Effects of Incubation Temperature on Juvenile Morphology of the Endangered Streamside Salamander (*Ambystoma barbouri*)

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Background: Urbanization around the globe has caused substantial damage to the environment and organisms that live in it, with negative direct and indirect effects such as habitat destruction and thermal stress from the urban heat island effect. [1] Streamside salamanders (*Ambystoma barbouri*), an endangered species in Tennessee, are affected because they nest in water systems that experience temperature elevation by urbanization and embryos are highly sensitive to heat stress.[2] Given these temperature changes, it is crucial to understand how temperature influences the morphology of these salamanders.

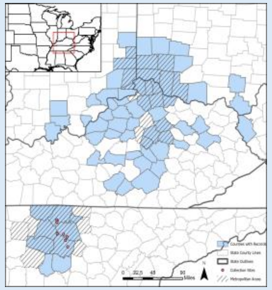

Figure 1. Map of egg collection sites adjacent to urban areas.

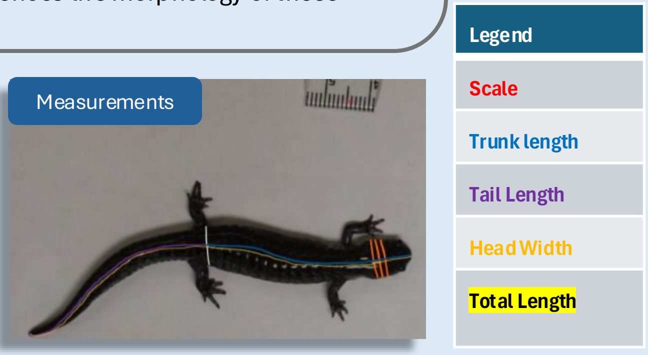

Figure 2. Measurements taken at six months, using ImageJ.

Methods: We collected eggs across the salamander's range in Tennessee (Fig 1) and incubated them at one of three incubation temperatures (5 , 10, and 20 °C). We monitored morphological changes after the larval period by measuring body size at metamorphosis and six months post metamorphosis. ImageJ was used to take measurements from photographs of individuals that had reached six months (Fig 2). We analyzed metrics of the salamander's body using linear mixed models, with the lmerTest package in R studio.

Results: Body mass was lower for salamanders incubated at 5 °C (Fig 3A). Head width, trunk, tail and total body length showed no conclusive dependence on incubation temperature (Fig 3 & Fig 4).

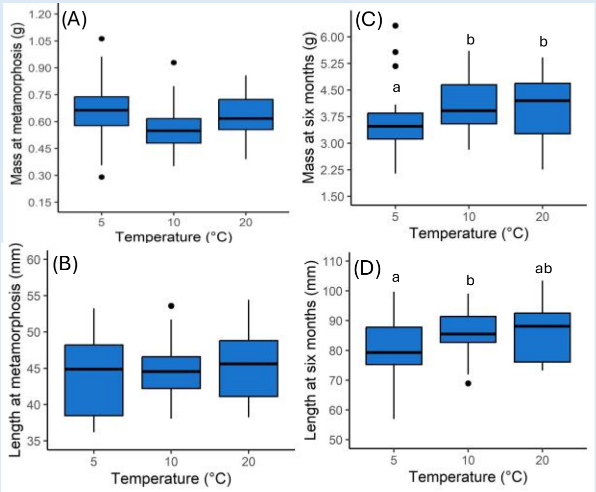

Figure 3. Size of individuals at metamorphosis (3A and 3B), and at six months (3C and 3D). For models of mass and length at six months, incubation temperature and size at metamorphosis were fixed effects.

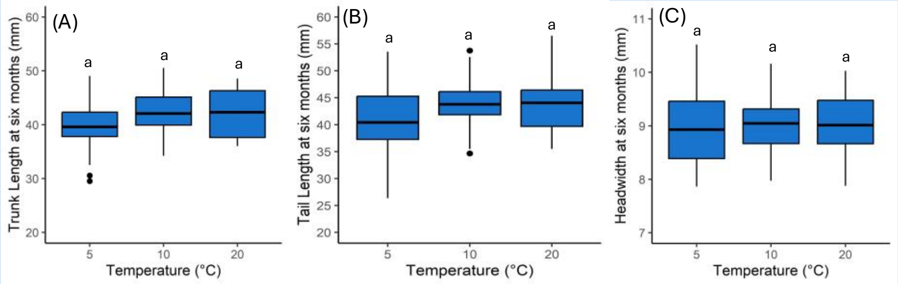

Figure 4. Trunk length, tail length, and head width at six months. Incubation temperature and total body length were fixed effects.

Conclusions: Eggs and larvae can develop at a wide range of temperatures without severe, long term effects on body size; however, the lower body mass of salamanders incubated at 5 ° C indicates they were in poorer body condition which might reflect plastic changes to their early-life metabolism in response to cold temperature. One caveat is that survival was low for eggs and larvae at 20 °C treatment; therefore, our data only represent the survivors.

Acknowledgements

Dr. Joshua Hall from Tennessee Technological University was awarded funds from Tennessee Wildlife Resources Agency for this project, along with a Tennessee Herpetological Society grant awarded to Julia Thulander. The research was approved by the Tennessee Technological University Committee for the Care and Use of Laboratory Animals in Experimentation (Protocol 22-23-002), the Tennessee Department of Environment and Conservation (permit #2022-041), and the Tennessee Wildlife Resources Agency (permit #5669). And to everyone involved with the care of the salamanders.

(1) Hall, J. M., & Warner, D. A. (2017). Body size and reproduction of a non-native lizard are enhanced in an urban environment. *Biological Journal of the Linnean Society, 122*(4), 860–871. https://doi.org/10.1093/biolinnean/blx109

(2) Hall, J. M., & Sun, B. (2021). Heat tolerance of reptile embryos: Current knowledge, methodological considerations, and future directions. *Journal of Experimental Zoology. Part A, Ecological and Integrative Physiology, 335*(1), 45–58. https://doi.org/10.1002/jez.2402

(3) Thulander, J.M. (2024). Embryonic temperature has carry-over effects on body size, development time, and larval survival in the streamside salamander (*Ambystoma barbouri*). TN Tech Thesis