A Novel Application of Membrane Inlet Mass Spectrometry to Study Larval Physiology of the Streamside Salamander (*Ambystoma barbouri*)

Kaitlyn Darnell, Julia Thulander, Trevor Crawford, Justin Murdock, Joshua Hall

Tennessee Technological University

Introduction

The streamside salamander (*Ambystoma barbouri)* is a state-endangered species that is threatened by urbanization around the Nashville Metro area. Urbanization results in an increase in environmental temperature due to an abundance of heat-absorbing substrates (e.g. asphalt) and a reduction in shade cover due to deforestation (Tiatragul et al. 2019). Early life stages (e.g. eggs, larvae) are particularly vulnerable to warming temperatures due to a reduced ability to thermoregulate (Hall & Sun 2020). Therefore, it is important to understand how *A. barbouri* metabolism responds to warming temperatures. To evaluate how incubation temperature and environmental temperature affect larval salamander metabolism, we used a MIMS (Membrane Inlet Mass Spectrometer) to measure the O_2 consumption of larva from two incubation environments (5 vs 10 °C) at each of two test temperatures (10 vs 20 °C). This represents a novel application of MIMS. The MIMS measures small traces of gas that are dissolved in water, and prior to this study, the MIMS has typically been used to measure gas exchange in aquatic plants, algae, and microorganisms.

Methods

Eggs incubated at 5 or 10 °C

Resultant larvae fasted for 72 hours

Acclimation of larva at target temperature (10 or 20 °C)

Placement of larva in airtight jars

Measurement period of 1.5 hours at target temperature

Respiration measurements via MIMS

Data collection: incubation time, atmospheric pressure, temperature, oxygen consumption, and sampling time, larval mass

Data analysis

Results

Mixed effects linear models indicate that *Ambystoma barbouri* have greater metabolic rate at 20 vs 10 °C with a Q10 of 2.6. ($F_{1,17}$ =86.3; p <0.0001) (Figure 2). Neither body mass ($F_{1,15}$=0.002; p = 0.97) nor egg incubation temperature ($F_{1,15}$ =1.5; p = 0.24) affected metabolic rate during testing.

Conclusions

Some results indicate our measures were accurate, others do not. Absolute O_2 consumption was similar to other larval amphibians (Rowe & Funck 2017). A Q10 of 2.6 is recorded for larval salamanders (Brown et al. 2003) and is within the range of expected values for biochemical reactions. However, we expected metabolism to scale positively with body size. For future research, we suggest running the MIMS for 48 hours instead of 24 when running cold samples due to time taken to stabilize. Additionally, samples could be incubated for longer periods of time to increase precision of measurements. Finally, our results indicate these temperatures are not stressful for *A. barbouri* larva and this corresponds with field temperature data.

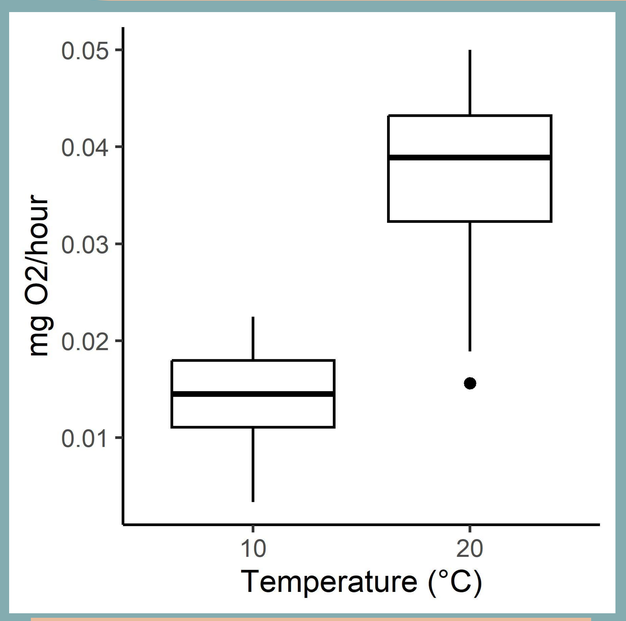

Figure 1. Oxygen consumption rate vs. temperature

Tiatragul, Sarin. "Nestled in the City Heat: Urban Nesting Behavior Enhances Embryo Development of an Invasive Lizard.", 2020, Arthur V. Brown a, et al. "Thermal Acclimation and Metabolism in the Gray-Bellied Salamander, Eurycea Multiplicata Griseogaster (Plethodontidae)." *Comparative Biochemistry and Physiology Part A: Physiology*, 17 Mar. 2003, Hall, Joshua M, and Bao-jun Sun. *The Thermal Ecology and Physiology of Reptiles*, 2020.

Acknowledgements

Funding was provided by a Tennessee tech university CISE grant awarded to **Kaitlyn Darnell**. Research 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).