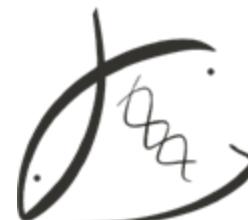




Stress Hardening and Metabolic Screening: Tools to Assess Oyster Performance and Resilience

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

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University of Washington
School of Aquatic and Fisheries Sciences

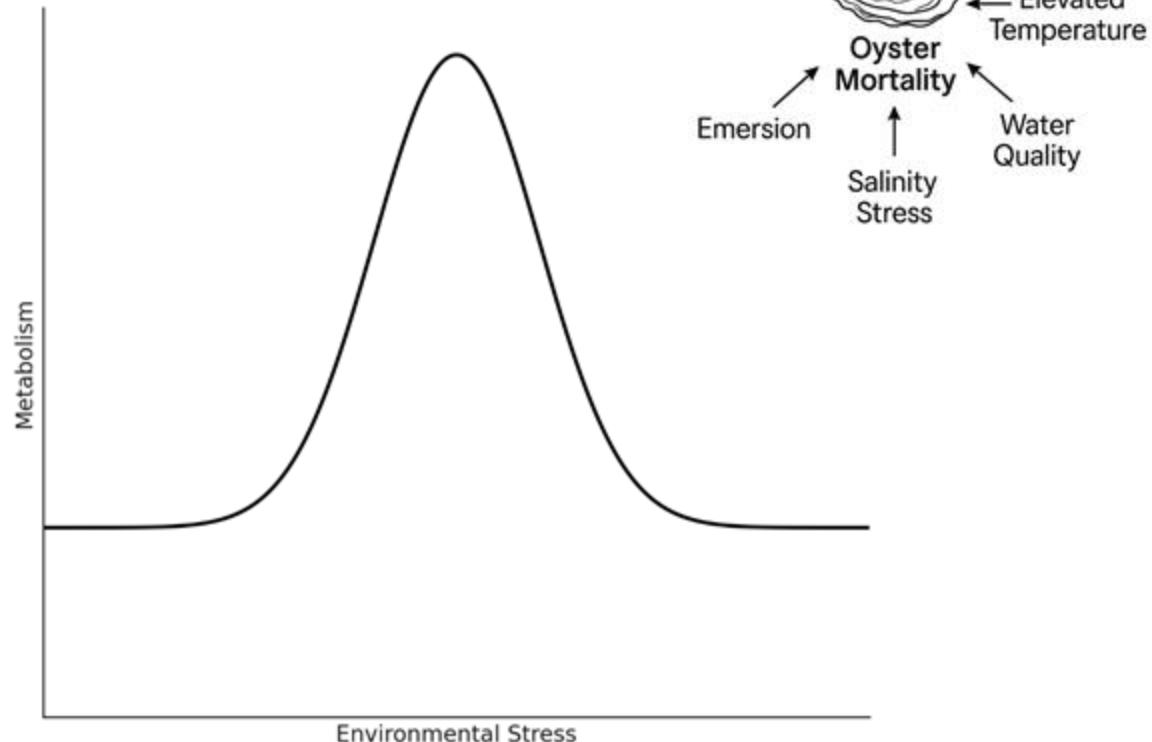


oyster.pink

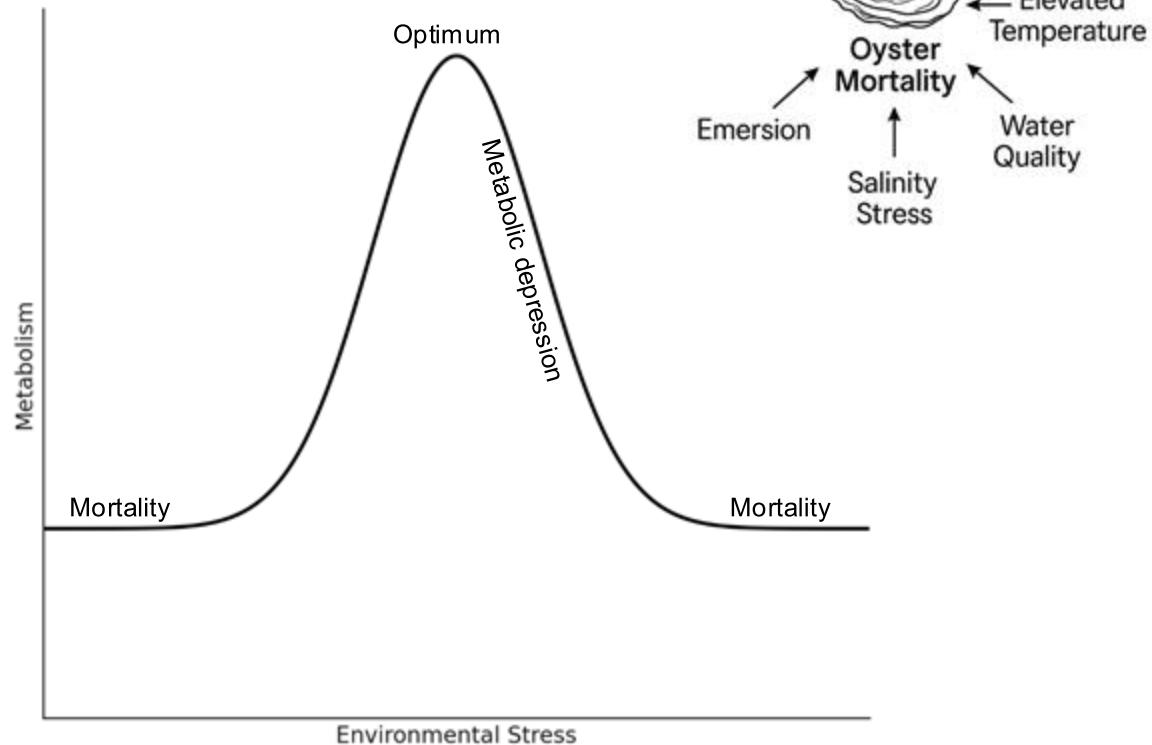
Resilience through the lens of metabolism



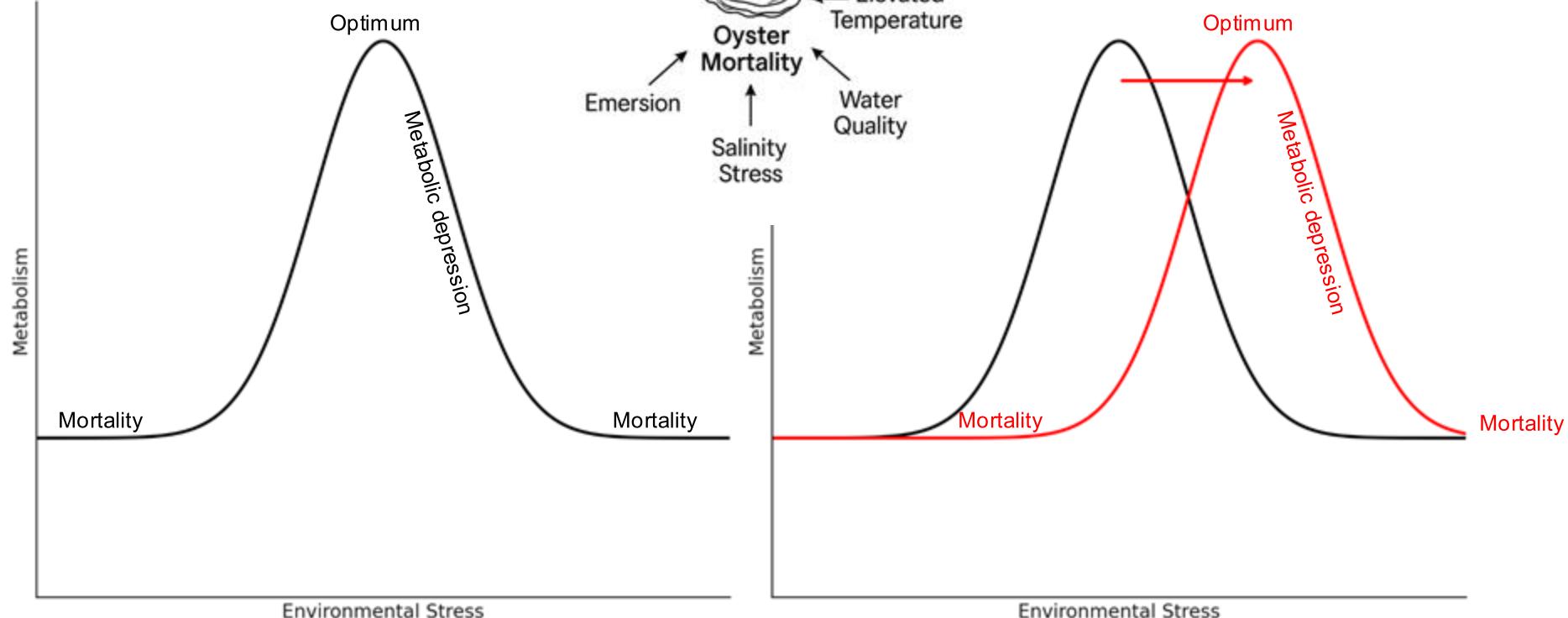
Resilience through the lens of metabolism



Resilience through the lens of metabolism

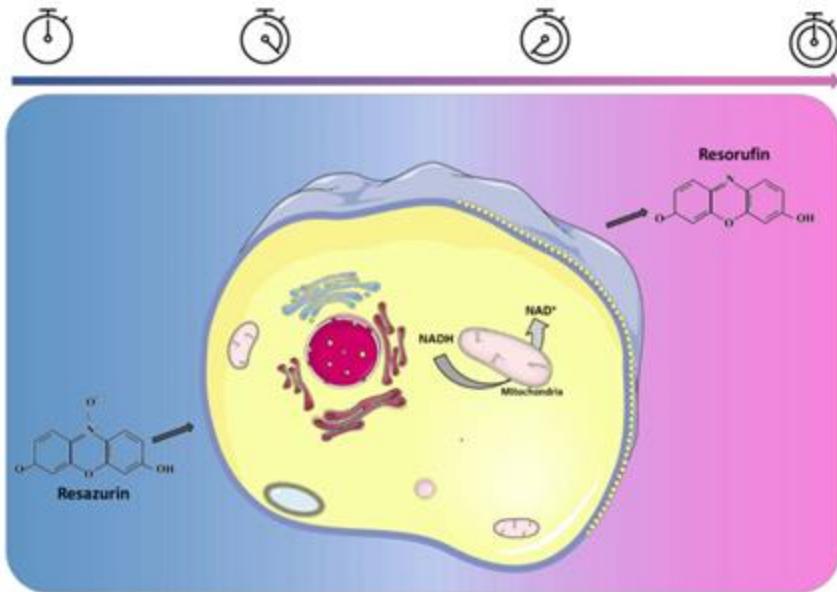


Resilience through the lens of metabolism

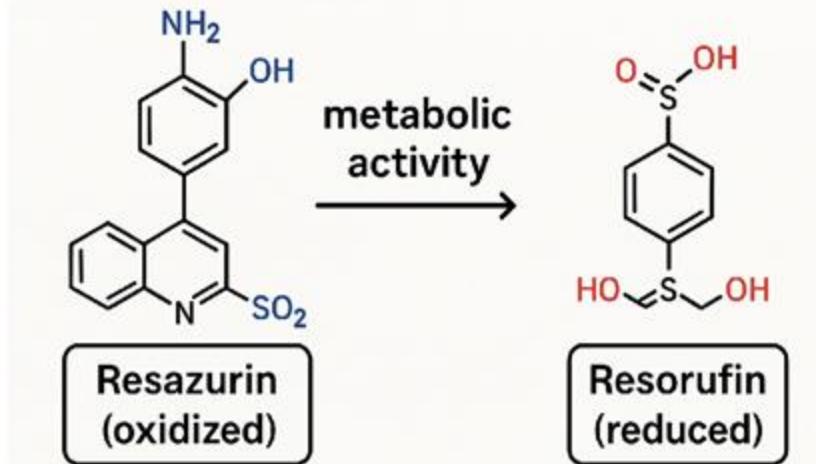


Modified from Sokolova et al. 2012

Resazurin Cell Viability Assay

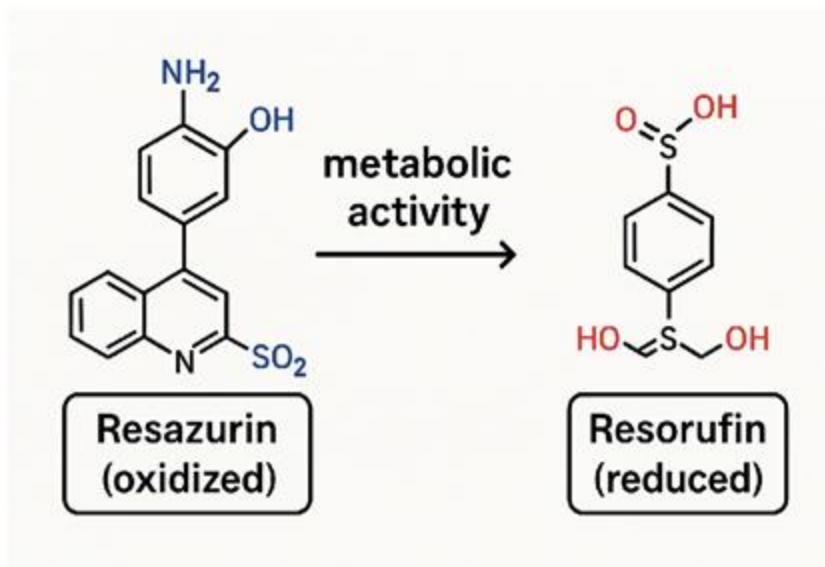
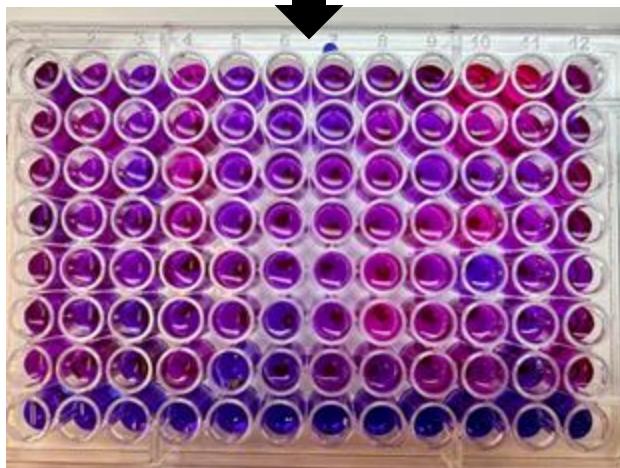
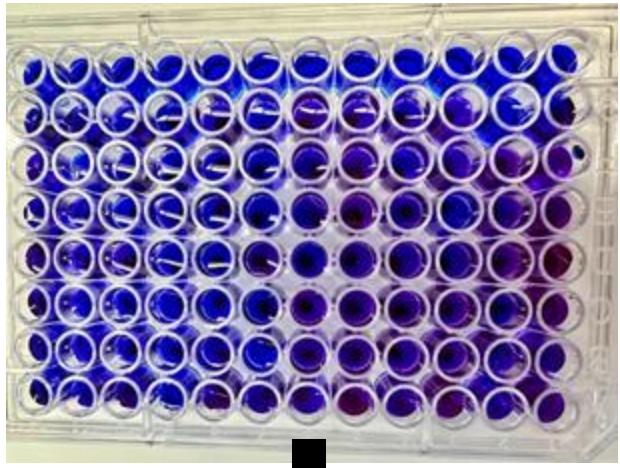


Petiti et al. 2024



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



Stream Ecology

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Fai & Grant 2009

Zare et al. 2015



Cytotoxicity

Petiti et al. 2024

Pace & Burg 2015

O'Brien et al. 2000

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

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MODIFIED

August 28, 2025

DOI [10.5281/zenodo.16973287](https://doi.org/10.5281/zenodo.16973287)

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](#).



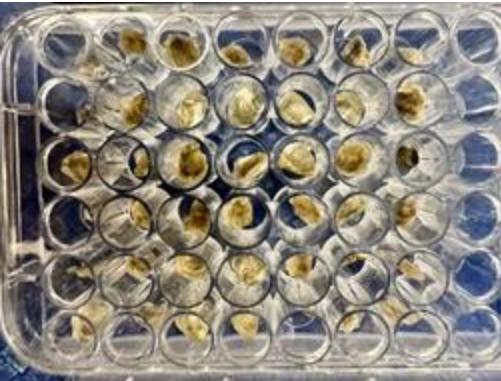
New: Public Summary Available

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



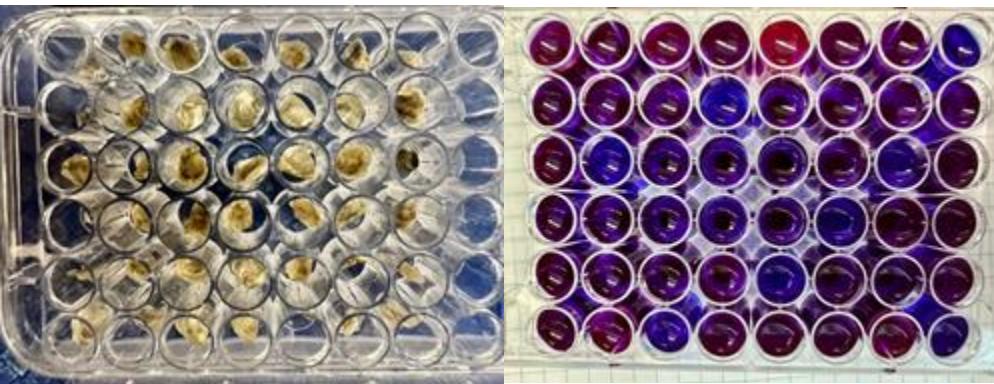
1 - Animal Selection

- 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)

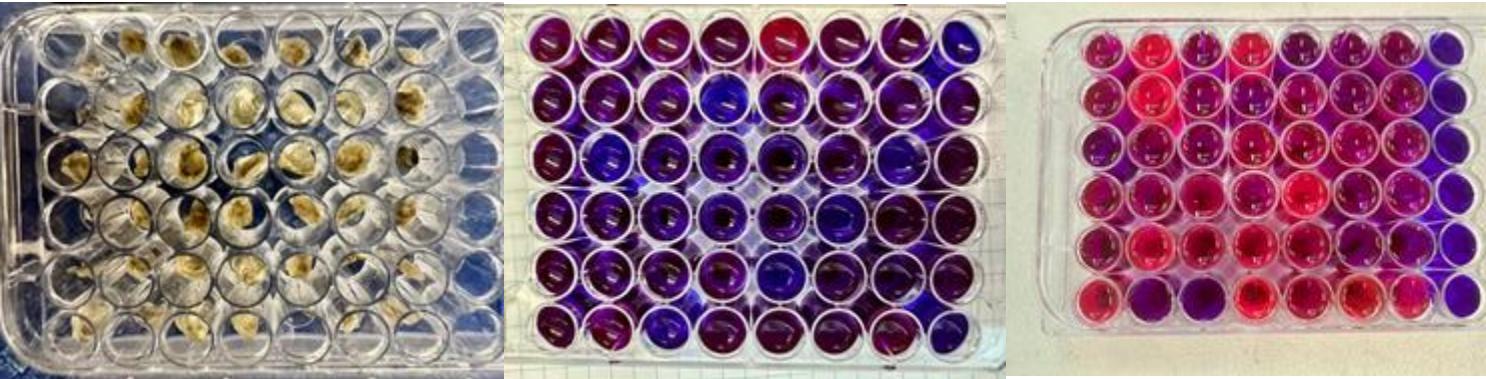
Safety margin: +10% extra volume

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

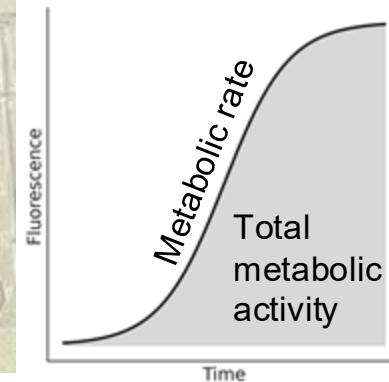
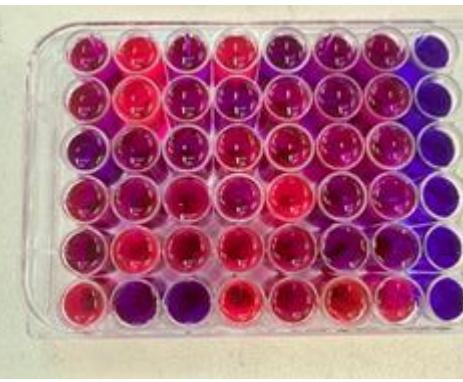
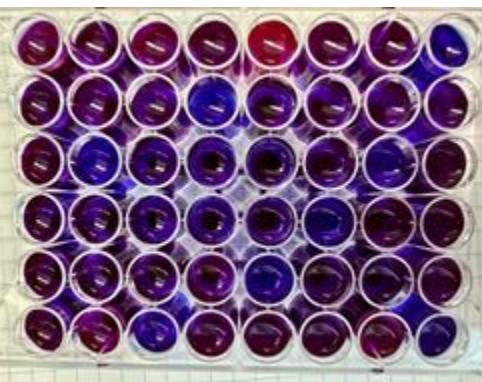
3 - Treatment

- Stressor incubation
- Hourly fluorescence readings



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Resazurin Assay Development



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- 2mm spat - adult oysters
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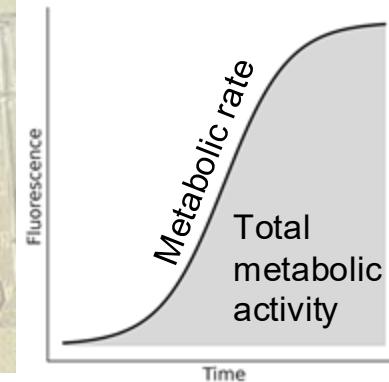
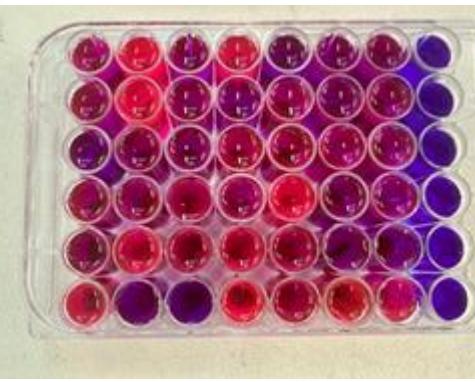
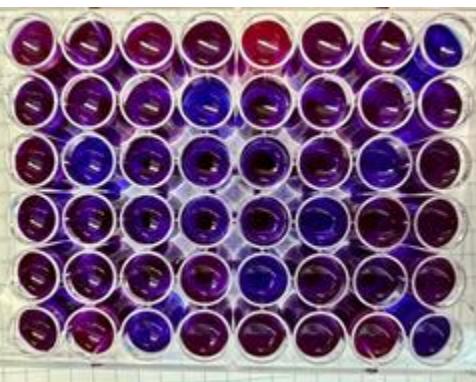
4 - Analysis

- Metabolic rates
- Total metabolic activity



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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

3 - Treatment

- Stressor incubation
- Hourly fluorescence readings

4 - Analysis

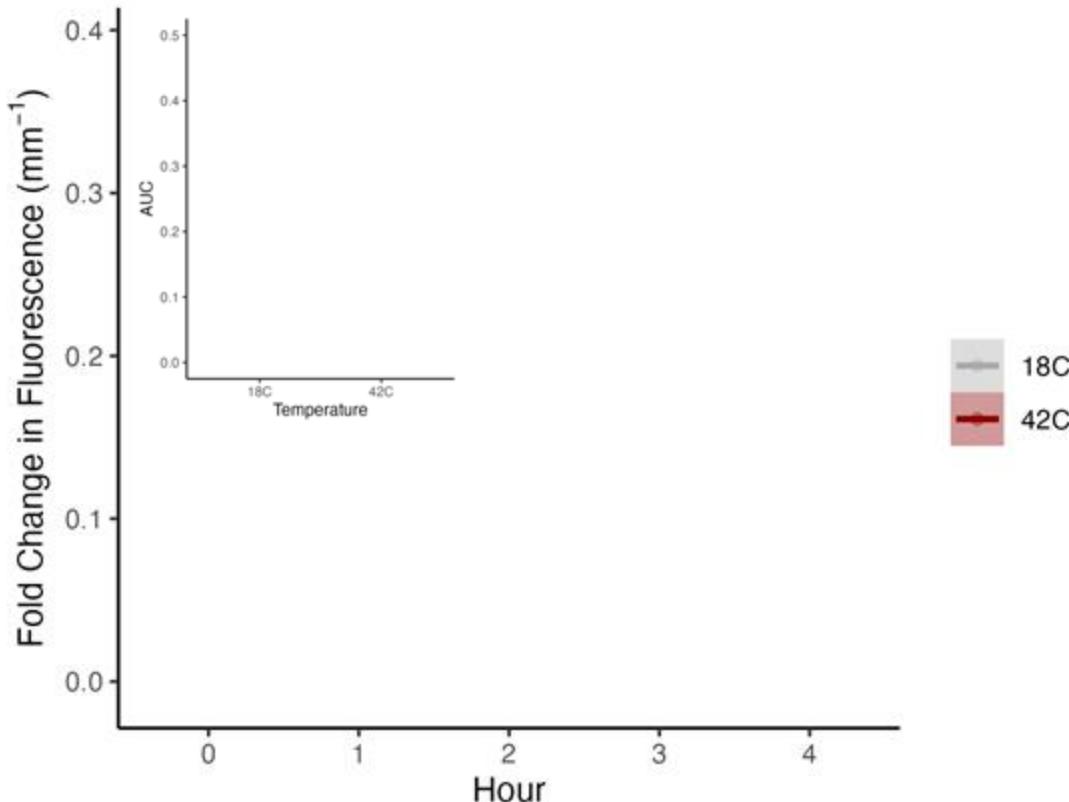
- Metabolic rates
- Total metabolic activity

- ✓ 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



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

1 - Rapid assessment of oyster acute stress response

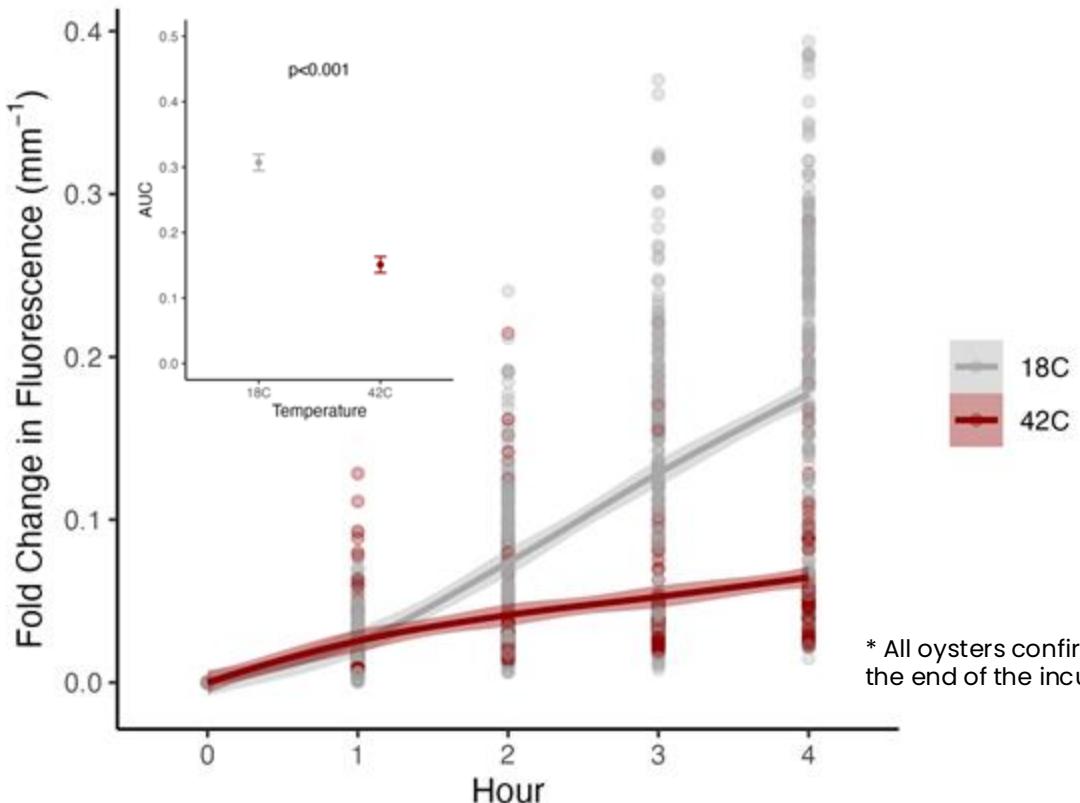


Metabolic rate = fold change in fluorescence normalized to shell length (mm)

- Signal normalization
- Blank correction

Total metabolic activity = area under the curve (AUC)

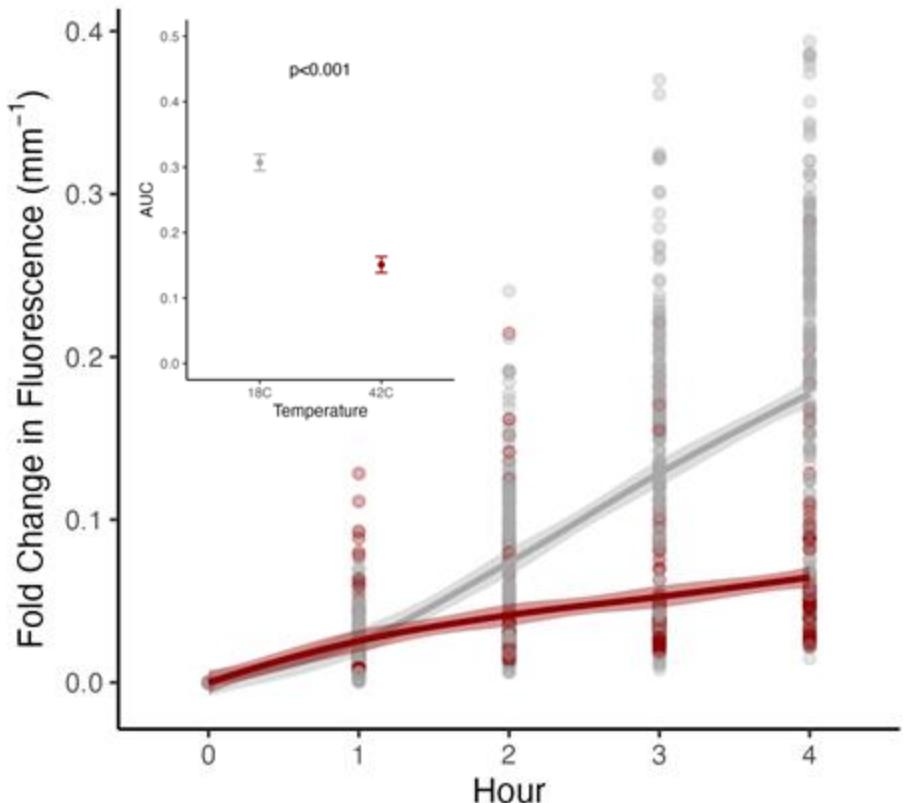
1 - Rapid assessment of oyster acute stress response



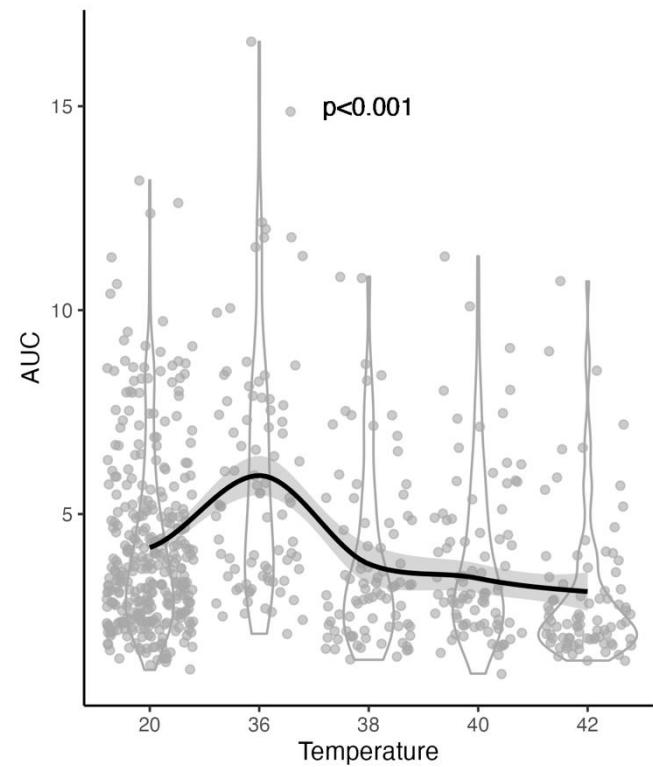
Metabolic activity was **lower** in oysters under acute heat stress

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

1 - Rapid assessment of oyster acute stress response

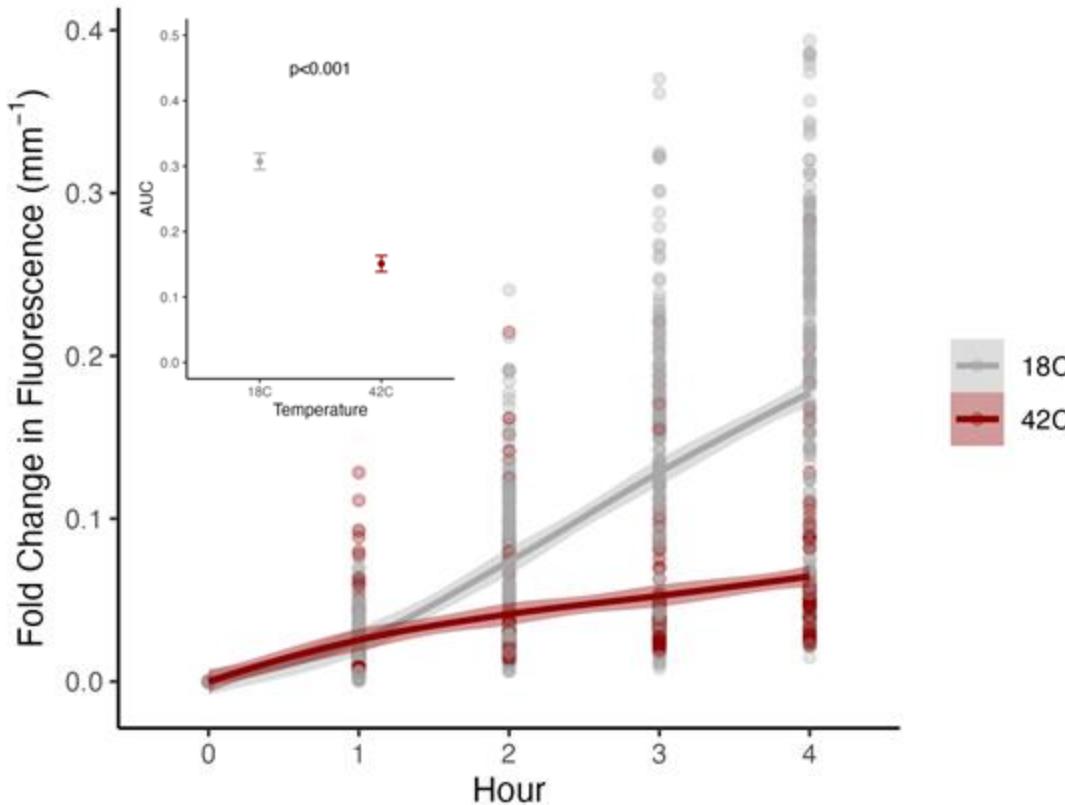


N=507 oysters
Format: 20 mL cups

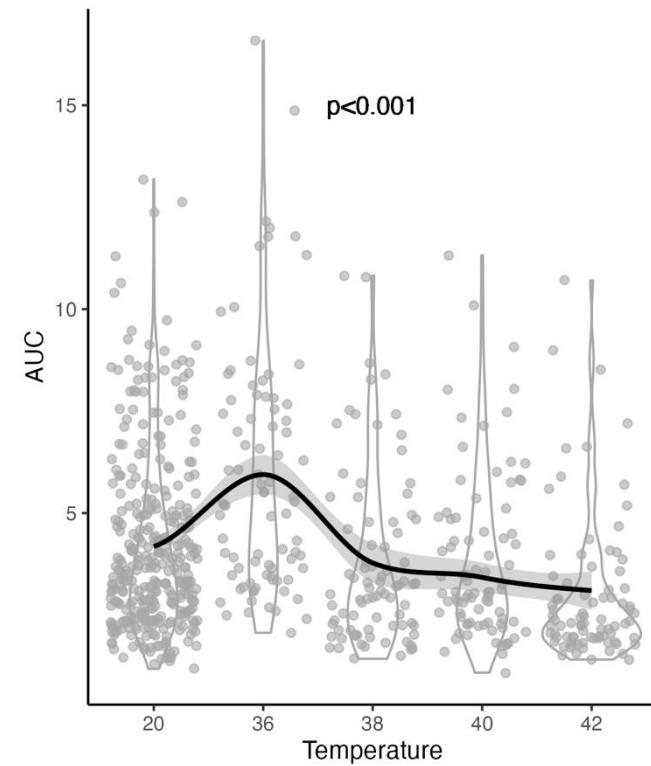


2-8 mm *C. gigas* seed
4 h exposures

1 - Rapid assessment of oyster acute stress response

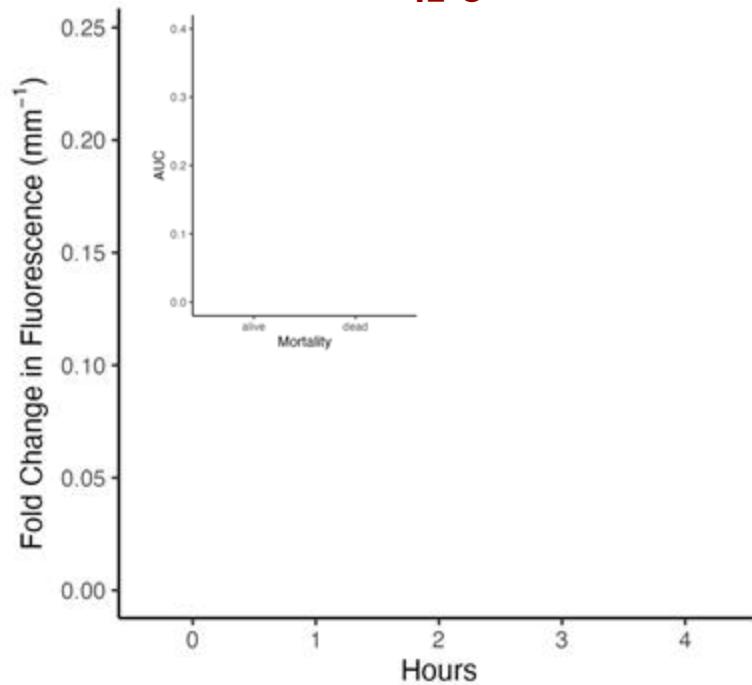


Resazurin assay provides a **rapid, high throughput** approach to assess metabolic response to stress

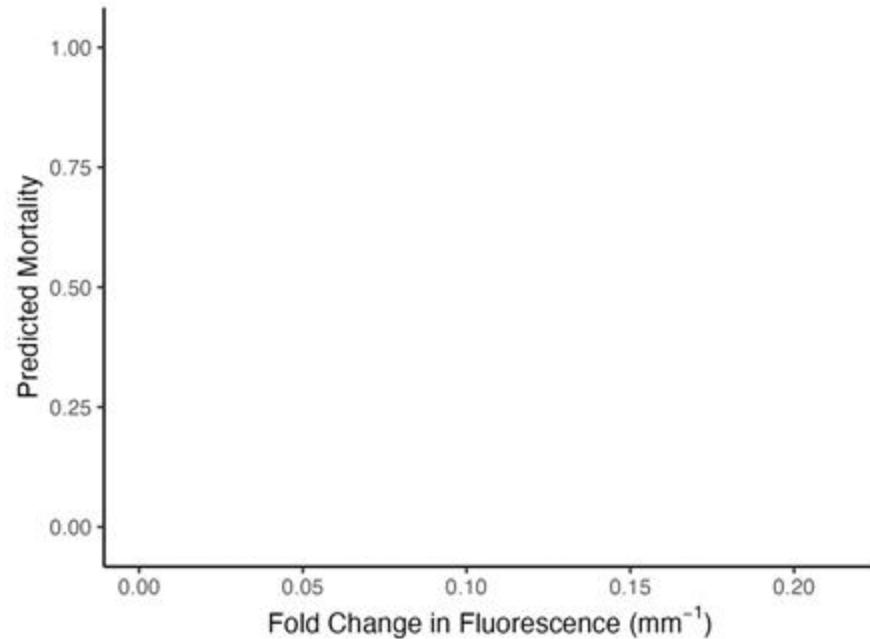


2 - Metabolic rate as indicator of stress tolerance

42°C



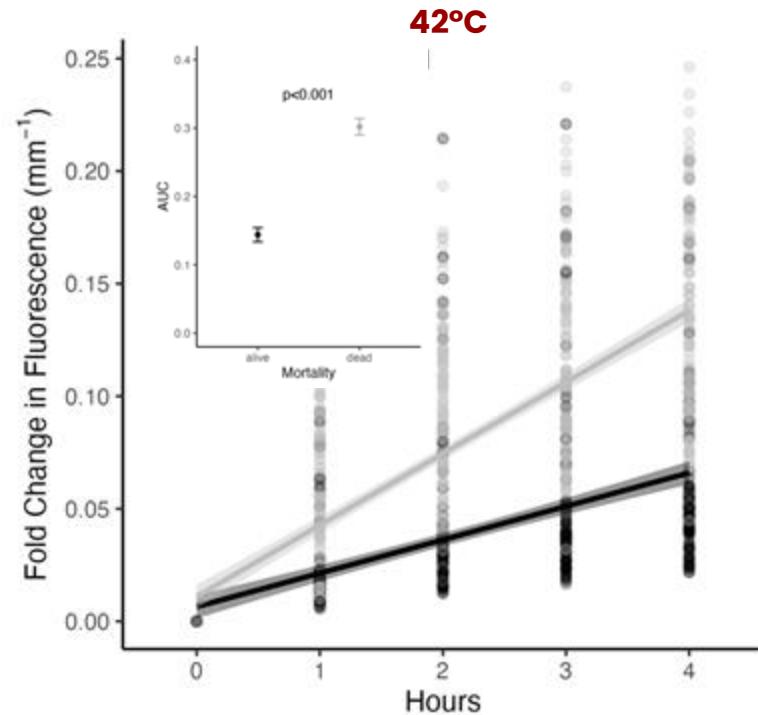
Mortality
alive
dead



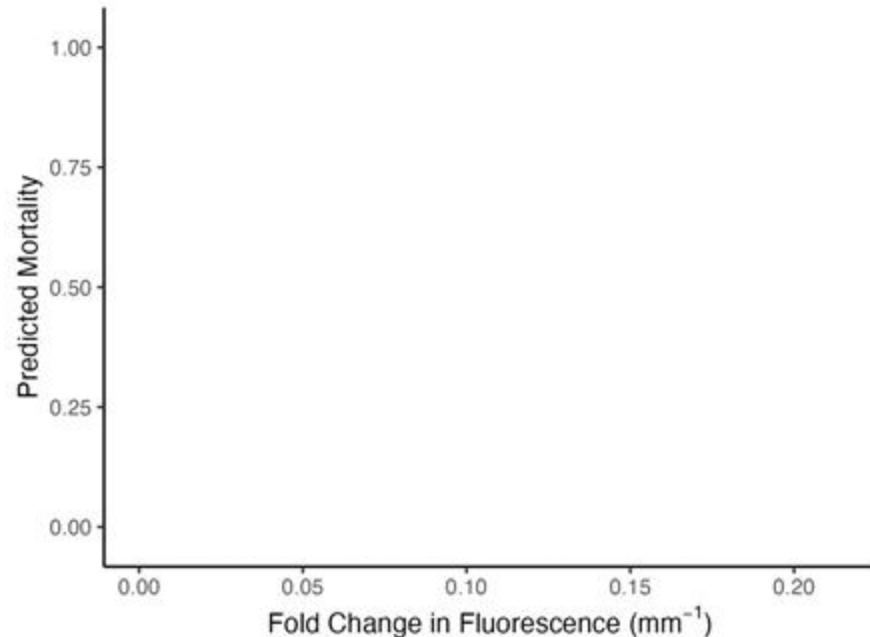
N=507 oysters
Format: 20 mL cups

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

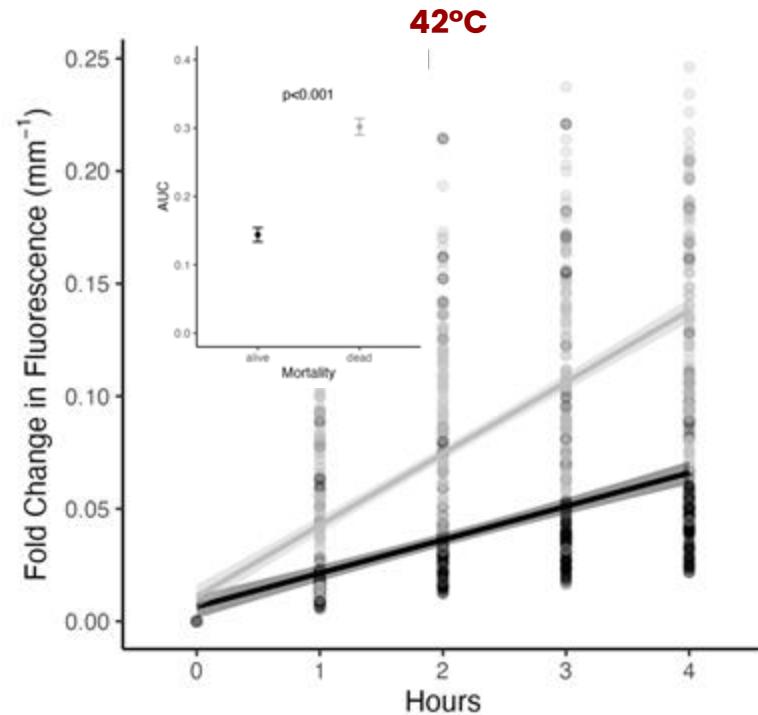
2 - Metabolic rate as indicator of stress tolerance



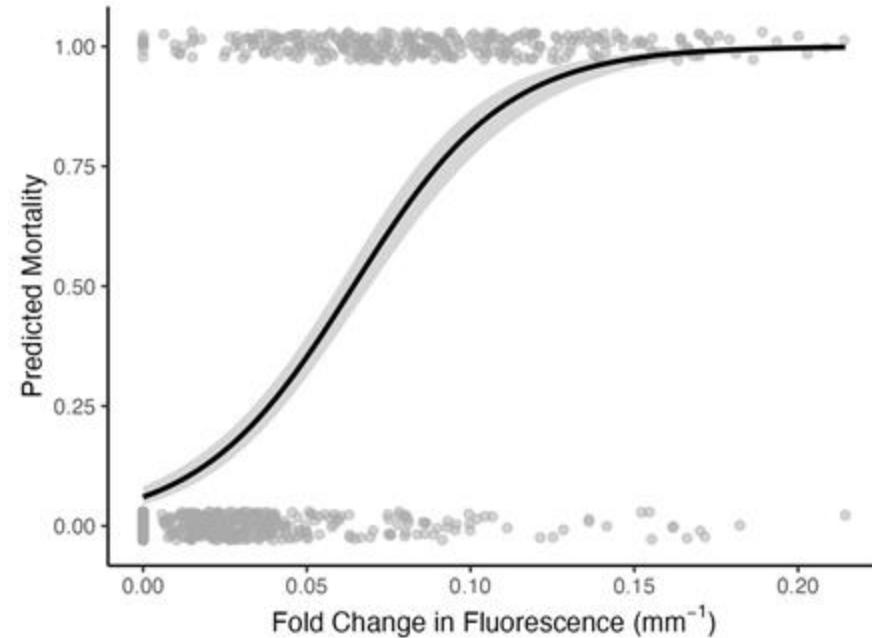
Oysters that survived exposure to acute stress had ***lower*** metabolic activity.



2 - Metabolic rate as indicator of stress tolerance

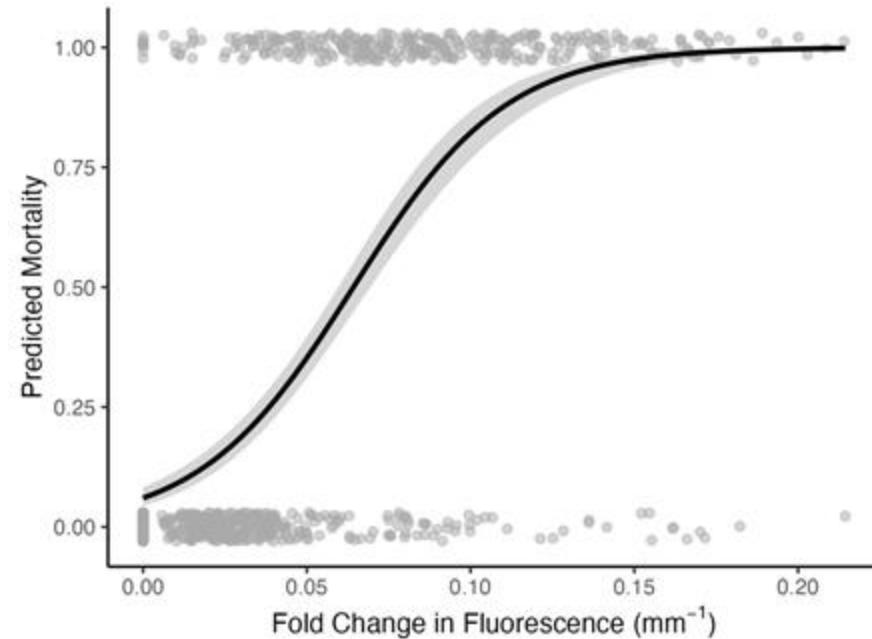
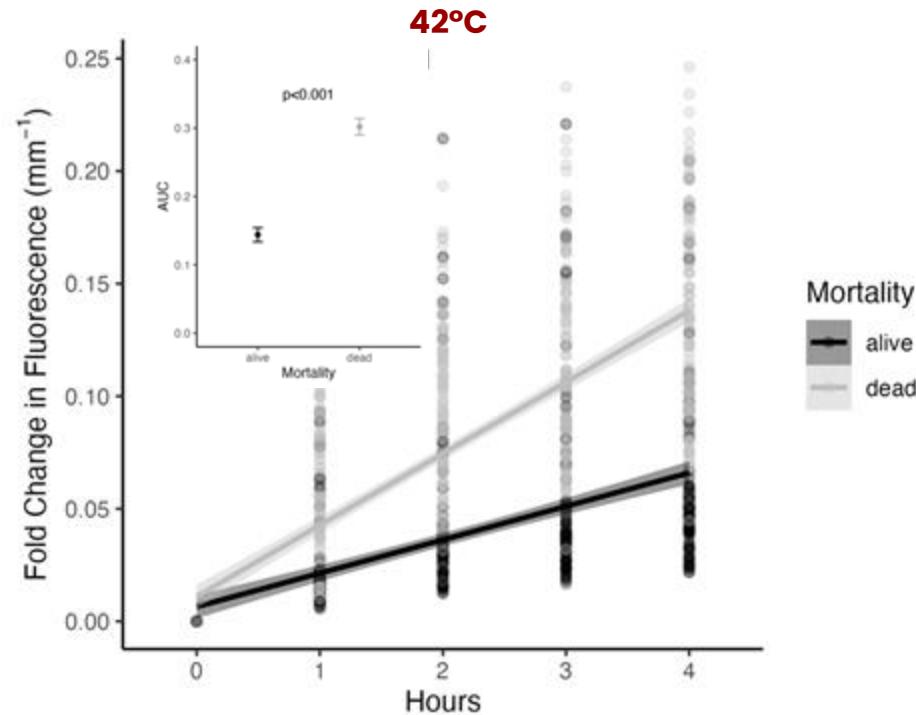


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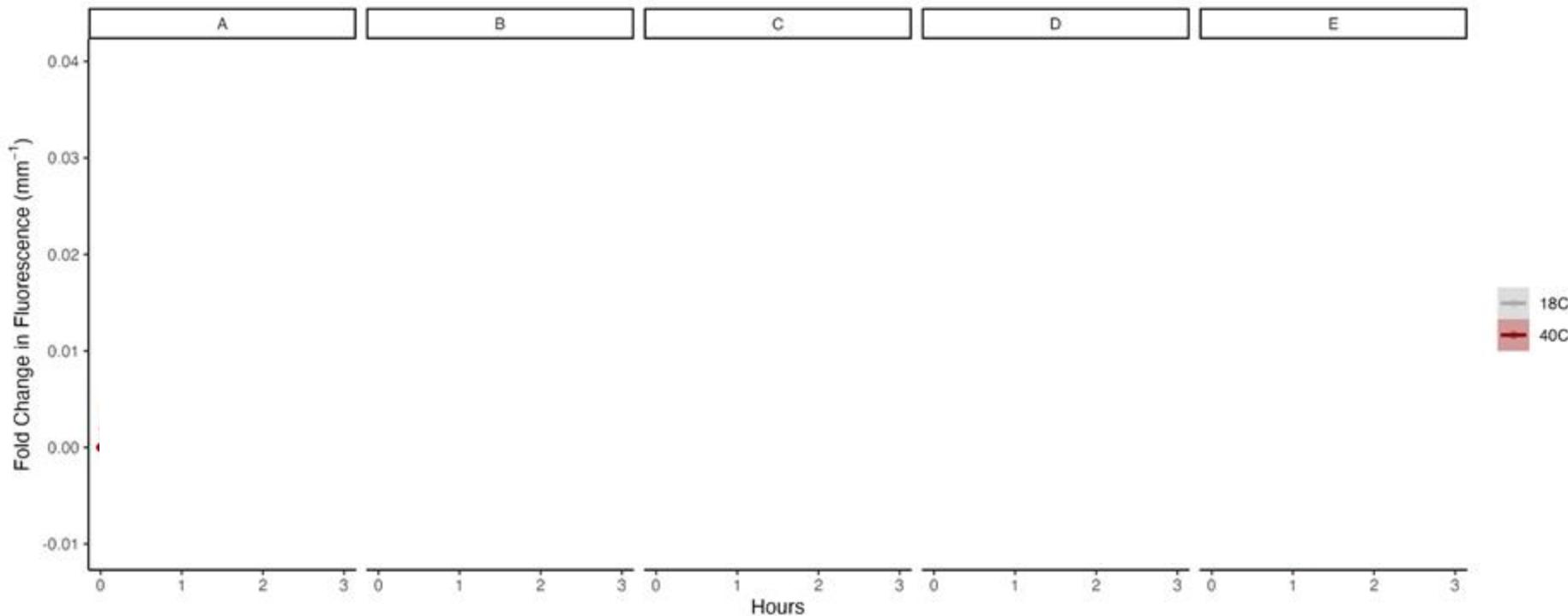
Increased metabolic activity led to a **higher mortality risk**.

2 - Metabolic rate as indicator of stress tolerance



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

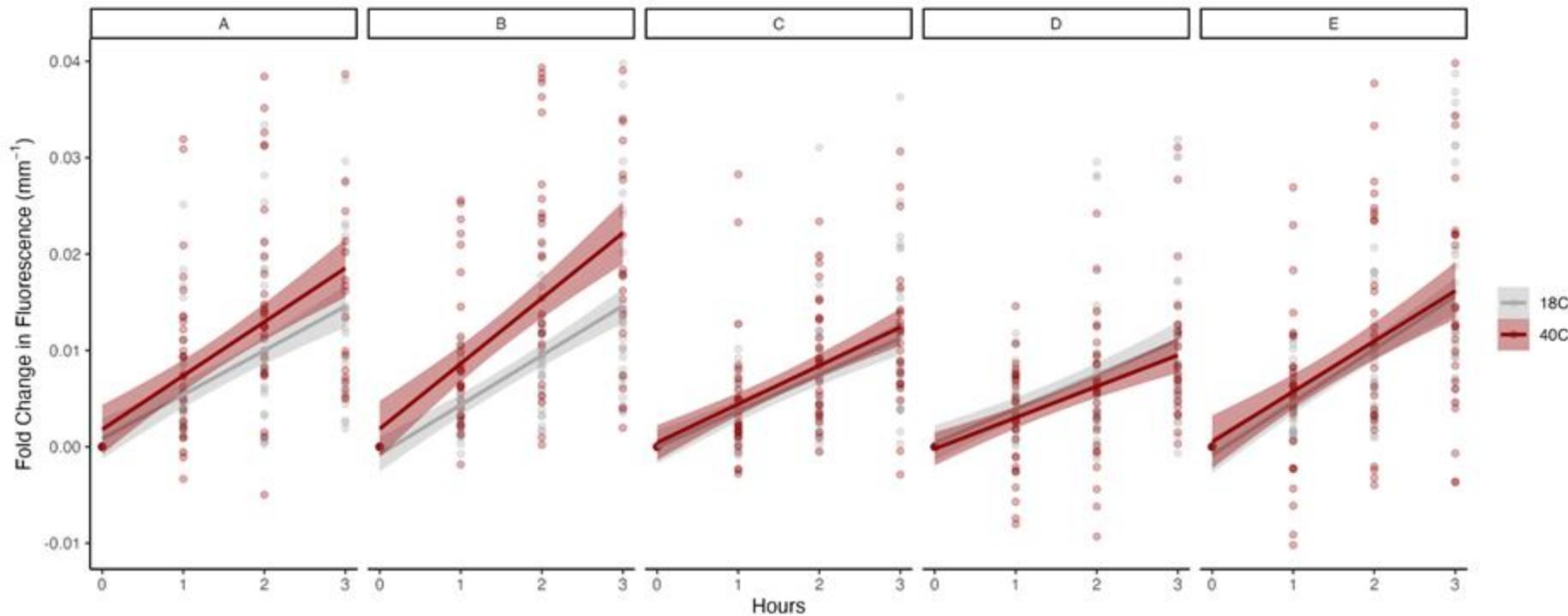
3 - Genetic background drives metabolic response



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

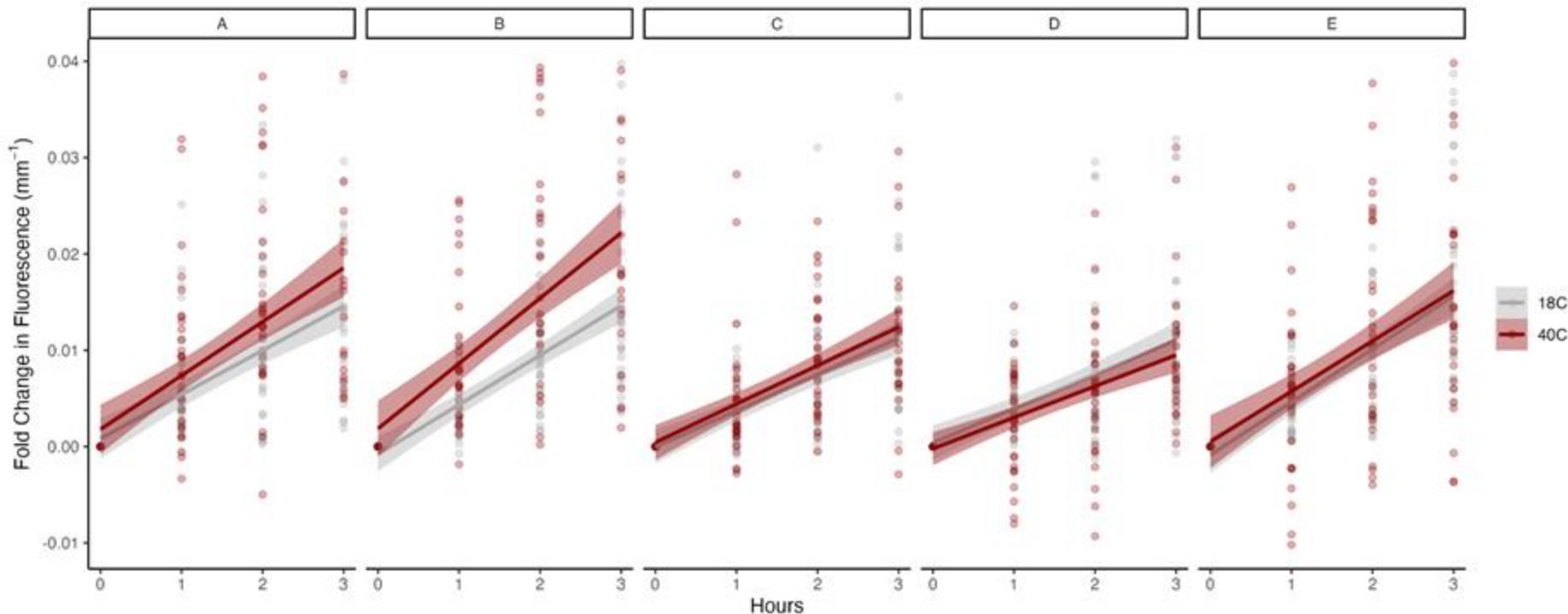
3 - Genetic background drives metabolic response



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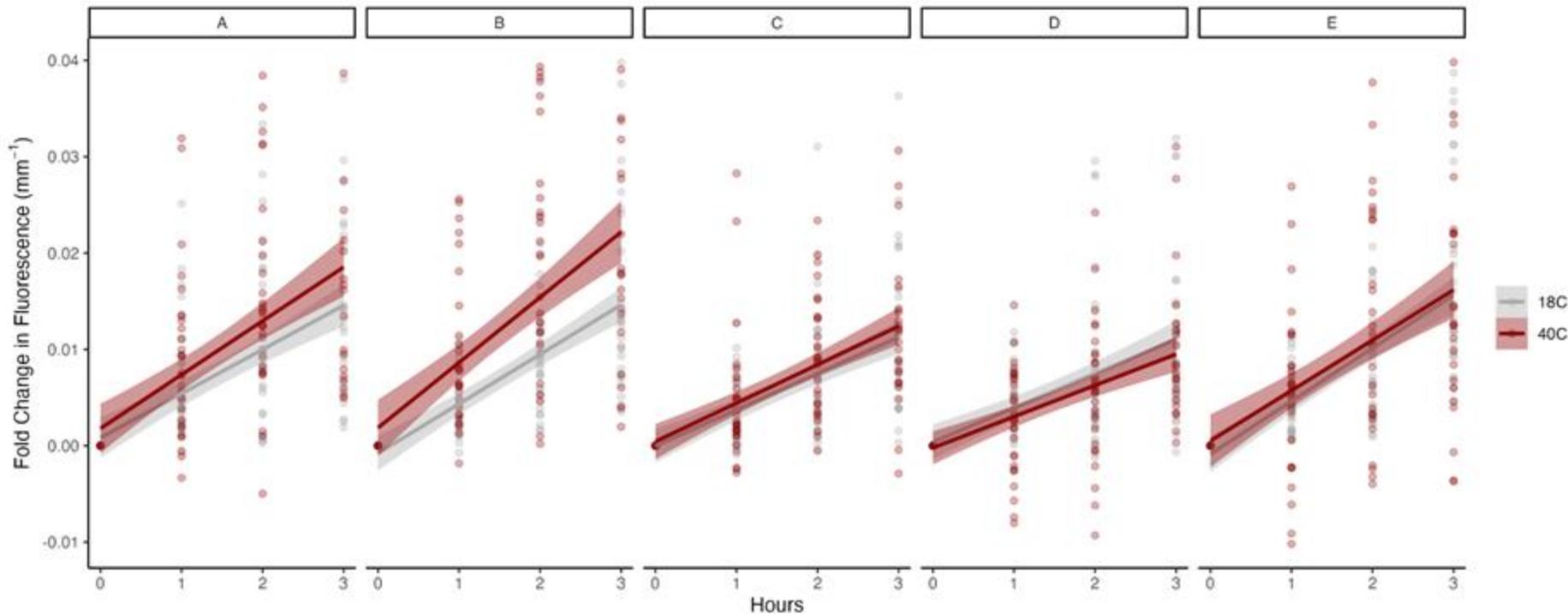
13–25 mm *C. gigas* seed
3 h exposure to 18°C followed by 3 h exposure to 40°C

3 - Genetic background drives metabolic response



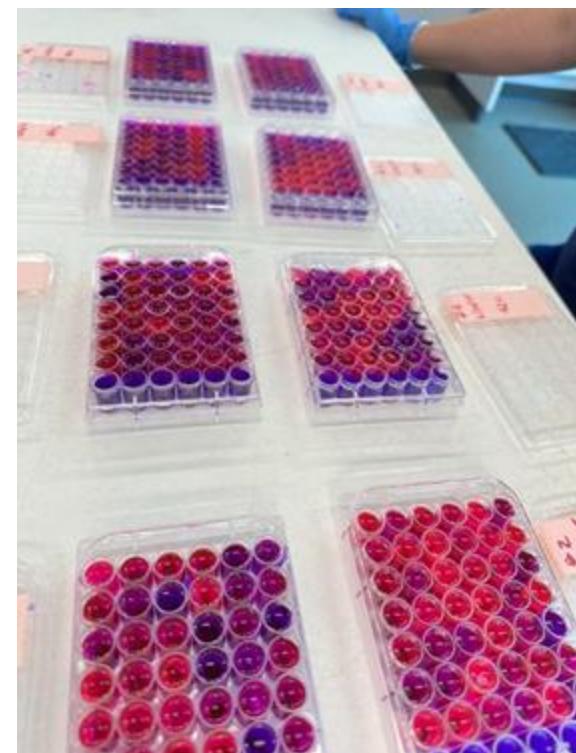
There is significant ***family-level variation*** in metabolic activity and metabolic response to stress.

3 - Genetic background drives metabolic response



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

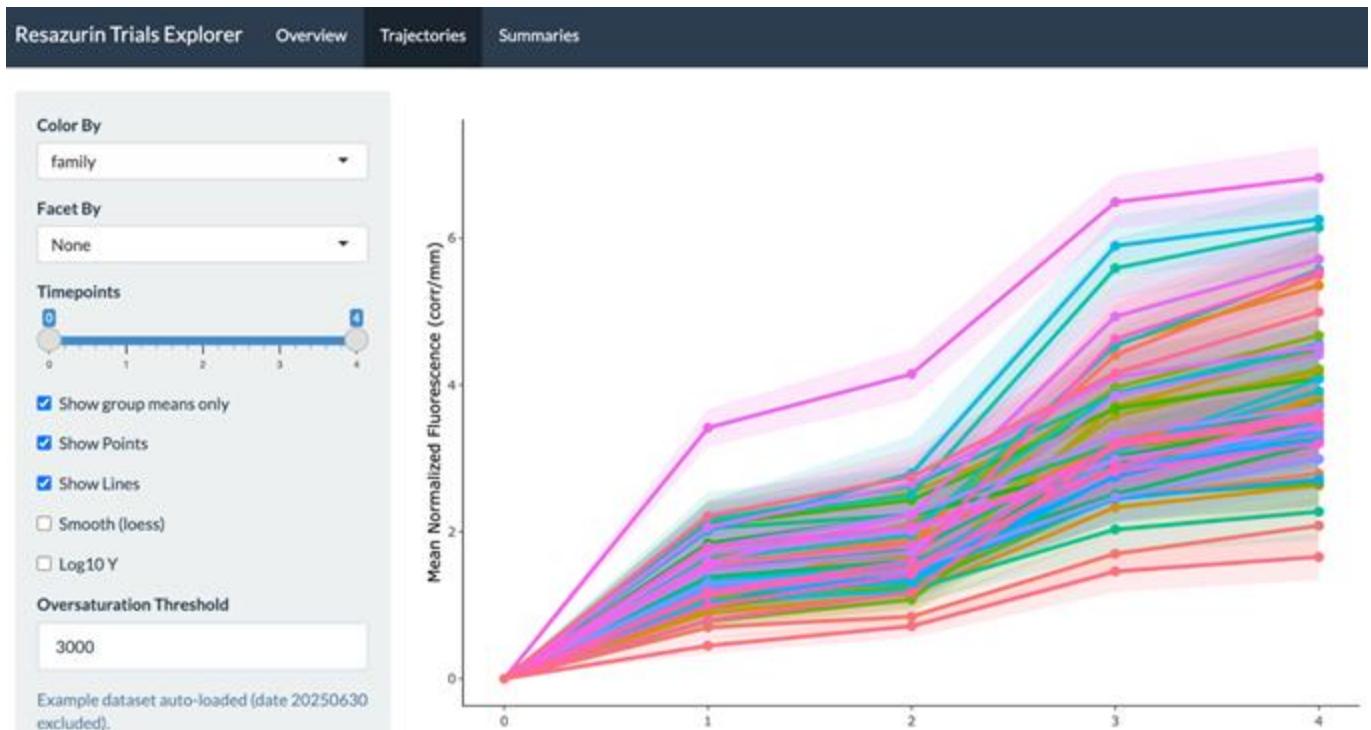
Case Study: Metabolic activity as a predictor of performance in *C. virginica*



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*

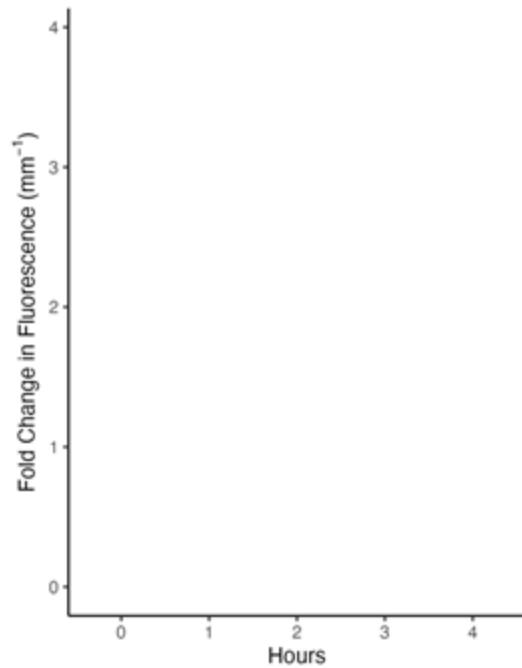


Explore the data here!
oyster.pink

Selective breeding drives metabolic response

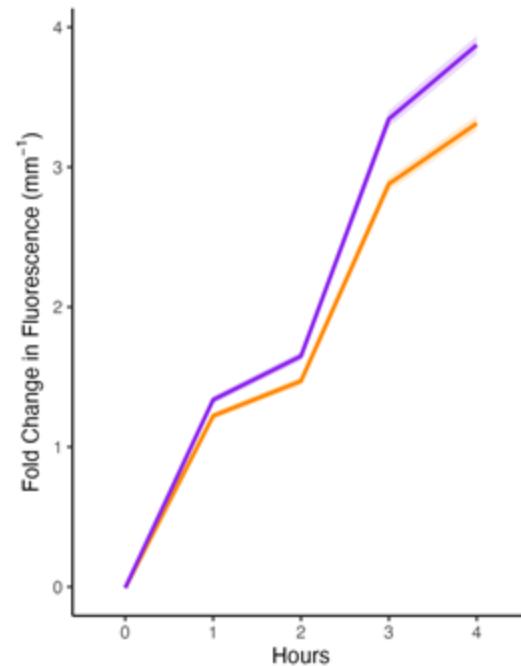
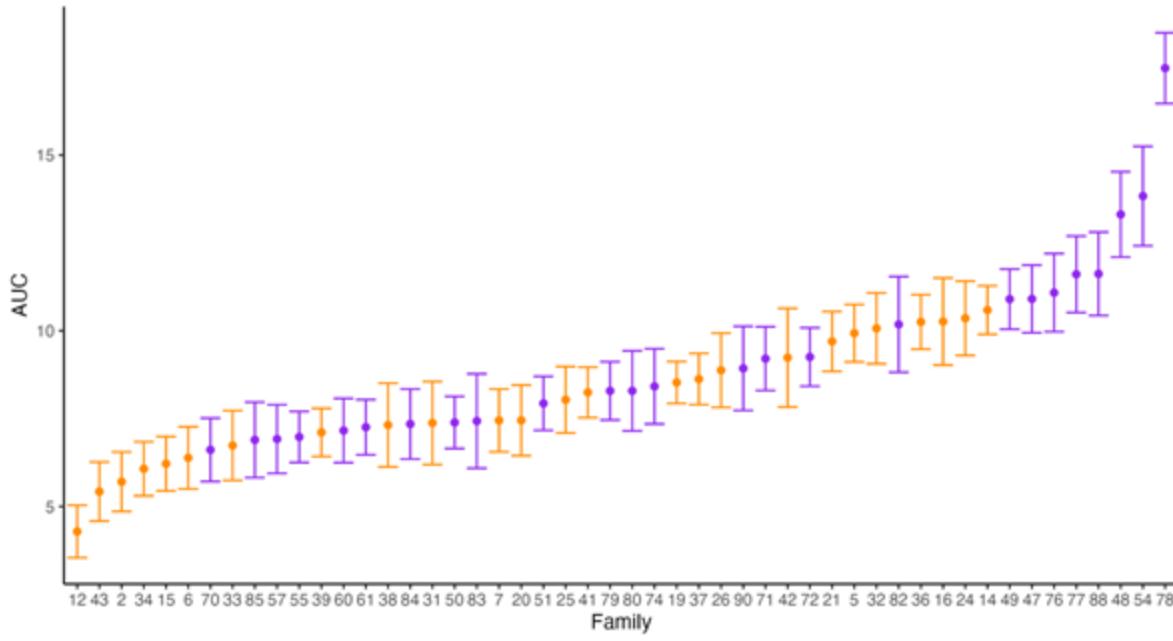


High Salinity = Selectively bred for performance in high/moderate salinity (18-23 psu) environments



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

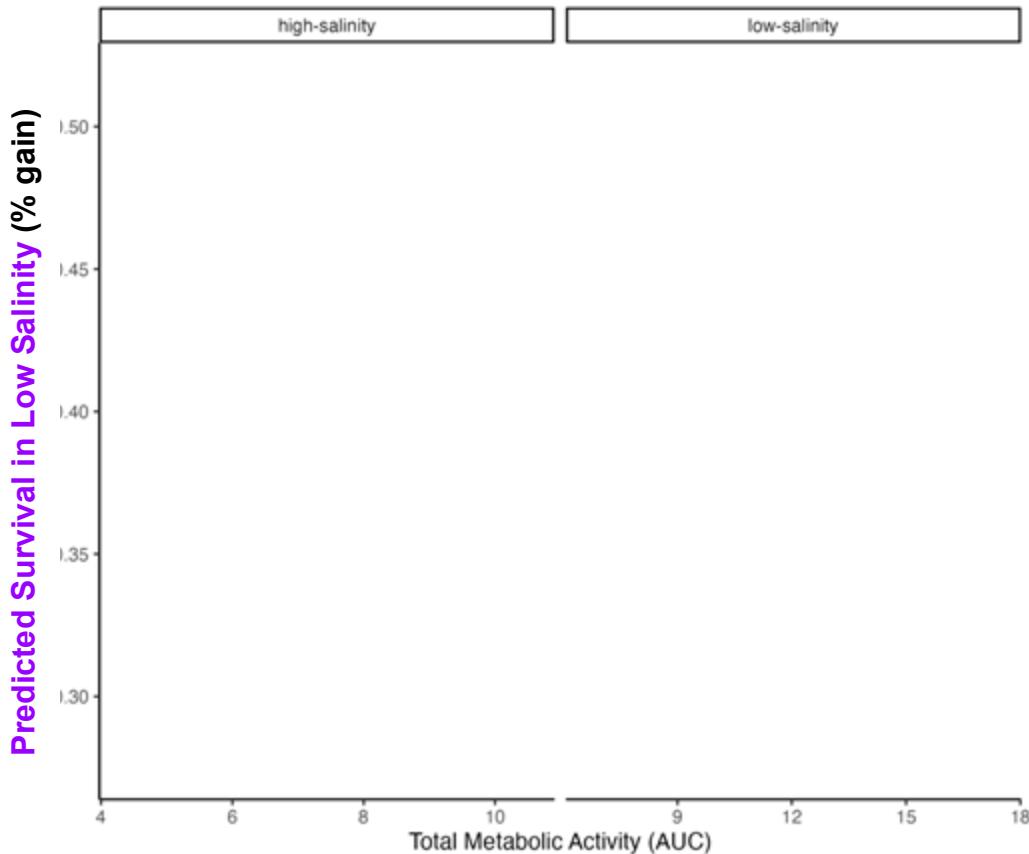
~

Metabolism

Predicted Growth

- High salinity
- Low salinity

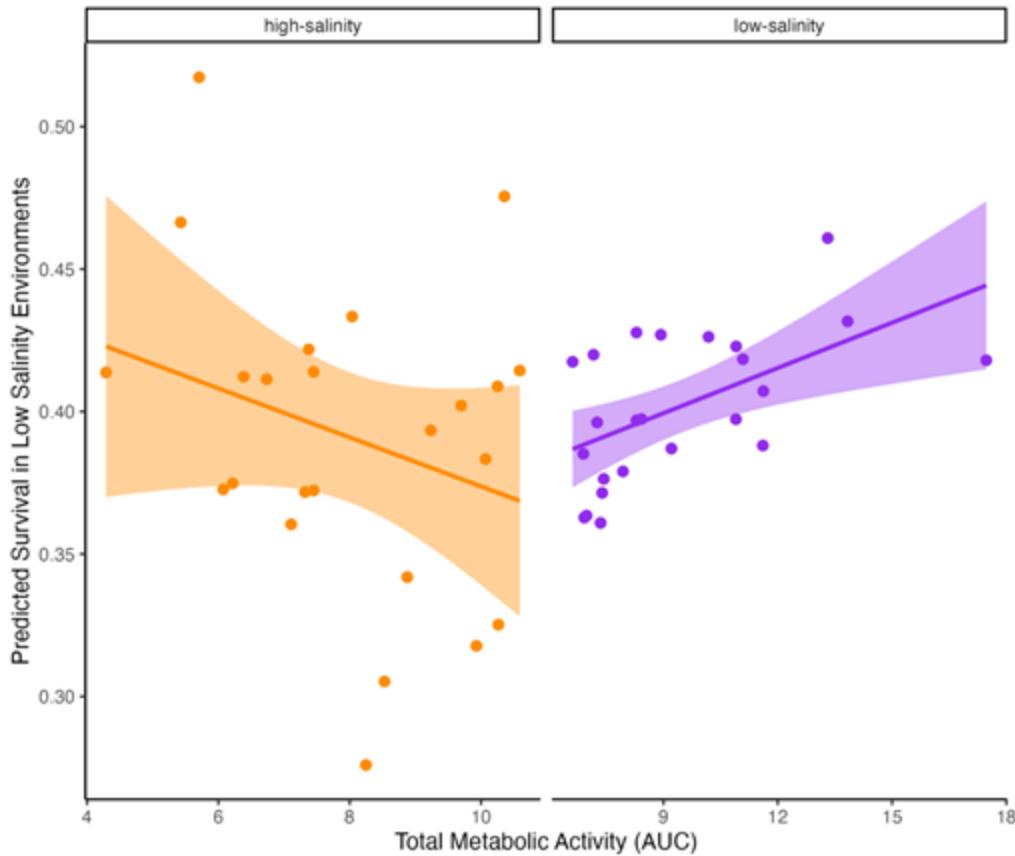
Metabolism is correlated to predicted performance



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

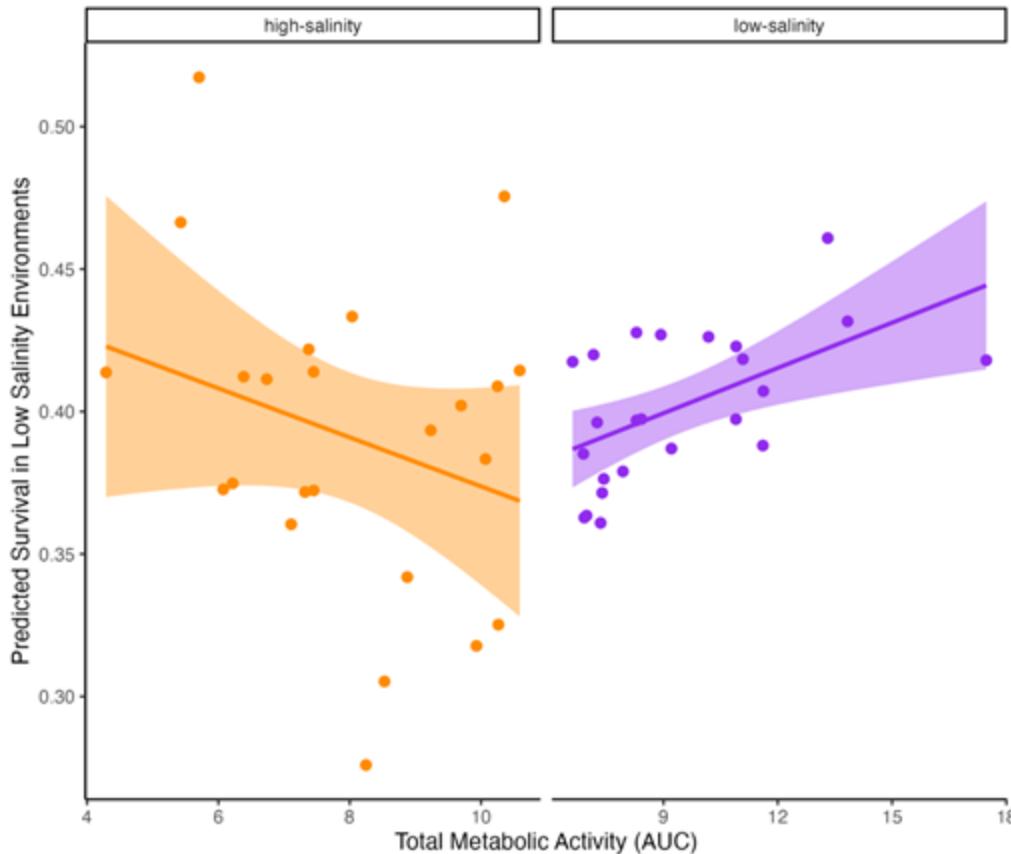
Low Salinity = Selectively bred for performance in low salinity (6-15 psu) environments

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



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

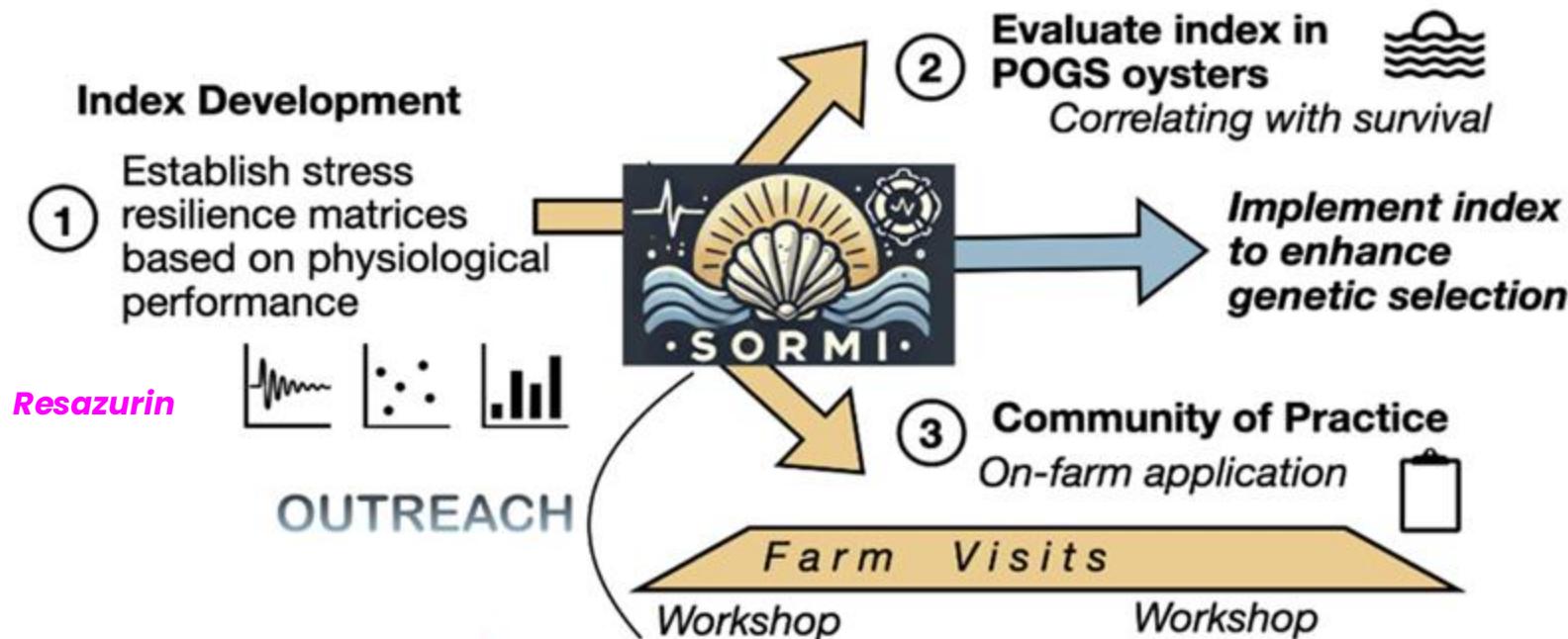
Metabolic activity correlates with predicted performance

Ongoing Efforts

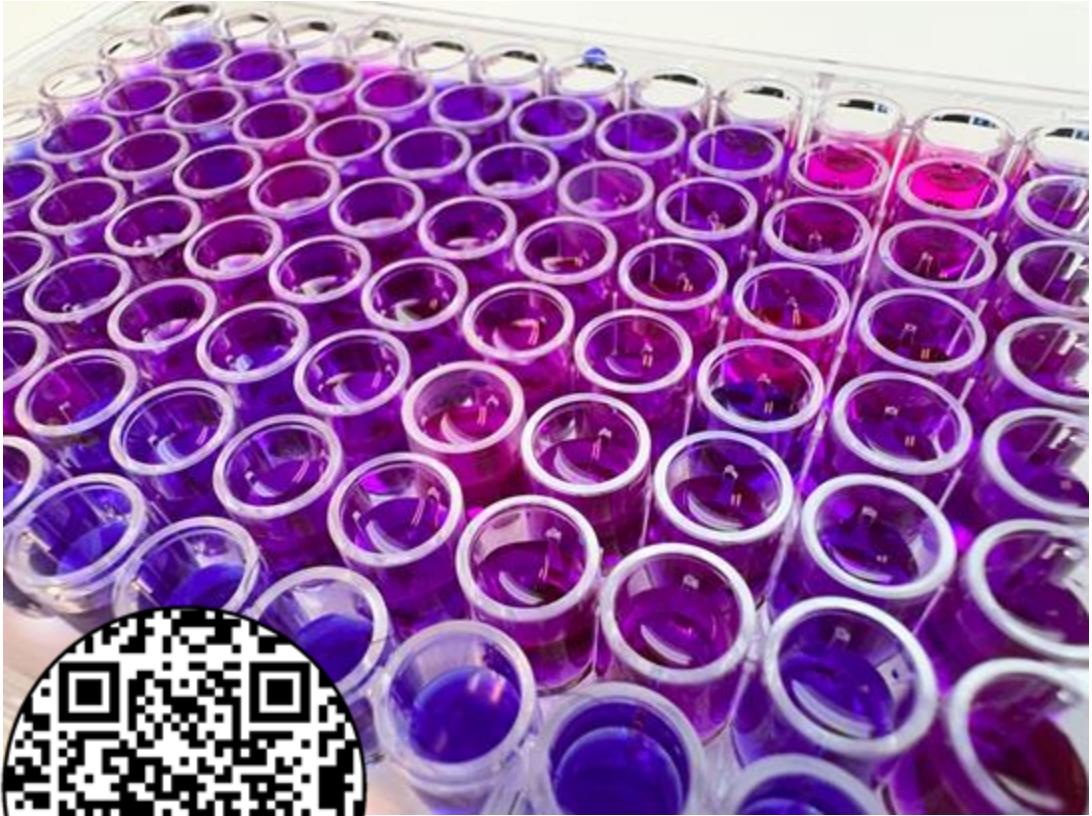
- Family screenings
- Performance measurements
- Expanding protocol applications
- Developing field-friendly tools

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

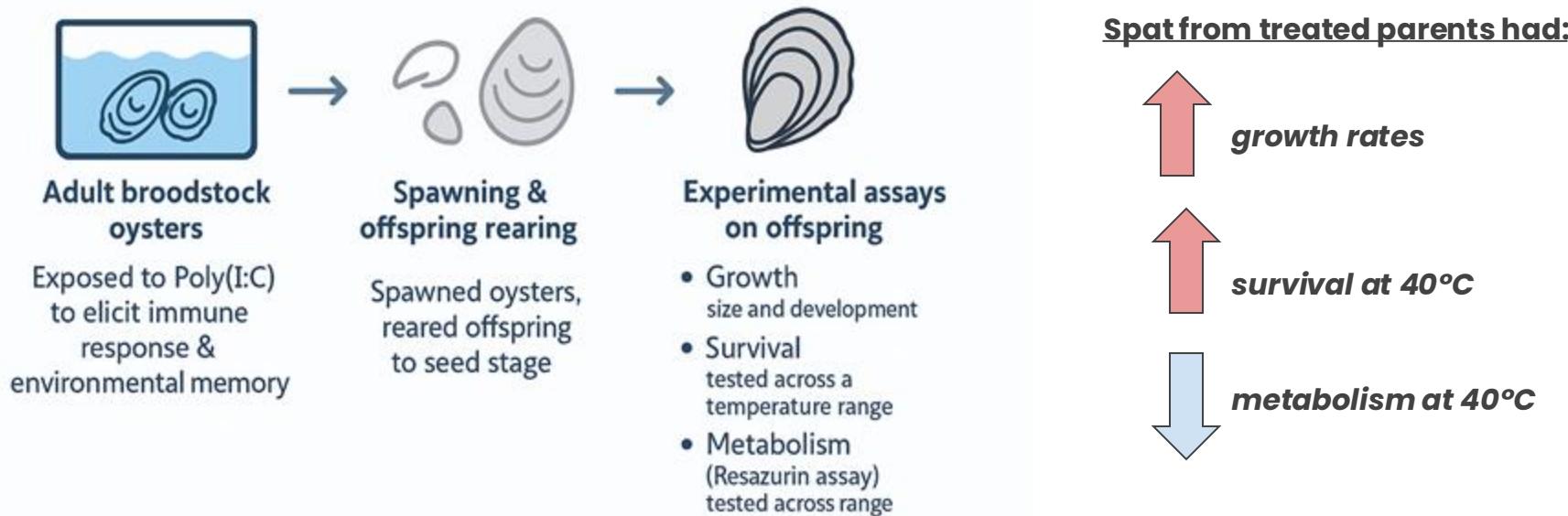
Steven Roberts: sr320@uw.edu

robertslab.info

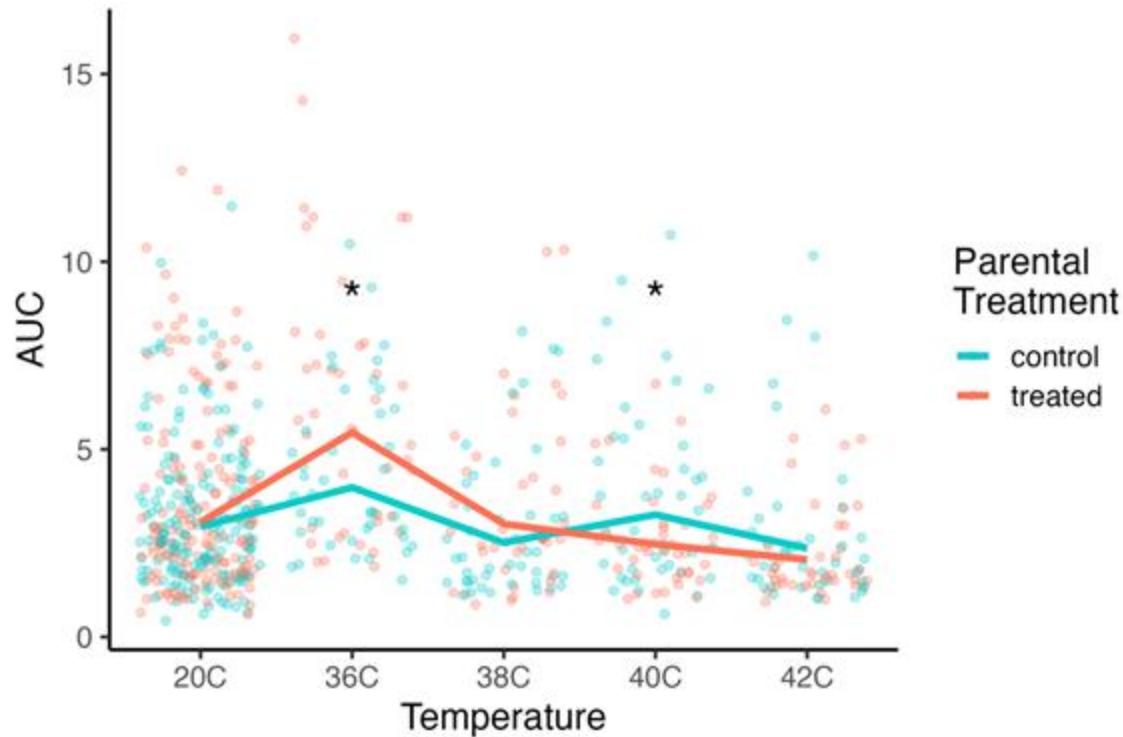


4 – Parental history affects metabolic stress response

EXPERIMENTAL OVERVIEW



4 – Parental history affects metabolic stress response

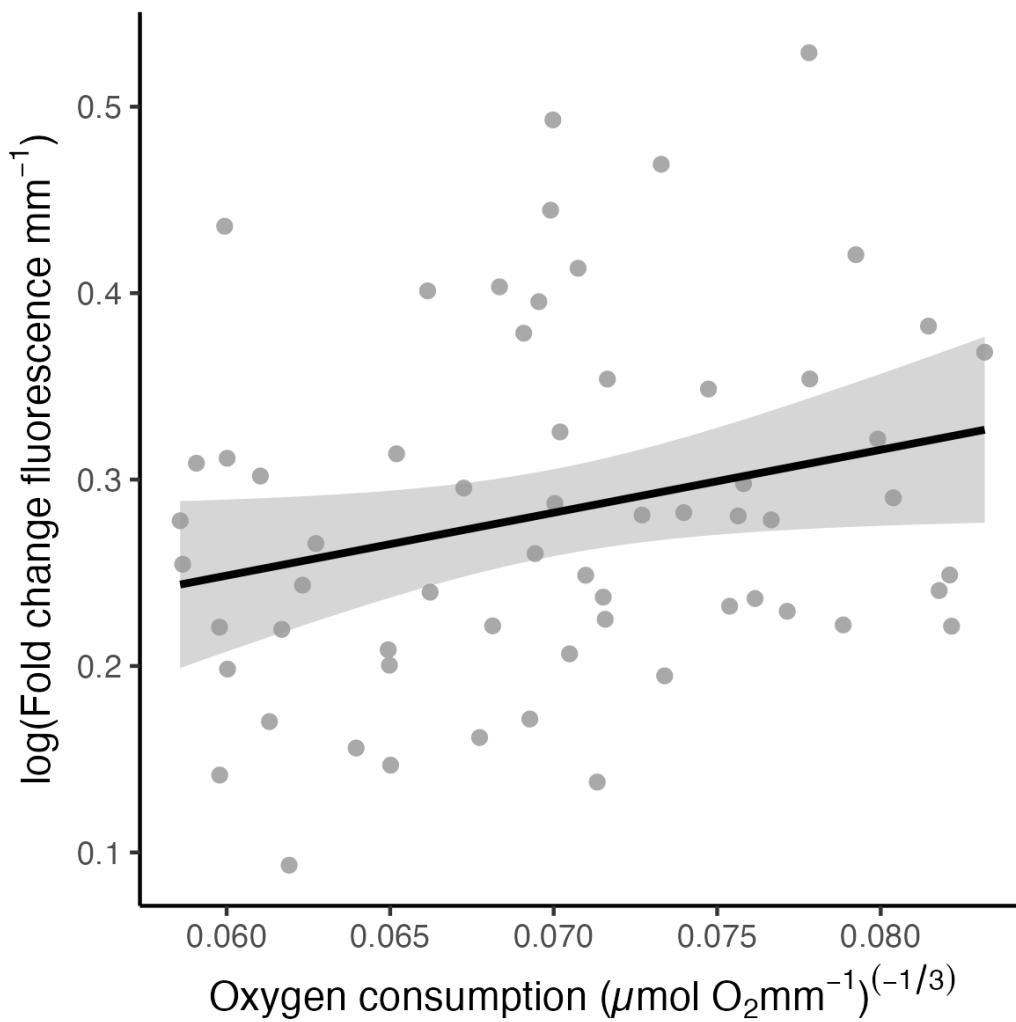


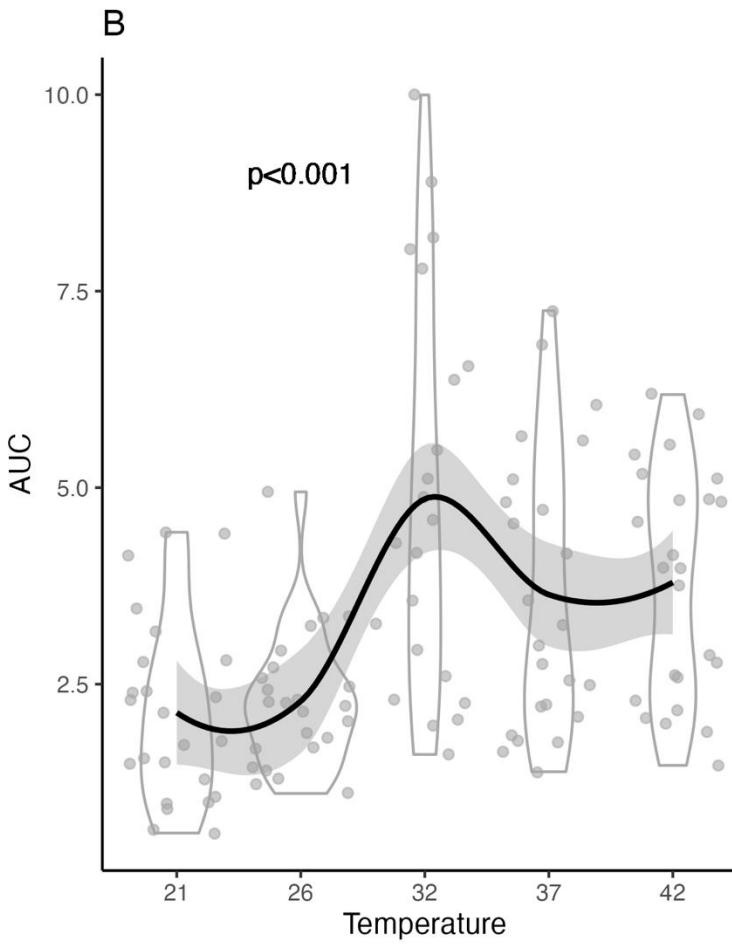
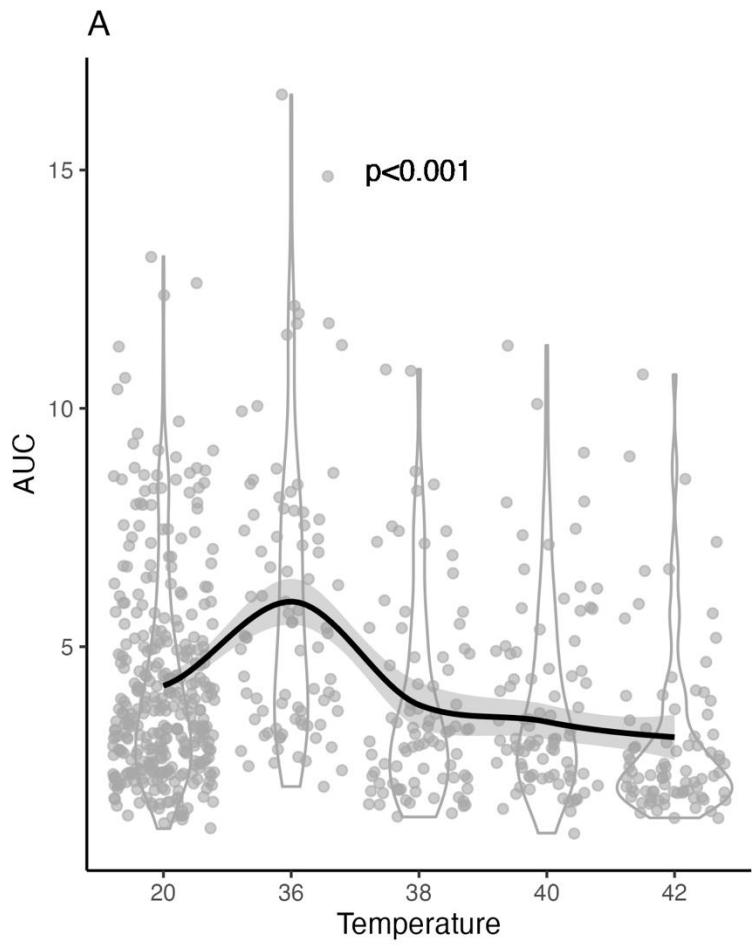
Offspring from parents that were immune challenged had **lower metabolism** at 40°C

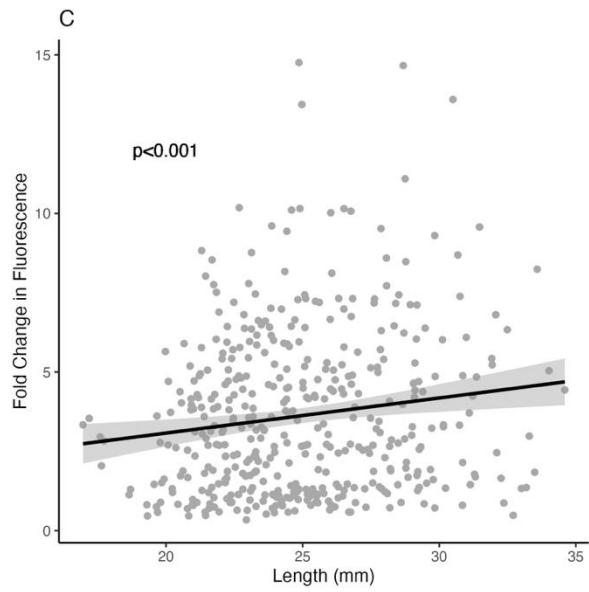
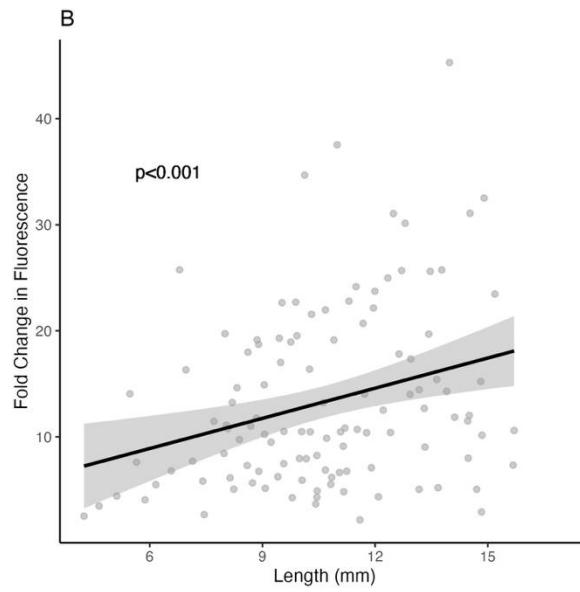
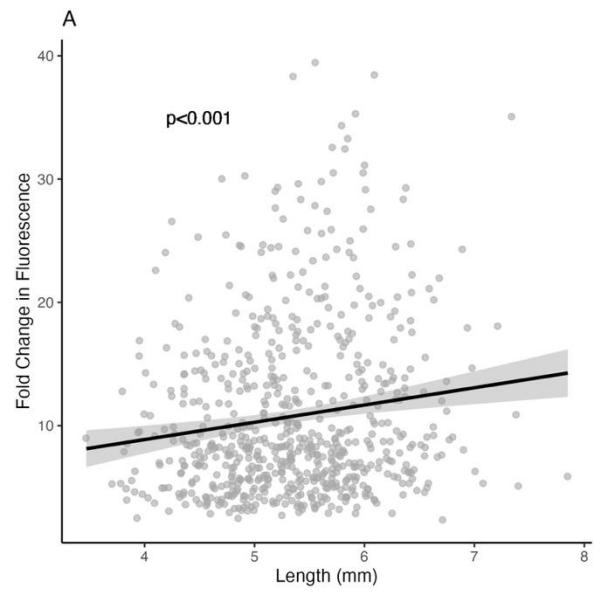
- Energy savings supporting survival

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

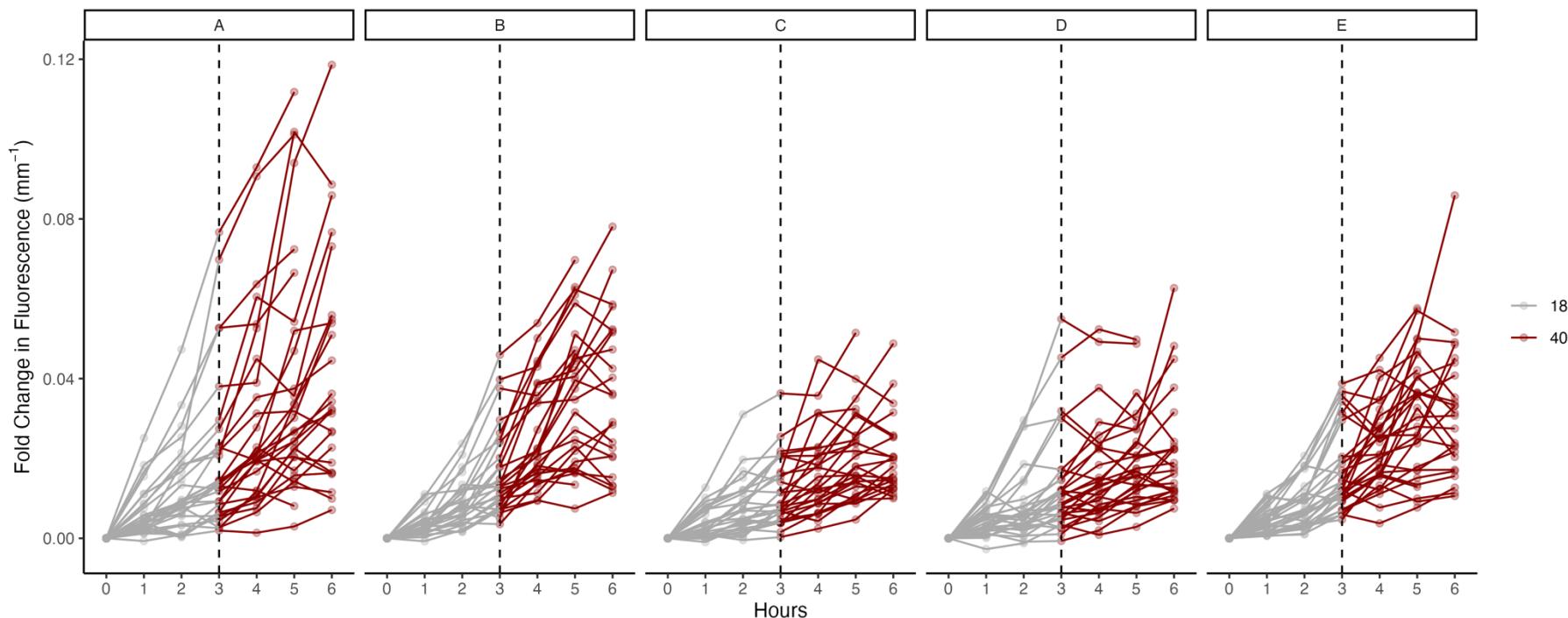
- Metabolic capacity for growth







A



Modeled Metabolic Rate

