

EFFECTS OF HYDROLOGIC VARIABILITY ON THE DEVELOPMENT OF *LITHOBATES SPHENOCEPHALUS*

KONSTANTINOS ANDRIOTIS¹, KEVIN L. FOUTS², AND KRISTEN K. CECALA^{1,3}

¹Department of Biology, University of the South, 735 University Avenue, Sewanee, Tennessee 37383, USA

²Office of Environmental Stewardship and Sustainability, University of the South, 735 University Avenue, Sewanee, Tennessee 37383, USA

³Corresponding author email: kkcecala@sewanee.edu

Abstract.—Organisms use environmental cues to make phenotypic changes that maximize fitness. As humans manipulate the environment, environmental variation can become so extreme that it exceeds the range of conditions to which the organism can respond effectively. As humans affect climate and modify rivers, hydrologic conditions become increasingly variable. Organisms, such as anurans that require water for their early life stages seek to maximize size at metamorphosis while facing the risks of predation, competition, and pond drying. Our study investigated the impact of hydroperiod variation on the survival and development of Southern Leopard Frogs (*Lithobates sphenoccephalus*) in *ex-situ* pond mesocosms. We manipulated mesocosm water levels to simulate different rates of pond volume fluctuations and examined their impact on tadpole survival, time to metamorphosis, and morphology. We did not find an effect of hydrologic variability on larval development, survival, time to metamorphosis, or morphology. Anurans may not be able to respond to habitat variability that occurs at temporal scales of days to weeks as in this study, or their response to environmental fluctuations may be conditional on additional factors. Alternatively, our treatments with low water levels may not have changed the benthic area or resource availability and, thus, may have had little impact on growth. This work emphasizes the complexities of frog responses to hydrologic variability and the importance of considering several interacting elements when measuring phenotypic plasticity in changing contexts.

Key Words.—anuran; hydroperiod; metamorphosis; phenotypic plasticity; Southern Leopard Frog; stress; tadpole

INTRODUCTION

Organisms use predictable environmental cues to initiate key life-history transformations or behaviors to maximize fitness, often demonstrating a high degree of phenotypic plasticity in their responses (Stearns 1976; Thorpe et al. 1998; Miner and Vonesh 2004). Human interventions are altering natural settings and disrupting the ability of organisms to use cues effectively (Nyingi et al. 2013; Fehlmann et al. 2017; Wilson et al. 2020). When connections between cue and outcome are disrupted, adaptive behaviors or physiological changes may no longer improve fitness and result in declining survival or reproductive success (Reed et al. 2010; Donaldson-Matasci et al. 2013; Fehlmann et al. 2017). In addition to increased ranges of environmental conditions, the rates of change are also increasing, which minimizes the ability of organisms to mount behavioral or physiological changes that allow them to respond appropriately to shifting cues (Jacobs and Wingfield 2000; Goldberg et al. 2012). For example, hydroelectric operations for daily power

needs result in daily shifts in high and low flow that have no natural analog (Piman et al. 2013; Bobrowiec and Tavares 2017). In these instances, the changes may occur so rapidly that an organism cannot mount a physiological response or select an alternative habitat that would allow them to be successful in either extreme condition (Piman et al. 2013). Thus, the organism may not respond to the cue and exhibit average responses across the spectrum of conditions, resulting in poor performance during maximal or minimal conditions (Gervasi and Foufopoulos 2008; Piccinetti et al. 2017; Brannelly et al. 2019). Alternatively, the organism could experience strain from constantly being in a state of physiological stress or may select refuge habitats that do not serve all life-history needs (Miner and Vonesh 2004; Reed et al. 2010).

Freshwater biodiversity is among the most threatened globally due to increased human use and modification of freshwater systems (McAllister et al. 1997; Nyingi et al. 2013; Fehlmann et al. 2017). Hydroperiod and the natural flow regime are the strongest drivers of the ecological conditions in

freshwater and are highly modified for human needs (Ficetola and De Bernardi 2003; Dudgeon et al. 2006; Gido et al. 2013). Increased variation in precipitation and hydrologic control by small and large dams have well-understood impacts on rivers, but floodplains, oxbows, and slackwater ponds where many amphibians live are impacted in similar ways (Eskew et al. 2012; Guzy et al. 2018). Modified hydrologic regimes provide an opportunity to examine how habitat variability and the rate of environmental change affect key indicators of success (Freeman et al. 2001; Craven et al. 2010; Kupferberg et al. 2012). *In-situ* systems are undergoing daily fluctuations from peaking power operations of hydrologic dams, and we expect that climate change will increase the frequency of drought and large rain events amplifying the variability of hydrologic regimes (Kupferberg et al. 2012) and the speed with which systems achieve minimum and maximum depth or flow (Knapp et al. 2008).

Amphibians are a model organism for understanding how environmental variation contributes to the developmental plasticity of organisms with biphasic life histories (Newman 1992; Miner et al. 2005; Blaustein et al. 2012). During their larval phase, these organisms must inhabit an aquatic environment, transitioning to a terrestrial environment only after undergoing metamorphosis. They subsequently return to the aquatic environment for reproduction. (Brühl et al. 2011). As a result, amphibians use different habitat types throughout their life cycle, and fitness is closely tied to the success they achieve during their larval phase, which is tightly linked to conditions experienced in the larval environment (Amburgey et al. 2012, 2016; Blaustein et al. 2012). The transition between life stages and habitat requires considerable energy and is predicted to occur when growth rates relative to body size slow (Werner 1986; Altwegg and Reyer 2003; Amburgey et al. 2012). Thus, changes in resource availability, intraspecific competition, or the introduction of predators that all reduce energy intake can accelerate this transition between habitats (Werner 1986; Xiao-Li et al. 2014). Ultimately, transformation at smaller sizes contributes to smaller adult body size, which is associated with lower survival and fecundity (Berven 1990; Newman 1992; Altwegg and Reyer 2003; but see Earl and Whiteman 2015).

Pond drying affects many of these conditions that can influence the rate of larval development and timing of metamorphosis (Semlitsch 1987; Semlitsch and Wilbur 1988; Leips et al. 2000). Drying reduces

pond volume, which decreases resource availability as the area of the pond can decrease, increases intraspecific density and competition, and increases thermal variability in the pond (Semlitsch and Wilbur 1988; Skelly 1996). Additionally, as ponds undergo desiccation, the substances and elements (e.g., waste products, hormones, or irritants) present in them become more concentrated, which may trigger metamorphosis (Schmuck et al. 1994; Castellano et al. 2022). The rate of drying, however, could influence the strength of amphibian responses to these cues. Fast drying may accelerate cues to transform because if ponds dry completely, the larvae run the risk of dying before metamorphosis (Cooke 1985; Loman 1999). Likewise, rapid filling and drying associated with highly variable precipitation or even peaking power generation may interfere with the environmental cues that amphibians would use to transform, which could have negative consequences at the larval or adult life stages (Newman 1992; Stallard 2001; Earl and Whiteman 2015). Previous work has demonstrated that water volume manipulations induce shifts in larval anuran development (e.g., Pintar and Resetarits 2018a), but it is less clear how variation in the rate of water level change might impact anuran responses to fluctuating conditions.

The Southern Leopard Frog (*Lithobates sphenoccephalus*) is a widespread species in the southeastern U.S. (Butterfield et al. 2005). Tadpoles transform into terrestrial juveniles at approximately 20–33 mm long (SUL) after 3 mo (Butterfield et al. 2005). They prefer to live in shallow freshwater aquatic environments, including ponds, creeks, reservoirs, swamps, and sloughs, and they can live for about 3 y (Butterfield et al. 2005). They are also common in Sewanee, Tennessee, USA, and have been successfully reared in mesocosms (Zeitler et al. 2019, 2021).

We used the Southern Leopard Frog as a model organism to determine the effects of rapid environmental change via changing hydroperiod variation on the development and survival of amphibians using *ex-situ* pond mesocosms with different hydrologic regimes. Relative to two stable treatments with little change in depth at either high or low depth, we compared four rates of water level change between high and low depths. We quantified differences in tadpole survival, time to metamorphosis, and morphology among treatments. Overall, we expected that more stable treatments with less variability would have higher survival, growth, and shorter time to metamorphosis.

MATERIALS AND METHODS

We set up 24 *ex-situ* pond mesocosms using 1,325 L cattle tanks placed at the Sewanee Utility District in Sewanee, Tennessee, USA (Fig. 1). We implemented four replicates of six treatments, incorporating one treatment with a consistently highwater depth of 50 cm and another one with a constant low water depth of 30 cm, serving as our control groups, where water depth remained unchanged. These water levels were selected to represent a 40% reduction in water volume within the range represented in previous studies of pond drying (e.g., Amburgey et al. 2016; Pintar and Resetarits 2018a). We then simulated fill and dry cycles of 3 d, 6 d, 15 d, and 30 d between 50 cm and 30 cm in depth. Every 3 d, we adjusted water levels over a period of a few hours to depths assigned by each treatment. For example, the 6-d cycle would be adjusted 10 cm every 3 d whereas the 30-d cycle would be adjusted 2 cm every 3 d. The treatments were randomly assigned to the 24 mesocosms.

At first, we filled mesocosms with water from Lake O'Donnell, the lake providing water to the municipality of Sewanee on 24 February 2022. Then, we inoculated each mesocosm with 1 L water from Cheston Lake with native communities of microorganisms and 1 kg of dried upland Sugar Maple (*Acer saccharum*) leaf litter on 28 February 2022. After that, we refilled mesocosms with dechlorinated

tap water as necessary to compensate for evaporation or as appropriate for the assigned treatment. Next, we covered all mesocosms with a 5 mm pond netting mesh covering to prevent unwanted predation by birds and colonization by non-target organisms. We also added a float to each mesocosm to allow transforming individuals to rest at the surface of the water. To easily manipulate water depth, we installed standpipes with regularly drilled holes filled with plugs. To lower water levels, we simply removed plugs to the assigned water depth after covering the holes with window screen mesh to prevent the loss of tadpoles. To fill the mesocosms, we inserted plugs to allow water depth to increase and filled mesocosms via gravity from 1,135 L tanks where tap water was allowed to dechlorinate for 48 h prior to introduction to the mesocosms. We allowed mesocosms to sit for four weeks before the introduction of tadpoles.

We collected five egg masses of *L. sphenoccephalus* after dark on 7 March 2022 from Lake Cheston. We transferred egg masses to the lab (10 min trip) in 19 L buckets and checked them daily. We housed the egg masses individually in a 38 L aquarium with pond water from their collection site. We filled the water to a depth of 5 cm and kept it well-oxygenated using a bubbler with an ambient photoperiod at 21° C. Then, on 15 March 2022, after approximately 75% of the eggs hatched, we randomly selected 10 tadpoles (Gosner stages 23 through 25) from each egg mass

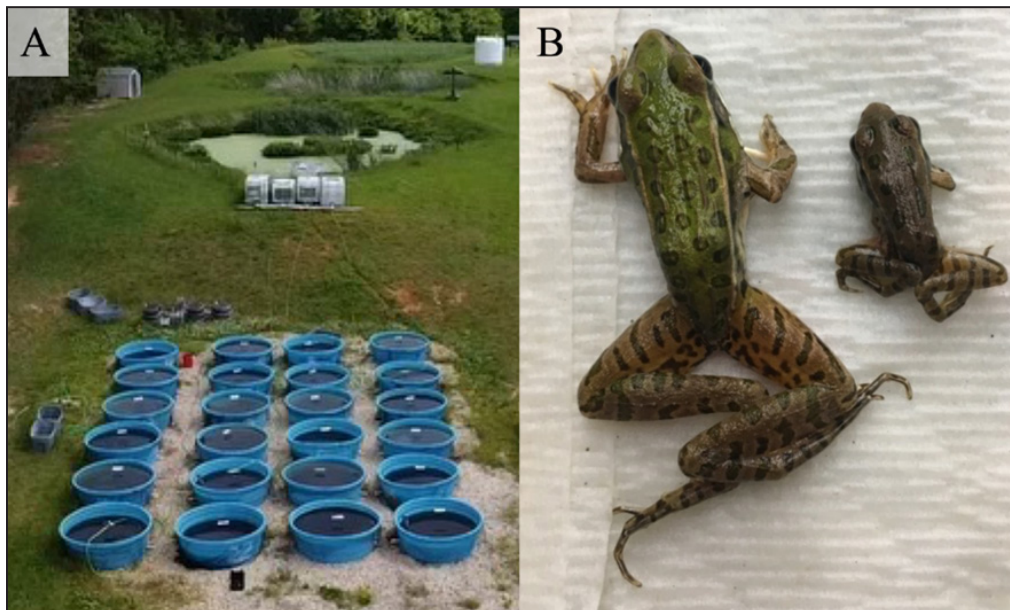


FIGURE 1. (A) Pond mesocosm array and (B) the study species, the Southern Leopard Frog (*Lithobates sphenoccephalus*), at the Sewanee Utility District in Franklin County, Tennessee, USA. (A photographed by Konstantinos Andriotis and B by Kristen Cecala).

to be introduced into each mesocosm for a total of 50 tadpoles per mesocosm. We introduced the tadpoles to outdoor mesocosms after being allowed to acclimate to the outdoor temperatures. After their introduction to the mesocosms, we checked daily to ensure that the mesocosms had not flooded or drained accidentally. We did not provide supplemental food to the mesocosms. As tadpoles approached Gosner stage 41, which is considered the stage at which they have reached metamorphosis (Gosner 1960), we removed them from the mesocosm using dipnets and minnow traps and transported them to the lab.

In the lab, we euthanized froglets in an overdose of MS-222 (3 gL⁻¹ of buffered water). We weighed froglets, measured snout-urostyle length (SUL), right femur length, and maximal head width, and we inspected froglets for any malformations or injuries before we placed them in 75% ethanol. We kept specimens at the University of the South for future studies. For analysis, we used mass and length measurements to calculate the scaled mass index for each individual, which is a metric to represent body fat proportion in small vertebrates (Peig and Green 2009; MacCracken and Stebbings 2012). We standardized head and leg length by body size by dividing the measurements by the SUL before proceeding with statistical analyses. These morphological variables are associated with amphibian success including survival and fecundity, which both contribute directly to fitness (Werner 1986; Zeitler et al. 2019).

Data analysis.— Our survival data and model residuals (time to metamorphosis, SUL, mass, scaled mass index) met expectations of normality ($W > 0.972$, $P > 0.145$) and equality of variances (K-squared > 8.22 , $P > 0.145$), and we evaluated the effects of treatment on survival data using an Analysis of Variance (ANOVA). We calculated survival as the proportion of the initial 50 tadpoles that were recovered as froglets. Because survival was measured at the mesocosm scale, we calculated the mean body size, body condition, leg length, and head width per mesocosm and evaluated if mesocosm survival influenced morphology using a series of ANOVAs. If an ANOVA indicated a significant effect of survival, we included survival as a covariate in the morphological mixed model described below. We evaluated the effects of treatment on the morphology of froglets at metamorphosis using Linear Mixed Models with mesocosm as a random effect and repeated the model for body size (SUL), body condition (scaled mass index: SMI), size-

corrected leg length (femur length/SUL), and size-corrected maximum head width (head width/SUL). We evaluated the normality of the residuals of these models to confirm use of this model structure. We repeated a similar model structure to evaluate the potential for differences among treatments in the length of time necessary to reach metamorphosis (calculated as the number of days between the introduction to the mesocosm and removal from the mesocosm). Linear Mixed Models were evaluated using package lme4 (Bates et al. 2015). Because of the long period of larval development relative to another similar study in this location (Zeitler et al. 2021), we assessed whether morphological characteristics were associated with time to metamorphosis using Linear Mixed Models with mesocosm as a random effect. We used Satterthwaite approximations to assess the significance of all Linear Mixed Models. For any significant outcomes in these models, we used package emmeans to evaluate pairwise *post-hoc* comparisons among treatments with Tukey corrections for multiple comparisons (Lenth 2019). All analyses were performed in R and evaluated with $\alpha = 0.05$.

RESULTS

We recovered 354 individuals with survival ranging from 0% to 66% per mesocosm. Two stable high water level replicates and one 30 d replicate had 0% survival, and a stable low water level treatment had 66% survival (Table 1). Survival to metamorphosis did not differ significantly among the treatments in this study ($F_{5,18} = 1.73$, $P = 0.179$), nor did time to metamorphosis ($F_{6,324} = 2.65$, $P = 0.068$). We performed a *post-hoc* analysis on this result to evaluate if there was one treatment driving this result, but there were no significant differences ($t = 3.23$, $P = 0.059$; Fig. 2). The proportion of animals in each mesocosm surviving to metamorphosis had no effect on head width ($F_{1,19} = 0.84$, $P = 0.367$), leg length ($F_{1,19} = 3.65$, $P = 0.071$) or body condition ($F_{1,19} = 2.35$, $P = 0.142$). Body size (SUL) was negatively associated with mesocosm survival rate and was included as a covariate in the mixed model used to evaluate the effects of treatment on body size ($F_{1,19} = 6.74$, $P = 0.018$).

We did not observe any treatment effects on morphology. In the Linear Mixed Model for body size (SUL), we did not observe a significant effect of the covariate, mesocosm survival ($F_{1,323} = 0.744$, $P = 0.414$); however, we did note the variation in

TABLE 1. Survival rates of Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpoles reaching metamorphosis after being reared in outdoor mesocosms with variable water level in Sewanee, Tennessee, USA (n = 4 mesocosms per hydrologic treatment). Means as well as minimum and maximum values are presented. The terms High and Low represent constant water levels throughout the experiment at depths of 50 and 30 cm respectively whereas x-day indicates the length of time mesocosms took to transition from high and low water levels and present different treatment rates of water level change.

Treatment	Mean	Minimum	Maximum
High	0.26	0.00	0.58
Low	0.41	0.20	0.66
3-day	0.15	0.08	0.30
6-day	0.35	0.26	0.46
15-day	0.46	0.32	0.60
30-day	0.15	0.00	0.54

how morphology changed relative to the length of the larval period. Likewise, body condition, size-corrected leg length, and size-corrected head width were not different among treatments ($F_{6,324} = 0.485$, $P = 0.776$, $F_{6,324} = 1.296$, $P = 0.322$, $F_{6,324} = 0.198$, $P = 0.959$; respectively) We did not find significant associations between time to metamorphosis and body condition ($F_{1,328} = 1.69$, $P = 0.195$) or between time to metamorphosis and size-corrected leg length ($F_{1,328} < 0.001$, $P = 0.983$). Conversely, we observed a negative relationship between time to metamorphosis and body size (SUL; $r^2 = 0.639$; $F_{1,328} = 13.96$, $P < 0.001$, Fig. 3), and a positive relationship between time to metamorphosis and size-corrected head width ($r^2 = 0.142$; $F_{1,328} = 5.41$, $P < 0.001$, Fig. 3).

DISCUSSION

Our data suggest that the hydrologic variability in our study had little impact on the larval development of *L. sphenoccephalus*. We found no significant relationships between variability and morphology, survival, or time to metamorphosis. We propose three potential explanations for the lack of discernible patterns that we elaborate on below. First, the variability in water depths we simulated could be within the range of natural variation experienced by this species, and therefore, tadpoles had the adaptive capacity to respond appropriately to these shifts in hydroperiod. Secondly, the effects of habitat variability may only emerge as a synergistic threat, which were eliminated in our *ex-situ* study. Lastly, shifts in water volume in this study may not have been extreme enough to simulate the threat of drying resulting in minimal physiological responses to our

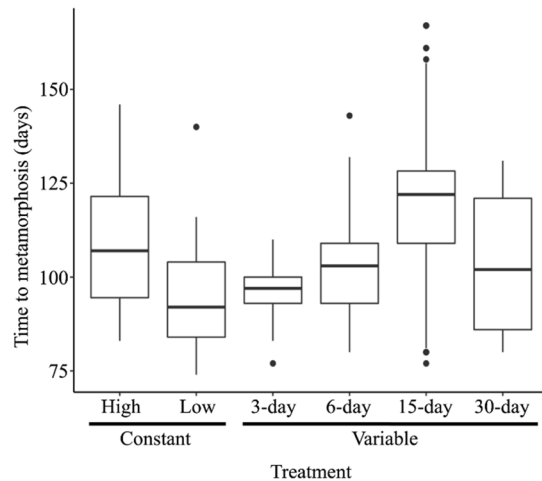


FIGURE 2. Days to metamorphosis (means and quartiles) of Southern Leopard Frog (*Lithobates sphenoccephalus*) tadpoles reared in outdoor mesocosms (Sewanee, Tennessee, USA) with manipulations of water level.

treatments.

As pools dry, the concentration of materials and compounds within them intensifies (Schmuck et al. 1994). Increased waste products, hormones, or irritants could induce metamorphosis to avoid the adverse effects of lowered water levels (Marques et al. 2013). Alternatively, less surface area for foraging and more densely packed tadpoles could slow growth rates to induce metamorphosis (Denver et al. 1998; Castellano et al. 2022). Unlike other studies investigating these particular drivers of time to metamorphosis or size at metamorphosis, we evaluated rapid versus slow shifts in water levels and found little evidence to suggest that even 30-d cycles between low and high-water levels were sufficient to induce changes in metamorphosis. It is possible that the repeated filling of the tanks diluted these compounds that inhibit growth, eliminating individual responses to decreasing volume. Alternatively, the majority of tadpole activity and foraging took place in the leaf litter rather than in the water column (Stoler and Relyea 2013, 2020). Therefore, changing water volume may not be a realistic cue to indicate drying where water volume but also pond area would decrease. In our study, low water volumes were only experienced for 3 d, limiting the time in which tadpoles were exposed to these low-water conditions and able to mount a physiological response.

Despite using similar methods established in another study on pond drying with a 60% reduction in water volume (Pintar and Resetarits 2018a), our results differed from their observations of declining size in fluctuating environments. Their

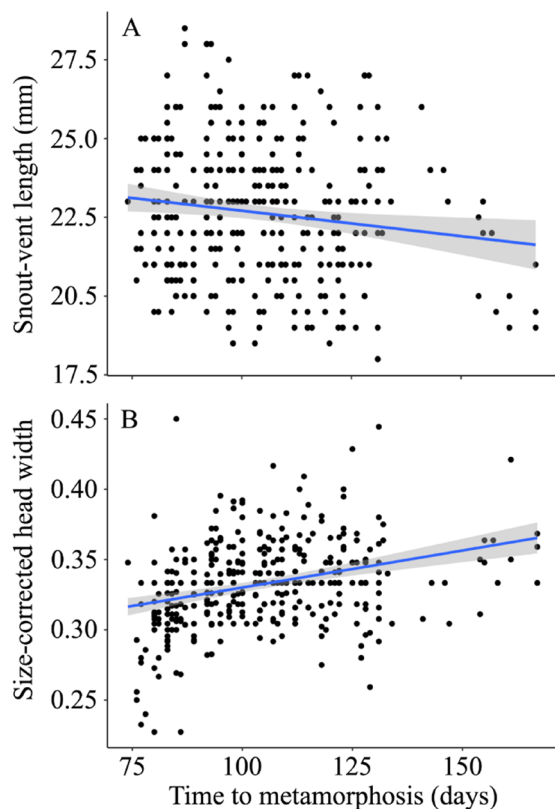


FIGURE 3. Relationships between morphological development of Southern Leopard Frog (*Lithobates sphenocephalus*) and time to metamorphosis for body size (A; snout-urostyle length) and size-corrected head width (B). Lines represent the predicted linear relationships with the shaded areas representing the 95% confidence intervals.

fluctuating treatment was roughly equivalent to our 30-d fluctuating treatment, for which we expected the effects would be magnified in more variable hydrologic regimes. Although the two treatments were adjusted at different frequencies (3 d in the present study versus 7 d in Pintar and Resetarits 2018a), the largest difference between the present study and Pintar and Resetarits (2018a) was the seasonality of the study and the refilling rate. We collected egg masses from spring-breeding *L. sphenocephalus* and manipulated water levels over the summer rather than the winter as in Pintar and Resetarits (2018a), so the water level fluctuations were applied throughout the study period rather than for limited time early in development or late in development. Alternatively, Pintar and Resetarits (2018b) demonstrated compensatory growth when experimental ponds were filled, particularly at the early stages of development in a species with faster developmental rates. It could be that there is a critical window in early development where the water level is

particularly important for setting a growth trajectory for tadpoles (Frisch and Santer 2004; Dmitriew 2011). Because we began the experiment with full mesocosms, all of them experienced draining in the first month of development, which could have set the trajectory for all the tadpoles and minimized differences among treatments. Investigating the potential for critical windows of drying cues would provide more insight into the potential effects of drought on anuran success.

Although we did not measure temperature throughout the experiment and temperature was not presented in Pintar and Resetarits (2018a), we think that our mesocosms may have attained higher temperatures relative to Pintar and Resetarits (2018a), which could have overwhelmed the effects of hydrologic variation (Newman 1998; Rose 2005; Wheeler et al. 2015). Repeated mixing of water in the mesocosms every 3 d may have also reduced the potential for vertical stratification in temperature and accelerated metamorphosis of all individuals in our study. We also noted that all our metamorphosed froglets had body sizes below the mean reported for the species (Luhring 2013; Pintar and Resetarits 2018a). It is possible that this effect of temperature overwhelmed the effect of hydrologic variability or that climate change impacts on pond hydroperiod could impact anuran development indirectly via temperature (Atkinson 1994; Newman 1998; Dastansara et al. 2017).

The metamorphosed froglets in our experiment were smaller than froglets from similar *ex-situ* experiments and took longer to transform (e.g., Dole 1971; Luhring 2013; Zeitler et al. 2021). More specifically, the metamorphic *L. sphenocephalus* sizes in nature vary and range from 20–33 mm (Butterfield et al. 2005). The mean size of metamorphosing tadpoles in our experiment was 22.6 ± 0.11 mm, which is smaller than mean sizes at transformation. Also, tadpoles in this experiment took 15% longer to metamorphose compared to the ones raised in similar experiments (Ashton and Ashton 1988). Our results highlighted a positive relationship between time to metamorphosis and size-corrected head width that could be interpreted as a signal of successful foraging in the larval environment. Research has shown that narrowed heads in individual tadpoles are associated with reduced foraging success of the frogs at a juvenile stage (Emerson 1985; Emerson and Bramble 1993; Tejedo et al. 2010; Zeitler et al. 2021). Positive relationship between time to metamorphosis and size-corrected head width as

well as the negative relationship between survival and size at metamorphosis suggest food resources may have become limited in our study (Emerson 1985; Emerson and Bramble 1993; Zeitler et al. 2021). In the future, we recommend that researchers evaluating variation in water level quantify primary productivity and temperature and consider adding additional resources like algal wafers to maintain consistent food availability and faster growth rates than we observed. Low survival and density-dependent growth may have inhibited our ability to detect treatment effects. Despite using mixed models to capture as much variability among treatments as possible, we were restricted to four replicate mesocosms per treatment. If the effects of hydrologic variability are small relative to other environmental factors affecting larval anuran development, the low power in this experiment may have also caused us to fail to detect a small to moderate treatment effect.

As climate changes, we expect hydrographs to become more variable and depart from long-term patterns (Arnell and Gosling 2013). Identifying if and how populations of aquatic organisms will respond to these changes is essential to understanding the long-term success of populations and the functions they provide. Although our study found no phenotypic changes associated with highly variable water levels in larval anurans, other studies have found that fluctuating water levels can induce responses by larval anurans (Pintar and Resetarits 2018a). Determining whether conditions like temperature or seasonality contribute to differences in our findings will provide insight into how human alterations of freshwater hydrologic regimes will impact anuran success. Likewise, the effect of water levels alone may be insufficient to change tadpole phenotype, but it may instead depend more directly on other factors or how declining water volume interacts with mitigating factors like predator presence or density dependence.

Acknowledgments.—We thank Kabir Menon, Molly Almon, Laura Kemper, Nneka Okolo, and Summer Smith for assistance with animal collection, research setup, and data collection. We also thank the Sewanee Utility District for providing the space for setting up the mesocosms and running the research experiment. The Animal Care and Use Committee of the University of the South (Cecala-7-2022) and the Tennessee Wildlife Resources Agency (#1296) approved all protocols.

LITERATURE CITED

- Altwegg, R., and H.U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* 57:872–882.
- Amburgey, S.M., W.C. Funk, M. Murphy, and E. Muths. 2012. Effects of hydroperiod duration on survival, developmental rate, and size at metamorphosis in Boreal Chorus Frog tadpoles (*Pseudacris maculata*). *Herpetologica* 68:456–467.
- Amburgey, S.M., M. Murphy, and W.C. Funk. 2016. Phenotypic plasticity in developmental rate is insufficient to offset high tadpole mortality in rapidly drying ponds. *Ecosphere* 7:1–14. <https://doi.org/10.1002/ecs2.1386>.
- Arnell, N.W., and S.N. Gosling. 2013. The impacts of climate change on river flow regimes at the global scale. *Journal of Hydrology* 486:351–364.
- Ashton, R.E., and P.S. Ashton. 1988. *Handbook of Reptiles and Amphibians of Florida: Part Three: The Amphibians*. Windward Publishing, Miami, Florida, USA.
- Atkinson, D. 1994. Temperature and organism size—a biological law for ectotherms? *Advances in Ecological Research* 25:1–58.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the Wood Frog (*Rana sylvatica*). *Ecology* 71:1599–1608.
- Blaustein, A.R., S.S. Gervasi, P.T. Johnson, J.T. Hoverman, L.K. Belden, P.W. Bradley, and G.Y. Xie. 2012. Ecophysiology meets conservation: understanding the role of disease in amphibian population declines. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367:1688–1707.
- Bobrowiec, P.E.D., and V.D.C. Tavares. 2017. Establishing baseline biodiversity data prior to hydroelectric dam construction to monitoring impacts to bats in the Brazilian Amazon. *PLoS ONE* 12:1–18. <https://doi.org/10.1371/journal.pone.0183036>.
- Brannelly, L.A., M.E. Ohmer, V. Saenz, and C.L. Richards-Zawacki. 2019. Effects of hydroperiod on growth, development, survival and immune defenses in a temperate amphibian. *Functional Ecology* 33:1952–1961.
- Brühl, C. A., S. Pieper, and B. Weber. 2011. Amphibians at risk? Susceptibility of terrestrial

- amphibian life stages to pesticides. *Environmental Toxicology and Chemistry* 30:2465–2472.
- Butterfield, B.P., M.J. Lannoo, and P. Nanjappa. 2005. *Rana sphenocephala*. Pp. 586–587 *In* Amphibian Declines: The Conservation Status of United States Species. Lannoo, M.J. (Ed.). University of California Press, Berkeley, California, USA.
- Castellano, S., D. Seglie, A. Gazzola, L. Racca, S. Ciaralli, and O. Friard. 2022. The effects of intra- and interspecific competitions on personality and individual plasticity in two sympatric Brown Frogs. *Behavioral Ecology and Sociobiology* 76:1–16.
- Cooke, A.S. 1985. The deposition and fate of spawn clumps of the Common Frog *Rana temporaria* at a site in Cambridgeshire, 1971–1983. *Biological Conservation* 32:165–187.
- Craven, S.W., J.T. Peterson, M.C. Freeman, T.J. Kwak, and E. Irwin. 2010. Modeling the relations between flow regime components, species traits, and spawning success of fishes in warmwater streams. *Environmental Management* 46:181–194.
- Dastansara, N., S. Vaissi, J. Mosavi, and M. Sharifi. 2017. Impacts of temperature on growth, development, and survival of larval *Bufo (Pseudepidalea) viridis* (Amphibia: Anura): implications of climate change. *Zoology and Ecology* 27:228–234.
- Denver, R.J., N. Mirhadi, and M. Phillips. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondi* tadpoles to habitat desiccation. *Ecology* 79:1859–1872.
- Dmitriew, C.M. 2011. The evolution of growth trajectories: what limits growth rate? *Biological Reviews* 86:97–116.
- Dole, J.W. 1971. Dispersal of recently metamorphosed Leopard Frogs, *Rana pipiens*. *Copeia* 1971:221–228.
- Donaldson-Matasci, M.C., C.T. Bergstrom, and M. Lachmann. 2013. When unreliable cues are good enough. *American Naturalist* 182:313–327.
- Dudgeon, D., A.H. Arthington, M.O. Gessner, Z.-I. Kawabata, D.J. Knowler, C. Lévêque, R.J. Naiman, A.-H. Prieur-Richard, D. Soto, M.J. Stiassny, and C.A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81:163–182.
- Earl, J.E., and H.H. Whiteman. 2015. Are commonly used fitness predictors accurate? A meta-analysis of amphibian size and age at metamorphosis. *Copeia* 103:297–309.
- Emerson, S.B. 1985. Skull shape in frogs: correlations with diet. *Herpetologica* 41:177–188.
- Emerson, S.B., and D.M. Bramble. 1993. Scaling, allometry, and skull design. *Skull* 3:384–421.
- Eskew, E.A., S.J. Price, and M.E. Dorcas. 2012. Effects of river-flow regulation on anuran occupancy and abundance in riparian zones. *Conservation Biology* 26:504–512.
- Fehlmann, G., M.J. O’Riain, C. Kerr-Smith, S. Hailes, A. Luckman, E.L. Shepard, and A.J. King. 2017. Extreme behavioural shifts by baboons exploiting risky, resource-rich, human-modified environments. *Scientific Reports* 7:1–8. <https://doi.org/10.1038/s41598-017-14871-2>.
- Ficetola, G.F., and F. De Bernardi. 2003. Influence of hydroperiod, sun exposure and fish presence on amphibian communities in a human dominated landscape. *Herpetologica Petropolitana* 12:140–142.
- Freeman, M.C., Z.H. Bowen, K.D. Bovee, and E.R. Irwin. 2001. Flow and habitat effects on juvenile fish abundance in natural and altered flow regimes. *Ecological Applications* 11:179–190.
- Frisch, D., and B. Santer. 2004. Temperature-induced responses of a permanent-pond and a temporary-pond cyclopoid copepod: a link to habitat predictability? *Evolutionary Ecology Research* 6:541–553.
- Gervasi, S.S., and J. Foufopoulos. 2008. Costs of plasticity: responses to desiccation decrease post-metamorphic immune function in a pond-breeding amphibian. *Functional Ecology* 22:100–108.
- Gido, K.B., D.L. Propst, J.D. Olden, and K.R. Bestgen. 2013. Multidecadal responses of native and introduced fishes to natural and altered flow regimes in the American Southwest. *Canadian Journal of Fisheries and Aquatic Sciences* 70:554–564.
- Goldberg, T., E. Nevo, and G. Degani. 2012. Phenotypic plasticity in larval development of six amphibian species in stressful natural environments. *Zoological Studies* 51:345–361.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- Guzy, J.C., E.A. Eskew, B.J. Halstead, and S.J. Price. 2018. Influence of damming on anuran species richness in riparian areas: a test of the serial discontinuity concept. *Ecology and Evolution* 8:2268–2279.
- Jacobs, J.D., and J.C. Wingfield. 2000. Endocrine control of life-cycle stages: a constraint on response to the environment? *Condor* 102:35–51.

- Knapp, A.K., C. Beier, D.D. Briske, A.T. Classen, Y. Luo, M. Reichstein, M.D. Smith, S.D. Smith, J.E. Bell, P.A. Fay, et al. 2008. Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience* 58:811–821.
- Kupferberg, S.J., W.J. Palen, A.J. Lind, S. Bobzien, A. Catenazzi, J.O.E. Drennan, and M.E. Power. 2012. Effects of flow regimes altered by dams on survival, population declines, and range-wide losses of California river-breeding frogs. *Conservation Biology* 26:513–524.
- Leips, J., M.G. McManus, and J. Travis. 2000. Response of treefrog larvae to drying ponds: comparing temporary and permanent pond breeders. *Ecology* 81:2997–3008.
- Lenth, R. 2019. Emmeans: estimated marginal means, aka least-squares means. R package V 1.4.3.01. <https://CRAN.R-project.org/package=emmeans>.
- Loman, J. 1999. Early metamorphosis in Common Frog *Rana temporaria* tadpoles at risk of drying: an experimental demonstration. *Amphibia-Reptilia* 20:421–430.
- Luhring, T.M. 2013. Complex life-histories and biogeochemical cycles; interactions between amphibian life-history strategies and elemental cycling. Ph.D. Dissertation. University of Missouri, Columbia, Missouri, USA. 139 p.
- MacCracken, J.G., and J.L. Stebbings. 2012. Test of a body condition index with amphibians. *Journal of Herpetology* 46:346–350.
- McAllister, D.E., A.L. Hamilton, and B. Harvey. 1997. Global freshwater biodiversity: striving for the integrity of freshwater ecosystems. *Sea Wind* 11:1–139.
- Miner, B.G., and J.R. Vonesh. 2004. Effects of fine grain environmental variability on morphological plasticity. *Ecology Letters* 7:794–801.
- Miner, B.G., S.E. Sultan, S.G. Morgan, D.K. Padilla, and R.A. Relyea. 2005. Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution* 20:685–692.
- Marques, S. M., S. Chaves, F. Gonçalves, and R. Pereira. 2013. Evaluation of growth, biochemical and bioaccumulation parameters in *Pelophylax perezi* tadpoles, following an *in-situ* acute exposure to three different effluent ponds from a uranium mine. *Science of the Total Environment* 445:321–328.
- Newman, R.A. 1992. Adaptive plasticity in amphibian metamorphosis. *BioScience* 42:671–678.
- Newman, R.A. 1998. Ecological constraints on amphibian metamorphosis: interactions of temperature and larval density with responses to changing food level. *Oecologia* 115:9–16.
- Nyingi, D.W., N. Gichuki, and M.O. Ogada. 2013. Freshwater ecology of Kenyan highlands and lowlands. *Developments in Earth Surface Processes* 16:199–218.
- Peig, J., and A.J. Green. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* 118:1883–1891.
- Piccinetti, C.C., L. Grasso, F. Maradonna, G. Radaelli, C. Ballarin, G. Chemello, J.O. Evjemo, O. Carnevali, and I. Olivotto. 2017. Growth and stress factors in Ballan Wrasse (*Labrus bergylta*) larval development. *Aquaculture Research* 48:2567–2580.
- Piman, T., T.A. Cochrane, M.E. Arias, A. Green, and N.D. Dat. 2013. Assessment of flow changes from hydropower development and operations in Sekong, Sesan, and Srepok rivers of the Mekong Basin. *Journal of Water Resources Planning and Management* 139:723–732.
- Pintar, M.R., and W.J. Resetarits, Jr. 2018a. Variation in pond hydroperiod affects larval growth in Southern Leopard Frogs, *Lithobates sphenoccephalus*. *Copeia* 106:70–76.
- Pintar, M.R., and W.J. Resetarits, Jr. 2018b. Refilling temporary ponds has timing-dependent effects on *Hyla gratiosa* performance. *Freshwater Biology* 63:1550–1559.
- Reed, T.E., R.S. Waples, D.E. Schindler, J.J. Hard, and M.T. Kinnison. 2010. Phenotypic plasticity and population viability: the importance of environmental predictability. *Proceedings of the Royal Society B: Biological Sciences* 277:3391–3400.
- Rose, C.S. 2005. Integrating ecology and developmental biology to explain the timing of frog metamorphosis. *Trends in Ecology & Evolution* 20:129–135.
- Schmuck, R., W. Geise, and K. E. Linsenmair. 1994. Life cycle strategies and physiological adjustments of reedfrog tadpoles (Amphibia, Anura, Hyperoliidae) in relation to environmental conditions. *Copeia* 1994:996–1007.
- Semlitsch, R.D. 1987. Pedomorphosis in *Ambystoma talpoideum*: effects of density, food, and pond drying. *Ecology* 68:994–1002.
- Semlitsch, R.D., and H.M. Wilbur. 1988. Effects of pond drying time on metamorphosis and survival in the salamander *Ambystoma talpoideum*. *Copeia* 1988:978–983.

- Skelly, D.K. 1996. Pond drying, predators, and the distribution of *Pseudacris* tadpoles. *Copeia* 1996:599–605.
- Stallard, R.F. 2001. Possible environmental factors underlying amphibian decline in eastern Puerto Rico: analysis of US government data archives. *Conservation Biology* 15:943–953.
- Stearns, S.C. 1976. Life-history tactics: a review of the ideas. *Quarterly Review of Biology* 51:3–47.
- Stoler, A.B., and R.A. Relyea. 2013. Bottom-up meets top-down: leaf litter inputs influence predator–prey interactions in wetlands. *Oecologia* 173:249–257.
- Stoler, A.B., and R.A. Relyea. 2020. Reviewing the role of plant litter inputs to forested wetland ecosystems: leafing through the literature. *Ecological Monographs* 90:1–23. <https://doi.org/10.1002/ecm.1400>.
- Tejedo, M., F. Marangoni, C. Pertoldi, A. Richter-Boix, A. Laurila, G. Orizaola, A.G. Nicieza, D. Álvarez, and I. Gomez-Mestre. 2010. Contrasting effects of environmental factors during larval stage on morphological plasticity in post-metamorphic frogs. *Climate Research* 43:31–39.
- Thorpe, J.E., M. Mangel, N.B. Metcalfe, and F.A. Huntingford. 1998. Modelling the proximate basis of salmonid life-history variation, with application to Atlantic Salmon, *Salmo salar* L. *Evolutionary Ecology* 12:581–599.
- Werner, E.E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *American Naturalist* 128:319–341.
- Wheeler, C.A., J.B. Bettaso, D.T. Ashton, and H.H. Welsh, Jr. 2015. Effects of water temperature on breeding phenology, growth, and metamorphosis of Foothill Yellow-legged Frogs (*Rana boylei*): a case study of the regulated mainstem and unregulated tributaries of California's Trinity River. *River Research and Applications* 31:1276–1286.
- Wilson, M.W., A.D. Ridlon, K.M. Gaynor, S.D. Gaines, A.C. Stier, and B.S. Halpern. 2020. Ecological impacts of human-induced animal behaviour change. *Ecology Letters* 23:1522–1536.
- Xiao-Li, F.A.N., L.I.N. Zhi-Hua, and W.E.I. Jie. 2014. Effects of hydroperiod duration on developmental plasticity in Tiger Frog (*Hoplobatrachus chinensis*) tadpoles. *Zoological Research* 35:124–131.
- Zeitler, E.F., K.K. Cecala, D.A. McGrath. 2019. Tertiary wastewater treatment wetlands provide suitable habitat for amphibians. *Journal of Freshwater Ecology* 33:475–488.
- Zeitler, E.F., K.K. Cecala, and D.A. McGrath. 2021. Carryover effects minimize positive effects of treated wastewater on anuran development. *Journal of Environmental Management* 289:1–11. <https://doi.org/10.1016/j.jenvman.2021.112571>.



KOSTAS ANDRIOTIS is an undergraduate student from Greece studying in the Department of Biology at the University of the South, Sewanee, Tennessee, USA. He is interested in pursuing research at the intersection of animal health and environmental challenges. (Photographed by Kristen Cecala).



KEVIN FOUTS is the Wildlife Research Coordinator at the University of the South, Sewanee, Tennessee, USA. He received his B.A. and M.Sc. from the University of Georgia, Athens, USA. Kevin works with undergraduate students to coordinate long-term monitoring and management as well as faculty research. (Photographed by Buck Butler).



KRISTEN CECALA is an Associate Professor in the Department of Biology at the University of the South, Sewanee, Tennessee, USA. She received her Ph.D. in the Warnell School of Forestry and Natural Resources at the University of Georgia, Athens, USA, and her B.S. in Biology at Davidson College, Davidson, North Carolina, USA. Kristen works with undergraduate researchers to understand patterns of amphibian behavior and ecology in response to environmental change. (Photographed by Kristen Cecala).