Murray State University | Office of Research and Creative Activity
ORCA Research Grant Information 2023-24

Last updated 8/16/2023

ORCA has a modest amount of funds available to assist faculty-mentored, student research projects. To be eligible for a research grant, a student must be enrolled as a full-time Murray State student. The project must have a faculty mentor and should be completed within the academic year (may include summer) in which the award is granted.

Research awards provide a maximum total of $1,000 per individual student or project. A student may only be awarded one ORCA Research Grant per academic year. No more than one grant will be awarded toward the same project in the same year.

Funding is preferably distributed *via interdepartmental reimbursements* to your campus department, *or* billing may be directed to ORCA from an established Murray State vendor. These should occur before May 31, 2023.

Applications are reviewed by ORCA staff and members of the ORCA Advisory Board. Selection will be made on the merits of the project and the likelihood of the project being successfully completed. Awarding of grants is dependent on ORCA funding; ORCA reserves the right to adjust the funding levels prior to award.

Although faculty mentor guidance is required, the student applicant is expected to take significant ownership of the project. A campus presentation or publication related to the work is expected.

Please do not hesitate to contact AJ Boston at msu.orca@murraystate.edu or aboston@murraystate.edu should you have any questions regarding the program or application process.

Instructions & Information

For Students:

- Download a copy of this application
    - At the top of this Google Docs page, click **File**, select **Download as**, and select **Microsoft Word**

- Save your file as "ORCAResearch_[yourfirst.yourlastname]"
    - ex. "ORCAResearch_RobertJackson.docx"

- Fill out the application as instructed
    - Sections A-G to be completed by the Student
    - Section H to be completed by the Faculty Mentor, then emailed to aboston@murraystate.edu

- Submit basic information at Murray State's Digital Commons
    - Go to: http://digitalcommons.murraystate.edu/orcagrants/
    - Click 'Submit' in the Author Corner on the left-hand
        - *You will need to create a new account, if you have not used the site before*
    - Complete the registration form
        - *Include a public-facing summary or abstract*
        - *View information about successful past grants at this site*
        - *You will not need to upload any documents*
    - You will receive an immediate automated email from Digital Commons, notifying that your application has been submitted

For Faculty Mentors:

- After you receive this document, review Sections A-G, consult with the student and make any necessary revisions
- Complete your section in Section H
- Email the completed document to AJ Boston at aboston@murraystate.edu with the subject line "ORCA Grant: [Student_Lastname]"

For Students and Faculty Mentors:

- Once the Student has submitted basic information through Digital Commons and the Faculty Mentor has emailed the completed application, the ORCA Coordinator will send an email to the ORCA Advisory Board.
- The body of this email will contain general information about the grant and the completed application will be included as an attachment. Both the Student and Faculty Mentor will be blind-copied to this email to keep as a record that the Grant Review process has begun.
- After 10-14 business days, the Student and Faculty Mentor both should receive an email from the ORCA Coordinator with a decision (approve, revise, reject), a summary of advisory board feedback, and instructions for next steps.

ORCA Research Grant Proposal Document 2023-24

Sections A-G prepared by the Student. Section H prepared by the Faculty Mentor and sent to the ORCA Coordinator. Delete highlighted instructions from the final document.

A.) TIMETABLE

I have collected corticosterone and chytrid samples from Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) in the summers of 2022 and 2023. These samples are currently being processed by myself in the lab at Murray State University. I will process these samples in the lab from September 2023 - March 2024, and all data to date will be statistically analyzed by May of 2024. Further sampling will occur May 25th - August 5th, 2024 and those samples processed from August 2024 - January 2025. This data will be analyzed by January 2025, and I will defend my thesis in April of 2025. I plan to graduate With a Master's in Biology/Watershed Sciences in May 2025.

B.) PROPOSAL NARRATIVE

Assessing the interaction of stress physiology and *Bd* infection in Arizona tiger salamanders (*Ambystoma mavortium nebulosum*)

Abstract

Amphibian biodiversity has greatly diminished in recent years due to disease. Panzootic pathogenic fungi *Batrachochytrium dendrobatidis* (*Bd*) causes the deadly disease chytridiomycosis (chytrid), a primary driver of catastrophic global amphibian declines and extinctions. The pathogenesis of chytrid is still unclear, as certain species and individuals within a species are differentially affected. Susceptibility and mortality of *Bd* are influenced by prolonged chronic stress. Prolonged glucocorticoid activity deleteriously affects many of the same physiological processes as Bd infections, and corticosterone is the primary glucocorticoid released by amphibians in response to stress. Thus, the objective of our study is to assess the relationship of corticosterone variation and *Bd* spore load in Arizona tiger salamanders (ATS; *Ambystoma mavortium nebulosum*). We used a non-invasive skin swabbing method to collect baseline corticosterone from paedomorph (aquatic morph), metamorph (terrestrial morph), and larval (immature) ATS in June and July 2022. Baseline samples were collected within three minutes of capture. *Bd* samples were then collected via skin swabbing, and biometrics for individual animals measured. Additional samples will be collected in summer of 2023, and will allow us to consider corticosterone and *Bd* spore load variation by morph, sex, location, and body condition. My results will develop the use of corticosterone as a predictor of *Bd* susceptibility and severity of infection. My study will provide a greater understanding of the pathogenesis of *Bd* and

the interacting effects of glucocorticoid production and polyphenic life history on amphibian disease resistance, which will aid in the study of other fungal diseases that affect aquatic taxa.

Introduction

Amphibian biodiversity has greatly diminished in recent years due to human-induced habitat disturbance (Hossack et al., 2009), invasive species (Crowl et al., 2008), and disease (Lips et al., 2016; Scheele et al., 2019). Panzootic pathogenic fungi *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*) cause the deadly disease chytridiomycosis (chytrid), a primary driver of catastrophic global amphibian declines and extinctions (Lips et al., 2006; Lips et al., 2016; Jancovich et al., 2005). The *Bd* pathogen causes death in affected individuals by invading skin cells of the semipermeable epidermis of amphibians (Fisher et al., 2021). This hinders many processes of the skin such as gaseous exchange, electrolyte balance, and water intake, which in turn prevents maintenance of metabolism and homeostasis, and often results in heart failure (Fisher et al., 2021). Globally, 400+ species of amphibians are now threatened due to *Bd* (Scheele et al., 2019). The pathogenesis of chytrid is still unclear, as certain species and individuals within a species are differentially affected (Searle et al., 2011). There are also environmentally-specific effects on the severity of the disease, including temperature (Forrest et al., 2011; Daskin et al., 2014) and microbiota (Lam et al., 2011), that may interact with *Bd*'s effects on immune function and hormone production.

Susceptibility and mortality of *Bd* are influenced by prolonged chronic stress (Wingfield et al., 2013; Gabor et al., 2015). Glucocorticoids are steroids released by vertebrates in response to a stressor to increase the chances of short-term survival, but chronic stress has adverse effects on long-term individual health that can hinder immune response and increase mortality (Wingfield et al., 2013; Sapolsky et al., 2000). Corticosterone (CORT) is the primary glucocorticoid released by amphibians in response to stress (Jungreis et al., 1970). Prolonged stress hormone activity deleteriously affects many of the same physiological processes as *Bd* infections (e.g., appetite, white blood cell count, metabolic rate, homeostasis); therefore, elevated stress in response to *Bd* infections may help explain the lethality of this devastating disease (Lam et al., 2011; Gabor et al., 2015; Romero et al., 2018).

Arizona Tiger Salamanders (ATS; *Ambystoma mavortium nebulosum*) are a species that is facultatively paedomorphic, a polyphenism that is expressed through metamorphism as a result of environmental factors (Whiteman et al.,, 1997). Individuals that undergo metamorphosis, known as metamorphs, develop physiological adaptations to allow terrestriality. Paedomorphosis is the alternative life history for this species; paedomorph

individuals retain their larval characteristics, such as gills, and remain obligately aquatic even after sexual maturity (Whiteman et al., 1997).

Metamorphic salamanders may be more susceptible to becoming infected with *Bd* than their paedomorphic counterparts. As a result of metamorphosis, these animals develop a layer of alpha keratin in their epidermis to reduce desiccation on land (Becker et al., 2017). This increased keratinization may exacerbate the negative impact of *Bd* infections, through decreased osmoregulatory capabilities (Becker et al., 2017). Metamorphs may also be more vulnerable to stress, as they have differential pressures of predation, resource availability, and habitat use (Millikin et al., 2019). Furthermore, differential energy expense of reproduction between the sexes may cause variation in CORT concentration (Millikin et al., 2019). In some organisms, males experience immunocompetence compared to females (Stoehr et al., 2006). These factors may all synergistically drive variation in glucocorticoid production as well as variation in susceptibility to *Bd* infections.

Boreal Toads (BT; *Anaxyrus boreas*) are highly susceptible to and have undergone widespread population declines and extirpations due to *Bd*. ATS is present in many of the same locations, and serves as pathogenic reservoirs for *Bd*, potentially causing disease outbreaks in the threatened BT populations without experiencing fatality themselves (Davidson et al., 2003). While ATS do not experience mortality as a result of *Bd*, there may be a variety of sub-lethal effects that negatively impact their fitness (Retallick et al., 2007). While it is difficult to study BT due to their threatened status, ATS can be used as a model system to study the synergistic effects of *Bd* infection and chronic stress. My previous research found that terrestrial ATS express higher concentrations of CORT than their aquatic counterparts. Further research could clarify the cause of CORT variation in this salamander species, as well as reveal relationships between stress, disease presence/intensity, and any climate-driven changes in such relationships (Mendelson et al., 2006).

Objectives and Predictions

I will determine if *Bd* infection status and intensity is associated with glucocorticoid activity, morph, sex, and abiotic factors related to climate change in adult ATS. I will survey corticosterone concentration, *Bd* infection status and intensity, and abiotic factors related to climate change in wild ATS populations under a range of natural conditions.

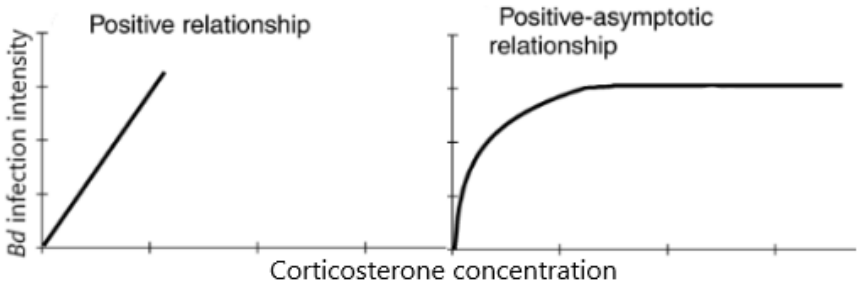

Figure 1. Predicted relationships of *Bd* and CORT.

I predict that *Bd* pathogenesis and intensity will be positively related to CORT (Fig. 1), either linear or positively asymptotic. I also predict that metamorphs will have higher concentrations of *Bd* zoospore infection, due to the keratinization of their skin.

Significance

My study will provide a greater understanding of the pathogenesis of *Bd* and the interacting effects of glucocorticoid production and polyphenic life history on disease resistance. My results will develop the use of CORT as a predictor of *Bd* susceptibility and severity of infection. This will benefit amphibian conservation including threatened BT through improved disease mitigation, as well as novel information about the effect of stress on disease susceptibility and population viability. CORT as a predictor of *Bd* may be beneficial for identifying salamander populations or species most vulnerable to *Bsal*.

The knowledge discerned from my research may be applicable to other taxa that suffer from pathogenic infections, particularly aquatic animals such as fish. The waterborne mold *Saprolegnia* spp. is an infectious oomycete responsible for global population declines in various freshwater fish species, especially in aquaculture (Yanong, 2003). This pathogen is commonly a secondary infection and its severity exacerbated by immunosuppression (Pickering and Willoughby, 1982). Ichthyophoniasis is another fungal pathogen that widely infects freshwater fish in North America and causes muscle tissue damage and lesions on internal organs (Kocan et al., 2004). Given the immunocompromisation of extended glucocorticoid production, corticosterone may have a significant effect on the disease resistance and mortality of fish.

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C.) METHODS & EXPECTED OUTCOMES

Methods

I will sample 20 adult salamanders from 15 known *Bd*+ and *Bd*- ponds during each sampling year (2022, 2023, and 2024), and collect abiotic environmental data such as water temperature, water depth, and elevation. Animals will be caught using dipnets and seines, and handled with nitrile gloves to prevent sample contamination. I will measure biometrics such as body condition (wet mass divided by snout to vent length) and reproductive status.

Baseline CORT will be sampled through a standardized, non-invasive dermal swabbing technique (Santymire et al., 2018) that I validated for ATS in my previous research. Samples will be collected within three minutes of capturing the animal, a period shown to accurately represent resting glucocorticoid concentration (Romero et al., 2007). These samples will be stored in 70% ETOH and frozen at -20 degrees Celsius. CORT will be measured via enzyme-linked immunosorbent assays designed for this hormone. I will collect *Bd* dermal samples by swabbing left and right of the vent, each hind foot, left and right ventral surface, left and right flank of the tail, and left and right cloacal grooves 5 times each, resulting in 50 swipes per swab/animal (Bishop et al., 2009). These samples will be stored in 70% ETOH and frozen at -20 degrees Celsius. I will utilize quantitative PCR techniques to determine presence and infection intensity (Torres, 2017).

Dissemination

Dissemination of this project began in fall of 2022 at the SUPERB Summer Institute, and the Tennessee Herpetological Society meeting. In 2023 I presented this research at the Southeast Partners in Amphibian and Reptile Conservation meeting, Spring 2023 Scholar's week, and the annual Midwest Ecology and Evolution Conference. Future plans for dissemination in 2023 include the Midwest Partners in Amphibian and Reptile Conservation meeting, Tennessee Herpetological Society meeting, and the Wildlife Society 30th Annual Conference. I intend to publish this research in a peer-reviewed journal upon successful defense of my thesis project in 2025.

D.) RESEARCH GRANTS: EQUIPMENT, SUPPLIES, AND RESOURCES

Sampling budget

Name of Item	Quantity	Cost per item/unit	Total Cost
Arbor Assays Corticosterone ELISA kit, 5-plate	2	$810.00	$1,620.00

Arbor Assays Corticosterone ELISA kit, 5-plate (Dr. Andrea Darracq, a collaborator on my project, has a discount with this company that reduces the item's price to $810 per kit).

E.) BUDGET JUSTIFICATION

Corticosterone sampling: Arbor Assays kits, required for sample processing, estimated at $1620.00. These kits are not available to me in my lab, so I must pay for them with external grant funding.

F.) INVOLVEMENT OF ANIMALS, HUMANS, OR SPECIAL MATERIALS

IACUC "Evolutionary Ecology & Conservation Biology of Tiger Salamanders", protocol number: RMBL-8

Approved for the Rocky Mountain Biological Station on June 17, 2023

G.) LOCAL PRESENTATION - Funded students are expected to share their expertise with campus. Use an **X** to indicate at least one of the following options.

Live Presentation Options:

[ ] Fall Scholars Week (November 13-17, 2023)
[X] Spring Scholars Week (April 15-19, 2024)
[ ] Posters-at-the-Capitol (March 7, 2024)

ORCA Journal *Steeplechase* Options:

[ ] Submit a full paper for peer review
[ ] Write a ~2,500 word research "snapshot"
[ ] Record an interview

Students: After Sections A-G have been completed by you, save and send the document to your Faculty Mentor to complete Section H.

H.) FACULTY MENTOR RECOMMENDATION

Faculty Member Name: Howard Whiteman
Faculty Member Email: hwhiteman@murraystate.edu
Academic College/Department: Biological Sciences/WSI
Contact Name for Interdepartmental Reimbursement: Gerry Harris
Contact Email for Interdepartmental Reimbursement: gharris@murraystate.edu

Please comment on the following seven questions.

i. Describe the merit of this project and its potential impact on the student.

Megan's project is both ambitious and has the potential to revolutionize the way we think about disease in amphibians, and, for that matter, in humans as well. It has already had an amazing impact on her, given her growth from her undergraduate work to the current sophistication of her research.

ii. Describe the student's ability to carry out the proposed project, in your view.

I have no doubt that Megan will complete this project successfully, particularly with her very supportive committee members.

iii. Describe the nature of past and present experiences you have had with this student (e.g., in a class, supervising this student as a research assistant, or in a directed study or scholarly project).

I first met Megan in my *Introduction to Wildlife and Conservation Biology* class (Fall 2019). Megan was a transfer student from a community college, and thus quite advanced compared to the incoming freshmen. She was one of the best students and by far the most active participant in the class. She was also one of the few students to ask me about getting involved in research (and she persisted, inquiring multiple times), and after discussing her interests, I offered her an opportunity. Her first job was simple—picking aquatic insect samples for my graduate students—but she took to it with a fervor, learned the insects quickly and became one of the best pickers in the lab. She worked hard, was always on time, and was diligent and careful about her work. Additionally, Megan was a regular participant at our weekly lab meetings, which is rare for our undergraduates, but continues to this day. She built on this success by earning one of the highest A's in my *Vertebrate Natural History* class during the spring of 2020, and this excellence continued through her undergraduate years, as she earned a solid 3.41 GPA.

Based on her initial performance and her interests, I suggested she might get more involved in my research, and she immediately began caring for an axolotl population that my former postdoc, Dr. Kelsey Reider, and I are using to help us understand better ways to study salamanders in the field. From her first day to the present, she has been diligent in her work, making sure that the animals are cared for and sending us updates. She also helped us with experimental surgeries, and **her work was so instrumental to the project that she was as a coauthor on the paper that Dr. Reider and I published in the *Journal of Experimental Zoology***. Today she supervises the axolotl colony and oversees three undergraduate workers, while sharing husbandry duties. I could not ask for a better RA.

Given this amazing progress and work ethic, Dr. Reider and I worked with Megan to develop an independent project, but it was Megan that came up with the idea of focusing on stress hormones. Megan initiated and developed these ideas with little guidance, and I have been impressed by her insight, ability to navigate and interpret the literature, and writing skills. She is an excellent student and a great thinker.

During the summer of 2021, Megan was awarded an NSF REU (Research Experience for Undergraduates) position at the Rocky Mountain Biological Laboratory (RMBL). With this support, Megan's work at Murray State paid off into an excellent field season. She conducted a series of experiments looking at how life history and sex influence stress hormones in salamanders. This question is broadly important for both basic (evolutionary ecology) and applied (climate change, disease) reasons, but is particularly important as it may help us better manage and restore endangered Boreal Toad populations. Megan set up a system by which she could sample multiple individuals, and kept meticulous notes of everything she did, which has proven useful as she analyzes her samples. Although her efforts were hampered by the pandemic, she persisted and presented her results at two local and one regional meeting in 2020, and, after building upon this work with **another field season in 2021 that was supported by grants that she wrote, including generous funding from WSI, won awards for her presentations at one local, one regional, and one national meeting**. We are currently working on finishing up analysis of her samples and writing a manuscript based on her undergraduate research.

Over the past year, Megan has built upon her excellent undergraduate work by taking her prowess in measuring stress in amphibians and combining it with disease, all within a climatically-susceptible elevation gradient. It is an ambitious project which has become her M.S. thesis, but one that I know she can complete because of her continued success in my laboratory.

iv. Describe your involvement in the project and your anticipated working relationship with the student, including your anticipated role in any direct outcomes of this research. If a conference or journal paper is an outcome of this study/project, how will the student's contribution be credited?

Megan is the primary on this work, but I have helped her with the design and implementation of her research and will continue to guide her until she is finished. Megan has and will continue to be first author of all presentations and papers that come from this project.

v. If the student has requested physical equipment or supplies (listed in part C.), please verify that these items are not otherwise made available to students through your department or school/college easily or freely, or make the case justifying the need for this supplement to pre-existing resources.

They are not available and are quite specialized, and yet they are critical to her research efforts.

vi. In section G, the student should have indicated how they intend to share knowledge back with the campus community. Are you able to support the student in the venue indicated?

Yes, Megan has presented her research each Scholar's Week and will continue to do so.

vii. Detail any other ORCA-funded research occurring this academic year that you are involved with.

Good question. I am not sure if Melissa Ocampo has current ORCA funding, but I am pretty sure her research was funded in 2022.

Faculty: Once completed, email this document to aboston@murraystate.edu with the subject line "ORCA Research Grant: [Student_Lastname]"