of 57, were collected at that time. The length of the remaining salamander larvae was recorded using the methods mentioned above. All salamanders were returned to the Savannah River Ecology Lab on 25 June.

Analyses

To understand the impact of size structure on the survivorship of *A. opacum* and *A. tigrinum*, I calculated the instantaneous mortality rate by dividing the number of surviving salamanders at the end of the experiment by the initial number of salamanders in each treatment. Then added one and log10 to transform the proportion (Billick & Case 1994, Ruehl et al. 2018).

Differences in survivorship between *A. opacum* and *A. tigrinum* were determined by using planned contrasts. To determine the effect of size manipulation of *A. opacum*, I compared tanks with small individuals of both species (StSm) to tanks with small *A. tigrinum* and large *A. opacum* (StLm). Mortality between the mixed species treatments (StLm, StSm, Fig. 1) was compared to the control treatments (Lm, Sm, Fig.1) respectively to see if the differences in survivorship were due to *A. tigrinum* or intraspecific competition between *A. opacum*. Lastly, the two control treatments Lm and Sm were compared to see if there was a difference in survivorship based on size.

Growth rate was calculated by subtracting the average length of salamanders within a tank according to species from the average starting length. Differences in salamander growth at the middle of the experiment were determined using a one-way ANCOVA with survivorship as the covariate. I tested for heterogeneity of slopes for the survivorship covariate by including an interaction term between treatment and survivorship and found no difference, so the interaction term was removed from the final model. Growth data were analyzed for each species separately. To analyze the difference in the growth of *A. opacum* in the presence of *A. tigrinum,* based on size, the two experimental treatments (StLm, StSm) were compared using a planned contrast. The larval growth in the two experimental treatments (StLm, StSm, Fig. 1) was also compared to the control treatments (Lm, Sm, Fig. 1) respectively to see if the difference in growth was due to *A. tigrinum* or intraspecific competition among *A. opacum*. Lastly, the two control treatments Lm, Sm were compared to see if there was a difference in growth based on initial size differences of *A. opacum*.

Growth differences of *A. tigrinum* larvae at the end of the experiment were determined using a one-way ANCOVA with survivorship as the covariate. I tested for heterogeneity of slopes for the covariate by including an interaction between treatment and survivorship and found no difference. The interaction term was removed from the final model. This analysis compared treatments St, StLm, and StSm together. Variation in the growth of *A. opacum* at the end of the experiment could be conducted because of low survivorship in treatments with small *A. opacum* and small *A. tigrinum*.

Time to metamorphosis was calculated as the days from the start of the experiment to the date of metamorphosis for each metamorphosed salamander in the mesocosm and then taking the average. These averages were then analyzed using a one-way ANOVA that compared treatments Sm, Lm, and StLm together. The treatment StSm could not be included due to low survivorship in tanks with both species at the same size. All analyses were conducted using JASP (Version 0.14).

RESULTS

Mortality

Mortality of *A. opacum* depended on their size when they occurred with *A. tigrinum* (StSm vs. StLm: t_{11}=3.233; P= 0.008, Fig. 2). Small *A. opacum* mortality was 80% greater than large *A. opacum* mortality when they resided with *A. tigrinum* (StLm vs. StSm). Mortality of large *A. opacum* did not depend on the occurrence of *A. tigrinum* (StLm vs Lm: t_{11}=0.3; P =0.770). Between treatments of small *A. opacum*, mortality depended on the occurrence of *A. tigrinum* (StSm vs. Sm: t_{11}= 3.046; P = 0.011). Small *A. opacum* had 60% more mortality when they occurred with *A. tigrinum* compared to they were with conspecifics. Mortality of *A. tigrinum* did not depend on the occurrence of *A. opacum* at either size or with other *A. tigrinum* (StSm vs. StLm vs. St: $F_{2,9}$= 0.03; P =0.971). There were no differences in mortality among single-species treatments (St vs. Sm vs. Lm: $F_{2,9}$= 0.150; P = 0.863, Fig. 2).

Growth

Small *A. opacum* growth was not different than large *A. opacum* growth when they occurred with *A. tigrinum* (StSm vs. StLm: t_{10}=0.903; P = 0.388, Fig. 3). Small *A. opacum* grew faster than large *A. opacum* when they occurred alone (Sm vs Lm tanks: t_{10}=2.847; P=0.017).

Growth of *A. tigrinum* did not depend on the occurrence of large or small *A. opacum*, or with other *A. tigrinum* (StSm vs. StLm vs. St: $F_{2,7}$=0.32; P = 0.74, Fig. 3). Experiment-wide growth of *A. tigrinum* did not depend on the presence of *A. opacum* at either size or with conspecifics (StLm vs. StSm vs. St: $F_{2,5}$= 2.38; P = 0.19). Overall *A. tigrinum* grew faster than *A. opacum* among all treatments ($F_{1,24}$ = 48.794, P=<0.001).

Time to metamorphosis

Overall, 35 *A. opacum* metamorphosed. Out of these 35 salamanders, 17 large *A. opacum* larvae metamorphosed prior to the initiation of the drying treatment. Eighteen *A. opacum* went through metamorphosis after the drying treatment was initiated, six of them being small *A. opacum*. Out of the six small *A. opacum* that metamorphosed, only one of them was from the treatment that included small *A. tigrinum*, and that one represented one of the two surviving *A. opacum*. There was no difference in time to metamorphosis in *A. opacum* among treatments (Lm vs. Sm vs. StLm $F_{2,7}$= 1.926; P = 0.216). *Ambystoma tigrinum* did not go through metamorphosis during the experiment.

DISCUSSION

I examined the potential outcomes of varying size structures on two species of larval salamanders that live in temporary ponds. Predation was asymmetric when the two species were similar sizes. *Ambystoma tigrinum* was the dominant predator resulting in strong partial intraguild interactions instead of the predicted full intraguild interactions (both species eating each other and competing) in StSm treatments. Conversely, we found no evidence of partial intraguild interactions between large *A. opacum* and *A. tigrinum* as predicted. Large *A. opacum* did not consume *A. tigrinum* suggesting that the only species interaction between these different size classes was competition. There was no difference in survivorship of *A. tigrinum* among treatments (St vs. StLm vs. StSm) and they grew faster than *A. opacum* regardless of treatment suggesting that *A. tigrinum* is a superior intraguild predator to *A. opacum*.

Breeding Phenology

Changes in breeding time can impact size-structured interactions in pond communities. *Ambystoma opacum* typically breeds in the fall and *A. tigrinum* breeds in the spring resulting in nearly a two-fold difference in size (~1.7 cm difference) between the two species in ponds where they co-occur. This size advantage for *A. opacum* enables them to both avoid predation and feed on larger prey items, but comes with risks (Petranka, 1989, Scott, 1990, Boone et al., 2002). *Ambystoma opacum* females lay their eggs in the fall and guard them where they risk freezing, desiccation, and pathogen infections while they wait until the pond fills. (Petranka, 1998, Todd et al., 2011, Rudolf & Singh, 2013). Alternatively, *A. tigrinum* females avoid the many abiotic environmental risks by laying eggs after ponds fill but face greater competition for resources and threats of predation. Changes in temperature and the timing of rainfall will impact the relative success of these species over the years and affect intraguild interactions between them.

I predicted that partial intraguild predation would impact survivorship in tanks with large *A. opacum* and small *A. tigrinum* larvae (StLm) because both would compete for the same resources and the large *A. opacum* would not be gape limited and therefore able to eat the smaller *A. tigrinum* (Crumrine 2005). However, my findings did not support this prediction as *A. tigrinum* exhibited no difference in growth or survivorship when they occurred with large *A. opacum* (StLm vs. St). Moreover, *A. tigrinum* grew faster than *A. opacum* across all treatments (StLm vs. StSM vs. St). The lack of intraguild interactions between large *A. opacum* and small *A. tigrinum* is an important finding because it demonstrates that *A. tigrinum* can successfully avoid predation by larger *A. opacum* and quickly grow into a size refuge from many other predators. These results also demonstrate the advantage that *A. opacum* has by arriving at ponds early and gaining a head start on development. In the treatment where both species were similar in size (StSm), *A. opacum* did not have a head start and *A. tigrinum* grew faster and gained a size advantage over *A. opacum*. Warmer temperatures and changes in rainfall patterns may shift the breeding time of *A. opacum* and *A. tigrinum* in ways that would result in the two species breeding closer together yielding aquatic communities with similarly sized individuals of both species that could negatively impact *A. opacum* but benefit *A. tigrinum*.

My second prediction was that similar-sized *A. opacum* and *A. tigrinum* would compete for resources and eat each other. Behavioral studies show that *A. opacum* responds to the presence of similarly sized *A. tigrinum* by hiding in vegetation longer than when they occur with conspecifics, but that *A. tigrinum* does not change behavior when they occur with *A. opacum* (Brodman & Jaskula, 2002). Additionally, other studies indicate that cannibalistic morphs of *A. tigrinum* grow faster and result in a large size advantage over conspecifics and other salamander species (Holomuzki & Collins, 1981, Lannoo et al., 1989). In this study, I found evidence for partial intraguild predation between the two species where *A. tigrinum* drastically reduced *A. opacum* survivorship. Given these results, *A. tigrinum* larvae appear to be much better predators and competitors than *A. opacum.*

Historically, *A. opacum* has enjoyed less competition for resources as they are among the first inhabitants of temporary ponds (Boone et al. 2002). Studies show that *A. opacum* in high densities reduces prey resources in temporary ponds ecosystems (Scott, 1990). Depletion of prey resources could slow the development of *A. tigrinum*. However, the current study shows that *A. tigrinum* grew faster than *A. opacum* in all of the treatments, demonstrating that they were strong competitors. To understand the impact of prey depletion in temporary pond ecosystems due to *A. opacum* and other fall breeding amphibians on *A. tigrinum* and other spring breeding amphibians, surveys of food web structure during winter and early spring in these systems should be done, and are needed, to inform response surface competition that will flesh out the mechanisms driving patterns from the field.

Competition

Larval salamanders grow slower due to competition for resources and decreased activity to avoid predation (Scott, 1990, Brodman & Jackula, 2002, Boone et al., 2002, Davis, 2012). I found that halfway through the experiment that large *A. opacum* grew less than small *A. opacum.* Even though there was the potential for stronger competition between small *A. opacum* and small *A. tigrinum* than there was with large *A. opacum* and small *A. tigrinum* (StLm vs. StSm) because of size structure. With both species at the same size, there is a greater opportunity for competition since they are both trying to compete for prey to reach the minimum size for metamorphosis. Since large *A. opacum* began the experiment close to the minimum size for metamorphosis they didn't need to compete as much for resources, while small *A. opacum* did in order to grow.

Through all of the treatments, *A. tigrinum* grew more than *A. opacum* even in the treatment where both species are the same size. It could be that *A. tigrinum* is better at processing and metabolizing prey items, as they are known to grow faster than *A. opacum* (Jensen, 2008). But it could also be that *A. tigrinum* was the stronger competitor. Since A. tigrinum grew at faster rates they were able to have a larger gape size providing them the opportunity to become cannibalistic and consume both other *A. tigrinum* and *A. opacum* larvae (Lannoo et al., 1989).

Metamorphosed salamanders

Salamanders go through metamorphosis when they reach a minimum size or enough resources to successfully transform, but transformation occurs across a range of sizes because waiting to transform until a larger size usually results in greater success as an adult (Semlitsch & Wilbur, 1988, Scott, 1990). Salamanders will transform at smaller sizes when environmental conditions become inhospitable (Petranka, 1998). For example, pond drying will stimulate salamanders to transform so they can avoid dying (Semlitsch & Wilbur, 1988). I implemented a drying regime to more closely mimic the natural environment of a temporary pond in the southeast and this encouraged larval salamanders to transform. I found no differences in time to metamorphosis among treatments. Small *A. opacum* most likely needed more time in the mesocosm to grow and reach the minimum size to metamorphose, which could explain why so few of the small *A. opacum* metamorphosed before I ended the experiment (Scott, 1990, Semlitsch & Wilbur, 1988). Whereas large *A. opacum* was already at the right size to metamorphose at the beginning of the experiment and most of them transformed during the experiment. The drying regime decreased space but this did not impact salamander growth for either species (Wilbur, 1987, Scott, 1990, Boone et al., 2002). This gave salamander larvae a reason to leave the mesocosm through metamorphosis and escape the potentially competitive environment. I ended the experiment before more salamanders could transform because visual surveys of the tanks suggested that *A. opacum* mortality in treatments with *A. tigrinum* was high and survivorship was the most important response variable in the study.

Conclusion

As the climate warms, the breeding time of amphibians, songbirds, and butterflies changes as well (Beebee, 1995, Gibbs & Breisch, 2001, Visser et al. 2006, Chadwick et al., 2006, Todd et al., 2011). Climate change has already directly impacted the breeding time of amphibians across the globe (Beebee, 1995, Gibbs & Breisch, 2001, Todd et al. 2011). This study has shown that shifts in size structure that results in *A. opacum* breeding later and *A. tigrinum* breeding early has the potential to shift species interactions between *A. opacum* and *A. tigrinum* in ways that would benefit *A. tigrinum* populations and result in declines in *A. opacum* populations where the two species overlap. Currently, *A. tigrinum* populations are declining throughout the southeast. These results suggest that climate change could benefit *A. tigrinum* populations in the region. Researchers should consider factors impacting *A. tigrinum* at other development stages to gain a broader picture of how climate change will impact this species.

Table

			Start length (cm)	Mid-length (cm)	Growth (cm)	Survivorship
Tank	Treatment	Species	Mean (SD)	Mean (SD)	(Mid-Start)	(Final-Initial)/Initial
A2	StSm	*A. opacum*	1.75 ±0.13	3.5 ±0.31	1.75	0
A2	StSm	*A. tigrinum*	1.79 ±0.22	4.65 ±0.64	2.86	0.25
A3	Sm	*A. opacum*	3.03 ±0.22	3.91 ±0.64	0.88	0.625
A4	Lm	*A. opacum*	3.44 ±0.55	4.11 ±0.31	0.67	0.625
A5	St	*A. tigrinum*	1.66 ±0.25	5.5 ±0	3.84	0.375
B1	St	*A. tigrinum*	1.63 ±0.12	4.37 ±0.70	2.74	0.75
B3	Sm	*A. opacum*	1.69 ±0.23	3.13 ±0.37	1.44	0.75
B4	StLm	*A. opacum*	3.73 ±0.42	4.77 ±0.50	1.04	0.75
B4	StLm	*A. tigrinum*	1.67 ±0.17	4.85 ±0.74	3.18	0.5
B5	StSm	*A. opacum*	1.61 ±0.17	3.37 ±0.23	1.76	0.5
B5	StSm	*A. tigrinum*	1.72 ±0.26	4.33 ±0.38	2.61	0.5
B6	Lm	*A. opacum*	3.55 ±0.75	4.46 ±0.46	0.91	0.88
C1	StLm	*A. opacum*	3.76 ±0.48	4.95 ±0.07	1.19	1
C1	StLm	*A. tigrinum*	1.62 ±0.13	5.1 ±0	3.48	0.5
C2	Sm	*A. opacum*	1.71 ±0.21	3.25 ±0.35	1.54	0.88
C3	St	*A. tigrinum*	1.84 ±0.22	3.86 ±0.46	2.02	0.88
C4	StSm	*A. opacum*	1.69 ±0.07	3.7 ±0	2.01	0
C4	StSm	*A. tigrinum*	1.74 ±0.24	5.62 ±0.38	3.88	0.75
C6	Lm	*A. opacum*	3.46 ±0.47	4.17 ±0.24	0.71	0.38
D1	Lm	*A. opacum*	3.29 ±0.65	4.25 ±0.35	0.96	0.88
D3	St	*A. tigrinum*	1.83 ±0.11	3.87 ±0.65	2.04	0.5
D4	StSm	*A. opacum*	1.7 ±0.02	3.2 ±0	1.5	0
D4	StSm	*A. tigrinum*	1.97 ±0.15	4.66 ±0.69	2.69	1
D5	Sm	*A. opacum*	1.72 ±0.17	4.11 ±0.28	2.39	0.38
D6	StLm	*A. opacum*	3.09 ±0.49	4.37 ±0.21	1.28	0.75
D6	StLm	*A. tigrinum*	1.69 ±0.18	4.17 ±0.15	2.48	0.75

Table 1: Mean salamander total length, growth, and survivorship in tanks among treatments for both species. Growth was calculated as the difference between length at the midpoint and at the beginning of the experiment, while survivorship was calculated as the proportion of salamanders from the start of the experiment that lived to the end of the experiment. The control treatments are small *A. opacum* (Sm), large *A. opacum* (Lm), and small *A. tigrinum* (St). The experimental treatments are large *A. opacum* and small *A. tigrinum* (StLm) which represents the current size structure, while small *A. opacum* with small *A. tigrinum* (StSm) represent the shift in size structure.

Figures

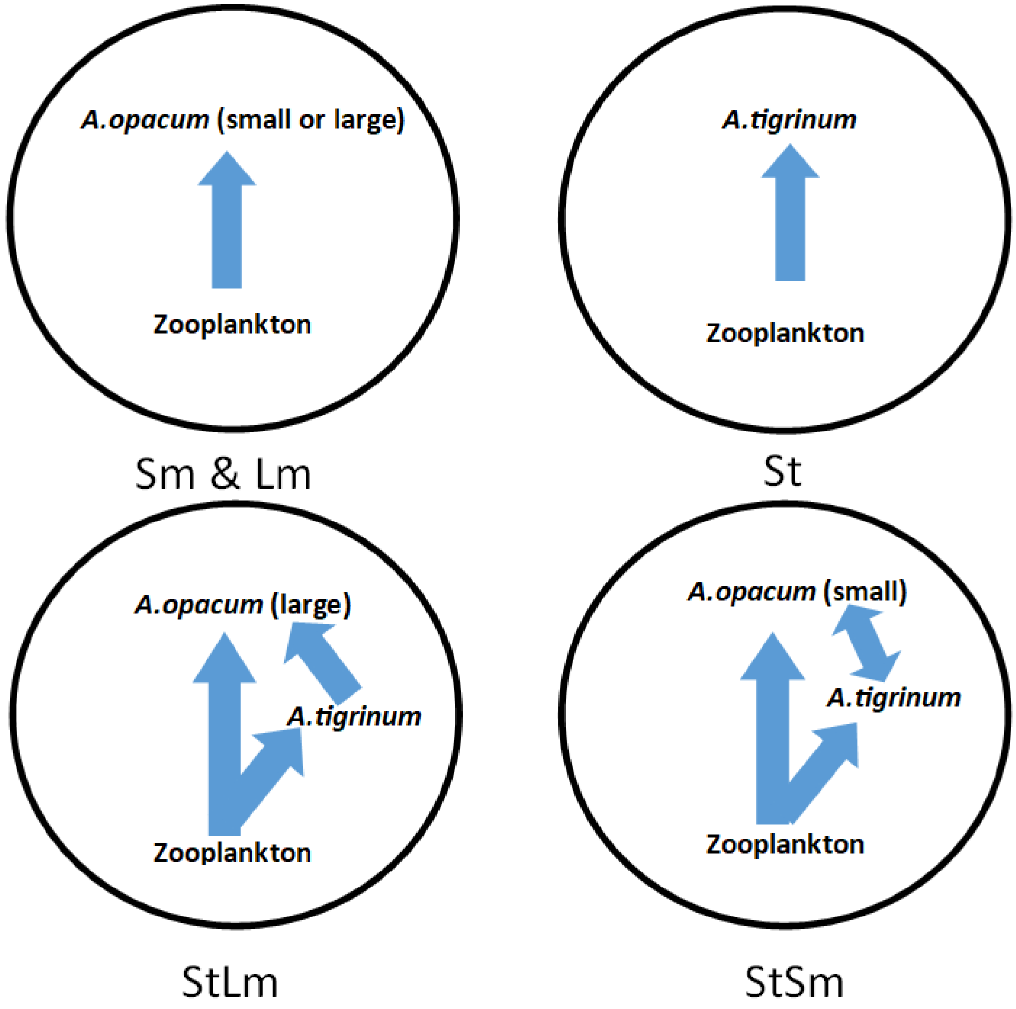

Fig. 1: Experimental communities used to test for size-structured interactions between larval salamanders. Control treatments had large *A. opacum* (Lm), small *A. opacum* (Sm), or *A. tigrinum* (St). Experimental treatments included a partial intraguild predation web with large *A. opacum* and small *A. tigrinum* (LmSt) or an intraguild predation food web with *A. opacum* and small *A. tigrinum* (SmSt) (i.e., Davenport and Chalcraft, 2012).

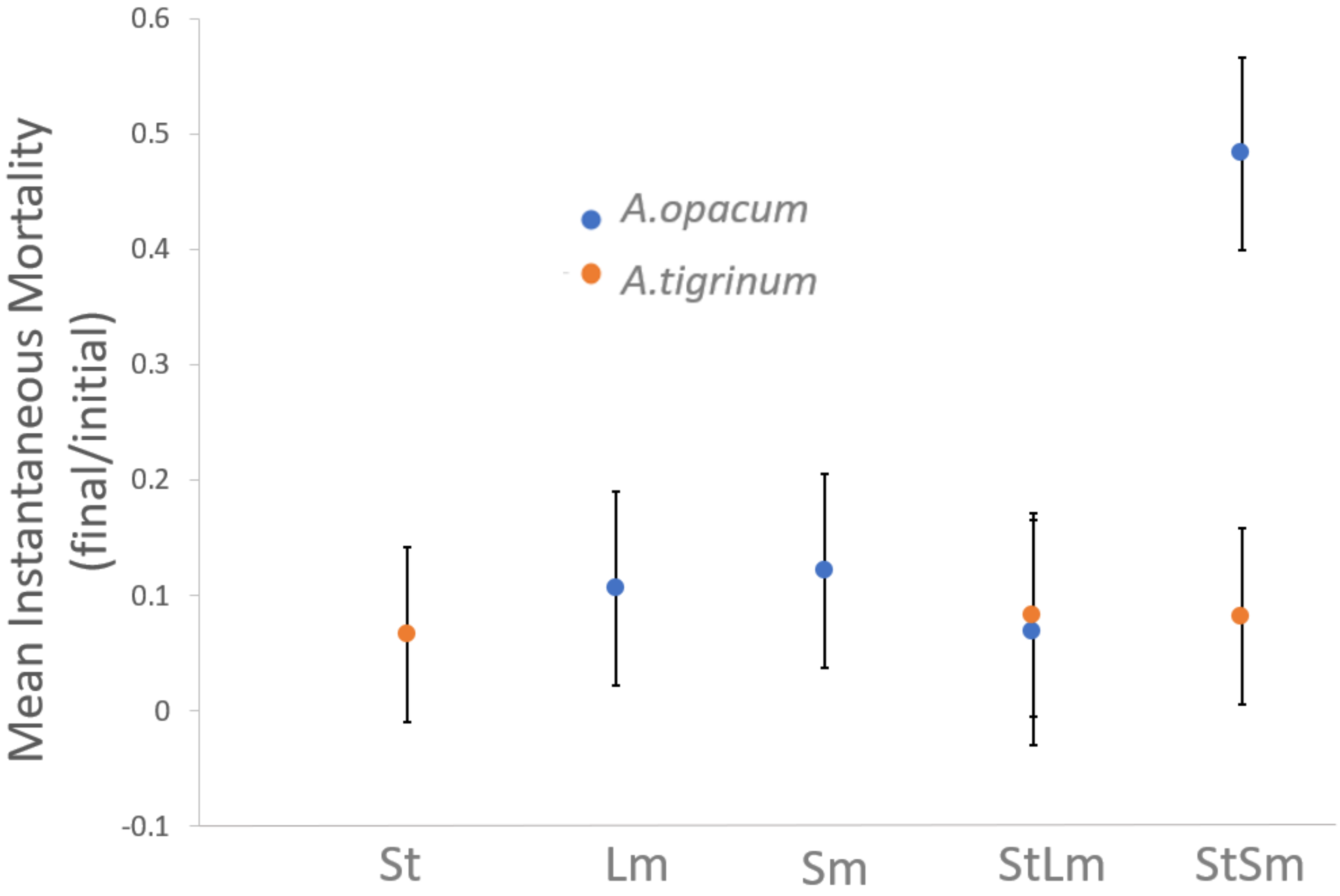

Fig. 2: Mean instantaneous mortality rate of salamanders (mean ± S.E) among the five treatments. There was a significant difference in the mortality of small *A. opacum* in the presence of *A. tigrinum* (StSm) compared to when *A. opacum* was large (StLm) or small *A. opacum* were alone (Sm).

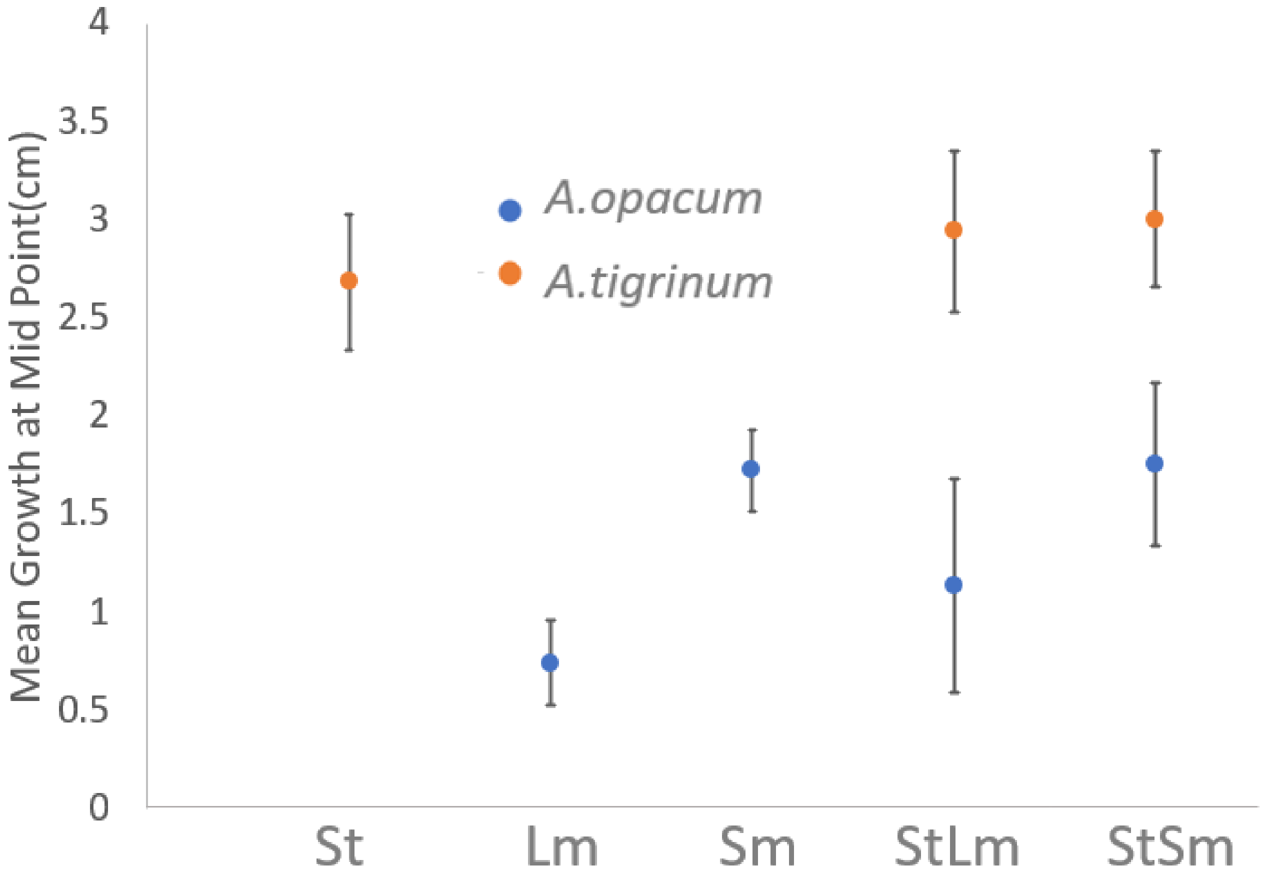

Fig. 3: Larval salamander growth (mean ± SE) halfway through the experiment with survivorship used as a covariate. Small *A. opacum* (Sm) grew faster in both the control (Lm and Sm) and experimental groups (StLm and StSm) There was no significant difference between treatments for *A. tigrinum*.

LITERATURE CITED

Anderson, T. L., Mott, C. L., Levine, T. D., & Whiteman, H. H. (2013). Life cycle complexity influences intraguild predation and cannibalism in pond communities. *Copeia*, *2013*, 284-291.

Bartley, T. J., McCann, K. S., Bieg, C., Cazelles, K., Granados, M., Guzzo, M. M., MacDougall, A.S., Tunney, T.D., & McMeans, B. C. (2019). Food web rewiring in a changing world. *Nature Ecology & Evolution*, *3*, 345-354.

Beebee, T. J. (1995). Amphibian breeding and climate. *Nature*, *374*(6519), 219-220.

Billick, I., & Case, T. J. (1994). Higher-order interactions in ecological communities: What are they and how can they be detected? *Ecology*, 75, 1529–1543.

Brodman, R., & Jaskula, J. (2002). Activity and microhabitat use during interactions among five species of pond-breeding salamander larvae. *Herpetologica*, *58*, 346-354.

Boone, M. D., Scott, D. E., & Niewiarowski, P. H. (2002). Effects of hatching time for larval ambystomatid salamanders. *Copeia*, *2002*, 511-517.

Carter, S. K., Vodopich, D., & Crumrine, P. W. (2018). Heterogeneity in body size and habitat complexity influence community structure. *Journal of Freshwater Ecology*, *33*, 239-249.

Chadwick, E. A., Slater, F. M., & Ormerod, S. J. (2006). Inter-and intraspecific differences in climatically mediated phenological change in coexisting Triturus species. *Global Change Biology*, *12*, 1069-1078.

Crumrine, P. W. (2005). Size structure and substitutability in an odonate intraguild predation system. *Oecologia*, *145*, 132-139.

Davenport, J. M., & Chalcraft, D. R. (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.

Davis, A. (2012). Investigating the Optimal Rearing strategy For *Ambystoma* Salamanders Using a Hematological Stress Index. *Herpetological Conservation and Biology*, 7, 95-100.

Gibbs, J. P., & Breisch, A. R. (2001). Climate warming and calling phenology of frogs near Ithaca, New York, 1900–1999. *Conservation Biology*, *15*, 1175-1178.

Gibbons, J. W., & Semlitsch, R. D. (1991). *Guide to the reptiles and amphibians of the Savannah River Site*. University of Georgia Press.

Hawley, T. J. (2009). The ecological significance and incidence of intraguild predation and cannibalism among anurans in ephemeral tropical pools. *Copeia*, *2009*, 748-757.

Holt, R. D., & Polis, G. A. (1997). A theoretical framework for intraguild predation. *The American Naturalist*, *149*, 745-764.

Holomuzki, J. R., & Collins, J. P. (1987). Trophic dynamics of a top predator, *Ambystoma tigrinum nebulosum* (Caudata: *Ambystomatidae*), in a lentic community. *Copeia*, 949-957.

JASP Team (2020). JASP (Version 0.14) [Computer software].

Jensen, J. B. (Ed.). (2008). *Amphibians and Reptiles of Georgia*. University of Georgia Press.

Kelly, A., & Goulden, M. (2008). Rapid Shift in plant distribution with recent climate change.*PNAS* 105, 11823-11826.

Lannoo, M. J., Lowcock, L., & Bogart, J. P. (1989). Sibling cannibalism in noncannibal morphs *Ambystoma tigrinum* larvae and its correlation with high growth rates and early metamorphosis. *Canadian Journal of Zoology*, *67*, 1911-1914.

Lurgi, M., López, B. C., & Montoya, J. M. (2012). Novel communities from climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*, 2913-2922

Lund, J. O., Wissinger, S. A., & Peckarsky, B. L. (2016). Caddisfly behavioral responses to drying cues in temporary ponds: implications for effects of climate change. *Freshwater Science*, *35*, 619-630.

Peacor, S. D., & Werner, E. E. (2000). Predator effects on an assemblage of consumers through induced changes in consumer foraging behavior. *Ecology*, 81, 1998-2010.

Petranka, J. W. (1990). Observations on nest site selection, nest desertion, and embryonic survival in marbled salamanders. *Journal of Herpetology*, 229-234.

Petranka, J., (1998). Salamanders of The United States and Canada.

Polis, G. A. (1998). Stability is woven by complex webs. *Nature*, *395*, 744-745.

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

Rudolf, V. H., & Singh, M. (2013). Disentangling climate change effects on species interactions: effects of temperature, phenological shifts, and body size. *Oecologia*, *173*, 1043-1052.

Ruehl, C. B., Vance-Chalcraft, H., & Chalcraft, D. R. (2018). Cooccurrence of prey species alters the impact of predators on prey performance through multiple mechanisms. *Ecology and Evolution*, *8*, 8894-8907.

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

Semlitsch, R. D., & Wilbur, H. M. (1988). Effects of pond drying time on metamorphosis and survival in the salamander *Ambystoma talpoideum*. *Copeia*, 978-983.

Shepard, I. D., Wissinger, S. A., Wood, Z. T., & Greig, H. S. (2021). Predators balance consequences of climate-change-induced habitat shifts for range-shifting and resident species. *Journal of Animal Ecology*.

Schneider, C. A.; Rasband, W. S., & Eliceiri, K. W. (2012), "NIH Image to ImageJ: 25 years of image analysis", *Nature Methods* 9(7): 671-675.

Sours, G. N., & Petranka, J. W. (2007). Intraguild predation and competition mediate stage-structured interactions between wood frog (*Rana sylvatica*) and upland chorus frog (*Pseudacris feriarum*) larvae. *Copeia*, *2007*, 131-139.

Todd, B. D., Scott, D. E., Pechmann, J. H., & Gibbons, J. W. (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(1715), 2191-2197.

Vance-Chalcraft H.D., Rosenheim J.A., Vonesh J.R., Osenberg C.W., & Sih A. (2007). The influence of intraguild predation on prey suppression and prey release: a meta-analysis. *Ecology*, 88, 2689-96.

Visser, M. E., Holleman, L. J., & Gienapp, P. (2006). Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia*, *147*, 164-172.

Wellborn, G. A., Skelly, D. K., & Werner, E. E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. *Annual review of ecology and systematics*, *27*, 337-363.

Whiteman, H. H., Howard, R. D., & Whitten, K. A. (1995). Effects of pH on embryo tolerance and adult behavior in the tiger salamander, *Ambystoma tigrinum tigrinum*. *Canadian Journal of Zoology*, 73, 1529-1537.

Wilber, H. (1987) Regulation of structure in complex systems: experimental temporary pond communities. *Ecology*, 68, 1437-1452.

Wissinger, S. A. (1992). Niche overlap and the potential for competition and intraguild predation between size-structured populations. *Ecology*, *73*, 1431-1444.

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

Zaifman, J., Shan, D., Ay, A., & Jimenez, A. G. (2017). Shifts in bird migration timing in North American long-distance and short-distance migrants are associated with climate change. *International Journal of Zoology*, *2017*.

Zellweger, F., Roth, T., Bugmann, H., & Bollmann, K. (2017). Beta diversity of plants, birds, and butterflies is closely associated with climate and habitat structure. *Global Ecology and Biogeography*, *26*, 898-906.