

Vertically Integrated Project Theme

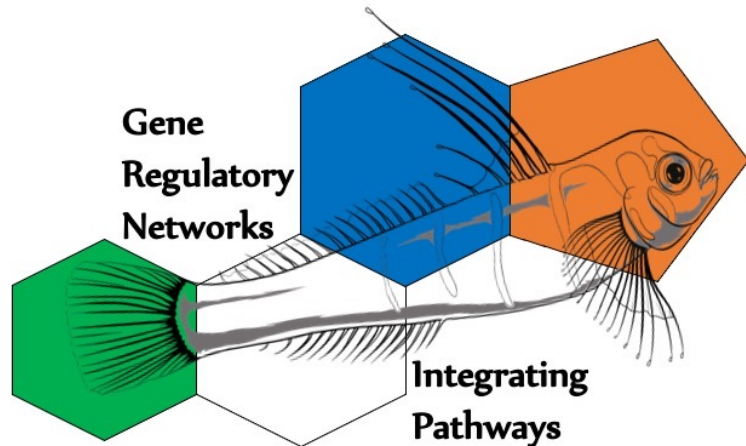
Gene Regulatory Networks in a sexually plastic fish: *Empowerment through Research Engagement*

Devaleena Pradhan

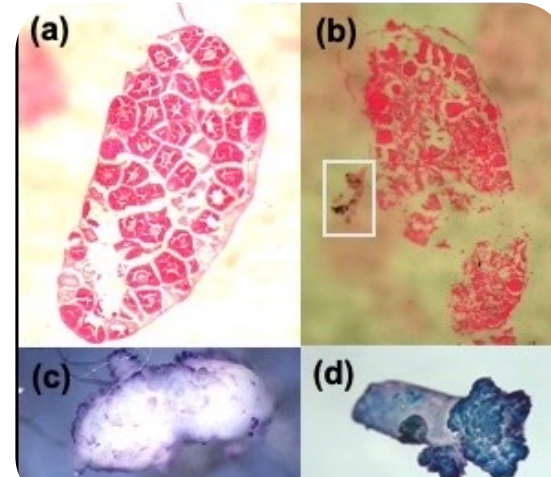
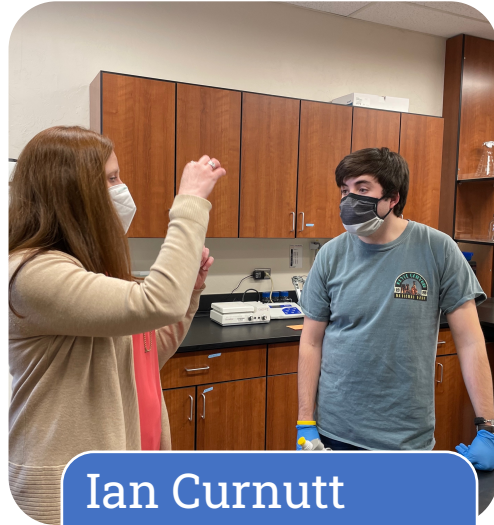
Associate Professor

Department of Biological Sciences

Idaho State University



Building a collaboration: two early career PIs



**Behavioral
Endocrinologist and
Developmental Biologist**

Photographed in our new joint lab space

Used our startup funding to initiate project

**Ian Curnutt
SARE Summer
2022**

Traveled to Atlanta to present poster

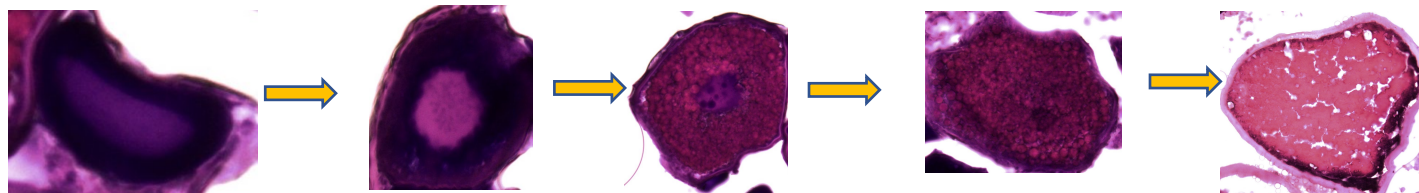
Pradhan: NSF CAREER 2022-2026

Ray and Pradhan:

GEM3 VIP, ISU new faculty seed grant in Dec 2021

Upcoming: Society for Developmental Biology: emerging research organisms

**"Gene Regulatory
Networks"**
endocrine and developmental genes involved in sexual plasticity



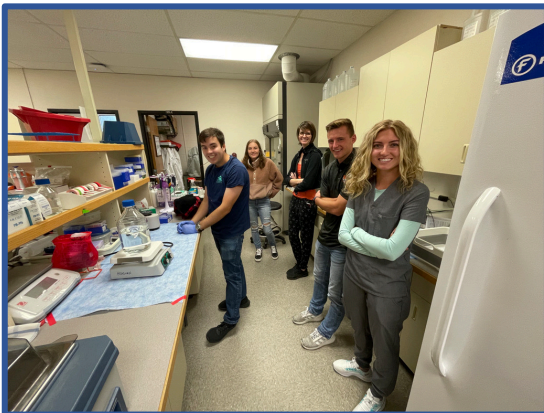
Workforce Development Support:

- SARE students Summer 2022 (1), 2023 (2) helped launch and make progress on the VIP
- Idaho State Board of Education (SBOE) Grant – paid 1 undergrad for GEM3 research (~\$3000)
- ISU small grant program Co-PI with Heather Ray in Dec 2021 (\$10,000)
- Received GEM3 VIP Co-PI with Heather Ray in May 2022 (\$12,000)
- Received NSF CAREER award in June 2022 (AIM 3 uses the VIP approach)

3 Semesters of VIP

Fall 2022

- 6 Undergrads
- 2 Grad students
- Course layout:
 - Weekly classroom meetings
 - Research check-in, planning
 - Process of science
 - Training on research methods – tissue embedding, record keeping, quantification methods
 - Purchase of equipment, supplies (cryostat)
 - Setting up a new lab space



Spring 2023

- 7 Undergrads
- 3 Grad students
- Course layout:
 - Weekly classroom meetings
 - Research check-in, planning
 - Reading and discussing primary literature
 - Training on research methods with increased peer training – cryosectioning, H&E staining, microscopy
 - Data collection and analysis
 - Increased student ownership of project

Fall 2023

- 6 Undergrads
- 4 Grad students
- Course layout:
 - Weekly classroom meetings
 - Research check-in, H&E staining, microscopy, counting
 - Data collection and analysis
 - Senior students leading experiments and workflow
 - Senior students expressing interest in wanting to participate in writing manuscript
 - Interest in having their own project

2 Summers of SARE

Summer 2022 1 student

Summer 2023 2 students

Highlights

- We gave in person informational presentations at Bio 1102 labs in Spring 2022 to recruit students
- We worked together on a syllabus that helped align our collaborative goals and schedule
- Skill oriented research techniques: preparing tissues for cryostat sectioning, *in situ* hybridizations, fluorescence microscopy
- Weekly team meetings emphasizing the process of science including writing in notebooks, reading articles, bioethics, setting goals, presentations, discussing time management, communication
- Students teaching others

Challenges

- Identifying students have different levels of interest/commitment
- How to keep students accountable
- Keeping students motivated when unexpected setbacks occur (equipment breakdown)
- Students learning how to schedule their weekly research time and coordinate schedules
- Continuity in notes – collaborated on spreadsheets, Box folders, Slack

Mid-semester and Final Reflections each semester helped us get feedback

→ Prompted questions to help guide
the content.

"I enjoyed spending time in the lab, and it all culminated with the end of the year poster presentation. It was pleasing to see everyone work together to bring about the course's first group poster. I found that it was gratifying to be able to go through each part of the poster and understand it."



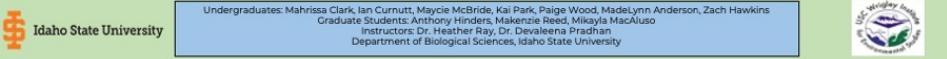
Research Roundup, Spring 2023

Presentations

- Society for Behavioral Neuroendocrinology and ICUR (2022) – Curnutt et al “Examining follicular development in a bidirectional hermaphroditic fish”
- GEM3 Annual Meeting (2022) – Workforce Development session (DP)
- Society for Integrative and Comparative Biology (2023) – Dirickson et al “Ovarian Follicular development and AMH distribution in the hermaphroditic fish *Lythrypnus dalli*”
- Research Roundup (2023) – entire class
- ICUR (2023) (by two SARE funded students)
 - Clark et al “Ovarian morphology of the female *Lythrypnus dalli*”
 - Cain et al “The expression of aromatase in *Lythrypnus dalli* during protogynous sex change”
- Society for Integrative and Comparative Biology (Jan 2024 – forthcoming oral presentation)
 - Ray, Anderson, Clark, Curnutt, Hawkins, Park, Pradhan “Using a Vertically Integrated Project to characterize ovarian follicles in a sexually plastic fish”

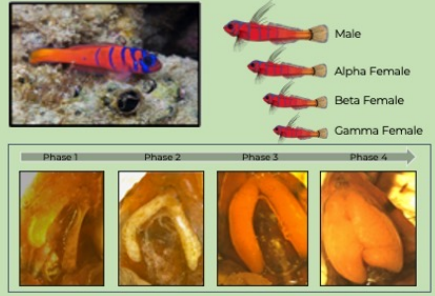
Analyzing the Ovarian Cycle in Sex Changing Gobies Conducted through a Vertically Integrated Project: GRN

Undergraduates: Mahrissa Clark, Ian Curnutt, Maycie McBride, Kai Park, Paige Wood, MadeLynn Anderson, Zach Hawkins
Graduate Students: Anthony Henders, Makenzie Reed, Mikayla MacAluso
Instructors: Dr. Heather Ray, Dr. Devaleena Pradhan
Department of Biological Sciences, Idaho State University



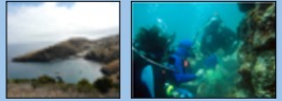
About the Bluebanded Goby

- *Lythrypnus dalli*, also known as the Bluebanded Goby, is a hermaphroditic fish capable of bidirectional sex change based on its social status.
- As females go through the reproductive cycle, morphological changes of the ovaries can be observed and categorized into ovarian phases and follicle stages.
- These changes can then be quantified by measuring the area of the follicles.
- The data is used to analyze the relationship between follicle area, follicle stage, and ovarian phase.




Capturing Fish

- Fish were caught off the coast of Santa Catalina Island in 2019.
- Fish were dissected and ovaries were fixed for long term storage.



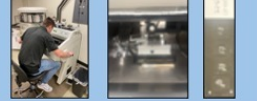
Embedding and Rehydrating

- Fixed tissues were rehydrated and embedded in a mold to prepare for sectioning.
- Embedded tissue is placed in OCT and frozen to prevent morphological damage.




Sectioning

- A cryostat slices frozen tissue into thin sections that can be fixed onto slides for histological staining and microscopic analysis.



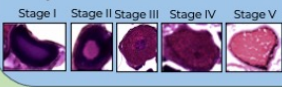
About Vertically Integrated Project (VIP): Gene Regulatory Networks (GRN)

- This class has a VIP approach, allowing for increased diversity and preparation of students and their skills.
- In this class, undergraduates, graduate students, Dr. Heather Ray, and Dr. Devaleena Pradhan work together in a team-based approach.
- Students receive opportunities to experience long-term research and more involved mentorship. The aim of this project is to study the GRN that govern sex change in the Bluebanded Goby.



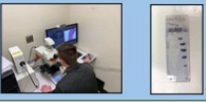
Quantification

- Follicles were classified by stage and their ovarian phase.
- Follicle area was measured by tracing the perimeter using a program called Fiji.
- Using this data, the relationship between follicle stage and ovarian phase can be analyzed.




Microscopy

- Stained sections were imaged using the Molecular Core Facility's Brightfield Microscope, LEICA DM6 B model.
- Leica Application Suite X imports the exact dimensions of the image to obtain accurate measurements.



Histological Staining

- Slides are stained for histological analysis.
- Hematoxylin stains nuclei and lipids purple, while Eosin stains connective tissue pink.
- Coverslips are placed on the slides to preserve the tissue.



Conclusions

- Our work characterizes markers of stable females among a sex-changing species.
- The number and area of each follicle stage can be used to estimate the levels of estradiol in the individuals used in this experiment.
- As the ovarian cycle phases progress, the ovary increases in size and the amount of developed follicles that are present.
- Future projects will focus on characterizing male and sex transitioning individuals in comparison to this project's findings.

Acknowledgements

- Molecular Core Research Facility at Idaho State University
- This project was supported by grants from NSF award number OIA-1757324 from the NSF-Idaho EPSCoR Program and ISU Office for Research to HDR and DSP, and NSF CAREER #2145398 to DSP.

QUOTES FROM STUDENTS: MID SEMESTER REFLECTIVE ESSAYS –Fall 2023

“In science, it really is a group effort to find information about how things work”

“I have found it a learning experience to teach techniques and skills I did a year ago to students who have never learned lab techniques in a research setting”

“I feel like so far in my journey in the lab, I have a much better chance of getting a job after I graduate. With having contributed to three posters, I can show to employers that I have some major production in a year”

“This is my first time working with an organism like the bluebanded goby and it has been fascinating to learn about because I can easily see how the things we are learning translate into the real world”

QUOTES FROM STUDENTS: FINAL SEMESTER REFLECTIVE ESSAYS – Spring 2023

“As a scientist, I have started to understand the scientific process and why it is so vital experimentally and intellectually”

“I grew as a scientist because I learned how to ask questions and how to go about answering them”

“My expectation coming into this course was to get some lab experience, not only to determine whether lab work/research was something I wanted to pursue in the future, but to grow as a scientist as well. I do think those expectations were met.”

“Being in a lab gave me opportunities to learn how science truly works, lead, and perform science. Research wasn’t always easy, good things never should be, but it was easier with great friends and great guidance”

Feedback from NSF Reviews regarding VIP:

STRENGTHS:

- *“Aim 3 will generate a number of target genes but leverages an innovative educational strategy to maximize the impact of the data that will be generated.”*
- *“Aim 3.1 nicely lays out an integrated education and research program for students with multiple mentors (PI, other faculty, postdoc, graduate students). A postdoc will be recruited to implement the VIP while at the same time evaluating and assessing efficacy educational activities.”*
- *“The VIP project is an excellent integration of the PI’s research with undergraduate training.”*

WEAKNESSES:

- *“While the PI does not have experience on techniques needed for aim 3, a collaboration has been set up at ISU with a molecular biologist faculty member (Ray) to oversee student training for these methods.”*
- *“The proposed VIP project is extremely ambitious and though there is departmental support for its development, it is still ambitious – this concern is mitigated by the PI’s amazing prior track record and pilot efforts. Though not a weakness or limitation of the current proposal, it would be exciting to make the findings from the VIP project available for other researchers and students.”*

Vision for the next few years

- More tiered VIP structure to facilitate the group meetings
- Publish paper on ovarian cycles – entirely undergrad driven
- Starting a couple new projects – at least one with a grad student
- Better workflow separation
- Add ISU to the worldwide VIP consortium
- Program assessment – collaboration with Dr. Anna Grinath
 - Recruited MS student Kasey Wozniak Fall 2023
- Future funding, especially for summer support



Acknowledgements

Collaborators

Heather Ray (co-leader)

Anna Grinath (biology education assessment research)

Undergraduate students

Kai Park, Ian Curnutt, Calvin Dirickson, Paige Wood, Maycie McBride, MadeLynn Anderson, Zach Hawkins, Andrew Christensen, Mahrissa Clark, Kaitlyn Thornsberry-Bistline, Grace Cain

Graduate students

Anthony Hinders, Makenzie Reed, Mikayla MacAluso, Kasey Wozniak (course assessment)

ISU Support

Janet Loxterman, Kitty Griswold



Faculty Early Career
Development Program
(CAREER) Award



GEM3
Genes by Environment
Modeling · Mechanisms · Mapping



**Idaho State
University**
Biological Sciences

College of Science and Engineering
Office for Research

