

# Hydroperiod Impacts on the Fall-Breeding Ambystomatid Salamanders *Ambystoma annulatum* and *Ambystoma opacum*

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**Wetland hydroperiod affects the ecology and evolution of numerous freshwater organisms. Pond-breeding amphibians are particularly affected by hydroperiod, with their life histories and distributional patterns often resulting from how long ponds remain inundated. Substantial variability exists among species in their responsiveness to fluctuations in hydroperiod, making taxon-specific investigations needed to understand its general impacts. We tested whether two fall-breeding salamanders, *Ambystoma annulatum* and *A. opacum*, each of which have long (> 6 month) larval period durations, respond to variation in hydroperiod length. We manipulated hydroperiod in outdoor experimental mesocosms under three scenarios (short hydroperiod, medium hydroperiod, and constant water levels), focusing specifically on how hydroperiod affected their life history traits (survival, size, and time to metamorphosis). We found that in the shortened hydroperiod treatments, few individuals of either species completed metamorphosis and would have died, whereas nearly all surviving individuals underwent metamorphosis under longer hydroperiod treatments. After correcting for differences in survival, body size at metamorphosis was greater in constant hydroperiod treatments for only *A. annulatum*. Larval periods were on average longer in constant hydroperiods for both species. The relationship between size and time to metamorphosis was positive for both *A. annulatum* and *A. opacum*, with limited differences between hydroperiod treatments in this relationship for either species. Overall, these results indicate these fall-breeding species are indeed affected by hydroperiod variation, but in different ways, depending on the life history response. These diverse responses highlight the complexities of building generalized responses of amphibians to a shared stressor like hydroperiod variation, as taxon- and trait-specific responses appear to be common. Future work should consider other aspects of hydroperiod as a structuring abiotic force (e.g., onset of drying), or the life history traits of amphibians (e.g., their breeding phenology), to better understand the ecology and evolution of pond-breeding amphibians, as well as provide insights into management actions for their conservation.**

**H**YDROPERIOD, or the duration of time a body of water remains inundated, is one of the most critical abiotic gradients for many freshwater organisms, and has shaped their distribution, ecology, and evolution (Schneider and Frost, 1996; Wellborn et al., 1996; Babbitt, 2005). Water bodies that are inundated for short time periods often contain organisms with adaptations that allow them to persist in an ephemeral habitat, such as high individual growth rates and/or short development times (Newman, 1989). Rapidly drying water bodies exclude species who cannot complete their life cycle within this short duration (Snodgrass et al., 2000; Werner et al., 2009; Semlitsch et al., 2015). In contrast, bodies of water that are permanently inundated can contain species that require aquatic conditions to persist, such as fish or some amphibians. Additionally, some odonates (Corbet, 1999) and amphibians (Scott, 1990; Cook et al., 2013; Johnson et al., 2013) that have longer development times exploit ponds that are inundated for longer periods of time over more ephemeral habitats to ensure a stable environment of sufficient duration for development of their larval stages. Some aquatic organisms also demonstrate high levels of plasticity during development, such that they can accelerate growth and development under drying conditions, allowing them to use water bodies that span the gradient of hydroperiod duration (Johansson et al., 2001). In some cases, however, this phenotypic plasticity is still insufficient to overcome rapidly drying ponds (Amburgey et al., 2016).

Understanding how organisms respond to variation in hydroperiod is important because one of the major

projected impacts of climate change is altered hydrological cycles. In particular, climate change may alter the length of time that water bodies remain inundated and thus the availability of bodies of water with suitable hydroperiods (Brooks, 2009; Walls et al., 2013; Lee et al., 2015). For organisms adapted to specific hydroperiod conditions, this may pose a substantial threat to their persistence in many areas, though the exact nature of how organisms will respond remains unclear for many taxa. Highly plastic species may have little difficulty persisting in the face of new abiotic constraints (Denver, 1997). Other species that are less plastic in their development may be extirpated from certain locations (Cook et al., 2013). Plasticity in development rates may also affect other demographic traits, such as body size or reaction norms of age and size at metamorphosis (Skelly, 1996; Reques and Tejedo, 1997; Gomez-Mestre et al., 2013), leading to fitness impacts. Thus, it is important to determine how freshwater organisms will respond to hydroperiod changes to determine which species may be most at risk.

While it is generally known that larvae of many pond-breeding amphibians can readily mount developmental responses to variation in hydroperiod, the bulk of this work comes from anurans (e.g., Wilbur, 1987; Reques and Tejedo, 1997; Ryan and Winne, 2001; Boone and Semlitsch, 2002; Rudolf and Rödel, 2007; Richter-Boix et al., 2011; Gomez-Mestre et al., 2013). The response of pond-breeding salamanders to hydroperiod variation is less well understood, with only a few species having been tested experimentally. Of these, some species respond developmentally to reductions

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in hydroperiod including *Ambystoma talpoideum* (Semlitsch and Wilbur, 1988), *A. texanum* (Ryan, 2007), *A. t. melanostictum* (McMenamin and Hadly, 2010), and *A. californiense* (Johnson et al., 2013; Searcy et al., 2015), while other species, including *A. tigrinum mavortium* (Johnson et al., 2013) do not respond. This species disparity is noteworthy because *A. t. mavortium* is adapted to long hydroperiod wetlands (Ghioca and Smith, 2008), suggesting a lack of plasticity in development rate. In some cases, there are also trade-offs among amphibians when development is accelerated, namely that individuals undergo metamorphosis at a smaller size (Phillips et al., 2002; Morey and Reznick, 2004). This is important because size at metamorphosis is correlated with future fitness (Semlitsch et al., 1988; Earl and Whiteman, 2015), meaning hydroperiod variation that induces trade-offs in size and development rate could have a substantial effect on population dynamics.

Ringed (*Ambystoma annulatum*) and Marbled (*A. opacum*) Salamanders are two species of pond-breeding salamanders that present a unique example for studying the impacts of altered hydroperiod and life history traits. Both of these species breed in the fall in semi-permanent wetlands (Petranka and Petranka, 1981; Urban, 2007; Ousterhout et al., 2015; Anderson et al., 2015). Their larvae then overwinter in ponds, prior to undergoing metamorphosis the following spring (Petranka and Petranka, 1980; Peterson et al., 1991; Timm et al., 2007; Semlitsch et al., 2014). This life history strategy leads to these species having substantially longer larval development times than most other pond-breeding amphibians, with both requiring 6+ months in most cases. Because of this, these organisms require water bodies that are inundated for similar lengths of time. This is often much longer than water bodies used by many spring-breeding species, which have much shorter development times (e.g., 2–4 months for *Ambystoma maculatum* or *A. texanum* [Petranka, 1998; Ryan, 2007]). Whether this life history difference (i.e., fall-breeding and larvae that overwinter in longer hydroperiod wetlands) has altered their evolution, in terms of their plasticity in development rates in response to hydroperiod variation, is unclear. Additionally, because *A. opacum* require inundation of their terrestrially laid eggs (Petranka and Petranka, 1981), their evolution may have been shaped more by hydroperiod than *A. annulatum*. It is also unknown whether trade-offs in age and size at metamorphosis exist for these taxa in response to hydroperiod variation. Understanding this balance is important for these taxa because at least *A. annulatum* has shown an opposite pattern to other salamanders (e.g., Semlitsch et al., 1988; Semlitsch and Anderson, 2016), where size and age at metamorphosis are negatively correlated (Semlitsch et al., 2014), though at least one other ambystomatid also shows this pattern (Searcy et al., 2016). As such, hydroperiod may affect the size–age relationship differently than for other amphibians.

In this study, we experimentally manipulated the hydroperiod that *A. annulatum* and *A. opacum* experienced during larval development to examine the degree to which they could respond developmentally to changes in water level. We monitored both species for development time, survival, and size at metamorphosis to understand the potential fitness impacts of varying hydroperiods (Earl and Whiteman, 2015). Because of their need for ponds with longer periods of inundation, we expected that both species would have limited capacity to respond to a quickly drying pond, in

terms of individuals successfully completing metamorphosis. We also expected that *A. opacum* would be better suited to accelerate growth and development in a slowly drying pond because of their life history adaptations to a fluctuating hydroperiod environment (Petranka and Petranka, 1981). Thus, we expected stronger trade-offs in *A. opacum* in terms of size and time to metamorphosis in the different hydroperiod treatments than *A. annulatum*.

## MATERIALS AND METHODS

We set up 16 1000 L cattle tank mesocosms (hereafter, tanks) in a fenced-in research area at Southern Illinois University Edwardsville (SIUE) in September 2021. Each tank was filled with tap water and allowed to age and dechlorinate for several days. We added approximately 1.25 kg of dried leaves (mainly *Populus tremuloides*) collected in Madison County, Illinois, to create a nutrient base. Tanks were inoculated with 250 mL of zooplankton collected from natural ponds near the SIUE campus approximately twice a week from 5 October to 11 November 2021 for total of 11 zooplankton additions. Tanks were covered with 70% shade cloth lids to exclude predatory insects. We recorded water temperature by placing HOBO Pendant loggers (Onset) in six tanks, with each logger recording temperature every four hours for the duration of the experiment (Fig. S1; see Data Accessibility).

We collected eggs of *A. opacum* and *A. annulatum* from semi-permanent ponds at Fort Leonard Wood, Missouri on 27 September 2021. Eggs were transported to an incubator at the SIUE campus and held at 12°C with a 13:11 light/dark cycle. For each species, we mixed clutches of eggs together to homogenize the genetic variation and waited for the eggs to hatch to ensure viability of the larval salamanders. On 19 October (Day 1), 21 (Day 3), and 28 (Day 10), we randomly assigned eight hatchling *A. annulatum* to each tank (24 total larvae of *A. annulatum* per tank) and eight hatchling *A. opacum* to each tank on 26 October (Day 8). Hatchling salamanders were added on different dates due to hatching asynchronies and at unequal densities of each species per tank due to differential hatching success.

To manipulate hydroperiod, we randomly assigned each tank to one of three treatments: short (209 days; 15 May 2022), medium (229 days; 4 June), or constant water level (full experiment duration: 239 days; 14 June). The shortest drying time corresponded approximately to when metamorphosis is initiated and/or early in the metamorphosis period of these species, based on previous experiments (Anderson and Semlitsch, 2014, 2016; Anderson et al., 2021), and should provide a strong selection pressure on metamorphosis timing. In contrast, the medium drying treatment represented ponds drying toward the middle-end of the typical metamorphosis period. The short and medium treatments were replicated five times and the constant water level six times. We began reducing water levels for the short and medium treatments on Day 155 (21 March 2022) of the experiment. Water level changes occurred every five days following a drying curve in which the short and medium treatments were lowered by different amounts to achieve different drying times (Fig. S2; see Data Accessibility). We reduced water levels by bailing water using a 5-gallon bucket with a protective mesh screen attached to allow only the removal of water and exclude larval salamanders. We

repeated the disturbance caused by the bailing in the constant water level tanks by submerging the bucket in the tank and gently pouring water back in. The short treatment tanks were lowered to approximately 10 cm after 50 days of drying, and the medium treatment tanks were lowered to same level after 70 days from the initiation of drying (Fig. S2; see Data Accessibility). Tanks were maintained for one day at that lowest depth before destructively sampling them (see below).

We began checking tanks for metamorphosing individuals on Day 195 (1 May 2022) at least every other night until Day 238 (14 June 2022) when the constant hydroperiod tanks were taken down. We removed individuals that had fully absorbed gills and recorded snout-vent length (SVL), mass, species, and date of metamorphosis for all individuals. When treatments became “dry,” we searched the leaf litter for remaining individuals and recorded stage (larva or metamorph), species, and body size data for any remaining salamanders. We calculated survival in each tank by counting the number of metamorphs as a proportion of the initial number added. Three individuals were discovered dead while searching for metamorphs, two of which were clearly metamorphs and one whose stage was unclear. Whether we counted these individuals in our calculation of tank survival did not qualitatively affect our conclusions, so we counted these as survivors as they appeared to have completed metamorphosis and were simply not captured before drowning. All surviving animals were humanely euthanized in MS222 solution following data collection in compliance with IACUC protocol 992.

**Statistical analysis.**—All analyses were conducted in R (R Core Team, 2020). We analyzed survival (i.e., proportioned metamorphosed) for each species separately using generalized linear models with quasibinomial error structure to account for overdispersion in the data. Hydroperiod treatment was our only predictor variable, though we had to exclude the short hydroperiod treatment from this analysis because nearly all individuals remained in a larval state, resulting in nearly complete separation of the data. Thus, we only compared the medium and constant treatments. We also analyzed the proportion of individuals recovered (larvae + metamorphs) from each tank using a similar model structure.

Tank was our experimental unit for most analyses described below (but see trade-offs analysis). We tested for differences in tank-averaged SVL, mass, and date of metamorphosis between treatments using one-way ANOVA, analyzing each species separately. Because of the slight asynchrony in *A. annulatum* additions, we could not calculate a true larval period duration, as the additions were not individually tracked. For *A. opacum*, date of metamorphosis did reflect larval period length, as they were added on only one date. The short hydroperiod treatments were not included in this analysis because only two individuals (both *A. annulatum*) metamorphosed in this treatment. Because survival was highly variable both within and among treatments, we then compared the same set of response variables for metamorphosed individuals using ANCOVA, with total animals recovered across both species as an additional covariate.

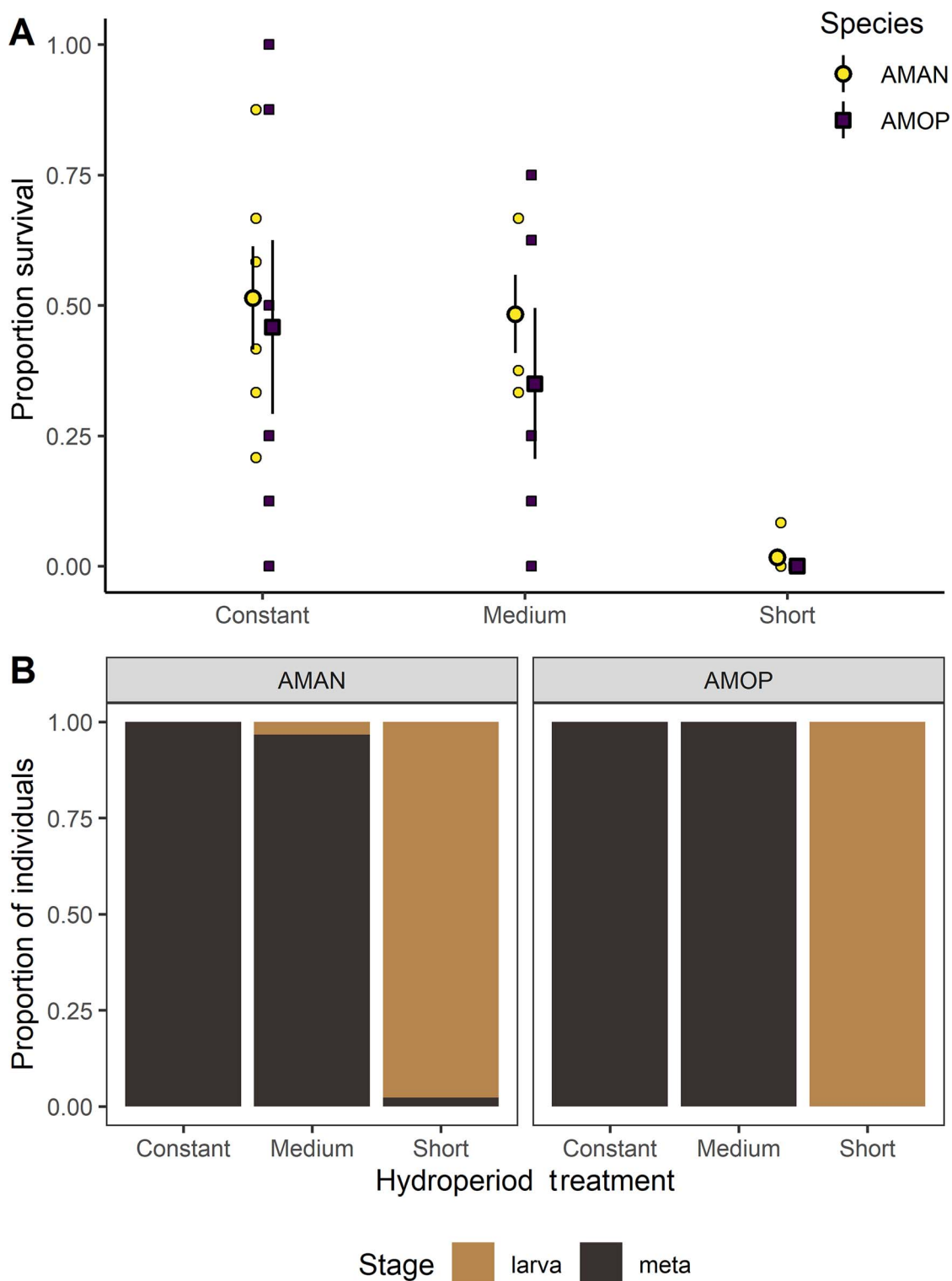
We analyzed trade-offs in size and date of metamorphosis for *A. annulatum* and *A. opacum* in two ways. First, we used linear mixed effects models, with mass as our response

variable and date of metamorphosis, treatment (medium and constant water levels only), the interaction of date and treatment, and total animals recovered as fixed effects; tank was a random effect to control for non-independence of individuals from each tank. These models were executed using the ‘nlme’ package (Pinheiro et al., 2020). For our second approach, we ran linear regression models for each tank separately, as each tank constituted a “population.” Mass was again our response variable and date of metamorphosis our predictor variable. From these models, we then extracted the slope values for each regression, and used ANOVA to analyze whether there were differences across our two treatments that contained metamorphs (constant and medium hydroperiods). We did not perform these analyses for *A. opacum* because of too few metamorphs in some tanks to generate meaningful regression lines at the tank level, though we graphically show the results as a comparison to *A. annulatum*.

## RESULTS

Survival (i.e., the proportion of individuals that underwent metamorphosis) differed by treatment, where survival was substantially lower in the short hydroperiod treatment compared with the constant water level and medium hydroperiod treatments (Fig. 1). Only two individuals (both *A. annulatum*) successfully metamorphosed from the short hydroperiod treatment. There were no significant treatment differences between the constant water level and medium hydroperiod treatments for either species (*A. annulatum*:  $F_{1,9} = 0.06$ ,  $P = 0.82$ ; *A. opacum*:  $F_{1,9} = 0.23$ ,  $P = 0.64$ ). The total number of animals recovered (larvae + metamorphs) for *A. annulatum* was marginally higher in the short hydroperiod treatment ( $F_{2,13} = 0.14$ ,  $P = 0.09$ ) and did not vary between treatments for *A. opacum* ( $F_{2,13} = 0.73$ ,  $P = 0.50$ ). Variability in the number recovered within certain treatments was substantial, especially in the constant water treatment, where the percent recovered ranged from 16–91% for *A. annulatum* and 0–100% for *A. opacum*. The average ( $\pm$  SE) proportion recovered for *A. annulatum* was  $0.51 \pm 0.10$ ,  $0.50 \pm 0.09$ , and  $0.73 \pm 0.04$  in the short, medium, and constant hydroperiod treatments. For *A. opacum*, the average proportion recovered was  $0.46 \pm 0.17$ ,  $0.35 \pm 0.15$ , and  $0.63 \pm 0.14$ , respectively, across the same three treatments.

Larval size (mean  $\pm$  SE) in the short hydroperiod treatment when the tanks were “dry” was  $32.3 \pm 0.36$  mm for *A. annulatum* and  $26.0 \pm 0.55$  mm for *A. opacum*. Average SVL did not differ between the constant and medium hydroperiod treatments for either *A. annulatum* ( $F_{1,9} = 2.48$ ,  $P = 0.45$ ) or *A. opacum* ( $F_{1,7} = 0.46$ ,  $P = 0.52$ ; Fig. 2A). After controlling for survival differences among tanks using ANCOVA, treatments significantly differed for *A. annulatum*, with average SVL being larger in the constant water treatment. Survival negatively affected SVL for *A. annulatum* ( $F_{1,8} = 9.13$ ,  $P = 0.02$ ), but not *A. opacum*. Mass did not differ statistically between treatments for *A. annulatum*, though metamorphs tended to be heavier in the constant hydroperiod treatments ( $F_{1,9} = 3.63$ ,  $P = 0.09$ ; Fig. 2B). After controlling for survival, this relationship was significant ( $F_{1,8} = 9.27$ ,  $P = 0.01$ ). Average mass did not differ between treatments for *A. opacum* ( $F_{1,7} = 1.42$ ,  $P = 0.27$ ; Fig. 2B), even after controlling for survival. Average date of metamorphosis was earlier in the medium hydroperiod than the constant treatment for

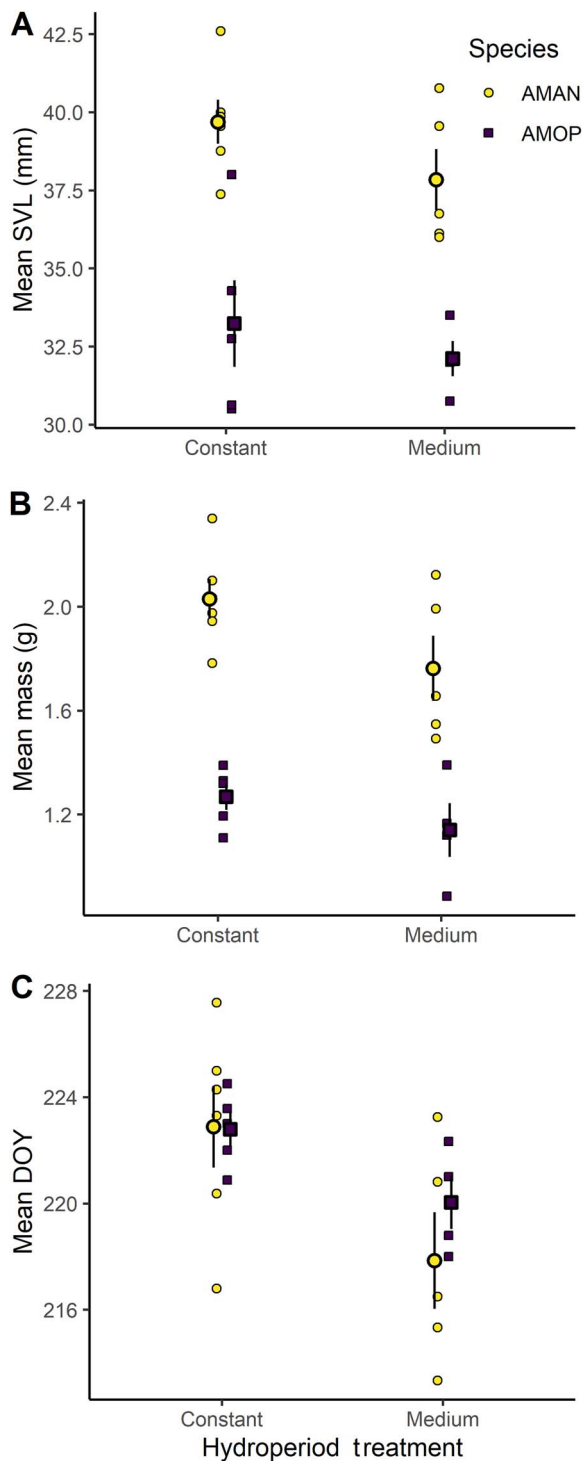


**Fig. 1.** Mean survival (A) and proportion metamorphosed (B) by hydroperiod treatment for *A. annulatum* (AMAN) and *A. opacum* (AMOP). In (A), large circles/squares and error bars represent treatment means and standard errors, and smaller circles/squares represent individual tanks.

both species (*A. annulatum*:  $F_{1,9} = 4.51$ ,  $P = 0.06$ ; *A. opacum*:  $F_{1,7} = 5.98$ ,  $P = 0.04$ ; Fig. 2C). After controlling for survival, these relationships remained similar (*A. annulatum*:  $F_{1,8} = 8.82$ ,  $P = 0.02$ ; *A. opacum*:  $F_{1,6} = 4.88$ ,  $P = 0.06$ ). Date of metamorphosis was significantly and positively related to total survival for *A. annulatum* ( $F_{1,8} = 13.65$ ,  $P = 0.006$ ) but not *A. opacum* ( $F_{1,6} = 0.08$ ,  $P = 0.78$ ).

Overall, mass at metamorphosis and date of metamorphosis were positively related for both species (Fig. 3; *A. annulatum*:  $\chi^2 = 47.97$ ,  $P < 0.001$ ; *A. opacum*:  $\chi^2 = 34.82$ ,  $P < 0.001$ ). After controlling for survival and non-independence of individual data points, there was a marginal interactive effect of treatment and date on mass in *A. annulatum*, where the mass-age relationship was stronger (more positive)





**Fig. 2.** Snout–vent length (A), mass (B), and DOY of metamorphosis (C) by hydroperiod treatment for metamorphosed individuals of *A. annulatum* (AMAN) and *A. opacum* (AMOP). Large circles/squares and error bars represent treatment means and standard errors, and smaller circles/squares represent tank averages. The short hydroperiod treatment is not shown because only two individuals metamorphosed from that treatment.

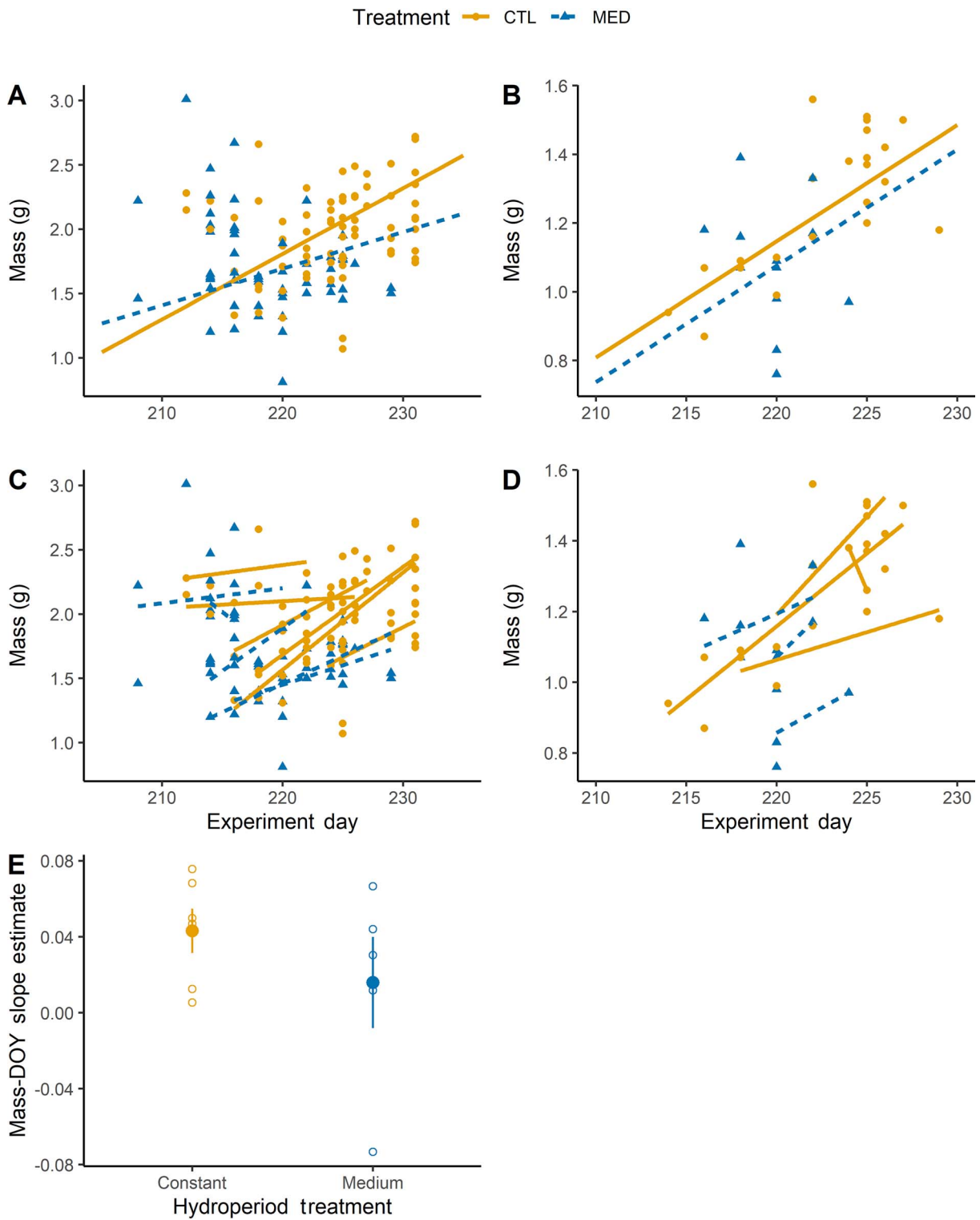
under constant water conditions ( $\chi^2 = 3.06$ ,  $P = 0.08$ ). This result is generally confirmed by analyzing population (i.e., tank) responses, with the majority of the slope estimates being more positive (Fig. 3C) and higher in the constant vs. medium treatment (Fig. 3E). However, the ANOVA between treatments for the slope effect was not statistically

significant ( $F_{1,9} = 1.16$ ,  $P = 0.31$ ). No interactive effect was present in *A. opacum*, meaning both treatments generally changed at the same rate (Fig. 3B, D).

## DISCUSSION

Hydroperiod is one of the most critical abiotic factors for pond-breeding amphibians, influencing both their ecology and evolution. Understanding how variation in hydroperiod influences these and other aquatic organisms is important because it can help predict what and how demographic responses may change under future climatic conditions, which are expected to change hydrological patterns (Brooks, 2009). Here, we investigated demographic responses of two pond-breeding salamanders, *A. annulatum* and *A. opacum*, to experimental manipulation of hydroperiod duration. These taxa both have relatively longer hydroperiod requirements compared to other pond-breeding amphibians (i.e., spring-breeding species), as their larval stages overwinter in ponds (Petranka and Petranka, 1980; Peterson et al., 1991). This led us to *a priori* predict they may have limited capacity to respond to variation in hydroperiod. We found that survival varied among hydroperiod treatments for both taxa, with shortened hydroperiods limiting completion of metamorphosis. Reduced survival (i.e., metamorphosis) in this treatment occurred despite individuals having reached the size threshold necessary to metamorphose (Scott, 1990; Ousterhout and Semlitsch, 2016). Some demographic traits like size and age at metamorphosis also varied by treatment. Thus, these species may be negatively impacted if hydroperiods shift to more ephemeral conditions, as is predicted (Brooks, 2009; Walls et al., 2013).

Similar to other studies on pond-breeding salamanders (e.g., Semlitsch and Wilbur, 1988; Ryan, 2007), rates of metamorphosis in our study differed under the varying hydroperiod regimes. Only two individuals (both *A. annulatum*) completed metamorphosis in the short hydroperiod treatment (209 days), where drying took place over approximately a 50-day period. In contrast, all but one individual that survived underwent metamorphosis in the medium and constant water treatments. Thus, in years where warmer spring and early summer temperatures result in premature drying of ponds, *A. annulatum* and *A. opacum* may have limited recruitment. Field studies have reported reproductive failure when hydroperiods are not sufficient for metamorphosis for both of our focal taxa (Semlitsch et al., 1996; Anderson et al., 2015). Our study indicates that an inability to accelerate development under rapidly drying conditions may contribute to reproductive failure in these species. This lack of response may pose a significant challenge for these taxa, given their need for a longer minimum hydroperiod and the predicted climatic shift toward more ephemeral habitats (Brooks, 2009; Walls et al., 2013). At the same time, our tanks were lowered to a depth of approximately 10 cm, which is deeper than other hydroperiod manipulations (e.g., Semlitsch and Wilbur, 1988). Thus, our focal taxa may not have responded in terms of metamorphosis because they did not yet perceive the pond to be in imminent danger of drying; had we lowered the tanks to even lower water levels, the salamanders may have initiated metamorphosis, which was possible given that the larval *A. annulatum* that were alive at the conclusion of the short hydroperiod treatment did exceed the size limit necessary to achieve metamorphosis (Anderson and Semlitsch, 2014; Semlitsch et al., 2014). No individuals showed any signs of initiating



**Fig. 3.** Relationships between mass at metamorphosis and day of metamorphosis for *A. annulatum* (A, C, E) and *A. opacum* (B, D) for constant water levels (circles, solid lines) and medium hydroperiod treatments (triangles, dashed lines). In A–D, each point represents an individual salamander. In A and B, lines are predicted relationships based on linear mixed effects models (see *Analysis*). In C and D, lines are predicted relationships for each tank. In E, the y-axis shows the slope estimate for an individual tank (open circles) for the relationship of mass and date of metamorphosis, and the filled circles and error bars are the mean  $\pm$  SE.

metamorphosis (e.g., reduced gills), however, so this transition would have had to occur rapidly to successfully escape the deteriorating conditions; the rate of metamorphosis in salamanders is not well understood, however, at least compared to anurans (Landberg and Azizi, 2010).

Survival of *A. annulatum* and *A. opacum* varied quite substantially among replicates, suggesting that other factors beyond our treatments also influenced these patterns. It is likely that overwintering conditions played at least some role in survival. Tanks were set up over the winter, when

these species naturally occur in ponds, and were covered in ice for several weeks, which could have limited oxygen and resource inputs (Herstoff and Urban, 2014). Tanks did not freeze solid, however, permitting some survival. Our slight addition asynchronies may have enhanced intraguild predation during the winter period by creating body size asymmetries (Brodman, 2004; Anderson et al., 2016), though if this was the case, survival likely would still have been more consistent across tanks and simply lower, on average. Similar patterns of stochastic survival have been observed in some (Anderson and Semlitsch, 2014), but not all, experimental studies of these species (Anderson and Semlitsch, 2016; Anderson et al., 2021). We had to control for variation in survival for most of our analyses, indicating density dependence played an important mediating role in our study, which confirms numerous field and experimental studies where life history traits at metamorphosis of salamanders are influenced by larval densities (Wilbur and Collins, 1973; Scott, 1990; Ousterhout and Semlitsch, 2016).

We found that, after controlling for survival, metamorphic *A. annulatum* were on average longer, heavier, and completed metamorphosis later in the constant water level treatment than in the medium hydroperiod treatment. This makes sense, as larvae were able to continue growing under relatively stable conditions with constant water levels. Reductions in hydroperiod result in numerous physical changes to the environment that could lead to reduced sizes at metamorphosis. For example, larval densities increased as tank volumes were reduced, increasing competition. Changes in temperature profiles, including increased variability and maximum temperatures, occur (Fig. S1; see Data Accessibility), which can limit physiological aspects of development and metamorphosis (Keen et al., 1984). Conversely, reduced water volumes also consolidate prey items and increase reproductive rates in species like zooplankton due to warmer water temperatures, potentially mitigating any effects of larval densities and reducing competition. Overall, our study indicates that it is advantageous to remain as a larva as long as possible, as individuals who undergo metamorphosis at a larger size have higher potential fitness in the terrestrial environment (Semlitsch et al., 1988; Earl and Whiteman, 2015).

Understanding the relationship between size and age at metamorphosis has received considerable attention over the last 50 years (Wilbur and Collins, 1973; Werner, 1986; Alford and Harris, 1988). The majority of this work is centered around what current growth conditions indicate about that environment to developing larvae, as well as mortality risks faced in aquatic versus terrestrial environments. A drying pond is a classic example of a stressor that imposes strong selection to exit ponds early, assuming that the necessary size has been achieved for metamorphosis, as remaining in such a pond could lead to mortality (Shoop, 1974; Semlitsch et al., 1996). However, early metamorphosis often occurs at a reduced body size, which can affect future fitness patterns (Smith, 1987; Semlitsch et al., 1988). Individuals that remain within the pond can continue growth, leading to larger sizes at metamorphosis. Thus, age and size at metamorphosis should show a positive relationship in a drying pond (Rudolf and Rödel, 2007). Our results for *A. annulatum* are consistent with this finding, where individuals in the medium hydroperiod treatment that were undergoing metamorphosis later were doing so at larger sizes. However,

the constant hydroperiod treatment showed a slightly stronger positive relationship, meaning hydroperiod had only a small effect on the direction of reaction norms in this study. Other studies of ambystomatids have found hydroperiod duration impacted the relationship of age and size at metamorphosis, depending on natal pond hydrology (Thomas, 2020). Our results on size–age relationships are especially interesting because in natural populations of *A. annulatum*, this life history pattern at metamorphosis was negative (Semlitsch et al., 2014); it is not entirely clear why we observed different results for this relationship, though population-specific reaction norms of metamorphosis have been noted in other pond amphibians (Berven and Gill, 1983; Semlitsch and Anderson, 2016). Focused attention on the causal mechanisms of variation (e.g., populations or species) in size–age relationships would be an interesting area of future research.

We expected some species-level differences between *A. annulatum* and *A. opacum*, due to differences in their egg-laying strategies. However, the primary difference we observed was simply a lack of effect of hydroperiod in *A. opacum*, which was opposite of our expectations. The lack of response is intriguing, as this species typically inhabits environments with fluctuating water levels (Petranka and Petranka, 1981; Ousterhout et al., 2015). However, extrapolating any species-level differences should be tempered by the fact that we had a more limited number of *A. opacum* available for study as compared to *A. annulatum*, leading to lower statistical power. Further comparative studies of amphibians across species in response to abiotic cues like hydroperiod may help elicit a better understanding of the similarities or differences among taxa.

Given that hydrology is expected to change under most future climate scenarios (Brooks, 2009), having an *a priori* understanding how organisms will respond to such change will aid conservation efforts of pond-breeding amphibians. Construction or modification of wetlands that retain water long enough for these species to complete metamorphosis may be needed in some areas, as well as preservation of existing habitats that satisfy these requirements (Snodgrass et al., 2000; McMenamin et al., 2008). Furthermore, developing an understanding of hydrology impacts beyond population recruitment, such as body size at metamorphosis (Phillips et al., 2002), may be especially necessary, as such effects may also influence population persistence. Experimental approaches similar to those employed here can be a powerful method to build quantitative predictions for different population parameters in response to different future conditions expected under climate change scenarios.

#### DATA ACCESSIBILITY

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