



Shellfish environmental memory

Implications and opportunities

Ariana Huffmyer
Steven Roberts

















Industry Concerns



Priming Within
Across generation

Industry Concerns



Priming

*Within
Across generation*

Mechanism

Industry Concerns



Current work

Mechanism

Priming

*Within
Across generation*

Industry Concerns

*Assays for assessment
of memory and stress*

Current work

Priming *Within
Across generation*

Mechanism

Industry Concerns



Industry Concerns

Grower Survey:

Perceptions of threats and proposed adaptation strategies

- 2024 requested input from industry
- 20-30 min interview:
 - Ranking threats
 - Input on leveraging environmental memory as an adaptation strategy
- *Huge thank you to those who participated!* Manuscript is in review

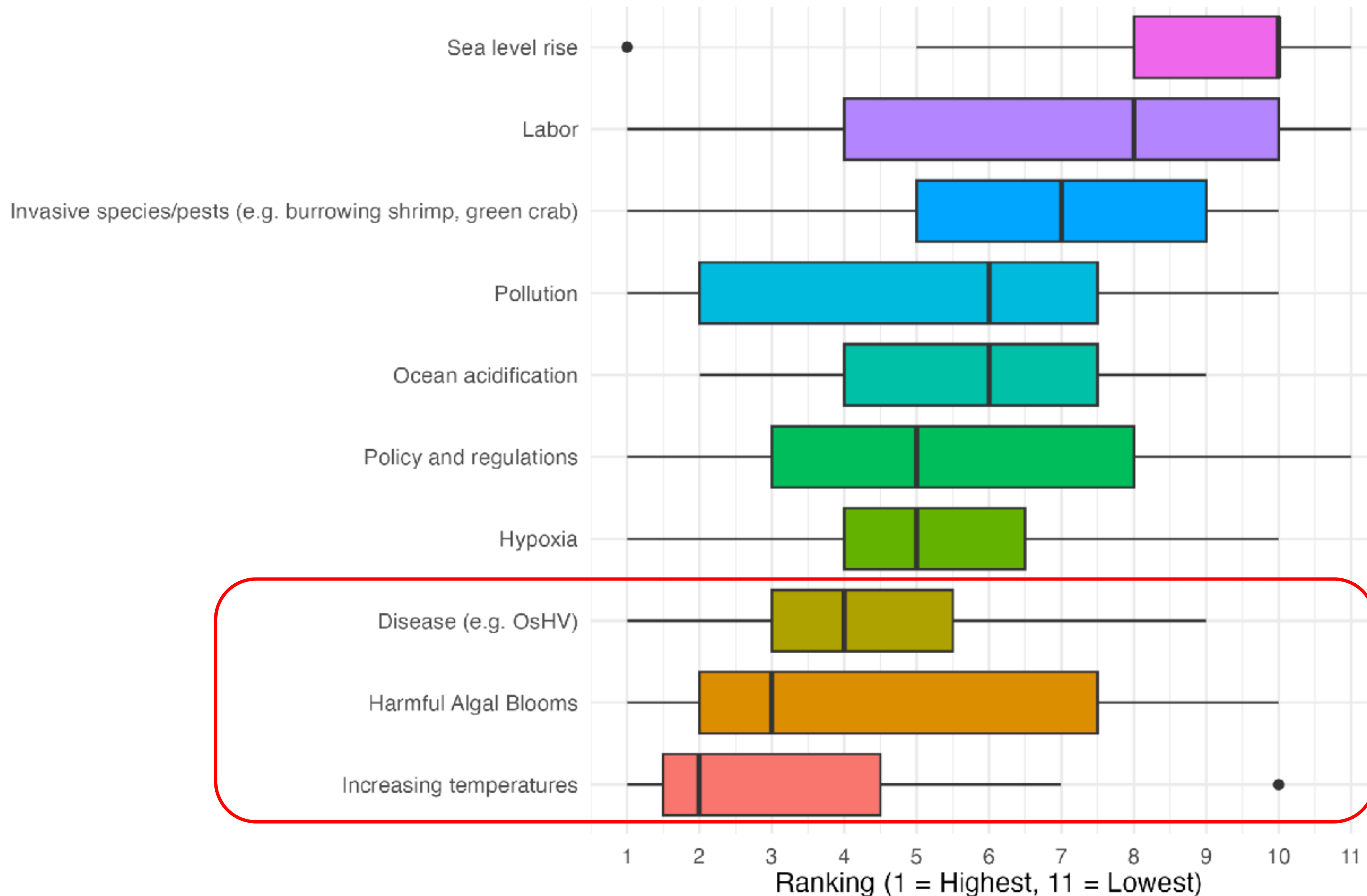


AQUATIC AND FISHERY SCIENCES
UNIVERSITY of WASHINGTON

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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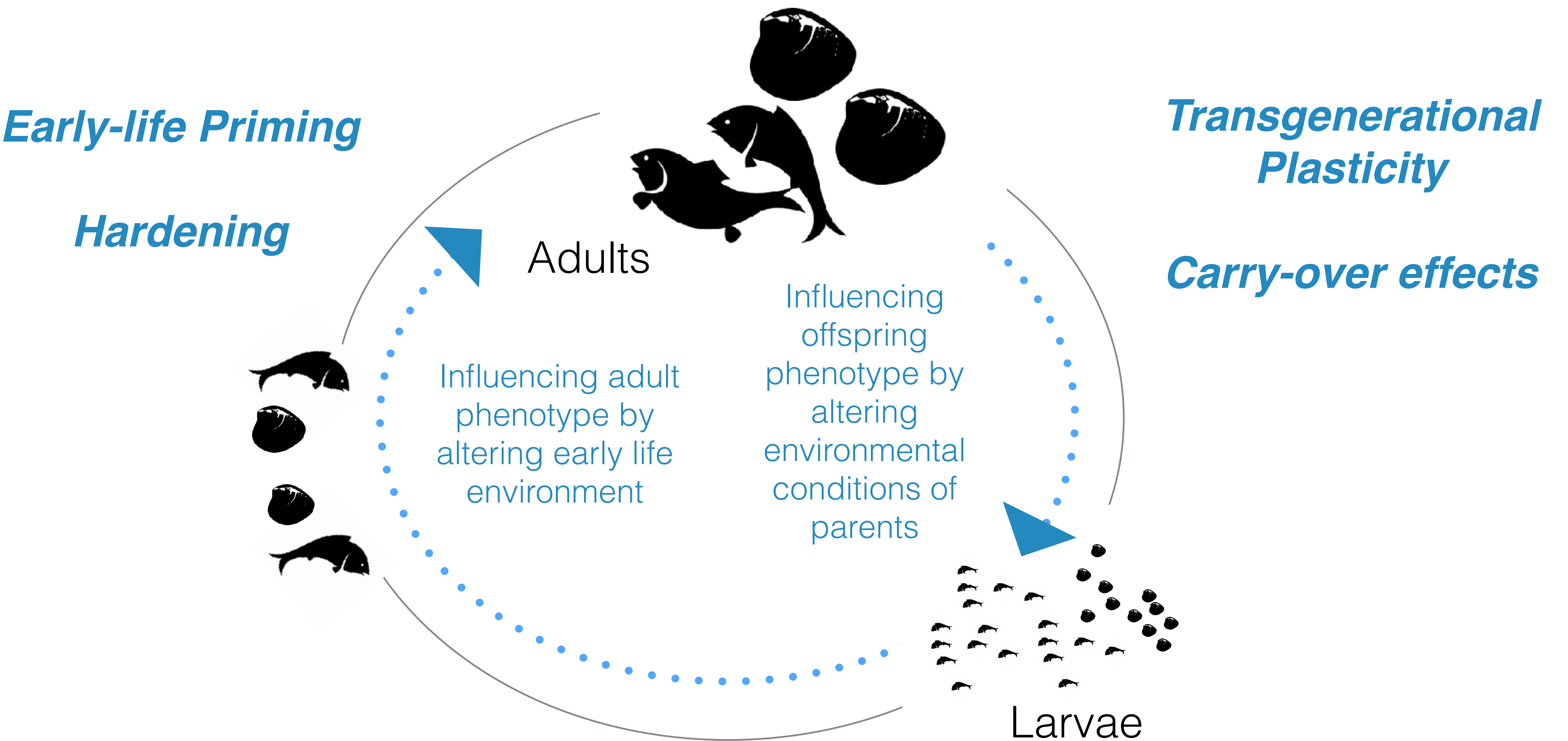
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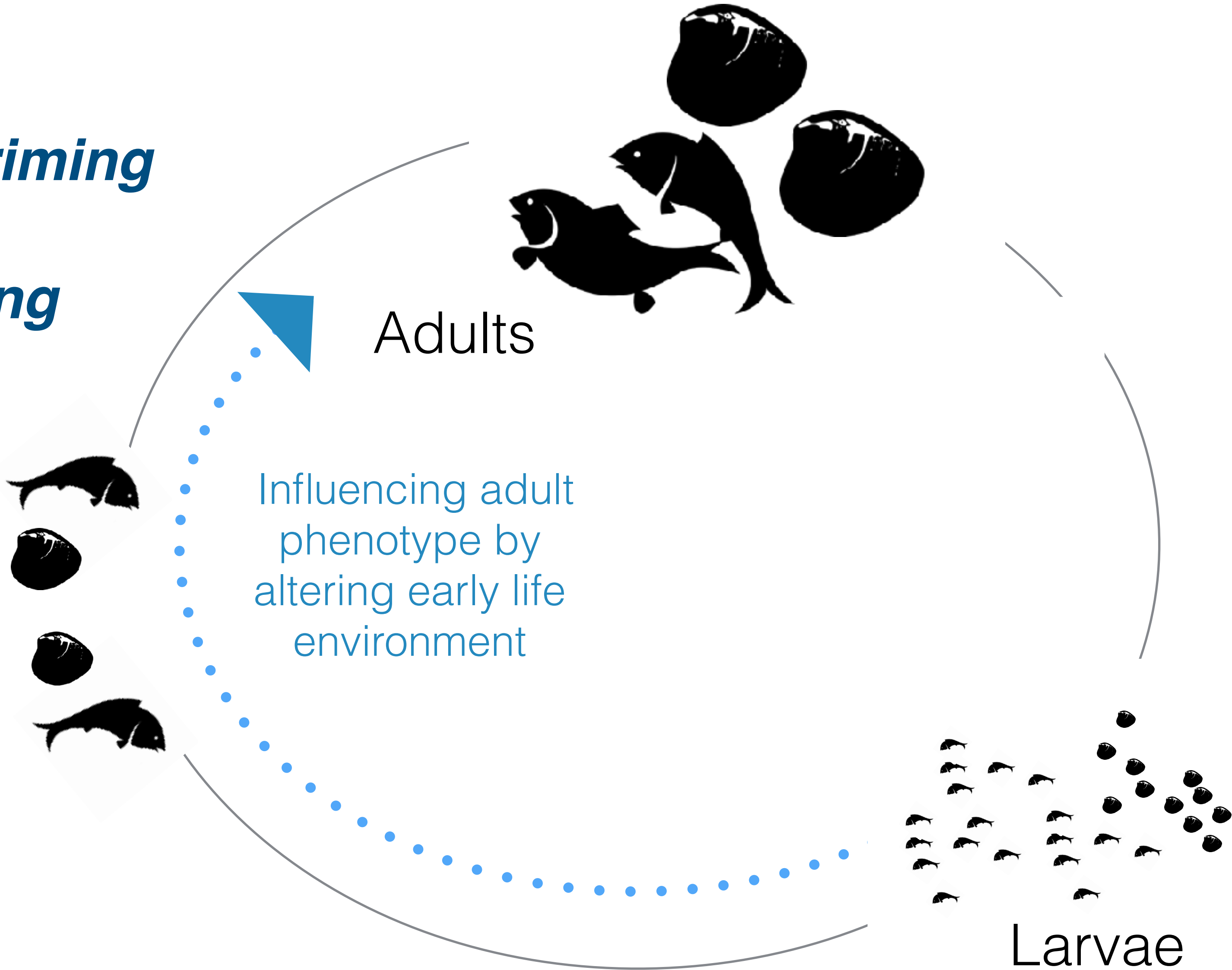
Priming

Industry Concerns



Early-life Priming

Hardening



Early-life Priming

Hardening



**Trends in
Plant Science**

Special issue: Climate change and sustainability I

Review

Priming crops for the future: rewiring stress memory

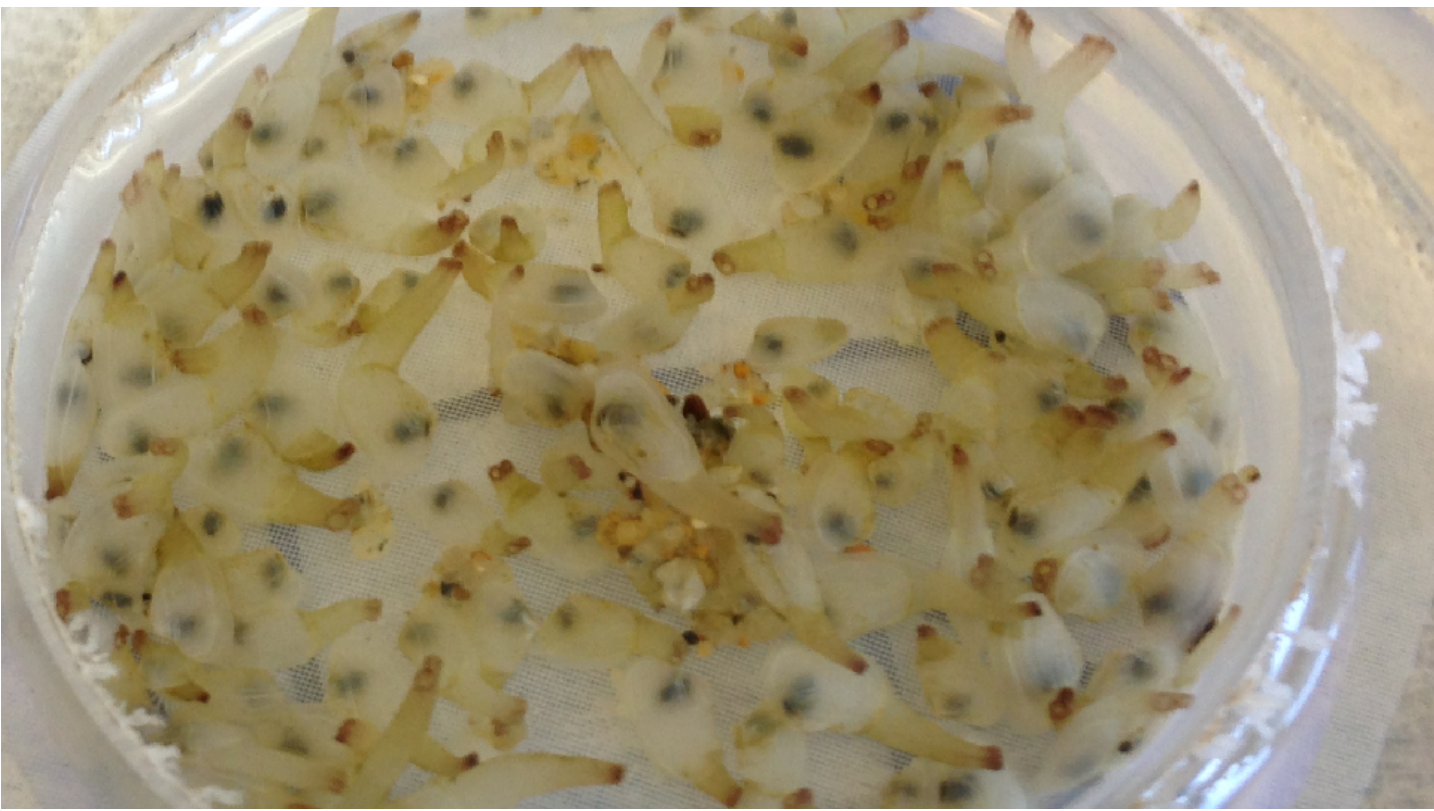
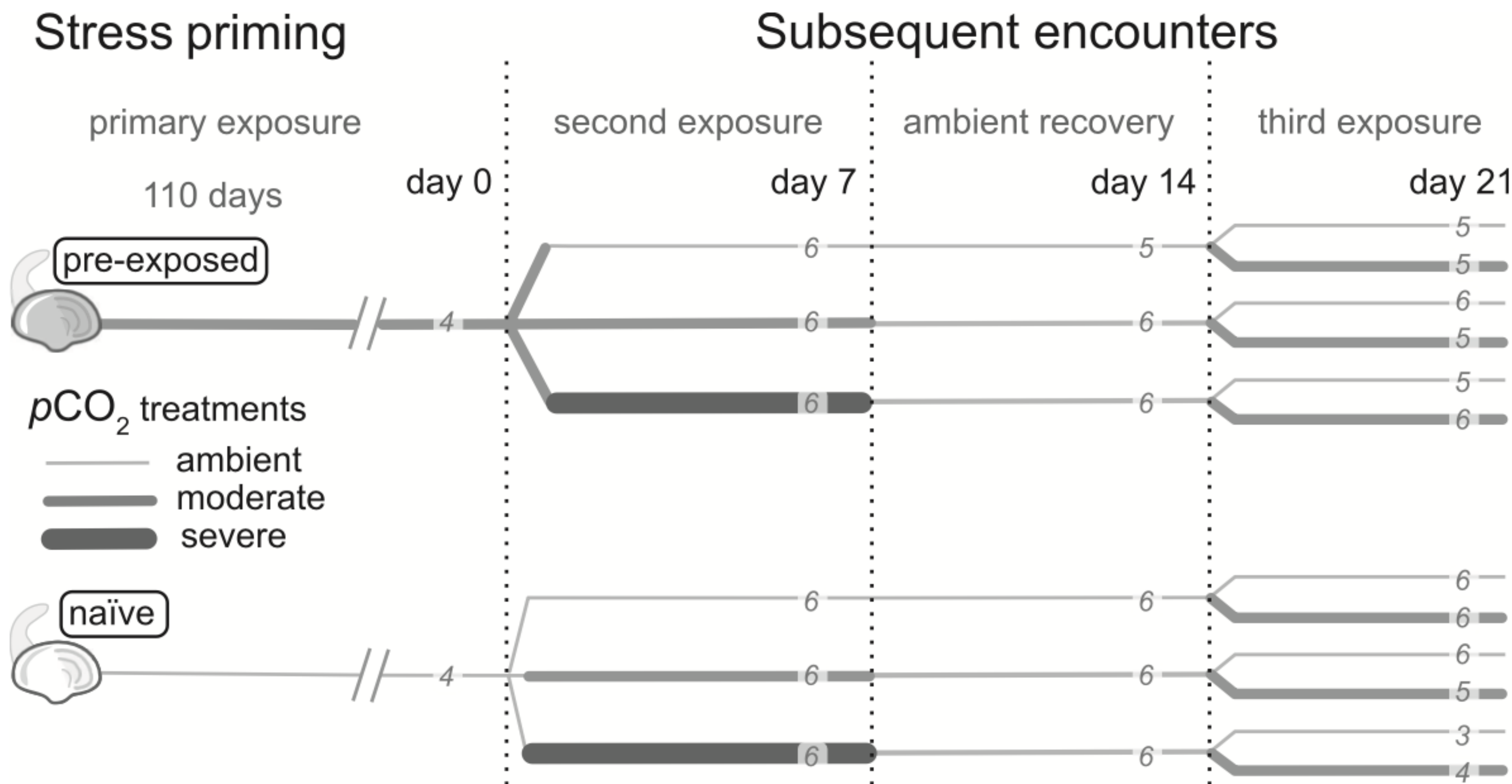
Haipei Liu ¹, Amanda J. Able ¹ and Jason A. Able ^{1,@,*}

Cross-stress priming success relies on synergistic stress signaling pathways being shared across stresses varying in nature and intensity.

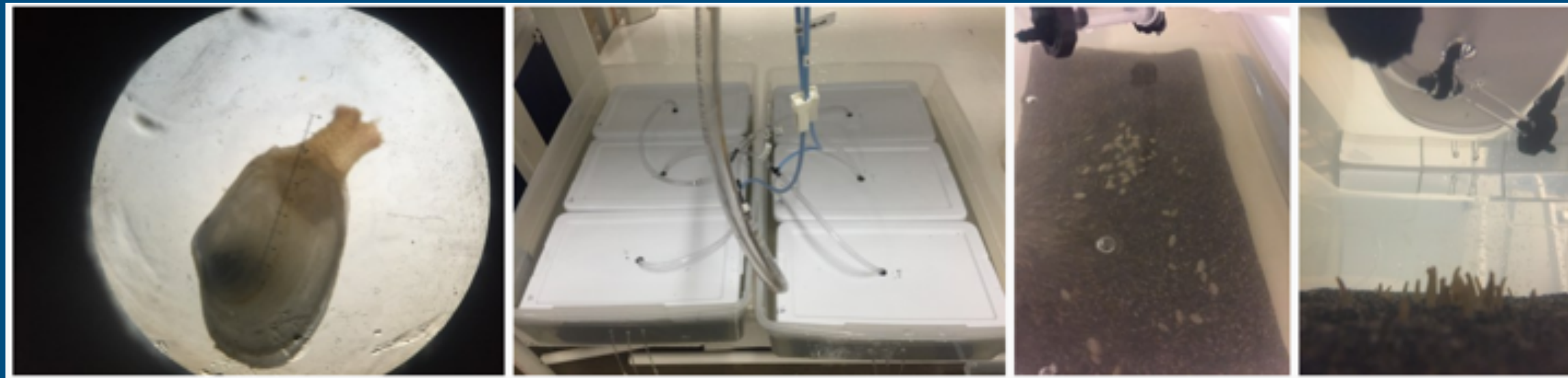
Geoduck Clams

Repeat exposure to hypercapnic seawater modifies growth and oxidative status in a tolerant burrowing clam

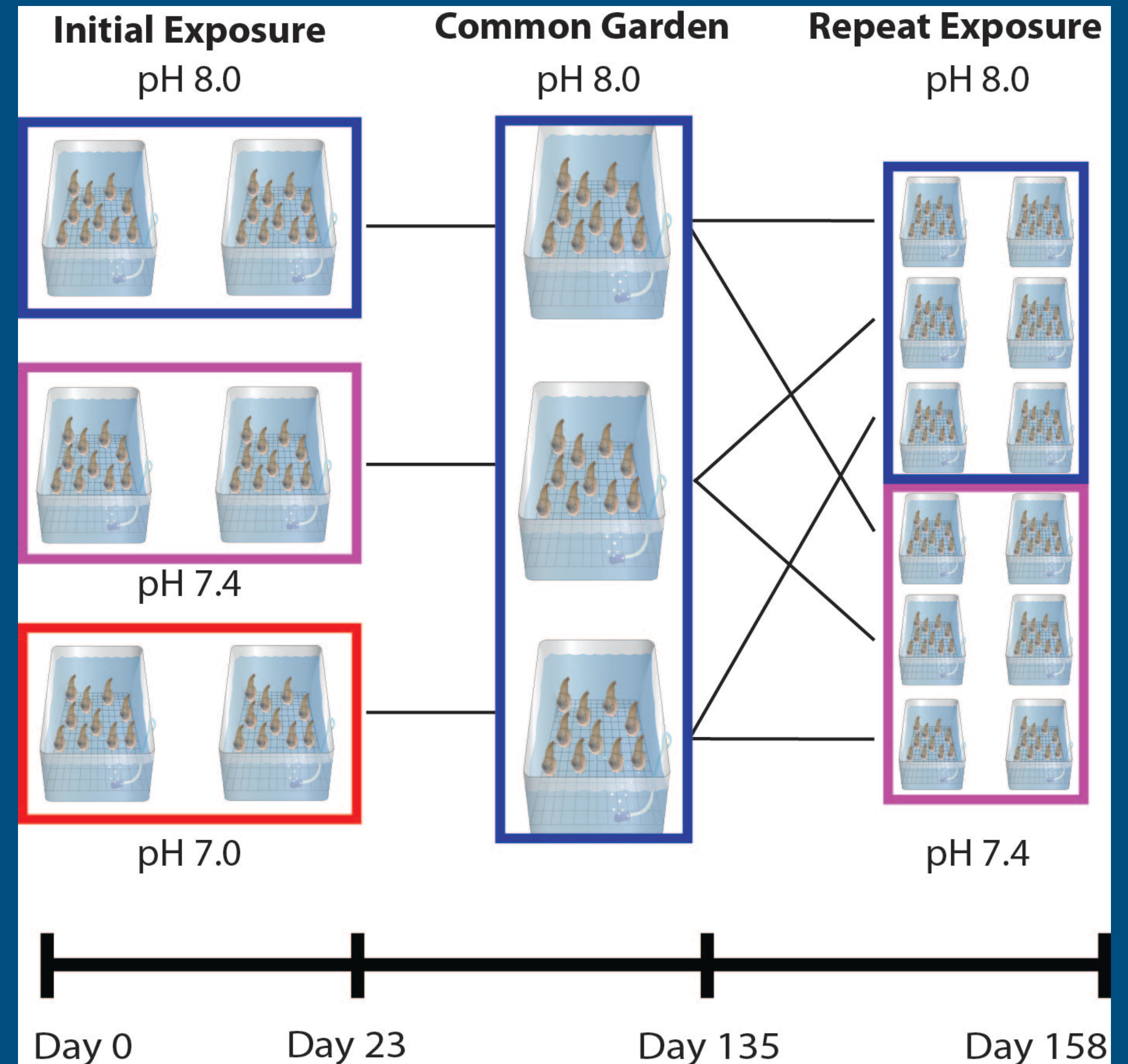
Samuel J. Gurr^{1,*}, Shelly A. Wanamaker², Brent Vadopalas³, Steven B. Roberts² and Hollie M. Putnam¹



GEODUCKS CLAMS

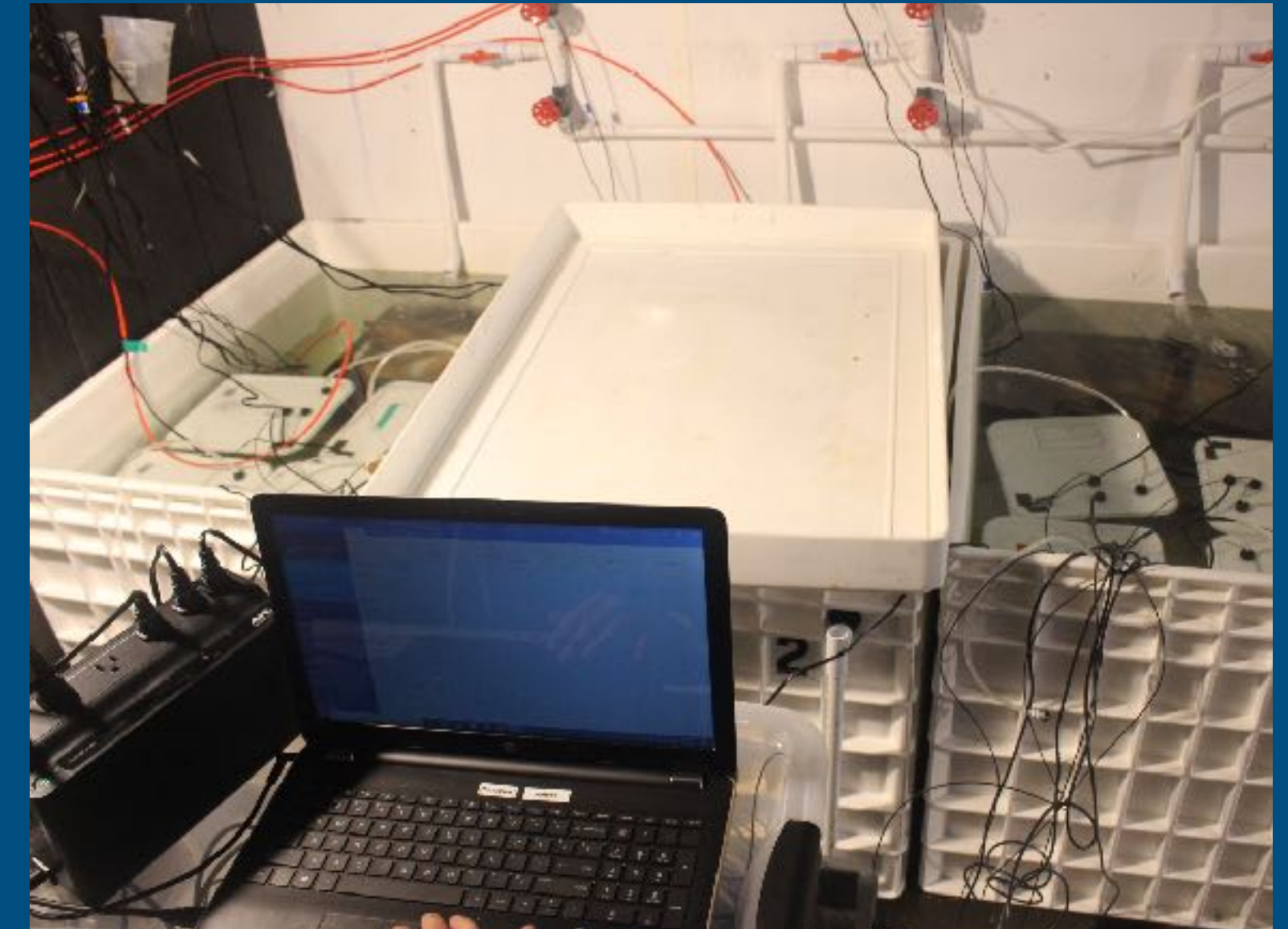


- ▶ Does conditioning to low pH confer tolerance within a generation?



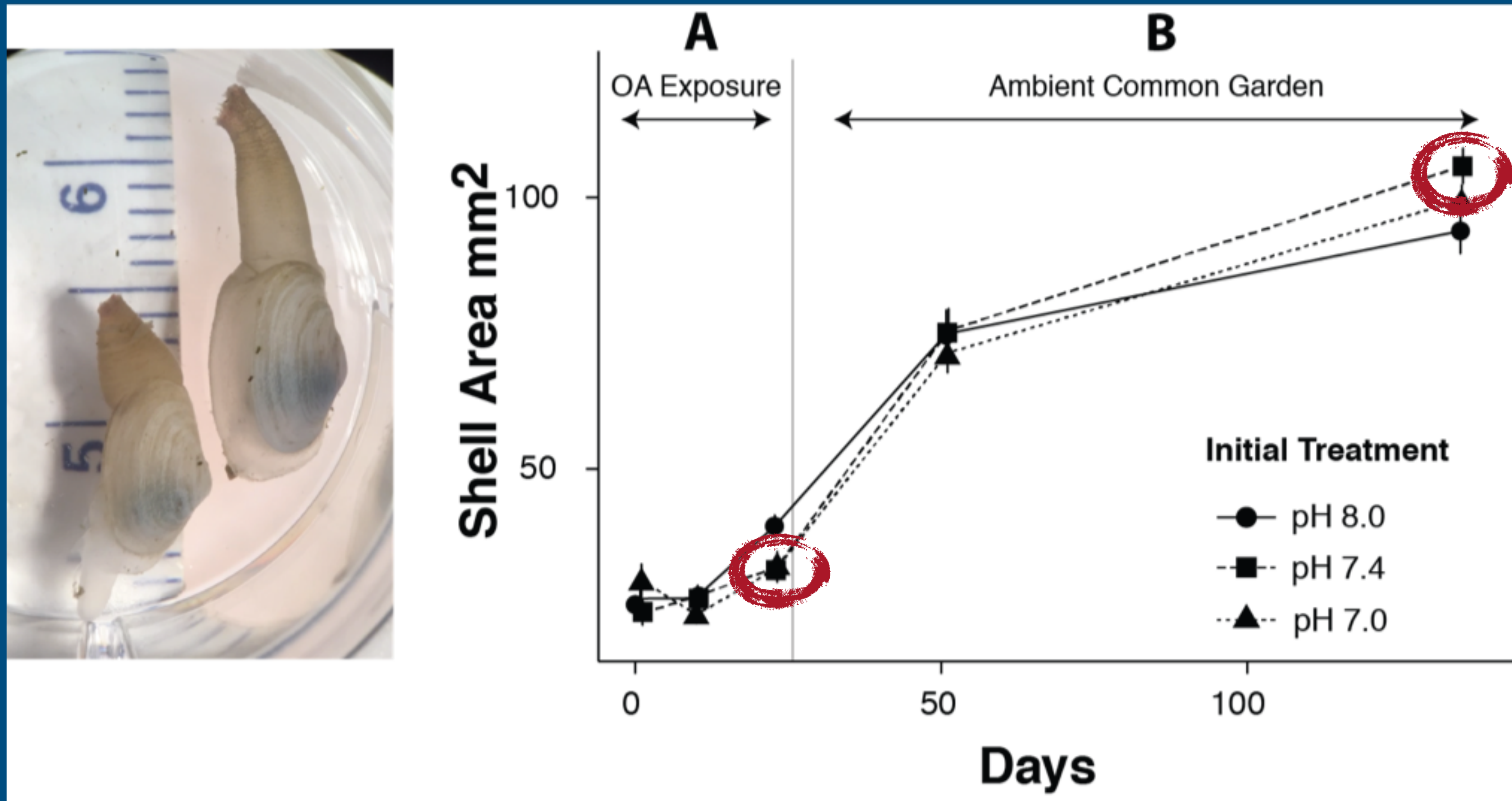
HOLLIE PUTNAM, SAM GURR, BRENT VADOPALAS, SHELLY TRIGG, JAMESTOWN S'KLALLAM TRIBE

GEODUCKS CLAMS



GEODUCKS CLAMS

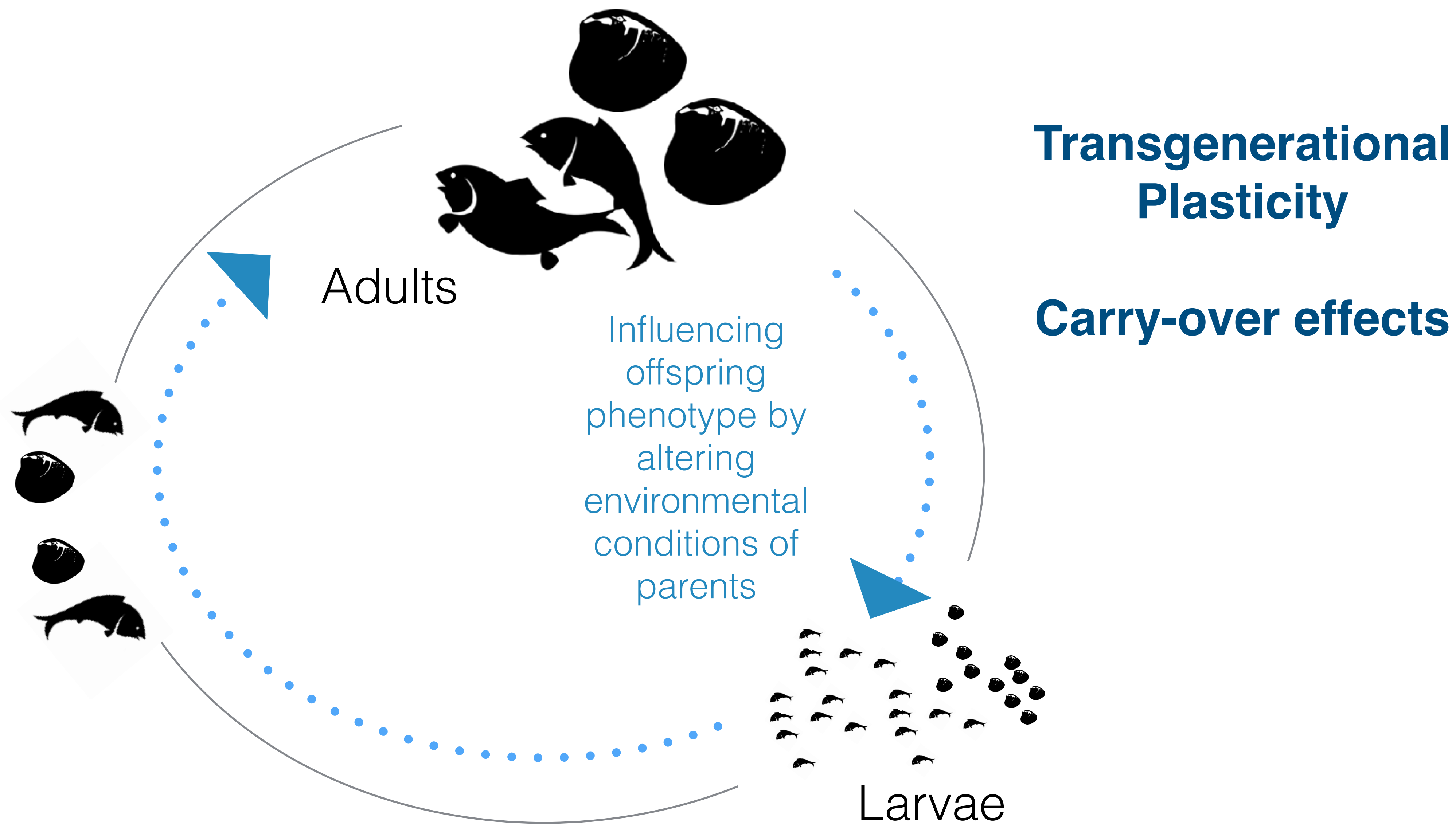
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Priming Across generation

Industry Concerns



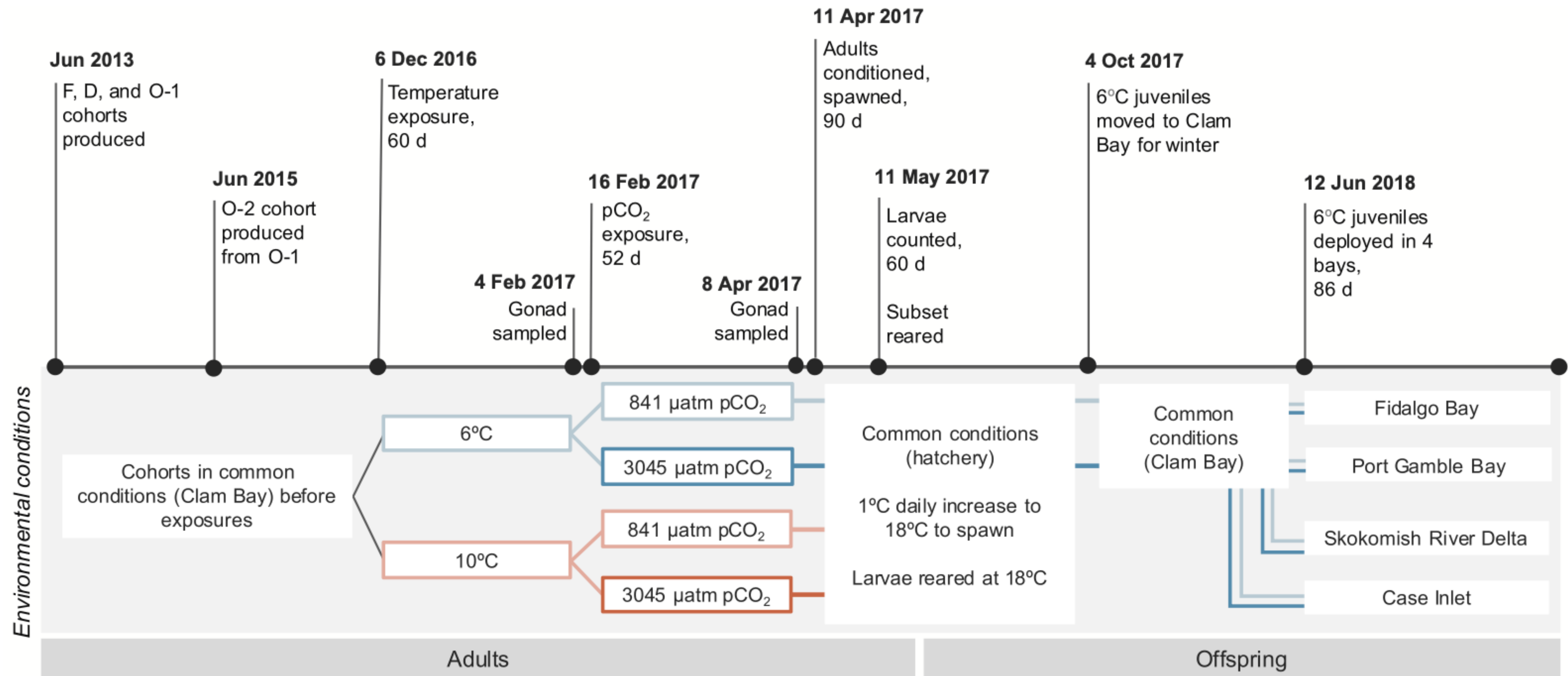
EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS



- ▶ Oysters were held at two temperature regimes (6°C and 10°C) for 60 days in December
- ▶ A differential pCO₂ exposure was carried out after the temperature treatment ended. Held at ambient pCO₂ (841 µatm) or high pCO₂ (3045 µatm) for 52 days, during the Winter.

Carryover effects of temperature and pCO₂ across multiple Olympia oyster populations

LAURA H. SPENCER,¹ YAAMINI R. VENKATARAMAN,¹ RYAN CRIM,² STUART RYAN,² MICAH J. HORWITH,³ AND STEVEN B. ROBERTS^{1,4}

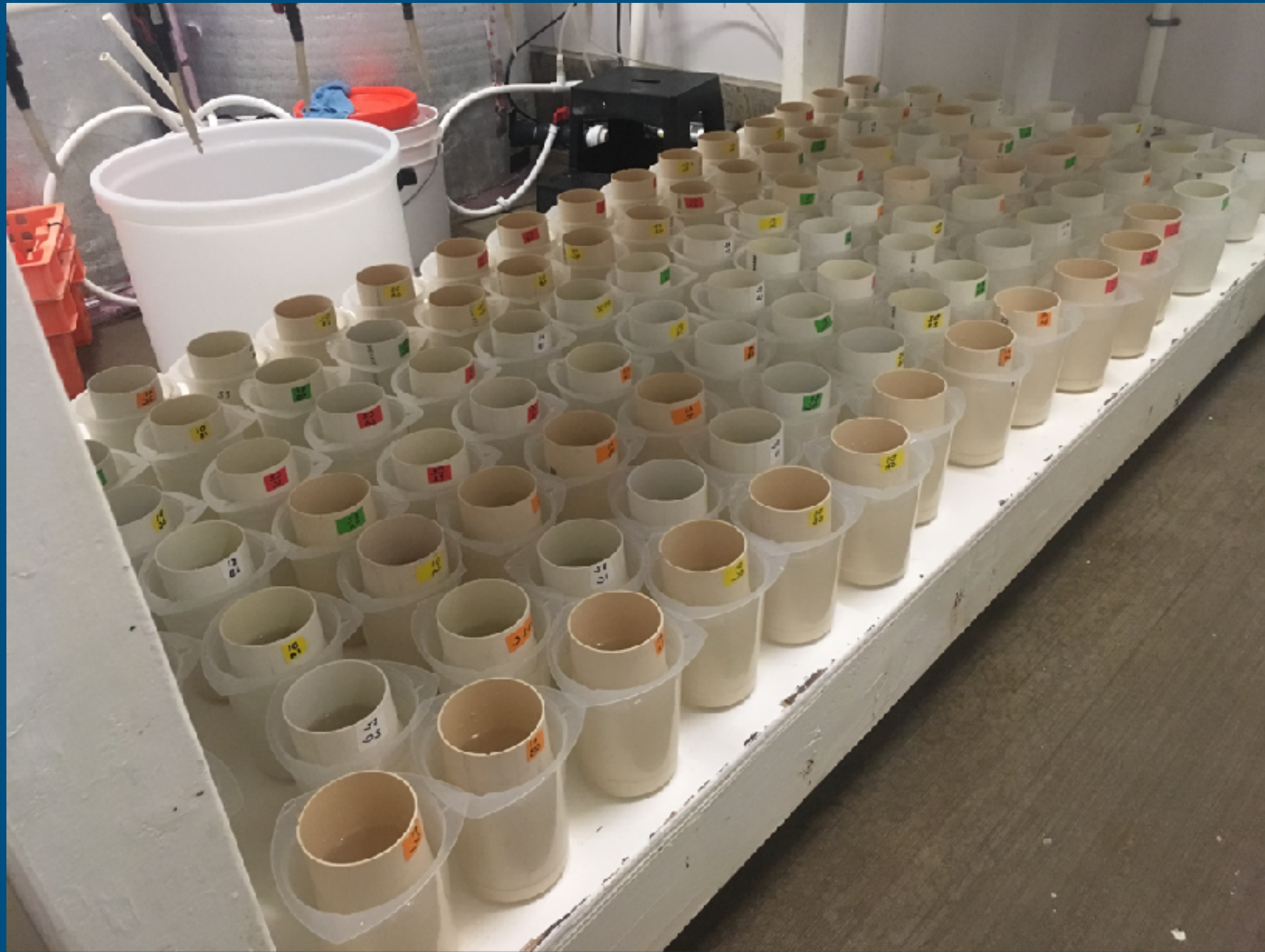


LAURA SPENCER

EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS

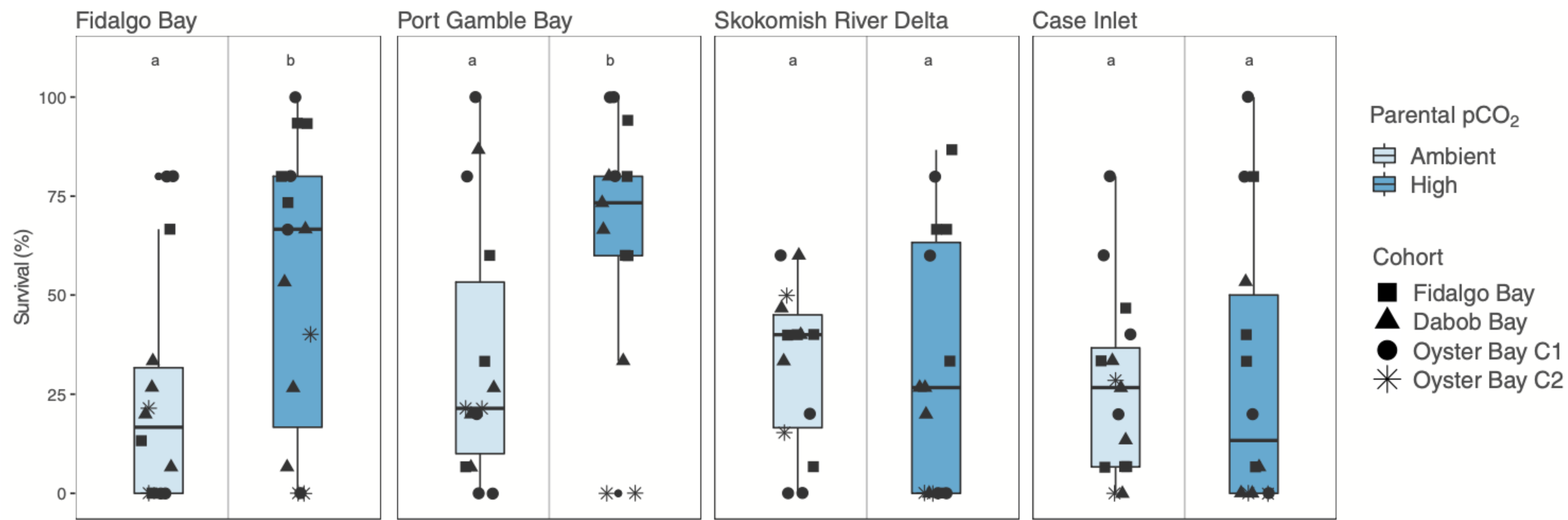


EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS



- ▶ 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 pCO₂ had higher survival rates in the natural environment**

EFFECTS OF OA IN OLYMPIA OYSTER POPULATIONS



Eastern Oyster

LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 67, 2022, 1732–1745
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doi: 10.1002/lno.12162

Parental exposure of Eastern oysters (*Crassostrea virginica*) to elevated $p\text{CO}_2$ mitigates its negative effects on early larval shell growth and morphology

Elise M. McNally ,* Alan M. Downey-Wall, F. Dylan Titmuss, Camila Cortina, Kathleen Lotterhos ,
Justin B. Ries

Department of Marine and Environmental Sciences, Marine Science Center, Northeastern University, Nahant, Massachusetts

Transgenerational plasticity in early larval shell growth and morphology, but not in survival, in response to the parental $p\text{CO}_2$ exposure. Larvae from parents exposed to elevated $p\text{CO}_2$ exhibited faster shell growth rates than larvae from control parents, with this effect being significantly larger when larvae were grown under elevated $p\text{CO}_2$



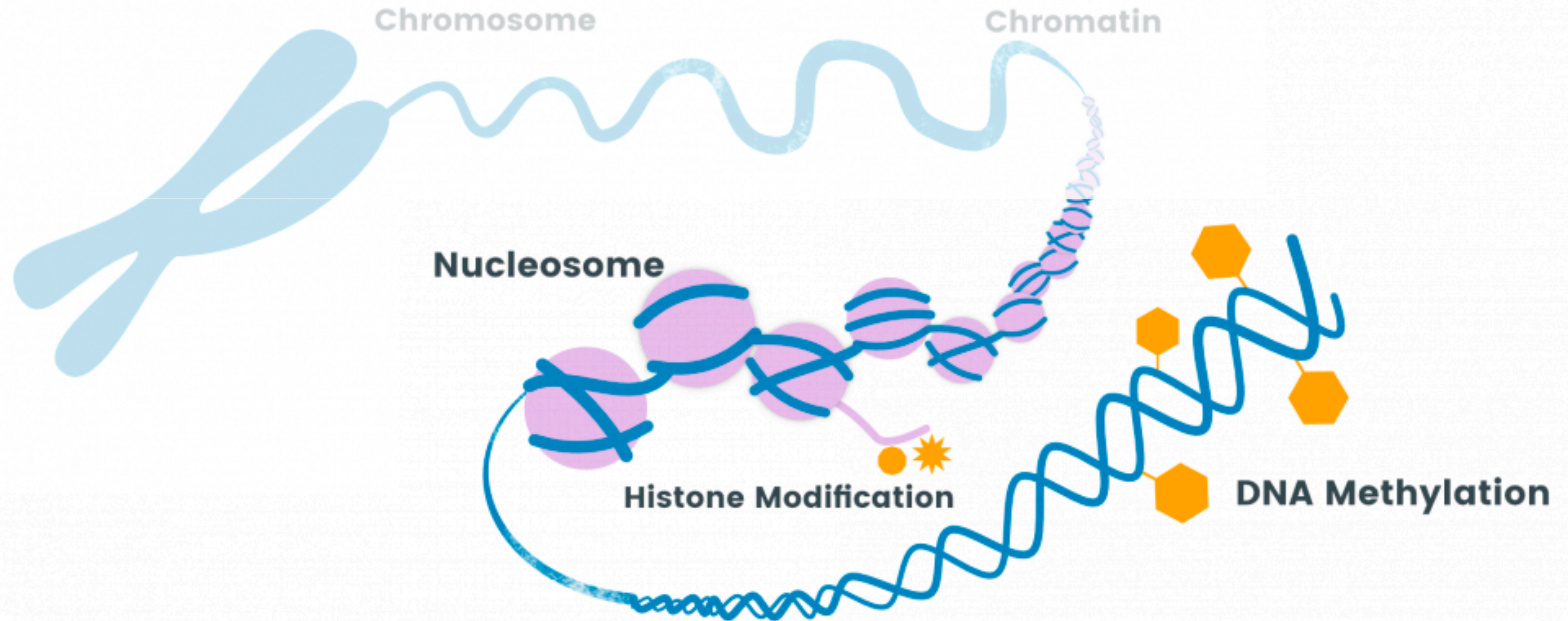
Priming

*Within
Across generation*

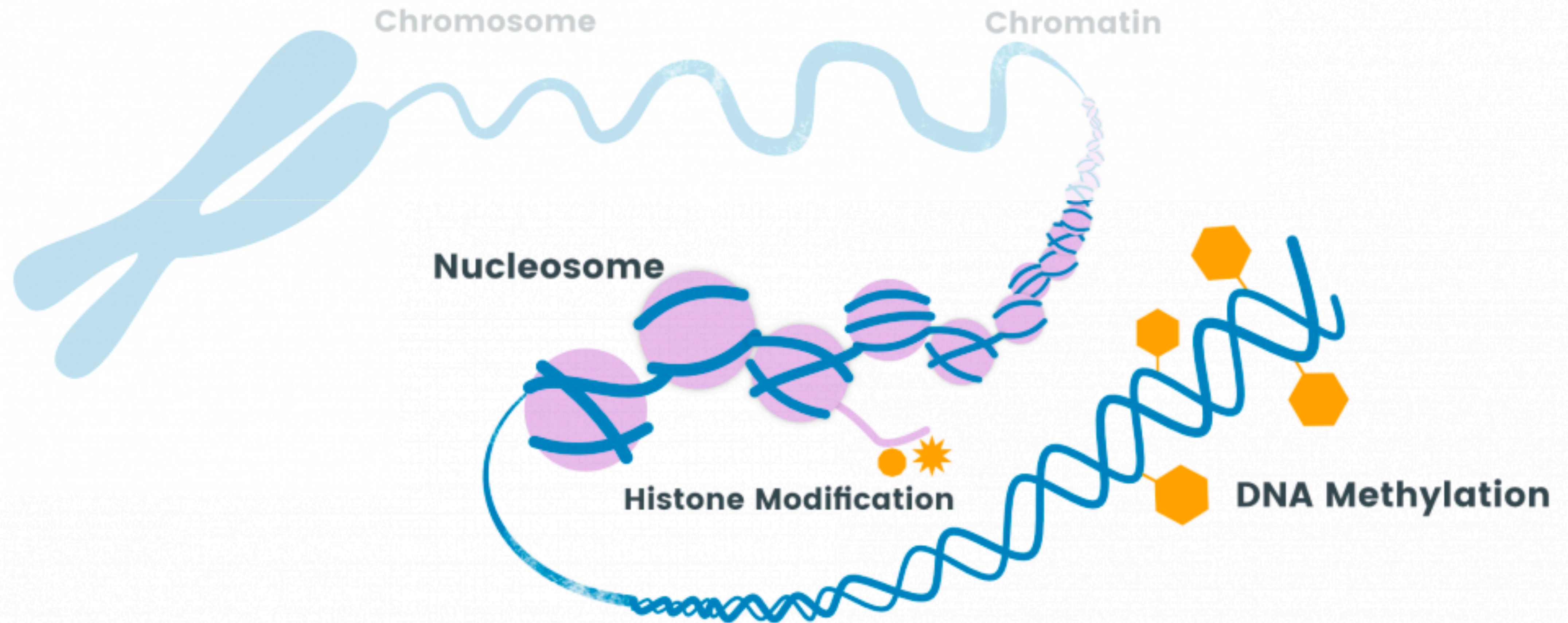
Mechanism

Industry Concerns

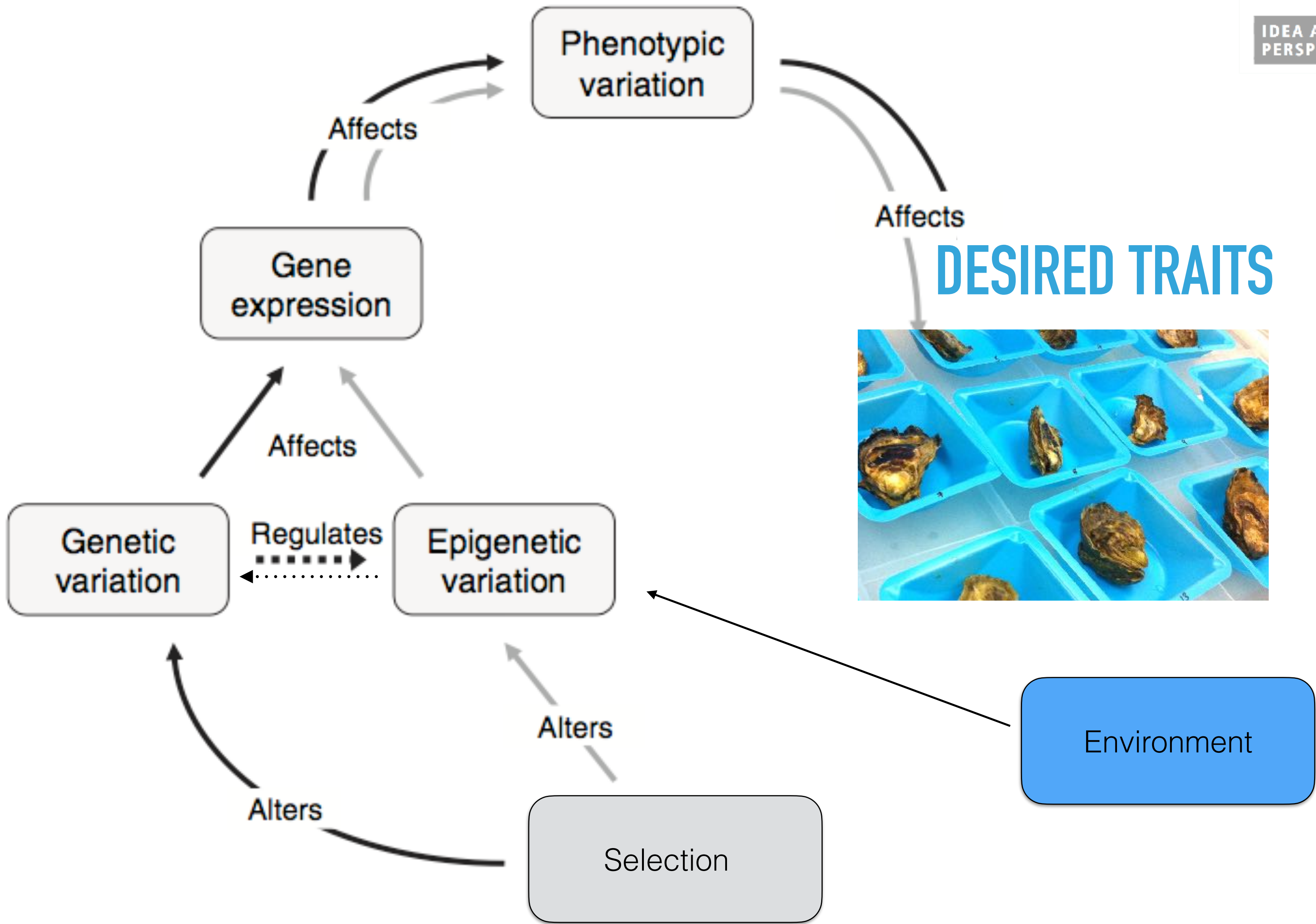
ALTERS THE PHENOTYPE (WITHOUT CHANGING DNA CODE); HERITABLE



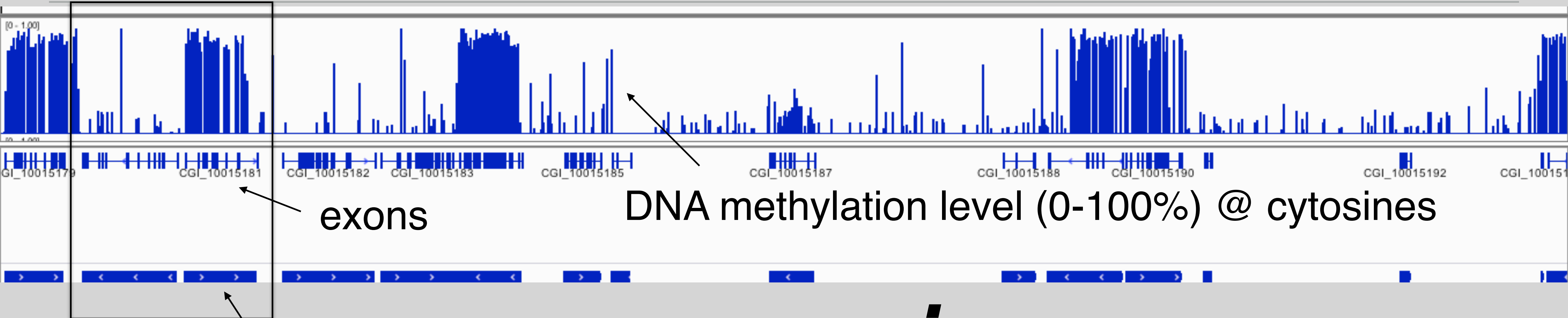
ALTERS THE PHENOTYPE (WITHOUT CHANGING DNA CODE); HERITABLE



CAN BE INDUCED WITH ENVIRONMENTAL MANIPULATION



METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



DNA methylation level (0-100%) @ cytosines

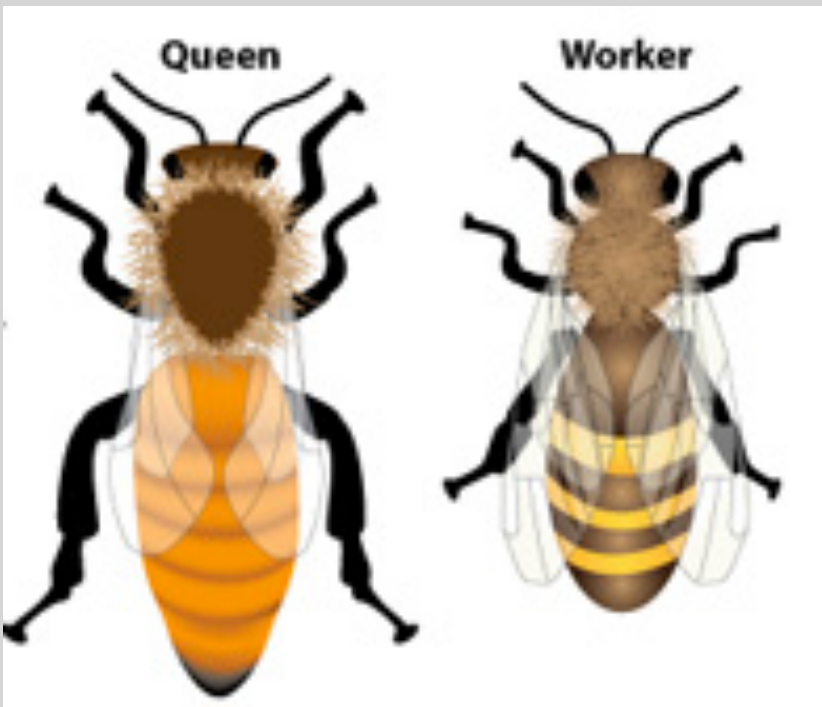
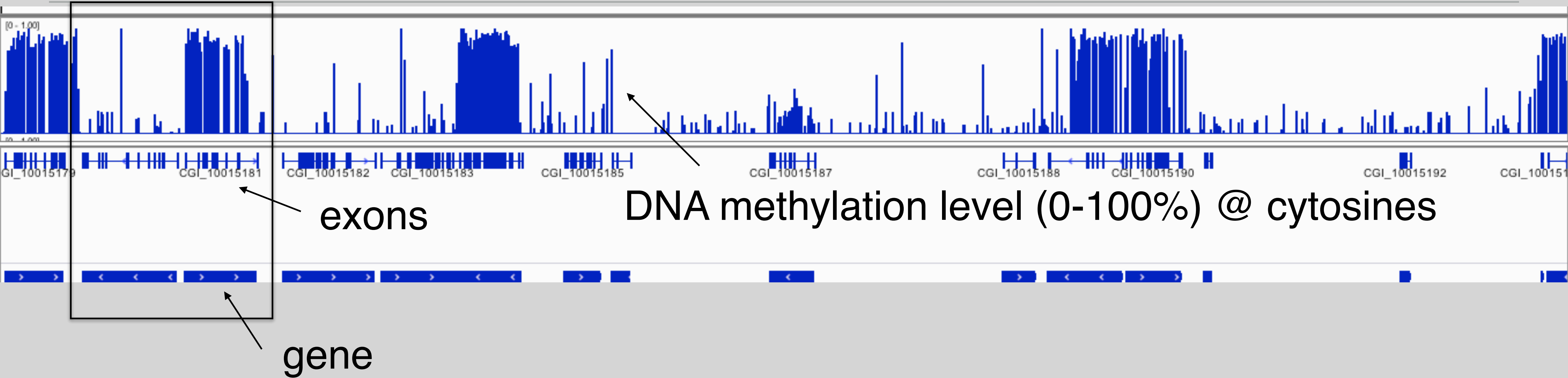
exons

gene

mosaic

associated with gene bodies

METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



These Two Mice are Genetically Identical and the Same Age

While pregnant, both of their mothers were fed Bisphenol A (BPA) but DIFFERENT DIETS:

The mother of this mouse received a **normal mouse diet**

The mother of this mouse received a diet **supplemented** with choline, folic acid, betaine and vitamin B12

High Nurtured

Low Nurtured

These mothers come from a large line of inbred rats, so their genomes are highly similar. But they care for their pups very differently.

AUDIO

Four Dimensionalities



Targeted Regulation

Stochastic Regulation

Reliable Transcription

Spurious Transcription

Four Dimensionalities

- Evolutionary
- Life History Driven
- Constitutive

Reliable Transcription

Spurious Transcription

Four Dimensionalities

- Distinct Lineage
- Experiential
- Inducible

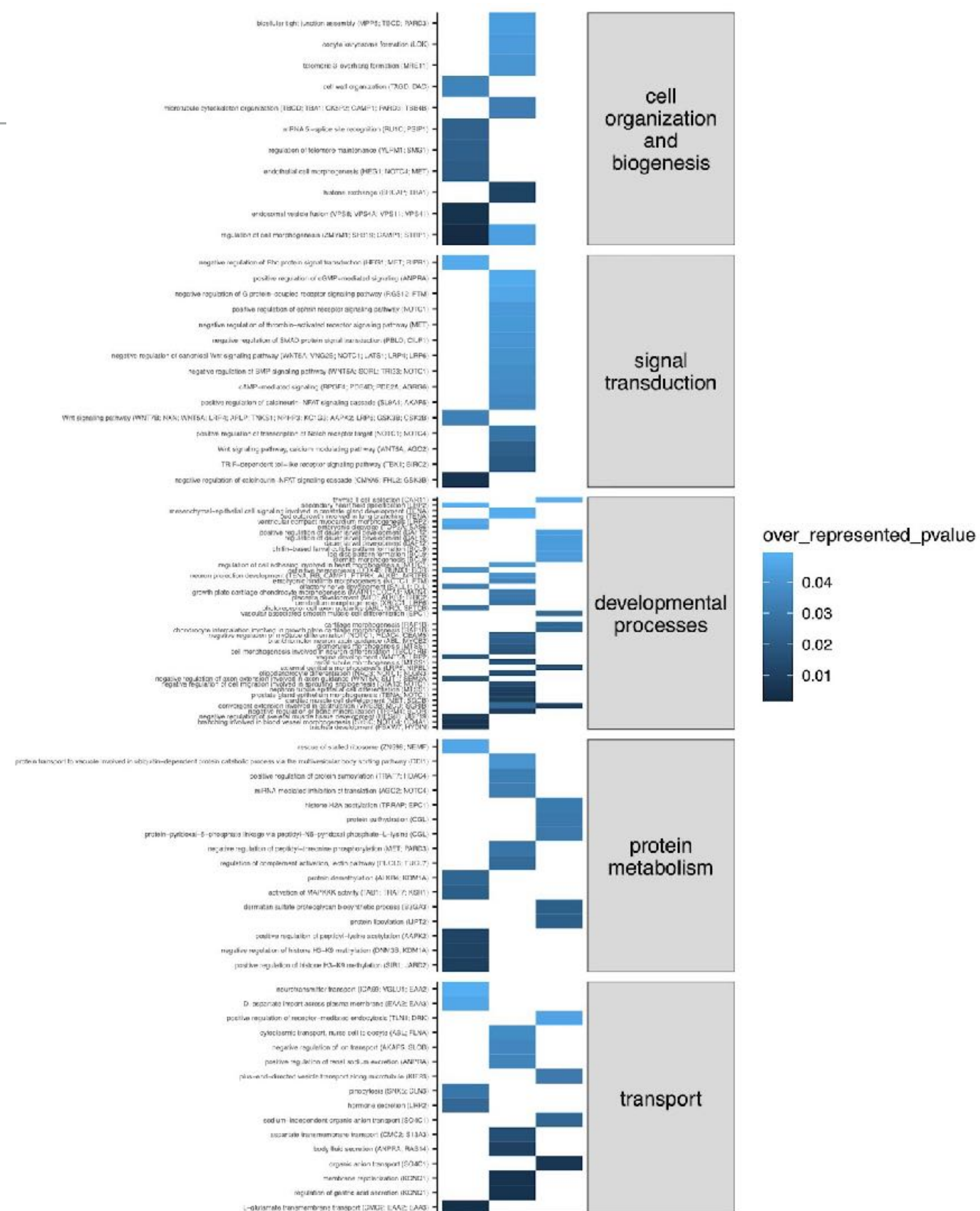
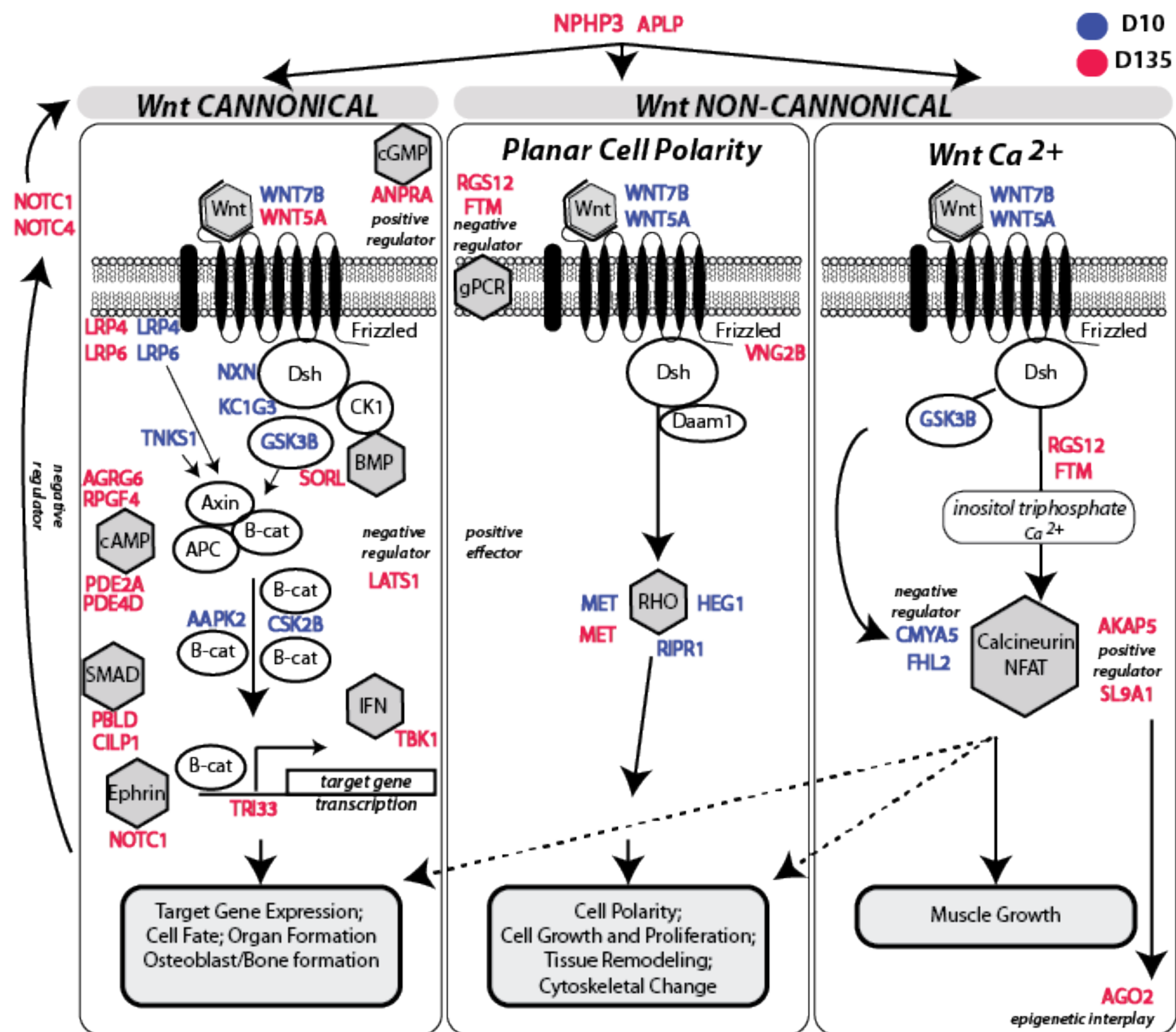
Targeted Regulation

Stochastic Regulation

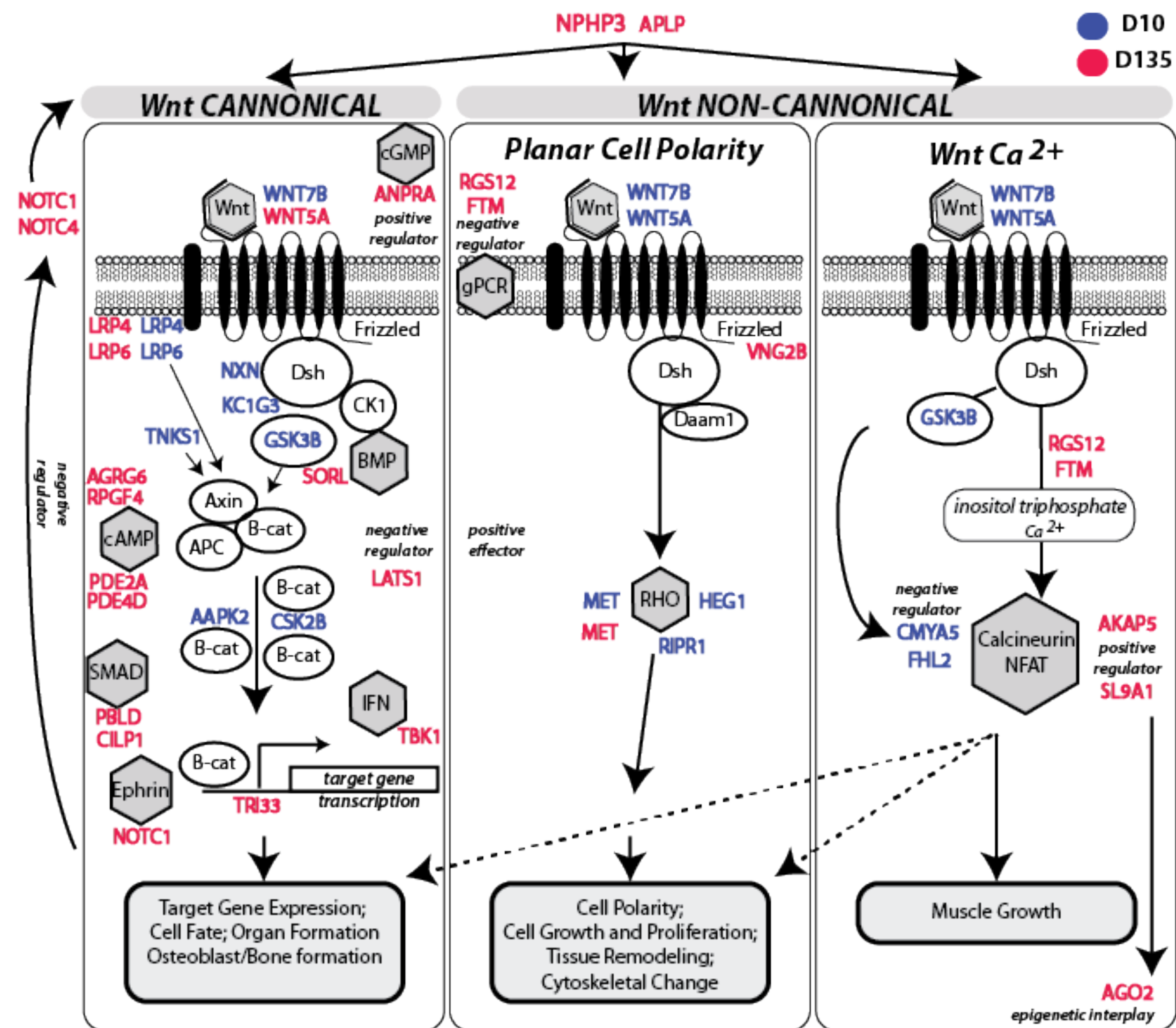
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Reliable Transcription

Spurious Transcription



GEODUCKS AND OA



New Results

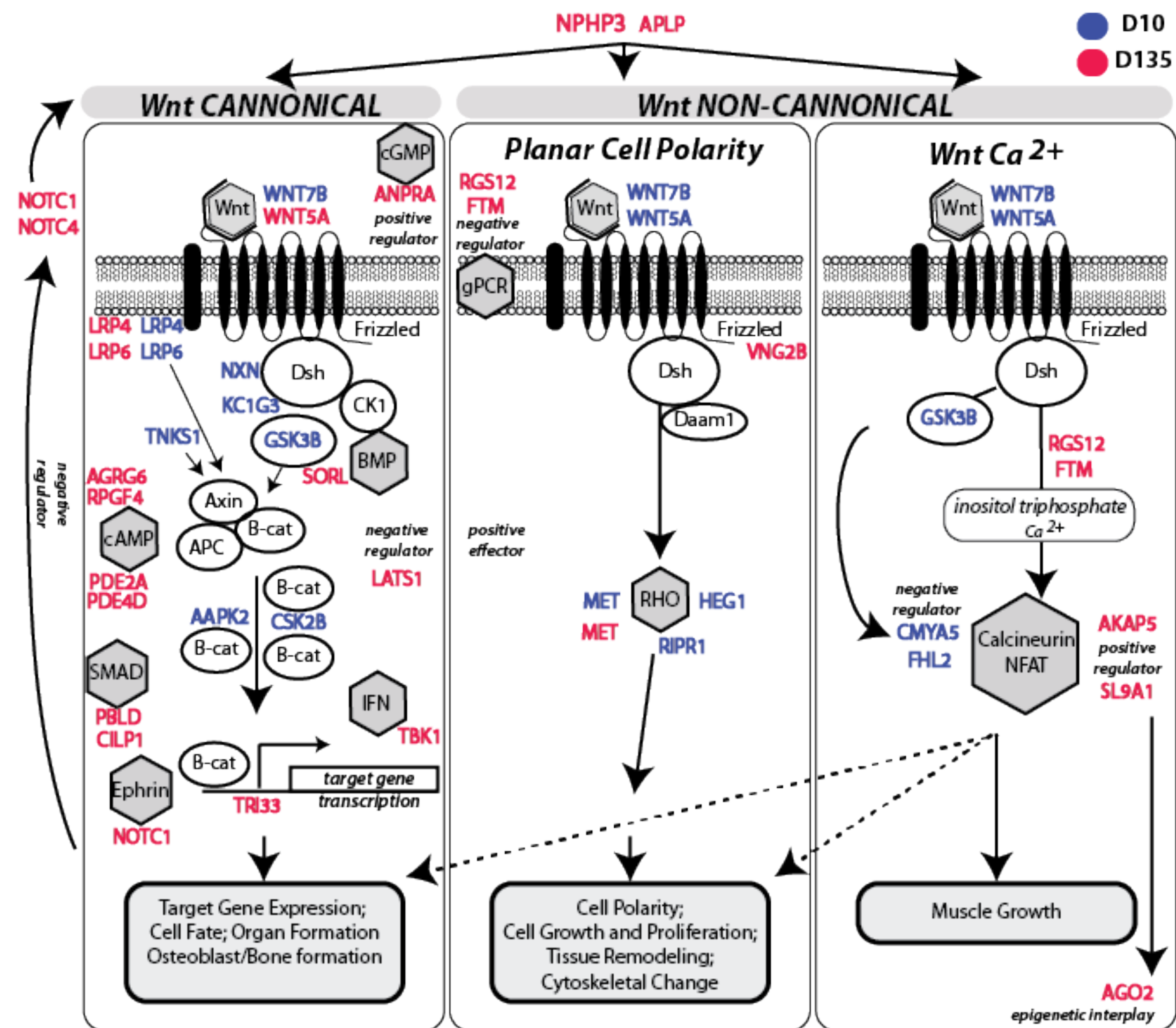
[Follow this preprint](#)

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.

EPIGENETIC MECHANISM INVOLVED?

LIMNOLOGY
and
OCEANOGRAPHY

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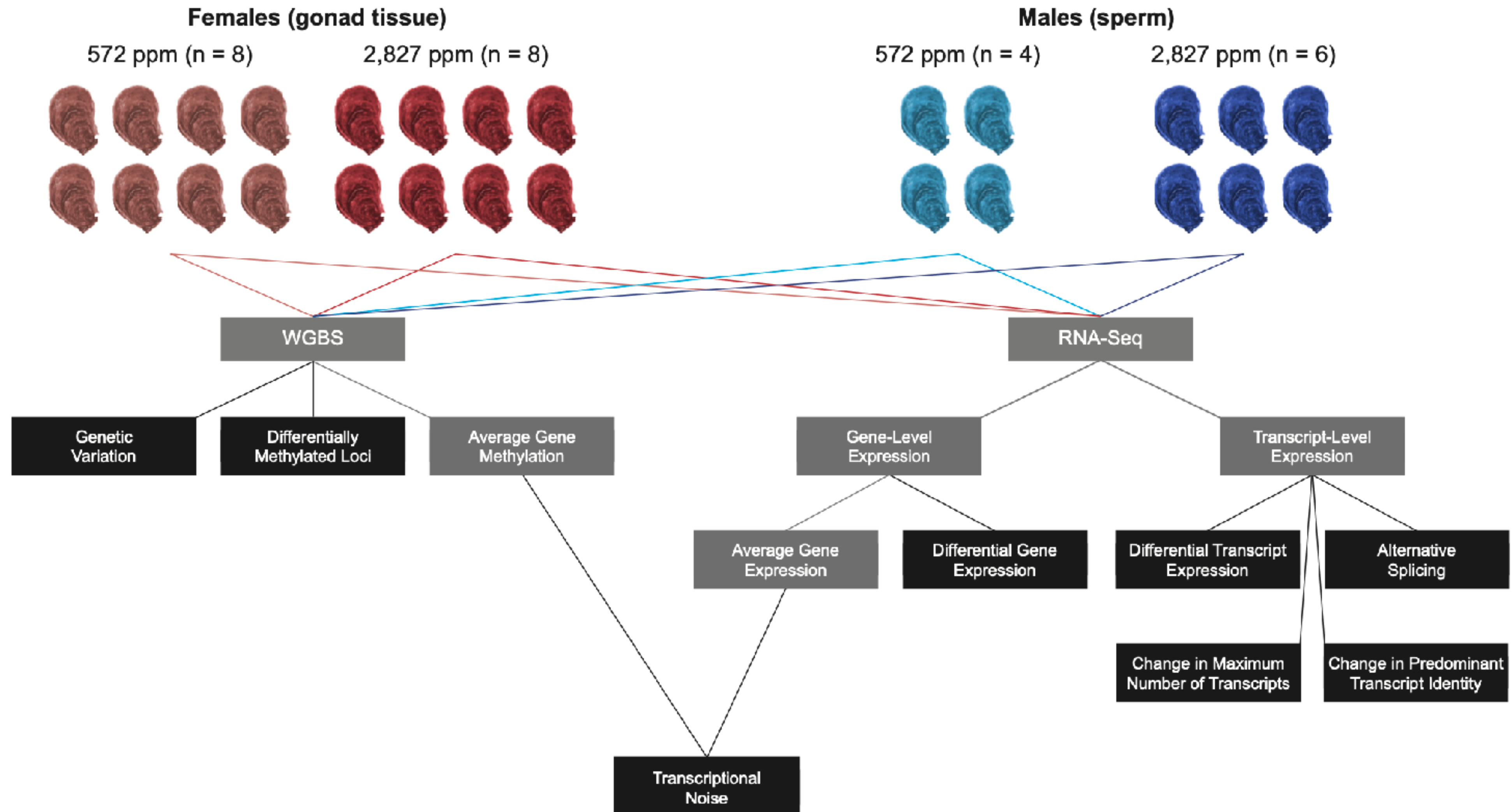
EPIGENETIC MECHANISM INVOLVED?

DNA methylation correlates with transcriptional noise in response to elevated pCO₂ in the eastern oyster (*Crassostrea virginica*)

Yaamini R. Venkataraman^{1*}, Ariana S. Huffmyer^{2,3}, Samuel J. White², Alan Downey-Wall⁴, Jill Ashey³, Danielle M. Becker³, Zachary Bengtsson², Hollie M. Putnam³, Emma Strand^{3,5}, Javier A. Rodríguez-Casariago⁶, Shelly A. Wanamaker⁵, Kathleen E. Lotterhos⁷, Steven B. Roberts²

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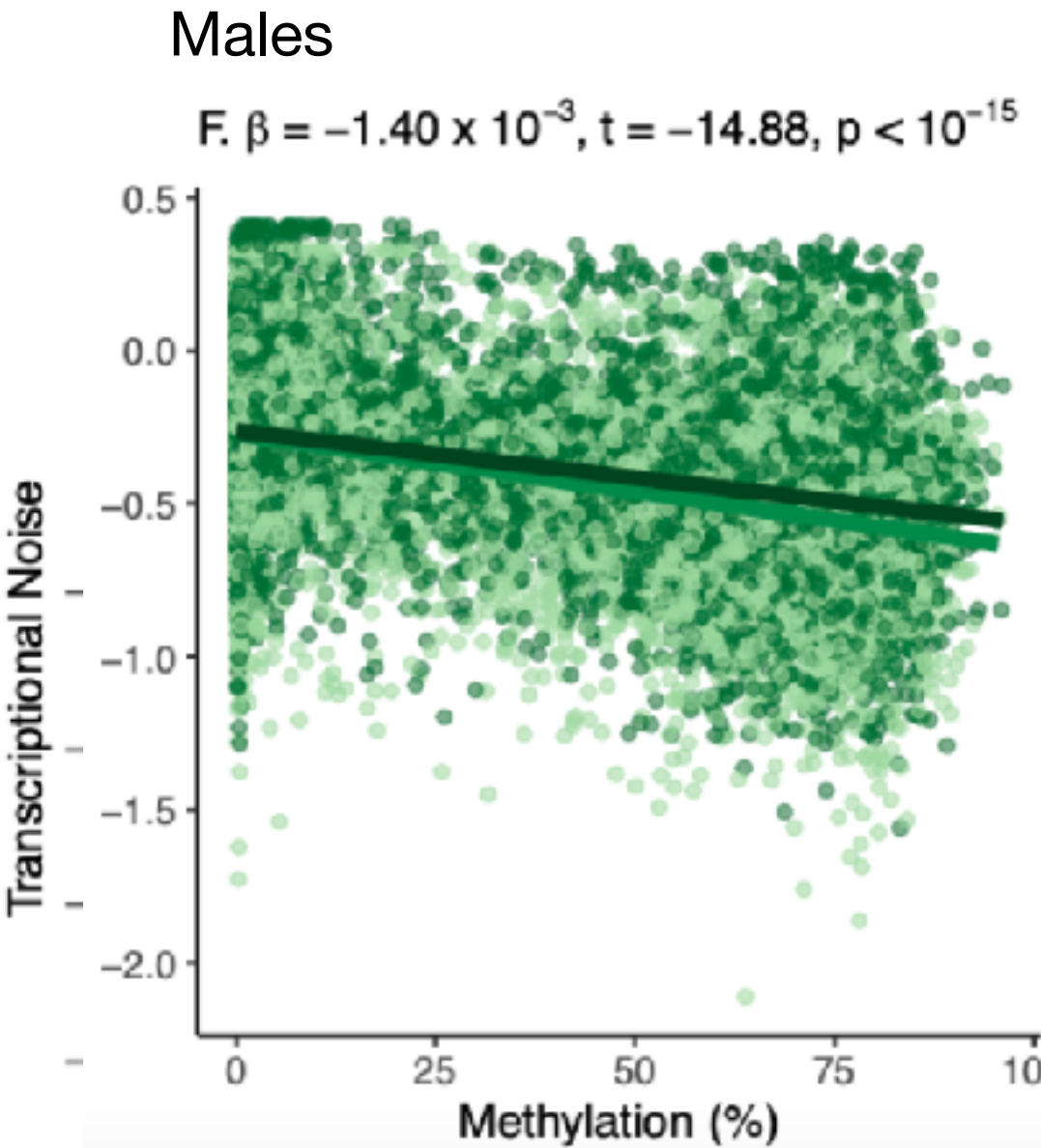
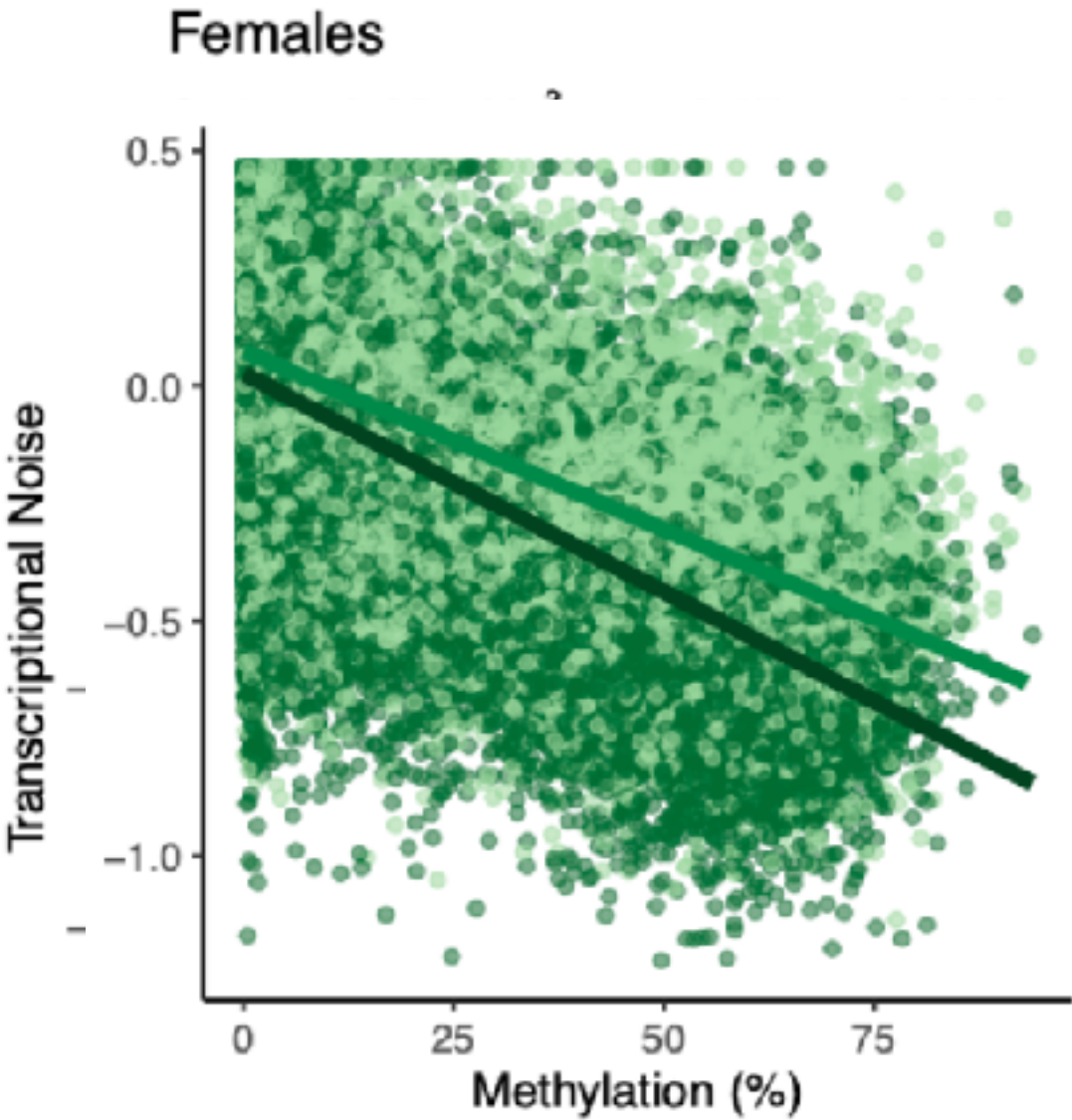
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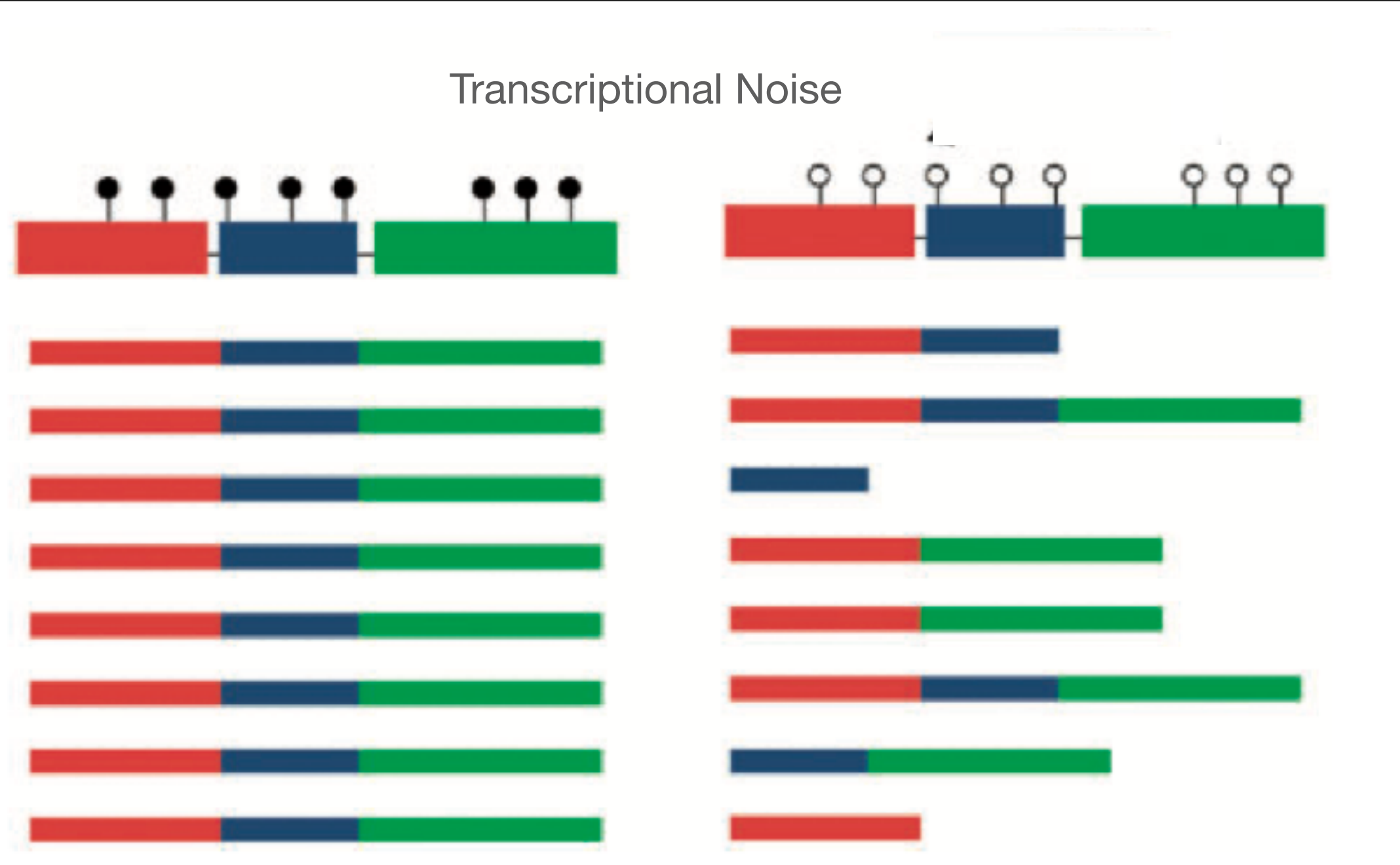
Gene body methylation impacts maximum number of transcripts expressed per gene and changes in the predominant transcript expressed. Elevated pCO₂ exposure increased gene expression variability (transcriptional noise) in males but decreased noise in females, suggesting a sex-specific role of methylation in gene expression regulation.

VENKATARAMAN: DNA METHYLATION CORRELATES WITH TRANSCRIPTIONAL NOISE

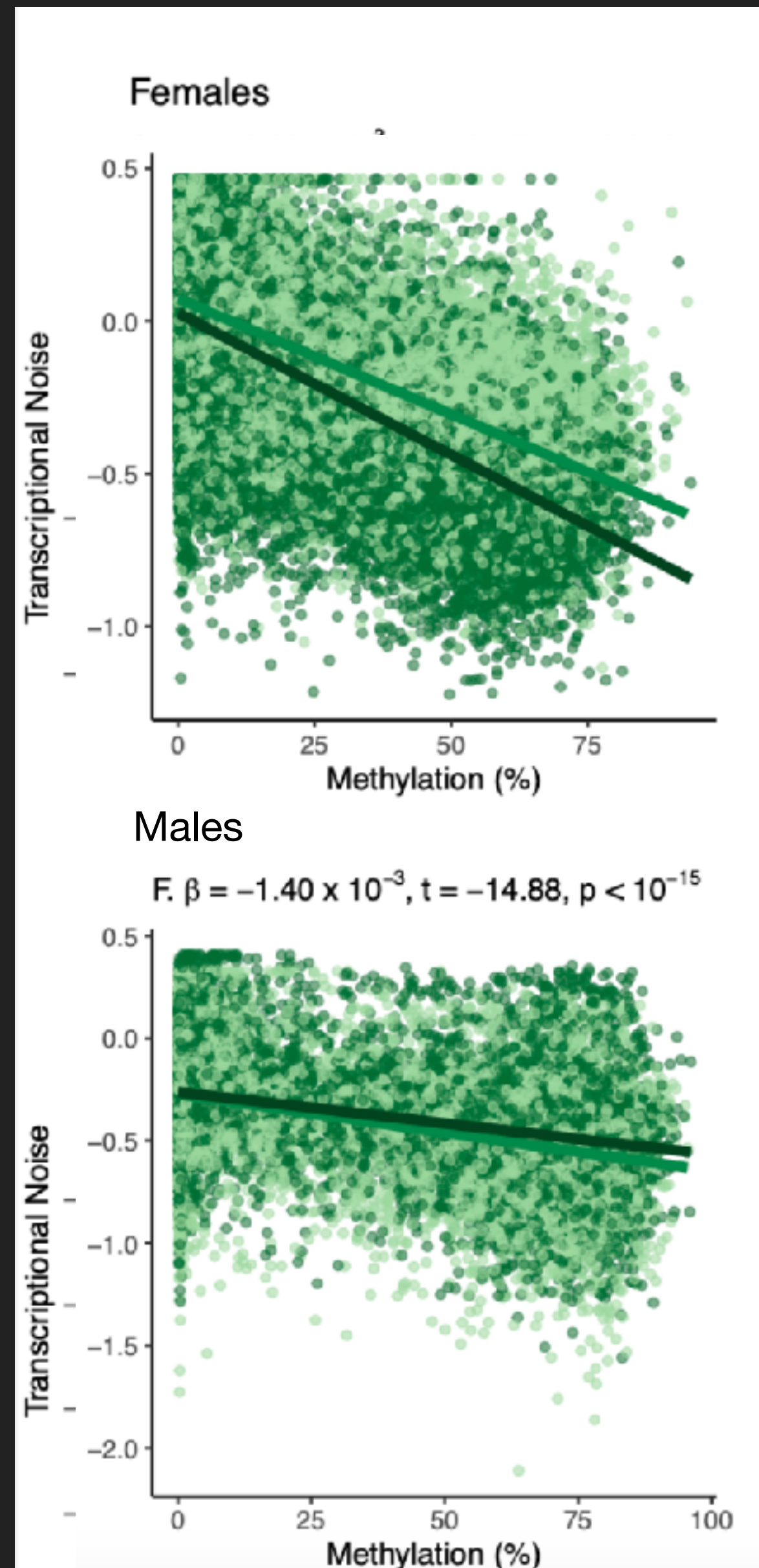


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Methylation has a genome-wide regulatory role, effectively maintaining gene expression homeostasis in reproductive tissues under elevated pCO₂ by reducing transcriptional noise.

The relationship between methylation and transcriptional noise was different between female reproductive tissue and sperm: male oysters required higher levels of methylation to achieve similar reductions in transcriptional noise when compared to female oysters.

Epigenetic maintenance of reproduction could confer intergenerational resilience to environmental perturbations.

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Males

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Looking at Offspring now

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EXCITING?
COMPLEX
'LAYER' OF RESILIENCE

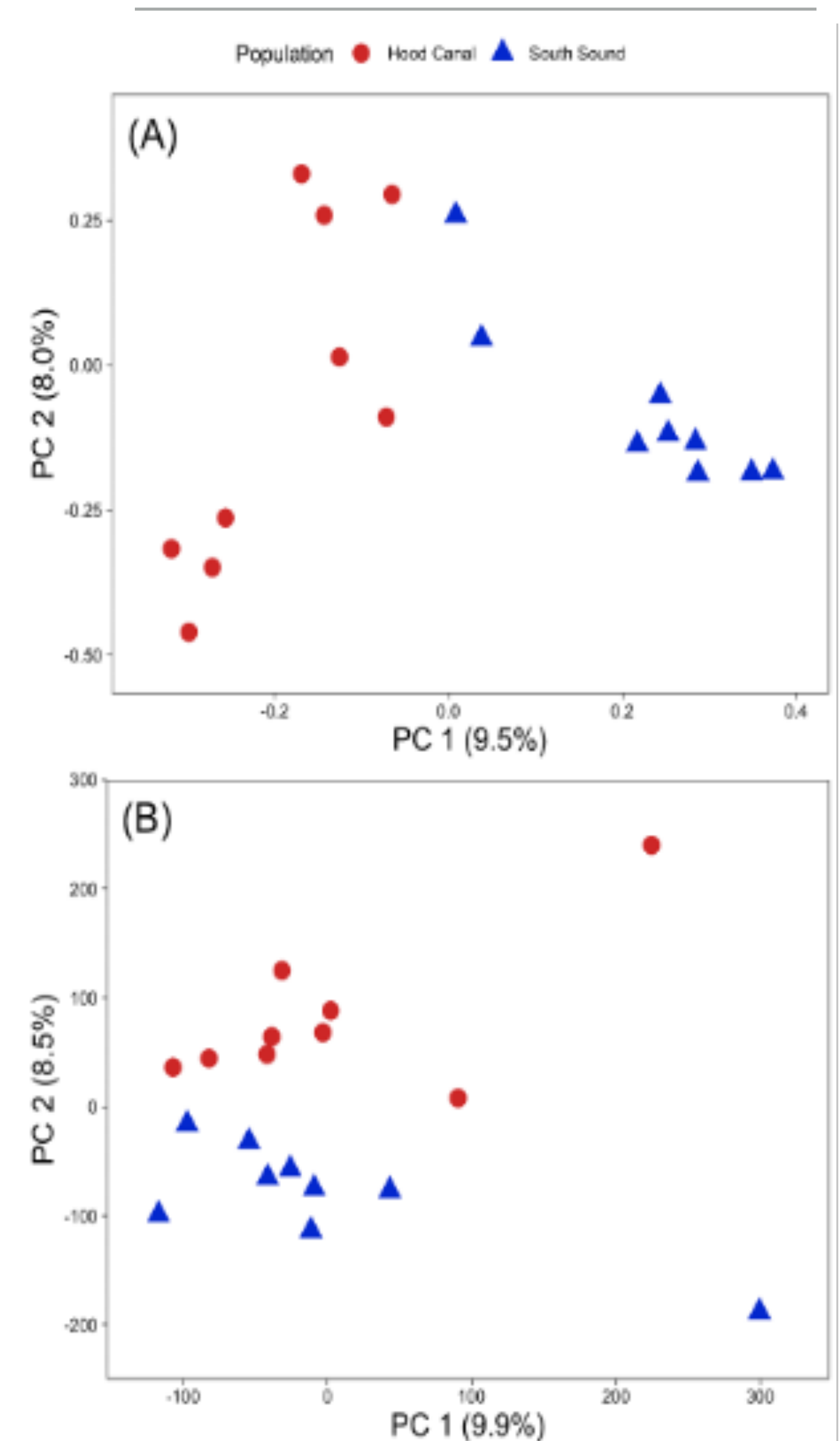
Epigenetic and Genetic Population Structure is Coupled in a Marine Invertebrate

Katherine Silliman ^{1,†}, Laura H. Spencer ^{2,†}, Samuel J. White², and Steven B. Roberts ^{2,*}

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

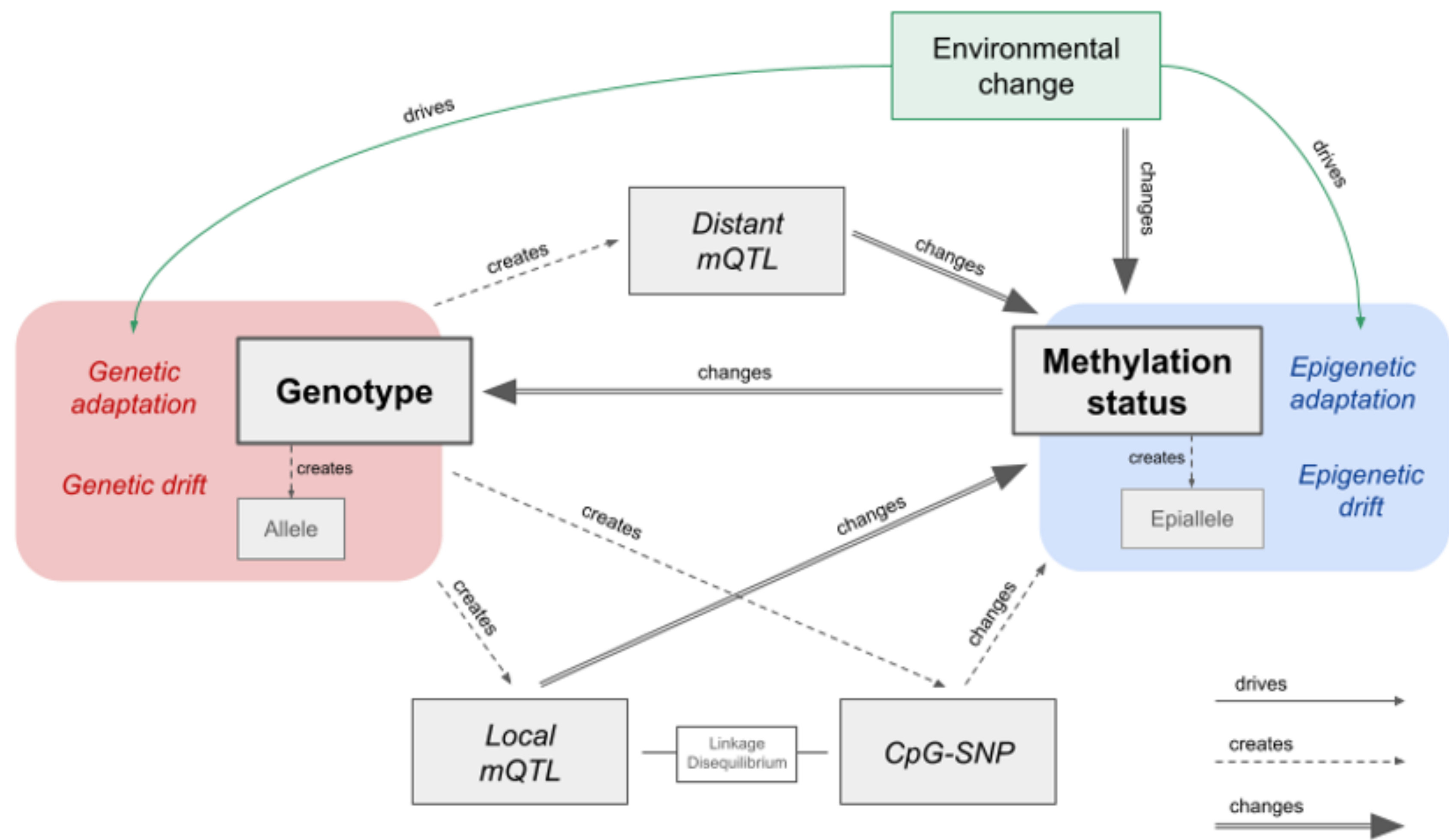
Identified 3,963 differentially methylated loci between populations. Clear coupling between genetic and epigenetic patterns of variation, **with 27% of variation in inter-individual methylation differences explained by genotype.**

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





Current work

Mechanism

Priming

*Within
Across generation*

Industry Concerns

Pacific Oyster

Ongoing Efforts



Emily Carrington - UW Biology



Pacific Oyster

Ongoing Efforts

- **Priming**
 - Immune Challenge
 - Temperature
 - Salinity
 - Multiple
- **Performance Testing**
 - Survival
 - Growth
 - Metabolism*



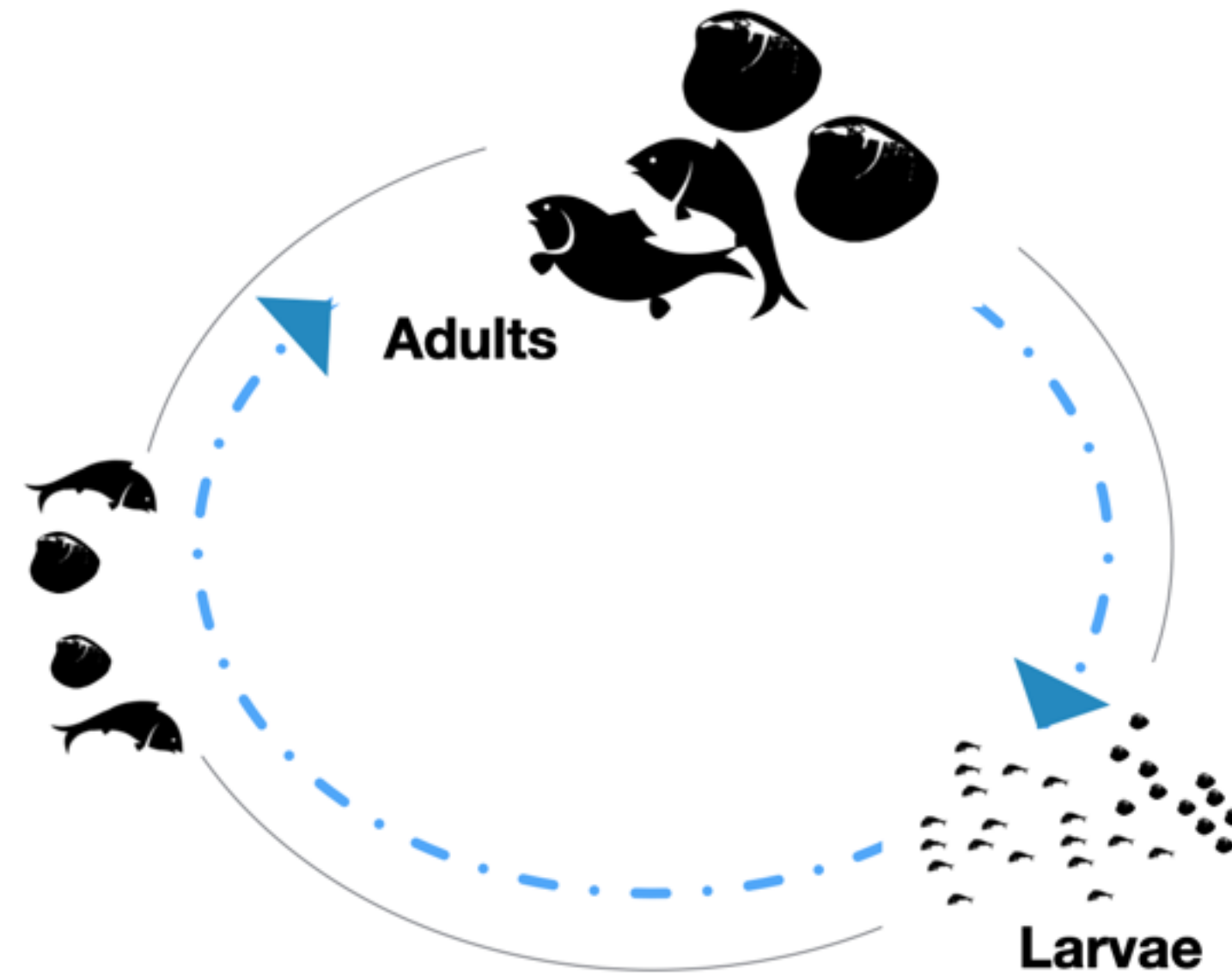
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Pacific Oyster

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Pacific Oyster

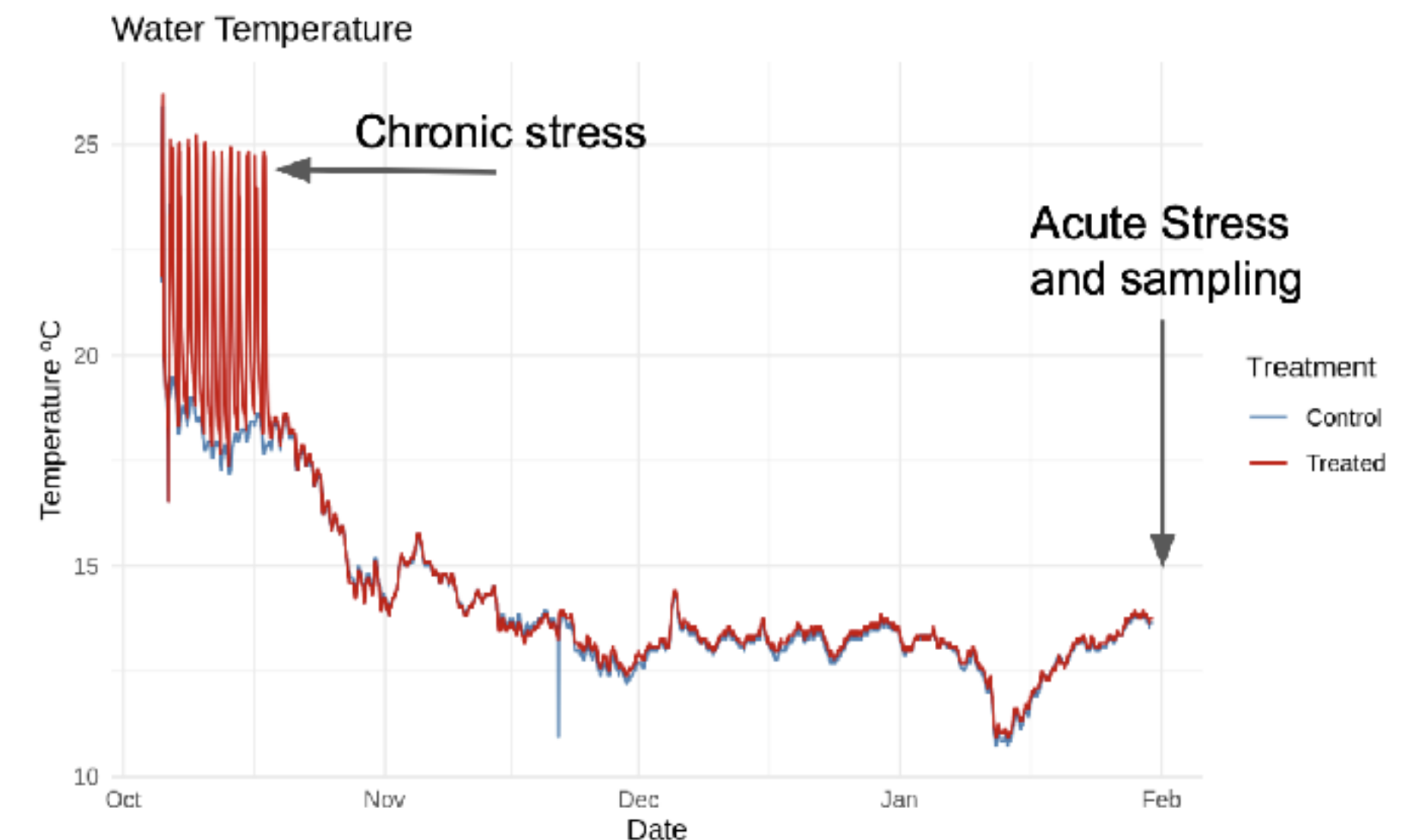
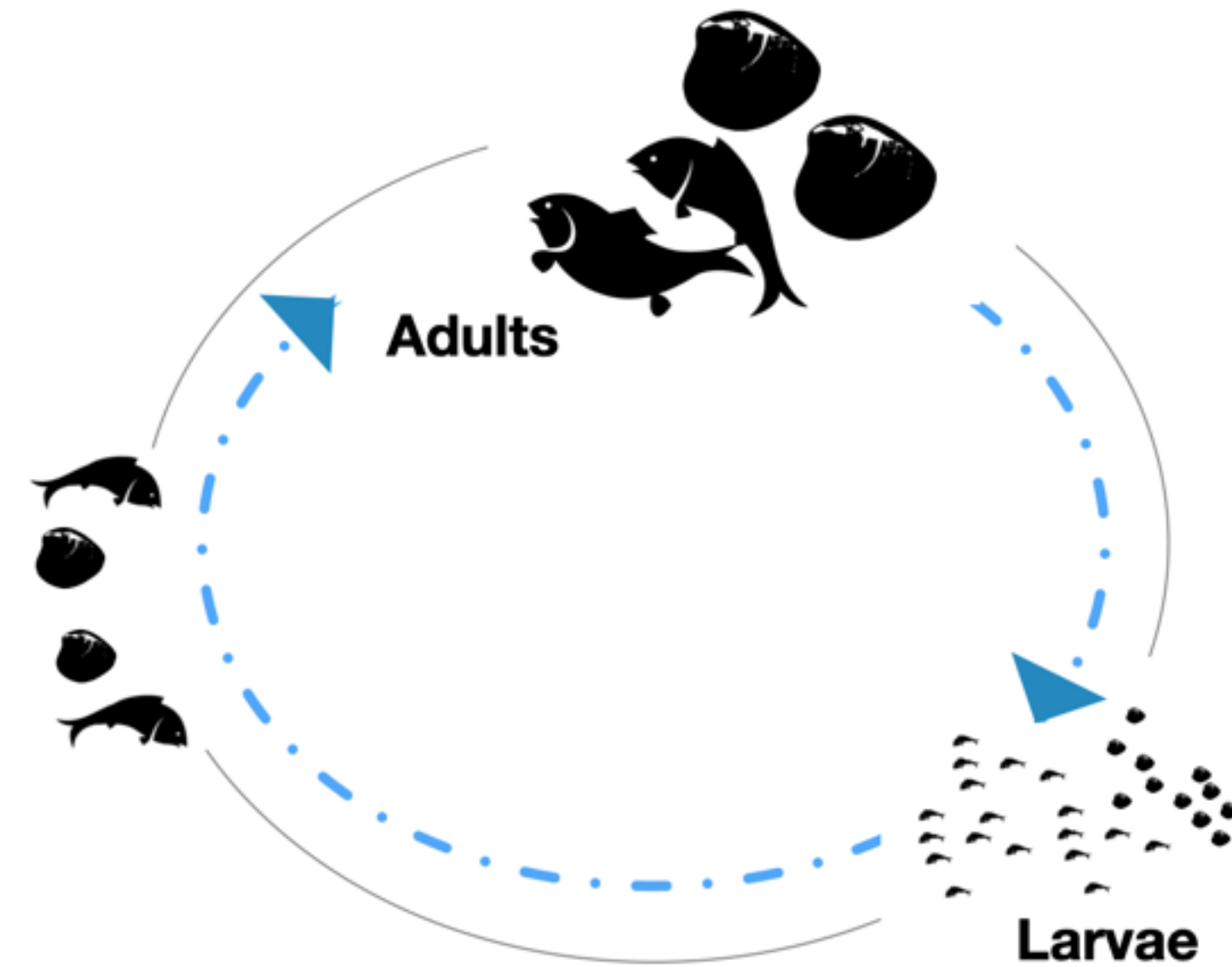
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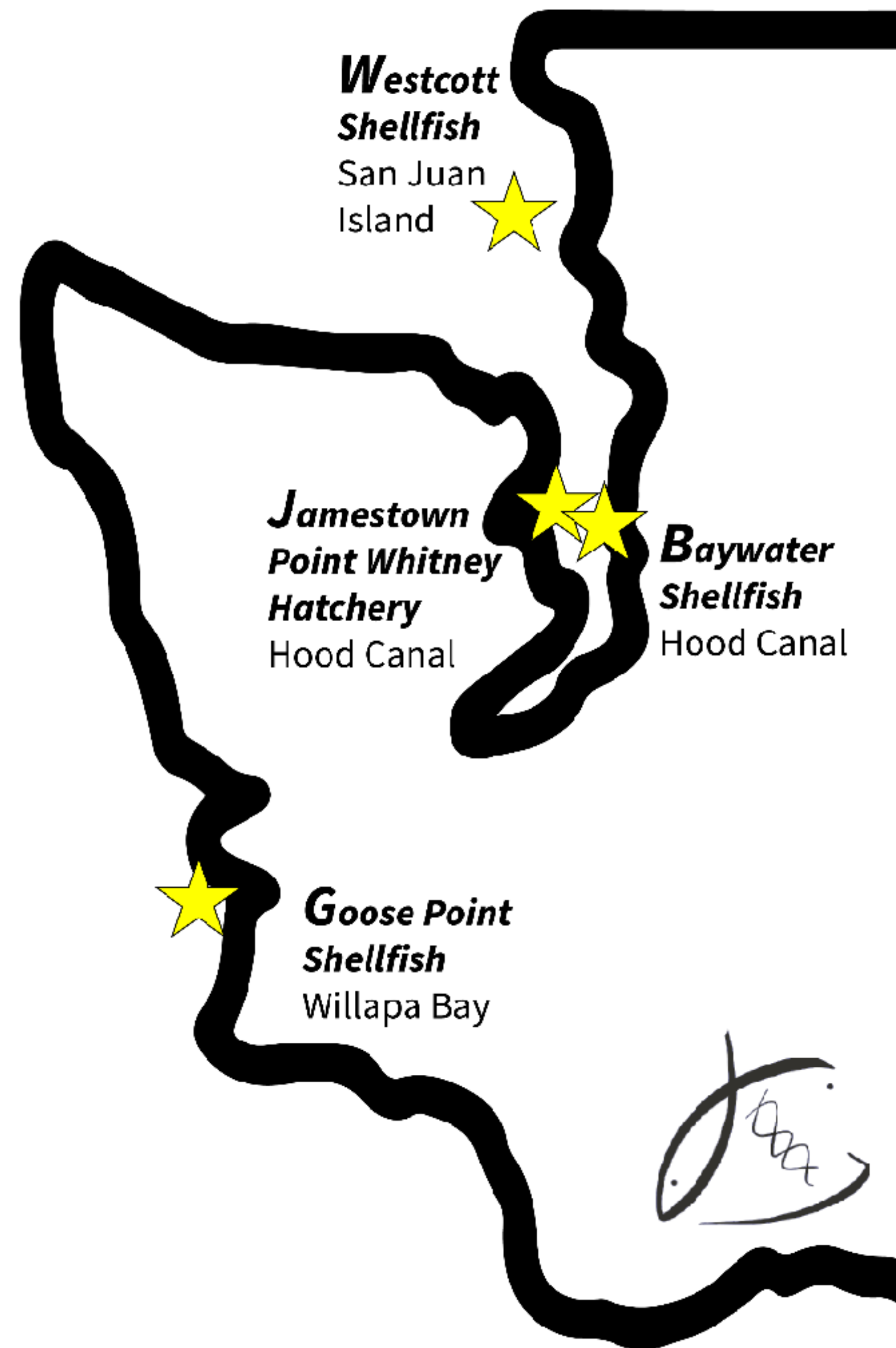
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Pacific Oyster

Ongoing Efforts

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Pacific Oyster

Ongoing Efforts

- **Priming**

- Immune Challenge
- Temperature
- Salinity
- Multiple

- **Performance Testing**

- Survival
- Growth
- Metabolism*

Core Partner:
Jamestown S'Klallam







*Assays for assessment
of memory and stress*

Current work

Priming

*Within
Across generation*

Mechanism

Industry Concerns

Pacific Oyster

Ongoing Efforts

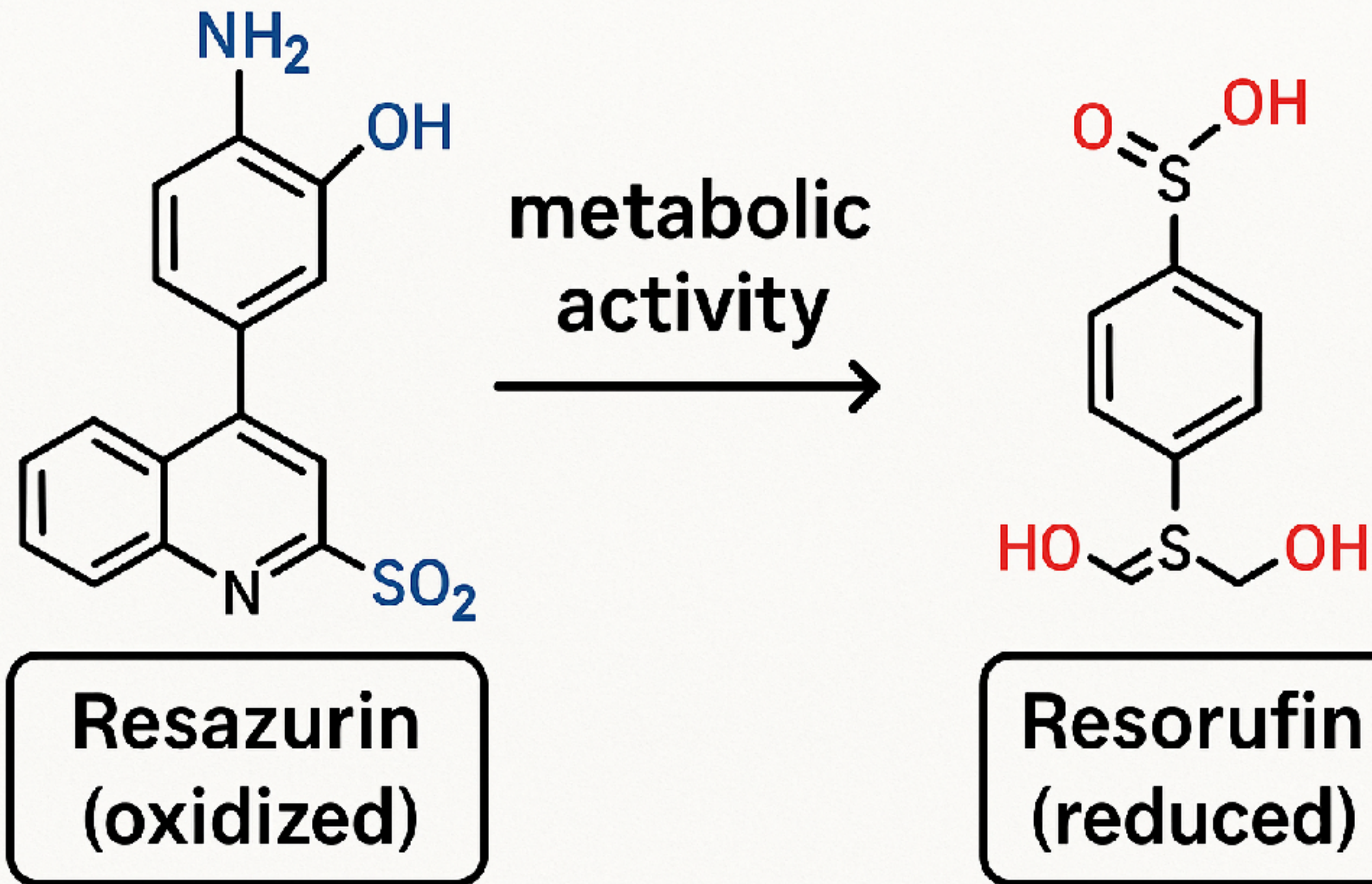
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Predicting Performance

Pacific Oyster

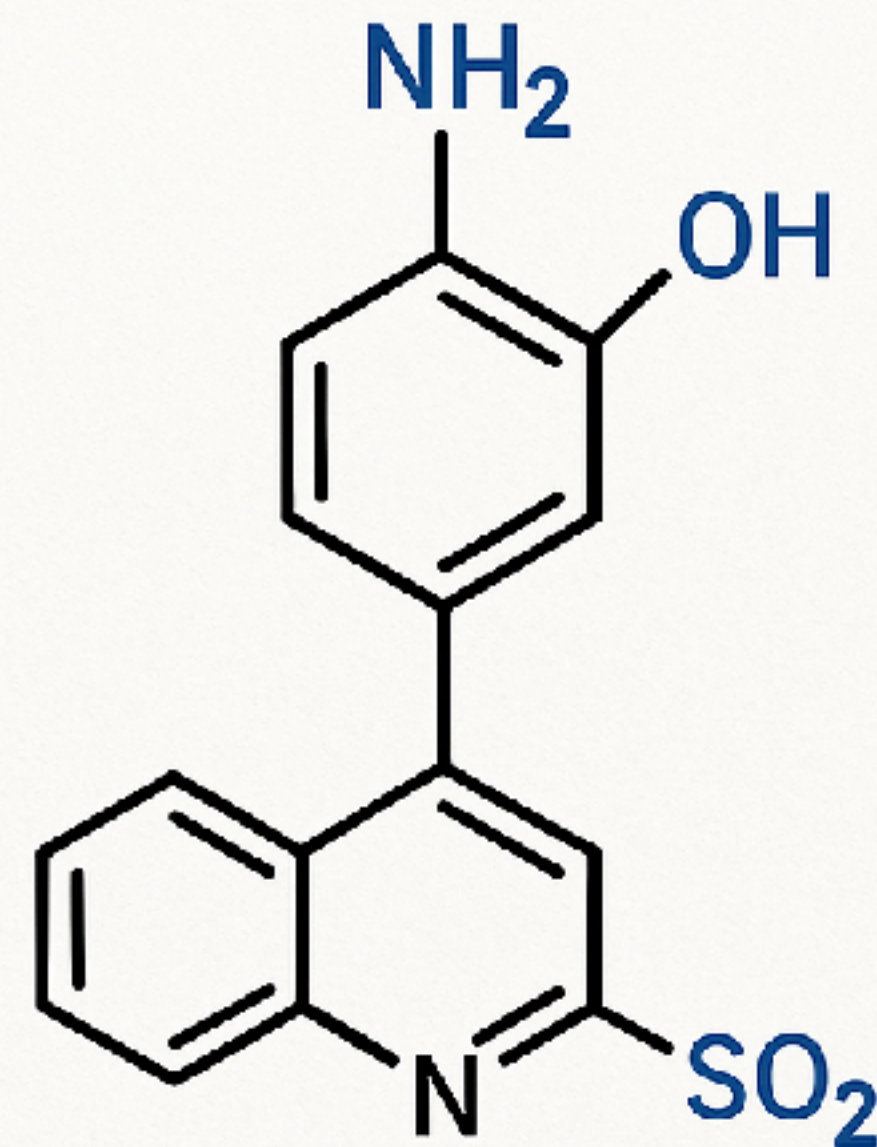
Resazurin

Louis Plough - USDA ARS



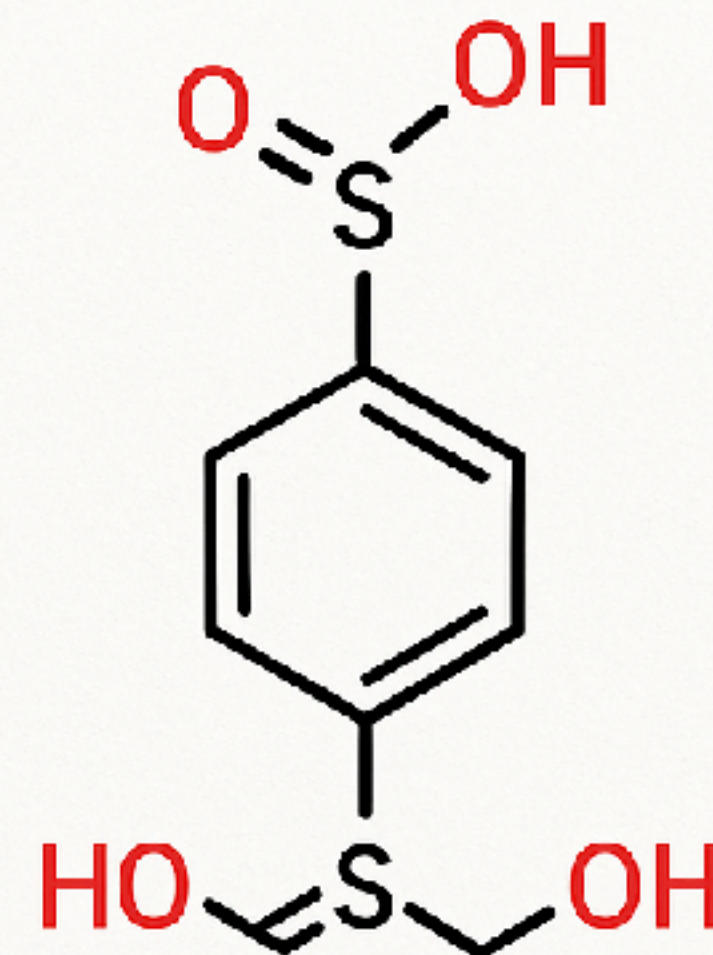
Pacific Oyster

Resazurin



**Resazurin
(oxidized)**

metabolic
activity



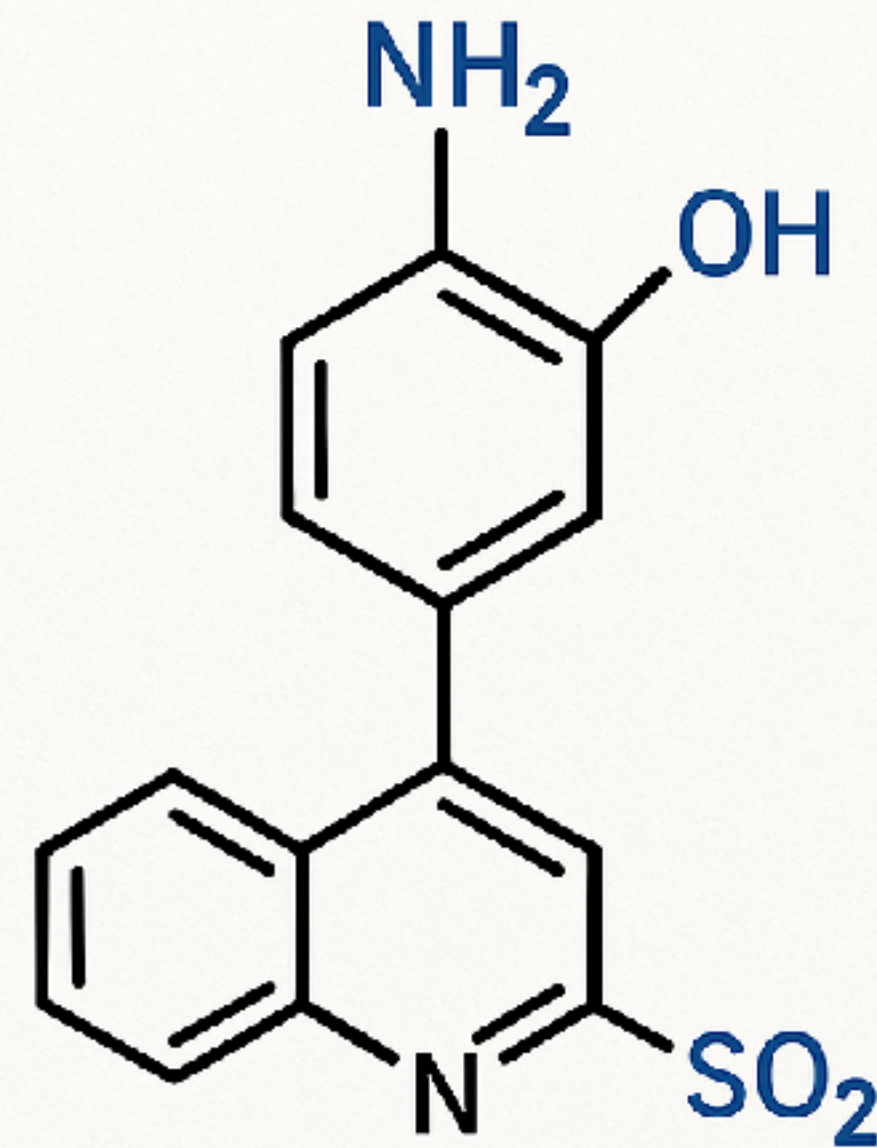
**Resorufin
(reduced)**

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

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

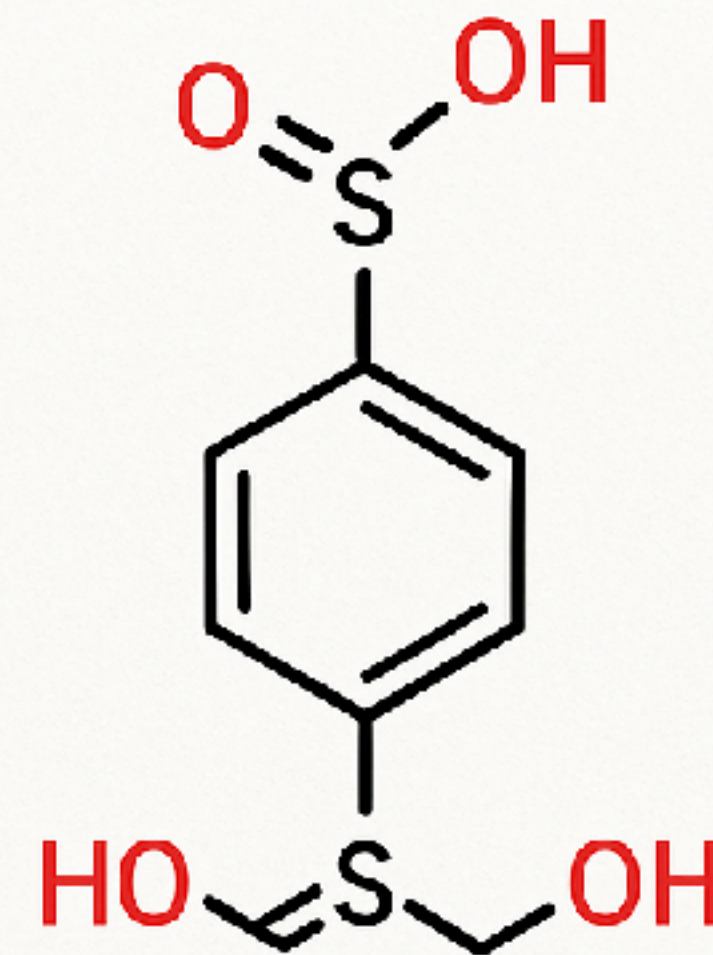
Pacific Oyster

Resazurin

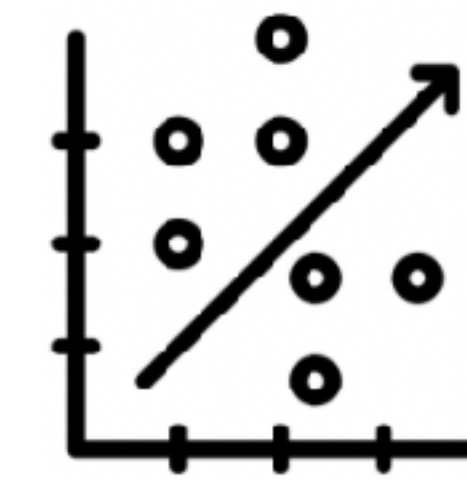


**Resazurin
(oxidized)**

metabolic
activity



**Resorufin
(reduced)**



**Correlated with oxygen
consumption**

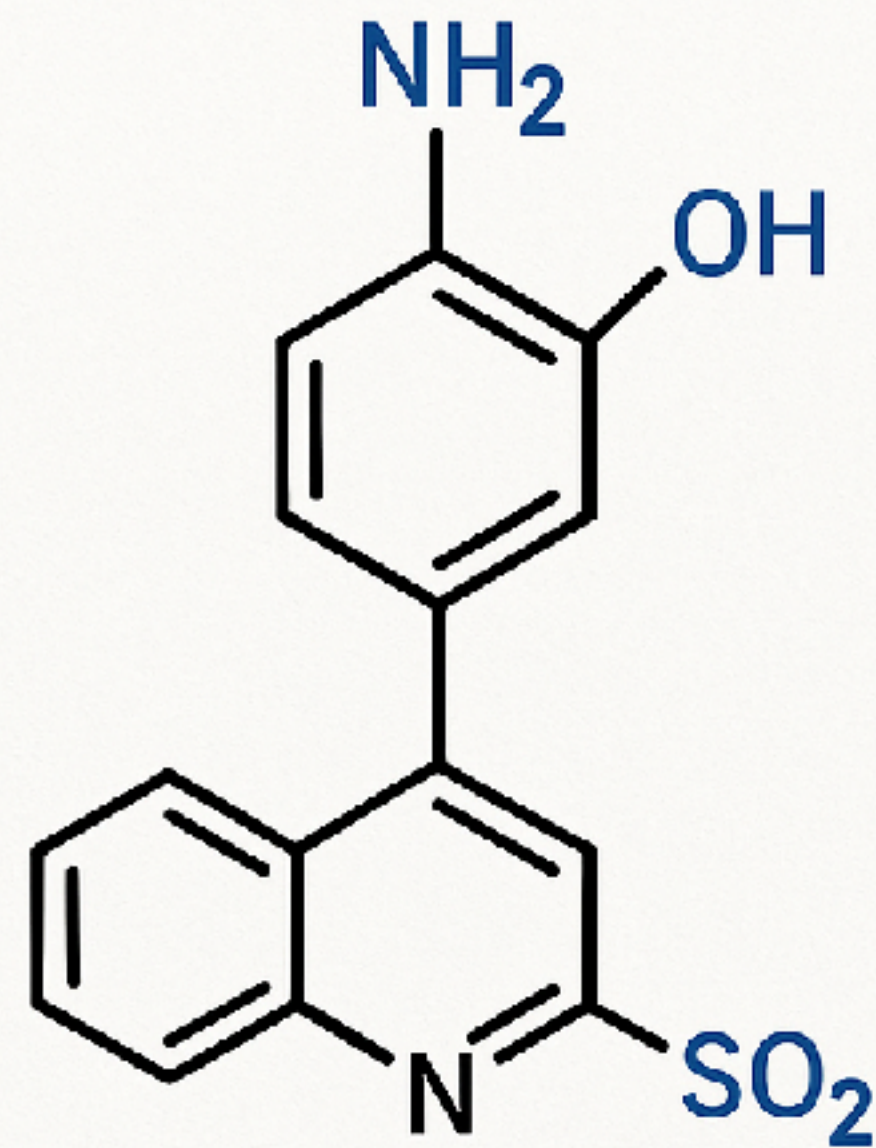
E.g., González-Pinzón et al. 2012,
Ricciardi et al. 2014

Correlation with respiration in oysters
(L. Plough, USDA, data analysis in
progress)

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

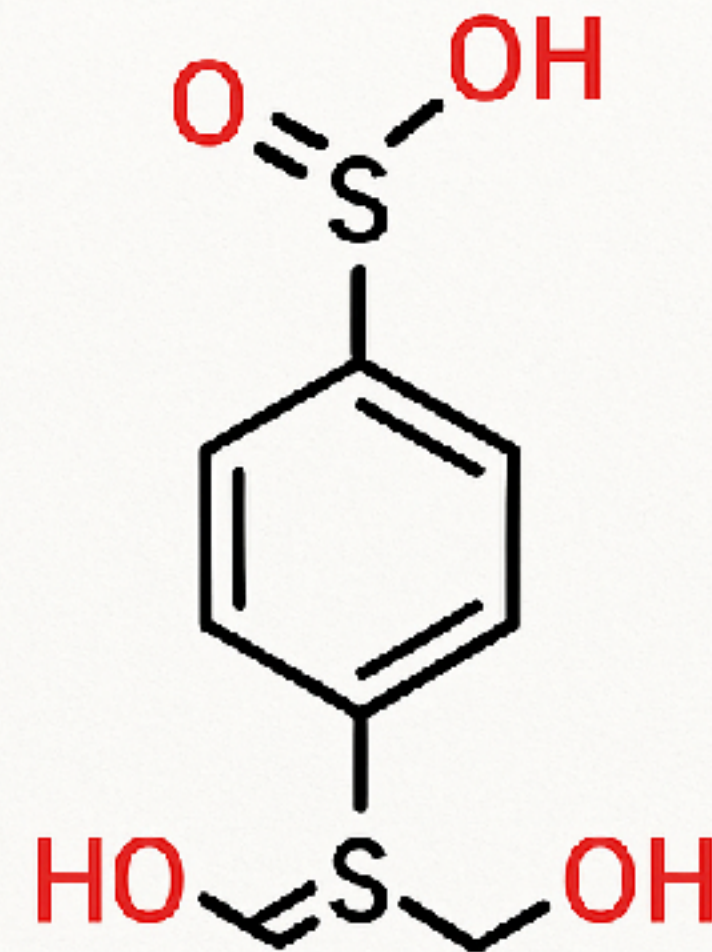
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Pacific Oyster



**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.

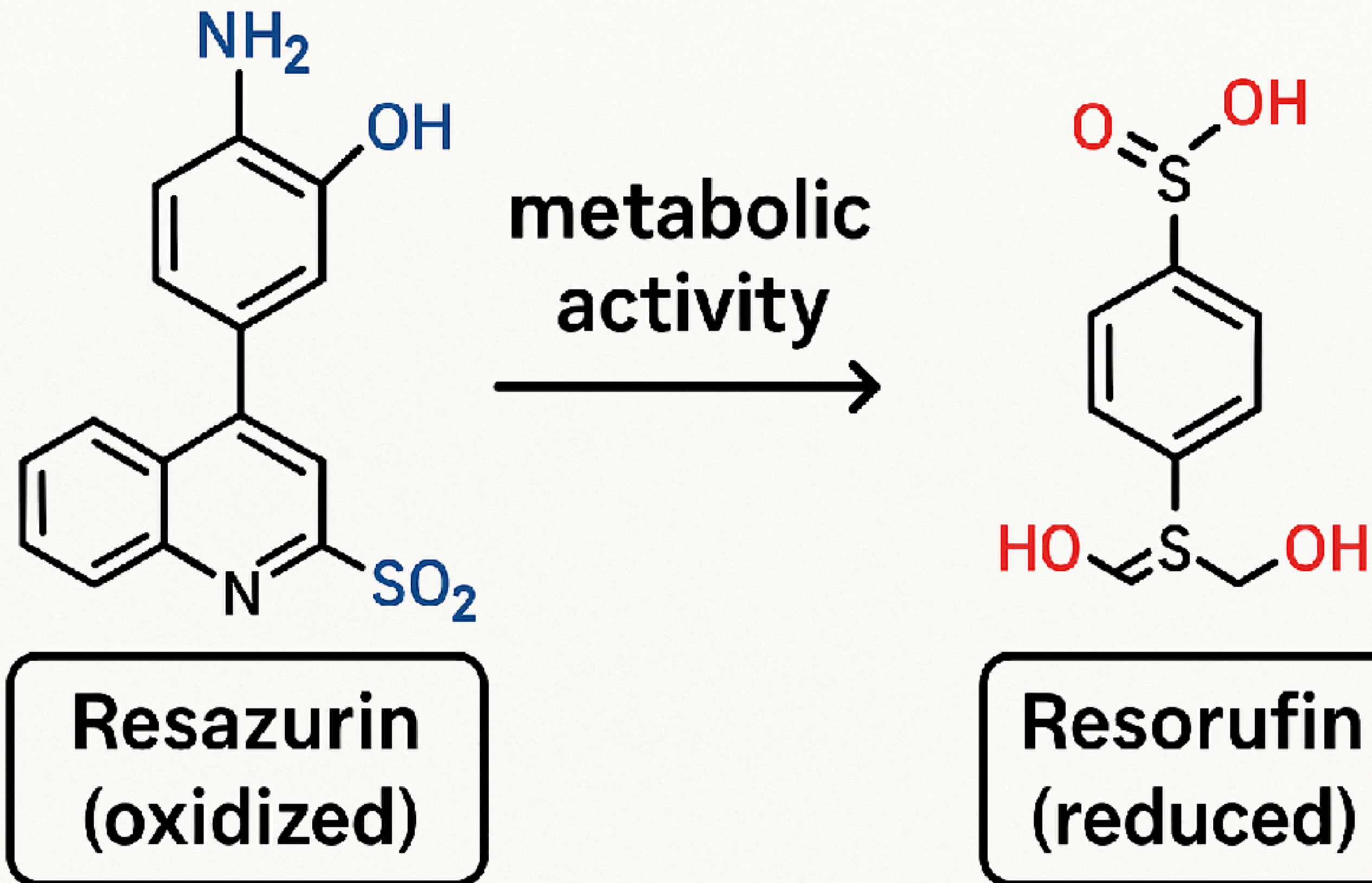


Testing the Efficacy of Diets or Nutritional Supplements

Compare metabolic output of oysters fed different diets

Applications

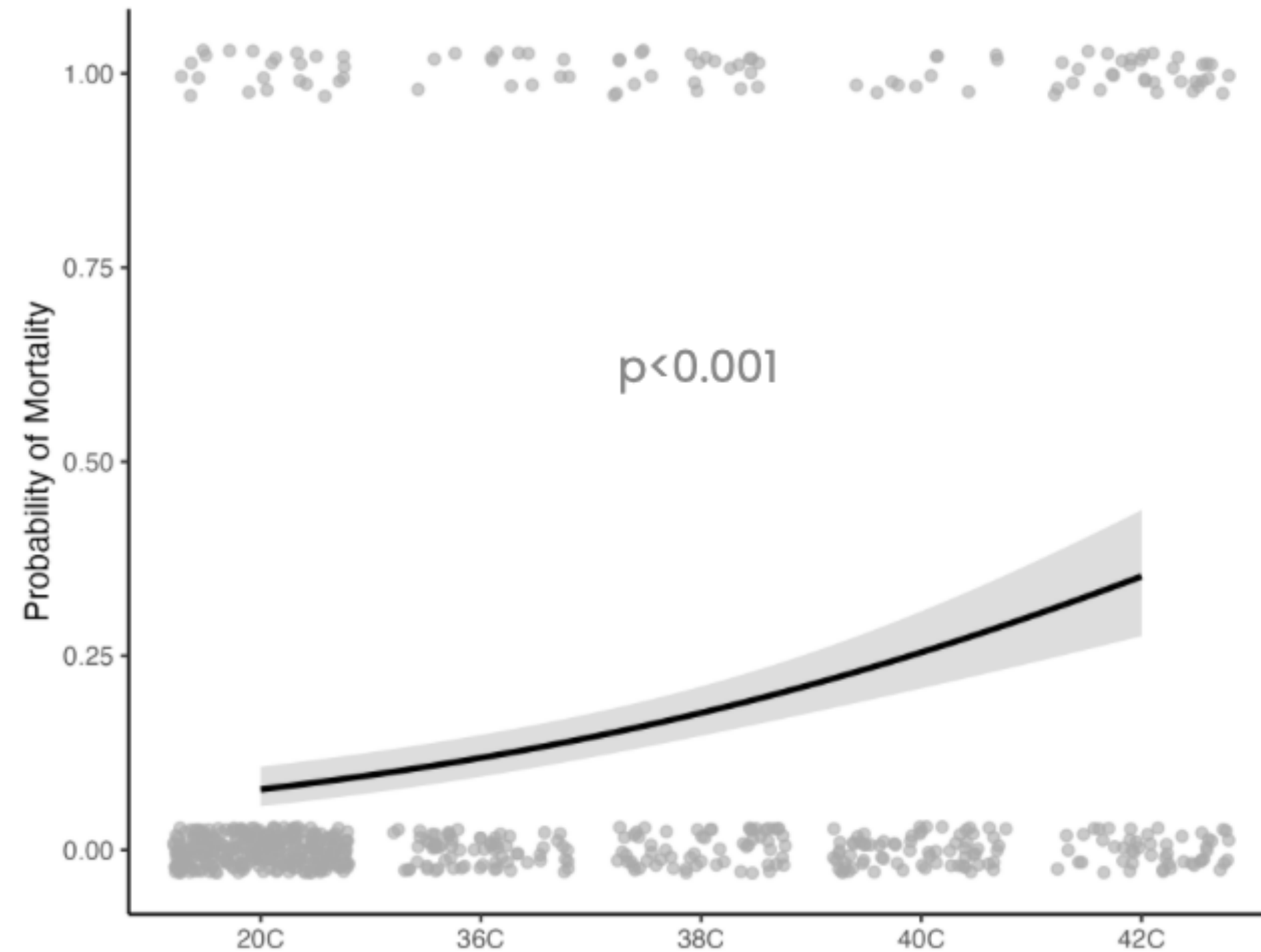
Resazurin



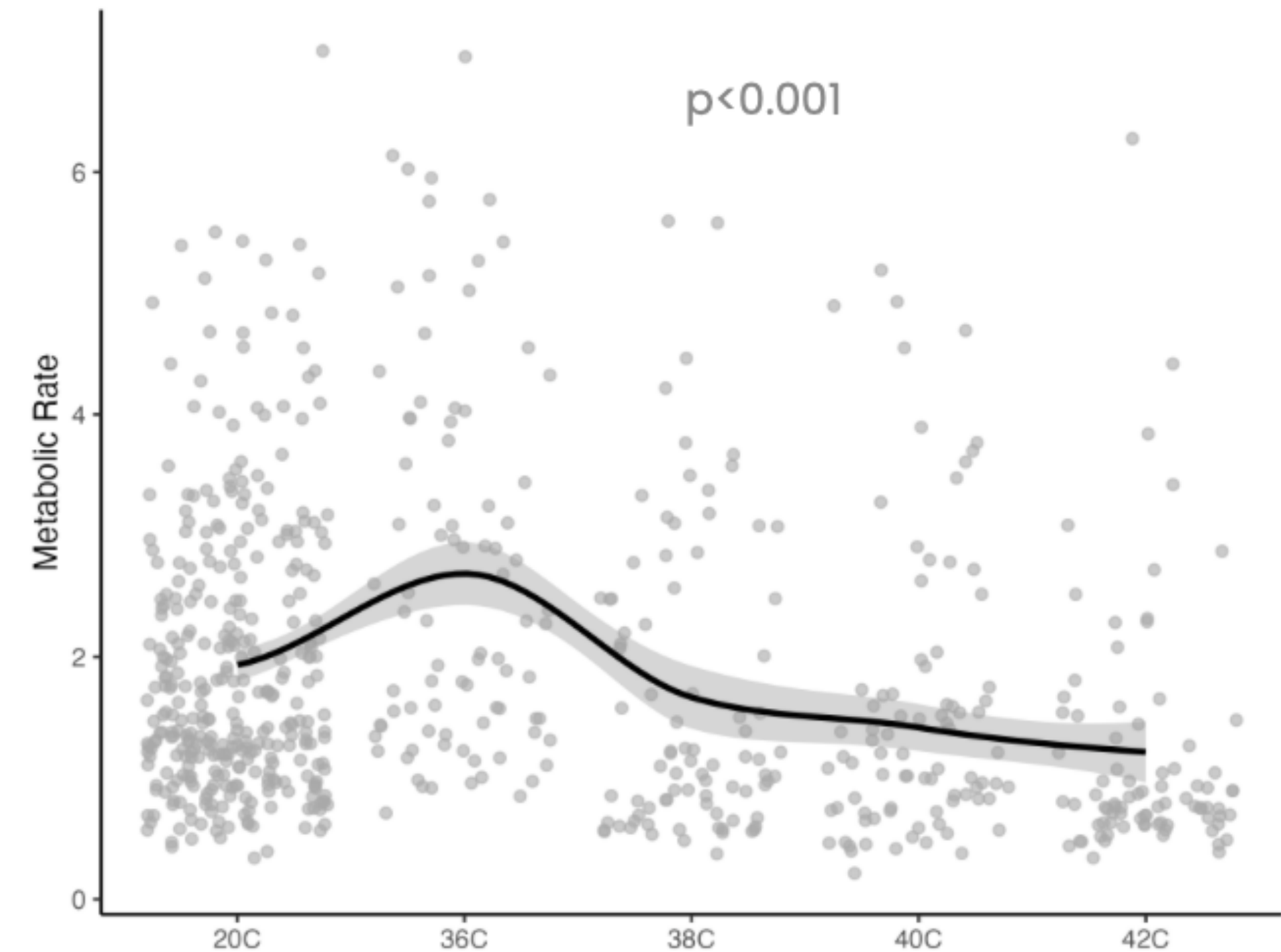
Is metabolic activity associated with a stress event different when oyster had previously experienced a stress? (Is there evidence for functional priming?)

Applications

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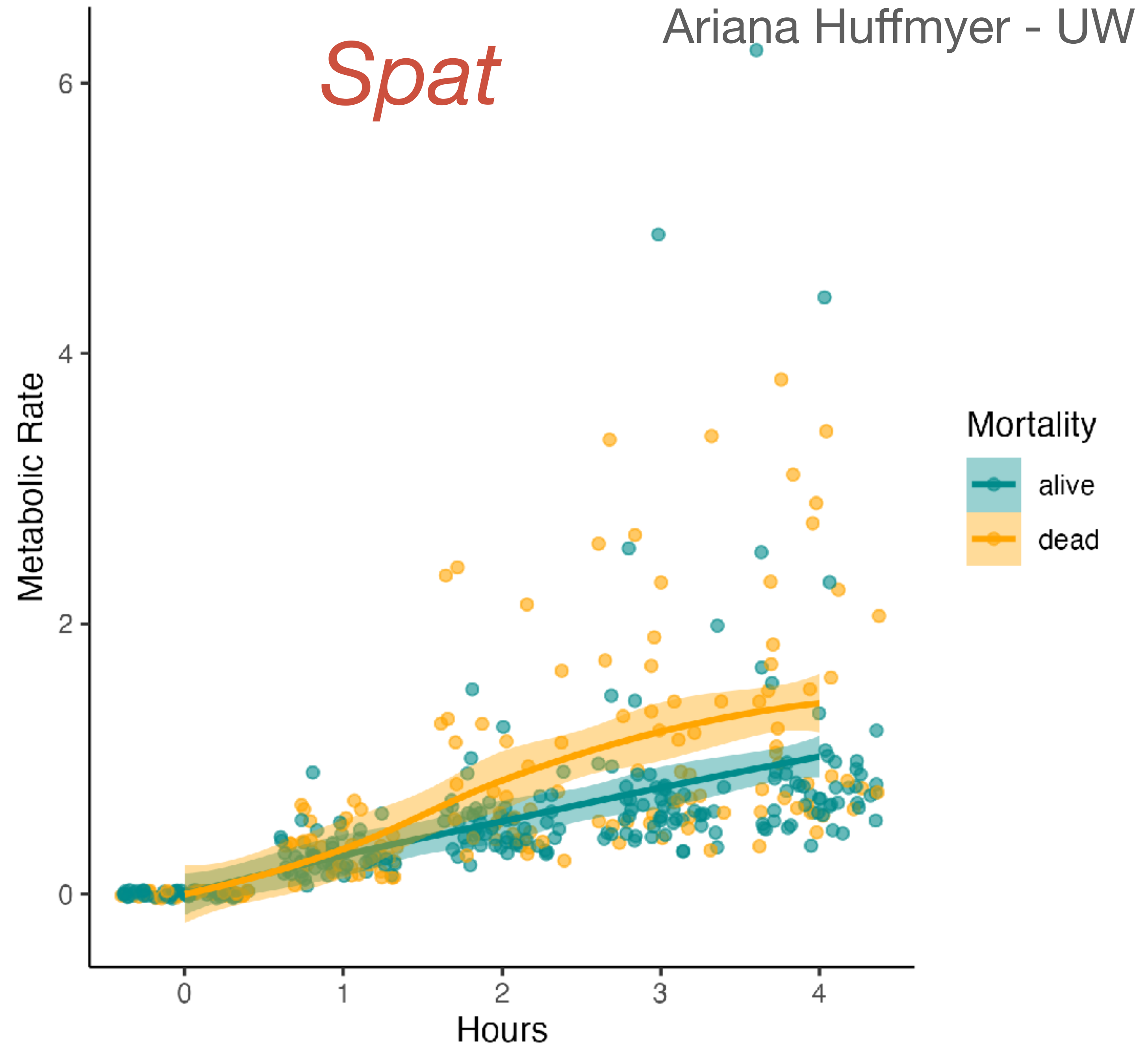
Mortality **increases** with temperature



Metabolic rates **decrease** at high temperatures

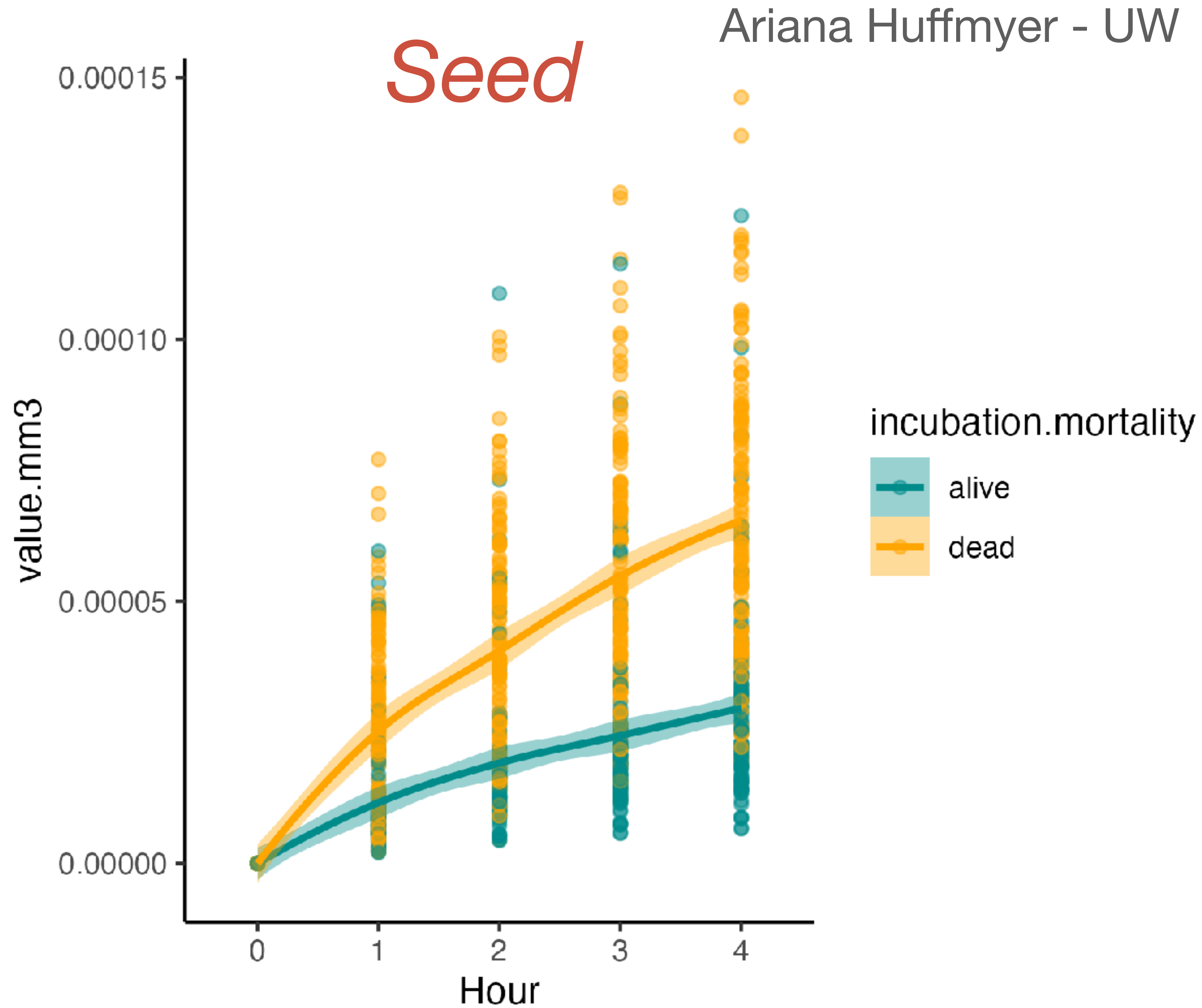
Applications

Oysters that ***survived stress trials*** had a greater capacity for metabolic depression



Applications

Oysters that ***survived stress trials*** had a greater capacity for metabolic depression

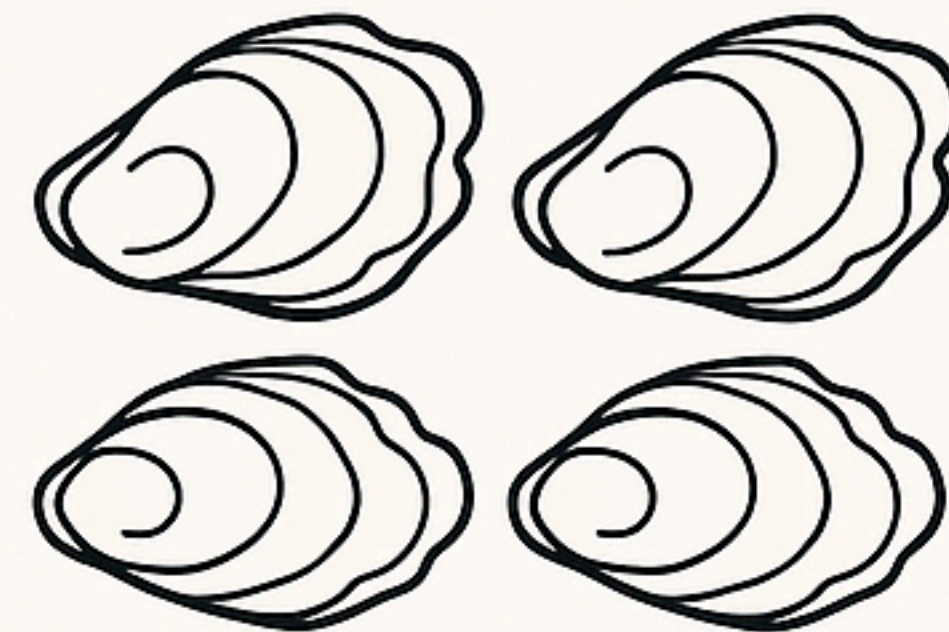


Applications

Elevated Metabolic Response to Stress



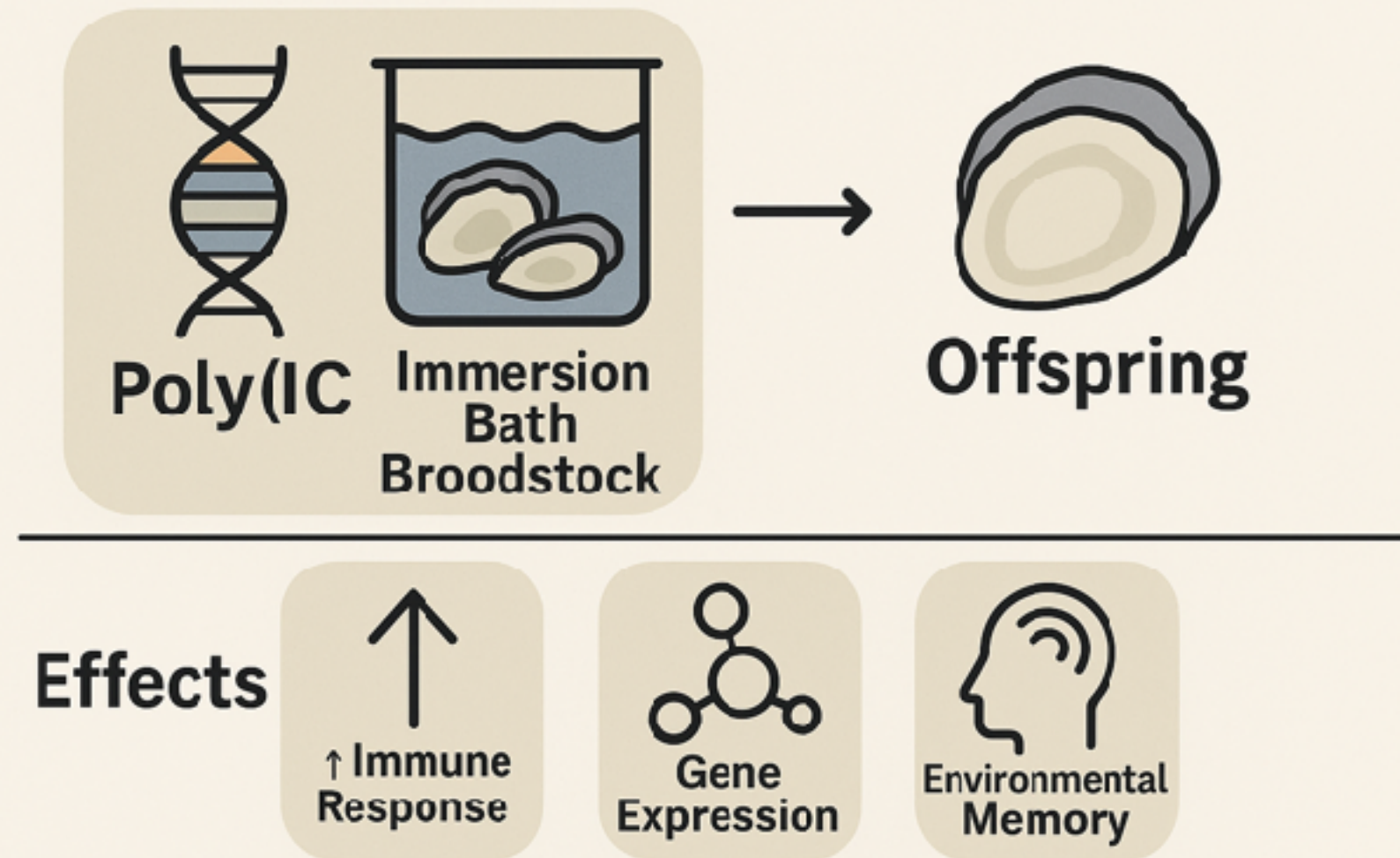
Survival



Mortality

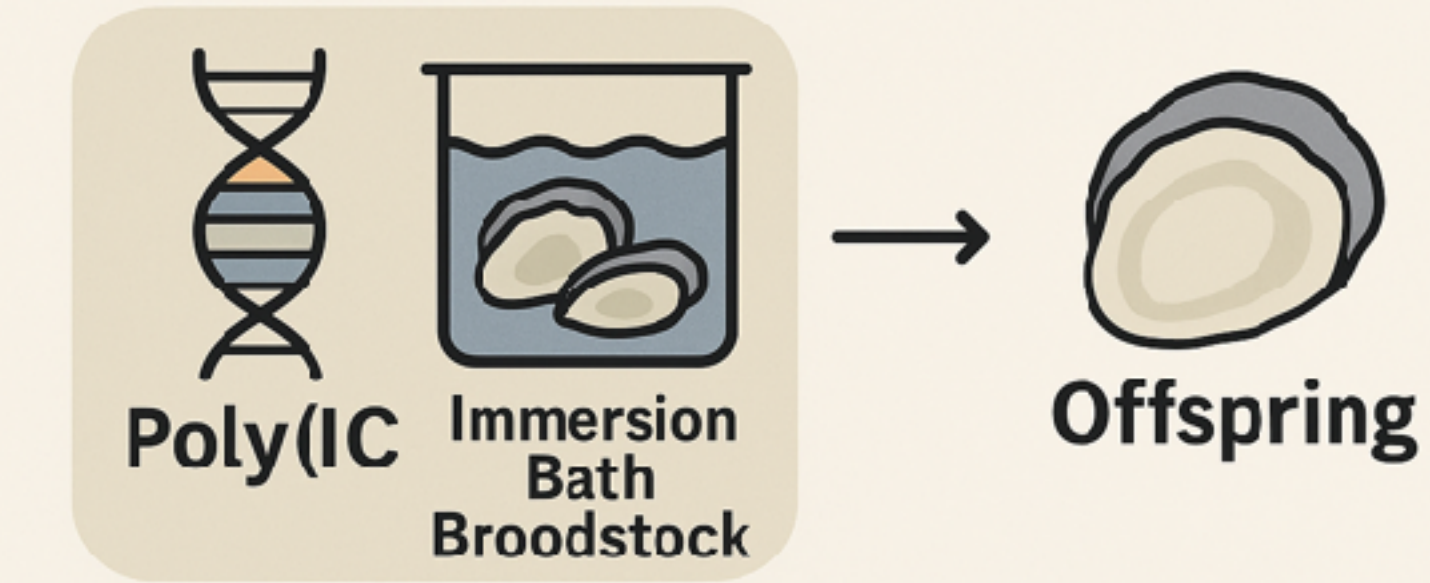


POLY(I:C) EXPERIMENT TO INDUCE ENVIRONMENTAL MEMORY



Ariana Huffmyer - UW

POLY(I:C) EXPERIMENT TO INDUCE ENVIRONMENTAL MEMORY



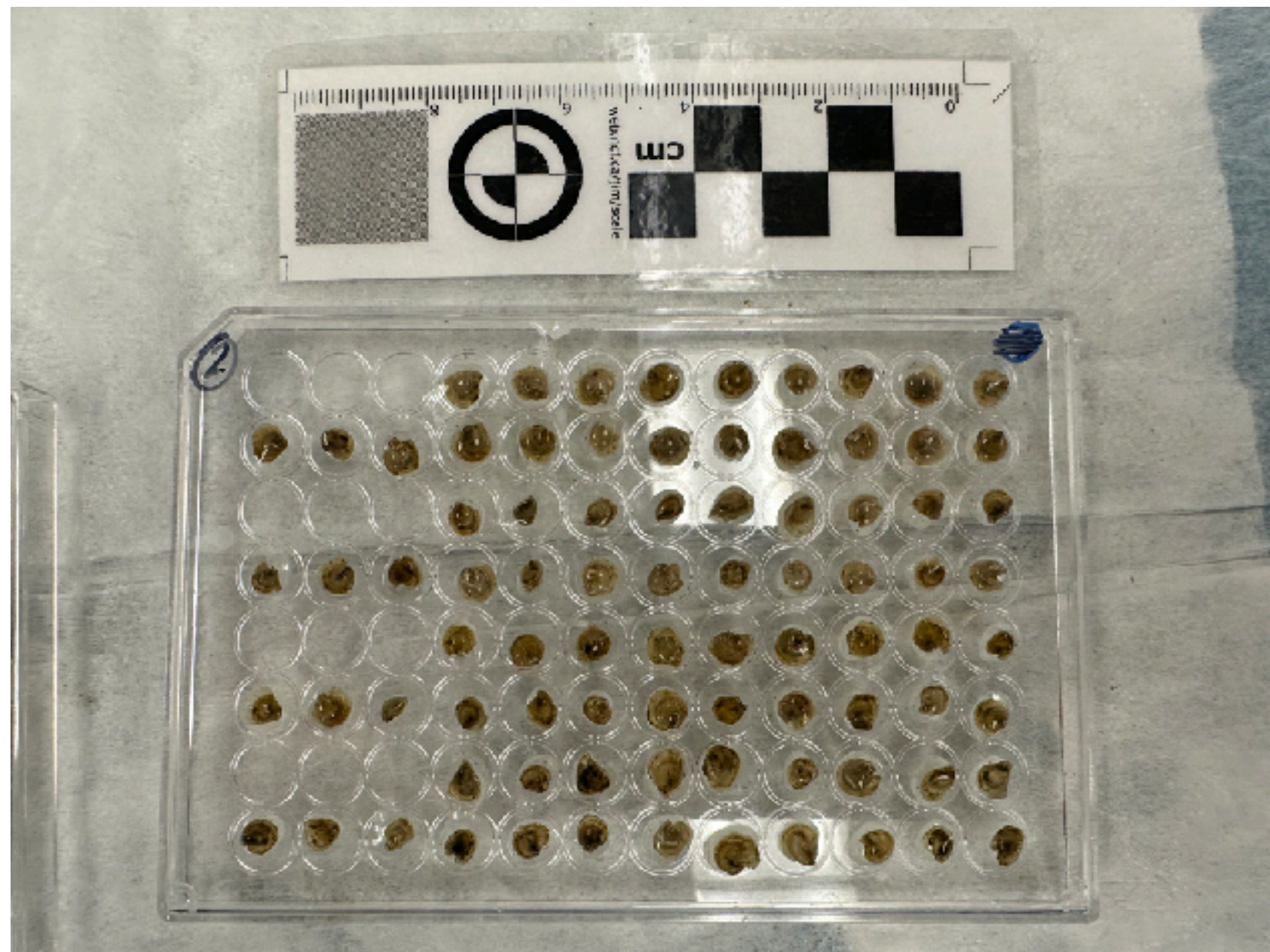
Effects

↑
Immune
Response

Gene
Expression

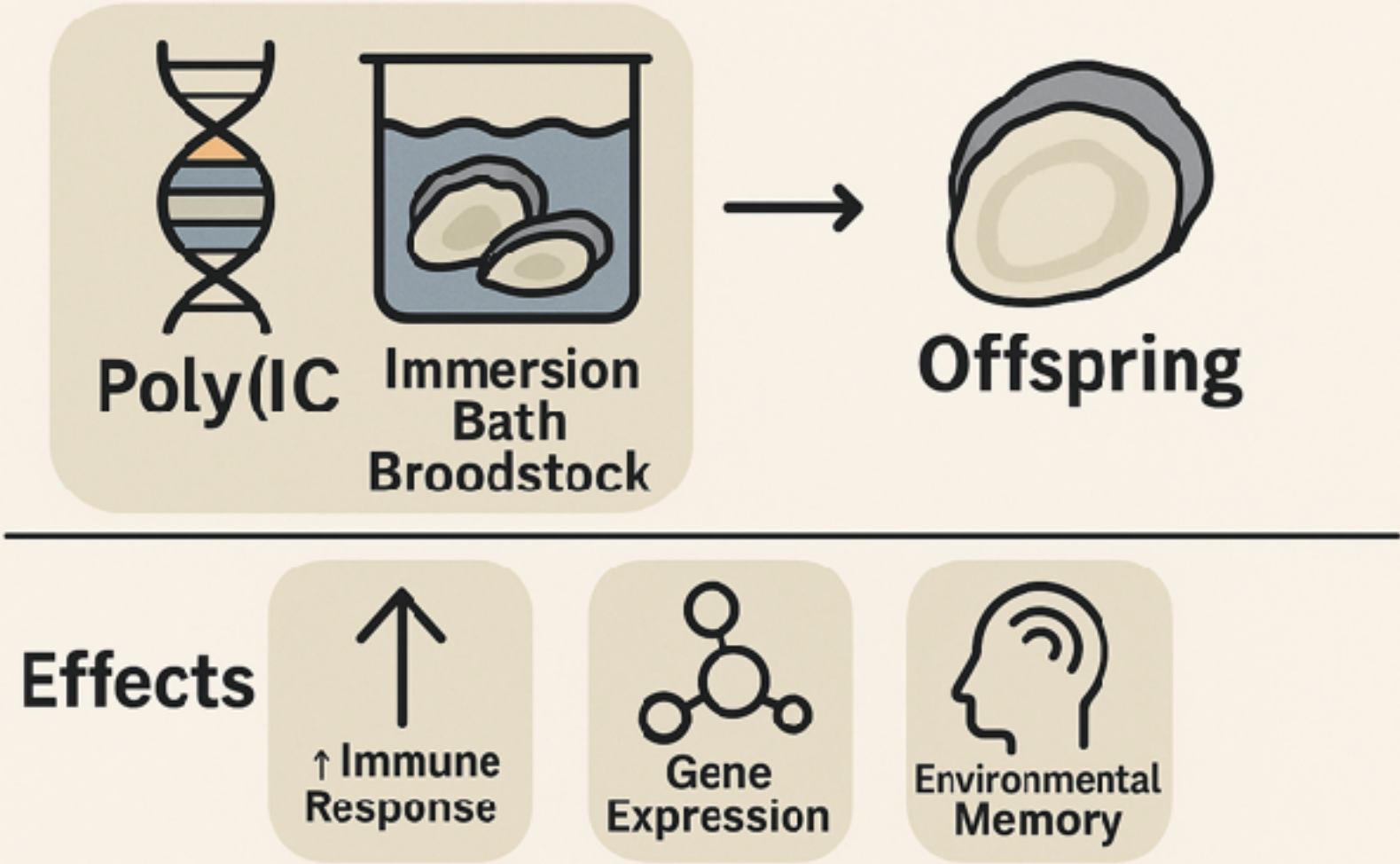
Environmental
Memory

Ariana Huffmyer - UW

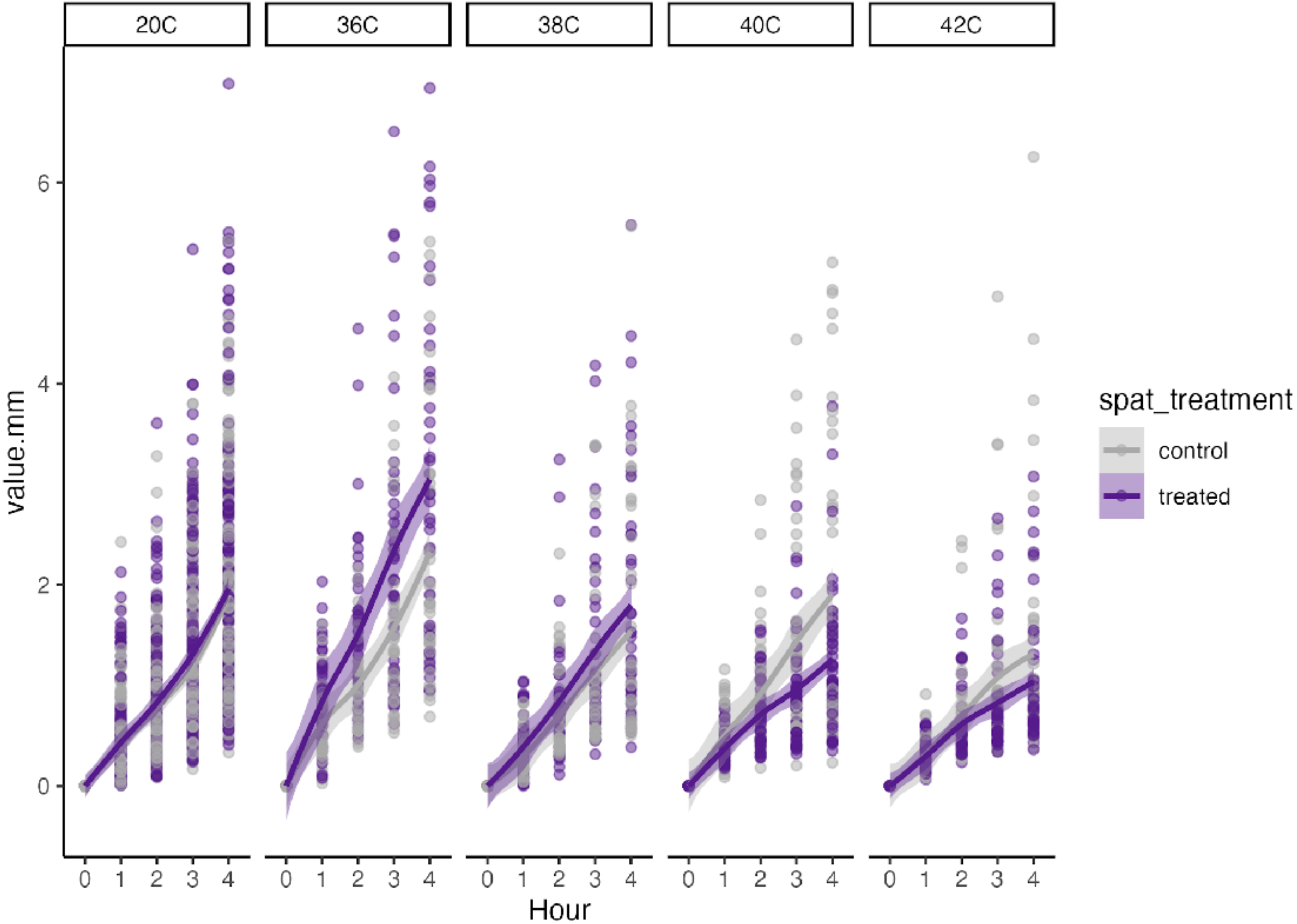


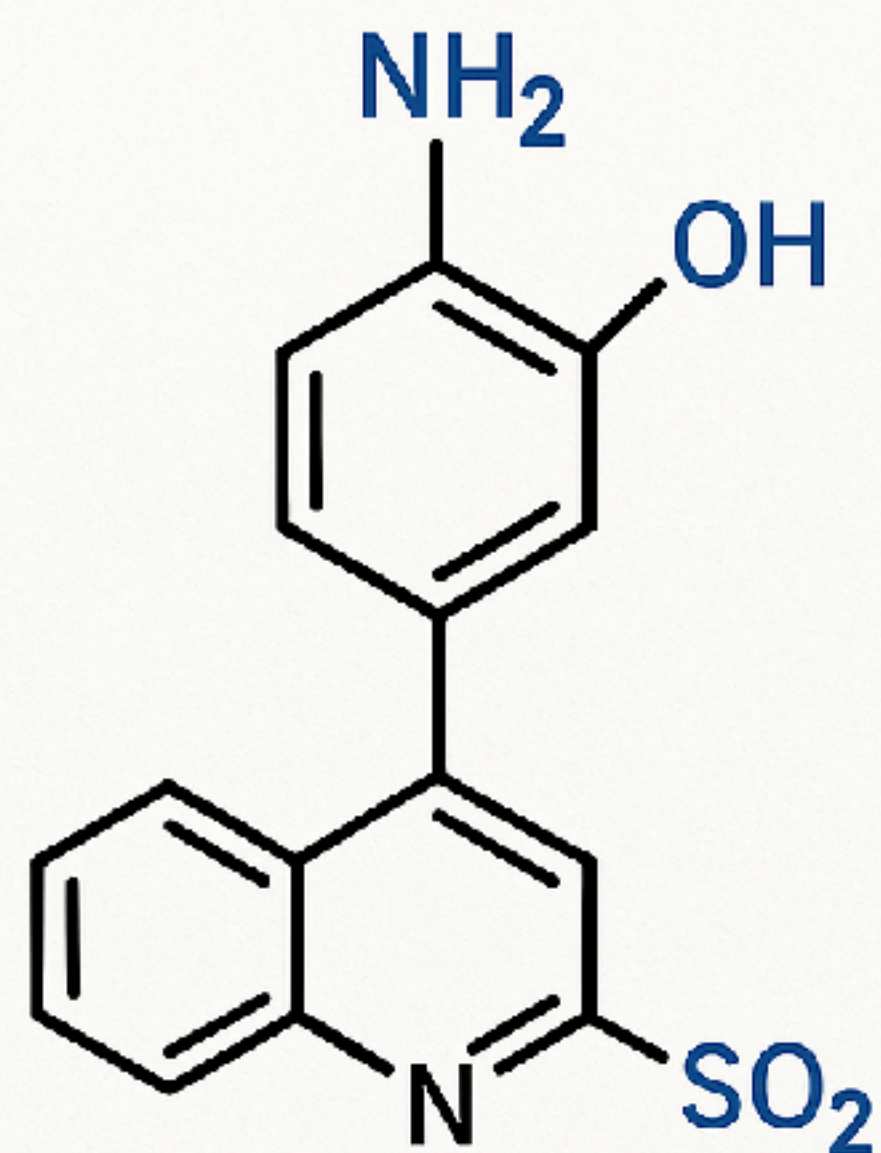
POLY(I:C) EXPERIMENT TO INDUCE ENVIRONMENTAL MEMORY

Ariana Huffmyer - UW



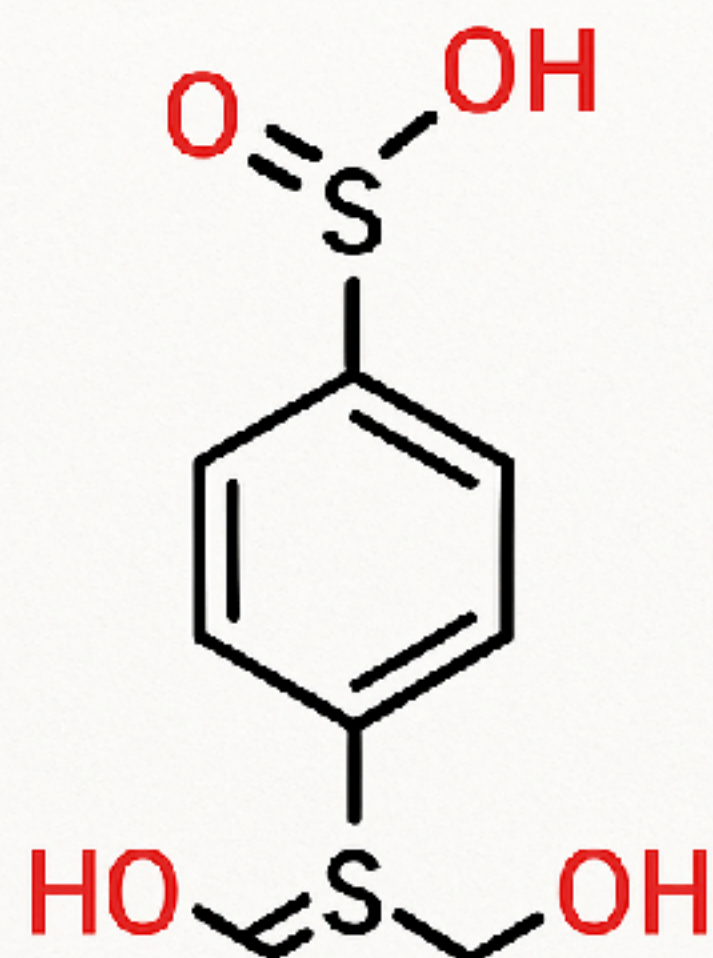
Offspring from immune-challenged parents exhibited *greater metabolic flexibility*





**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



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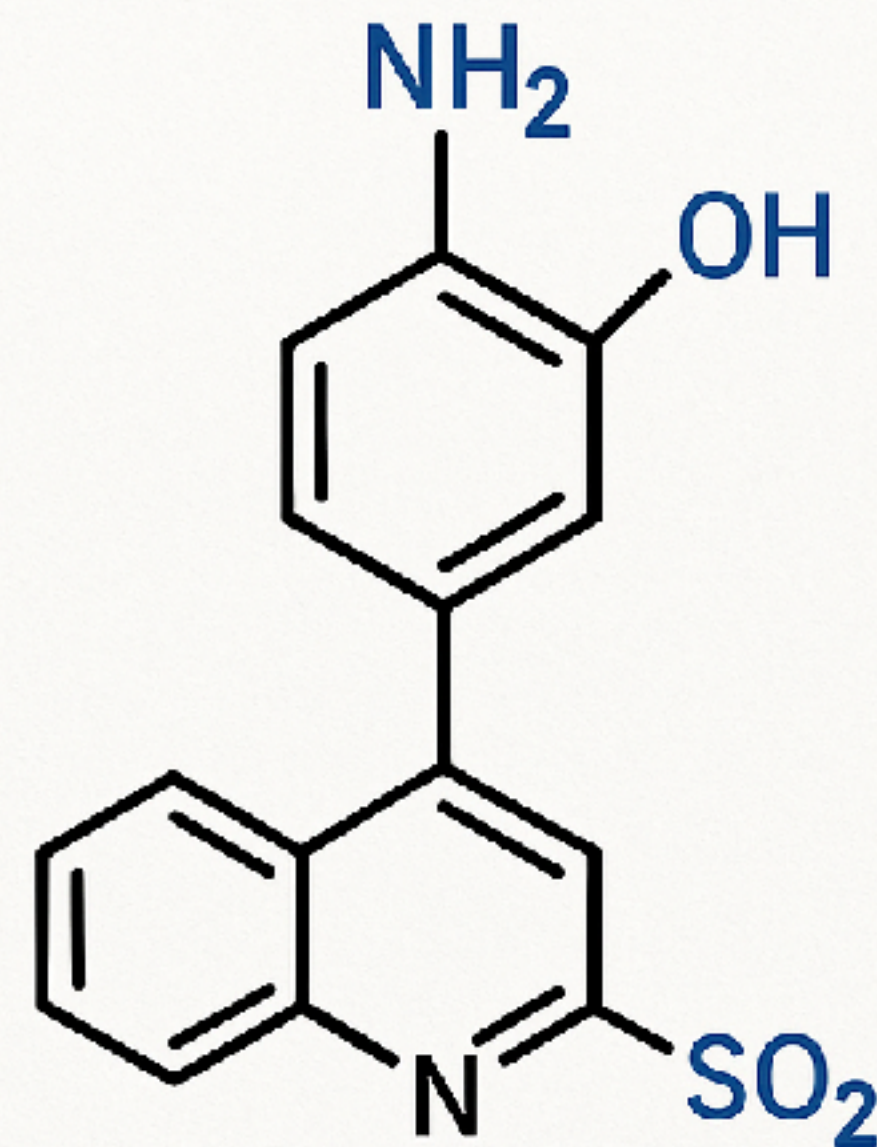
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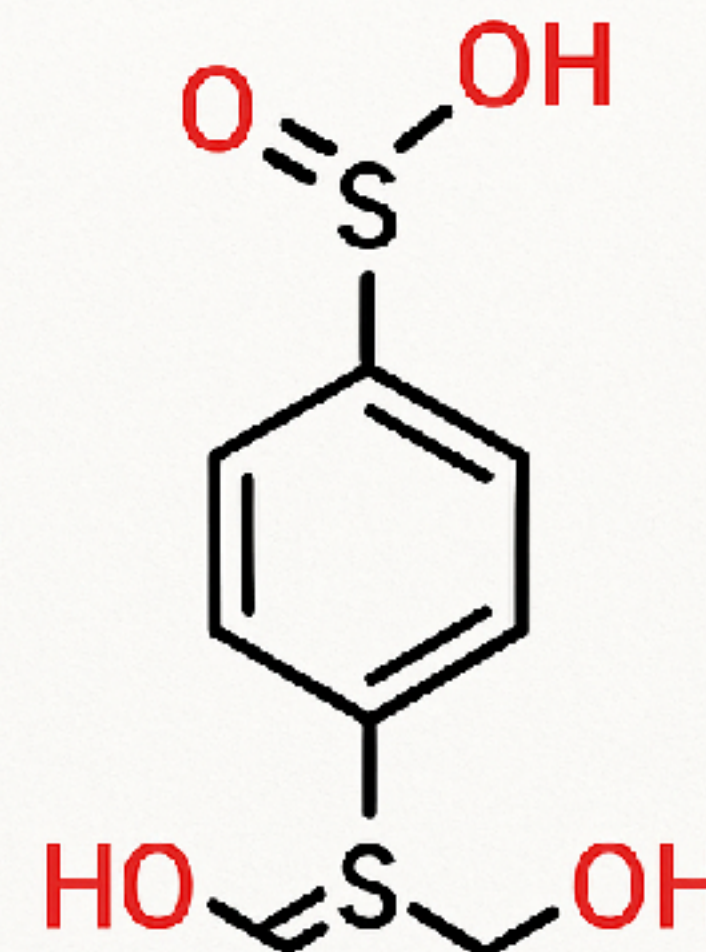
Testing the Efficacy of Diets or Nutritional Supplements

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**Resazurin
(oxidized)**

metabolic
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**Resorufin
(reduced)**

Next Step



Performance Testing

Development of SORMI (Summer Oyster Resilience and Mortality Index)

A quantitative tool for improving field survival

Bobbi Hudson - Pacific Shellfish Institute (Lead)

Neil Thompson - USDA-ARS Pacific Shellfish Breeding Center

Mackenzie Gavery - NOAA Northwest Fisheries Science Center

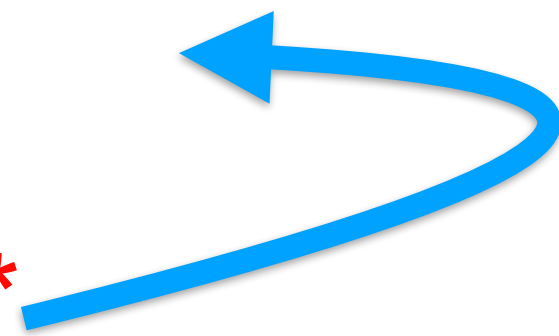
Kevin Marquez Johnson - California Polytechnic State University and
California Sea Grant

- Performance Testing

- Survival

- Growth

- Metabolism*

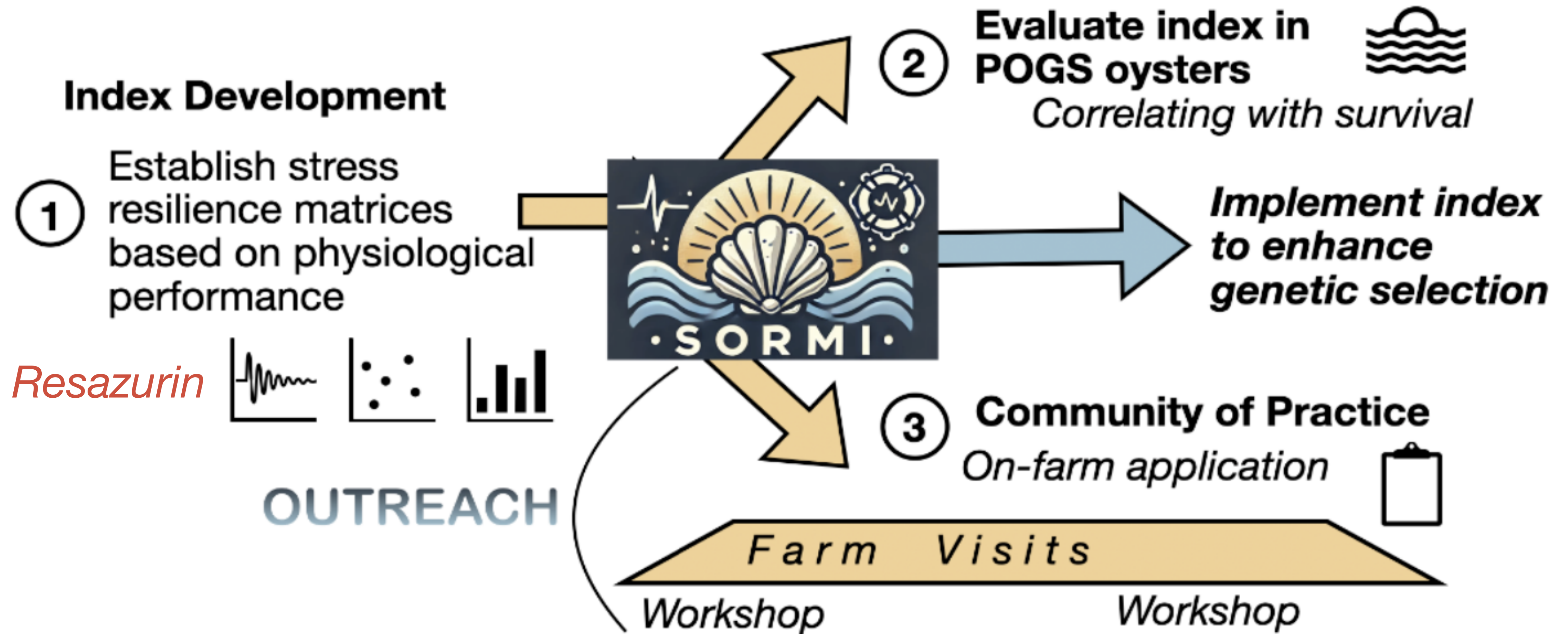


Predicting Performance

Performance Testing

Development of SORMI (Summer Oyster Resilience and Mortality Index)

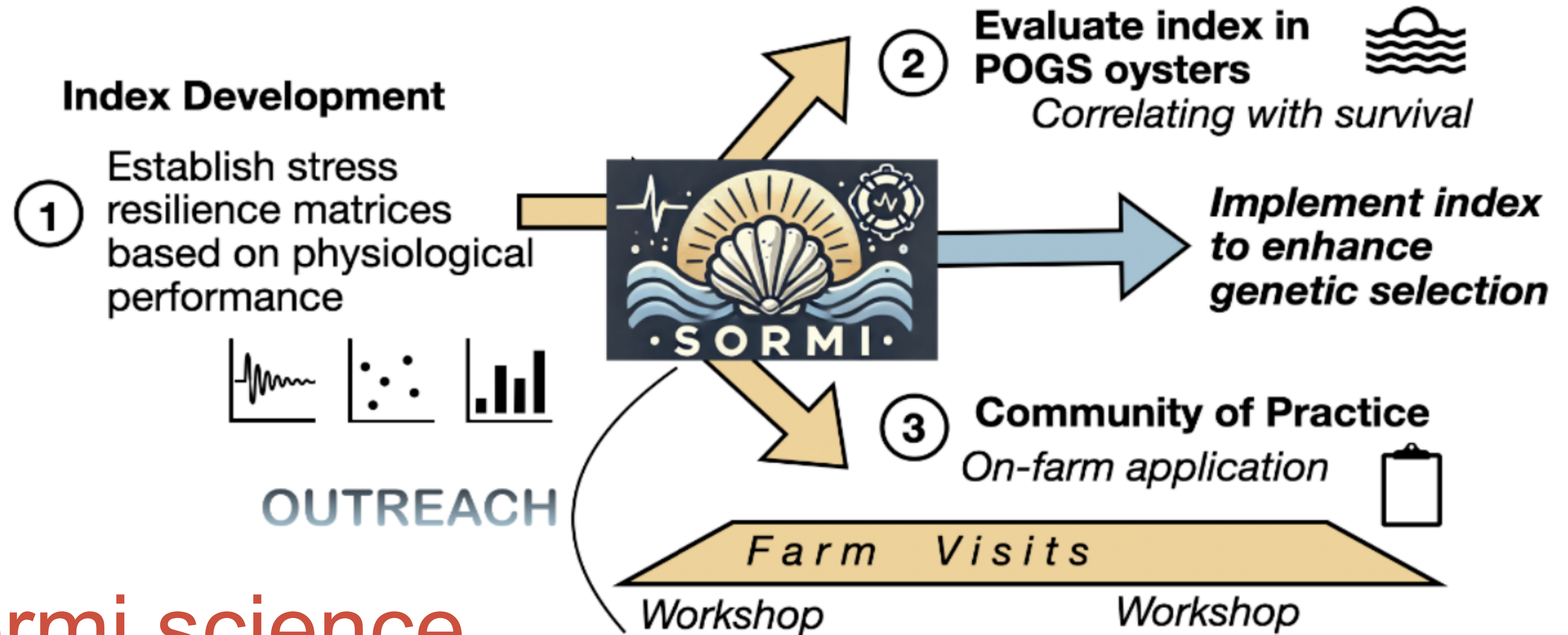
A quantitative tool for improving field survival



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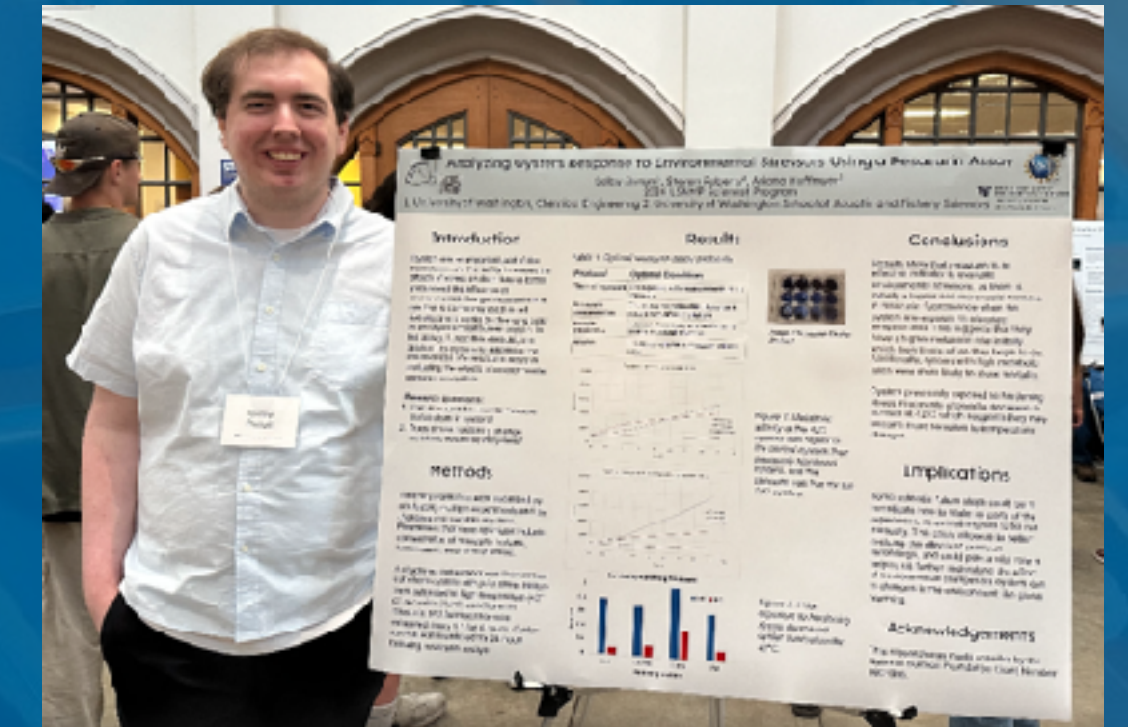
A quantitative tool for improving field survival



sormi.science

ACKNOWLEDGEMENTS

- Sam White (UW), Brent Vadopalas (UW), Shelly Wanamaker (GMGI), Sam Gurr (NOAA), Hollie Putnam (URI), Laura Spencer (UW), Katherine Silliman (NOAA), Yaamini Venkataraman (WHOI), Katie Lotterhos (NEU)



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