



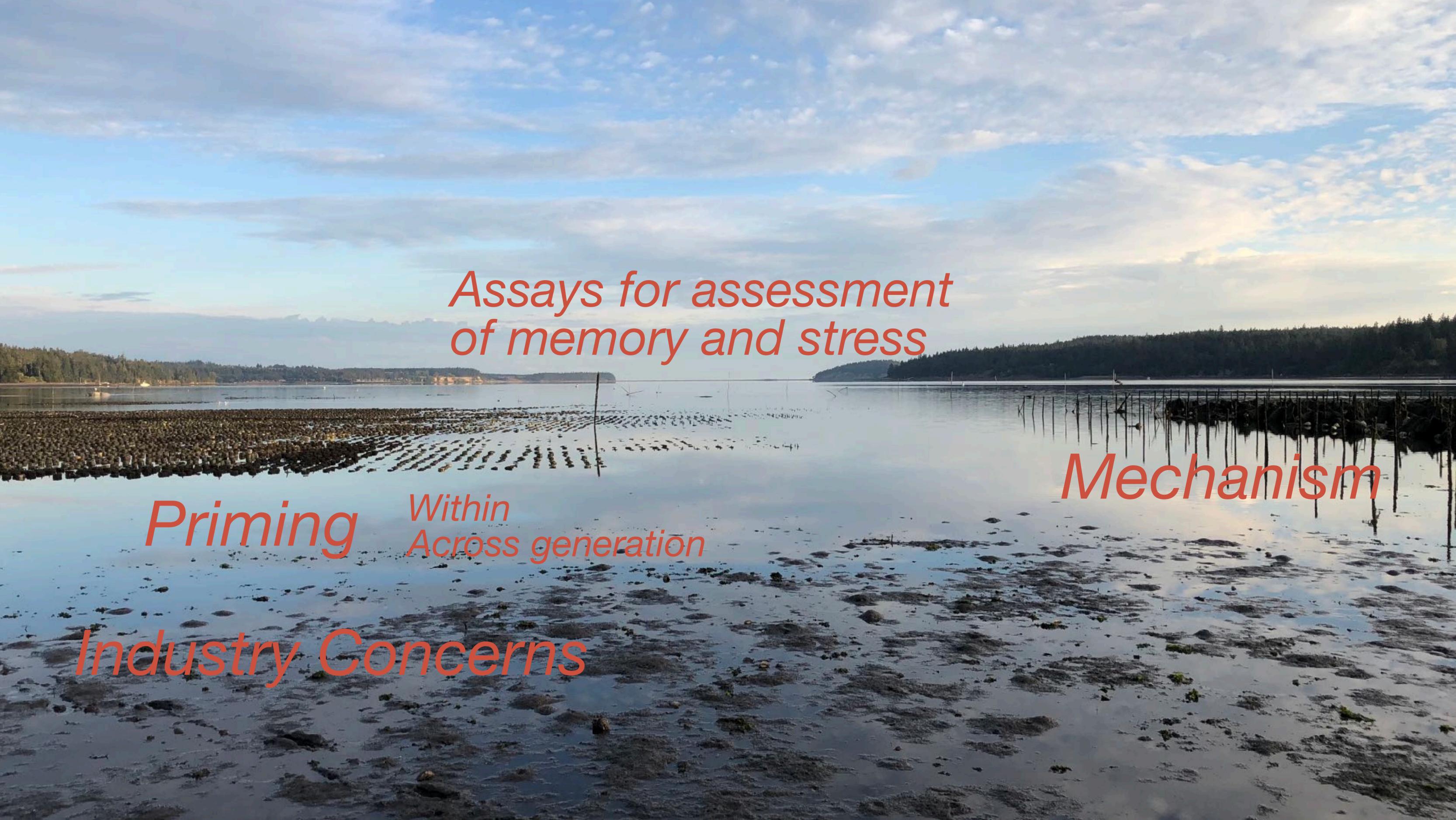
# Shellfish Environmental Memory

*Beyond Epigenetics*

Steven Roberts

[github.com/sr320/talk-EpiAqua-2026](https://github.com/sr320/talk-EpiAqua-2026)





*Assays for assessment  
of memory and stress*

*Priming*

*Within  
Across generation*

*Mechanism*

*Industry Concerns*



*Industry Concerns*

# Grower Survey: Perceptions of threats and proposed adaptation strategies

- 20-30 min interview:
  - Ranking threats
  - Input on leveraging environmental memory as an adaptation strategy

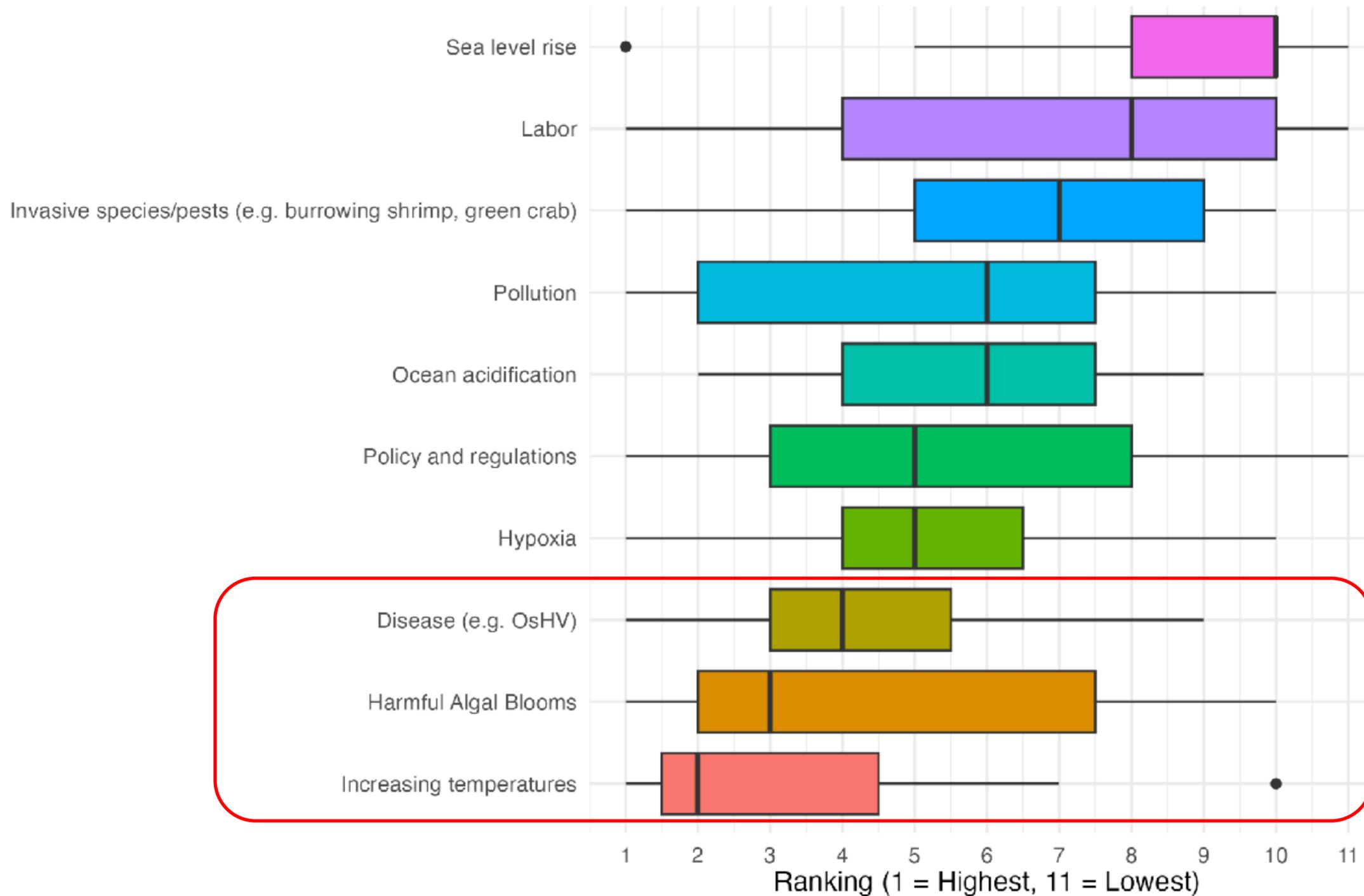


AQUATIC AND FISHERY SCIENCES  
UNIVERSITY *of* WASHINGTON

Connor Lewis-Smith



# Grower Survey: Perceptions of threats and proposed adaptation strategies



# Environmental Priming

Would you consider implementing environmental priming in your hatchery?

Response	Percentage
Yes, immediately	7%
Yes, but after other growers try it	7%
Yes, but after reviewing literature supporting the practice	64%
No	21%

Would you pay more for primed seed?

Response	Percentage
Yes, immediately	0%
Yes, starting with a test plot	17%
Yes, but after other growers try it	7%
Yes, but after reviewing data supporting the practice	53%
No	23%

# Environmental Priming

78% of hatcheries would consider adopting environmental priming practices, most only after reviewing supportive literature

70% of respondents would be willing to pay more for primed seed, most only after a test plot or supportive literature

Would you consider implementing environmental priming in your hatchery?

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Yes, immediately	7%
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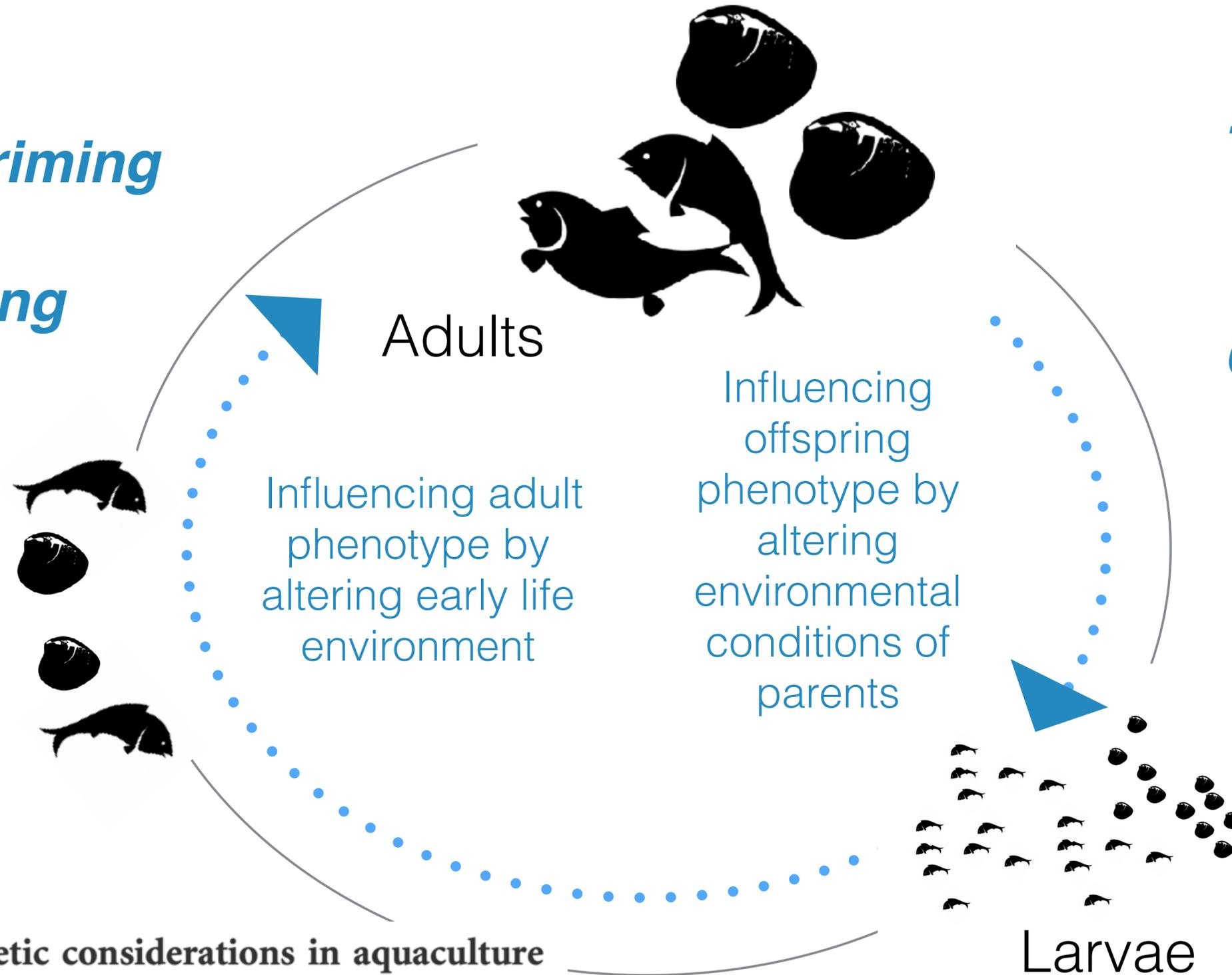


*Priming*

*Industry Concerns*

**Early-life Priming**

**Hardening**



**Transgenerational Plasticity**

**Carry-over effects**

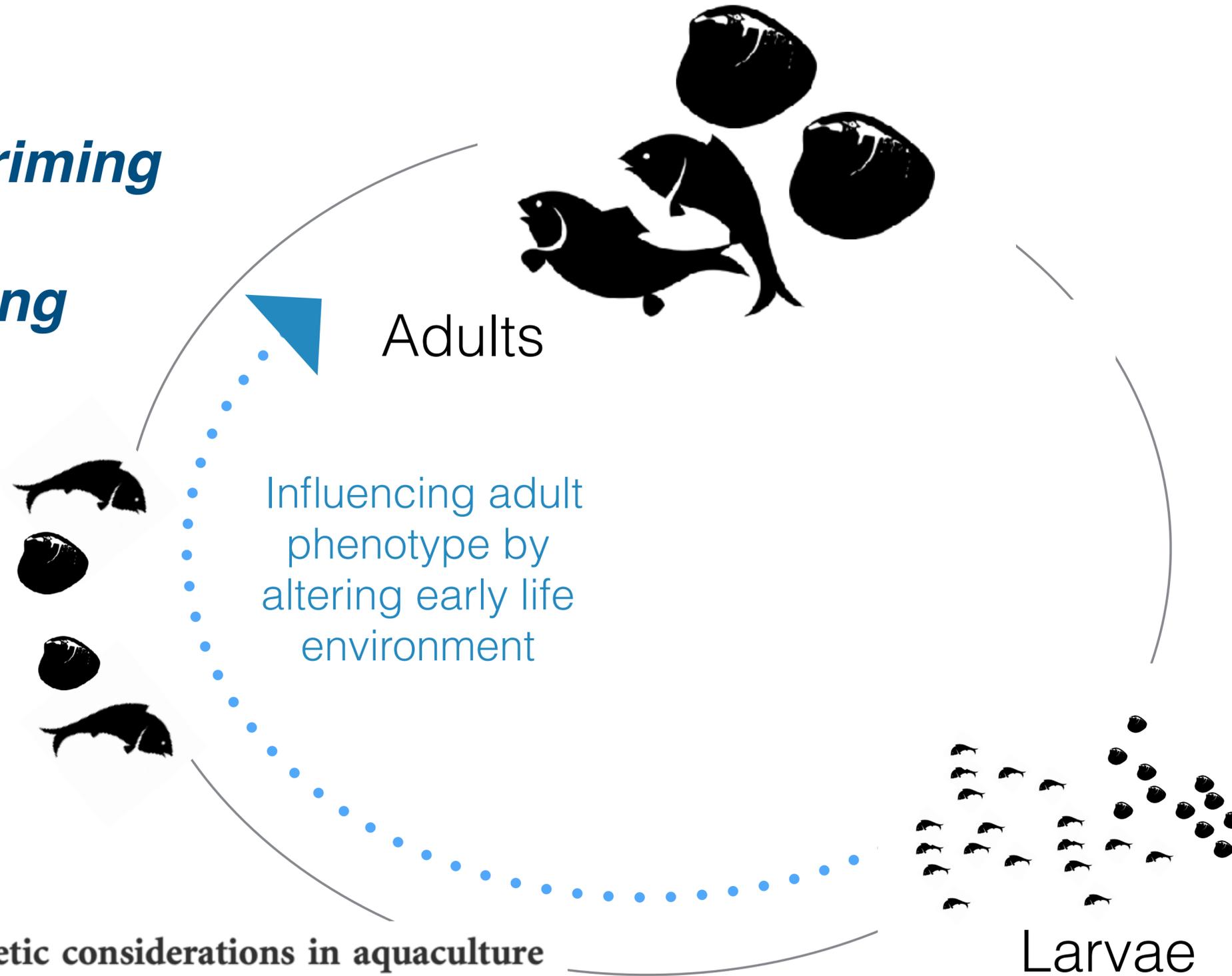
**Epigenetic considerations in aquaculture**

Mackenzie R. Gavery and Steven B. Roberts

School of Aquatic & Fishery Sciences, University of Washington, Seattle, WA, USA

# Early-life Priming

## Hardening



### Epigenetic considerations in aquaculture

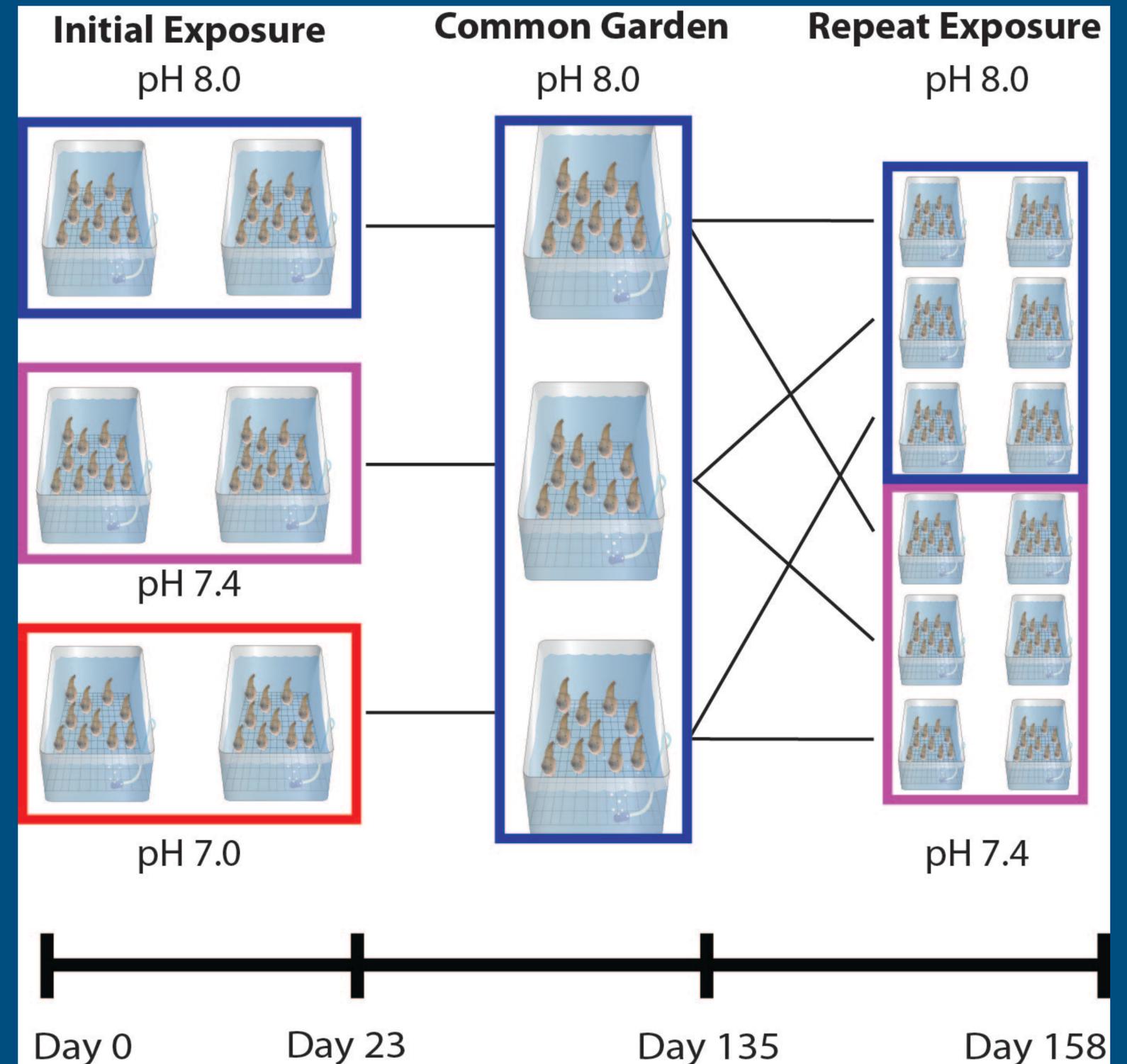
Mackenzie R. Gavery and Steven B. Roberts

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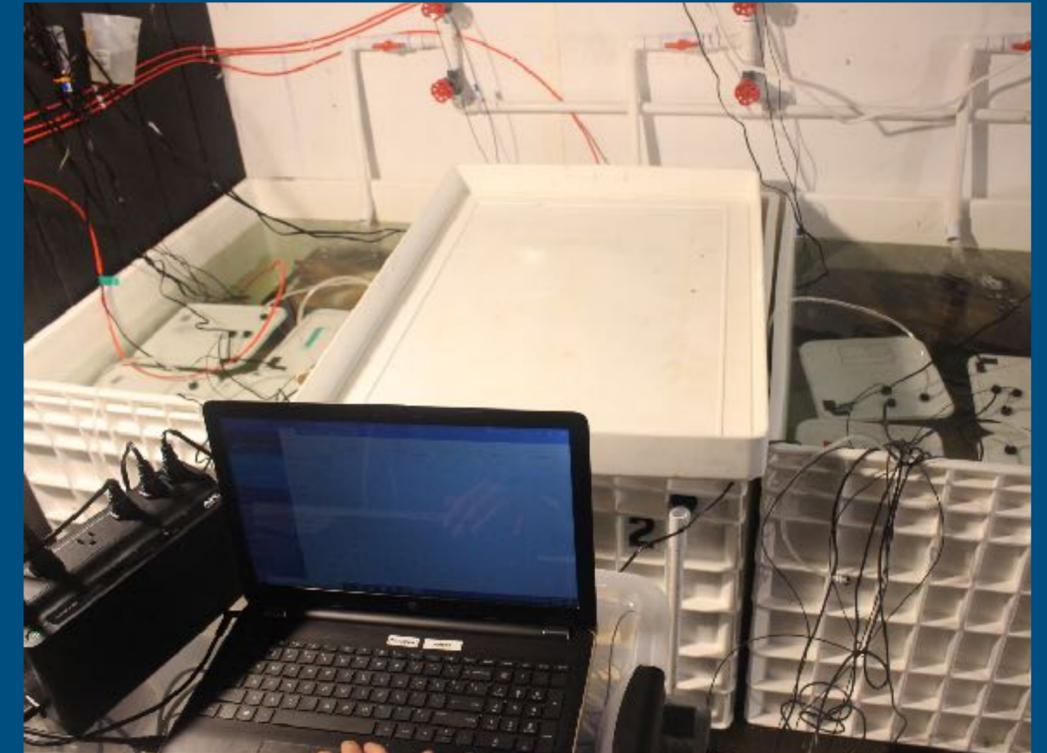
# GEODUCKS CLAMS



- ▶ Does conditioning to low pH confer tolerance within a generation?

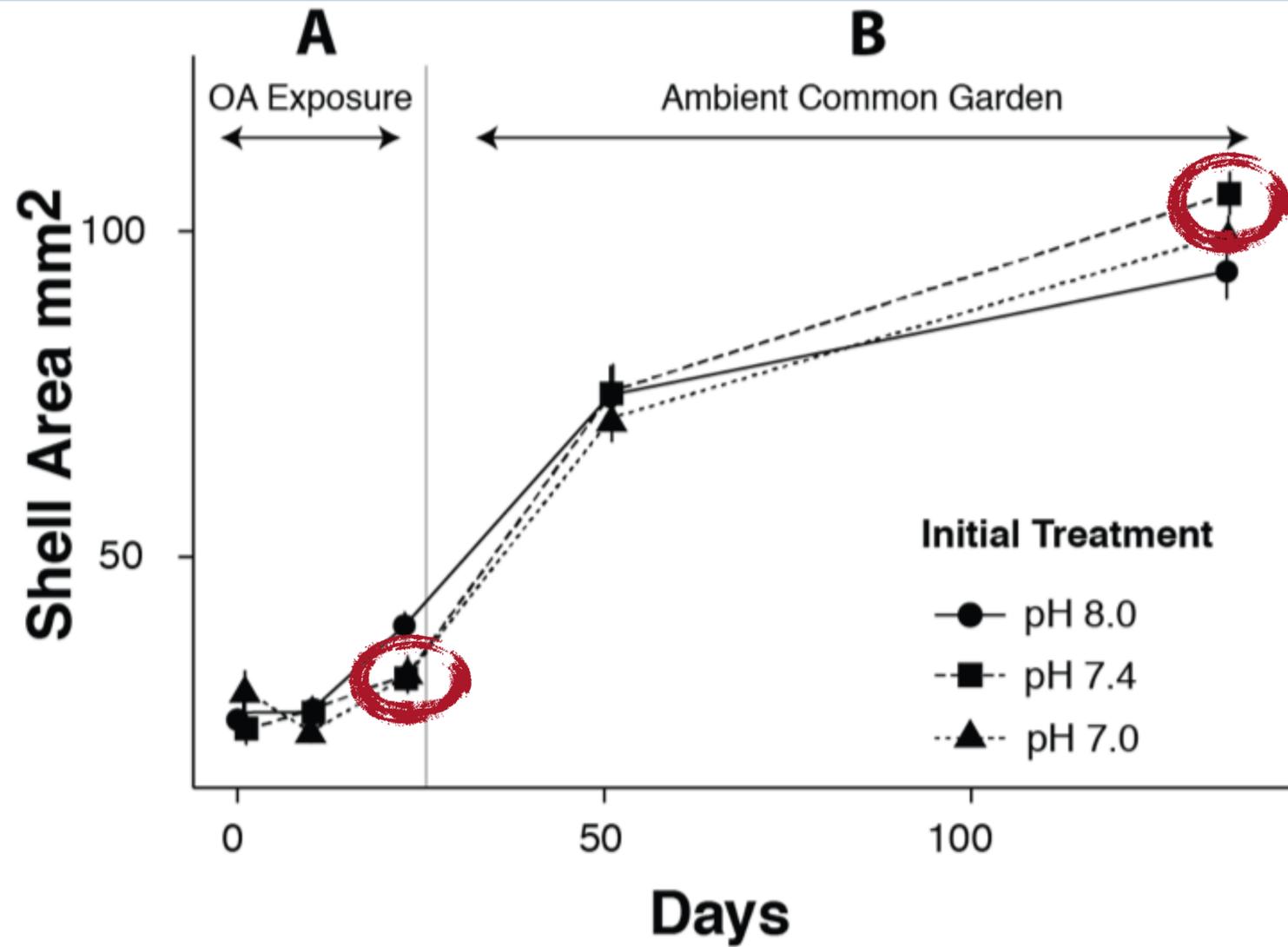


# GEODUCKS CLAMS



# GEODUCKS CLAMS

- ▶ Does conditioning to low pH confer tolerance within a generation?



# Heat-Priming Pacific Oysters: Field Performance vs. Lab Stress Tolerance

Results from an 18-month Sea Grant experiment at Westcott Bay Shellfish

Project Leads: Emily Carrington & Steven Roberts

Research Team: Grace Leuchtenberger & Ariana Huffmyer



# Pacific Oyster



- Priming

- Temperature

- Performance

- Survival
- Growth
- Metabolism\*

## 1. Daily Mild Heat Priming (Chronic)

 25°C  4 hours

MON TUE WED THU FRI SAT SUN

		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

PRIMING  
EFFECT  
ANALYSIS

→ **8 WEEKS** →  
TOTAL DURATION

## 2. Weekly Strong Heat Priming (Acute/Intermittent)

 35°C  30–60 minutes

MON TUE WED THU FRI SAT SUN

		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

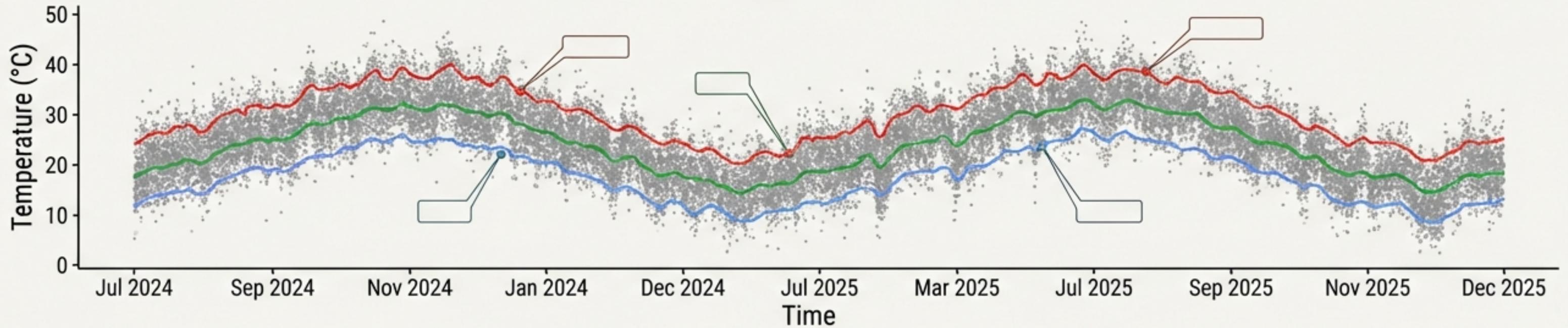
PRIMING  
EFFECT  
ANALYSIS

→ **8 WEEKS** →  
TOTAL DURATION

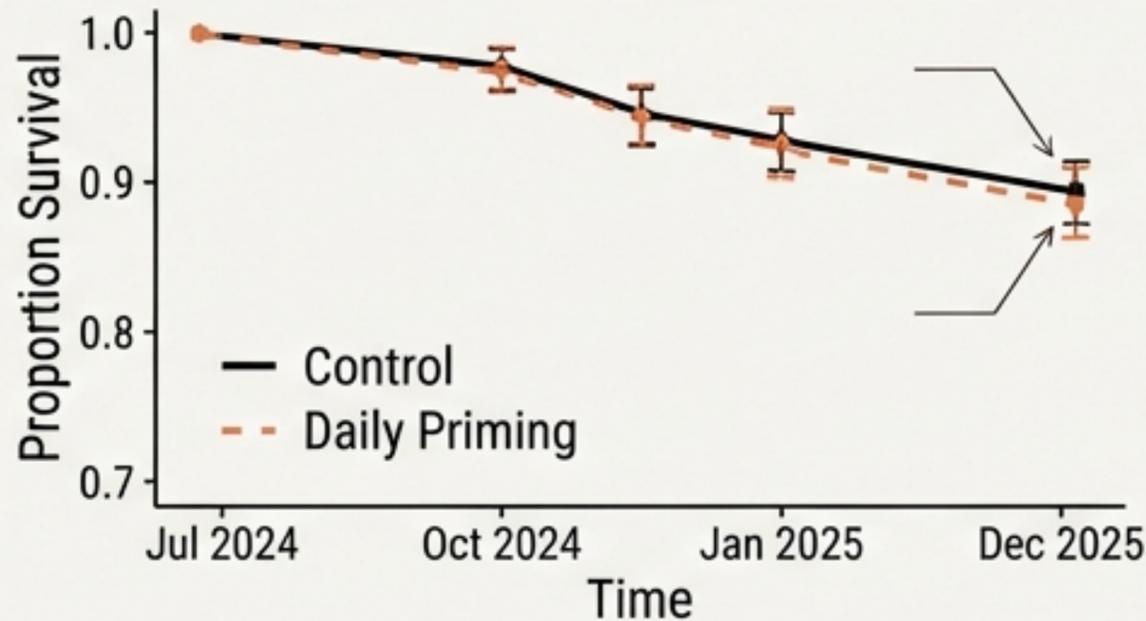


# Heat hardening did not improve survival under standard farm conditions.

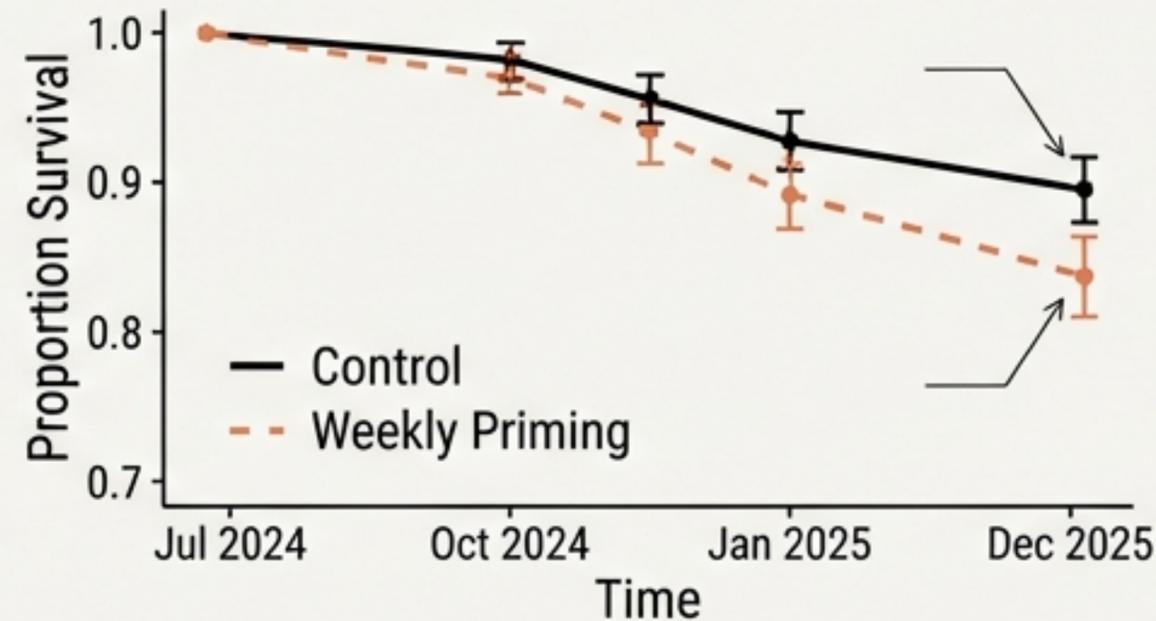
## Temperature Variability (Standard Farm Conditions)



## Daily Priming Survival



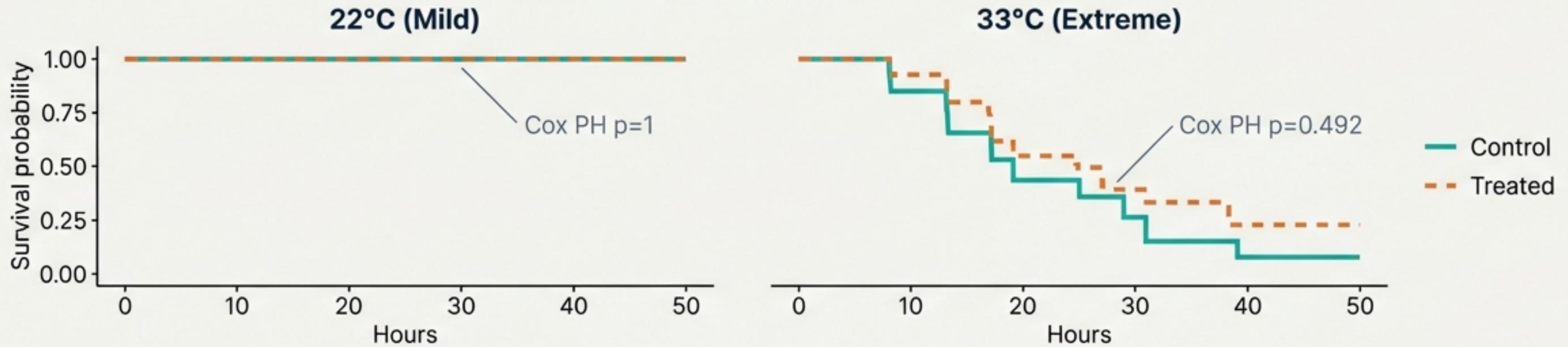
## Weekly Priming Survival



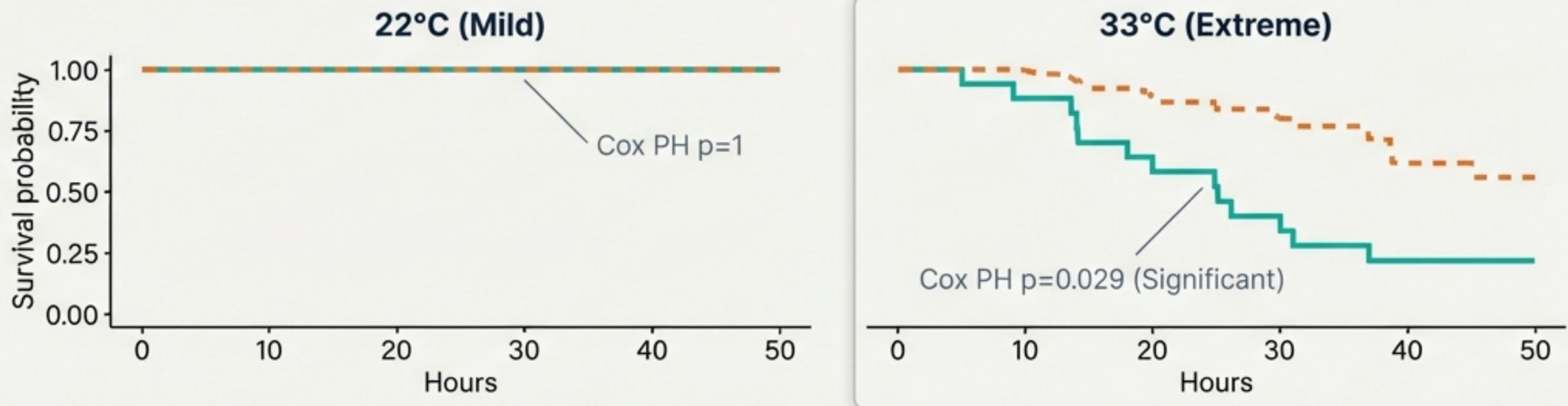
**RESULT:**  
Weekly heat-hardened oysters survived slightly worse (~5% lower) than controls. The energetic cost of priming may have been a disadvantage.

# Hardening worked—but only revealed itself under extreme acute stress.

## Daily Hardening



## Weekly Hardening

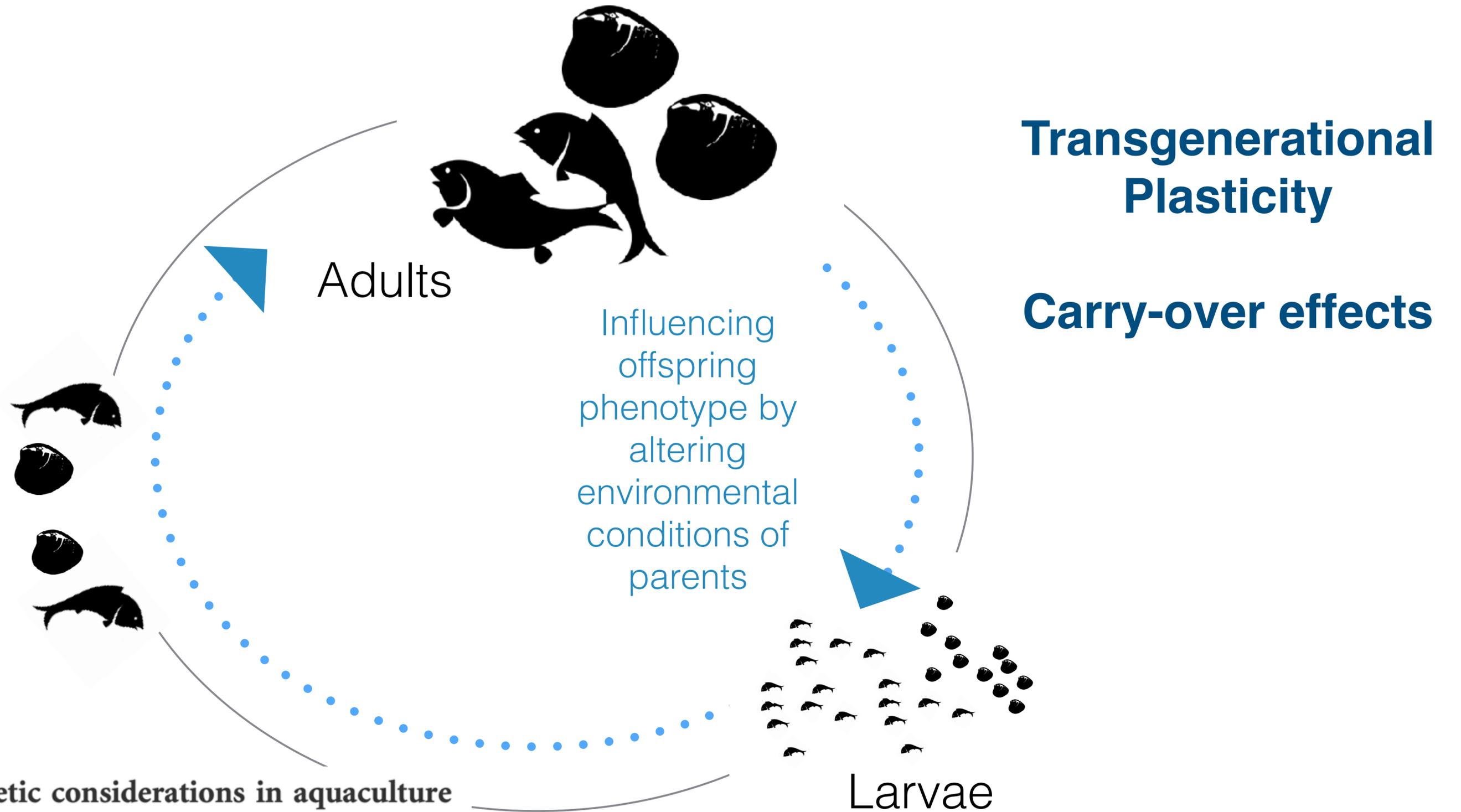


**SUCCESS:**  
Weekly primed oysters showed significantly higher survival during a simulated heat wave.



*Priming Across generation*

*Industry Concerns*

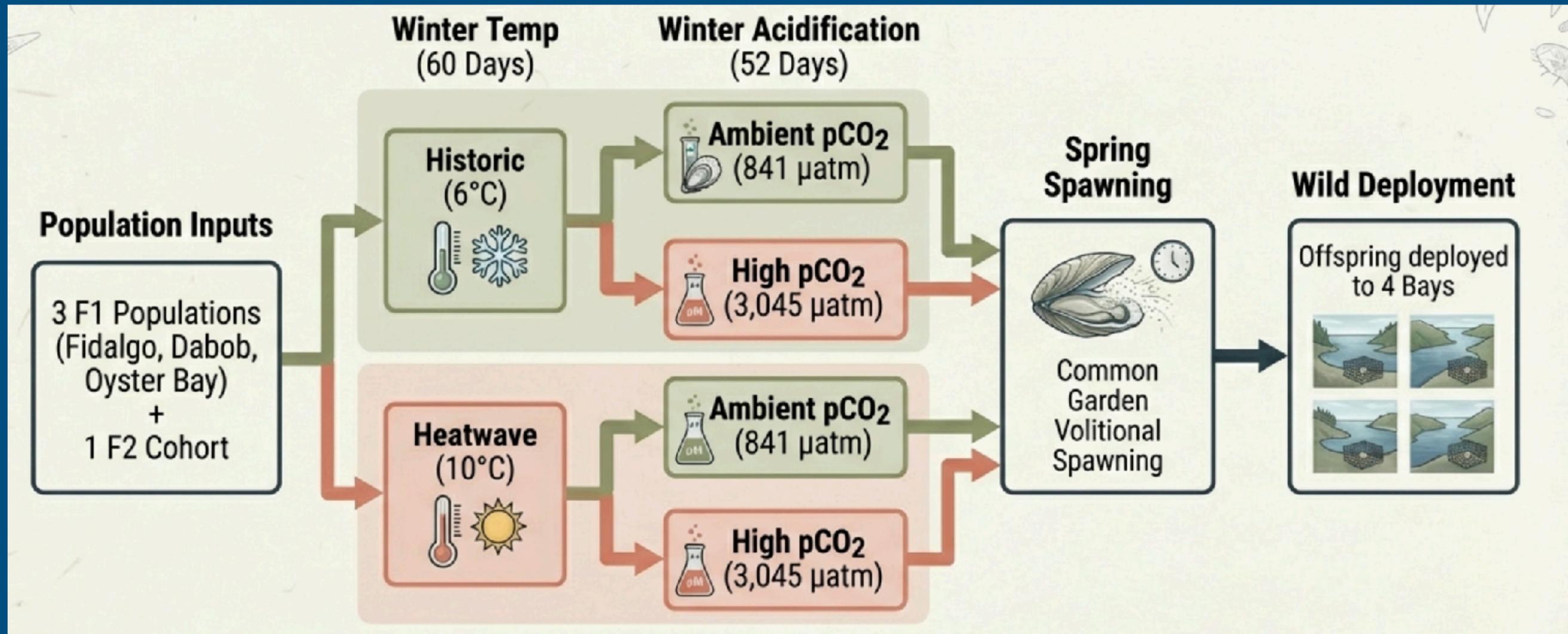


**Epigenetic considerations in aquaculture**

Mackenzie R. Gavery and Steven B. Roberts

School of Aquatic & Fishery Sciences, University of Washington, Seattle, WA, USA

# EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS



Carryover effects of temperature and pCO<sub>2</sub> across multiple Olympia oyster populations

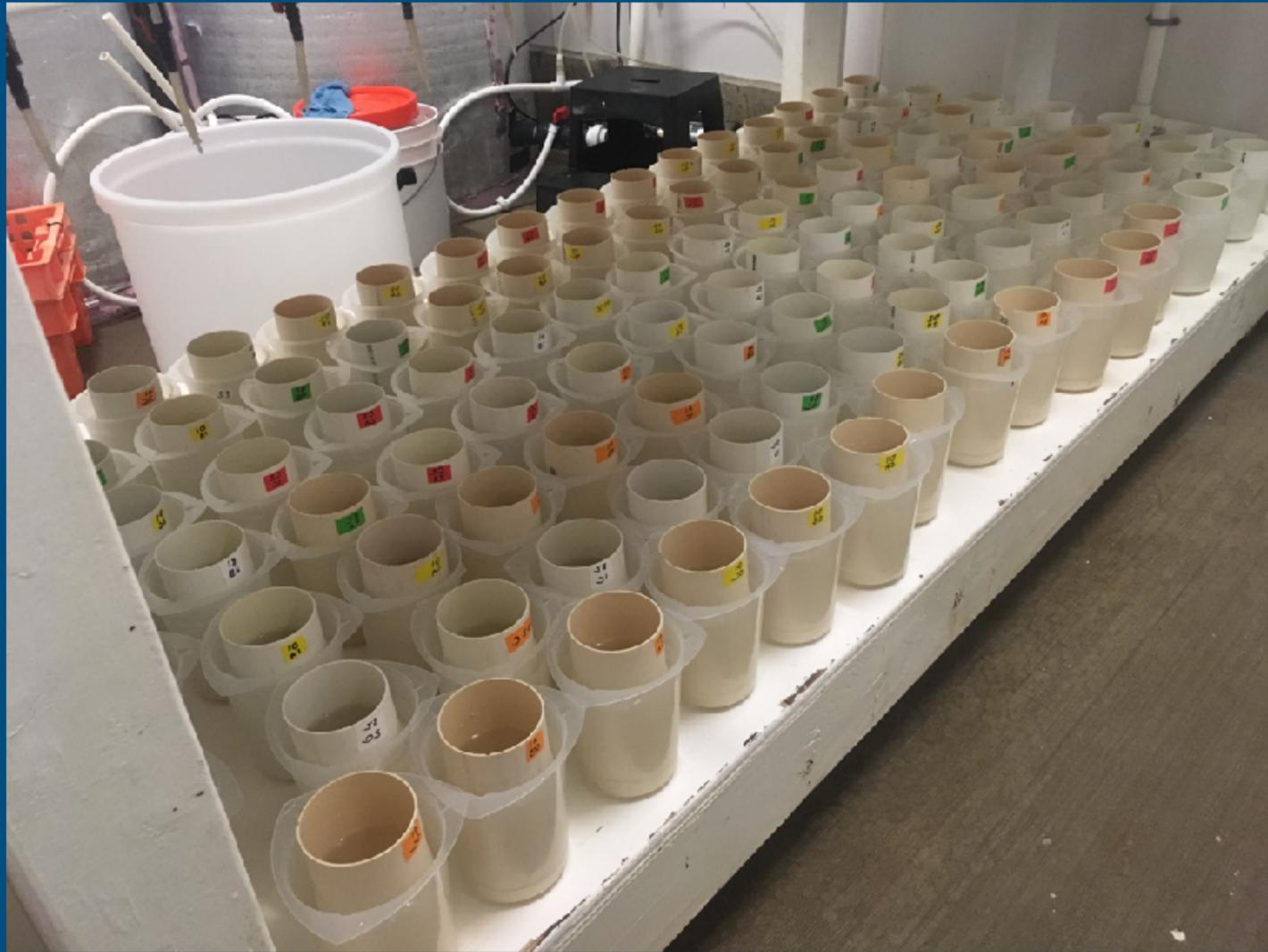
LAURA H. SPENCER,<sup>1</sup> YAAMINI R. VENKATARAMAN,<sup>1</sup> RYAN CRIM,<sup>2</sup> STUART RYAN,<sup>2</sup> MICAH J. HORWITH,<sup>3</sup> AND STEVEN B. ROBERTS<sup>1,4</sup>

LAURA SPENCER

# EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS

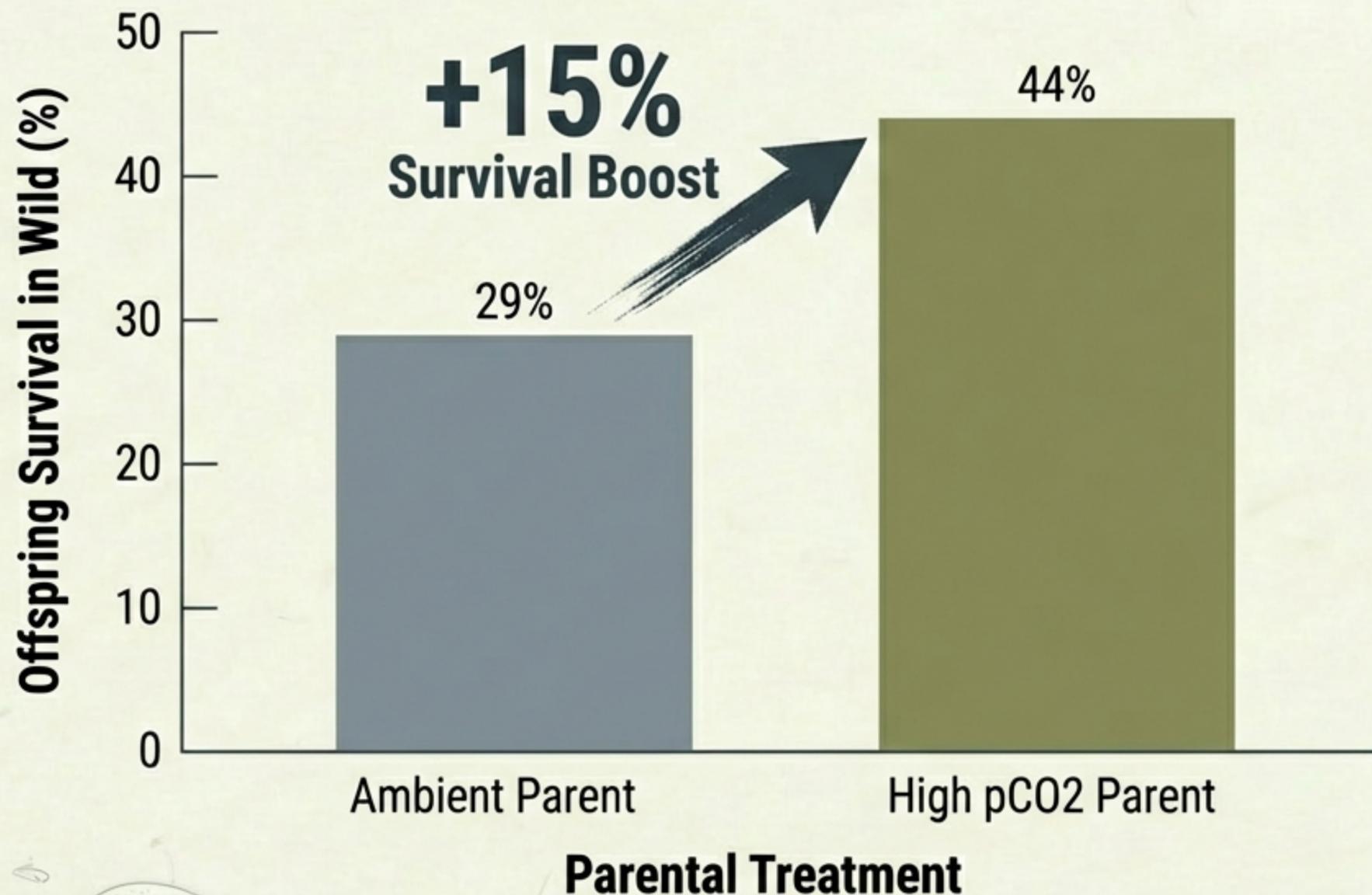


# EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS



- ▶ Larval release occurred earlier in warm-exposed oysters
- ▶ Winter warming conditions increased larval production
- ▶ No effects on larval survival were detected
- ▶ **Juveniles of parents exposed to elevated pCO<sub>2</sub> had higher survival rates in the natural environment**

## Stress as Preparation: The Beneficial Carryover



- **Finding:** Offspring of parents exposed to High pCO<sub>2</sub> had significantly higher survival rates.
- **Interpretation:** Intergenerational Plasticity. The parents “primed” the offspring for a tough environment.

# Resilience is Context-Dependent



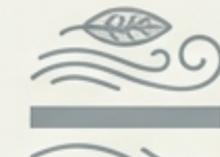
Significant Survival Boost.  
(Low pH / High Salinity Bays)



Significant Survival Boost.



Matching Stressful Environment



No Significant Benefit.



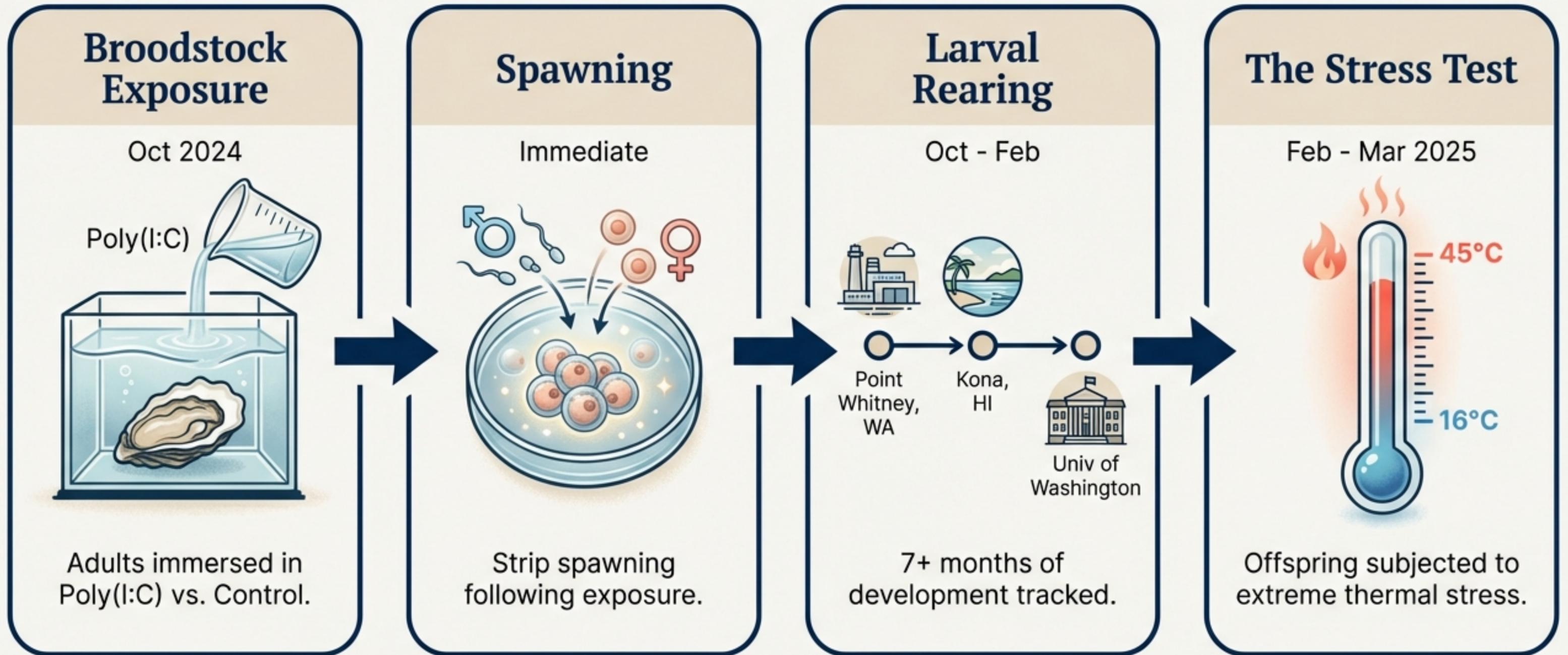
Mismatch



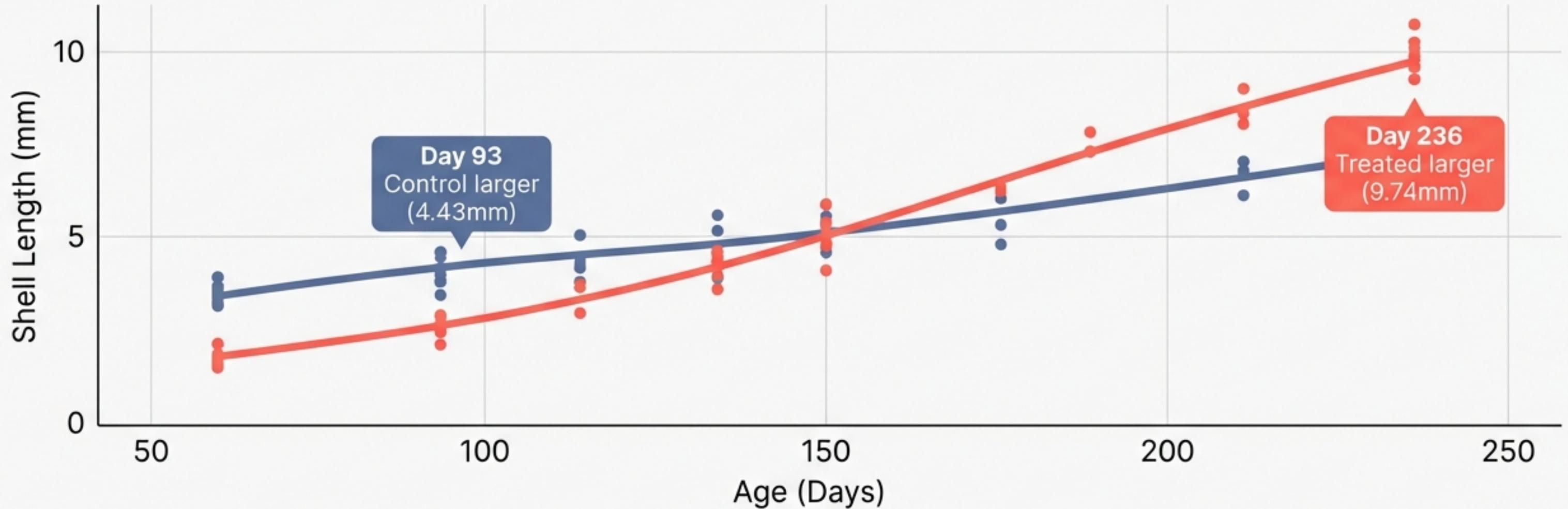
The benefit wasn't universal. The "preparation" provided by parents is most useful when offspring face a matching stressful environment.



# The Experiment: Engineering Resilience

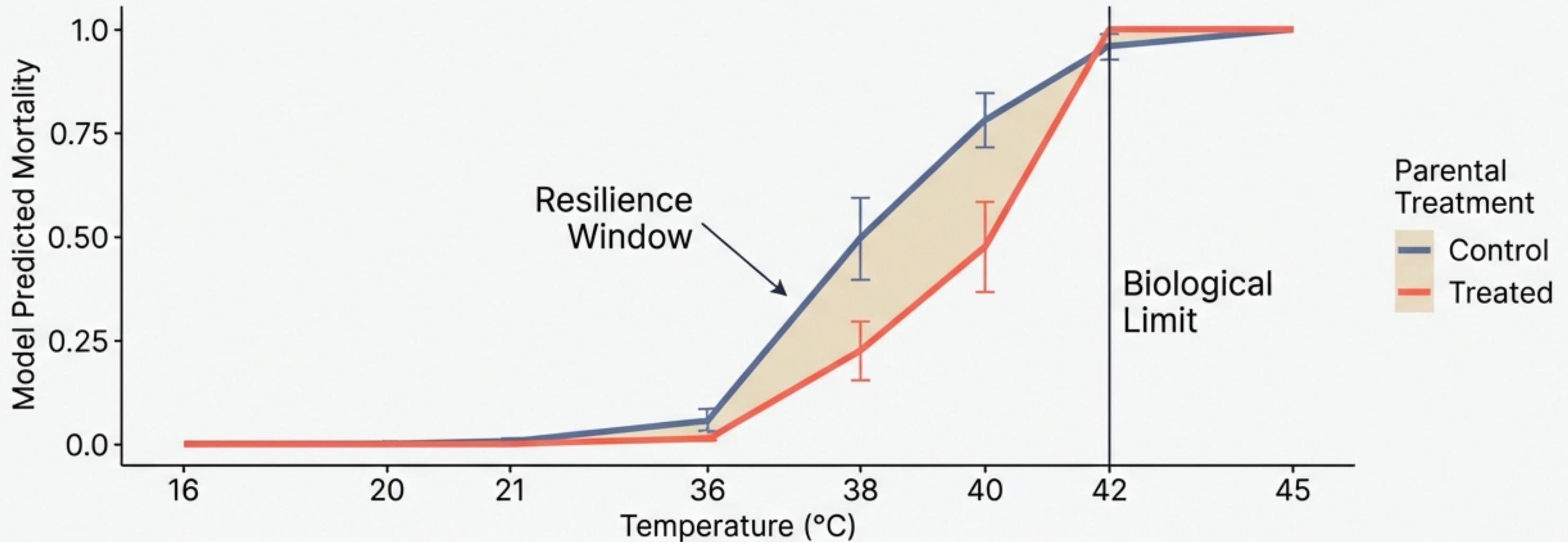


# Growth Dynamics: The 'Catch-Up' Phenomenon



**Insight:** Treated offspring started slower—likely investing energy in immune defense—but **accelerated later** to become significantly larger.

# Thermal Tolerance: The Resilience Window



**Insight:** Priming widens the survival window up to 40°C, creating a 'buffer zone' for heatwaves. However, this defense mechanism has a biological breaking point at 42°C.

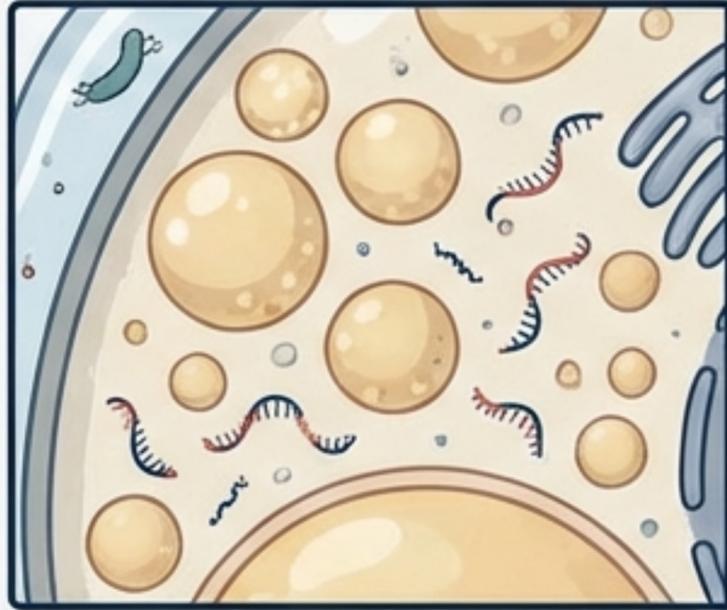
# Cross-Protection: Immune Inputs, Thermal Outputs



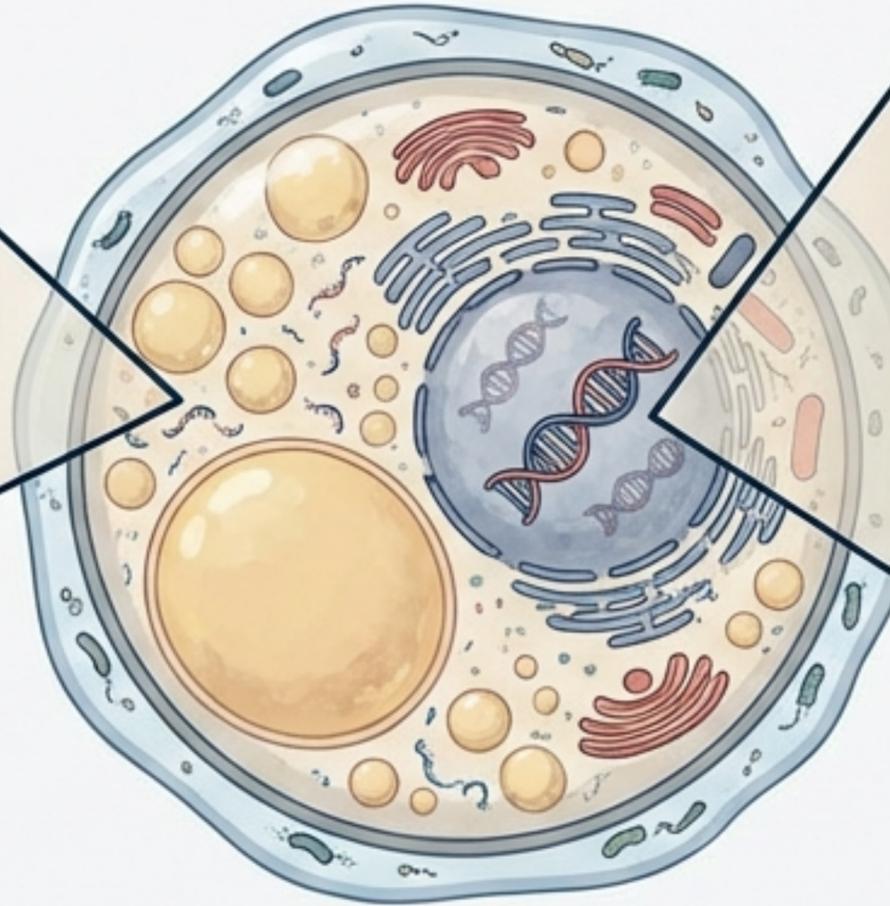
**The Surprise:** Parents were treated with a viral mimic, yet offspring showed heat tolerance. Why? The 'fire drill' prepared the cellular machinery for both fire (heat) and intruders (virus).

# How is the Message Passed?

## Maternal Provisioning

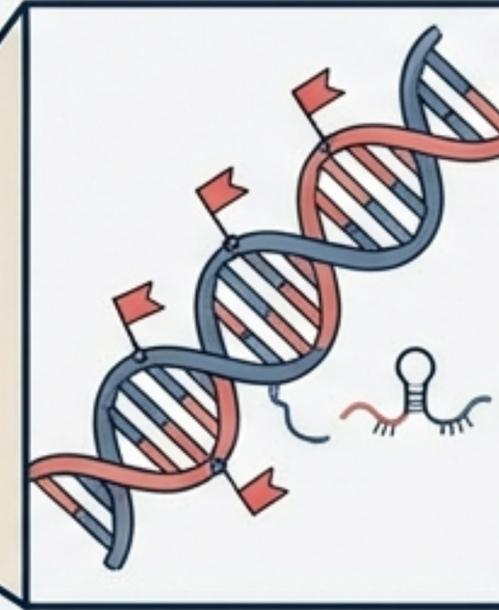


## Oyster Egg Cell



## Oyster Egg Cell

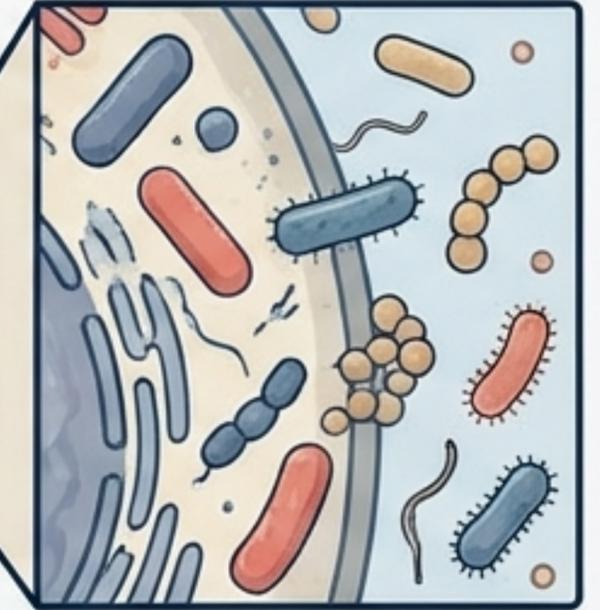
## Epigenetics



## Epigenetics

DNA Methylation & Non-coding RNAs.  
Switches that turn stress genes On/Off.

## Microbiome



## Microbiome

Microbial Transfer  
Shifts in bacterial community passed to seed.

## Maternal Provisioning

Investment of Lipids & mRNA  
A survival 'starter pack'.

Status: Observed effects persisted >7 months, strongly suggesting epigenetic mechanisms beyond simple energy provisioning.



*Priming*

*Within  
Across generation*

*Mechanism*

*Industry Concerns*

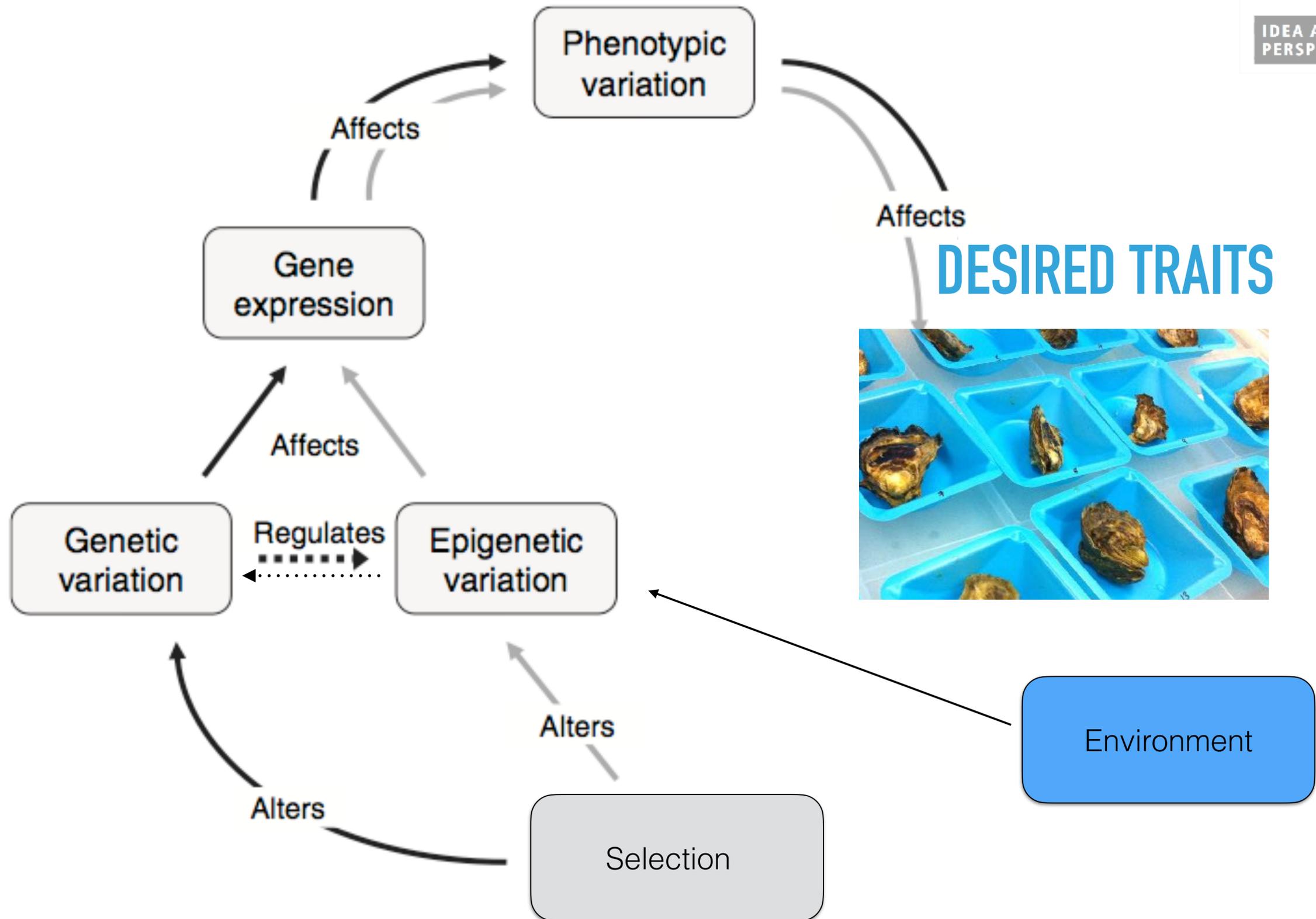
# ECOLOGICAL EPIGENETICS

Ecology Letters, (2008) 11: 106–115

doi: 10.1111/j.1461-0248.2007.01130.x

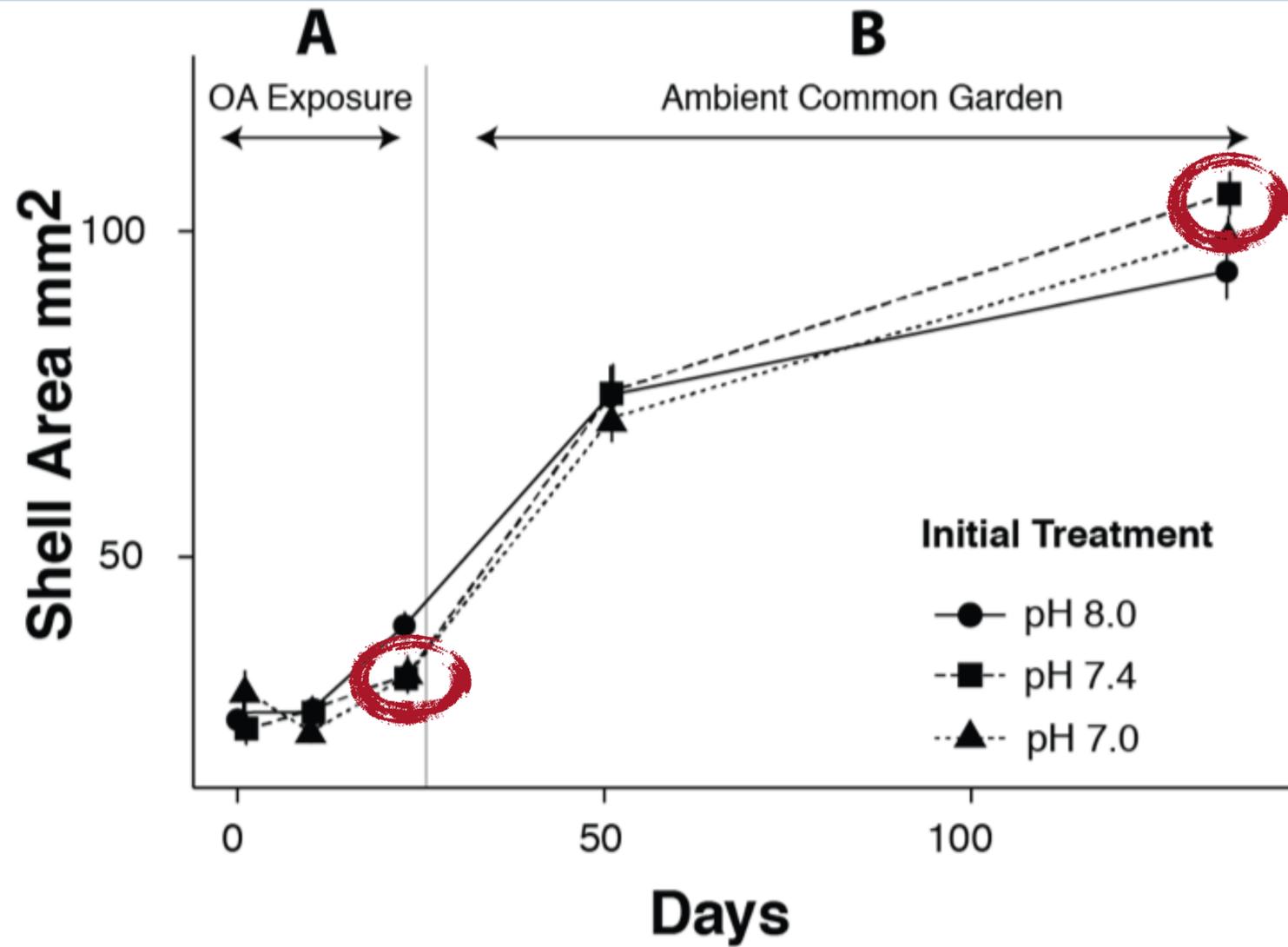
IDEA AND  
PERSPECTIVE

Epigenetics for ecologists



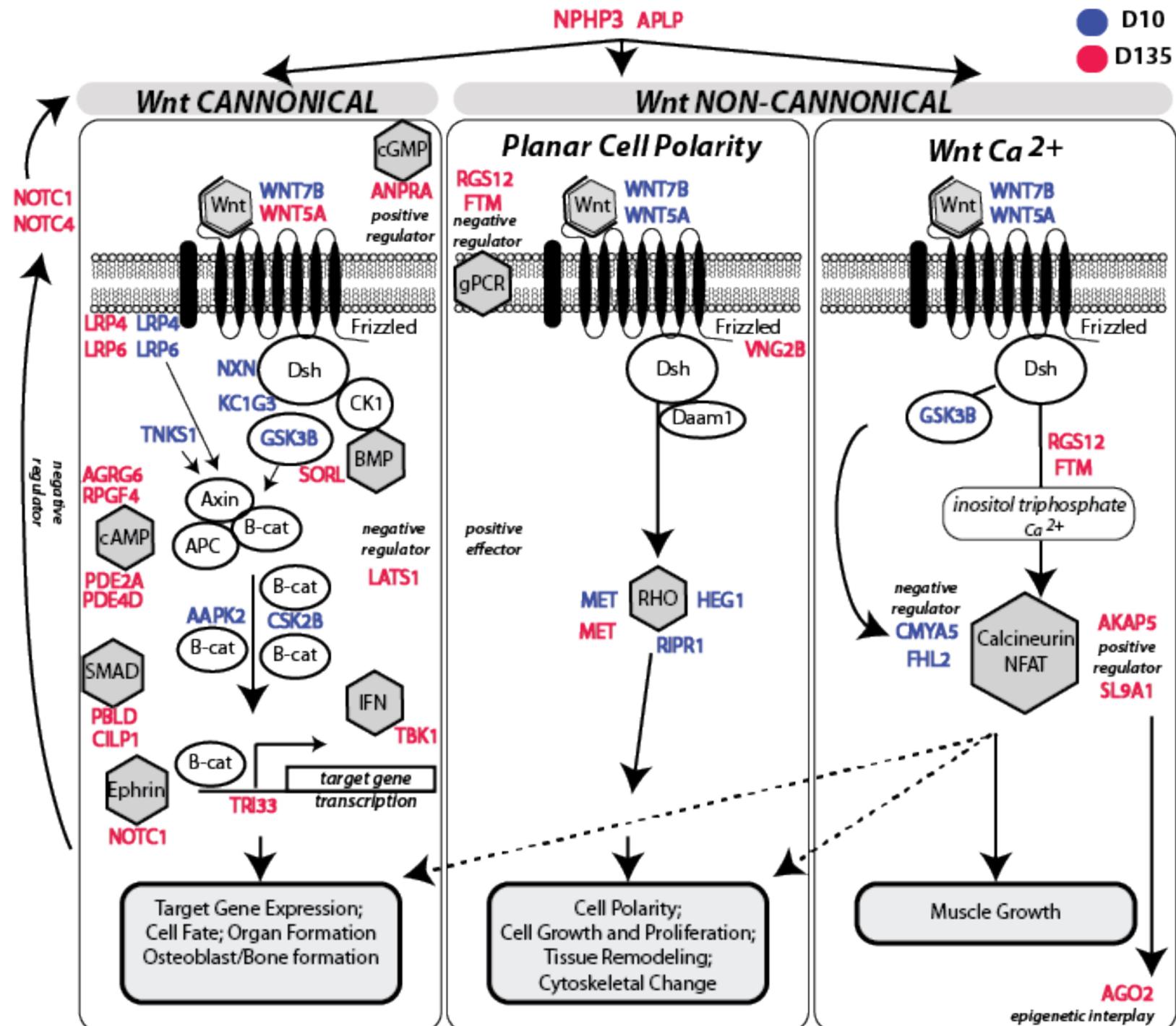
# GEODUCKS CLAMS

- ▶ Does conditioning to low pH confer tolerance within a generation?





# GEODUCKS AND OA



New Results

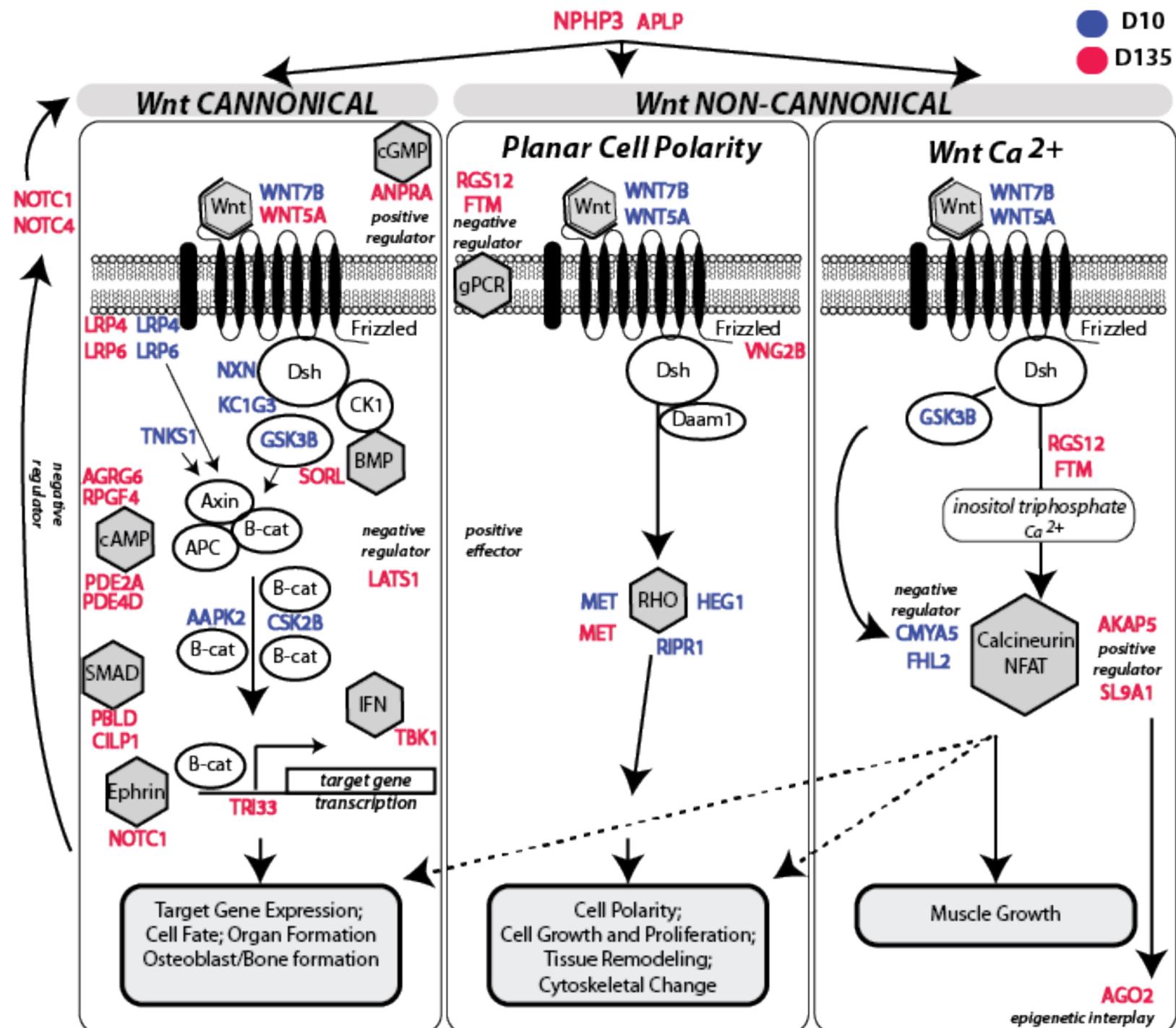
[Follow this preprint](#)

## Dynamic DNA methylation contributes to carryover effects and beneficial acclimatization in geoduck clams

Hollie M. Putnam, Shelly A. Trigg, Samuel J. White, Laura H. Spencer, Brent Vadopalas, Aparna Natarajan, Jonathan Hetzel, Erich Jaeger, Jonathan Soohoo, Cristian Gallardo-Escárate, Frederick W. Goetz, Steven B. Roberts

doi: <https://doi.org/10.1101/2022.06.24.497506>

# GEODUCKS AND OA



Following four months of ambient common-garden conditions, **juveniles initially exposed to low pH compensatorily grew larger**, with DNA methylation indicative of these phenotypic differences, demonstrating epigenetic carryover effects persisted months after initial exposure.



**EXCITING?  
COMPLEX  
'LAYER' OF RESILIENCE**

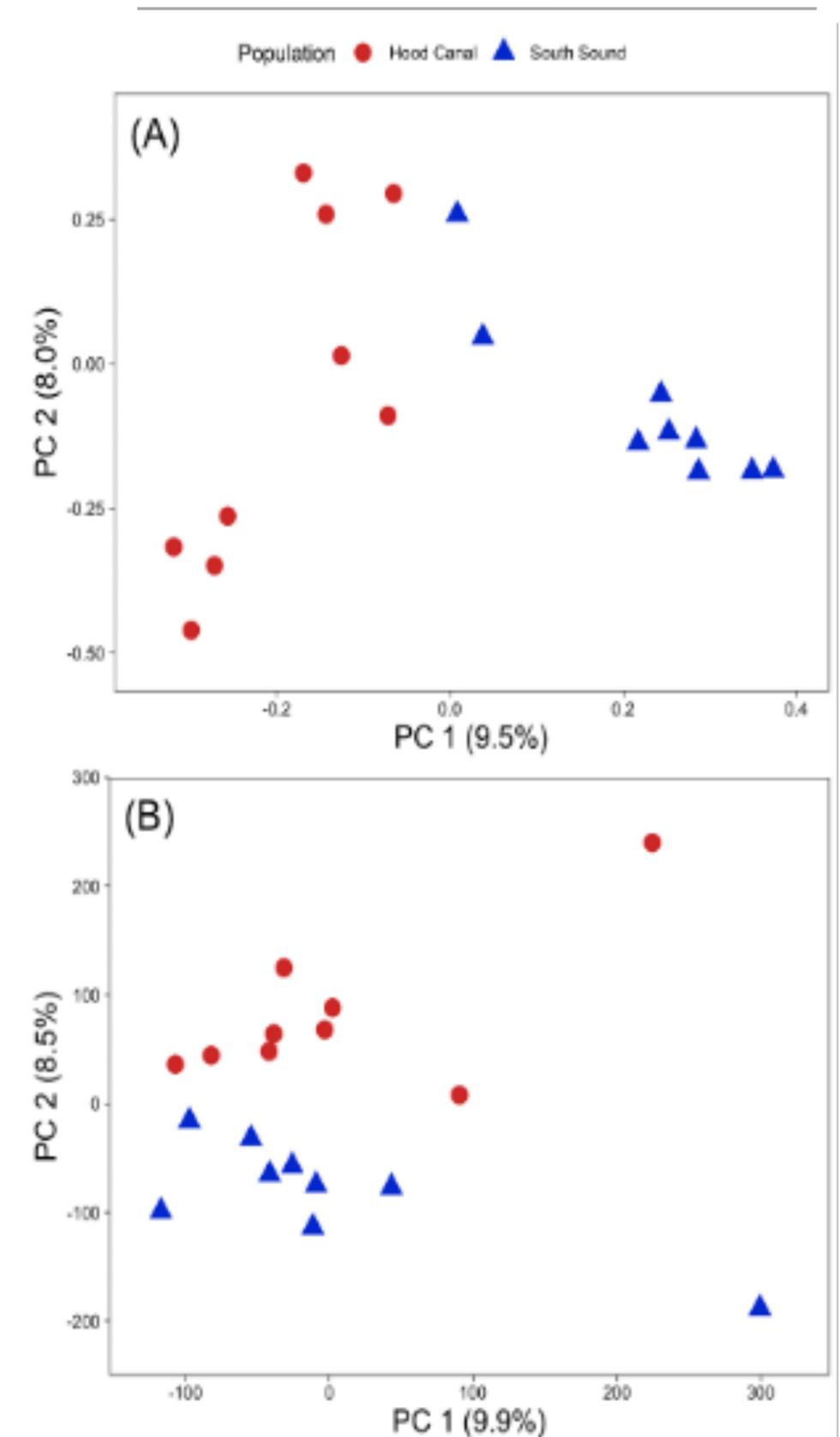
# Epigenetic and Genetic Population Structure is Coupled in a Marine Invertebrate

Katherine Silliman <sup>1,†</sup>, Laura H. Spencer <sup>2,†</sup>, Samuel J. White<sup>2</sup>, and Steven B. Roberts <sup>2,\*</sup>

First characterization of genome-wide DNA methylation patterns in the oyster genus *Ostrea*

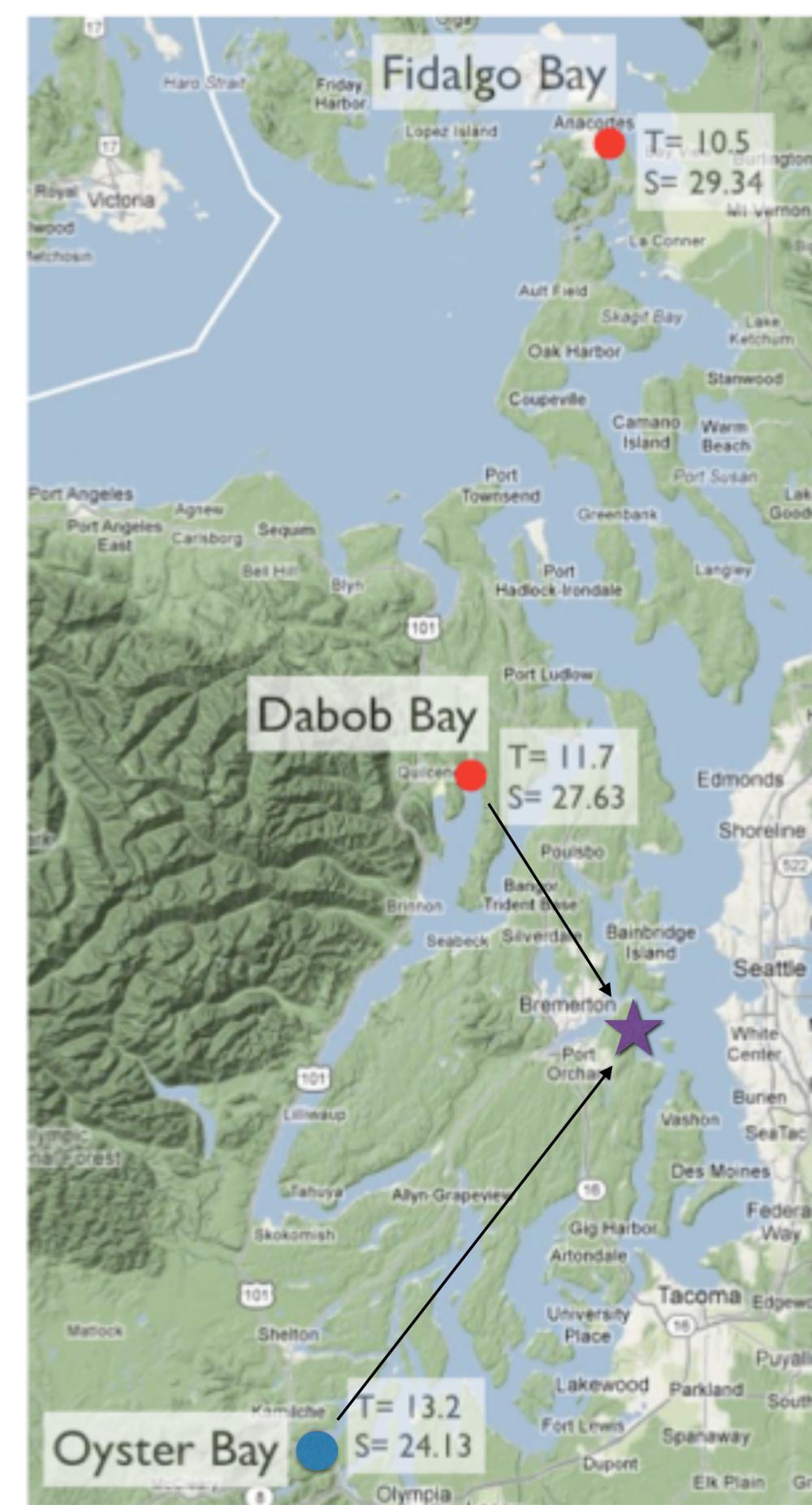
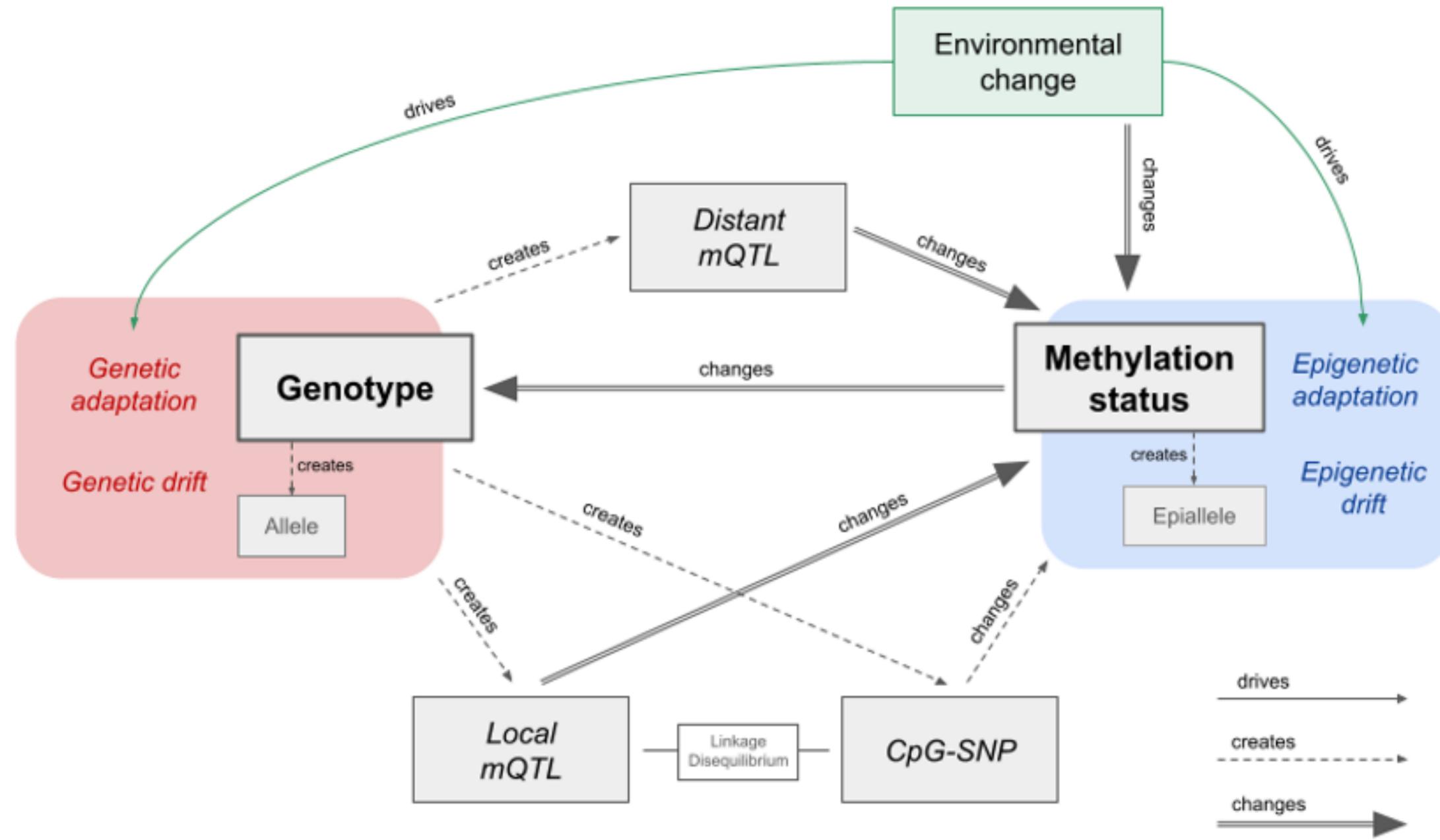
Identified 3,963 differentially methylated loci between populations. Clear coupling between genetic and epigenetic patterns of variation, **with 27% of variation in inter-individual methylation differences explained by genotype.**

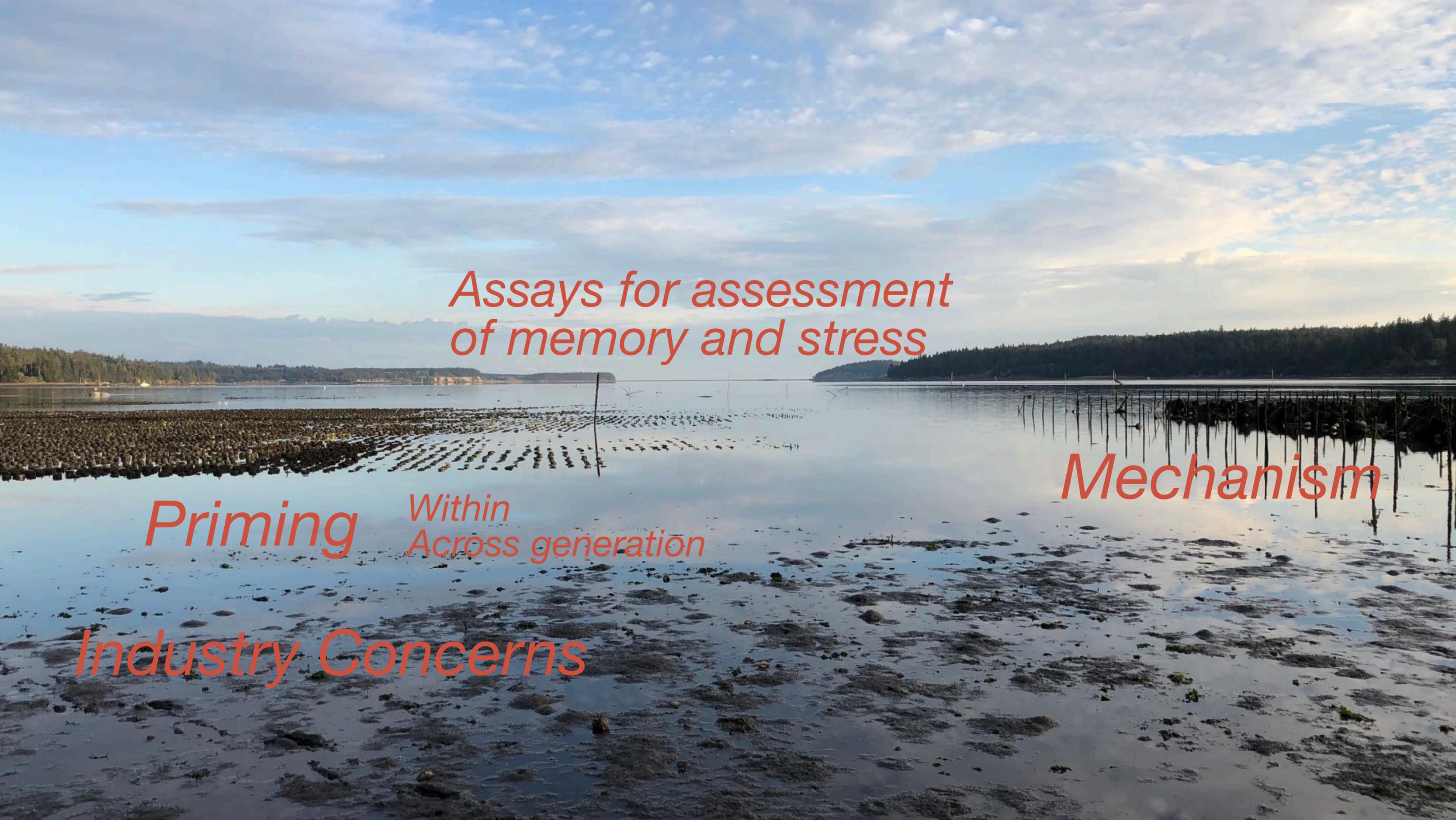
Underlying this association are both direct genetic changes in CpGs (CpG-SNPs) and genetic variation with indirect influence on methylation (mQTLs).



# IMPLICATIONS

# EPIGENETIC AND GENETIC POPULATION STRUCTURE





*Assays for assessment  
of memory and stress*

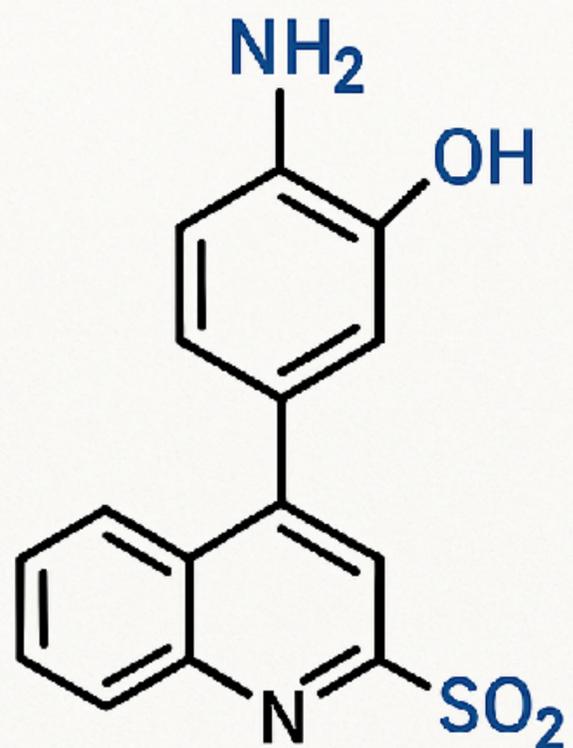
*Priming*

*Within  
Across generation*

*Mechanism*

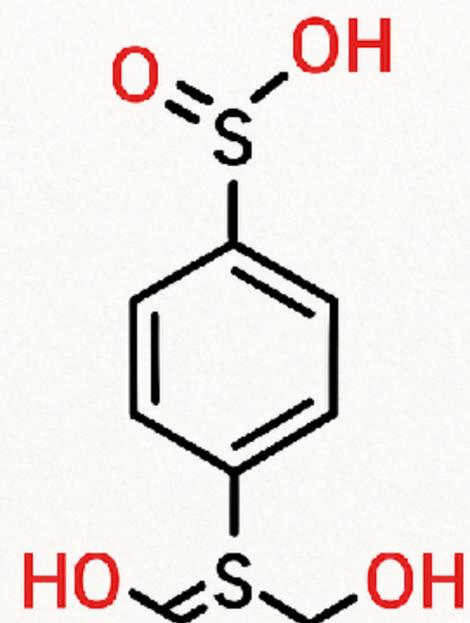
*Industry Concerns*

# Resazurin



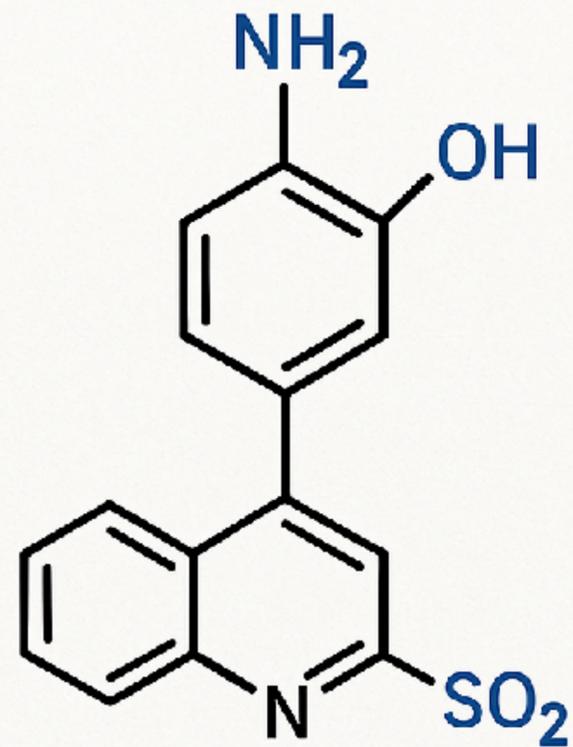
**Resazurin  
(oxidized)**

metabolic  
activity



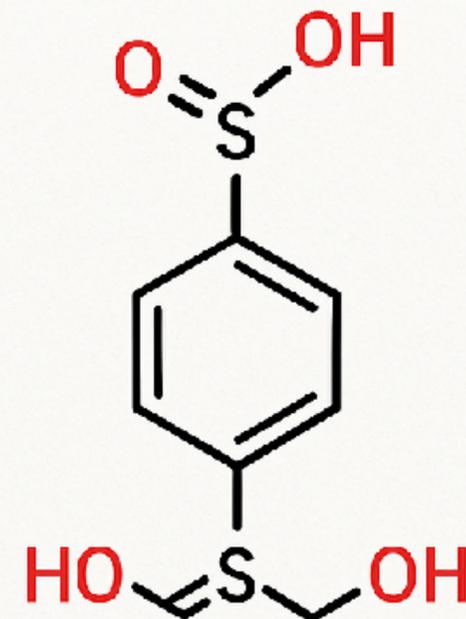
**Resorufin  
(reduced)**

# Resazurin



**Resazurin  
(oxidized)**

metabolic  
activity



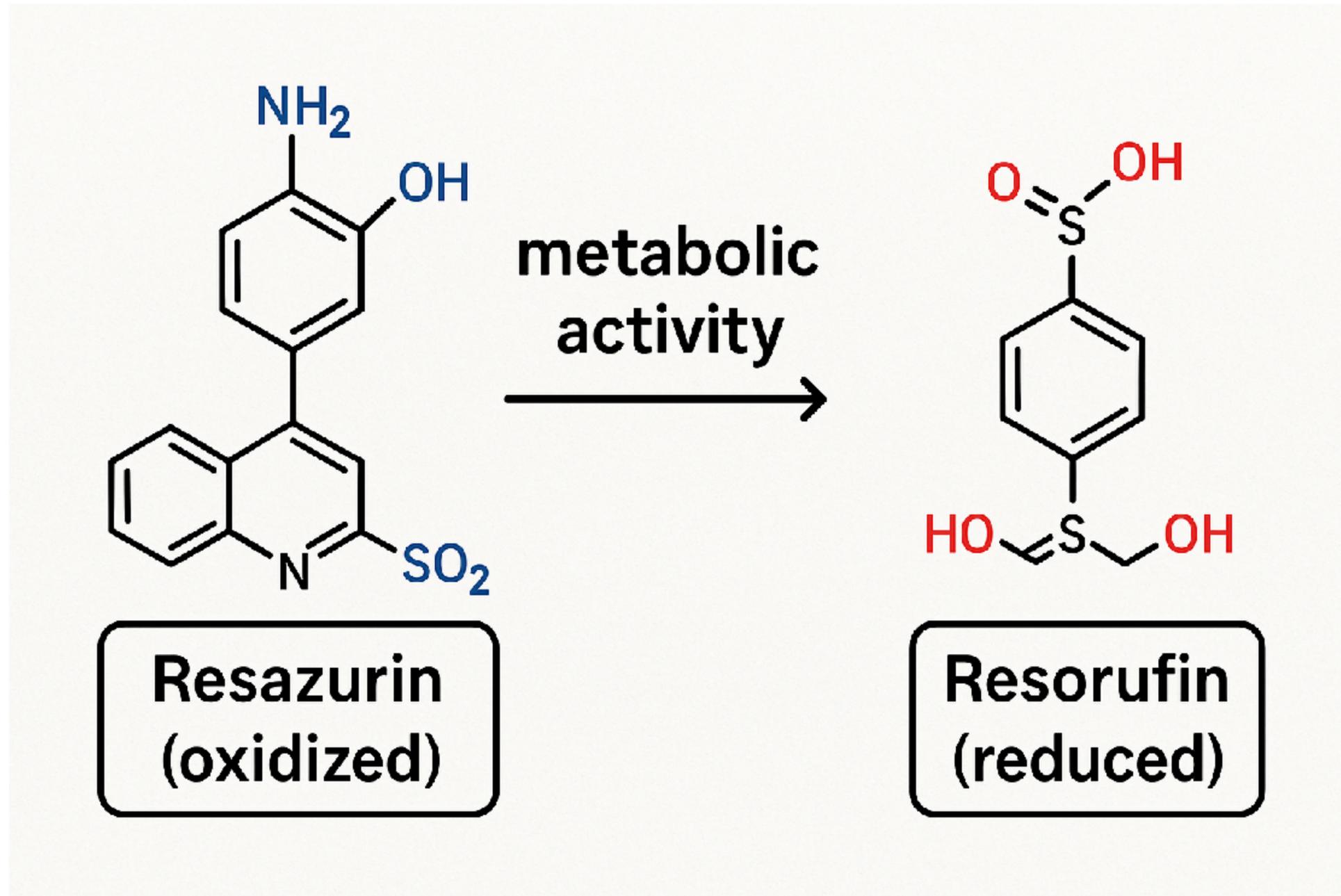
**Resorufin  
(reduced)**

Highly fluorescent (excitation ~570 nm, emission ~585 nm)

The amount of resorufin produced is directly proportional to the metabolic activity of the cells.

# Applications

## Resazurin



Is metabolic activity associated with a stress event different when oyster had previously experienced a stress? (Is there evidence for functional priming?)

# Applications

Ariana Huffmyer - UW

## Heat-Priming Pacific Oysters: Field Performance vs. Lab Stress Tolerance

Results from an 18-month Sea Grant experiment at Westcott Bay Shellfish

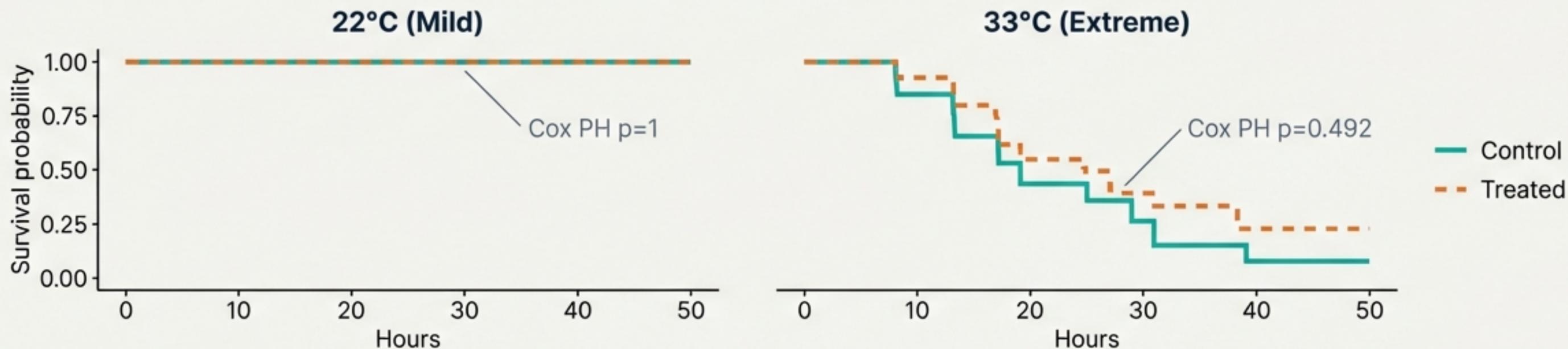
Project Leads: Emily Carrington & Steven Roberts

Research Team: Grace Leuchtenberger & Ariana Huffmyer

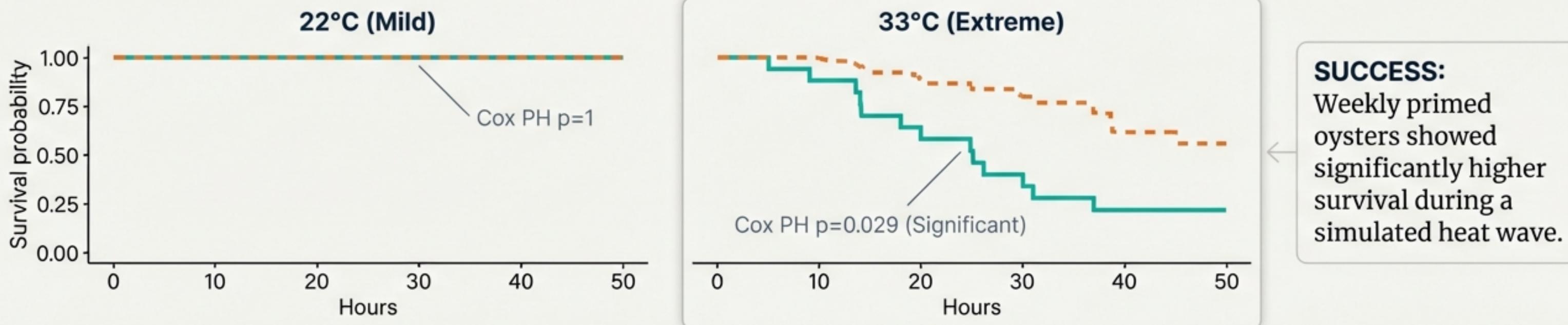


# Hardening worked—but only revealed itself under extreme acute stress.

## Daily Hardening

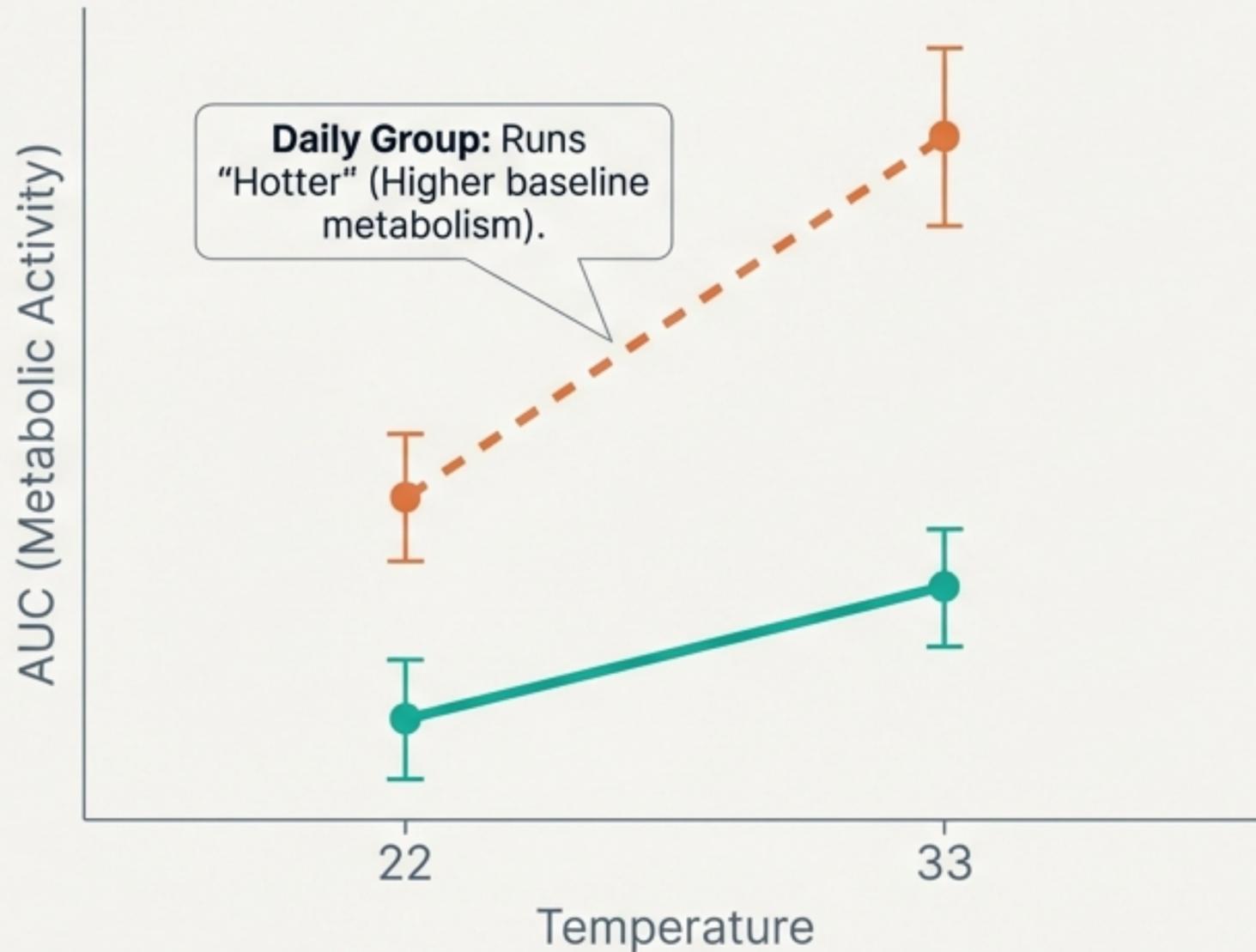


## Weekly Hardening

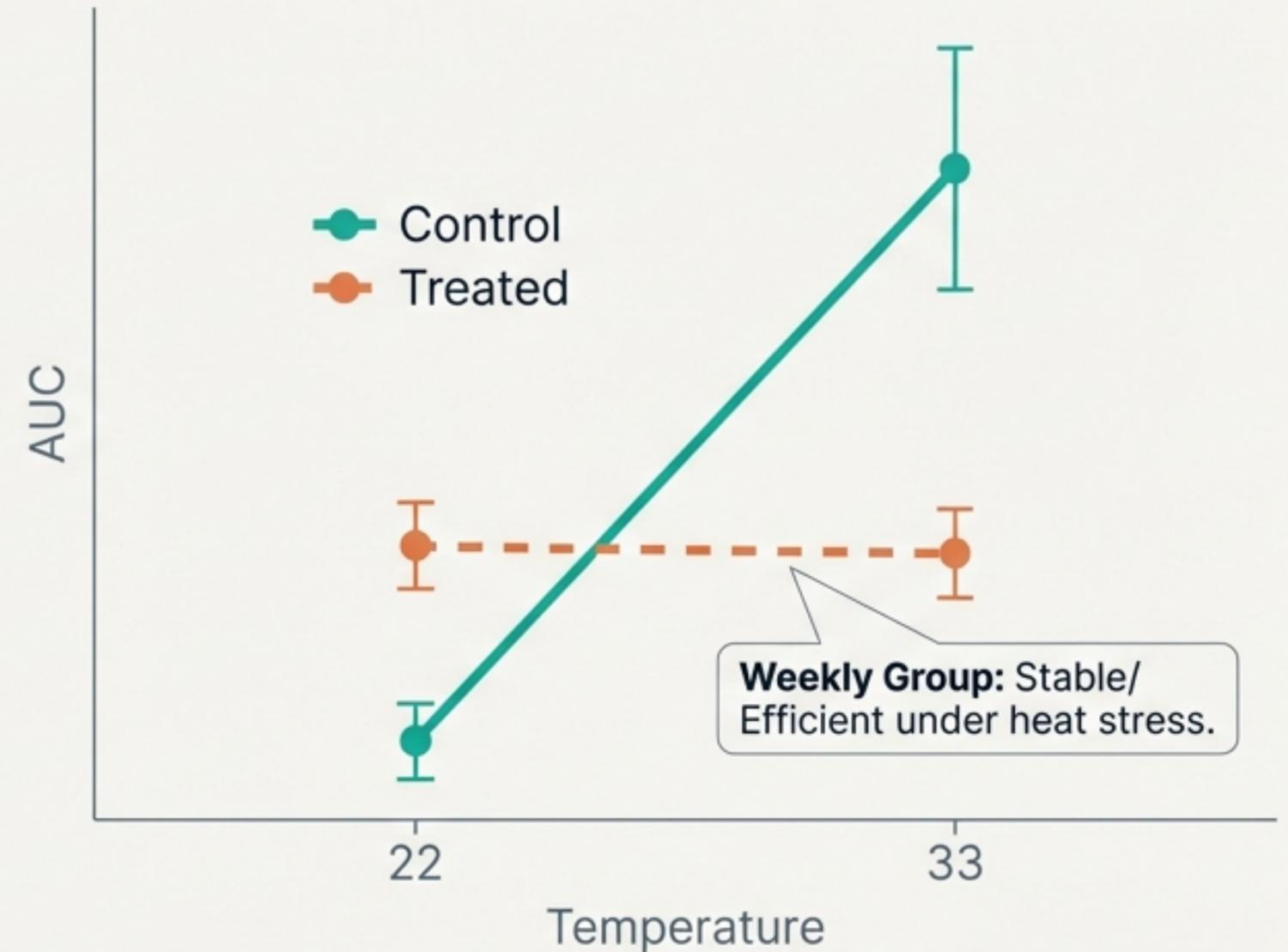


# Priming fundamentally altered oyster metabolic strategies.

## Daily Hardening Metabolism



## Weekly Hardening Metabolism

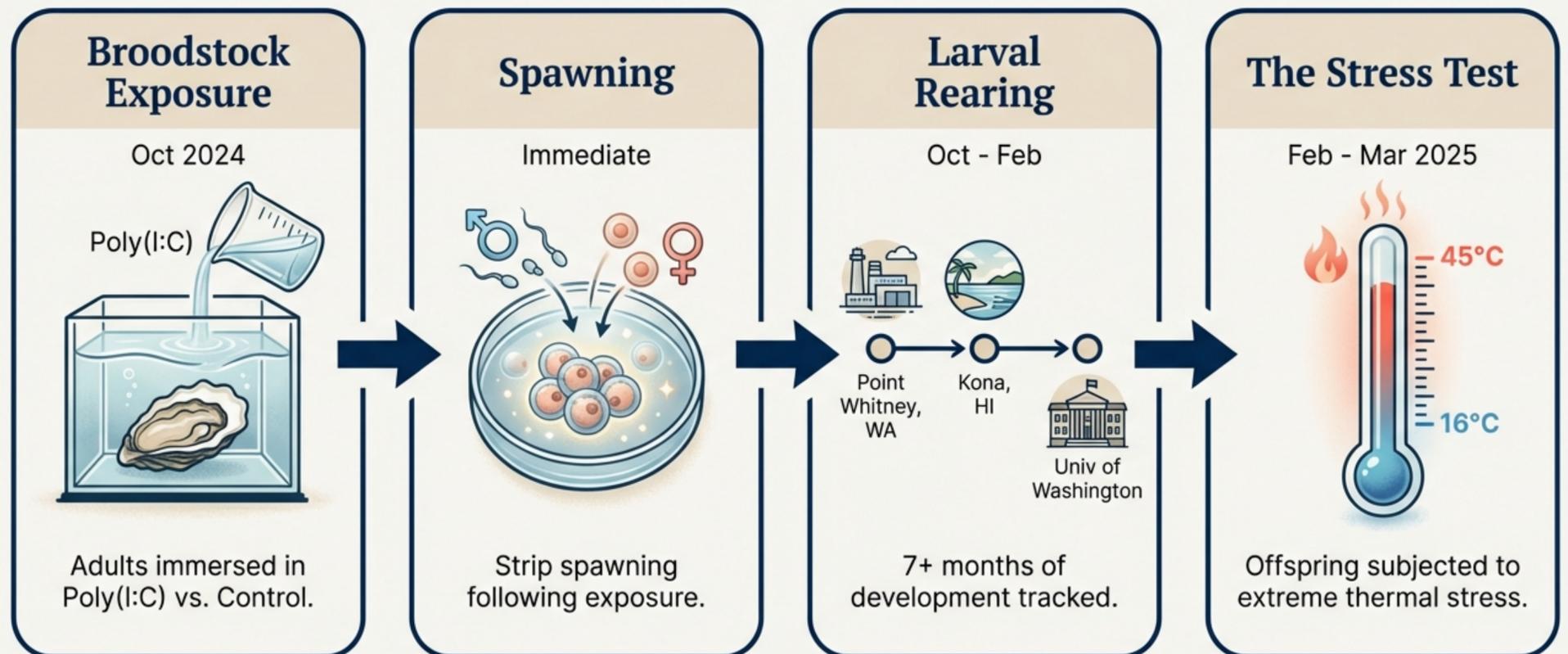


**Conclusion:** The hatchery treatments successfully programmed long-term changes in energy management.

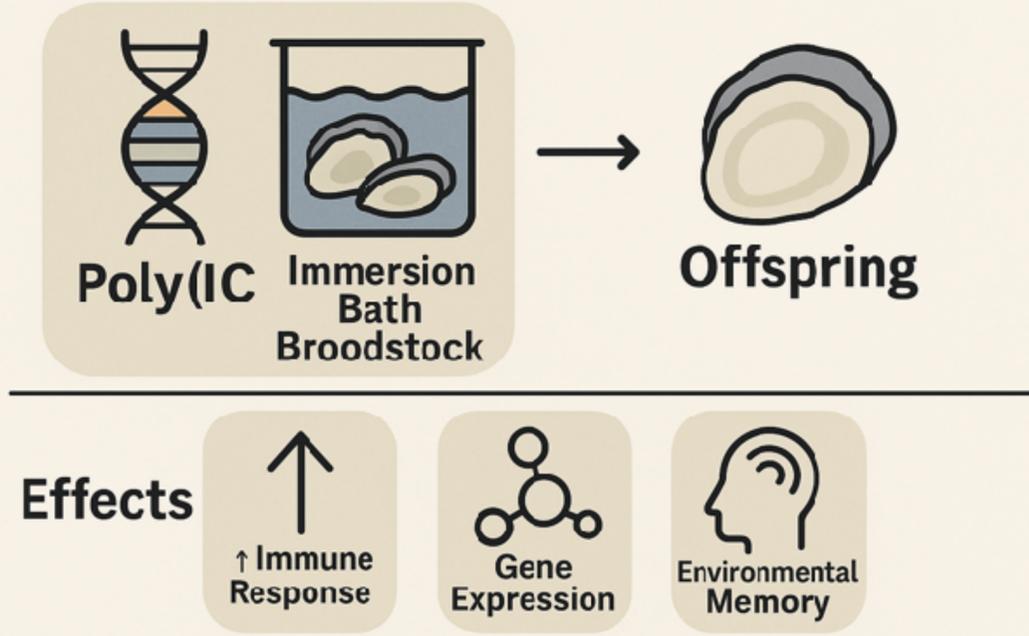
# Applications

Ariana Huffmyer - UW

## The Experiment: Engineering Resilience

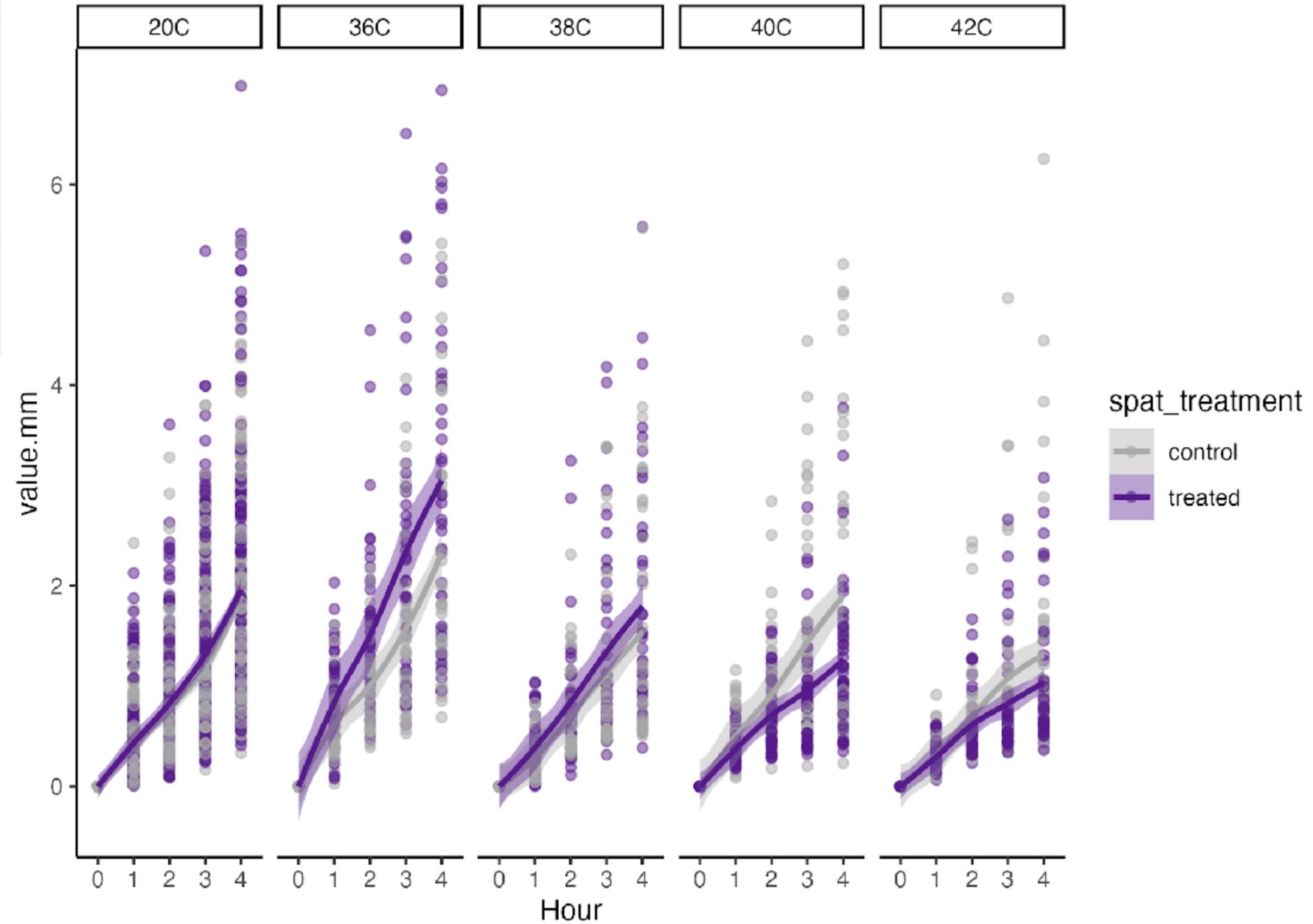


# POLY(I:C) EXPERIMENT TO INDUCE ENVIRONMENTAL MEMORY

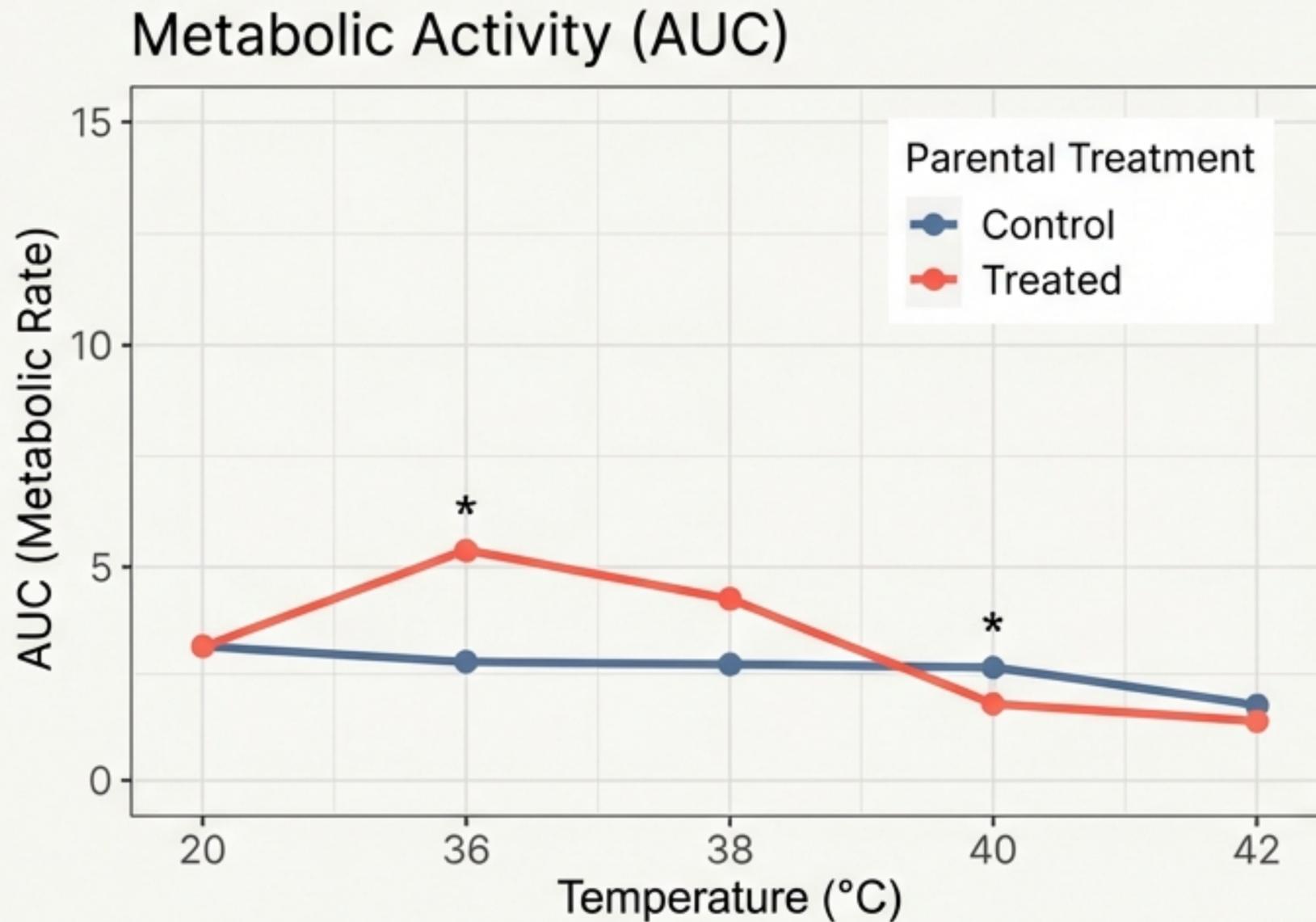


Offspring from immune-challenged parents exhibited **greater metabolic flexibility**

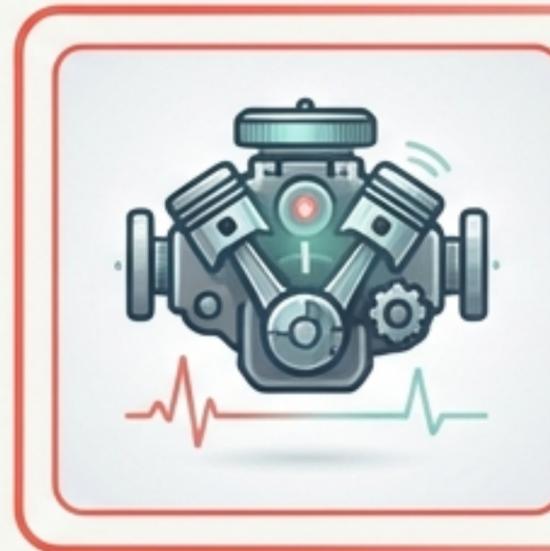
Ariana Huffmyer - UW



# The Hidden Mechanism: Metabolic Flexibility



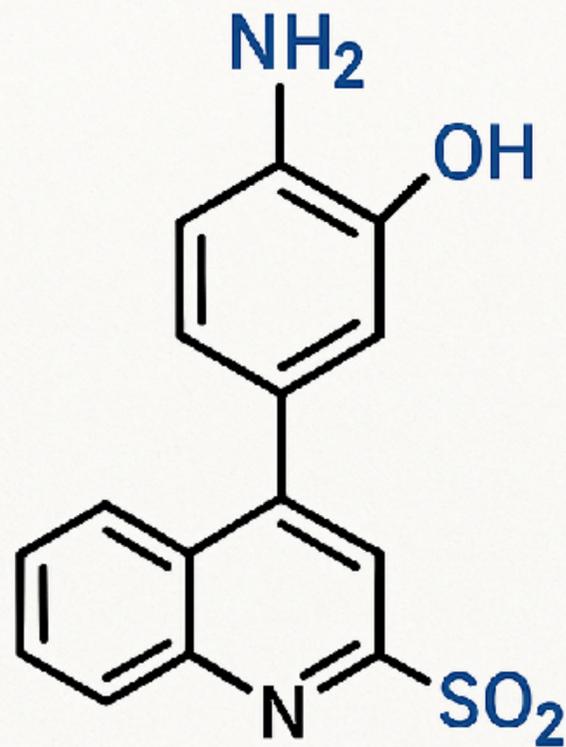
**36°C: Ramping Up Defenses**



**40°C: Metabolic Depression (Conservation Mode)**

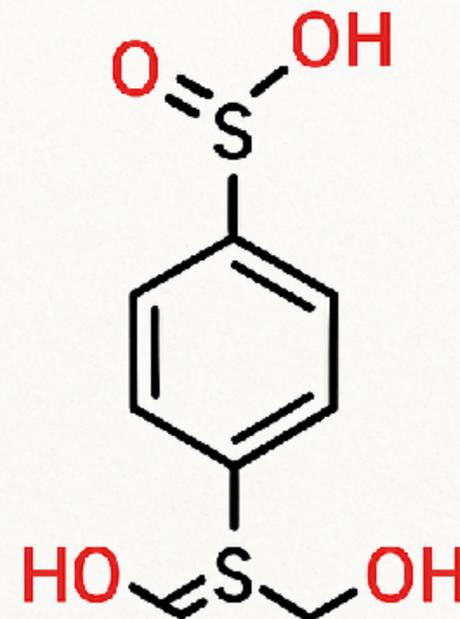
**Insight:** Treated offspring were not just 'tougher'; they were metabolically flexible. They revved up early to prepare, then idled down to survive the peak heat.

# More Applications



**Resazurin  
(oxidized)**

metabolic  
activity



**Resorufin  
(reduced)**

## Using Resazurin Assay to Study Oysters and Improve Aquaculture



### Screening for Stress-Resilient Oyster Lines

Test metabolic activity under thermal, salinity, or pH stress



### Assessing the Effects of Microbiome Manipulation

Measure host metabolic health changes from microbiome modifications



### Monitoring Larval and Juvenile Health in Hatcheries

Rapid assessment of viability and metabolic status



### Evaluating Impact of Environmental Exposures

Measure metabolic response to pollutants, harmful algal blooms, etc.



### Testing the Efficacy of Diets or Nutritional Supplements

Compare metabolic output of oysters fed different diets

# Conclusions and Next Steps

**Environmental Memory (*Epigenetics*) can be useful for aquaculture**

- Environmental conditions can influence later phenotypes without genetic selection.
- In many cases a protective phenotype is prevalent under extreme conditions.
- Cross-protection makes priming practice amenable in aquaculture.
- There is a complex genetic - epigenetic relationship
- Inexpensive physiological assays can be used to assess how priming can improve performance.

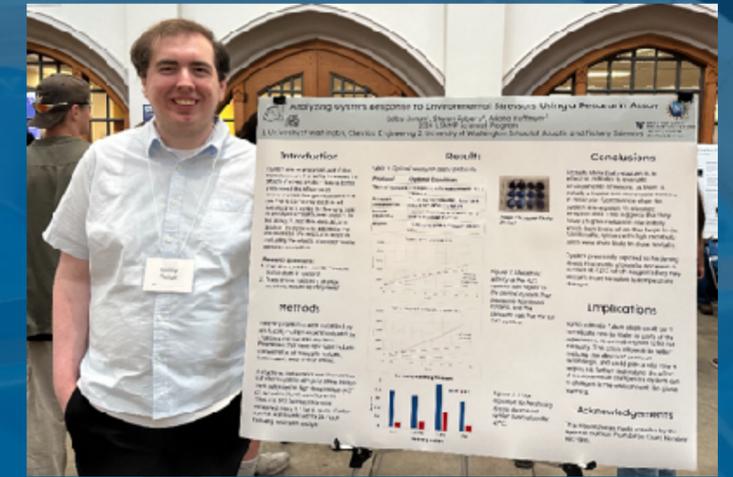
# Conclusions and Next Steps

## More Questions

- What stress, intensity, timing, developmental stage is best for priming?
- How does DNA methylation, non-coding RNA, histone modification work together to imprint a memory?
- What is the role of the microbiome?
- Can epigenotype selection be useful tool in aquaculture industry?

# ACKNOWLEDGEMENTS

- ▶ Ariana Huffmyer (UW), Sam White (UW), Brent Vadopalas (UW), Shelly Wanamaker (UW), Valentina Valenzuela (U Concepcion), Sam Gurr (OSU), Hollie Putnam (URI), Laura Spencer (UW), Katherine Silliman (NOAA), Yaamini Venkataraman (WHOI), Katie Lotterhos (NEU)



[github.com/sr320/talk-EpiAqua-2026](https://github.com/sr320/talk-EpiAqua-2026)