

# Asymmetric density-dependent competition and predation between larval salamanders

Thomas L. Anderson<sup>1,2</sup>  | Jacob J. Burkhardt<sup>1</sup>  | Jon M. Davenport<sup>1</sup> 

<sup>1</sup>Department of Biology, Appalachian State University, Boone, NC, U.S.A.

<sup>2</sup>Department of Biology, Southern Illinois University Edwardsville, Edwardsville, IL, U.S.A.

## Correspondence

Thomas L. Anderson, Department of Biology, Southern Illinois University Edwardsville, Edwardsville, IL, U.S.A.  
Email: thander@siue.edu

## Abstract

1. Body size differences among consumers often lead to asymmetric interactions, with larger individuals typically being stronger competitors and/or predators on small individuals. These types of interaction are particularly exemplified in freshwater pond communities, where substantial size variation exists both within and among species of top consumers. We investigated whether density dependence can modify the outcome of size-structured interactions between larval stages of two pond-breeding salamanders, *Ambystoma annulatum* and *Ambystoma opacum*. Size structure exists in populations of these species due to variation in the timing of breeding, which we hypothesised would amplify predation rates and competitive asymmetries from the early-arriving species (*A. annulatum*) on the later-arriving species (*A. opacum*).
2. We manipulated the relative densities of both *A. annulatum* and *A. opacum* in outdoor mesocosms. We maintained the experiment through metamorphosis, and analysed size at metamorphosis, larval period length and survival of each species.
3. *Ambystoma annulatum* imparted a strong density-dependent effect on *A. opacum* through a combination of predation and competition. Survival of *A. opacum* was negatively related to the density of *A. annulatum*. For the *A. opacum* that survived, body size was reduced and larval period lengthened at higher *A. annulatum* densities, indicative of interspecific competition that was partly explained by resource pre-emption. In contrast, *A. annulatum* was only affected by intraspecific density-dependent competition.
4. Our results suggest that density-dependent effects reinforce asymmetric interactions among larval salamanders. However, the intensity of the asymmetric interactions is mediated by the arrival time and size of conspecifics. Specifically, earlier-arriving species can negatively affect the later-arriving species via size-mediated predation and competition.
5. The interactive effects of density dependence and arrival time of community members are probably a common mechanism generating size variability in ecological communities. Yet, most studies only evaluate one mechanism or the other. By interweaving these two processes, our work displays the importance of understanding context-dependence in species interactions.

## KEYWORDS

amphibian, body size, ontogeny, phenology, pond

## 1 | INTRODUCTION

Body size often plays a critical role in structuring populations and communities in freshwater ecosystems (Woodward & Warren, 2007), in part because it can influence species interaction strengths. For instance, large individuals are typically stronger competitors than smaller individuals because of their greater motility in obtaining resources and ability to generate stronger interference effects (Persson, 1985; Schoener, 1983), leading to asymmetric intra- and interspecific competition. Additionally, increasing size differences between predators and prey greatly impacts predation rates and survival of prey (Kalinkat et al., 2013). Body size variability can also lead to changes in food web stability (Emmerson & Raffaelli, 2004; Woodward et al., 2005), and trophic structure (e.g. trophic cascades; DeLong et al., 2015).

Two factors that can generate variability in body size among individuals in aquatic communities are differences in ontogeny and population density. Many aquatic organisms exhibit ontogenetic variability in their timing of entry into aquatic communities (Wilbur, 1980). For example, many species exhibit biphasic life cycles with terrestrial adults and aquatic larval stages, with the timing of adult breeding differing between either individuals of the same species (i.e. the degree of synchrony) or different species (e.g. priority effects). Larval stages of early-arriving individuals can then grow to attain larger sizes by the time later-arriving individuals enter a community, resulting in asymmetric interaction strengths, i.e. affecting the persistence or life history (e.g. growth rates) of the relatively smaller offspring of later-arriving individuals (Rasmussen et al., 2014). In addition, population density can affect body size through intra- or interspecific competition, slowing growth rates and increasing size variability. This effect has been repeatedly demonstrated in numerous freshwater taxa (Wilbur, 1997; Wissinger, 1989). Population density is especially important when both predators and prey are simultaneously growing, as it can limit when species reach size refuges by limiting growth of either the predator or prey (Anders, 2001; Wilbur, 1988). Ontogeny and population density can also affect species interactions via their joint impacts on body size. For instance, the size advantage gained by early arrivers is dependent on suitable growing conditions (Alford & Wilbur, 1985; Wilbur & Alford, 1985). If there is strong negative density dependence in early arrivers, individual growth opportunities may be limited, reducing size asymmetries. For predatory species, this could ultimately shift interactions from predation to competition when body size differences are minimised (Boone et al., 2002; Yang & Rudolf, 2010). Alternatively, if species breed in synchrony but exhibit different growth rates (Griffiths et al., 1994), then population densities may be more critical. Additionally, high densities of later-arriving species from synchronised breeding efforts may satiate early-arriving predators, increasing prey survival relative to when they are at lower densities (Ims, 1990). Higher individual growth rates for later-arriving species may also reverse predator and prey roles once they surpass size thresholds necessary for consuming the early-arriving species (Rasmussen et al., 2014). Overall, there is a complex interplay between body size, population

density, and ontogeny that can be difficult to discern *in situ*, requiring manipulation in experimental studies.

The importance of ontogeny and density in size-dependent interactions are particularly well exemplified in pond-breeding amphibians. Differences in arrival times to breeding sites, both within and among species, permits offspring of early-arriving adults to hatch before subsequent cohorts arrive, which allows early larvae to attain body sizes large enough to outcompete or prey upon later-arriving individuals (Alford, 1989; Alford & Wilbur, 1985; Anderson et al., 2017; Anderson & Semlitsch, 2014; Boone et al., 2002; Lawler & Morin, 1993; Segev & Blaustein, 2007; Wilbur & Alford, 1985). However, because individual growth rates are often density-dependent (Anderson & Whiteman, 2015; Scott, 1990; Semlitsch & Caldwell, 1982) and species-specific (Griffiths et al., 1994), the size disparities between species could be altered depending on the relative densities and identities of each species. For carnivorous taxa (e.g. larval salamanders), such differences are key to determining predation rates (Anderson et al., 2016), and ultimately whether species predominantly interact as competitors or predator/prey (Boone et al., 2002; Van Buskirk, 2007; Griffiths et al., 1994).

The goal of this study was to determine how density dependence influenced the outcome of size-structured species interactions. Specifically, we tested whether the effects of early-arriving ringed salamanders (*Ambystoma annulatum*) on demographic traits of individuals (e.g. body size) and survival of later-arriving marbled salamanders (*Ambystoma opacum*) were dependent on each species' density. Our focal taxa share many similar life history traits, including a complex life cycle where adults breed in the autumn, larvae overwinter in ponds prior to undergoing metamorphosis in late spring to early summer (Hassinger et al., 1970; Semlitsch et al., 2014), and are often found in sympatry in semi-permanent to permanent ponds in the Ozark and Ouachita Mountains (Anderson et al., 2021; Ousterhout et al., 2015; Shaffer, 2010). Larval densities are highly variable among ponds (Ousterhout et al., 2015), as are body size distributions (Anderson et al., 2016). They also generally have overlapping breeding phenologies, with both species having the potential to breed before the other, although their breeding phenology patterns have not been thoroughly explored when occurring in sympatry. Thus, this system is well-suited for understanding how ontogeny and density impact size structure and ultimately species interactions.

Because *A. annulatum* was added before *A. opacum*, we expected them to act as predator and prey, respectively. However, we expected that the density of each species may influence the interaction strength and type (i.e. predation vs. competition). As such, we predicted that when *A. annulatum* arrives early at low abundances, reduced intraspecific competition would lead to larger body sizes and thus higher predation rates on *A. opacum*. In contrast, when *A. annulatum* arrives early but at high abundances, intense intraspecific competition would lead to less disparities in size with *A. opacum*, shifting the interaction type towards competition, with reduced predatory impacts. This effect would be more pronounced with low densities of *A. opacum*, as they would have higher growth potential. Additionally, when *A. opacum* occurred at high abundances, predator

swamping effects may occur (Ims, 1990), especially when *A. annulatum* were at low abundances.

## 2 | METHODS

### 2.1 | Experimental design

We tested for density-dependent effects from larval *A. annulatum* on *A. opacum* using a response surface design in outdoor mesocosms (Inouye, 2001). We used 16 different combinations of larval abundances, some of which were replicated while others were not (Figure S1). Starting at an abundance of six larvae per tank for each species, we increased in increments of six larvae for each species so that all combinations of numbers between six and 24 were used. We had three replicates of the corners of the response surface (*A. annulatum*:*A. opacum*; 6:6, 6:24, 24:6, 24:24) whereas inner treatments (6:12, 6:18, 12:6, 12:12, 12:18, 12:24, 18:6, 18:12, 18:18, 18:24, 24:12, 24:18) were not replicated. The lack of replication for some treatments does not hinder inference because we relied on regression approaches (Cottingham et al., 2005; Inouye, 2001). We also had two treatments that only contained *A. opacum* to control for the minimum ( $n = 12$ ) and maximum ( $n = 48$ ) overall number of salamanders in each mesocosm. These densities fall within the observed densities in natural ponds for each species (Ousterhout et al., 2015). We randomly assigned treatments to mesocosms within the array.

### 2.2 | Experimental setup

The study was conducted in 28 outdoor mesocosms (1,000-L cattle watering tanks; hereafter, tanks) set up at the University of Missouri from September 2018 to June 2019. We filled tanks with water on 15 September 2018 and let tanks sit for 2 days to allow chlorine to evaporate. We then added 1.5 kg of dry leaf litter (primarily *Quercus* sp., *Carya* sp., and *Platanoides occidentalis*) on 17 September. We added c. 1 L of concentrated pond water to each tank to initiate plankton communities. We drilled drain holes in the top lip of each tank to limit water overflowing the top of the tanks during rain events. Tanks remained uncovered to permit natural colonisation by prey insects (e.g. Diptera). While this could have allowed for predatory invertebrates to also colonise the tanks, we did not observe any in the tanks, probably because the experiment took place primarily over the winter months.

We collected c. 30 partial clutches of *A. annulatum* on 20 September 2018 from a pond at Fort Leonard Wood, Missouri, U.S.A. We brought the eggs back to the lab and let them hatch in plastic containers, which was fully completed by 26 September. We haphazardly caught hatchlings and placed them in individual containers, and then added them to their assigned tanks on 27 September. We collected seven nests of *A. opacum* from several ponds at Fort Leonard Wood on 18 October. We placed the nests on moist soil

substrate inside plastic containers. We then flooded the containers with water on 21 October. After the eggs hatched, we again haphazardly caught individuals, added them to individual containers, and then added them to their assigned tanks on 26 October. Thus, there was a 29-day difference in addition dates between *A. annulatum* and *A. opacum*, matching the relative timing of breeding in the year we conducted this experiment. The natural phenology that year did not permit a test of adding *A. opacum* first, or both species at the same time to gauge priority effects. All hatchlings were visually similar in size, although we did not measure them.

We captured larvae from each tank in mid-March 2019 to assess the strength of density dependence midway through the experiment. We dorsally photographed individuals over a ruler to obtain total length (TL), snout-vent length (SVL), and head width measurements, using ImageJ (Rasband, 1997). All three measures were highly correlated ( $r > 0.95$ ); thus, we used TL which had the fewest missing values. While our goal was to catch at least three individuals of each species from each tank to calculate an average size, we were sometimes unsuccessful due to individuals being inactive or not alive, especially *A. opacum* (i.e. *A. opacum* had already been consumed by *A. annulatum*). Thus, we only compared larval body size differences across density treatments for *A. annulatum*, as well as larval size across species.

We took zooplankton samples from each mesocosm on 3 November 2018 to determine if *A. annulatum* had reduced prey availability by the time *A. opacum* arrived into the community (i.e. the effect of exploitative competition). We took two integrated samples from the water column along the wall of each tank in a random cardinal direction, and filtered the samples through an 80- $\mu$ m net. We preserved zooplankton in ethanol, and then subsampled to count approximately 50% of the volume of each sample. We categorised and counted zooplankton as cladocerans, adult copepods, juvenile copepods, and rotifers under a dissecting scope.

We began checking each tank for metamorphosing animals in early May 2019, which corresponded to the timing of metamorphosis in previous experiments (Anderson et al., 2017, 2020; Anderson & Semlitsch, 2014). We removed individuals that had reabsorbed their gills, and recorded their mass (g) and date of metamorphosis. We again dorsally photographed individuals over a ruler to obtain SVL and TL measurements using ImageJ (Rasband, 1997). We approximated SVL by measuring to the distal junction of the tail and hind legs. We only report results for SVL as it was highly correlated with TL ( $r = 0.97$ ).

Beginning the second week of June 2019, we drained all tanks and carefully sifted through the leaf litter to find any remaining individuals; only one larval *A. annulatum* was captured during the deconstruction process, meaning nearly all surviving individuals had metamorphosed. We determined survival of each species by counting the total number of individuals (both larvae and metamorphs) that had been recovered alive. During the larval sampling (March 2019), we discovered four tanks where larvae ( $n = 1$  in three tanks, and  $n = 4$  in one tank) had died due to stranding on the upper lip of the tank when the drain holes became clogged. Based on relative

size, these individuals were likely to be *A. opacum*, so we subtracted these numbers from the initial number of *A. opacum* in those tanks when analysing survival.

## 2.3 | Analysis

Because our primary objective was to assess interspecific interactions, we first analysed the density combinations containing both species (i.e. excluding the tanks containing only *A. opacum*). We used multiple linear regression to analyse four response variables for each species: SVL, mass, larval period length, and survival. The two predictors in each model were the initial abundance of *A. annulatum* and *A. opacum*. We also tried including quadratic terms of each species' density to account for non-linear effects, but they were all non-significant so we do not report them here. We averaged SVL, mass, and larval period over all individuals that survived in each tank. In cases where only one individual survived ( $n = 6$  tanks for *A. opacum*), we used that one data point. For survival, we used different model structures for each species. For *A. opacum*, we used a generalised linear mixed model with a binomial error distribution and an individual level random effect to correct for overdispersion. We compared this model to a generalised linear model with a quasibinomial errors, but they qualitatively had the same result, so we only report the mixed model. We used a generalised linear model with binomial errors for *A. annulatum*, as no evidence of overdispersion was present. We assessed significance by generating bootstrap confidence intervals ( $n = 1,000$  samples). Because both predictors have the same units, the regression coefficients can be compared as approximate effect sizes on each response. To tease apart overall density effects versus species identity effects on *A. opacum*, we conducted a separate set of ANOVAs comparing their responses in tanks with total densities of 12 (6 *A. annulatum*: 6 *A. opacum* vs. 0:12) and 48 salamanders (24:24 vs. 0:48). We analysed the same response variables of SVL, mass, larval period length, and survival.

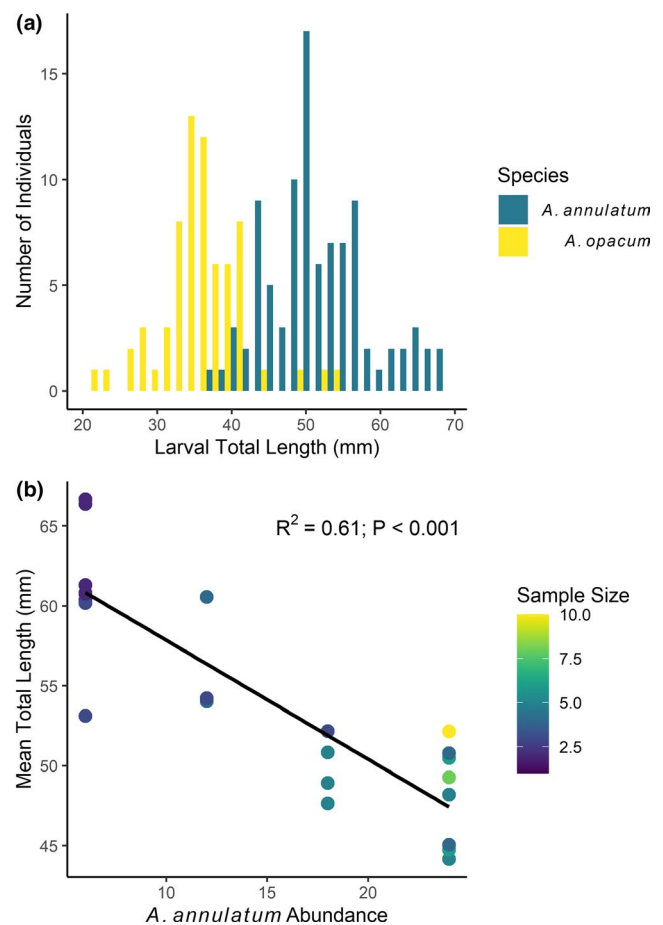
We analysed differences in larval TL between species using ANOVA. We examined the effects of density dependence on TL in larval *A. annulatum* using linear regression. Because of the potential for low sample sizes influencing mean values for each tank, we reran the analysis on a subset of data where we caught at least three larval *A. annulatum* per tank, with the results being qualitatively similar; therefore, we report only the results using all the data.

We used linear regression to test for effects of initial *A. annulatum* abundance on prey resource (zooplankton) levels at the time *A. opacum* was added to tanks. We analysed groups separately (adult cladocerans, adult copepods or juvenile copepods) and total zooplankton abundance. We did not analyse rotifers as they occurred too infrequently. We log or  $\log(x + 1)$  transformed each group to improve the normality of model residuals. All analyses were conducted in R (R Core Team, 2020).

## 3 | RESULTS

### 3.1 | Larval size

On average, TL in mid-March of larval *A. annulatum* was 51.9 mm and 35.5 for *A. opacum*, with the difference being statistically significant ( $F_{1,39} = 101.00$ ,  $p < 0.001$ ; adj.  $r^2 = 0.71$ ). The range of sizes for each species were nearly completely non-overlapping, with only a few *A. opacum* being as large as *A. annulatum*, primarily from the 0:12 treatment (Figure 1a). Larval body size of *A. annulatum* was negatively related to intraspecific density ( $p < 0.001$ ; adj.  $r^2 = 0.61$ ), with *A. annulatum* being about 22% (12 mm) longer at the lowest densities ( $n = 6$ ) compared the highest densities ( $n = 24$ ; Figure 1b).



**FIGURE 1** Larval size (total length, in mm) of *Ambystoma annulatum* and *Ambystoma opacum* caught in mid-March 2019 across all density combinations (a), and the average total length of larval *A. annulatum* in relation to the initial number of *A. annulatum* (b). Samples sizes for larval measurements in (a) were  $n = 94$  for *A. annulatum* and  $n = 68$  for *A. opacum*. Based on the average total length for each species (*A. annulatum* = 51.9; *A. opacum* = 35.5), *A. annulatum* had higher average growth rates, 0.31 mm/day, than *A. opacum*, 0.26 mm/day. Colour scale in (b) represents the sample size per tank over which the average total length was taken

### 3.2 | *Ambystoma annulatum*

Overall, *A. annulatum* was affected by intraspecific density, and unaffected by *A. opacum* density (Table 1). Average SVL at metamorphosis was negatively related to *A. annulatum* abundance (Table 1, adj.  $r^2 = 0.53$ ) with SVL decreasing 0.23 mm for each increase in *A. annulatum* abundance (Figure 2a). Similarly, mass at metamorphosis for *A. annulatum* was negatively affected by conspecific abundance (Table 1; adj.  $r^2 = 0.43$ ), with mass declining 0.036 g for each increase in *A. annulatum* abundance (Figure 2b). Larval period length was positively related to conspecific abundance (Table 1, adj.  $r^2 = 0.62$ ; Figure 2c). Larval period was lengthened by 0.60 days for each increase in conspecific abundance. Survival of *A. annulatum* was unrelated to either conspecific or *A. opacum* abundance (Table 1; Figure 2d).

### 3.3 | *Ambystoma opacum*

Intra- and interspecific effects both contributed to explaining the responses of *A. opacum* (Table 2). The SVL at metamorphosis for *A. opacum* decreased with increasing abundances of *A. annulatum*, and was unrelated to conspecific abundance (Table 2; adj.  $r^2 = 0.23$ ). SVL decreased 0.17 mm for each increase in *A. annulatum* abundance (Figure 3a). Mass at metamorphosis for *A. opacum* was negatively affected by both conspecifics and *A. annulatum* abundances, with the effect of *A. annulatum* being slightly greater (Table 2; adj.  $r^2 = 0.36$ ). *Ambystoma opacum* mass decreased approximately 0.026 and 0.019 g for each increase in abundance of *A. annulatum* and *A. opacum*, respectively (Figure 3b). Larval period length of *A. opacum* was positively affected by the abundance of *A. annulatum* (Table 2; adj.  $r^2 = 0.19$ ). Larval periods increased by 0.28 days for each increase in *A. annulatum* abundance (Figure 3c). Across all

densities, the larval period length of *A. opacum* was also on average 26 days faster than *A. annulatum*, showing that *A. opacum* overcame the 29-day difference in addition dates to metamorphose at roughly the same time as *A. annulatum*. Survival of *A. opacum* was only affected by the number of *A. annulatum* (Table 2; Figure 3d). The odds of survival decreased by 55% with each increase in the number of *A. annulatum* (Table 2).

Comparisons of tanks that controlled for overall low and high abundances (comparisons of 6:6 – 0:12 and 24:24–0:48, respectively) showed our results were driven by *A. annulatum* density (Figure S2). At low overall abundances, *A. opacum* survival was 100% in two of the three replicates of the 6:6 treatment, and 100% in each of the two replicates of the 0:12 treatment. Survival was 0% in the third replicate of 6:6 treatment, although the reason is unclear; the statistical results are similar whether we include or exclude this data point. At high overall abundances, survival of *A. opacum* was nearly zero with *A. annulatum* present, whereas survival was nearly 100% without them. Thus, our observed low survival of *A. opacum* with high abundances of *A. annulatum* is not simply due to numeric effects. Body size and larval period length in these same comparisons respond primarily to total abundance and not in response to whether *A. annulatum* was present (Figure S2).

### 3.4 | Prey resources

Total zooplankton density was negatively related to the abundance of *A. annulatum* ( $p = 0.06$ ; adj.  $r^2 = 0.13$ ; Figure S3), indicating some evidence of exploitative competition. Examining individual prey species revealed this relationship was primarily driven by the relationship between *A. annulatum* and cladocerans ( $p = 0.02$ ; adj.  $r^2 = 0.17$ ; Figure S3). Adult copepods and juvenile copepods were not related the abundance of *A. annulatum* (Figure S3).

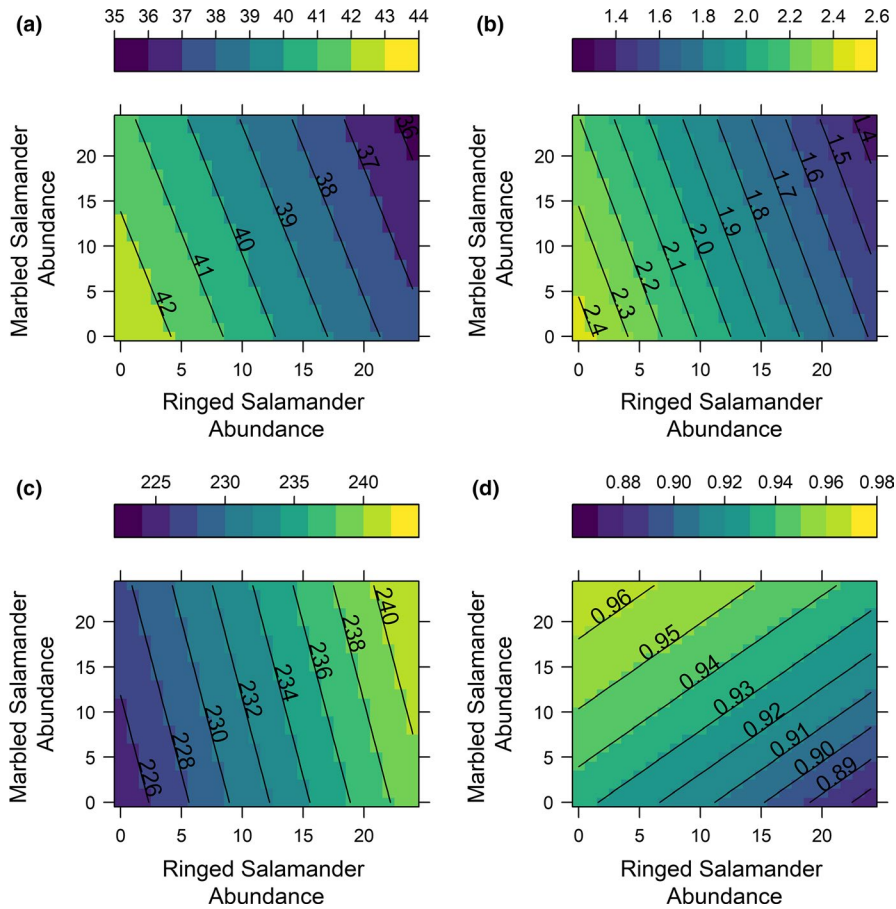
**TABLE 1** Parameter estimates and bootstrapped confidence intervals (CIs) for regression models of *Ambystoma annulatum* responses

Response	Coefficient	Estimate	Lower CI	Upper CI
SVL	Intercept	42.967	40.851	45.082
	<i>A. annulatum</i>	<b>-0.233</b>	<b>-0.330</b>	<b>-0.136</b>
	<i>Ambystoma opacum</i>	-0.070	-0.165	0.025
Mass	Intercept	2.444	2.063	2.824
	<i>A. annulatum</i>	<b>-0.036</b>	<b>-0.053</b>	<b>-0.018</b>
	<i>A. opacum</i>	-0.010	-0.027	0.007
Larval period length	Intercept	224.602	220.073	229.131
	<i>A. annulatum</i>	<b>0.604</b>	<b>0.396</b>	<b>0.812</b>
	<i>A. opacum</i>	0.118	-0.085	0.321
Survival	Intercept	2.632	1.249	4.266
	<i>A. annulatum</i>	-0.029	-0.099	0.033
	<i>A. opacum</i>	0.030	-0.021	0.083

Note: All models are general linear models, except survival which is a generalised linear model with a binomial error distribution. The coefficients for survival are log-odds ratio estimates. Bolded values indicate where the CIs do not overlap zero.

Abbreviation: SVL, snout-vent length.





**FIGURE 2** Predicted relationships for (a) snout-vent length, (b) mass, (c) larval period length, and (d) percent survival of *Ambystoma annulatum*. The x-axis shows the effect of *A. annulatum* (intraspecific effects), and the y-axis shows the effect of *Ambystoma opacum* (interspecific effects). Numbers indicate predicted values for a given isocline. Vertical isoclines indicate only intraspecific effects, horizontal isoclines only interspecific effects and diagonal (45 degree) lines would indicate intra- and interspecific effects are equal. Darker colour shading indicates smaller body lengths and masses, reduced days to metamorphosis, and lower survival. Legends above each panel show the range of values for each response. Only *A. annulatum* abundance was a significant predictor for snout-vent length, mass and larval period; survival was unaffected by either species

Response	Coefficient	Estimate	Lower CI	Upper CI
SVL	Intercept	36.323	32.985	39.661
	<i>Ambystoma annulatum</i>	<b>-0.175</b>	<b>-0.336</b>	<b>-0.013</b>
	<i>A. opacum</i>	-0.077	-0.227	0.073
Mass	Intercept	1.931	1.476	2.385
	<i>A. annulatum</i>	<b>-0.026</b>	<b>-0.048</b>	<b>-0.004</b>
	<i>A. opacum</i>	-0.019	-0.040	0.001
Larval period length	Intercept	204.483	199.019	209.947
	<i>A. annulatum</i>	<b>0.281</b>	<b>0.017</b>	<b>0.545</b>
	<i>A. opacum</i>	0.054	-0.192	0.300
Survival	Intercept	3.004	0.621	6.082
	<i>A. annulatum</i>	<b>-0.234</b>	<b>-0.397</b>	<b>-0.121</b>
	<i>A. opacum</i>	-0.101	-0.232	0.019

**TABLE 2** Parameter estimates and bootstrapped confidence intervals (CIs) for regression models of *Ambystoma opacum* responses

Note: All models are general linear models, except survival which is a generalised linear mixed model with a binomial error distribution. The coefficients for survival are log-odds ratio estimates. Bolded values indicate where the CIs do not overlap zero.

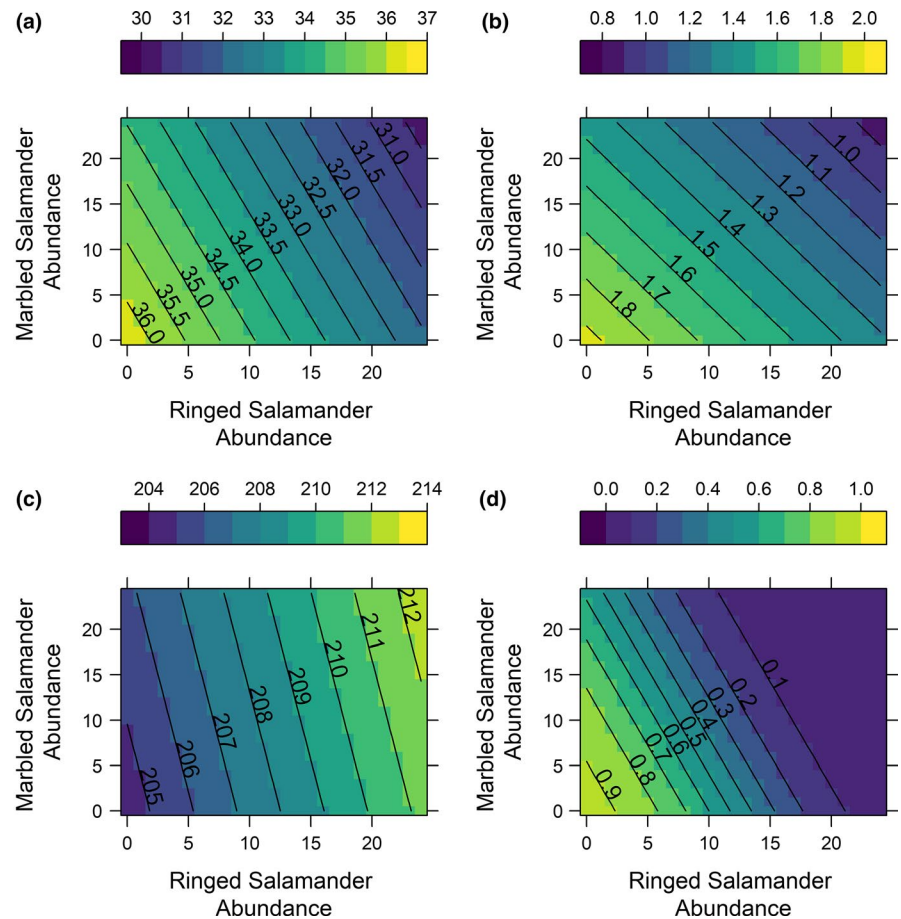
Abbreviation: SVL, snout-vent length.

## 4 | DISCUSSION

Larger body size often confers advantages in competitive and predator-prey interactions (Kalinkat et al., 2013; Persson, 1985; Schoener, 1983). Developing a better understanding of the underlying mechanisms, such as variation in population density and ontogenetic timing, that

produce size variation is therefore critical. We experimentally tested how population densities influence size advantages gained through ontogenetic differences by manipulating the relative abundances of two larval salamanders that were introduced a month apart into pond communities. As expected, we found strong density-dependent effects of the early arriver, *A. annulatum*, on the late-arriving species,

**FIGURE 3** Predicted relationships for (a) snout-vent length (SVL), (b) mass, (c) larval period length, and (d) percent survival of *Ambystoma opacum*. Only *Ambystoma annulatum* abundance was a significant predictor for SVL, larval period length, and survival. Numbers, colours, and isoclines are as in Figure 2. Only *A. annulatum* abundance was a significant predictor for SVL, mass and larval period; survival was unaffected by either species. Mass was affected relatively equally by both *A. annulatum* and *A. opacum*



*A. opacum*, that were from a combination of competition and predation, depending on the demographic trait. Further, the competitive effects are at least partially attributable to density-dependent resource limitation imposed by *A. annulatum*. Finally, species interactions were highly asymmetric, where *A. annulatum* was unaffected by *A. opacum* and instead was only limited by intraspecific density. As this system operates through a combination of competition and predation, it could be classified as intraguild predation (Polis et al., 1989), although we discuss below when and how these interactions may shift. Overall, we show strong support for the hypothesis that the magnitude of size-dependent species interactions can be determined through asymmetric effects of population densities.

The primary factor driving the results of our study was the density of *A. annulatum*. This species arrived early into the experimental communities, which we hypothesised would allow it to act as a predator and superior competitor of *A. opacum* due to the size advantages gained from early arrival. Correspondingly, increasing densities of *A. annulatum* resulted in higher mortality of *A. opacum*. Additionally, for individual *A. opacum* that did survive, we observed strong density-dependent responses for body size and development, indicative of interspecific competition from *A. annulatum*: metamorphs emerged smaller and took longer to develop at higher densities of *A. annulatum* compared to *A. opacum*. Because these life history traits are correlated with adult fitness metrics (Scott, 1994; Semlitsch et al., 1988), the effects of *A. annulatum*

would be expected to translate into population-level effects on *A. opacum*.

The density-dependent interactions we observed were highly asymmetric, as prey density (*A. opacum*) largely did not affect many of the responses of either species. In particular, high prey density did not result in swamping effects on the predator that would have increased their survival (Ims, 1990). It is possible that our prey densities were not large enough to satiate the number of *A. annulatum* present in each tank, although they did match densities found in natural pond communities (Ousterhout et al., 2015). Additionally, the larger *A. annulatum* may have consumed enough *A. opacum* to eliminate any density-dependent effects on either themselves or *A. opacum* in the mesocosms. Evidence from natural ponds indicates that the density of *A. opacum* does matter for their interactions, as it was the best predictor of larval *A. annulatum* size (Anderson et al., 2021), although this information was collected in the absence of any information on breeding phenology patterns. As both species can breed earlier than the other, it could be that density dependence only matters for the earliest-arriving species, which would subsequently affect their individual growth rates and predation rates on later-arriving species. We were not able to manipulate additions of *A. opacum* before *A. annulatum* or concurrent arrival due to the natural phenology in the year of the study. However, we suspect that if *A. opacum* were to enter ephemeral ponds first, they would impart equivalent density-dependent mortality upon *A. annulatum*,

given their similarity as predators on other amphibians (Anderson et al., 2016; Stemp, 2017).

We hypothesised that high densities of early-arriving species would limit individual growth rates, minimise their size advantage, and result in a switch in interaction type from predation to competition. While we found a strong density-dependent effect, this did not result in a change of interaction type. Our study used densities that fell within those observed in natural populations (Ousterhout et al., 2015), although it could be that the densities of *A. annulatum* we used were still not high enough to reduce their ability to prey on *A. opacum*. Thus, only under extreme density dependence may such a shift in interaction type occur. The duration of the phenological timing differences could also play a role. In years where species breed more synchronously, density dependence may be more likely to cause a switch in interaction type. Anderson et al., (2017) also found a lack of switch in interaction type, although this work explored species that breed in different seasons (autumn vs. spring), which would be even less likely to observe a shift in interaction type. Because size-mediated interactions are common in univoltine organisms occupying seasonal aquatic habitats (e.g. larval stages of aquatic invertebrates and amphibians), further work should investigate the mechanisms that might result in switches in interaction type, although we emphasise that the outcomes we observed here are equally likely to occur in many freshwater taxa.

It should be noted that any density-dependent effects we postulate to have occurred assume that the densities in our response surface remained constant throughout the experiment. This was not true for *A. opacum*, however, as their survival decreased with increasing *A. annulatum* densities. *Ambystoma annulatum* survival was very high on average so their final densities were effectively the same as the initial densities. Therefore, our regression-based approach may have limited our ability to detect any density-dependent effects from *A. opacum*. Using the number of survivors of each species as a predictor variable resulted in similar conclusions to using the initial densities (results not shown), so this potential limitation probably does not alter our overall conclusions.

In some situations, predators can reduce intraspecific competition between prey individuals, resulting in high individual growth (e.g. thinning effects) (Anderson & Semlitsch, 2014; Van Buskirk & Yurewicz, 1998; Davenport & Chalcraft, 2012). We did not observe that here, probably because of the long temporal duration over which the predator and prey overlapped after prey individuals entered a size refuge, suggesting a long subsequent period of competitive interactions. Larval salamanders are often considered to be gape-limited as predators (Smith & Petranksa, 1987) and can grow fast enough in ponds to reach size refuges as prey (Urban, 2008). Thus, the temporal overlap as a consequence of ontogeny may mitigate or intensify thinning effects of predators.

While predation is the likely mechanism by which *A. annulatum* limited *A. opacum* persistence in experimental communities, we did find some evidence for exploitative competition for shared resources. Zooplankton densities at the time *A. opacum* entered the communities were negatively related to the abundance of *A. annulatum*, and

are the primary food of early larval stages of salamanders (Taylor et al., 1988). Other studies have similarly found resource preemption by early-arriving species in pond communities (Hernandez & Chalcraft, 2012; Segev & Blaustein, 2007), which can sometimes persist well beyond when the predator that initiated the prey reduction had left the community (i.e. legacy effect through metamorphosis) (Rudolf & Van Allen, 2017). We do not expect that *A. opacum* starved to death, and instead suspect such resource limitation slowed their growth or development rates, resulting in the lower average larval sizes and slower growth rates we observed midway through the experiment. This probably matches what would happen in natural communities, where limited food resources are unlikely to lead to starvation. Further support for this observation comes in our previous work on *A. annulatum* (Anderson et al., 2017), where resource depletion by *A. annulatum* did not result in mortality of their prey (*A. maculatum*), which was instead linked to predation. Because we did not perform behavioral observations, we cannot determine the role of interference in limiting access to food resources, although other studies have suggested only weak interference between our focal species (Stemp, 2017). Future research can combine behavioural observations of taxa with phenology manipulations to uncover the exact mechanisms by which species may exclude each other.

Understanding the mechanistic basis of ontogeny and variability in body size among species is imperative to determine how such factors contribute to structuring both populations and communities (Woodward et al., 2005; Woodward & Warren, 2007; Yang & Rudolf, 2010). Here we show that species densities can play a mediating role in the strength of interactions between species that vary in body size. At the highest densities, this resulted in a combination of predatory and competitive interactions that negatively impacted the smaller species, which in our case was due to phenological differences. Further studies that tease apart how other factors, such as temperature or food web structure, might also modulate the mechanisms of priority effects (e.g. habitat modification or resource preemption) are warranted. Studies of this sort are especially needed because many species' phenologies are shifting (Cohen et al., 2018; Parmesan, 2007) and phenological shifts are likely to impact how and which size classes interact (Yang & Rudolf, 2010), as well as species coexistence (Rudolf, 2019). Furthermore, not all species' phenologies are changing in the same way (e.g. different shifts in the shapes of phenological distributions; Carter et al., 2018), which could also impact density dependence and/or body size distributions and subsequent species interactions. Without baseline information on the mediating factors of body size variability and priority effects, it will be difficult to predict how historical contingency may change with phenological shifts or climate change more generally.

#### ACKNOWLEDGMENTS

We thank the Division of Biological Sciences, B. Sonderman, and M. Leal at the University of Missouri for support and access to facilities; K. Stemp and D. Burton for zooplankton collection and enumeration; and K. Stemp, J. Hardy, S. Sheth, A. Maddox, A. Knutson, A. Ward, and J. Kent for helping collect metamorphs. Animals were collected



with Missouri Department of Conservation approval (#17673) and experiments conducted with IACUC approval (Missouri: #9297; Appalachian State: #18-19).

#### DATA AVAILABILITY STATEMENT

Data is archived in the Zenodo digital repository (<http://doi.org/10.5281/zenodo.4661640>).

#### ORCID

Thomas L. Anderson  <https://orcid.org/0000-0001-7479-2192>

Jacob J. Burkhart  <https://orcid.org/0000-0002-7913-6220>

Jon M. Davenport  <https://orcid.org/0000-0002-9911-2779>

#### REFERENCES

- Alford, R. A. (1989). Variation in predator phenology affects predator performance and prey community composition. *Ecology*, *70*, 206–219. <https://doi.org/10.2307/1938427>
- Alford, R. A., & Wilbur, H. M. (1985). Priority effects in experimental pond communities: Competition between *Bufo* and *Rana*. *Ecology*, *66*, 1097–1105. <https://doi.org/10.2307/1939161>
- Anders, N. P. (2001). Predator behaviour and prey density: Evaluating density-dependent intraspecific interactions on predator functional responses. *Journal of Animal Ecology*, *70*, 14–19. <https://doi.org/10.1111/j.1365-2656.2001.00472.x>
- Anderson, T. L., Linares, C., Dodson, K., & Semlitsch, R. D. (2016). Variability in functional response curves among larval salamanders: Comparisons across species and size classes. *Canadian Journal of Zoology*, *94*, 23–30. <https://doi.org/10.1139/cjz-2015-0149>
- Anderson, T. L., Ousterhout, B. H., Rowland, F. E., Drake, D. L., Burkhart, J. J., & Peterman, W. E. (2021). Direct effects influence larval salamander size and density more than indirect effects. *Oecologia*, *195*(1), 173–186. <https://doi.org/10.1007/s00442-020-04820-8>
- Anderson, T. L., Rowland, F. E., & Semlitsch, R. D. (2017). Variation in phenology and density differentially affects predator-prey interactions between salamanders. *Oecologia*, *185*, 475–486. <https://doi.org/10.1007/s00442-017-3954-9>
- Anderson, T. L., & Semlitsch, R. D. (2014). High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations. *Population Ecology*, *56*, 265–273. <https://doi.org/10.1007/s10144-013-0419-9>
- Anderson, T. L., Stemp, K. M., Ousterhout, B. H., Burton, D. L., & Davenport, J. M. (2020). Impacts of phenological variability in a predatory larval salamander on pond food webs. *Journal of Zoology*, *310*, 95–105. <https://doi.org/10.1111/jzo.12733>
- Anderson, T. L., & Whiteman, H. H. (2015). Non-additive effects of intra- and interspecific competition between two larval salamanders. *Journal of Animal Ecology*, *84*, 765–772. <https://doi.org/10.1111/1365-2656.12335>
- Boone, M. D., Scott, D. E., & Niewiarowski, P. H. (2002). Effects of hatching time for larval ambystomatid salamanders. *Copeia*, *2002*, 511–517. [https://doi.org/10.1643/0045-8511\(2002\)0020511:EOHTFL2.O.CO;2](https://doi.org/10.1643/0045-8511(2002)0020511:EOHTFL2.O.CO;2)
- Carter, S. K., Saenz, D., & Rudolf, V. H. (2018). Shifts in phenological distributions reshape interaction potential in natural communities. *Ecology Letters*, *21*, 1143–1151. <https://doi.org/10.1111/ele.13081>
- Cohen, J. M., Lajeunesse, M. J., & Rohr, J. R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, *8*, 224. <https://doi.org/10.1038/s41558-018-0067-3>
- Cottingham, K. L., Lennon, J. T., & Brown, B. L. (2005). Knowing when to draw the line: Designing more informative ecological experiments. *Frontiers in Ecology and the Environment*, *3*, 145–152.
- 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. <https://doi.org/10.1111/j.1365-2656.2011.01906.x>
- DeLong, J. P., Gilbert, B., Shurin, J. B., Savage, V. M., Barton, B. T., Clements, C. F., ... O'Connor, M. I. (2015). The body size dependence of trophic cascades. *The American Naturalist*, *185*, 354–366. <https://doi.org/10.1086/679735>
- Emmerson, M. C., & Raffaelli, D. (2004). Predator-prey body size, interaction strength and the stability of a real food web. *Journal of Animal Ecology*, *73*, 399–409. <https://doi.org/10.1111/j.0021-8790.2004.00818.x>
- Griffiths, R. A., Wijer, P. D., & May, R. T. (1994). Predation and competition within an assemblage of larval newts (*Triturus*). *Ecography*, *17*, 176–181. <https://doi.org/10.1111/j.1600-0587.1994.tb00091.x>
- Hassinger, D. D., Anderson, J. D., & Dalrymple, G. H. (1970). The early life history and ecology of *Ambystoma tigrinum* and *Ambystoma opacum* in New Jersey. *American Midland Naturalist*, *84*(2), 474–495. <https://doi.org/10.2307/2423862>
- Hernandez, J. P., & Chalcraft, D. R. (2012). Synergistic effects of multiple mechanisms drive priority effects within a tadpole assemblage. *Oikos*, *121*, 259–267. <https://doi.org/10.1111/j.1600-0706.2011.19221.x>
- Ims, R. A. (1990). On the adaptive value of reproductive synchrony as a predator-swamping strategy. *The American Naturalist*, *136*, 485–498. <https://doi.org/10.1086/285109>
- Inouye, B. D. (2001). Response surface experimental designs for investigating interspecific competition. *Ecology*, *82*, 2696–2706.
- Kalinkat, G., Schneider, F. D., Digel, C., Guill, C., Rall, B. C., & Brose, U. (2013). Body masses, functional responses and predator-prey stability. *Ecology Letters*, *16*, 1126–1134. <https://doi.org/10.1111/ele.12147>
- Lawler, S. P., & Morin, P. J. (1993). Temporal overlap, competition, and priority effects in larval anurans. *Ecology*, *74*, 174–182. <https://doi.org/10.2307/1939512>
- Ousterhout, B. H., Anderson, T. L., Drake, D. L., Peterman, W. E., & Semlitsch, R. D. (2015). Habitat traits and species interactions differentially affect abundance and body size in pond-breeding amphibians. *Journal of Animal Ecology*, *84*, 914–924. <https://doi.org/10.1111/1365-2656.12344>
- Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, *13*, 1860–1872. <https://doi.org/10.1111/j.1365-2486.2007.01404.x>
- Persson, L. (1985). Asymmetrical competition: Are larger animals competitively superior?. *American Naturalist*, *126*, 261–266. <https://doi.org/10.1086/284413>
- Polis, G. A., Myers, C. A., & Holt, R. D. (1989). The ecology and evolution of intraguild predation: Potential competitors that eat each other. *Annual Review of Ecology and Systematics*, *20*, 297–330. <https://doi.org/10.1146/annurev.es.20.110189.001501>
- R Core Team (2020). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing.
- Rasband, W. S. (1997). *ImageJ*. US National Institutes of Health.
- Rasmussen, N. L., Van Allen, B. G., & Rudolf, V. H. W. (2014). Linking phenological shifts to species interactions through size-mediated priority effects. *Journal of Animal Ecology*, *83*, 1206–1215. <https://doi.org/10.1111/1365-2656.12203>
- Rudolf, V. H. W. (2019). The role of seasonal timing and phenological shifts for species coexistence. *Ecology Letters*, *22*, 1324–1338. <https://doi.org/10.1111/ele.13277>
- Rudolf, V. H. W., & Van Allen, B. G. (2017). Legacy effects of developmental stages determine the functional role of predators. *Nature Ecology & Evolution*, *1*, 38. <https://doi.org/10.1038/s41559-016-0038>
- Schoener, T. W. (1983). Field experiments on interspecific competition. *The American Naturalist*, *122*, 240–285.
- Scott, D. E. (1990). Effects of larval density in *Ambystoma opacum*: An experiment in large-scale field enclosures. *Ecology*, *71*, 296–306. <https://doi.org/10.2307/1940269>

- Scott, D. E. (1994). The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology*, *75*, 1383–1396. <https://doi.org/10.2307/1937462>
- Segev, O., & Blaustein, L. (2007). Priority effects of the early breeding fire salamander on the late breeding banded newt. *Hydrobiologia*, *583*, 275–283. <https://doi.org/10.1007/s10750-006-0565-6>
- Semlitsch, R. D., Anderson, T. L., Osbourn, M. S., & Ousterhout, B. H. (2014). Structure and dynamics of ringed salamander (*Ambystoma annulatum*) populations in Missouri. *Herpetologica*, *70*, 14–22. <https://doi.org/10.1655/HERPETOLOGICA-D-13-00074>
- Semlitsch, R. D., & Caldwell, J. P. (1982). Effects of density on growth, metamorphosis, and survivorship in tadpoles of *Scaphiopus holbrookii*. *Ecology*, *63*, 905–911. <https://doi.org/10.2307/1937230>
- Semlitsch, R. D., Scott, D. E., & Pechmann, J. H. (1988). Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology*, *69*, 184–192. <https://doi.org/10.2307/1943173>
- Shaffer, C. L. (2010). *Demographics and Terrestrial Ecology of an Amphibian Community in a Large Ephemeral Pond in the Ozark Mountains*. Arkansas State University.
- Smith, C. K., & Petranka, J. W. (1987). Prey size-distributions and size-specific foraging successes of *Ambystoma* larvae. *Oecologia*, *71*, 239–244. <https://doi.org/10.1007/BF00377290>
- Stemp, K. M. (2017). *Examining potential keystone effects of an endemic pond-breeding salamander*. Southeast Missouri State University.
- Taylor, B. E., Estes, R. A., Pechmann, J. H. K., & Semlitsch, R. D. (1988). Trophic relations in a temporary pond: Larval salamanders and their microinvertebrate prey. *Canadian Journal of Zoology*, *66*, 2191–2198. <https://doi.org/10.1139/z88-326>
- Urban, M. C. (2008). Salamander evolution across a latitudinal cline in gape-limited predation risk. *Oikos*, *117*, 1037–1049. <https://doi.org/10.1111/j.0030-1299.2008.16334.x>
- Van Buskirk, J. (2007). Body size, competitive interactions, and the local distribution of *Triturus* newts. *Journal of Animal Ecology*, *76*, 559–567. <https://doi.org/10.1111/j.1365-2656.2007.01218.x>
- Van Buskirk, J., & Yurewicz, K. L. (1998). Effects of predators on prey growth rate: Relative contributions of thinning and reduced activity. *Oikos*, *82*, 20–28. <https://doi.org/10.2307/3546913>
- Wilbur, H. M. (1980). Complex life cycles. *Annual Review of Ecology and Systematics*, *11*, 67–93. <https://doi.org/10.1146/annurev.es.11.110180.000435>
- Wilbur, H. M. (1988). Interactions between growing predators and growing prey. *Size-Structured Populations* (pp. 157–172). Springer.
- Wilbur, H. M. (1997). Experimental ecology of food webs: Complex systems in temporary ponds. *Ecology*, *78*, 2279–2302.
- Wilbur, H. M., & Alford, R. A. (1985). Priority effects in experimental pond communities: Responses of *Hyla* to *Bufo* and *Rana*. *Ecology*, *66*, 1106–1114. <https://doi.org/10.2307/1939162>
- Wissinger, S. A. (1989). Seasonal variation in the intensity of competition and predation among dragonfly larvae. *Ecology*, *70*, 1017–1027. <https://doi.org/10.2307/1941370>
- Woodward, G., Ebenman, B., Emmerson, M., Montoya, J. M., Olesen, J. M., Valido, A., & Warren, P. H. (2005). Body size in ecological networks. *Trends in Ecology & Evolution*, *20*, 402–409. <https://doi.org/10.1016/j.tree.2005.04.005>
- Woodward, G., & Warren, P. (2007). Body size and predatory interactions in freshwaters: Scaling from individuals to communities. *Body Size: The Structure and Function of Aquatic Ecosystems* (pp. 98–117). Cambridge University Press.
- Yang, L. H., & Rudolf, V. H. (2010). Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecology Letters*, *13*, 1–10. <https://doi.org/10.1111/j.1461-0248.2009.01402.x>

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Anderson TL, Burkhart JJ, Davenport JM. Asymmetric density-dependent competition and predation between larval salamanders. *Freshwater Biology*. 2021;00:1–10. <https://doi.org/10.1111/fwb.13721>