



# Stress Hardening and Metabolic Screening: Tools to Assess Oyster Performance and Resilience

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**Ariana S. Huffmyer**, Noah Ozguner, Madeline Baird,  
Colby Elvrum, Carolyn Kounellas, Dash Dickson,  
Samuel J White, Louis Plough, Mackenzie Gaverty, Noah  
Krebs, William Walton, Jessica Small, Madeline  
Pitsenbarger, Healy Ealy-Whitfield, Steven Roberts

**Email:** [ashuff@uw.edu](mailto:ashuff@uw.edu)  
[robertslab.info](mailto:robertslab.info)

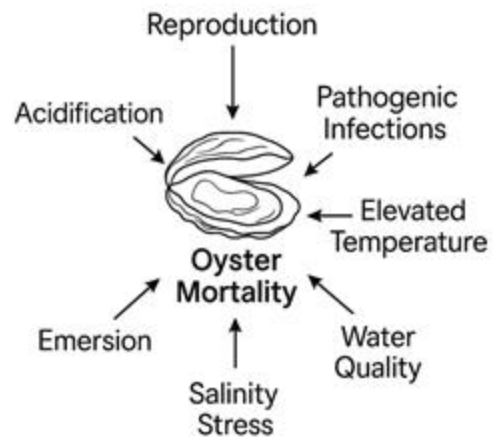


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School of Aquatic and Fisheries Sciences

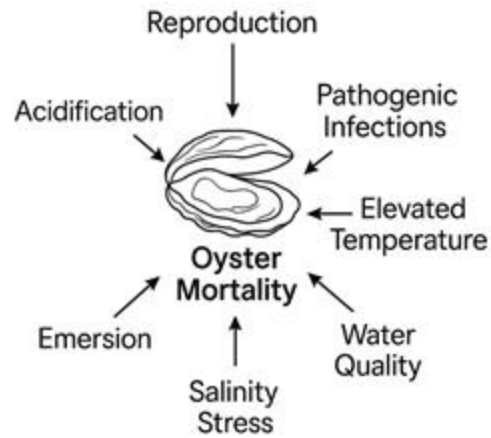
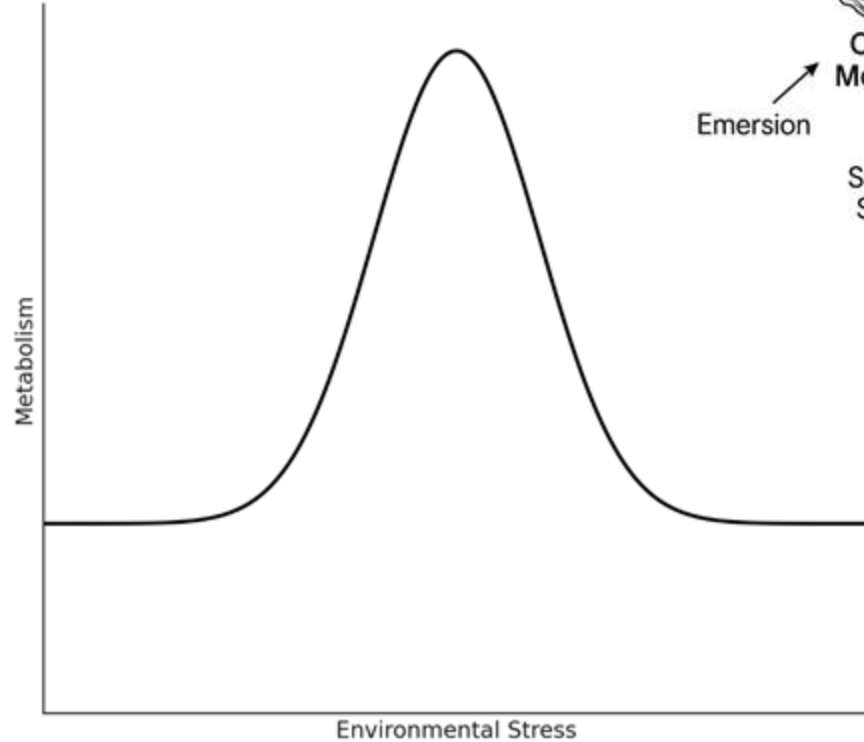


[oyster.pink](http://oyster.pink)

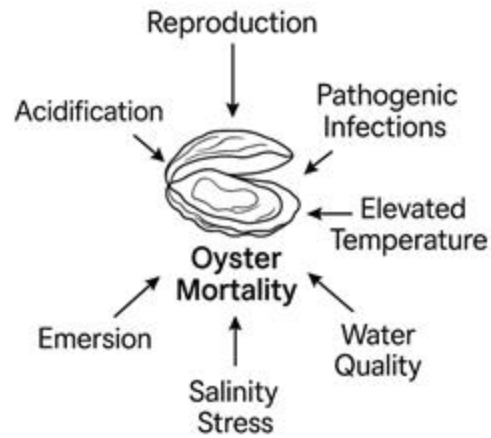
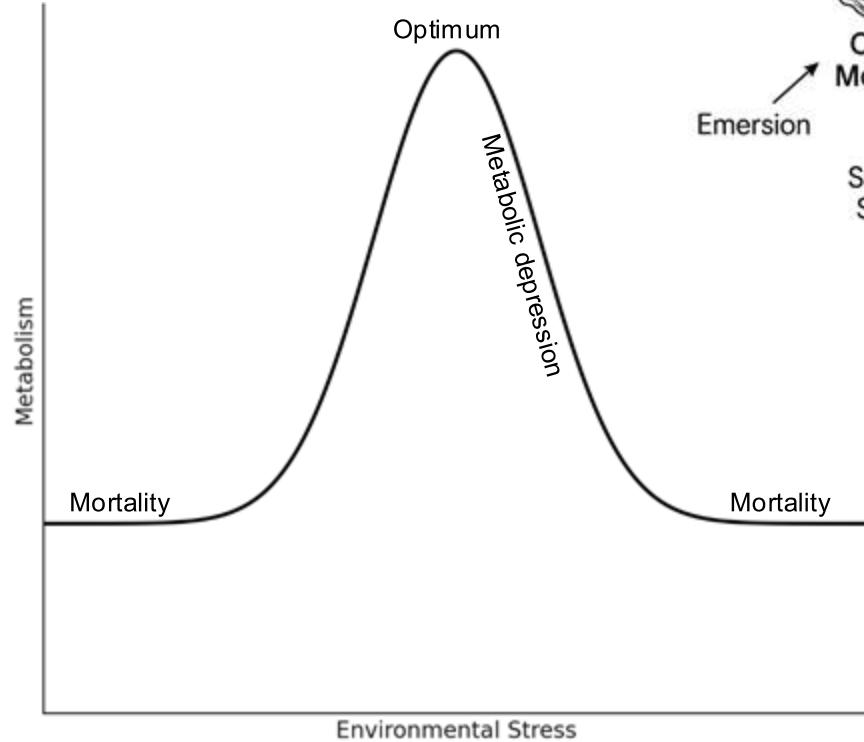
# ***Resilience through the lens of metabolism***



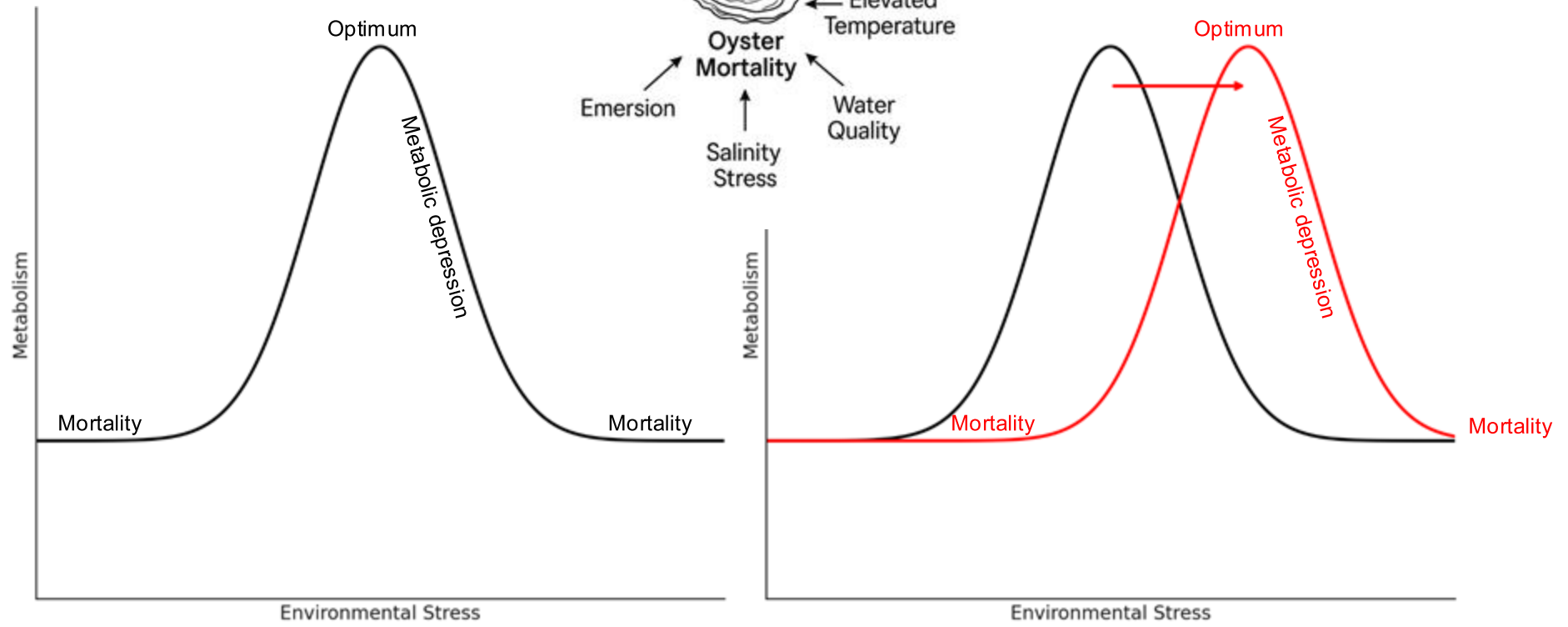
# ***Resilience through the lens of metabolism***



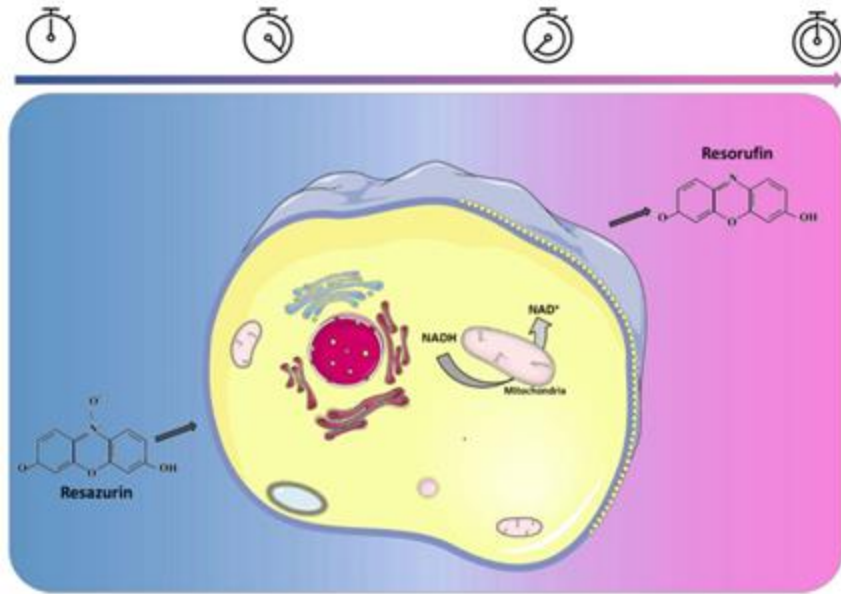
# ***Resilience through the lens of metabolism***



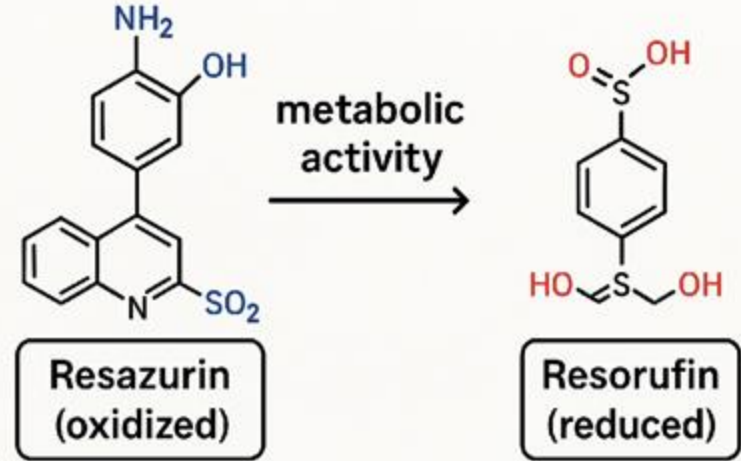
# Resilience through the lens of metabolism



# Resazurin Cell Viability Assay

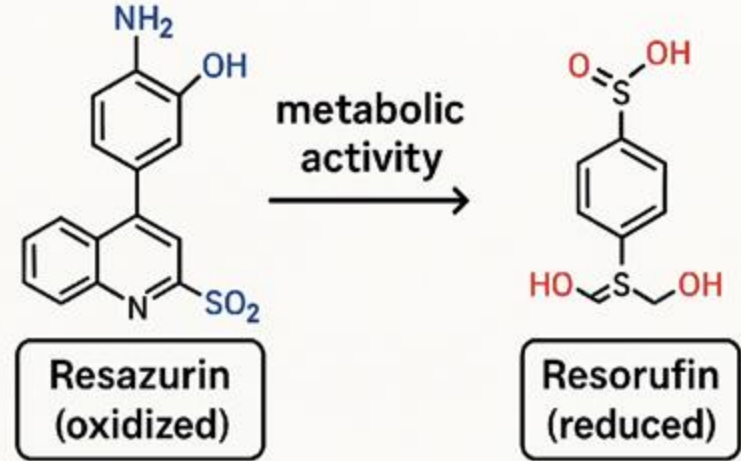
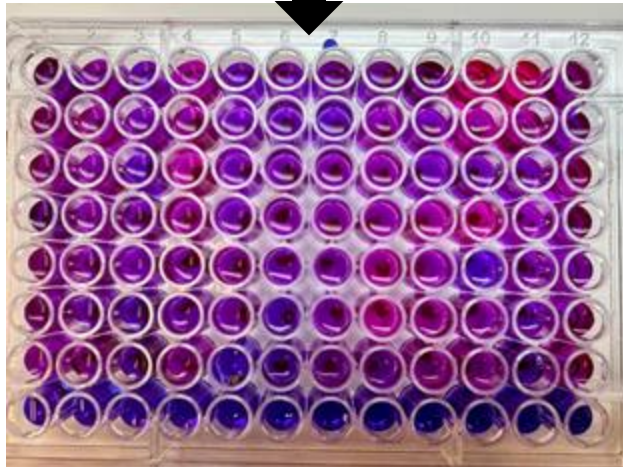
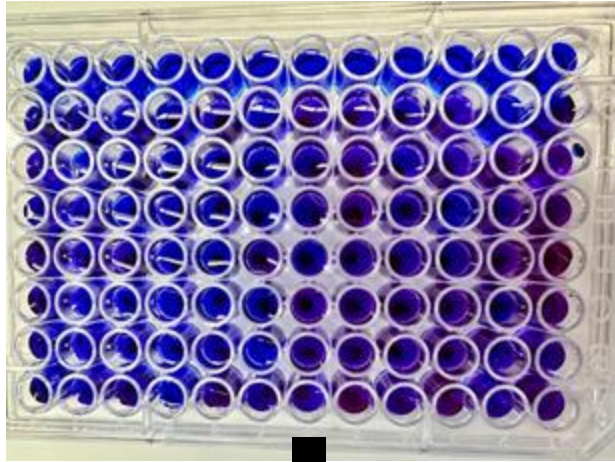


Petiti et al. 2024



**Metabolic activity is directly proportional to the fluorescence of resorufin**

# Resazurin Cell Viability Assay



**Metabolic activity is directly proportional to the fluorescence of resorufin**



# Applications of Resazurin



## Stream Ecology

González-Pinzón et al. 2012



## Microbial Metabolism

Ricciardi et al. 2014

Fai & Grant 2009

Zare et al. 2015

Van den Driessche et al. 2014



## Cytotoxicity

Petiti et al. 2024

Pace & Burg 2015

O'Brien et al. 2000



## Biomedical

Anoopkumar-Dukie et al.

2005

McMilliam et al. 2002



## Fish Metabolism

Petiti et al. 2024

Pace & Burg 2015

O'Brien et al. 2000

Renquist et al. 2013



## Oyster Hemocyte Viability

Estrada et al. 2021



# Applications of Resazurin



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**Objective: Optimize and test the resazurin assay to assess whole-organism oyster metabolism**



## Biomedical

Anoopkumar-Dukie et al.

2005

McMilliam et al. 2002



## Fish Metabolism

Petiti et al. 2024

Pace & Burg 2015

O'Brien et al. 2000

Renquist et al. 2013



## Oyster Hemocyte Viability

Estrada et al. 2021

# Blue Notes and Bivalves

Exploring oyster metabolism with a jazzy *resazurin* twist

## AUTHORS

Ariana Huffmyer

Louis Plough (USDA)

Steven Roberts

## MODIFIED

August 28, 2025

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Landing page for quick info on where we are with **implementing easy resazurin metabolism assays** for oysters. Please explore the [Github Repo](#), glimpse real-time activity in from the lab [here](#), and dive deeper with the [Canonical Protocol](#).

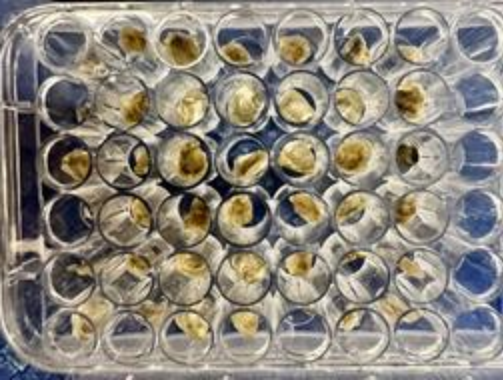
### New: Public Summary Available

For a general audience overview of this research, including implications for the aquaculture industry and key findings to date, see our [Public Summary: Resazurin Assay for Oyster Health Assessment](#).



Find our full protocol and interactive tools to plan experiments on our website!

# Resazurin Assay Development



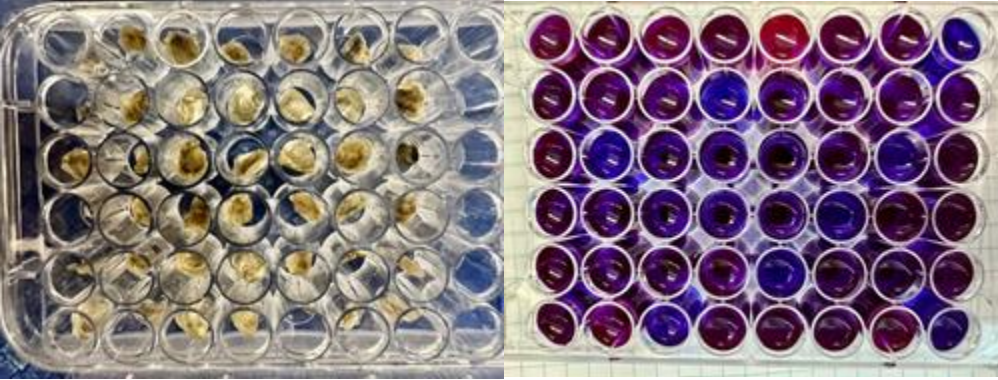
## 1 - Animal Selection

- 2mm spat - adult oysters
- Image for size normalization



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# Resazurin Assay Development



## 1 – Animal Selection

- 2mm spat – adult oysters
- Image for size normalization

## 2 – Resazurin Addition

- Prepare solutions
- Initial fluorescence readings



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## Interactive Recipe Calculator

Use this calculator to automatically determine the required amounts of each ingredient based on your experimental setup.

Plate type:

Number of plates:

Custom total volume (mL):

Extra volume for safety (%):

### Recipe for 38.5 mL Working Solution

**Experimental setup:** 1 × 96-well plate (35.0 mL base volume)

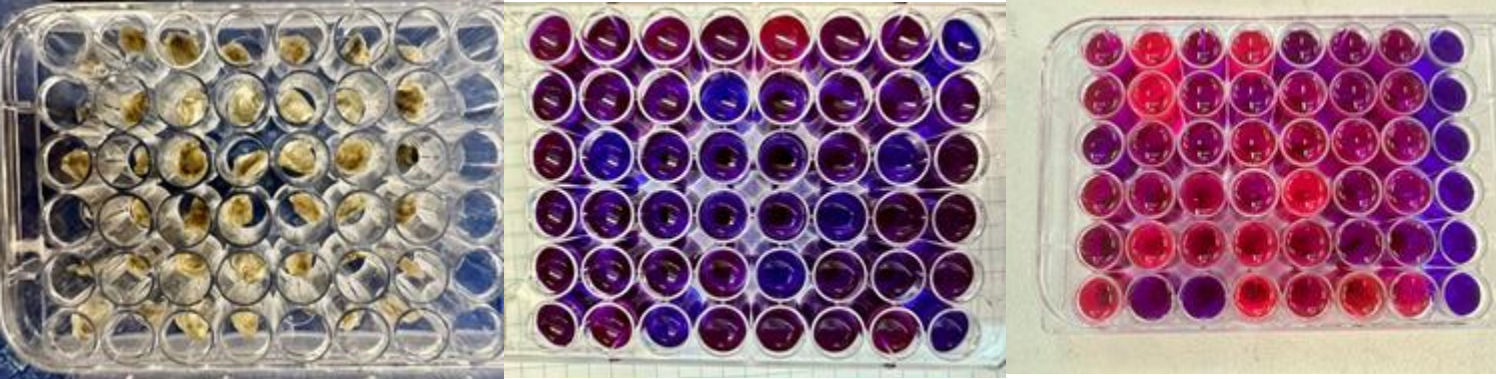
**Safety margin:** +10% extra volume

#### ✓ Required Ingredients:

- 37.99 mL filtered seawater (DI water with Instant Ocean adjusted to 23-25 ppt or filtered <1µm seawater)
- 85 µL resazurin stock solution (from step 1 above)
- 39 µL DMSO
- 385 µL antibiotic solution (100x Penn/Strep & 100x Fungizone)

**Tip:** Remember to thaw the antibiotic solution in the dark before use, and store the resazurin stock solution in a dark fridge or freezer.

# Resazurin Assay Development



## 1 - Animal Selection

- 2mm spat - adult oysters
- Image for size normalization

## 2 - Resazurin Addition

- Prepare solutions
- Initial fluorescence readings

## 3 - Treatment

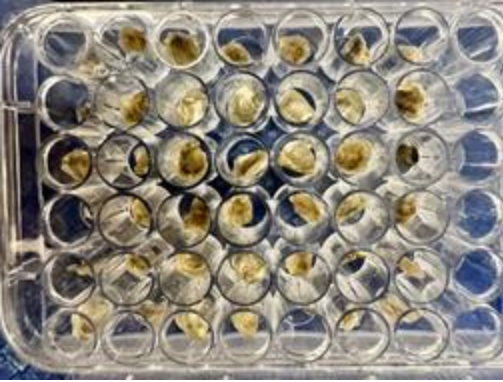
- Stressor incubation
- Hourly fluorescence readings



Find our full protocol and interactive tools to plan experiments on our website!

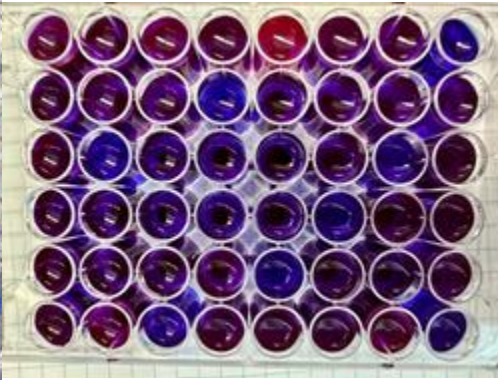


# Resazurin Assay Development



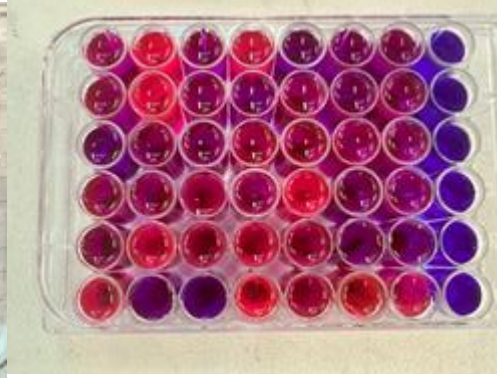
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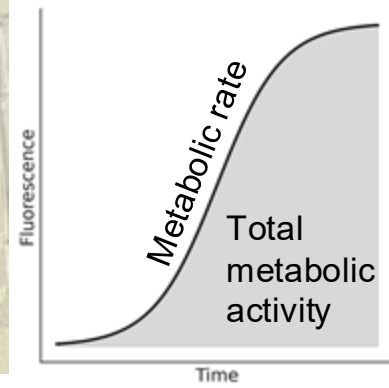
## 2 - Resazurin Addition

- Prepare solutions
- Initial fluorescence readings



## 3 - Treatment

- Stressor incubation
- Hourly fluorescence readings



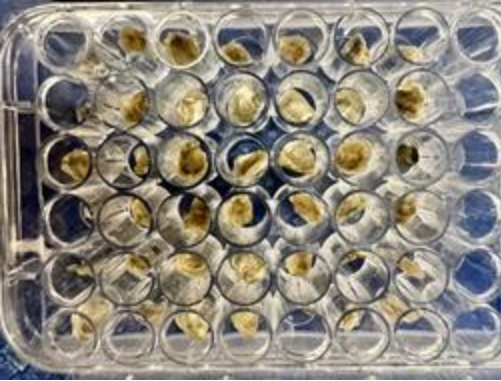
## 4 - Analysis

- Metabolic rates
- Total metabolic activity



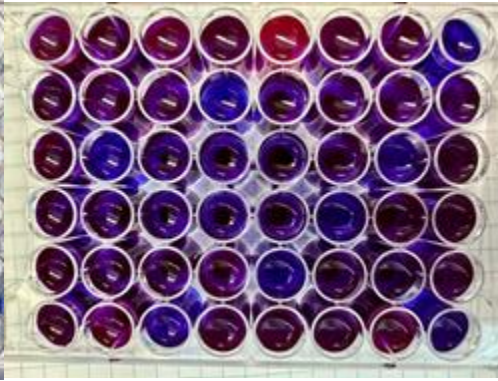
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# Resazurin Assay Development



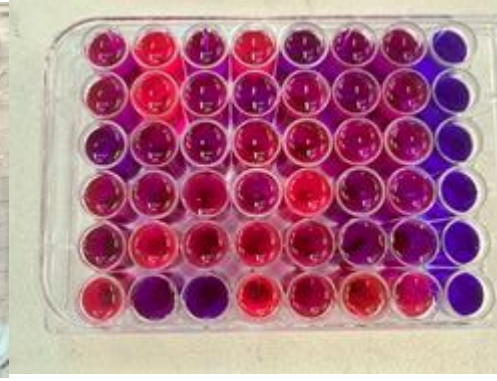
## 1 - Animal Selection

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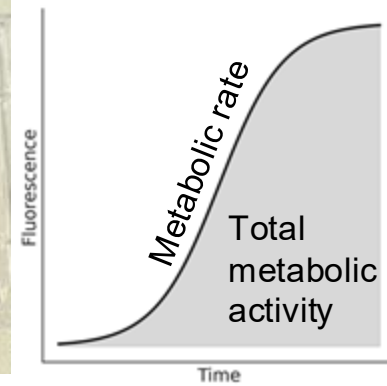
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## 4 - Analysis

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- Total metabolic activity

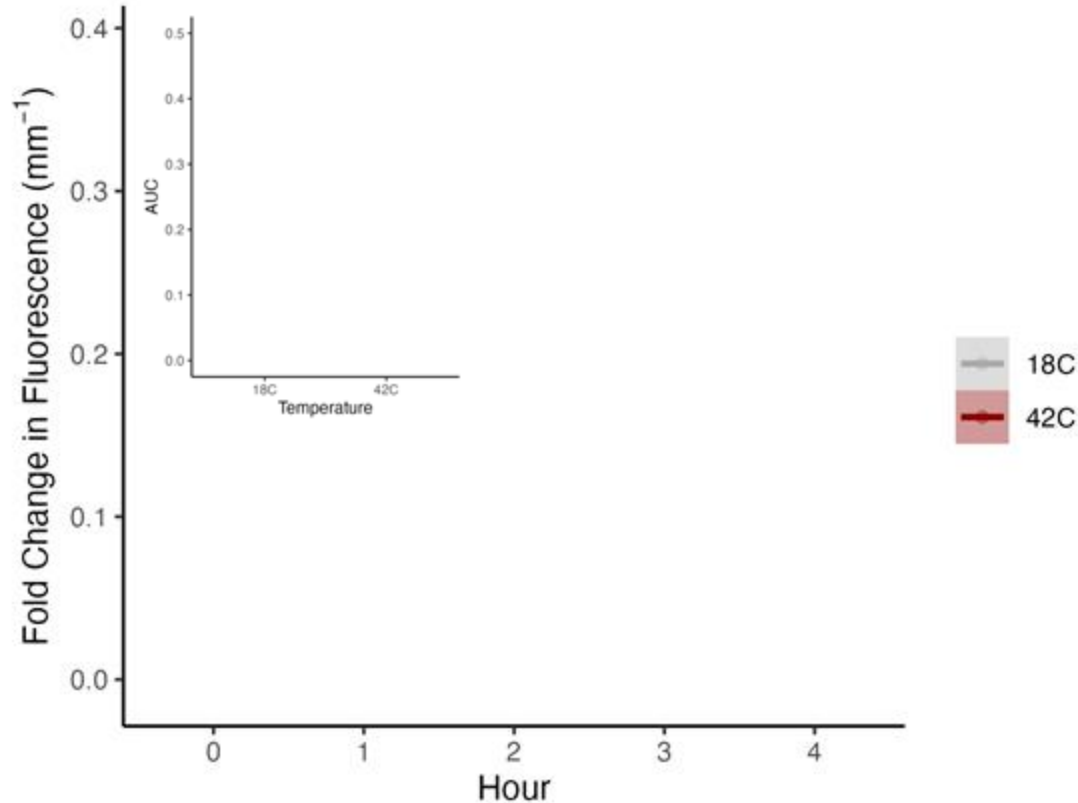


Find our full protocol and interactive tools to plan experiments on our website!

- ✓ **Metabolism scales positively with oyster size**
- ✓ **Signals are produced by live oysters, even when closed**
- ✓ **Metabolic rate is responsive to temperature**
- ✓ **Effective across wide range of sizes and stressors**
- ✓ **Non-destructive, sensitive measurements**



# 1 - Rapid assessment of oyster acute stress response

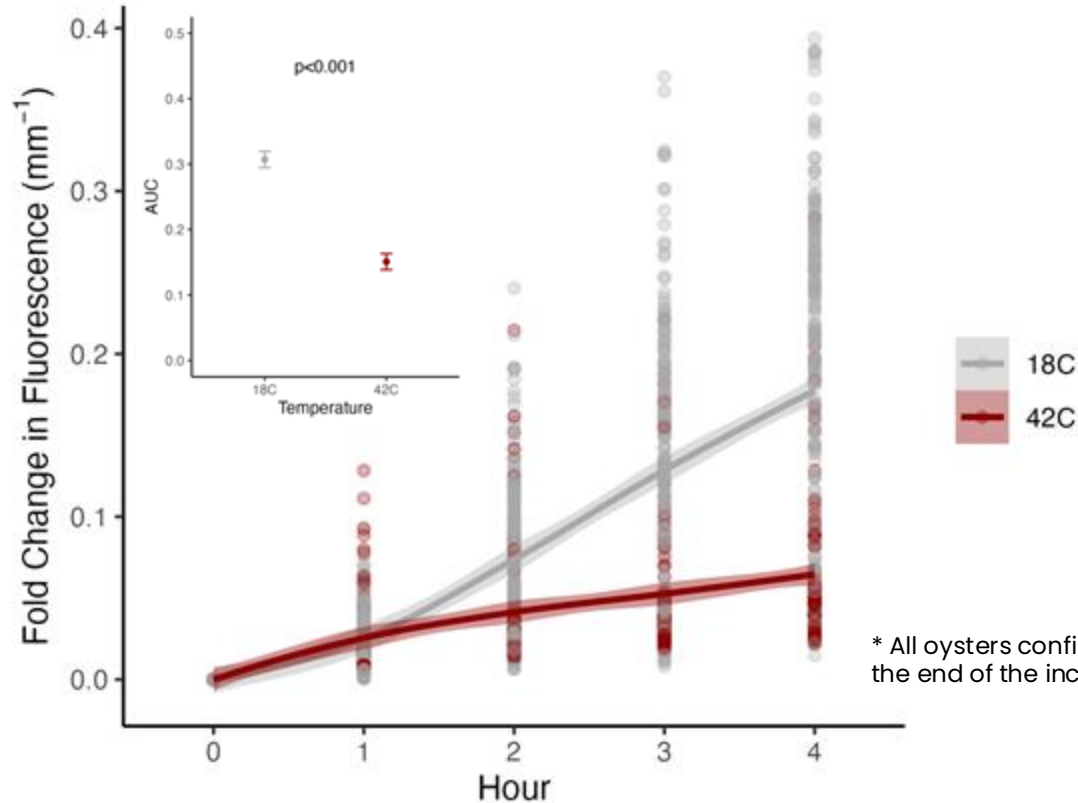


**Metabolic rate** = fold change in fluorescence normalized to shell length (mm)

- Signal normalization
- Blank correction

**Total metabolic activity** = area under the curve (AUC)

# 1 - Rapid assessment of oyster acute stress response

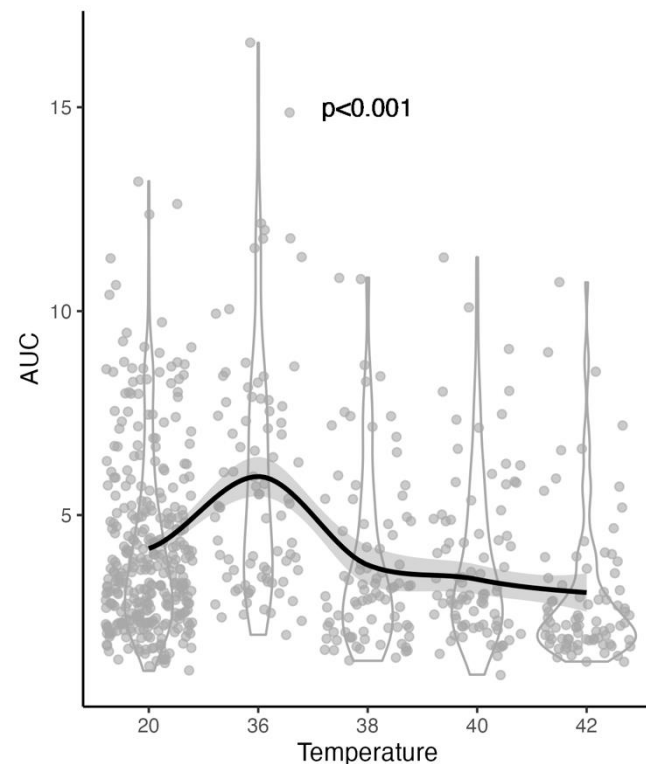
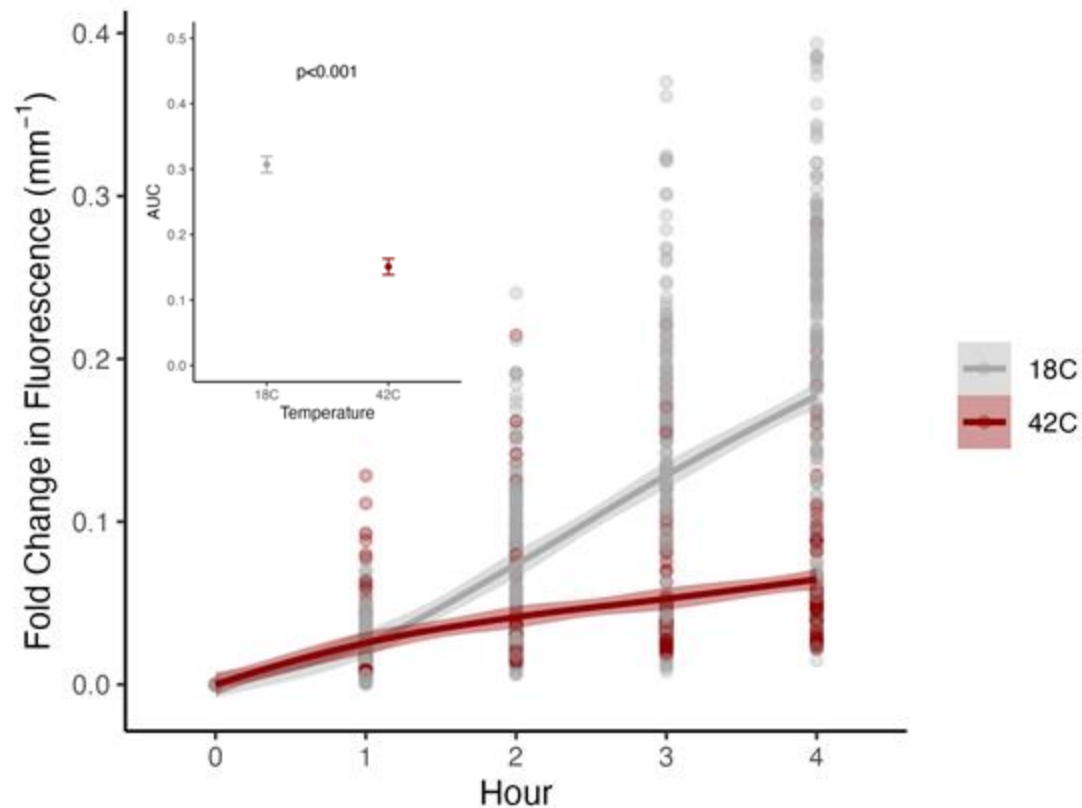


Metabolic activity was **lower** in oysters under acute heat stress

Oysters exhibit **metabolic depression** under acute heat stress

\* All oysters confirmed alive at the end of the incubation period

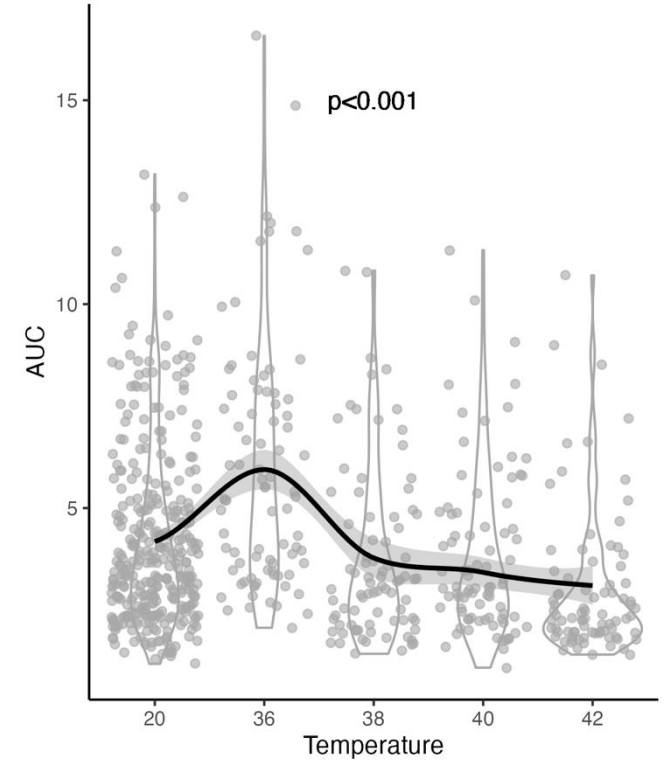
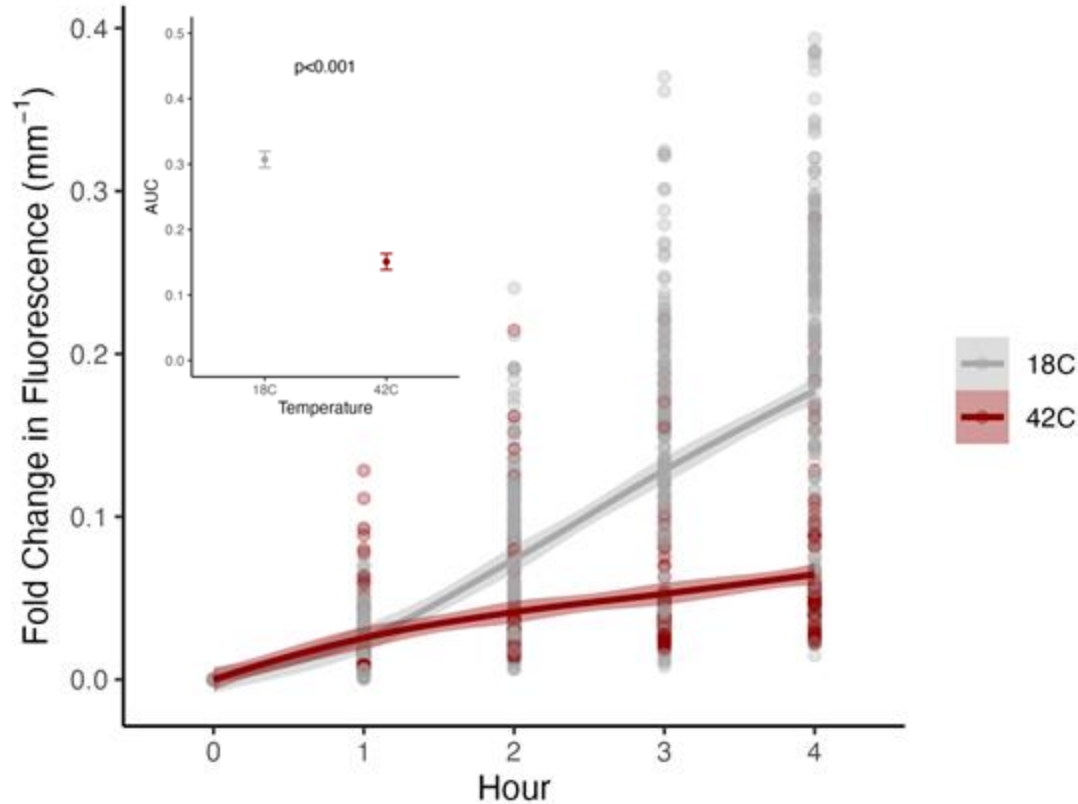
# 1 - Rapid assessment of oyster acute stress response



N=507 oysters  
Format: 20 mL cups

2-8 mm *C. gigas* seed  
4 h exposures

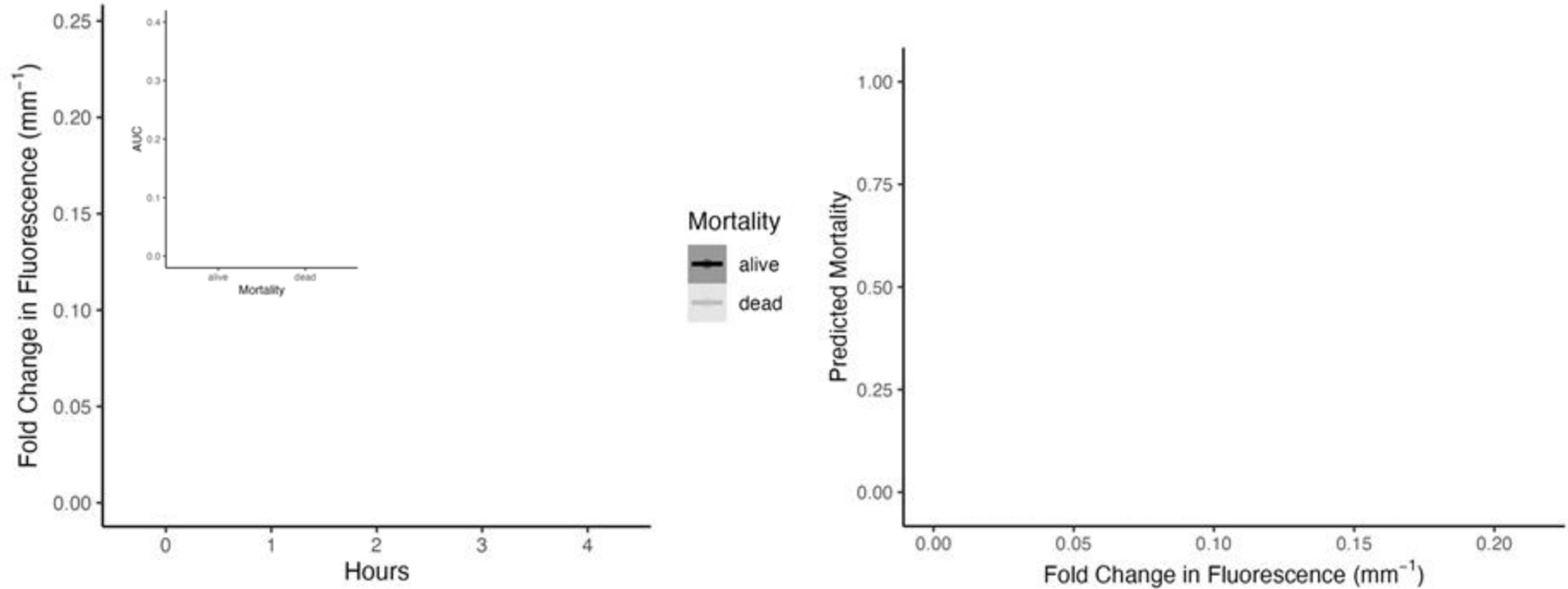
# 1 - Rapid assessment of oyster acute stress response



Resazurin assay provides a **rapid, high throughput approach** to assess metabolic response to stress

# 2 – Metabolic rate as indicator of stress tolerance

42°C

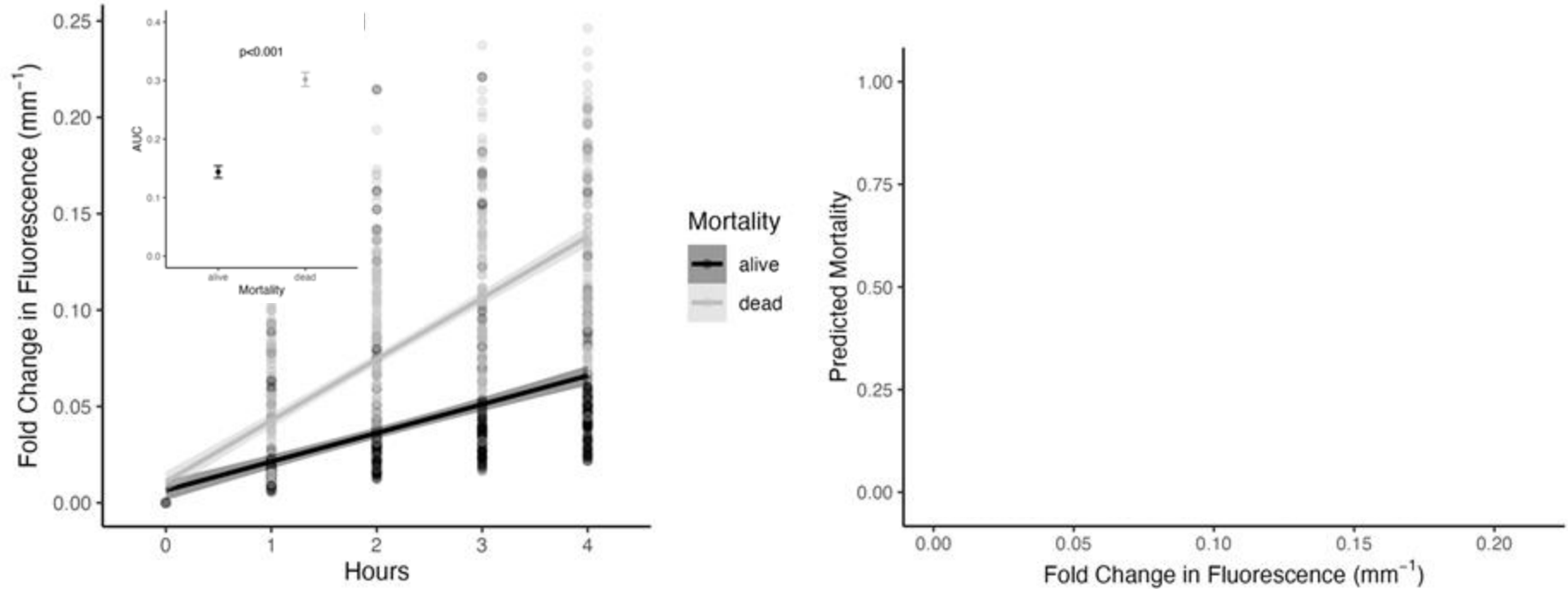


N=507 oysters  
Format: 20 mL cups

15–30 mm *C. gigas* seed  
4 h exposure to 42°C

## 2 – Metabolic rate as indicator of stress tolerance

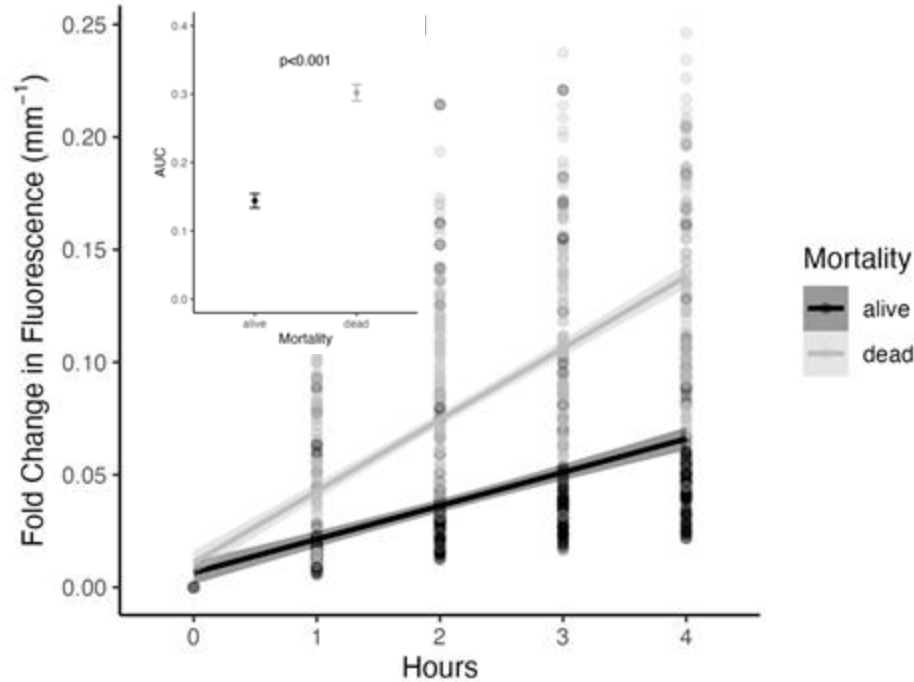
42°C



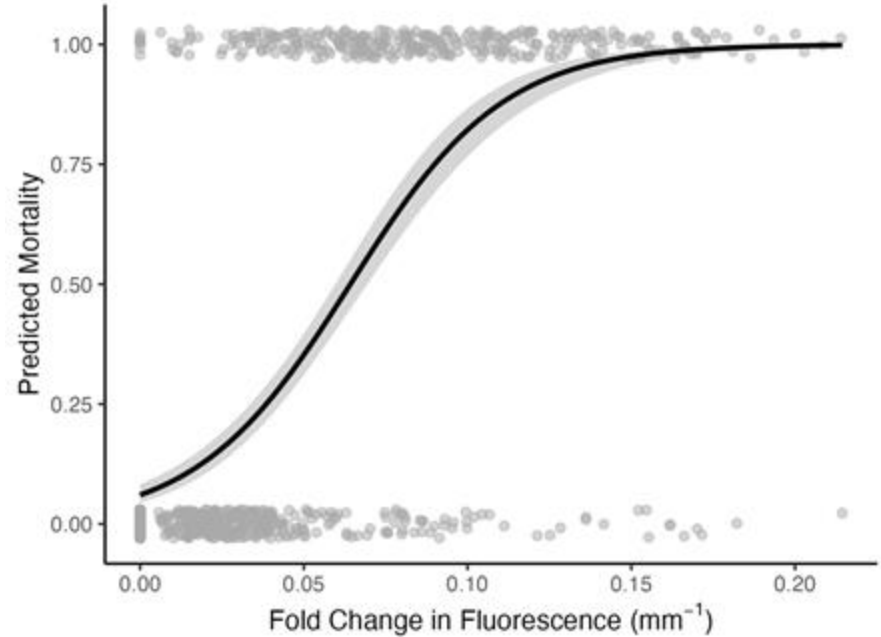
Oysters that survived exposure to acute stress had **lower** metabolic activity.

## 2 – Metabolic rate as indicator of stress tolerance

42°C



Oysters that survived exposure to acute stress had **lower** metabolic activity.

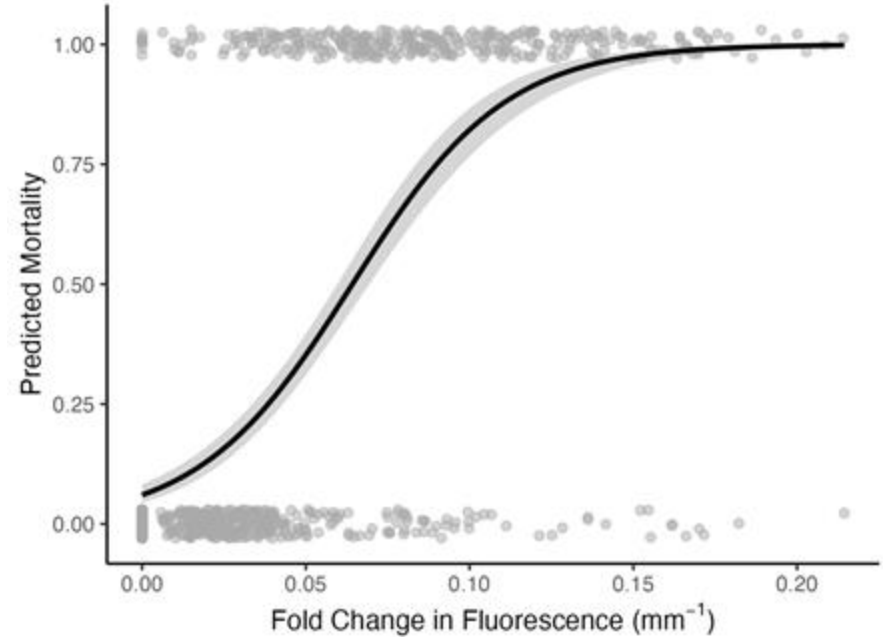
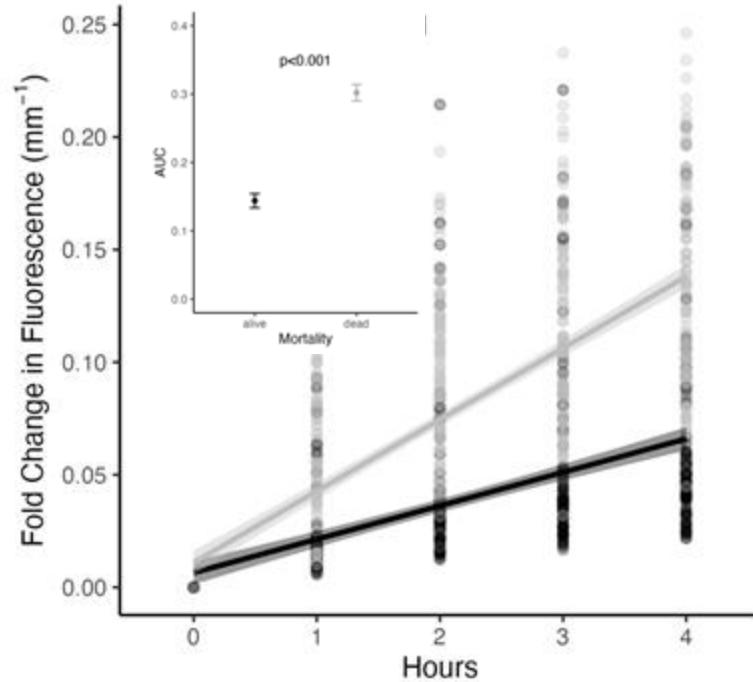


Increased metabolic activity led to a **higher mortality risk**.



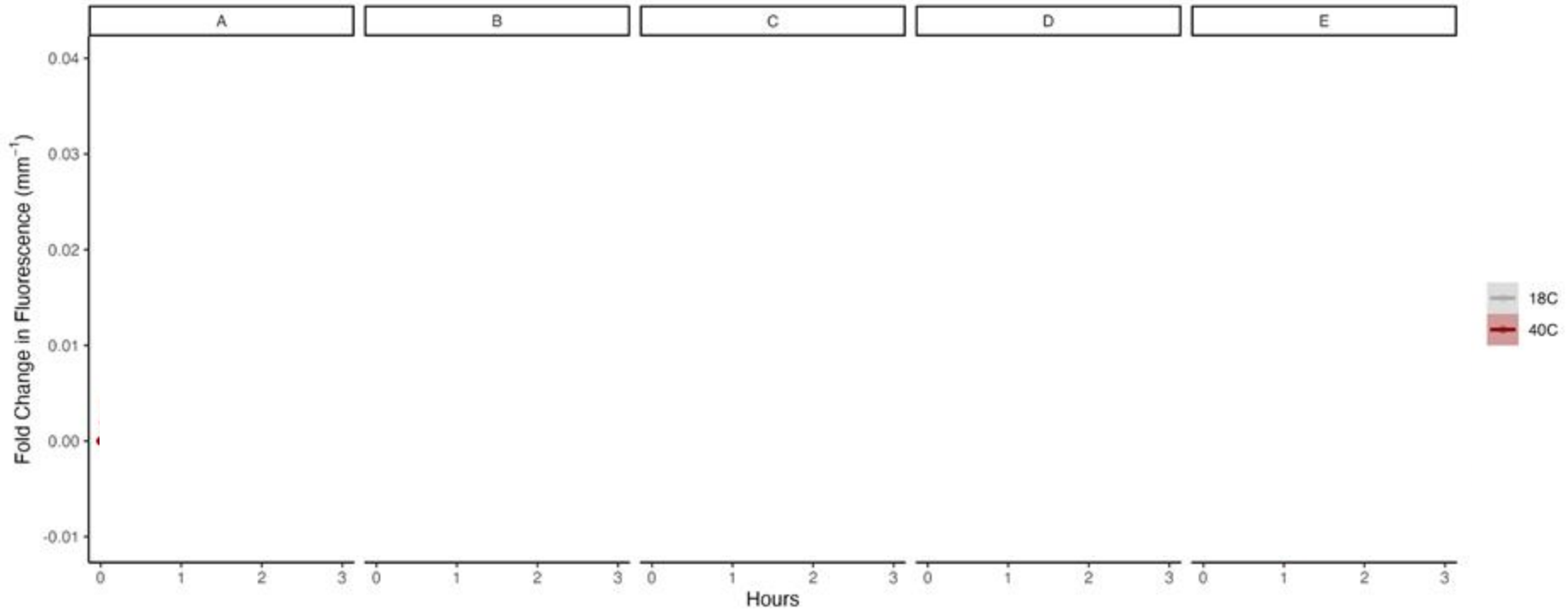
## 2 – Metabolic rate as indicator of stress tolerance

42°C



Capacity for metabolic depression was associated with **higher thermal tolerance**, providing a tool to assess indicators of tolerance.

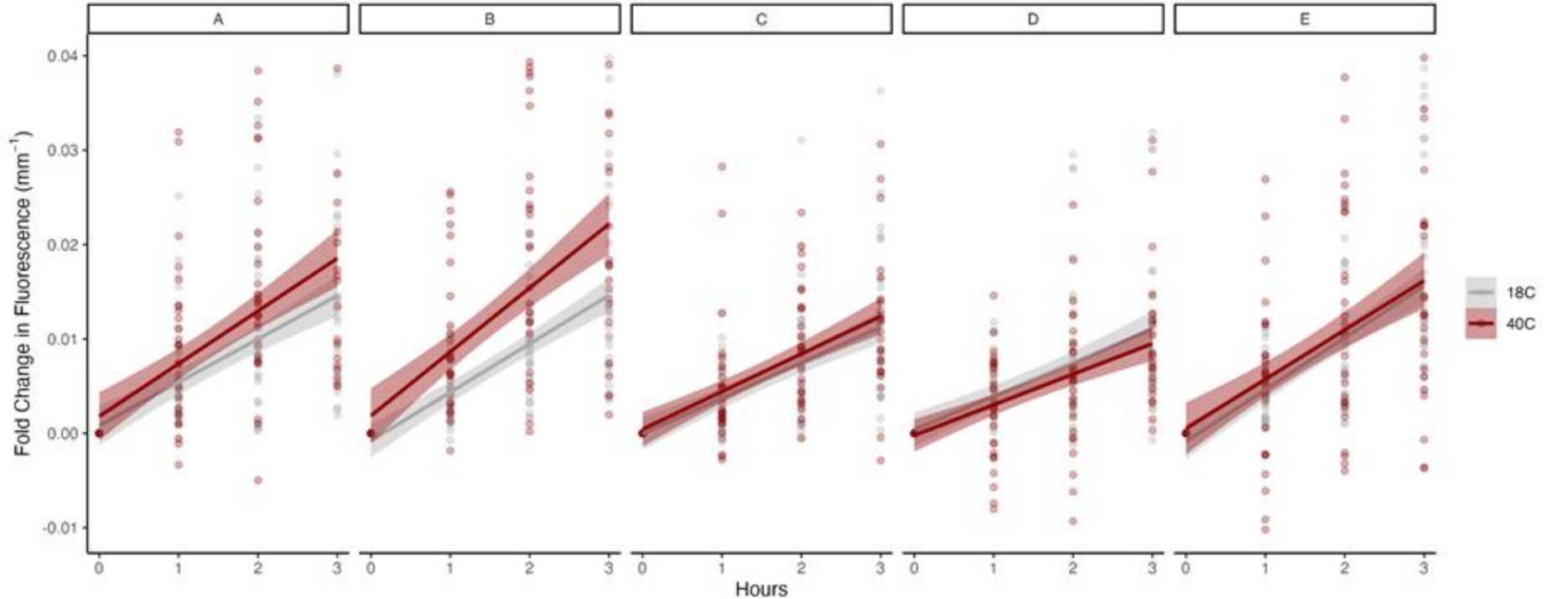
# 3 - Genetic background drives metabolic response



N=180 oysters  
Format: 20 mL cups

13–25 mm *C. gigas* seed  
3 h exposure to 18°C followed by 3 h exposure to 42°C

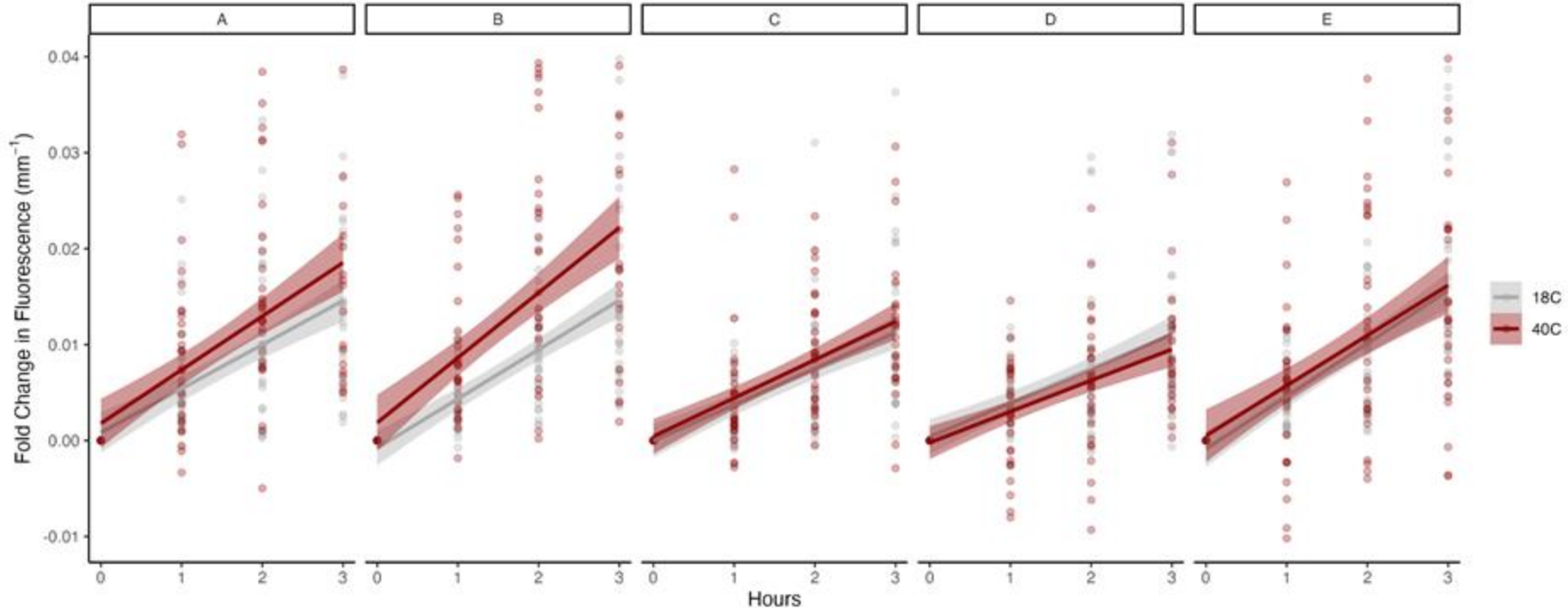
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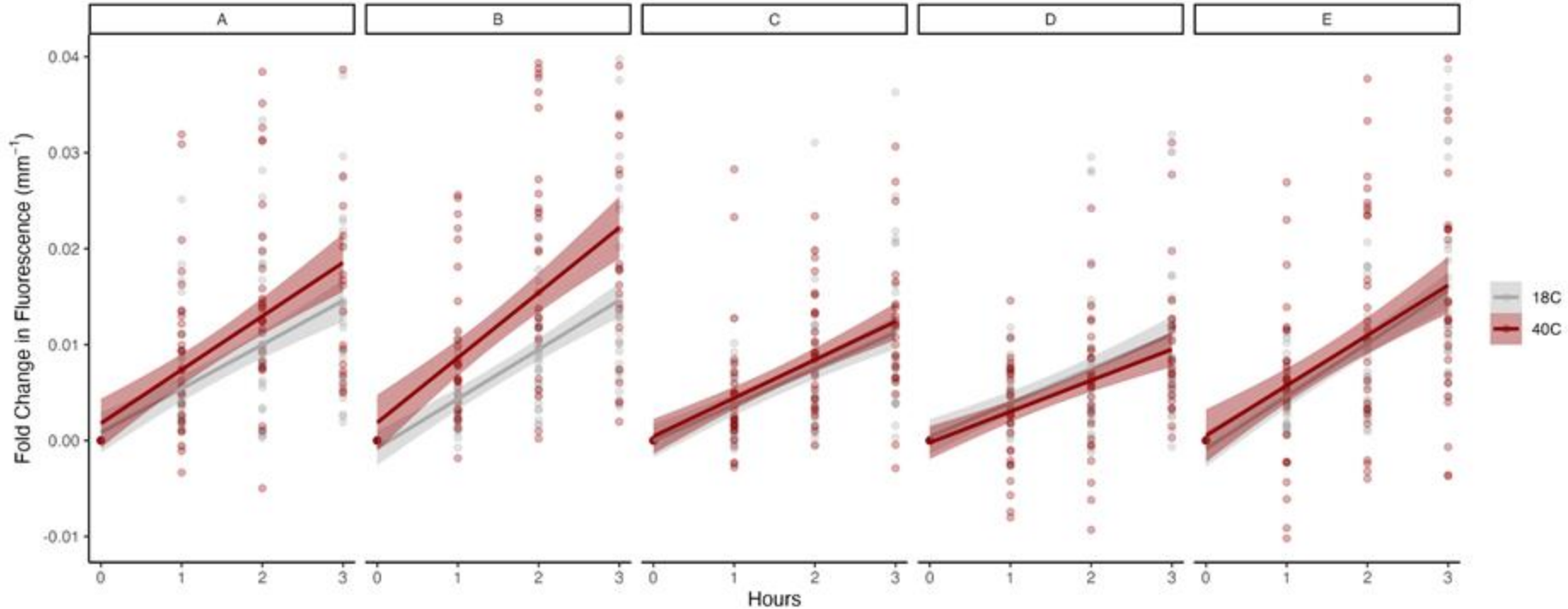
13–25 mm *C. gigas* seed  
3 h exposure to 18°C followed by 3 h exposure to 40°C

### 3 - Genetic background drives metabolic response



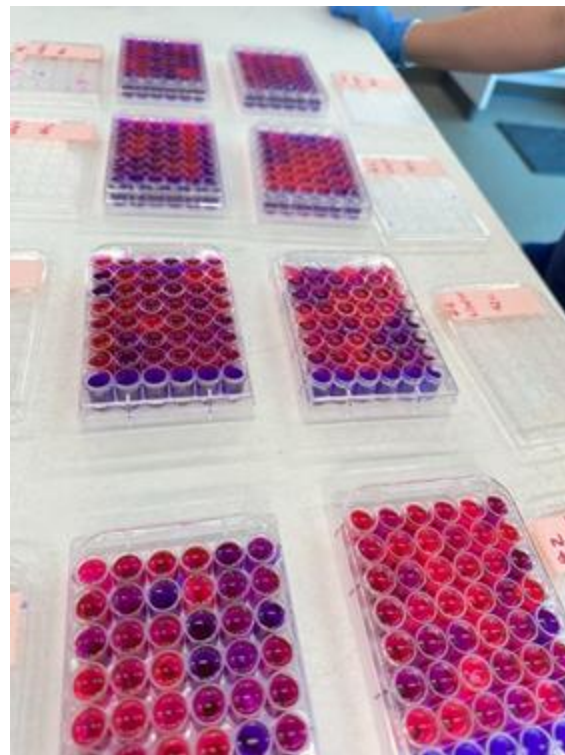
There is significant ***family-level variation*** in metabolic activity and metabolic response to stress.

### 3 - Genetic background drives metabolic response



Resazurin assays provide a ***tool to assess metabolic responses*** across families and lines.

# Case Study: Metabolic activity as a predictor of performance in *C. virginica*



N=50 families; 1,400 oysters  
Format: 24-well plates

10–18 mm *C. gigas* seed  
2.5 h exposure at 40°C; 1.5 h recovery



# Case Study: Metabolic activity as a predictor of performance in *C. virginica*

Resazurin Trials Explorer

Overview

Trajectories

Summaries

Color By

family

Facet By

None

Timepoints



☒ Show group means only

☒ Show Points

☒ Show Lines

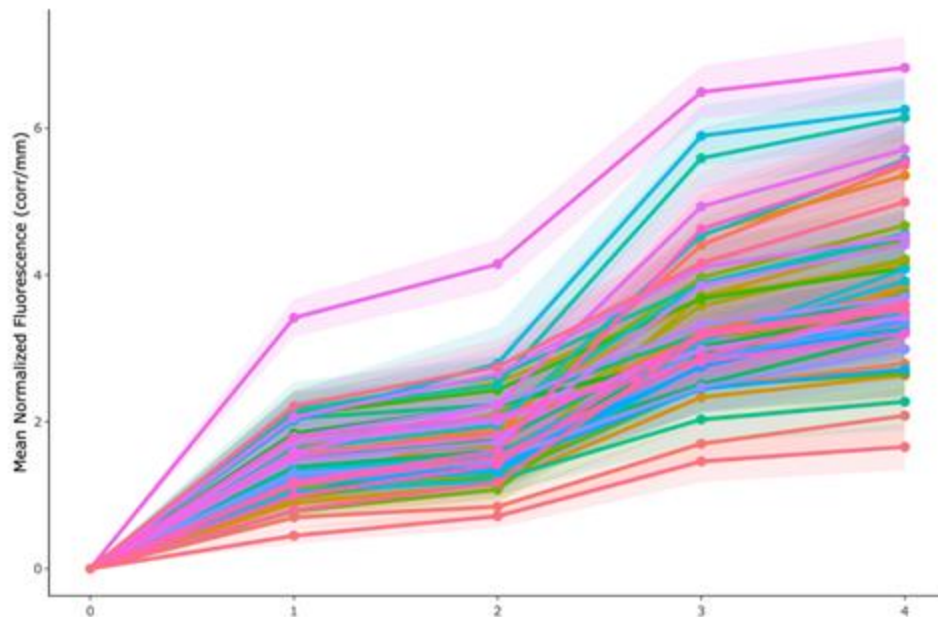
☐ Smooth (loess)

☐ Log10 Y

Oversaturation Threshold

3000

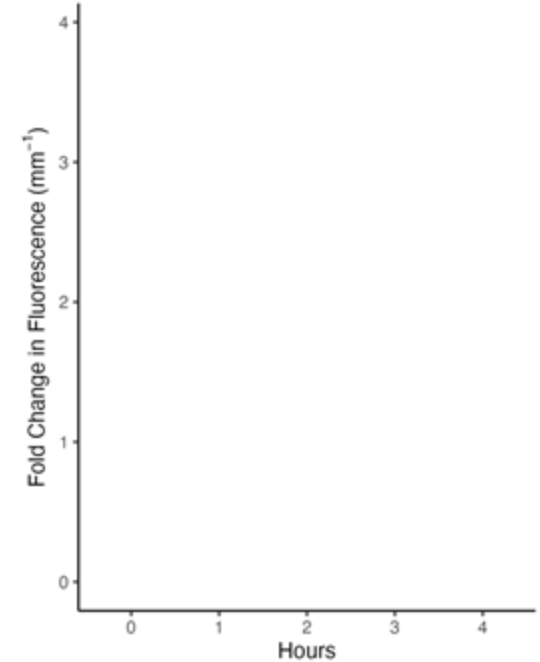
Example dataset auto-loaded (date 20250630 excluded).



Explore the data here!  
oyster.pink



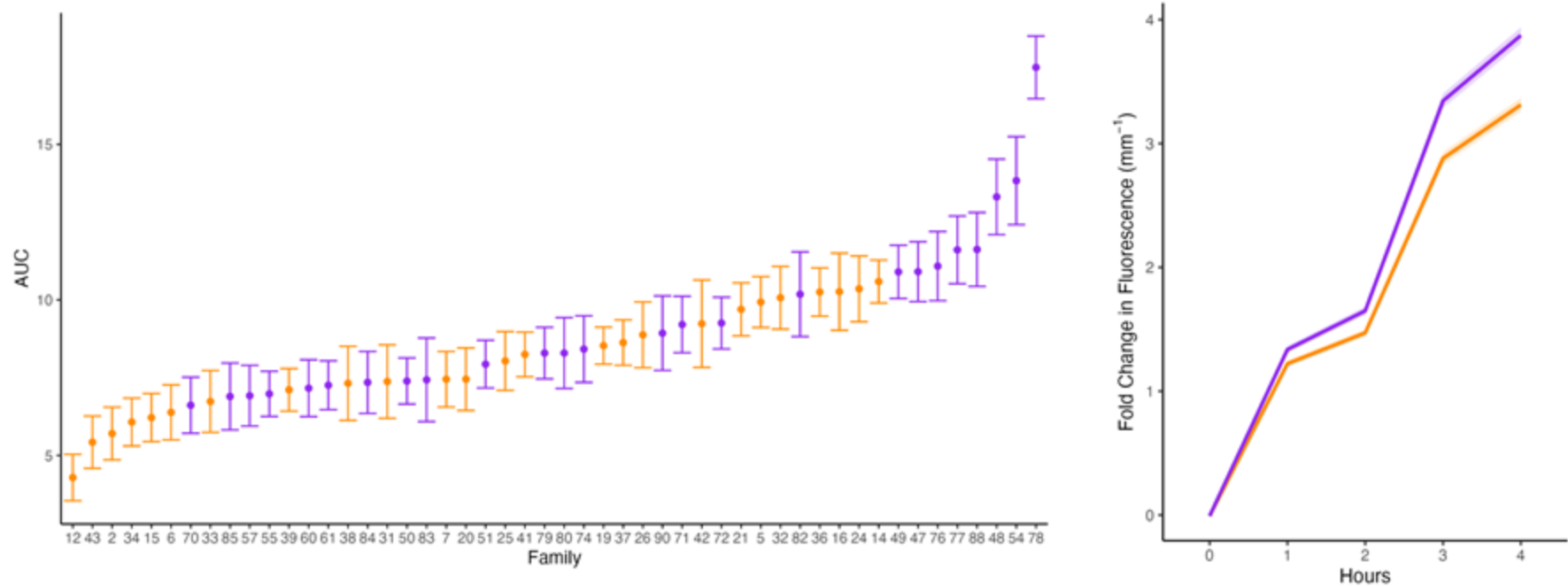
# Selective breeding drives metabolic response



**High Salinity** = Selectively bred for performance in high/moderate salinity (18-23 psu) environments

**Low Salinity** = Selectively bred for performance in low salinity (6-15 psu) environments

# Selective breeding drives metabolic response



*Low Salinity* selectively bred families exhibit higher metabolic activity

# Metabolism is correlated to predicted performance

## Predicted Survival

- High salinity
- Low salinity

~

Metabolism

## Predicted Growth

- High salinity
- Low salinity

# Metabolism is correlated to predicted performance

## Predicted Survival

- High salinity
- Low salinity

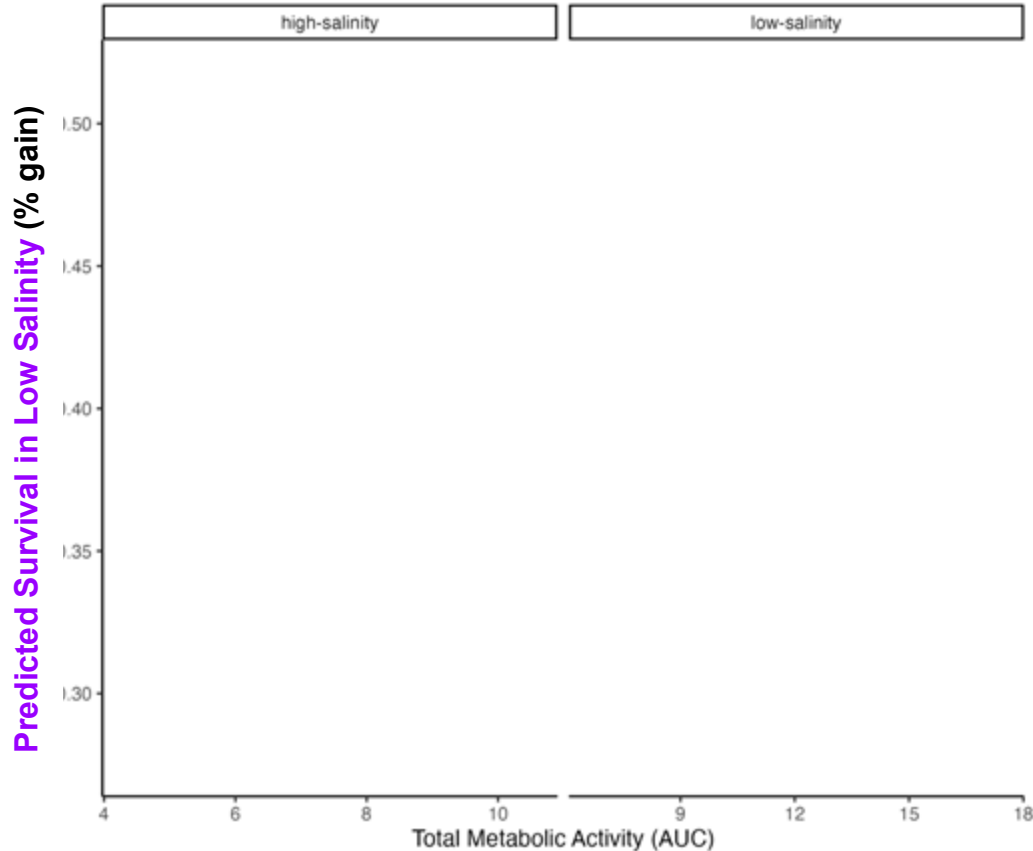
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Metabolism

## Predicted Growth

- High salinity
- Low salinity

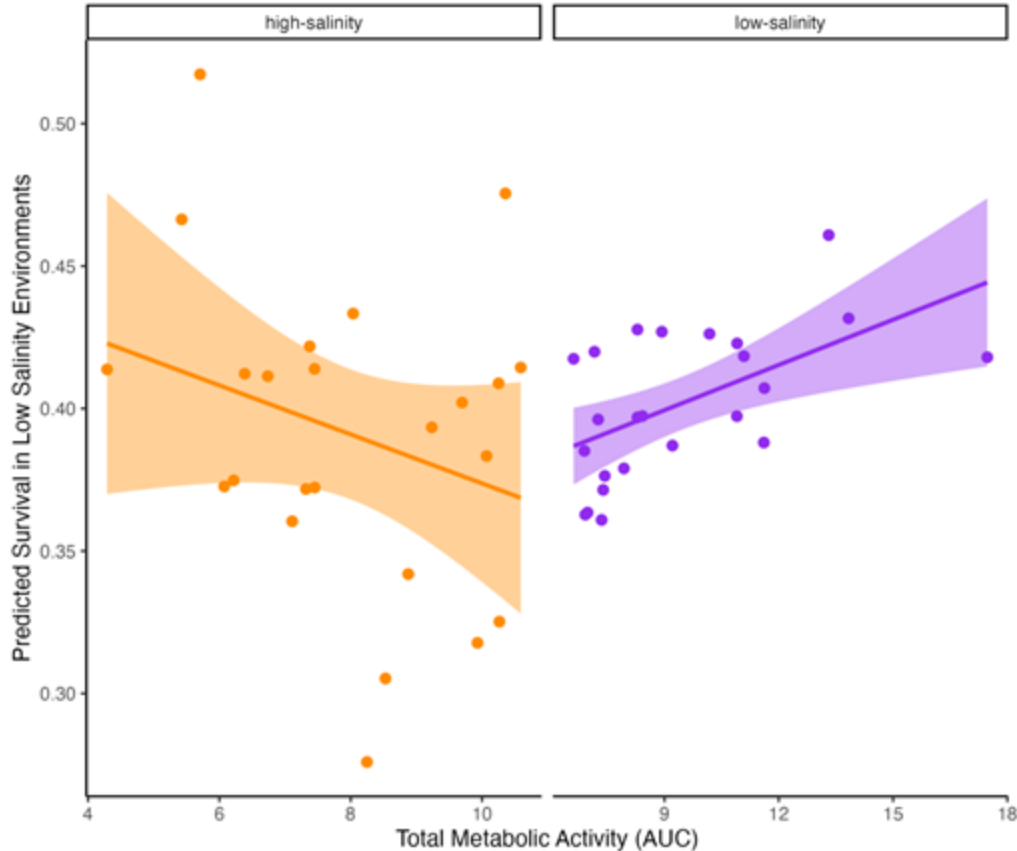
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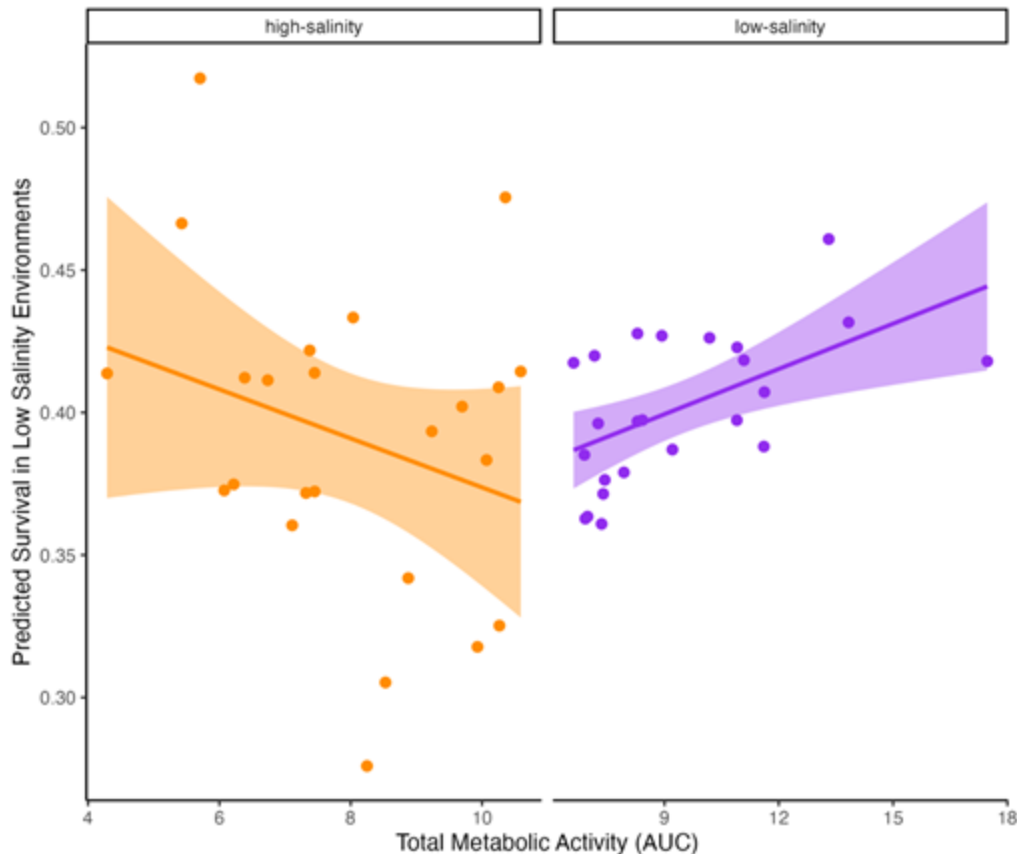
**Low Salinity** = Selectively bred for performance in low salinity (6-15 psu) environments

# Metabolism is correlated to predicted performance



Metabolic activity is positively correlated with predicted survival in **Low Salinity** environments in **Low Salinity** selected families

# Metabolism is correlated to predicted performance



Resazurin assays may provide a rapid, high throughput metabolic ***indicator of field performance***

*Ongoing: field performance measurements to validate the capacity to predict actual performance from metabolism*





## Key Findings from Resazurin Oyster Work

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## Key Findings from Resazurin Oyster Work



### Rapid assessment of stress responses

Resazurin provides quick, non-destructive readouts.



### Metabolic rate = indicator of stress tolerance

Higher rates linked to better acute performance



### Genetic background drives performance

Variation in metabolic response reflects lineage



### Parental history shapes offspring metabolism

Parental history alters stress response



### Metabolism predicts performance

Metabolic activity correlates with predicted performance

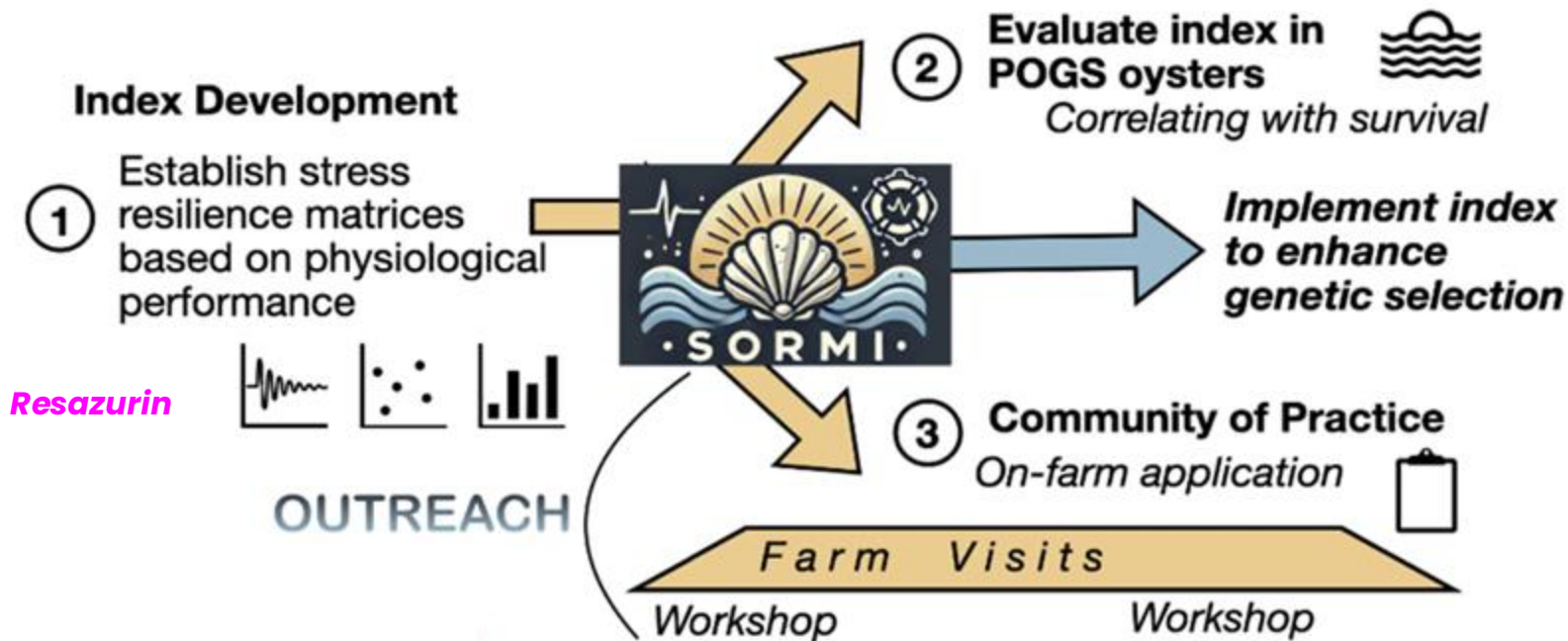
## Ongoing Efforts

- Family screenings
- Performance measurements
- Expanding protocol applications
- Developing field-friendly tools

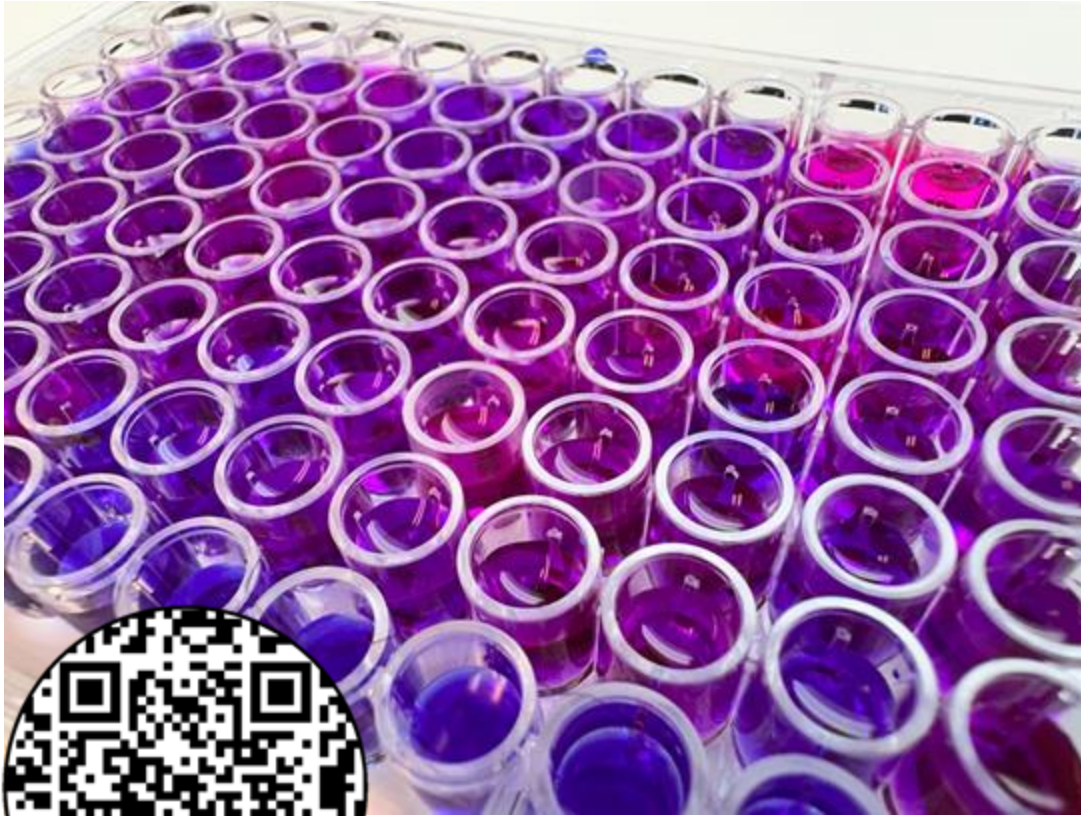
# Performance Testing

Development of SORMI (Summer Oyster Resilience and Mortality Index)

A quantitative tool for improving field survival



# Let's collaborate!



oyster.pink

Ariana Huffmyer: [ashuff@uw.edu](mailto:ashuff@uw.edu)

Steven Roberts: [sr320@uw.edu](mailto:sr320@uw.edu)

[robertslab.info](http://robertslab.info)



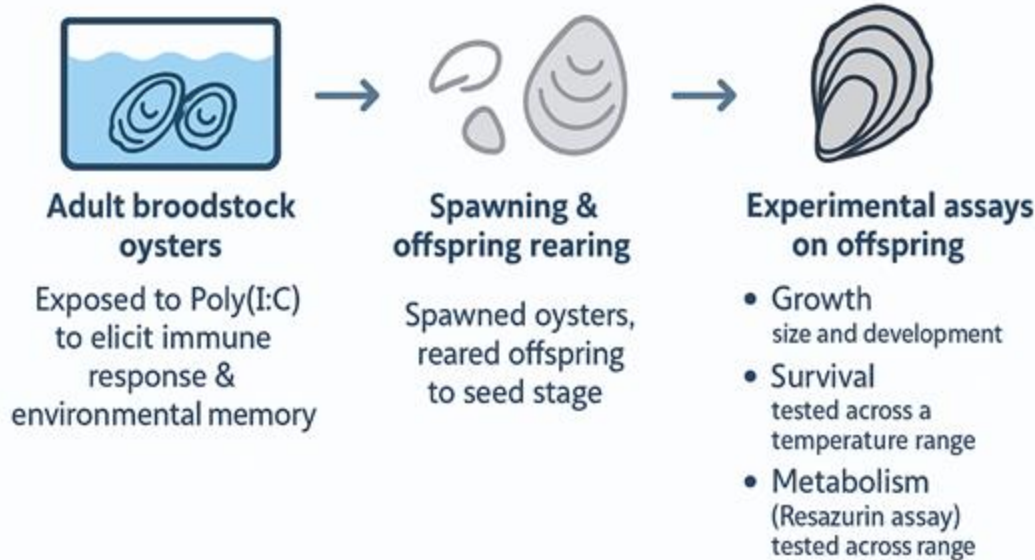
**VIMS** | WILLIAM & MARY  
VIRGINIA INSTITUTE OF MARINE SCIENCE





# 4 – Parental history affects metabolic stress response

## EXPERIMENTAL OVERVIEW



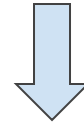
Spat from treated parents had:



*growth rates*

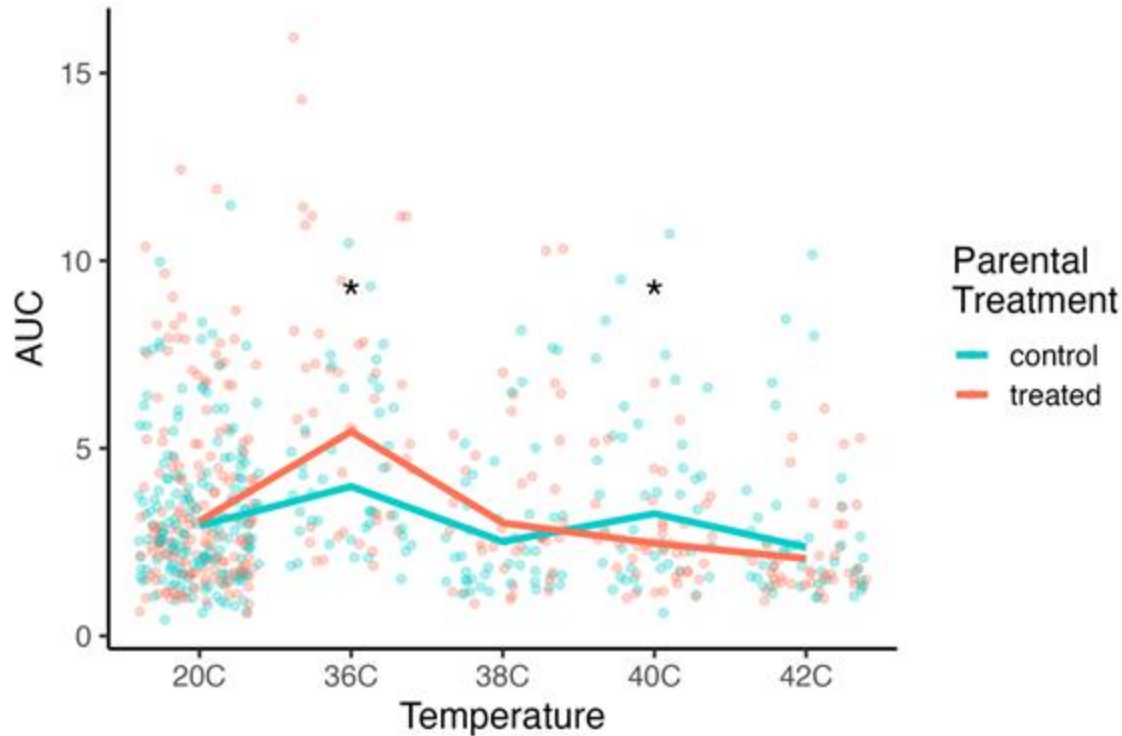


*survival at 40°C*



*metabolism at 40°C*

# 4 – Parental history affects metabolic stress response

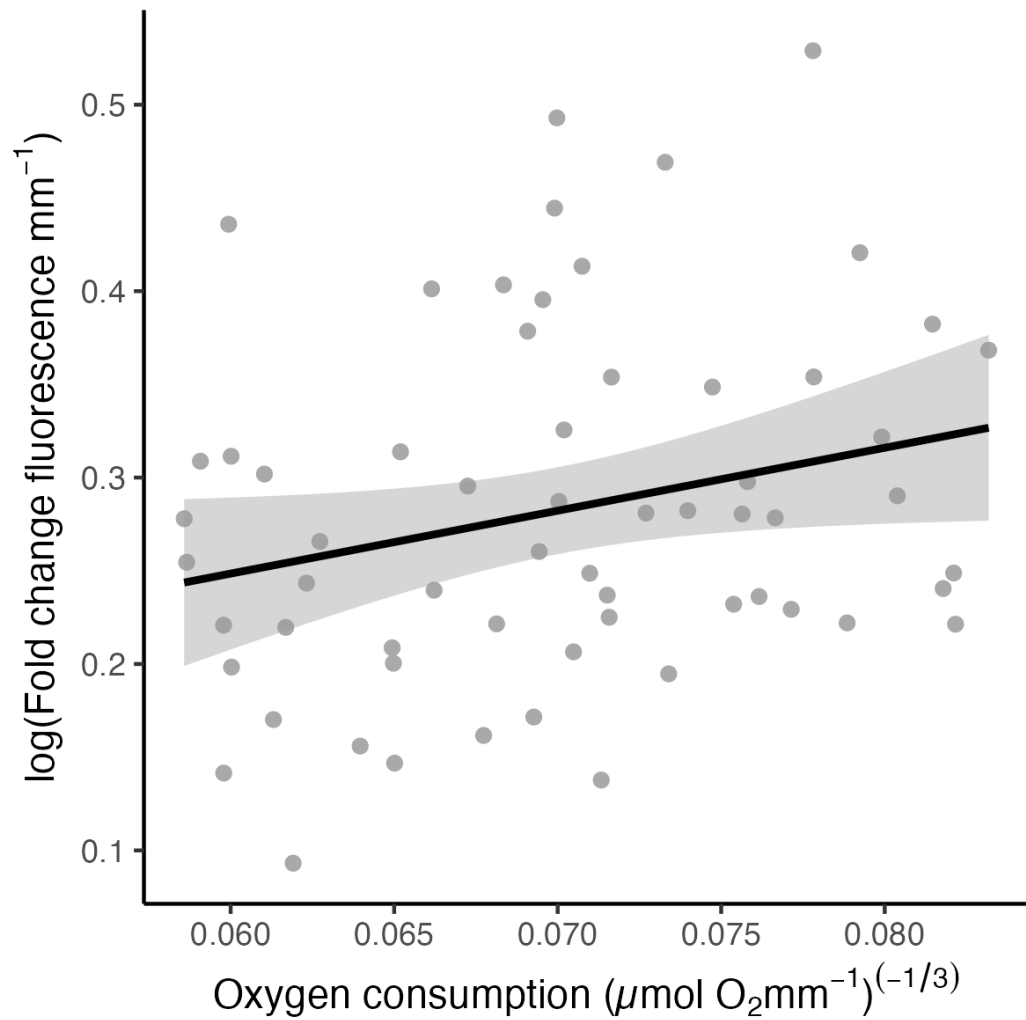


Offspring from parents that were immune challenged had **lower metabolism** at 40°C

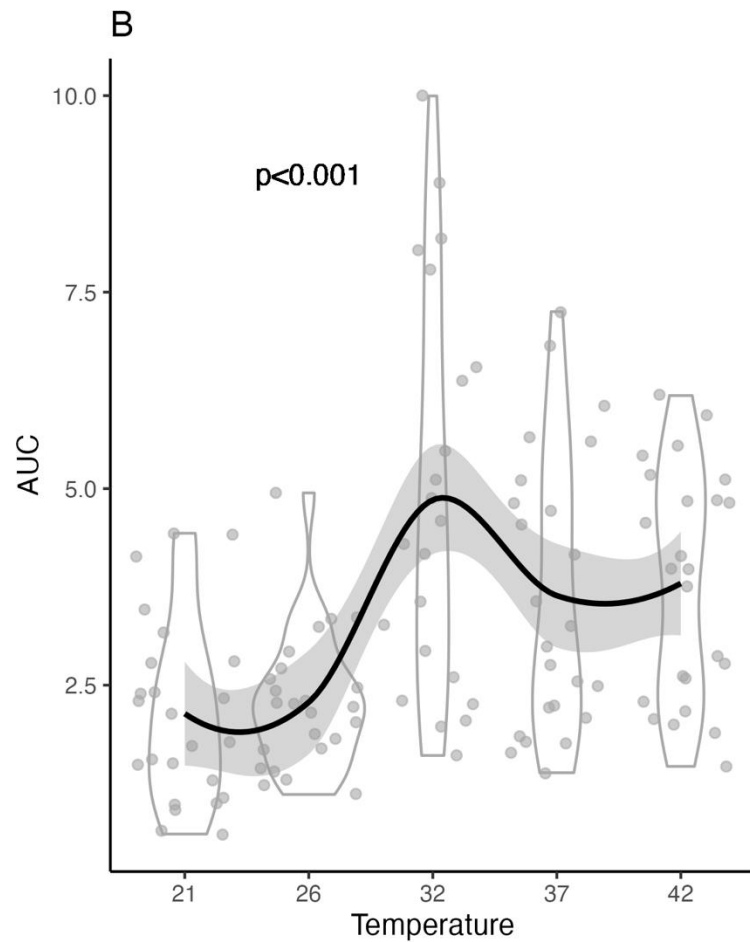
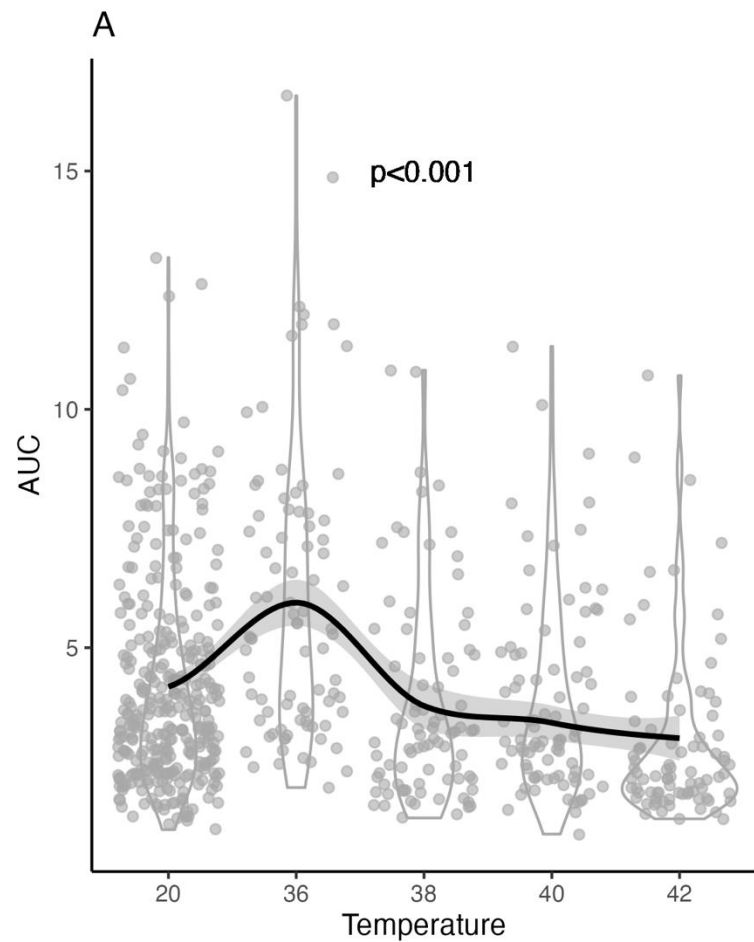
- Energy savings supporting survival

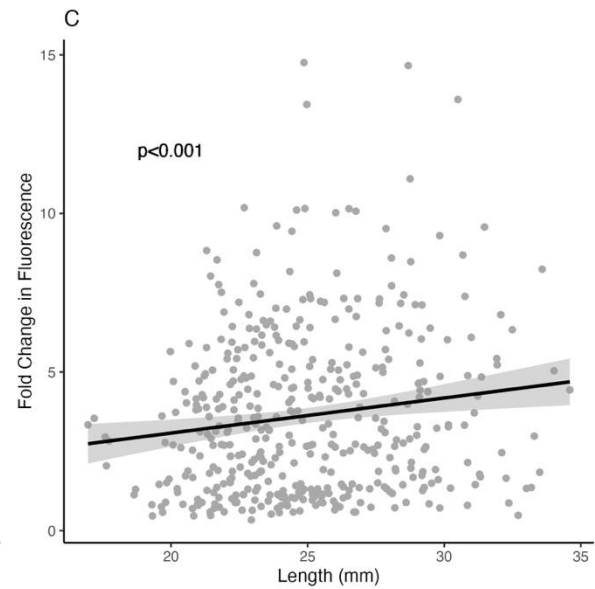
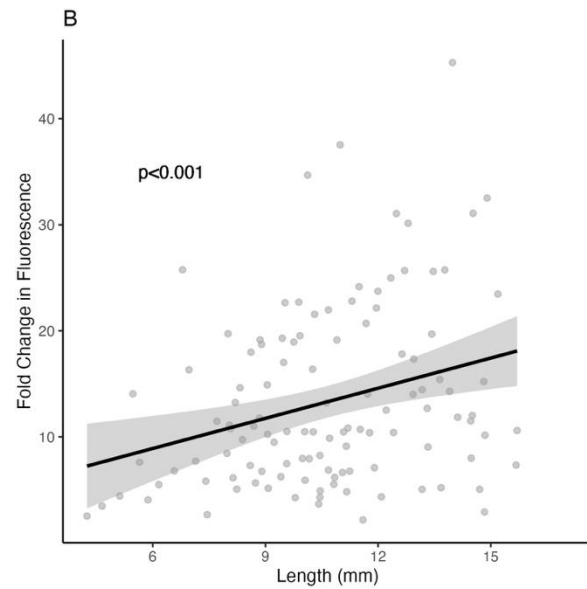
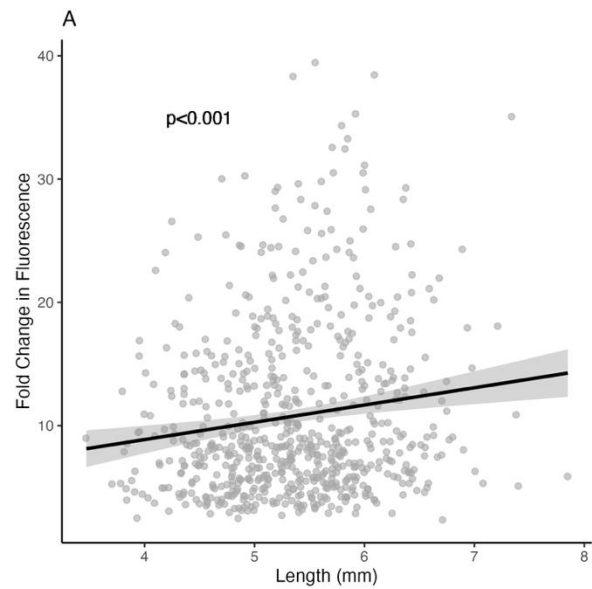
Offspring from immune-challenged parents had **greater metabolic activity** at moderate temperatures

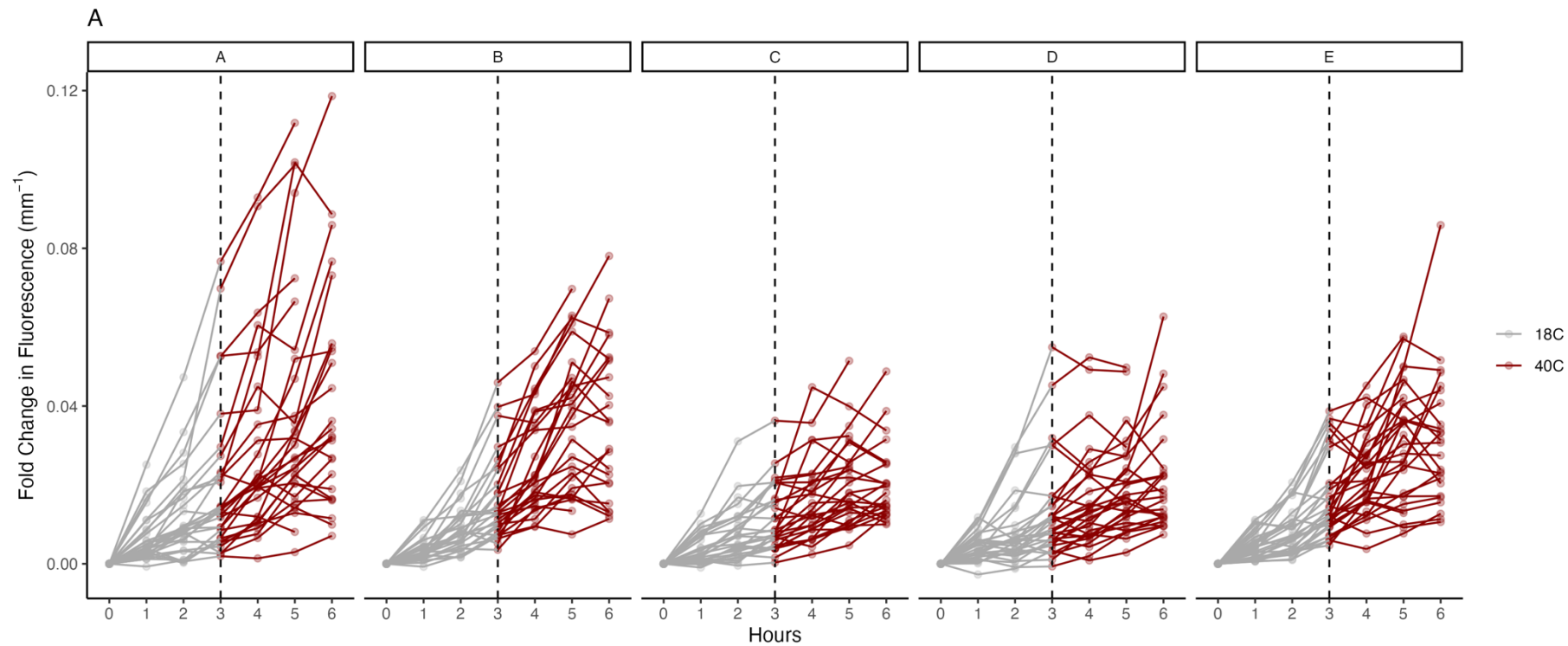
- Metabolic capacity for growth











Modeled Metabolic Rate

