



The effects of intraguild interactions (or lack of) on prey diversity in experimental ponds food webs

K.M. Stemp^{a,b}, T.L. Anderson^{b,c}, J.M. Davenport^{a,b,*}

^a Department of Biology, Southeast Missouri State University, Cape Girardeau, MO 63701, USA

^b Department of Biology, Appalachian State University, Boone, NC 28608, USA

^c Department of Biology, Southern Illinois University Edwardsville, Edwardsville, IL 62026, USA

ARTICLE INFO

Keywords:

Ambystoma
Amphibian
Aeshnidae
Diversity
Food web
Intraguild
Predation

ABSTRACT

The interactions between top- and intermediate-level predators can have significant effects on community structure of food webs. The direct and indirect effects of top predators not only affect prey communities both negatively (e.g. predation) and positively (e.g. thinning effects), but can also impact intermediate-level predators (via intraguild predation [IGP]). These IGP interactions can have especially far-reaching implications when predators of increased ecological importance, such as keystone predators, are involved. Yet, the strength of these interactions are often context-dependent with relationships that are highly variable, and little is known about the factors that shape the outcomes. We conducted a mesocosm experiment to test for the potential IGP interactions among top (invertebrate) and intermediate (amphibian) predators and their effects on a diverse prey community. We used larval *Boyeria vinosa* (Fawn Darner), a rarely studied member of the family Aeshnidae, and *Ambystoma opacum* (Marbled Salamander), a known keystone predator in parts of its range, in a mesocosm study with an additive experimental design. Each mesocosm received an equal assemblage of five anuran prey species. Total prey survival was lowest when both predators were present, but *A. opacum* overwhelmingly reduced the survival of three prey species, lowering species diversity in all food webs when present. Species diversity was highest in the *B. vinosa* food web, with one prey species (*Anaxyrus americanus*) emerging smaller and sooner than from other food webs. We did not detect any interactions between predator species, indicating that *B. vinosa* may not be as voracious as other members of Aeshnidae. Our findings suggest that density mediated IGP interactions are non-existent among our focal species. Ultimately, we also demonstrate the importance of examining variability in keystone predators and how abundant, but less-known predator species affect community structure.

1. Introduction

Top predators can have strong top-down effects that structure food webs and shape community dynamics. The top-down effects not only affect prey negatively but also impact intermediate predators (via intraguild predation, IGP) (Heithaus, 2001; Vance-Chalcraft et al., 2007). Intraguild interactions among predators can manifest directly by consumption, interference competition, or indirectly via scramble competition over shared prey resources (Finke and Denno, 2005; Holt and Huxel, 2007; Polis et al., 1989; Wissinger et al., 1996). Indeed, reviews suggest that intraguild interactions are generally common in nature (Arim and Marquet, 2004). However, while IGP is well understood in many ecosystems, a better understanding of how predator-prey dynamics shift when ecologically important predators are included is

needed.

Some predators can also fill keystone species roles, where they have disproportionately strong effects relative to their biomass by consuming competitively dominant prey, which increases overall prey diversity (Paine, 1966; Power et al., 1996). Several species have been identified as keystone predators (e.g., *Pisaster*) but interestingly keystone predators are often not the top predators in a community (Estes et al., 1998; Libralato et al., 2006; Navarrete and Menge, 1996). Therefore, keystone predators and their effects on prey diversity have the potential to be influenced by intraguild interactions with top predators (Fauth and Resetarits, 1991; Witman et al., 2017).

In freshwater pond ecosystems, prior research has implicated some salamander species (adult eastern newts and larval Ambystomatid salamanders) as keystone predators (Chalcraft and Resetarits, 2003; Fauth,

* Corresponding author at: Department of Biology, Southeast Missouri State University, Cape Girardeau, MO 63701, USA.

E-mail address: davenportjm@apstate.edu (J.M. Davenport).

<https://doi.org/10.1016/j.fooweb.2021.e00207>

Received 15 January 2021; Received in revised form 21 July 2021; Accepted 1 August 2021

Available online 8 August 2021

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1999; Morin, 1995, 1983; Petranka, 1998; Smith, 2006). All species appear to reverse competitive outcomes of larval anurans in experimental pond communities (Chalcraft and Resetarits, 2003; Morin, 1995, 1983). Yet, only one study has evaluated how intraguild interactions influences the strength of keystone predation on prey diversity (Fauth and Resetarits, 1991). In that study, siren (*Siren intermedia*) adults prevented newts from consuming all of the larval amphibian prey community, thus altering the context of keystone predation. Adult newts were not susceptible to consumption by siren, which indicated that competition was likely the mechanism for the changes in prey diversity. Aside from the siren-newt research, few studies have explored how intraguild interactions can affect keystone salamander predators and any cascading effects to prey diversity in natural communities.

Freshwater pond ecosystems provide an excellent study system to explore intraguild interactions among predators and prey diversity. For example, relationships between hydrological permanence, top predator identity, and amphibian diversity have been well documented (Anholt et al., 2000; Skelly, 2001; Skelly et al., 1999; Welborn et al., 1996; Werner et al., 2007). Briefly, amphibian diversity is lowest in permanent ponds, because fish are present (Hecnar and M'Closkey, 1997; Semlitsch et al., 2015). Fish are voracious predators on larval amphibians and insects and can lead to local extinctions of populations upon colonization (McPeck, 1998; Semlitsch et al., 2015). In semi-permanent and ephemeral ponds without fish, amphibian diversity can be higher and larval dragonflies and insects are often the top predators (Wilbur, 1997). Often body size determines top predator status in fishless ponds, with larger individuals or species with larger gape widths filling that role (Crumrine, 2005; Van Buskirk, 1992; Wissinger, 1988). Indeed, large larvae of dragonflies from the family Aeshnidae have minor gape limitations and are capable of restructuring entire pond food webs (Caldwell et al., 1980; Relyea, 2003; Relyea and Yurewicz, 2002; Rudolf and Rasmussen, 2013; Turner and Chislock, 2007). Data suggest that *Anax*, one of primary genera studied in North America, will consume one keystone salamander species (larval *A. opacum* [marbled salamanders]) and likely influences consumptive effects on other amphibian prey by changing behavior, morphology and growth (Anderson and Semlitsch, 2016; Davenport and Chalcraft, 2014, 2012). Yet, we currently know little about how other groups of larval Aeshnids (excluding *Aeshna* and *Anax*) affect larval amphibian food webs. Therefore, examination of other Aeshnid dragonfly species in food web studies should be a priority for future research to broaden scope of inference.

To test the effects of a dragonfly top predator on the survival and keystone effects of *A. opacum* and on a shared prey assemblage, we conducted a mesocosm (modified cattle tanks) experiment. Since *A. opacum* are susceptible to predation by larval Aeshnid dragonflies, we tested how the presence of a co-occurring dragonfly species, *Boyeria vinosa* (Fawn Darner), affected *A. opacum* survival, growth, and consumptive effects on a tadpoles assemblage. As *B. vinosa* is closely related to other species of predatory dragonfly and has similar life history traits, we predicted that *B. vinosa* would reduce survival of both *A. opacum* and an assemblage of larval anurans. In the absence of *B. vinosa*, we predicted that *A. opacum* would serve as top predators and survival would be high, and prey diversity would also be highest due to keystone predation. In food webs with only *B. vinosa*, we expected prey survival to be equivalent to mixed predator food webs due to IGP.

2. Materials and methods

2.1. Study system

Ambystoma opacum are a common and wide-ranging species found across eastern North America, with populations ranging from Texas to New Hampshire (Petranka, 1998). *Ambystoma opacum* breed in the fall, unlike most Ambystomatids, and overwinter in ponds as larvae (Petranka, 1998). Overwintering allows larvae to grow large enough to serve as important predators on and superior competitors to other spring-

breeding amphibians (Anderson and Semlitsch, 2014; Cortwright and Nelson, 1990; Davenport and Chalcraft, 2012; Urban, 2007). As well, *A. opacum* has been identified as a keystone predator in parts of its range, in some cases restructuring entire pond communities (Chalcraft and Resetarits, 2003; Morin, 1995; Stemp et al., 2021).

Larval dragonflies are also voracious predators of amphibian tadpoles (Wilbur, 1997). Members of the family Aeshnidae are often top predators in ponds because of hinged mouthparts that allow them to consume prey much larger than their gapes (Stoks and McPeck, 2003; Welborn et al., 1996; Wilbur, 1997). If oviposition occurs early in the breeding season and temperatures are high, these larvae can grow fast enough within one season to metamorphose; alternatively, in colder growing conditions they may overwinter as larvae, which can amplify already strong top-down effects. *Boyeria vinosa* is a relatively common species in streams and floodplain habitats across North America (Needham et al., 2014) that breeds in mid- to late summer and often overwinters in larval form. *Boyeria vinosa* are ambush predators with strong effects on aquatic insect communities in streams and floodplain wetlands (Galbreath and Hendricks, 1992; Vance-Chalcraft et al., 2004; Vance-Chalcraft and Soluk, 2005). Larval *B. vinosa* overlap frequently with larval amphibians, including *A. opacum*, in floodplain wetlands in southern Illinois (Davenport, unpub. Data). To date, little is known on the predatory effects of *B. vinosa* on larval amphibian assemblages.

2.2. Experimental design

We conducted our mesocosm experiment at Southeast Missouri State University (hereafter SEMO). We filled sixteen 1100 L mesocosms with tap water and added water conditioner (AmQuel by Kordon®) to eliminate chloramine and chlorine. Each mesocosm received two 500 mL inoculations of concentrated zooplankton pond water to establish a zooplankton and periphyton community as well as 1.5 kg of dry leaf litter to serve as substrate (primarily *Quercus* spp.) (Anderson and Whiteman, 2015). We left all mesocosms open to allow natural deposition of additional prey items and drilled holes into the lips to allow for drainage of excess rainwater (Anderson et al., 2020; Anderson and Whiteman, 2015; Garig et al., 2020). All predatory macroinvertebrates that were observed in mesocosms were too small to interfere with our experiment (larval *Gyrinidae* spp. and *Dytiscidae* spp. ≤ 1.5 cm in total length) and were removed daily.

Larvae of both predator species and anuran prey were collected in spring 2016. All *A. opacum* larvae and *B. vinosa* were collected from Jackson County, Illinois in an area where they co-occur; we purposefully collected each predator from different ponds within the area to avoid preconditioning of predators to each other. *Ambystoma opacum* larvae were collected on 22 March 2016 and *B. vinosa* were collected on 24 March 2016 and 26 March 2016. Tadpole prey were acquired either by collecting partial egg masses (*Anaxyrus americanus* [American toad], *Pseudacris feriarum* [upland chorus frog], *Rana sylvatica* [wood frog], and *Rana sphenoccephala* [southern leopard frog]) or amplexant pairs of adults that then laid eggs in captivity (*Pseudacris crucifer* [spring peeper]) from ponds in Bollinger, Cape Girardeau, Wayne, and Scott counties of Missouri. We collected several egg masses of each prey species to limit any possible genetic effects. All egg masses and organisms were transported to SEMO, where they were held in an animal care facility that had large windows, resulting in natural 12:12-light:dark cycle. Air temperature was constant throughout holding at 19°- 21 °C. We held egg masses individually in plastic containers (30 × 15 × 11.5 cm) filled to a standard volume and changed the water every other day until hatching. Upon hatching, we counted tadpoles from each egg mass equally for each mesocosm and added them to the mesocosms. All predators were housed separately and individually before being added to the experiment.

All organisms were randomly assigned to one of four different experimental food webs as follows: 1) Control with only tadpole prey, 2) Six *A. opacum* with tadpole prey, 3) One *B. vinosa* with tadpole prey, and

4) Six *A. opacum* and one *B. vinosa* with tadpole prey (hereafter 'mixed'). Each food web was replicated four times in a 2×2 factorial design for a total of 16 mesocosms arranged in four experimental blocks (one replicate per block). All predators were randomly assigned to food webs in densities similar to those observed in nature and used in other mesocosm studies (Davenport and Chalcraft, 2013; Vance-Chalcraft et al., 2004; Vance-Chalcraft and Soluk, 2005). Dragonfly predator biomass ranged from 0.11–1.51 g (mean 0.563 g \pm 0.45 g) while total salamander predator biomass ($n = 6$ individuals per mesocosm) ranged from 3.19–6.64 g (mean 5.01 g \pm 1.11 g). All individual salamander larvae used in a single mesocosm were \pm 0.09 g. Dragonfly larvae were 19.36–43.32 mm total length (mean = 32.37 mm, SD = 8.34) and head width 4.29–10.43 (mean = 7.03, SD = 1.98). Additionally, every mesocosm received the same assemblage of larval spring-breeding amphibian prey: 100 American toads (*Anaxyrus americanus*), 160 southern leopard frogs (*Rana sphenoccephala*), 140 wood frogs (*Rana sylvatica*), 15 upland chorus frogs (*Pseudacris feriarum*), and 125 spring peepers (*Pseudacris crucifer*). The densities of both tadpole prey and predators used were within naturally occurring conditions and previous mesocosm experiments (Morin, 1983; Smock, 1988; Van Buskirk, 1988; Scott, 1990; Alford, 1999).

Rana sphenoccephala, *R. sylvatica*, *P. feriarum*, and *P. crucifer* tadpoles were added to mesocosms on 24 March 2016. *Anaxyrus americanus* tadpoles were added on 27 April 2016 to replicate natural phenological conditions for the year we conducted this experiment. All salamander predators were added on 26 March 2016 and dragonfly larvae were added on 27 March 2016. Mesocosms were monitored daily and maintained until metamorphosis was completed by most species (only one prey species overwinters as larvae, *R. sphenoccephala*). We were able to recover five of the eight *B. vinosa* at the experiment's end; we did not detect any exuvia at the time of experimental breakdown, but assumed the three remaining larvae all emerged from mesocosms. As *B. vinosa* likely emerged very near the end of the experiment as temperatures were warming, individuals were not replaced and natural phenology was allowed to progress. Metamorphosis for amphibians was defined by forelimb emergence for tadpoles or gill absorption for salamanders. Time to metamorphosis was defined as the number of days from the date of addition to the mesocosm to date of total tail or gill absorption. Size at metamorphosis (dry mass and snout-vent length, hereafter SVL) was measured upon complete reabsorption of the tail or gills in the lab. Size at and time to metamorphosis were measured because they serve as important predictors of reproductive fitness for amphibians (Berven, 1990; Semlitsch et al., 1988). At the conclusion of the experiment, all surviving metamorphs and larvae were euthanized and specimens were placed in the Natural History Museum at SEMO.

2.3. Data analysis

We used the program R (v3.3.3) to assess the predatory effects of salamanders and dragonflies on each experimental food web (R Core Team, 2019). We performed ANOVAs for each response variable of a prey species: survival, size at metamorphosis, and time to metamorphosis. Survival for both predator larvae and tadpoles is defined as the sum of 1) the number of individuals that underwent metamorphosis, and 2) the number of larvae that survive to the experiment's end date, as a proportion of initial input. It is assumed that tadpoles surviving to the end of the experiment would have reached size refugia from predators and would have emerged eventually or overwintered as larvae (e.g. *R. sphenoccephala*). If no individuals survived from a given food web, that food web was removed from the analysis because it would create complete separation in the data. We logit-transformed percent survival values prior to conducting ANOVA analyses (Warton and Hui, 2011). We also tried analyzing survival data using generalized linear mixed models with binomial errors, with food web and block as predictors and an individual level random effect to account for overdispersion in the data; these analyses produced identical results to logit transformed values,

thus only transformed survival data is reported (Warton and Hui, 2011). Block effects were significant ($P < 0.05$) for *P. crucifer* survival; therefore, block was retained for all prey models.

We calculated Simpson's Diversity Index (hereafter, diversity) for anuran prey for each tank, providing a measure of species evenness in each food web (Chalcraft and Reseratis, 2003, R package "vegan"). We also calculated the Shannon Wiener Index of Evenness for each tank; however both indices produced qualitatively identical results, thus only the Simpson's Index is reported (Appendix 1). We analyzed diversity using ANOVA, with predator food web and block as factors.

To test predator responses, we conducted ANOVAs for survival and size of *A. opacum*, and *B. vinosa*. Larval period length was unknown for both predator species as both were collected as free-swimming larvae. Predator survival was analyzed in the same manner as prey survival. We also examined both initial mass and mass at metamorphosis for *A. opacum* and initial total length for *B. vinosa* among predator food webs. Additionally, because initial predator size covered a range of sizes, we examined the effects of initial predator size on overall prey survival and prey diversity; however, neither of these terms were significant and thus were not included in any other analyses. Block effects were not included in predator models. All ANOVAs used the Tukey multiple comparison of means with 95% family-wise confidence levels to assess food web differences.

3. Results

3.1. Prey survival and diversity

Total prey survival was lowest in the Mixed food web, as compared to any other food webs, while the remaining three food webs had equivalent survival ($F_{3,9} = 9.012$, $p = 0.004$, $R^2 = 0.593$, Fig. 1). *Anaxyrus americanus* survival was higher in the *A. opacum* only food web than in the *B. vinosa* only or Mixed food webs, but the Control food web did not differ from any other food webs ($F_{3,9} = 5.401$, $p = 0.021$, $R^2 = 0.433$, Fig. 2a). *Pseudacris crucifer* survival was higher in the *B. vinosa* only food web than in any other food webs, which were all equivalent ($F_{3,9} = 10.718$, $p = 0.003$, $R^2 = 0.715$, Fig. 2b). *Pseudacris feriarum* survived only in the *B. vinosa* only and Control food webs, with survival the

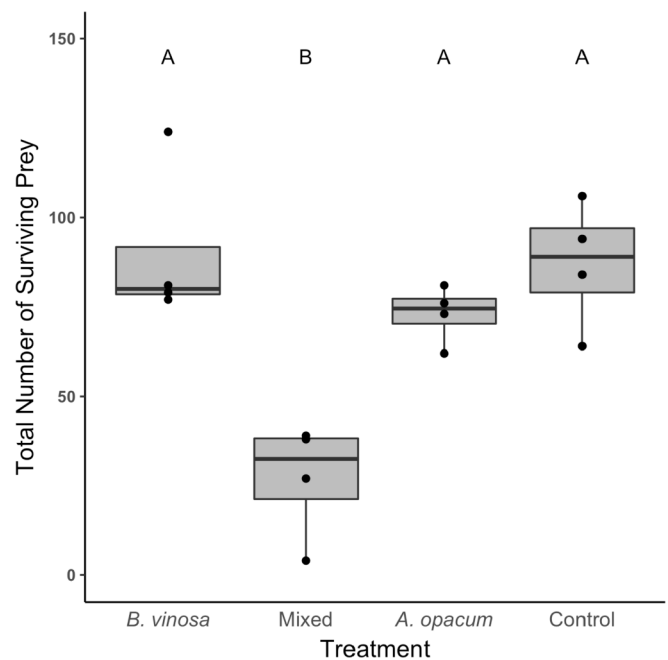


Fig. 1. Total number of surviving prey that emerged from each food web. Circles represent actual data points. Capital letters above boxes identify pairs of means that are significantly different. $N = 4$ in all cases.

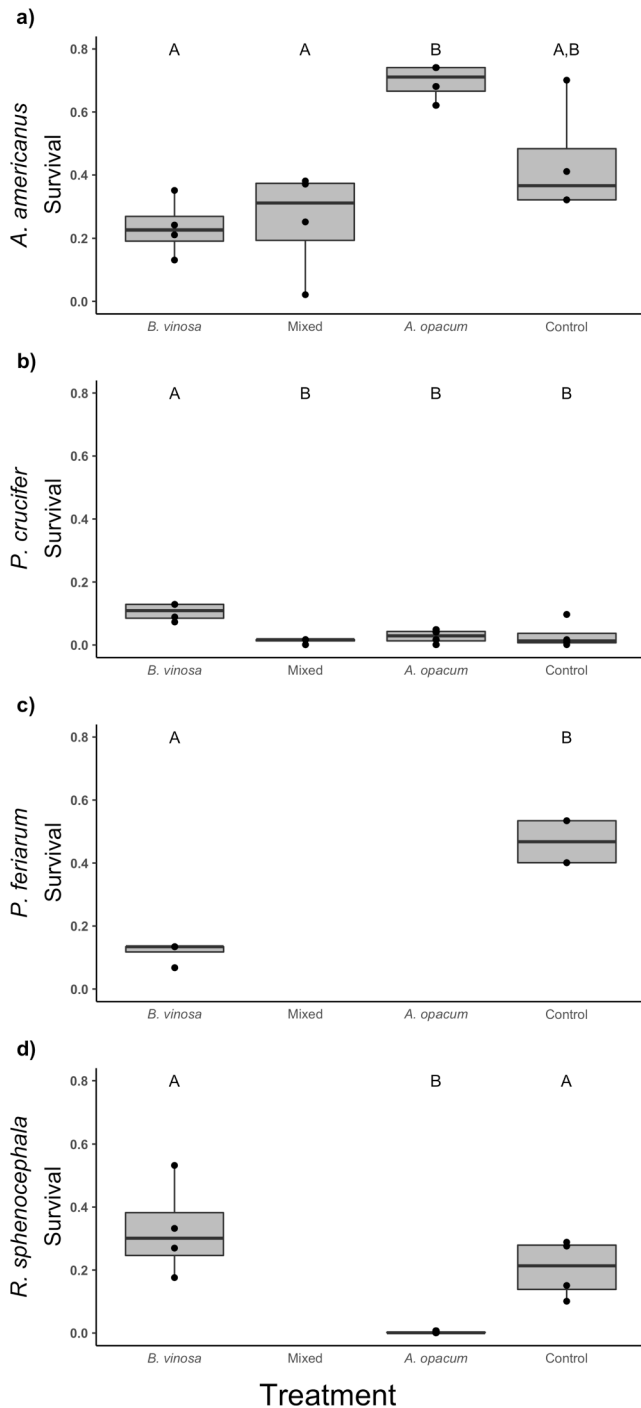


Fig. 2. Mean survival of a) *A. americanus*, b) *P. crucifer*, c) *P. feriarum*, and d) *R. sphenocéphala* across predator food webs. Circles represent actual data points. Capital letters above boxes identify pairs of means that are significantly different. $N = 4$ in all cases.

highest in the Control food web ($F_{1,3} = 159.2$, $p = 0.001$, $R^2 = 0.960$, Fig. 2c). Lastly, *R. sphenocéphala* survival was higher in *B. vinosa* only and Control food webs than in any food webs with *A. opacum*, with no survivors in Mixed food web and only a single metamorph emerging from one replicate of the *A. opacum* only food web ($F_{2,6} = 26.192$, $p = 0.001$, $R^2 = 0.813$, Fig. 2d). Pairwise contrasts are reported in Appendix 1: Tables S3, S4. Overall, prey diversity was lower in both Mixed and *A. opacum* only food webs than in *B. vinosa* only or Control food webs ($F_{3,9} = 15.534$, $p < 0.001$, $R^2 = 0.741$, Fig. 3).

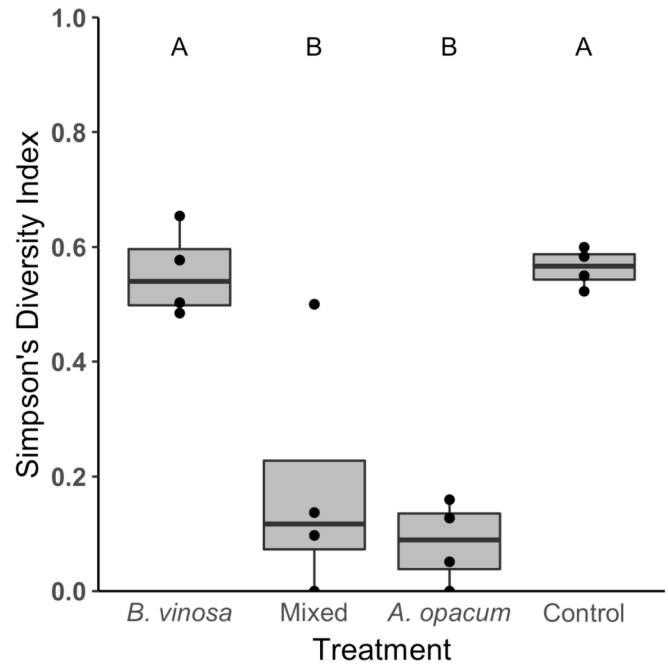


Fig. 3. Mean species diversity across predator food webs as represented by the Simpson's diversity index. Circles represent actual data points. Capital letters above bars identify pairs of means that are significantly different. $N = 4$ in all cases.

3.2. Prey traits

Anaxyrus americanus emerged from Mixed and *A. opacum* only food webs significantly larger than from other food webs, both in regards to mass at metamorphosis and SVL at metamorphosis ($F_{3,9} = 11.920$, $p = 0.002$, $R^2 = 0.677$ and $F_{3,9} = 17.247$, $p < 0.001$, $R^2 = 0.761$ respectively, Fig. 4a,b). Additionally, *A. americanus* emerged from Mixed and *A. opacum* only food webs in less time than from *B. vinosa* only or Control food webs ($F_{3,9} = 16.838$, $p < 0.001$, $R^2 = 0.767$, Fig. 4c). Neither mass at metamorphosis, SVL at metamorphosis, or larval period varied with any food web for *P. crucifer*, *P. feriarum*, or *R. sphenocéphala* (Appendix 1: Fig. S1-S3).

3.3. Predator response

Ambystoma opacum survival did not vary by food web, and in all cases at least 50% of larval *A. opacum* survived to metamorphosis ($F_{1,6} = 0.087$, $p = 0.777$, $R^2 = -0.150$). Additionally, initial mass did not vary by food web ($F_{1,6} = 0.415$, $p = 0.543$, $R^2 = -0.091$), nor did mass at metamorphosis ($F_{1,6} = 0.321$, $p = 0.592$, $R^2 = -0.108$, Appendix 1: Table S11, Fig. S4). We also did not detect any differences in *B. vinosa* traits; *B. vinosa* survival did not vary by predator food web ($F_{1,6} = 0.429$, $p = 0.537$, $R^2 = -0.089$) nor did initial starting total length ($F_{1,6} = 0.019$, $p = 0.895$, $R^2 = -0.163$, Appendix 1: Table S12, Fig. S4). There was no effect of predator size on overall prey survival or prey diversity.

4. Discussion

The predators in our study do not appear to engage in intraguild interactions. Despite the wealth of data on strong top-down and intraguild effects of Aeshnid dragonflies on other pond predators (Crumrine and Crowley, 2003; Davenport and Chalcraft, 2012; Ramos and van Buskirk, 2012; Relyea and Yurewicz, 2002; Yurewicz, 2004), survival of both predators was high in the Mixed food web, suggesting a lack of negative consequences. Both predators did affect prey survival and diversity, but not in the ways originally predicted. *Ambystoma opacum* did

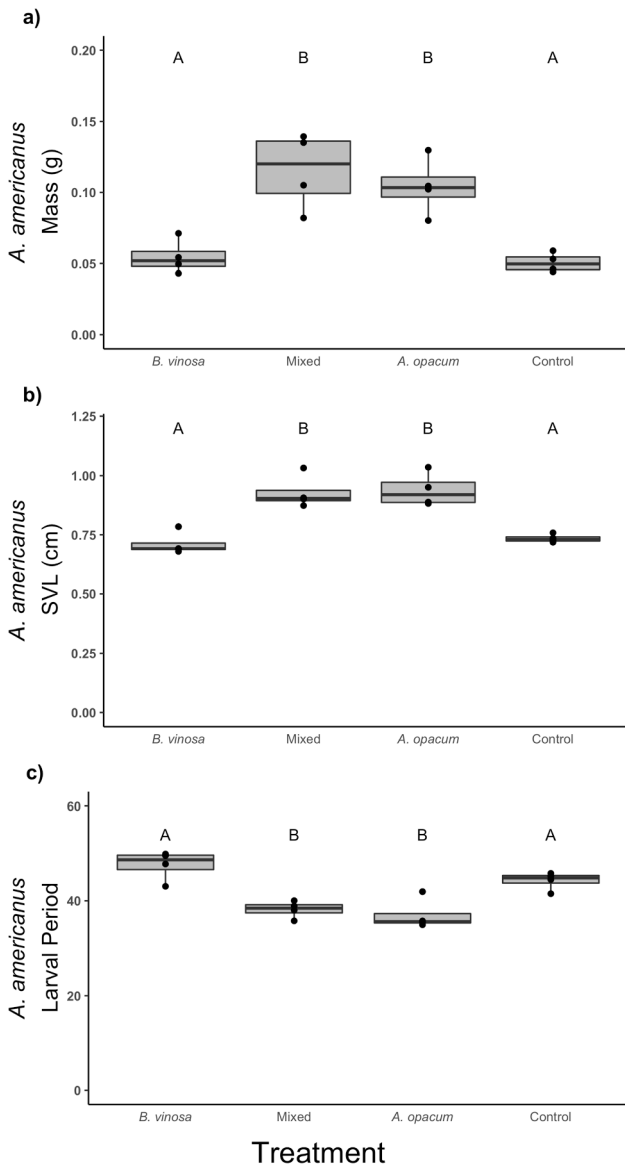


Fig. 4. *Anaxyrus americanus* trait specific responses for a) mass at metamorphosis b) SVL at metamorphosis and c) larval period across predator food webs. Circles represent actual data points. Capital letters above boxes identify pairs of means that are significantly different. $N = 4$ in all cases.

not enhance prey diversity via keystone predation (Fig. 3). Indeed, prey diversity was lowest in food webs where *A. opacum* was present. *Boyeria vinosa* did lower overall prey survival to similar levels as *A. opacum*, and when both predators were present, very few prey survived. But, prey diversity was substantially higher when *B. vinosa* was the only predator present (Fig. 3). The differences in prey diversity seem to manifest from *A. opacum* reducing prey survival for all species except *A. americanus* while *B. vinosa* significantly affected *A. americanus* (Figs. 2, 4). These predator specific effects on *A. americanus* survival are also likely responsible for *A. americanus* being larger in *A. opacum* food webs and emerging sooner (i.e. thinning of interspecific competitors, Fig. 4).

The top predator in our experiment, *B. vinosa*, did not reduce survival of the intermediate predator, *A. opacum* as expected. Larval Aeshnid dragonflies are known to be voracious predators on larval amphibians (Wilbur, 1997). However, most studies have focused on a subset of genera (*Anax* or *Aeshna*) when experimentally manipulating pond food webs (Relyea, 2007). To our knowledge, our study is one of the first to use *B. vinosa* in experimental ponds with larval amphibians. In

floodplain ponds of southern Illinois, *B. vinosa* is one of the largest and most commonly encountered aquatic invertebrates (Davenport, unpubl. data). Therefore, we had hypothesized that it may have strong top-down effects in these food webs, similar to *Anax* or *Aeshna*, though this was unsupported by the data. While it is possible that *B. vinosa* did not identify *A. opacum* as a prey species in our study, we doubt this is the case because *B. vinosa* did consume other larval amphibians as prey (*P. crucifer* and *A. americanus*). With an abundance of smaller prey available, *A. opacum* may not have been the primary target of *B. vinosa*. In addition, *B. vinosa* is a sit-and-wait predator, so it is possible that larval *A. opacum* were more active and able to evade *B. vinosa* as a predator. Activity shifts and behavioral avoidance are common for larval *Ambystoma* (Holomuzki, 1986; Walls, 1995) and could also be the mechanism responsible for their high survival with *B. vinosa*. *Rana sphenoccephala*, one of the largest tadpole prey species, also had the highest survival among shared prey in food webs with *B. vinosa* (Fig. 2). This suggests that it may have behavioral shifts in activity, like other larval amphibians, to physically avoid predation by larval dragonflies (Davenport et al., 2014; Relyea, 2007). We did not measure activity levels of prey or predators in our current experiment so we currently cannot identify the mechanism for our observed patterns.

The intermediate predator in our study system, *A. opacum*, has been suggested as a potential keystone predator species in pond food webs (Chalcraft and Resetarits, 2003; Morin, 1995; Stemp et al., 2021). However, we did not detect positive effects on prey diversity based on their co-occurrence with a tadpole prey assemblage (Fig. 3). Indeed, food webs with larval *A. opacum* present had the lowest prey diversity. Several scenarios may explain why our results are different from other published studies. First, the individual larval *A. opacum* that were added to our experiment were smaller (0.51–1.15 g) than those from previously reported experiments (1.12–1.178 g and 1.826–2.566 g from Chalcraft and Resetarits, 2003 and Morin, 1995, respectively). This could have led to our larval *A. opacum* growing in size and gape along with tadpole prey, thus prolonging the window for our larval salamanders to consume prey. For example, it is possible larger *A. opacum* from other studies may have selected for larger prey and would likely have had smaller windows of predation to due to earlier metamorphosis from ponds. Second, the keystone effect of a predator may not exist across the geographic range of the species (Kurzava and Morin, 1994; Paine et al., 1985). A recent experiment suggests that larval *A. opacum* from several other populations (OH, NC, and MS) also do not increase tadpole diversity in experimental pond food webs (Stemp et al., 2021). The specific mechanism for this is unknown, and could be related to any number of differences including variation in activity levels or local adaptation in foraging among the studied populations, or physiological differences (e. g. metabolic rates). Future work should consider such population level differences of larval *A. opacum* to explain variation in foraging.

Total prey survival was reduced the greatest in our Mixed predator food web, with diversity also being the lowest in that food web. This was driven by *A. opacum*, as some prey species (*R. sphenoccephala* and *P. feriarum*) were completely eliminated from food webs with *A. opacum* (Fig. 2). The only prey species with high survival in food webs with *A. opacum* was *A. americanus* tadpoles (Fig. 4). This was likely due to the later addition of *A. americanus* tadpoles and the early metamorphosis of *A. opacum*. Our experimental additions followed the natural phenology of our region, suggesting that fall-breeding *Ambystoma* larvae may have less impact on late spring-breeding amphibians (Anderson et al., 2020; Anderson and Whiteman, 2015). One benefit for *A. americanus* was a higher mass at metamorphosis and shorter time to metamorphosis in food webs with *A. opacum*, likely because of the reduced competition from fewer conspecific competitors. Alternatively, *A. americanus* had lower survival in food webs with *B. vinosa* and in the Control food webs. This was likely due to the greater overlap in phenology with *B. vinosa*. *Pseudacris crucifer* has previously been considered an inferior competitor with very low survival in mixed assemblages of larval amphibians (Morin, 1983; Skelly, 1996). Nevertheless, *P. crucifer* tadpoles had

higher survival in *B. vinosa* food webs, which suggests that either *B. vinosa* was a poor predator on *P. crucifer* tadpoles or *B. vinosa* may have focused more on other tadpole species. Future research could evaluate priority effects of our specific prey assemblage along with predator specific effects on mixed tadpole assemblages.

Our study advances the understanding of pond food webs by assessing potential intraguild interactions with a different Aeshnid species and their effects on a keystone predator. First, some larval Aeshnid dragonflies may influence intermediate predators in pond food webs more than others (e.g. *Boyeria* versus *Anax* or *Aeshna*). Intraguild predation of larval salamanders by larval Aeshnids is frequently documented in experimental studies (Anderson and Semlitsch, 2016; Davenport and Chalcraft, 2012; Kishida et al., 2009) and expansion of other species in experiments is vital to broadening inference. Based on our results, *B. vinosa* likely does not affect larval *A. opacum* growth and survival. This suggests that, in some freshwater ponds, larval salamanders may coexist with larval Aeshnid dragonflies and may still serve as top predators. Second, *A. opacum* from southern Illinois populations do not appear to have a keystone predator effect on tadpole prey diversity. This finding is in line with work on two populations of *N. viridescens* that differed in top-down effects in experimental ponds (Kurzava and Morin, 1994). While our study did not detect any impacts of a top predator on a perceived intermediate predator, more research is needed to understand the context-dependence of predator-predator interactions. Specifically, intraguild interactions are common in freshwater systems and aquatic ecologists should make expanding estimates of prey diversity because of those interactions a priority.

Acknowledgments

We would like to thank D. Siegel, S. N. Hwang, J. Bukhart, D. Garig, L. Taylor, S. Bishir, A. Feltmann, J. Bumpass, C. Weible, J. Mosebach, and H. Midkiff for their help running experiments and providing guidance. Research was conducted under Missouri Department of Conservation Permit Numbers 15992 and 16821, Illinois Department of Natural Resources Permit Number XNH16.5874, University of Missouri ACUC #7403, and Southeast Missouri State IACUC protocol 16-001. All animals were handled and housed in accordance with Institutional Animal Care and Use Committee (IACUC) policies. Funding for the writing of this manuscript was also provided by SERDP RC-2703. The authors have no conflicts of interest to declare.

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fooweb.2021.e00207>.

References

- Alford, R.A., 1999. Ecology: Resource Use, Competition, and Predation. In: McDiarmid, R.W., Altig, R. (Eds.), *Tadpoles: The Biology of Anuran Larvae*. The University of Chicago Press, Chicago, pp. 240–278.
- Anderson, T.L., Semlitsch, R.D., 2014. High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations. *Popul. Ecol.* 56, 265–273. <https://doi.org/10.1007/s10144-013-0419-9>.
- Anderson, T.L., Semlitsch, R.D., 2016. Top predators and habitat complexity alter an intraguild predation module in pond communities. *J. Anim. Ecol.* 85, 548–558. <https://doi.org/10.1111/1365-2656.12462>.
- Anderson, T.L., Whiteman, H.H., 2015. Asymmetric effects of intra- and interspecific competition on a pond-breeding salamander. *Ecology* 96, 1681–1690.
- Anderson, T.L., Stemp, K.M., Ousterhout, B.H., Burton, D., Davenport, J.M., 2020. Impacts of phenological variability in a predatory salamander on pond food webs. *J. Zool.* 310, 95–105.
- Anholt, B.R., Werner, E., Skelly, D.K., 2000. Effect of food and predators on the activity of four larval Ranid frogs. *Ecology* 81, 3509–3521. <https://doi.org/10.2307/177510>.
- Arim, M., Marquet, P.A., 2004. Intraguild predation: a widespread interaction related to species biology. *Ecol. Lett.* 7, 557–564.
- Berven, K.A., 1990. Factors affecting population fluctuations in larval and adult stages of the Wood Frog (*Rana sylvatica*). *Ecology* 71, 1599–1608.
- Caldwell, J.P., Thorp, J.H., Jevey, T.O., 1980. Predator-prey relationships among larval dragonflies, salamanders, and frogs. *Oecologia* 46, 285–289.
- Chalcraft, D.R., Resetarits, W.J.J., 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? *Ecology* 84, 2407–2418.
- Cortwright, S.A., Nelson, C.E., 1990. An examination of multiple factors affecting community structure in an aquatic amphibian community. *Oecologia* 83, 123–131. <https://doi.org/10.1007/BF00324643>.
- Crumrine, P.W., 2005. Size structure and substitutability in an odonate intraguild predation system. *Oecologia* 145, 132–139.
- Crumrine, P.W., Crowley, P.H., 2003. Partitioning components of risk reduction in a dragonfly-fish intraguild predation system. *Ecology* 84, 1588–1597.
- 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. *J. Anim. Ecol.* 81, 242–250. <https://doi.org/10.1111/j.1365-2656.2011.01906.x>.
- Davenport, J.M., Chalcraft, D.R., 2013. Nonconsumptive effects in a multiple predator system reduce the foraging efficiency of a keystone predator. *Ecol. Evol.* 3, 3063–3072. <https://doi.org/10.1002/ece3.691>.
- Davenport, J.M., Chalcraft, D.R., 2014. Increasing conspecific density weakens the ability of intermediate predators to develop induced morphological defences to top predators. *Freshw. Biol.* 59, 87–99. <https://doi.org/10.1111/fwb.12248>.
- Davenport, J.M., Hossack, B.R., Lowe, W.H., 2014. Partitioning the non-consumptive effects of predators on prey with complex life histories. *Oecologia* 176, 149–155. <https://doi.org/10.1007/s00442-014-2996-5>.
- Estes, J.A., Tinker, M.T., Williams, T.M., Doak, D.F., 1998. Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science* (80-.) 282, 473–476. <https://doi.org/10.1126/science.282.5388.473>.
- Fauth, J.E., 1999. Identifying potential keystone species from field data – an example from temporary ponds. *Ecol. Lett.* 2, 36–43.
- Fauth, J.E., Resetarits, W.J., 1991. Interactions between the salamander *Siren intermedia* and the keystone predator *Notophthalmus viridescens*. *Ecology* 72, 827–838.
- Finke, D.L., Denno, R.F., 2005. Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecol. Lett.* 8, 1299–1306.
- Galbreath, G.H., Hendricks, A.C., 1992. Life history characteristics and prey selection of larval *Boyeria vinosa* (Odonata: Aeshnidae). *J. Freshw. Ecol.* 7, 201–207.
- Garig, D.F., Ennen, J.R., Davenport, J.M., 2020. The effects of Common Snapping Turtles on a freshwater food web. *Copeia* 108, 132–139. <https://doi.org/10.1643/ce-19-258>.
- Hecnar, S.J., M'Closkey, R.T., 1997. The effects of predatory fish on amphibian species richness and distribution. *Biol. Conserv.* 79, 123–131.
- Heithaus, M.R., 2001. Habitat selection by predators and prey in communities with asymmetrical intraguild predation. *Oikos* 92, 542–554.
- Holomuzki, J.R., 1986. Predator avoidance and diel patterns of microhabitat use by larval Tiger Salamanders. *Ecology* 67, 737–748.
- Holt, R.D., Huxel, G.R., 2007. Alternative prey and the dynamics of intraguild predation: theoretical perspectives. *Ecology* 88, 2706–2712.
- Kishida, O., Trussell, G.C., Nishimura, K., 2009. Top-down effects on antagonistic inducible defense and offense. *Ecology* 90, 1217–1226.
- Kurzava, L.M., Morin, P.J., 1994. Consequences and causes of geographic variation in the body size of a keystone predator, *Notophthalmus viridescens*. *Oecologia* 99, 271–280.
- Libralato, S., Christensen, V., Pauly, D., 2006. A method for identifying keystone species in food web models. *Ecol. Model.* 195, 153–171.
- McPeck, M.A., 1998. The consequences of changing the top predator in a food web: a comparative experimental approach. *Ecol. Monogr.* 68.
- Morin, P.J., 1983. Predation, competition, and the composition of larval Anuran guilds. *Ecol. Monogr.* 53, 119–138.
- Morin, P.J., 1995. Functional redundancy, non-additive interactions, and supply-side dynamics in experimental pond communities. *Ecology* 76, 133–149. <https://doi.org/10.2307/1940637>.
- Navarrete, S.A., Menge, B.A., 1996. Keystone predation and interaction strength: interactive effects of predators on their main prey. *Ecol. Monogr.* 66, 409–249.
- Needham, J.G., Westfall, M.J., May, M.L., 2014. Dragonflies of North America: The Odonata (Anisoptera) Fauna of Canada, the Continental United States, Northern Mexico and the Greater Antilles, 3rd, editio. ed. Scientific Publishers, U.S.A.
- Paine, R.T., 1966. Food web complexity and species diversity. *Am. Nat.* 100, 65–75.
- Paine, R.T., Castillo, J.C., Cancino, J., 1985. Perturbation and recovery patterns of starfish-dominated intertidal assemblages in Chile, New Zealand, and Washington State. *Am. Nat.* 125, 679–691.
- Petranka, J.W., 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington DC.
- Polis, G.A., Myers, C.A., Holt, R.D., 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annu. Rev. Ecol. Syst.* 20, 297–330.
- Power, M.E., Tilman, D., Estes, J.A., Menge, B.A., Bond, W.J., Mills, S., Daily, G., Castilla, J.C., Lubchenco, J., Paine, R.T., 1996. Challenges in the quest for keystones. *Bioscience* 46, 609–620. <https://doi.org/10.2307/1312990>.
- R Core Team, 2019. R: A Language and Environment for Statistical Computing.
- Ramos, O., van Buskirk, J., 2012. Non-interactive multiple predator effects on tadpole survival. *Oecologia* 169, 535–539. <https://doi.org/10.1007/s00442-011-2208-5>.
- Relyea, R.A., 2003. How prey respond to combined predators: A review and an empirical test. *Ecology* 84, 1827–1839.
- Relyea, R.A., 2007. Getting out alive: how predators affect the decision to metamorphose. *Oecologia* 152, 389–400. <https://doi.org/10.1007/s00442-007-0675-5>.
- Relyea, R.A., Yurewicz, K.L., 2002. Predicting community outcomes from pairwise interactions: integrating density- and trait-mediated effects. *Oecologia* 131, 569–579. <https://doi.org/10.1007/s00442-002-0910-z>.

- Rudolf, V.H.W., Rasmussen, N.L., 2013. Ontogenetic functional diversity: Size structure of a keystone predator drives functioning of a complex ecosystem. *Ecology* 94, 1046–1056.
- Scott, D.E., 1990. Effects of larval density in *Ambystoma opacum*: an experiment in large-scale field enclosures. *Ecology* 71, 296–306.
- Semlitsch, R.D., Scott, D.E., Pechmann, J.H.K., 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* 69, 184–192.
- Semlitsch, R.D., Peterman, W.E., Anderson, T.L., Drake, D.L., Ousterhout, B.H., 2015. Intermediate pond sizes contain the highest density, richness, and diversity of pond-breeding amphibians. *PLoS One* 10, 1–20. <https://doi.org/10.1371/journal.pone.0123055>.
- Skelly, D.K., 1996. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. *Copeia* 1996, 599–605.
- Skelly, D.K., 2001. Distributions of pond-breeding anurans: an overview of mechanisms. *Isr. J. Zool.* 47, 313–332.
- Skelly, D.K., Werner, E.E., Cortwright, S.A., 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology* 80, 2326–2337.
- Smith, K.G., 2006. Keystone predators (eastern newts, *Notophthalmus viridescens*) reduce the impacts of an aquatic invasive species. *Oecologia* 148, 342–349. <https://doi.org/10.1007/s00442-006-0370-y>.
- Smock, L.A., 1988. Life histories, abundance and distribution of some macroinvertebrates from a South Carolina, USA coastal plain stream. *Hydrobiologia* 157, 193–208. <https://doi.org/10.1007/BF00004197>.
- Stemp, K.M., Anderson, T.L., Davenport, J.M., 2021. Macrogeographic variation of a pond predator's top-down effects in a common garden environment. *Popul. Ecol.* 63, 5–13.
- Stoks, R., McPeck, M.A., 2003. Predators and life histories shape *Lestes* damselfly assemblages along a freshwater habitat gradient. *Ecology* 84, 1576–1587.
- Turner, A.M., Chislock, M.F., 2007. Dragonfly predators influence biomass and density of pond snails. *Oecologia* 153, 407–415.
- Urban, M.C., 2007. Predator size and phenology shape prey survival in temporary ponds. *Oecologia* 154, 571–580. <https://doi.org/10.1007/s00442-007-0856-2>.
- Van Buskirk, J., 1988. Interactive effects of dragonfly predation in experimental pond communities. *Ecology* 69, 857–867. <https://doi.org/10.2307/1941035>.
- Van Buskirk, J., 1992. Competition, cannibalism, and size class dominance in a dragonfly. *Oikos* 65, 455–464.
- Vance-Chalcraft, H.D., Soluk, D.A., 2005. Estimating the prevalence and strength of non-independent predator effects. *Oecologia* 146, 452–460. <https://doi.org/10.1007/s00442-005-0201-6>.
- Vance-Chalcraft, H.D., Soluk, D.A., Ozburn, N., 2004. Is prey predation risk influenced more by increasing predator density or predator species richness in stream enclosures? *Oecologia* 139, 117–122. <https://doi.org/10.1007/s00442-003-1484-0>.
- 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, 2689–2696.
- Walls, S.C., 1995. Differential vulnerability to predation and refuge use in competing larval salamanders. *Oecologia* 101, 86–93. <https://doi.org/10.1007/BF00328904>.
- Warton, D.I., Hui, F.K.C., 2011. The arcsine is asinine: the analysis of proportions in ecology. *Ecology* 92, 3–10. <https://doi.org/10.1890/10-1922.1>.
- Welborn, G.A., Skelly, D.K., Werner, E.E., 1996. Mechanisms creating community structure across a freshwater habitat gradient. *Annu. Rev. Ecol. Syst.* 27, 337–363.
- Werner, E.E., Yurewicz, K.L., Skelly, D.K., Relyea, R.A., 2007. Turnover in an amphibian metacommunity: the role of local and regional factors. *Oikos* 116, 1713–1725.
- Wilbur, H.M., 1997. Experimental ecology of food webs: complex systems in temporary ponds. *Ecology* 78, 2279–2302.
- Wissinger, S.A., 1988. Life history and size structure of larval dragonfly populations. *J. North Am. Benthol. Soc.* 7, 13–28.
- Wissinger, S.A., Sparks, G.B., Rouse, G.L., Brown, W.S., Steltzer, H., 1996. Intraguild predation and cannibalism among larvae of detritivorous caddisflies in subalpine wetlands. *Ecology* 77, 2421–2430.
- Witman, J.D., Smith, F., Novak, M., 2017. Experimental demonstration of a trophic cascade in the Galápagos rocky subtidal: Effects of consumer identity and behavior. *PLoS One* 12, e0175705.
- Yurewicz, K.L., 2004. A growth/mortality trade-off in larval salamanders and the coexistence of intraguild predators and prey. *Oecologia* 138, 102–111.