


Impacts of phenological variability in a predatory larval salamander on pond food webs

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Keywords

predation; body size; anuran; salamander; *Ambystoma*; food webs; community dynamics; phenology.

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Editor: Mark-Oliver Rödel

Received 15 January 2019; revised 2 August 2019; accepted 5 August 2019

doi:10.1111/jzo.12733

Abstract

Phenological shifts are expected to affect species interactions, in part by influencing which size classes, life stages or species occur simultaneously in a community. Yet, changes in phenology beyond shifts to the first, mean or median date of an ontogenetic event are underexplored in their importance to community dynamics. Using outdoor mesocosms, we experimentally mimicked increasing variability in breeding phenology of a top predatory salamander in pond food webs (*Ambystoma annulatum*) to assess its impacts on its own demographic traits and survival. We also tested whether variability in predator breeding would cascade to impact survival and diversity of lower trophic levels (intermediate salamander predators, anuran tadpoles, zooplankton and periphyton). We found that only variability in body size at metamorphosis of *A. annulatum* was impacted by phenological manipulations, with size variability being greater at higher levels of phenological variability. Because size at metamorphosis is often correlated with adult fitness, covarying variability in body size and phenology may lead to altered population dynamics. We also found that the density and size of *A. annulatum* were better predictors of overall survival and diversity of amphibian prey compared to phenological variability. We speculate that overwintering mortality of *A. annulatum* due to pond freezing modulated the impacts of phenological variability, such that changes in demographic traits and cascading effects throughout the food web were mollified.

Introduction

Changes in the timing of ontogenetic events, or phenological shifts, can affect both population and community dynamics (Miller-Rushing *et al.*, 2010; Yang & Rudolf, 2010; Nakazawa & Doi, 2012). Variation in these dynamics occurs in part when phenological shifts impact the outcome of species interactions through processes such as match–mismatch scenarios (Cushing, 1990; Durant *et al.*, 2007) or monopolization of resources by earlier arriving individuals or species, that is priority effects (Connell & Slatyer, 1977; Rasmussen, Van Allen & Rudolf, 2014; Murillo-Rincón *et al.*, 2017). Understanding the consequences of phenological shifts has become imperative, as the timing of key life history events of many taxa is changing worldwide in response to climate change (Parmesan, 2006). Yet, the effects of these phenological shifts for species interactions, and thus population and community dynamics, remain poorly understood.

The subtle details of how phenologies are changing are particularly critical to understanding how any shifts may impact species interactions. For example, numerous studies have documented that shifts in the phenological mean or median alter

the amount of temporal overlap, and thus interaction strength, between species (Morin, 1987; Alford, 1989; Lawler & Morin, 1993). More recent studies have shown that shifts in a single time point (e.g. the first or last date) alone do not accurately encapsulate how phenologies are changing, and that changes in variability or the shape of a phenological distribution are more important than shifts in the mean in population and community dynamics (CaraDonna, Iler & Inouye, 2014; Thackeray *et al.*, 2016; Carter, Saenz & Rudolf, 2018). Additionally, shifts in multiple aspects of phenology may not be mutually exclusive (CaraDonna, Iler & Inouye, 2014; Rasmussen & Rudolf, 2016). The impacts phenological shifts have on interaction strengths, and ultimately community dynamics, also likely depends on the trophic position of a species (e.g. top vs. intermediate predators). For instance, changes in the phenology of top predators, especially those that serve keystone roles, may disproportionately impact species interactions across trophic levels (Alford, 1989; Deacy *et al.*, 2017). Therefore, there is a need for a greater understanding of how different aspects of phenology, such as variability or skewness in phenology, impact populations and communities, especially for predators that exert strong top-down control on communities.

Amphibian assemblages in pond ecosystems are a model system to investigate phenological variability because of the prominent role phenology has in determining community dynamics. Adults rely on temperature and rainfall cues to initiate breeding migrations (Todd & Winne, 2006), but vary in their relative timing both within and between species. Thus, interaction strengths among larval stages of amphibians can strongly depend on species timing and arrival order to ponds (Alford & Wilbur, 1985; Lawler & Morin, 1993; Petranka & Thomas, 1995; Boone, Scott & Niewiarowski, 2002; Eitam, Blaustein & Mangel, 2005; Segev & Blaustein, 2007; Murillo-Rincón *et al.*, 2017). This occurs largely due to body size asymmetries that develop between early and later breeders: larger larvae from earlier breeding adults outcompete and prey on smaller larvae from later breeding adults of the same or different species. Furthermore, increasing variability in body size, a proxy for increasingly variable phenologies, can affect interspecific interactions (Rasmussen & Rudolf, 2016). As climate variability is expected to increase (Thornton *et al.*, 2014), breeding will potentially become more variable, in addition to shifts in mean or median date (Blaustein *et al.*, 2001, Todd *et al.*, 2011), and manifest as increased larval size structure. Finally, while some species can act as keystone predators of pond communities (Morin, 1995; Davenport & Chalcraft, 2012), whether shifts to their phenology impact their ability to act as keystones is unclear.

The goal of this study was to experimentally test how different scenarios of phenological variability of a top predator affect its own demographic traits and overall community structure. We hypothesized that greater variability in breeding phenology of the ringed salamander (*Ambystoma annulatum*, Cope 1886) would increase cannibalism rates among larvae due to greater body size variability (Nyman, Wilkinson & Hutcherson, 1993; Petranka & Thomas, 1995), and ultimately result in increased body size at metamorphosis, a correlate of future adult fitness (Semlitsch, Scott & Pechmann, 1988; Scott, 1994; Earl & Whiteman, 2015). Because *A. annulatum* are a top predator of spring-breeding amphibians (Anderson & Semlitsch, 2014; Anderson *et al.*, 2016a; Anderson, Rowland & Semlitsch, 2017), we also predicted that when more cannibalism occurred, the reduced number of salamander larvae would have less of an impact on the anuran community, resulting in greater amphibian diversity. Lastly, we expected that greater cannibalism in *A. annulatum* from increased breeding variability would cascade to other trophic levels, that is higher abundances of zooplankton due to decreased predation pressure and subsequently lower biomass of periphyton, the zooplankton and anuran food resource.

Materials and methods

Study system

Ambystoma annulatum exhibit a complex life cycle characteristic of most pond-breeding amphibians (Wilbur, 1980), with aquatic eggs and larval stages and terrestrial juvenile and adult stages (Petranka, 1998). Breeding occurs in the fall over a period of 6–8 weeks, sometimes resulting in size variability that

induces cannibalism (Nyman, Wilkinson & Hutcherson, 1993). Larvae overwinter in ponds, and metamorphose in the late spring and early summer (Hocking *et al.*, 2008; Semlitsch *et al.*, 2014). Thus, *A. annulatum* larvae are typically large enough to be a top predator on aquatic stages of spring-breeding amphibians (Anderson & Semlitsch, 2014; Anderson *et al.*, 2016a; Anderson, Rowland & Semlitsch, 2017). This is important because *A. annulatum* are sympatric with at least six anurans and two other caudates at our collection sites (Semlitsch *et al.*, 2015).

Experimental design

We conducted an outdoor mesocosm experiment on the campus of Southeast Missouri State University from fall 2017 to early summer 2018. We set up mesocosms following established protocols (Semlitsch & Boone, 2009), which we briefly describe here. We filled 1000 L cattle watering tanks in early October and added 150 mL of a water conditioner (Amquel Plus, Kordon) to remove chlorine and chloramine. We added 1.5 kg of dry leaf litter to provide structural complexity and a nutrient base, and several inoculations of pond water that contained zooplankton, a major dietary item of larval salamanders. Mesocosms were left open, but colonization of the mesocosms by predatory insects was rare due to the experiment occurring primarily over the winter months. Some dragonflies (Libellulidae) colonized mesocosms in mid-June during the last weeks of the experiment. However, it is unlikely that the dragonflies unduly impacted amphibian survival because colonization occurred after most salamanders and anurans had already metamorphosed.

We collected egg masses of *A. annulatum* from natural populations in Pulaski County, Missouri, USA in October 2017. We stored eggs in plastic containers filled with aged tap water and stored them in a refrigerator at ~6°C to delay hatching of later addition dates. We pulled containers out of the refrigerator approximately a week before their addition date to permit hatching, prior to being added to mesocosms on the dates outlined below. Because of logistical difficulties of delaying developing eggs across a 6-week period (see below), we did not equalize genetic diversity across all additions periods but did use multiple clutches (3–5) within each addition.

The experiment consisted of a single *A. annulatum* density ($N = 36$ per tank) in three phenological treatments, with six replicates per treatment: (1) single addition (all 36 hatchlings added on one date, 5-Nov), (2) pulsed addition (12 hatchlings added on three dates; 22-Oct, 5-Nov, 26-Nov) or (3) continuous addition ($N = 6$ hatchlings added on 6 dates; 22-Oct, 29-Oct, 5-Nov, 12-Nov, 19-Nov, 26-Nov). These treatments manipulate different aspects of breeding variability (e.g. the degree of synchrony), while holding the mean breeding date constant. The treatments were designed to induce different degrees of cannibalism, where the single addition should have the lowest rate, followed by the continuous addition date, and the pulsed date having the greatest potential for cannibalism due to more potential predators added on the first overall addition date with greater time between the next addition. The extent of the breeding we were able to manipulate spans the

breadth and temporal extent of breeding variability in natural populations (Hocking *et al.*, 2008; Semlitsch *et al.*, 2014).

We collected all other amphibian egg masses from Scott, Cape Girardeau and Wayne Counties, Missouri, USA during March and April 2018. After allowing hatching to occur in the laboratory, all mesocosms received tadpoles (based on availability) of four different species of anurans, with the timing of additions matching the natural phenological patterns in Missouri that year. We added 200 southern leopard frogs (*Rana sphenoccephala*) on 5-Mar, 200 chorus frogs (*Pseudacris feriarum*) on 11-Mar, 37 American toads (*Anaxyrus americanus*) on 7-Apr and 200 Cope's gray treefrogs (*Hyla chrysoscelis*) on 9-May. We also added 45 hatchling *Ambystoma* larvae on 3-Apr, including spotted salamanders (*A. maculatum*) and small-mouthed salamanders (*A. texanum*). We refer to these as *Ambystoma* prey in all results. Each mesocosm received a total of 682 prey items, and all densities match those observed in natural populations (Morin, 1983; Ousterhout *et al.*, 2015).

We obtained larval body size measurements of *A. annulatum* by capturing a subset of individuals using aquarium nets from each mesocosm on three dates during the experiment: 21-Mar, 9-Apr and 26-Apr. After capture, larvae were placed in a pan of water and photographed next to a ruler, from which we obtained head width (HW) and snout-vent length (SVL) measurements using ImageJ (Rasband 1997). We attempted to capture at least five individuals per mesocosm but in some cases fewer were captured (e.g. when water was murky) which may have hindered our ability detect larval body size differences. Average number of larvae caught per tank, and date was 3.9 larvae (21-Mar, range: 1–7), 4.5 (9-Apr, range: 1–6) and 4.9 (26-Apr, range: 3–7). We hereafter focus on larval tank-averaged HW because it was not always possible to assess SVL from dorsal photographs if back legs were absent. However, it should be noted that HW and SVL are highly correlated in *Ambystoma* larvae (Anderson *et al.*, 2016b).

We determined the effects of *A. annulatum* and other amphibians on lower trophic levels by estimating zooplankton abundance in mesocosms by sampling with a Nunez pipe sampler on 30-Apr. We took two samples from each mesocosm near the wall at two haphazardly decided compass directions. Water volume was noted, and samples were suspended in 70% ethanol before being reconstituted to a standard volume equivalent to the highest volume acquired in a sample. We estimated zooplankton abundance from a 4 mL subsample from each sample counted under a dissecting scope. To estimate periphyton abundance, we deployed a periphytometer (PVC with white flagging) into each mesocosm on 14-Nov and removed it on 4-May. A standardized length of the flagging (10 cm) was cut from the same proportion of each periphytometer and suspended in mesocosm water for transportation to the laboratory. Periphyton growth was scraped off the flagging into the water suspension and then filtered in the laboratory. Samples were analyzed at the analytical laboratory of Southern Illinois University at Carbondale.

In early May, we began monitoring tanks for individuals undergoing metamorphosis. Metamorphosis was indicated by reabsorption of external gills for salamanders and emergence of a forelimb for anurans. We immediately measured salamander

SVL using calipers and recorded their wet mass. Anurans were placed in containers in the laboratory to permit tail reabsorption, after which SVL and mass measurements were taken. We also recorded the day on which individuals were measured to calculate larval period lengths. We ended the experiment on 25-Jun, at which point the tanks were drained through a dipnet and the leaf litter thoroughly searched to recover any remaining individuals. We counted the total number of recovered animals per mesocosm per species to calculate survival.

Analysis

One potential outcome of increased breeding variability is greater variability in demographic traits such as body size, in addition to differences in mean values, as differences in cannibalism rates would alter the strength of density-dependent competition. Therefore, we analyzed *A. annulatum* response variables in two ways (1) the mean across all individuals in each mesocosm and (2) the coefficient of variation (CV) of all surviving individuals in each mesocosm. We analyzed metamorph SVL and mass with phenological treatment as the primary predictor using ANOVA in R (R Core Team 2018). We initially included the number of surviving *A. annulatum* as an additional covariate, as this number would represent the functional density of competitors in each mesocosm, but it was not significant and therefore not included in final analyses. We analyzed mean and CV of date of metamorphosis, but because of the different addition dates, true measures of larval period length would be inaccurate. Thus, these tests would determine if our treatments influenced whether animals converged on a similar date of metamorphosis or affected the degree of emigration synchrony. Survival of *A. annulatum* was analyzed using a generalized linear mixed model with binomial errors and an individual-level random effect to correct for overdispersion. We also analyzed changes in mean HW and CV of HW (i.e. the degree of size structure) over time for larval *A. annulatum* using a linear mixed effects model that had random intercepts and slopes for each tank, that is day of measurement and tank as random effects. Phenology treatment and date were the only fixed effects. When significant treatment differences were present in all the above analyses, we examined pairwise differences using Tukey *post hoc* tests in the 'emmeans' package in R (Lenth, 2018).

We analyzed mean SVL, mass, larval period length and survival of *R. sphenoccephala* and *Ambystoma*, and survival only for *P. feriarum*. Too few individuals of *A. americanus* and *H. chrysoscelis* survived for demographic trait models to converge or to conduct meaningful statistical tests. We included all five amphibian prey to calculate Shannon diversity index of amphibians for each tank. For each response, we initially included phenology treatment and the number of surviving *A. annulatum* as predictors. The latter term was again not significant for all response variables and thus dropped, except for survival of *Ambystoma*; we kept the number of *A. annulatum* in that model. For survival of *Ambystoma*, *R. sphenoccephala*, *P. feriarum* and amphibian diversity, we also included mean larval *A. annulatum* HW on 21-Mar, a proxy for predator size at the time prey were added.

We analyzed total zooplankton abundance and chlorophyll-a biomass, a proxy for periphyton biomass, using a general linear model with Gaussian errors, with phenology treatment as the only predictor. We natural log-transformed chlorophyll-a and natural log-x + 1 transformed zooplankton abundances to improve normality of the residuals. For zooplankton, we also explored whether results changed with use of a generalized linear mixed model with Poisson errors and an individual-level random effect to correct for overdispersion; qualitatively, the results were the same, so we only report the Gaussian model. We initially included the number of surviving *A. annulatum* as an additional covariate, the number of surviving *Ambystoma* (zooplankton model) or the total number of surviving anurans (chlorophyll-a model). None of these additional covariates explained a significant portion of the variability and thus were removed. All raw data and R code to conduct analyses are available in the Zenodo online repository upon acceptance, and at <http://github.com/anderstl> (Anderson *et al.*, 2019).

Results

Ambystoma annulatum

Overall, individual traits of larvae did not vary by phenology treatment (Fig. 1). Mean larval HW increased through time, but treatments only showed marginally different trajectories (interaction term of date and treatment: $P = 0.07$). Larvae in the continuous addition treatment started out as the smallest, while larvae in the pulsed addition were the largest. Over the next five weeks, all treatments converged to a similar body size (Fig. 1a). CV in larval HW did not vary by treatment ($P = 0.61$) and did not change through time ($P = 0.72$; Fig. 1b).

Similar to larvae, traits of metamorphic *A. annulatum* were largely comparable across treatments (Fig. 2). There was a significant treatment effect for CV in SVL at metamorphosis ($F_{2,14} = 4.49$, $P = 0.03$; adj. $R^2 = 0.30$), where individuals from the pulsed addition treatment had greater variability in SVL than those in the single addition treatment (Fig. 2b). There were no significant treatment differences for mean SVL ($F_{2,14} = 0.36$, $P = 0.70$; adj. $R^2 = -0.09$; Fig. 2a), mean mass ($F_{2,14} = 0.36$, $P = 0.58$; adj. $R^2 = -0.05$; Fig. 2c), CV of mass ($F_{2,14} = 0.37$, $P = 0.69$; adj. $R^2 = -0.08$; Fig. 2d), mean date of metamorphosis ($F_{2,14} = 0.83$, $P = 0.46$; adj. $R^2 = -0.02$; Fig. 2e) or CV of metamorphosis date ($F_{2,14} = 0.18$, $P = 0.83$; adj. $R^2 = -0.11$; Fig. 2f). Overall, we recovered 150 ringed salamander metamorphs across all treatments, resulting in an average survival of only 23% (range: 0–69%). Survival did not significantly vary among phenology treatments ($\chi^2 = 2.71$; $P = 0.26$; Fig. 2g).

Prey community

Total survival of all amphibian prey items significantly varied with phenology treatment ($\chi^2 = 9.08$; $P = 0.01$; Fig. 3a), the number of surviving *A. annulatum* ($\chi^2 = 20.39$; $P < 0.001$; Fig 3b), and through an interaction of treatment and the mean HW

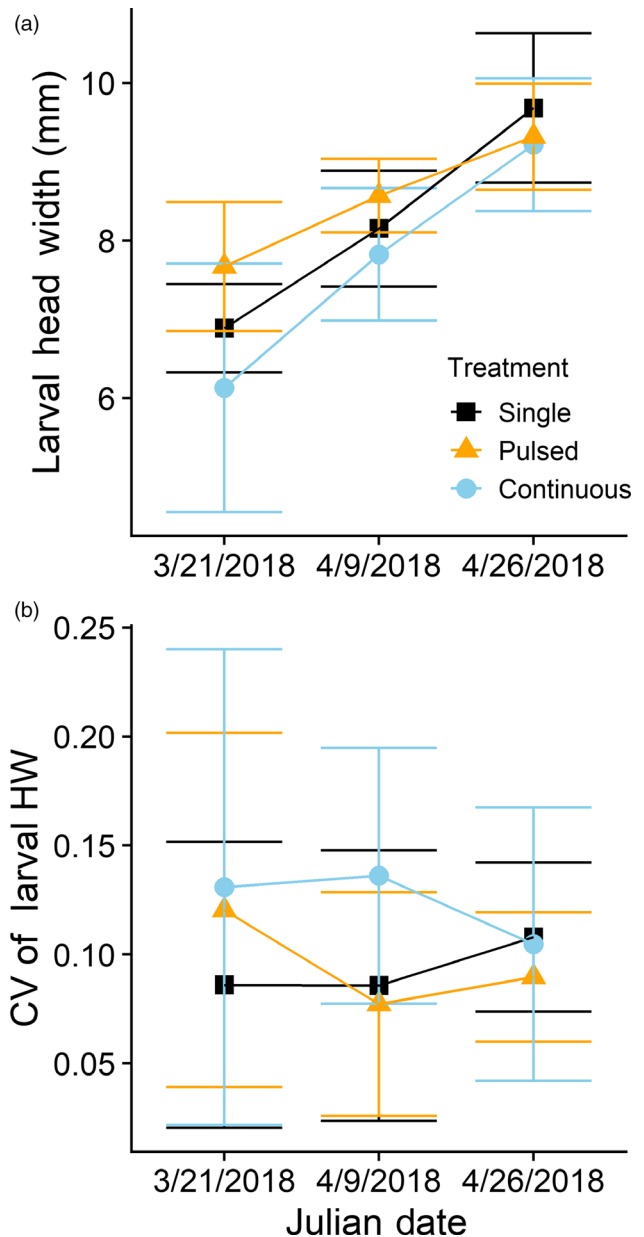


Figure 1 (a) Mean (\pm SD) larval head width (HW) and (b) variability (coefficient of variation, CV) in HW through time. Colors and symbols distinguish the different phenology treatments. No significant differences were observed between phenology treatments

of larval *A. annulatum* ($\chi^2 = 7.97$; $P = 0.02$; Fig. 3c). Survival declined with increasing numbers of *A. annulatum* (Fig. 3b). Total prey survival declined with increasing HW for all treatments, but the single addition treatment had the steepest decline (Fig. 3c). These patterns were robust to removing one potential outlier value for HW.

Survival rates of prey also varied by species (Figure 3d). *Ambystoma* had the highest overall survival at 25%, with individuals surviving in all tanks. Survival of *Ambystoma* was

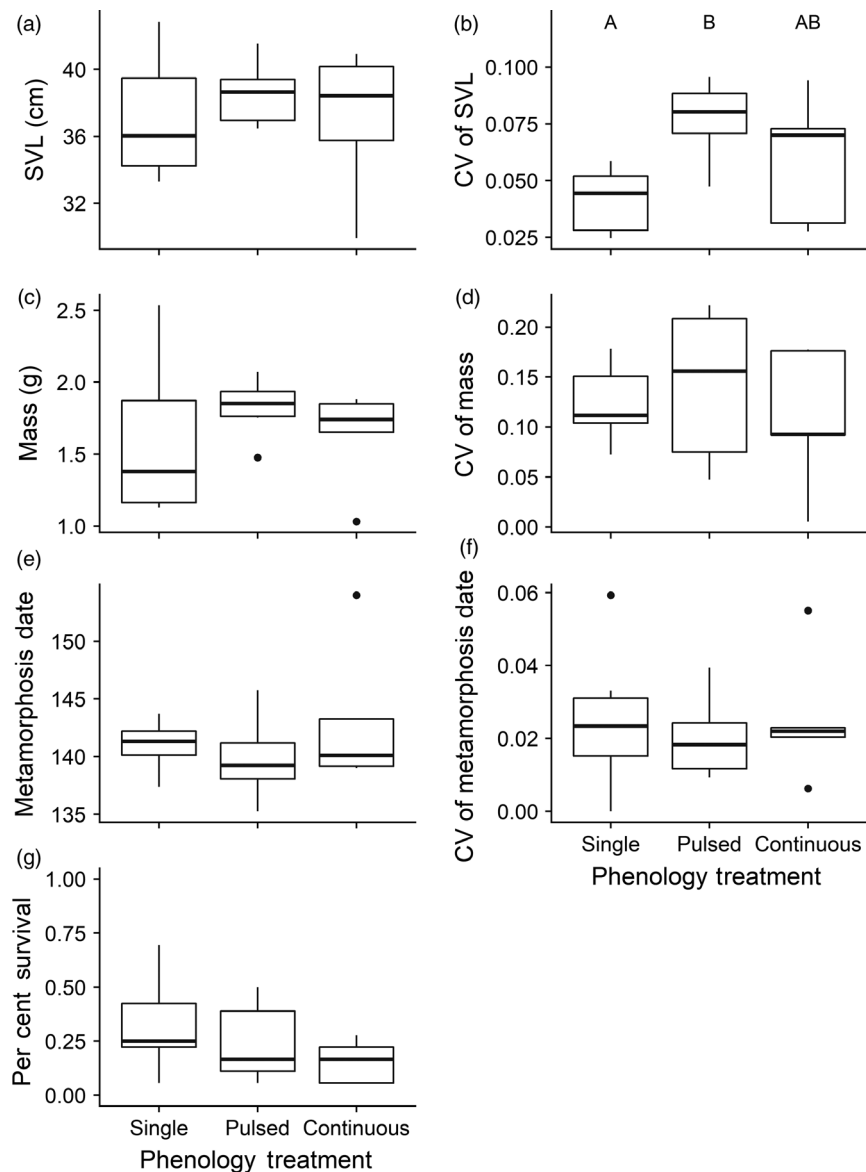


Figure 2 (a) Snout–vent length (SVL), (b) variability in SVL (measured as coefficient of variation, CV), (c) mass, (d) variability in mass, (e) date of metamorphosis (Julian), (f) variability in date of metamorphosis, and (g) per cent survival in *Ambystoma annulatum*. No treatment differences were significant except in (b), denoted by the letters

significantly predicted by *A. annulatum* survival ($\chi^2 = 18.19$, $P < 0.001$; Fig. 4a) and through an interaction of phenology treatment and larval HW ($\chi^2 = 6.82$, $P = 0.03$; Fig. 4b). *Ambystoma* survival declined with increases in both *A. annulatum* survival (Fig. 4a) and larval HW of *A. annulatum* (Fig. 4b). This latter effect was weakest in the continuous addition treatment (Fig. 4b). Survival of *R. sphenoccephala* and *P. feriarum* each averaged 2%, respectively but no metamorphs of either species were recovered in the pulsed addition treatment. Phenology treatment was not a significant predictor of survival for either species (comparisons between single and continuous additions only; *R. sphenoccephala*: $\chi^2 = 0.81$,

$P = 0.66$; *P. feriarum*: $\chi^2 = 0.32$, $P = 0.85$). Larval HW was a significant predictor of survival for *R. sphenoccephala* ($\chi^2 = 10.06$, $P = 0.002$), though we suggest caution with over-interpretation of this result, given the strong zero-inflation of these data (Appendix 1, Fig. A1). Only four *A.anaxyrus* (0.5%) and six *H. chrysoscelis* (0.1%) survived, each from only two tanks.

Prey diversity was not significantly predicted by phenology treatments (Fig 3e), but did show a significant negative relationship with larval *A. annulatum* HW (treatment: $F_{2,14} = 0.16$, $P = 0.85$; HW: $F_{1,14} = 7.29$, $P = 0.018$; adj. $R^2 = 0.34$; Fig 3f). After removing one potential outlier HW measurement,

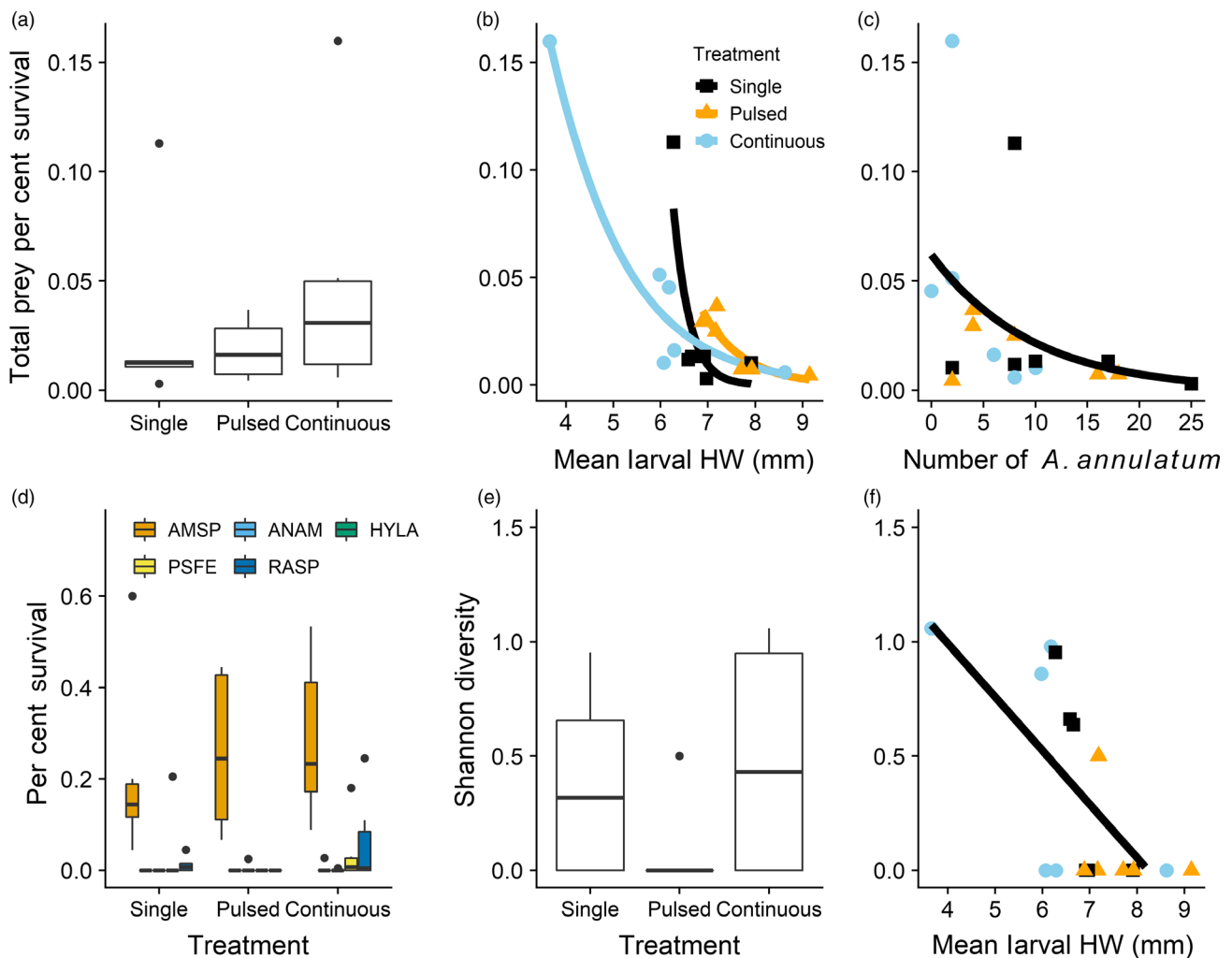


Figure 3 (a) Relationships of total prey survival to phenology treatments, (b) mean larval *Ambystoma annulatum* head width, and (c) number of surviving *A. annulatum*, (d) individual species survival by phenology treatment, and (e) Shannon diversity in relation to phenology treatments and (f) larval *A. annulatum* head width. In (b, c and f), symbols/colors correspond to the phenology treatment. Species codes in (d) are as indicated: AMSP = *Ambystoma*, ANAM = *A. americanus*, HYLA = *Hyla chrysoscelis*, PSFE = *Pseudacris feriarum*, and RASP = *Rana sphenoccephala*. Relationships were all significant ($P < 0.05$) in (b, c and f), with (c) and (f) remaining significant if the HW value < 4 was removed; (b) changed to $P = 0.07$

these patterns changed to a marginal relationship for diversity ($P = 0.07$; adj. $R^2 = 0.17$).

Phenology treatment was not a significant predictor of SVL or mass at metamorphosis for either *Ambystoma* (SVL: $F_{2,15} = 0.59$, $P = 0.57$; adj. $R^2 = -0.05$; mass: $F_{2,15} = 1.60$, $P = 0.23$; adj. $R^2 = 0.07$; Appendix 1, Fig. A2) or *R. sphenoccephala* (SVL: $F_{1,4} = 0.16$, $P = 0.85$; adj. $R^2 = -0.05$; mass: $F_{1,4} = 0.16$, $P = 0.85$; adj. $R^2 = -0.03$; Appendix 1, Fig. A3). Date of metamorphosis of *Ambystoma* was not significantly predicted by phenology treatment ($F_{2,15} = 0.23$, $P = 0.79$; adj. $R^2 = -0.10$; Appendix 1, Fig. A2), but varied significantly by treatment for *R. sphenoccephala* ($F_{1,4} = 17.15$, $P = 0.002$; adj. $R^2 = 0.90$). Metamorphosis emerged on average 8 days later in the continuous compared to single addition treatment (Appendix 1, Fig. A3).

Both periphyton biomass and zooplankton abundance were relatively similar across treatments (Appendix 1, Fig. A4). Periphyton biomass was not significantly affected by phenology treatment ($F_{2,15} = 0.57$, $P = 0.57$, adj. $R^2 = -0.05$). Similarly, total zooplankton did not significantly vary by treatment ($F_{2,14} = 1.28$; $P = 0.81$, adj. $R^2 = -0.11$).

Discussion

Phenological shifts are expected to alter species interactions, as well as population and community dynamics, in part by changing which size and stage classes co-occur in a given habitat (Yang & Rudolf, 2010). We manipulated phenological variability, an underexplored type of shift, that was expected to induce greater cannibalism rates in a salamander predator by creating

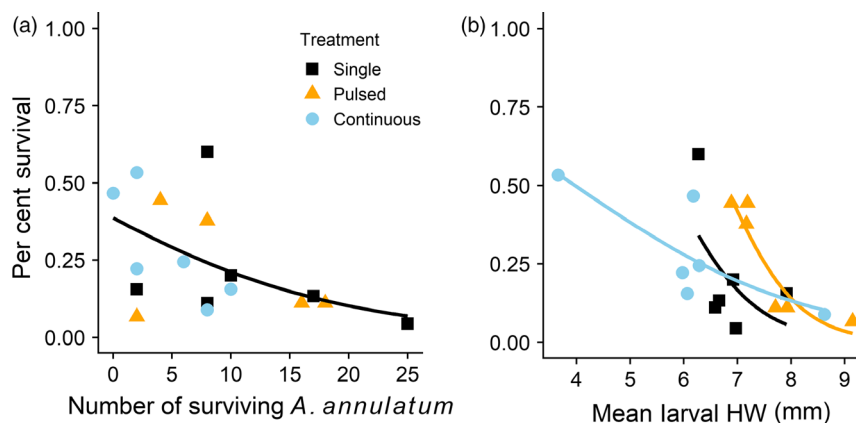


Figure 4 Survival of *Ambystoma* in relationship to (a) the number of surviving *A. annulatum* and (b) the mean larval *A. annulatum* head width (HW). Colors and symbols distinguish the different phenology treatments

greater body size asymmetries (Nyman, Wilkinson & Hutcherson, 1993; Walls *et al.*, 1993; Wissinger *et al.*, 2010). Changes to the survival rates of the top predators would then have cascading effects on the structure of the co-occurring amphibian and plankton prey communities (Morin, 1981; Alford, 1989; Morin, 1995). However, these expectations were only weakly supported: we failed to detect an effect of our phenology manipulations on survival of our top predator, *A. annulatum*, or most of its traits (e.g. SVL or mass). The only significant effect was increasing variability in SVL at metamorphosis when individuals were added over three addition dates. For the prey community, we similarly found that survival and diversity for the most part varied independently of our phenology manipulations and instead responded only to *A. annulatum* size or density (anurans) or did not vary at all with any covariate we tested (plankton).

One of the hypothesized mechanisms that induce body size asymmetries is variability in the timing of breeding, which could occur between species (fall- vs. spring-breeding species) (Urban, 2007) or within a species (multiple breeding cohorts) (Petranka & Thomas, 1995; Murillo-Rincón *et al.*, 2017). We found partial support for this hypothesis. Metamorphic *A. annulatum* displayed greater variability in SVL at metamorphosis when added on three dates as compared to one date. At the same time, we did not observe any effects on metamorphosis mass, or in larval body size leading up to metamorphosis, though CV of larval HW did tend to be higher in the pulsed addition treatment (Fig. 1b). As SVL at metamorphosis is associated with adult fitness (Semlitsch, Scott & Pechmann, 1988; Earl & Whiteman, 2015), increasing variability in phenology could potentially lead to altered population dynamics as fewer individuals would have mean fitness and more would be in the distributional tails (but no shift in the overall mean). Reconciling how increasing phenological variability can differentially impact different demographic traits warrants further investigation.

Asymmetries in body size often permit intraguild predation and/or cannibalism to occur among larval salamanders (Yurewicz, 2004; Wissinger *et al.*, 2010; Anderson *et al.*, 2013;

Anderson & Semlitsch, 2014), with phenological differences being one of the mechanism that produces such size variation (Boone, Scott & Niewiarowski, 2002). Yet, we failed to detect an effect of our phenology treatments on survival, similar to other studies that manipulated size structure (Doyle & Whiteman, 2008; Asquith & Vonesh, 2012). Our treatments spanned a 6-week time period, mimicking realistic variability in breeding and a length that matches the current temporal extent of breeding in *A. annulatum* (Hocking *et al.*, 2008; Semlitsch *et al.*, 2014). However, average or variability in larval size was not different between treatments at any time point we measured, indicating our phenology treatments did not induce substantial differences in larval size structure. Thus, a wider disparity in breeding times (>6 weeks) or overlapping cohorts (e.g. via pedomorphosis, Wissinger *et al.*, 2010) may be required to see an increase in size structure and cannibalism rates. However, our results were somewhat confounded by very low and atypical *A. annulatum* survival (Fig. 2e). We speculate the reason for their low survival was prolonged low winter temperatures and tank freezing. Larval *A. annulatum* overwinter in ponds that in natural systems can result in anoxia and death due to pond freezing, as has been documented in other fall-breeding *Ambystoma* (Herstoff & Urban, 2014), and thus our results could represent a natural biologically plausible scenario. Our experimental mesocosms were placed on top of a concrete pad that likely remained colder than other surfaces; as such, tanks froze nearly solid, resulting in the relatively low survival across all treatments, as compared to other studies on *A. annulatum* at a different location that had >70% survival (Anderson & Semlitsch, 2014; Ousterhout & Semlitsch, 2016; Anderson, Rowland & Semlitsch, 2017). We cannot confirm winter stress as the cause of low survival, as we would have needed tanks unaffected by winter conditions to confirm this hypothesis, which was not logistically possible; winter stress equally affected tanks across all treatments and thus should have not produced biased results for certain treatments. Additionally, we were unable to track cohorts or individuals in the pulsed and continuous addition treatments to know which hatchling addition(s) contributed most to the survivors. Nonetheless, this

outcome provides support for the hypotheses that sensitivity to climatological factors may modulate phenological effects in natural systems (Anderson, Rowland & Semlitsch, 2017, Inouye *et al.*, 2019).

The largest impacts on total anuran survival and survival of *Ambystoma* were from the number and size of *A. annulatum*. Such effects from larvae of fall-breeding salamanders have been reported previously in pond systems (Urban, 2007; Anderson & Semlitsch, 2014; Anderson, Rowland & Semlitsch, 2017). In particular, larval body size of *A. annulatum* influences their functional response parameters when consuming amphibian prey, where larger larvae have greater foraging rates with reduced handling times (Anderson *et al.*, 2016a). In support of this previous result, we observed higher survival of *Ambystoma* and total amphibian prey with smaller *A. annulatum*. Larval HW of *A. annulatum* was also negatively related to amphibian prey diversity: diversity was lower with larger *A. annulatum*. As body size is often used as a proxy for the timing of breeding (e.g. Rasmussen & Rudolf, 2015; Rasmussen & Rudolf, 2016), the differences in *A. annulatum* HW could also implicate the importance of phenology in this system. When the timing of breeding is shifted earlier for spring-breeding species (i.e. the prey), or later for *A. annulatum*, the relative size difference would then be diminished, potentially releasing prey from greater mortality and increasing diversity. However, previous experimental work suggests this shift would have to be rather large (several weeks) to observe a strong effect, especially when *A. annulatum* can exhibit compensatory growth (Anderson, Rowland & Semlitsch, 2017).

Phenological variability can influence the amount of temporal overlap between predators and prey in a habitat and is a critical factor in determining prey survival (Tiitsaar, Kaasik & Teder, 2013; Carter, Saenz & Rudolf, 2018). Our results indicate that overlap plays only a partial role in our system. Despite having comparable periods of overlap to *R. sphenoccephala*, *P. feriarum* and *B. americanus*, *Ambystoma* had substantially higher survival. This may be due to functional differences in swimming abilities or behavior of hatchling anurans versus caudates (Hoff *et al.*, 1989), the time when they are most vulnerable to gape-limited predators. Our study also shows that overlap can also be very short for impacts to occur. Based on average dates of metamorphosis for *A. annulatum*, they overlapped with *H. chrysoseleis* tadpoles for between 3–44 days, with an average overlap of 21 days and had nearly 100% mortality. While this length of time could be sufficient for *A. annulatum* to be the causal agent of mortality in *H. chrysoseleis*, it is also possible that *Ambystoma* larvae, which had 4 weeks of growth before *H. chrysoseleis* were added, were abundant and large enough to consume them as well, which our study cannot differentiate. In support of this hypothesis, no *H. chrysoseleis* survived in a separate experiment conducted at the same time and had the same food web structure except that *A. annulatum* was absent. Thus, considering both intermediate and top predators in food webs may help explain variability in interactions across trophic levels (Davenport & Chalcraft, 2012).

Cascading effects of phenological shifts across trophic levels have rarely been documented (Deacy *et al.*, 2017),

though they are likely to occur if pairwise interactions change in response to such shifts. We found that our phenology treatments had little impact on periphyton biomass and zooplankton densities. These patterns are consistent with the absence of a strong response in *A. annulatum* to our phenological manipulations: we anticipated greater breeding variability to induce cannibalism and thus lower survival in the pulsed and continuous addition treatments. When this did not occur, densities did not vary across treatments to induce a density-dependent trophic cascade (i.e. a positive and negative relationship with survival of *A. annulatum* for periphyton and zooplankton, respectively). As plankton population abundances fluctuate through time, our single time point may also have been insufficient to detect an effect. Alternatively, top-down effects and trophic cascades can be transient processes (Piovia-Scott, Yang & Wright, 2017; Rowland, Rawlings & Semlitsch, 2017), and our sampling time point may not have coincided with when the greatest impact of salamanders occurred, though it was right before metamorphosis when *A. annulatum* larvae would have been the largest in size. In any case, further tests of trophic cascades and altered trophic interactions beyond pairwise tests will likely improve our ability to predict the community-level consequences of phenological shifts.

Phenological shifts are expected to influence myriad processes in both populations and communities (Miller-Rushing *et al.*, 2010; Yang & Rudolf, 2010; Nakazawa & Doi, 2012). While we failed to detect strong effects of increasing variability in phenology on population and community dynamics, our results underscore the need to gauge the relative importance of phenology to other processes. In our case, we suspect but cannot confirm climatic conditions impacted top predator survival, negating any effects that our experimental phenological shifts may have imparted on both the predator or lower trophic levels. Yet, such an effect potentially represents a realistic scenario which could impact dynamics of ponds and many other communities. Species that display high levels of phenological variation might have more tempered changes in species interactions and population sizes because of environmental filtering. Further tests of how phenology and climate sensitivities covary will be very informative to understand their impacts on population and community dynamics (Rudolf & Singh, 2013, Inouye *et al.*, 2019). Because the phenologies of many species are changing (Parmesan, 2006), studies such as ours that assess how different contemporary phenological patterns (e.g. pulsed vs. continuous breeding) impacts species can provide important benchmarks against which future phenological shifts can be compared (Visser & Both, 2005). Future experiments that manipulate phenology beyond contemporary patterns can help unravel how changes may unfold. We also found that combinations of predator density and body size had strong impacts on pond ecosystems, similar to results shown in other studies (Segev & Blaustein, 2007; Urban, 2007; Anderson & Semlitsch, 2014). Further investigation of phenological variability and other aspects of phenological change will help unravel when and where such shifts are important to population and community dynamics.

Acknowledgements

We thank K. O'Donnell, S. Walls, T. Gorman and C. Haas for providing insightful discussions related to this project, T. Hastings C. Cannon, D. Kirsch, T. Simmonds, T. Recker, and B. McLaughlin for assisting with collecting metamorphs, L. Suchniak for assistance with counting zooplankton, M. Brooks at SIU-Carbondale for periphyton analysis and J. Burkhart for providing comments on previous versions of this manuscript. This work was supported by SERDP RC-2703 and conducted with Missouri Department of Conservation and SEMO-IACUC approval.

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Supporting Information

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

Figure S1. Survival of *Rana sphenoccephala* in relationship to mean larval *A. annulatum* head width (HW).

Figure S2. Snout-vent length (SVL), mass, date of metamorphosis and survival of *Ambystoma*.

Figure S3. Snout-vent length (SVL), mass, date of metamorphosis and survival of *Rana sphenoccephala*.

Figure S4. Phenology treatments differences in chlorophyll-a biomass (A) and zooplankton abundance (B) on 4-May and 30-Apr, respectively.