

GEOGRAPHIC VARIATION IN THE EFFECTS OF HYDROPERIODS ON WOOD FROG
(*RANA SYLVATICA*) TADPOLES

A Thesis
by
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Abstract

GEOGRAPHIC VARIATION IN THE EFFECTS OF HYDROPERIODS ON WOOD FROG (*RANA SYLVATICA*) TADPOLES

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Environmental change is increasing climate variability (e.g., precipitation and temperature) globally. This can be detrimental to taxa, such as amphibians, that are sensitive to changes to environmental factors. Changes to breeding habitats (e.g., hydroperiod of temporary ponds) can lead to local population declines with mass mortality of embryos and tadpoles. Even when metamorphosis is possible in these shorter hydroperiod ponds, the potential of negative carry-over effects manifested in size at metamorphosis can have long-lasting effects on population persistence. To determine how different populations of Wood frogs (*Rana sylvatica*) would respond to shortened hydroperiods, I examined the effects of pond drying on the growth and survival of *R. sylvatica* tadpoles in a common garden experiment. I predicted that tadpoles from northern populations are more adapted to environmental variability compared to southern populations. As such, I predicted that northern populations would metamorphose earlier in response to pond drying than southern populations but would exhibit trade-offs including smaller sizes at metamorphosis in shortened hydroperiod environments. Pond drying significantly affected both mean size at metamorphosis and survival of tadpoles to metamorphosis.

Metamorphs from the longest hydroperiods had a larger mean size and higher survival than metamorphs from replicate pond communities with the shortest hydroperiods. The origin of egg clutches had no significant effect on mean size at metamorphosis but did affect survival to metamorphosis. The northern populations had the highest survival, and the southern populations had the lowest survival. My results suggest that populations of a widespread amphibian may vary in their ability to respond to environmental stressors. My study is also one of the first to examine and predictions of how changes to environmental parameters (via pond hydroperiod) may affect wood frog persistence across a latitudinal gradient.

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Dedication

I would like to dedicate this thesis to my husband William Tyler Bigler for this constant support before, during and after my research. There is no way I would have succeeded in my research without his support.

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Foreword

This thesis will be submitted to Journal of Zoology, a peer-reviewed journal. It has been formatted according to the style guide for that journal.

Introduction

Human-induced changes are increasing environmental variability (e.g., temperature and precipitation) on a global-wide scale (Karl, 2003; IPCC, 2014). Although increased environmental variability may be a global issue, not all ecosystems are being affected proportionally (Woodward et al., 2010). Disproportionate environmental variability can cause organisms to respond differently in accordance with local selective forces (Laugen et al., 2003; Laurila, Pakkasmaa & Merilä, 2001). For example, northern latitudes of the North America may be experiencing less variability in precipitation than southern latitudes (Figure 1). These changes in precipitation can be detrimental to freshwater ecosystems that depend heavily on rainfall and snowpack to function (Sievers et al., 2018). To persist, taxa in more variable these environments will have to shift their home ranges (Woodward et al., 2010) modify growth rates (Mawdsley, O'Malley & Ojima, 2009) or access different resources to survive (Chu & Karr, 2017; Mawdsley et al., 2009).

Amphibians are particularly susceptible to environmental change and populations are declining across the globe (Bonebrake & Mastrandrea, 2010; Carey & Alexander, 2003; Lips et al., 2008; 2010; Rollins & Benard, 2020). As ectotherms, amphibian growth and development are directly linked to environmental factors including temperature and precipitation (Berven & Gill, 1983). All amphibians require moist habitats to forage in and many need water for breeding (Blaustein et al., 2001). Thus, environmental variability that alters when water is available could be detrimental to many populations (Blaustein, Wake & Sousa, 1994). The suitability of breeding ponds for amphibians depends on the hydroperiod duration. This hydroperiod sets the length of time available for larval development (Wilbur, 1987). Reductions in the hydroperiod of temporary ponds i.e., (early drying) may result in desiccation of eggs and failure of larvae to

metamorphose (Blaustein et al., 1994; Rowe & Dunson, 1995; Ryan & Winne, 2001). Previous studies have shown that pond drying can influence life history traits such as growth, recruitment into the population, and timing of and size at metamorphosis (Rowe & Dunson, 1995; Ryan & Winne, 2001; Semlitsch & Wilbur, 1988; Wilbur, 1987). These traits are important to adult reproductive success in most species of amphibians (Berven, 1990; Earl & Whiteman, 2015; Riha & Berven, 1991; Semlitsch & Wilbur) and are often correlated with environmental gradients (Riha & Berven, 1991).

Previous studies have shown that the *R. sylvatica* larval periods are shorter in northern populations and increase toward the southern portion of their geographic range (Berven & Gill, 1983; Davenport, Fishback & Hossack, 2020; Davenport & Hossack, 2016; Davenport, Hossack & Fishback, 2017; Riha & Berven, 1991; Rollins & Benard, 2020). Previous studies on amphibians have also shown that populations at different altitudes may differ substantially in embryonic developmental rates, sizes at metamorphosis, and size at first reproduction (Martof & Humphries, 1959).

Hypotheses and Predictions

In this study, I examine the effects of hydroperiod on mass at metamorphosis and survival of Wood Frog (*Rana* [=*Lithobates*] *sylvatica*) tadpoles along a latitudinal gradient (Figure 1). I hypothesize that tadpoles from northern populations are more adapted to variability in precipitation and shorter growing seasons than southern populations who have less variability in precipitation. I predict that individuals in faster drying ponds will metamorphose early but at a smaller body size than individuals in slower drying ponds. I also predict that tadpoles from the northern populations will metamorphose early but may not have a trade-off relative to size when compared to southern populations.



Figure 1. Three locations of *Rana sylvatica* populations used in this study shown by black stars. The geographic range of the Wood Frog (*Rana sylvatica*) is shown in brown (Map Source: IUCN [International Union for Conservation of Nature], Conservation International & NatureServe. 2014. *Rana sylvaticus*. The IUCN Red List of Threatened Species. Version 2021-3).

Materials and Methods

Rana sylvatica are a widely distributed amphibian in North America (Martof & Humphries, 1959). Wood frogs range from Georgia north to the Arctic Circle in Alaska and Canada (Dodd, 2013). Wood frogs are the only North America frog to inhabit land above the Arctic Circle. *R. sylvatica* breed after the first warm rains of late winter or early spring depending on the region. Breeding in North Carolina occurs from February-March. In Ohio and Connecticut breeding occurs from March and April. Emergence of juvenile frogs occurs between 65-130 days. *Rana sylvatica* are a medium sized frog and vary in shades from brown to pinkish depending on sex. Female *R. sylvatica* are often larger and more brightly colored than males.

Experimental Design

Two-three *R. sylvatica* egg masses were collected from temporary ponds in Boone North Carolina (36.2168° N, 81.6746° W); Athens, Ohio (39.3292° N, 82.1013° W); and Yale Forest Connecticut (41.9547° N, 72.1204° W). Egg masses were mailed from source population to ASU and were kept in small plastic tubs until hatched. Once tadpoles had fully hatched six tadpoles were chosen randomly from each of the egg masses and placed in 19 L white plastic buckets to create small replicate pond communities. Similar densities of *R. sylvatica* tadpoles have been used in other experimental studies on *R. sylvatica* and other larval amphibians (Amburgey et al., 2012; Mogali, Saidapur & Shanbhag, 2011; Rowe & Dunson, 1995).



Figure 2. Wood Frog egg masses in a temporary pond near Boone NC on March 02, 2021.

Each experimental bucket included approximately 4 grams of mixed hardwood leaf litter (primarily *Fagus grandifolia*, *Quercus alba*, and *Quercus rubra*) collected from Boone, NC. Leaf litter acted as cover for the tadpoles, and mimicked natural habitat conditions (Rowe & Dunson, 1995). Approximately 30% of the total water volume in each bucket was changed twice weekly (Laugen et al., 2003; Laurila et al., 2001;) to control water quality and removal of excessive metabolic waste produced by the tadpoles. The buckets were arranged so that each population was replicated independently in a climate-controlled room at the vivarium of Appalachian State University. Tadpoles were fed an 8:1 mixture of rabbit chow pellets and fish flakes once weekly for the first 3 feedings and then mixture was lowered to 5:1 rabbit pellets and fish flakes to control waste production and reduce uneaten food in the bucket (Laurila & Kujasalo, 1999; Rowe & Dunson, 1995).

I deployed a 3x3 factorial design where I introduced *R. sylvatica* tadpoles from North Carolina, Ohio, and Connecticut to three pond drying regimes. Each of these regimes were replicated eight times for a total of 24 experimental units per source population (except for Connecticut) due to space limitations in the ASU vivarium. Connecticut populations were replicated 6 times for a total of 18 experimental units due to lack of space in the vivarium. The three drying regimes in this experiment were 50 days, 65 days, and 80 days to imitate a fast, intermediate, and slow drying pond. This range of dates was chosen based on *R. sylvatica* growth and development data reported in previous research (Davenport et al., 2017; Rowe & Dunson, 1995; Ryan & Winne, 2001; Thompson & Popescu, 2021). Temperature was held constant at 18-19 degrees C°. The amount of water drained from each bucket to simulate pond drying was decided according to the expression $D_j = 1 - (j/t)^a P$. D_j is the desired depth on day j , j is the time in days since the start of the experiment, t is the target date for the depth in buckets to reach zero,

P is the maximum depth at the start of the experiment, and a is a shape parameter for the curves (Wilbur, 1987). This technique has been used in previous studies investigating the effects of drying on larval amphibians, including *R. sylvatica* (Davenport et al., 2017; Rowe & Dunson, 1995; Semlitsch & Wilbur, 1988; Wilbur, 1987). Since each geographic location has a different wood frog breeding time, the total number of concurrent replicates was staggered in the ASU Vivarium at one time. A random number generator was used to randomly assign experimental treatments to a spatial block.

Collection of Data

At the end of each experimental drying regime, measurements of tadpoles or metamorphs were collected. Data collection for tadpoles and metamorphs were conducted differently based on stage of metamorphosis. Measurements collected regardless of Gosner stage were mass or mass at metamorphosis, SVL (snout vent length) and Gosner stage. After an experimental period had ended, all remaining tadpoles were removed from the 19L buckets, counted, and transferred to individual deli cups for the measuring process. The tadpoles were then transferred to the lab in Rankin Hall at Appalachian State University. Mass for tadpoles was calculated by weighing the tadpoles suspended in water in grams. The tadpole was then anesthetized with liquid oragel and SVL was measured. Once mass and SVL were measured the tadpole was transferred to a specimen cup containing 70% ethanol and refrigerated for preservation.

Survival of metamorphs was defined by forelimb emergence and having a tail length of less than 0.2 mm (Rowe & Dunson, 1995). Metamorphs were collected from the buckets as forelimbs emerged to reduce risk of drowning. Metamorphs were then transferred to deli cups with a wet piece of paper towel until the tadpole tail fully absorbed. Once the tail fully absorbed, body mass and SVL (snout vent length) were measured. Body mass of metamorphs were

collected by blotting the individual dry and weighing them in grams (Riha & Berven, 1991; Rowe & Dunson, 1995). Metamorphs were then anesthetized with liquid orajel. After metamorphs had been anesthetized, SVL values were recorded. Metamorphs were then transferred to a specimen cup containing 70% ethanol for preservation.

Data Analysis

No tadpoles survived to metamorphosis in the 50-day drying regime. For this reason, I ran separate models for the 50-day drying regime and the 65-day/80-day drying regimes. For the 50-day drying regime, I used a general linear model to test hypotheses related to tadpole survival, Gosner stage and mass (g) at end of experiment for all populations. I also used a Tukey's post hoc test to examine treatment differences in mass among treatments.

I used generalized linear models to test how tadpole survival (proportion metamorphosed by the end of the experiment) and size at metamorphosis responded to variation in experimental drying regimes for the 65-day and 80-day drying regimes. Experimental blocks were treated as random effects because any effects are assumed to be random with the random assignment of experiment units. Non-significant blocks were removed from the analysis. The two experimental factors (drying regime and source population) were treated as fixed effects. For each population, my response variables included survival to metamorphosis and size at metamorphosis.

Results

50-Day Drying Regime

Only 1 tadpole out of the 125 total tadpoles metamorphosed from the 50-day drying treatments. The lone survivor to metamorphosis was from the Connecticut population. No statistical differences were detected in survival of tadpoles across all buckets ($F=1.057$, $P=0.284$;

Fig. 3a). Gosner stage was statistically different among populations ($F=2.670$, $P=0.024$; Fig. 3b). Mass at metamorphosis was also significantly different among populations ($F=11.200$, $P=0.004$; Fig. 3c). Tukey's post-hoc analysis indicated that North Carolina populations had significantly smaller mass in comparison to Ohio and Connecticut populations at the end of the 50-day drying regime.

65 and 80-Day Drying Regimes

Drying had a significant effect on mean survival to metamorphosis ($F=8.903$, $P=0.009$; Fig. 4) with more wood frog tadpoles surviving to metamorphosis in the 80-day drying vs. 65-day drying regime. Source population also had a significant effect on mean survival ($F=18.613$, $P<0.001$) with Connecticut population having the highest survival and North Carolina having the lowest survival (Fig. 5). The effect of source population and drying interaction had a significant effect on mean survival ($F=4.157$, $P=.028$; Fig. 6) with Connecticut having the highest survival in both the 65-day and 80-day drying regimes and North Carolina having the lowest survival in the 65-day and 80-day regimes.

Source population had no statistically significant effect on mass at metamorphosis ($F=1.160$, $P=0.455$; Fig. 7). However, drying had a significant effect on mass at metamorphosis ($F=5.537$, $P=0.021$; Fig. 8) with metamorphs from the 80-day drying regime having higher mass at metamorphosis compared to metamorphs from the 65-day drying regime. The interaction of source population and drying had no effect on mass at metamorphosis ($F=0.528$, $P=0.573$; Fig 9).

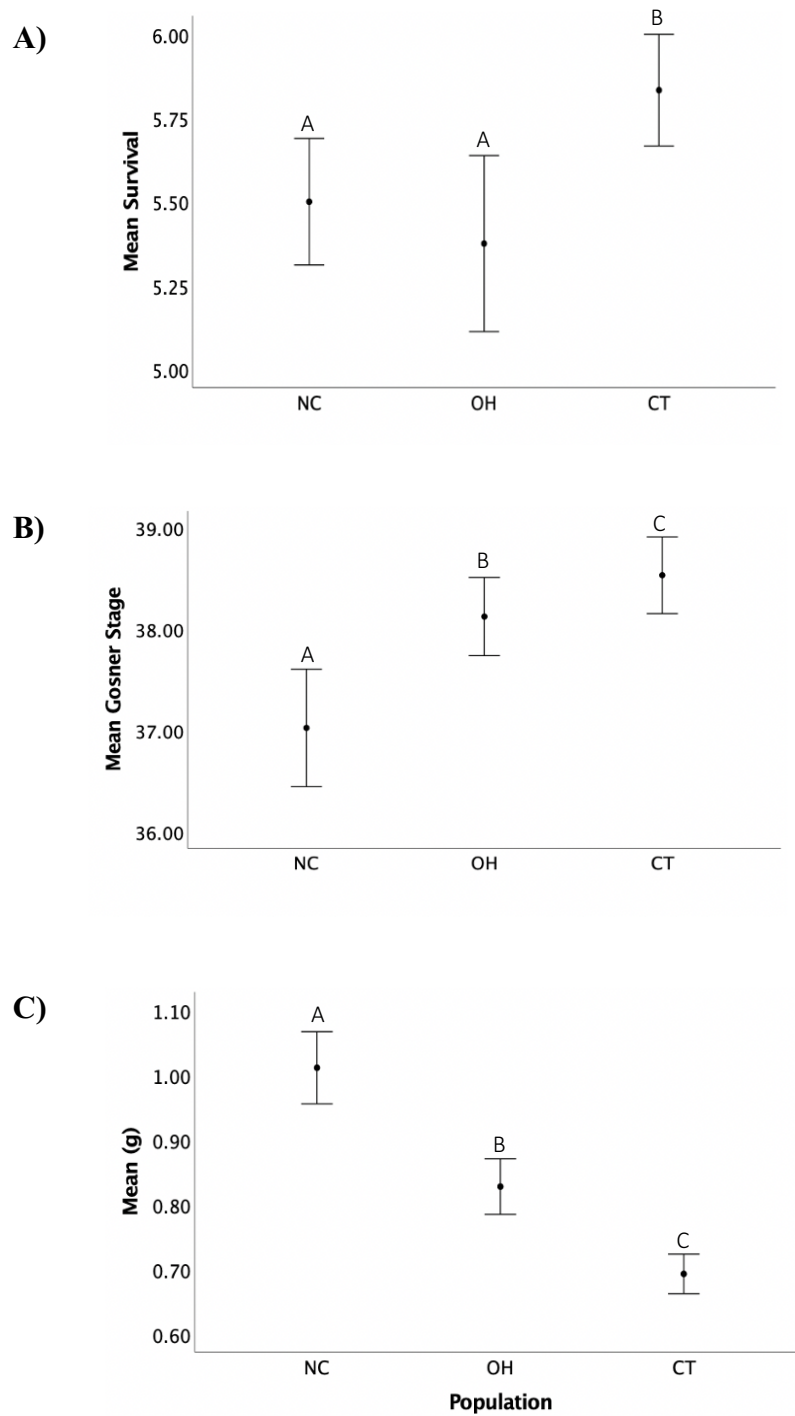


Figure 3. Mean (± 1 standard error) of wood frog tadpoles **A)** survival to metamorphosis; **B)** Gosner stage; **C)** wet mass (g) at end of 50-day hydroperiod treatment by source populations (NC=North Carolina, OH= Ohio and CT= Connecticut). N=8 except for CT where N=6.

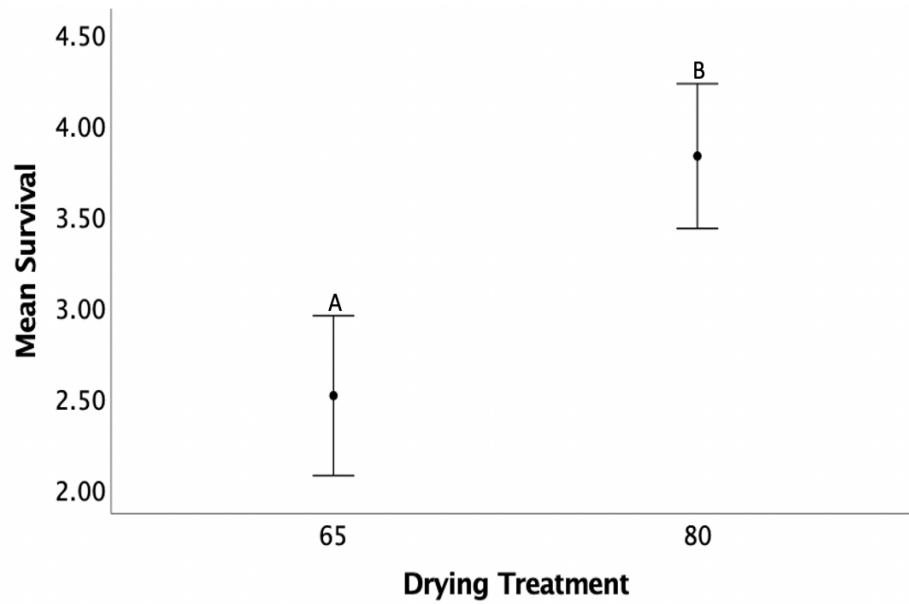


Figure 4. Mean wood frogs survival to metamorphosis (± 1 standard error) in two hydroperiod treatments (65= 65 days, 80= 80 days). Means are pooled across all three source populations. Different letters above error bars indicate significant differences among groups.

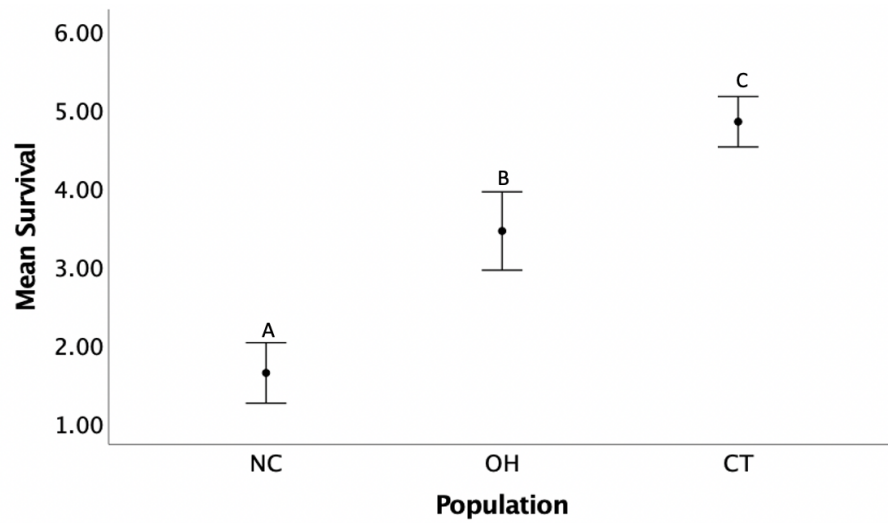


Figure 5. Mean wood frog survival to metamorphosis (± 1 standard error) by population (NC=North Carolina, OH= Ohio and CT= Connecticut). Different letters above error bars indicate significant differences among groups. N=8 except CT where N=6.

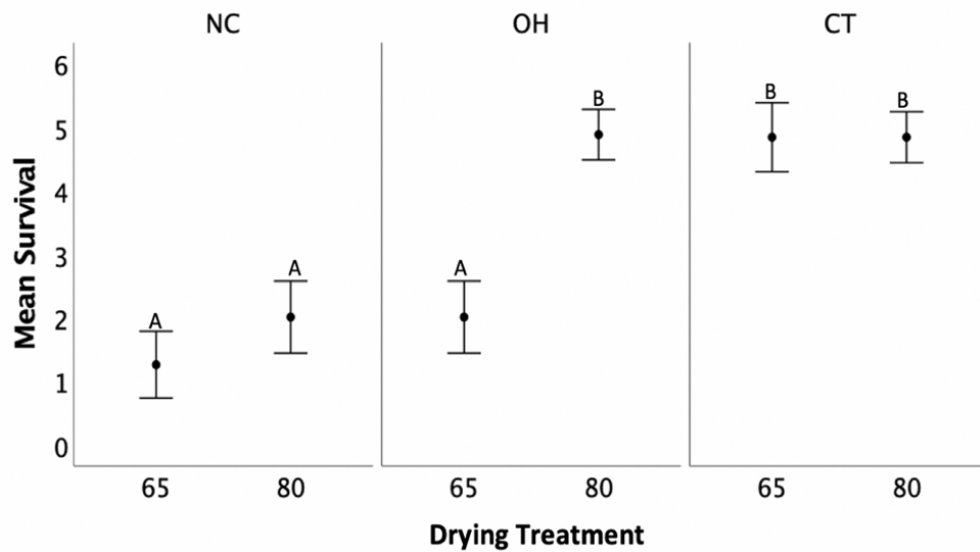


Figure 6. Mean wood frog survival to metamorphosis ± 1 (standard error) in two hydroperiod treatments (65= 65 days, 80= 80 days) by population (NC=North Carolina, OH=Ohio, CT=Connecticut). Different letters above error bars indicate significant differences among groups. N=8 except CT where N=6.

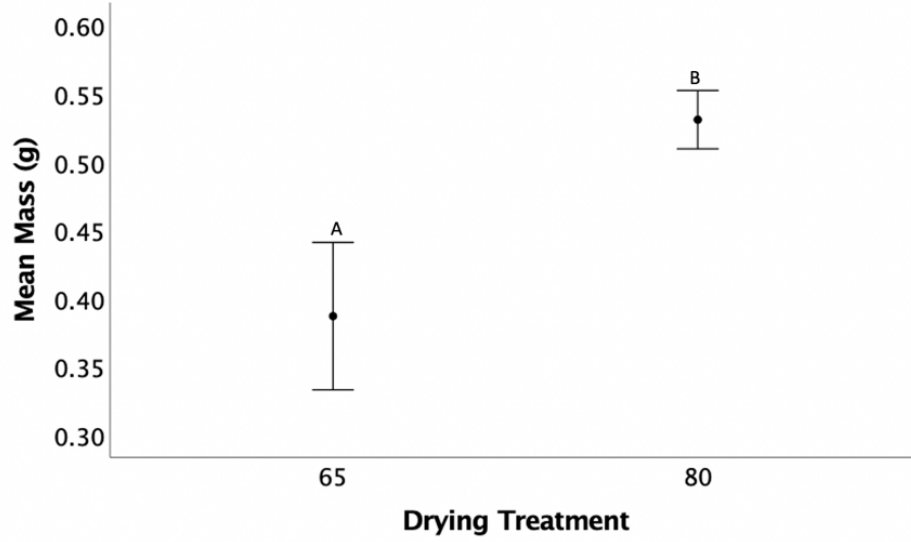


Figure 7. Mass (g) of larval wood frogs surviving to metamorphosis (mean and +/-1 standard error) in two hydroperiod treatments (65= 65 days, 80= 80 days). Different letters above error bars indicate significant differences among groups. N=8 except for CT = 6.

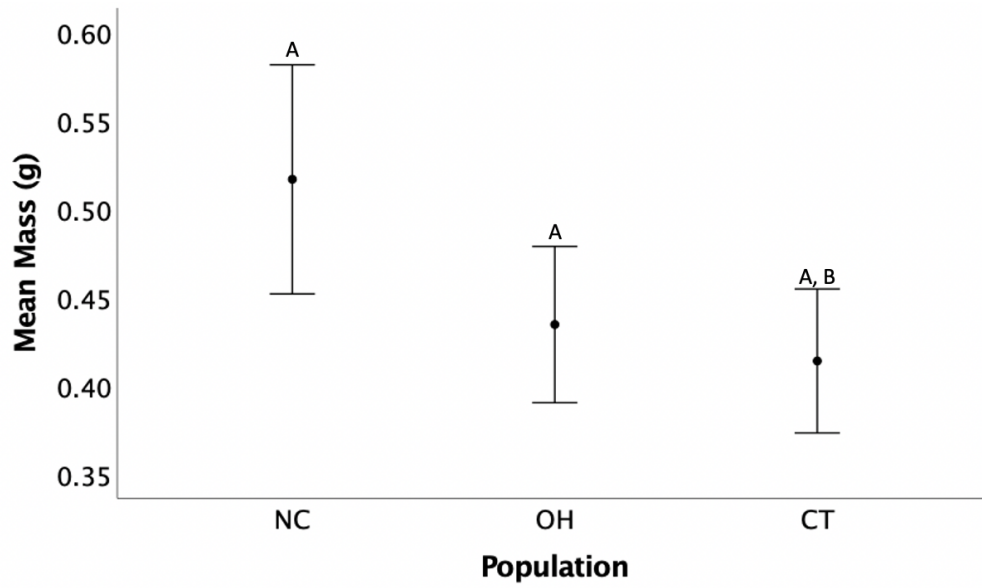


Figure 8. Mean mass (g) of wood frogs surviving to metamorphosis (± 1 standard error) by population (NC=North Carolina, OH= Ohio and CT= Connecticut). Different letters above error bars indicate significant differences among groups. N=8 except CT where N=6.

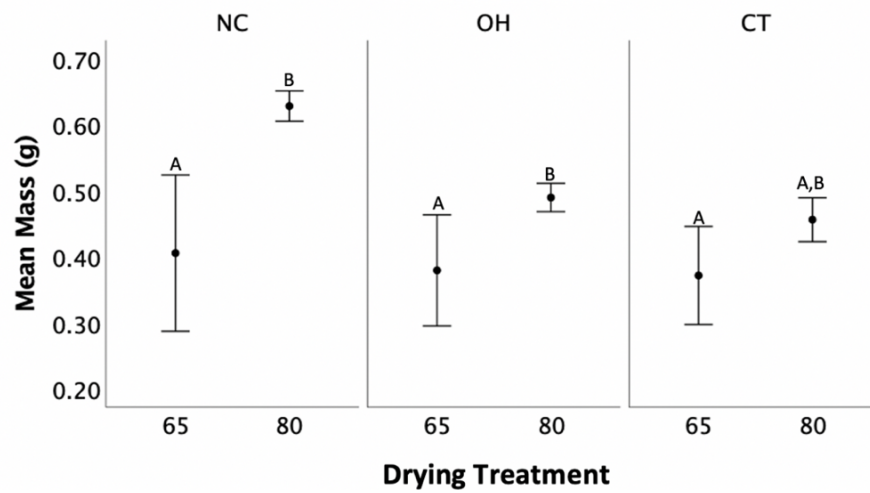


Figure 9. Mass (g) of larval wood frogs surviving to metamorphosis (mean and +/-1 standard error) in two hydroperiod treatments) 65=65 days, 80= 80 days) by population (NC=North Carolina, OH=OH, CT=Connecticut). Different letters above error bars indicate significant differences among groups. N=8 except for CT = 6.

Discussion

Human-induced environmental change is predicted to result in shorter and more variable hydroperiods in wetlands (Rowe & Dunson, 1995). To gain insight into the role of geographic variation and how a wide-ranging amphibian responds to more variable hydroperiods, I conducted a common garden experiment with *R. sylvatica* tadpoles from Boone, North Carolina; Athens, Ohio; and Yale Forest, Connecticut. The results from this experiment demonstrate a latitudinal cline in developmental rates and survival of *R. sylvatica* tadpoles in response to shortened drying regimes. The expected outcome that tadpoles in shorter experimental drying regimes would have a lower survival rate and smaller body size were met in this experiment. The amount of time water was held in experimental buckets significantly affected survival and showed a decrease in body size of *R. sylvatica* tadpoles and metamorphs. The expectation that northern populations would also have a higher survival rate, but would not have a trade-off relative to size, was also met in this experiment. Previous research on *R. sylvatica* has shown wood frogs exhibit counter-gradient variation within vertebrates and the results from this experiment support that the effects of hydroperiods follow this counter-gradient response to abiotic factors.

I found that drying regime can significantly affect *R. sylvatica* survival to metamorphosis of *R. sylvatica* tadpoles. Additionally, I found that survival to metamorphosis was higher when ponds held water longer and survival decreased in more rapidly drying ponds (Figure 4). Higher survival rates in ponds that held water for longer periods of time has been noted in previous research on *R. sylvatica* and congeners (Davenport et al., 2017; Lent & Babbitt, 2020; Rowe & Dunson, 1995; Thompson & Popescu, 2021). Rowe and Dunson (1995) investigated how three pond drying regimes (56 days, 84 days, and 158 days) affected larval *R. sylvatica*, *Ambystoma*

jeffersonianum, and *Ambystoma maculatum* larvae from temporary wetlands in central Pennsylvania. They found a similar trend in survival for *R. sylvatica* tadpoles with no metamorphic survival in ponds drying in 56 days and the highest survival in the 84-day drying regime. In the 158-day drying regime, only 14% of the *R. sylvatica* tadpoles survived to metamorphosis. The authors attributed lower survival rates to predatory *Ambystoma jeffersonianum*, and *Ambystoma maculatum* larvae. *R. sylvatica* egg masses from Zaleski State Forest, Ohio (near the population of Ohio tadpoles used in my experiment), were used to evaluate carryover effects of hydroperiod length (50–62 days) on *Rana sylvatica* tadpoles (Thompson & Popescu, 2021). Thompson and Popescu (2021) also found higher survival rates in the 62-day drying regime when compared to the 50-day drying regime. Results from my study along with results from previous studies support the hypothesis that *R. sylvatica* may be unable to survive to metamorphosis until they reach a certain pond drying threshold. These results reveal that wood frogs have some capacity to respond to environmental changes. However, the capacity of *R. sylvatica* to respond to changing drying regimes may be affected by geographic location.

R. sylvatica tadpoles from shorter drying regimes were smaller on average than those ponds with longer (Figure 7). This result supports existing studies of phenotypic plasticity of this species to environmental stressors. (Davenport & Hossack, 2016; Rowe & Dunson, 1995; Thompson & Popescu, 2021). Wilbur and Collins (1973) suggest that pond breeding amphibians escaping from a deteriorating environment, such as a quickly drying pond, should exhibit a trade-off with faster development but a smaller size at metamorphosis. Previous studies have shown that size and timing at metamorphosis is related to juvenile survival and adult reproductive fitness. (Berven & Gill, 1983; Earl & Whiteman, 2015; Riha & Berven, 1991; Semlitsch &

Wilbur, 1988). Larger metamorphs are more likely to be recruited into the population, survive to the age at which first reproduction occurs, and larger females lay more eggs (Berven, 1982; Davenport & Hossack, 2016; Wilbur, 1980). Therefore, larger body size at metamorphosis is beneficial to many amphibians (Berven & Gill, 1983; Bredeweg et al., 2019; Semlitsch & Wilbur, 1988).

Recent studies have also found that size at metamorphosis in amphibians may have carry-over effects on juvenile endurance and behavior (Bredeweg et al., 2019; Thompson & Popescu, 2021; Yagi & Green, 2018). Thompson and Popescu (2021) performed endurance trials to investigate juvenile locomotive performance on *R. sylvatica* metamorphs previously raised in differing drying regimes. From these trials, they found that metamorphs from shorter drying regimes had overall decreased endurance capability. Post-metamorphic frogs also had slower growth rates and remained smaller over time compared to frogs from longer drying regimes. This suggests that *R. sylvatica* tadpoles from ponds with longer hydroperiods are being recruited at a larger body size after metamorphosis and have higher survive rates to reproductive maturity with compared to their smaller counterparts. Bredeweg et al. (2019) evaluated movement behavior of juvenile *Rana aurora* (Northern Red-legged Frogs) after being raised in permanent or drying pools. Post metamorphic movement behavior is defined by the tendency to move away from the natal pools. Movement distance under a moist, low-stress terrain or a dry, high-stress terrain were tested in this experiment. Bredeweg et al. (2019) found that *R. aurora* from permanent water ponds metamorphosed at a larger size than individuals reared in water levels that were gradually lowered and that body size at metamorphosis is a strong predictor of behavioral movement. Movement from the start location and the distance traveled by post-metamorphic frogs can give insight into the success metamorphs post recruitment (Bredeweg et al., 2019).

R. sylvatica range from Georgia north to the Arctic Circle in Alaska and Canada (Dodd, 2013) and therefore populations are likely to experience wide variation to environmental conditions. The threshold needed to complete metamorphosis differs with source population. I found a significant interaction between drying regime and source population on survival of *R.* tadpoles and metamorphs. Survival to metamorphosis increased with latitude and longer drying regime (Figure 6). *R.* tadpoles from Manitoba, Canada (58.7381° N, 93.8225° W) and Durham, New Hampshire (43.1340° N, 70.9264° W) under shorter drying regimes (45 day/54 day in Canada and 50 days in New Hampshire) in both natural and experimental settings (Davenport et al., 2017; Dodd, 2013; Lent & Babbitt, 2020). In lower latitudes, such as Zaleski State Forest, Ohio (39.2905° N, 82.3901° W), *R. sylvatica* tadpoles were found to have low survival in shorter (50 days) drying regimes when compared to the 62-day drying regime (Thompson & Popescu, 2021).

Although my results suggest that northern populations have more adaptive potential to survive shorter growing periods, I did not find a significant interaction between source population and drying on size at metamorphosis. This suggests that northern populations may not exhibit a trade-off relative to size in response to drying. Riha and Berven (1991) found a similar pattern in *R. sylvatica* tadpoles from natural ponds in Michigan, Maryland, and Virginia. Their study revealed that MI tadpoles grew faster, had shorter larval periods, and were larger at metamorphosis than populations in either MD or VA. The rationale for this lack of trade-off in size may be that northern populations of *R. sylvatica* tadpoles are genetically adapted to withstand environmental pressures and have no ability to alter development rate with size at metamorphosis. In this case, *R. sylvatica* tadpoles demonstrate counter-gradient variation in response to drying ponds. Counter-gradient variation is a term used to explain a geographic

pattern of variation where genetic influences on a trait work against environmental influences across an environmental gradient. Thus, reducing phenotypic change across the environmental gradient (Laugen et al., 2003). This type of variation has been reported in research of *Rana sylvatica* and other ranid frogs (Berven, 1982; Davenport & Hossack, 2016; Laugen et al., 2003). Laugen et al. (2003) suggest that development is positively correlated with latitude but may be difficult to interpret without investigating local adaptation to a selective factor closely associated with latitude.

Changes in the Earth's climate are increasing environmental variability in temperature and precipitation and the impacts are widespread, particularly for freshwater ecosystems (Karl, 2003; Woodward et al., 2010). Shorter hydroperiods are among the many potential stressors negatively affecting freshwater ecosystems and subsequent larval amphibian survival (Amburgey et al., 2012; Blaustein et al., 1994; Thompson & Popescu, 2021). The range of climate variability across regions is disproportionate and likely results in geographic variation in local adaptation (Amburgey et al., 2018; Davenport & Hossack, 2016; Laugen et al., 2003). Differences in the adaptative potential of larval amphibians may have cascading carry-over effects on future reproductive success and population persistence. Therefore, it is important to identify the limits of adaptation to change in populations. The interaction between life-history traits and the environmental factors that increase susceptibility to stressors (including changing hydroperiods) have been noted as a possible cause for these declines (Davenport & Hossack, 2016; Sodhi et al., 2008). Though *R. sylvatica* is not an imperiled species, it is a great model organism with which to investigate interactions between geographic variation and environmental variation on life history traits because it is so widely distributed (Dodd, 2013). Identifying the adaptability of *R. sylvatica* tadpoles to changing hydroperiods and other abiotic factors, may help researchers

identify effects of hydroperiod affiliation on endangered taxa. Further investigation of sensitive taxa will prove useful in predicting the resiliency of local populations to climatic changes in the future.

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Vita

Anna Nicole Bricchetto was born in Denver, Colorado. Shortly after her parents, John Bricchetto and Lisa Bricchetto, moved to Atlanta, Georgia where Anna Bricchetto spent much her childhood. Her family then proceeded to live in Wyoming and finally settled in Knoxville, Tennessee. This is time that Anna grew to love the mountains and spent much of her childhood in the Great Smoky Mountains National Park. It was at ths National Park that Anna began to enjoy catching salamanders and frogs and her love for amphibians developed. Anna Bricchetto attended Maryville College in the fall of 2013, where she earned her Bachelor of Arts in 2017. Anna majored in Environmental Studies and minored in Environmental Science, Biology and Sociology while at Maryville College. After spending 4 years working various research assistant jobs in the field of biology, she decided to return to academia to pursue her passion for ecology, salamanders, and frogs. Anna started her study towards a Master of Science in Biology at Appalachian State University in 2020 and received this degree in May 2022.