Robustness & Resilience of Aquatic Biosystems REU Abstracts 2025

2025 R&R REU Cohort

Back: Chris Close (Eastern Washington University), Thomas Holloway (WSU). **Middle:** Adel Bordas (Oregon State University), Jing Garber (U. Texas, Austin), Nina Allen (U. Wisconsin, Madison), Nicholas Kessler (WSU). **Front:** Ana Rowley (Scripps College), Jessica Matthew (U. South Dakota), Sofia Yildirim (St. Mary's College, CA), Tamia Love (N.C. Agriculture and Technology University), Ruby Moore (U. Florida) [Tammi Morellano (U. Tulsa) and Tyler Robinson (WSU) not shown]

Back: Grace Curtis, R&R REU Program Asst. Director, Adele Bordas, Chris Close, Jing Garber, Nicholas Kessler, Nina Allen, Tyler Robinson, Ruby Moore. **Middle:** Tammi Morellano, Sofia Yildirim, Jessica Matthew, Ana Rowley, Tamia Love. [Thomas Holloway not shown]

Phenomics of 6PPD-quinone Exposure in *Xenopus tropicalis* Embryos

Undergraduate Researcher: Nina Allen, *University of Wisconsin - Madison*

Co-Authors: Jennifer Michal, Grace Curtis, Kourtnie Whitfield, Erica Crespi, Zhihua Jiang

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
A major source of aquatic pollution is road run-off, which affects the health of aquatic animals inhabiting freshwater ecosystems adjacent to urban areas. Tire tread wear particles accumulate on roads and leach chemicals into rainwater, contributing significantly to this toxic runoff. Among the many chemicals in road run-off is 6PPD-quinone (6PPD-q), which is the oxidized form of 6PPD, a chemical ubiquitously added to rubber tires to extend their life. Although harmless on its own, 6PPD reacts with ozone to form the much more toxic 6PPD-q. While the effects of 6PPD-q exposure are well documented in fish, which show increased mortality, decreased growth, and disrupted vascular development, there is little research regarding its impact on amphibians, or regarding early embryonic development. We tested the hypothesis that environmentally relevant concentrations of 6PPD-q will have conserved effects on amphibians, which often breed in road-side ponds, using the *Xenopus tropicalis* model. We exposed embryos to control, vehicle (acetonitrile), and 2000 ng/L 6PPD-q (3 replicates/treatment) immediately after fertilization. We collected embryos at stages 8 and 11 (before and after maternal-to-zygotic transition), as well as 24 and 72 hours post-fertilization. At each stage, survival and phenotypic traits were measured and bulk RNA was extracted for whole transcriptome termini site sequencing (WTTS-seq). Although survival was high in all groups, compared to control and vehicle embryos, those exposed to 6PPD-q were developmentally delayed with vascular abnormalities, shorter bodies, and shorter, thinner, curvier tails, with symptoms starting at 48 hours post fertilization. WTTS-seq results will be used to understand the mechanisms of these phenotypic changes. These findings support our hypothesis that the effects of 6PPD-q are conserved in amphibians, and they indicate that the consequences of exposure may apply to a much broader range of animals, including humans, than previous thought.

Investigating the Role of EGLN3 in Hypoxia Response and Cardiac Physiology in Rainbow Trout (*Oncorhynchus mykiss*)

Undergraduate Researcher: Adel Bordas, *Oregon State University*

Co-Authors: Chaya Gaberria, Thomas Holloway, Jingping Graber, Georgina Cox, Michael Phelps

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Hypoxia is a prevalent and intensifying stressor in aquatic systems due to climate change, eutrophication, and habitat alteration. While rainbow trout typically require cold, well-oxygenated water, they show population-level variation in hypoxia tolerance. This study investigates the role of the gene *EGLN3*, a regulator of the hypoxia-inducible factor (HIF) pathway, in mediating physiological and cardiac responses to hypoxia in rainbow trout (*Oncorhynchus mykiss*). We hypothesized that knocking out *EGLN3* would enhance HIF stability under low oxygen, leading to increased hypoxia tolerance. To test this, we used CRISPR to generate *EGLN3* knockout (KO) fish and compared them to albino controls. Fish were anesthetized and placed in a custom system for ECG monitoring during a controlled hypoxia trial. After establishing baseline ECG readings, heart rate was pharmacologically manipulated using atropine to block parasympathetic input and isoproterenol to stimulate sympathetic activity. Following this, nitrogen gas was bubbled into the tank to reduce oxygen levels in 10% stepwise increments until signs of cardiac dysfunction or arrhythmia were observed. After euthanasia, hearts were fixed and sectioned for later histological analysis, including measures of ventricular compaction and vacuolization to assess cardiac development, structural integrity, and cellular stress under hypoxia. Preliminary results show that control fish are outperforming knockouts under hypoxia, with longer times to arrhythmia and more stable heart rate responses. Statistical analysis is ongoing, and histological data have not yet been collected. These early findings suggest that *EGLN3* may have a more complex role in hypoxia response than predicted.

Testing the Efficacy of Biosecurity Practices in the Ranavirus-Lithobates sylvaticus System

Undergraduate Researcher: Chris Close, *Eastern Washington University*

Co-Authors: Bob Pearhill, Jesse Brunner, Nick Kessler

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Biosecurity practices- procedures to limit the spread of infectious disease- are used in captive animal production to protect animal health, protect against zoonotic infection, and reduce spillover to wild populations. However, recommended procedures can be costly in time and resources: farmers may be forced to forgo biosecurity completely or adopt partial biosecurity practices where possible. To determine if this latter option is worth it, we must understand to what extent partial practices can reduce pathogen transmission. In this study, we use ranavirus (Rv) infection in wood frogs (*Lithobates sylvaticus*) as a model system to broadly represent many aquaculture settings. We handled one focally infected tadpole, with bare hands for one treatment, and gloved hands for the second treatment, and used the same hand to consecutively handle three uninfected tadpoles (each housed separately prior to and after handling). However, for the gloved treatment, we also dipped the hand briefly (1 second) into a diluted chlorhexidine rinse. This structure was repeated 25 times for each treatment group. I hypothesized the amount of virus on the hand would decrease after each tadpole was handled, due to direct contact or loss to the environment. If we assume a dose dependent model of infection, the likelihood of infection should decrease sequentially for each tadpole in the replicate. Similarly, the chlorhexidine rinse should decrease the likelihood of infection by deactivating virions or washing them off. Preliminary results support this hypothesis. Our results also suggest that even when biosecurity practices as recommended are not possible, partial adoption may still help meaningfully limit transmission.

Effects of Casr Knockout on Cardiac Function, Calcium Regulation, and Bone Mineral Density in Rainbow Trout (*Oncorhynchus mykiss*)

Undergraduate Researcher: Jingping Graber, *University of Texas at Austin*

Co-Authors: Chaya Gaberria, Adel Bordas, Thomas Holloway, Georgina Cox, Michael Phelps

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Rising temperatures and hypoxia increasingly threaten salmonid populations in both wild and aquaculture contexts. Rainbow trout (*Oncorhynchus mykiss*), a key aquaculture species with several anadromous populations listed as threatened or endangered, may benefit from strategies that enhance physiological resilience to these stressors. The calcium-sensing receptor (CaSR), a G-protein-coupled receptor involved in calcium regulation and environmental stress responses, has been implicated in hypoxia tolerance in other teleosts. In tilapia, hypoxia-induced downregulation of CaSR via miR-92a reduces apoptosis and liver damage, suggesting that suppression of CaSR activity may confer protective effects. To test this in *O. mykiss*, we generated CaSR knockout (KO) individuals using CRISPR/Cas9 genome editing. CaSR KO and control fish were subjected to acute hypoxia via stepwise reduction of dissolved oxygen, with cardiac function monitored using electrocardiography (ECG) to detect arrhythmia or cardiac failure. Following hypoxic exposure, brain and head kidney tissues were collected to quantify expression of *stanniocalcin-1* (STC-1), a hormone involved in calcium homeostasis. Additionally, microCT scans of the cephalic region were performed to assess bone mineral density. This study aims to clarify the functional role of CaSR in mediating physiological responses to hypoxia in *O. mykiss*, with potential implications for genome editing strategies aimed at improving stress resilience in salmonids.

Examination of the Effects of Egln2 Knockout in Oncorhynchus Mykiss Cardiovascular Function

Undergraduate Researcher: Asher Holloway, *Washington State University*

Co-Authors: Chaya Gaberria

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Understanding how fish, in this case Oncorhynchus Mykiss (Rainbow Trout) respond to oxygen stress is vital as climate change provides the new challenge of increased water temperatures and its accompanied reduced ability to hold oxygen. Egln2 is a gene that encodes prolyl hydrolase 1 (PHD1), and this enzyme is a key regulator of Hypoxia Inducible Factor 1 alpha (HIF-1alpha). Here we took fish (already edited before the study) with both egln2 and slc45a2 (a gene co-edited to show a visual marker – albinism - of success or failure of the edit) and ran EKG's while simultaneously running hypoxia trials. Oxygen was decreased in a stepwise manner, until arrythmias were found. This will help to examine the effects of egln2 knockout on the cardiovascular function of these fish, specifically through its regulation of HIF-1alpha. A protein workup to show relative expression in sampled tissues (liver and spleen) will be performed via Western Blot, and the anti-bodies being tried have never been attempted in Rainbow Trout for this purpose. This will give a semi-quantitative answer to describe the control egln2 exerts over HIF-1alpha.

Sequential Contact and Disinfection: Testing Aquarium Net Fomite-mediated Transmission of Ranavirus in Wood Frogs

Undergraduate Researcher: Nicholas Kessler, *Washington State University*

Co-Authors: Bob Pearhill, Jesse Brunner

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Biosecurity practices are used in captive animal farming to protect the health of livestock and prevent zoonotic infections and spillover into wild populations. However, biosecurity practices can be prohibitive in terms of time or cost requirements, and some businesses may not have the resources to completely implement recommended interventions. Farmers are then left with two choices, partially adopting practices where possible or completely forgoing biosecurity. To determine whether partial biosecurity practices are worth the cost, it is first necessary to understand to what extent they reduce pathogen transmission. In this study we seek to answer this question for a simple biosecurity practice - disinfecting nets between animal contacts - using ranavirus (Rv) infection in wood frogs (*Lithobates sylvaticus*), a model system that is broadly representative of many aquaculture settings. A single replicate comprised using a net to capture an experimentally exposed tadpole followed by using the same net to consecutively capture three uninfected animals. This structure was repeated 25 times across two treatment groups: 1) no disinfectant between contacts, and 2) brief submersion (1 second as opposed to industry recommended 1 minute) of nets in a dilute chlorhexidine bath between all captures. I hypothesized that the amount of virus remaining on nets would decrease fractionally after each capture event due to direct contact with tadpoles and loss to the environment. This would lead to a reduction in the probability of transmission with each consecutive tadpole capture. Our preliminary results tentatively support this view, with the proportion of infected animals appearing to decrease after each subsequent capture. Additionally, the chlorhexidine treatment reduced the probability of transmission relative to no disinfectant. Our results lend credence to the notion that in contexts where complete biosecurity practices are prohibitive, partial measures may still be worth pursuing in order to limit transmission.

Mitigating *Batrachochytrium dendrobatidis* Infections in Juvenile Wood Frogs Through Dietary Short-Chain Fatty Acid Supplementation

Undergraduate Researcher: Tamia Love, *North Carolina A&T State University*

Co-Authors: Erica Crespi, Alexa Dulmage, Tyler Robinson, Robert Pearhill, Nicholas Kessler, Chris Close

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Amphibian populations globally are facing significant declines due to the fungal pathogen Batrachochytrium dendrobatidis (Bd), which causes lethal skin infections. Short-chain fatty acids (SCFAs), like butyrate and acetate, are known to influence immune responses and improve epithelial barrier integrity in other vertebrates; however, their effects on amphibian immunity remain unexplored. Wood frog juveniles are susceptible to Bd, yet the impact of SCFAs acquired during the larval stage on post-metamorphic infections is unknown. Preliminary data indicated that SCFAs, particularly butyrate, reduced ranavirus infection rates in these frogs. We hypothesized that increased SCFA production during the larval stage promotes physiological carry-over effects that enhance immunity and skin health in post-metamorphic frogs, thus reducing Bd susceptibility. To test this, late-stage wood frog larvae were given one of four diets during metamorphosis: alfalfa (control), alfalfa plus 1% butyrate, alfalfa plus 1% acetate, and alfalfa plus both 1% butyrate and 1% acetate (three replicate tanks with 4 treatment groups -10 larvae each). We measured the diets' effects on the time to metamorphosis, body size at metamorphosis, and monitored infection severity and survival in Bd-inoculated juveniles. Results showed that the group receiving both butyrate and acetate was significantly larger than the others and had higher survival rates post-Bd exposure. Conversely, the acetate, butyrate, and control groups exhibited similar mortality rates. These findings suggest a synergistic effect between butyrate and acetate in reducing mortality rates. Specifically, butyrate enhances immune function, while acetate may reduce stress through the brain-gut axis. This research introduces the concept of the “gut-skin axis” in amphibians and supports the idea that SCFAs could be a novel, safe, and cost-effective strategy for amphibian conservation.

Interspecific Variation in Growth Rate in the Cryptic Species Complex, Prymnesium parvum

Undergraduate Researcher: Jessica Matthew, *University of South Dakota*

Co-Authors: Timilehin Jeje, Jennifer Wisecaver

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:

Prymnesium parvum (Haptophyta) is a planktonic, single-celled eukaryote commonly associated with harmful algal blooms (HAB) that devastate aquatic ecosystems. *P. parvum* are mixotrophs, allowing them to produce energy through photosynthesis as well as ingestion of prey via phagocytosis. Through the production of toxins, *P. parvum* immobilizes and ruptures the cells of microbial and gill-breathing organisms. The *P. parvum* species is a genetically diverse species complex, with cryptic species producing unique toxins. Morphologically, *P. parvum* strains are indistinguishable from one another, but they exhibit considerable phenotypic diversity within the species complex. Previous studies conducted on different *P. parvum* strains show that strains grown with prey have a higher growth rate than strains grown without prey. We sought to repeat the experiment and tested the hypothesis that *P. parvum* of differing genotypes vary in their response to prey. Six strains from a previously collected 2020 HAB in the Possum Kingdom Reservoir (TX, USA) were grown in phosphate-limited media. Of the six strains, four were cryptic species AH and two were cryptic species B. Strain identity was confirmed through restriction digest analysis. To measure the growth rate of *P. parvum* with and without prey, daily flow cytometry measurements were taken to estimate cell densities of *P. parvum* and a prey/competitor species, *Dunaliella tertiolecta* (Chlorophyta). Growth rates of the AH cryptic species were higher in treatments grown with prey compared to their control treatments. However, in the B cryptic species, we found that the growth rates were higher in the control treatment group than in the prey treatment. These results suggest that there may be different growth strategies and consumption patterns among the strains of the *P. parvum* cryptic species.

The evolution of the intramandibular joint in reef fishes

Undergraduate Researcher: Ruby Moore, *Florida State University*

Co-Authors: Katherine A. Corn

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
The intramandibular joint, or IMJ, is a major evolutionary novelty among reef fishes used to access substrate-attached prey items like algae, coral, and sponges. An IMJ permits flexion between the two largest bones of the oral jaws, the articular and the dentary bones, which allows for longer jaw protrusion, increased jaw gape while mitigating the trade-off between force and gape, and more contact between jaw and substrate, all of which aid the removal of substrate-attached prey items. Yet the evolution of a novel jaw joint in an already working functional system poses a challenge for any lineage, suggesting that the evolution of IMJs should be rare. We explored the history of IMJ evolution and how IMJ presence affects the accumulation of body shape diversity in reef fishes. A literature review was conducted to identify IMJ presence and absence, and IMJ flexion was measured from cleared and stained specimens. We analyzed this data by simulating trait histories of the absence and presence of the IMJ, using joint discrete character mapping to compare transitions between feeding modes and the IMJ presence, and using multivariate disparity to compare the relative body shape diversity among IMJ-bearers and non-IMJ-bearers. We found that, contrary to our expectations, IMJ evolution is not unidirectional; and that IMJ is associated with constrained body morphology compared to both suction feeding reef fish as well as biting reef fish without IMJs.

Molecular Response to Hydrostatic Pressure Throughout Zebrafish Embryogenesis

Undergraduate Researcher: Jaralynn Tammi Morellano, *University of Tulsa*

Co-Authors: Jennifer Michal, Emery Edgar, Katelyn Mika, Zhihua Jiang

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
As aquatic organisms retreat from warming surface waters, they often migrate into deeper or cooler environments. These zones are characterized by darkness, limited food availability, and intense increases in hydrostatic pressure (HP). These conditions can disrupt cellular processes, preventing many aquatic species from venturing deeper into the waters. This study investigates how *Danio rerio* (Zebrafish) responds at the transcriptomic level to the stress of HP. Using publicly available RNA-seq datasets, we identified differentially expressed genes (DEGs) in adult zebrafish tissues (ovary, muscle, eye, and brain) under HP using a Kallisto-Sleuth pipeline. We then compared these results with control embryo datasets at timepoints that span the maternal-to-zygotic transition (MZT). By identifying shared DEGs between adult tissues and embryos, we aim to reveal candidate genes involved in conserved pressure responses across developmental stages. By observing stages across the MZT, we plan to determine whether maternal RNA is similar or different from zygotic RNA in the HP stress response. In addition, we are generating our own RNA-seq dataset from pressurized and control embryos to validate and extend these findings. Unlike past research, our experiments expose embryos to gradual increases in atmospheres (atms) to simulate natural increases in HP. Our RNA was extracted with Qiagen-RNeasy Minikits and sequenced via Whole Transcriptome Termini Site Sequencing (WTTS-Seq), allowing us to identify polyadenylation events and mRNA variations. This research will fill a major gap in environmental transcriptomics by studying how pressure responses occur in a model shallow-water species. It may reveal a previously uncharacterized stress-response pathway and offer novel perspectives into how current aquatic species might adapt to deep-sea environments or future ecological pressures.

Butyrate health effects on Batrachochytrium dendrobatidis infected wood frogs (*Lithobates sylvaticus*)

Undergraduate Researcher: Tyler Robinson, *Washington State University*

Co-Authors: Tamia Love, Alexa Dulmage, Robert Pearhill, Erica Crespi

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Globally, amphibian populations are falling to historically low numbers in part due to the deadly fungal pathogen *Batrachochytrium dendrobatidis* (Bd). The prevalence of Bd in amphibians worldwide is Bd has been observed in ~700 species of amphibians. Itraconazole, an antifungal agent, is currently used to reduce infection severity, but it requires multiple water baths and precise dosing because it can be toxic to smaller amphibians. A new approach is to leverage endogenous factors that strengthen the immune system to fight infections of Bd. In order to address this critical conservation problem, we hypothesize that dietary butyrate, a short chain fatty acid, will strengthen adaptive immunity and skin barrier integrity as it has been shown to do in mammals, thereby decreasing Bd zoospore load on skin (the site of infection for this pathogen). To test this, we exposed recently metamorphosed wood frogs to Bd then stratified them by weight and developmental stage into three dietary groups (n=37): a control group (flightless drosophila flies), a butyrate group (flies dusted with sodium butyrate) and a Reptivite group (flies dusted with this commonly used dietary supplement for amphibians). We showed that frogs ate flies dusted with butyrate as readily as flies of the other treatment groups, and we were able to confirm an increase of sloughing and increase in lethargic behavior in infected frogs (indicating successful inoculation). Frogs will be monitored for Bd-related health symptoms, BD zoospore load, and growth for 30 days after inoculation. If our hypothesis is supported, dietary butyrate supplements could become a more risk-free way of treating Bd and potentially other pathogen infections in amphibians.

Community Physiology in Splashpools: Interactions of Primary Producer and Consumer Responses to Thermal Stress

Undergraduate Researcher: Ana Rowley, *Scripps College*

Co-Authors: Murad Jah, Wes Dowd

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Rising temperatures threaten to push intertidal organisms beyond their thermal limits. Most stress physiology studies in this system have ignored the potential effects of other species' responses, yet such community-scale physiological interactions could be important, particularly for producer-consumer species pairs. Recent studies suggest that the thermal history of primary producers can also influence the physiology of their consumers, leading to changes in the consumers' polyunsaturated fatty acids (PUFAs). PUFAs play a critical role in shaping membrane composition and thermal stress responses. The intertidal copepod Tigriopus californicus is an ideal organism for exploring climate resilience due to its ability to survive its highly variable and extreme splashpool habitat, and this study extends our focus beyond abiotic factors to ask whether dietary intake of thermally stressed algae influences heat tolerance of copepods. We reared green algae (*Nannochloropsis*) at 17 or 27 °C and then fed these diets to adult and larval copepods raised at the same two temperatures. We assayed copepods' heat tolerance in each of the four conditions and will extract lipids from both the diet and the copepods for analysis of membrane fluidity and composition. Together, this work examines the impact of consuming thermally stressed primary producers on consumers. Many aquatic organisms consume primary producers that are also adjusting to ambient conditions, and this study can catalyze future studies on the effects of climate change on broader physiological relationships across food chains.

Feeding Performance Across Functional Niche Space

Undergraduate Researcher: Sofia Yildirim, *Saint Mary's College of California*

Co-Authors: Katherine Corn

Summer Research Program: Robustness and Resilience of Aquatic Biosystems

Abstract:
Ecomorphological specialization has the potential to bolster both an individual's performance at an ecological task and to improve their community's diversity and thus resilience to changing conditions. Functional diversity due to morphological differences in jaw structures can lend deeper insight into the patterns and processes of ecological diversification. To understand the full picture of a functional niche a clear understanding of the feeding performance of an organism is essential. In this study, we examined how feeding performance changes across an array of different prey types for the damselfish species, *Amphiprion ocellaris*. *A. ocellaris* use small jaws with a relatively blunt head to consume a mix of algae and plankton in the wild, so we expected that these fish would feed well on complex prey structures, perform moderately on flat prey, and perform poorly on divotted prey. We 3D printed five different prey type models with varying complexity, which were covered with a food paste consisting of seaweed, shrimp, garlic, and gelatin. The weight of the structure was taken before and after 20-minute feeding trials to measure the amount of food consumed by each fish. An ANOVA revealed significant differences in performance of prey removal among structures, with greater variance in feeding performance on complex structures compared to flat surfaces. Our results suggest that *A. ocellaris* are well suited for feeding on relatively simple prey, but their lack of elongated snout makes their capacity for prey capture off complex prey dependent on varying ability to maneuver their entire body to access prey that is tucked away in nooks and crannies. This provides some of the first evidence that variance in functional properties of prey, which range among damselfishes from algae to plankton, affect the total capacity of a damselfish to utilize a given resource on the reef.