



# Gene Expression of Cytochrome P4501A (CYP1A) in *Cyprinodon variegatus* when exposed to WAF and CEWAF in various Salinities

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# PowerPoint Summary

Gene Expression of Cytochrome of P4501A (CYP1A) in *Cyprinodon variegatus* when exposed to WAF and CEWAF with different Salinities

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**Special Thanks**  
To everyone I worked with

# Veronica Alston

## Background

**Student - Tuskegee University, College of Agriculture, Environment, and Nutrition Sciences**

### **Education:**

B.S., Tuskegee University, 2010 – Present, Animal and Poultry Science

-In my fourth year I will have a dual enrollment in Tuskegee University undergraduate program and Tuskegee University Veterinary Medicine.

### **Research Interests:**

I am interested in cancer and stem cell research through animals so that my work with animals will one day help people. Also I hope to one day either work for the Central Diseases Control (CDC) or travel to different parts of the world to help out the community and the people who live there with my knowledge of animal medicine.



# Joe Griffitt

## Background

**Assistant Professor - Department of Coastal Sciences, University of Southern Mississippi**

### **Education:**

Post-doc, University of Florida, 2006-08, Nano-toxicology

Ph.D., University of South Carolina, 2002-06, Environmental Health Sciences

C.A.S., University of South Carolina, 2003-05, Statistics

M.S., University of South Carolina, 2000-02, Marine Science

B.S., UNC Wilmington, 1993-97, Marine Biology



### **Research Interests**

The main interest of my laboratory centers around the study of the effect of anthropogenic contaminant on aquatic organisms. We focus on molecular approaches aimed at identifying the precise cellular and molecular pathways that are affected by different contaminants.

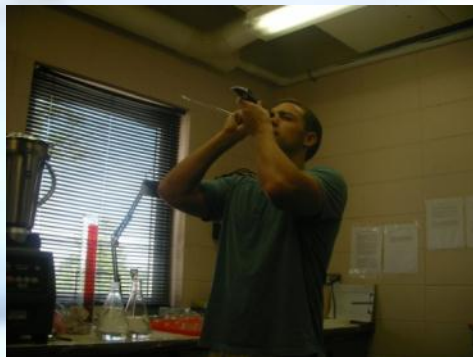
# Some people who helped

Wonderful friends



## Idrissa Boube

- Help me get use to the area and helped me get settled in the every day life at USM



## Bryan Hedgpeth

- Helped me with collecting data and showed me how do a lot of different things in the lab.

# Field Experience

## Overlook



Joe is helping create CEWAF as well as showing me how to measure everything out.



One day we went out to search for shrimp in the marshes near the lab.



A group picture of every one I met and worked with.

# Project Summary

Gene Expression of Cytochrome P4501A (CYP1A) in *Cyprinodon variegatus* when exposed to WAF and CEWAF with different Salinities

- The overall summary of my project was to test the changes in the CYP1A gene when *Cyprinodon variegatus* (Sheepshead Minnow) were exposed to different levels of salinities in WAF and CEWAF treatments.
- The salinities that were used in this experiment were 5 parts per thousands (ppt), 15ppt, and 25ppt.
- The purpose of the different salinities was to see if the salinities had an effect on the CYP1A gene while being exposed to oil, WAF and CEWAF,.**
- CYP1A1 is involved in phase I xenobiotic and drug metabolism. It is involved in the metabolic activation of aromatic hydrocarbons (polycyclic aromatic hydrocarbons, PAH).

A circular inset image showing a microscopic view of several small, yellowish, tadpole-like larvae of Cyprinodon. The larvae are scattered across a light-colored, slightly textured background.

Larval Cyprinodon

A circular inset image showing a group of adult Cyprinodon fish in a tank. The water is greenish, and the fish are visible near the bottom of the frame. A white pipe or ledge is visible in the lower-left corner of the circle.

Adult Cyprinodon

# What is WAF and CEWAF

The difference between the two.

## WAF

WAF is Water Accommodated Fraction. This means that the oil in the water has not been treated with anything and consists of just oil and water.

WAF is made in the lab by blending the oil for 30 seconds then pouring the compound into a Separatory funnel and covering with foil for one hour. After this the oil is drained and ready for use.



## CEWAF

CEWAF is Chemically Enhanced Water Accommodated Fraction. This means that the water that has oil in it has been treated with an oil dispersant. The water will contain oil and a dispersant with in it.

CEWAF is made in the lab by mixing oil and dispersant in a aspirator bottle with a magnetic stir bar. Covering with foil this spins for about 18 hours and let sit for an additional three hours.



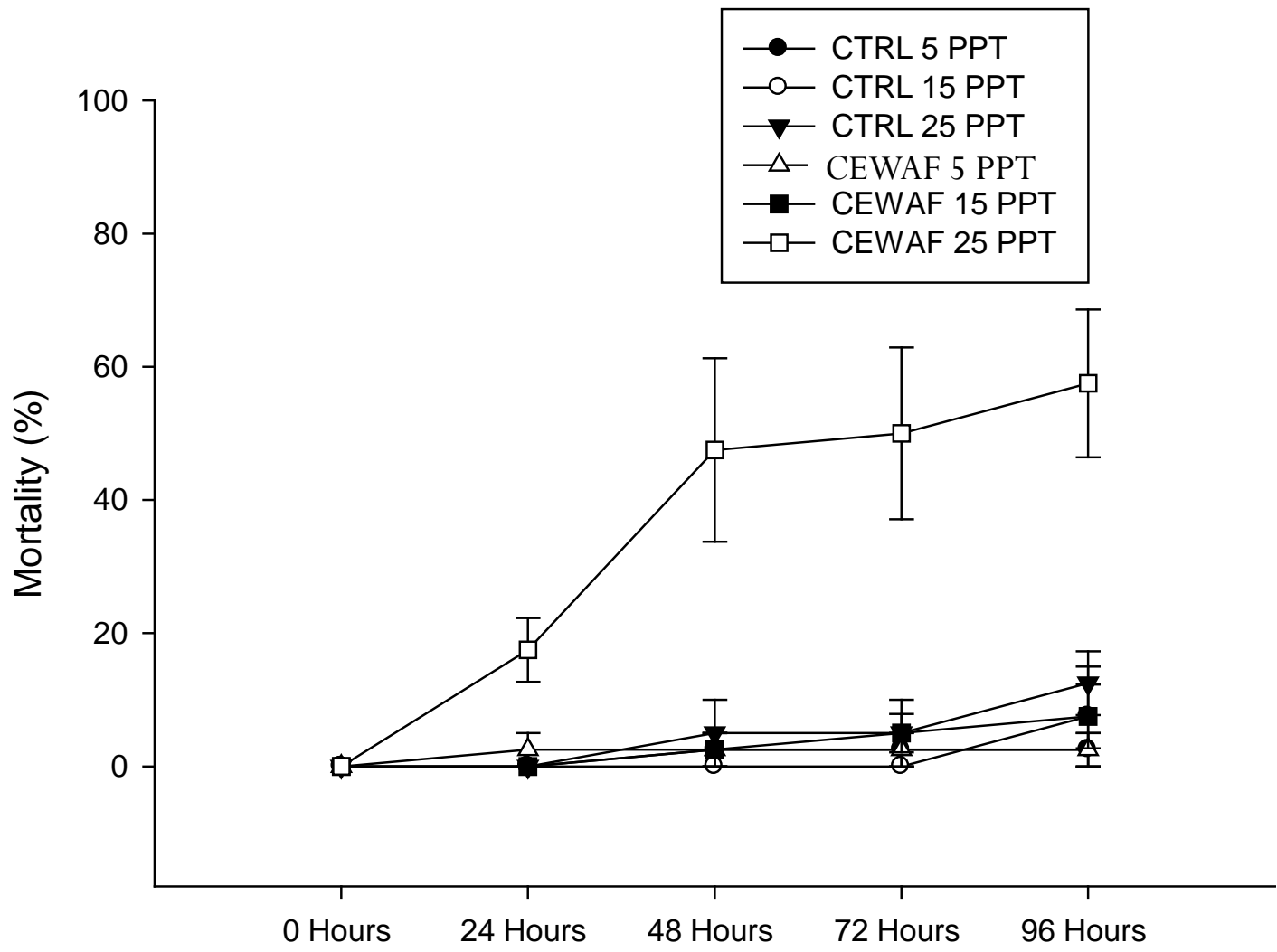


NOW THE

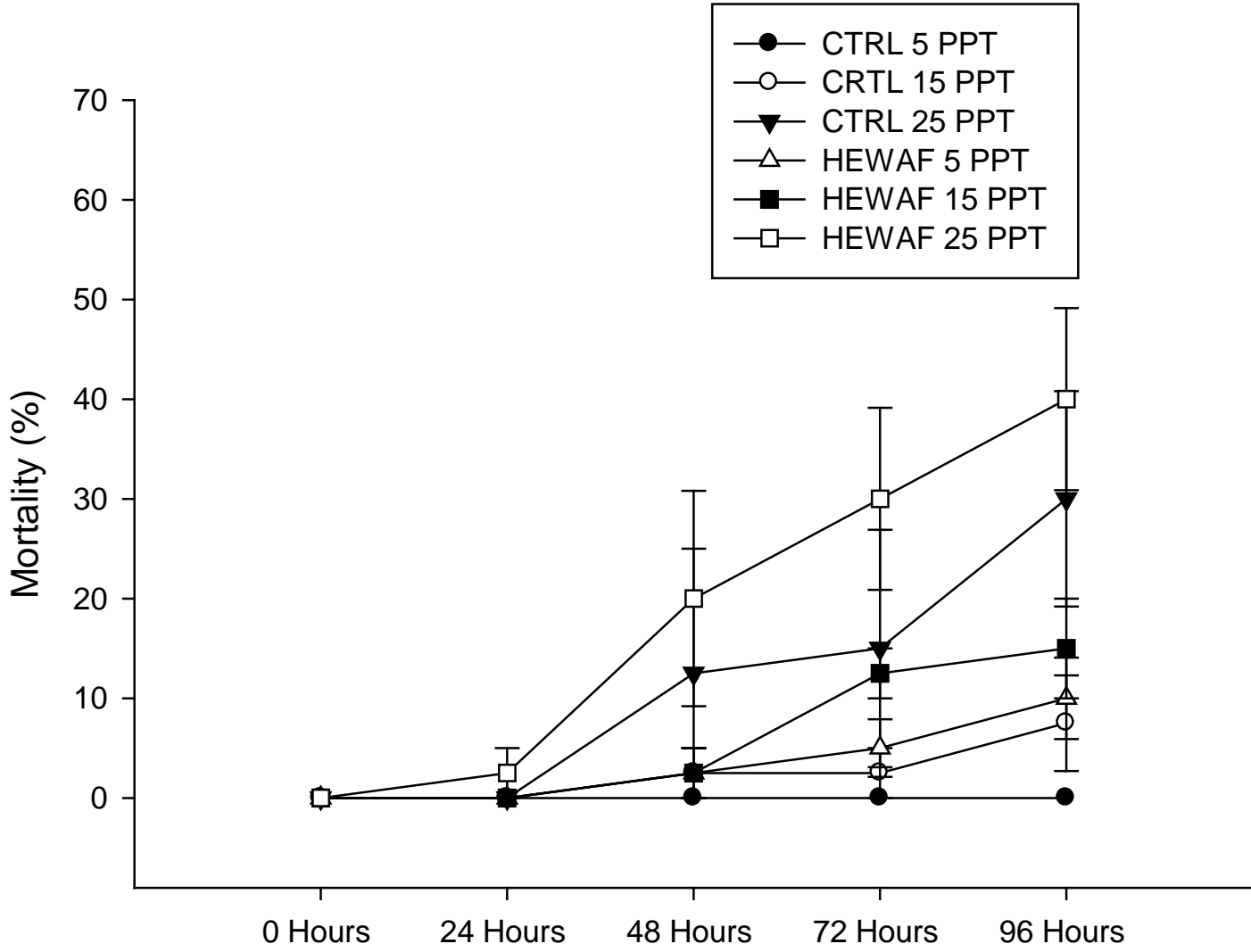
# **Results: Graphs and Charts**

Mortality, Water Chemistry, and qPCR

# Mortality of the CEWAF treatment



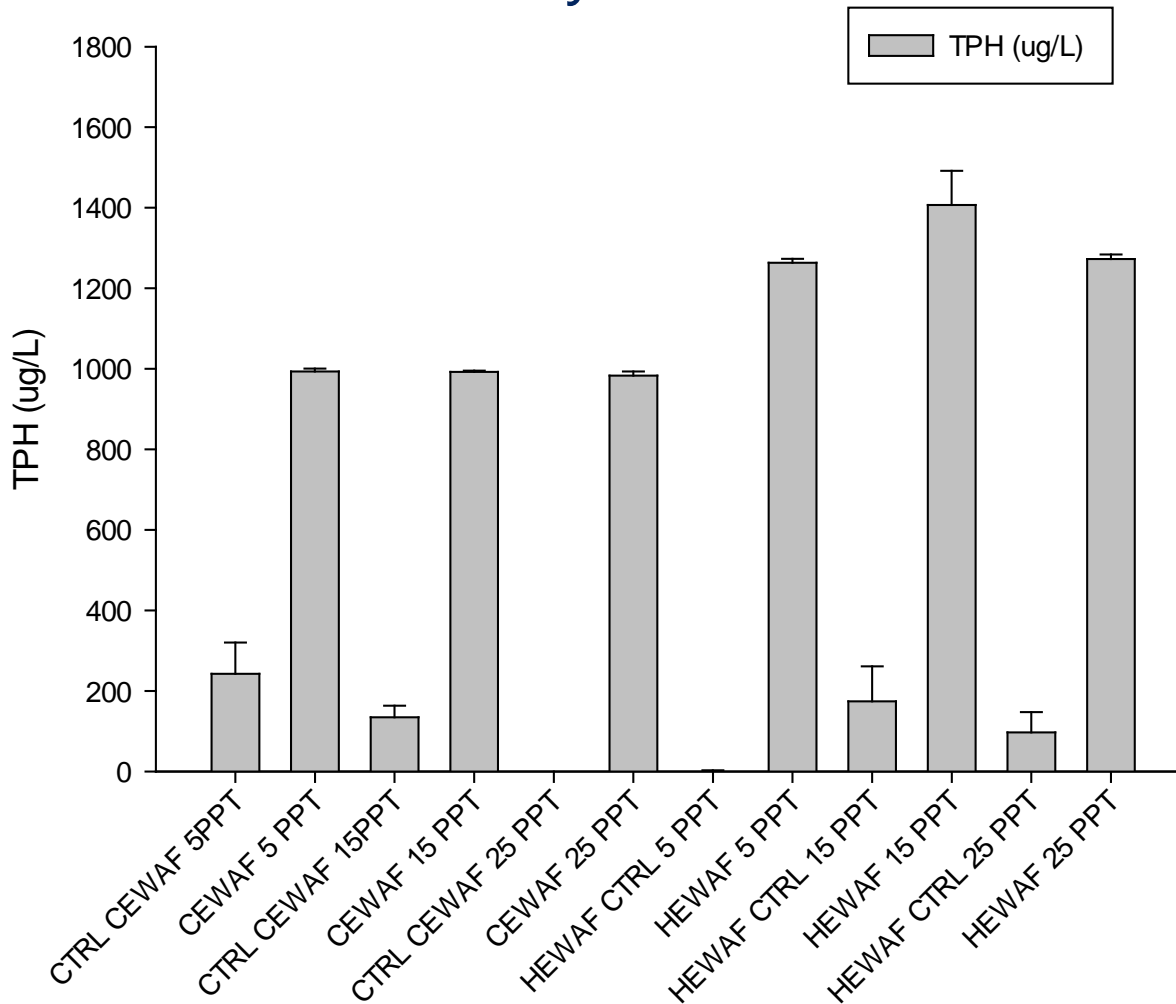
# Mortality of the WAF treatment



# Initiation Water Samples

Start: 0 hours Water Chemistry

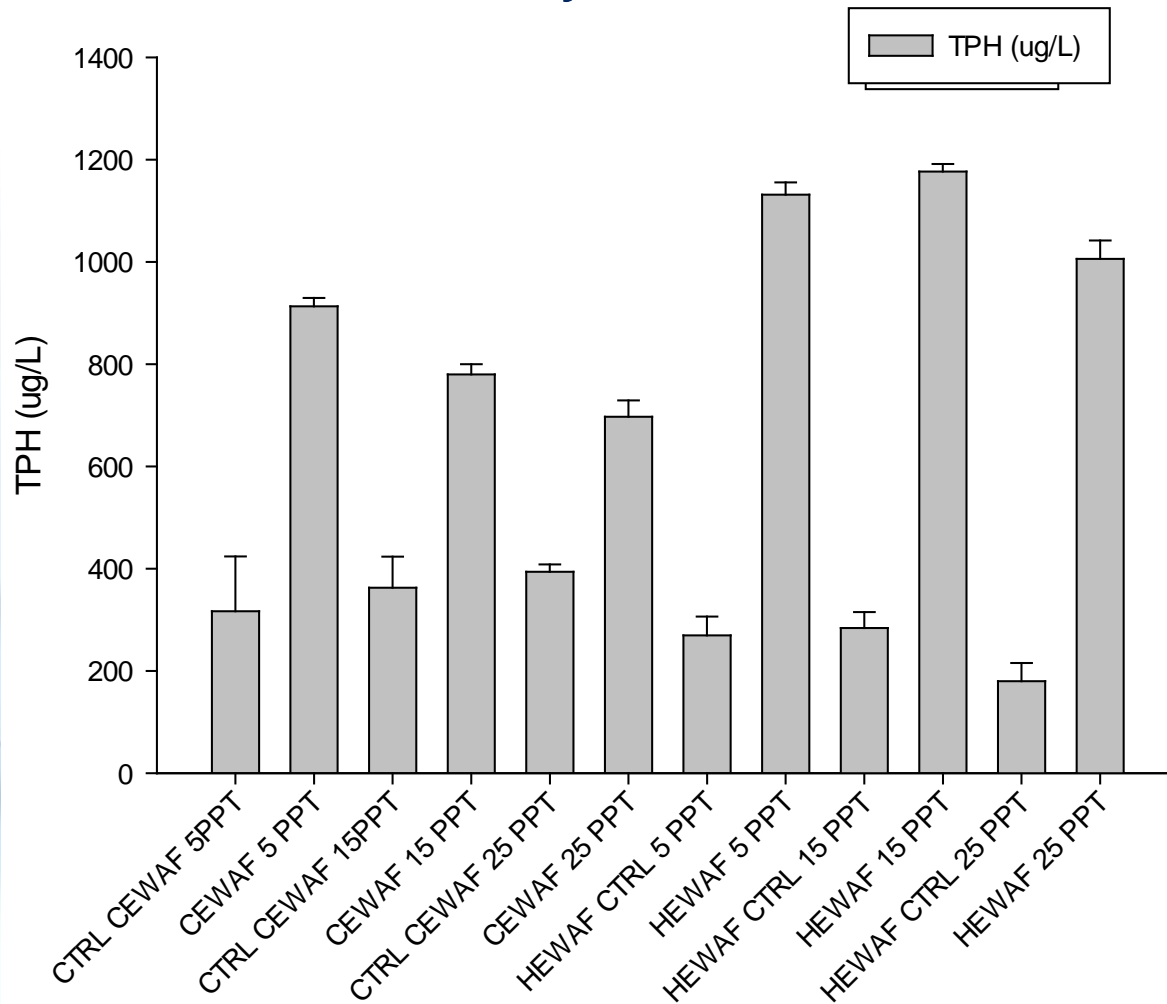
Due to some errors a small portion of oil was detected in the control groups. Thus the qPCR results may be effected by this.



# Take Down Water Samples

End: 96 hours Water Chemistry

Due to some errors a small portion of oil was detected in the control groups. Thus the qPCR results may be effected by this.



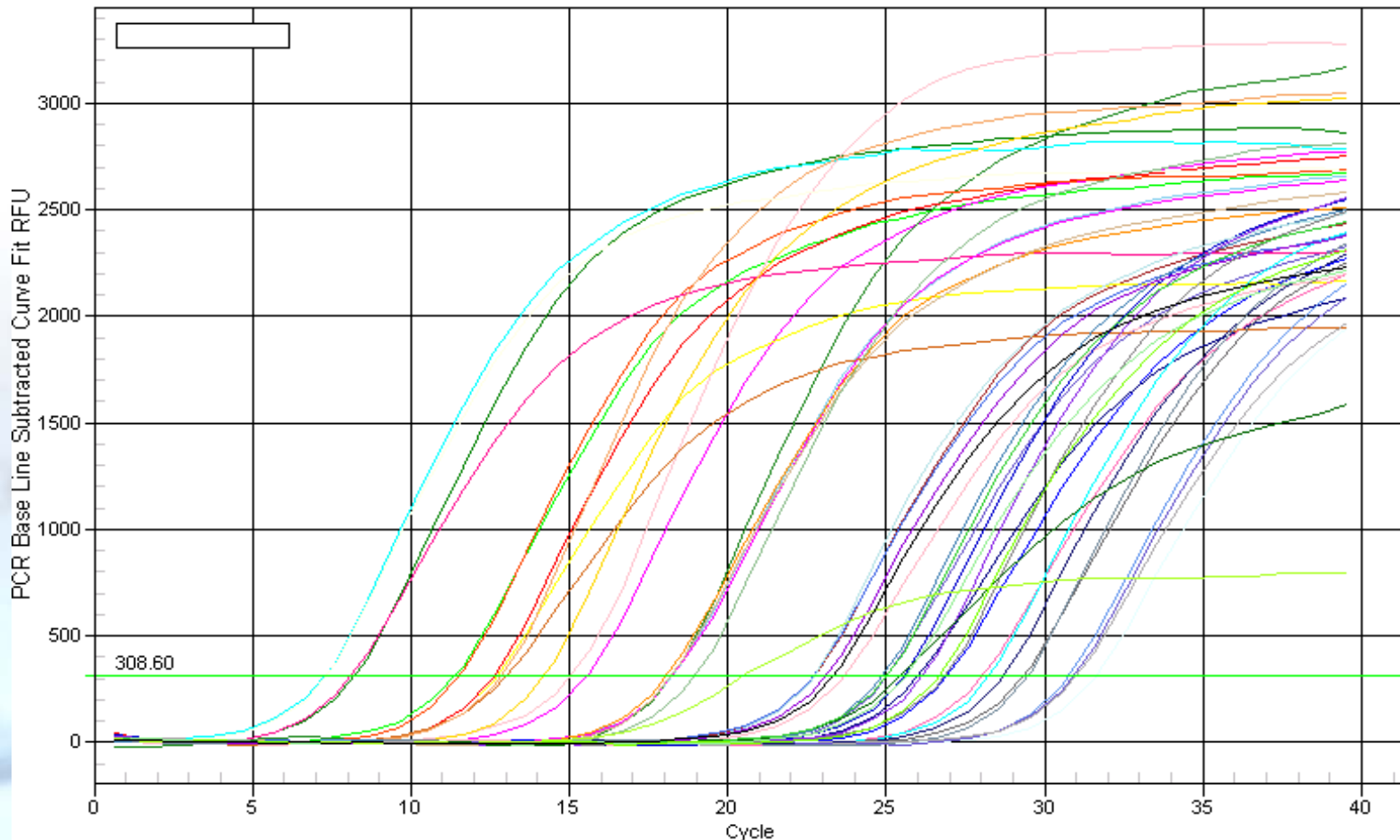
# Time in the lab

## qPCR

- ❑ In this picture I am preparing for my qPCR.
- ❑ qPCR stands for quantitative real time polymerase chain reaction
- ❑ Real Time-PCR enables both detection and quantification of DNA. The quantity can be either an absolute number of copies or a relative amount when normalized to DNA input or additional normalizing genes.



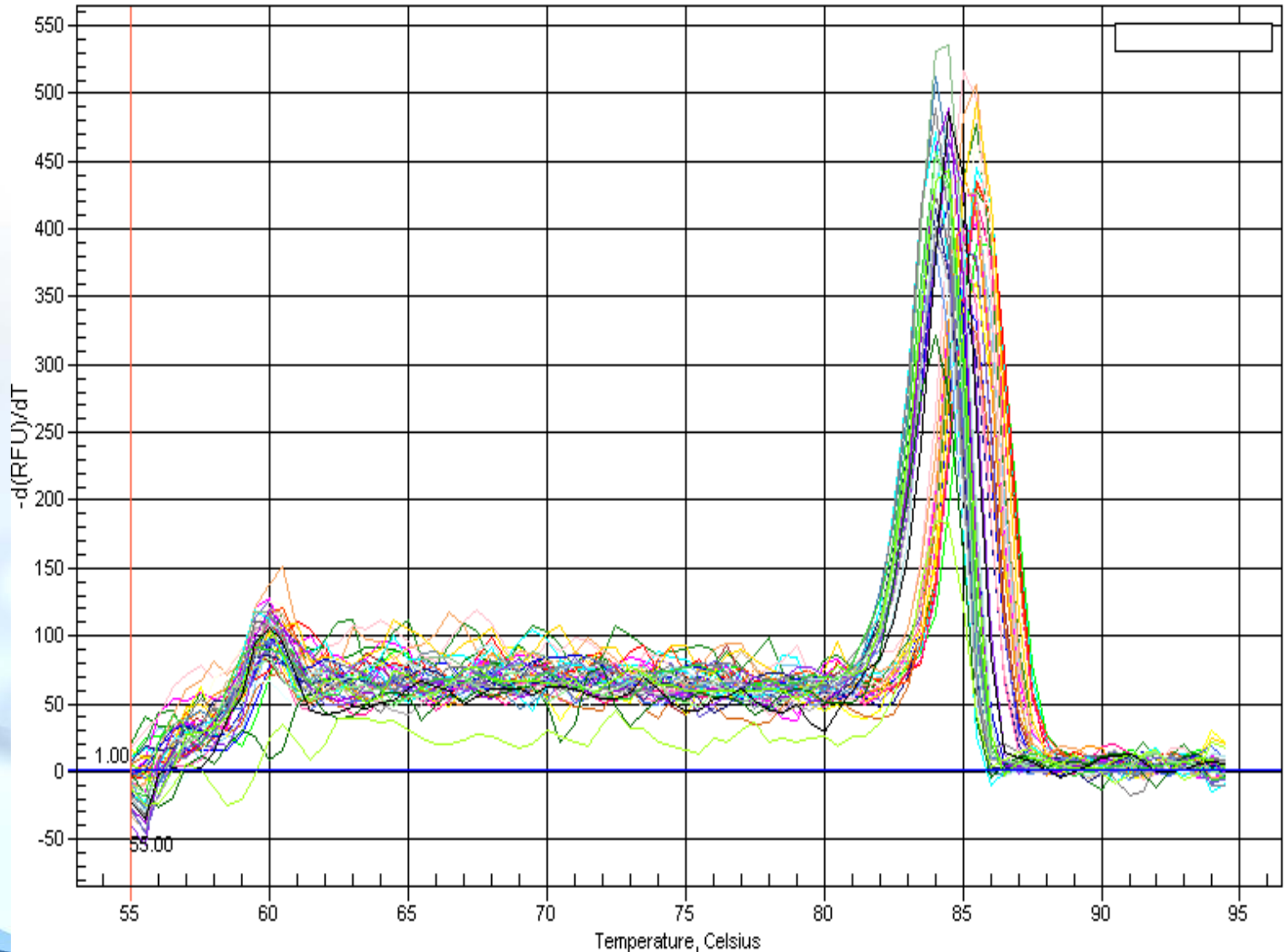
# Amplification Chart: WAF and CEWAF



Ct stands for Cycle threshold and it's a measure of the number of PCR cycles (in Real-time PCRs) needed to get a fluorescent signal. In other words, it's how many times you need to run the PCR to get enough product. It depends on how much DNA you began with

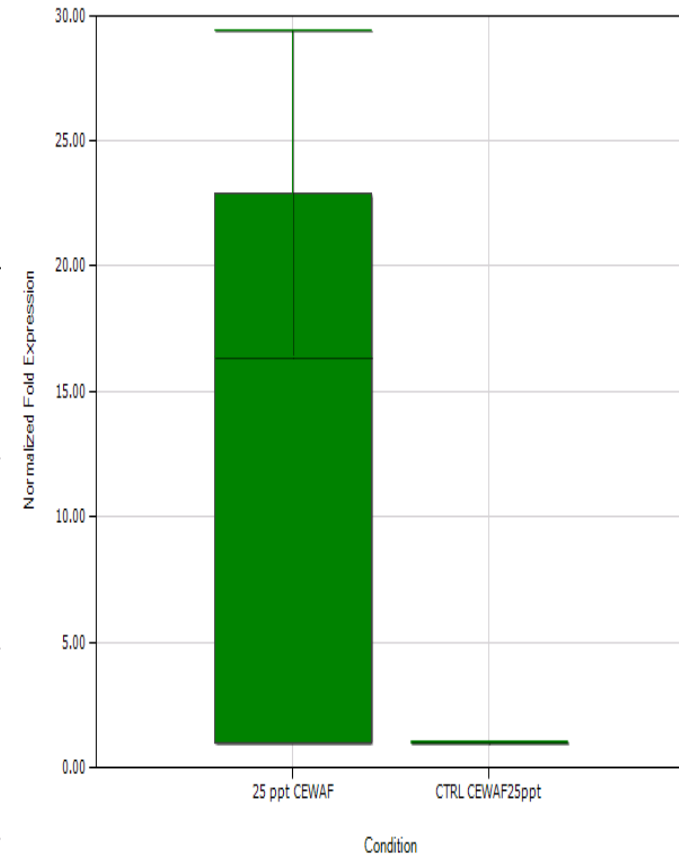
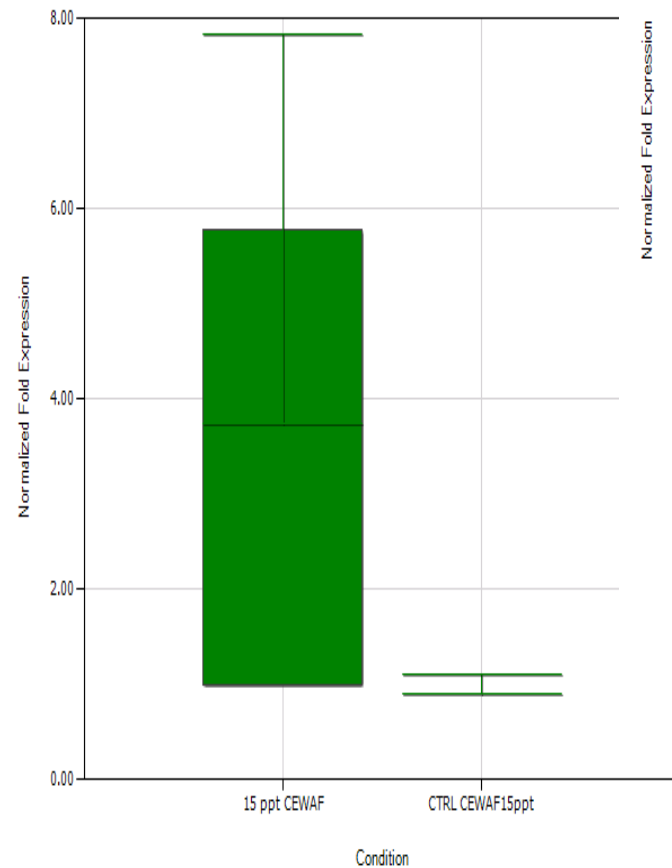
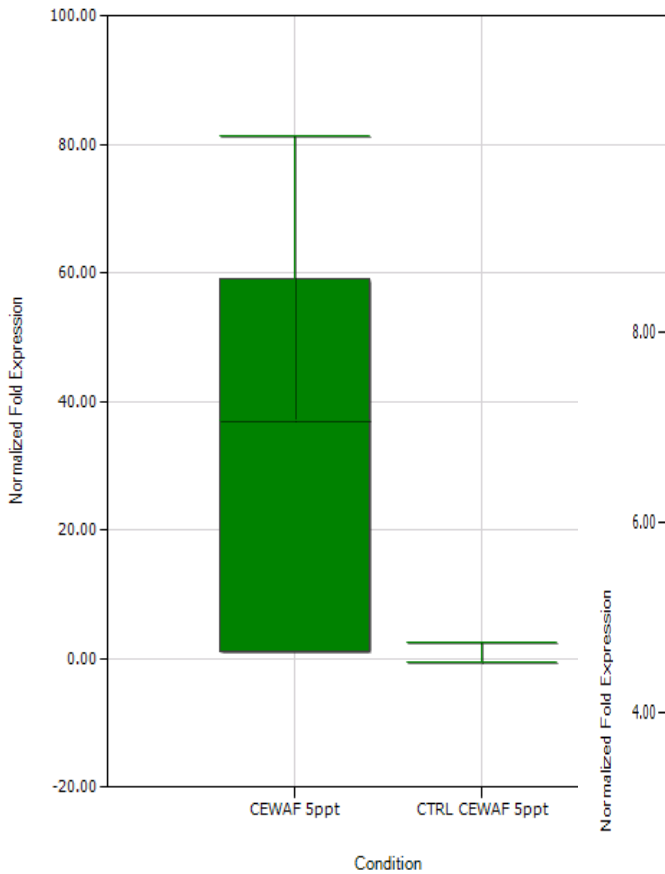
# Melt Peak Chart: WAF and CEWAF

It is apparent that the melting point occurs at 85 degrees Celsius

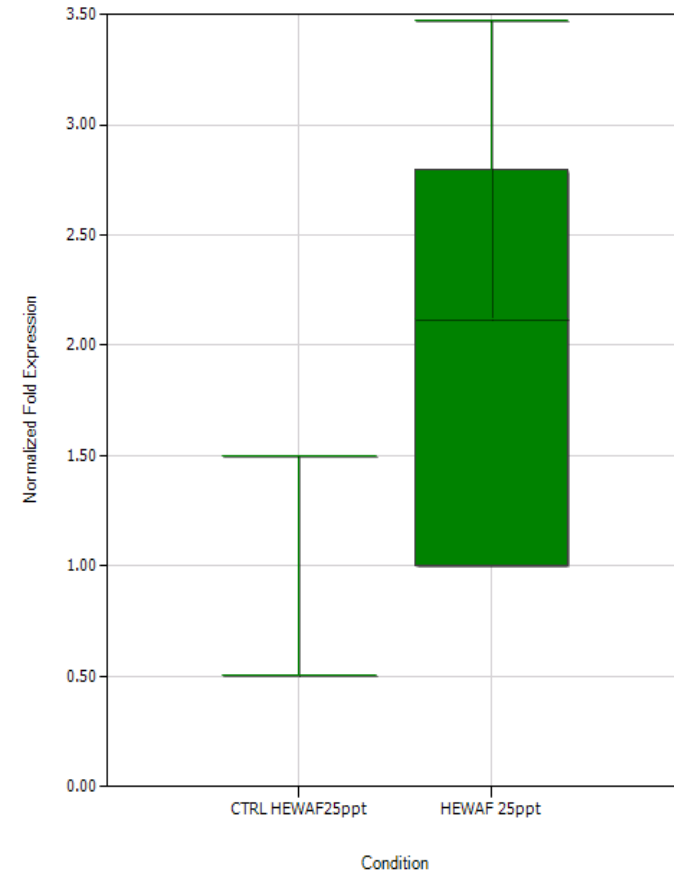
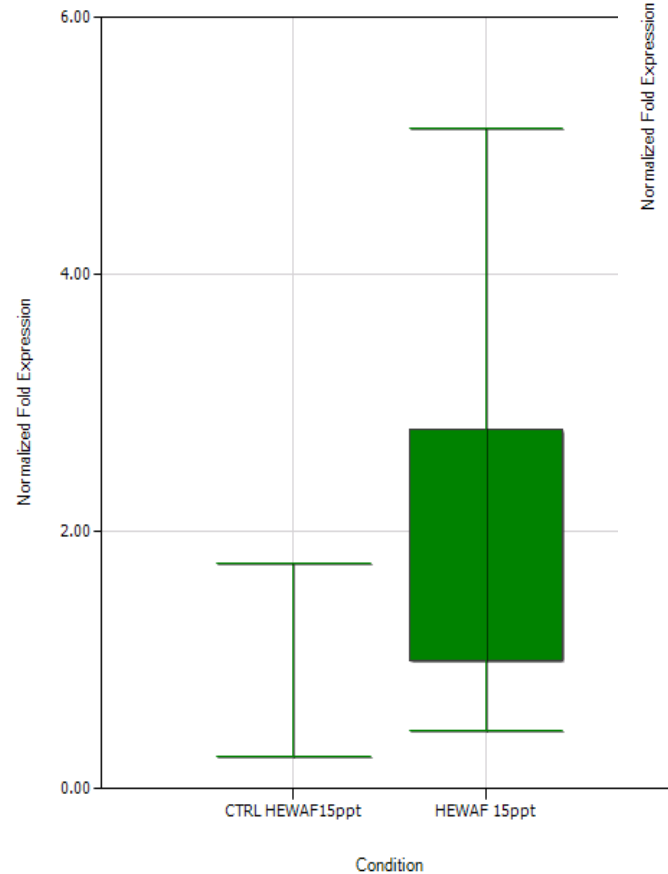
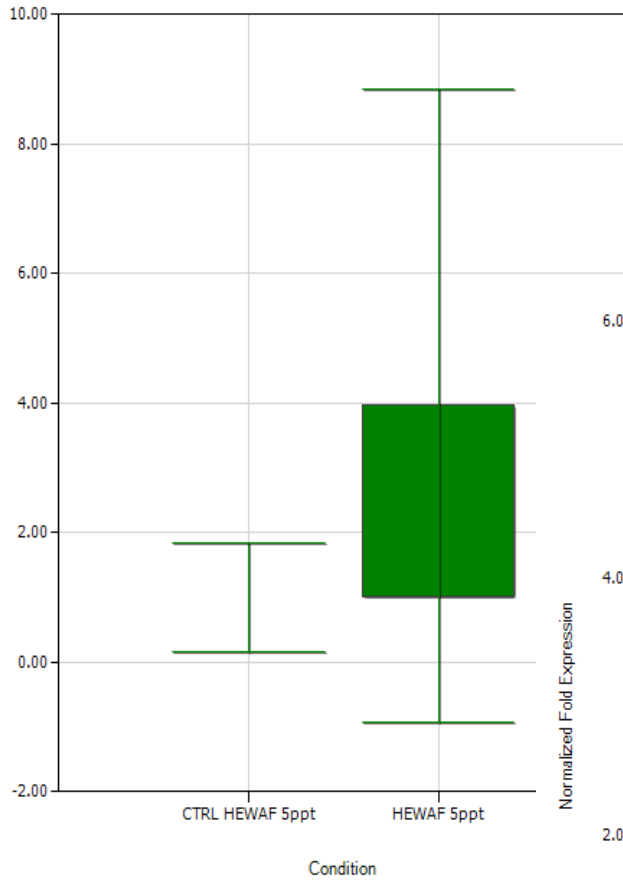




# CYP1A: qPCR CEWAF- 5, 15, 25 ppt



# CYP1A: qPCR WAF- 5, 15, 25ppt



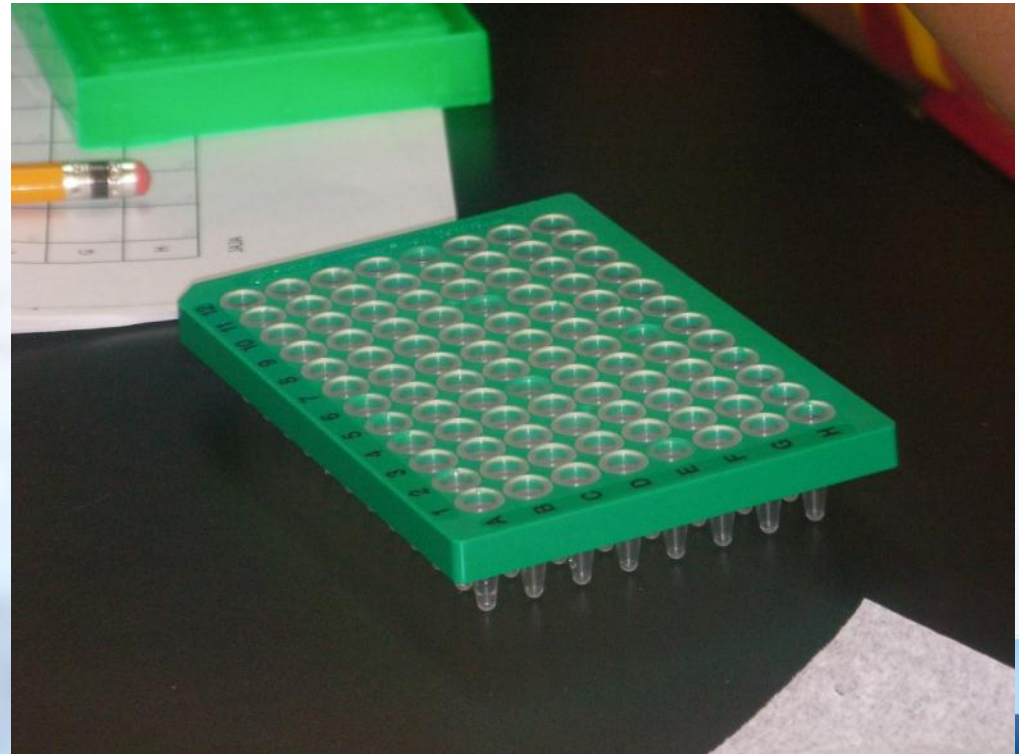
# Brief Conclusion from my work

## Summary

qPCR  
Plate.

In this experiment larval fish had a higher mortality rate in the higher salinities than in the lower ones. Also from the data larval fish that were exposed to the CEWAF had a less likely chance of surviving the exposure than the fish in the WAF treatments.

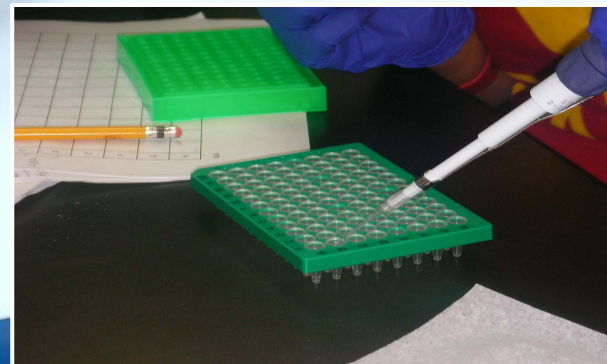
The qPCR showed that the higher salinities the higher the CYP1A gene was expressed. This has a relation with the mortality data that was collected.



# New Skills

I've learned so much

- Over the last two months I have learned many new skills. Many of these skills will help in my future medical career.
- Its hard to describe everything I have done in the past two months. This experience was very educational and I would love to say here longer and learn more.



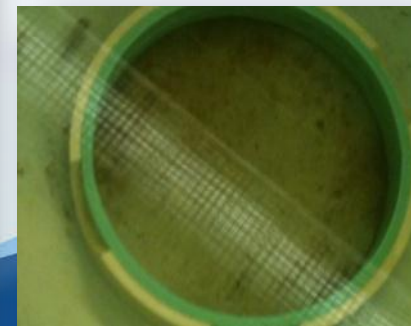
Sheepshead minnows (*Cyprinodon variegatus*)

Image is from [www.newswise.com/](http://www.newswise.com/)  
*Ecology Letters* (DOI: 10.1111/j.1461-0248.2011.01721.x)

# Challenges

## Overwhelmed

- At times I can say I was overwhelmed with everything that was going on. The lab is always busy and everyone is always working hard



# Overall Experience

## NOAA-NGI Summer Program

I love this summer program and I would be more than happy to do it again. I'm happy that I had the opportunity to meet such wonderful people and to be able to learn so much from everyone. I wish I can come back next year in research my project even deeper and learn even more. I can see my self doing this as a career. I love research and this program makes me want to study the world even more.



# THANKS TO EVERYONE WHO HELPED ME

Binnaz Bailey  
Idrissa Boube  
Nancy Brown-Peterson  
Joe Griffitt  
Bryan Hedgpeth  
Arthur Karels  
Jeremy Lindsey



Left to Right: (Nancy Brown-Peterson, Veronica Alston, Joe Griffitt, Idrissa Boube, Bryan Hedgpeth, Binnaz Bailey, Arthur Karels)



**QUESTIONS?**





THE UNIVERSITY OF SOUTHERN MISSISSIPPI



# University of Southern Mississippi Gulf Coast Research Lab

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Tuskegee University

