



# Marine invertebrate environmental memory

## Implications, mechanisms, and opportunities



Steven Roberts - University of Washington

Duke |

NICHOLAS SCHOOL *of*  
*the* ENVIRONMENT  
*Duke University Marine Lab*













## *Early-life Priming*

### *Hardening*



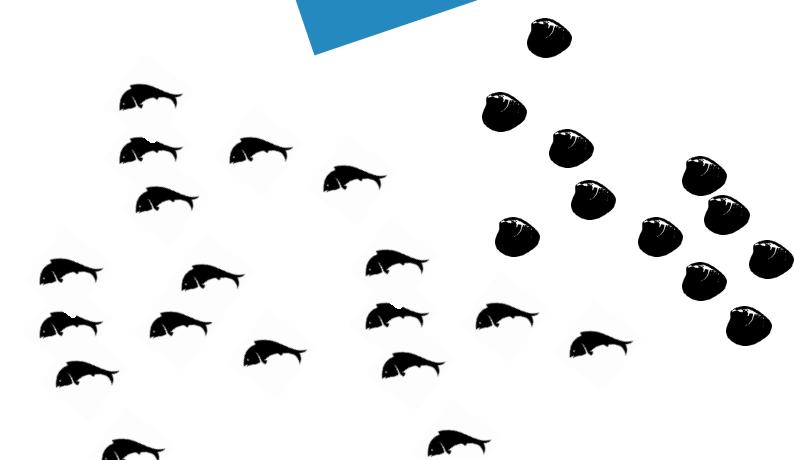
Adults

Influencing adult phenotype by altering early life environment



## *Transgenerational Plasticity*

### *Carry-over effects*



Larvae

Influencing offspring phenotype by altering environmental conditions of parents

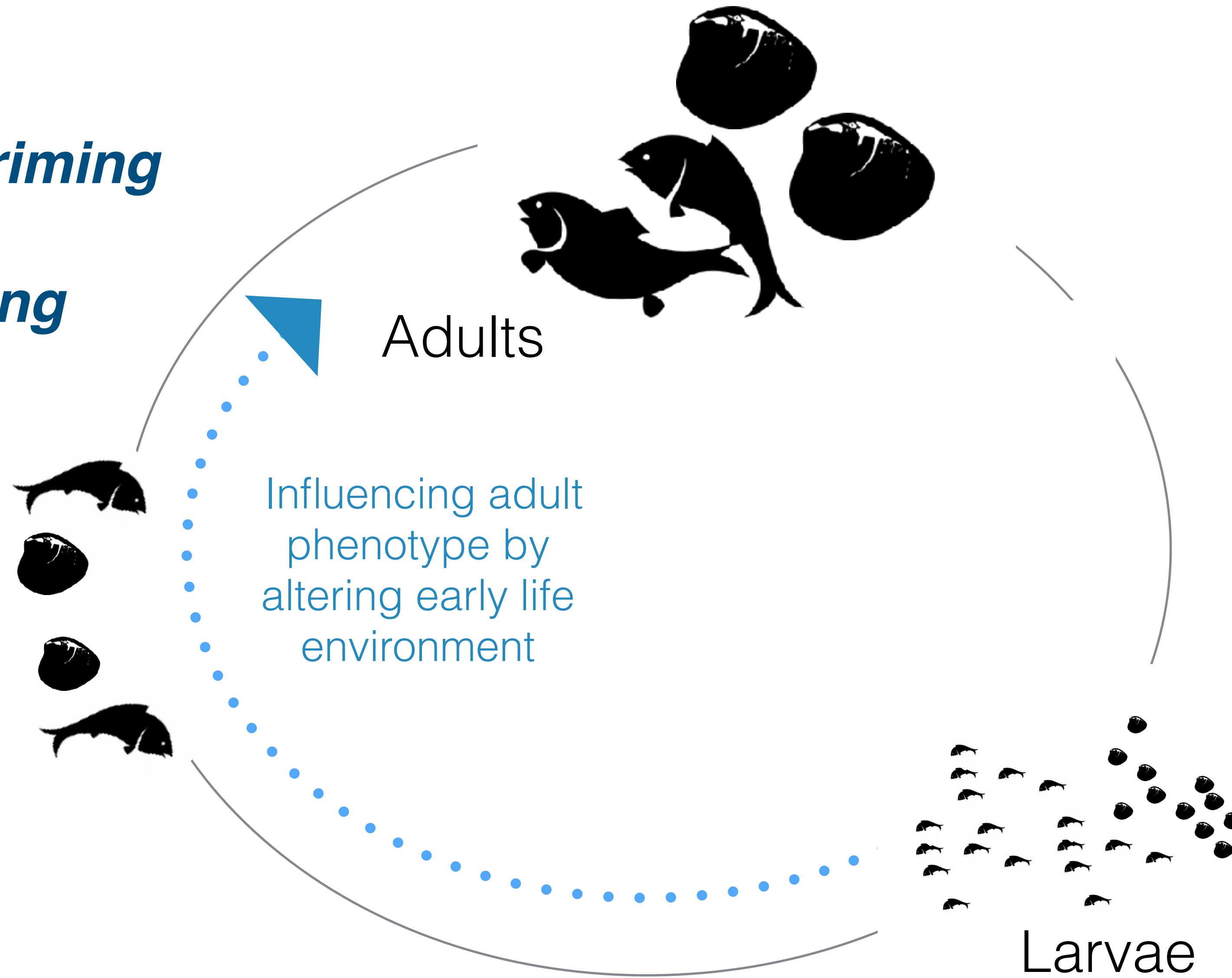
## *Early-life Priming*

## *Hardening*

Adults

Influencing adult  
phenotype by  
altering early life  
environment

Larvae



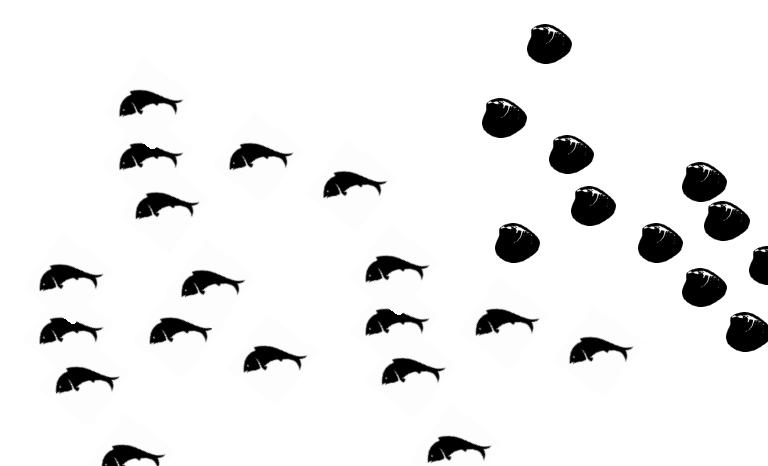
# Trends in Plant Science

## Early-life Priming

### Hardening



Haipei Liu ,<sup>1</sup> Amanda J. Able ,<sup>1</sup> and Jason A. Able  <sup>1,@,\*</sup>

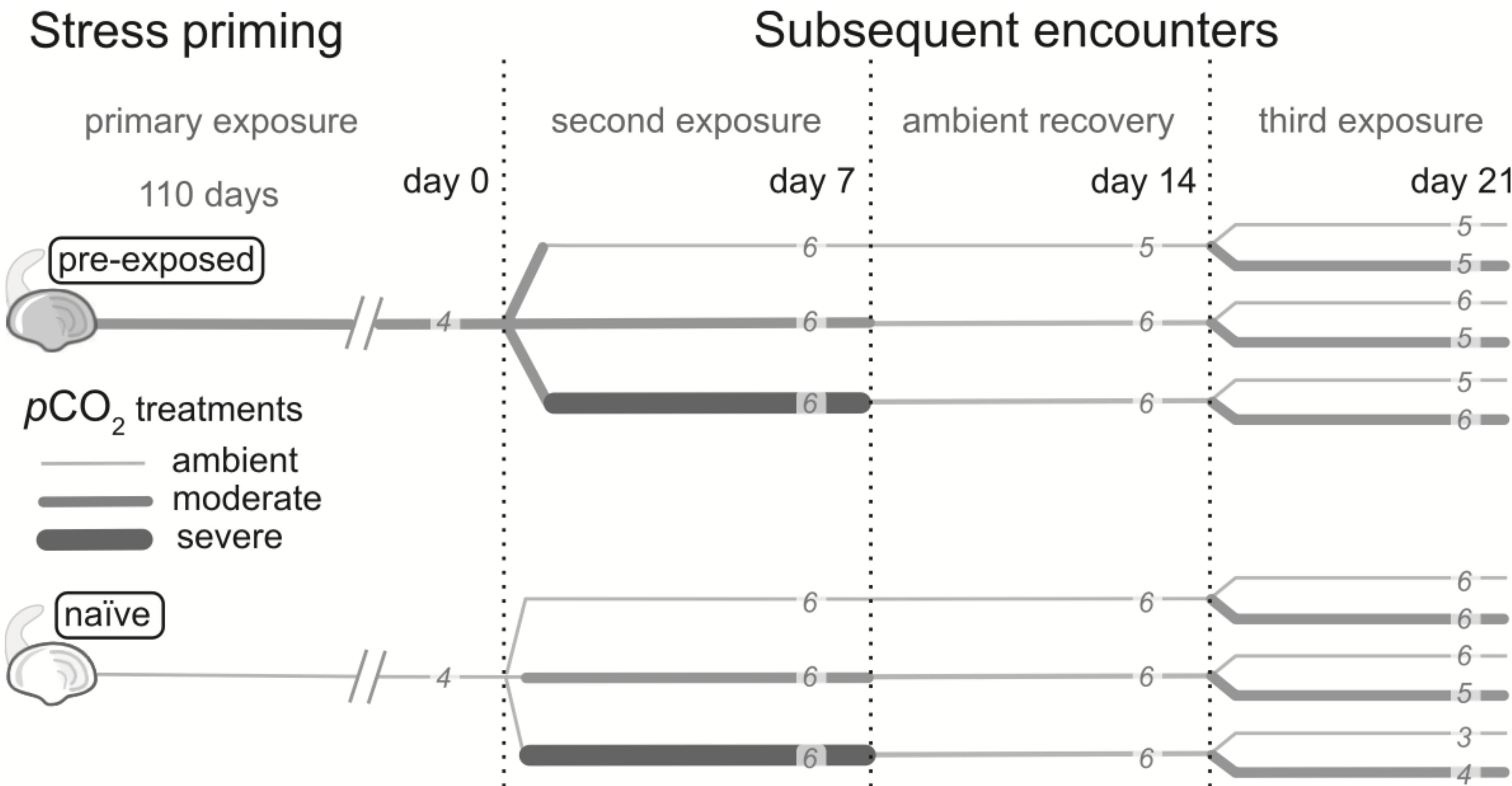


*Cross-stress priming success relies on synergistic stress signalling pathways being shared across stresses varying in nature and intensity.*

# Geoduck Clams

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

Samuel J. Gurr<sup>1,\*</sup>, Shelly A. Wanamaker<sup>2</sup>, Brent Vadopalas<sup>3</sup>, Steven B. Roberts<sup>2</sup> and Hollie M. Putnam<sup>1</sup>

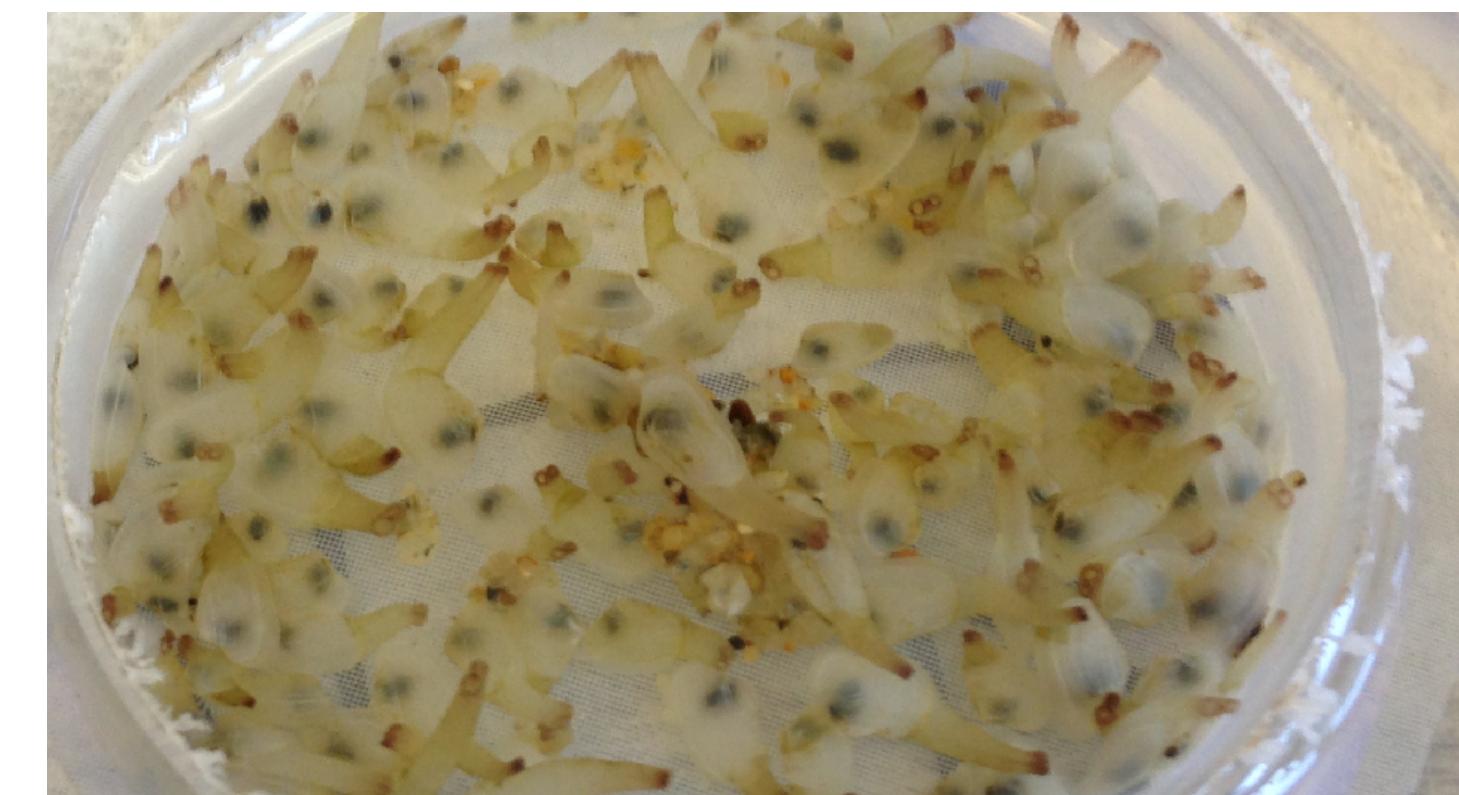


# Geoduck Clams

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

Samuel J. Gurr<sup>1,\*</sup>, Shelly A. Wanamaker<sup>2</sup>, Brent Vadopalas<sup>3</sup>, Steven B. Roberts<sup>2</sup> and Hollie M. Putnam<sup>1</sup>

- Initial conditioning followed by second and third exposure to severe and moderate PCO<sub>2</sub> stress increased respiration rate, organic biomass and shell size, suggesting a stress-intensity-dependent effect on energetics.
- Stress acclimated clams had lower antioxidant capacity compared with clams under ambient conditions, supporting the hypothesis that stress over postlarval-to-juvenile development affects oxidative status later in life.



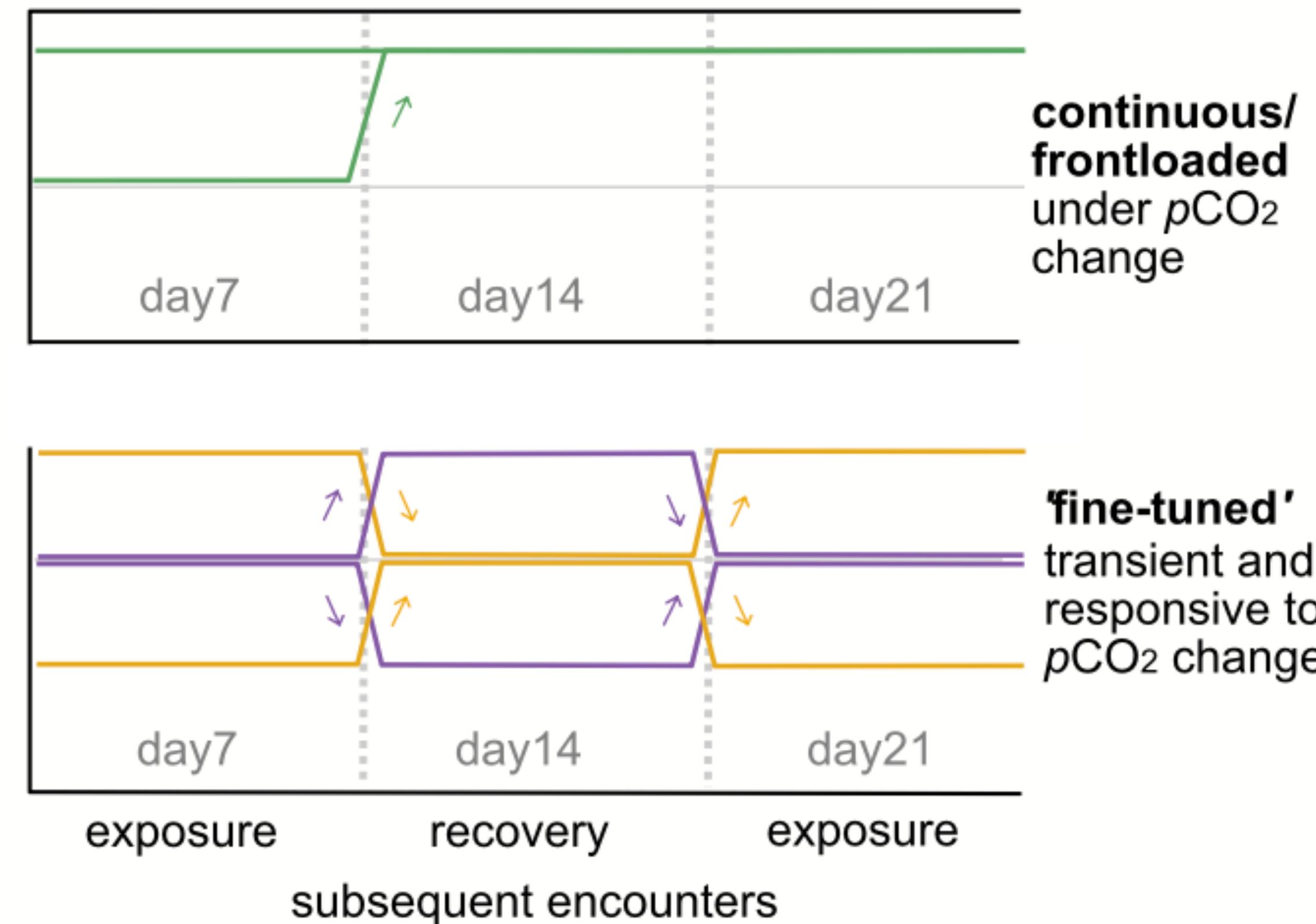
# Geoduck Clams

Acclimatory gene expression of primed clams enhances robustness to elevated  $p\text{CO}_2$  

Samuel J. Gurr<sup>1,2</sup>  | Shelly A. Trigg<sup>3,4</sup>  | Brent Vadopalas<sup>5</sup> | Steven B. Roberts<sup>3</sup>  
Hollie M. Putnam<sup>1</sup> 

(b)

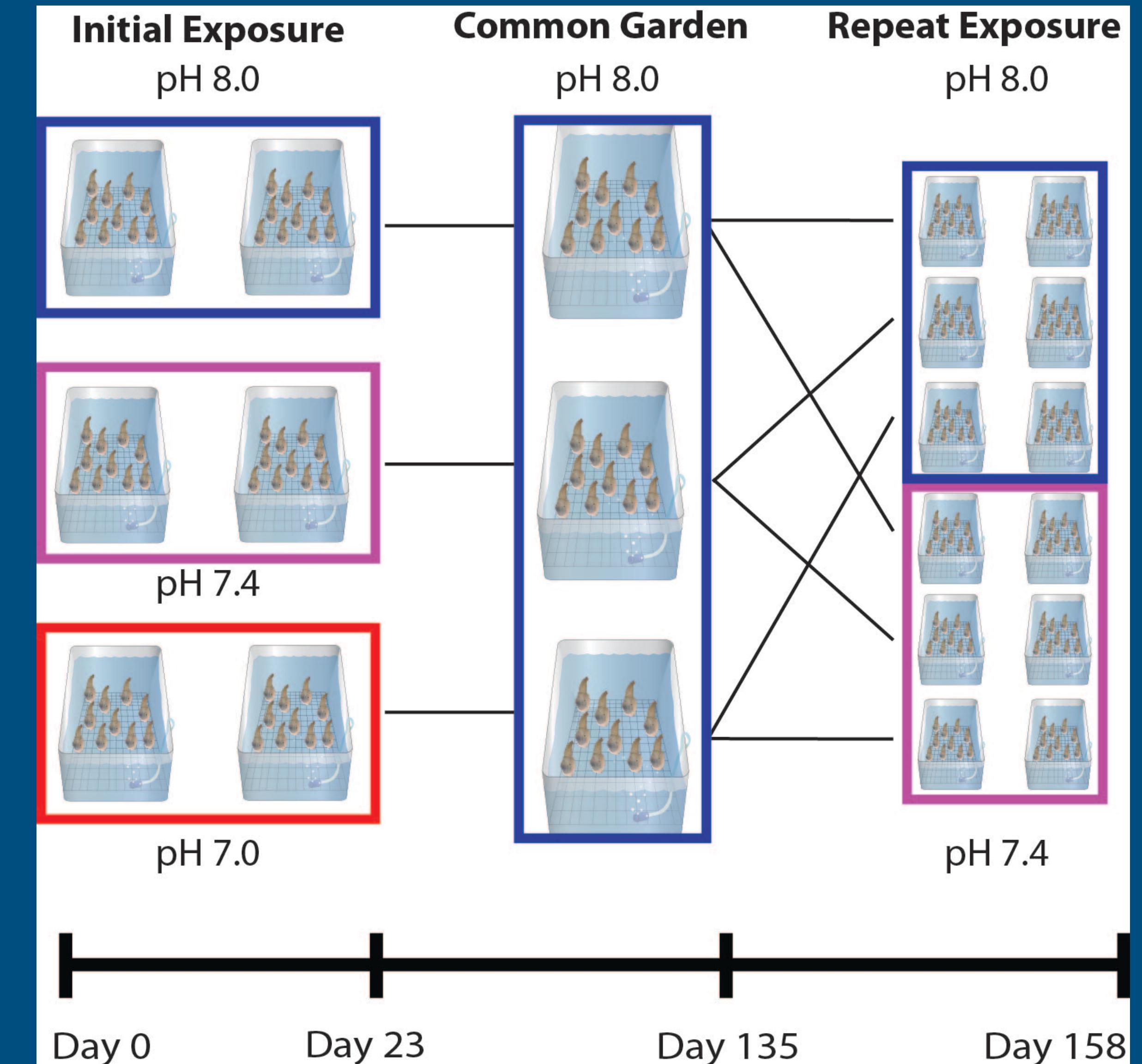
patterns in functional enrichment analysis



## GEODUCKS CLAMS



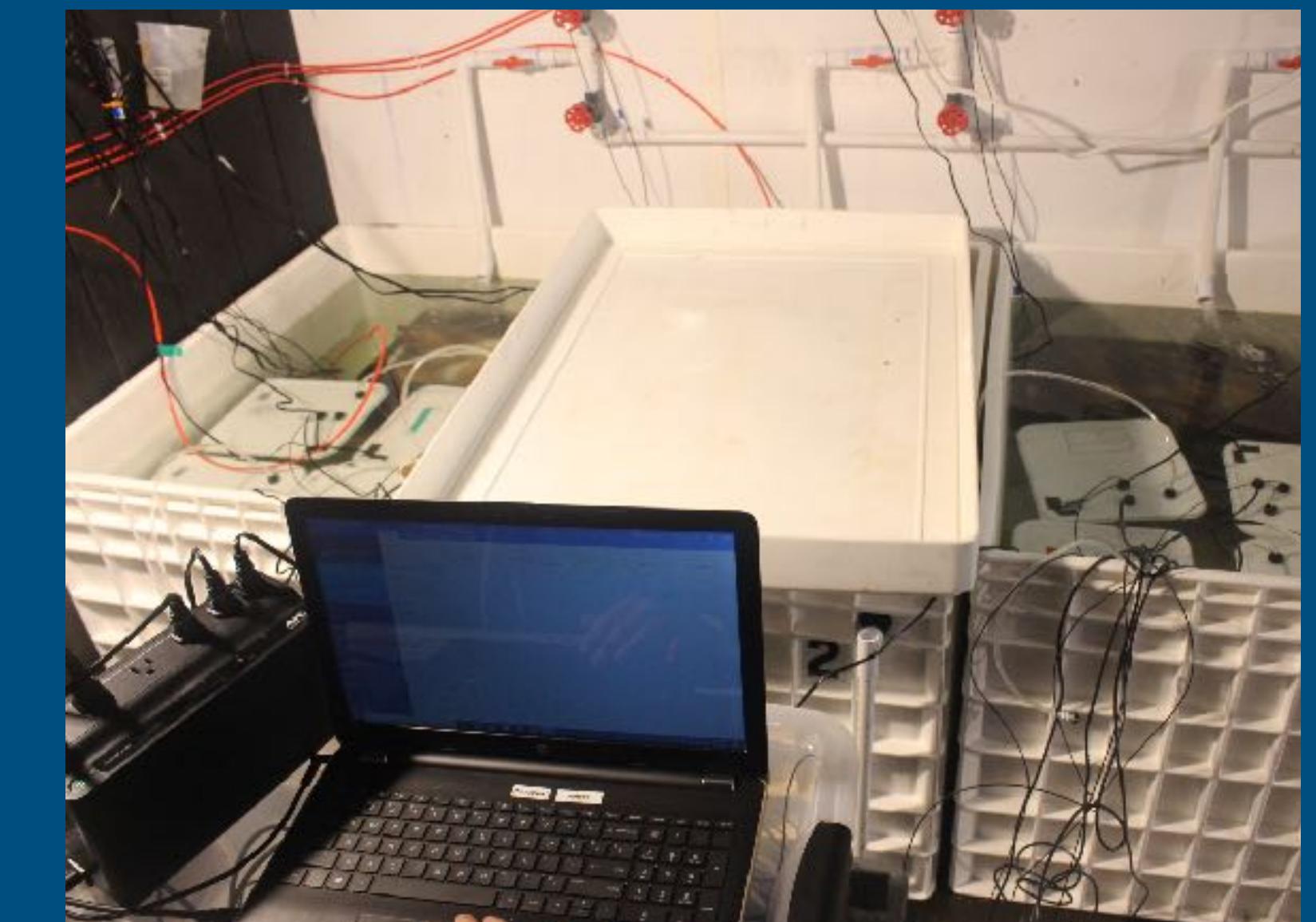
- ▶ Does conditioning to low pH confer tolerance within a generation?



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

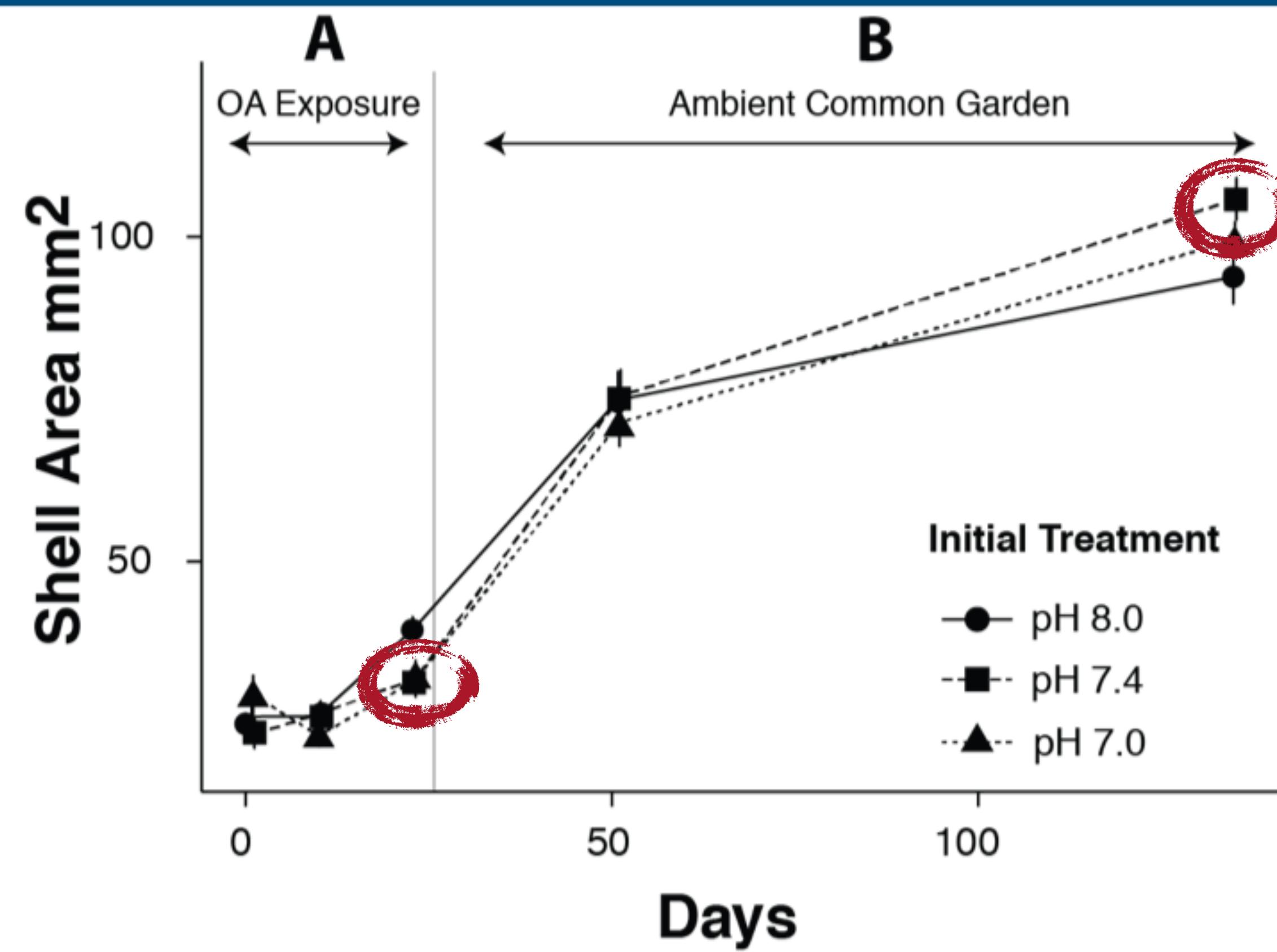
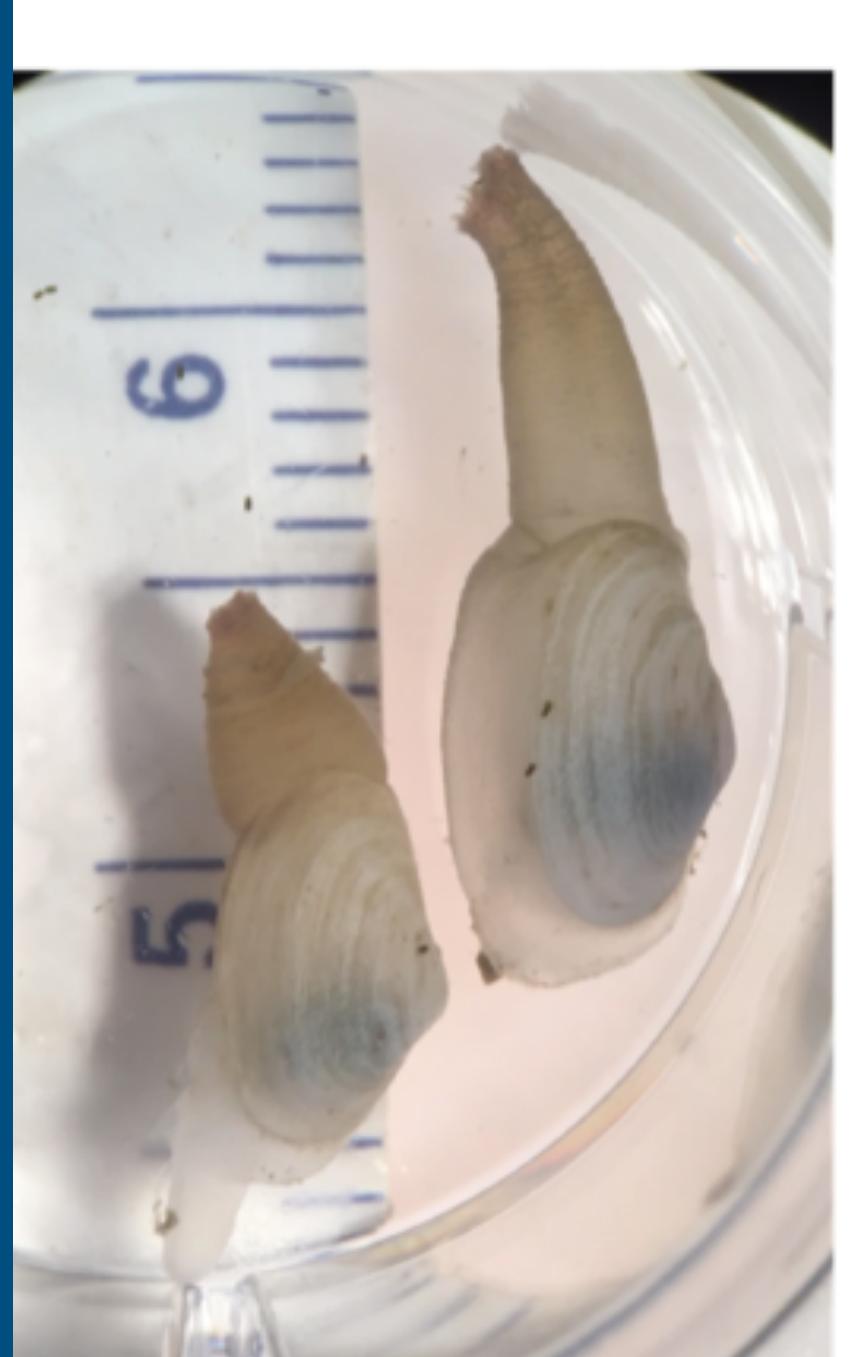
---

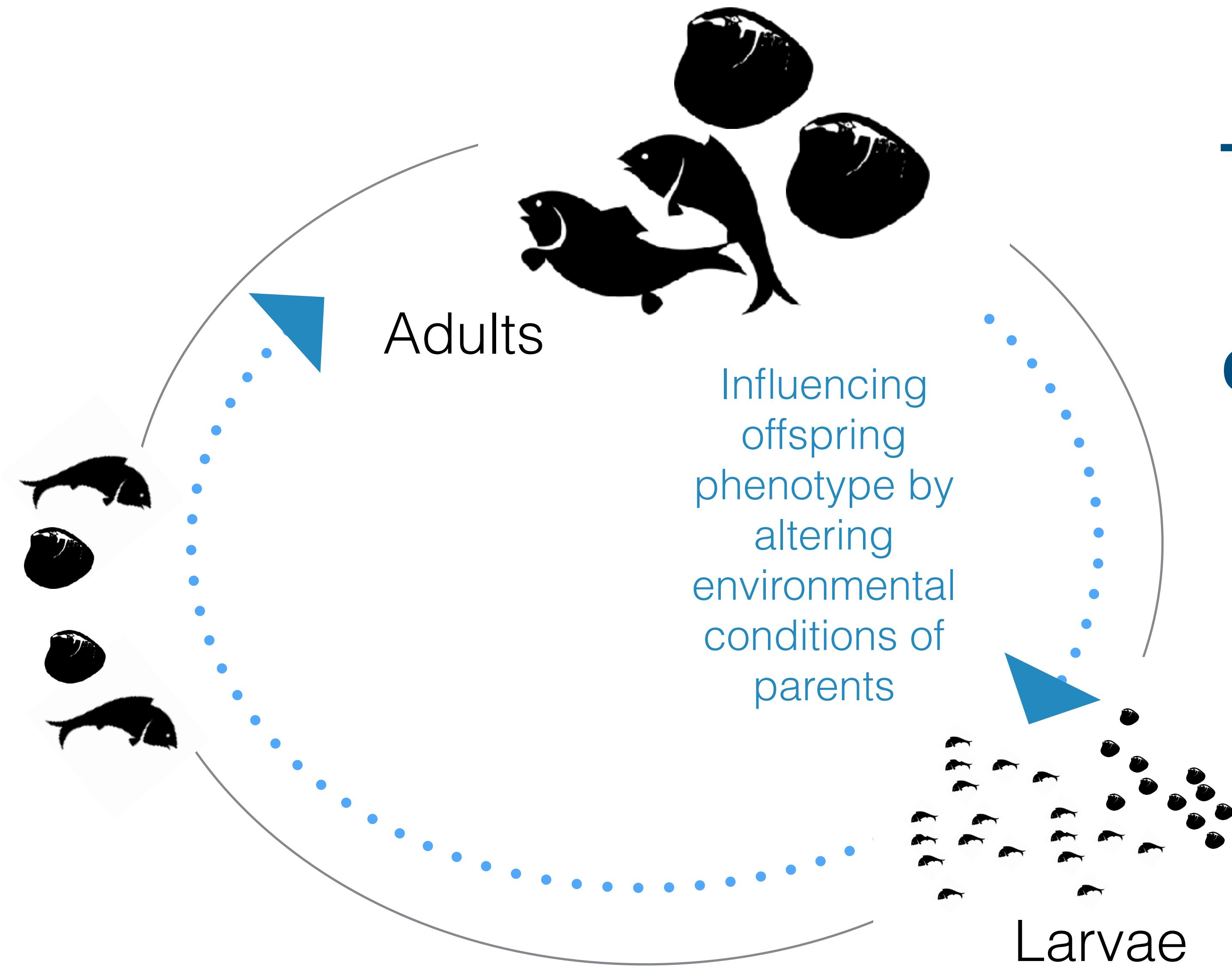
## GEODUCKS CLAMS



# GEODUCKS CLAMS

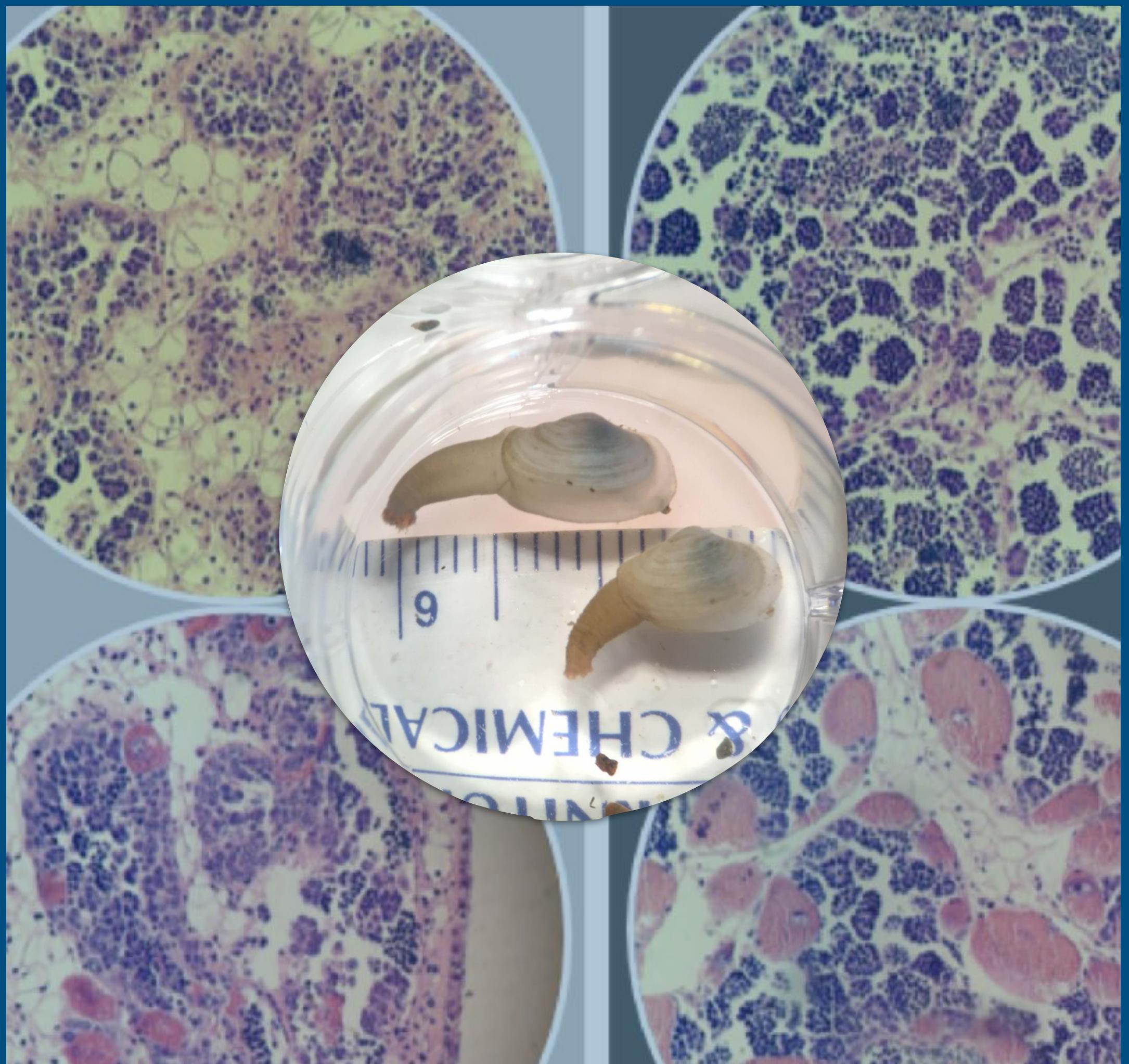
► Does conditioning to low pH confer tolerance within a generation?





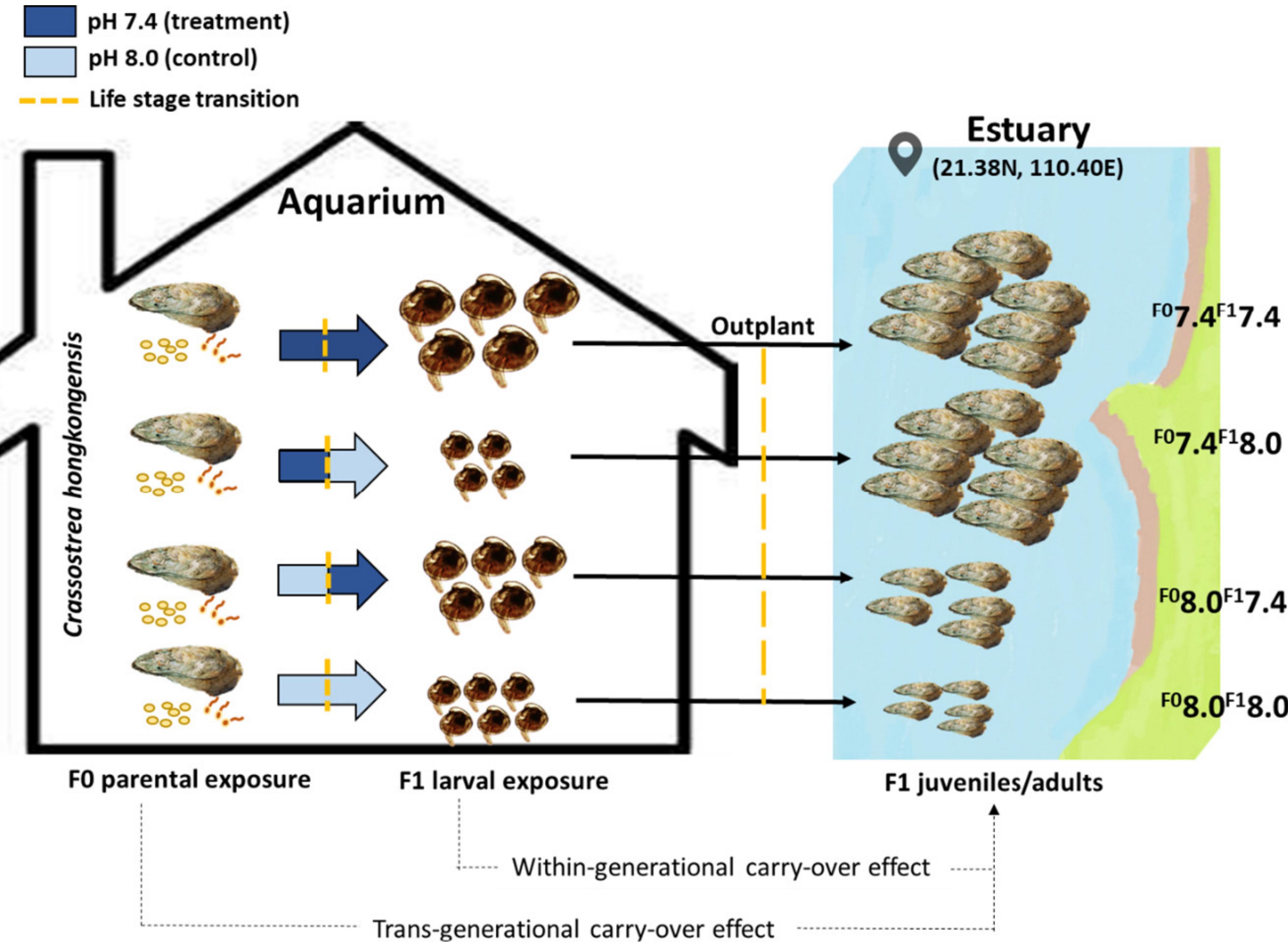
# INFLUENCE OF PARENTAL CONDITIONS

- ▶ Selection (various generations)
- ▶ Germ cells are present
- ▶ Maternal provisioning
- ▶ Paternal role?
- ▶ Beneficial versus detrimental
- ▶ *Mechanisms at play*



# Crassostrea hongokn

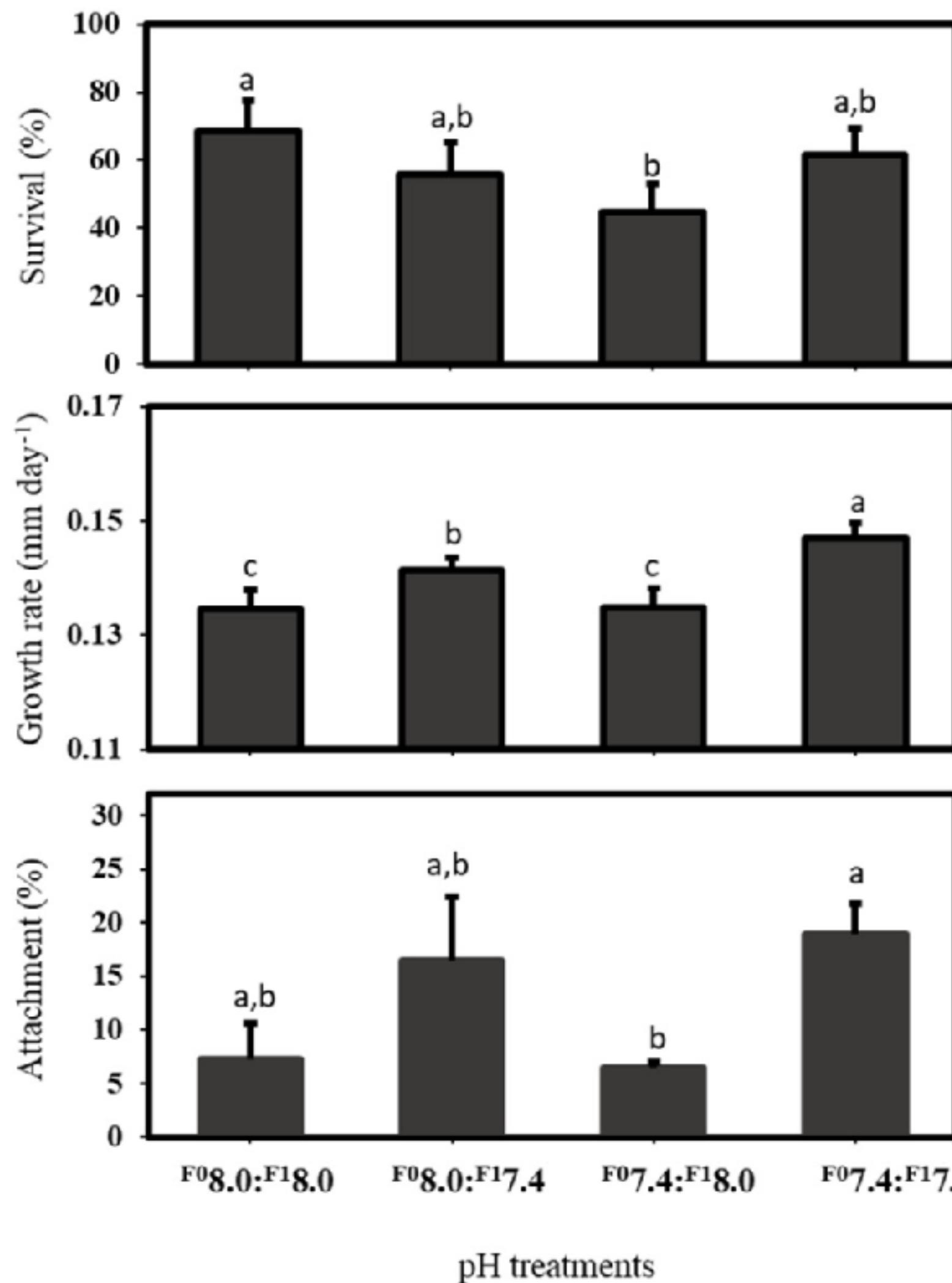
Transgenerational responses to seawater pH in the edible oyster, with implications for the mariculture of the species under future ocean acidification



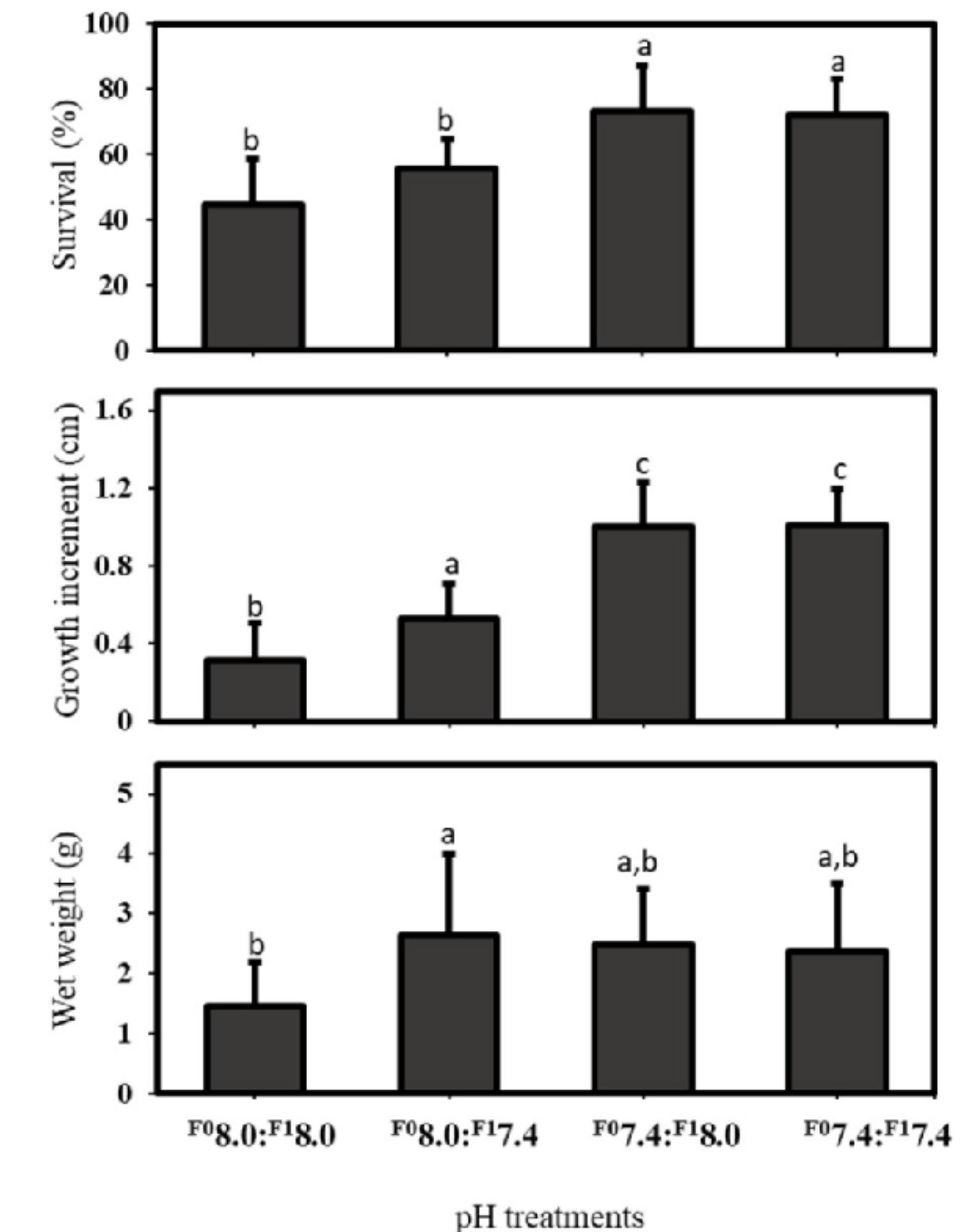
# Oysters



A. F<sub>1</sub> larvae



B. F<sub>1</sub> juveniles



Transgenerational responses to  
seawater pH in the edible oyster,  
with implications for the  
mariculture of the species under  
future ocean acidification

Yong-Kian Lim<sup>1</sup>, Xin Dang<sup>1</sup>, Vengatesen Thiagarajan  

# EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS

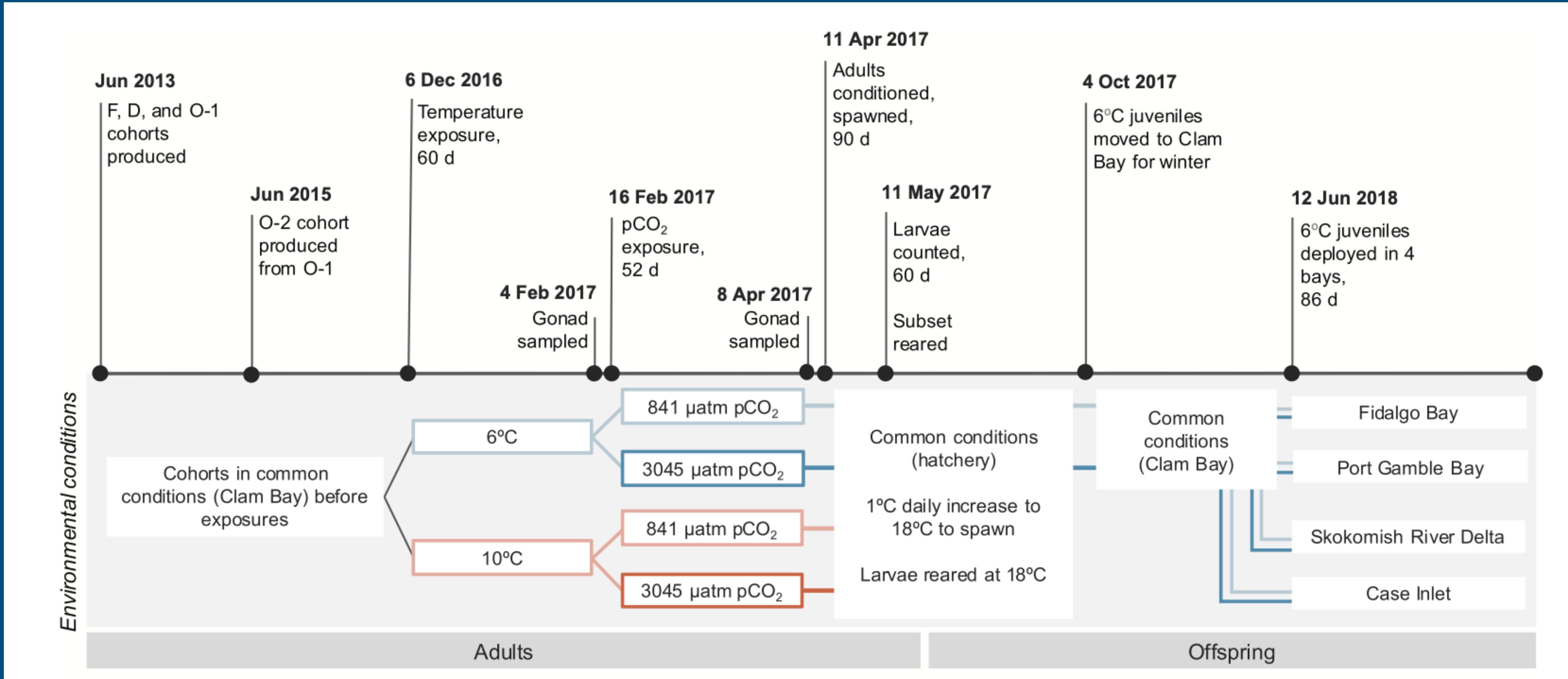


- ▶ Oysters were held at two temperature regimes (6°C and 10°C) for 60 days in December
- ▶ A differential pCO<sub>2</sub> exposure was carried out after the temperature treatment ended. Held at ambient pCO<sub>2</sub> (841 µatm) or high pCO<sub>2</sub> (3045 µatm) for 52 days, during the Winter.

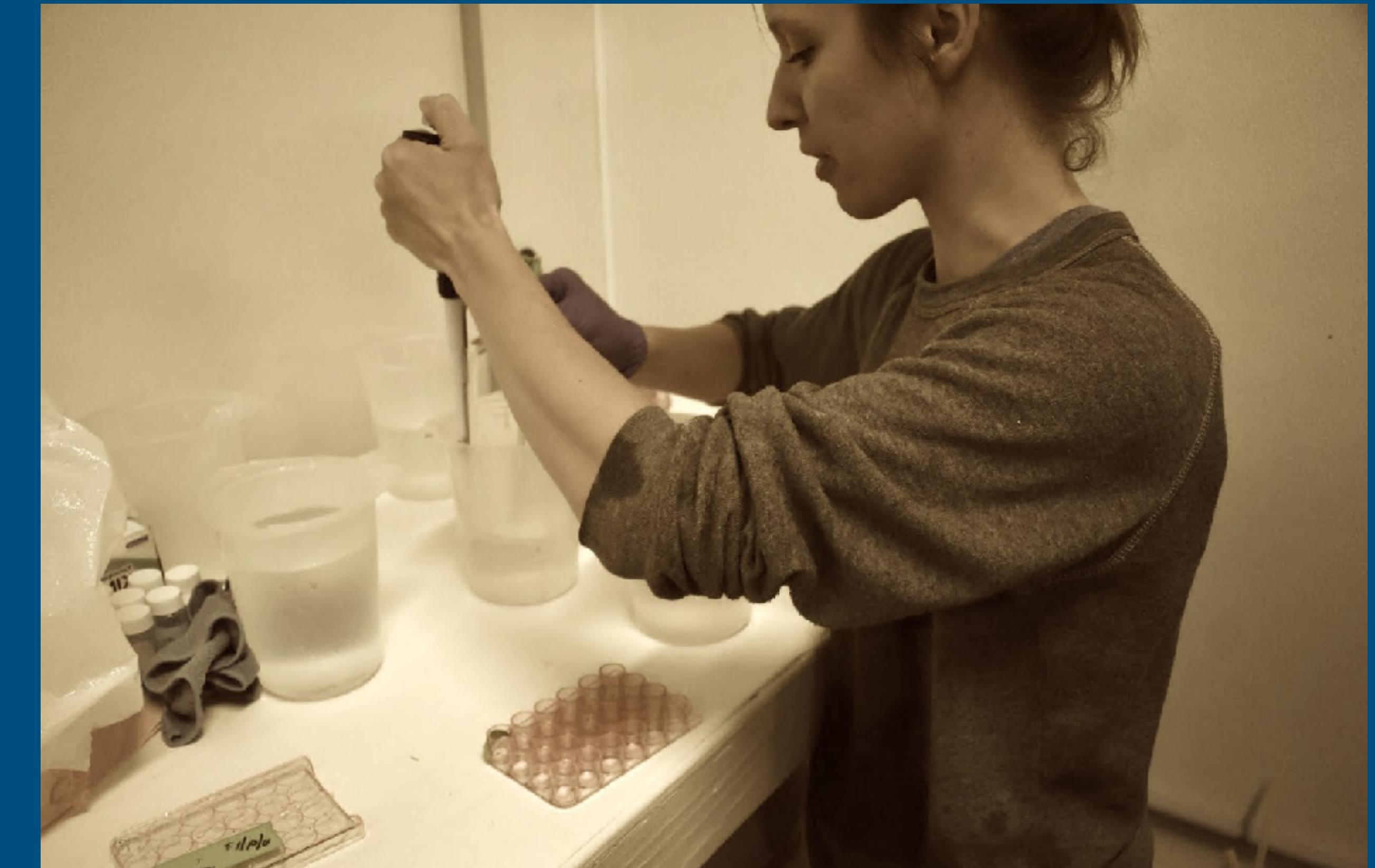
Carryover effects of temperature and pCO<sub>2</sub> across multiple Olympia oyster populations

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

# TEXT



# EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS

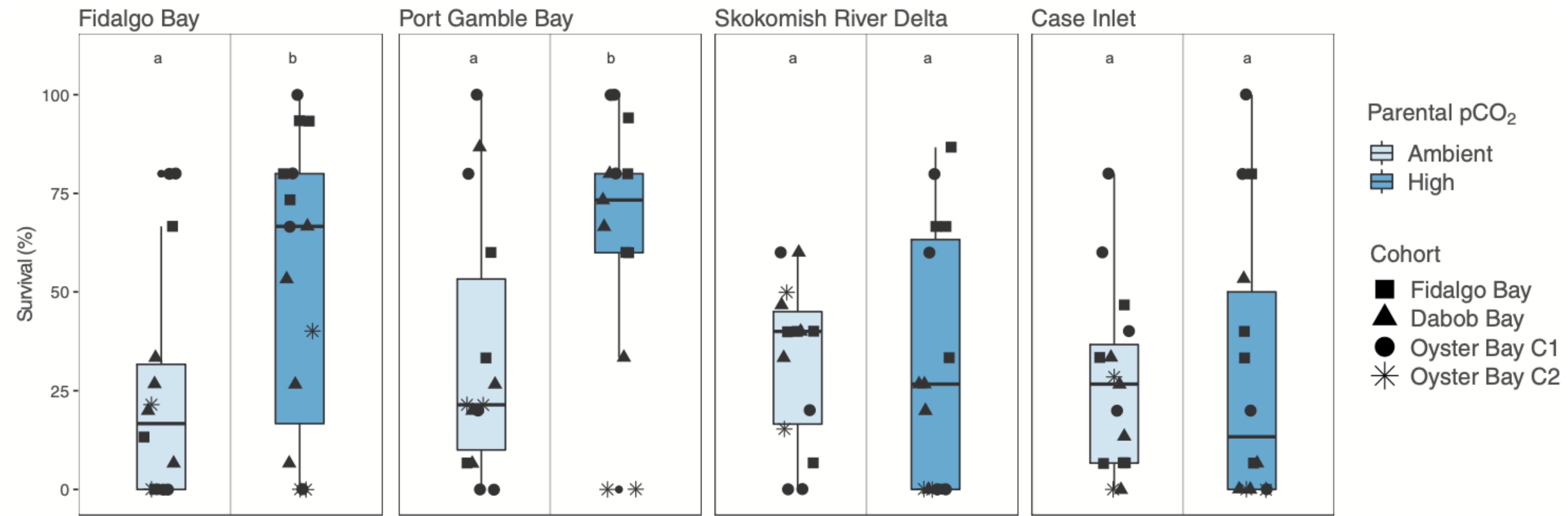


# EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS



- ▶ 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**

# EFFECTS OF OA IN OLYMPIA OYSTER POPULATIONS



# Latent effects of winter warming on Olympia oyster reproduction and larval viability

Laura H. Spencer <sup>a,\*</sup>, Erin Horkan <sup>b</sup>, Ryan Crim <sup>b</sup>, Steven B. Roberts <sup>a</sup>

<sup>a</sup> University of Washington, School of Aquatic and Fishery Sciences, 1122 NE Boat St, Seattle, WA 98105, United States

<sup>b</sup> Puget Sound Restoration Fund, 8001 NE Day Rd W, Bainbridge Island, WA 98110, United States

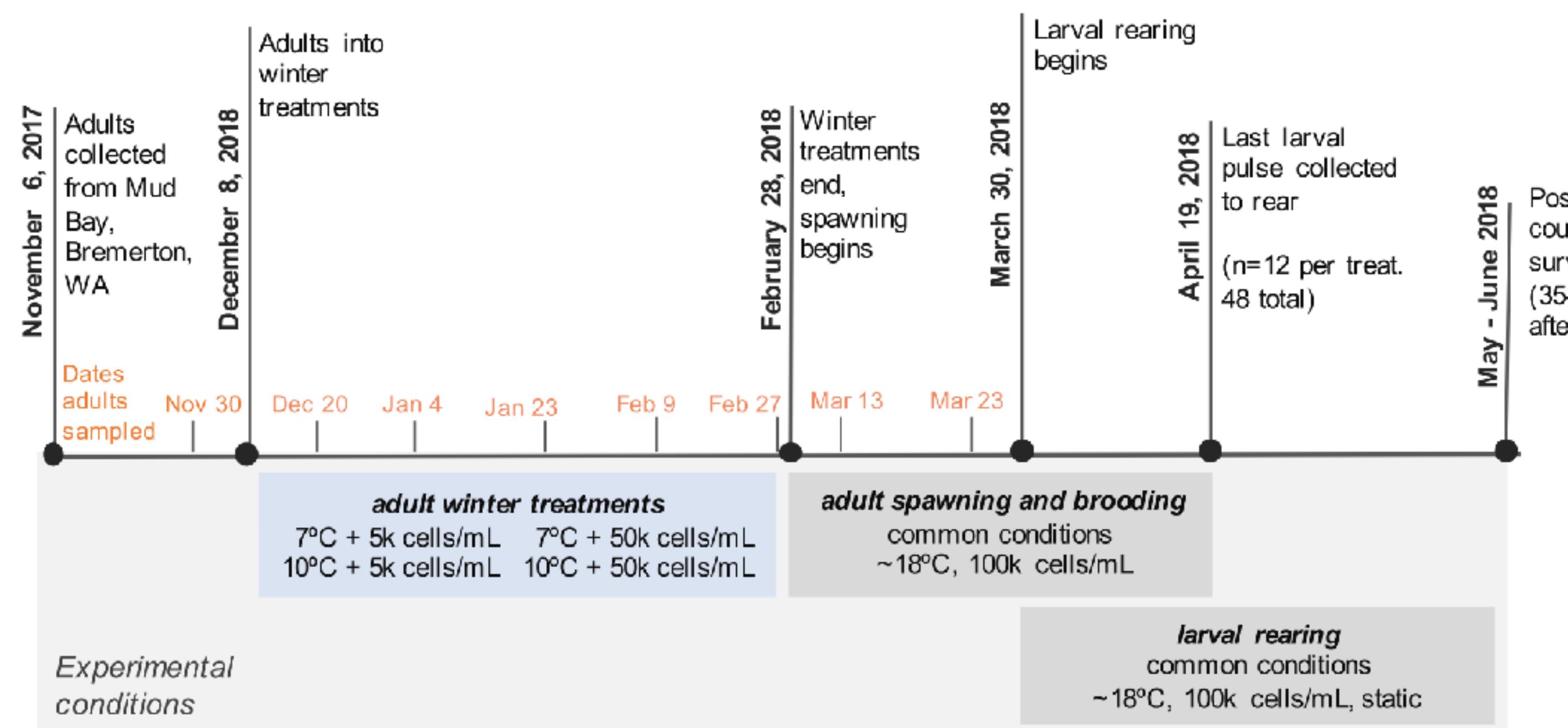
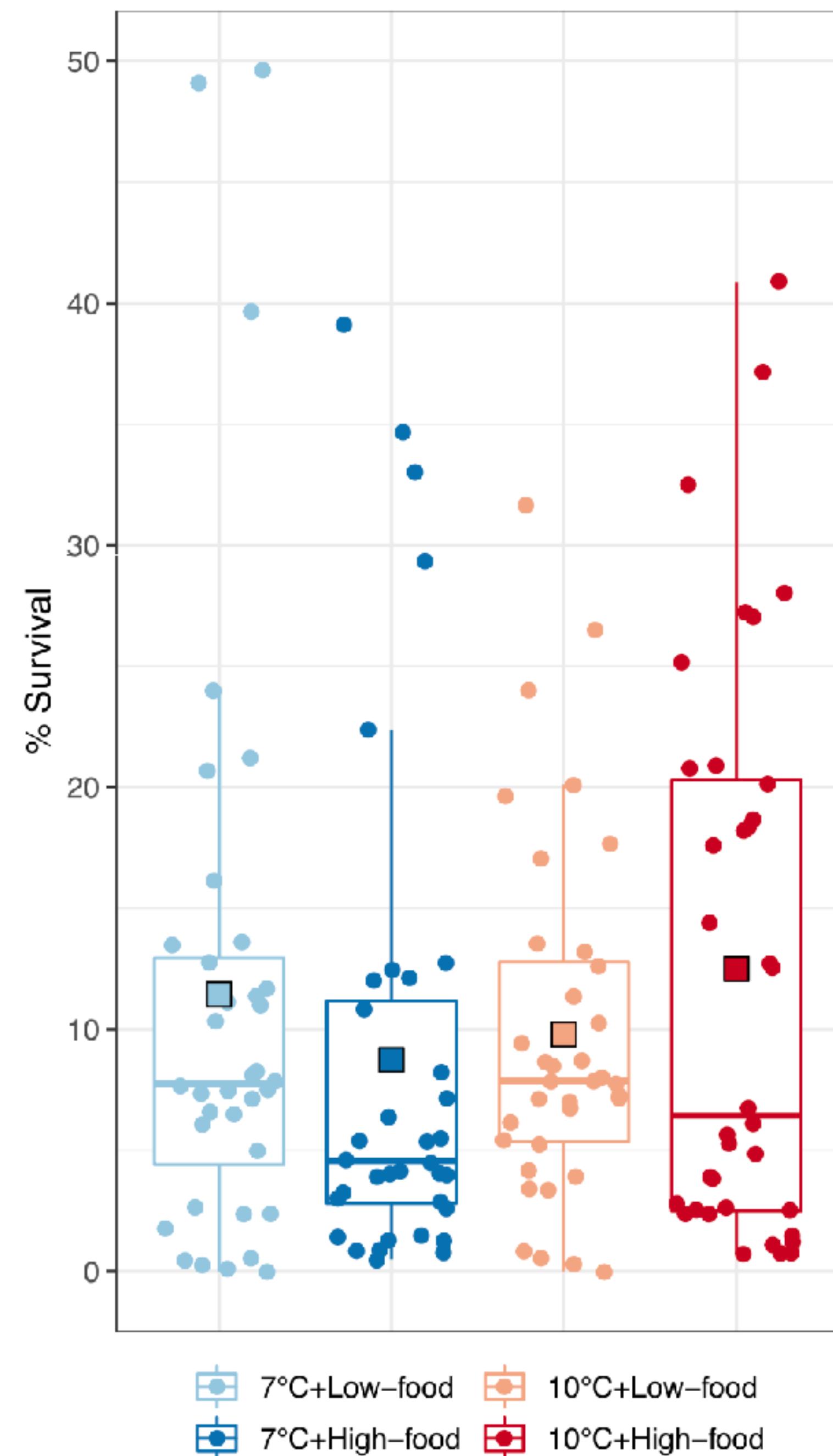


Fig. 1. Experimental timeline. Cells/mL indicate the concentrations of live algae given to oysters at various stages.

## Larval survival by parental treatment



# Environmental Memory



CAN BE BENEFICIAL  
STAGE DEPENDENT  
ACROSS GENERATION  
IMPLICATIONS ...

# Environmental Memory



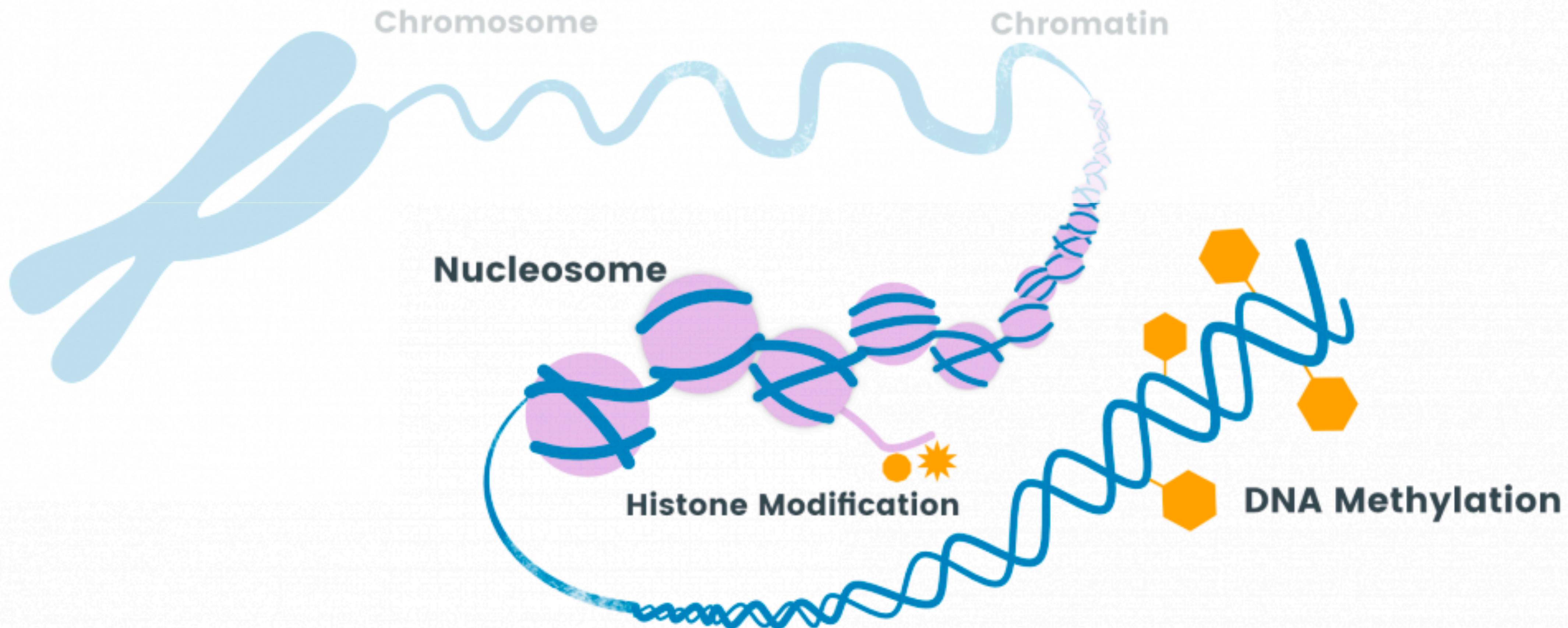
CAN BE BENEFICIAL  
STAGE DEPENDENT  
ACROSS GENERATION  
IMPLICATIONS . . .

**Epigenetic Mechanisms**

# Epigenetics

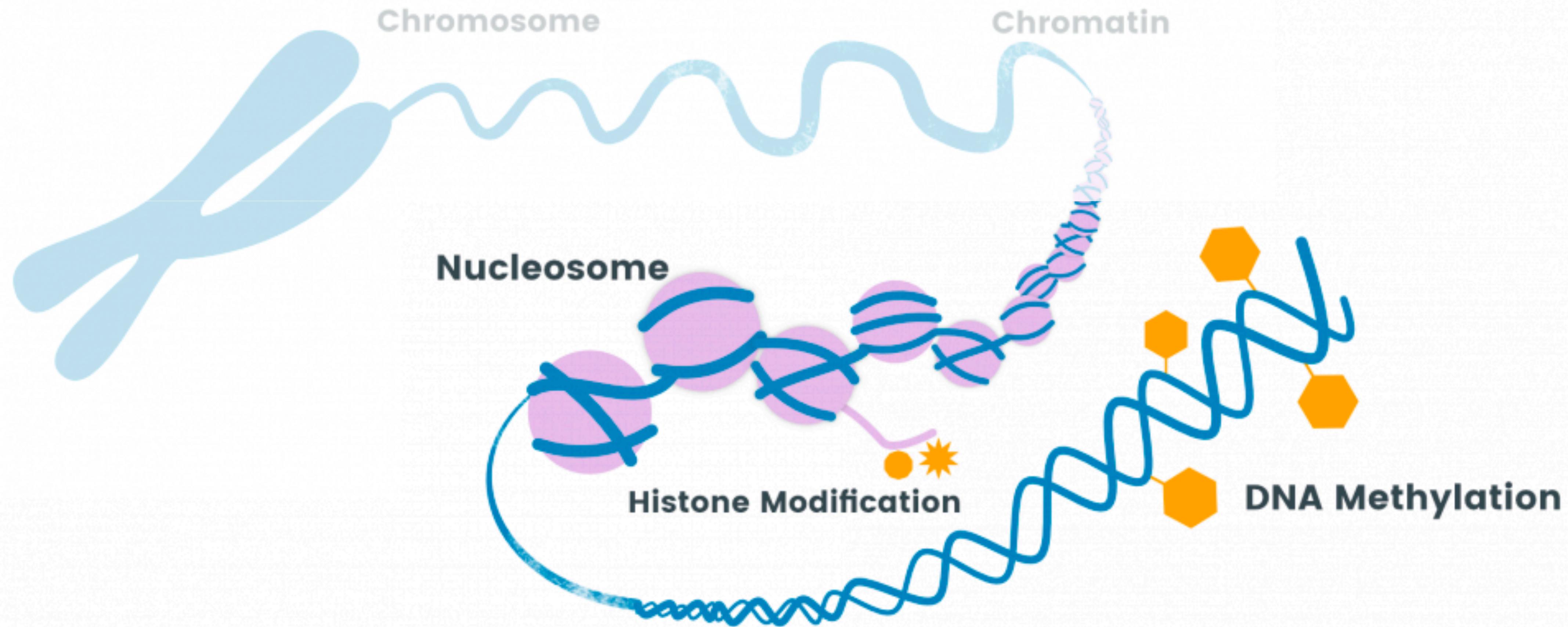
## WHAT IS EPIGENETICS?

**ALTERS THE PHENOTYPE (WITHOUT CHANGING DNA CODE); HERITABLE**

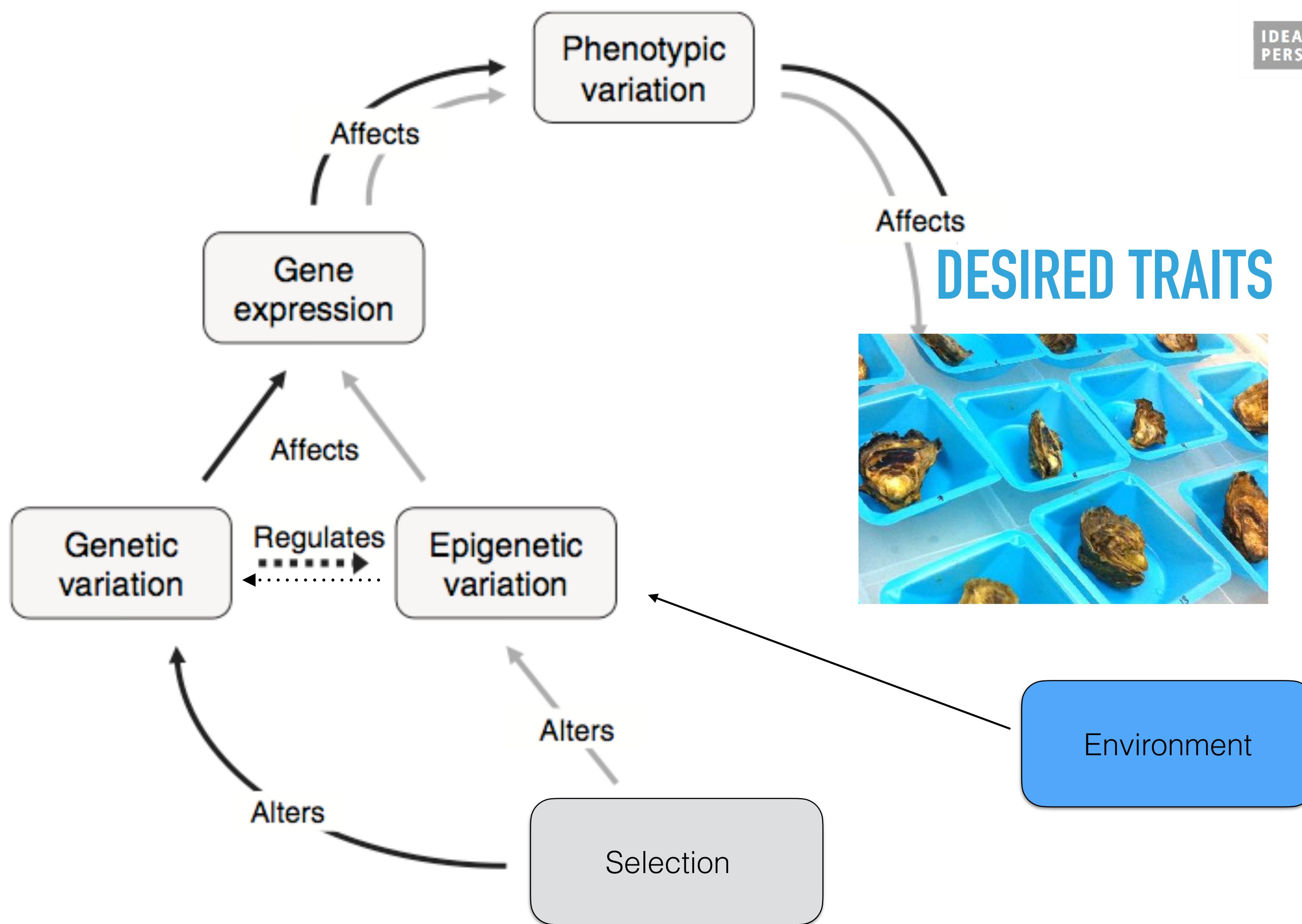


## WHAT IS EPIGENETICS?

**ALTERS THE PHENOTYPE (WITHOUT CHANGING DNA CODE); HERITABLE**



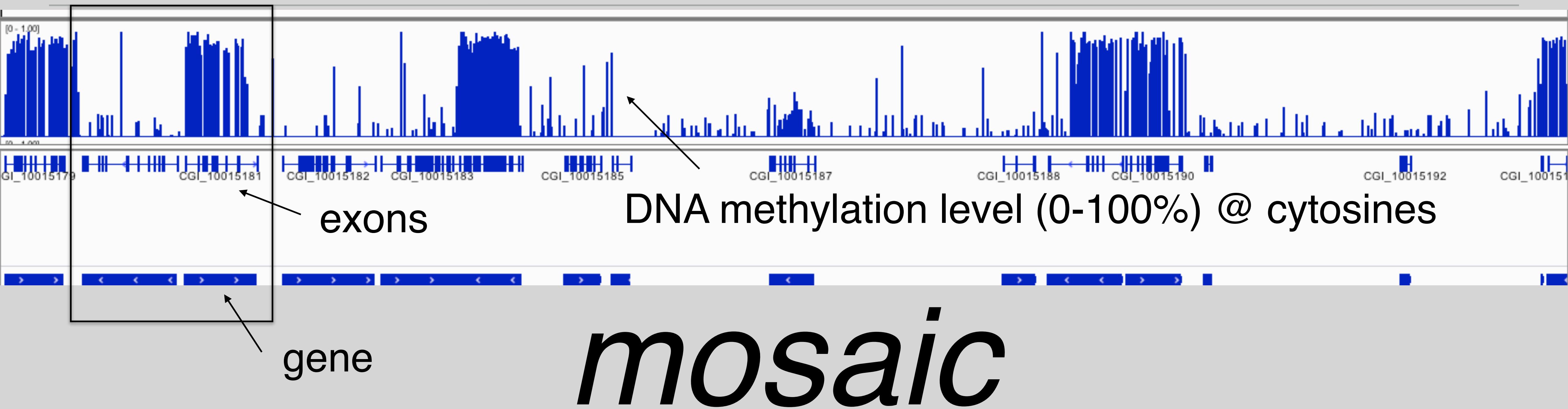
**CAN BE INDUCED WITH ENVIRONMENTAL MANIPULATION**



IDEA AND  
PERSPECTIVE

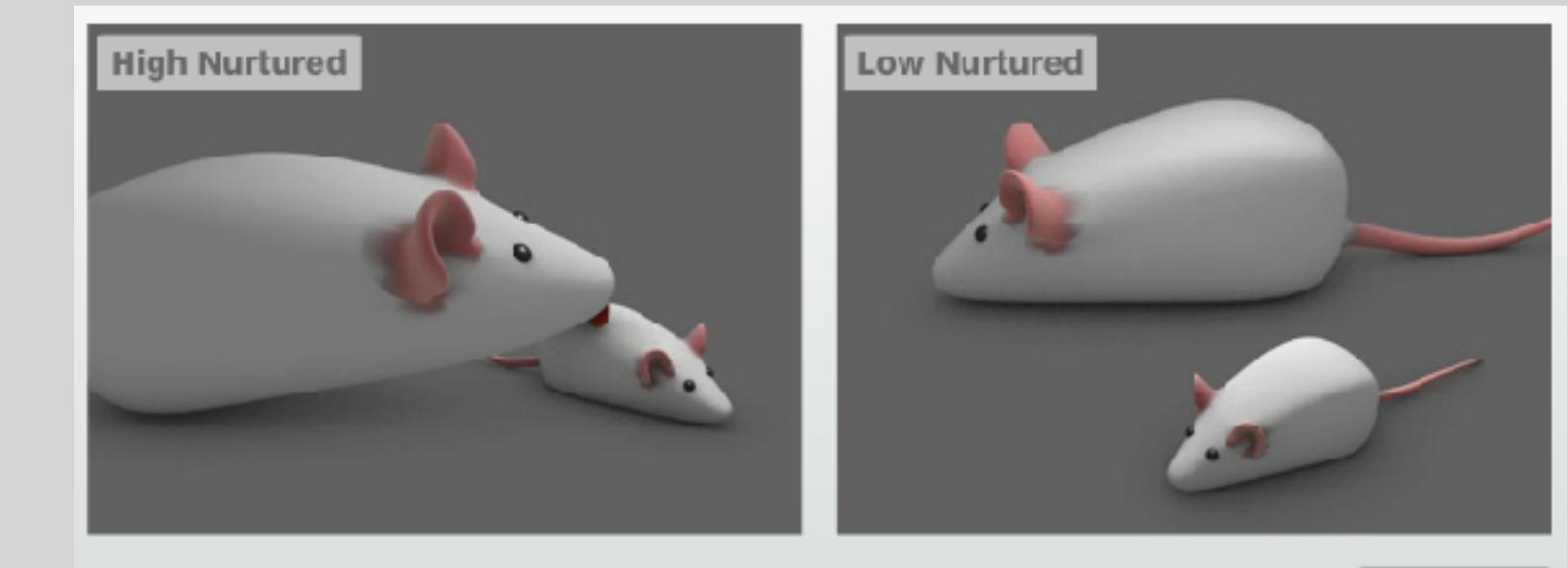
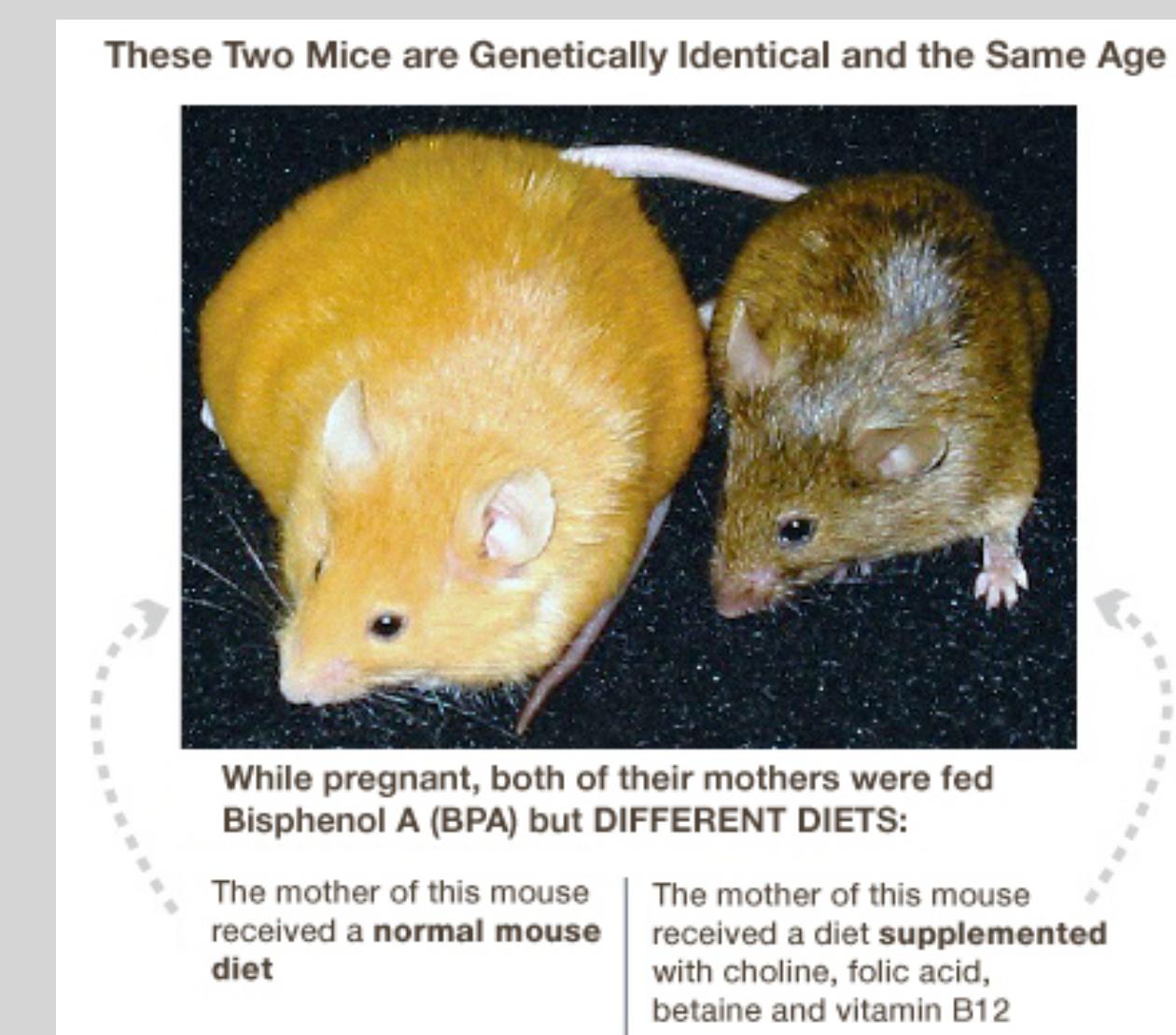
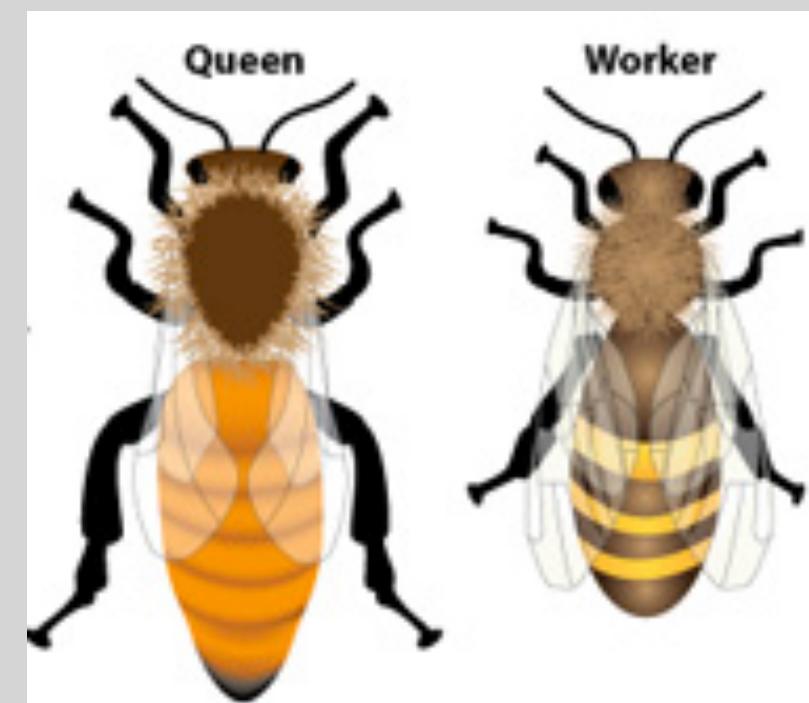
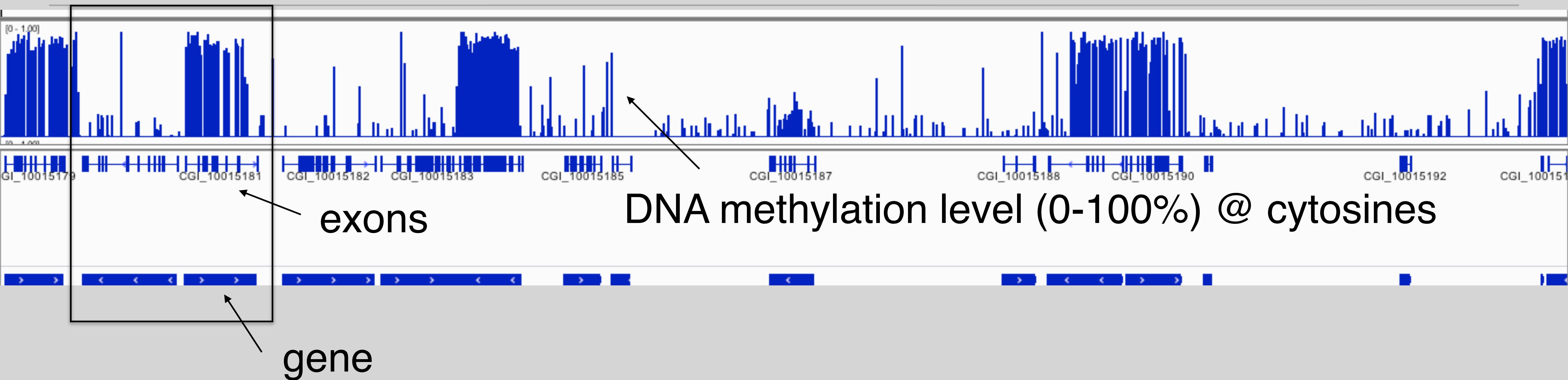
Epigenetics for ecologists

## METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



associated with gene bodies

# METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



# Four Dimensionalities



Reliable Transcription

Spurious Transcription

Targeted Regulation

Stochastic Regulation

# Four Dimensionalities

- Evolutionary
- Life History Driven
- Constitutive

Reliable Transcription

Spurious Transcription

- Distinct Lineage
- Experiential
- Inducible

Targeted Regulation

Stochastic Regulation

# Four Dimensionalities

- Evolutionary
- Life History Driven
- Constitutive

Reliable Transcription

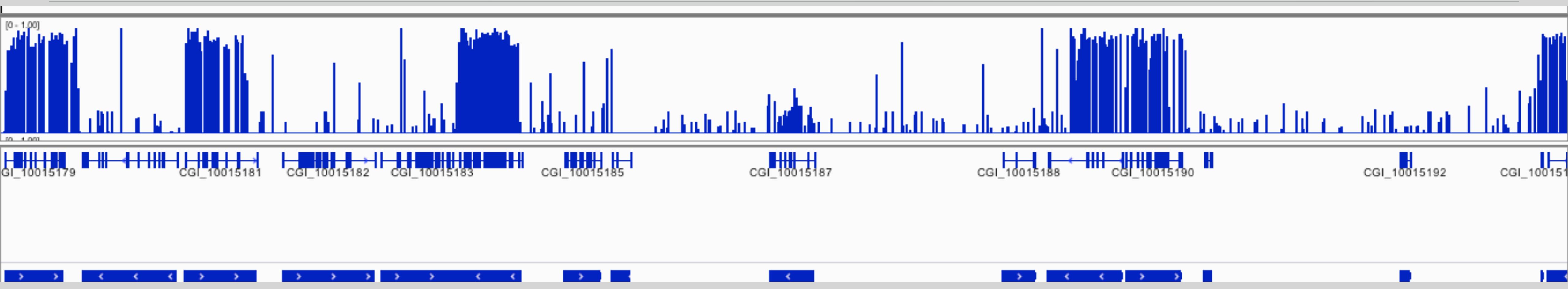
Spurious Transcription

- Distinct Lineage
- Experiential
- Inducible

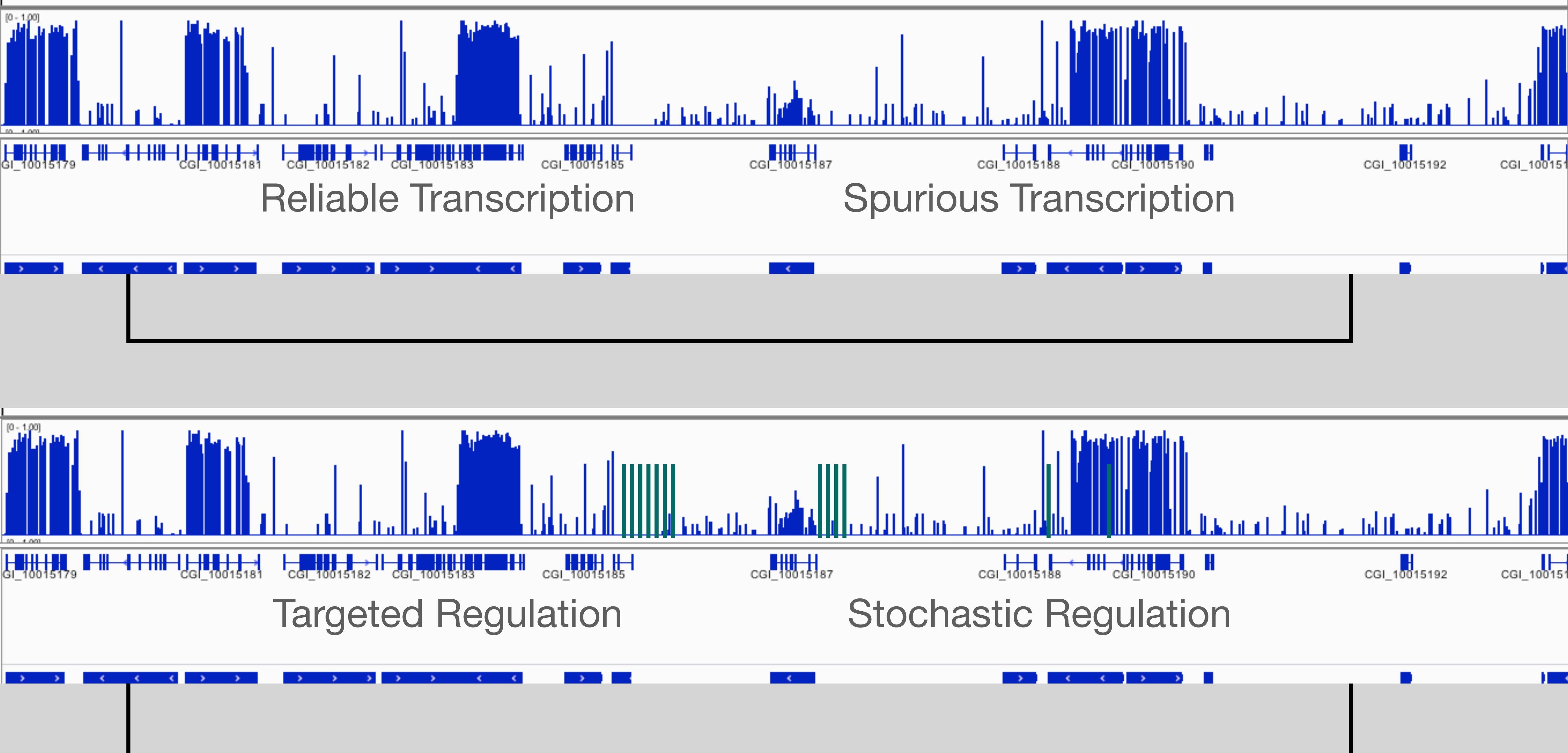
Targeted Regulation

Stochastic Regulation

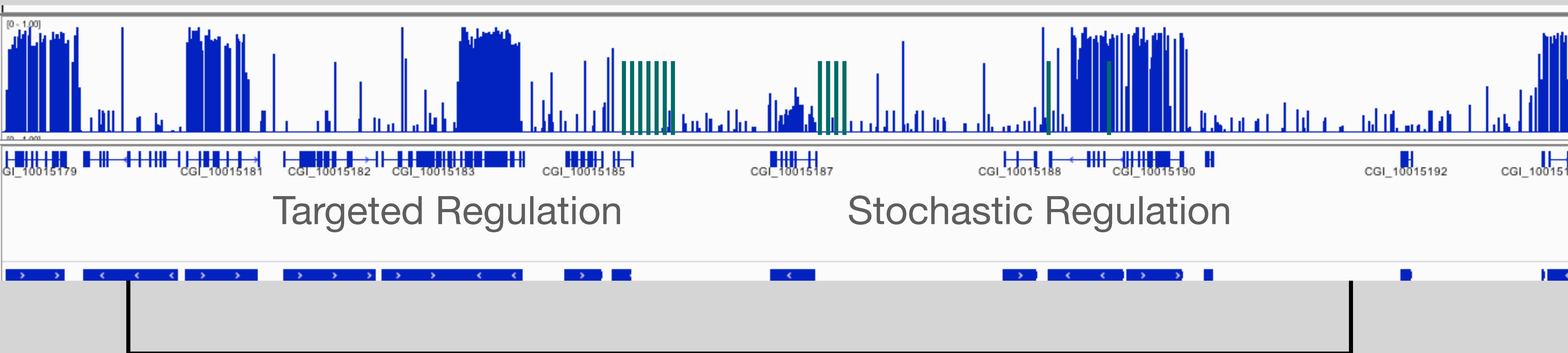
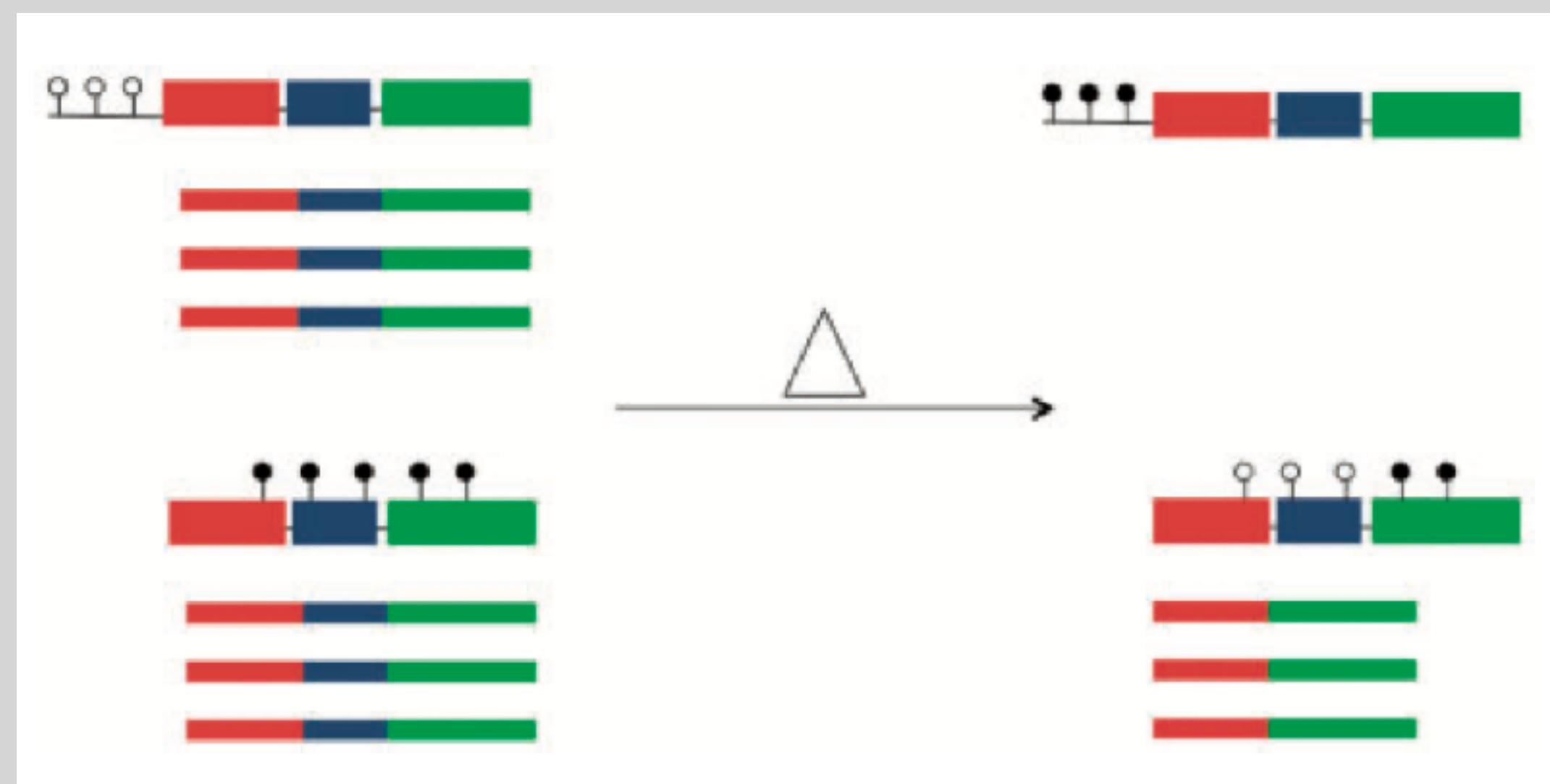
# METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



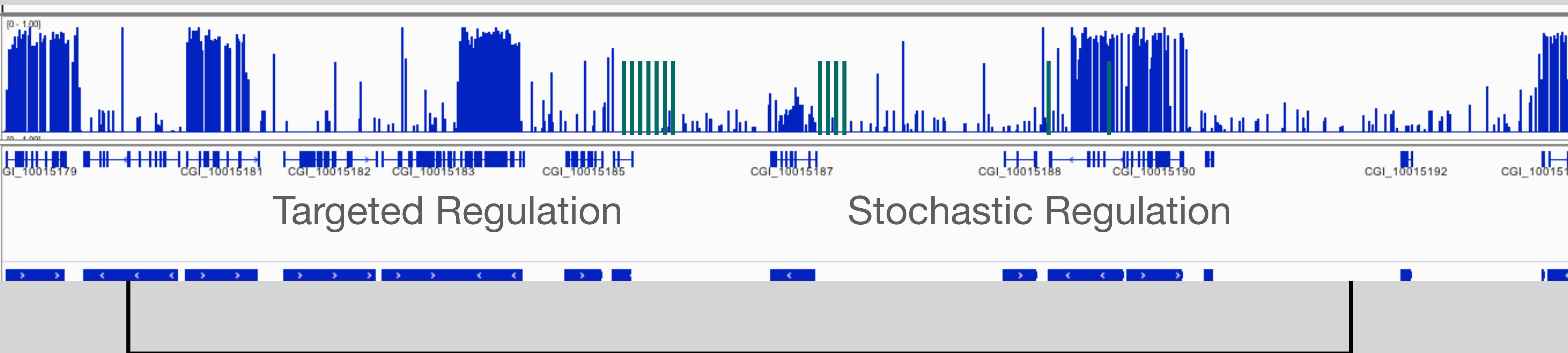
# METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



# METHYLATION LANDSCAPE IN MARINE INVERTEBRATES

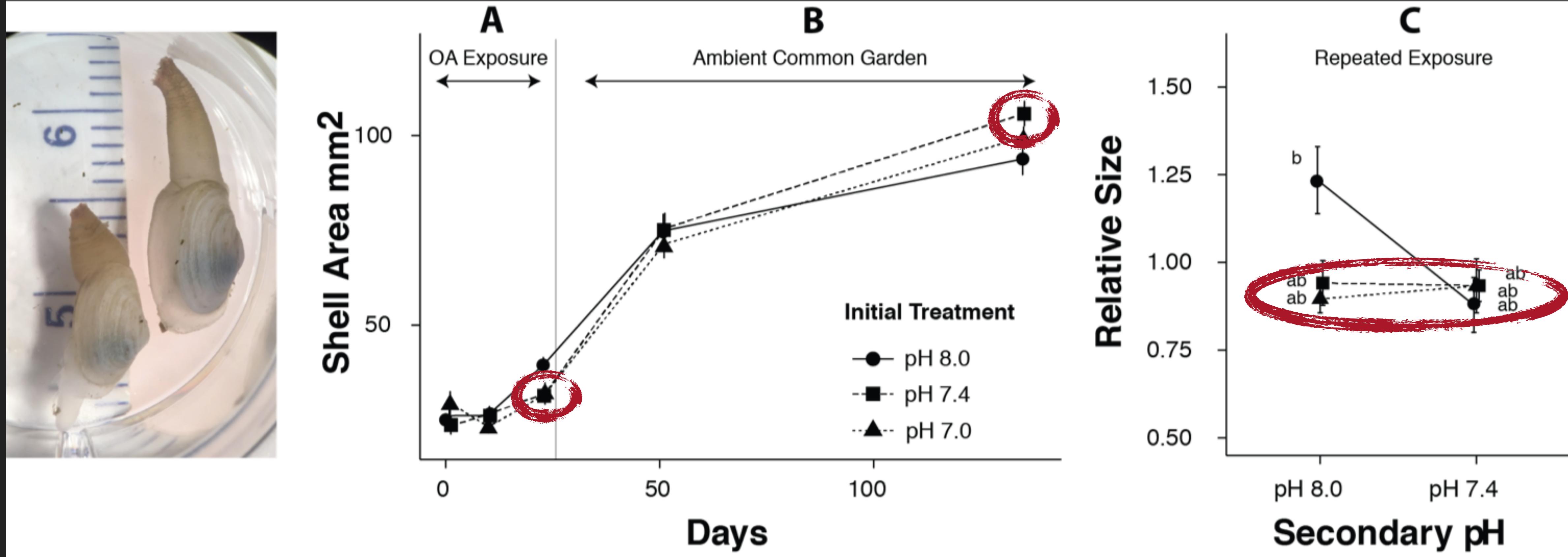


# METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



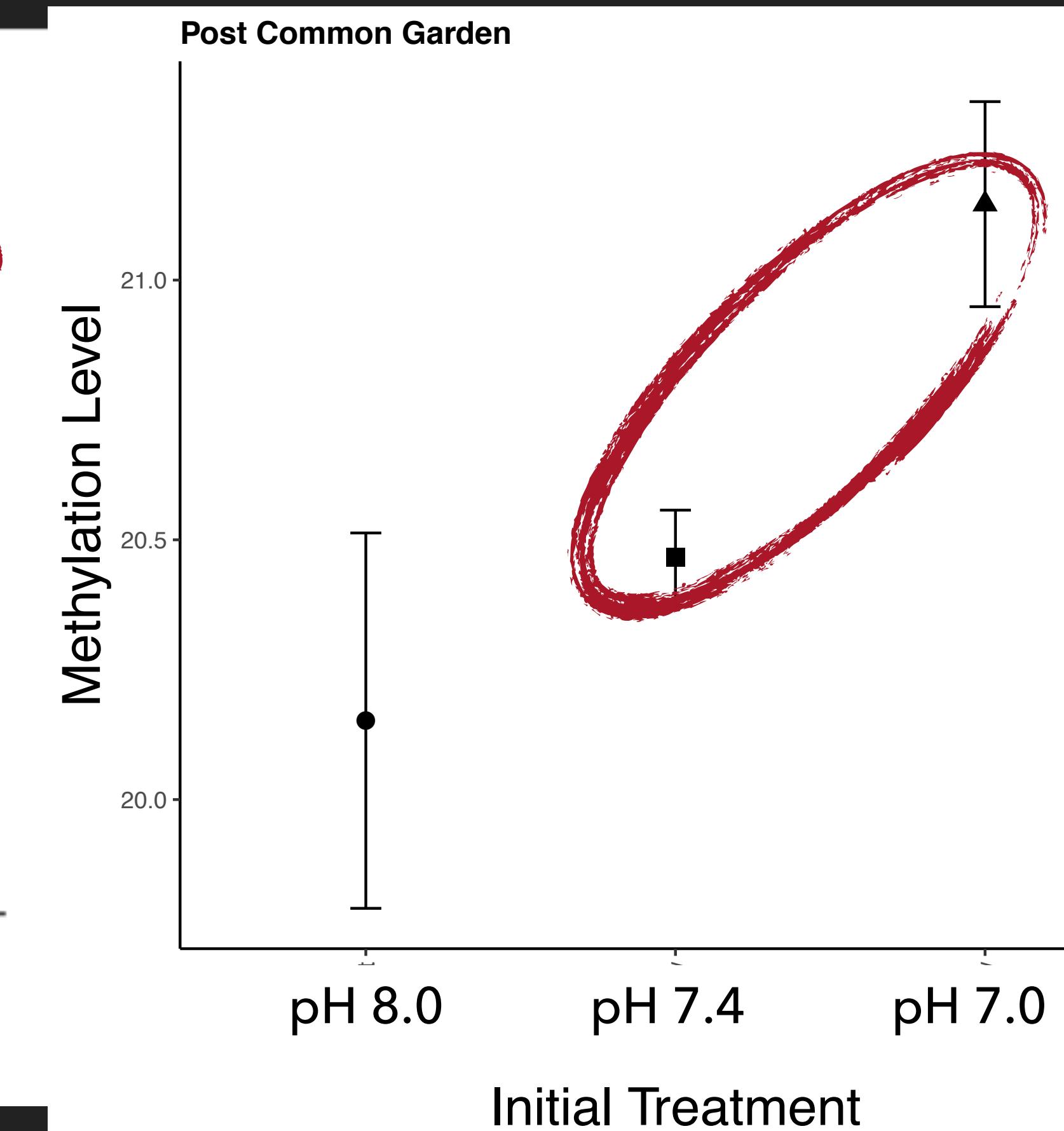
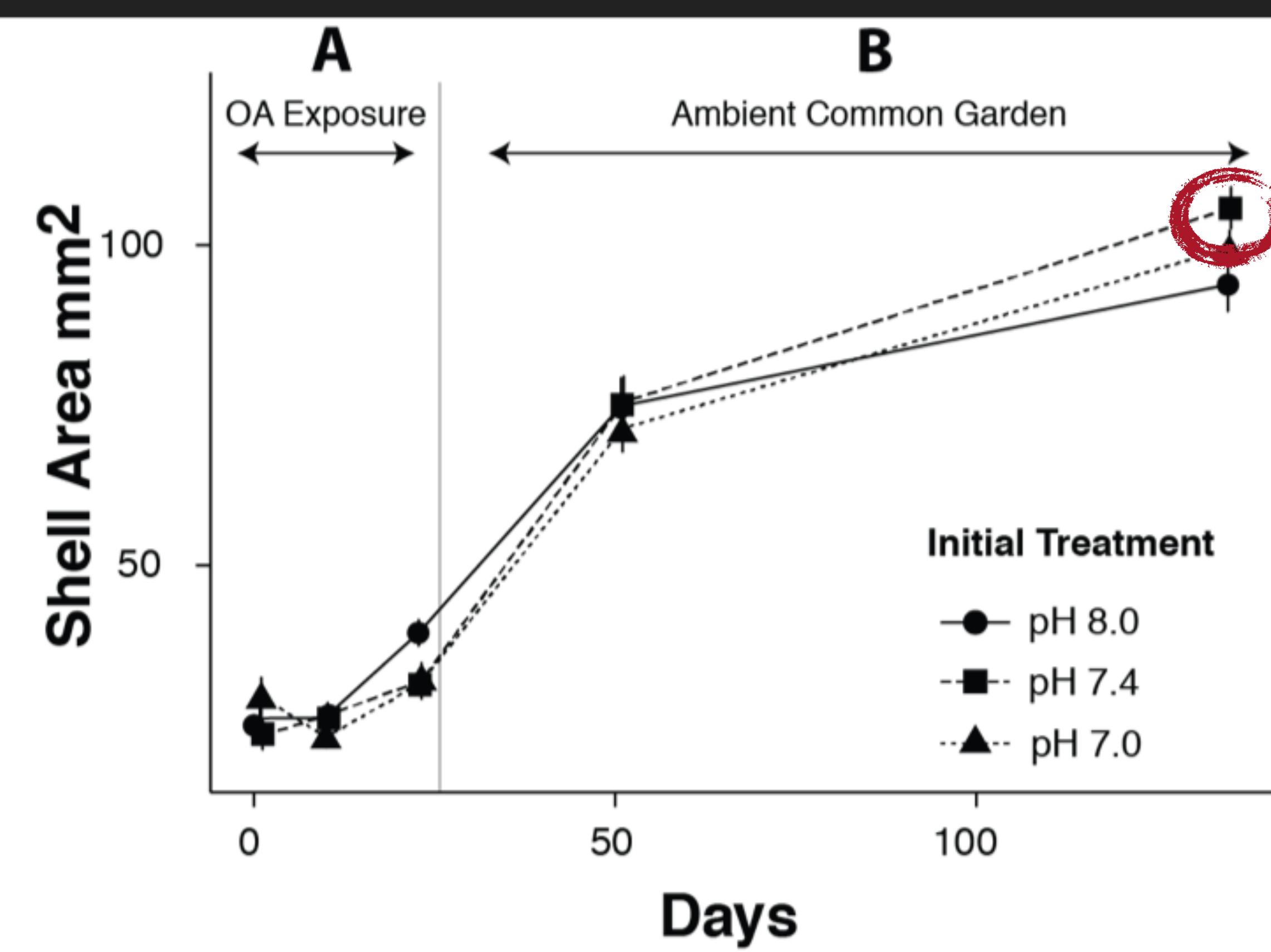
# GEODUCKS AND OA

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



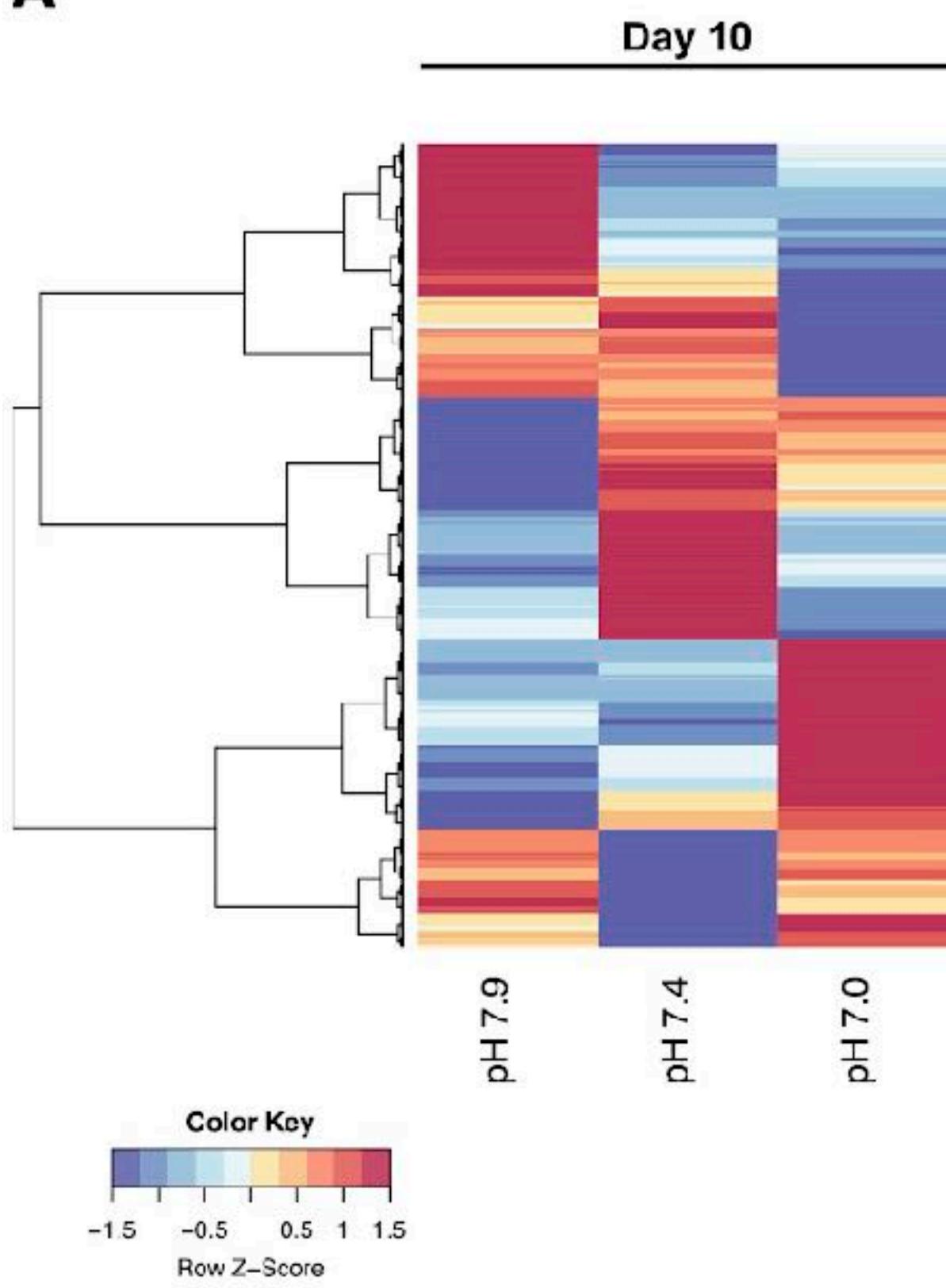
# GEODUCKS AND OA

# DNA METHYLATION

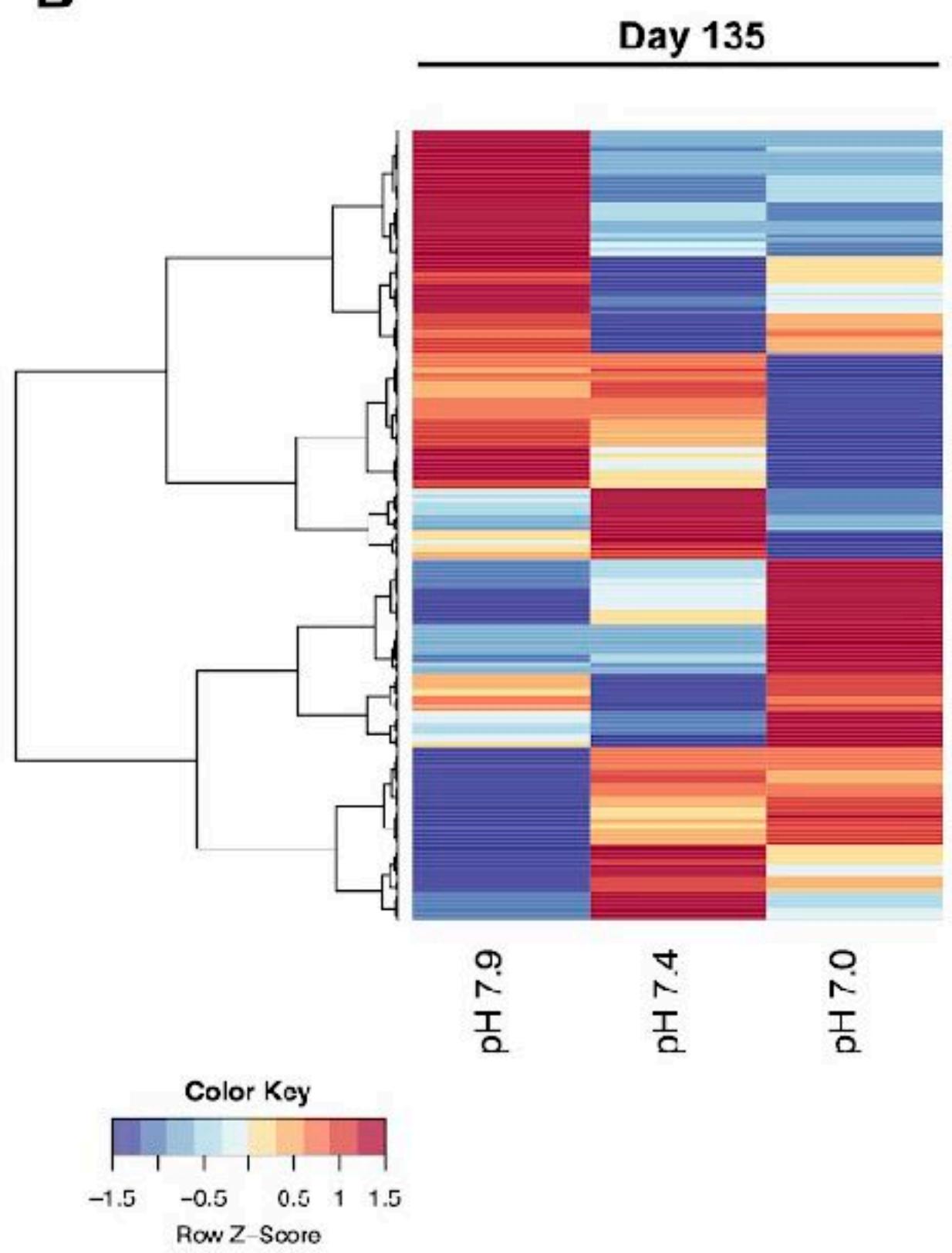


# GEODUCKS AND OA

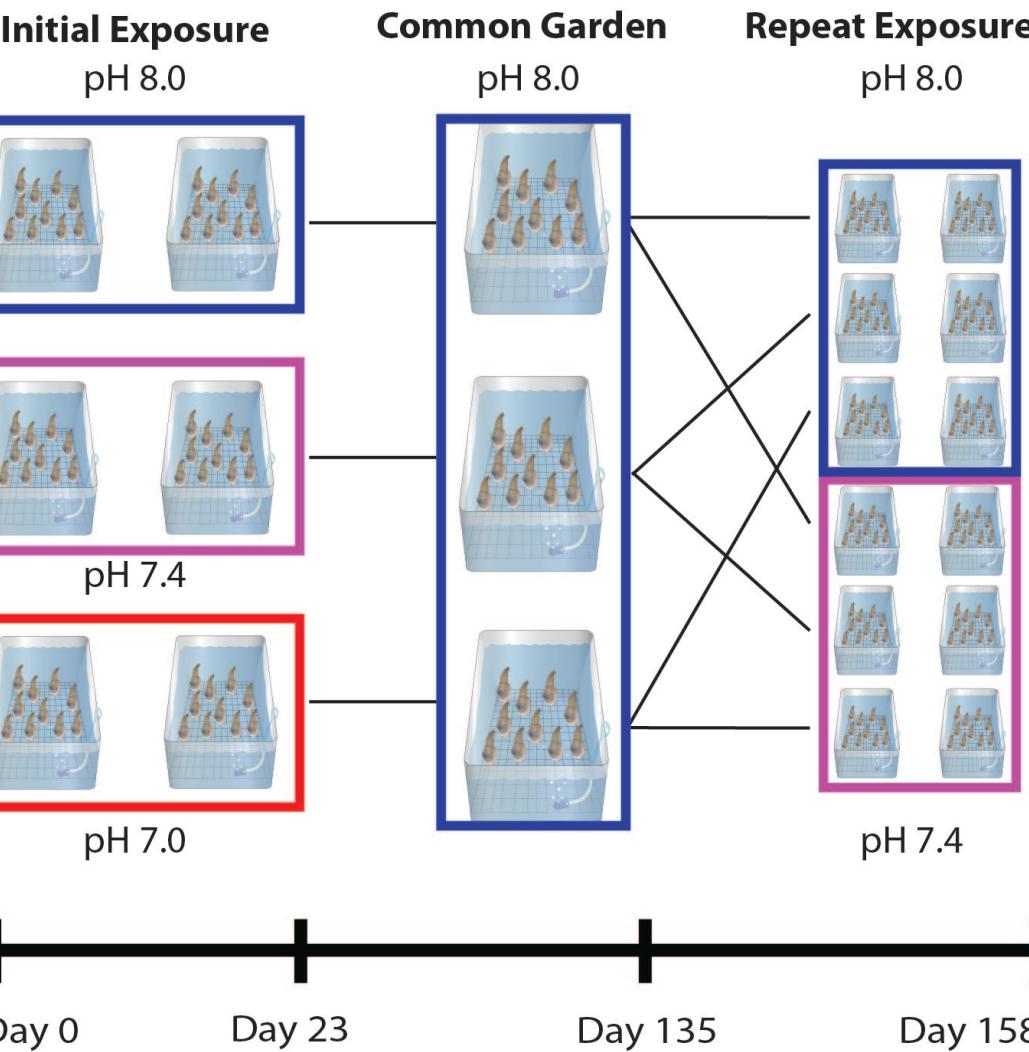
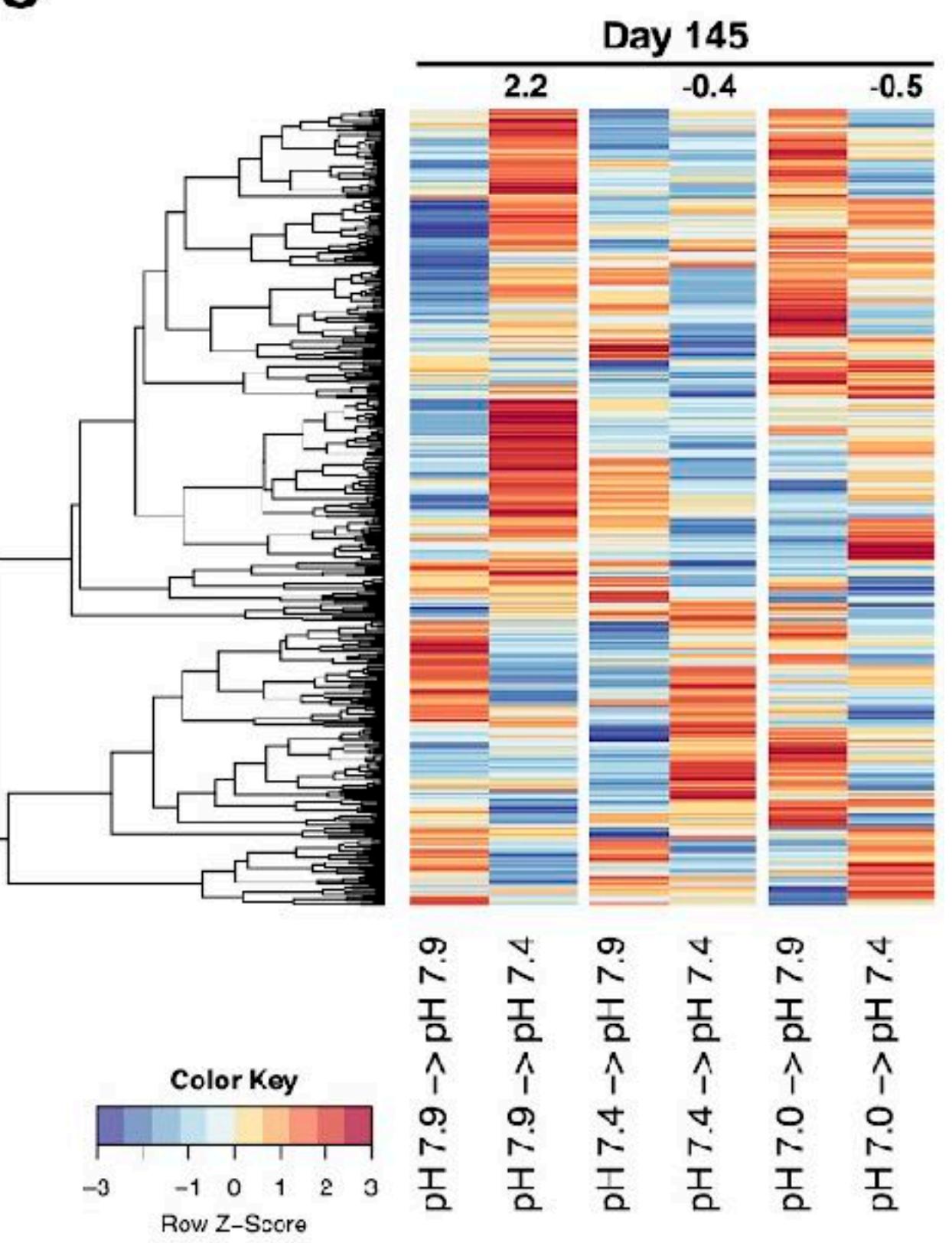
A



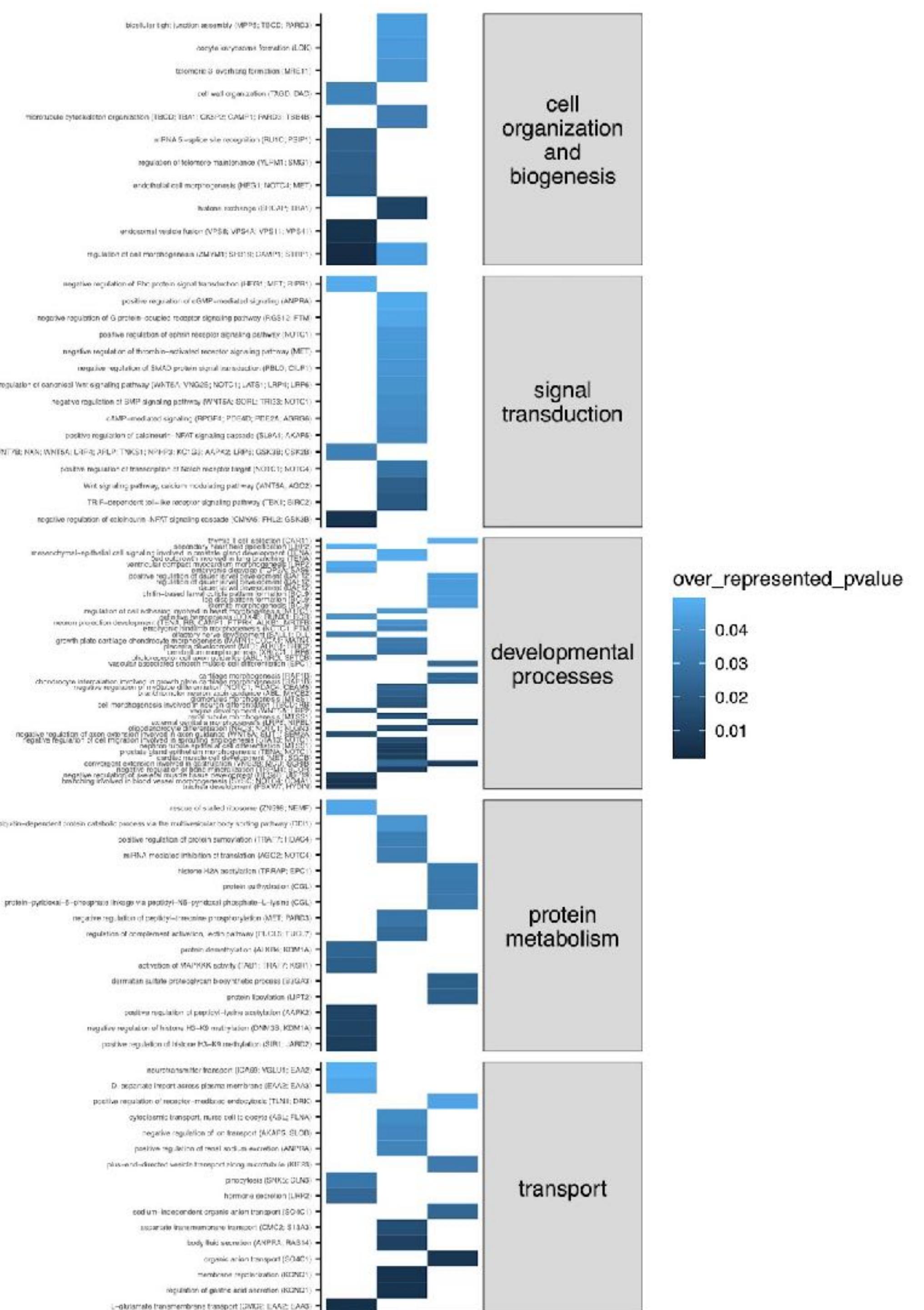
B



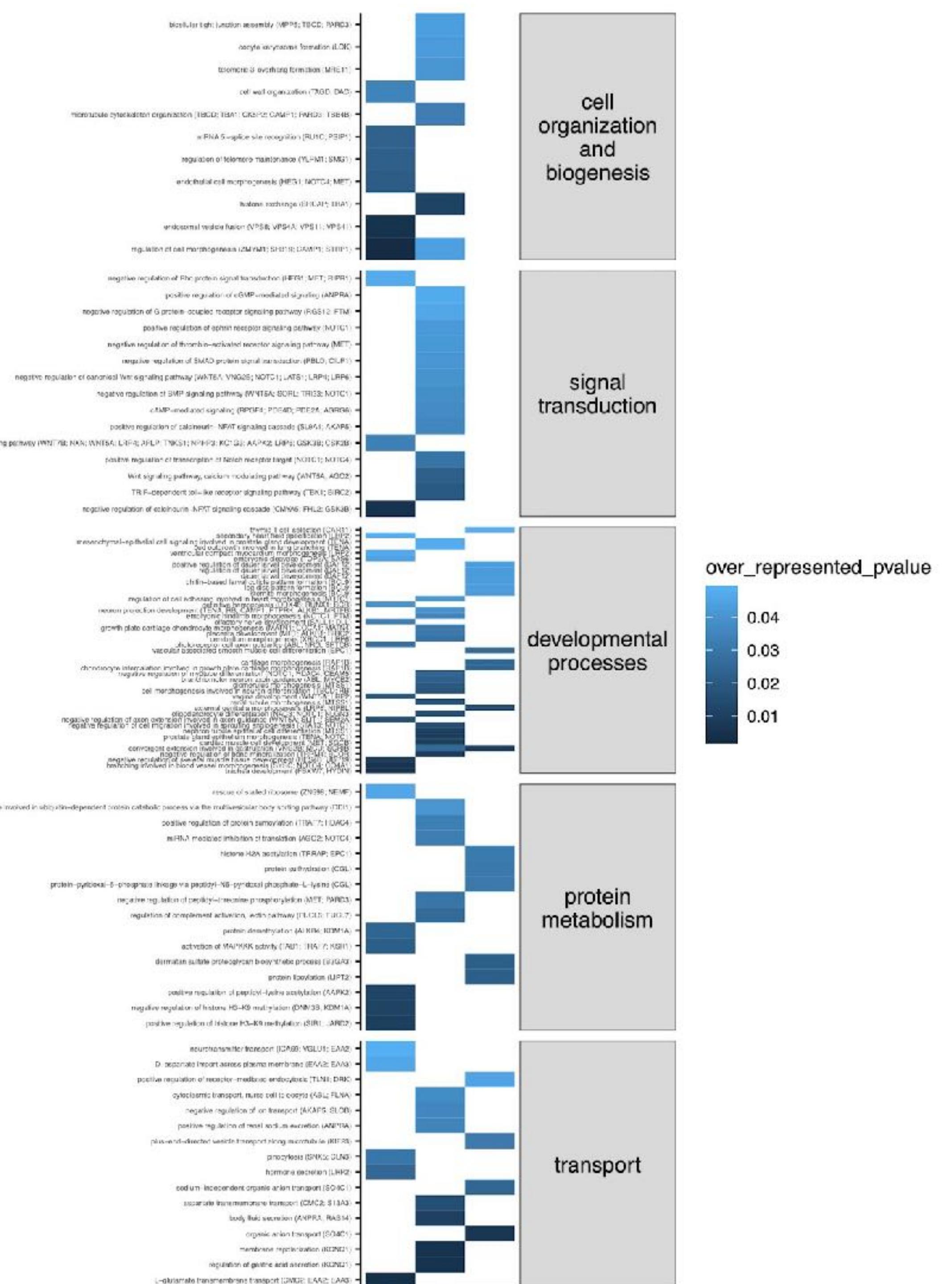
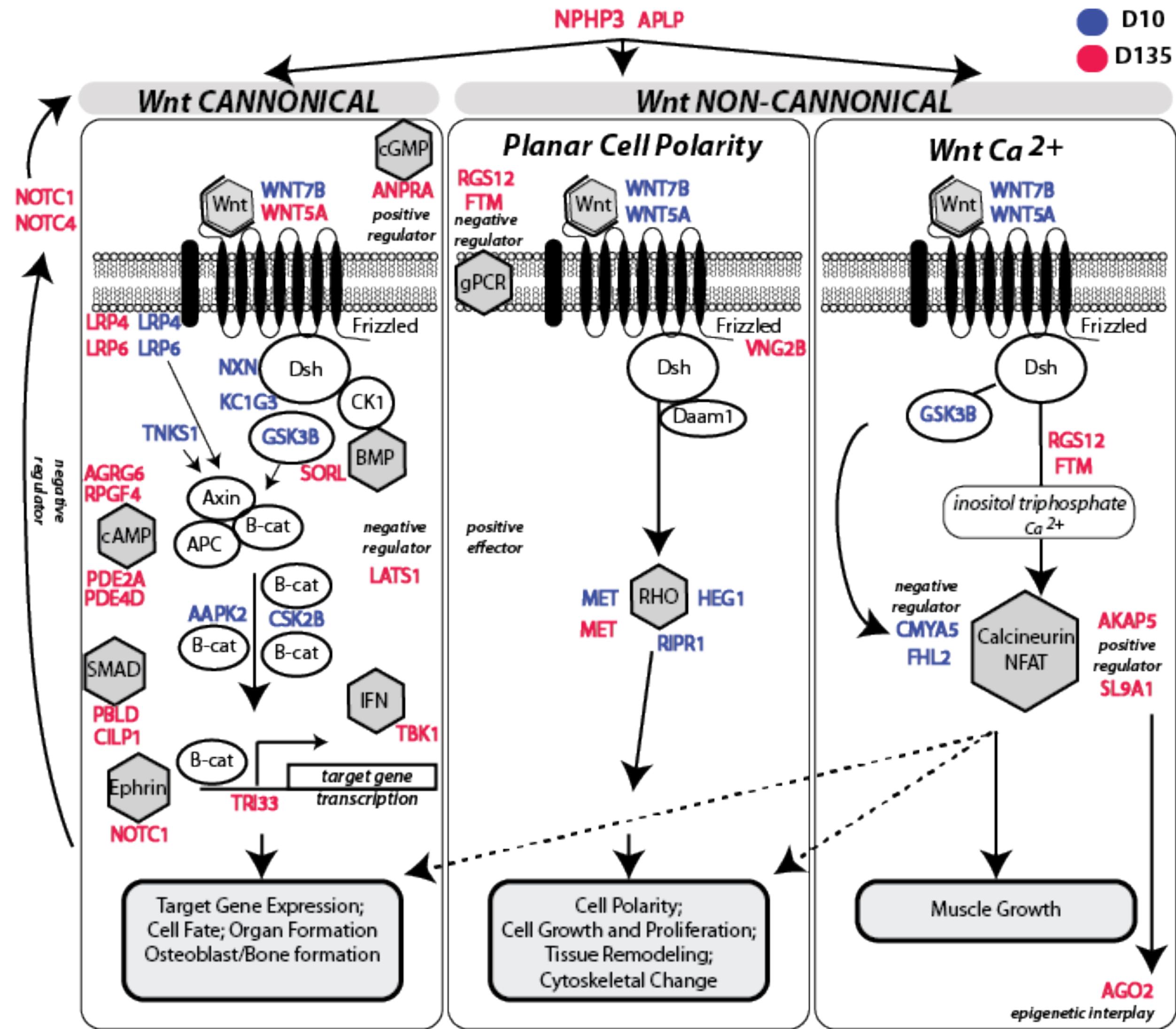
C



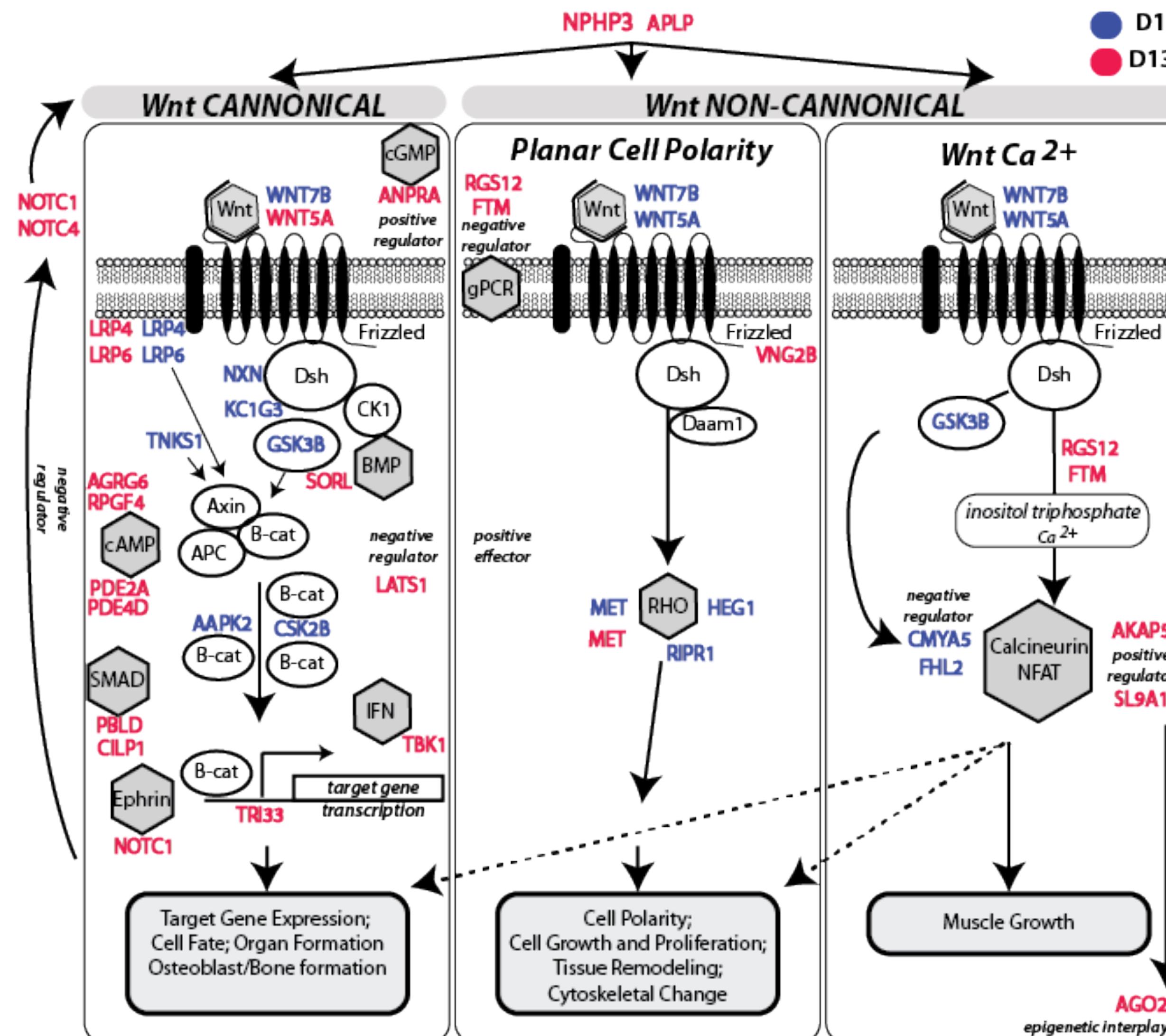
# GEODUCKS AND OA



# GEODUCKS AND OA



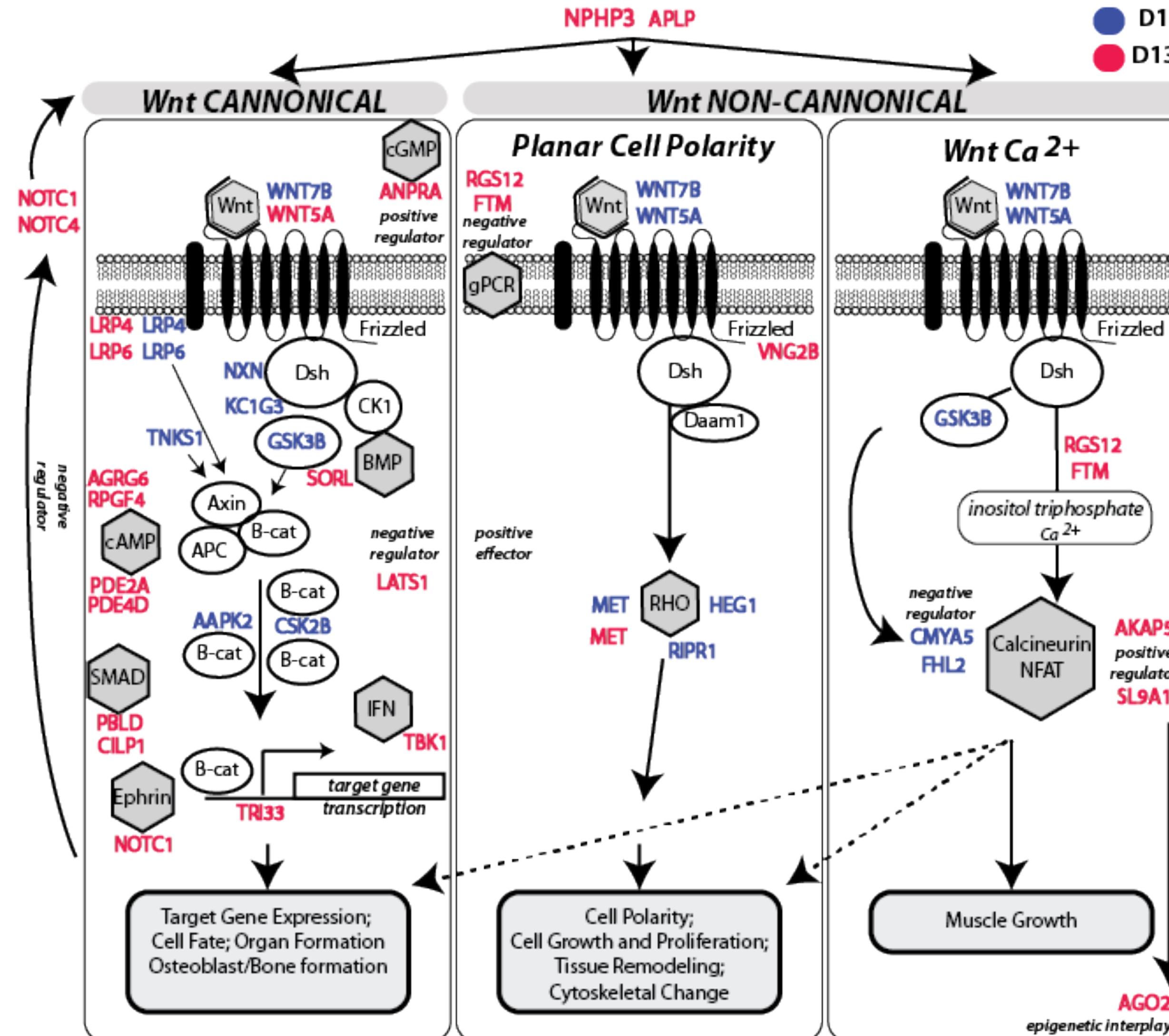
# GEODUCKS AND OA



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

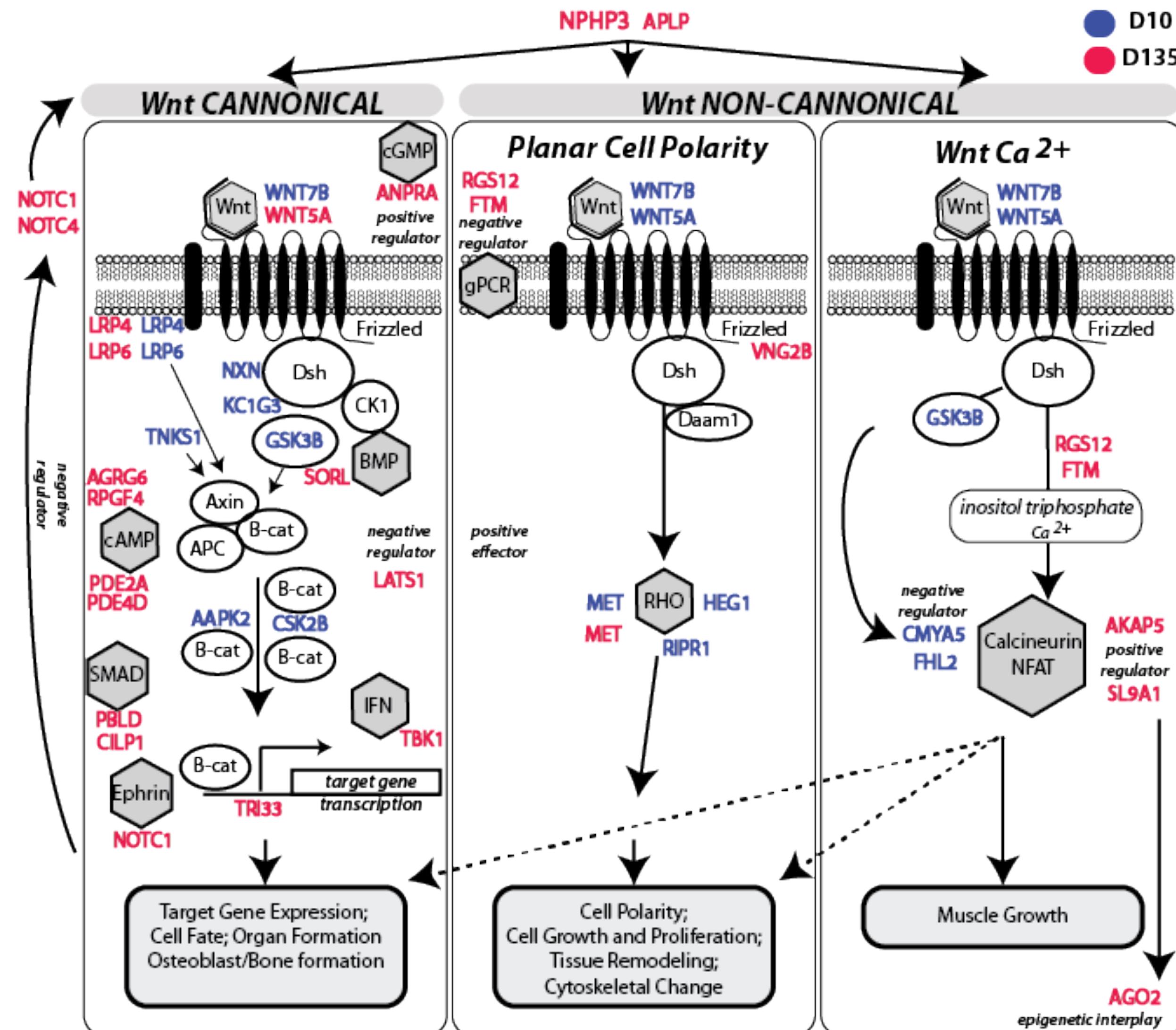
doi: <https://doi.org/10.1101/2022.06.24.497506>

# 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.

# GEODUCKS AND OA



Functional enrichment analysis of differentially methylated genes revealed regulation of signal transduction through widespread changes in the **Wnt signaling pathways that influence cell growth, proliferation, tissue and skeletal formation, and cytoskeletal change.**

## *Early-life Priming*

### *Hardening*

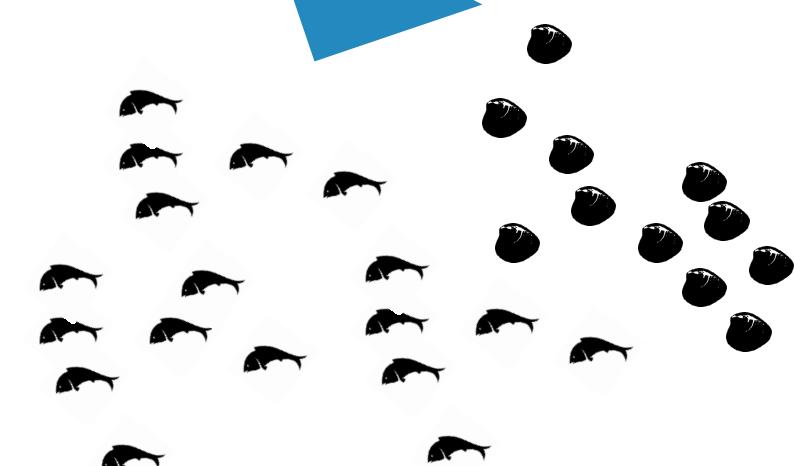


Adults

Influencing adult phenotype by altering early life environment



Influencing offspring phenotype by altering environmental conditions of parents



Larvae

## *Transgenerational Plasticity*

### *Carry-over effects*

Can we see an imprint in parents transmitted to the offspring?

## *Early-life Priming*

### *Hardening*

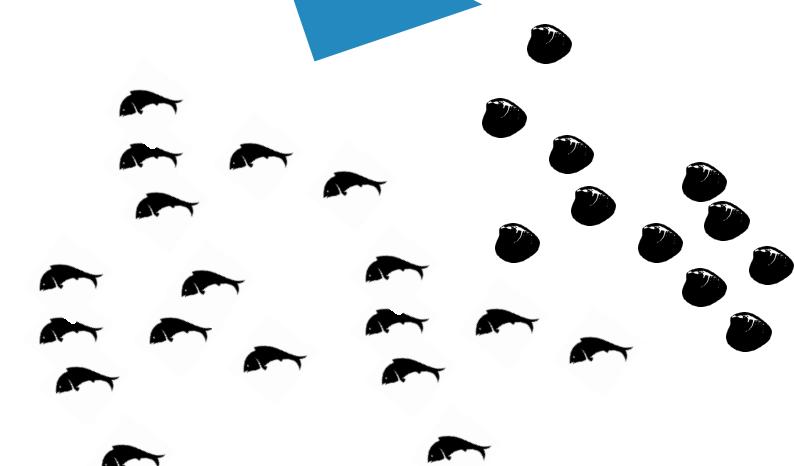


Adults

Influencing adult phenotype by altering early life environment



Influencing offspring phenotype by altering environmental conditions of parents



Larvae

## *Transgenerational Plasticity*

### *Carry-over effects*

Can we see an imprint in parents transmitted to the offspring?

**NO**



EXCITING?  
COMPLEX  
'LAYER' OF RESILIENCE



# EXCITING? COMPLEX 'LAYER' OF RESILIENCE

## Genetic Linkage

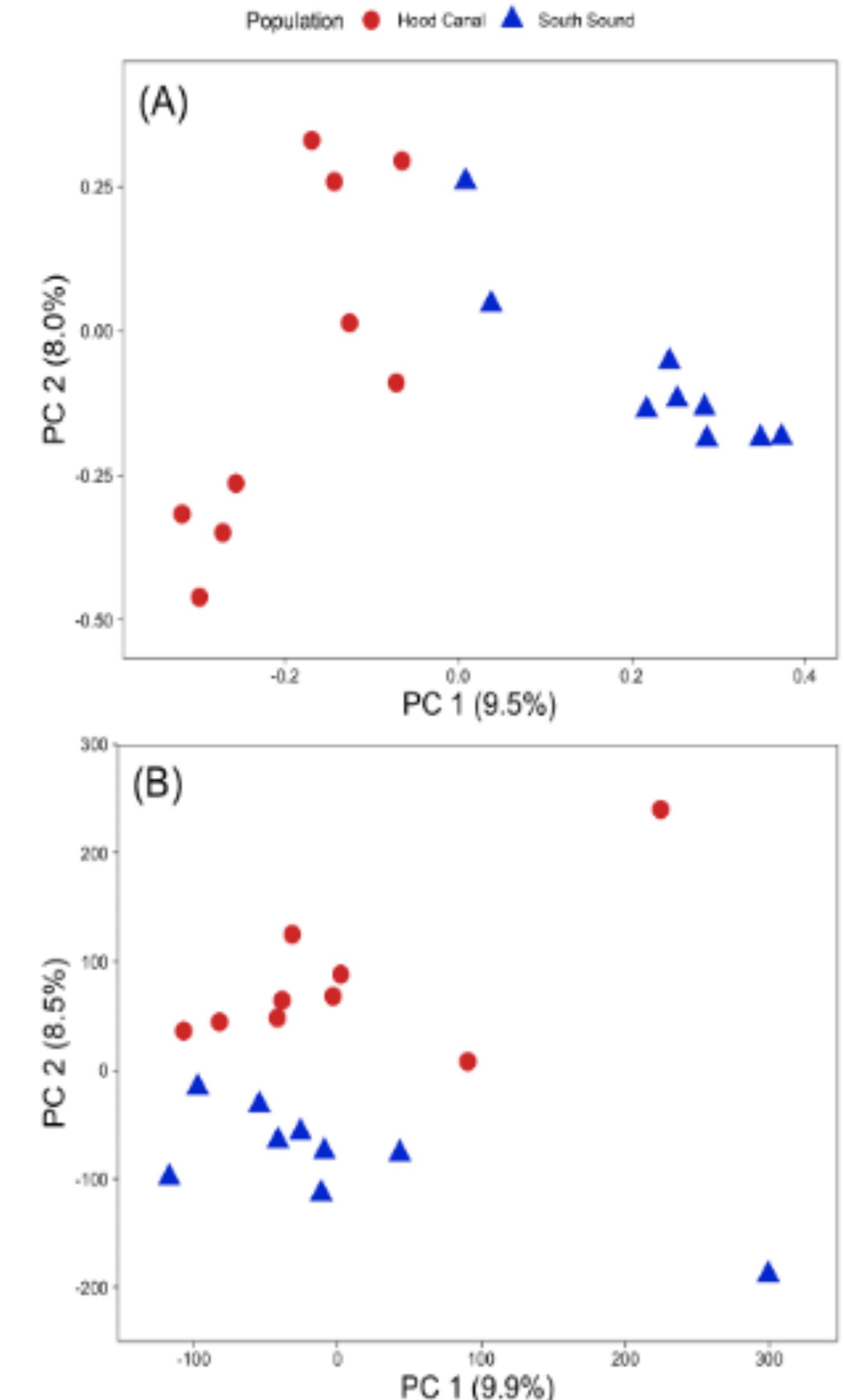
# Epigenetic and Genetic Population Structure is Coupled in a Marine Invertebrate

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*

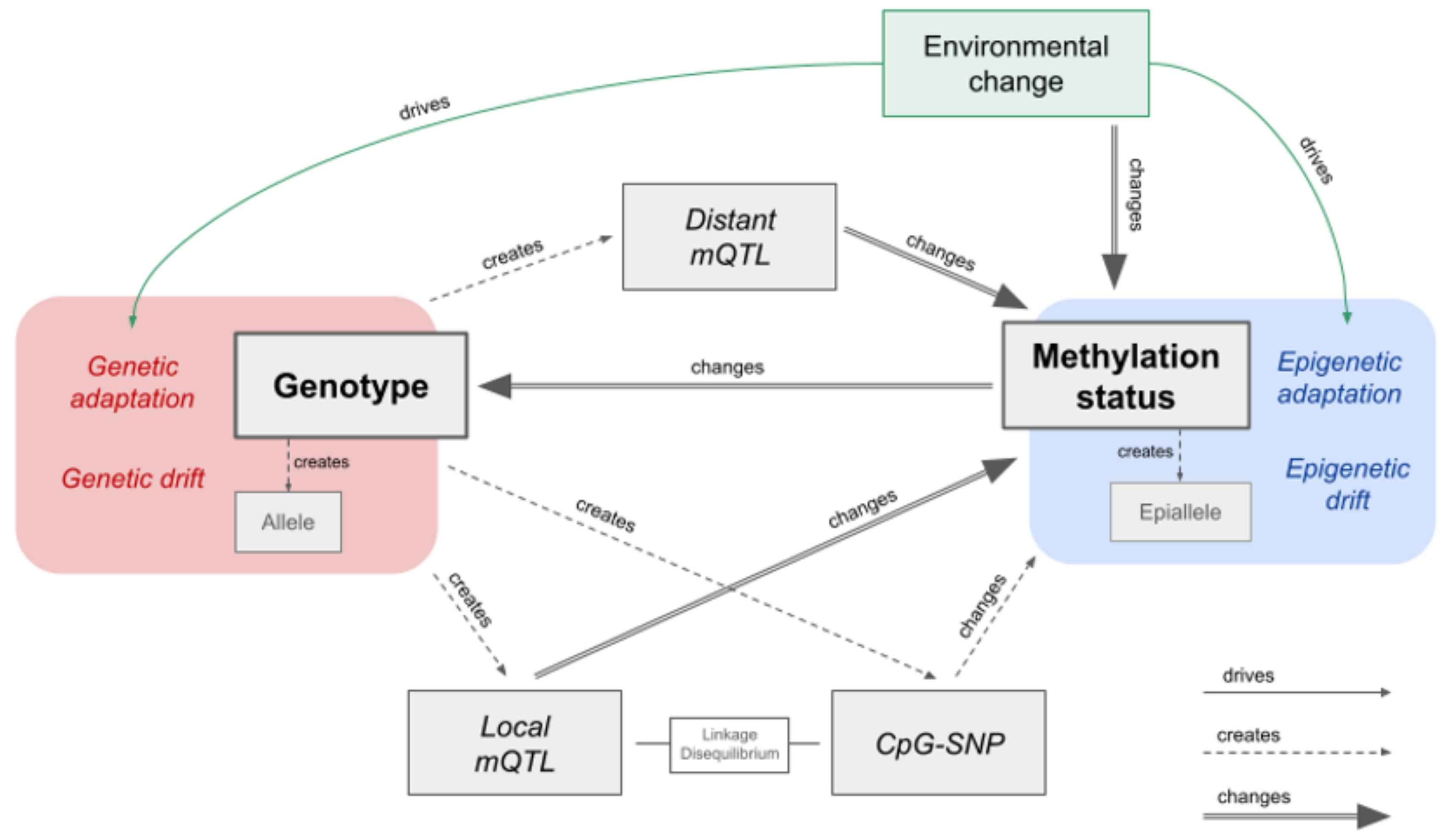
Identified 3,963 differentially methylated loci between populations. Clear coupling between genetic and epigenetic patterns of variation, **with 27% of variation in inter-individual methylation differences explained by genotype.**

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



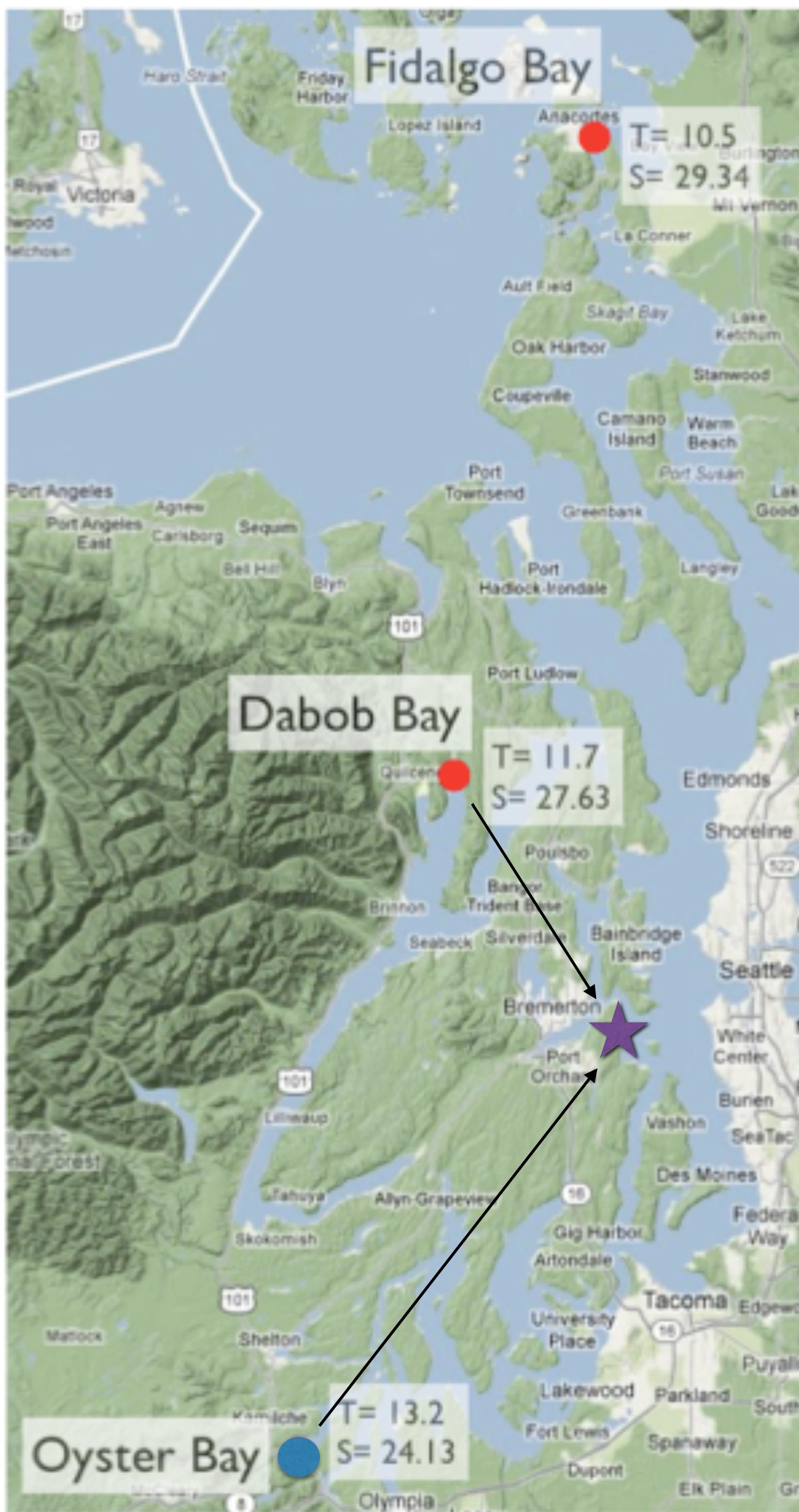
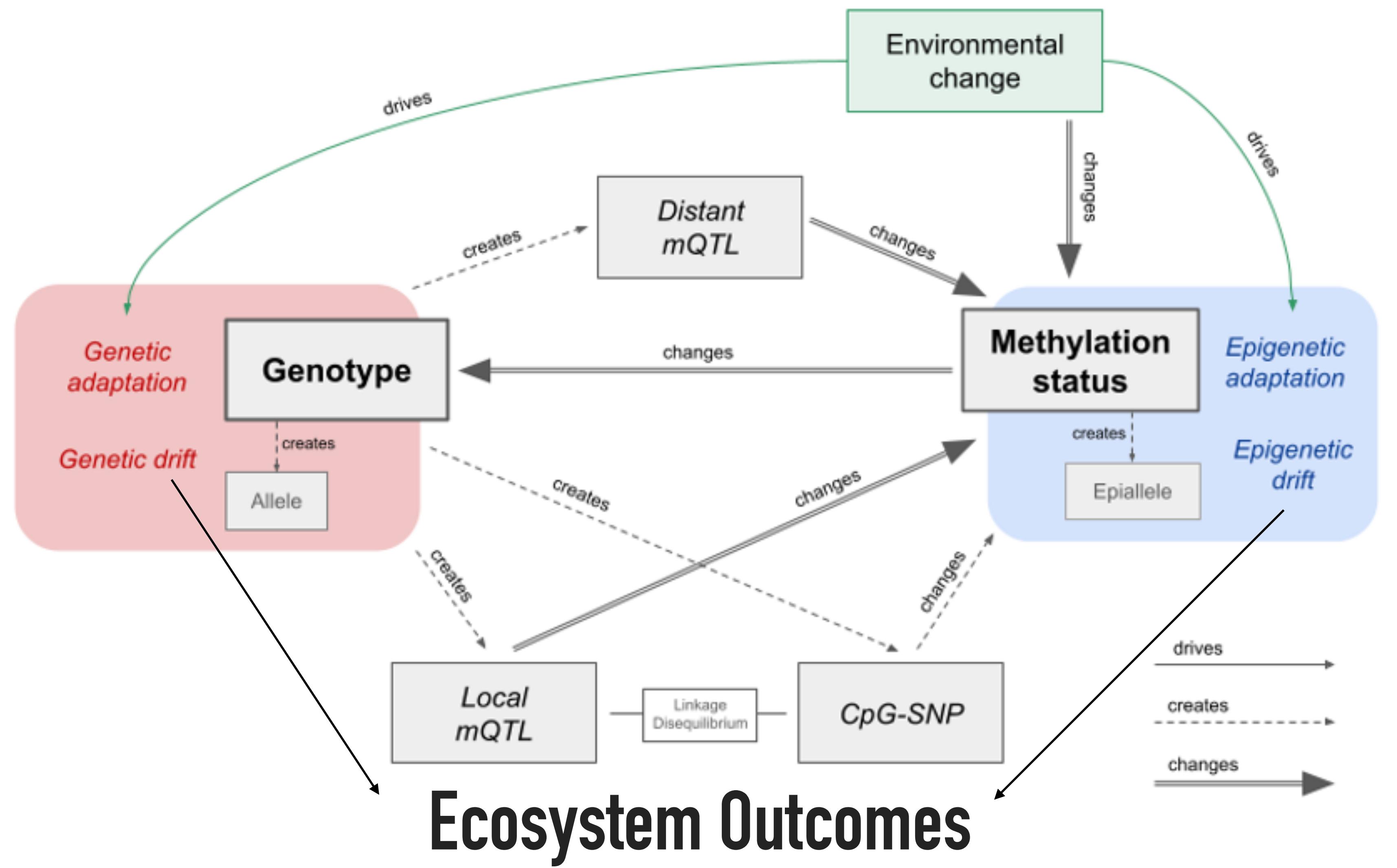
TEXT

# EPIGENETIC AND GENETIC POPULATION STRUCTURE



TEXT

# EPIGENETIC AND GENETIC POPULATION STRUCTURE



# Open Science



# Bioinformatic Approaches in non-model species

## marineomics.io

Received: 29 March 2023 | Accepted: 11 September 2023

DOI: 10.1111/2041-210X.14219

### APPLICATION

#### A dynamic web resource for robust and reproducible genomics in nonmodel species: marineomics.io

Samuel N. Bogan<sup>1</sup> | Jason Johns<sup>1</sup> | Joanna S. Griffiths<sup>2</sup> | Danielle Davenport<sup>3</sup> |  
Sara J. Smith<sup>4,5,6</sup> | Sara M. Schaal<sup>7</sup> | Alan Downey-Wall<sup>8</sup> | Runyang Nicolas Lou<sup>9,10</sup> |  
Katie Lotterhos<sup>11</sup> | Megan E. Guidry<sup>12</sup> | Hanny E. Rivera<sup>13</sup> | Joseph A. McGirr<sup>14</sup> |  
Jonathan B. Puritz<sup>12</sup> | Steven B. Roberts<sup>15</sup> | Katherine Silliman<sup>16,17</sup>

4 | Methods in Ecology and Evolution Open Access BRITISH  
ECOLOGICAL  
SOCIETY

MarineOmics

Best Principles Contributions ▾ Population Genomics ▾ Functional Genomics ▾ Genome-Phenome ▾ Panel Seminars Discussion Forum

Topics covered within RADSeq tutorial

RADseq

Katherine Silliman, Danielle Davenport

Description of tutorial steps

Setup for running code

Reduced Representation Sequencing (RADseq/GBS)

Considerations During Lab Work

Principles for Analyzing Your Data

Steps for a robust RAD analysis

First, look at the raw data!

Run an assembly pipeline

Evaluate potential sources of error

"Bad" samples

The power of PCA

Batch effects

Cryptic species/contamination/clones

Test a range of key parameters

Clustering threshold

Mapping parameters

If you would like to run the R code examples that are scattered throughout the guide (recommended but not required!), you will need to install some R packages. Only need to run this code once:

```
install.packages("tidyverse")
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")

BiocManager::install("SeqArray")
BiocManager::install("SNPRelate")
```

Example code to run using real data

Now load those packages, if using:

```
library(SeqArray) # efficient storage and filtering of genomic data
```

```
## Loading required package: gdsfmt
```

# ACKNOWLEDGEMENTS

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

GITHUB.COM/SR320/TALK-DUKE-2024

