



# Marine invertebrate environmental memory

## Implications, mechanisms, and opportunities

**Steven Roberts - University of Washington**

[github.com/sr320/talk-SZAN-2024](https://github.com/sr320/talk-SZAN-2024)













## *Early-life Priming*

### *Hardening*



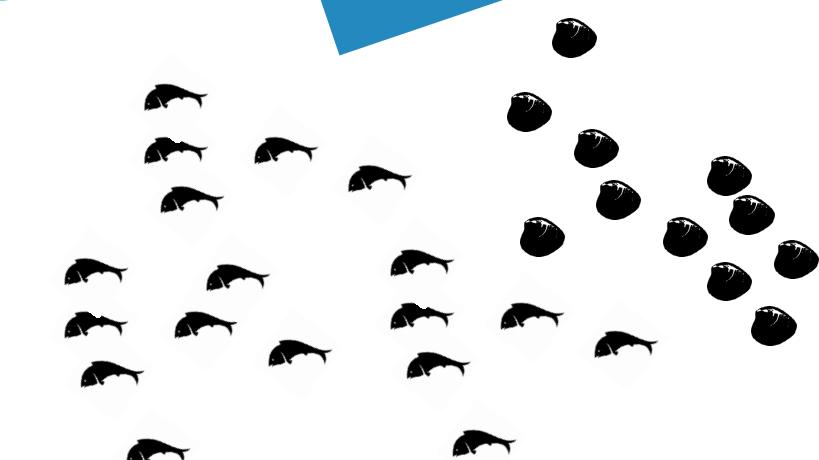
Adults

Influencing adult phenotype by altering early life environment



## *Transgenerational Plasticity*

### *Carry-over effects*



Larvae

Influencing offspring phenotype by altering environmental conditions of parents

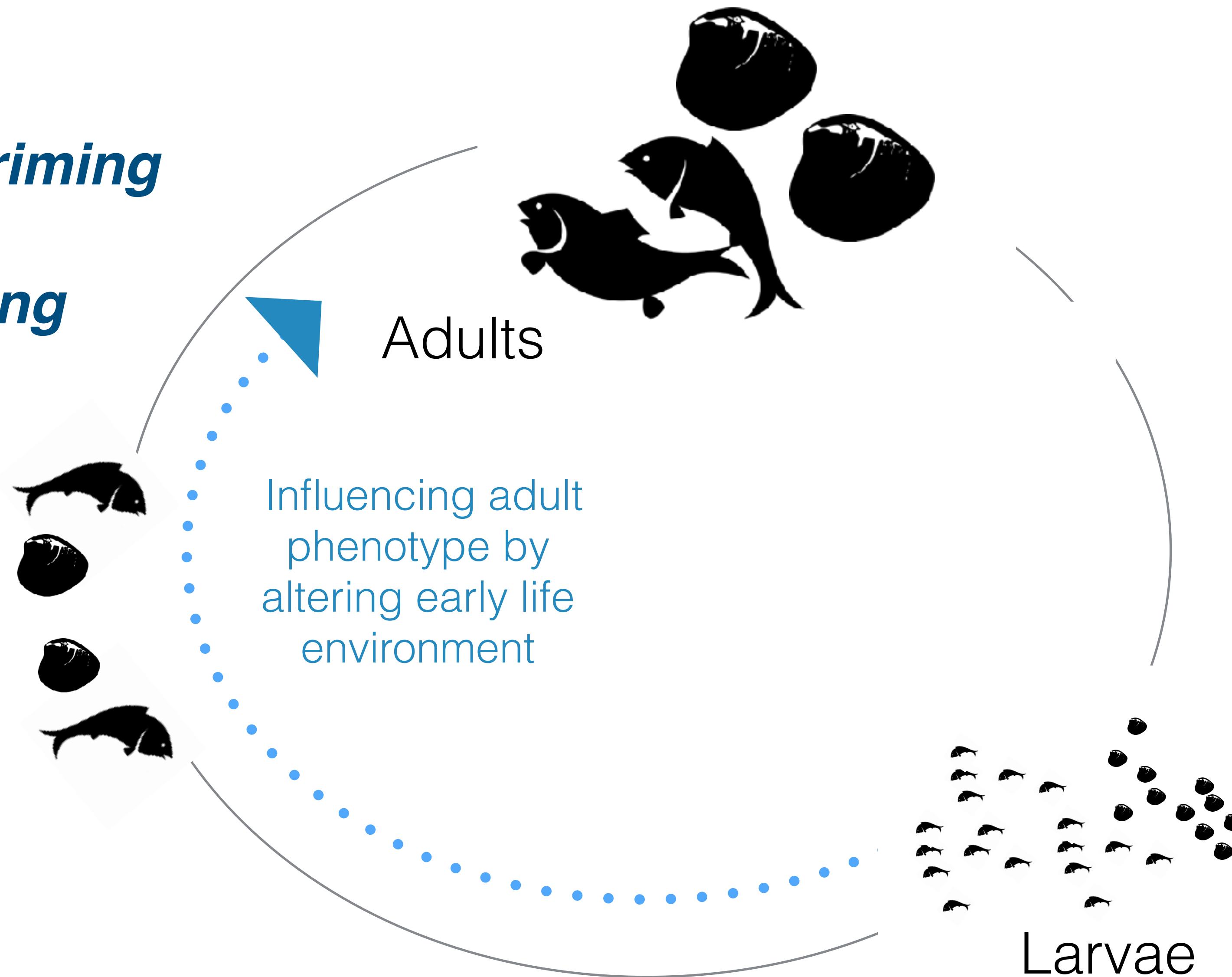
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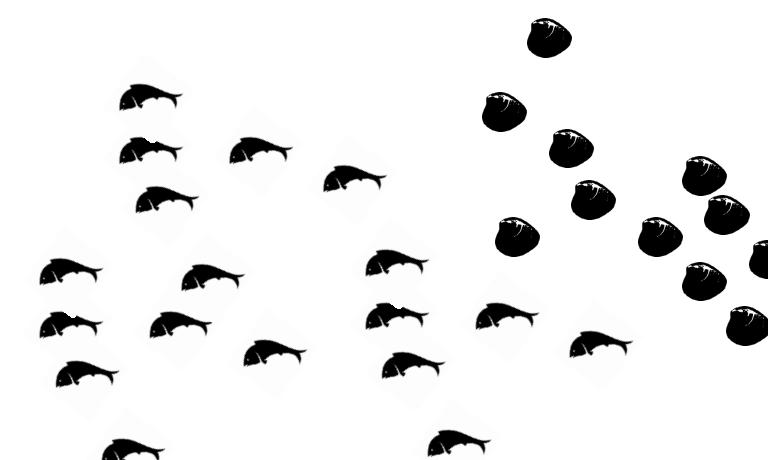
# Trends in Plant Science

## Early-life Priming

### Hardening



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

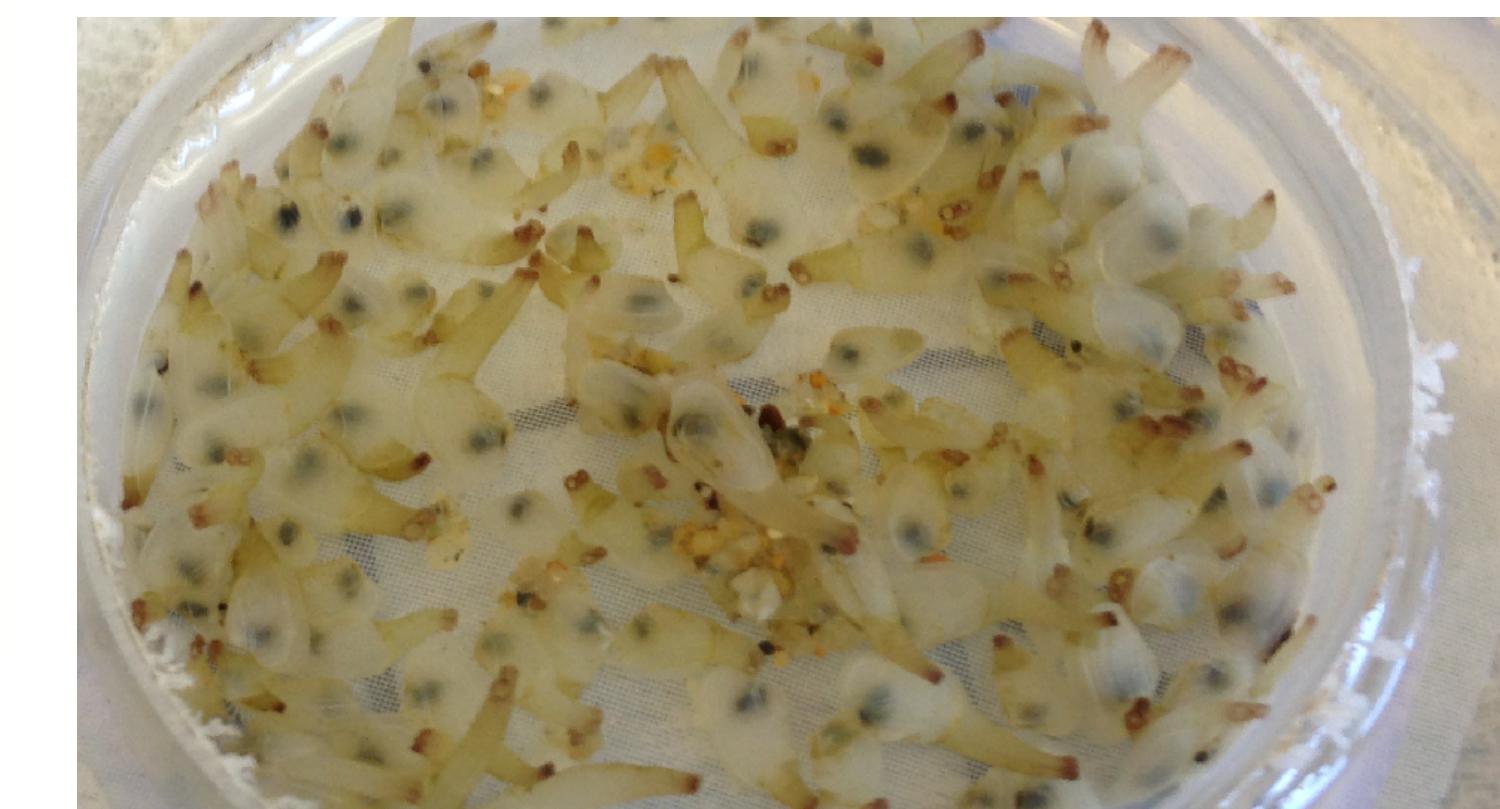
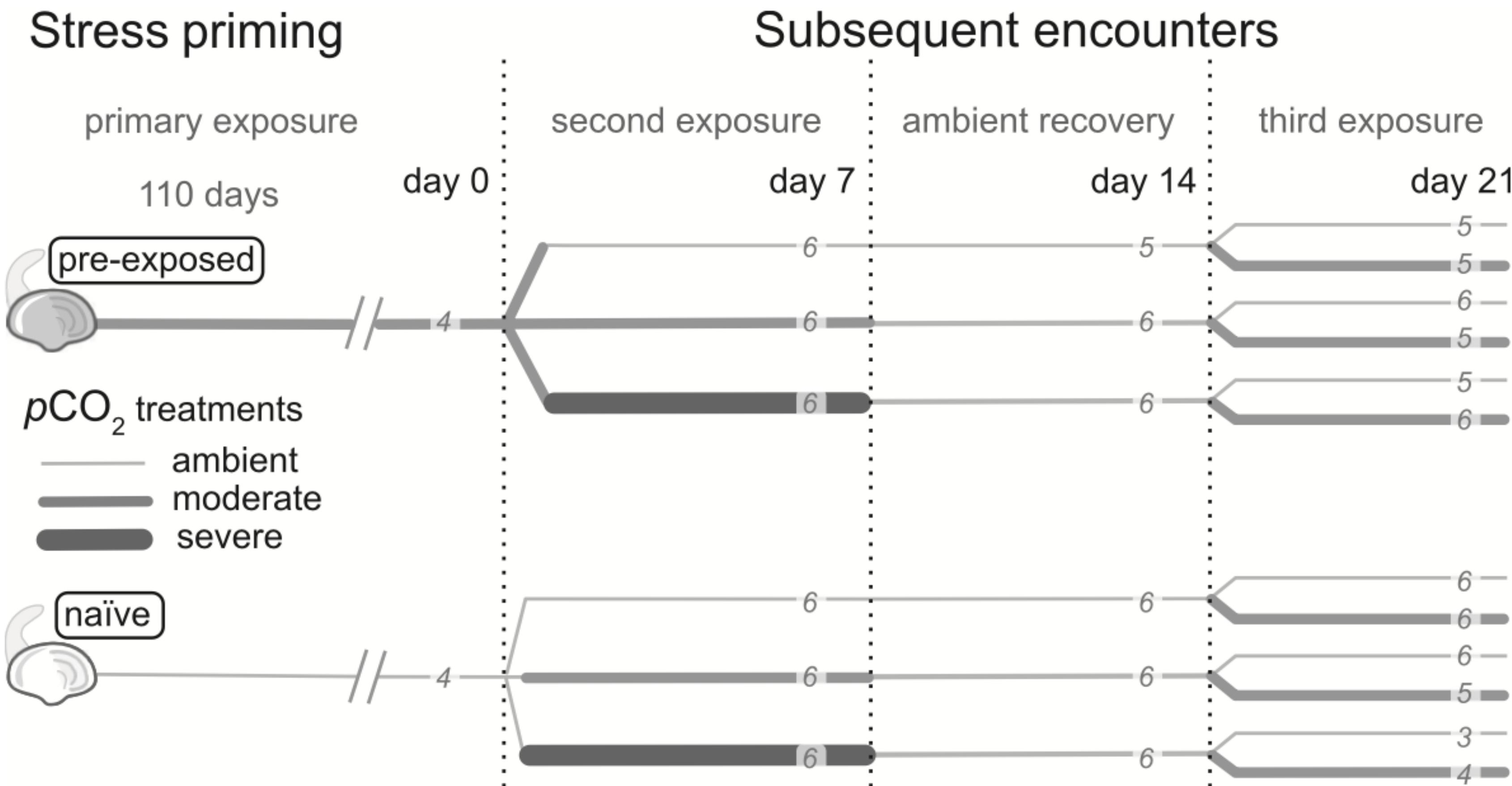


*Cross-stress priming success relies on synergistic stress signaling pathways being shared across stresses varying in nature and intensity.*

# Geoduck Clams

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

Samuel J. Gurr<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>



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- 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.



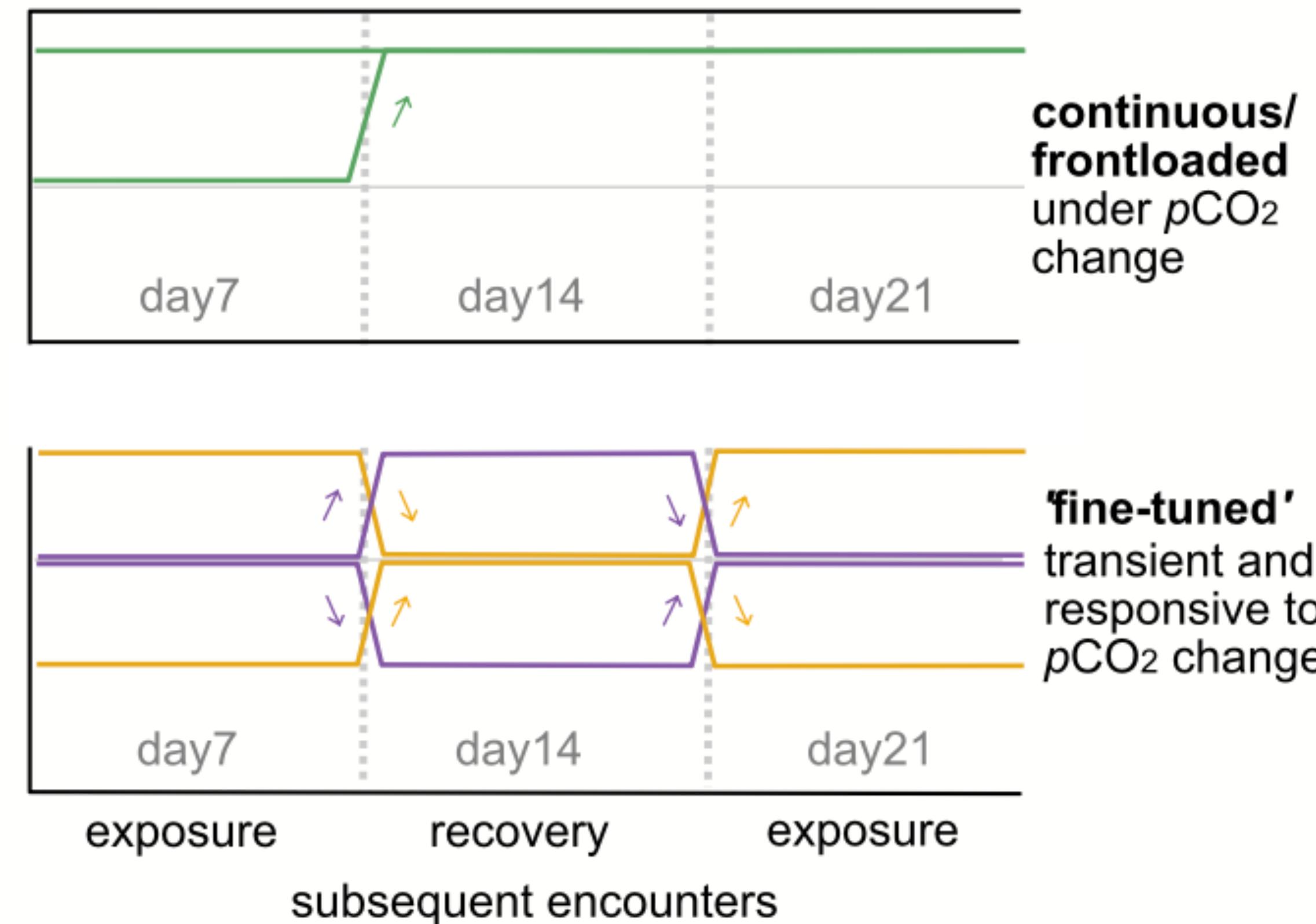
# Geoduck Clams

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)

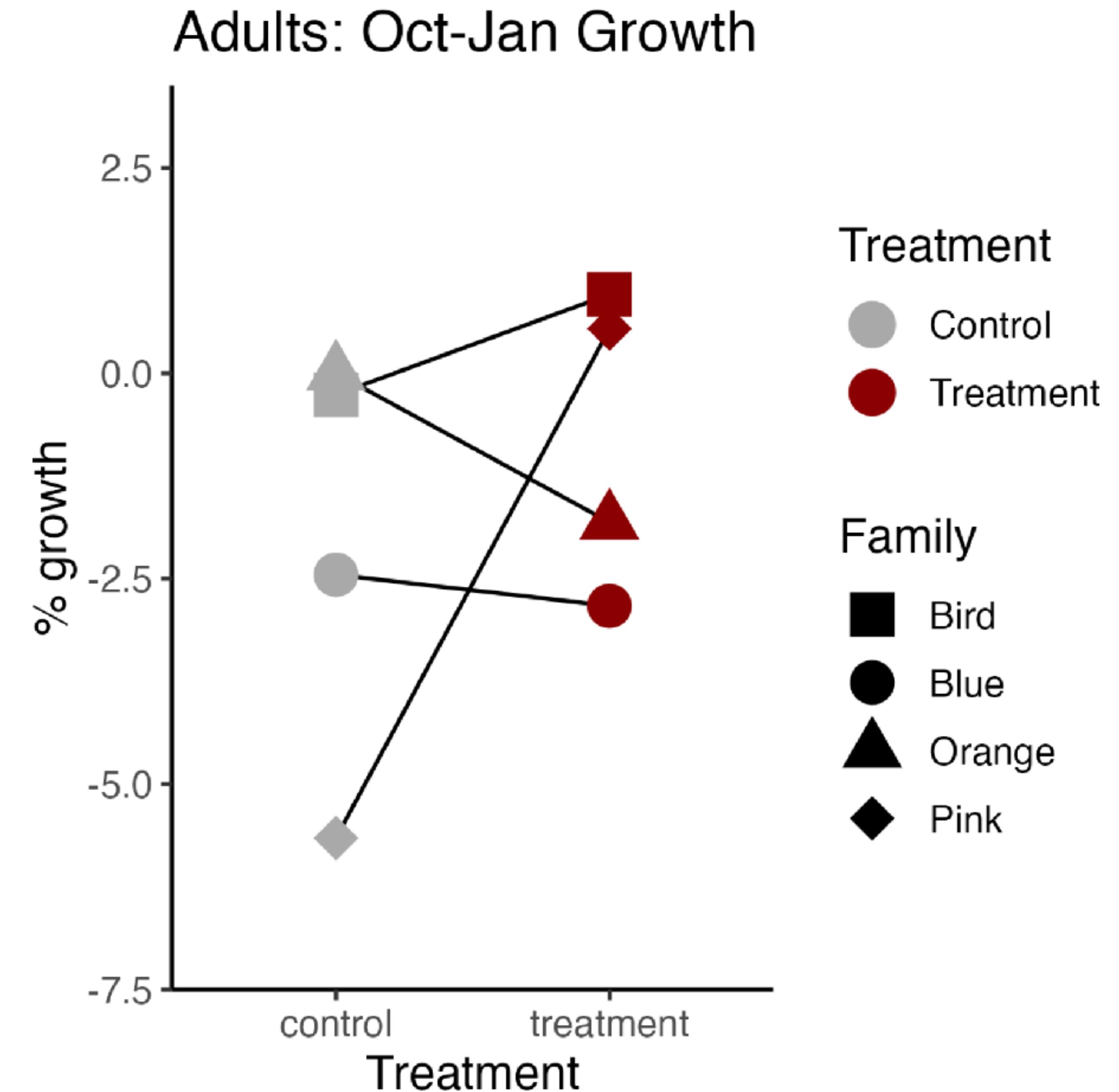
patterns in functional enrichment analysis



# *Crassostrea gigas*

Very new data!

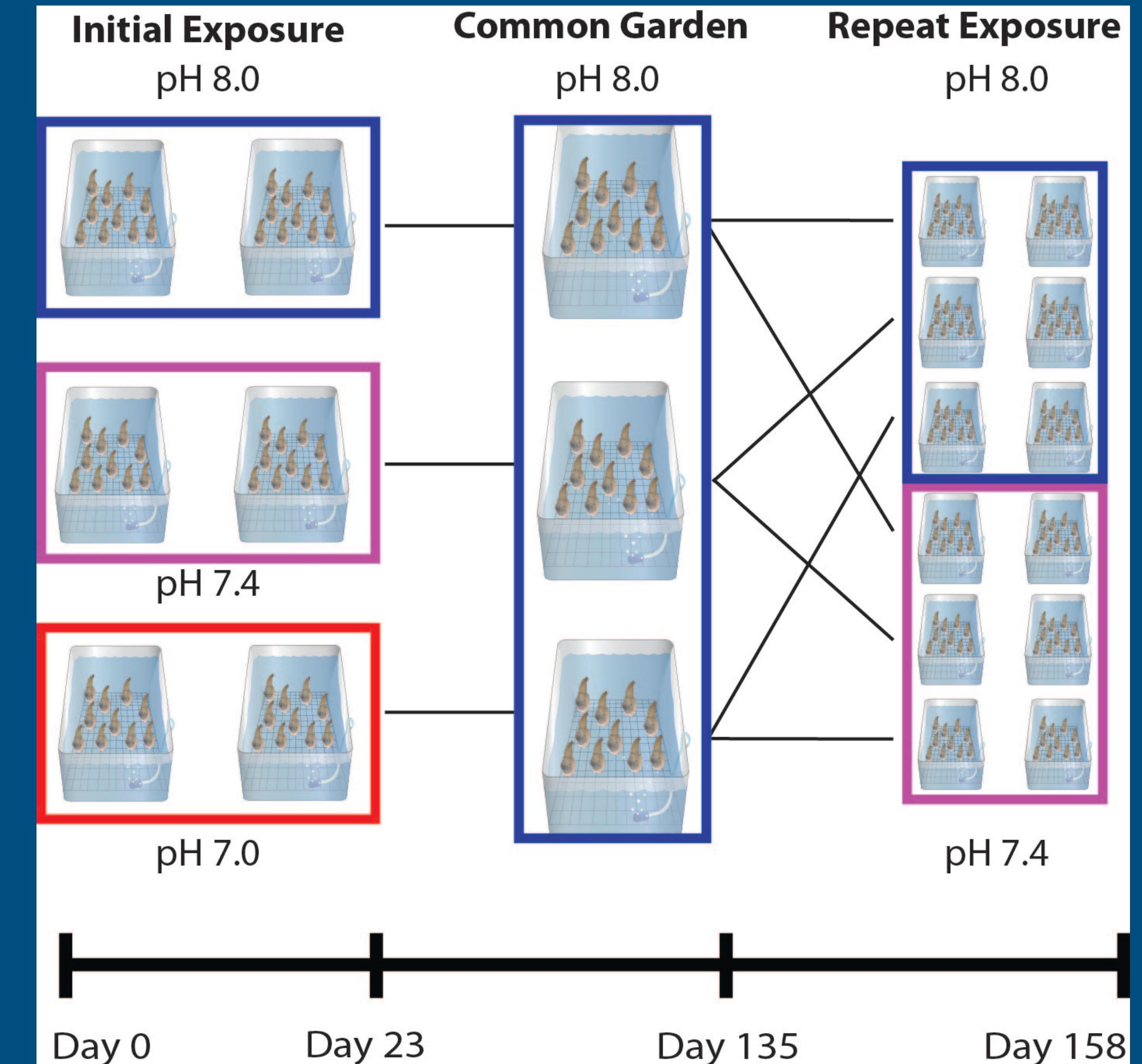
Combined temperature and  
mechanical stress hardening...



## GEODUCKS CLAMS

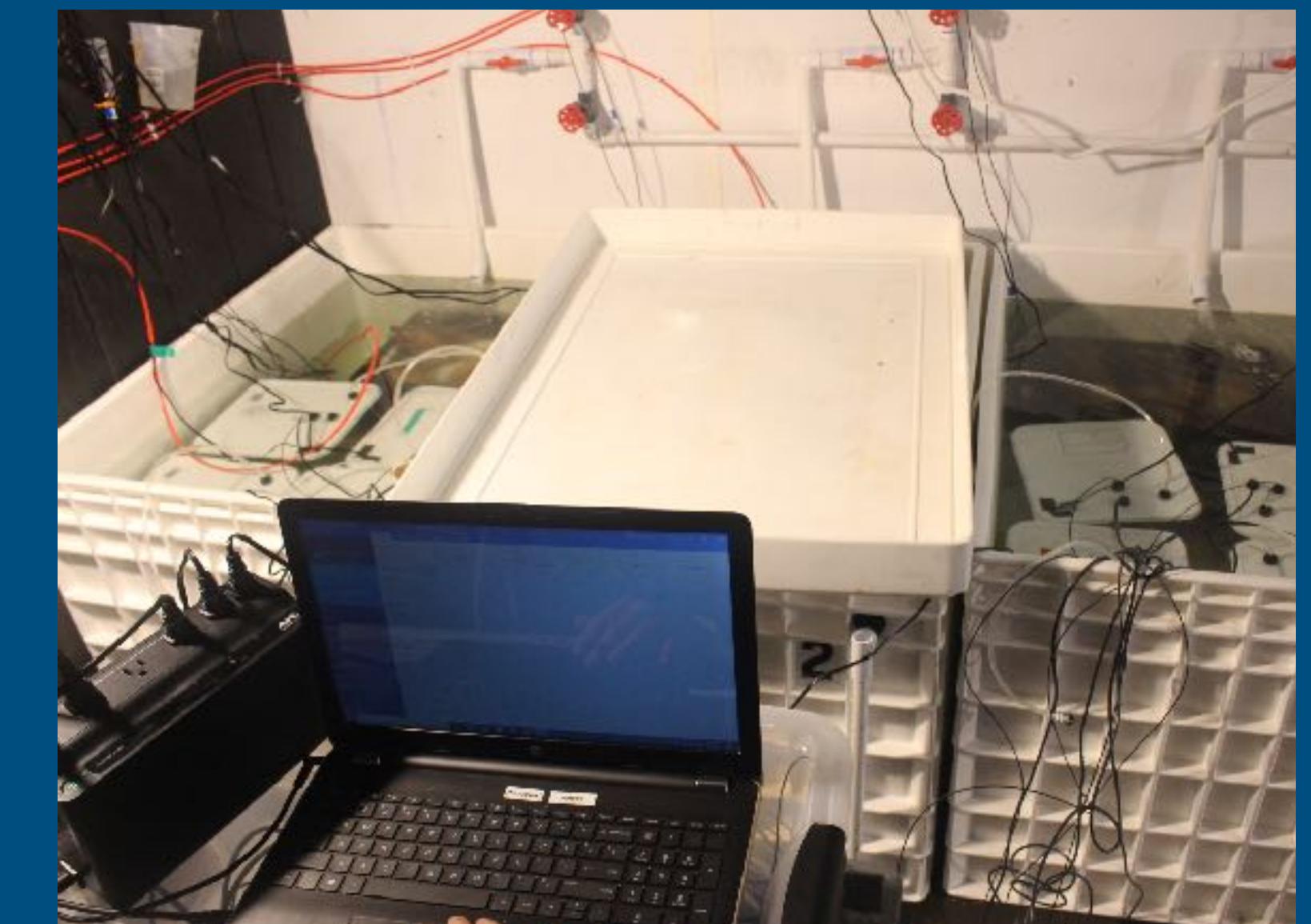


- ▶ Does conditioning to low pH confer tolerance within a generation?



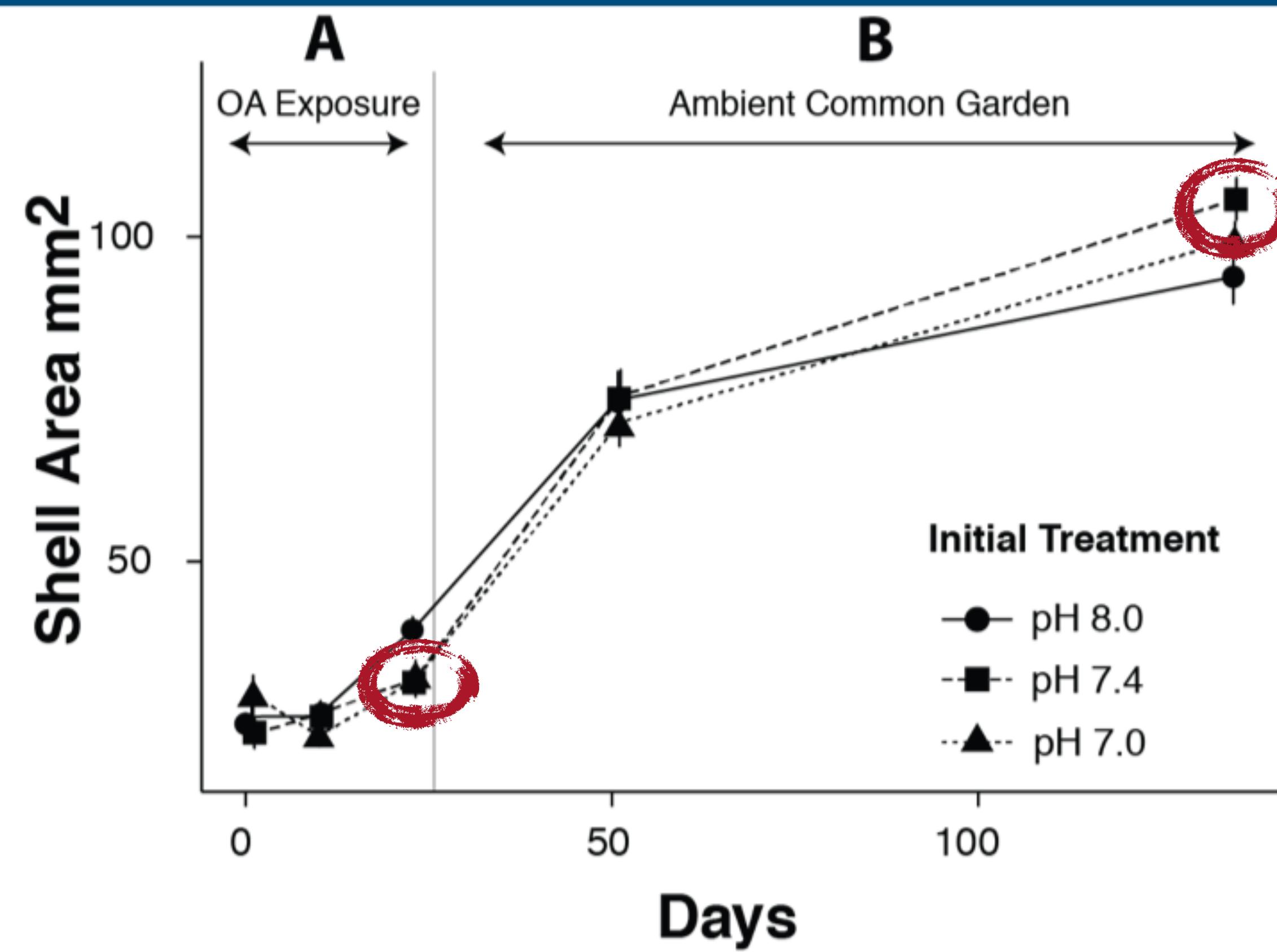
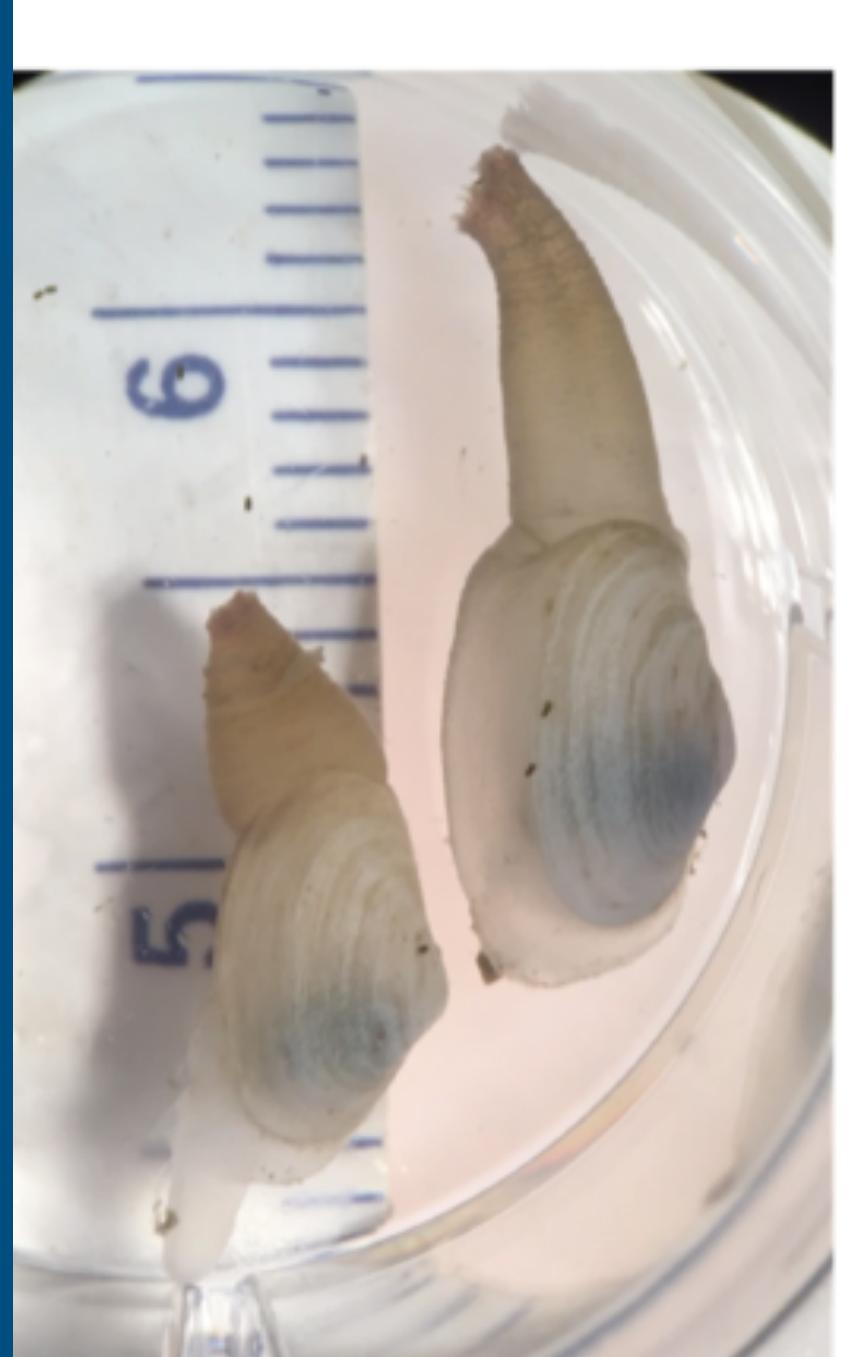
HOLLIE PUTNAM, SAM GURR, BRENT VADOPALAS, SHELLY TRIGG, JAMESTOWN S'KLALLAM TRIBE

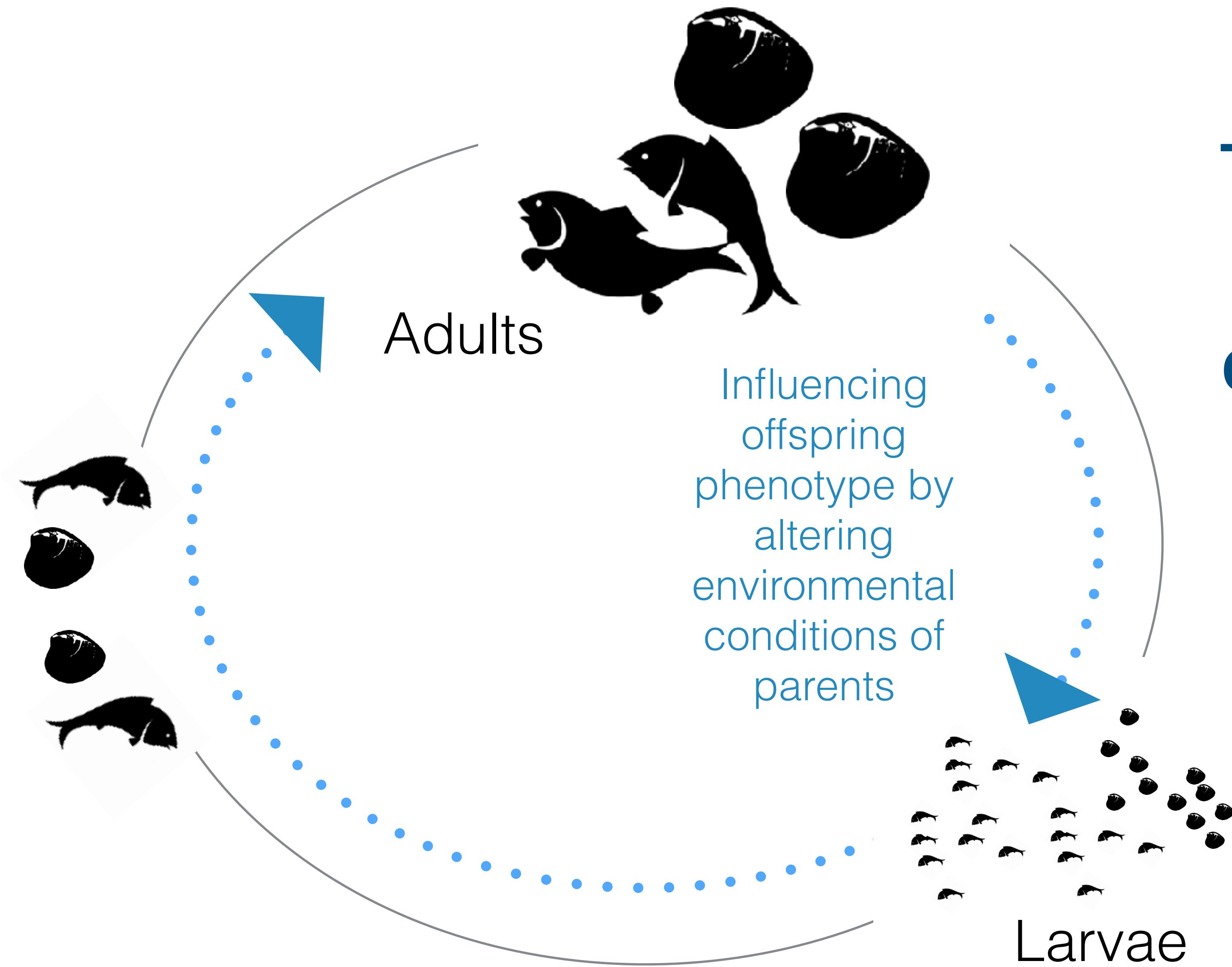
## GEODUCKS CLAMS



# GEODUCKS CLAMS

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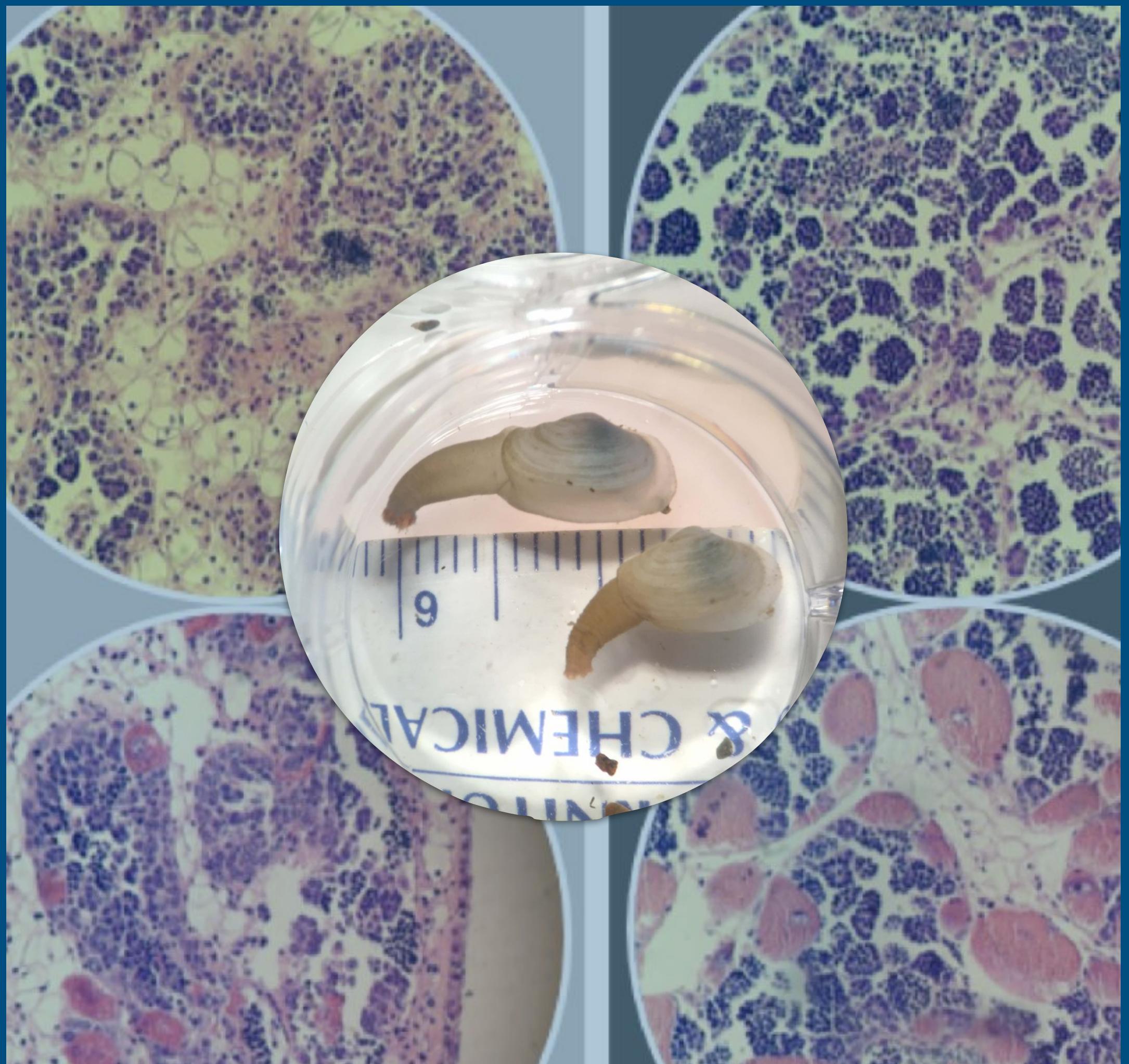




**Transgenerational  
Plasticity**  
**Carry-over effects**

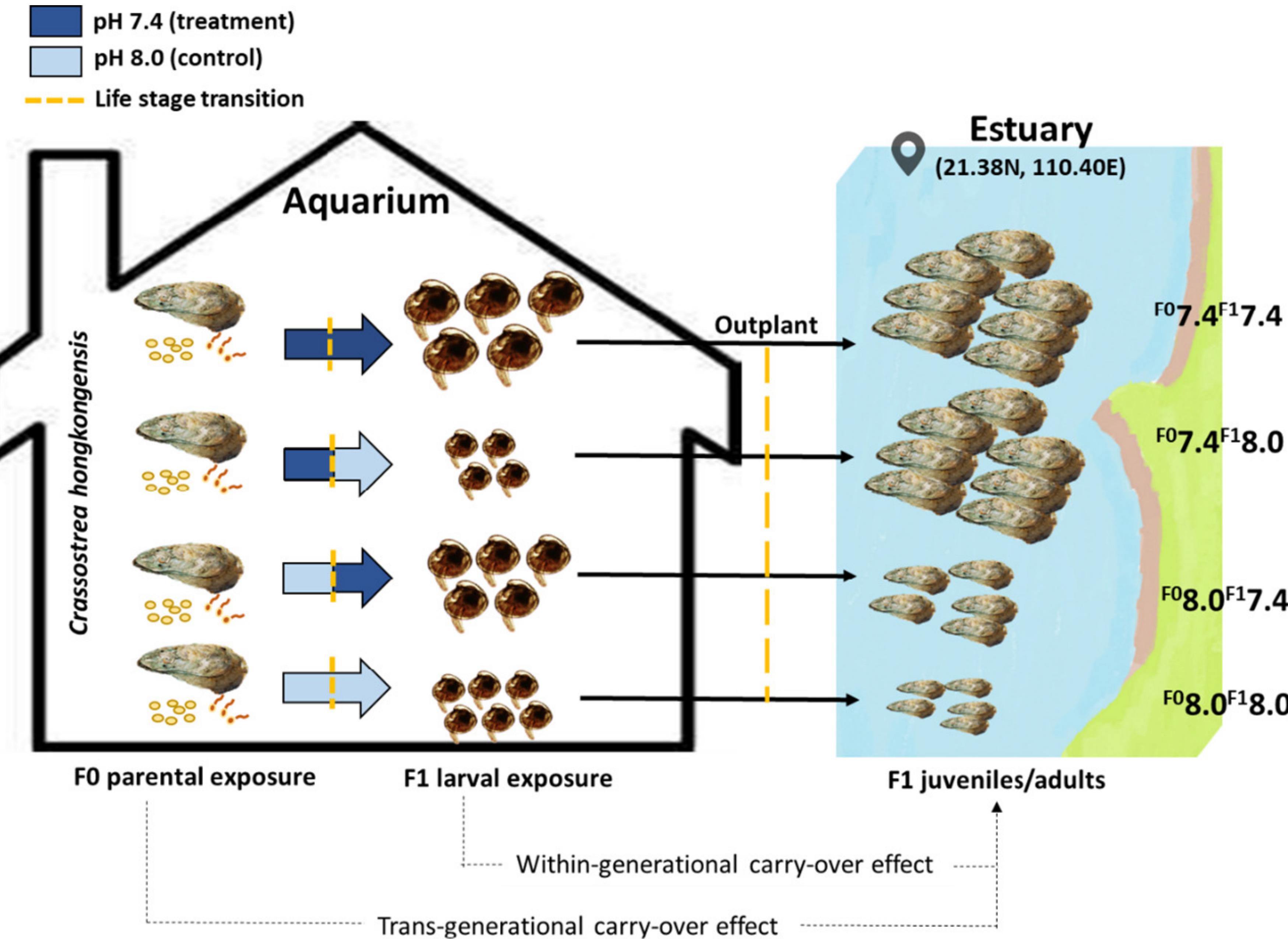
# INFLUENCE OF PARENTAL CONDITIONS

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



# *Crassostrea hongkongensis*

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



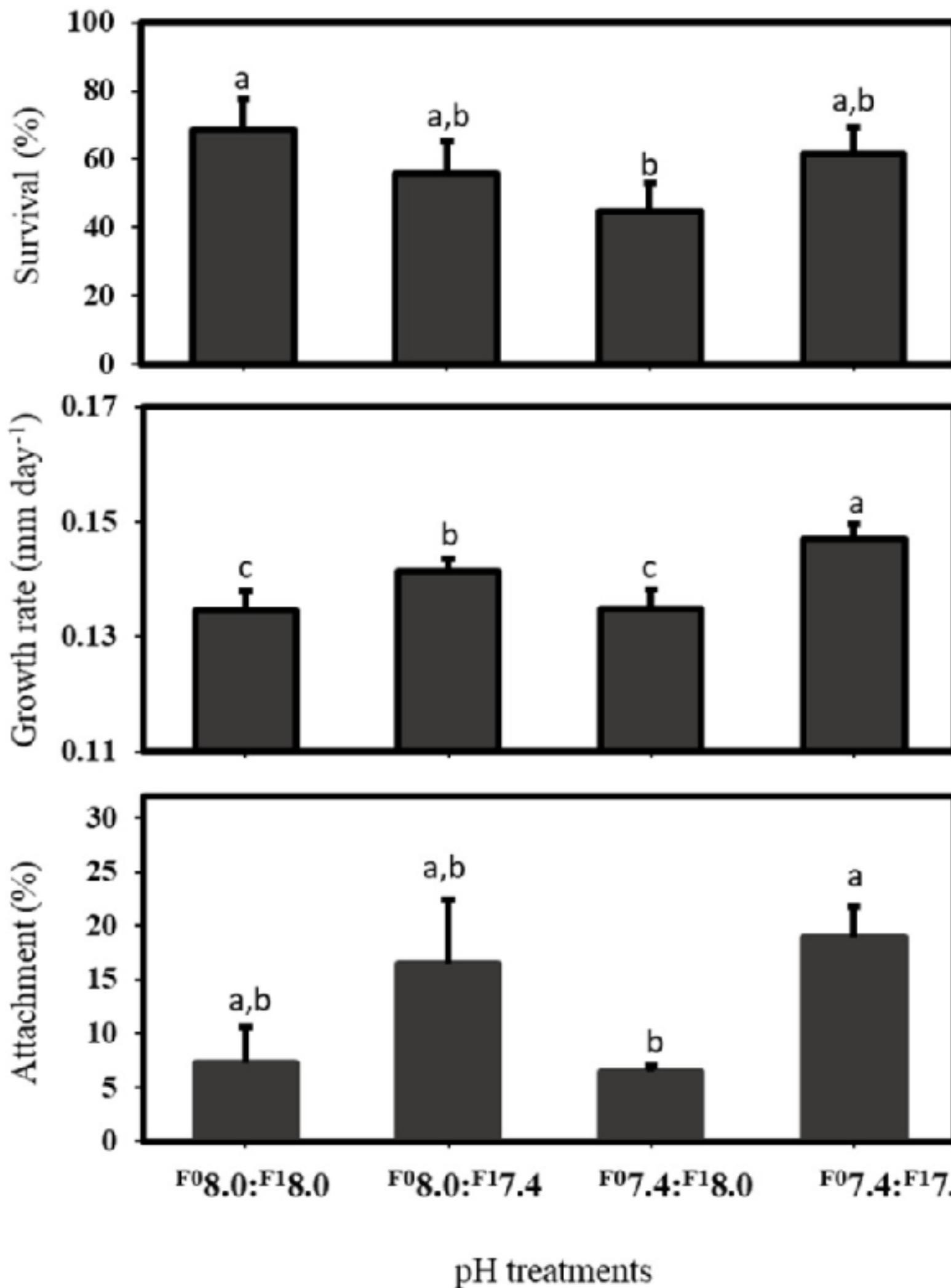
# *Crassostrea* *hongkongensis*

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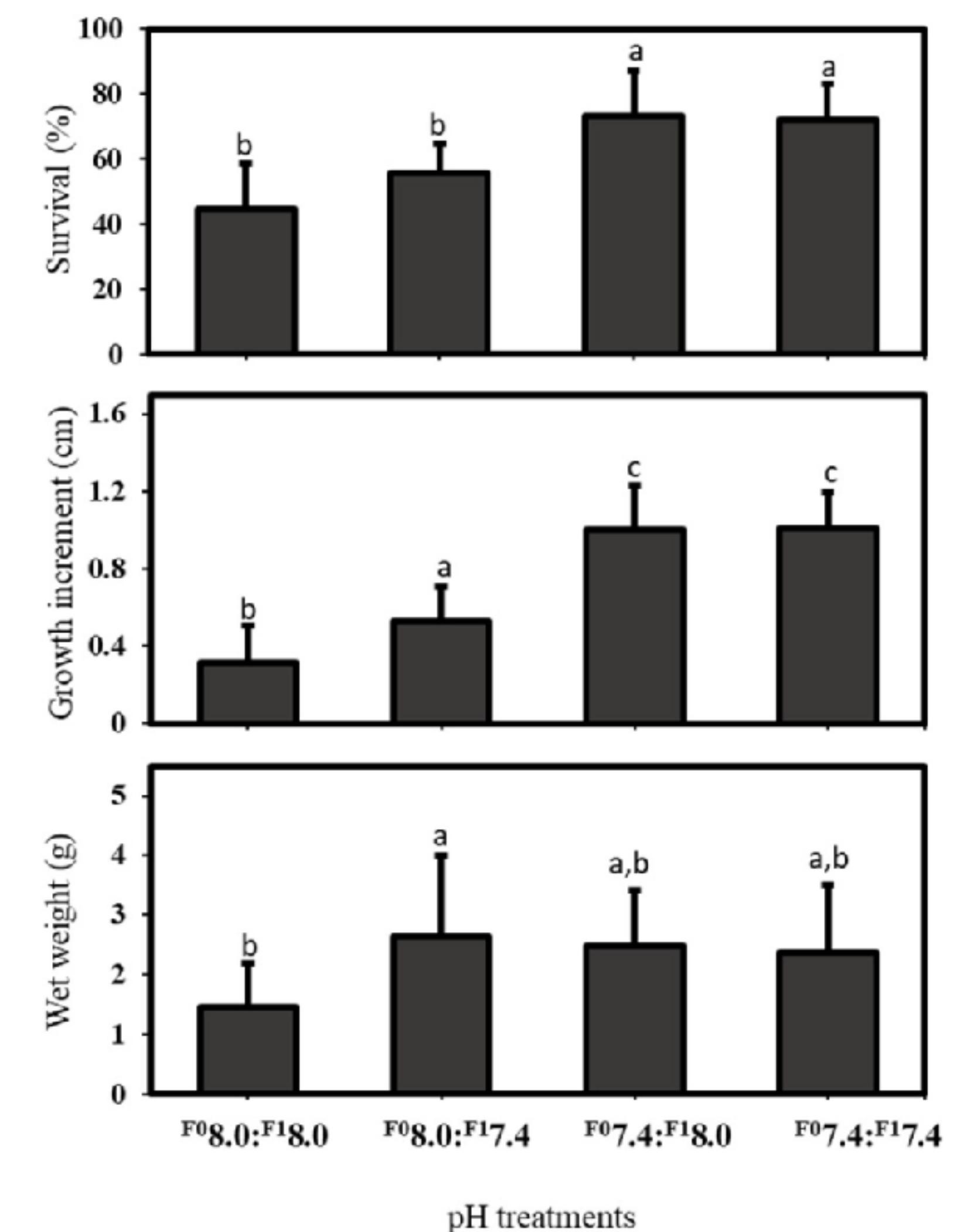
Yong-Kian Lim<sup>1</sup>, Xin Dang<sup>1</sup>, Vengatesen Thiagarajan  



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



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



# EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS

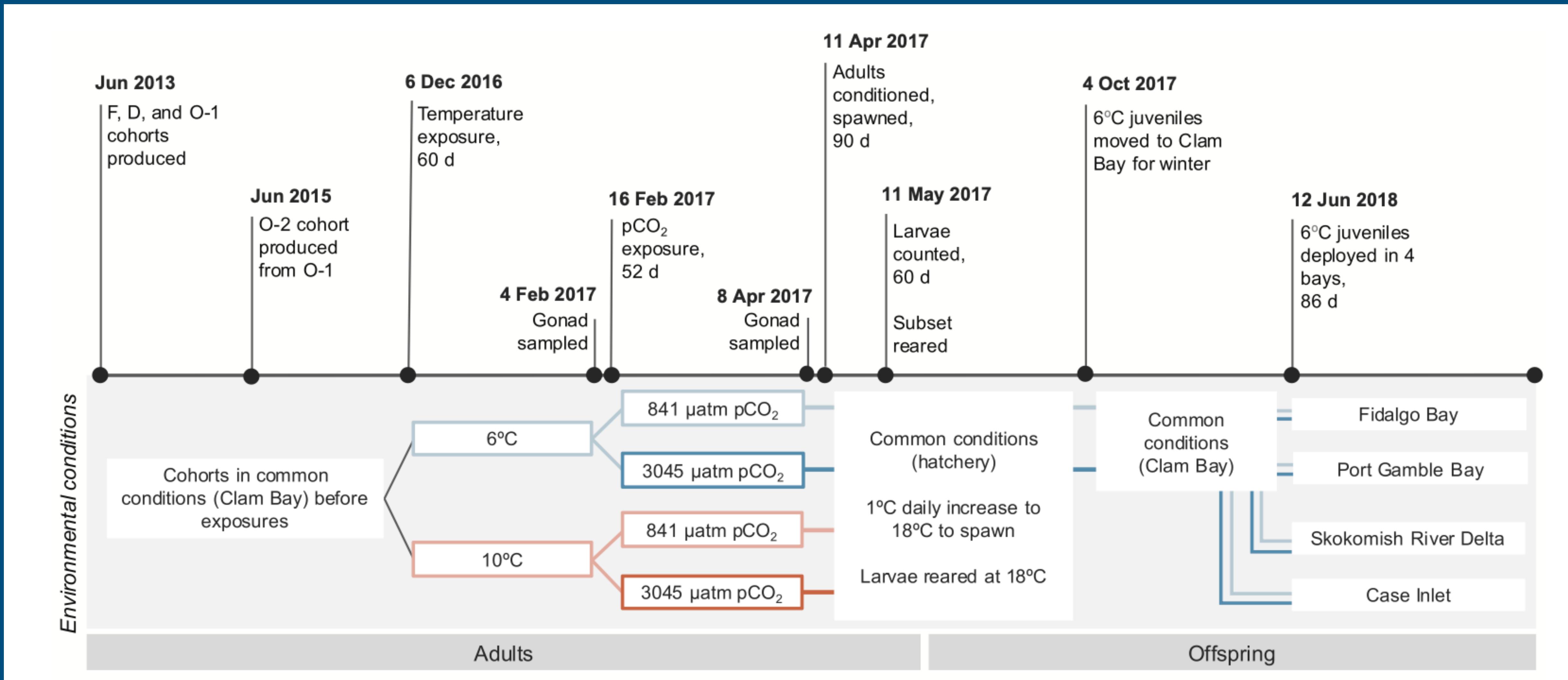


- ▶ Oysters were held at two temperature regimes ( $6^{\circ}\text{C}$  and  $10^{\circ}\text{C}$ ) for 60 days in December
- ▶ A differential pCO<sub>2</sub> exposure was carried out after the temperature treatment ended. Held at ambient pCO<sub>2</sub> (841  $\mu\text{atm}$ ) or high pCO<sub>2</sub>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

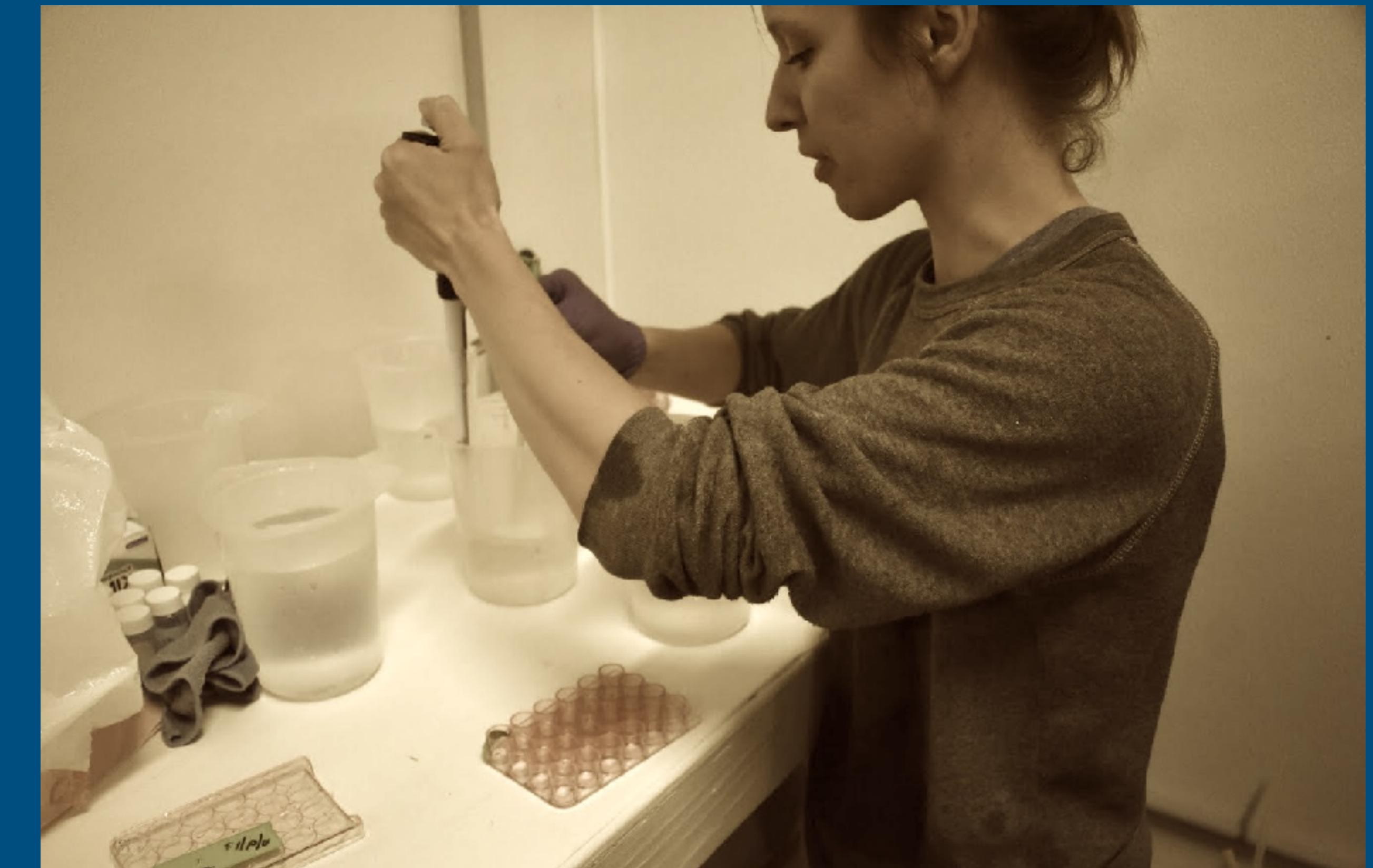
Carryover effects of temperature and pCO<sub>2</sub> across multiple Olympia oyster populations

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

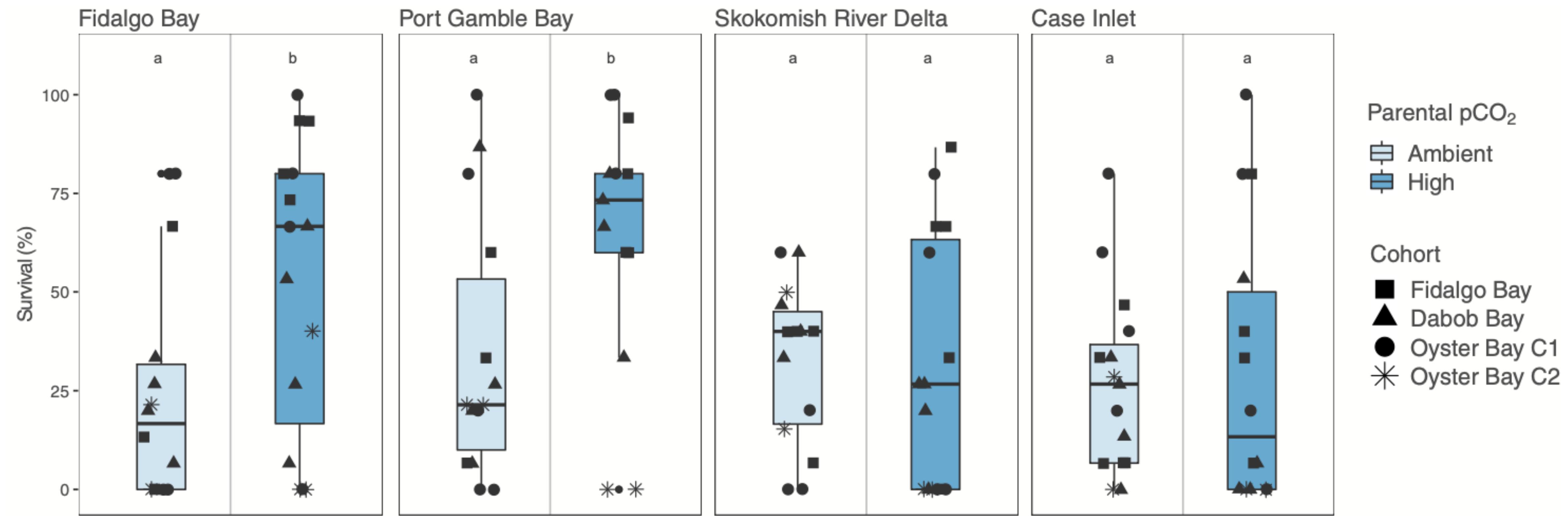


# EFFECTS OF TEMPERATURE AND OA IN OLYMPIA OYSTER POPULATIONS



- ▶ 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

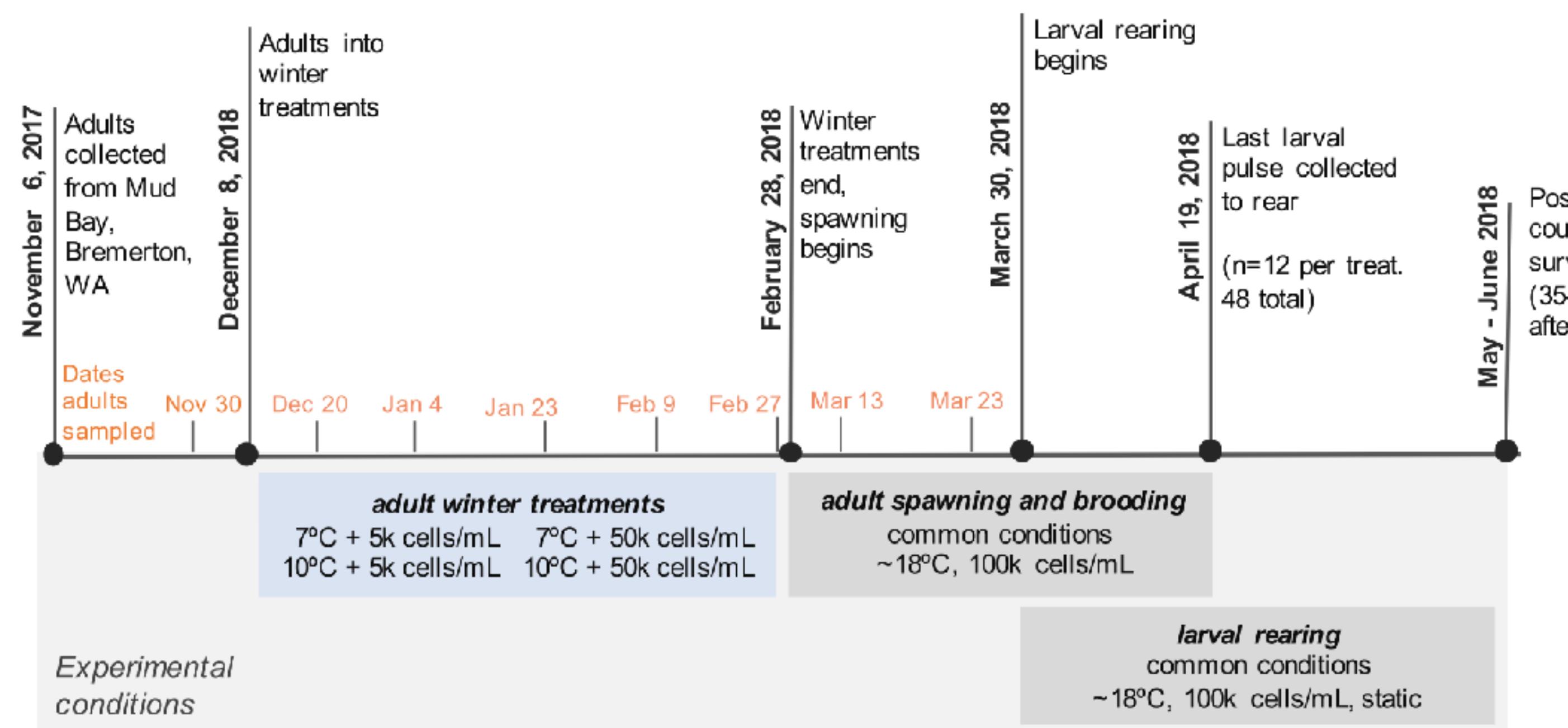
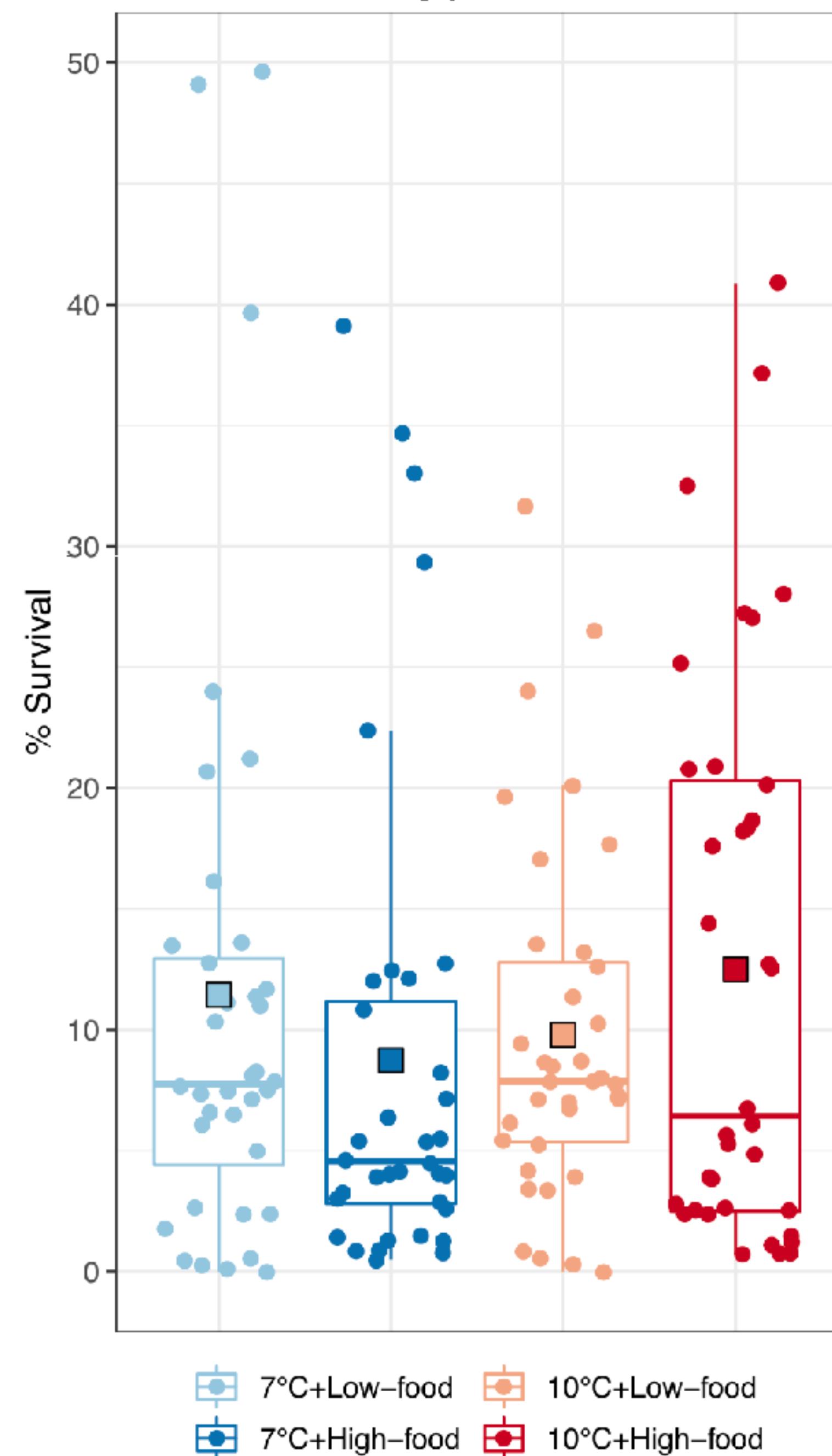


Fig. 1. Experimental timeline. Cells/mL indicate the concentrations of live algae given to oysters at various stages.

## Larval survival by parental treatment



# Environmental Memory



CAN BE BENEFICIAL  
STAGE DEPENDENT  
ACROSS GENERATION  
IMPLICATIONS ...

# Environmental Memory



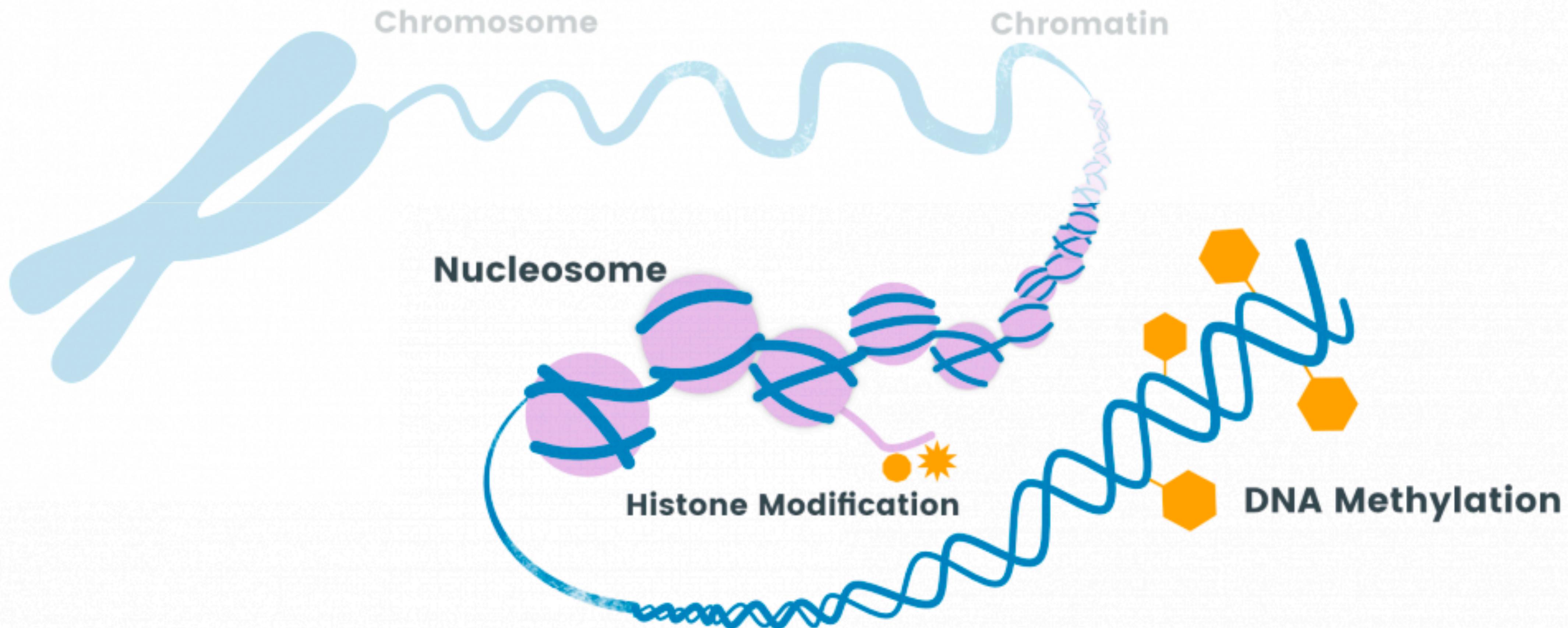
CAN BE BENEFICIAL  
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**Epigenetic Mechanisms**

# Epigenetics

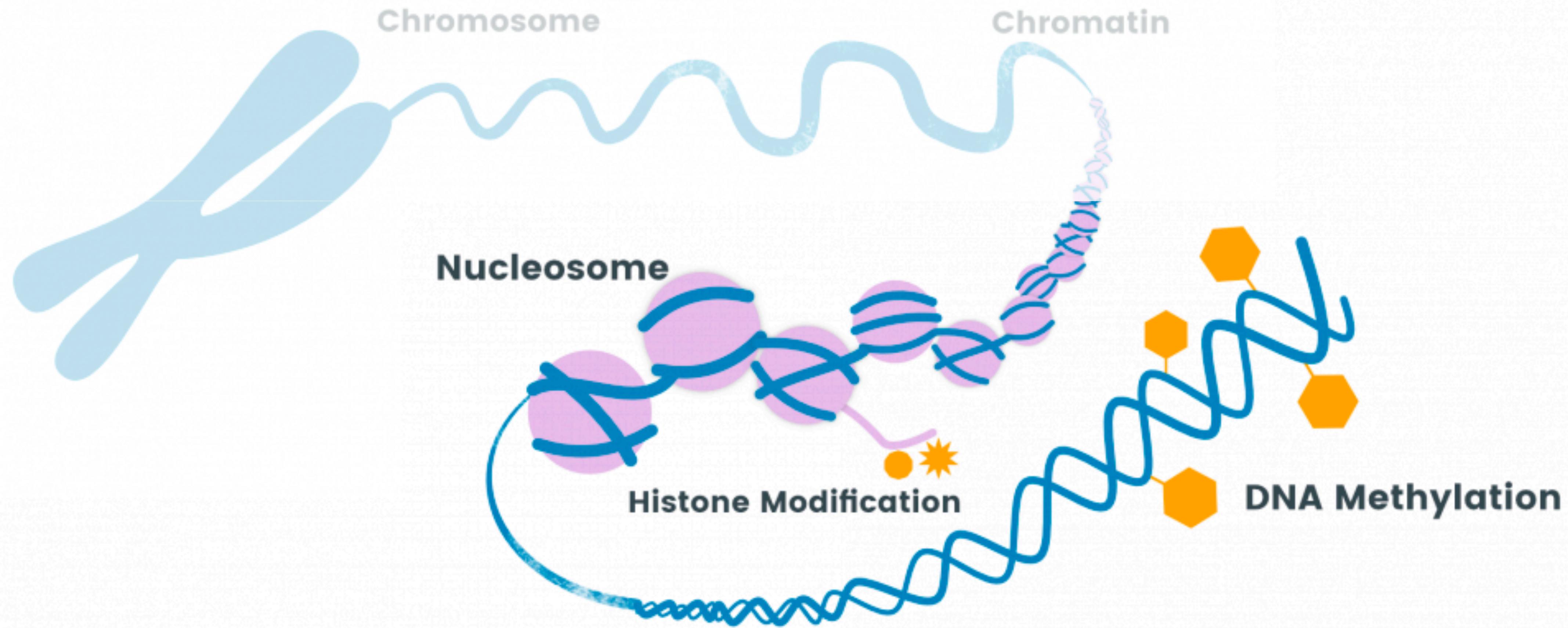
## WHAT IS EPIGENETICS?

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

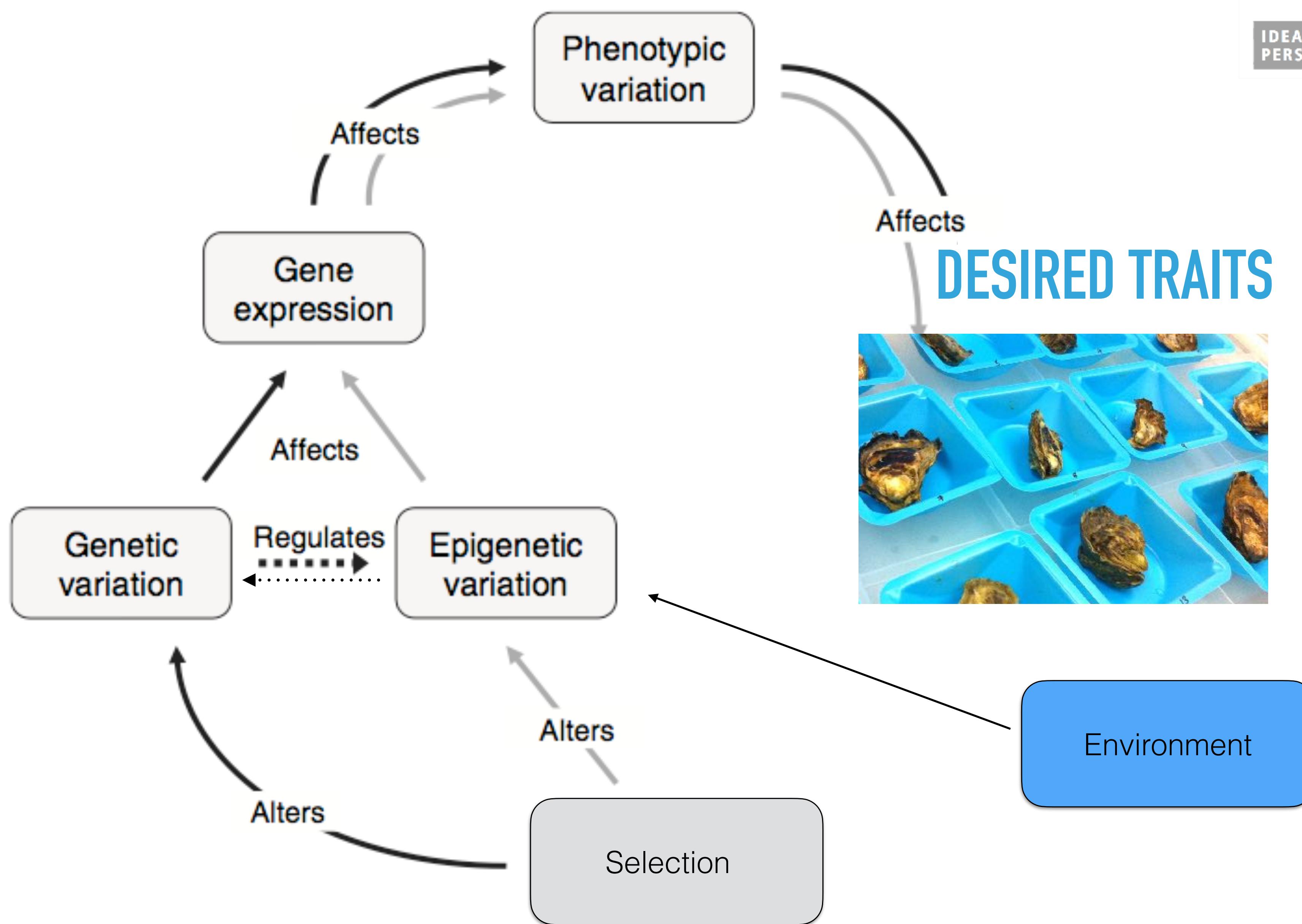


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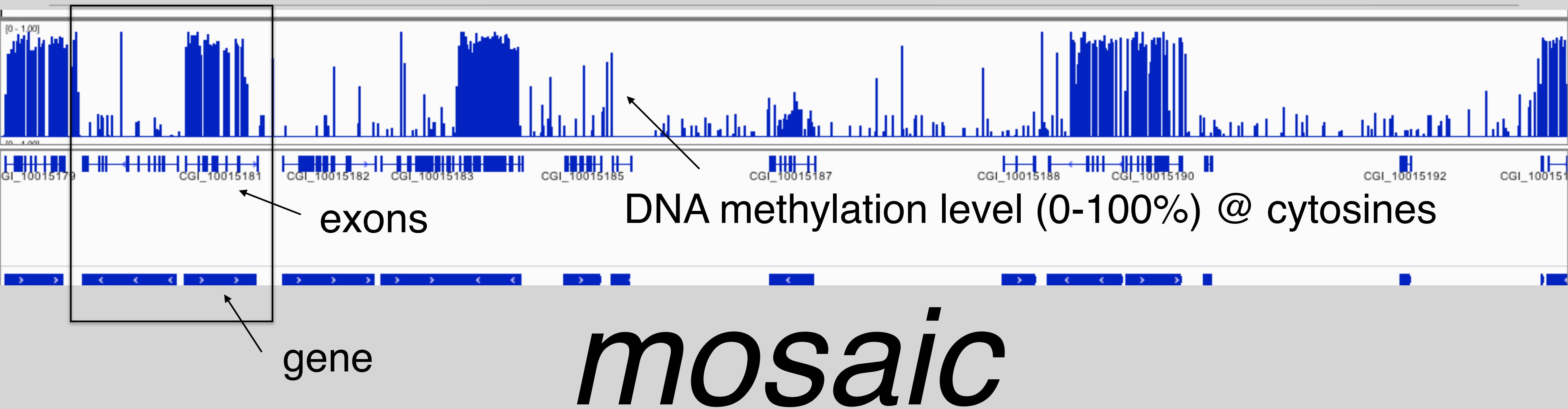
**CAN BE INDUCED WITH ENVIRONMENTAL MANIPULATION**



IDEA AND  
PERSPECTIVE

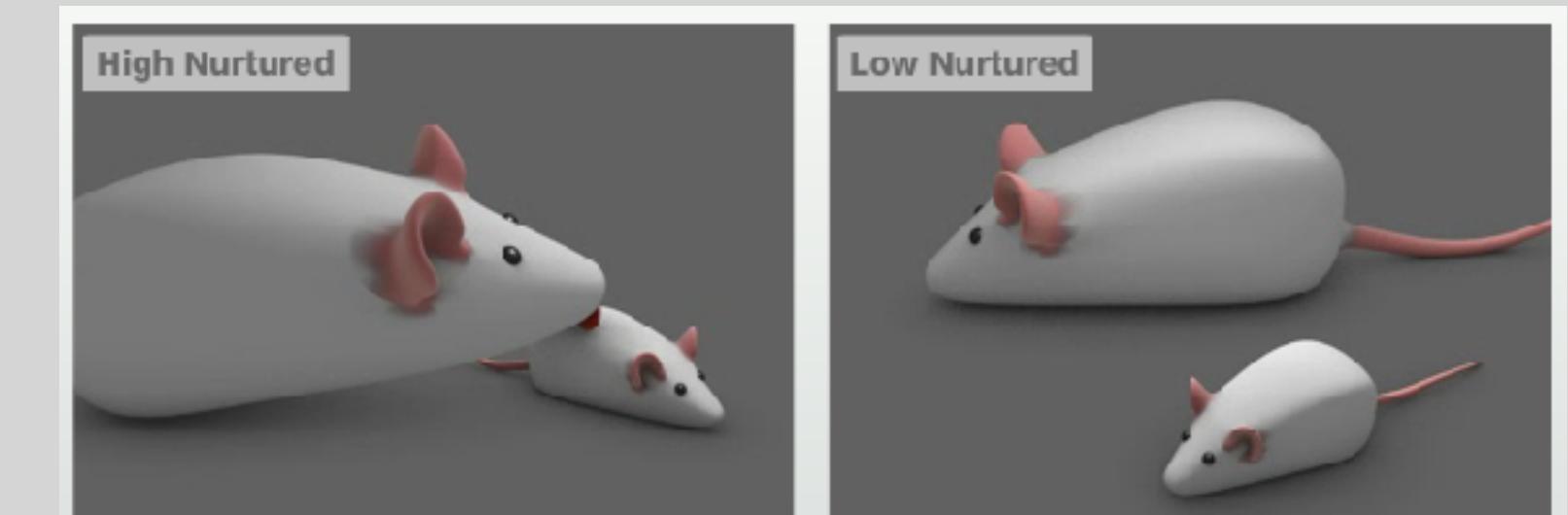
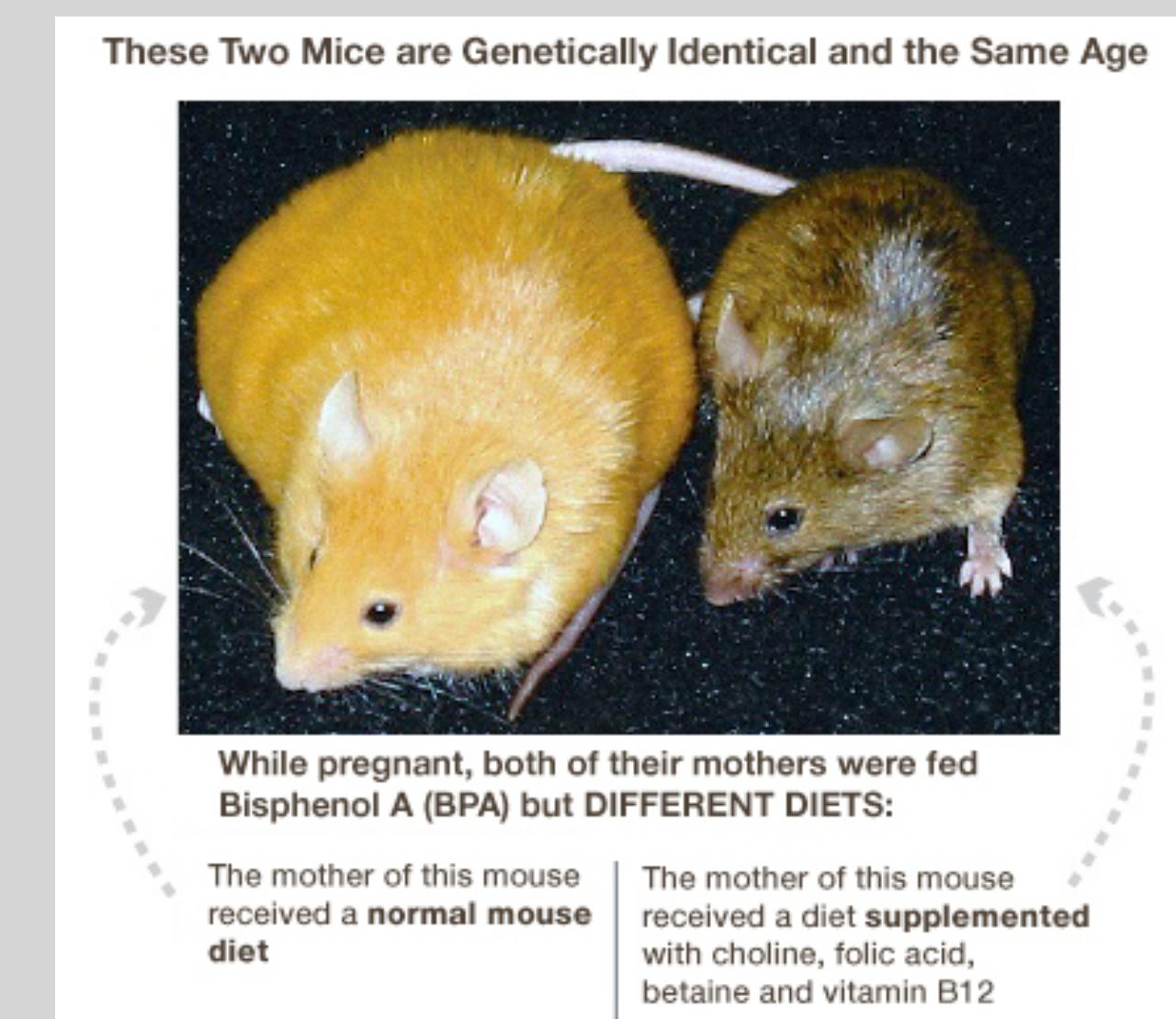
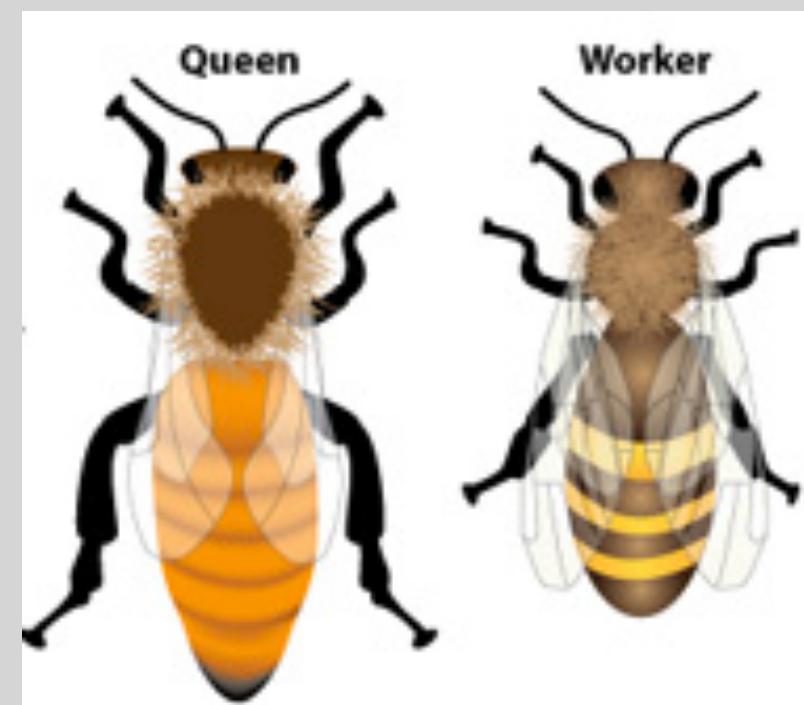
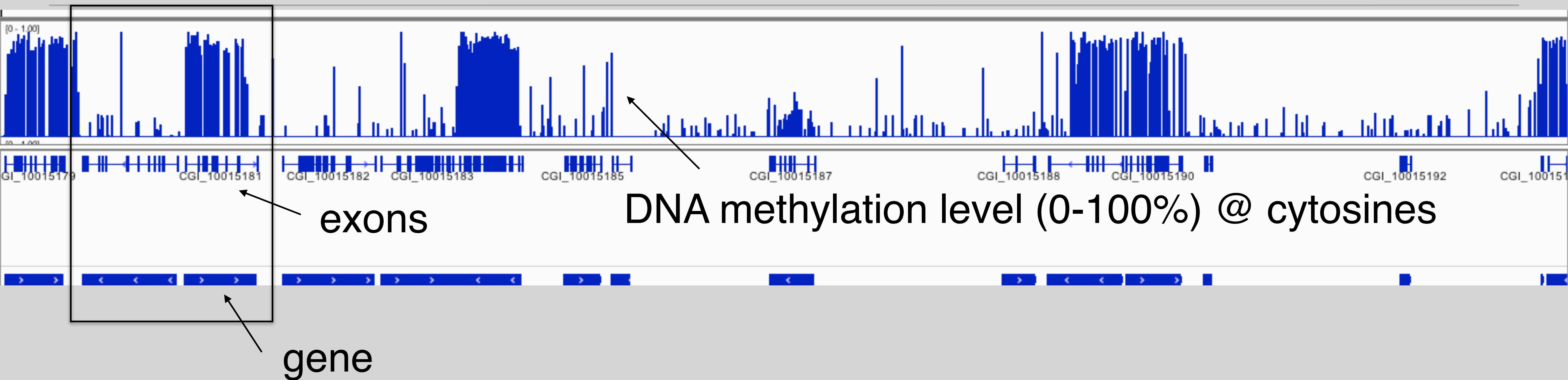
Epigenetics for ecologists

## METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



associated with gene bodies

# METHYLATION LANDSCAPE IN MARINE INVERTEBRATES



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

# Four Dimensionalities



Targeted Regulation

Stochastic Regulation

Reliable Transcription

Spurious Transcription

# Four Dimensionalities

- Distinct Lineage
- Experiential
- Inducible

Targeted Regulation

Stochastic Regulation

- Evolutionary
- Life History Driven
- Constitutive

Reliable Transcription

Spurious Transcription

# Four Dimensionalities

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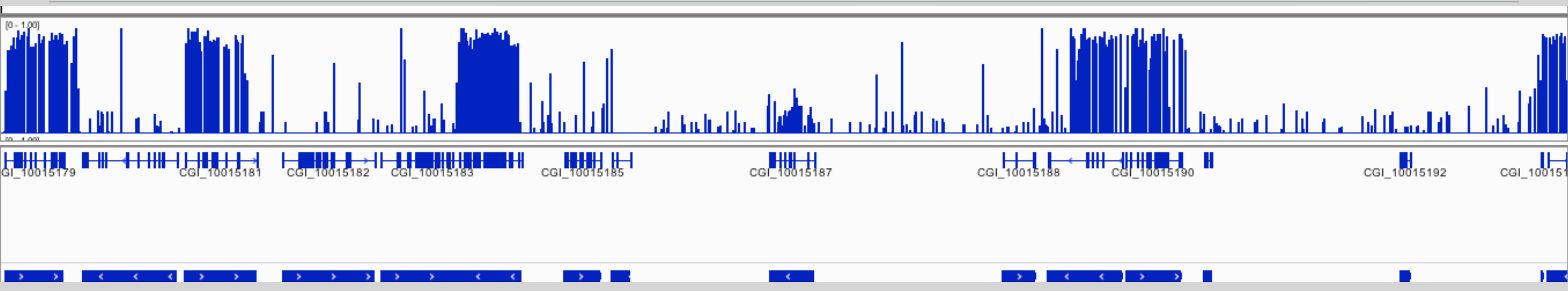
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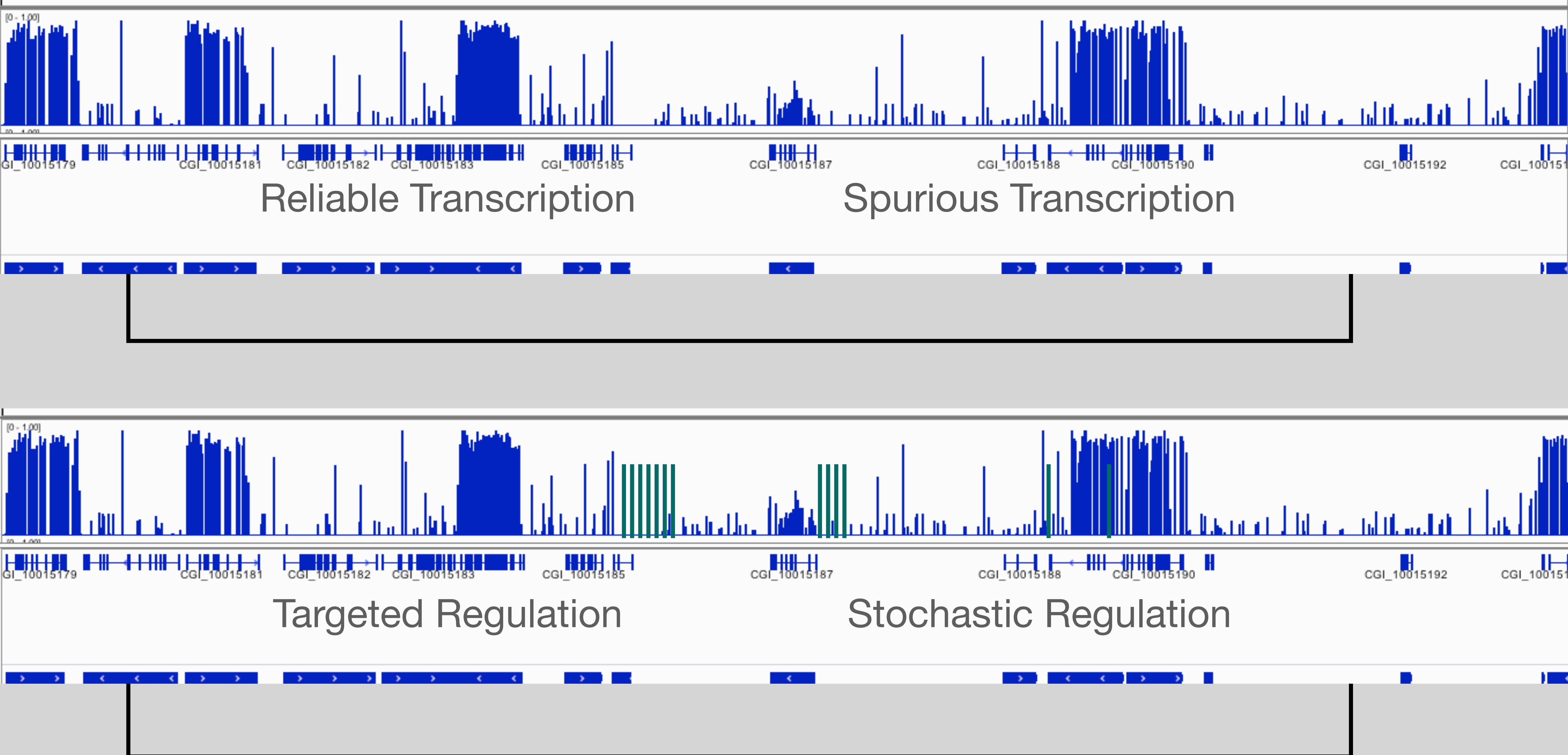
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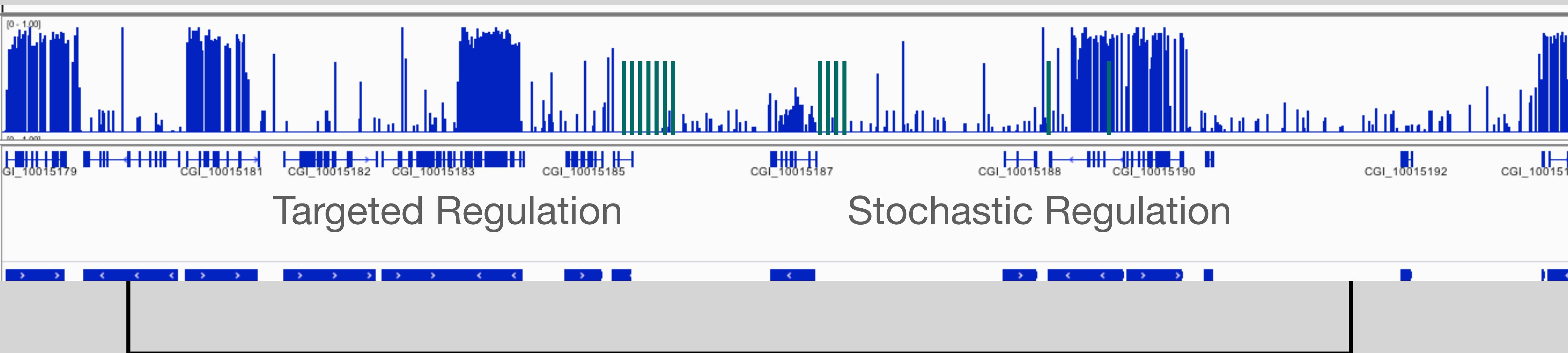
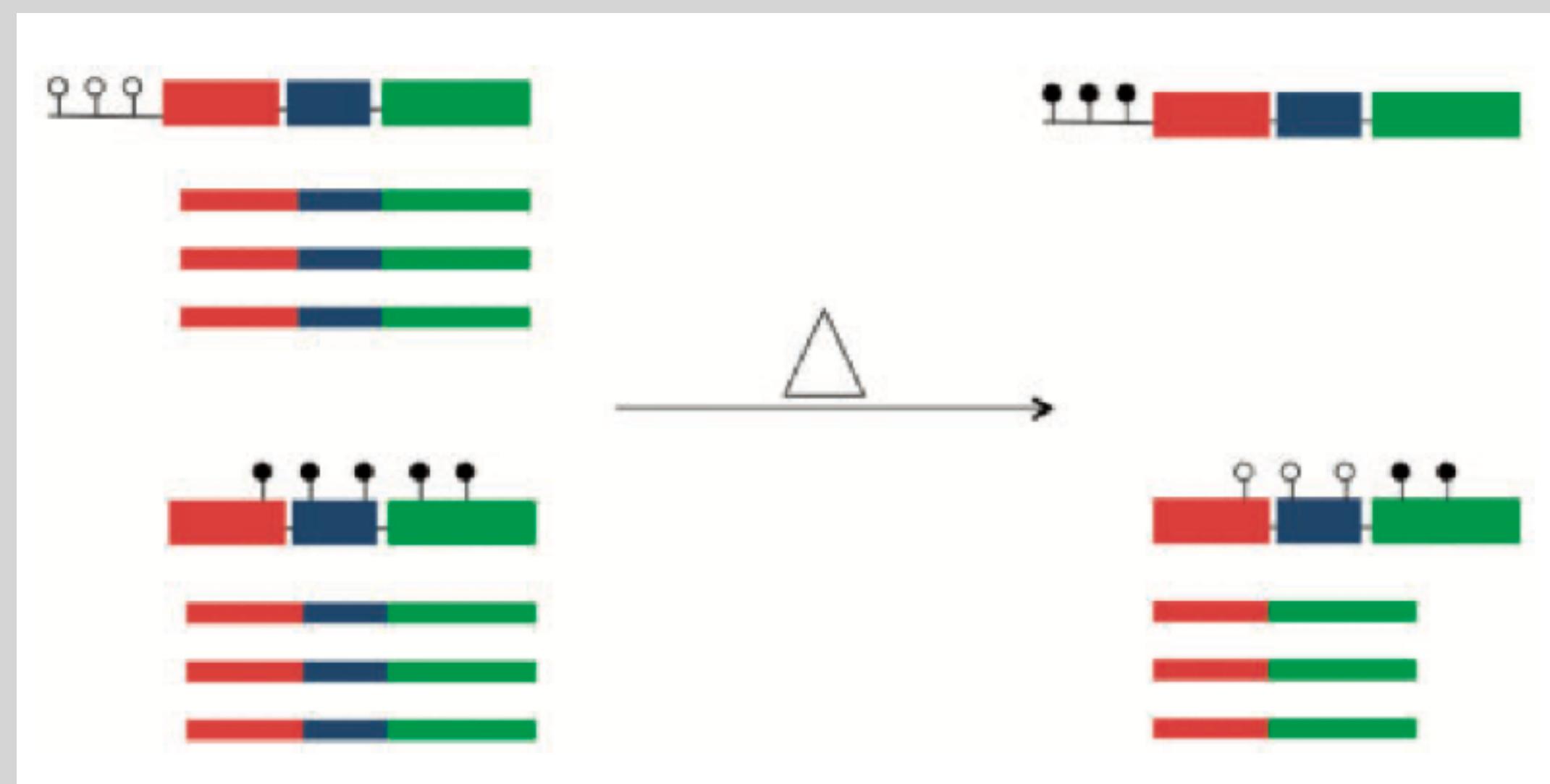
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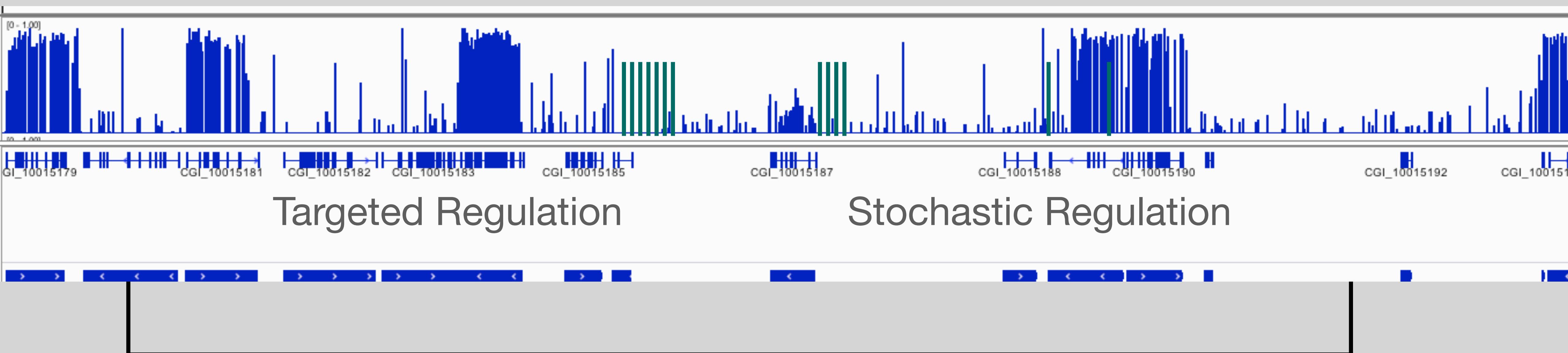
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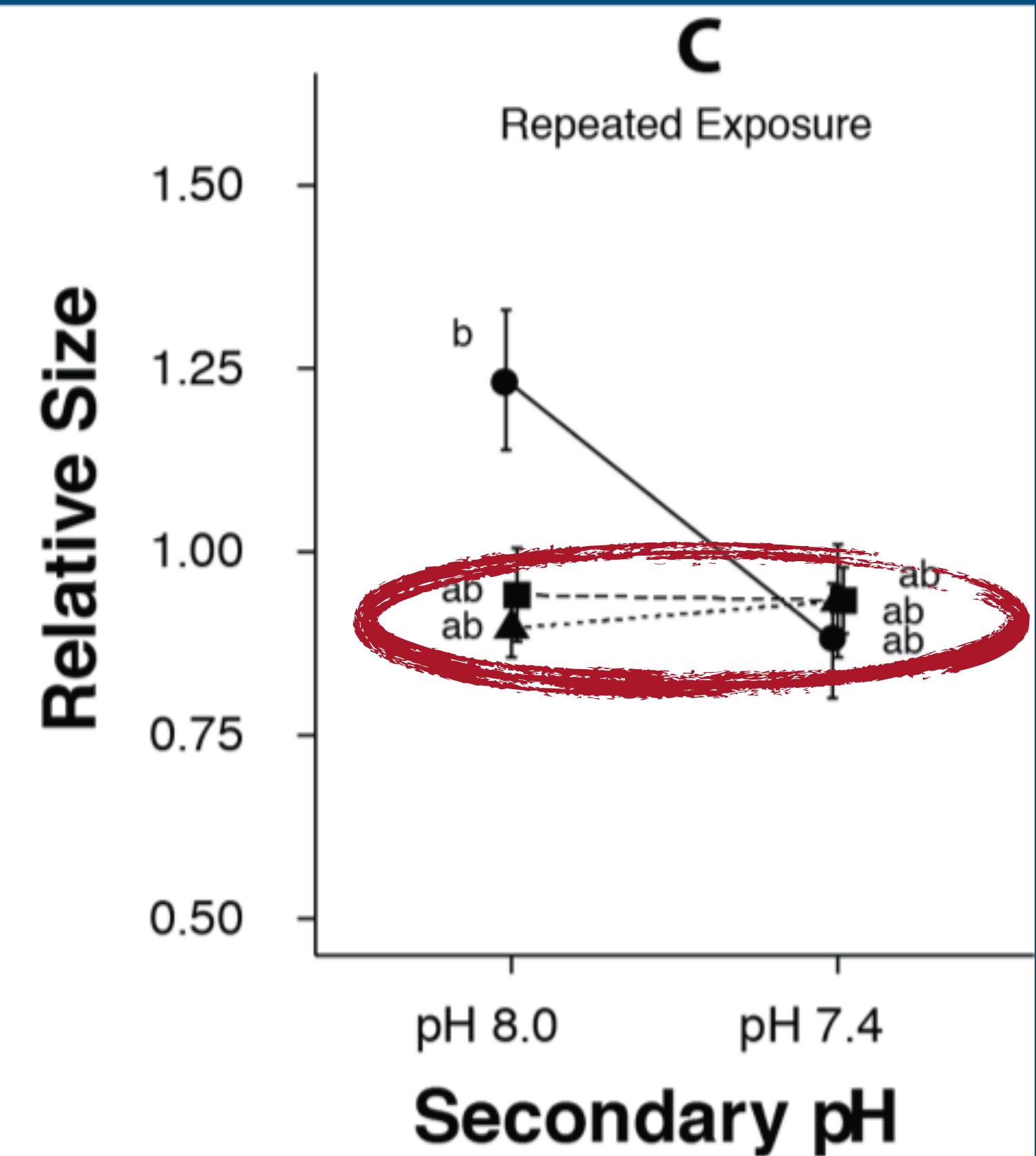
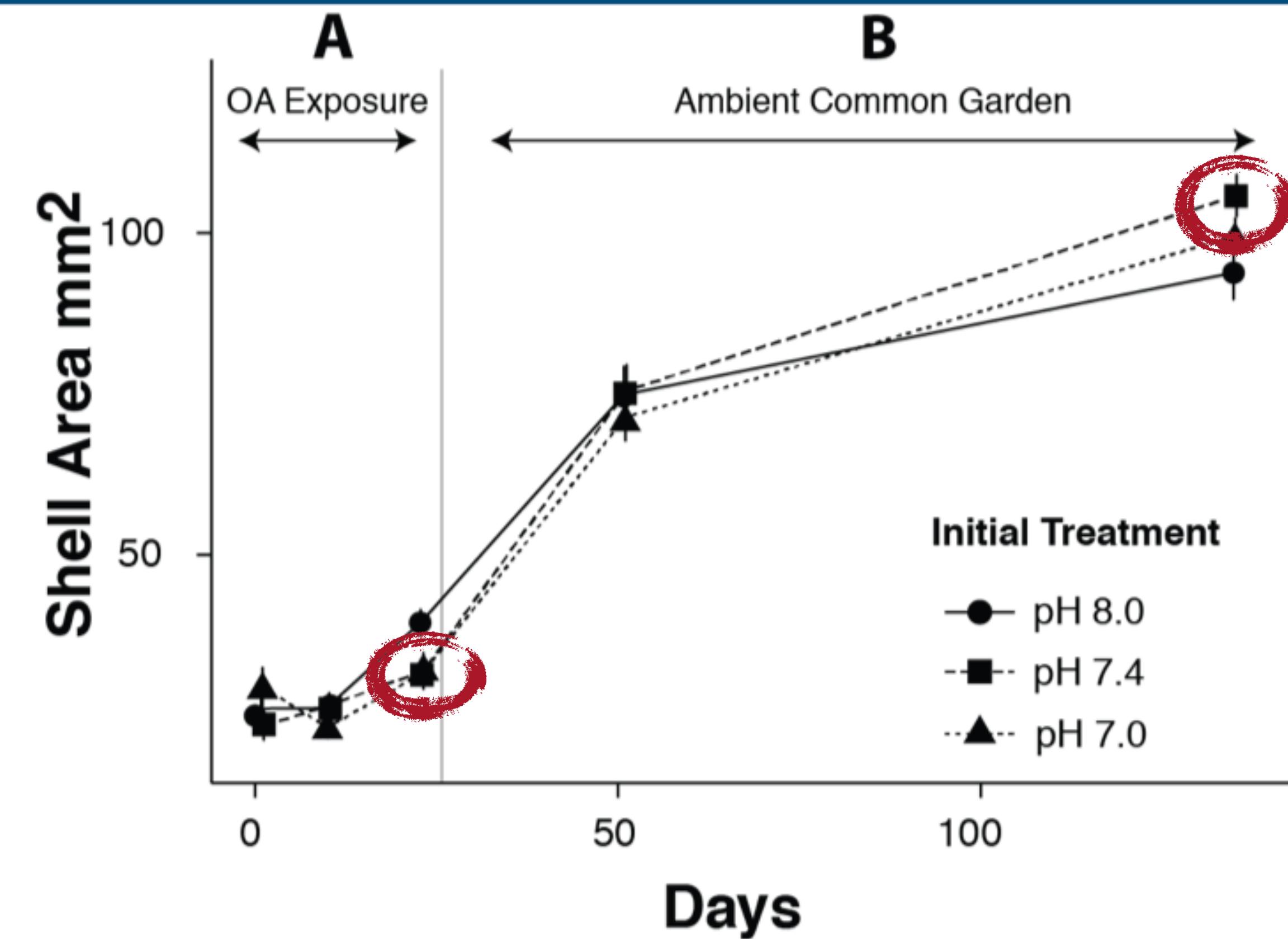


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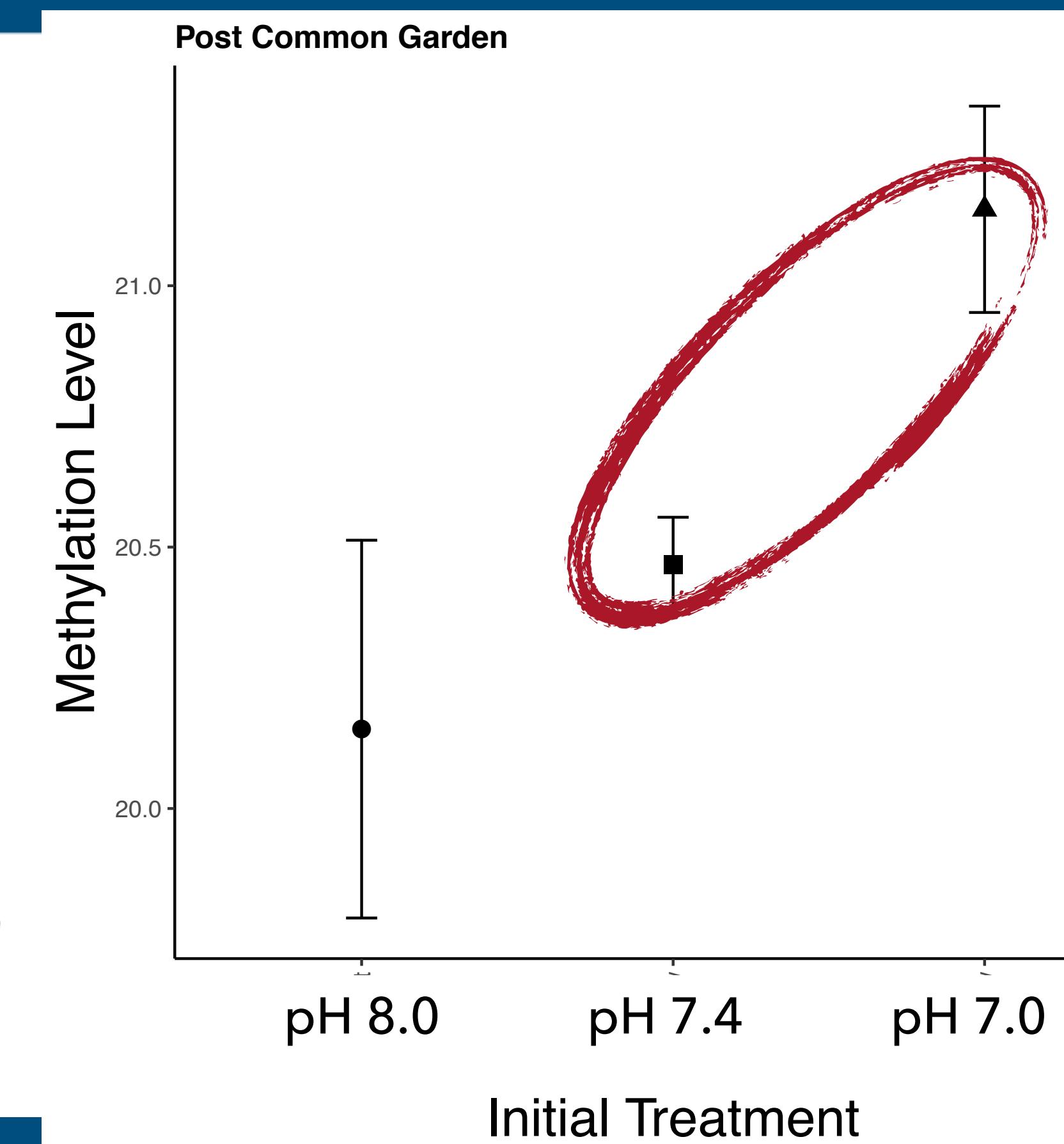
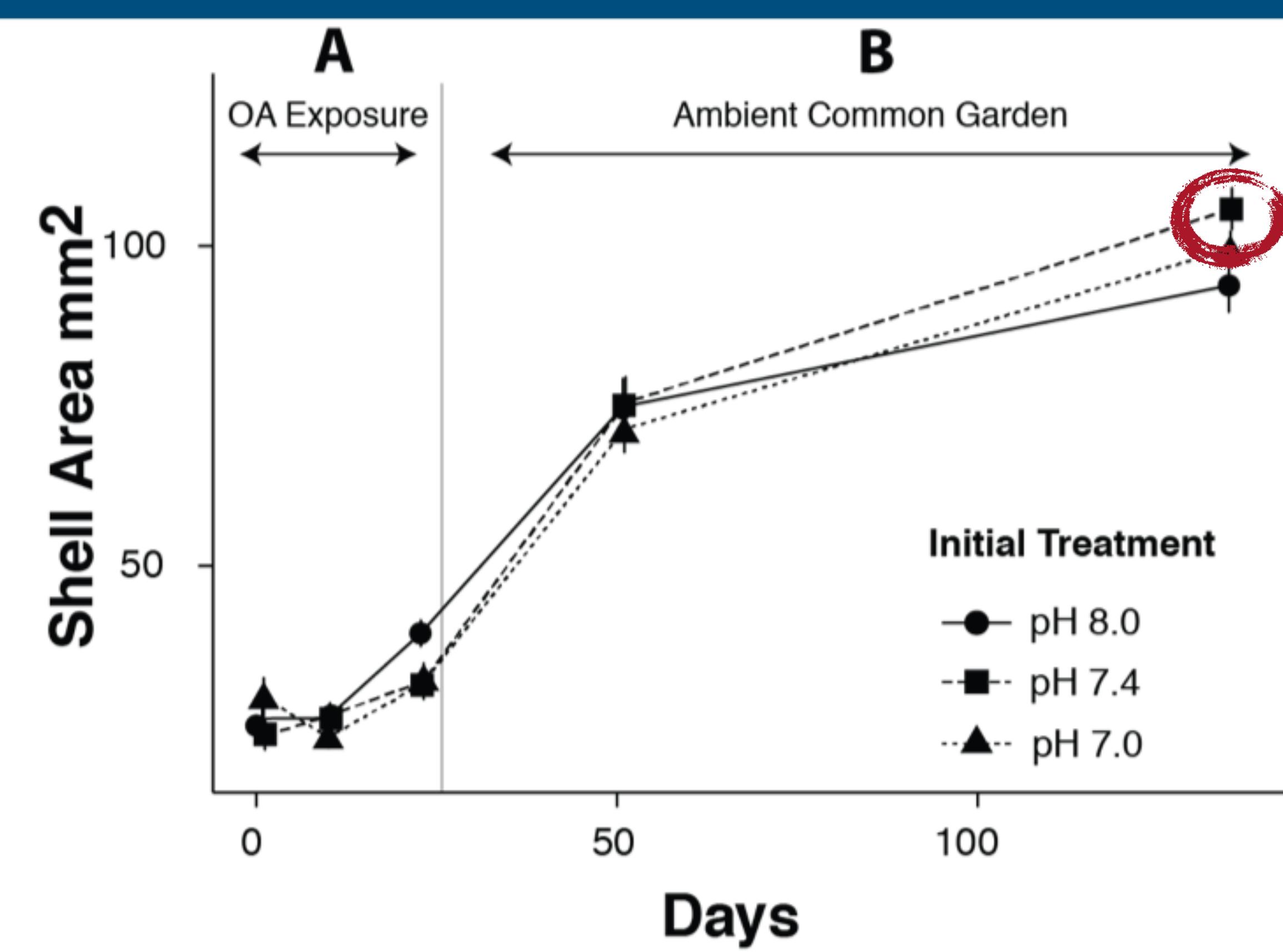
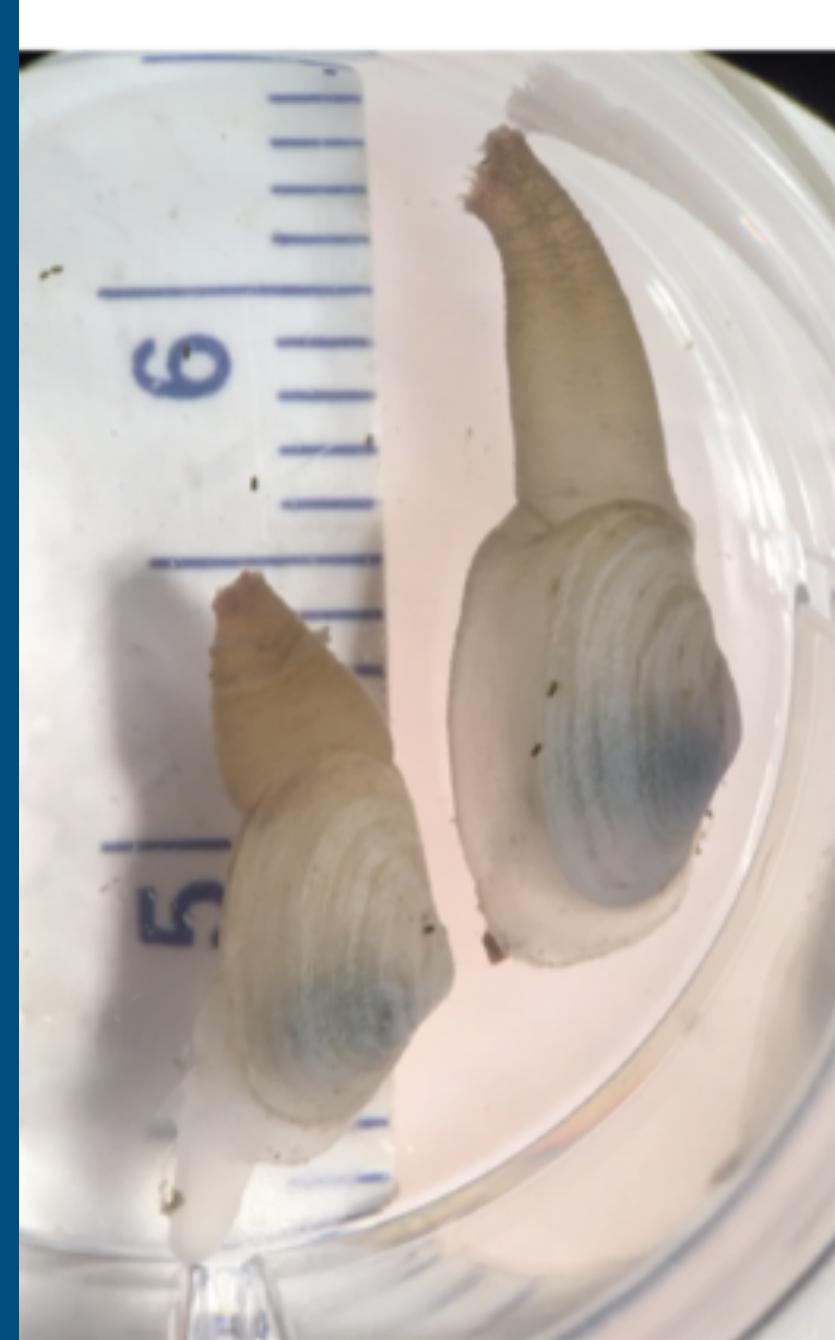
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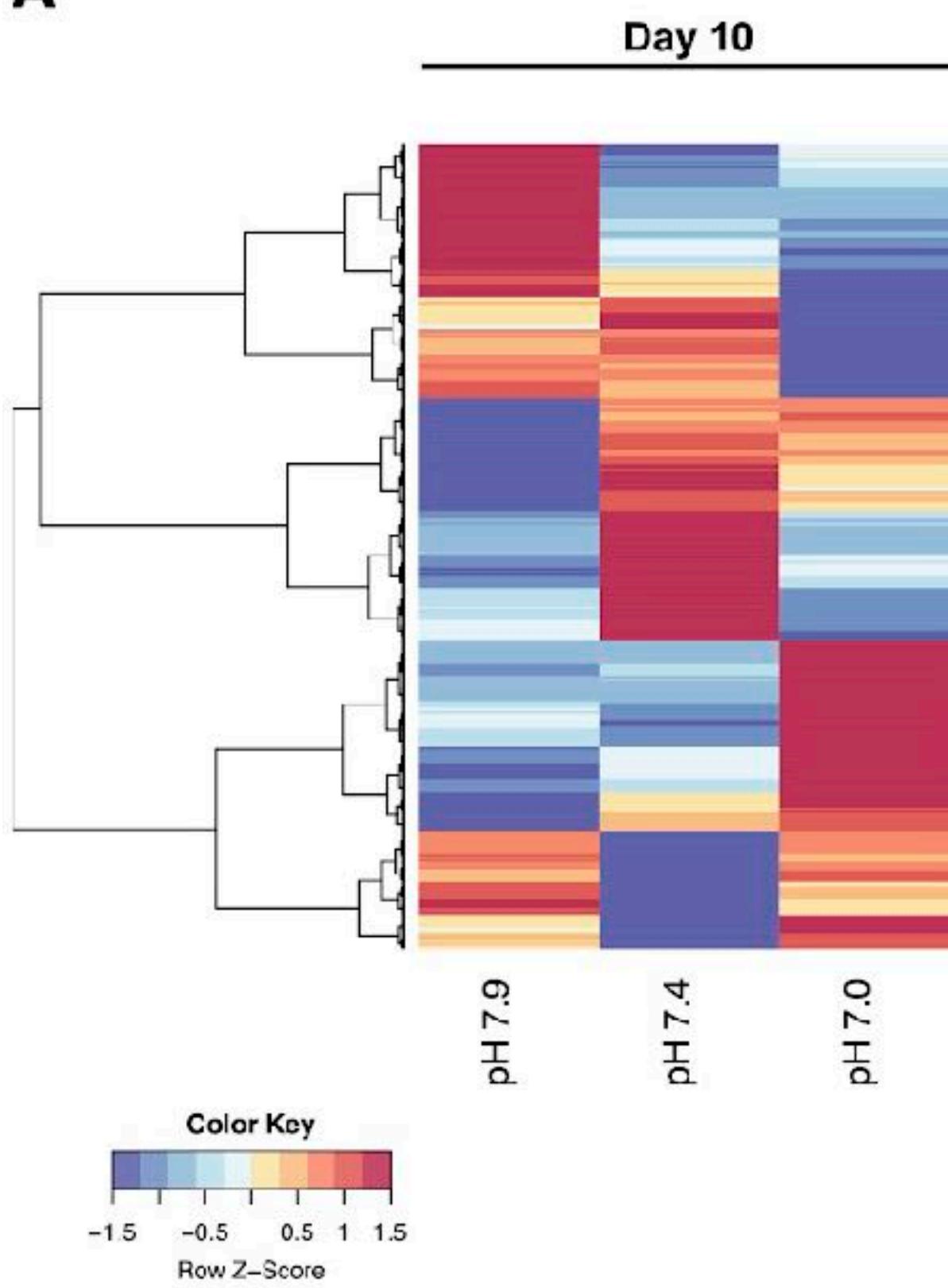
# GEODUCKS AND OA

# DNA METHYLATION

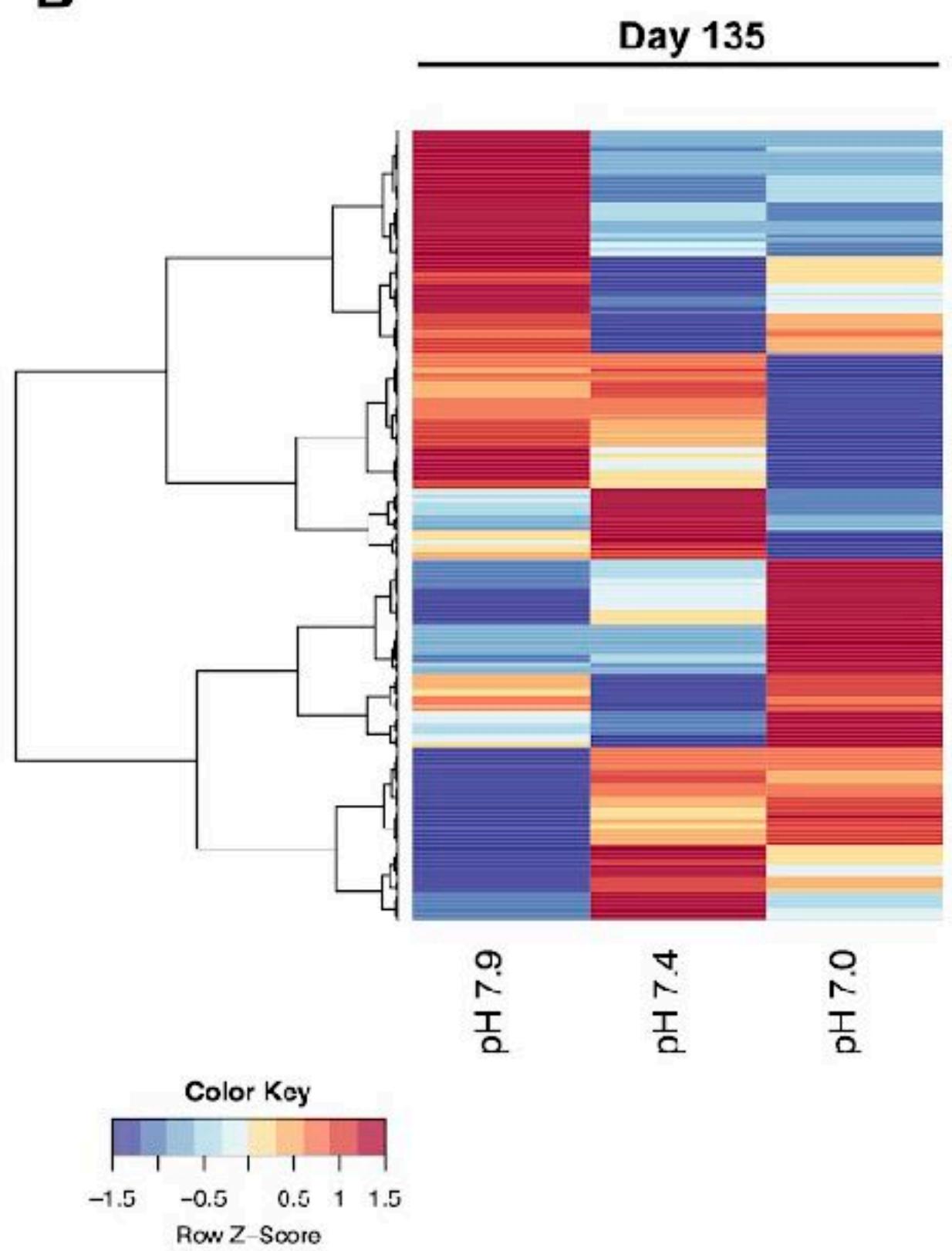


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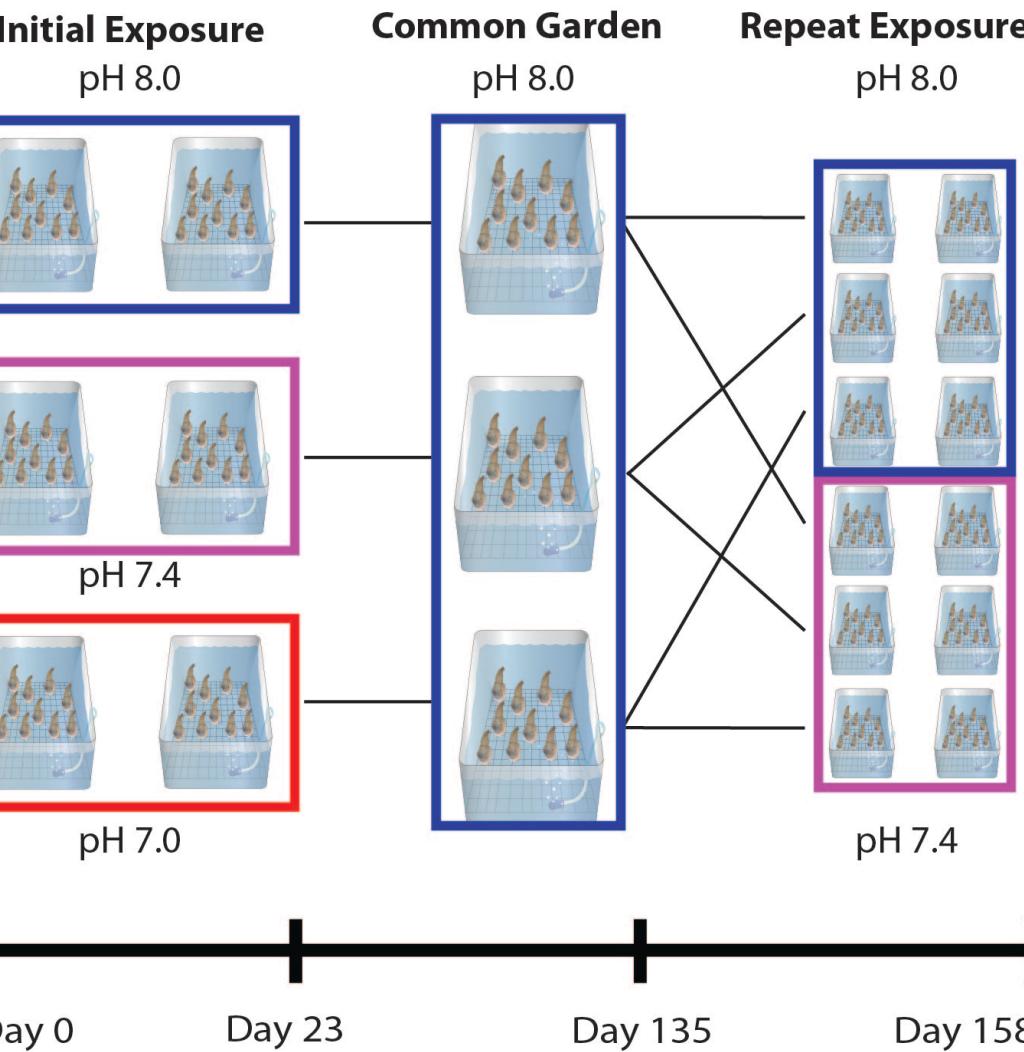
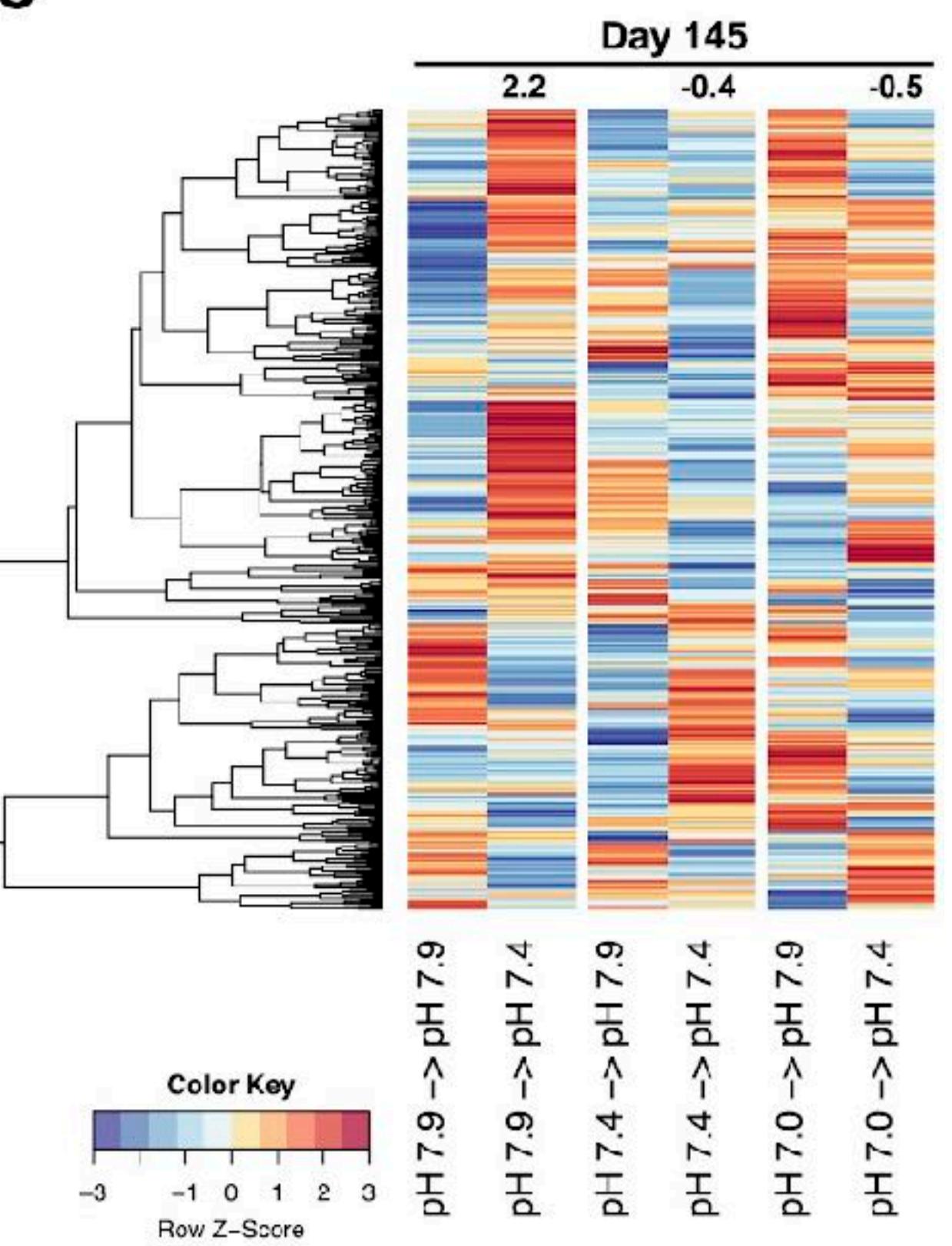
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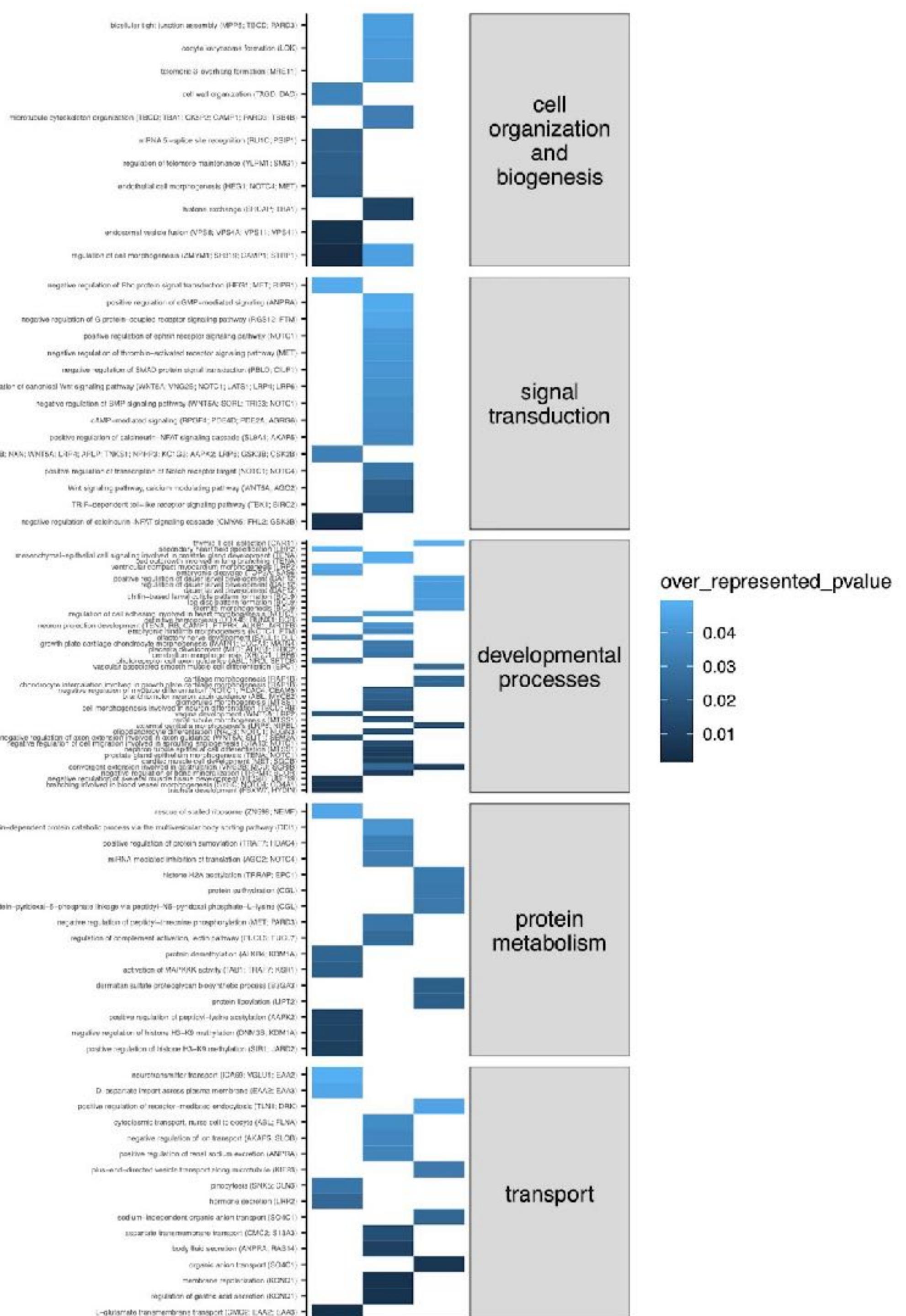
B



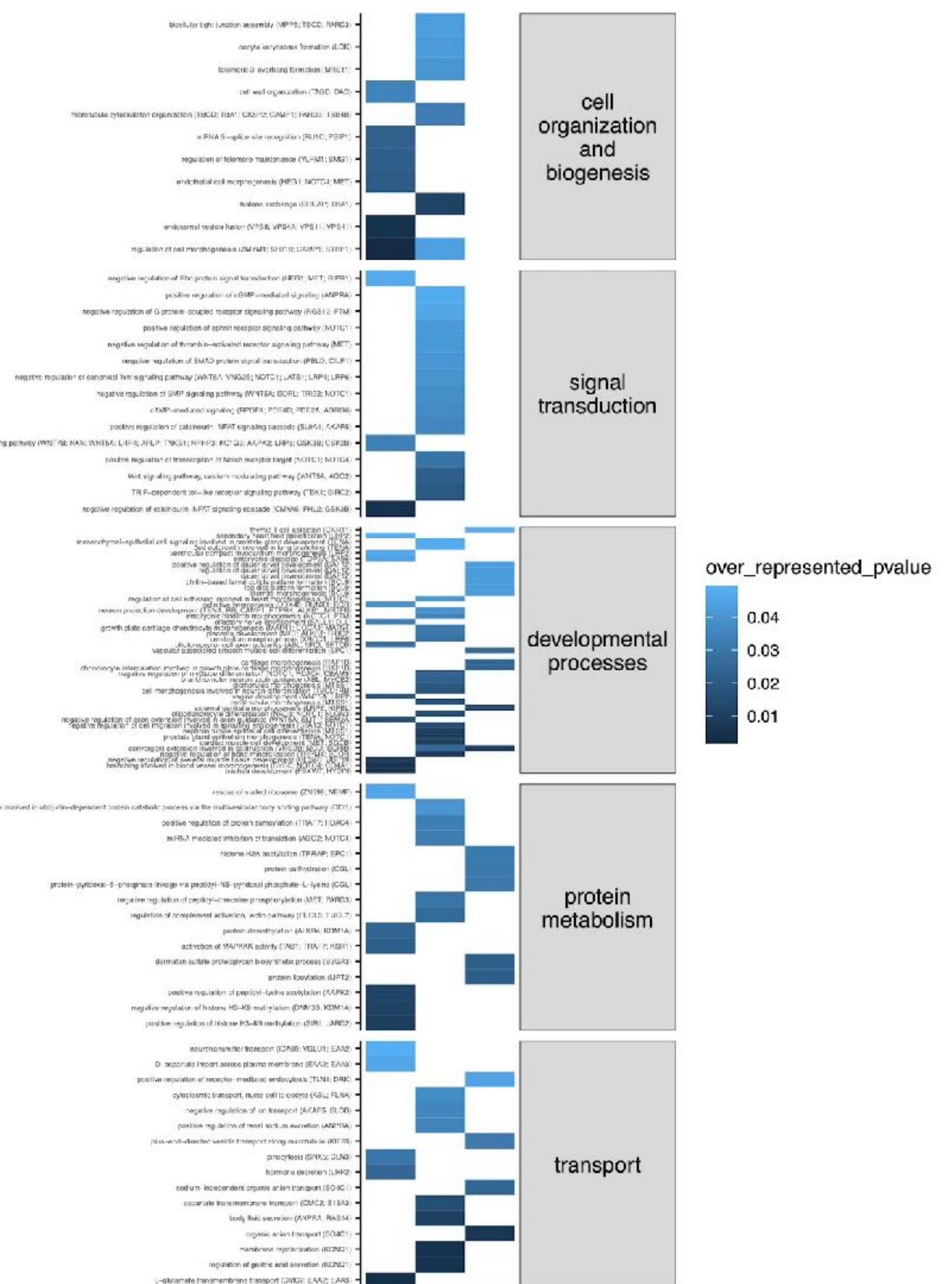
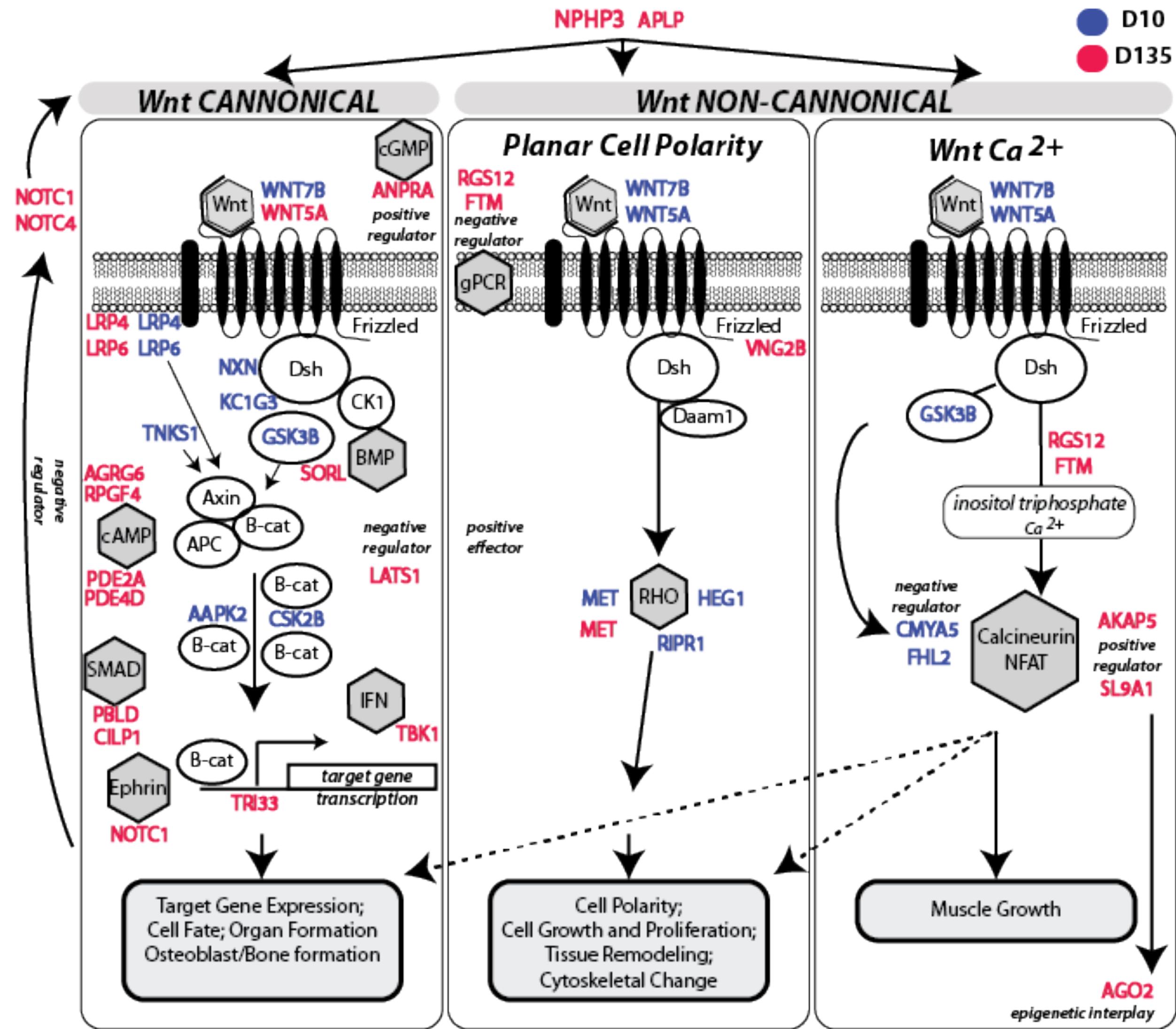
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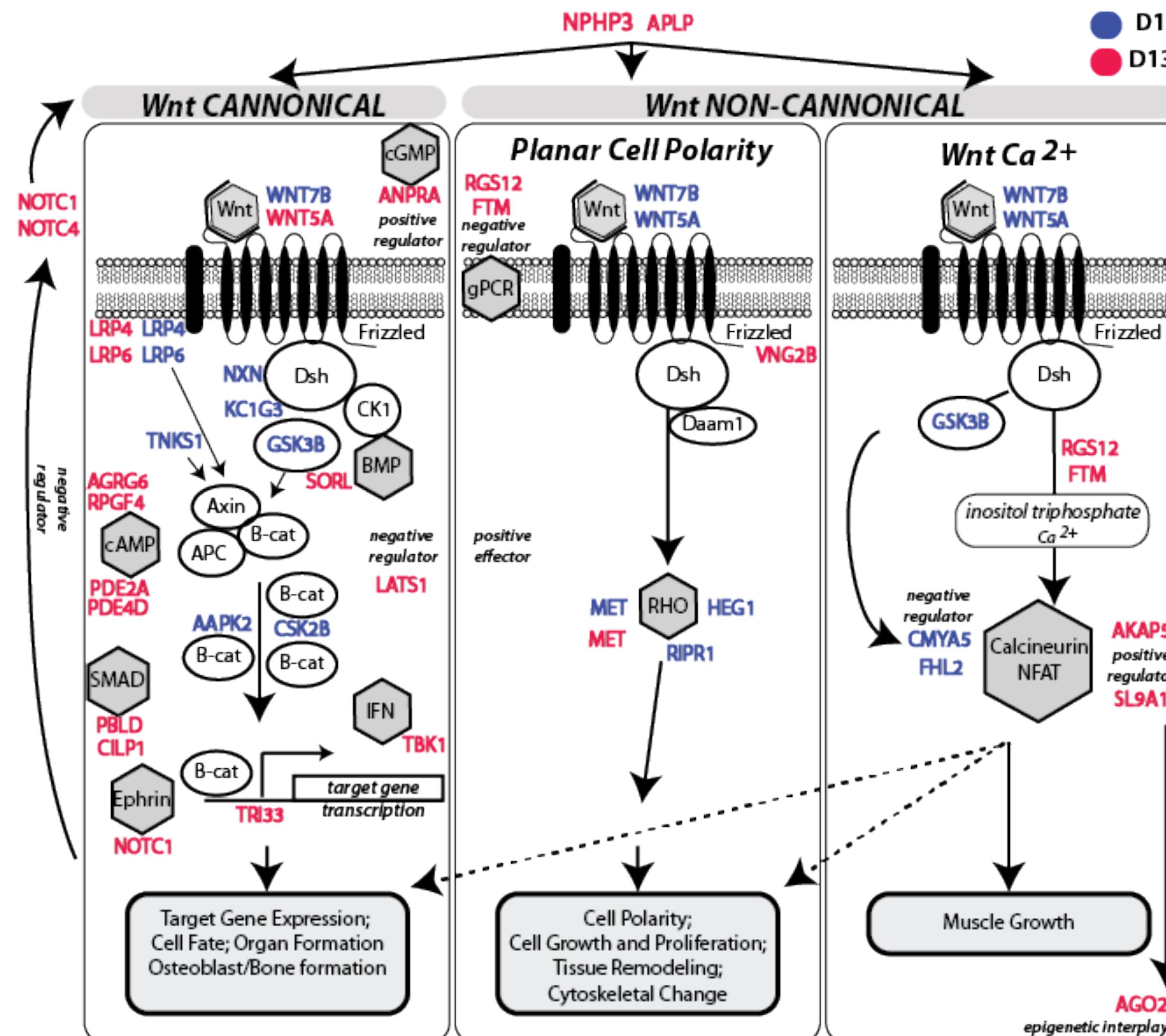
# GEODUCKS AND OA



# GEODUCKS AND OA

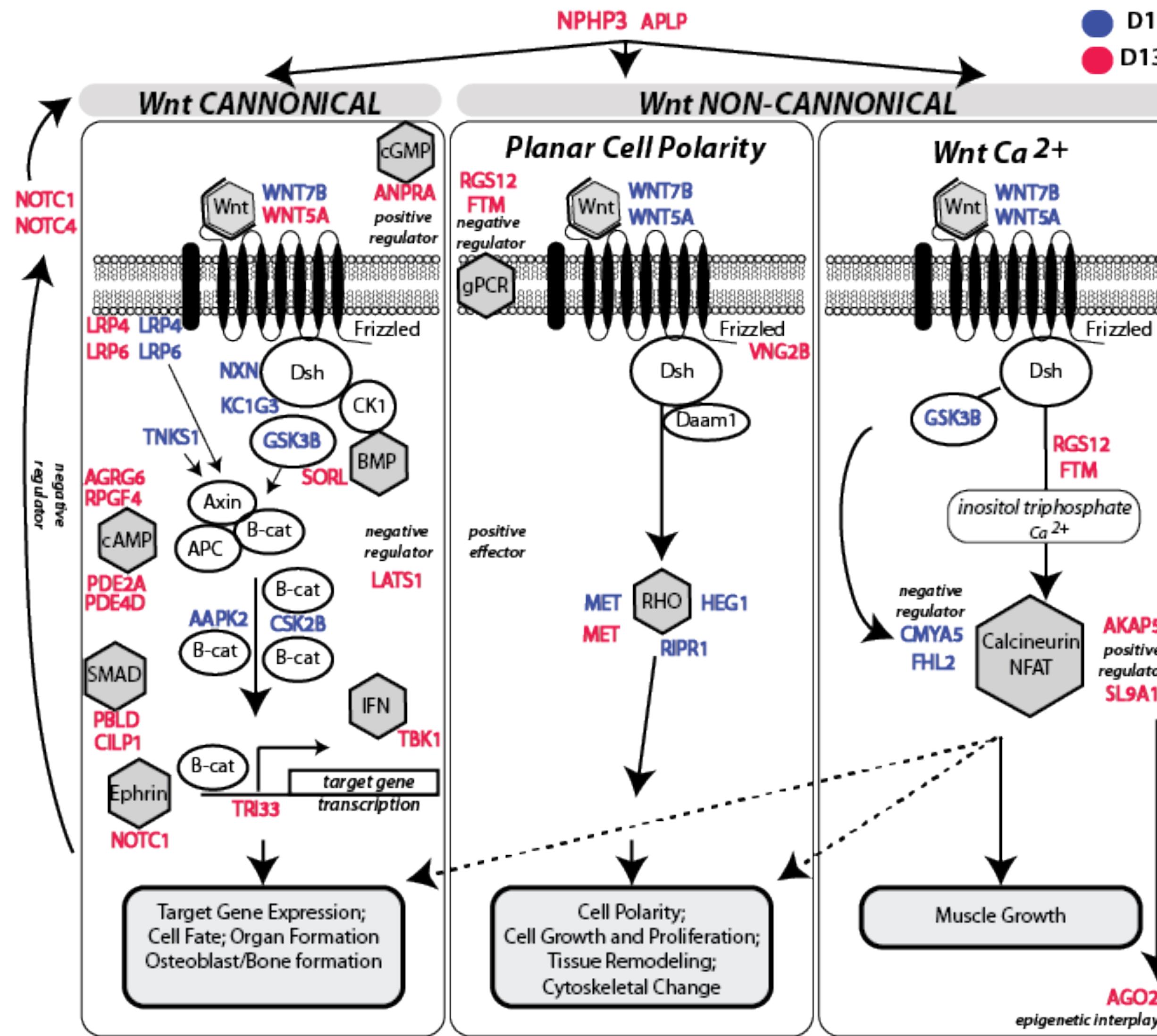


# GEODUCKS AND OA



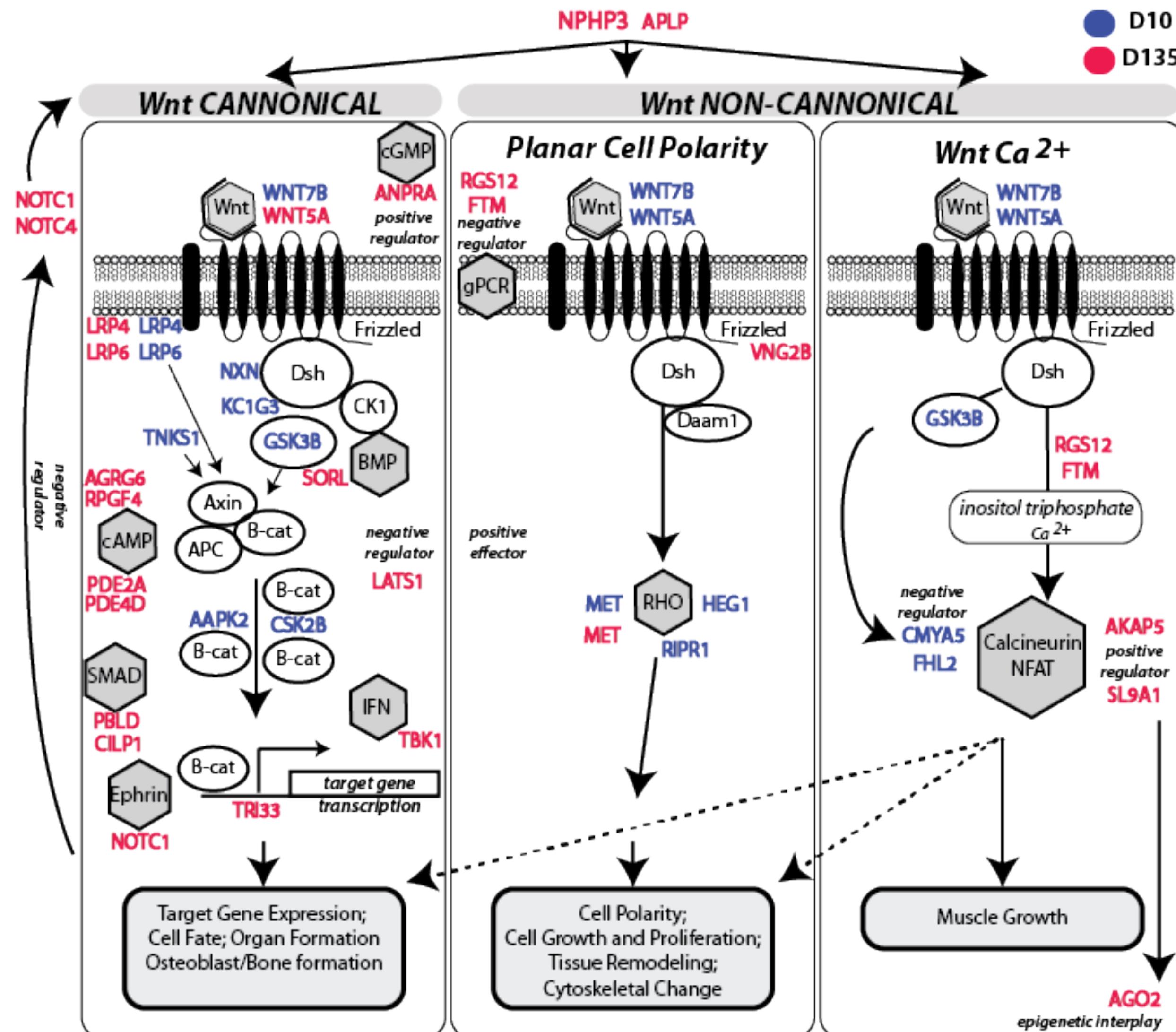
Hollie M. Putnam, Shelly A. Trigg, Samuel J. White, Laura H. Spencer, Brent Vadopalas, Aparna Natarajan, Jonathan Hetzel, Erich Jaeger, Jonathan Soohoo, Cristian Gallardo-Escárate, Frederick W. Goetz, Steven B. Roberts  
doi: <https://doi.org/10.1101/2022.06.24.497506>

# 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*

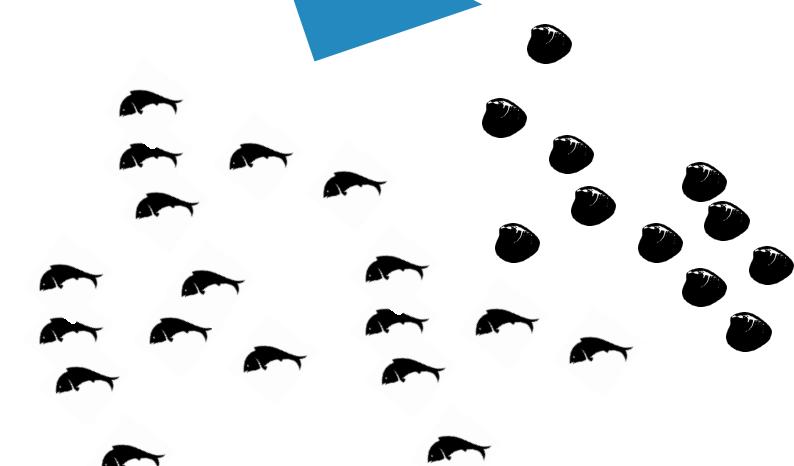


Adults

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*

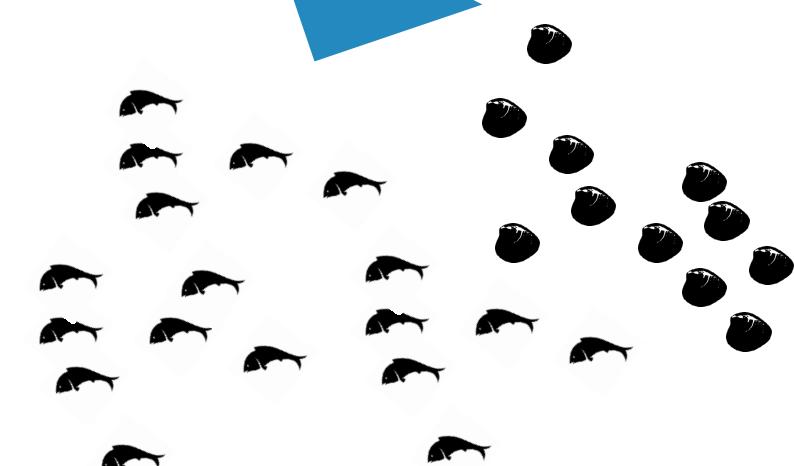


Adults

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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

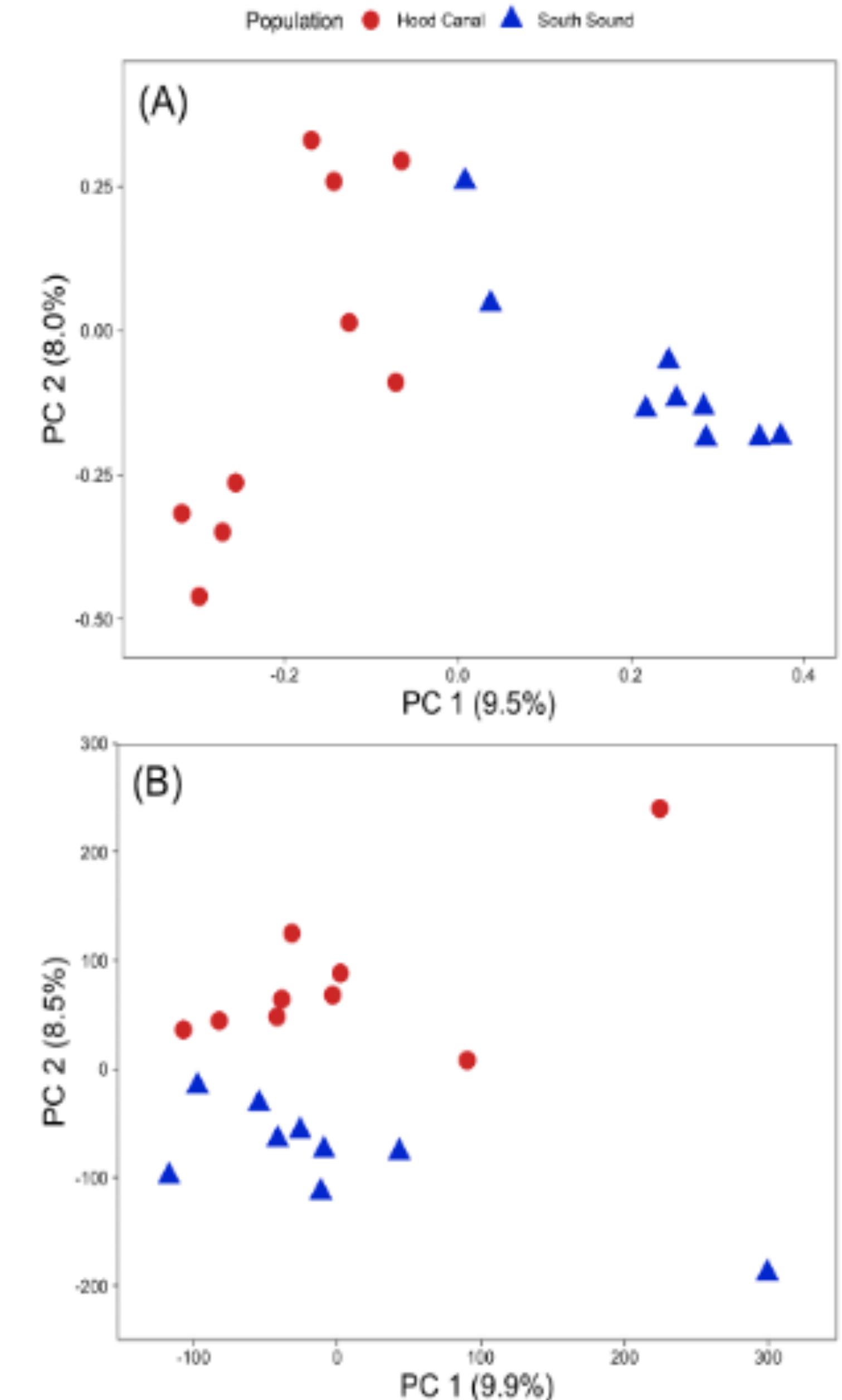
# Epigenetic and Genetic Population Structure is Coupled in a Marine Invertebrate

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.**

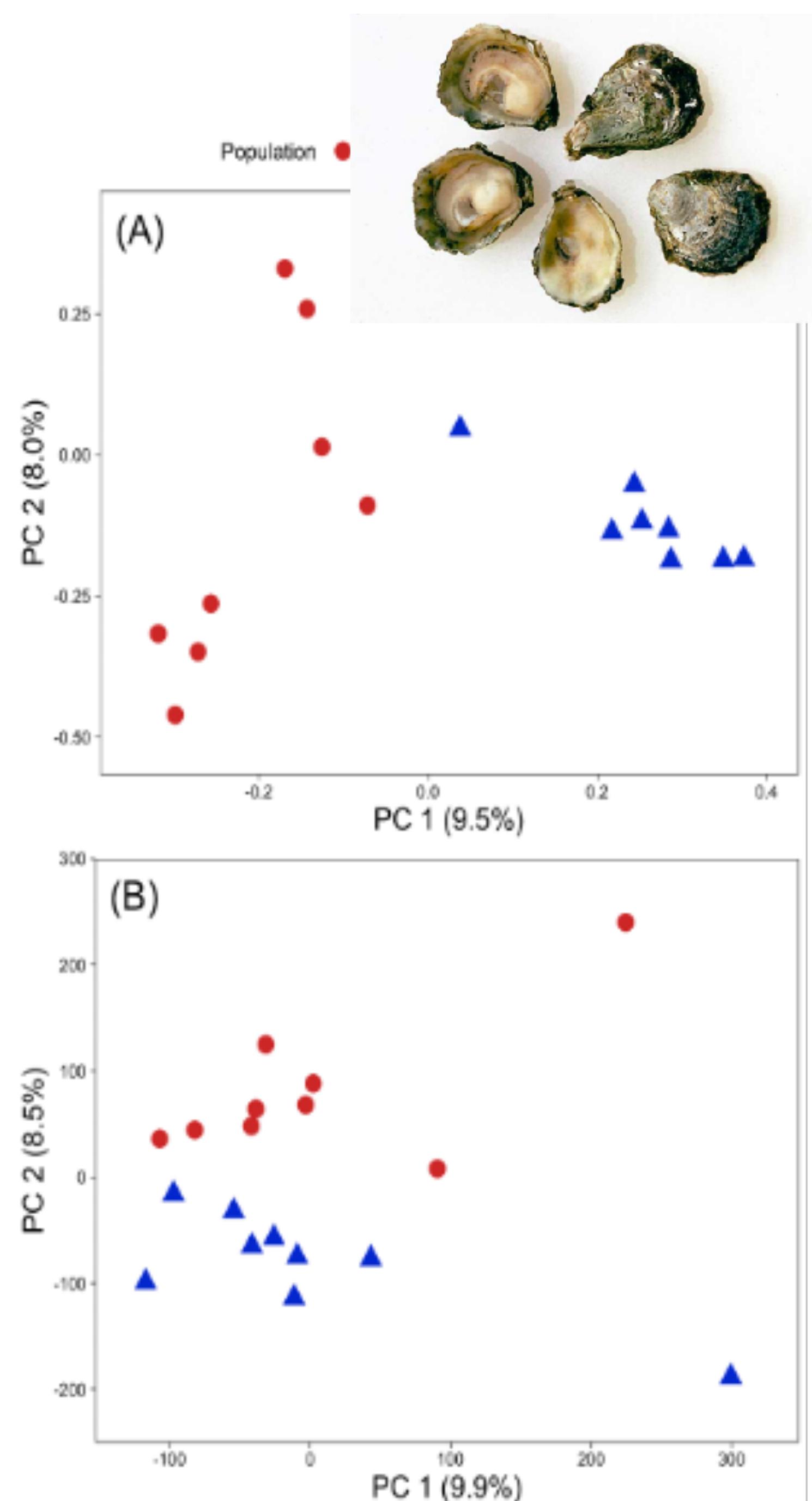
Underlying this association are both direct genetic changes in CpGs (CpG-SNPs) and genetic variation with indirect influence on methylation (mQTLs).



# Phenotype associated loci after taking genetics into consideration

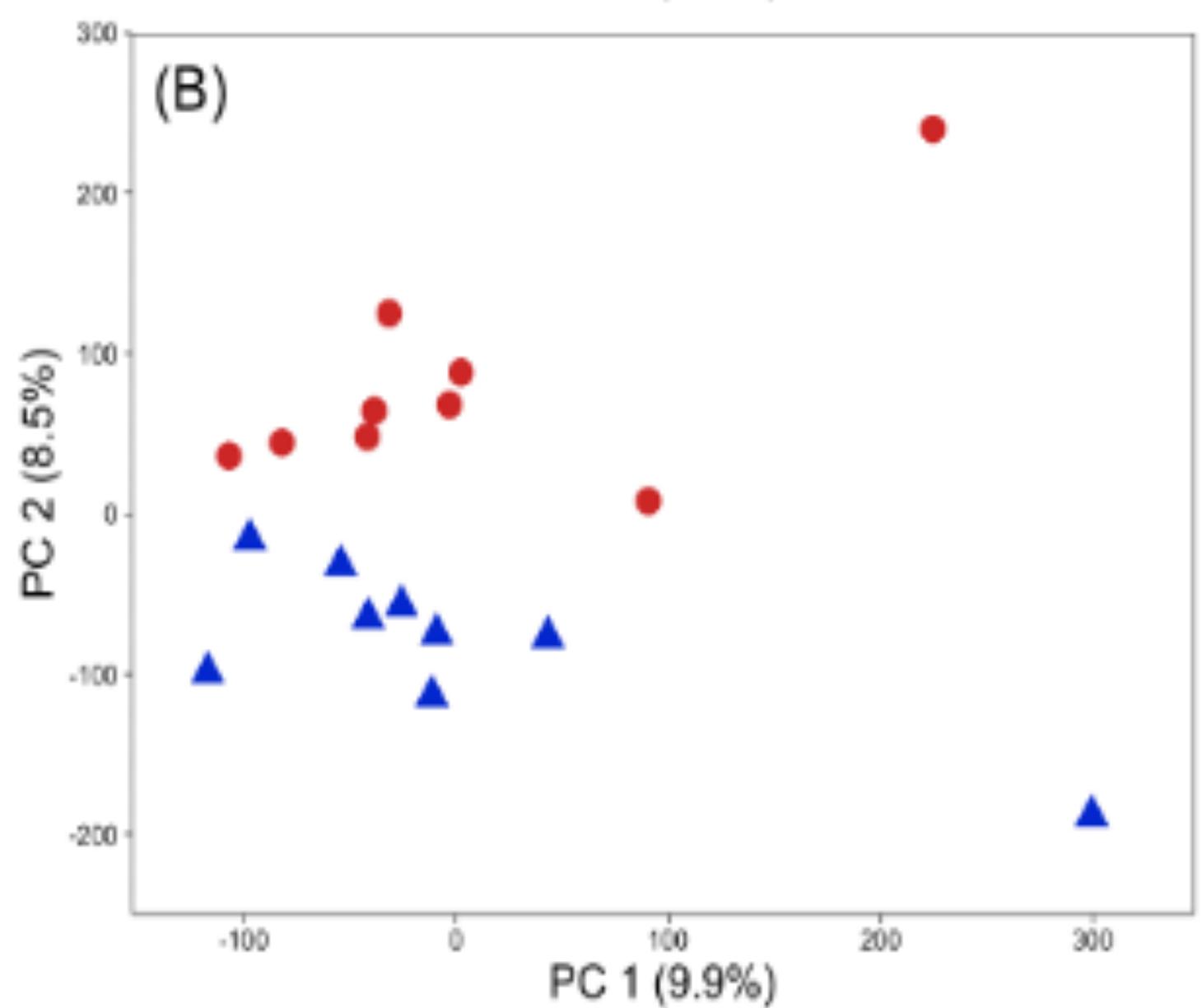
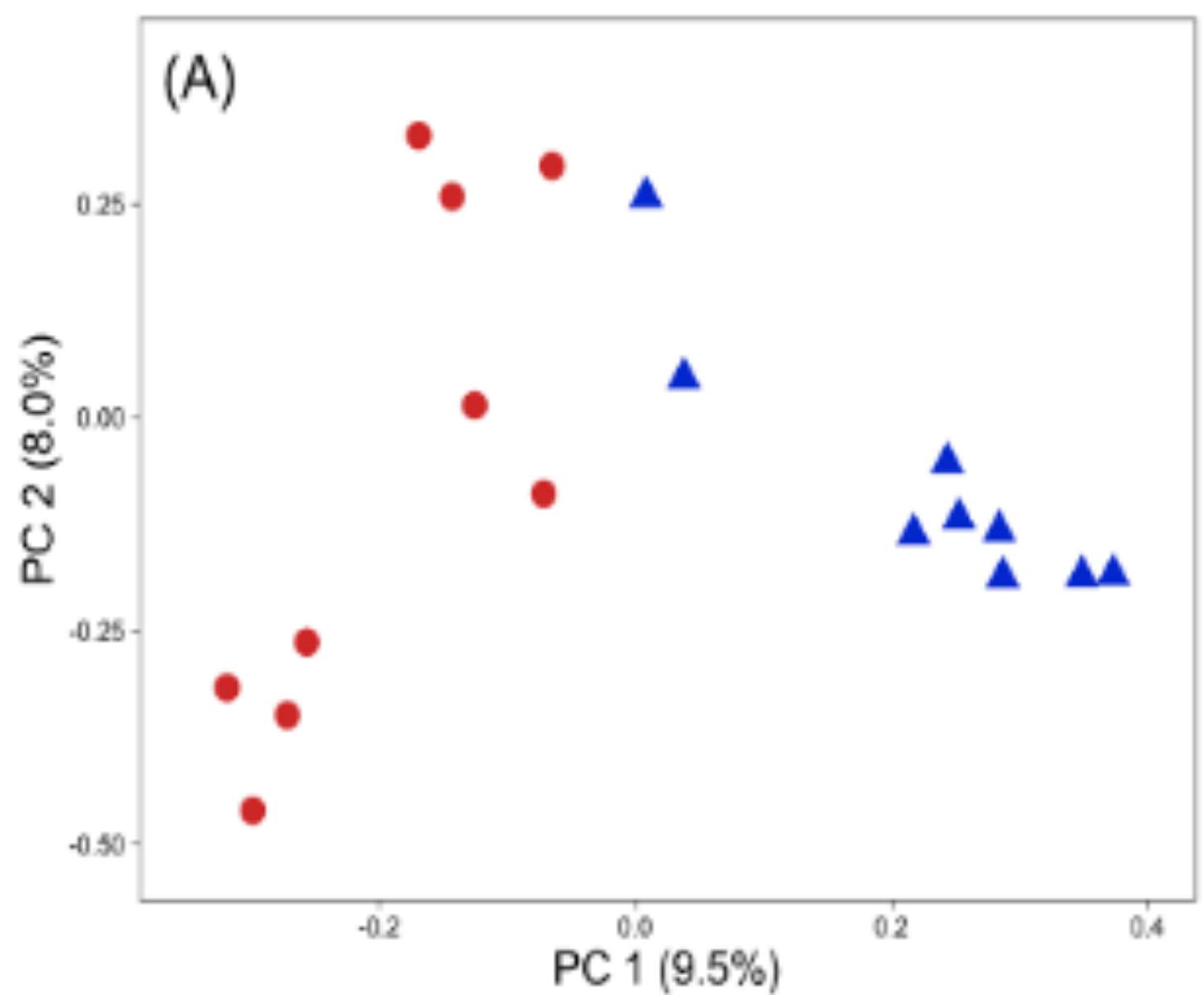
Table 2: Genes that contain loci in which methylation status is associated with oyster size (SALs)

Uniprot Accession	Gene Name	Protein Name
Q5W0Q7	USPL1	SUMO-specific isopeptidase
Q15937	ZNF79	zinc finger protein 79
Q9QXV3	Ing1	inhibitor of growth family, member 1
Q23551	unc-22	Twitchin
A4II09	eif3a	eukaryotic translation initiation factor 3 subunit A
Q3UCV8	Otulin	Ubiquitin thioesterase otulin
Q28I85	poc1a	POC1 centriolar protein A
Q8BGS3	Zkscan1	zinc finger with KRAB and SCAN domains 1
Q8BFY9	Tnpo1	transportin 1
H2QII6	RANBP2	E3 SUMO-protein ligase
Q14315	FLNC	filamin C



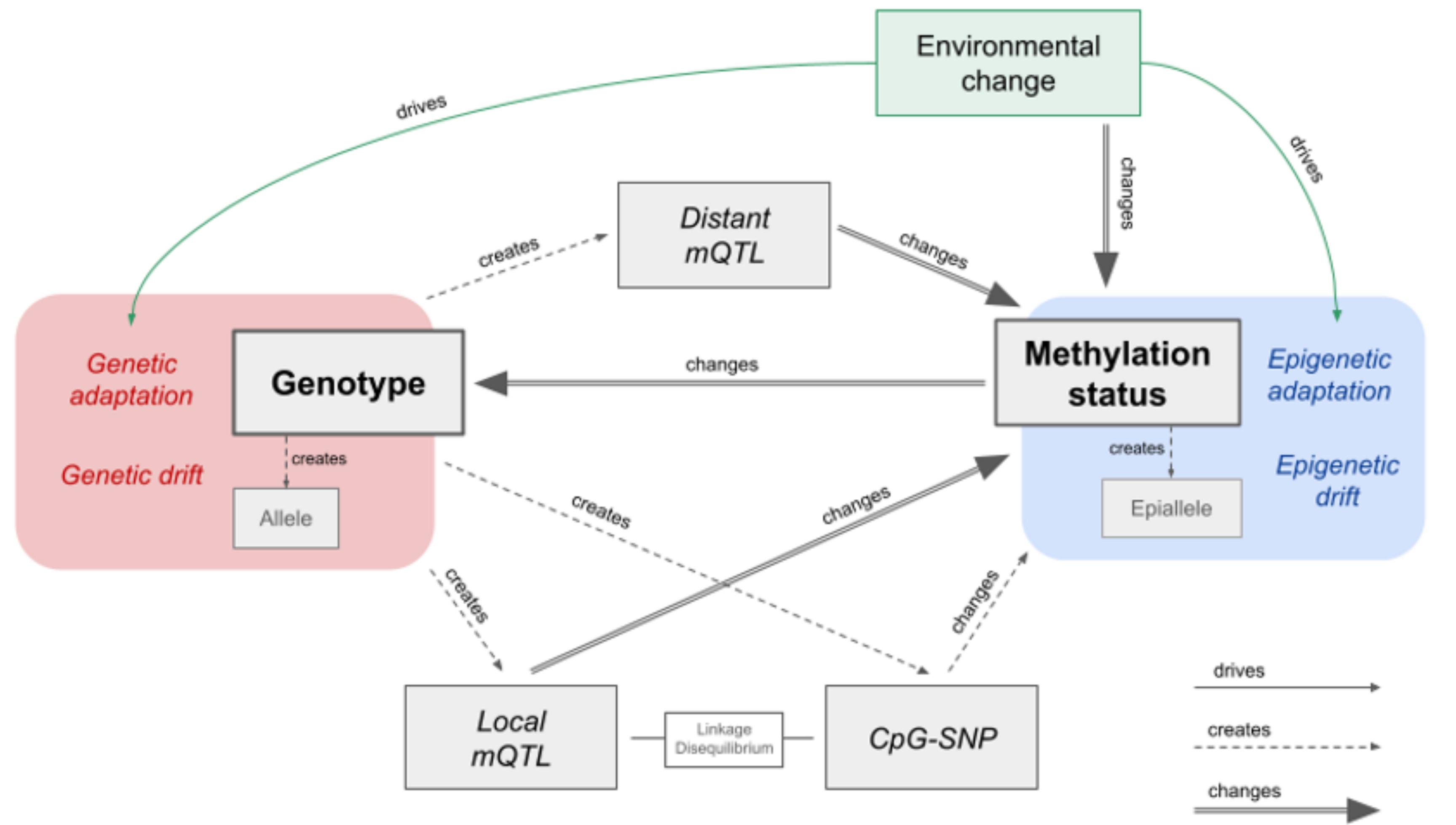
# EPIGENETIC AND GENETIC POPULATION STRUCTURE

Population ● Hood Canal ▲ South Sound



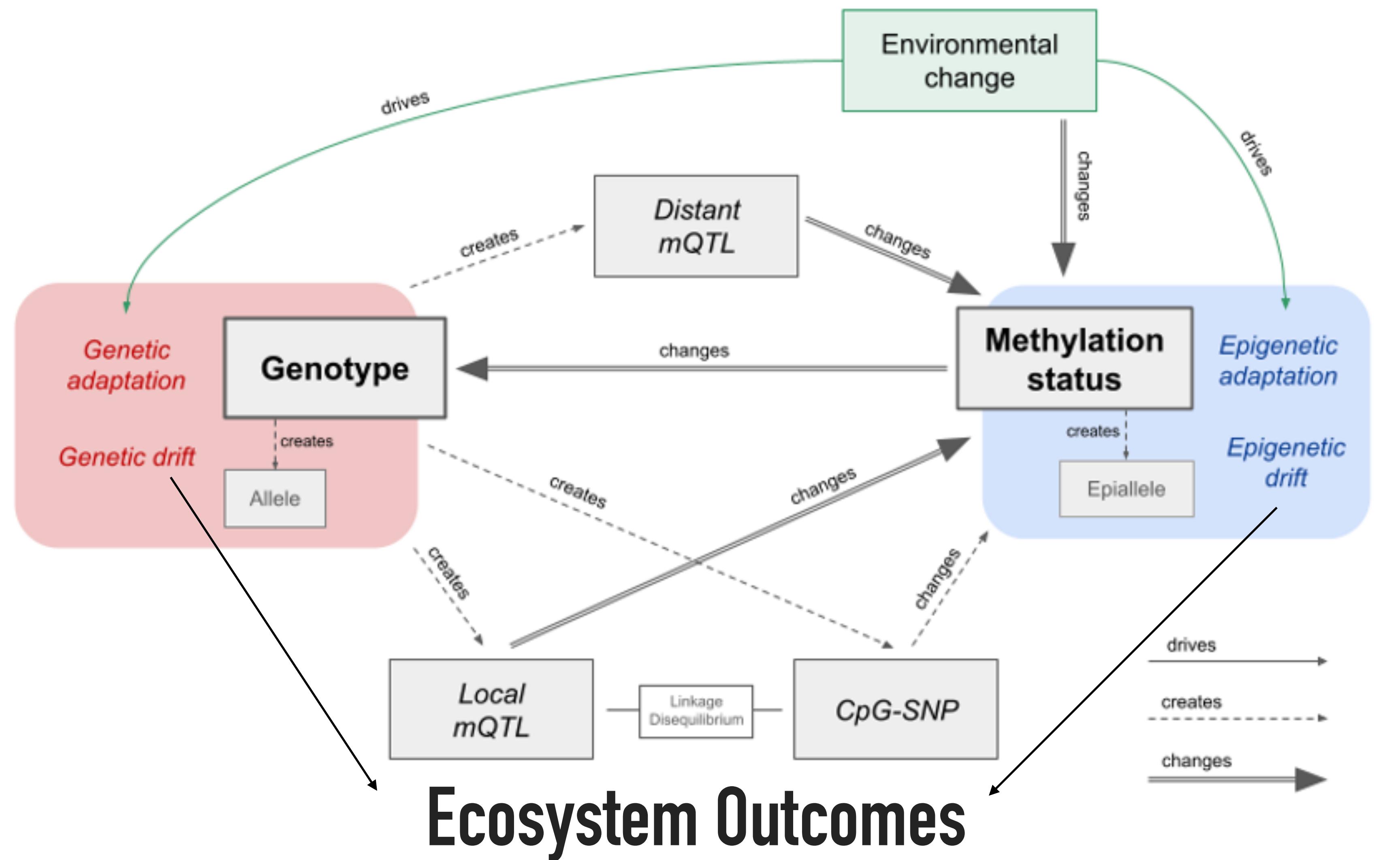
## IMPLICATIONS

# EPIGENETIC AND GENETIC POPULATION STRUCTURE



## IMPLICATIONS

# 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

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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> |  
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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
```

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