2013

The Effects of Temperature Frequency and Magnitude on Lithobates sylvaticus and Hyla versicolor

Diana Cristina Macklem
University of Connecticut - Storrs

The Effects of Temperature Frequency and Magnitude on *Lithobates sylvaticus* and *Hyla versicolor*

D. Cristina Macklem, Jason H. O'Connor, and Tracy A. G. Rittenhouse

Department of Natural Resources and the Environment,

University of Connecticut, Storrs, CT

2013

Introduction:

Amphibians are recognized as excellent indicators for the overall health of an ecosystem since they tend to be more susceptible to the effects of ecosystem alterations than other taxa (Guzy *et al* 2012; Niemi & McDonald 2004; Price *et al* 2007; Welsh & Ollivier 1998). Amphibians are more vulnerable because they are ectotherms with permeable skin and shell-less eggs that require suitable terrestrial and aquatic habitats (Duellman & Trueb 1986). Unfortunately, this susceptibility has caused amphibian populations to decline worldwide from habitat alterations, pollution, invasive species, and disease at rates higher than birds and mammals (GAA; IUCN et al. 2004). The changing global climate also poses a new threat to amphibian populations since amphibian growth and development is strongly influenced by temperature and moisture (Atkinson 1996, Rohr & Palmer 2013).

Temperature can induce chemical and morphological changes during the anuran larval period with warm temperatures typically causing faster growth and a smaller size at metamorphosis than colder temperatures (Smith-Gill & Berven 1979; Walsh, Downie, & Monaghan 2008). Predictive climate change models for the Northeastern part of the United States show increases in temperature by as much as 3 degrees Celsius by 2050 and almost 6 degrees Celsius by 2100 (Hayhoe *et al* 2008). The cause of amphibian declines worldwide is likely a combination of climate change and the aforementioned problems facing amphibians (Rohr & Palmer 2013; Kiesecker, Blaustein, & Belden 2001; Collins & Storfer 2003). With support for climate-related phonological shifts and for climate change exacerbating the detrimental effects from habitat use, pollution, and disease, it is imperative that we try to gain a better understanding of how climate change and its interactions will affect amphibian growth and survival (Li, Cohen, & Rohr 2013).

Much of the research on the effects of climate change on amphibians focuses on these predicted mean increases in temperature (Bustamante, Livo, & Carey 2010; Rohr & Palmer 2013), which, although useful, does not accurately represent the temperature regime amphibians are subjected to in nature. Temperatures vary daily, weekly, and seasonally and, with climate change, this variability is likely to increase in frequency and magnitude (Ganguly *et al* 2009). Thus, in order to truly grasp the implications of climate change, temperature variability should be a fundamental aspect of amphibian research. An experiment done by Niehaus *et al* 2006 tested the effects of diurnal temperature fluctuations compared to a constant temperature on the development of *Limnodynastes peronii.* Tadpoles from that experiment metamorphosed earlier, at a smaller mass, and in poorer condition compared to the tadpoles kept at a constant temperature. It is for this reason that we wanted to investigate the effect of a variable temperature environment on the growth of two larval frogs species: Wood Frogs (*Lithobates sylvaticus*) and Gray Tree Frogs (*Hyla versicolor*), which were chosen because they are readily abundant in the Northeast United States, and because they are contrasting spring and summer breeding species with larvae that do not overwinter.

In our experiment, we tested the effect of six-degree temperature fluctuations at either a weekly or biweekly interval on the age at metamorphosis and the mass at metamorphosis. We also tested the effect of a single, six-degree temperature spike on size and age at metamorphosis and examined whether the timing of the temperature spike (whether it occurs during early or late larval development) was important. Similar to the findings of Niehaus et al 2006, we predicted that increasing the temperature variability and increasing the magnitude of variability would act as a potential stressor, creating tadpoles that metamorphose earlier at a smaller final mass.

Methods:

Experimental Design

We performed two laboratory experiments to test the effect of temperature variability (5 factor levels) on the mass and age at metamorphosis of Wood Frogs and Gray Tree Frogs. The treatments included a constant control, a fluctuating weekly temperature regime, a fluctuating biweekly temperature regime, an extreme temperature fluctuation early in development, and an extreme temperature fluctuation late in development. The experiments were conducted in a cold room in the Aquatics Animal Care Facility at the University of Connecticut, Storrs, CT between 5 May 2013 and 17 July 17 2013.

We collected three late-stage Wood Frog egg masses from a vernal pool in the University of Connecticut Forest on 20 April 2013. We also collected 6 amplectant pairs of Gray tree frogs on May 21 from a wetland in the forest. Eggs of both species were hatched and raised in the laboratory until they were free swimming. We attempted to start the Wood Frog experiment on April 22, but difficulty reconstituting RO water resulted in high mortality. This forced us to restart the experiment on April 30 with surplus tadpoles from the initial collection. The Gray Tree Frog experiment began on May 30.

The set up for both experiments was similar. Ten tadpoles were haphazardly assigned to plastic cups, which were randomly assigned to 19-liter aquarium tanks filled with 17 liters of reconstituted RO water. RO water was reconstituted with a 10% Holtzfreter solution for the Wood Frog experiment and with RO Rite (Kent Marine®) for the Gray Tree Frog experiment.

Tanks were randomly assigned to one of five treatments and treatments were randomly assigned within spatial blocks so that each experimental block contained one tank from each treatment. Block location within the cold room was randomly reassigned every six days to minimize effects of temperature and light gradients within the room. Partial water changes were conducted every three days. Theses water changes unavoidably resulted in temperature fluctuation within tanks that lasted less than 3 hours. To minimize this disturbance, we replaced heaters as quickly as possible. All tanks were fed *ad libitum* a combination of 50% rabbit chow and 50% tetramin.

The Wood Frog control treatment started at 18 degrees Celsius with an ambient room temperature of 15 degrees Celsius. All wood frog temperature treatments, however, were subjected to a one-degree ambient air temperature increase every six days (until the room temperature reached 20 degrees Celsius, the maximum room temperature in the cold room) to reflect seasonal temperature increases. The control treatment, therefore, ended at 23 degrees Celsius. By the time the Gray Tree Frogs were added to the cold room, the maximum room temperature had already been reached, so the ambient air temperature was maintained at 20 degrees Celsius and the control treatment was maintained at 23 throughout the duration of that experiment.

The experimental treatments varied in the number of temperature fluctuations and the magnitude of the temperature fluctuations. The control treatment was kept at a constant three degrees above the ambient air temperature to allow the fluctuating treatments to fluctuate below the constant temperature. There were two of these fluctuating treatments that maintained the same mean temperature as the constant control temperature but fluctuated repeatedly between three degrees above and three degrees below the constant treatment temperature. The first treatment fluctuated every six days (fluc weekly) and the second treatment fluctuated every 12 days (fluc biweekly). Weekly and biweekly fluctuations were chosen to reflect average temperature fluctuations of vernal pools in the area from April to June. The finalized six- and 12-day fluctuation periods were selected to coincide with the three-day water change cycle.

We also included two treatments to look at the effect of a single, large-magnitude temperature fluctuation. The first treatment entailed a six-degree increase in temperature compared to the control for six days at the beginning of the experiment (extreme early). The second treatment entailed a six-degree increase in temperature compared to the constant control for six days at the end of the experiment as tadpoles neared metamorphosis (extreme late). Both of these treatments experienced a six-degree temperature spike and represent a situation in which a short-term, extreme weather pattern has a significant effect (Ganguly *et al* 2009).

To prevent drowning, tadpoles were removed from the tanks once their front legs protruded and placed into small plastic cups. Once their tails were fully absorbed, we recorded the date of metamorphosis and mass.

Data Analysis

For both experiments, we first analyzed the data using one-way ANOVAs to look for survival differences among treatments. We then used one-way ANOVA to test for block and treatment effects on age and mass at metamorphosis and we included the number of metamorphs as a continuous covariate to account for potential density differences within tanks due to mortality. Finally, we used Tukey-adjusted *post hoc* tests to make pairwise comparisons between treatments.

Results:

Survival (i.e., number of individuals that metamorphosed from a tank) affected mass ($F_{1,15}$= 33.79, $p < 0.0001$) and time to metamorphosis ($F_{1,15}$=10.26, $p = 0.0059$) of Wood Frogs.

Increased survival resulted in increased time to metamorphosis and decreased size at metamorphosis. Temperature did not significantly affect mass at ($F_{4,15}$ = 1.52, p = 0.2474) or time to metamorphosis ($F_{4,15}$ = 0.40, p = 0.8067). However, there was a tendency for tadpoles from fluctuating treatments to reach metamorphosis earlier (Figure 1) and at a larger size (Figure 2) than tadpoles from the constant temperature treatment.

For the Gray Tree Frog experiment, Tank 37 and 42 were identified as outliers for the age at metamorphosis analysis and Tank 50 was identified as an outlier for the mass analysis, so we conducted analyses both with and without outliers. Exclusion of outliers did not affect the interpretation of any of the statistics so we only present analyses with outliers removed because removal improved residual normality and homoscedasticity.

Tank survival had a significant effect on mass ($F_{1,14}$= 30.92, p < 0.0001) but not on age at metamorphosis ($F_{1,13}$ = 2.71, p = 0.1239) of Gray Tree Frogs. Mass tended to increase slightly with increasing survival. There was also a significant effect of temperature treatment ($F_{4,14}$ = 14.09, p < 0.0001) and age at metamorphosis ($F_{4,13}$ = 10.52, p = 0.0005). Tadpoles in the extreme late treatment were significantly smaller than tadpoles from the constant control, extreme early, fluctuating biweekly, and fluctuating weekly treatments. Tadpoles from the fluctuating weekly treatment were also significantly larger than tadpoles from both the constant control and the extreme early treatments (Figure 3). The fluctuating biweekly treatment metamorphosed significantly later than the constant control, extreme early, extreme late, and fluctuating weekly treatments. The fluctuating weekly treatment also metamorphosed significantly later the extreme late treatment (Figure 4). Furthermore, all treatments finished with similar final average temperatures, which confirms that the treatments all had similar mean temperatures and only differed in the frequency and magnitude of temperature variation.

Discussion:

Contrary to our initial hypothesis, our results indicate that temperature fluctuations increase growth and delay development in Gray Tree Frogs compared to the control. The frequency of fluctuations (i.e., biweekly or weekly) had an effect on age at metamorphosis but not mass at metamorphosis. Additionally, the effect of the magnitude of temperature variation is influenced by the time at which the temperature spike occurs with spikes later in development causing decreased mass and age at metamorphosis. The wood frog experiment also showed trends for increased mass with variability, but a decrease in time until metamorphosis.

For the Gray Tree Frog experiment, the increased growth and delayed metamorphosis observed in the fluctuating treatments might be the result of the decreased developmental rate during the depressed temperature periods that the control treatment did not experience. This could also explain why the fluctuating biweekly treatment had a significantly later metamorphosis date compared to the fluctuating weekly treatment. Since the fluctuating biweekly treatment was subjected to longer consecutive periods of decreased temperature than the fluctuating weekly treatment, it is possible that those prolonged periods of slowed development were enough to result in significantly delayed metamorphosis.

The results of our experiment on temperature variability at a weekly scale run counter to results on the effect of temperature variability at a daily scale reported by Niehaus *et al* 2006. One possibility is that there is considerable species-specific variability in response to temperature fluctuation. Niehaus *et al* 2006 performed the experiment with *Limnodynastes peronii*, a species found on the east coast of Australia. Different life history characteristics, habitats, and evolutionary adaptations could lead to contradictory results. The use of Wood Frogs in our experiment was supposed to provide a species comparison, but complications in the experiment may have confounded the results. Repeating the Wood Frog experiment, perhaps with an additional species, might clarify the possibility of a species-specific reaction to temperature variation.

Our results suggest that a temperature threshold may need to be met by the fluctuating treatments to induce early metamorphosis at a reduced size. While the Extreme Late treatment was not subjected to continuous temperature variations like the fluctuating treatments, it is interesting to note that a spike of three degrees Celsius greater than the fluctuating treatments was enough to induce early metamorphosis at a reduced mass relative to the fluctuating treatments. The idea of a temperature threshold that must be surpassed before growth is suppressed is supported by studies done on invertebrates (Hagstrum and Milliken 1991; An, Dong, & Dong 2009). Georges *et al* 2005 also performed a similar temperature variability experiment on reptile development that further supported a temperature threshold idea for temperature variability since the results showed increased developmental rates when fluctuations were below a certain threshold and decreased developmental rates above the temperature threshold. An experiment with many temperature levels would be needed to test for a threshold effect. Future research should include repeating the gray tree frog experiment on a larger scale with additional fluctuating treatments at varying magnitudes of temperature variation to look more in depth into the results gathered from this experiment.

The extreme fluctuating treatments also provided insight into the effect of high-magnitude temperature fluctuations. The extreme temperature fluctuation at the end of the larval period was enough of a temperature spike to encourage metamorphosis, at a decreased mass. This may be an evolutionary response to the spike in temperature in vernal pools as they dry; in which case, it is beneficial to metamorphose, even at the cost of growth in these situations (Atkinson 1996; Laurila & Kujassalo 1999). This result was not observed for the extreme temperature fluctuation that occurred early in the larval period because the tadpoles may not have reached the size threshold necessary for metamorphosis, which would explain why the extreme early temperature treatment was not significantly different from the control for either days-to-metamorphosis or for mass at metamorphosis.

We did not detect differences in time to or age at metamorphosis in the Wood Frog experiment; however, methodological errors warrant repeating this experiment before final conclusions are drawn. Tank densities (i.e., number of metamorphs) significantly affected time to and size at metamorphosis for Wood Frogs. The strong density effect seen in the wood frog experiment was most likely caused by the high number of mortalities early in the experiment that caused significant differences in tank densities (some tanks had as few as 5 tadpoles while others had the full 10). The inconsistency in tank densities combined with small size disparities among the tadpoles from the reset experiment also prevented accurate feeding to *ad libitum* at the beginning, which resulted in exacerbated size differences among the tadpoles in the tanks as the experiment progressed. Tail kinks also became apparent just before metamorphosis in about 32.6% of the surviving tadpoles. We began feeding 80% rabbit chow and 20% tetramin to prevent further tail kinks. This was also the reasoning behind changing our water reconstitution method for the Gray Tree Frog experiment since RO Rite (Kent Marine®) contains trace minerals. These stressors may explain why the Wood Frog tadpoles tended to metamorphose earlier instead of later like in the Gray Tree Frog experiment even though they showed a similar trend for increased mass with variability. There was also a significant survival effect for the Wood Frog experiment. The fluctuating weekly treatment had significantly decreased survival compared to the +3 treatment. However, this result should be considered very cautiously since it is impossible to know whether the treatments had any additional effect on the water solution complications that occurred early in the experiment.

Our results suggest that temperature variability is an important aspect of an amphibian's thermal environment and that examination of mean temperature changes alone may not be enough to accurately predict the effects of climate change on amphibian populations or its interactions within amphibian habitats (Li, Cohen, & Rohr 2013). Amphibians may appear to withstand the elevated mean temperatures and even the increased variability in temperatures predicted by climate change models, but once a certain temperature threshold is met, it is possible that the consequences of this variability will be more severe than those predicted at an elevated mean temperature. Defining temperature thresholds are important to the management of threatened species and to the conservation of species that are not immediately threatened. Additional research that incorporates temperature variability is strongly advised since it more accurately represents natural temperature regimes and the effects the natural climate has on the growth and fitness of amphibians.

Literature Cited

An, ZH, YW Dong, SL Dong. 2009. A high-performance temperature-control scheme: growth of sea cucumber Apostichopus japonicas with different modes of diel temperature fluctuation. *Aquaculture International* **17:** 459-467.

Atkinson, D. "Ectotherm Life-history Responses to Developmental Temperature." Ed. Ian A. Johnston and Albert F. Bennett. *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. N.p.: n.p., 1996. 183-204. Print.

Bustamante, H. M., L. J. Livo, and C. Carey. 2010. Effects of temperature and hydric environment on survival of the Panamanian Golden Frog infected with a pathogenic chytrid fungus. *Integrative Zoology* **5:** 143-153.

Collins, J. P., and A. Storfer. 2003. Global amphibian declines: sorting the hypothesis. *Diversity and Distributions* **9:** 89-98.

Duellman W., and L. Trueb. 1986. Biology of Amphibians. McGraw Hill, New York.

Ganguly, A. R., K. Steinhaeuser, D. J. Erickson, M. Branstetter III, E. S. Parish, N. Singh, J. B. Drake, and L. Buja. 2009. Higher trends but larger uncertainty and geographic variability in 21st century temperature and heat waves. *Proceedings of the National Academy of Sciences* **106:** 15555–15559.

Georges, A., K. Beggs, J. E. Young, and J. S. Doody. 2005. Modelling development of reptile embryos under fluctuating temperature regimes. *Physiological and Biochemical Zoology* **78**: 18-30.

Guzy, J. C., E. D. McCoy, A. C. Deyle, S. M. Gonzalez, N. Halstead, and H. R. Mushinsky. 2012. Urbanization interferes with the use of amphibians as indicators of ecological integrity of wetlands. *Journal of Applied Ecology* **49:** 941-952.

Hagstrum D.W. and G.A. Milliken. 1991. Modeling differences in insect development times between constant and fluctuating temperatures. *Annual Entomological Society of America* **84**: 369–379.

Hayhoe, K., C. Wake, B. Anderson, X. Liang, E. Maurer, J. Zhu, J. Bradbury, A. DeGaetano, A. M. Stoner, and D. Wuebbles. 2008. Regional climate change projections for the Northeast USA. *Mitigation and Adaption Strategies for Global Change* **13**: 425-36.

IUCN (International Union for the Conservation of Nature and Natural Resources), Conservation International, and NatureServe. 2004. Global Amphibian Assessment. http://www.globalamphibians.org.

Kiesecker, J. M., A. R. Blaustein, and L. K. Belden. 2001. Complex causes of amphibian population declines. *Nature* **410:** 681-684.

Laurila, A., and J. Kujassalo. 1999. Habitat duration, predation risk and phenotypic plasticity in common frog (Rana temporaria) tadpoles. *Journal of Animal Ecology* **68**: 1123-1132.

Li, Y., J. M. Cohen, and J. R. Rohr. 2013. Review and synthesis of the effects of climate change on amphibians. *Integrative Zoology* **8:** 145-161.

Niehaus, A. C., R. S. Wilson, and C. E. Franklin. 2006. Short- and long-term consequences of thermal variation in the larval environment of anurans. *Journal of Animal Ecology* **75**: 686-692.

Niemi, G. J., and M. E. McDonald. 2004. Application of ecological indicators. Annual Review of Ecology, Evolution, and Systematics **35:** 89-111.

Price, S. J., R. W. Howe, J. M. Hanowski, R. R. Regal, G. J. Niemi, and C. R. Smith. 2007. Are anurans of Great Lakes coastal welands reliable indicators of ecological condition. *Journal of Great Lakes Research* **33:** 211-222.

Rohr, J. R., and B. D. Palmer. 2013. Climate change, multiple stressors, and the decline of ectotherms. *Conservation Biology* **27:** 741-751.

Smith-Gill, S. J., and K. A. Berven. 1979. Predicting Amphibian Metamorphosis. *The American Naturalist* **113**: 563-585.

Walsh, P. T., J. R. Downie, and P. Monaghan. 2008. Temperature-mediated morphology changes during metamorphic climax in the African clawed frog, *Xenopus laevis. Journal of Thermal Biology* **33:** 244-249

Welsh, H. H., and L. M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: A case study from California's Redwoods. *Ecological Applications* **8:** 1118-1132.

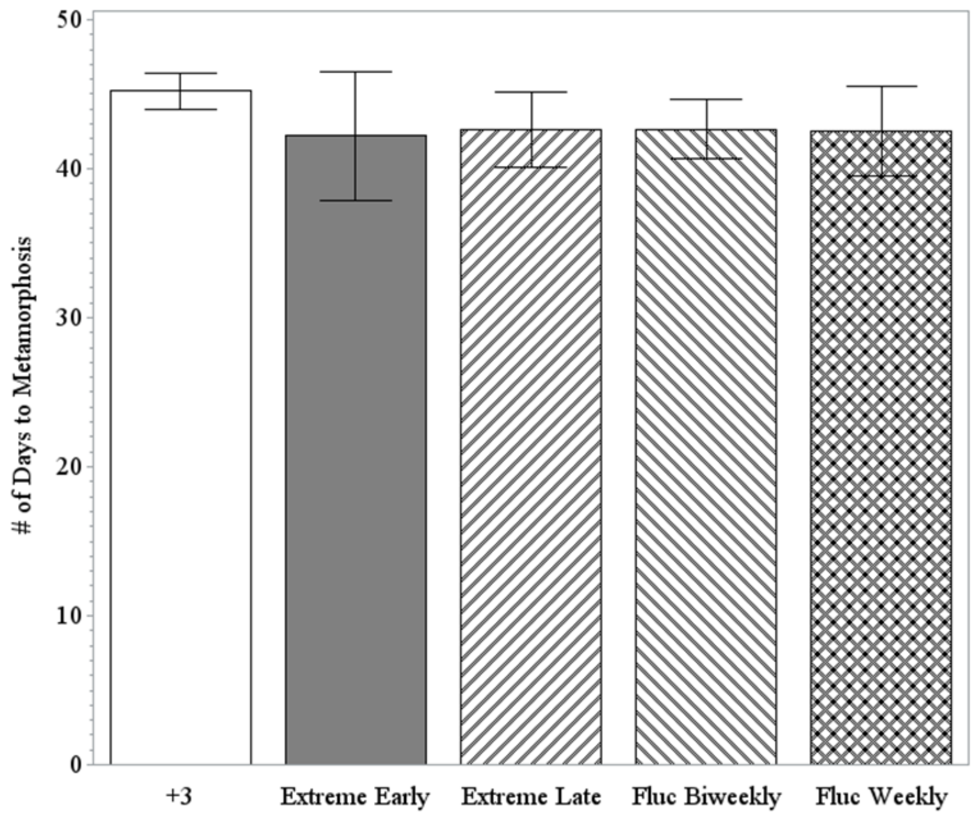

Figure 1: Average number of days to metamorphosis for wood frogs. No significant differences among treatments.

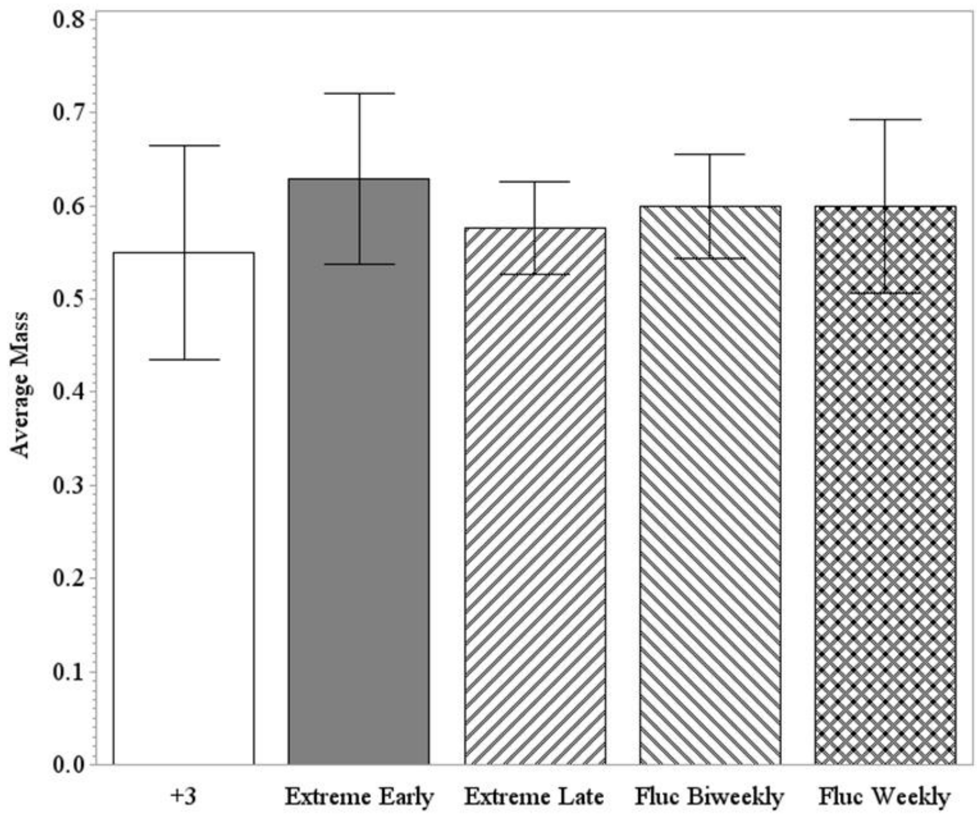

Figure 2: Average mass at metamorphosis for Wood Frogs. No significant differences among treatments.

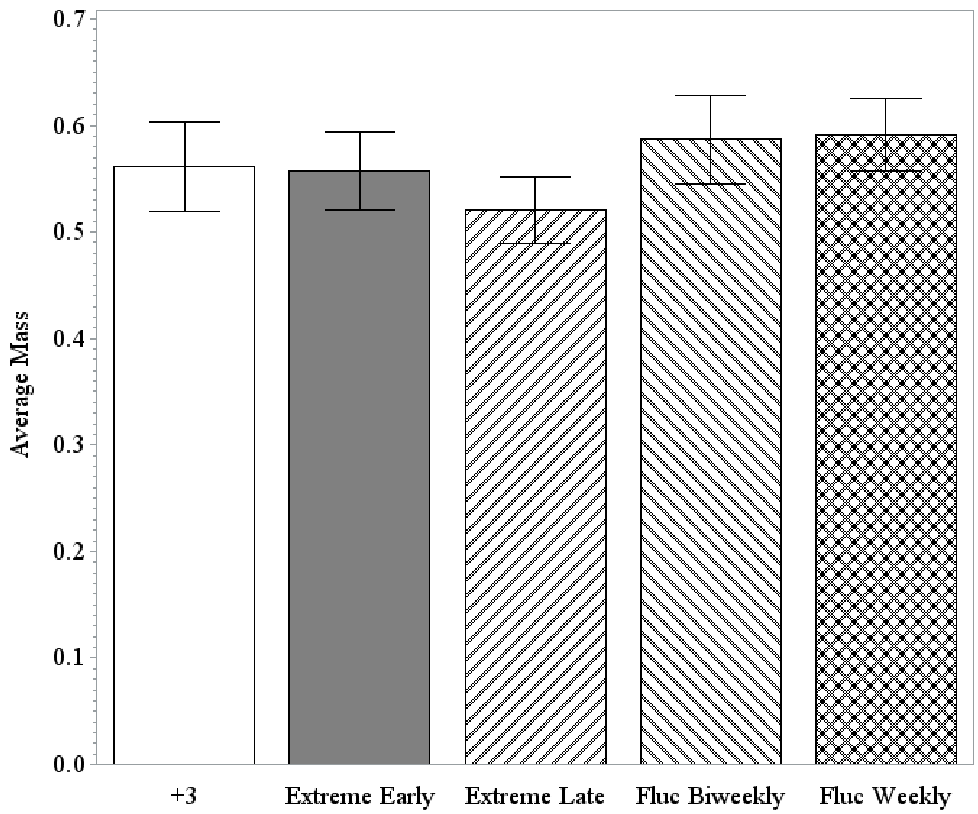

Figure 3: Average mass at metamorphosis for Gray Tree Frogs.

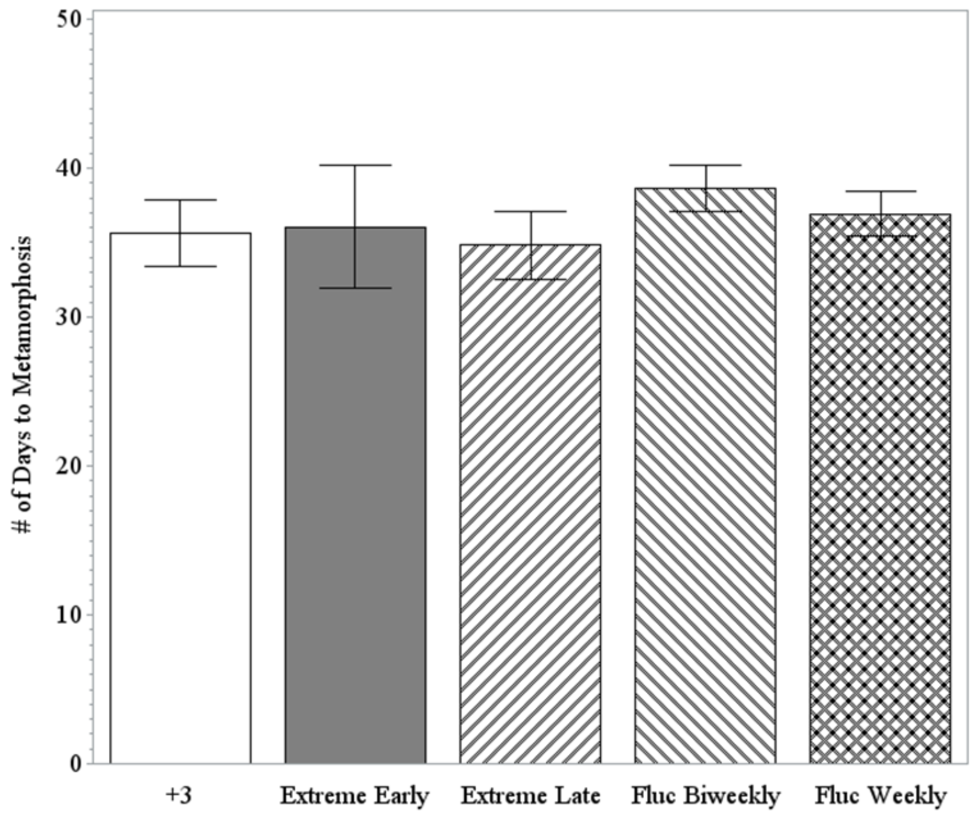

Figure 4: Average number of days to metamorphosis for Gray Tree Frogs.