



# Use of Resazurin to Analyze Potential Sex and Age Specific Metabolic Differences in Zebrafish (*Danio rerio*)



Addesyn R. Aderogba, Mason M. Strickland, and Peggy Biga  
The University of Alabama at Birmingham, Dept. Of Biology



# Background

## Greater Connection:

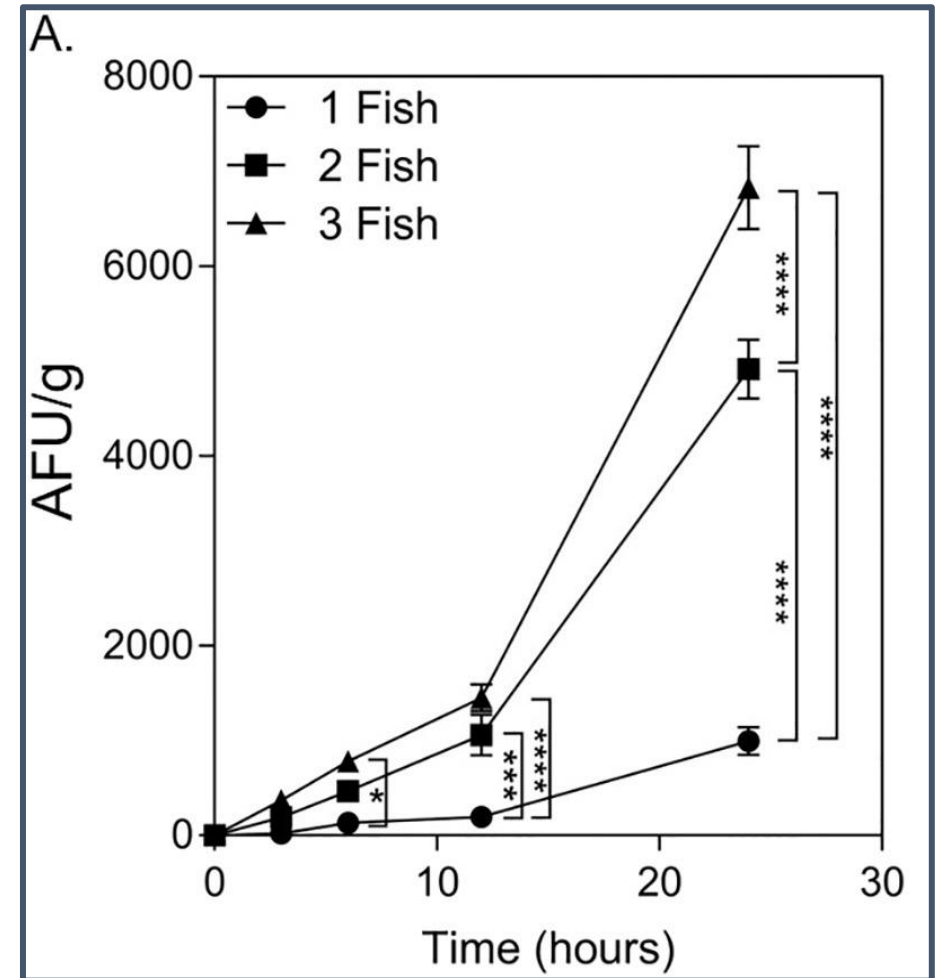
- An understanding of specific diseases such as autoimmune disorders, neurodegenerative diseases, and certain cancers.

## Background:

- Use of resazurin as an indicator of metabolic activity through the observation of the reduced fluorescent form, resorufin<sup>1</sup>.
- The zebrafish were grouped by young and old females and young and old males.
- Previous research has identified a positive trend between time in resazurin solution and resorufin concentration<sup>1</sup>.

## Hypothesis

- A correlation in metabolic changes between sexes and age groups of the zebrafish is expected
  - Young fish will have higher metabolic rates than old fish.



1) Reid, R. M., D'Aquila, A. L., & Biga, P. B. (2018). The validation of a sensitive, non-toxic in vivo metabolic assay applicable across zebrafish life stages. *Comparative biochemistry and physiology. Toxicology & pharmacology*, CBP, 108, 29-37.



# Methods

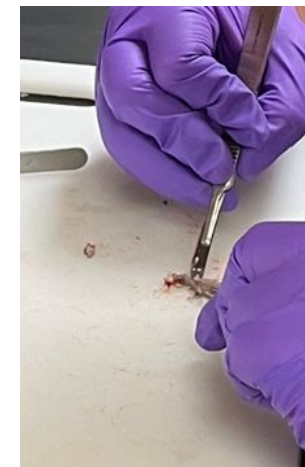
## Assay:

- Three experimental trials:
  - A control trial
  - Three-week fasted trial
  - Fish that were refed for one week
- One fish placed in conical tube with 50 mL resazurin solution, placed at 45° angle
- Four collection periods: 500 uL of resazurin solution collected four, eight-, and twelve-hours post exposure to the resazurin solution.



## Dissection:

- Performed on six fish from each sex and age group
- Tissues collected:
  - muscle
  - liver
  - whole fish
- Samples were flash frozen and histology techniques will be used to identify gonads of fasted fish





# Conclusion

## Next Steps:

- Analyze resazurin samples:
  - Use a microplate reader to collect resorufin concentrations in each sample
  - Analyze and interpret resorufin FI data
- Perform polymerase chain reaction (PCR) on all of the tissue dissection samples:
  - Target the mTOR, AMPK, and GCK genes with species-specific primers

## Skills Learned:

- Zebrafish related work
  - Basic husbandry
  - Dissection
- Conducting Assay
- Use of microplate reader
- Cryostat Histology Techniques

## Related Themes :

- Themes 1 and 2