

Effect of Acclimated Temperature on Skin Resistance to Water Loss in Long-Toed Salamanders (*Ambystoma macrodactylum*)

Project Statement: With the support and mentorship of the Sinervo Herpetology Lab, I will be testing long-toed salamanders (*Ambystoma macrodactylum*) for plasticity in skin resistance as a consequence of acclimation to different temperatures. Skin resistance is a value used to quantify the biological component of an organism's resistance to evaporative water loss independently of physical and environmental factors (Riddell et al. 2017). Understanding the natural plasticity and limits of skin resistance is key in determining how salamanders will respond to climate change. As terrestrial ectotherms, they will be exposed to rising temperatures, and as amphibians with permeable skin, they are sensitive to cutaneous water loss (Lertzman-Lepofsky et al. 2020). If skin resistance is a plastic trait, salamanders may be able to survive higher temperatures than previously estimated, so it is critical we understand this part of their natural history. I believe this project, and others like it, have the potential to provide a more complete, and possibly more optimistic, picture of the future prospects of amphibians.

I am well prepared for this project. I have experience with scientific research from my classes in college. In particular, I attended a Study Abroad program in Costa Rica which included the completion of an independent project on the response of male orchid bees to the presence of model predators at chemical baits. This required me to come up with a research question, devise a method for an experiment, collect and analyze data, and write a research paper. I also presented my findings to a general audience. Additionally, I have been volunteering with the Sinervo Lab for three years, so I can provide husbandry for the animals and I am familiar with the research protocols.

To date, I have made significant progress on this thesis. I have been discussing my topic and background information with my mentors since June. I have searched through the scientific literature and read several scientific papers. This quarter, I am also enrolled in a thesis writing course for EEB senior thesis topics. I am currently working on a draft of my thesis paper, as well as developing the details of my method.

Methods: My goal is to find out whether *A. macrodactylum* shows a positive correlation between skin resistance and acclimated temperature. I believe that individuals acclimated to higher temperatures will have higher skin resistance, which would demonstrate the plasticity of this trait. This particular salamander species has a high-elevation subspecies, *A. macrodactylum sigillatum*, found in the mountains of Central California (Pilliod and Fronzuto 2005). Adults have a unique annual migration pattern which makes them good candidates to study skin resistance (Pilliod and Fronzuto 2005). Individuals grow to adulthood in ponds, then migrate away from water to spend the dry season in hiding, only returning to the ponds to breed (Pilliod and Fronzuto 2005). This shows tolerance for a wide range of moisture levels, and suggests that they may already alter skin resistance within their lifespan.

I will test individuals from a group of *A. macrodactylum sigillatum* that were collected and brought to the Sinervo Lab, where they have been acclimating in one of three temperature-controlled rooms at 15, 18, and 21°C since the larval stage. I will randomly select 8 adult individuals from each room for a total of 24 individuals. Skin resistance will be measured at trial temperatures of 12, 15, 18, 21, and 24°C. Skin resistance will be measured as the time it takes animals to lose 3% of their body weight. Before each trial, salamanders will be weighed and sit in their trial temperature for approximately 2 hours to adjust. Agar models will also be provided,

weighed and paired with a salamander of similar mass. These agar models will serve as the control, since they offer a negligible skin resistance (Lertzman-Lepofsky et al. 2020, Navas and Araujo 2000).

I will use a flow-through system that controls the flow rate of air, pumping it through a container of Drierite dessicant to dehydrate it. The dry air will be directed into one of three chambers – one for a live salamander, one for an agar model, and one left empty to monitor temperature and humidity. Each chamber will contain a scale to continuously measure the mass inside, a logger to measure humidity level, and a flow meter to measure flow rate. At the start of a trial, the salamander and its agar model will be placed in their respective chambers. The flow-through system will be turned on and run until the salamander has lost 3% of its mass. Time, salamander's mass, and agar's mass will all be recorded. Salamanders will be returned to temperature-controlled rooms and allowed to recover from the stress of dehydration for one week between trial temperatures.

After I have collected my data, I will calculate the skin resistance for each trial and the average skin resistance for all salamanders from each acclimated temperature. I will determine whether there is any significant difference in skin resistance between individuals acclimated to different temperatures by using an ANOVA test. I will also construct thermal performance curves (TPCs) for all three groups, using the method outlined in Sinclair et al. 2016 and substituting skin resistance as the variable for performance or fitness.

I will use these TPCs and climate records to construct a species distribution model for the current range of *A. macrodactylum sigillatum*, and a future extinction risk model, following the methods of Sinervo et al. 2010. TPCs will be used to create “hours of restriction” functions, which determine under what conditions salamanders can be behaviorally active or restricted. I

will apply both projected environmental conditions and the restriction functions to the species distribution to determine what extinction risk the population faces.

At the end of this project, I will produce a complete thesis and present my work at the EEB Undergraduate Research Fair. The paper, presentation slides, and all my data will be made freely available to the Norris Center. I will also create a short video which can be added to the Norris Center's website. This film will explain skin resistance in *A. macrodactylum* in terms accessible to a general audience. Thus, every component of this project will be made available to the public to learn about my findings.

My timeline for this project spans the entire 2020-2021 school year. This fall, I am writing a preliminary draft of the paper and refining my methods. I plan to test run my experimental trial method before the end of the fall quarter. In winter quarter, I will run all trials and begin inputting the data into statistical models. I will also record footage of my trial methods and write a script for the video. In spring quarter, I will finish statistical modelling, interpret my results, and finish writing the discussion section of my paper. I will also narrate my script and edit together clips for the finished video, which I will give to the Norris Center. I will conclude by creating a slide presentation to show at the EEB Undergraduate Research Symposium.

References:

- Lertzman-Lepofsky, G.F., A.M. Kissel, B. Sinervo, and W.J. Palen. 2020. Water loss and temperature interact to compound amphibian vulnerability to climate change. *Global Change Biology* 26: 4868-4879.
- Navas, C.A., and C. Araujo. 2000. The Use of Agar Models to Study Amphibian Thermal Ecology. *Journal of Herpetology* 34: 330-334.
- Pilliod, D.S. and J.A. Fronzuto. 2005. *Ambystoma macrodactylum*. Pp. 617-621 *In* Amphibian Declines: The Conservation Status of United States Species. Lannoo, M. (Ed.) University of California Press, London, England.
- Sinclair, B.J., K.E. Marshall, M.A. Sewell, D.L. Levesque, C.S. Willett, S. Slotsbo, Y. Dong, et al. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters* 19: 1372-1385.
- Riddell, E.A., E.K. Apanovitch, J.P. Odom, and M.W. Sears. 2017. Physical calculations of resistance to water loss improve predictions of species range models. *Ecological Monographs* 87: 21-33.
- Spotila, J.R. and E.M. Berman. 1976. Determination of skin resistance and the role of the skin in controlling water loss in amphibians and reptiles. *Comparative Biochemistry and Physiology Part A: Physiology* 55: 407-411.

Budget Proposal

Many supplies are provided for me by the Sinervo Lab and thus are not included in the budget proposal. These include tanks to house the animals, pre-mixed water which contains the appropriate amount of salts and minerals, agar mixture and molds to create models, a container for the Drierite dessicant, digital scales, HOBO Pro v2 data loggers, and flow meters. However, I currently have no other funding for this project, as the lab cannot provide me with any and I have not received any other grants or awards.

Item	Amount	Justification
Crickets	\$40	I estimate that I will need approximately 250 crickets to feed salamanders for the duration of the project. The amount includes the cost of shipping live animals.
Paper Towels	\$60	I will need paper towels as an easily replaceable substrate to house the animals. Each package of 6 rolls will cost approximately \$15.
Drierite	\$80	I will need Drierite dessicant to dehydrate the air inside the flow-through system during skin resistance trials. The amount covers 2 jars of Drierite, which cost \$40 each.
Plastic Chambers	\$60	I will 3 new plastic containers to use as chambers in the flow-through system. Each chamber costs \$20.
Air Pump and Flow Rate Valve	\$60	I will need a Tetra AP300 aquarium air pump to power the entire flow-through system. This model can provide a sufficiently high flow rate. I will also need a flow rate valve to ensure the flow rate is consistent.
Gas tubing	\$30	I estimate that I will need 3 sets of 3-foot Tygon gas tubing to connect the different parts of the flow-through system. Each set costs \$8.25, plus shipping and tax.
Thread Sealant Tape	\$10	I will need 10 rolls of thread sealant tape to ensure the flow-through system is airtight. Each roll costs \$0.64, plus shipping and tax.

TOTAL: \$340.00