

# Stress conditioning to hypercapnic seawater in the Pacific geoduck *Panopea generosa*

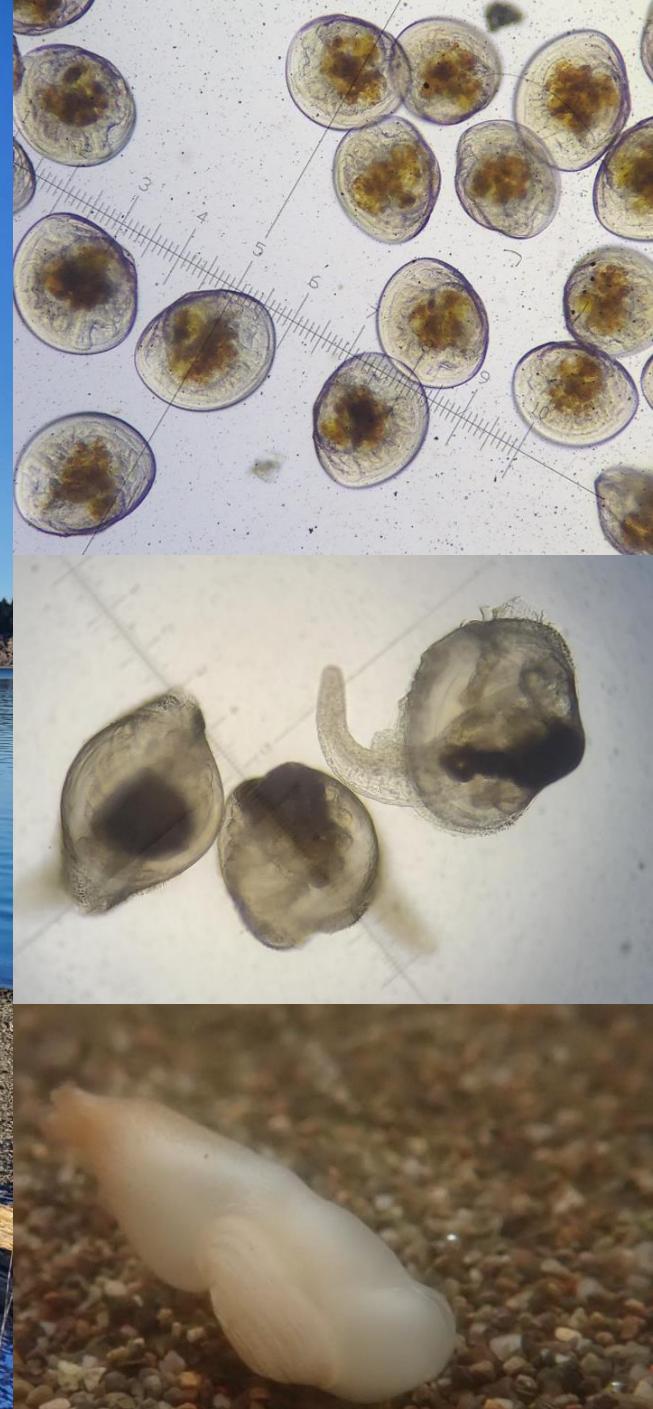


Samuel J. Gurr | University of Rhode Island

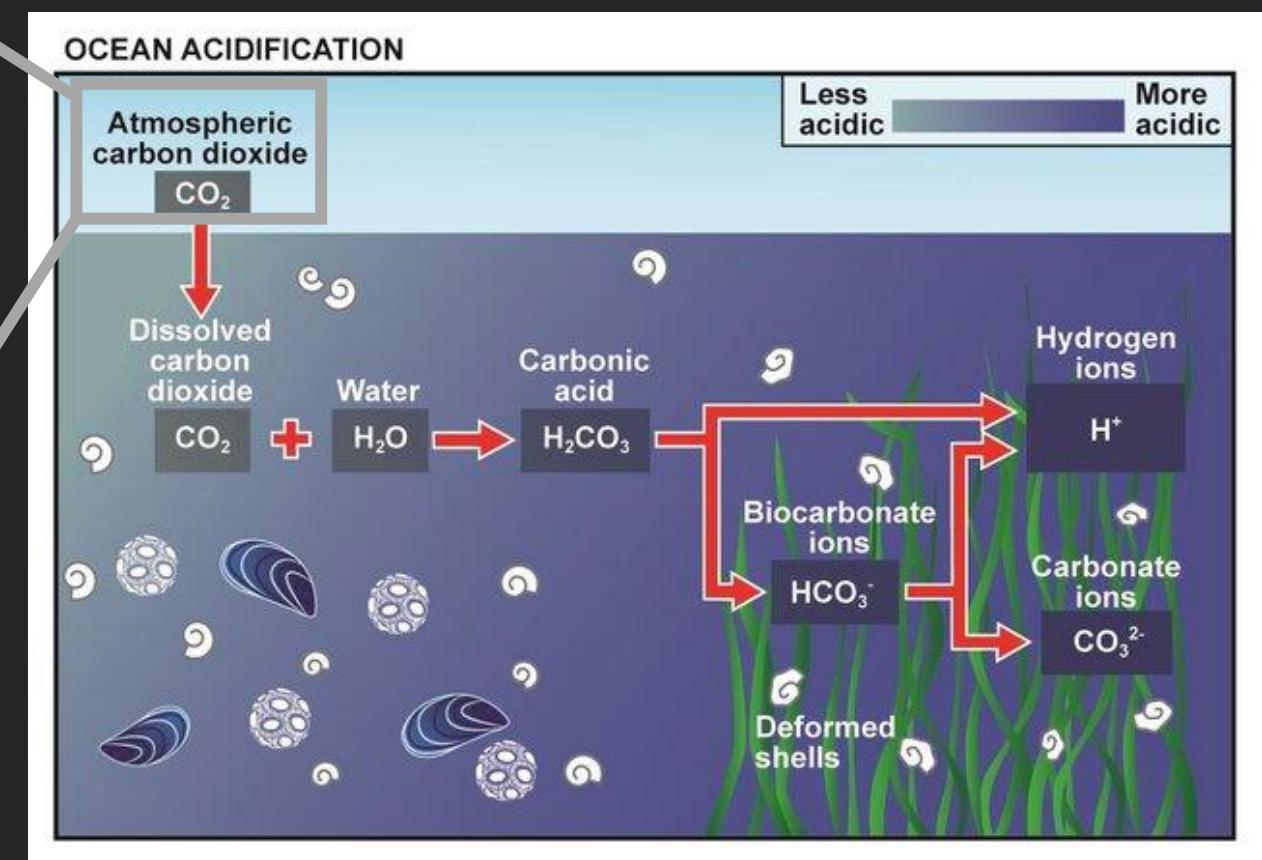
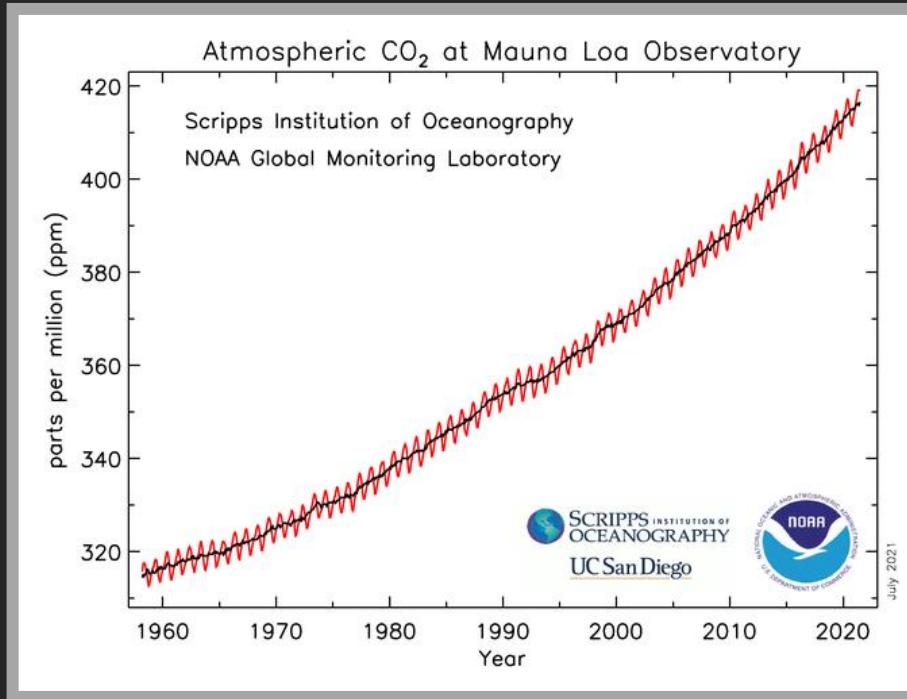
Major professor: Hollie Putnam

Committee members: Jon Puritz and Coleen Suckling

Chair: Marta Gomez-Chiarri



# Ocean acidification

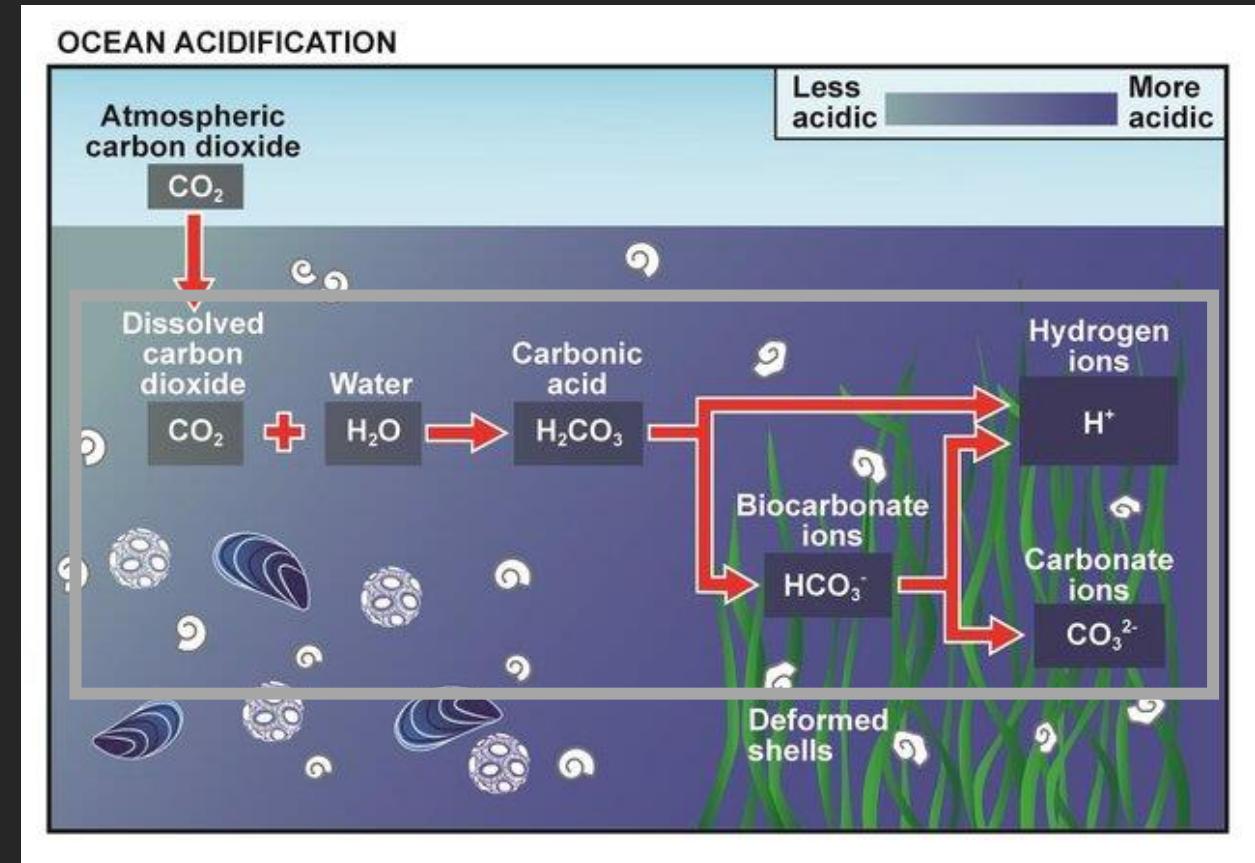


# Ocean acidification

**Elevated** partial pressure



**Hypercapnic** seawater



# Ocean acidification

Elevated partial pressure

↑CO<sub>2</sub> ( $p\text{CO}_2$ )



Hypercapnic seawater

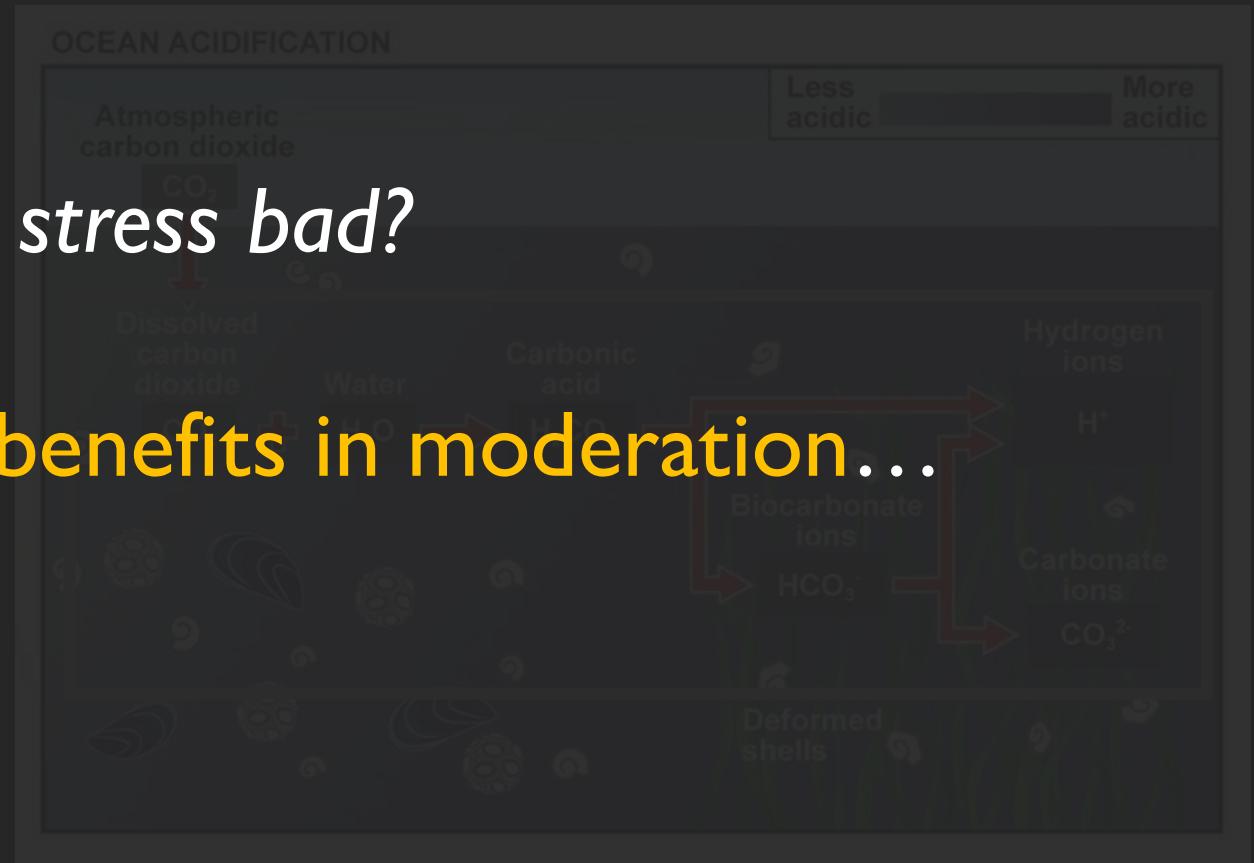
↓ pH

↓ CO<sub>3</sub><sup>2-</sup>

↓ CaCO<sub>3</sub> ( $\Omega$ )

*Is all stress bad?*

Perhaps there are **benefits in moderation...**



# What is conditioning...

**stress  
conditioning**

**Priming with sub-lethal exposure(s)**  
to increase stress resilience and performance  
under a **subsequent encounter**

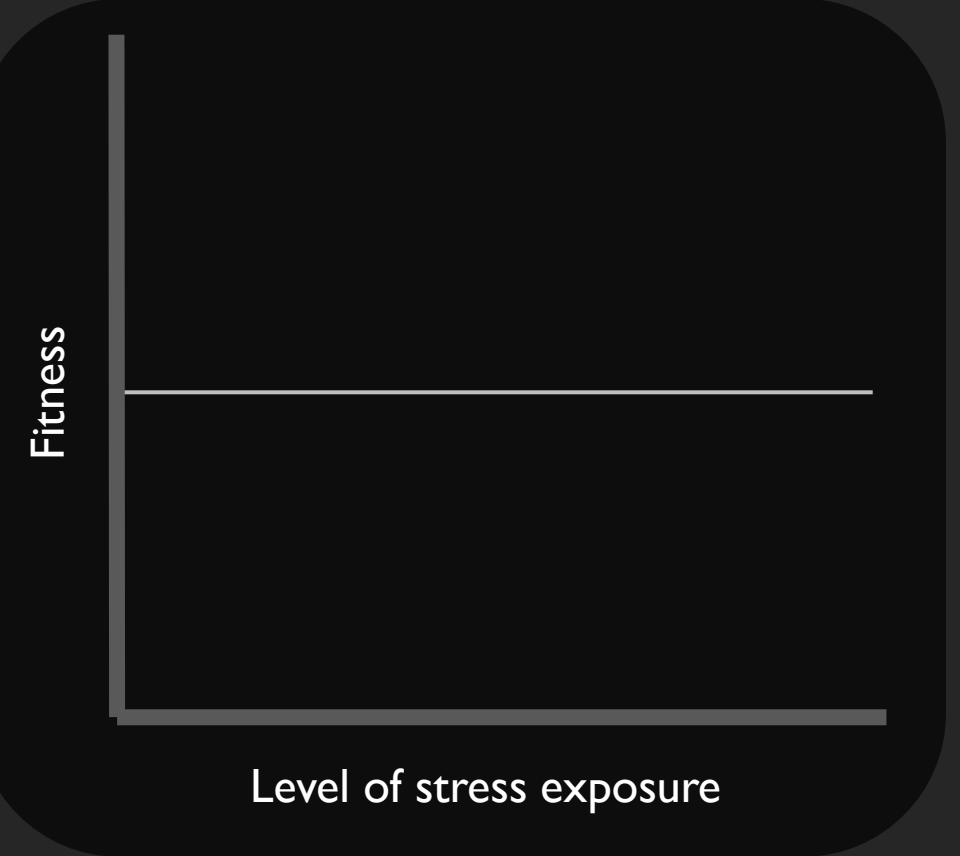
# What is conditioning...

**stress  
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**Priming with sub-lethal exposure(s)**  
to increase stress resilience and performance  
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*'rapid stress hardening'*  
*'hormetic conditioning'*

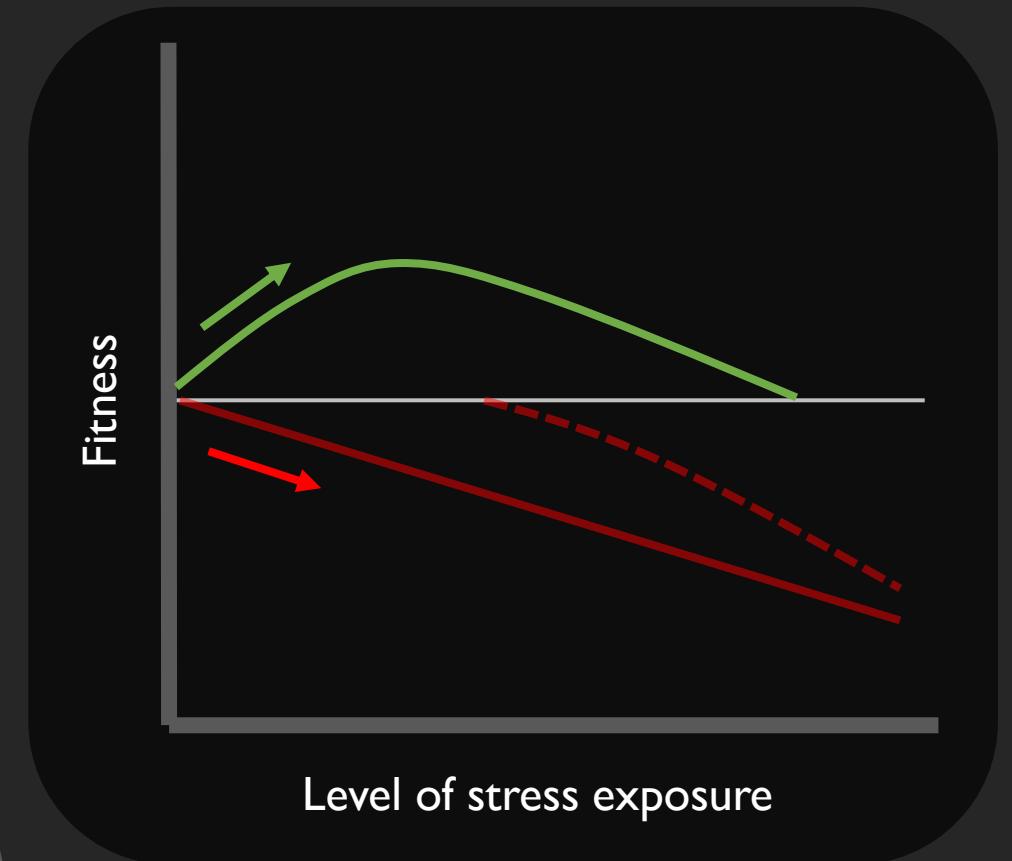
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**stress  
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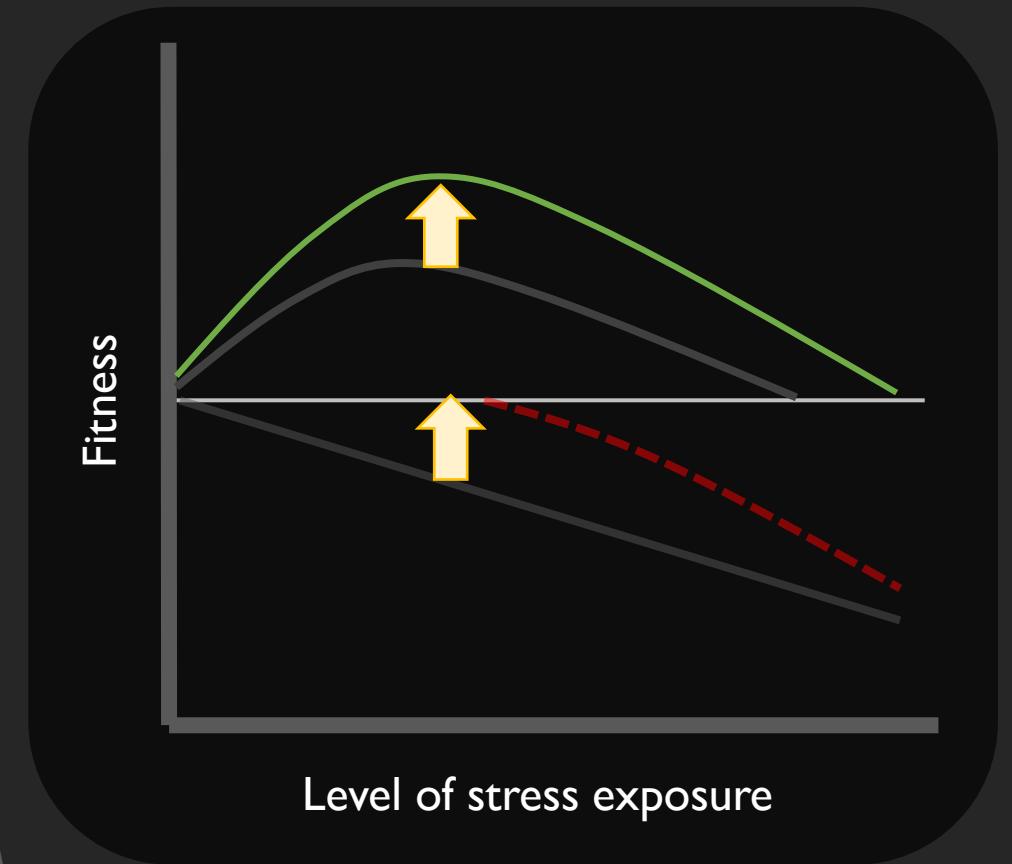
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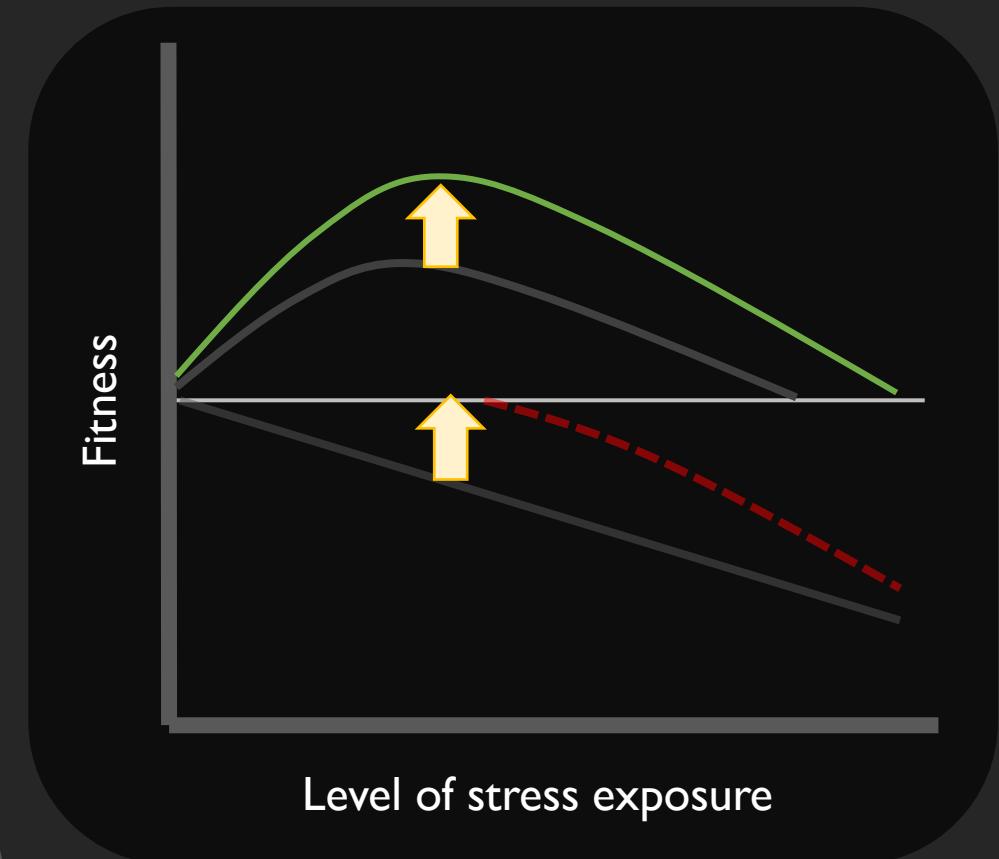
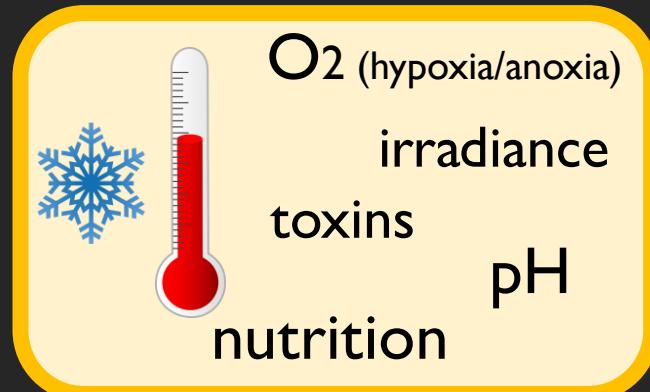


# What is conditioning...

**stress  
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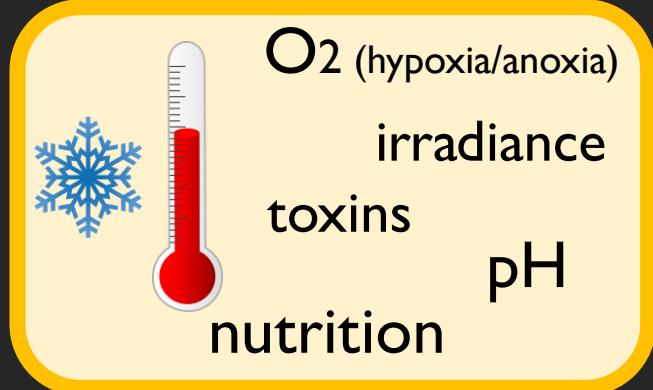


# A conserved phenomenon...



Visser et al. 2018; López-martínez et al. 2012; Costantini et al. 2012; Gecse et al. 2019; Bible et al. 2019; Zhang et all 2017

# A conserved phenomenon...



## Physiology

- Storage retention (lipids)
- Thermotolerance & survival
- Lifespan extension

## Cellular response

- Cryoprotection/cellular defenses
- Reduced cellular damage

O<sub>2</sub> (hypoxia/anoxia)

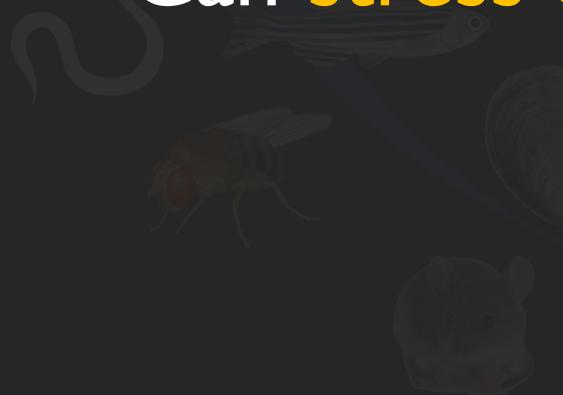


irradiance

toxins

pH

nutrition



*In light of environmental challenges today...*

Physiology

- greater storage retention (lipids)

induced thermotolerance (survival)

Cellular response

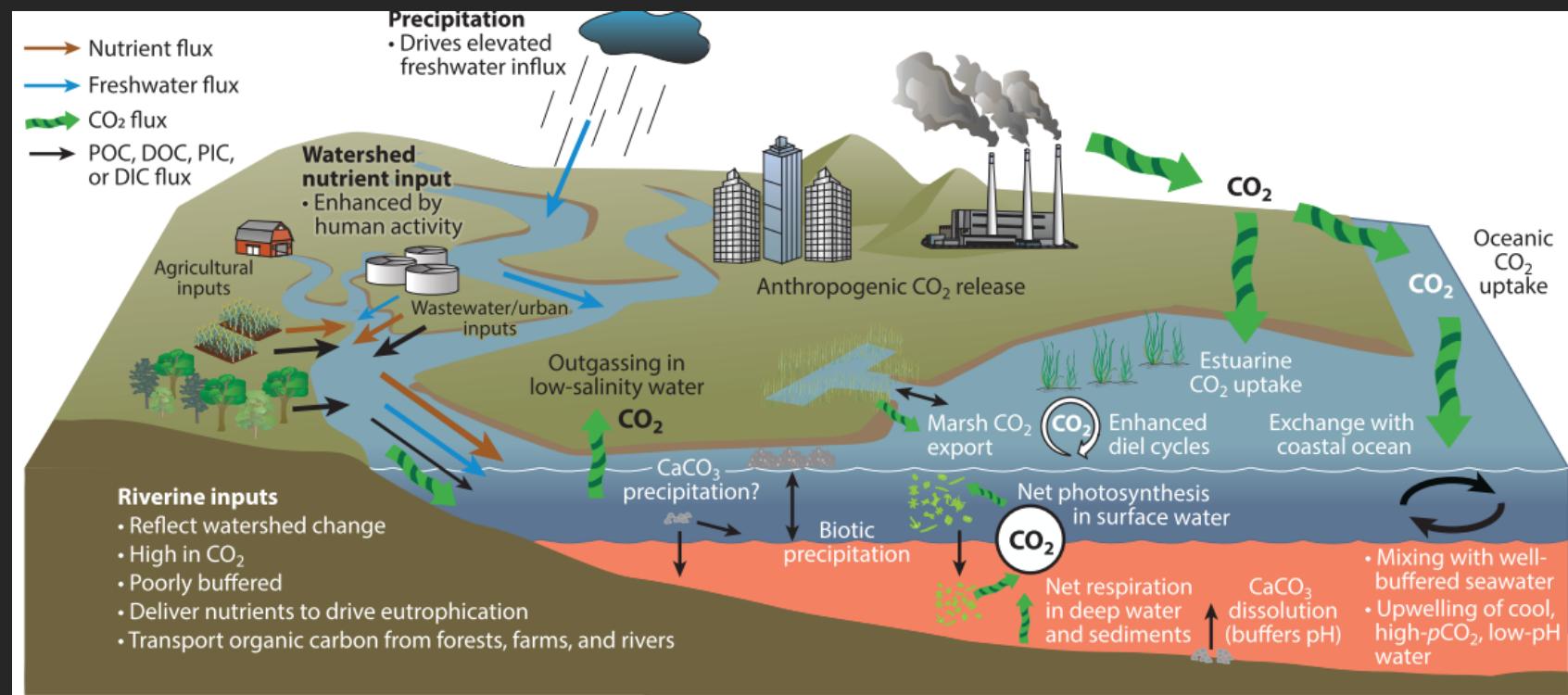
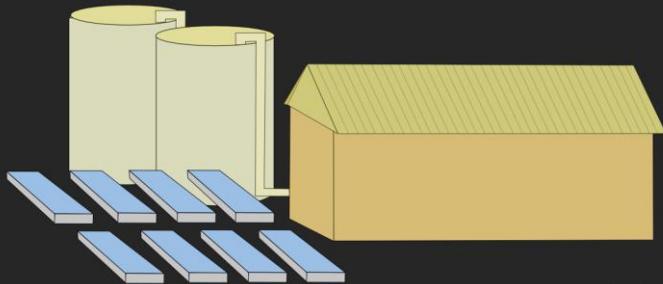
- cryoprotection/cellular defenses
- reduced cellular damage

**Can stress conditioning improve resilience?**

# Ocean acidification



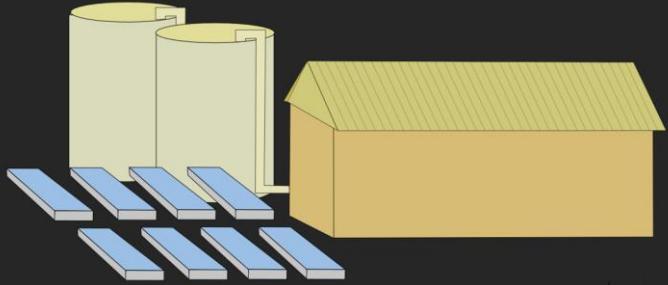
AQUACULTURE



# Ocean acidification



AQUACULTURE

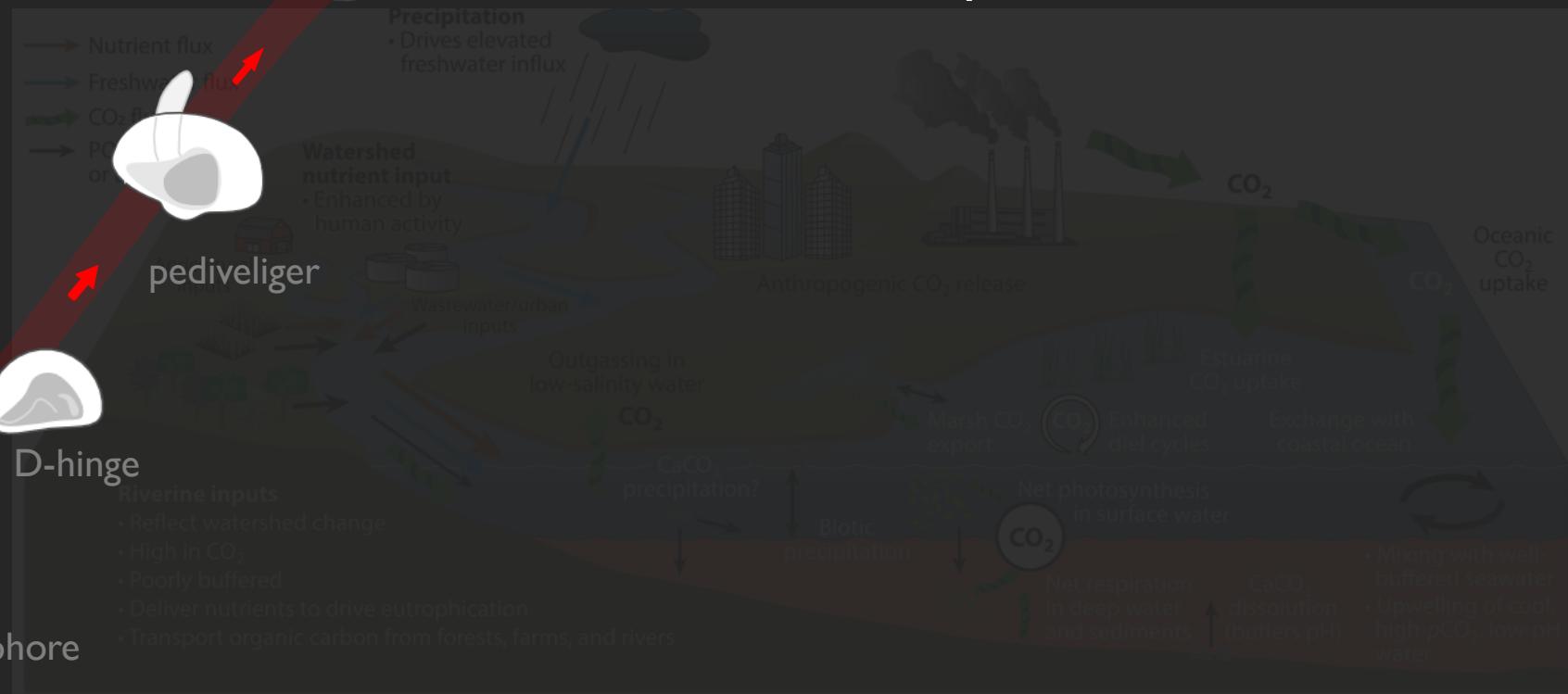


embryo

trochophore  
D-hinge

juvenile

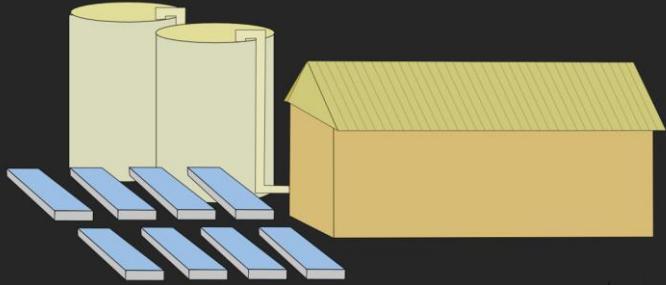
**Bottleneck for production!**



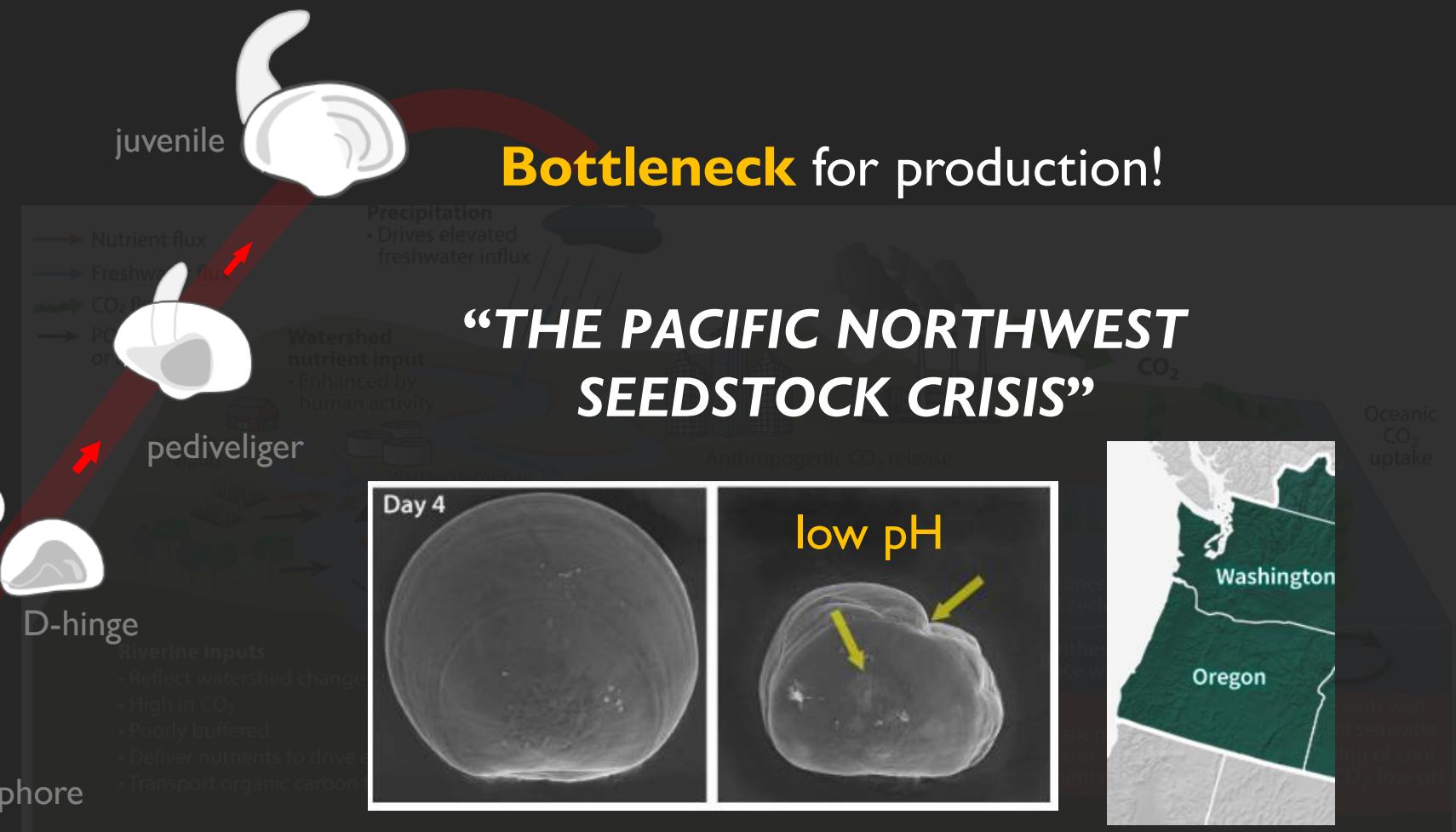
# Ocean acidification



AQUACULTURE

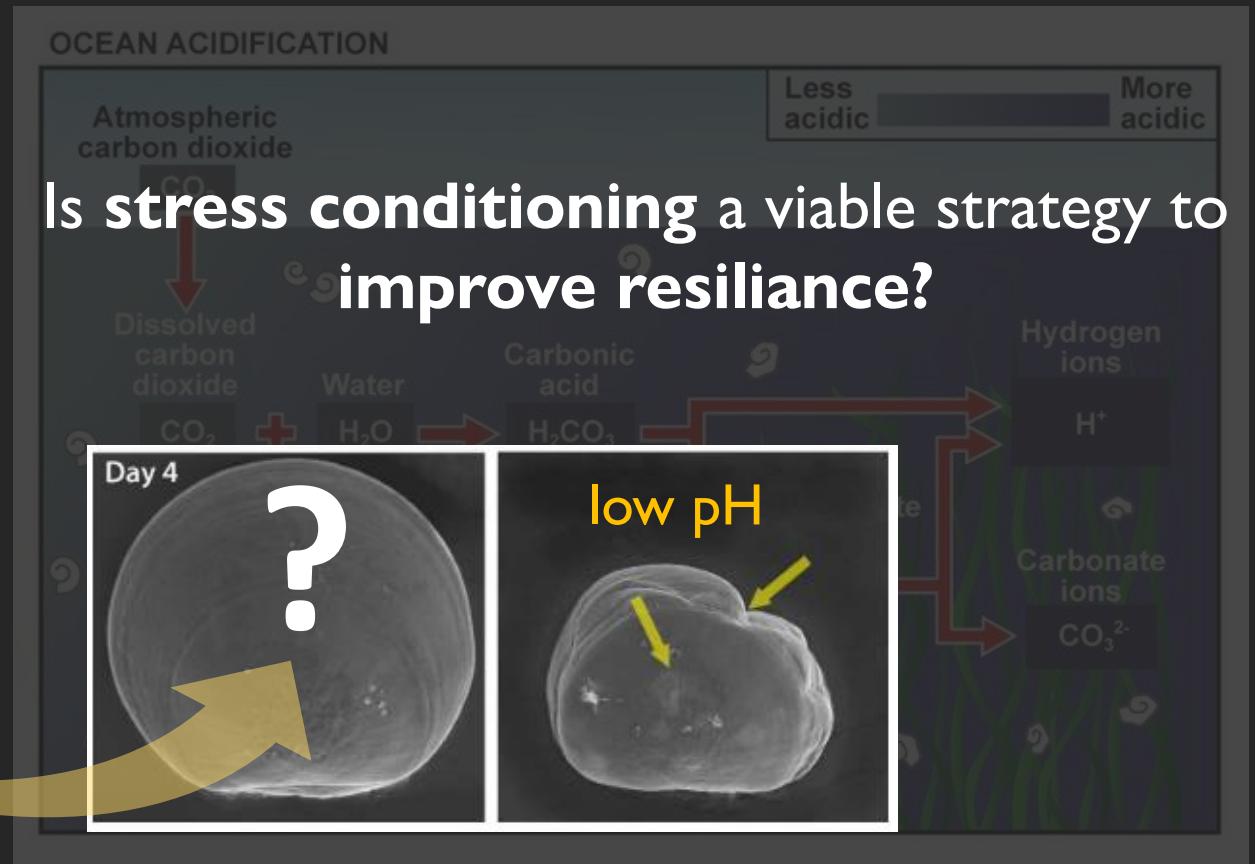


embryo



# Ocean acidification and stress conditioning

**stress  
conditioning**



# Ocean acidification and stress conditioning

**stress  
conditioning**



adult

An array of **benefits** following  
**exposure to low pH**



larval stages

# Ocean acidification and stress conditioning



# Ocean acidification and stress conditioning

**stress  
conditioning**

Is this **transferable** to other bivalve species?

What is the **timing (life stage)** and **intensity** to test stress conditioning?

What are the **mechanisms underlying** a **conditioned response**



# Ocean acidification and stress conditioning

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# Pacific geoduck *Panopea generosa*

**What are ‘gooey-duck’?**  
*meaning “dig deep”*

- Cultural and economic importance for tribal communities of PNW
- Largest known infaunal clam
  - *oldest recorded, 168 years of age!*



Photo credit: Mat Henderson

# Pacific geoduck *Panopea generosa*

What are ‘gooey-duck’?  
meaning “dig deep”

- Cultural and economic importance for tribal communities of PNW
- Largest known infaunal clam
  - *oldest recorded, 168 years of age!*

## Geoduck aquaculture

- ~90% global geoduck produced from Washington state **alone**
- \$24 mil annually; \$14 per pound



Photo credit: Mat Henderson

# Ocean acidification and stress conditioning

**stress  
conditioning**

Is this **transferable** to other bivalve species?

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# Relevance to life history and habitat..

Life stage?

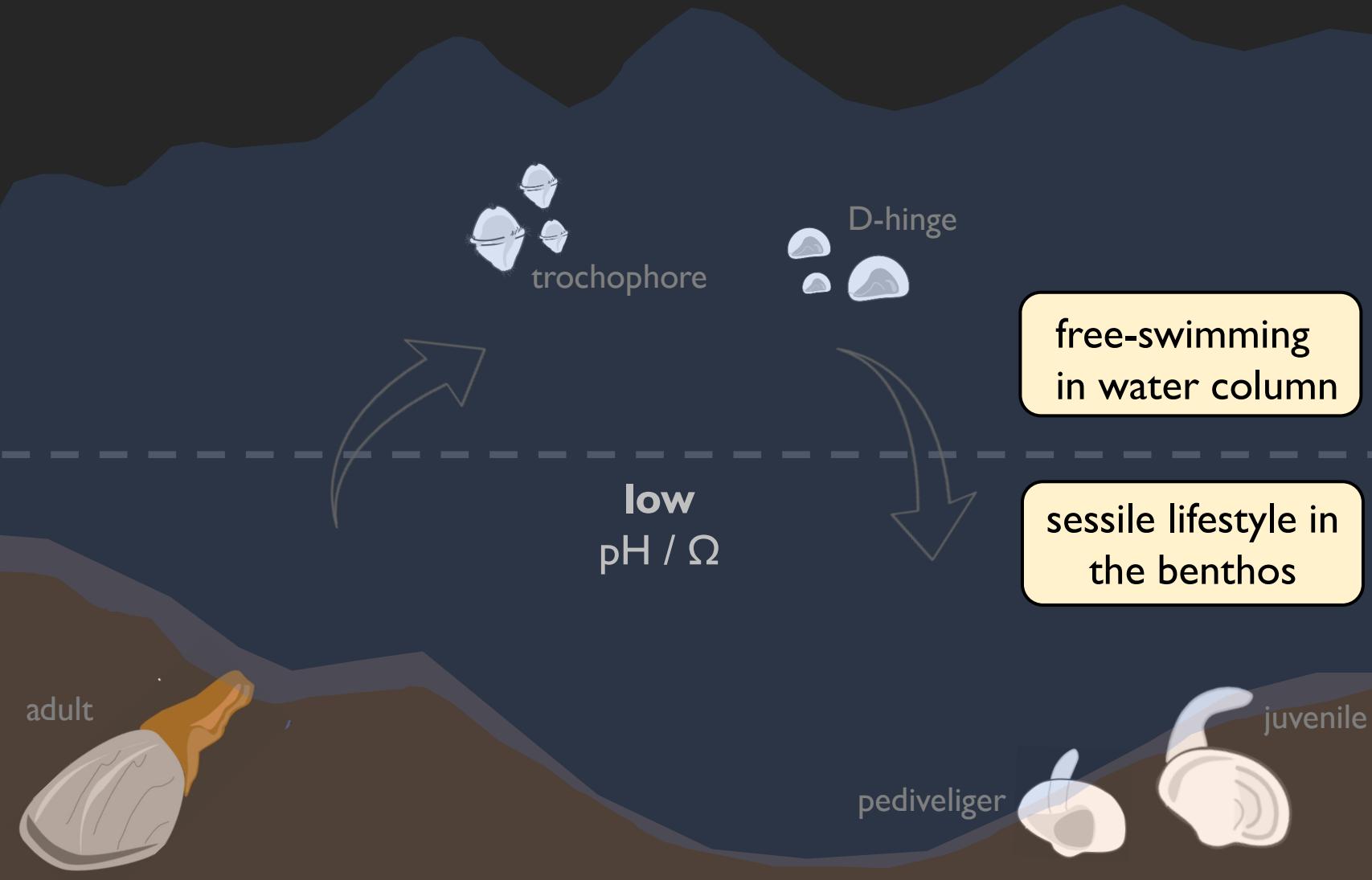
Intensity?



# Relevance to life history and habitat..

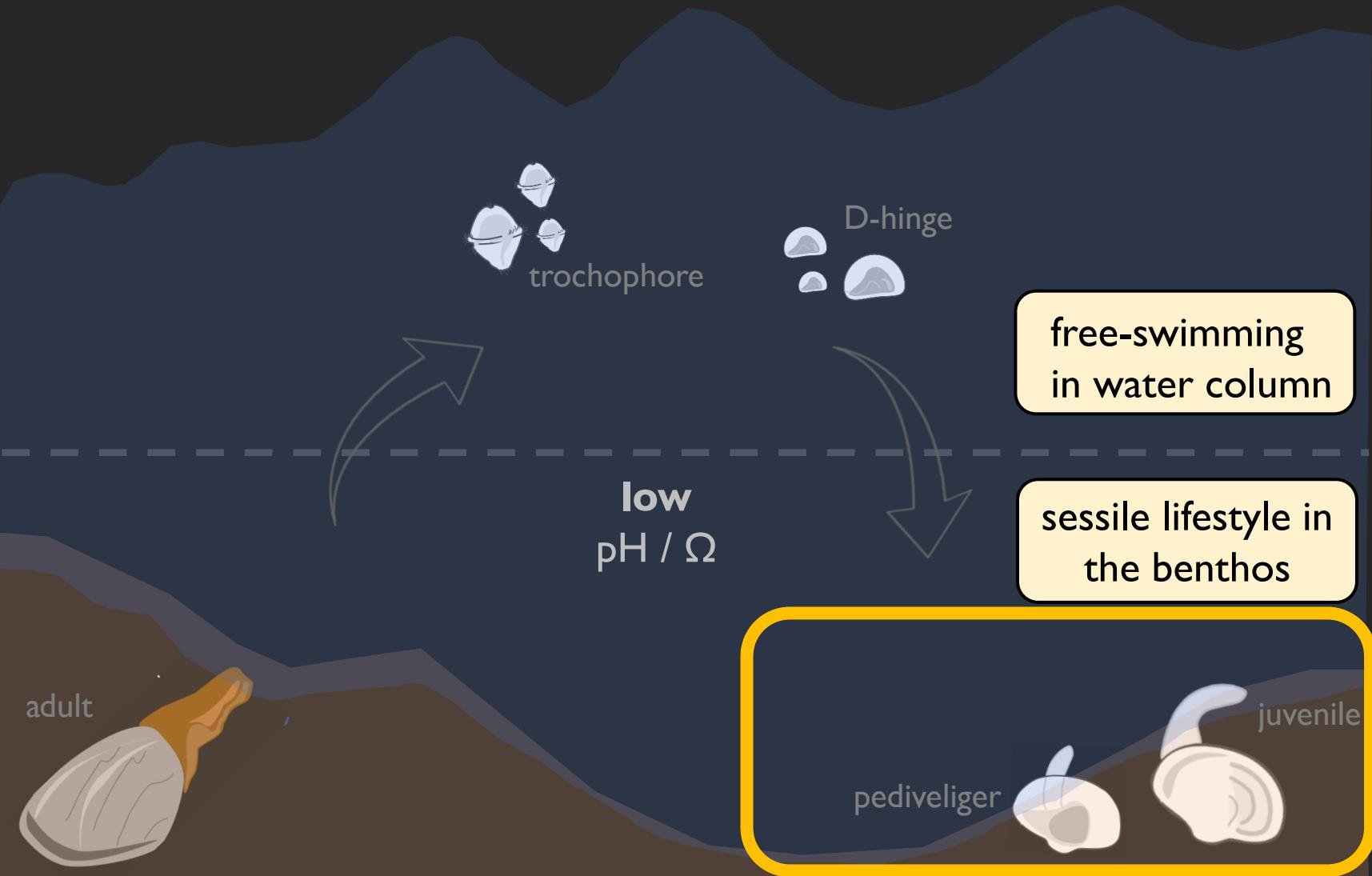
Life stage?

Intensity?



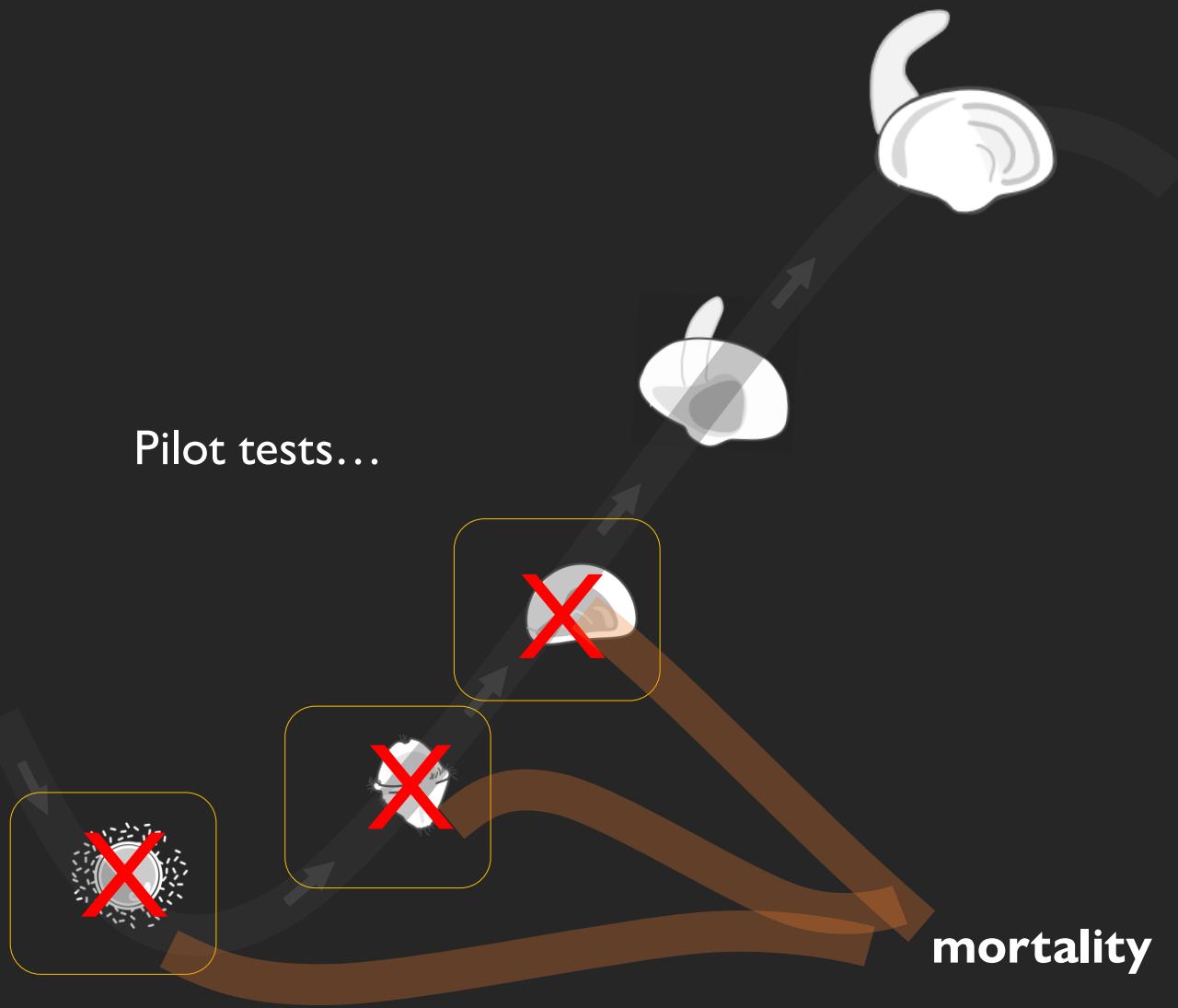
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Life stage?



# Relevance to life history and habitat..

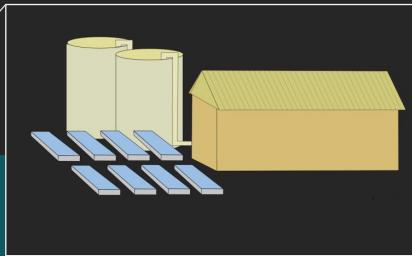
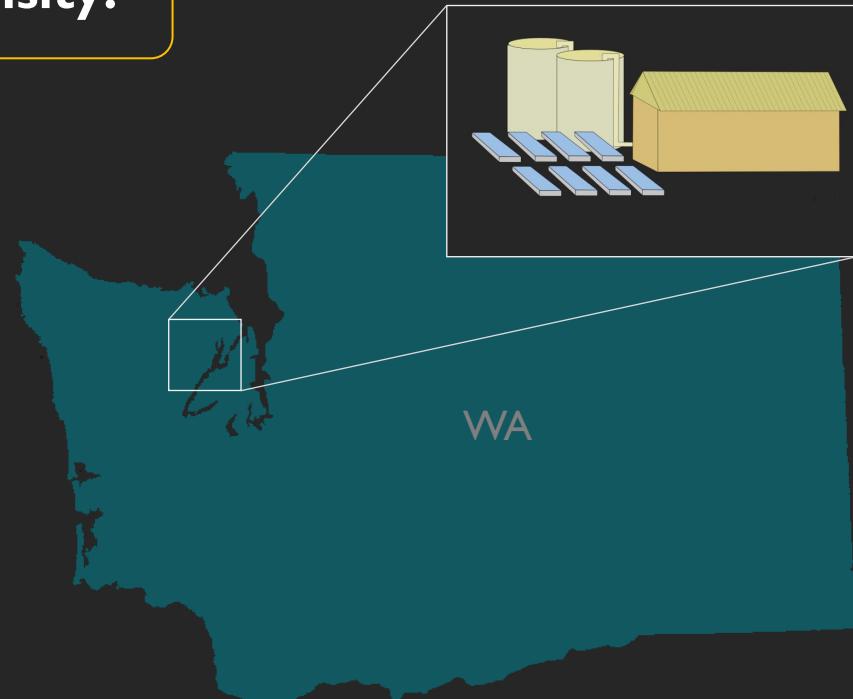
Life stage?



# Relevance to life history and habitat..

Life stage?

Intensity?



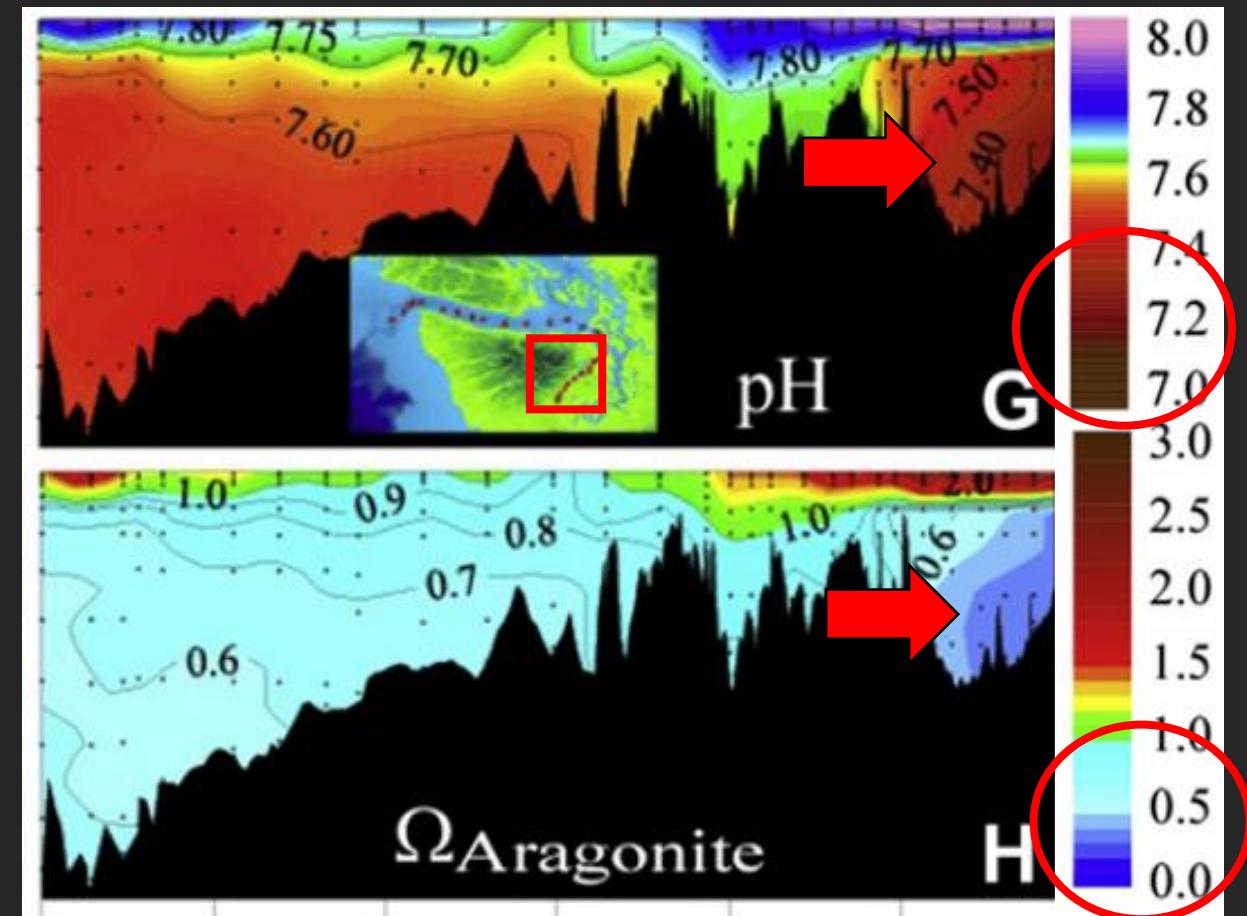
# Relevance to life history and habitat..

Life stage?

Intensity?



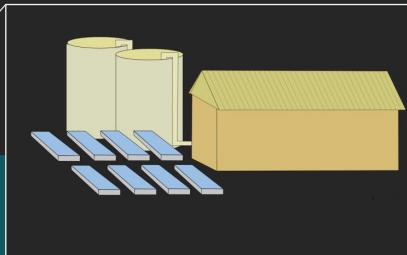
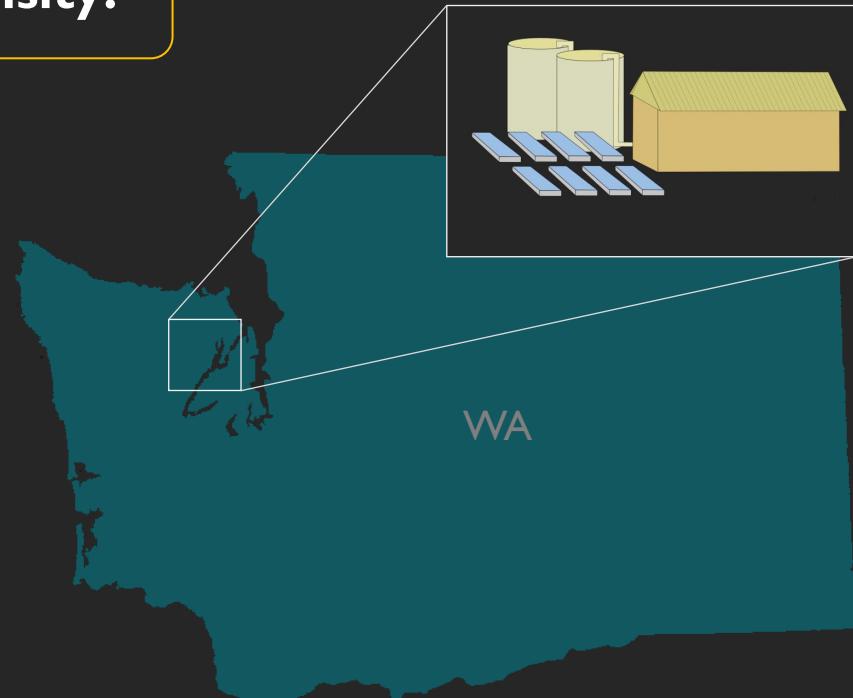
Transect in Hood Canal (Puget Sound, WA) August 2008



# Relevance to life history and habitat..

Life stage?

Intensity?



pH: <7.4  
 $\Omega$ : < 0.5

Relevant to habitat range  
and post-settlement stage

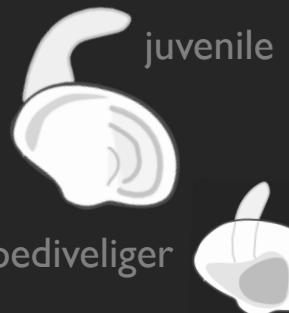
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**stress  
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Is this **transferable** to other bivalve species?

What is the **timing (life stage)** and **intensity** to test stress conditioning?

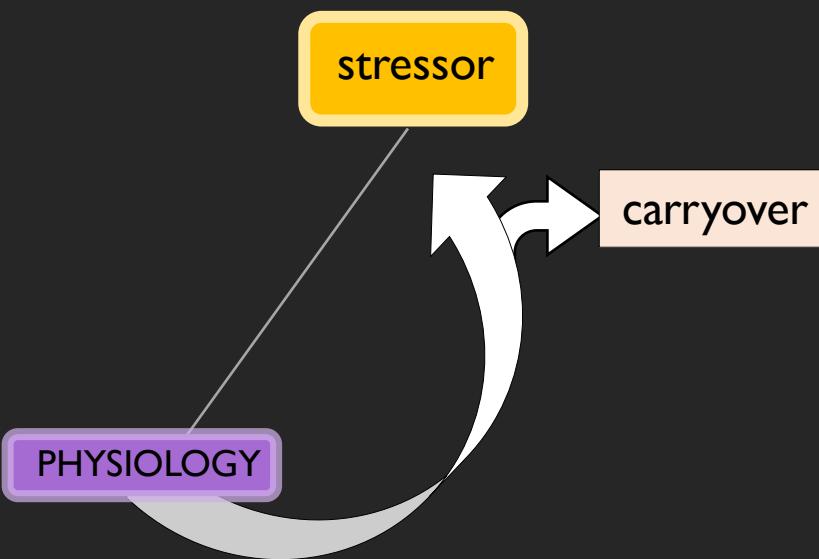
What are the **mechanisms underlying** a **conditioned response**



# Chapter I

# Chapter II

# Chapter III

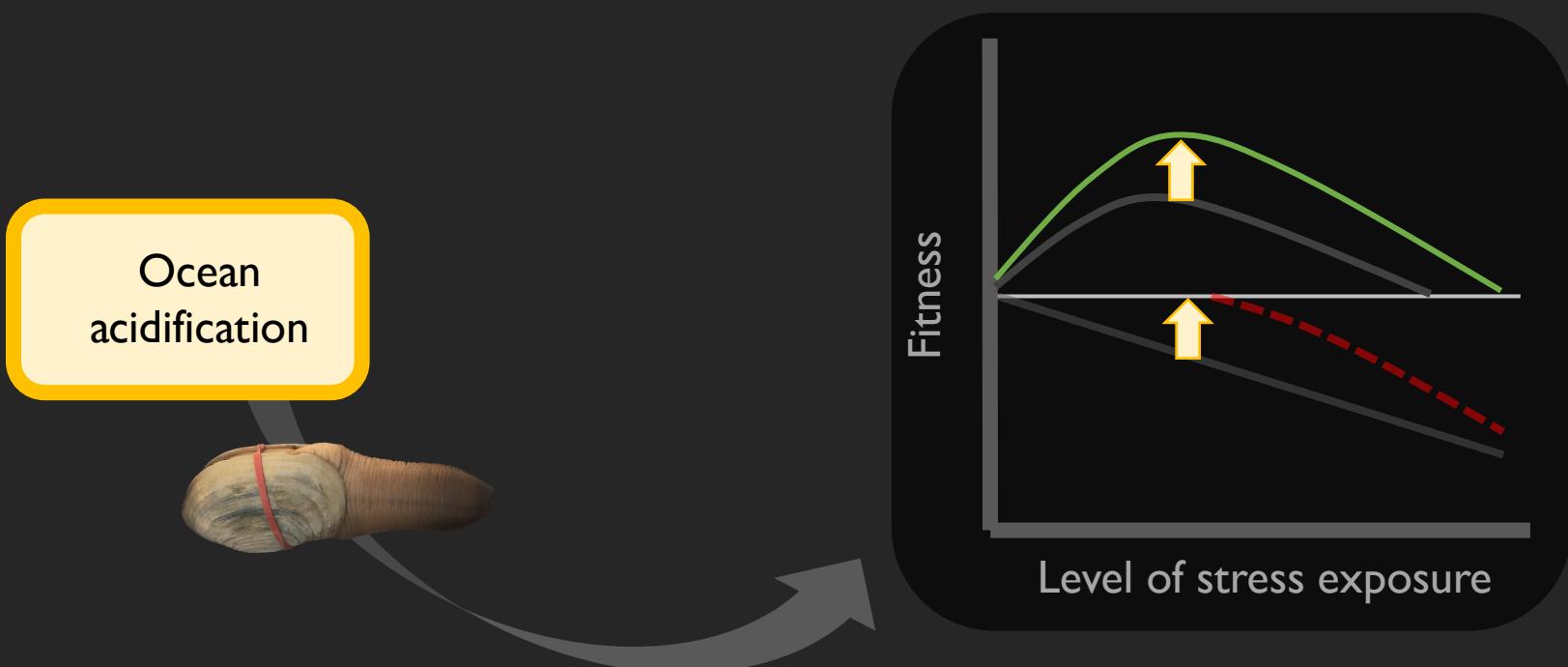


## Hypothesis

Repeated stress exposure under elevated  $p\text{CO}_2$  enhances intragenerational performance for Pacific geoduck.

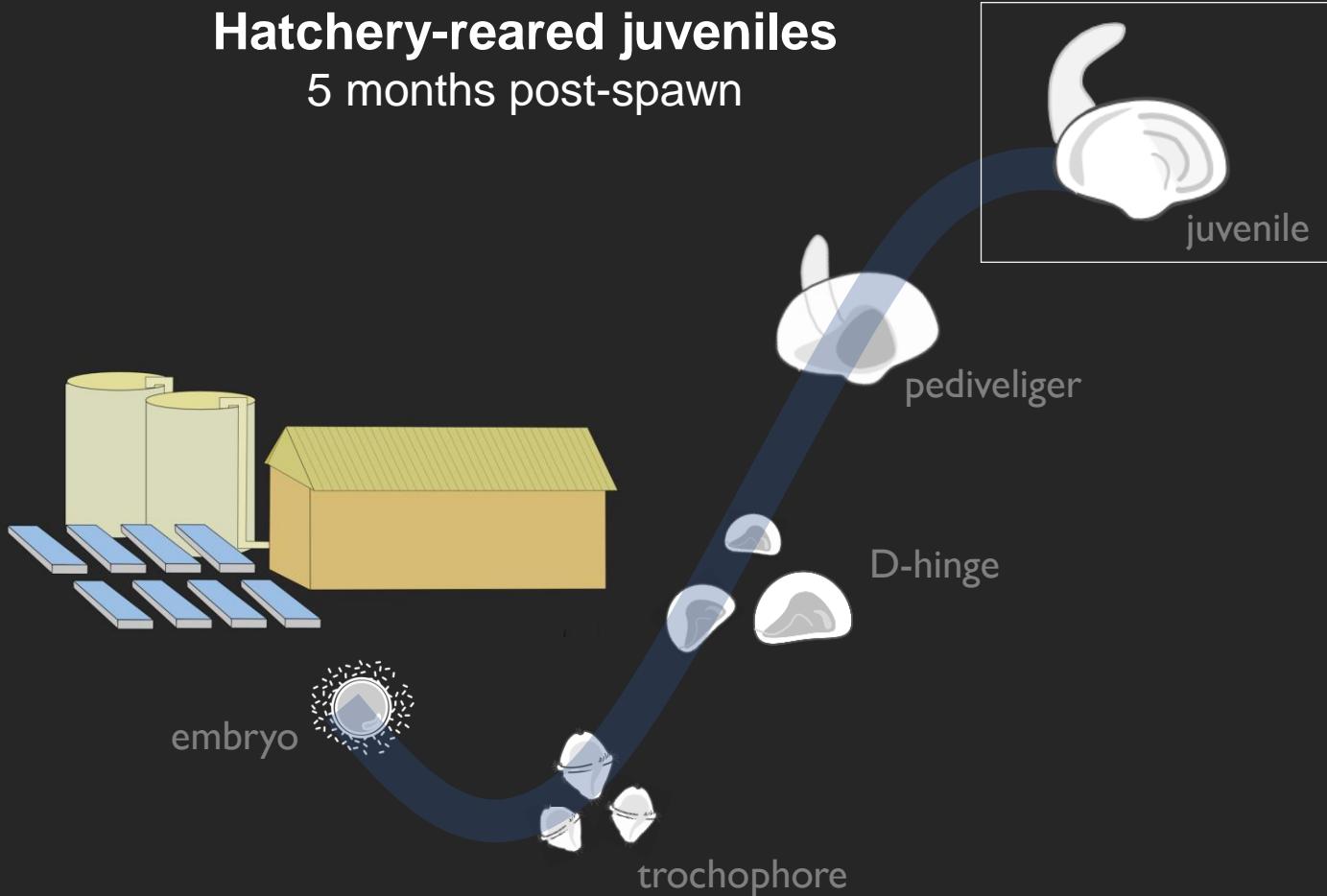
Q: Are there carryover effects of prior encounters?

- how is **metabolic rate** and **shell growth** affected by repeated stress



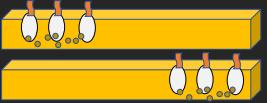
## Hatchery-reared juveniles

5 months post-spawn

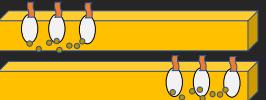




Ambient  
 $p\text{CO}_2$

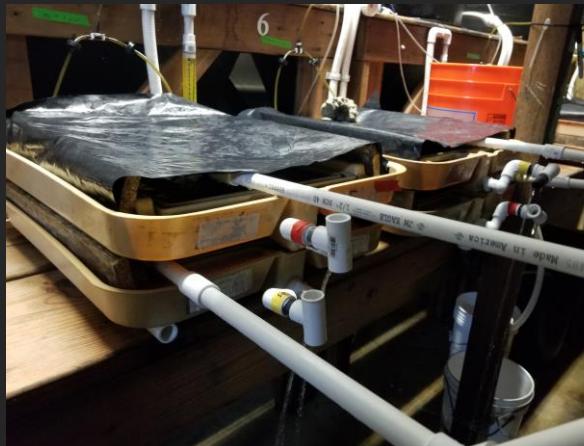
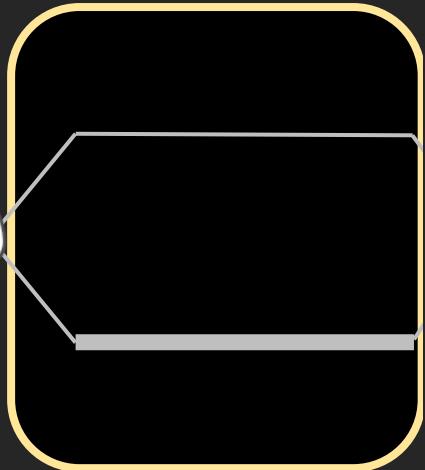
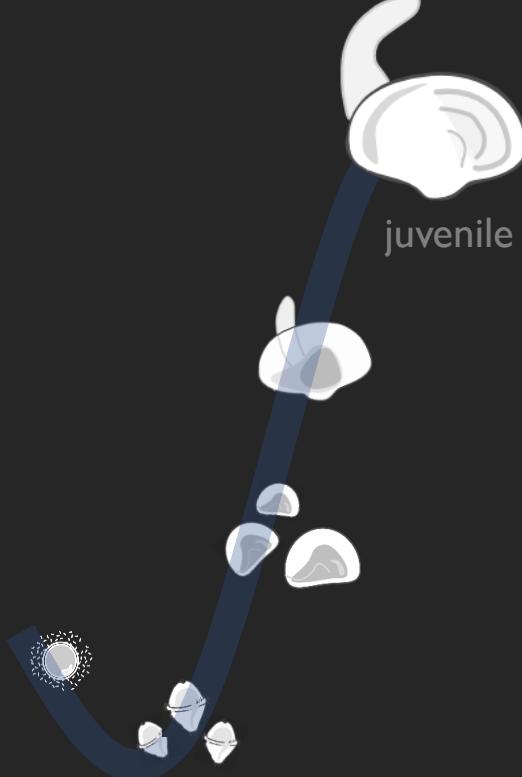


Elevated  
 $p\text{CO}_2$



# Initial exposure

10 days



*pCO<sub>2</sub>* treatments

—

Ambient: pH 7.9;  $\Omega > 1$

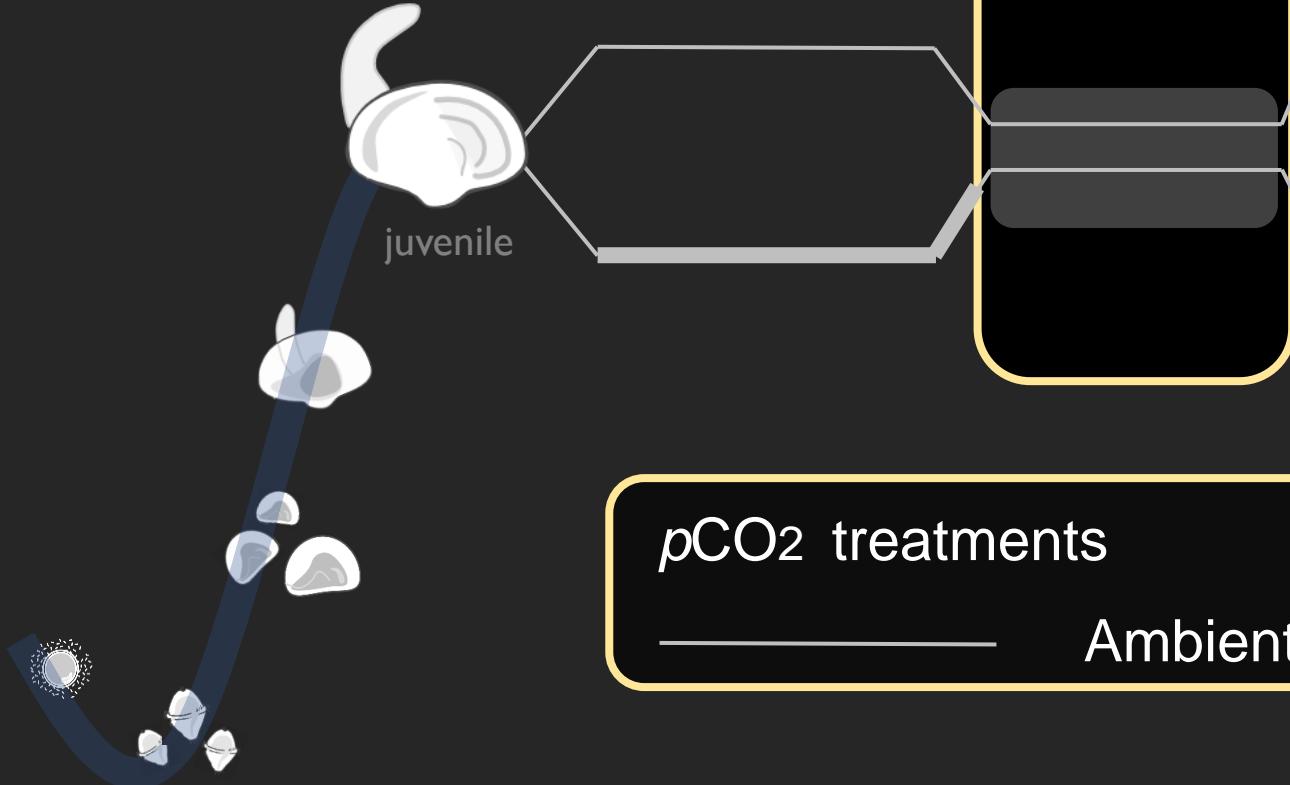
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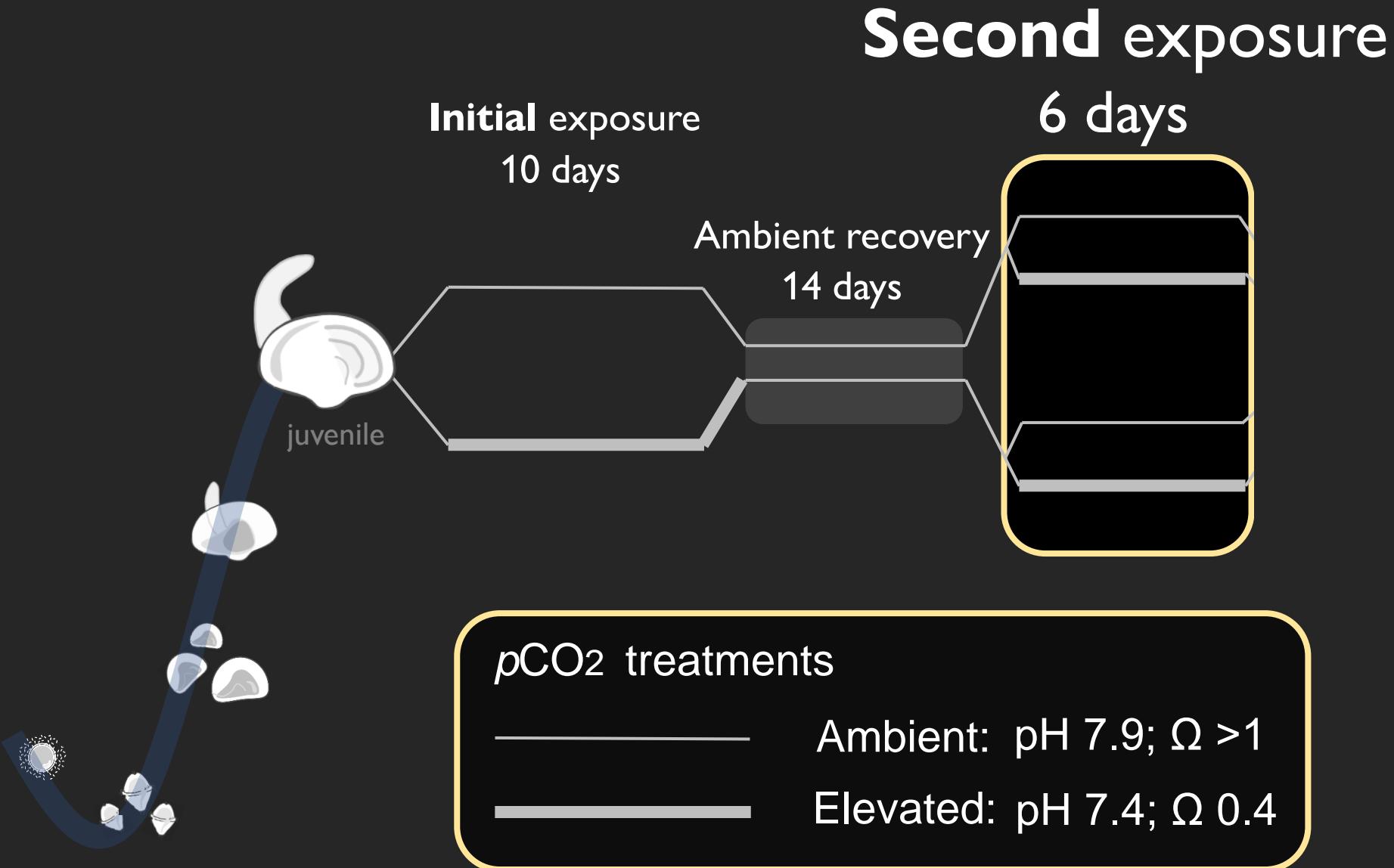
Elevated: pH 7.4;  $\Omega 0.4$

# Ambient recovery 14 days

Initial exposure

10 days





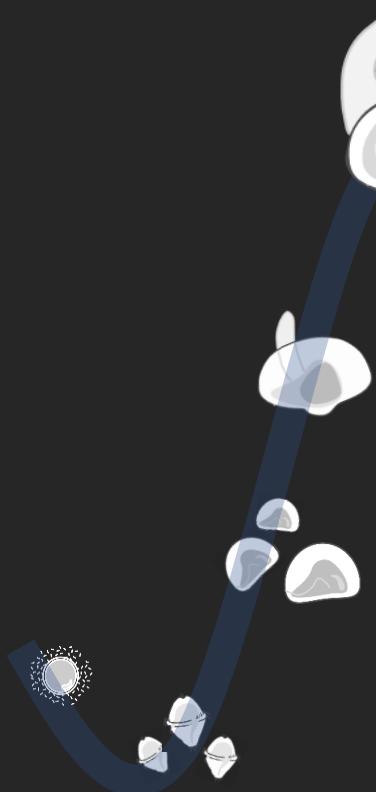
Ambient  
grow-out  
~5 months

**Initial exposure**  
10 days

**Second exposure**  
6 days

Ambient recovery  
14 days

juvenile

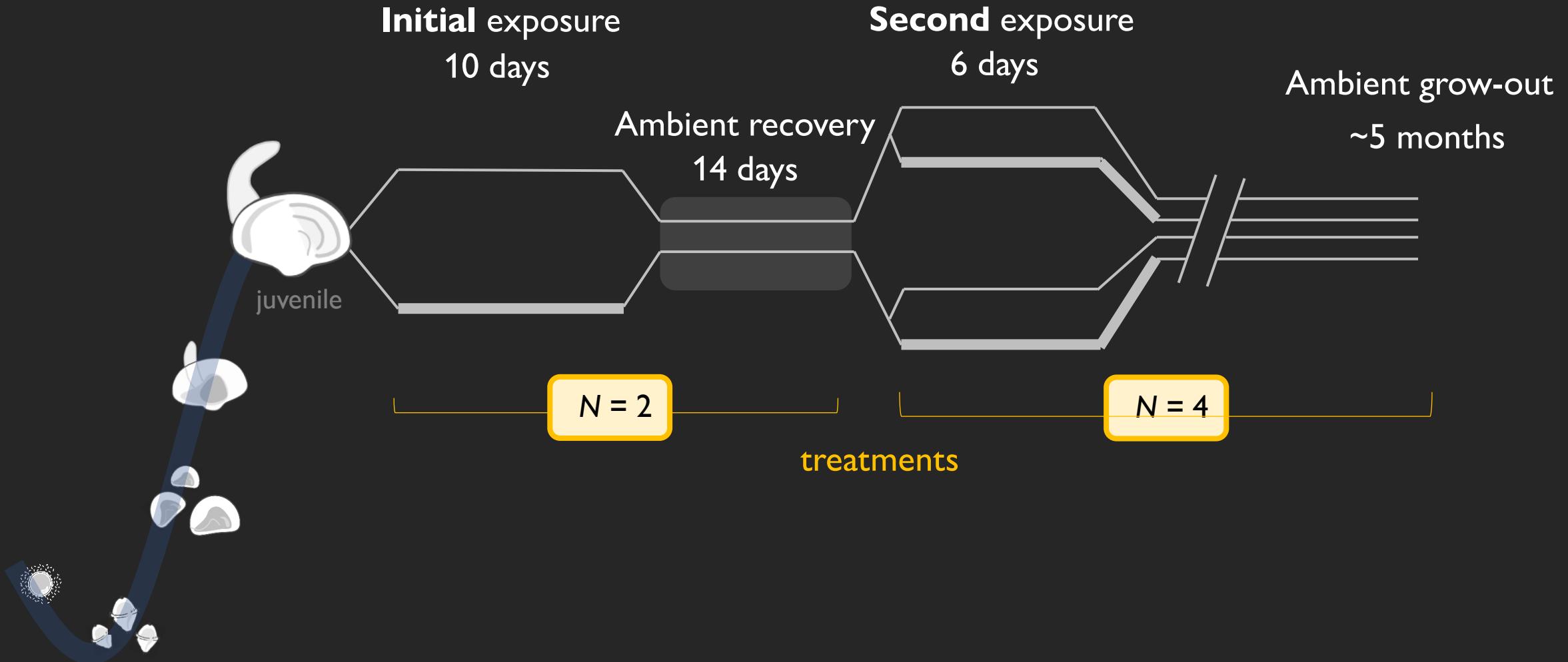


*pCO<sub>2</sub>* treatments

Ambient: pH 7.9;  $\Omega > 1$

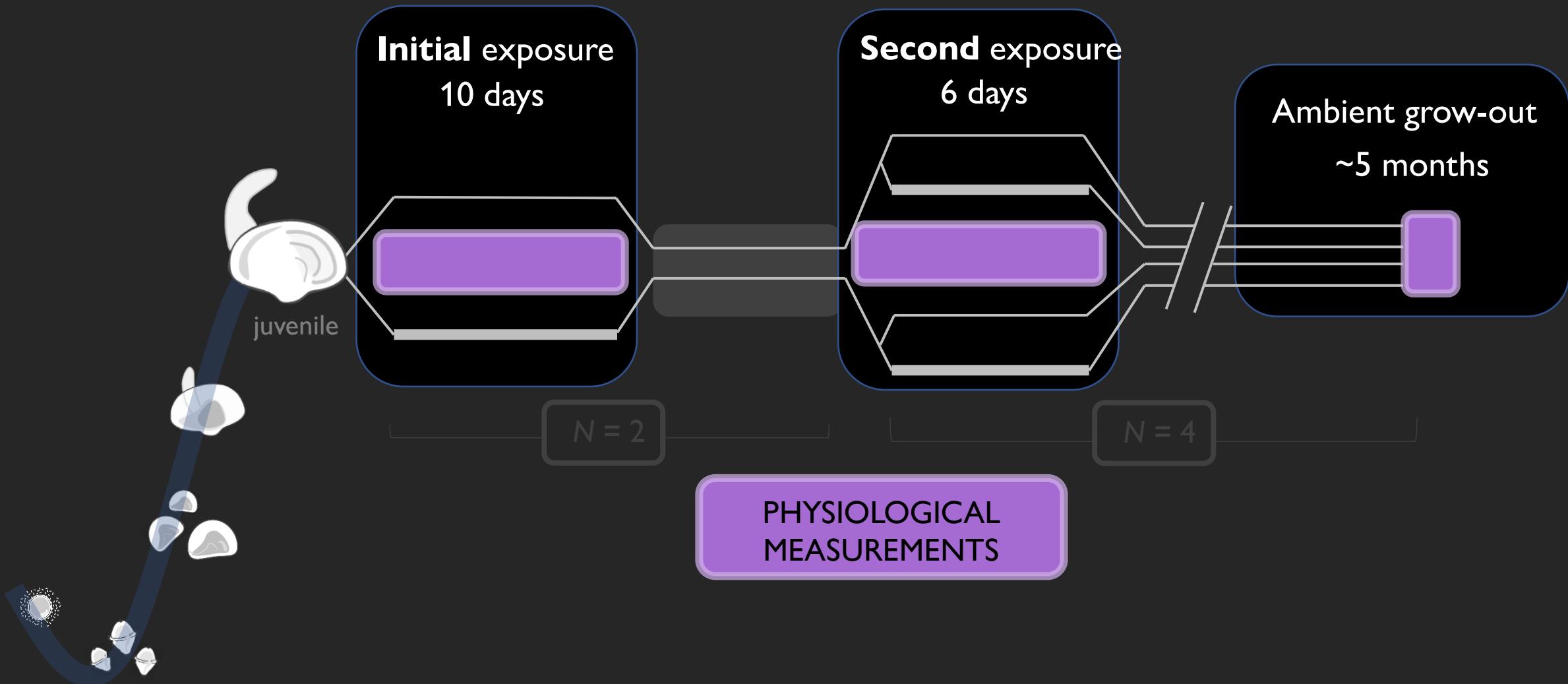
# Repeated acute exposures

# Long-term carryover



# Repeated acute exposures

# Long-term carryover



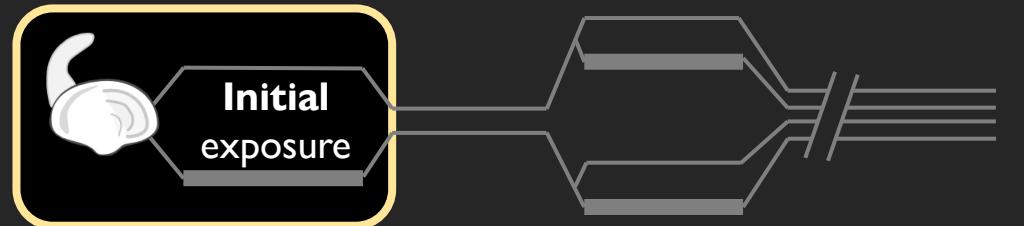
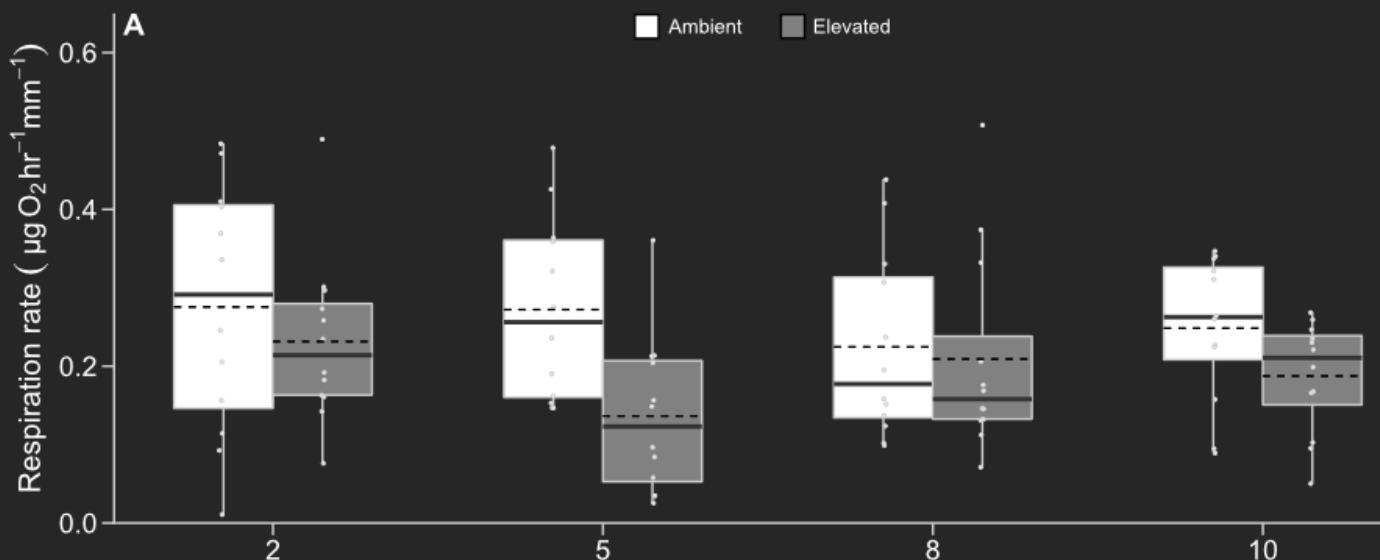
## RESPIRATION RATE SHELL LENGTH

Geoduck removed periodically to measure:

- respiration rate:  $\mu\text{g O}^2 \text{ hr}^{-1} \text{ mm}^{-1}$
- shell size: mm length



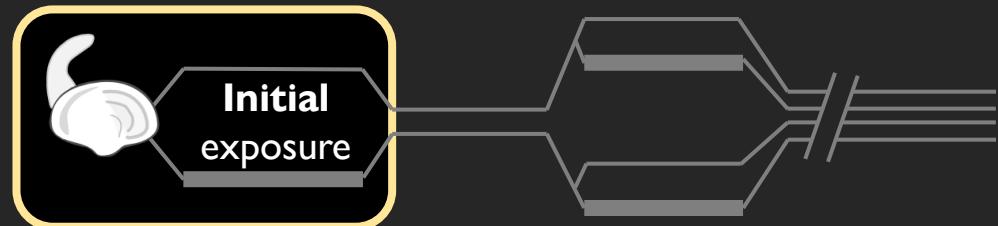
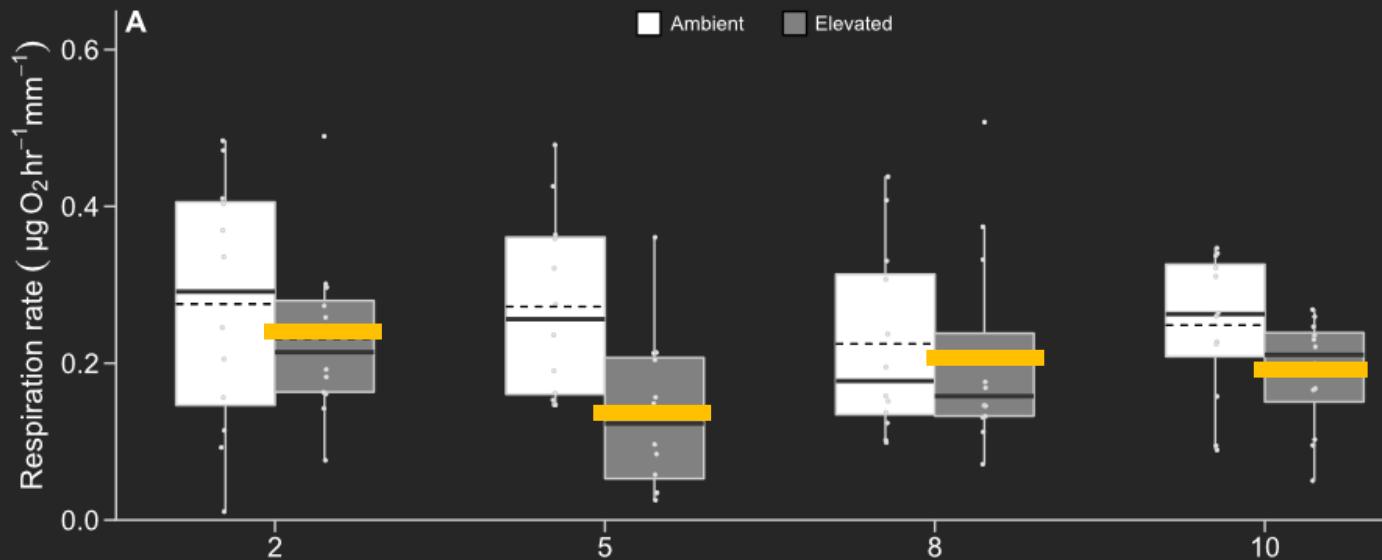
# Results: Initial exposure



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## Metabolic rate:

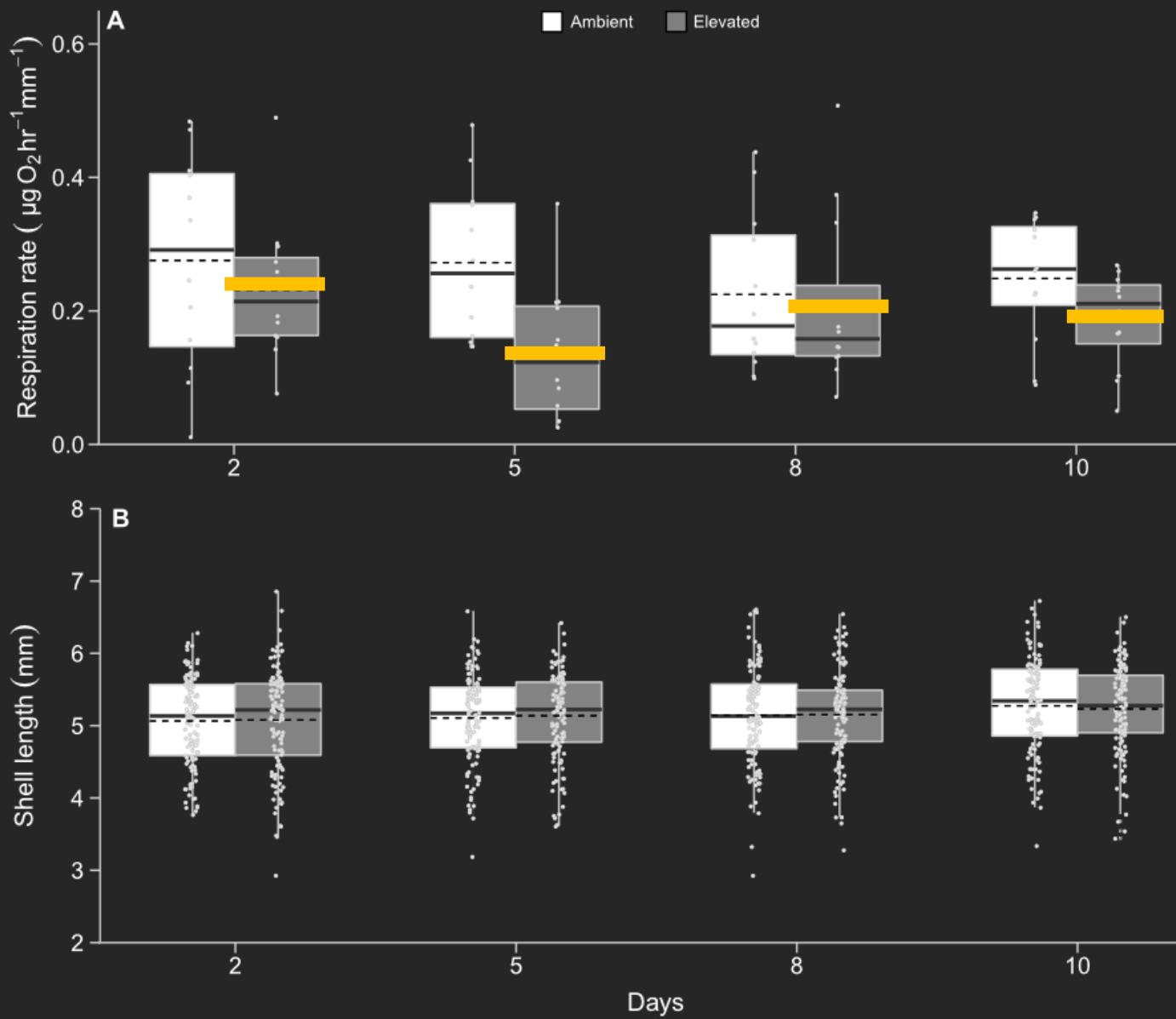
- 25% reduction in respiration rate under elevated  $p\text{CO}_2$



# Results: Initial exposure

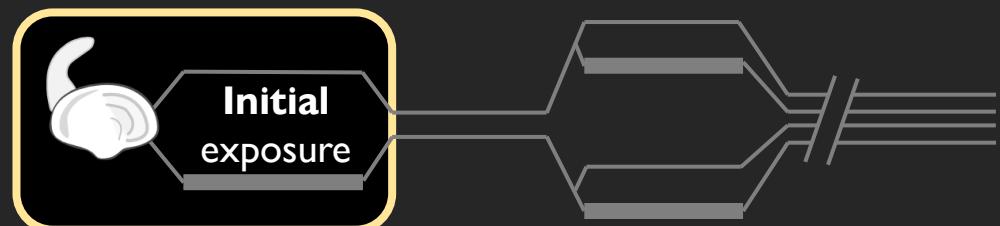
## Metabolic rate:

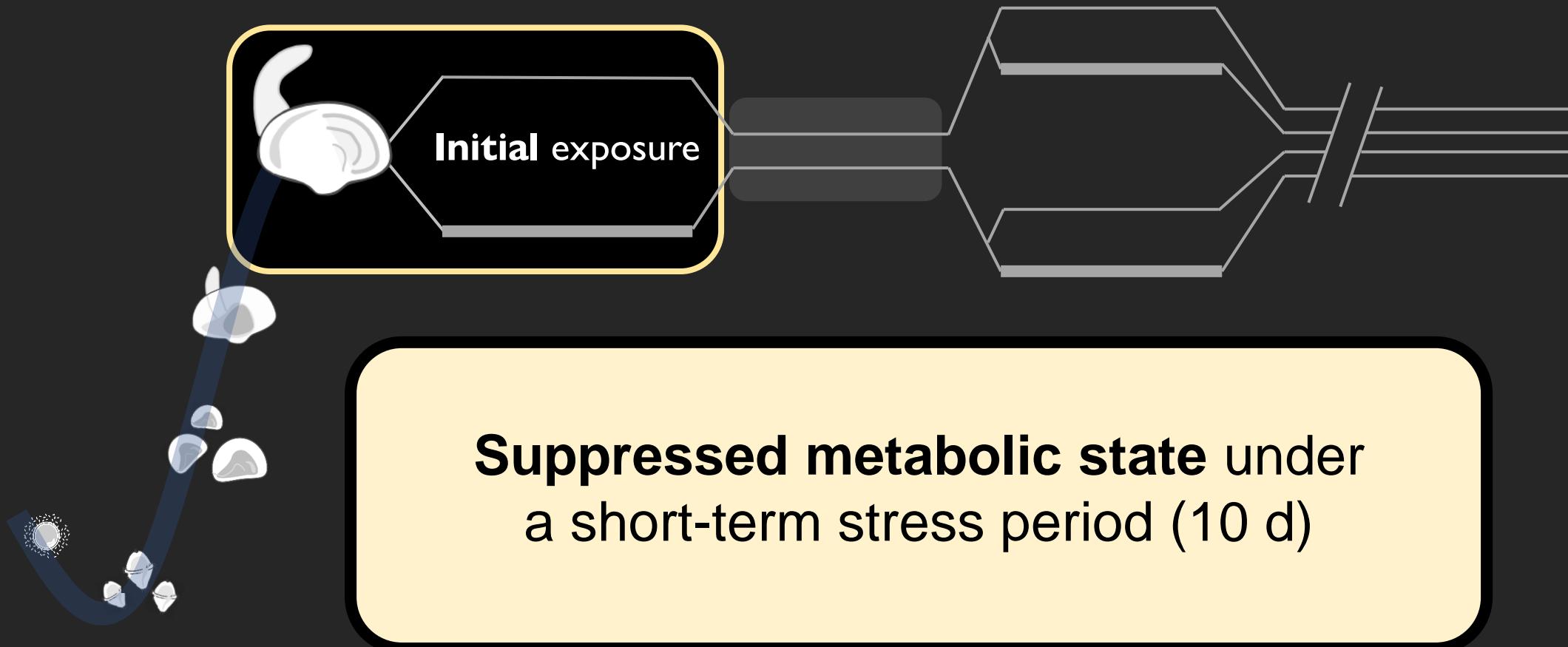
- **25% reduction** in respiration rate under **elevated  $p\text{CO}_2$**

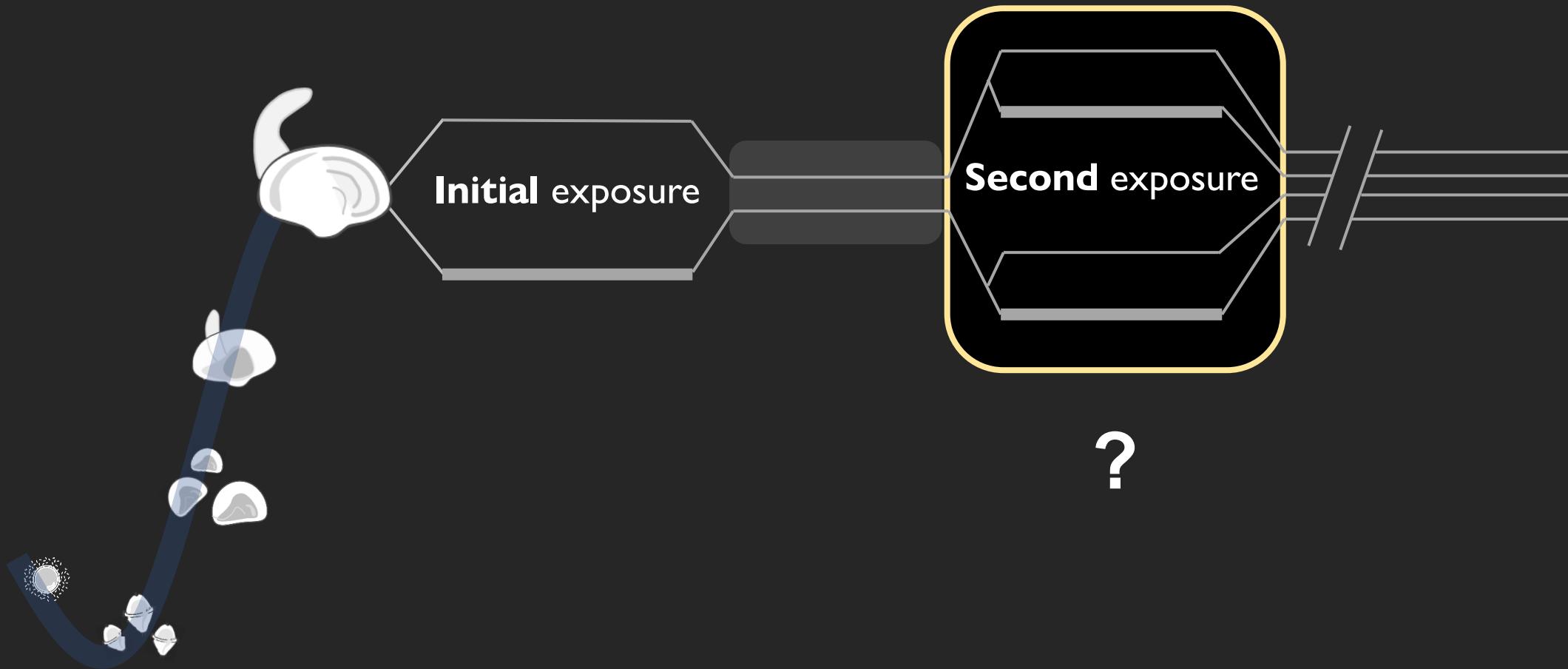


## Shell length:

- **No response** under to elevated  $p\text{CO}_2$



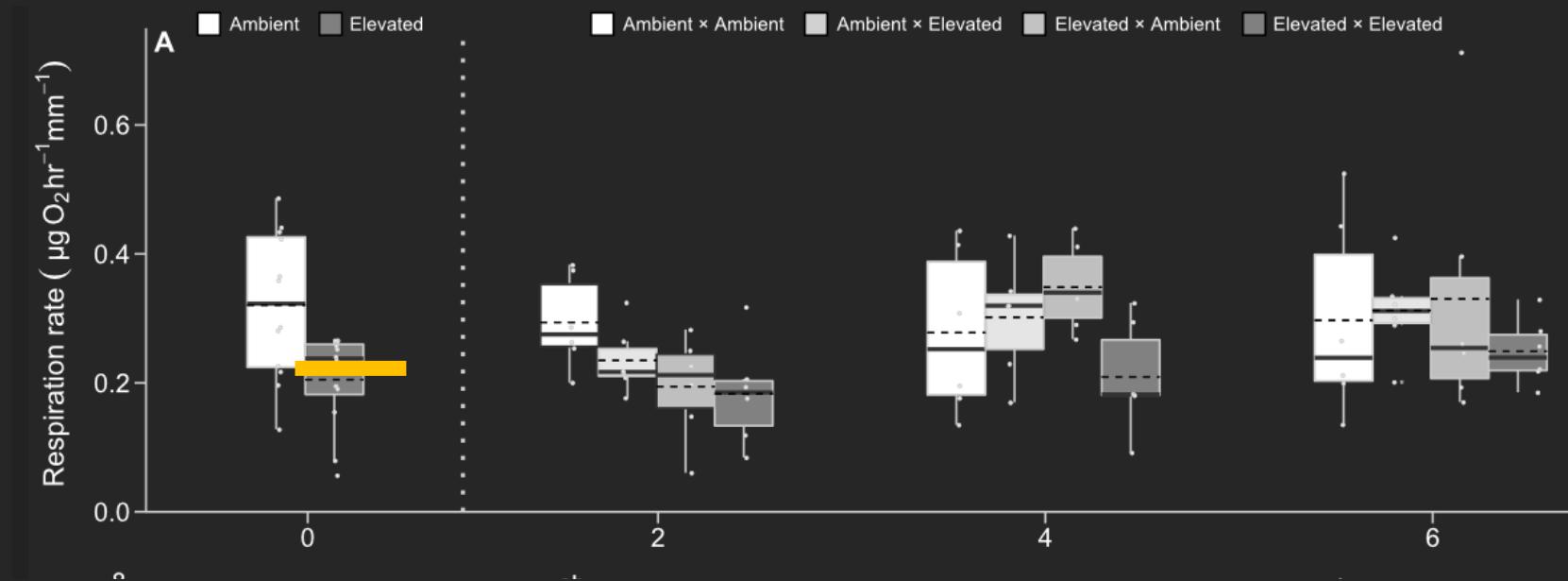




# Results: Secondary exposure

## Metabolic rate:

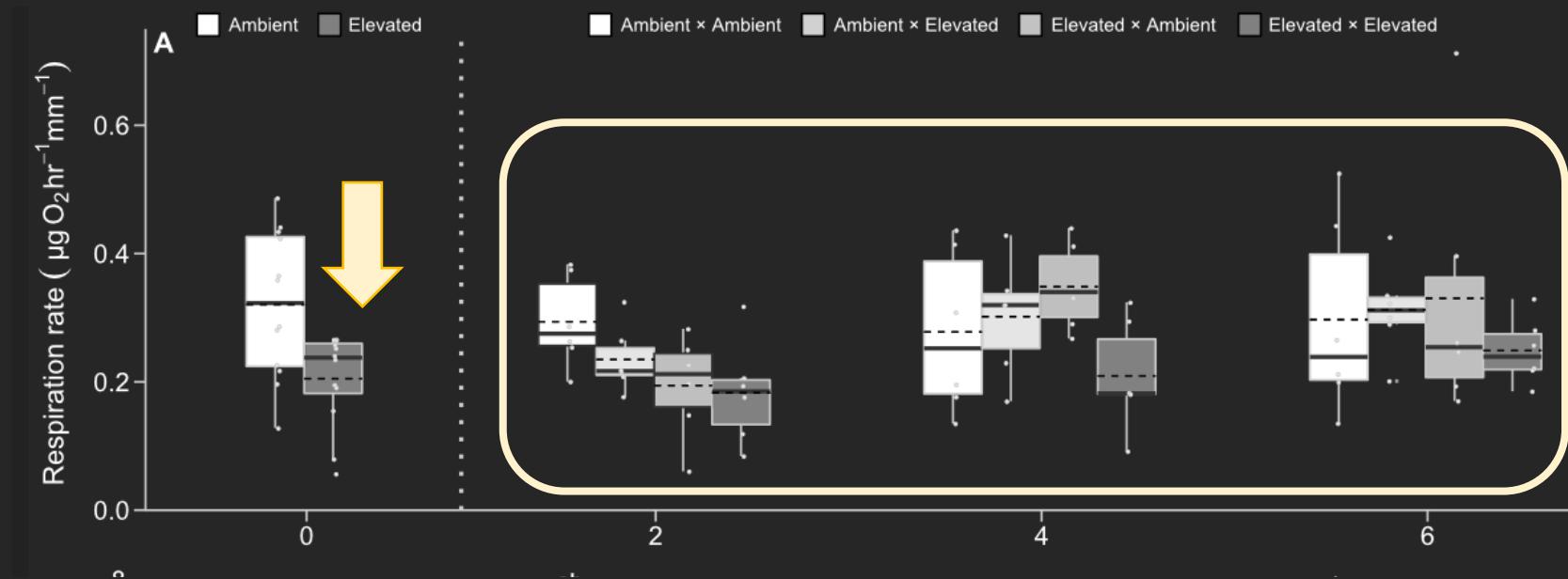
- Continued **metabolic suppression** prior to exposure



# Results: Secondary exposure

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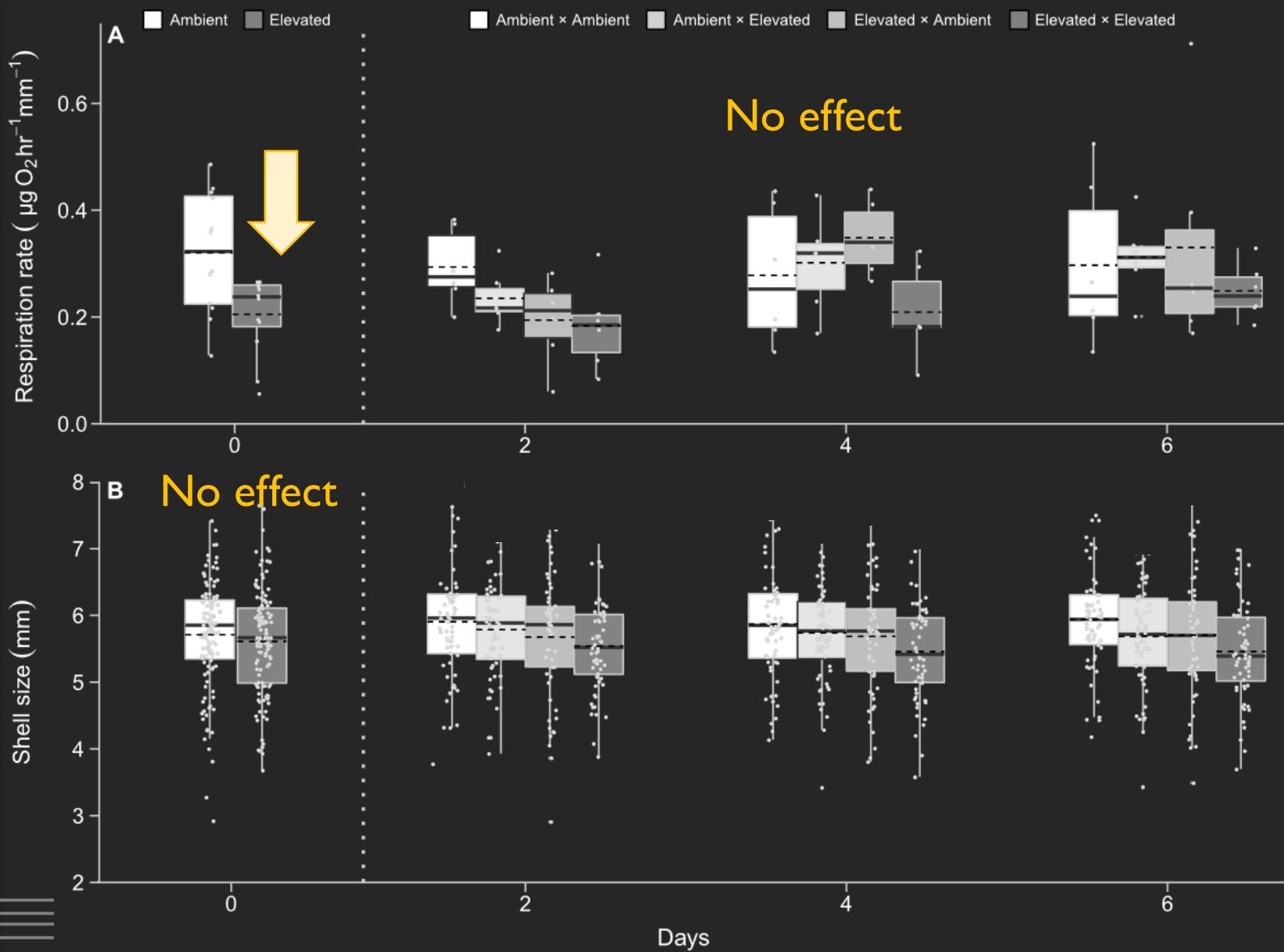
- Continued **metabolic suppression** prior to exposure
- No effect of treatment, **metabolic recovery** under subsequent encounter



# Results: Secondary exposure

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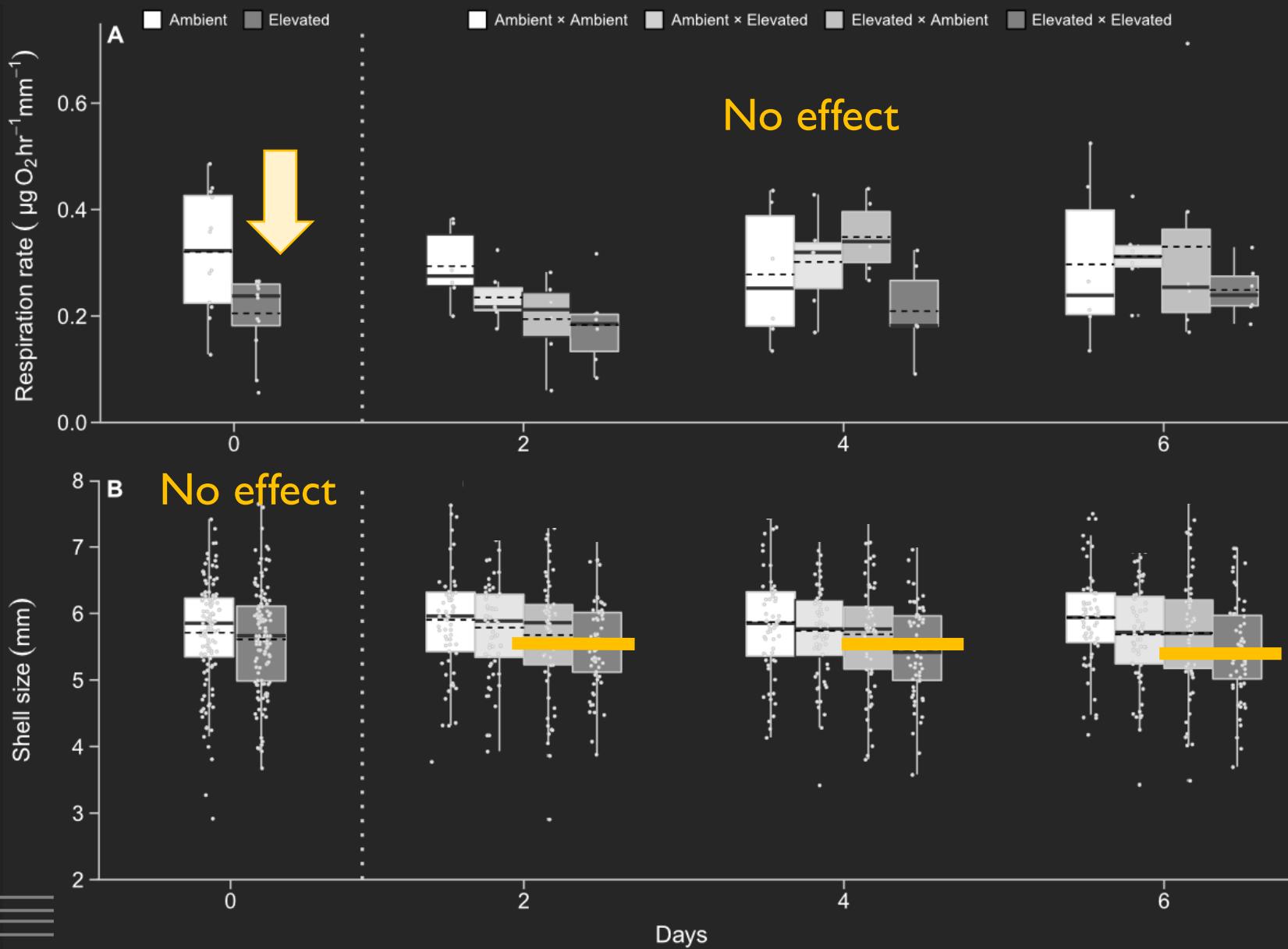
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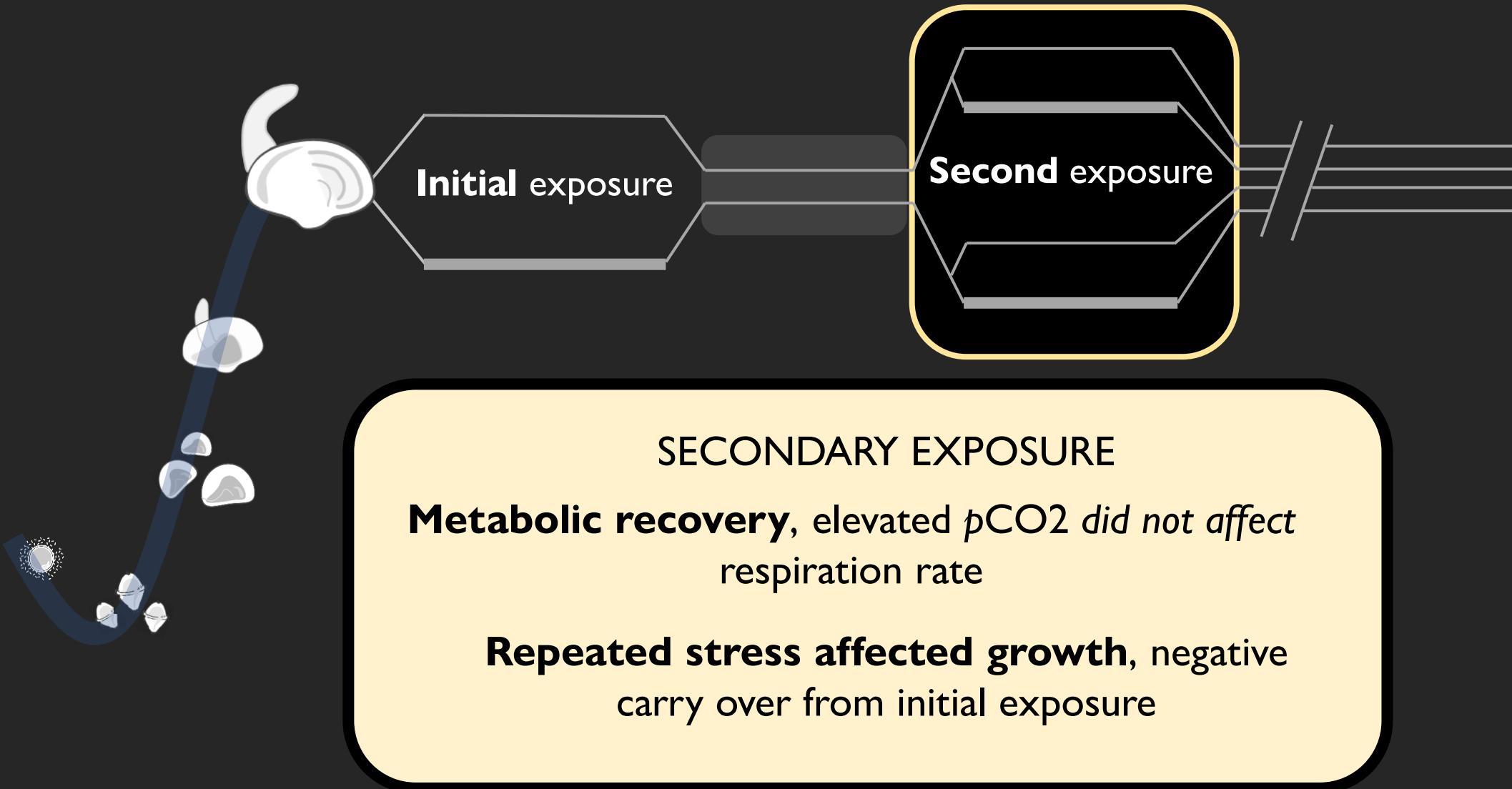
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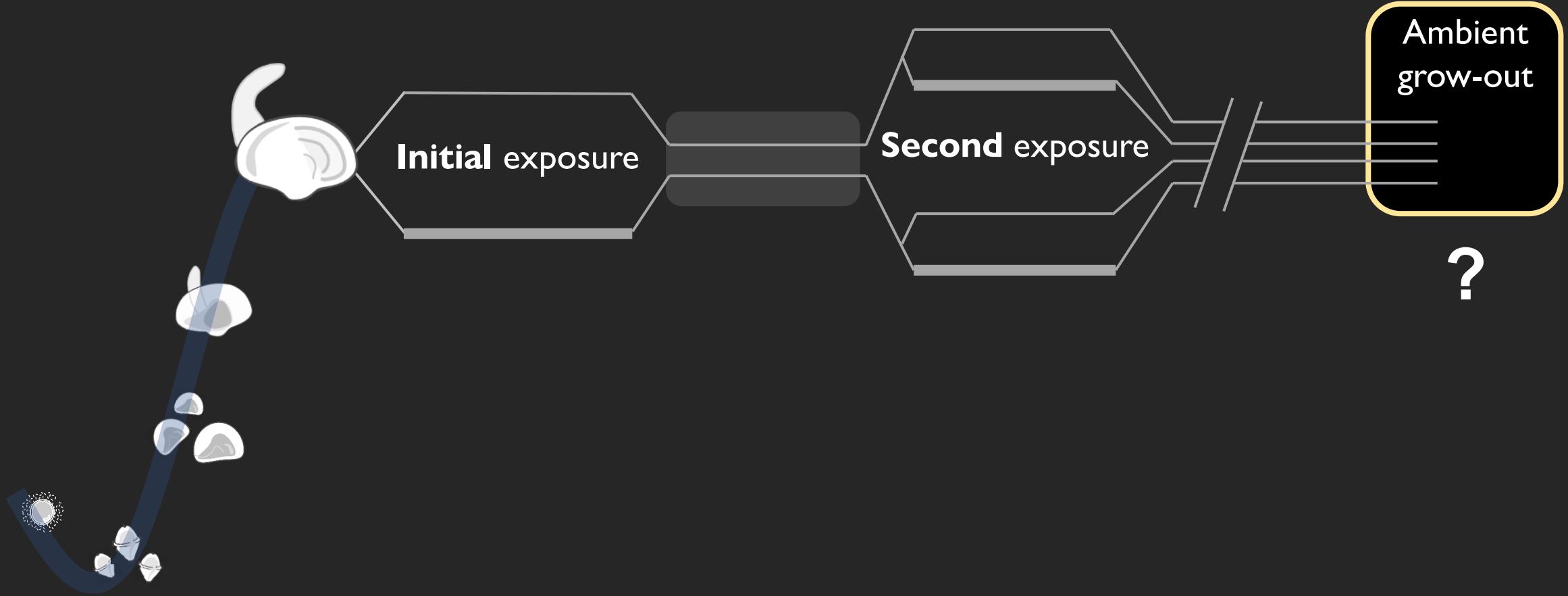


## Shell length:

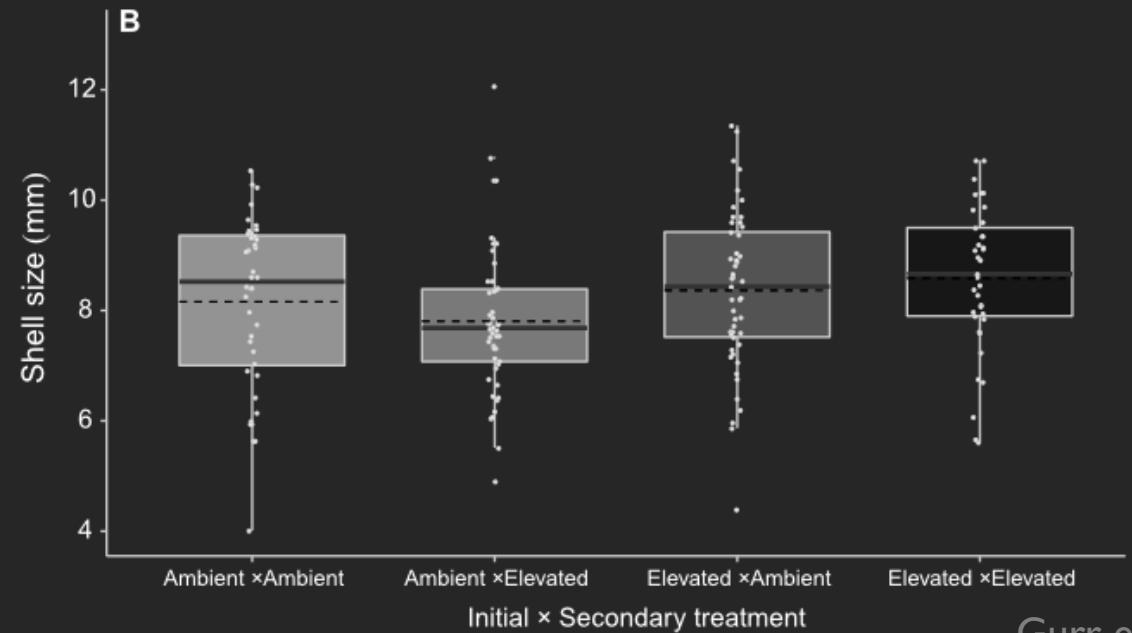
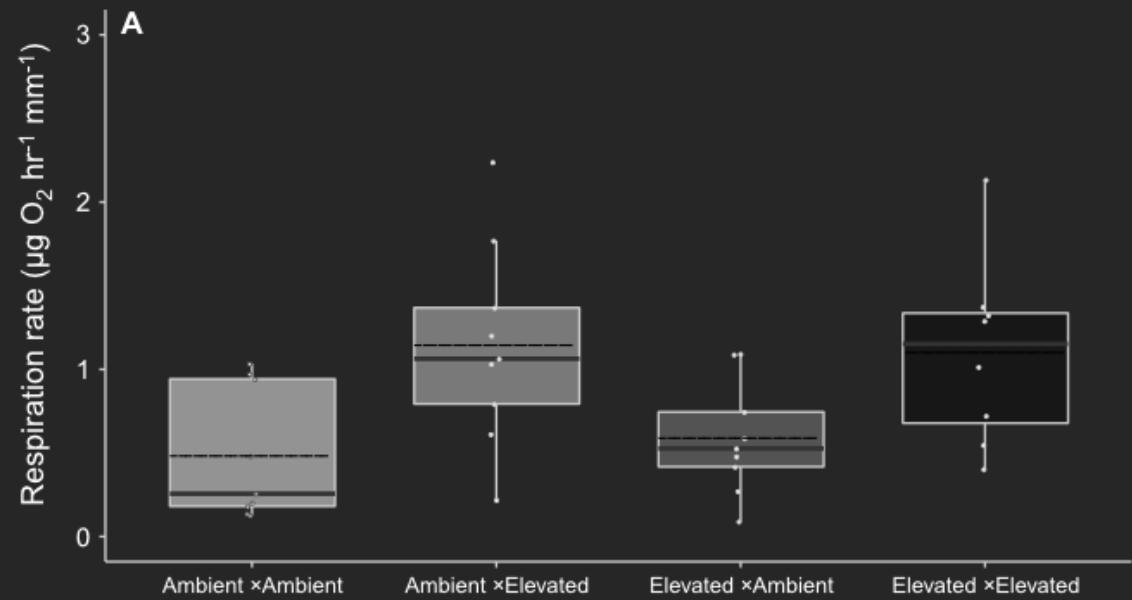
- No effect prior to exposure
- Initial treatment:** 4.02% (mm length) **smaller** under **elevated pCO<sub>2</sub>**







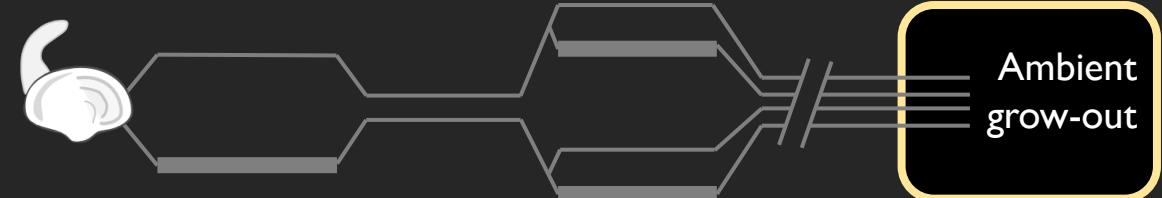
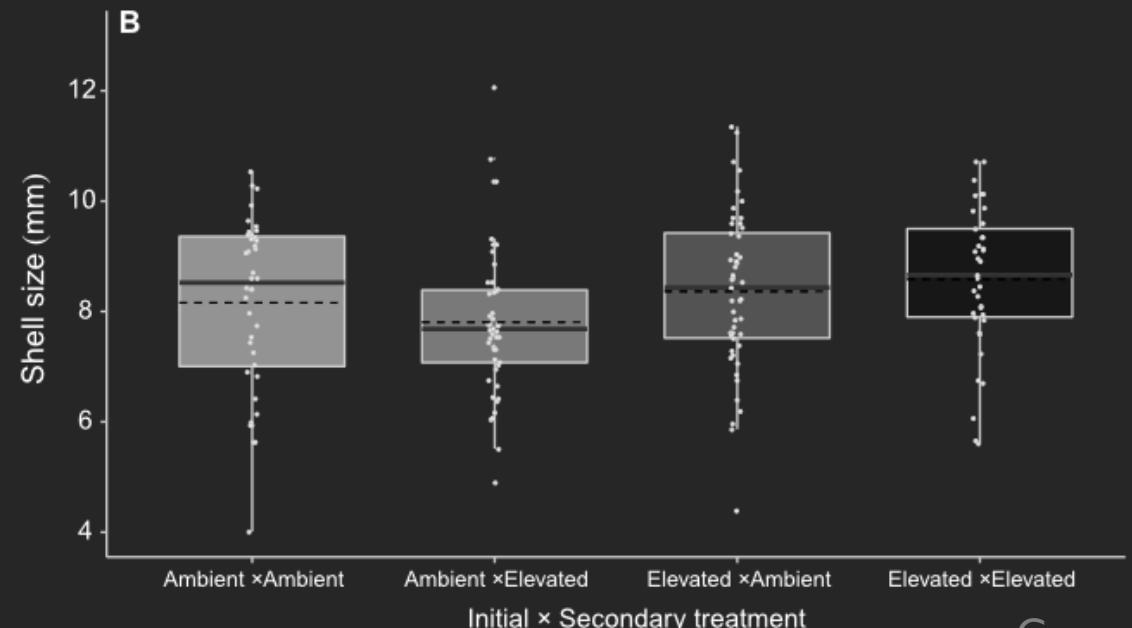
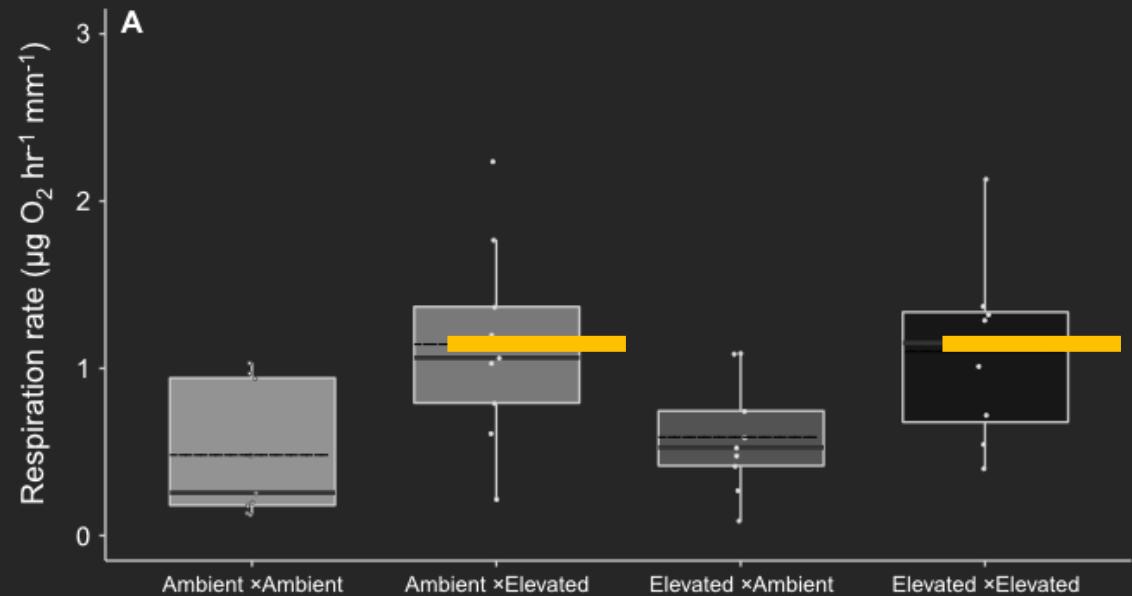
# Results: Long-term carryover



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## Metabolic rate:

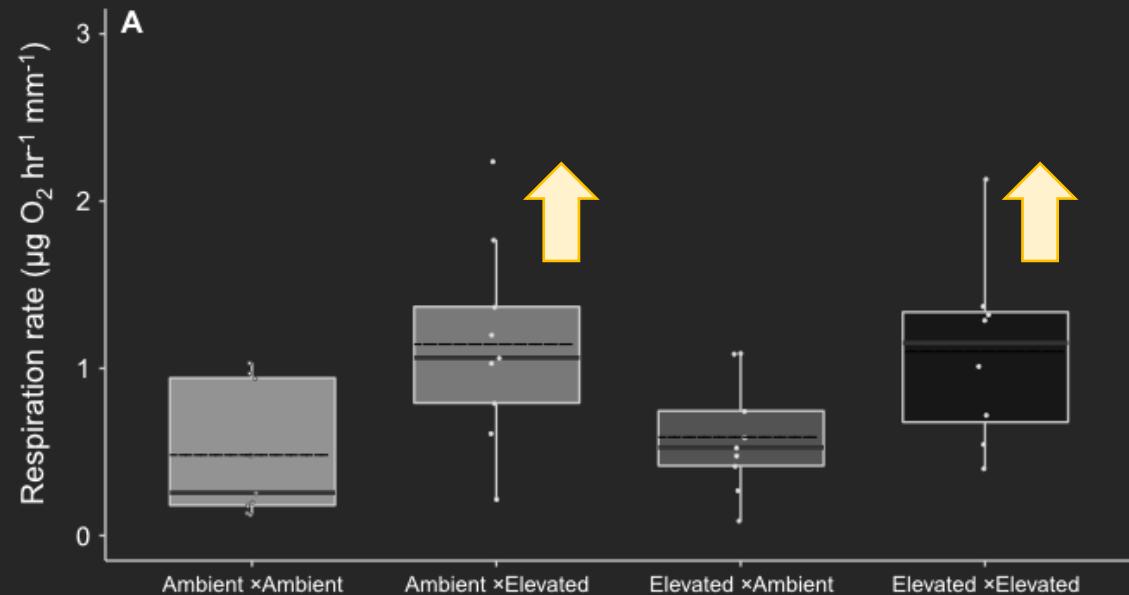
- Second treatment under elevated pCO<sub>2</sub>...  
**52% greater respiration rate**



# Results: Long-term carryover

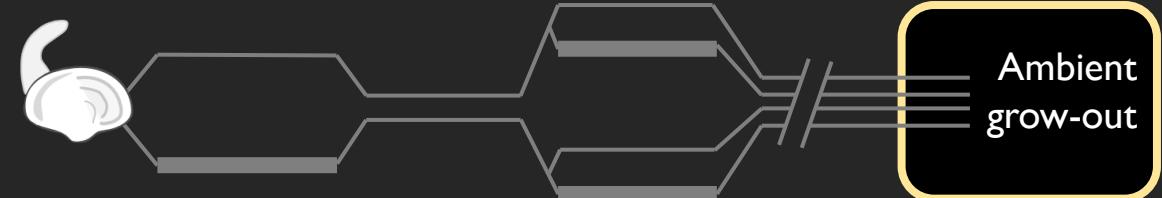
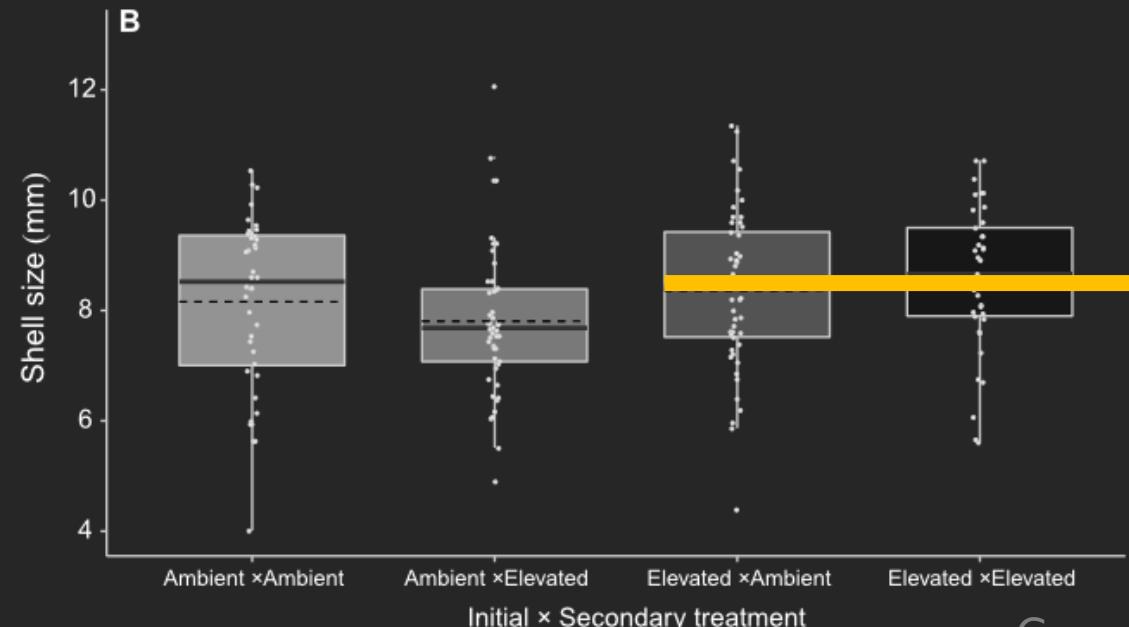
## Metabolic rate:

- Second treatment under **elevated pCO<sub>2</sub>**...  
**52% greater respiration rate**



## Shell length:

- Initial treatment under **elevated pCO<sub>2</sub>**...  
**~6% larger**



# Chapter 1: Conclusions

Are there carryover effects of prior encounters?

- how is **metabolic rate** and  
**shell growth** affected by repeated stress



# Chapter 1: Conclusions

Stress history (although initially negative) preceded  
**growth compensation** and **metabolic recovery**



- **Too early causes mortality...**

- post-settlement stage to survive low pH exposure and target stress conditioning

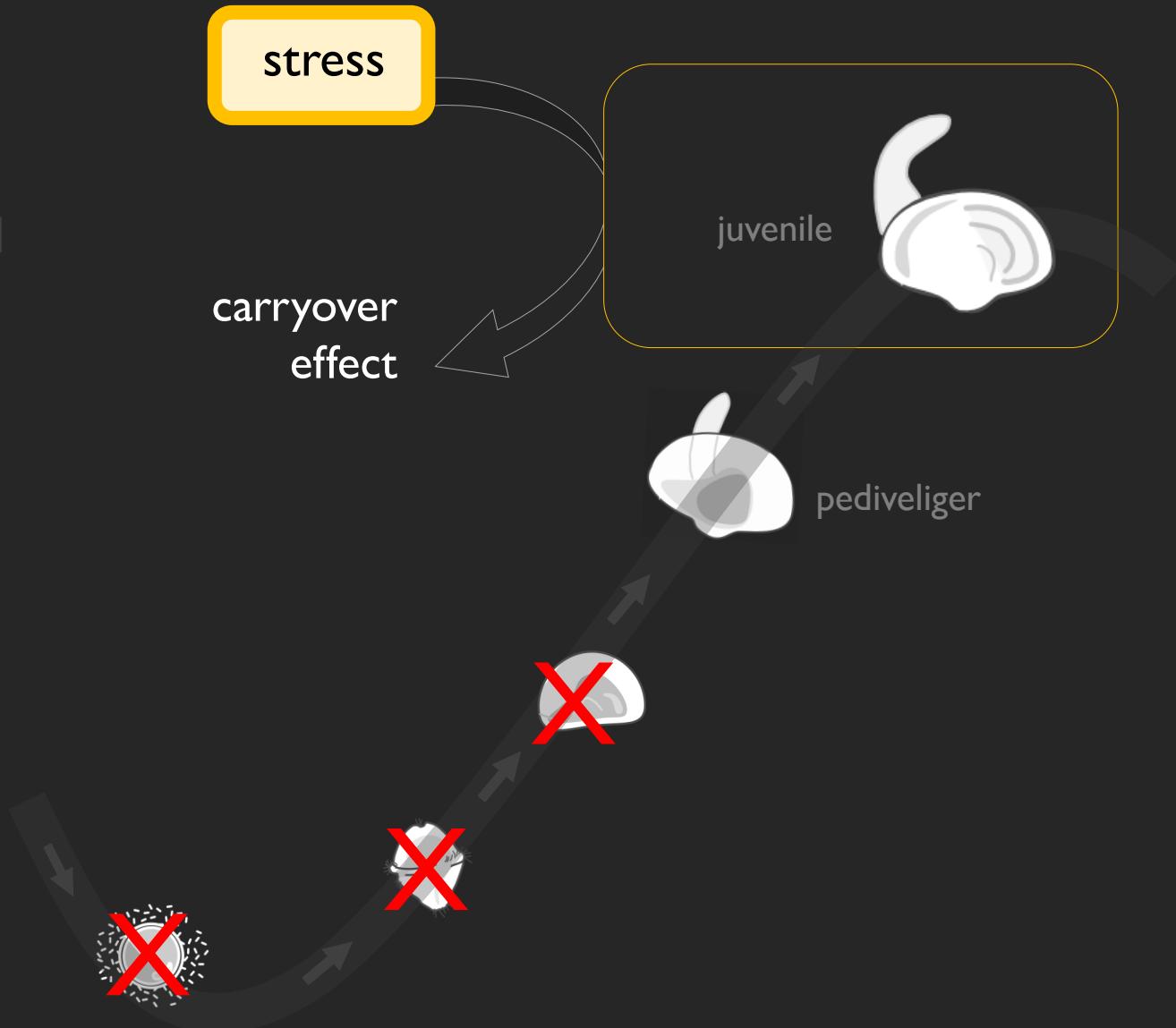


- Too early causes mortality...

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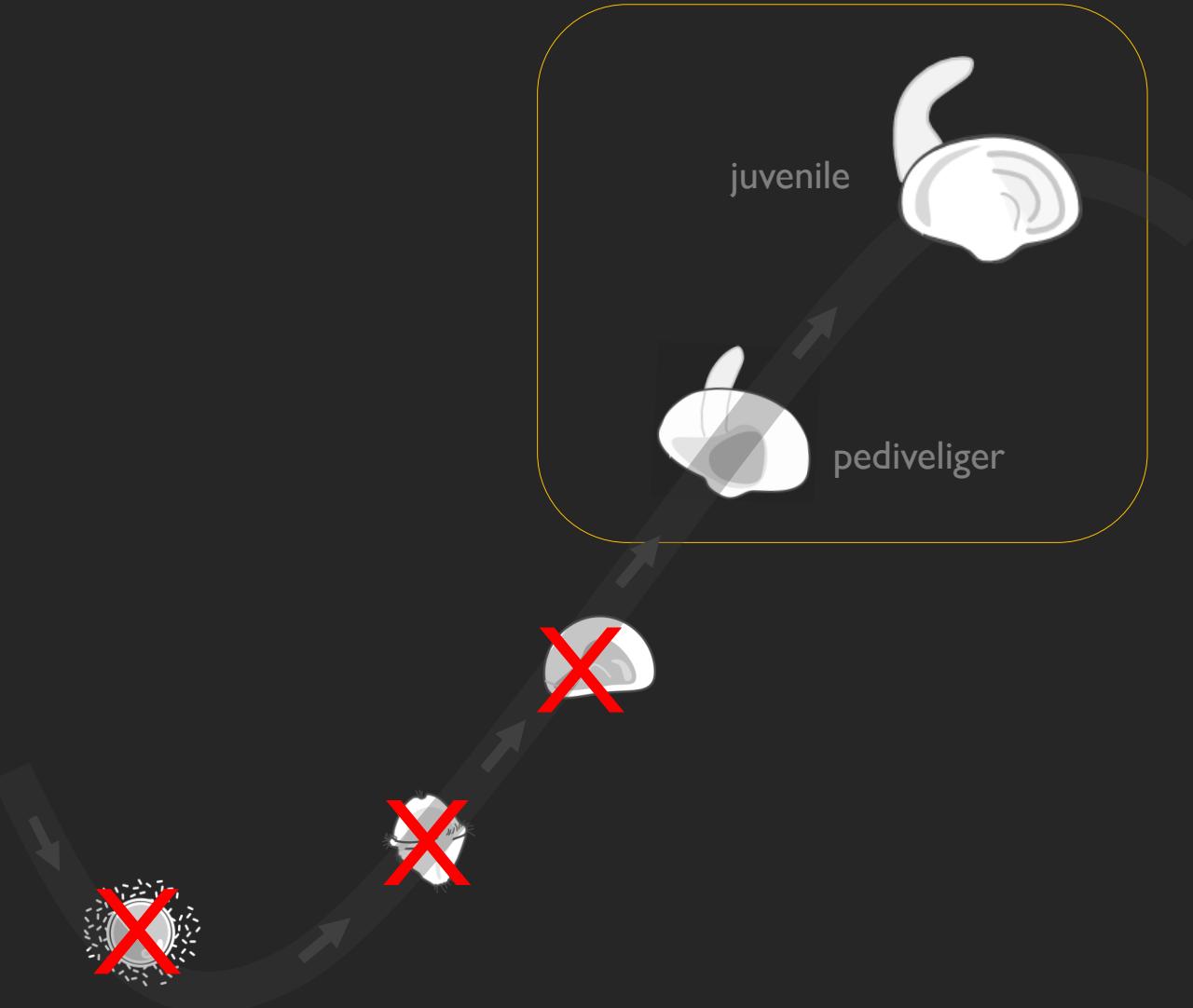
- Conditioning effect on juveniles

- phenotypic response to stress
- carry over to grow-out setting

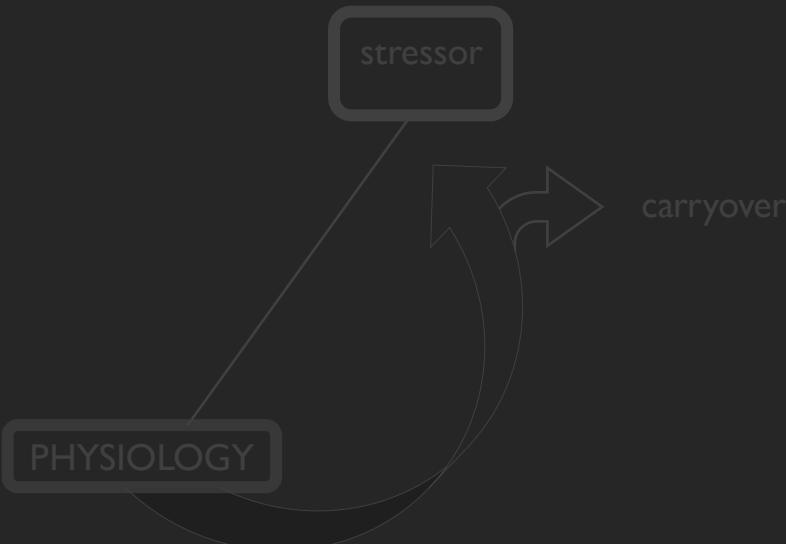


*Next step..*

Build upon  
mechanisms underlying a  
conditioned response



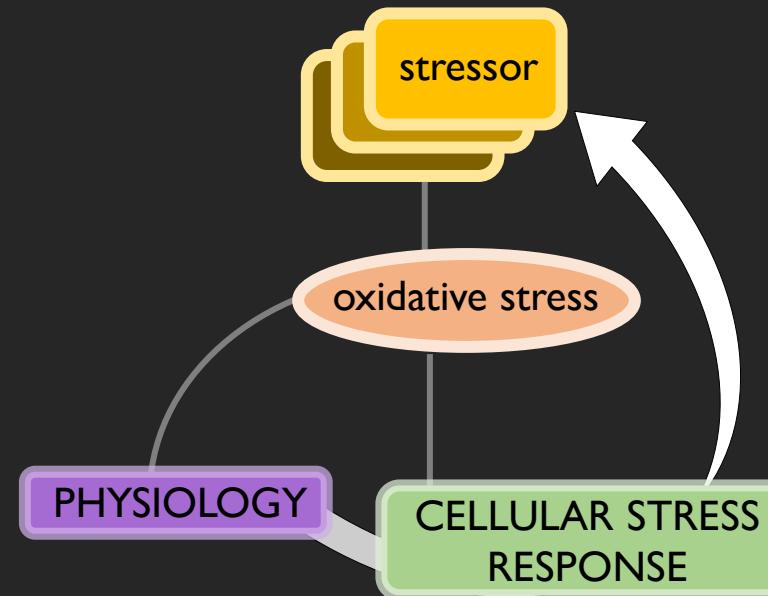
# Chapter I



## Hypothesis

Repeated stress exposure under elevated  $p\text{CO}_2$  enhances intragenerational performance for Pacific geoduck.

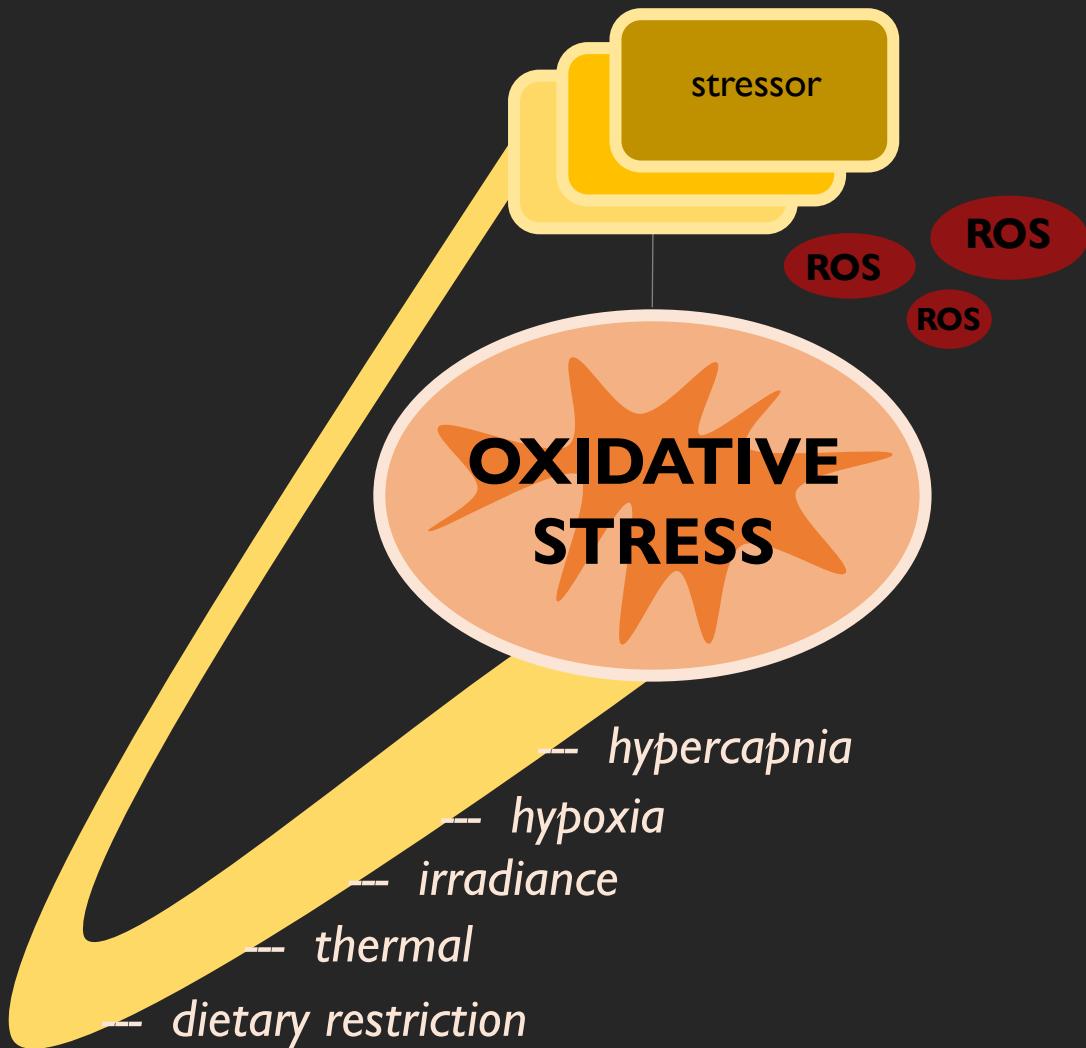
# Chapter II

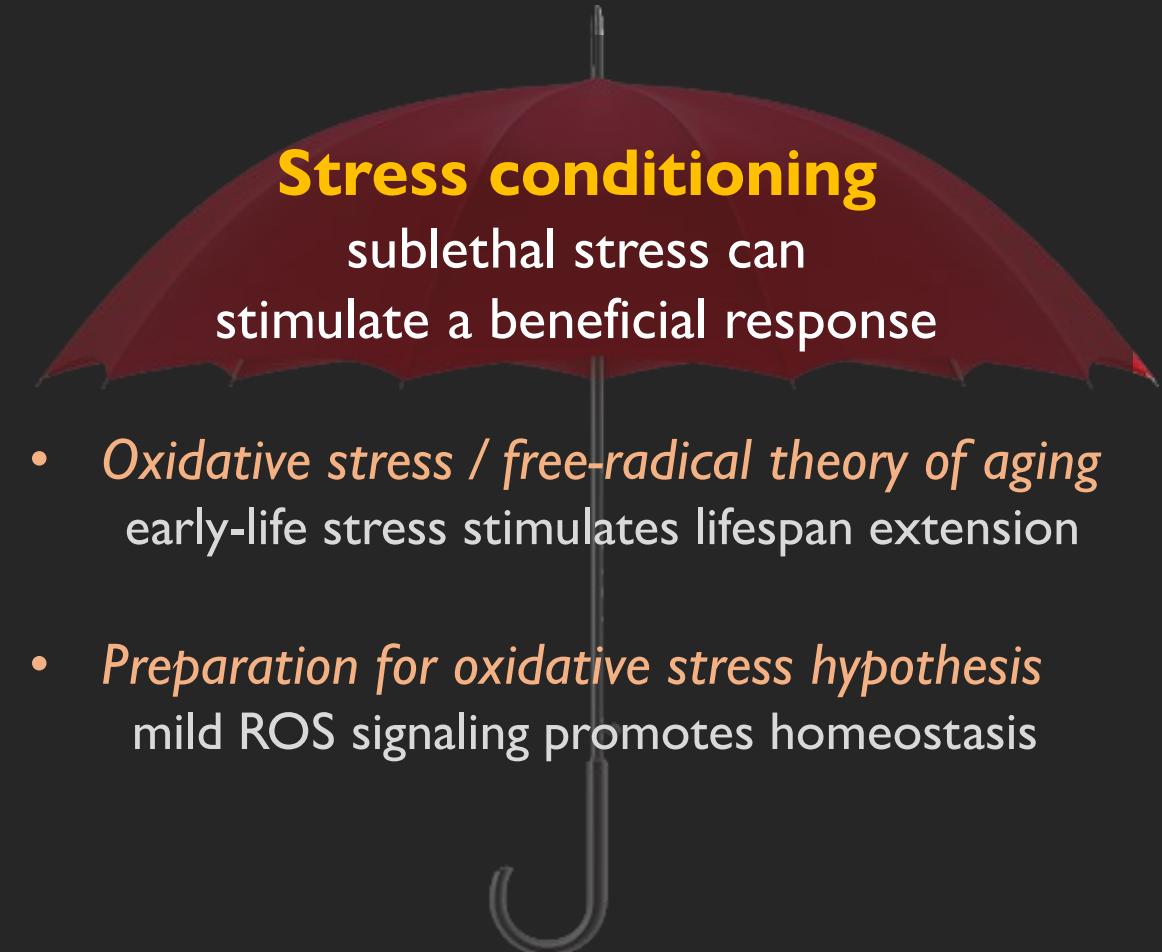
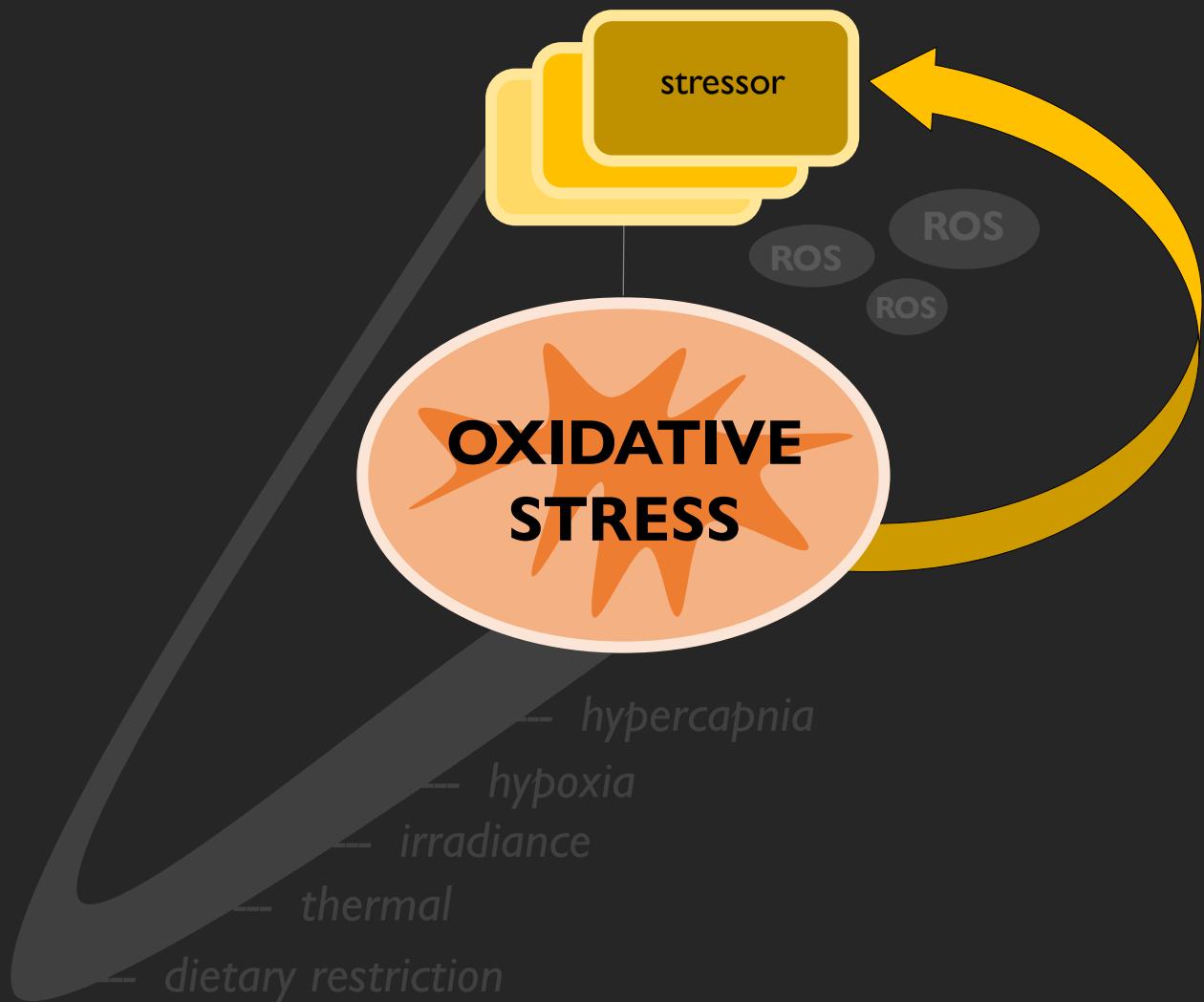


## Hypothesis

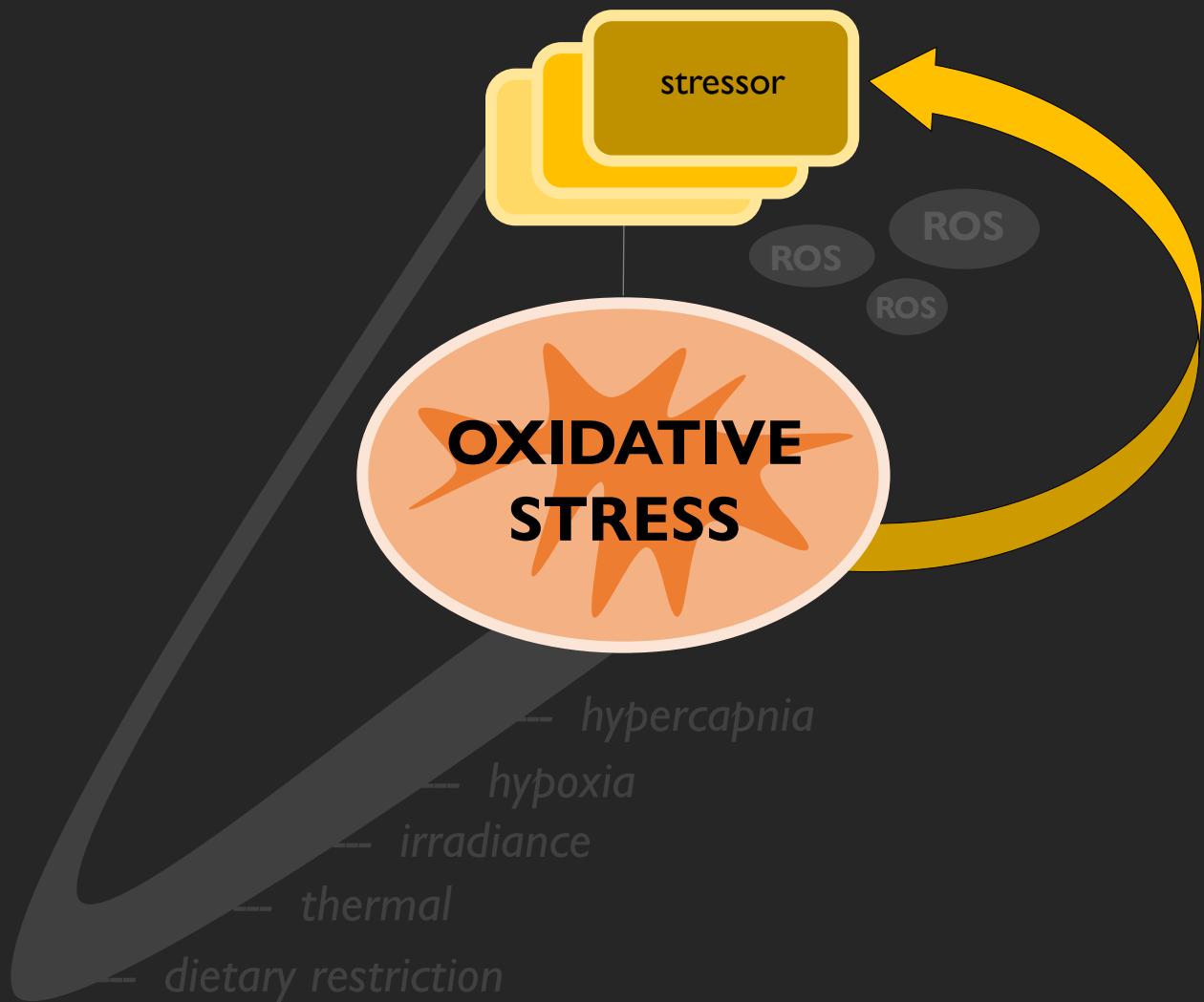
Early conditioning under elevated  $p\text{CO}_2$  enhances the ability to cope with subsequent exposures

# Chapter III





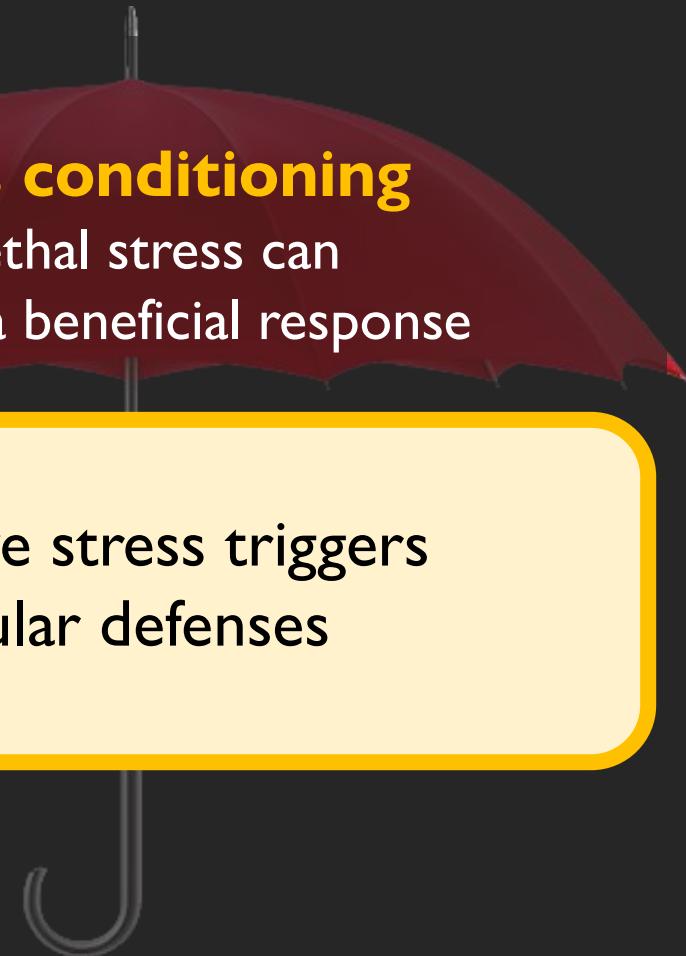
Wojtczyk-Miaskowska et al. 2018;  
Berry and López-Martínez 2020;  
Buttemer et al. 2010; Costantini et al. 2012



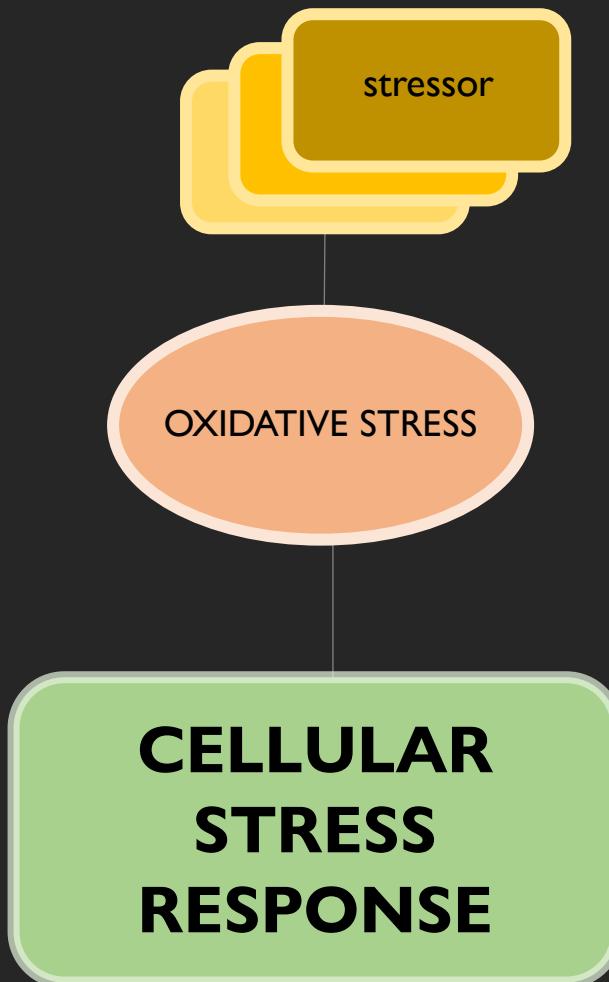
## Stress conditioning

sublethal stress can stimulate a beneficial response

oxidative stress triggers cellular defenses

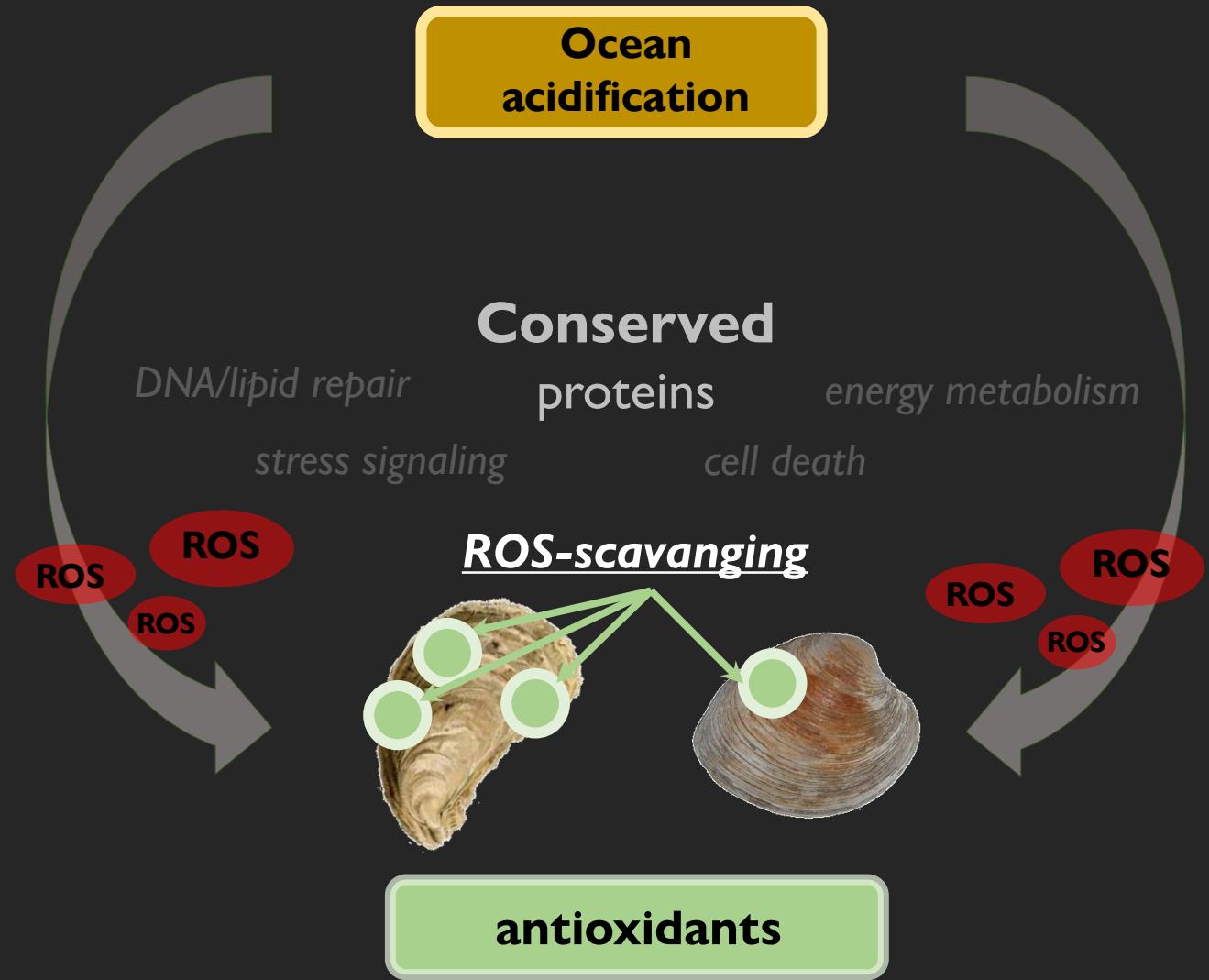
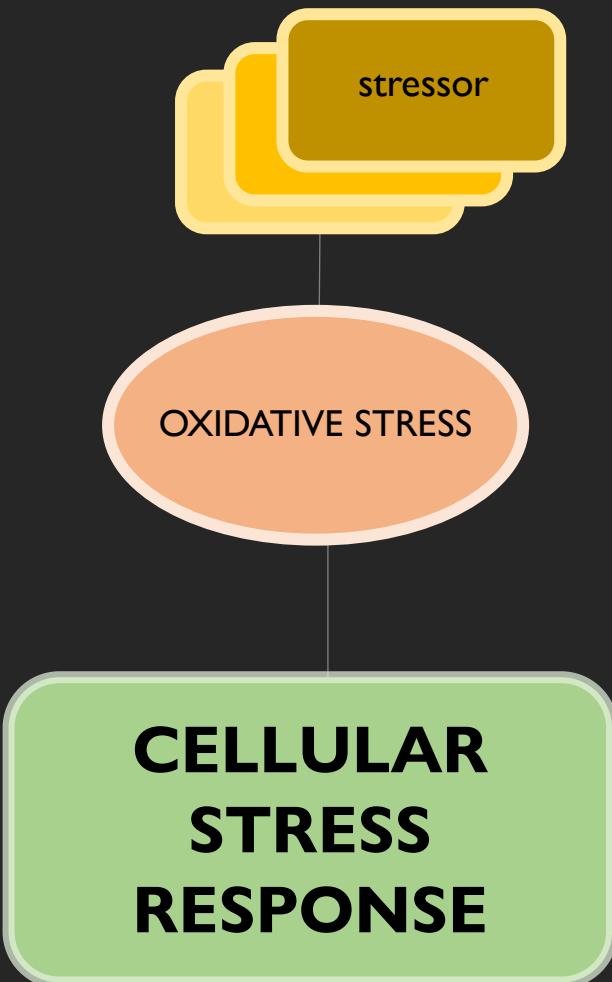


Wojtczyk-Miaskowska et al. 2018;  
Berry and López-Martínez 2020;  
Buttemer et al. 2010; Costantini et al. 2012



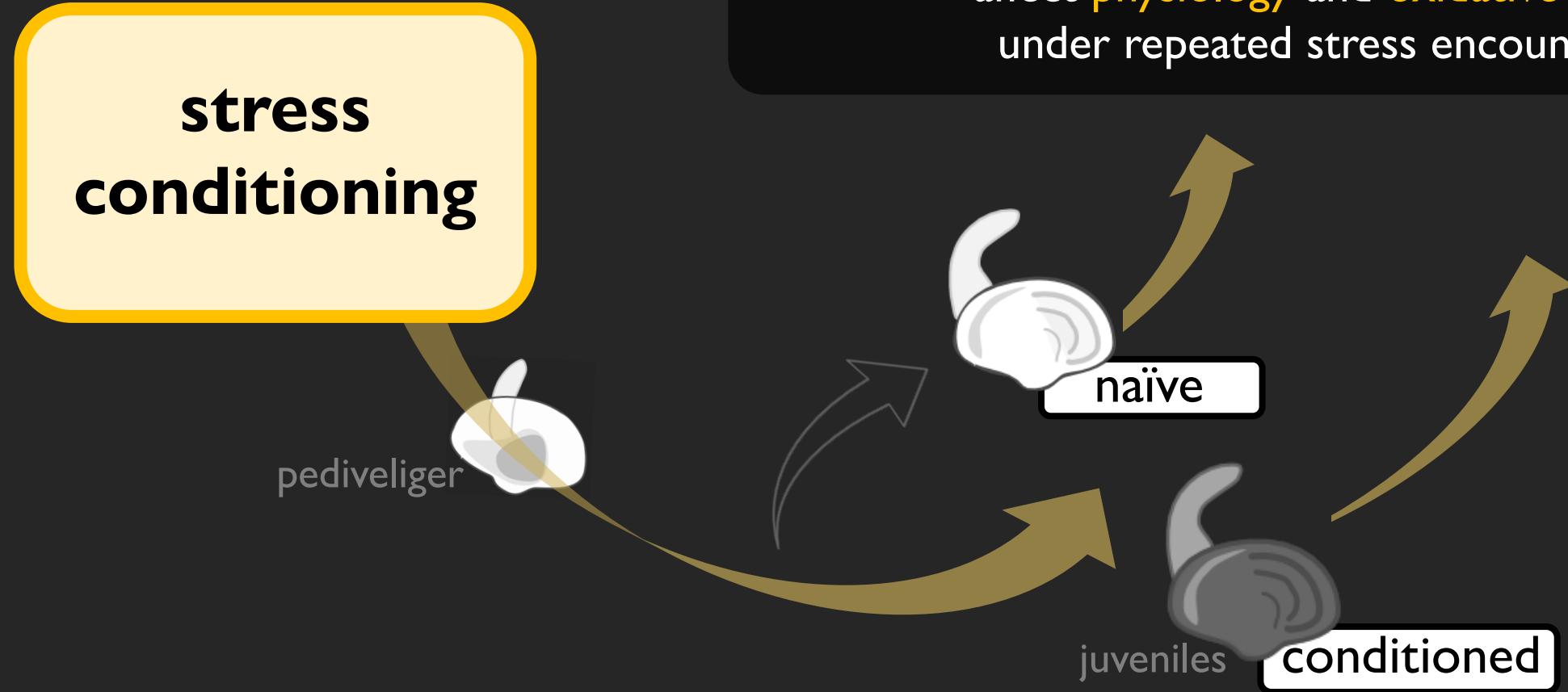
## Conserved

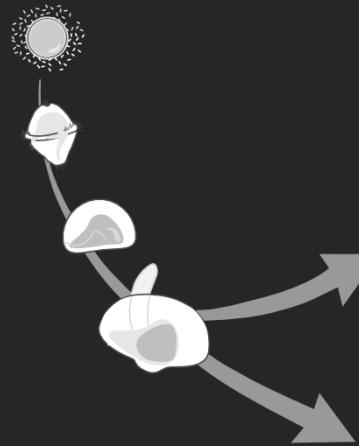
*DNA/lipid repair      proteins      energy metabolism*  
*stress signaling      cell death*  
*ROS-scavenging*



In this chapter...

Does **stress conditioning** over pediveliger-juvenile stages affect **physiology** and **oxidative status** under repeated stress encounters?



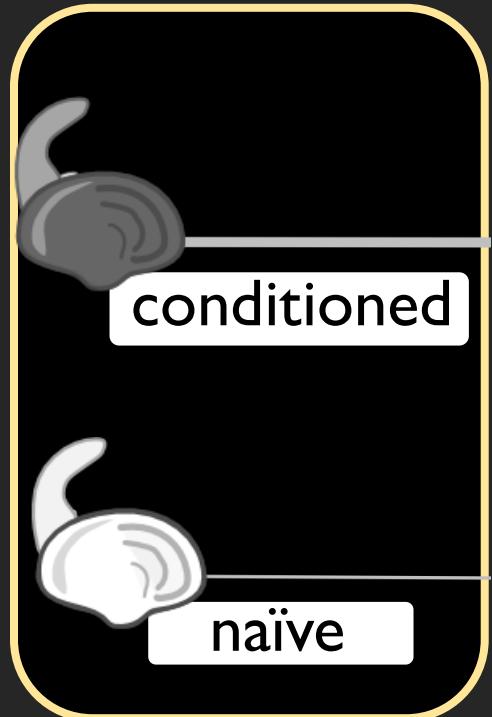


## stress conditioning



# Primary exposure

110 days



pCO<sub>2</sub> treatments

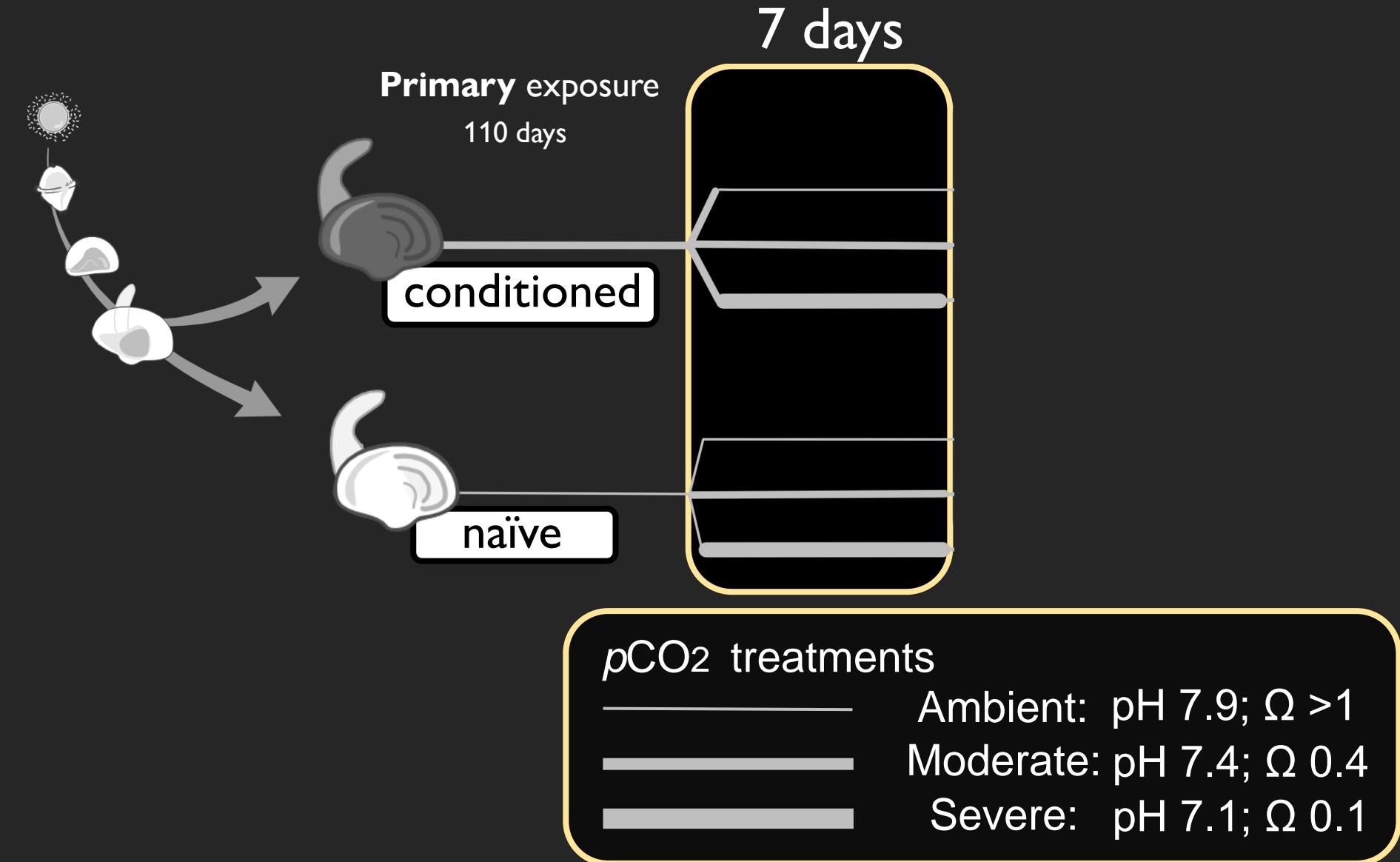
—

Ambient: pH 7.9;  $\Omega > 1$

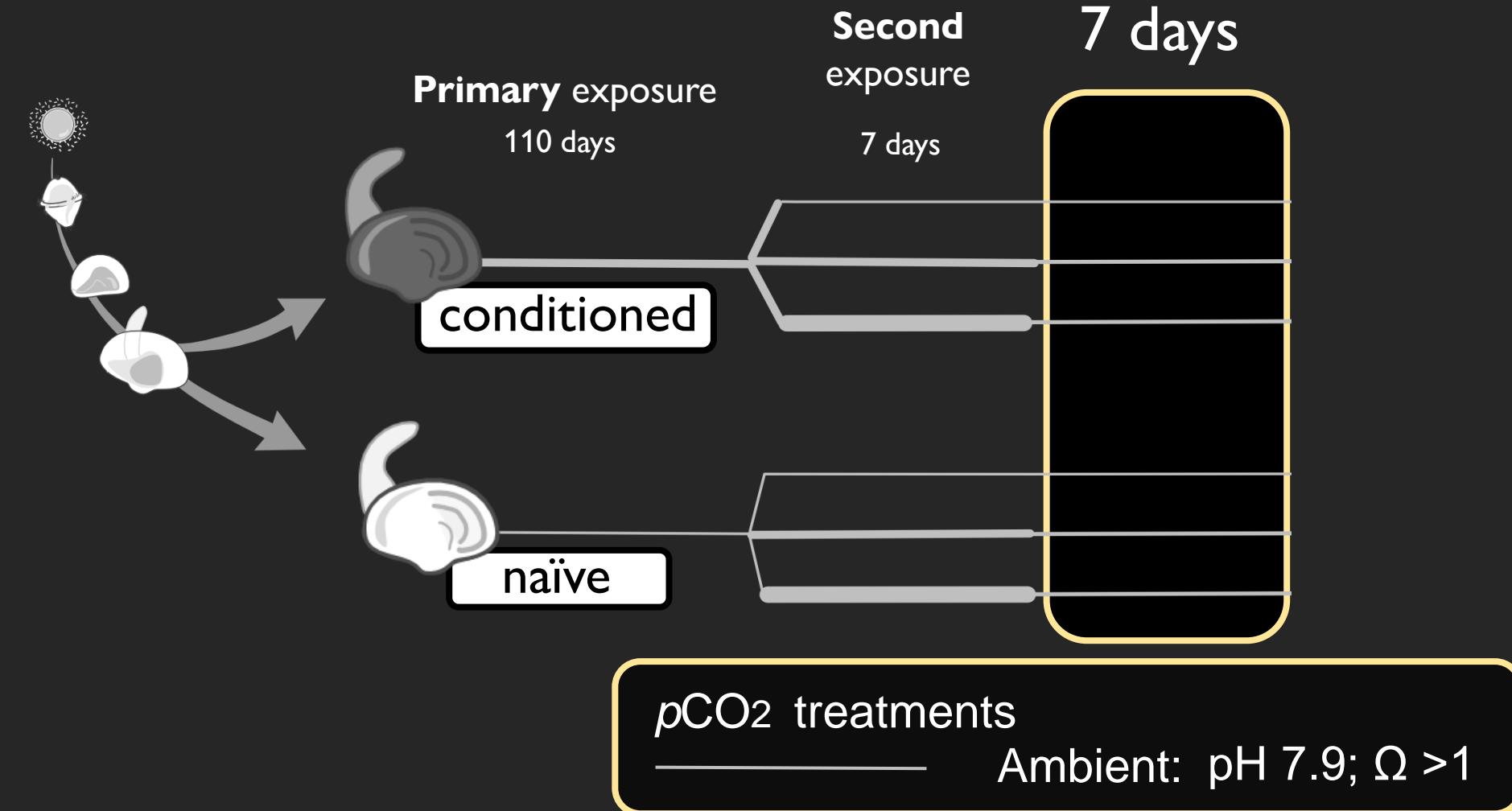
—

Moderate: pH 7.4;  $\Omega 0.4$

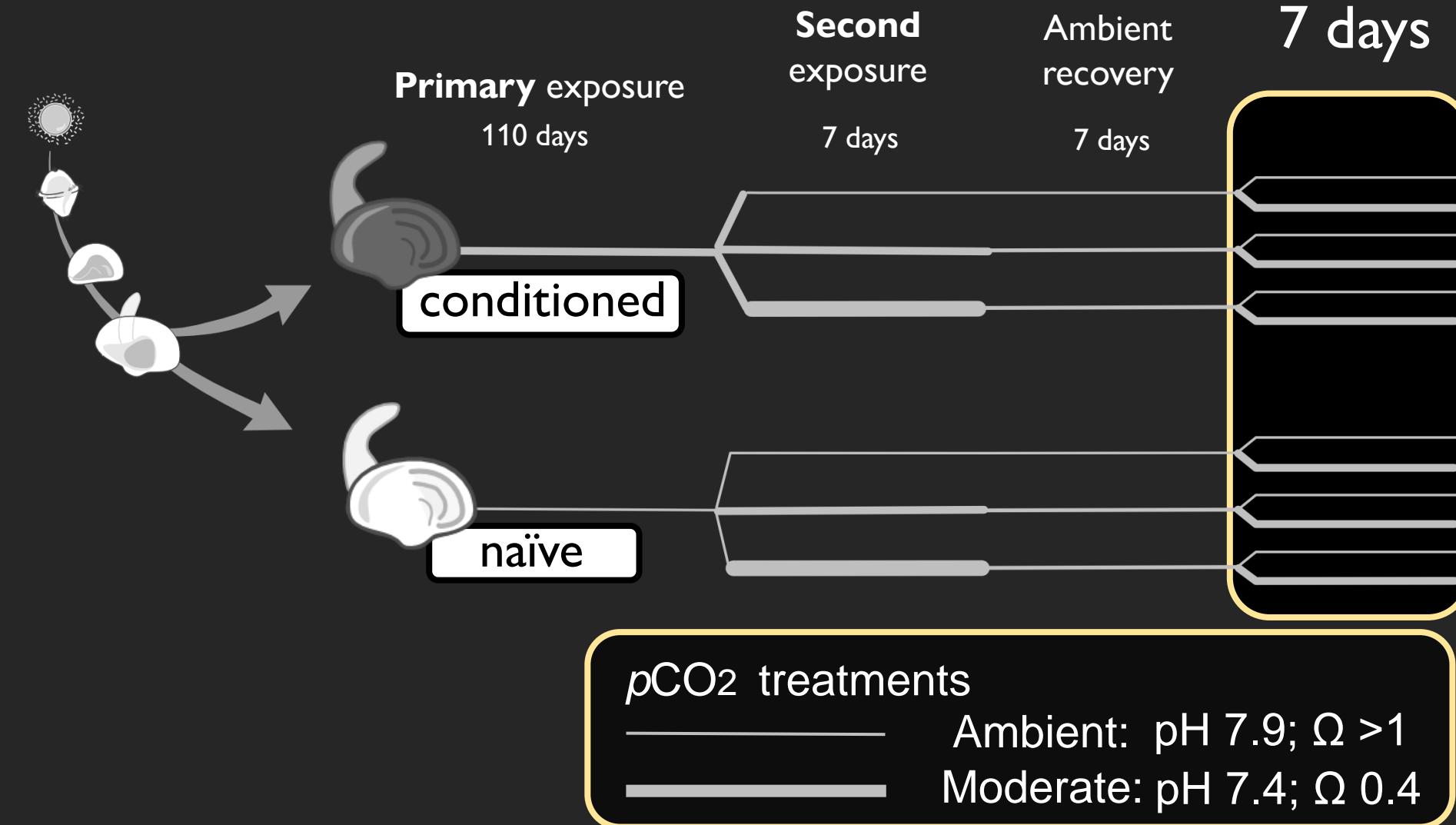
# Second exposure



# Ambient recovery

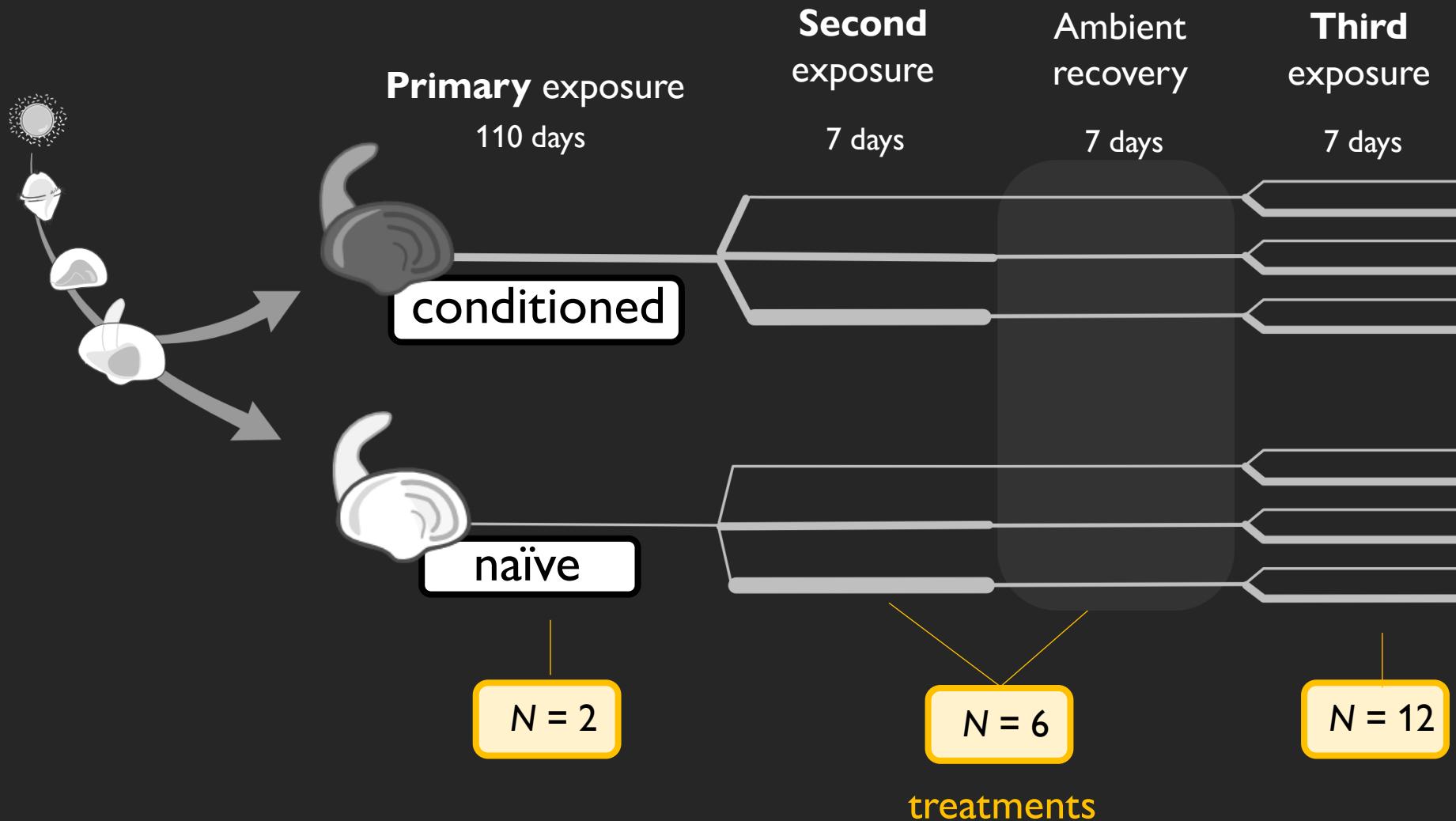


# Third exposure



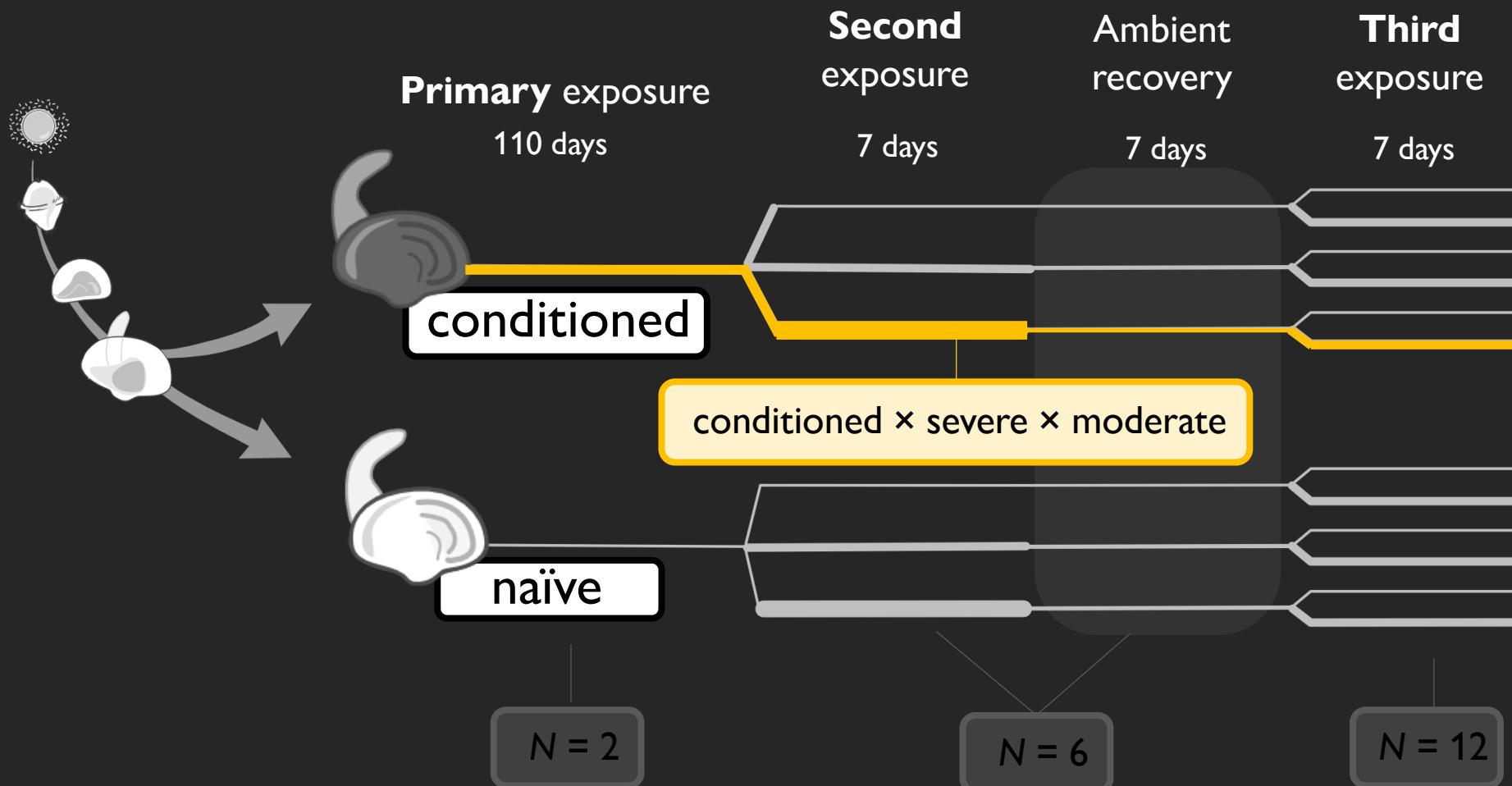
# Conditioning period

# Repeat exposures



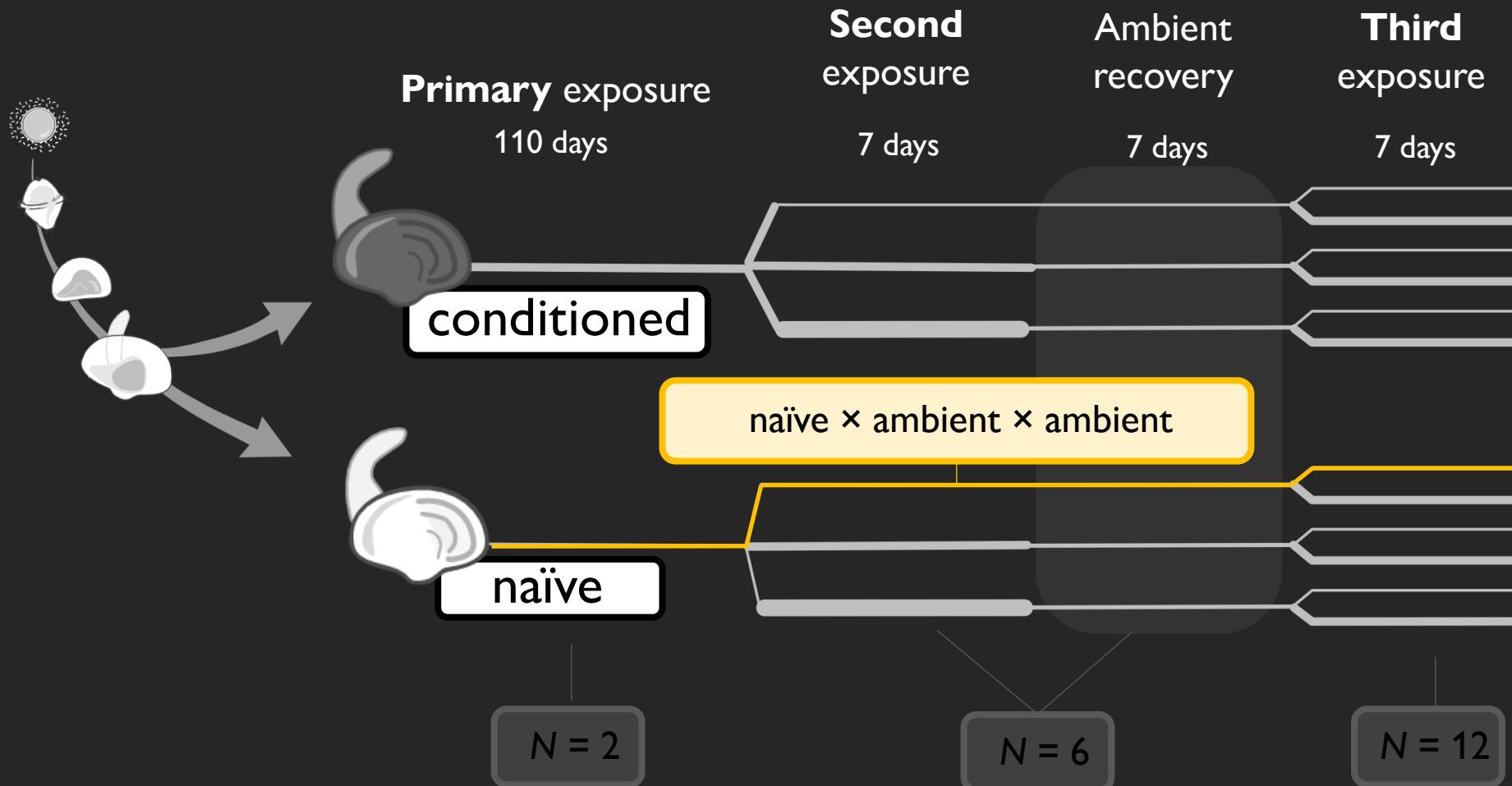
# Conditioning period

# Repeat exposures



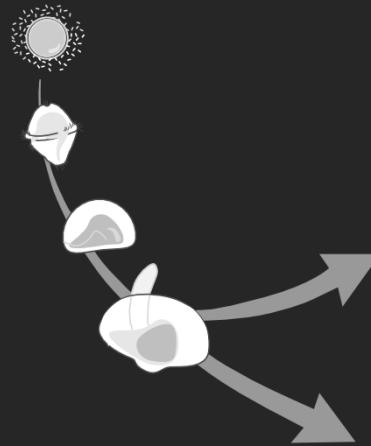
# Conditioning period

# Repeat exposures



# Conditioning period

# Repeat exposures



**Primary exposure**  
110 days

**conditioned**



**naïve**

$N = 2$

**Second**  
exposure

Ambient  
recovery

**Third**  
exposure

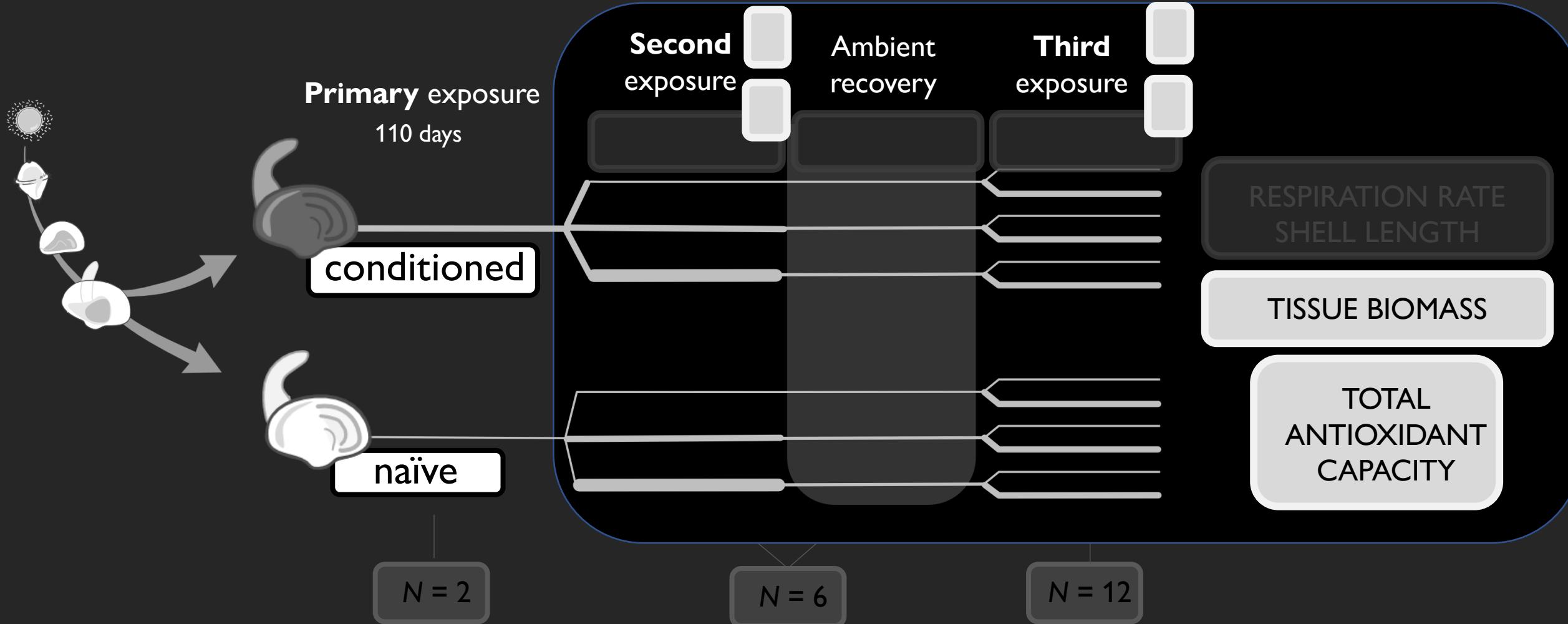
**RESPIRATION RATE  
SHELL LENGTH**

$N = 6$

$N = 12$

# Conditioning period

# Repeat exposures

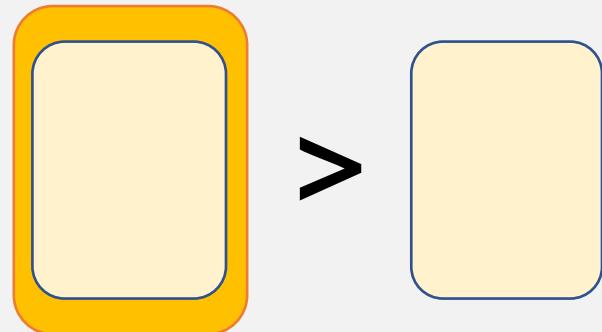


# RESULTS

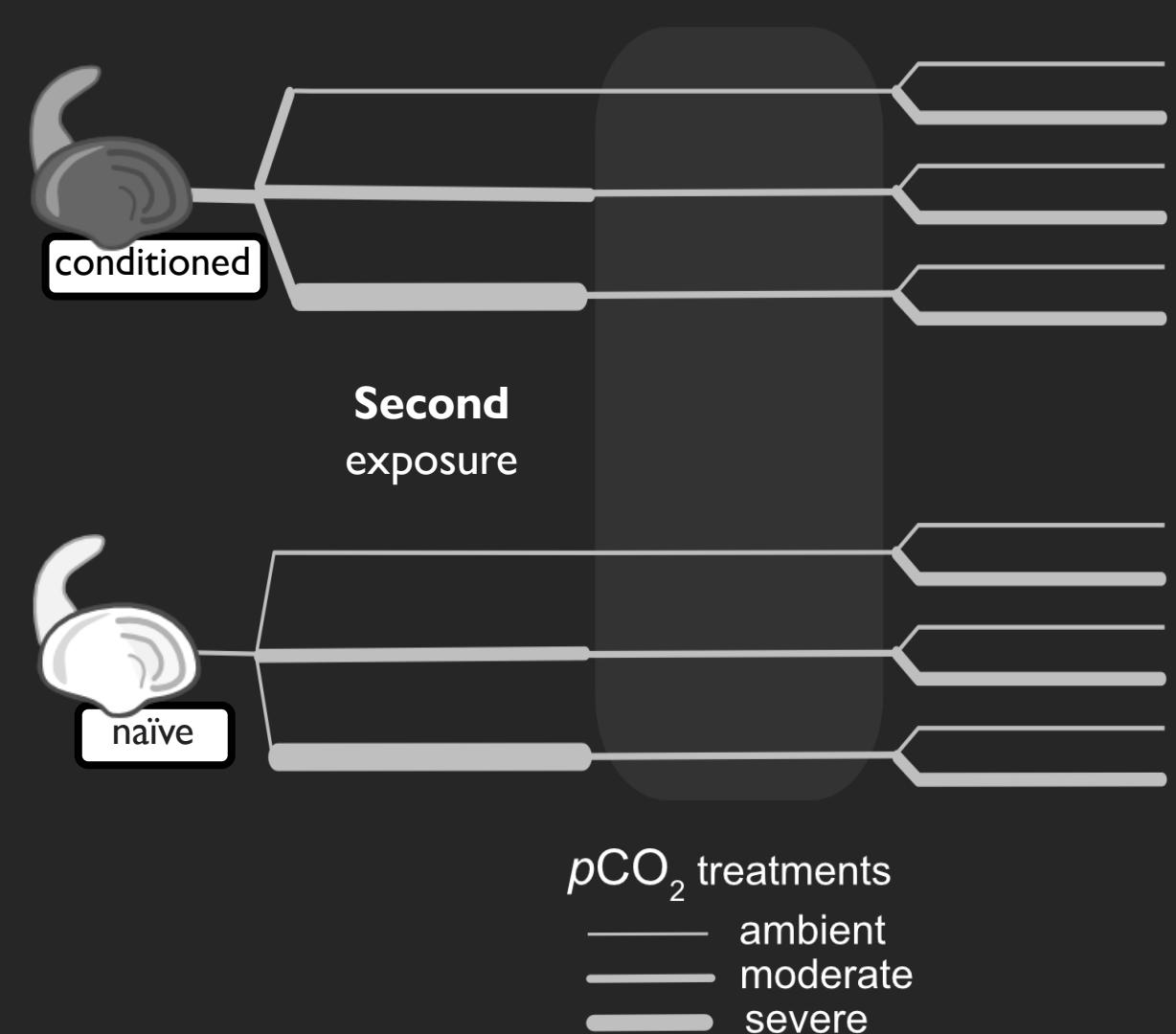
Schematic

Data

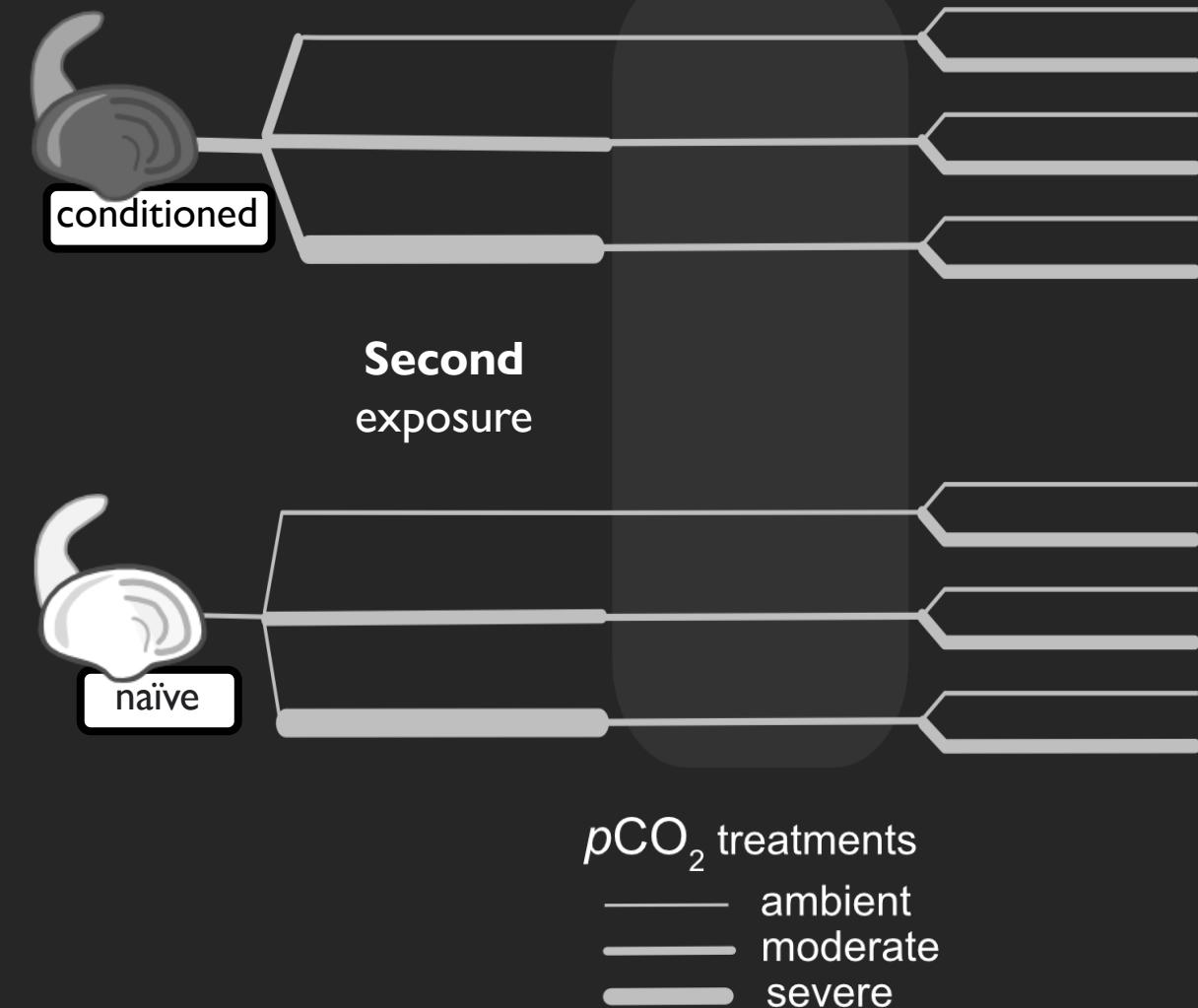
significant  
effects



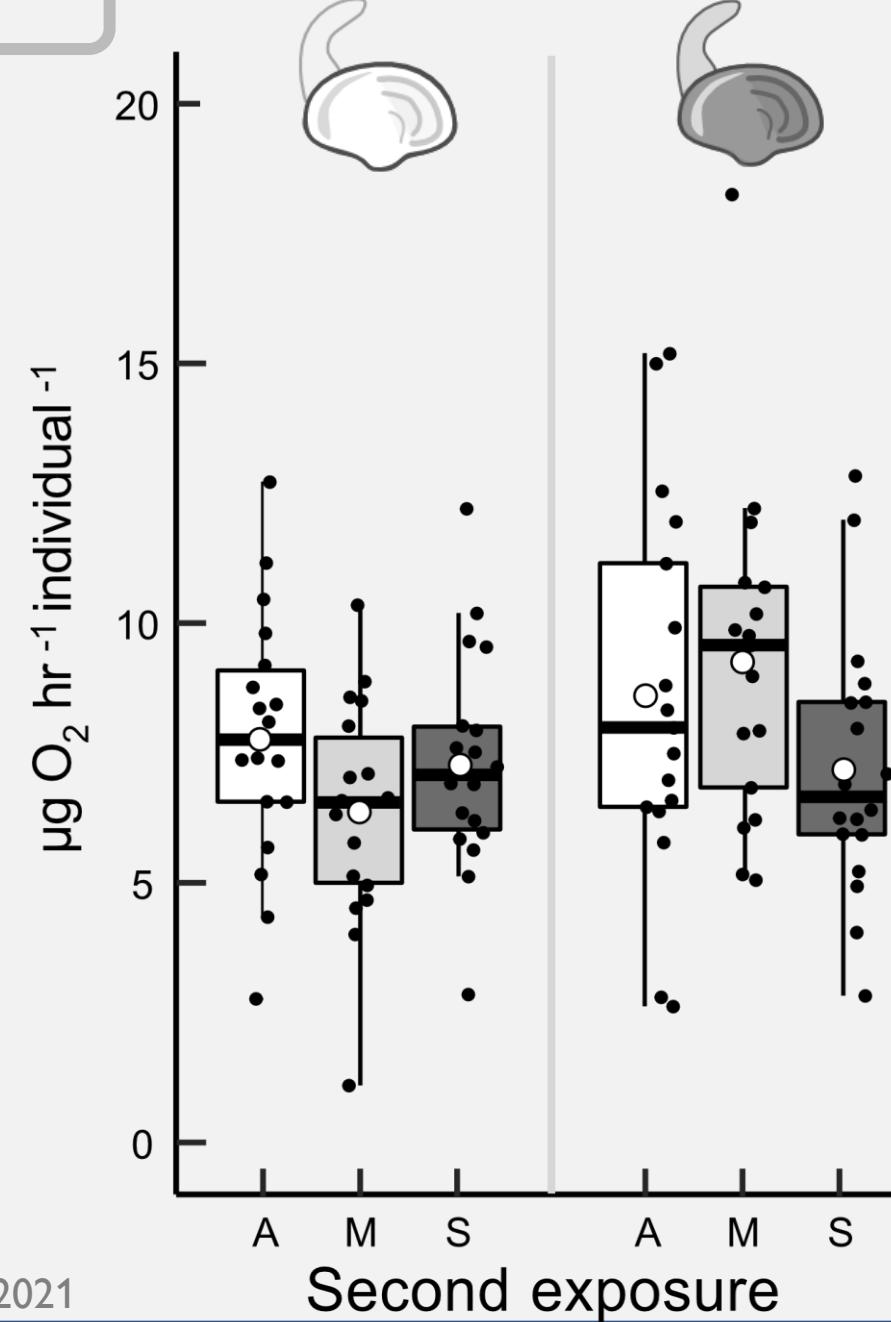
# PHYSIOLOGY



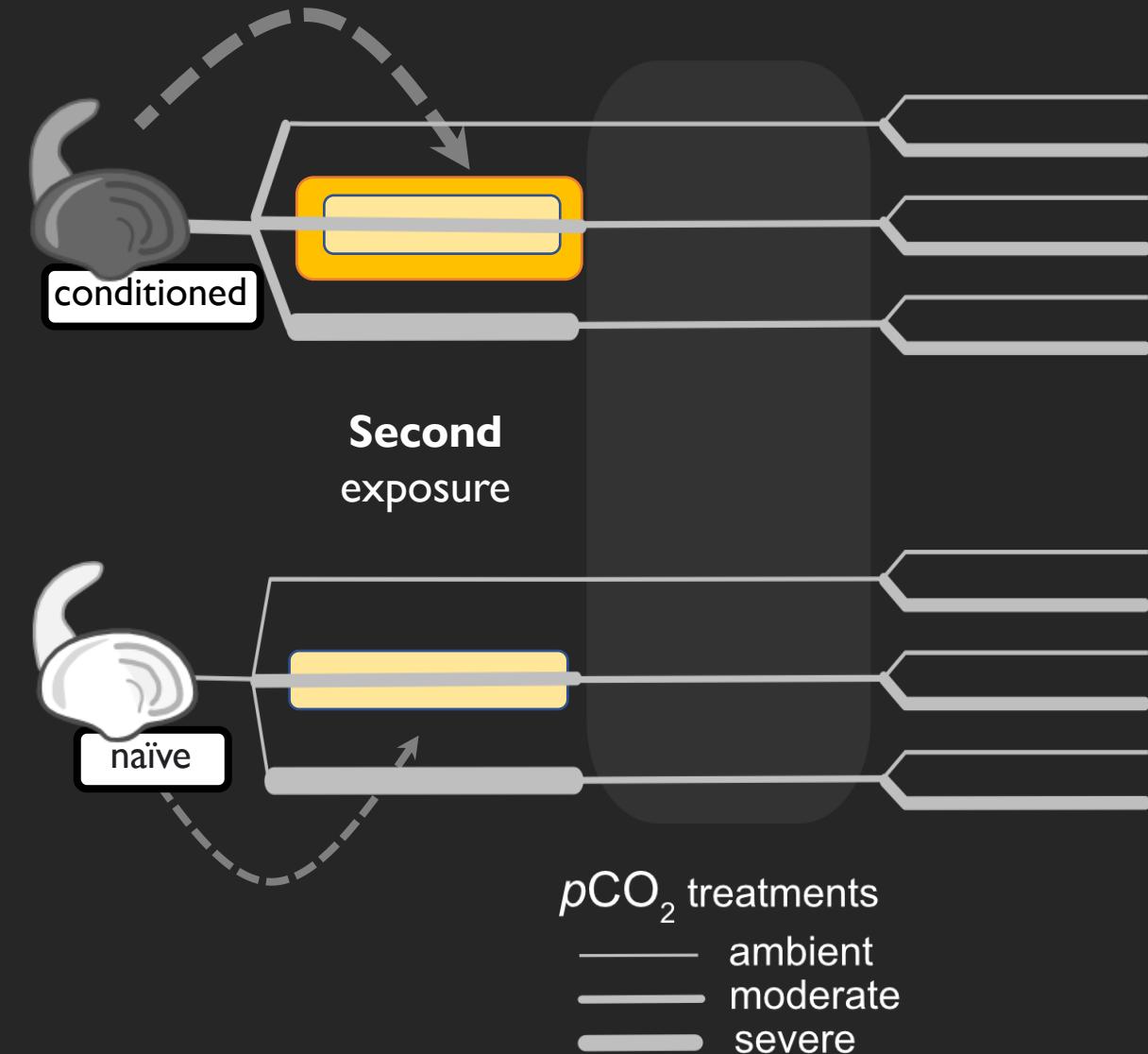
## PHYSIOLOGY



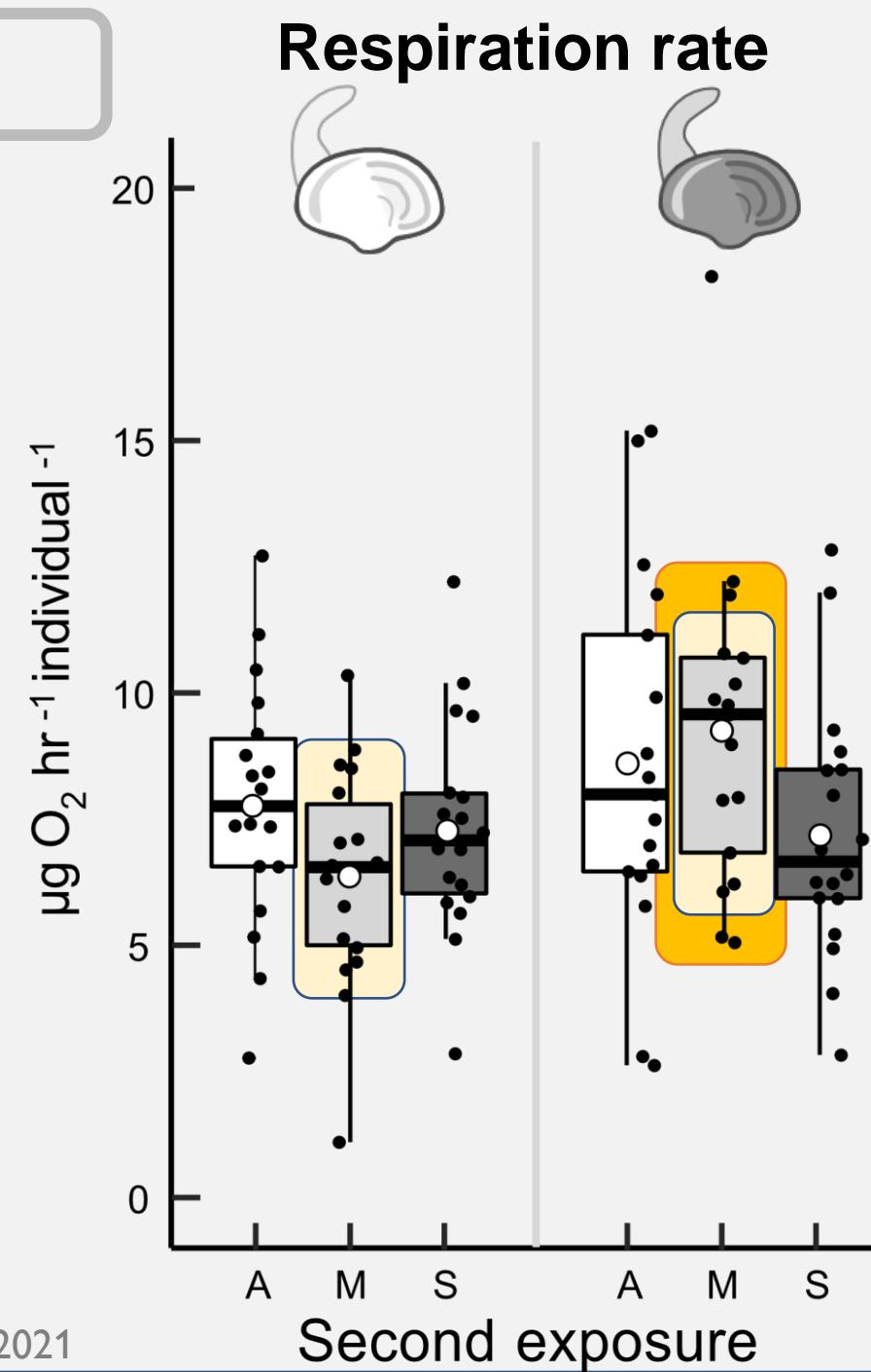
## Respiration rate



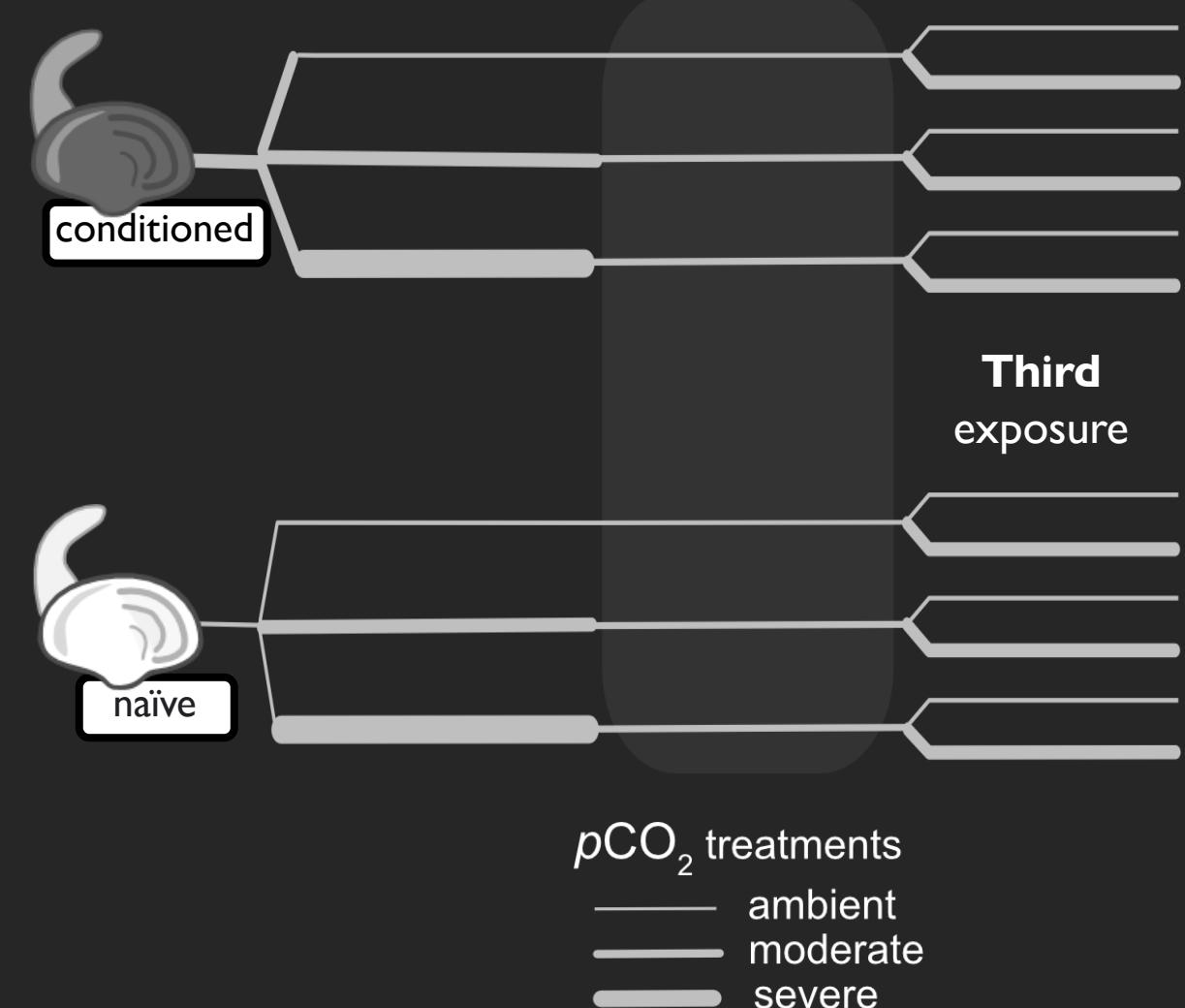
## PHYSIOLOGY



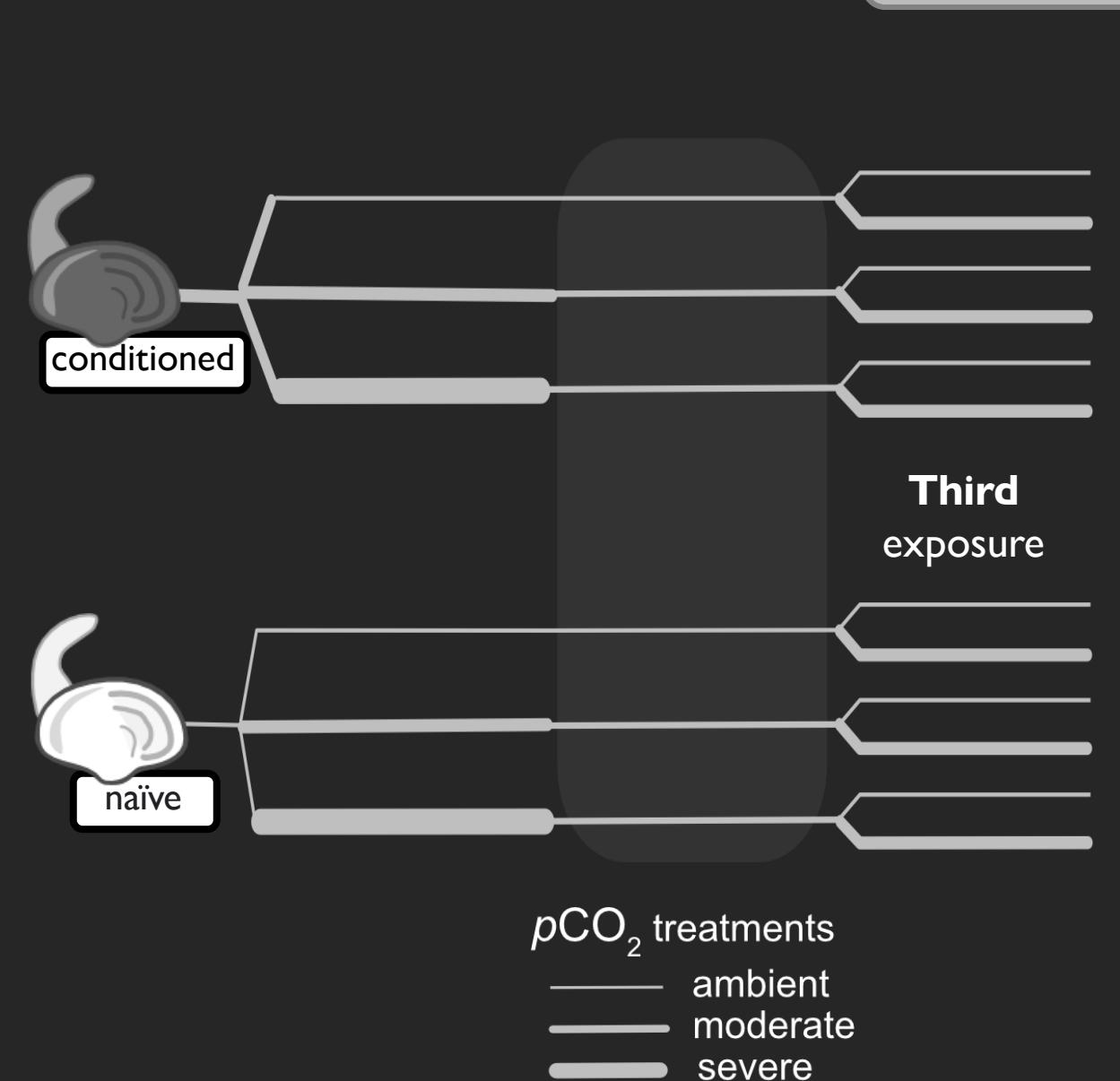
## Respiration rate



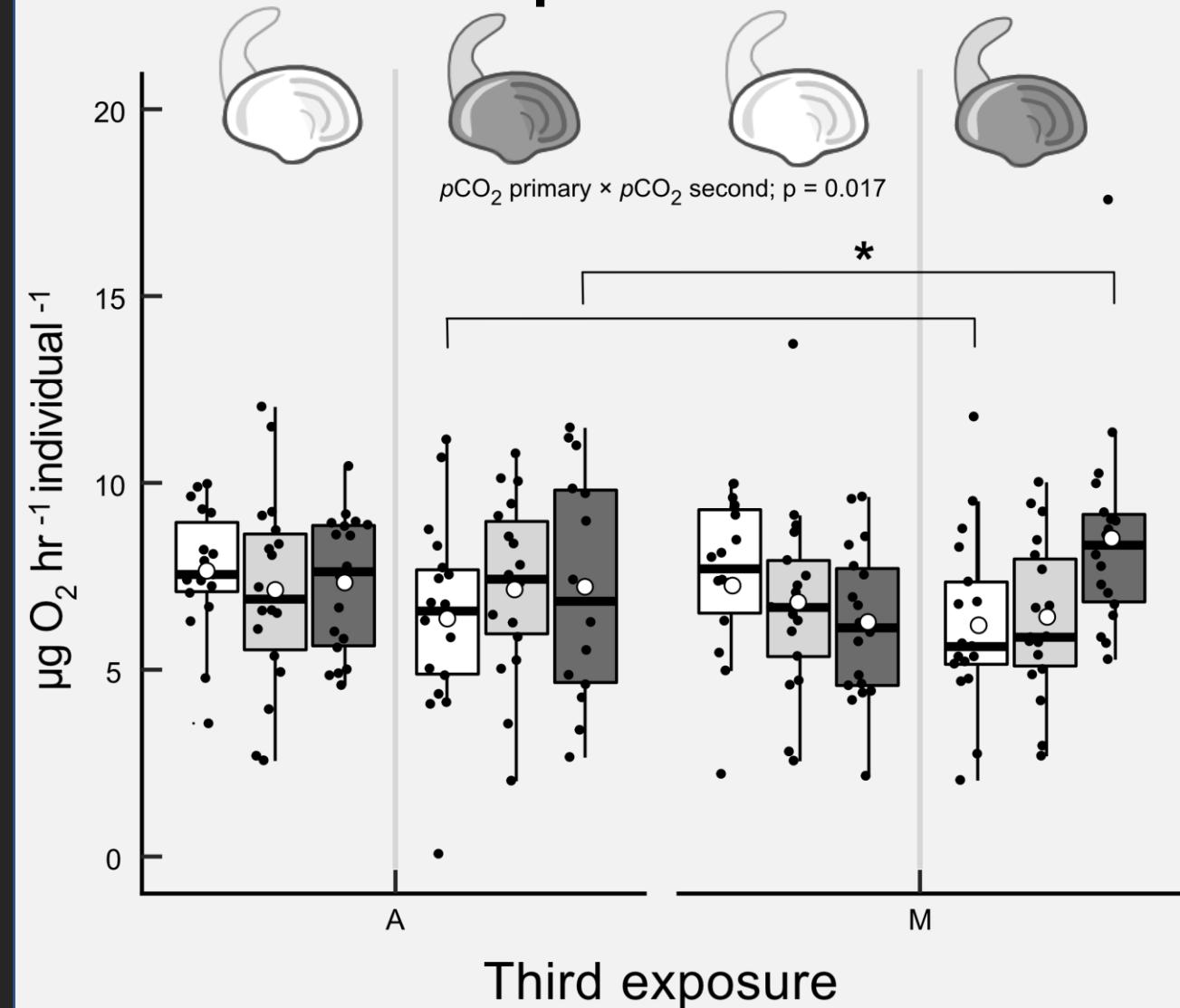
# PHYSIOLOGY



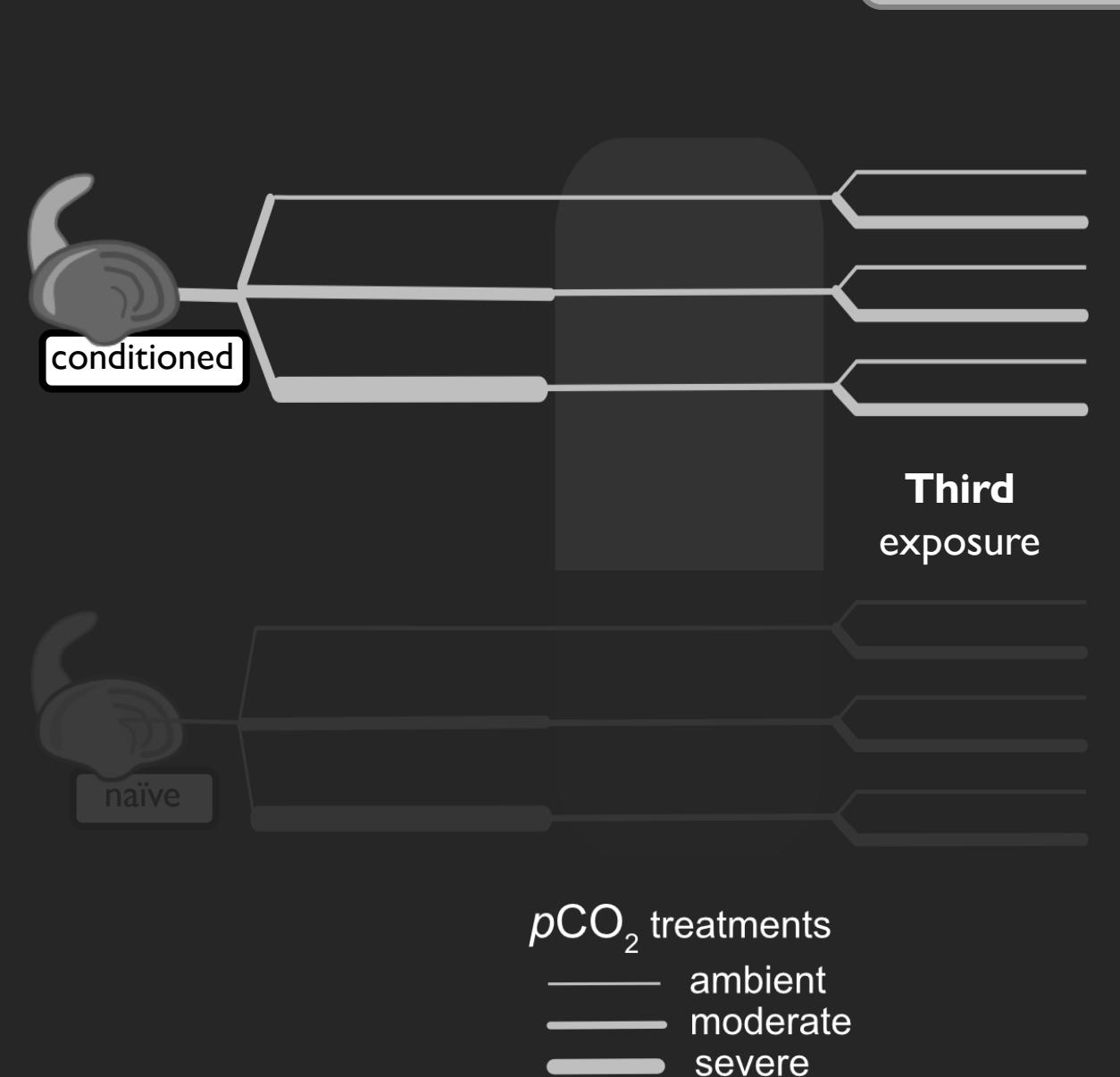
# PHYSIOLOGY



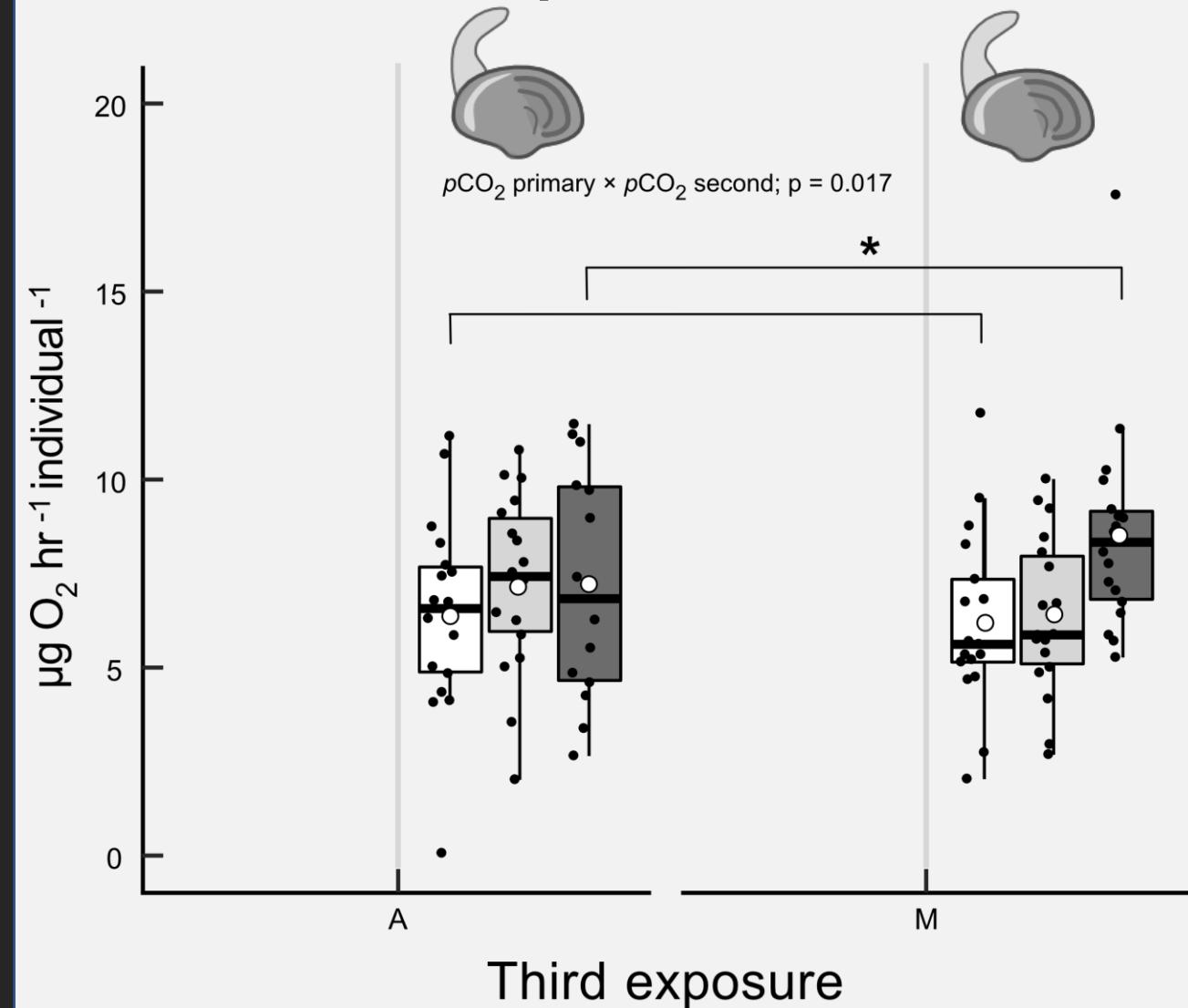
## Respiration rate



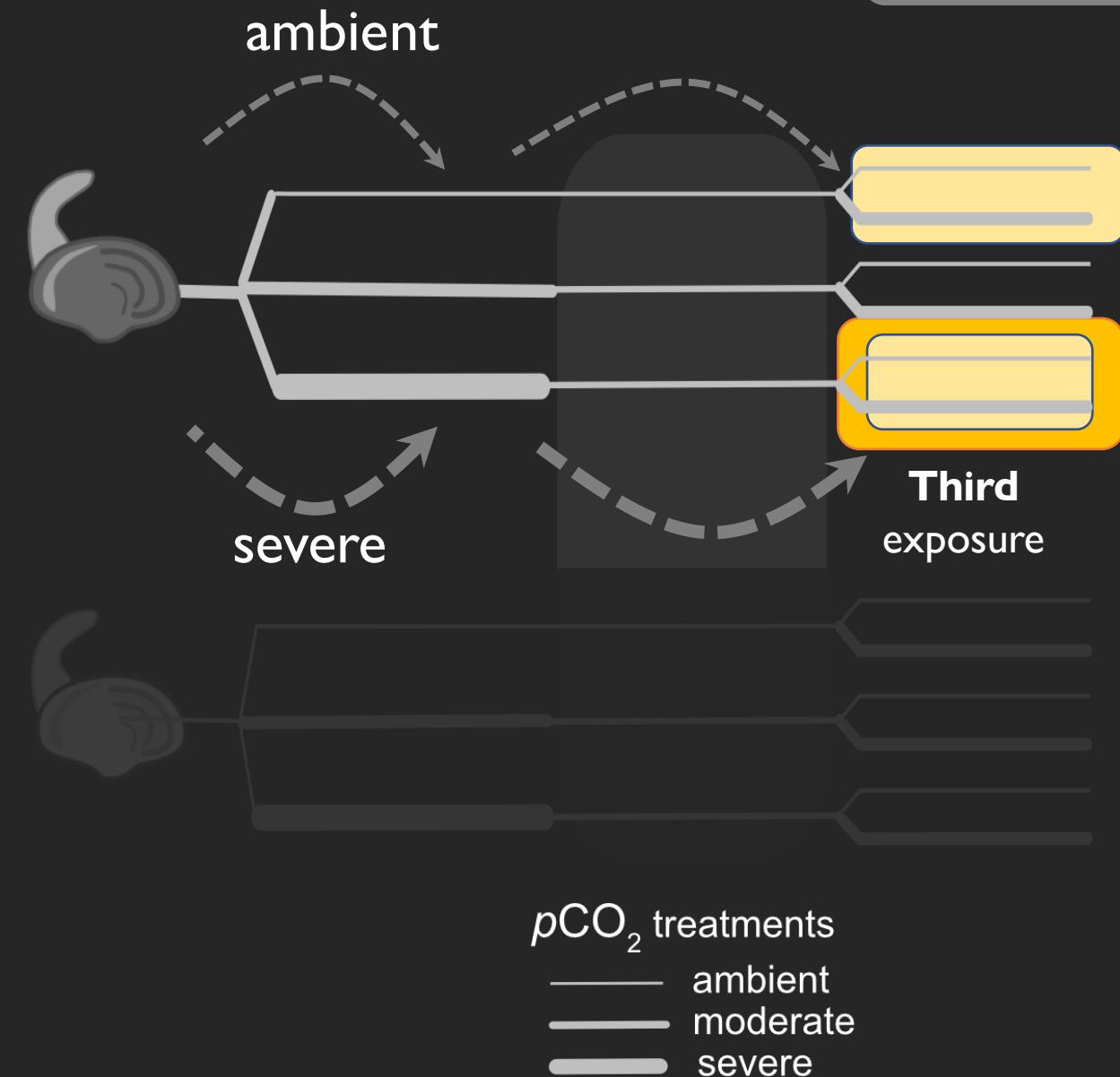
# PHYSIOLOGY



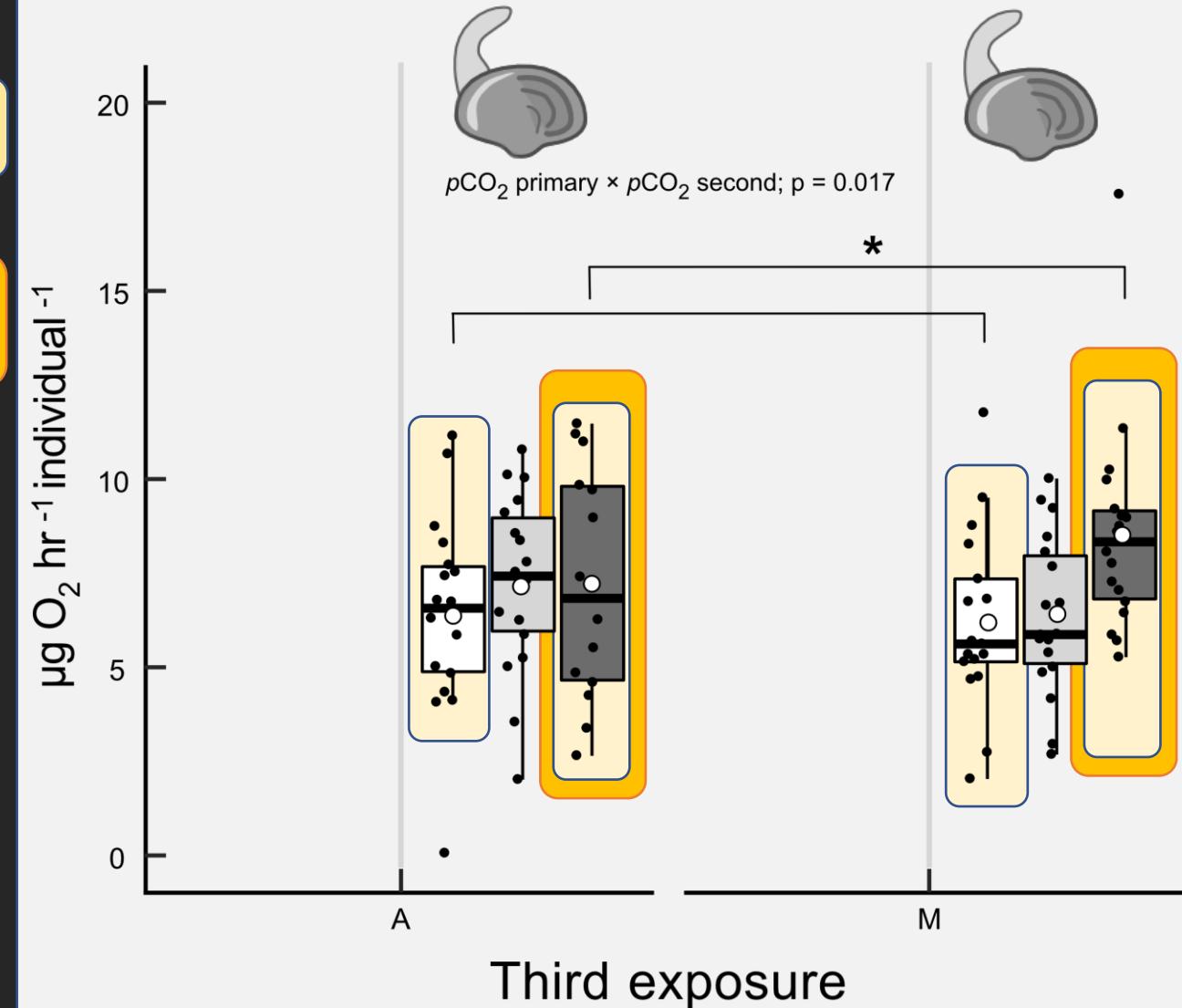
## Respiration rate



# PHYSIOLOGY



## Respiration rate

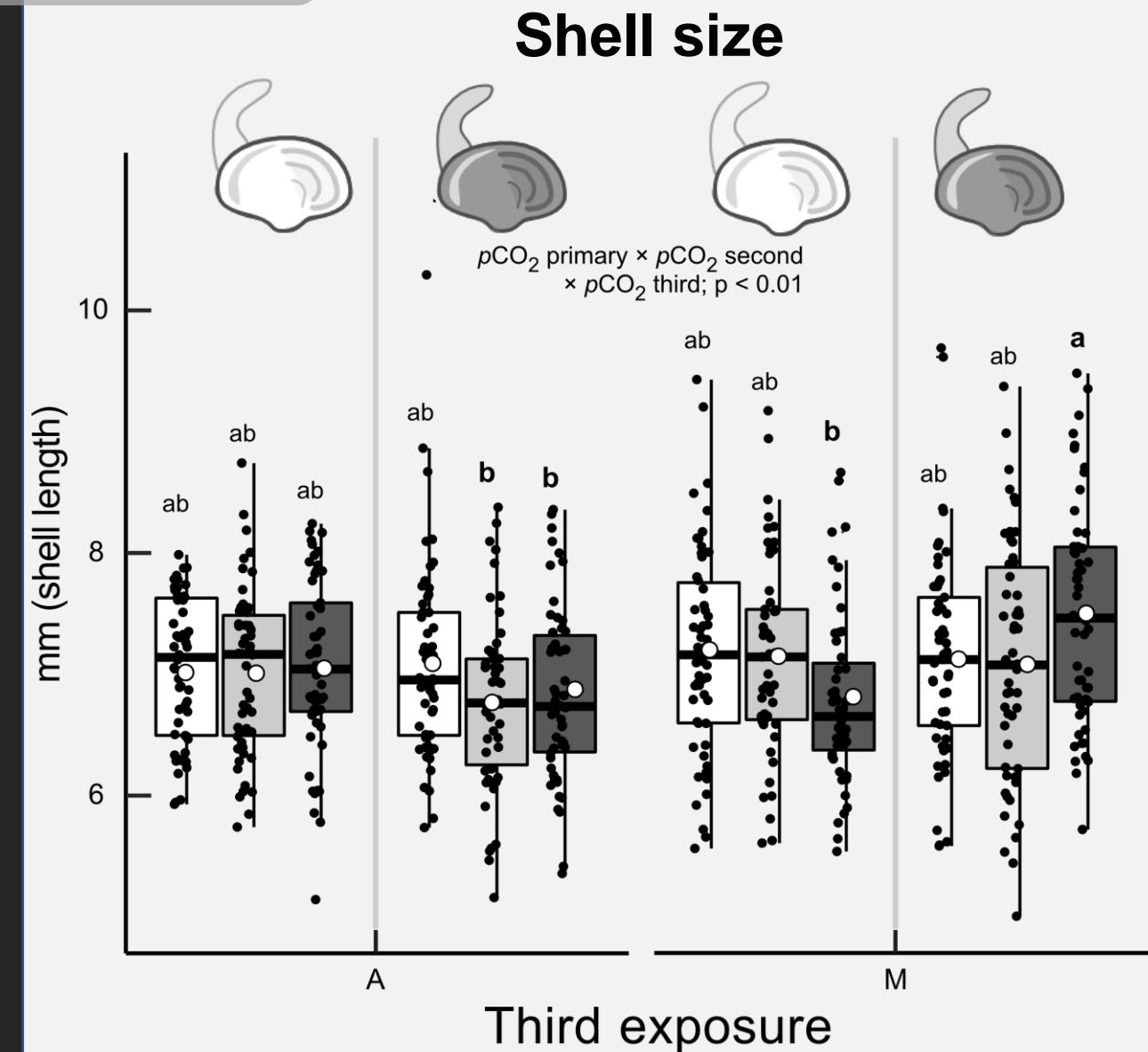
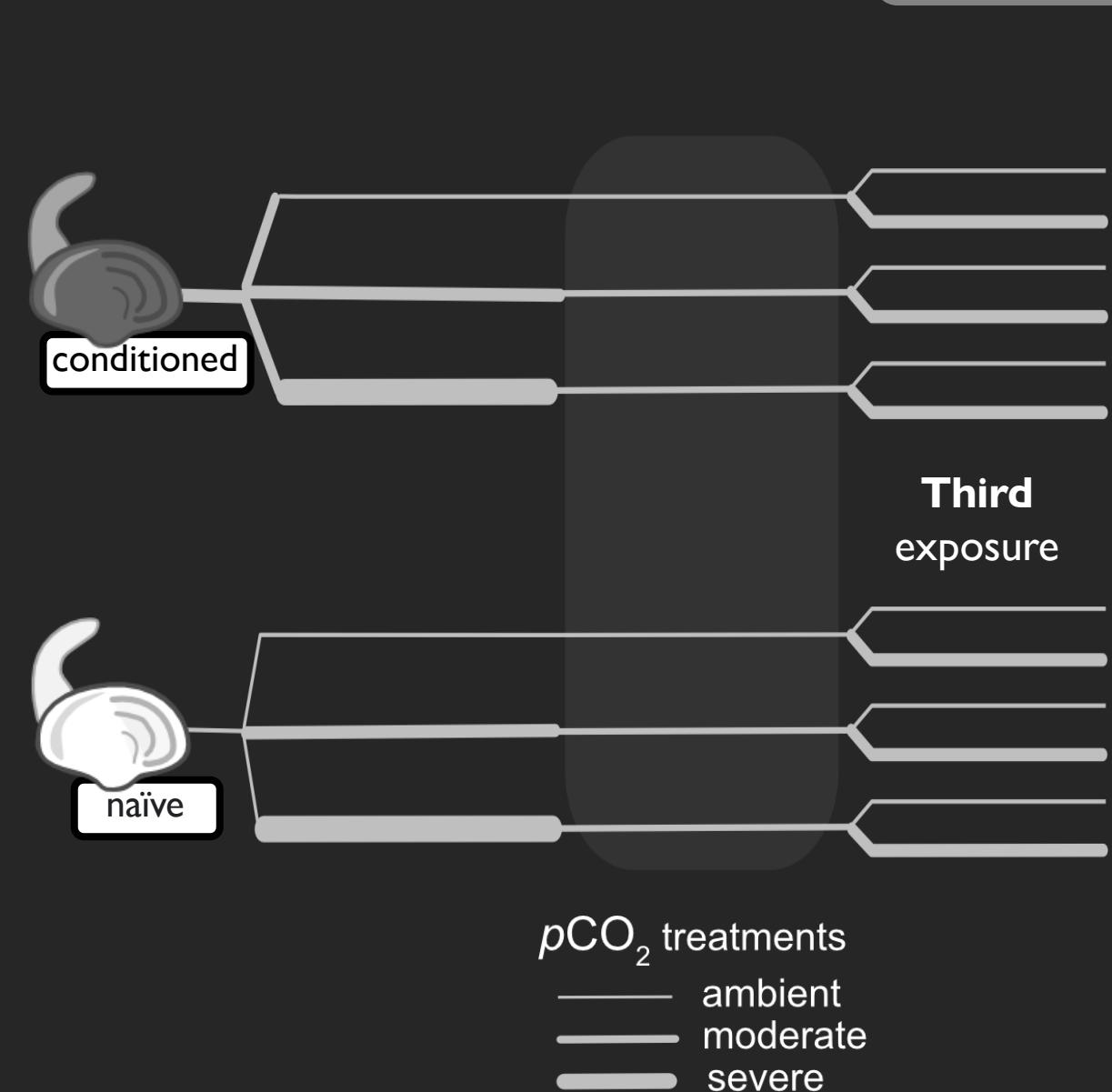


## PHYSIOLOGY

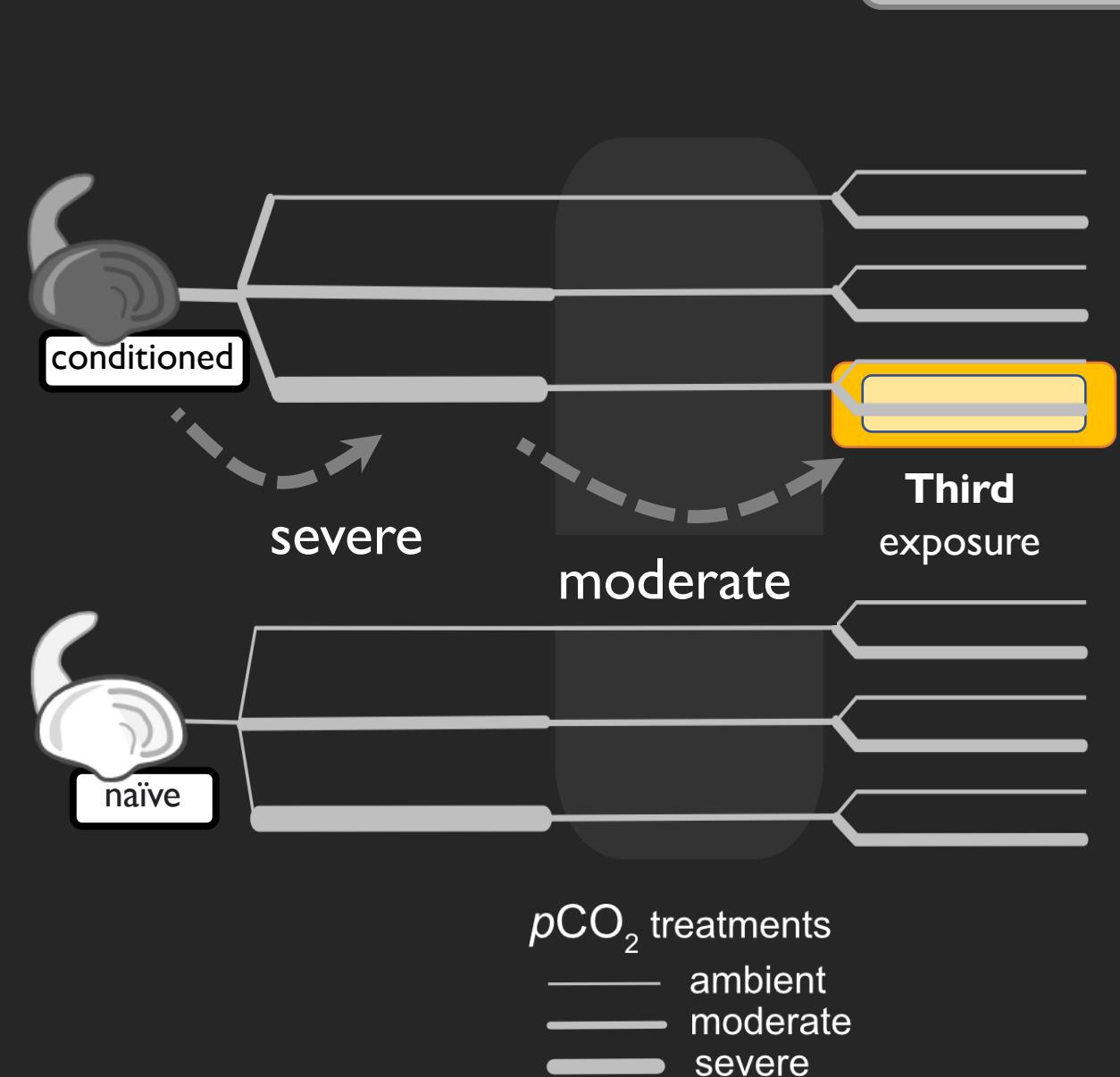
### Respiration rate

Post larval **stress conditioning** and repeated stress exposure  
to hypercapnic seawater **increases respiration rate**  
in juvenile clams

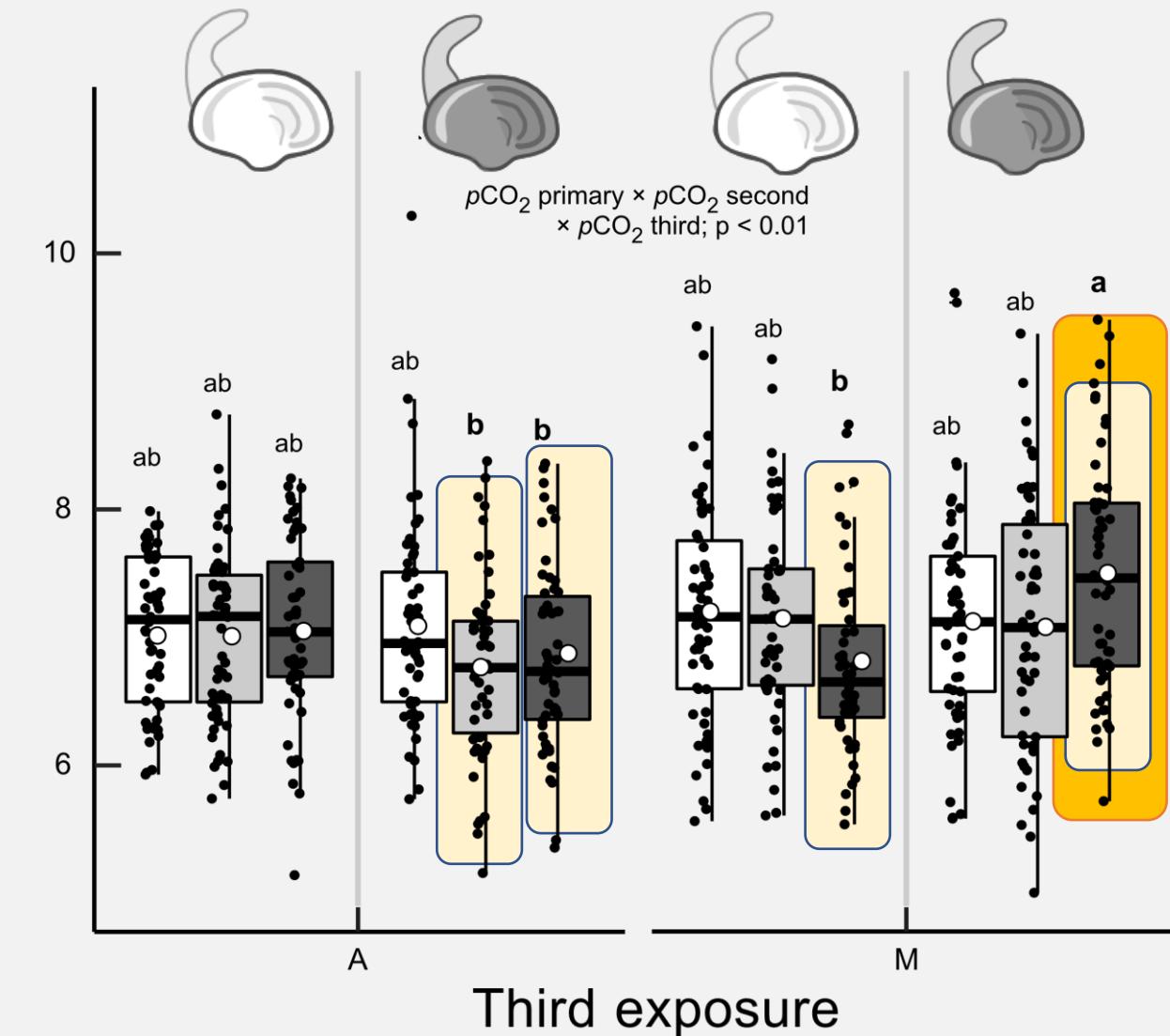
# PHYSIOLOGY



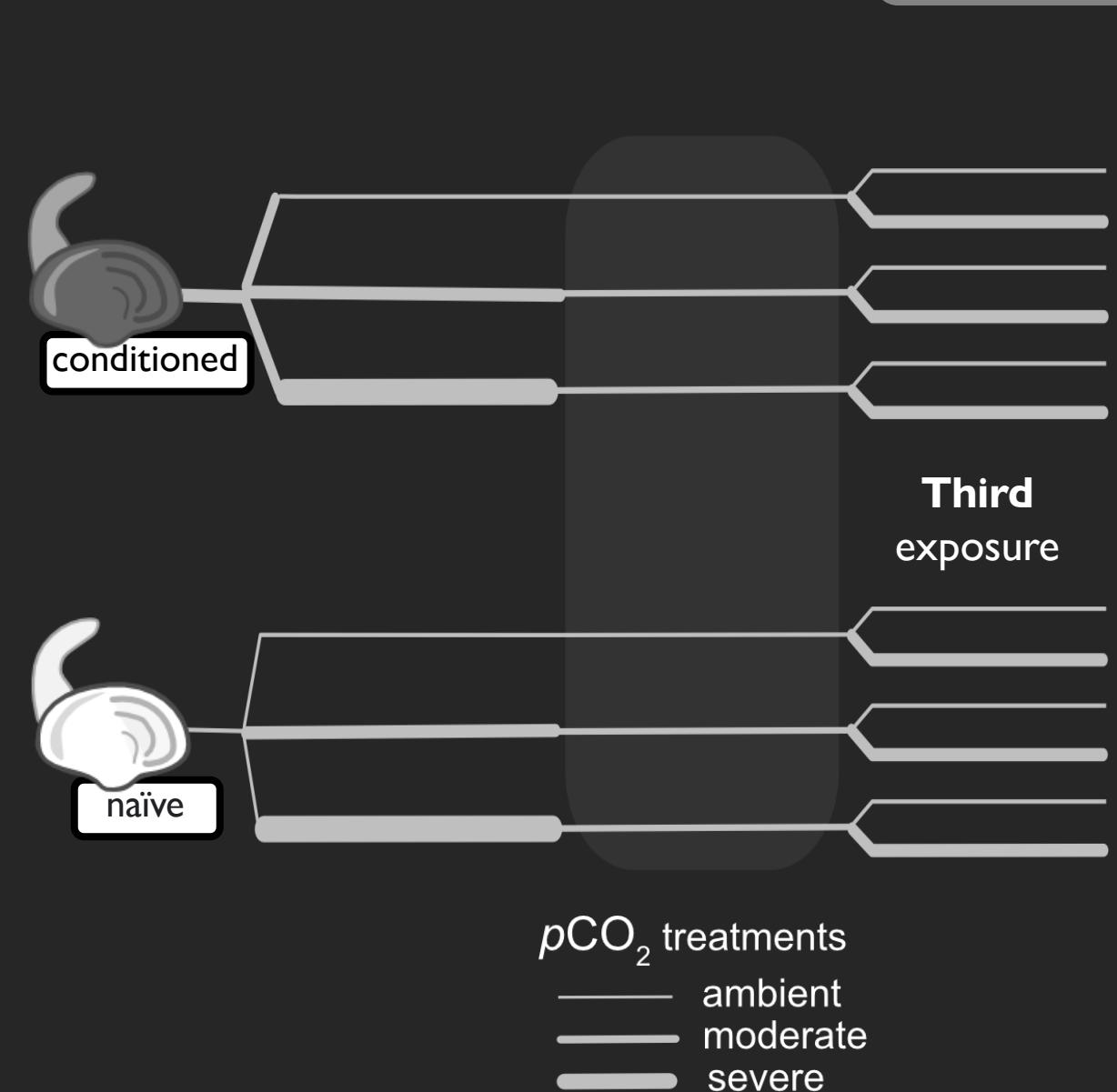
# PHYSIOLOGY



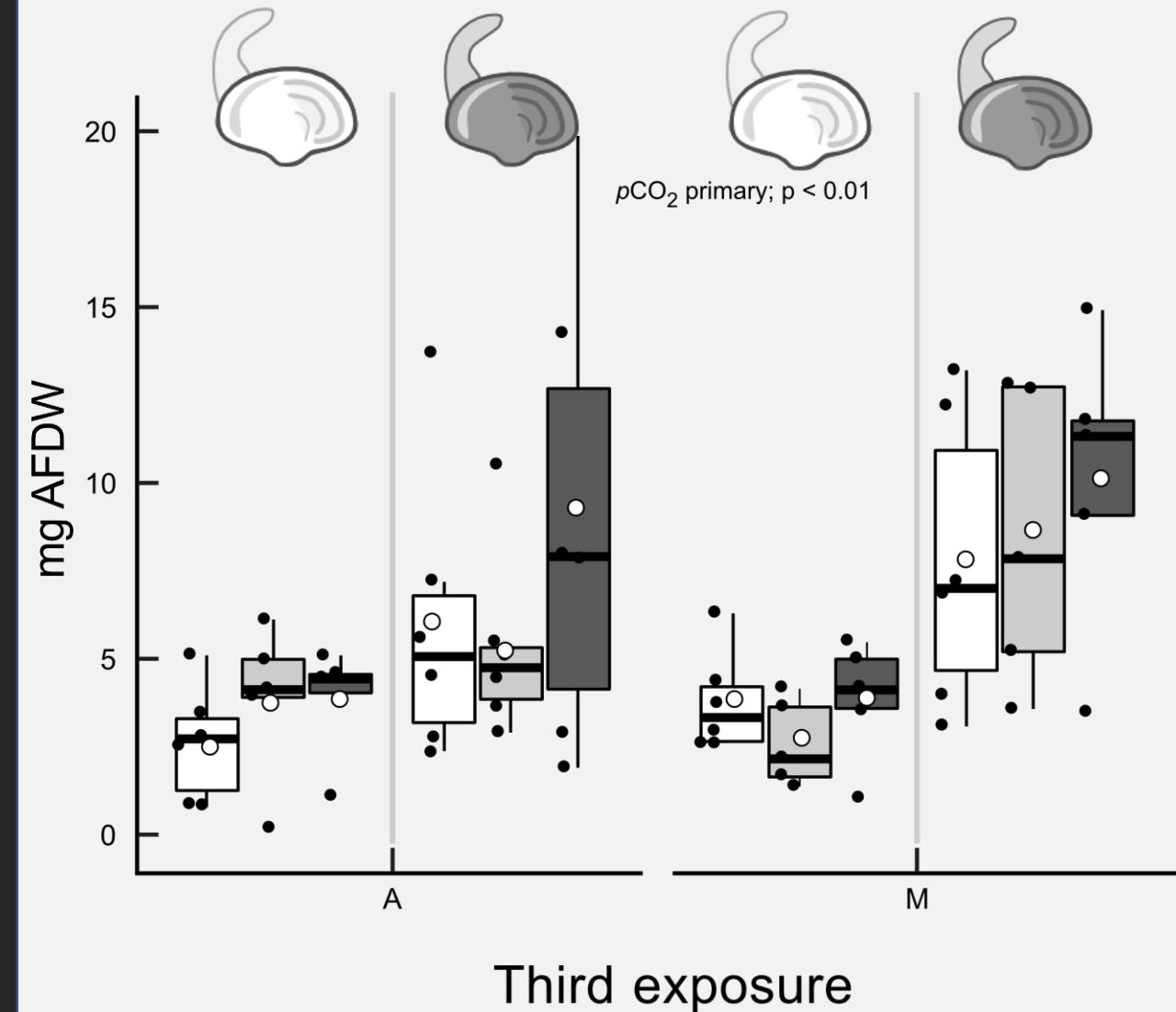
## Shell size



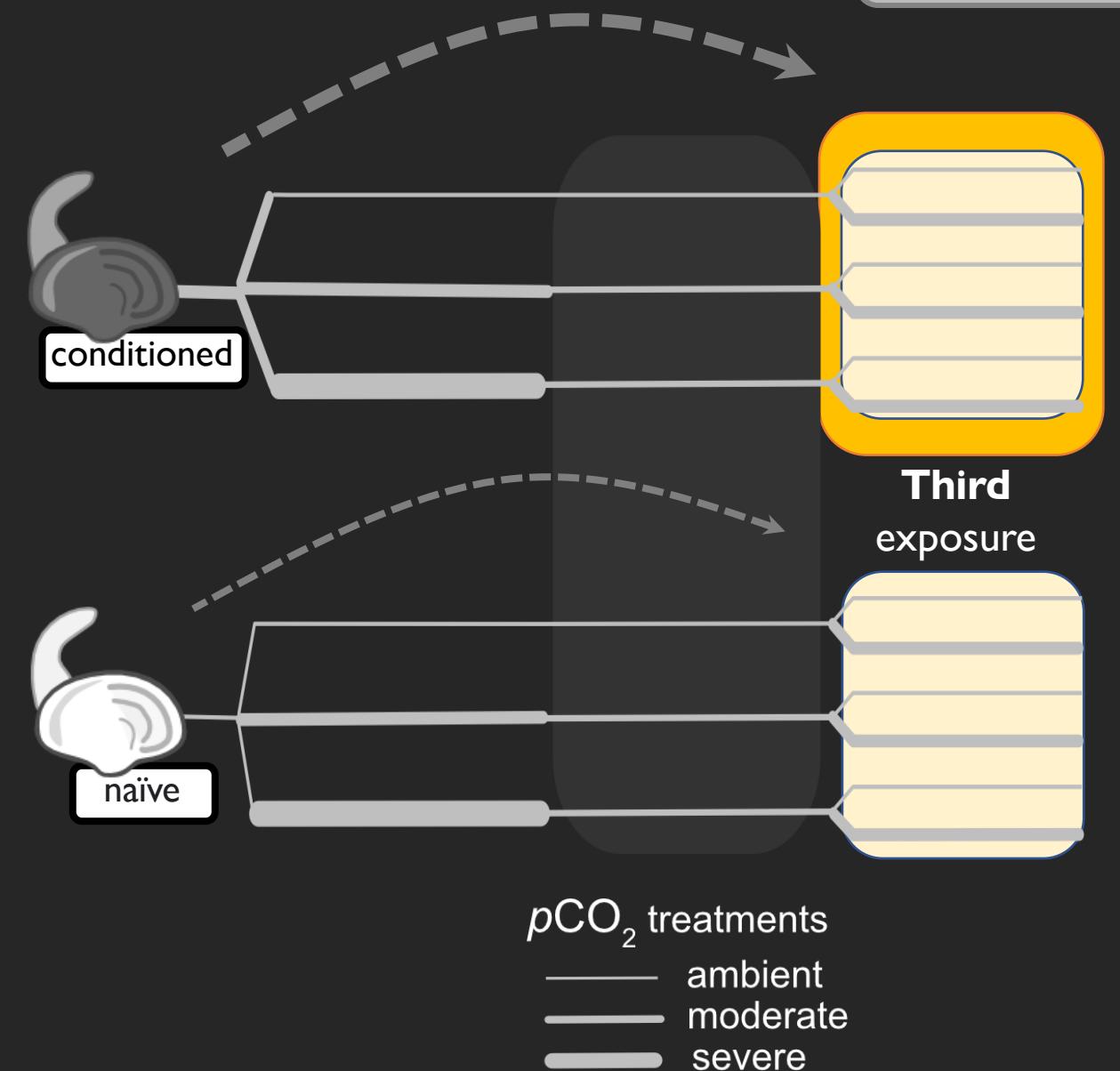
# PHYSIOLOGY



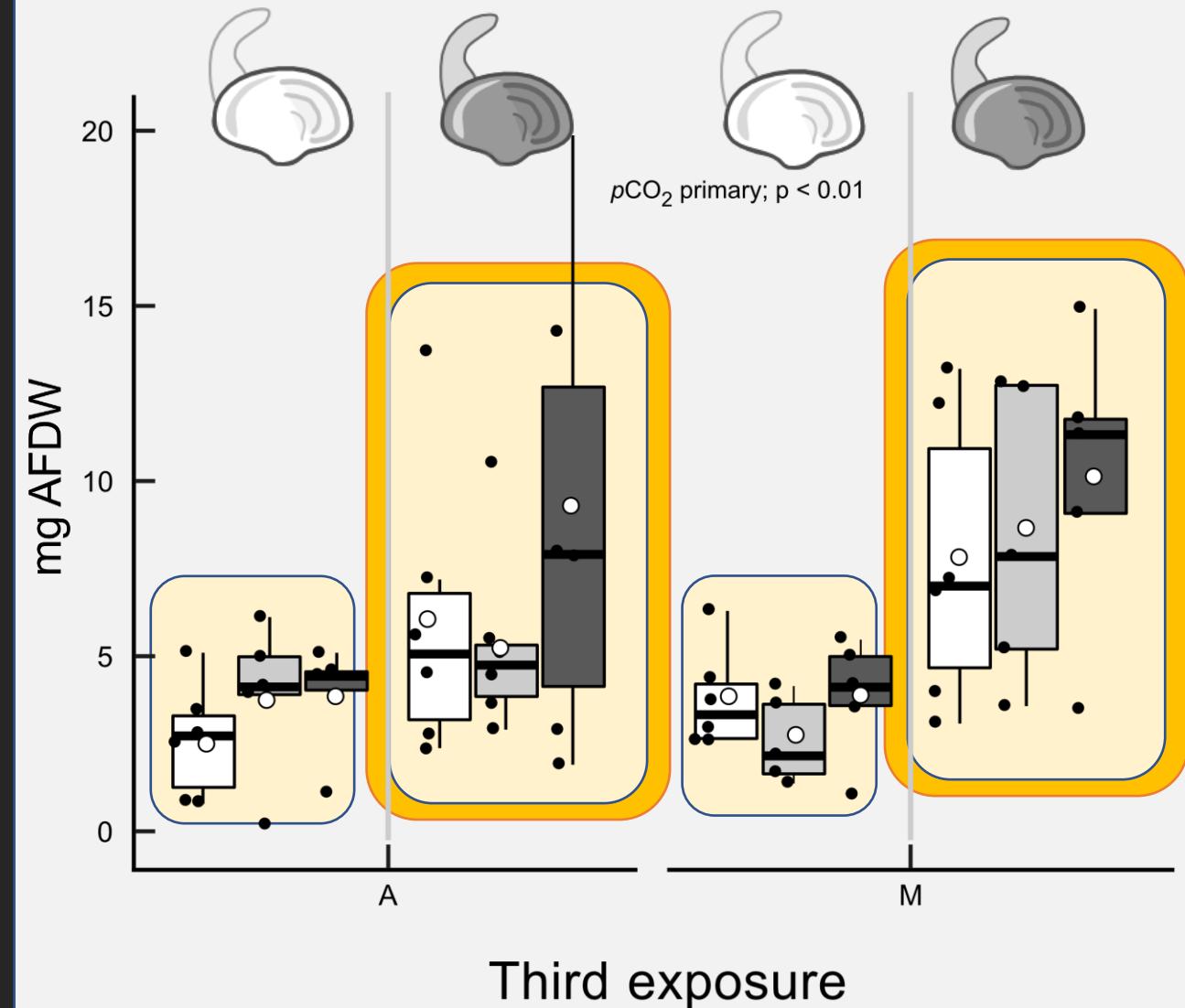
## Organic biomass



# PHYSIOLOGY



# Organic biomass

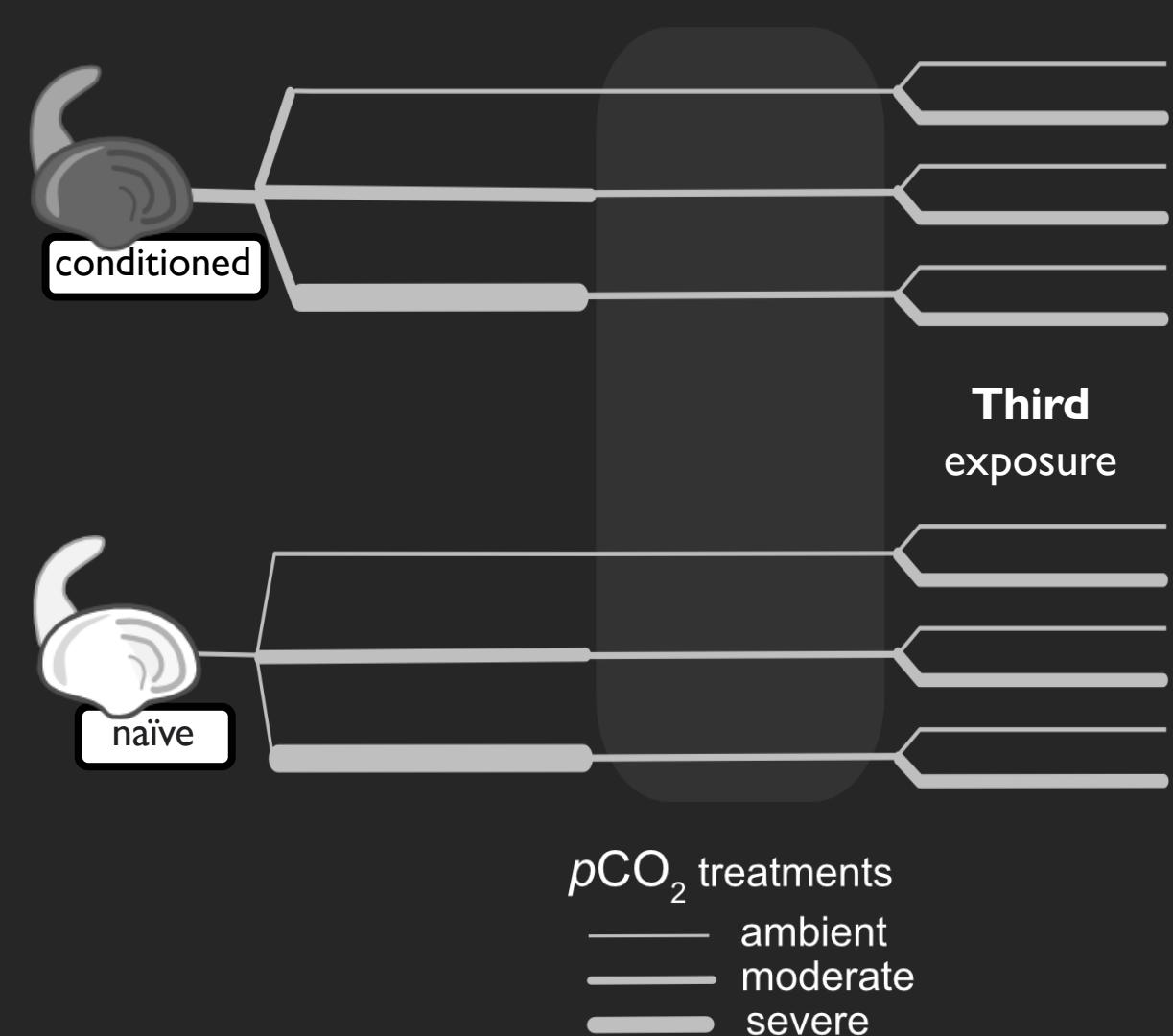


## PHYSIOLOGY

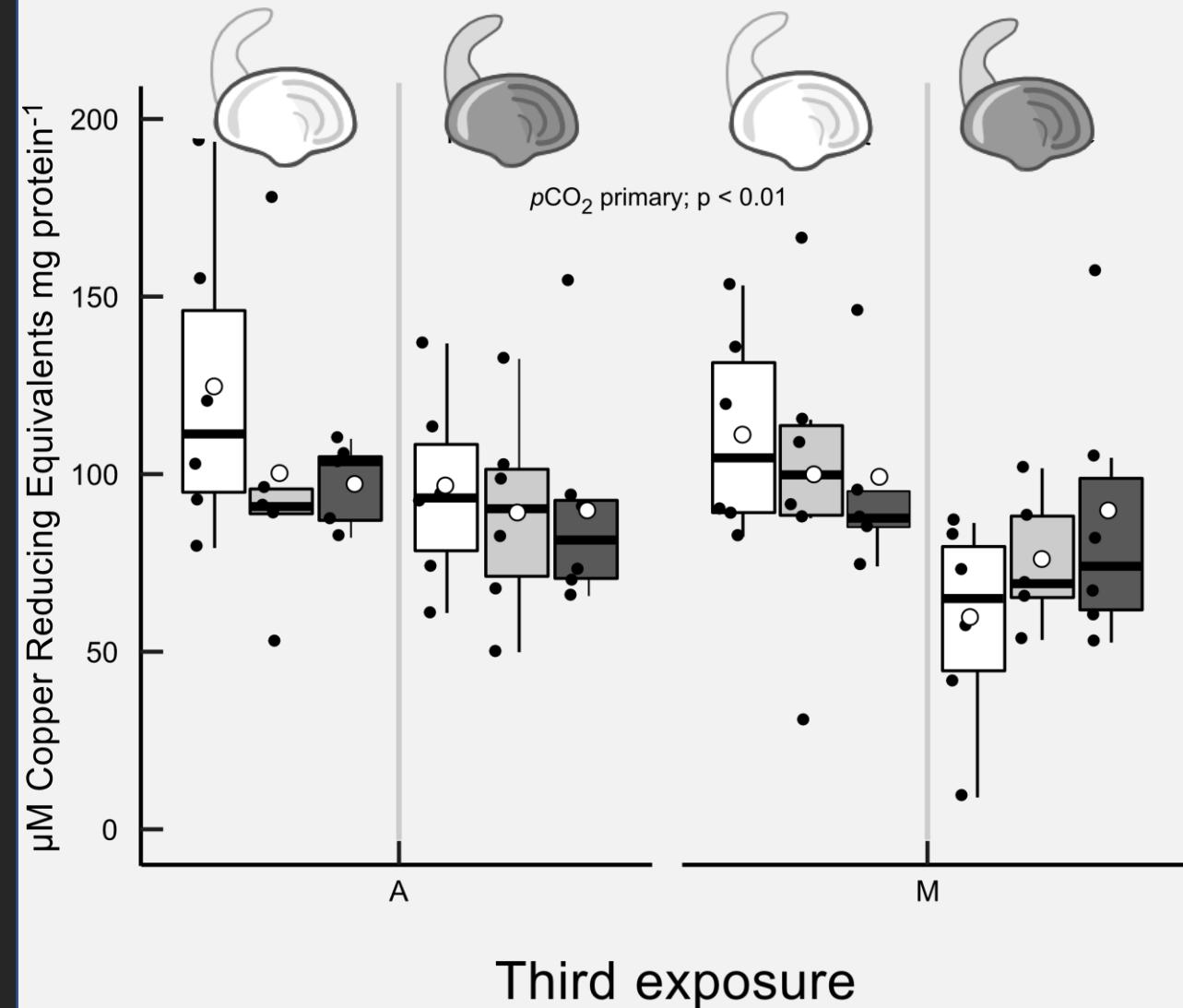
### **Shell size & organic biomass**

**Post larval stress conditioning and repeated stress exposure  
hypercapnic seawater increases size (shell & biomass)  
in juvenile clams**

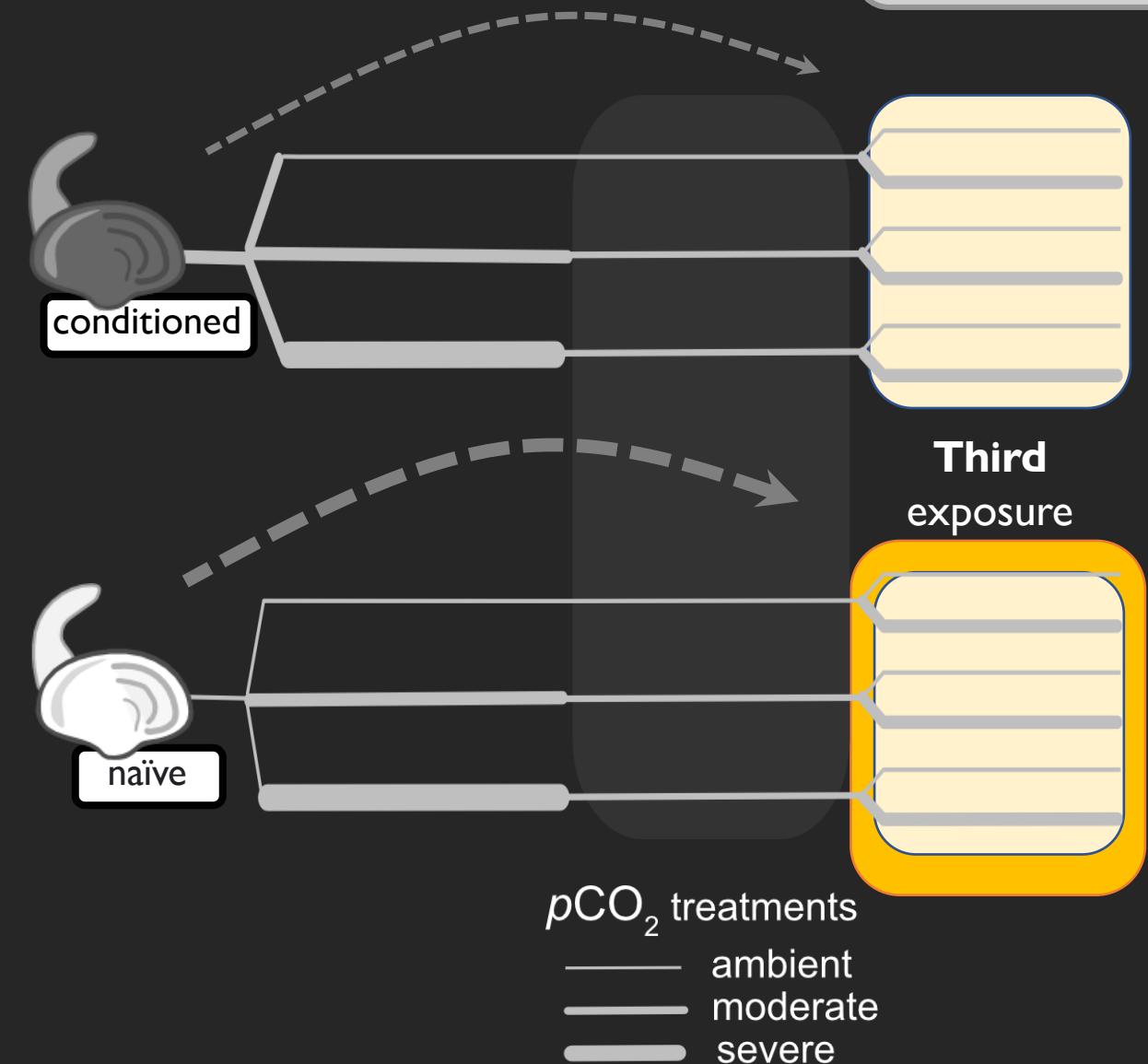
## CELLULAR STRESS RESPONSE



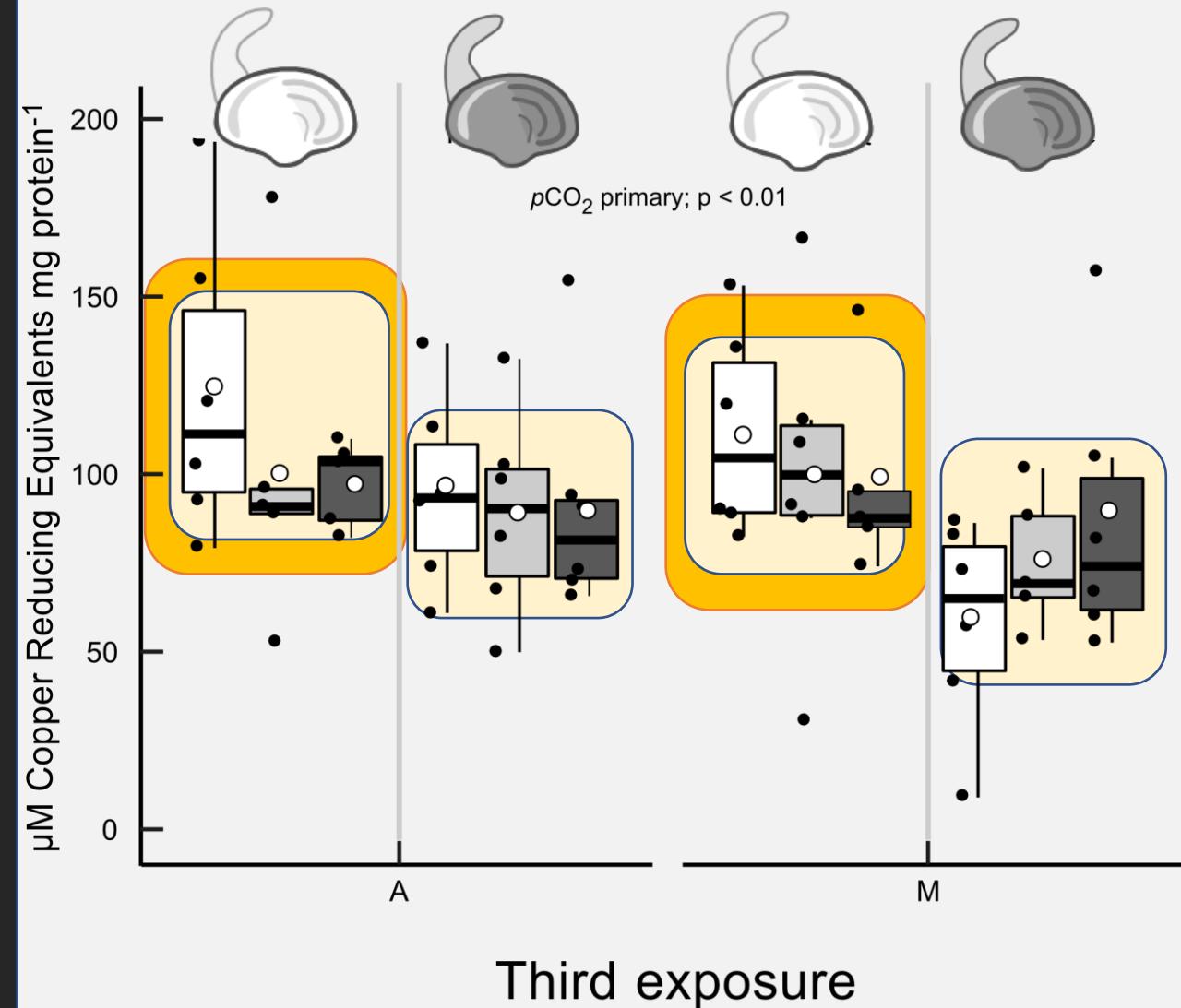
## Total Antioxidant Capacity



## CELLULAR STRESS RESPONSE



## Total Antioxidant Capacity



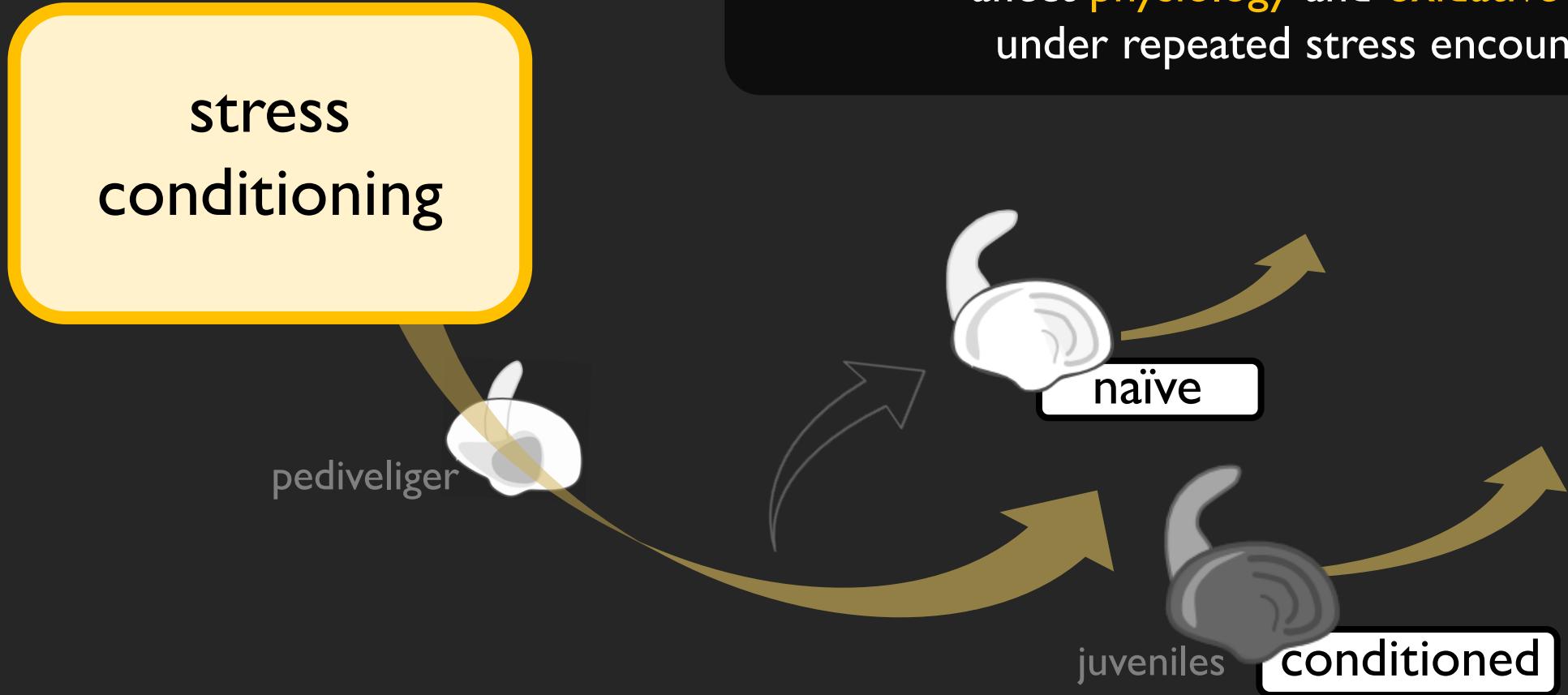
## CELLULAR STRESS RESPONSE

**Total antioxidant capacity**

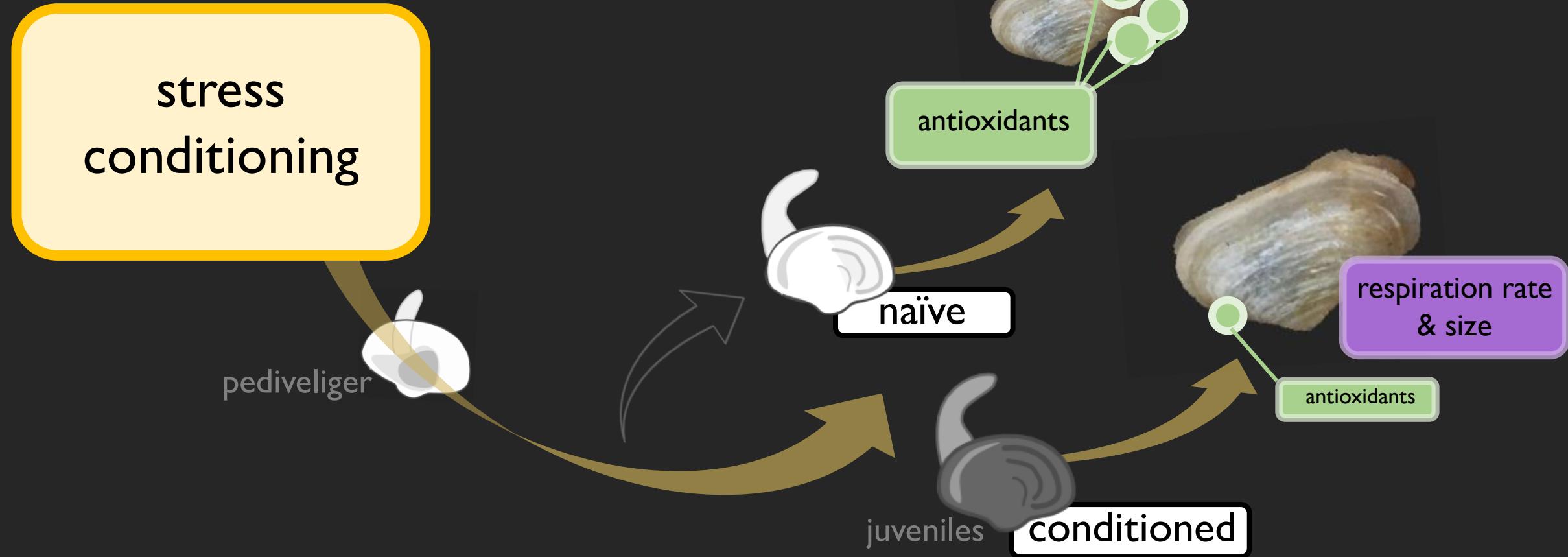
**Post larval stress conditioning to hypercapnic seawater  
reduced antioxidant capacity in juvenile clams**

# Chapter II: Conclusions

Does stress conditioning over pediveliger-juvenile stages affect physiology and oxidative status under repeated stress encounters?



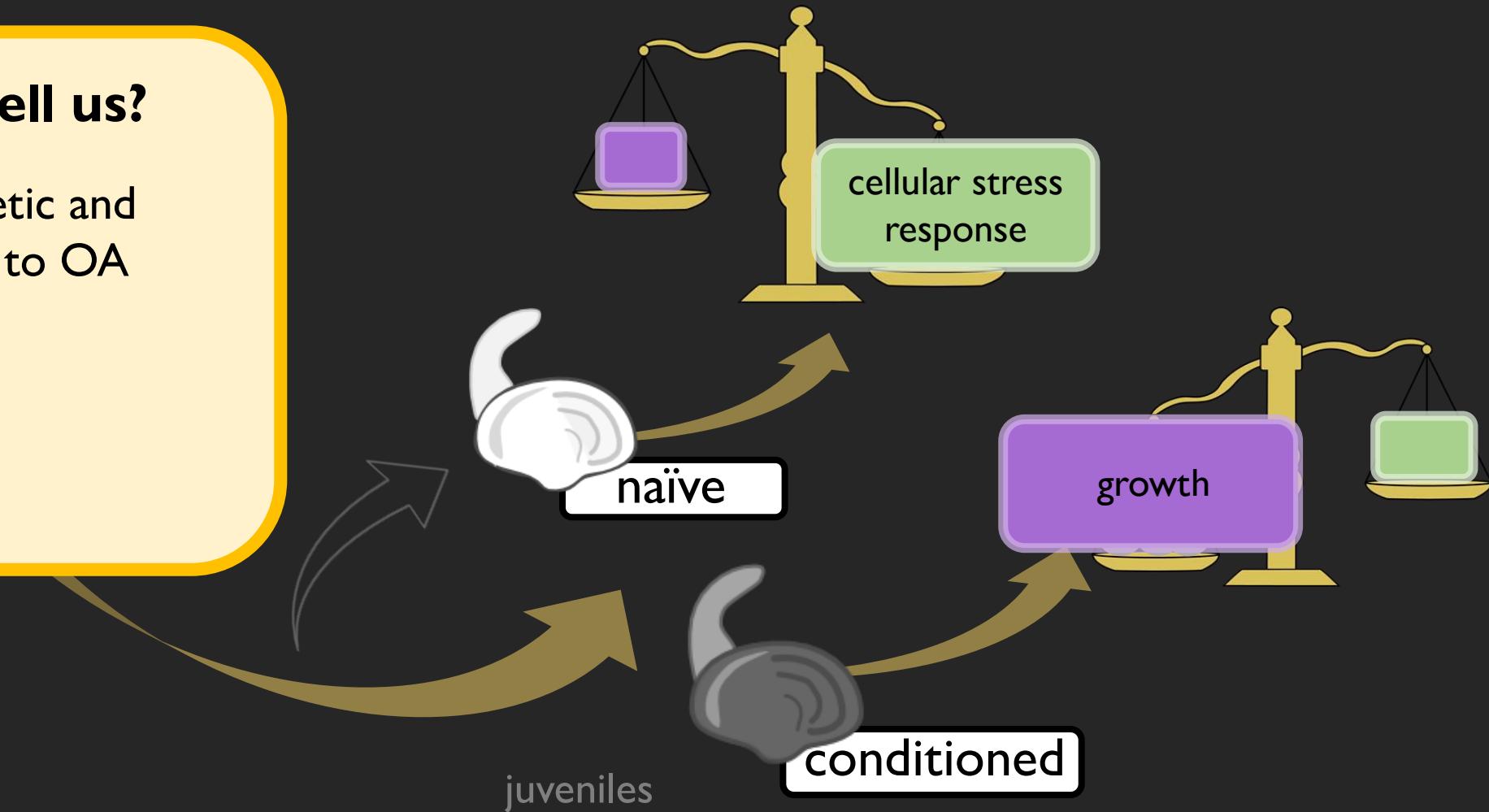
# Chapter II: Conclusions



# Chapter II: Conclusions

## What does this tell us?

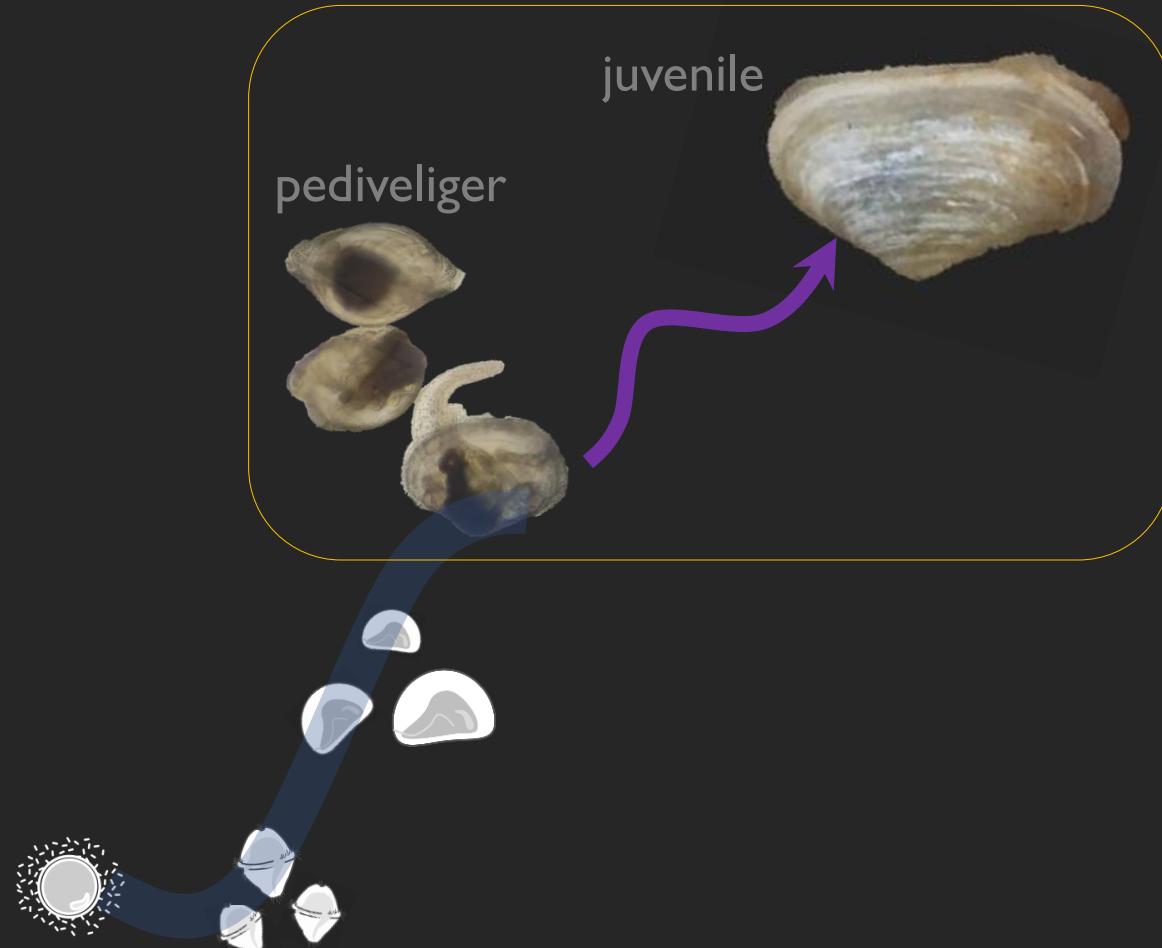
- Plasticity of bioenergetic and subcellular responses to OA



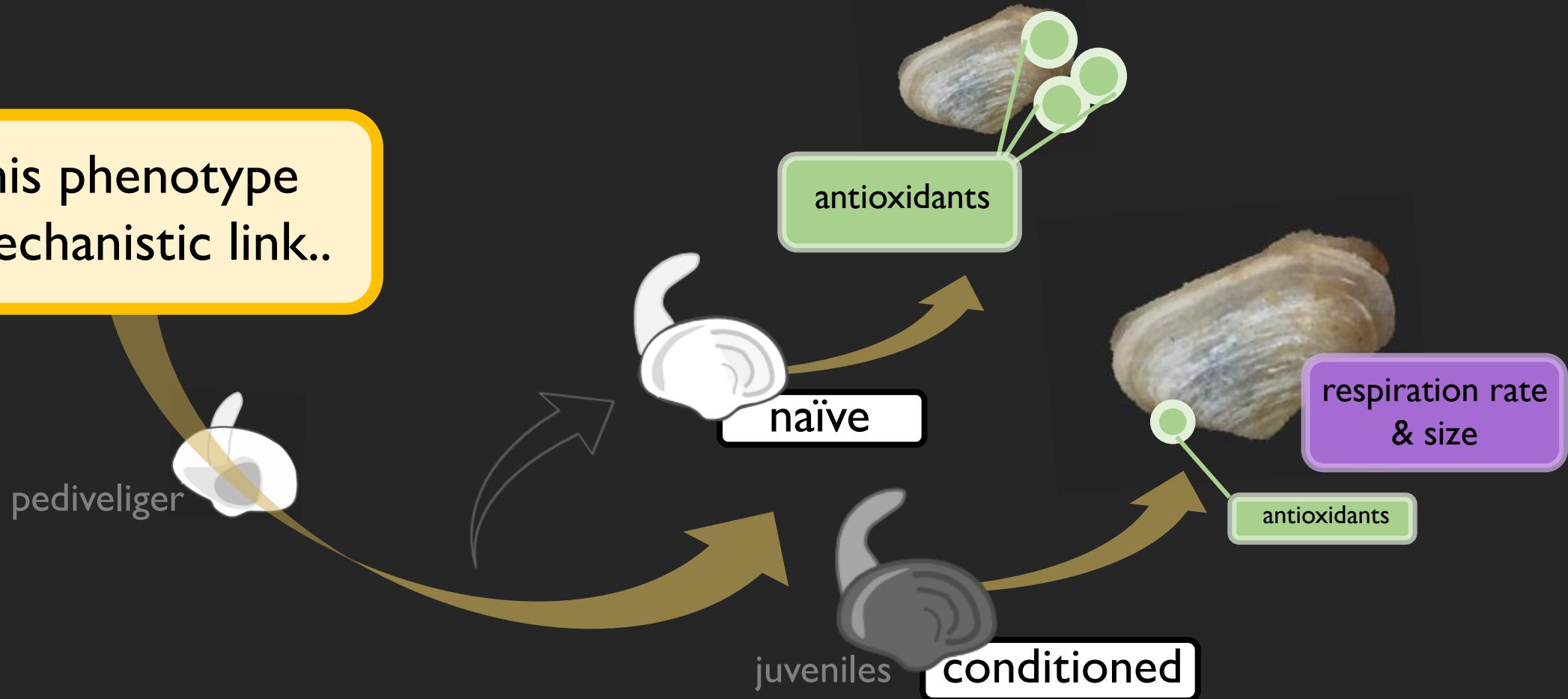
# Chapter II: Conclusions

## What does this tell us?

- Plasticity of bioenergetic and subcellular responses to OA
- Stress **post settlement** may elicit beneficial phenotypic variation



with this phenotype  
and a mechanistic link..



with this phenotype  
and a mechanistic link..

pediveliger

juveniles

conditioned

Illuminate underlying  
mechanisms on the  
**molecular level**



antioxidants

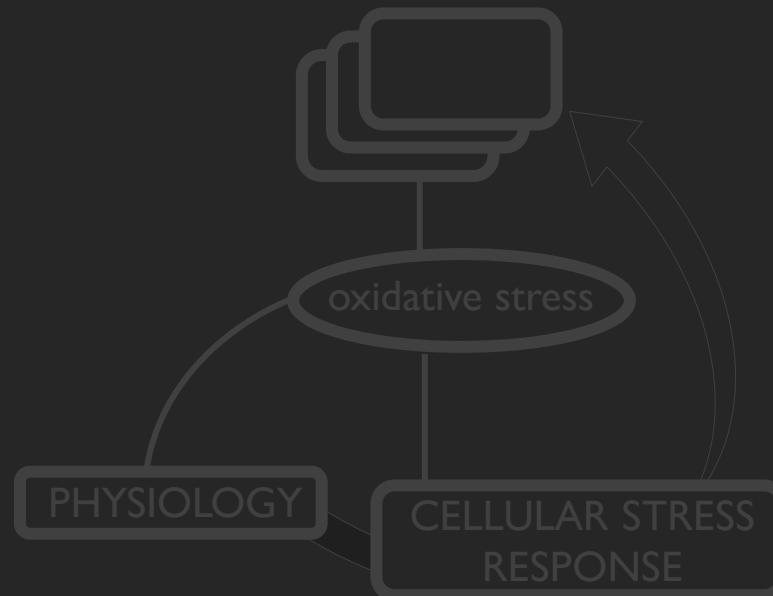
# Chapter I



## Hypothesis

Repeated stress exposure under elevated pCO<sub>2</sub> enhances intragenerational performance for Pacific geoduck.

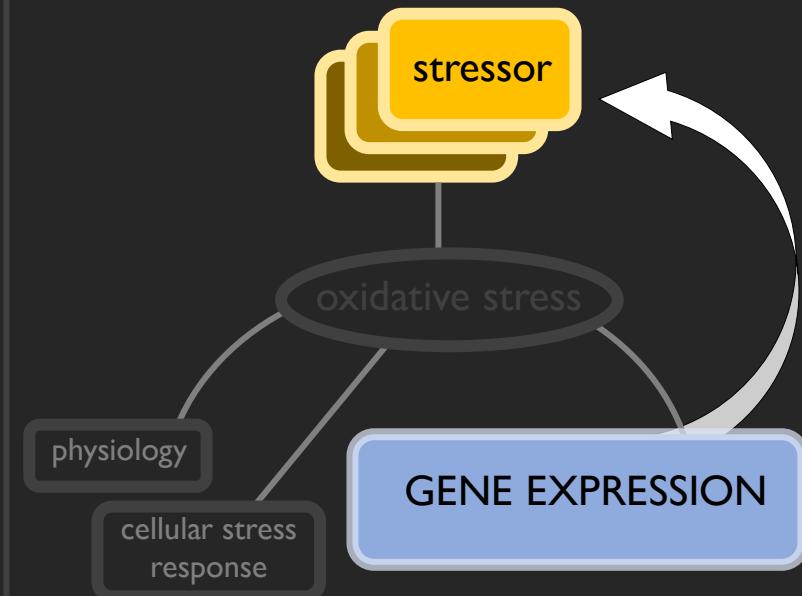
# Chapter II



## Hypothesis

Repeated stress exposure under elevated pCO<sub>2</sub> can enhance intragenerational performance for Pacific geoduck.

# Chapter III



## Hypothesis

Early acclimation generates distinct functions and pathways under repeated stress

# Gene expression and stress conditioning



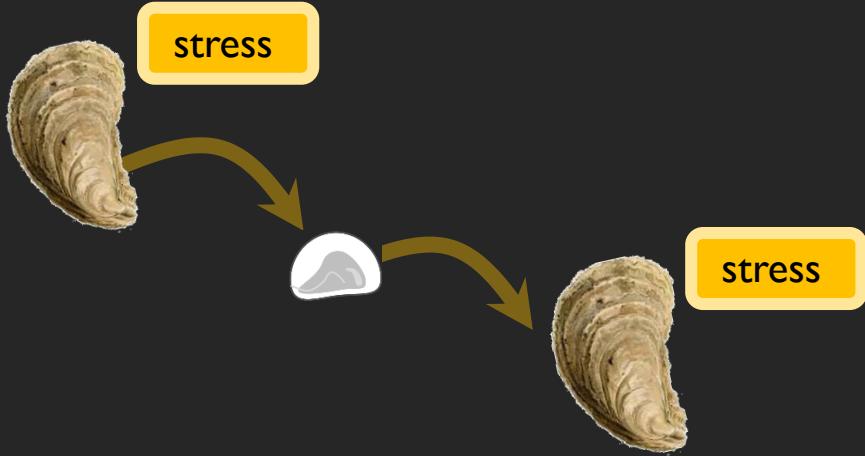
*'loaded'* expression of  
stress-related proteins



## Frontloading

Enables individuals to maintain **resilience** and  
**physiology** during **frequent stress encounters**

# Gene expression and stress conditioning



upregulated **fewer genes**, but...  
were coded for stress response

**Fine-tuned** gene expression  
responsive to subsequent environmental challenges

# Gene expression and stress conditioning



**Frontloading**

prepared

**Fine-tuned** gene expression  
rapidly responding

## Conditioning period

### Primary exposure

110 days

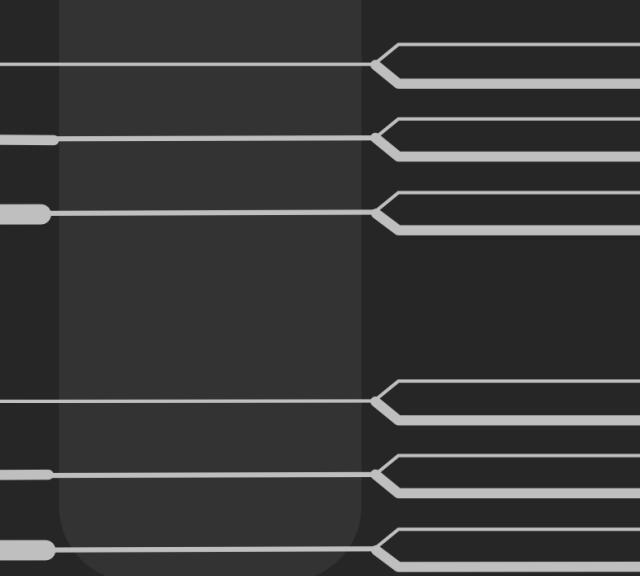


## Repeat exposures

### Second exposure

Ambient recovery

### Third exposure



# Conditioning period

**Primary exposure**

110 days

conditioned

naïve

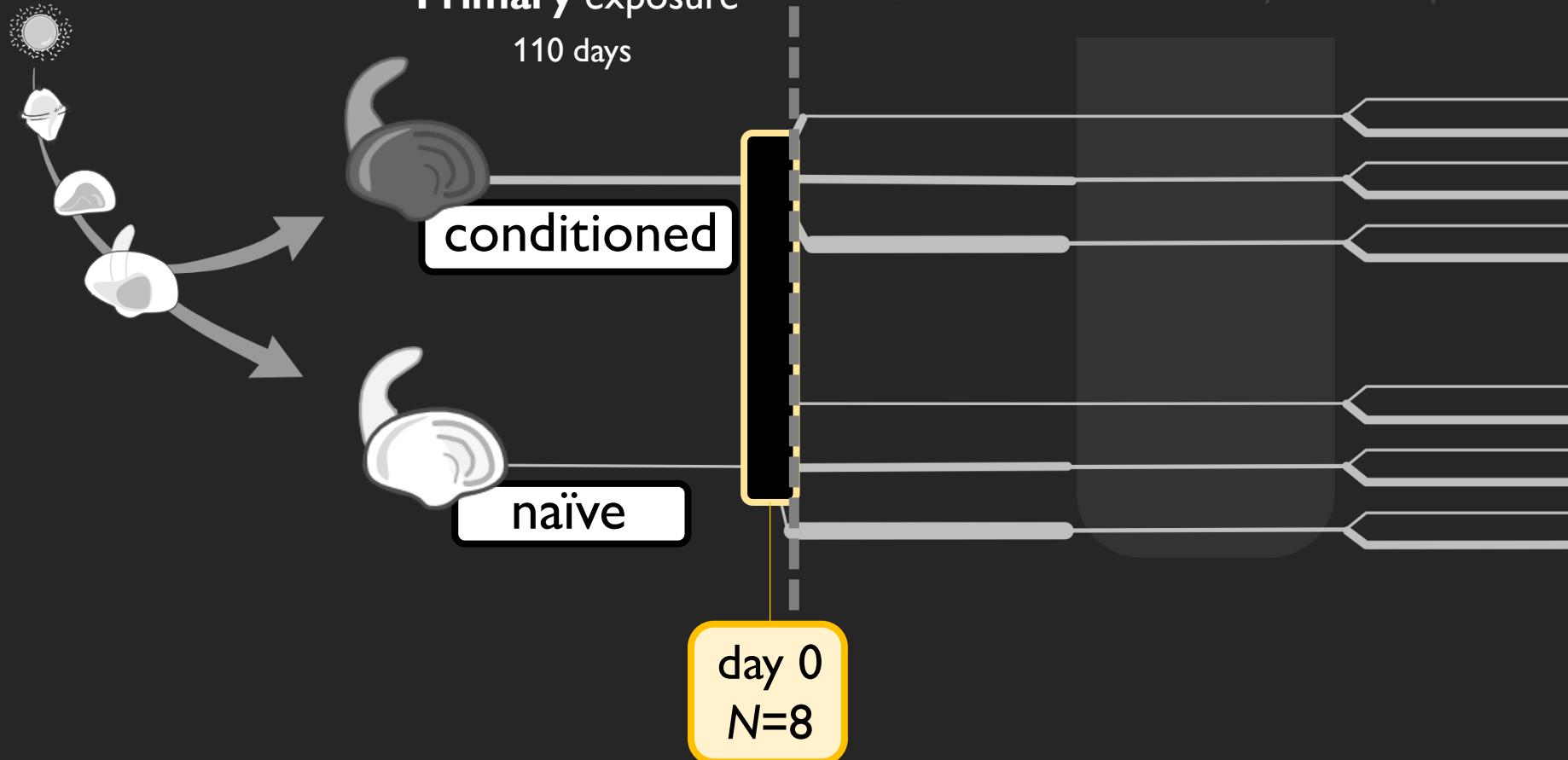
day 0  
 $N=8$

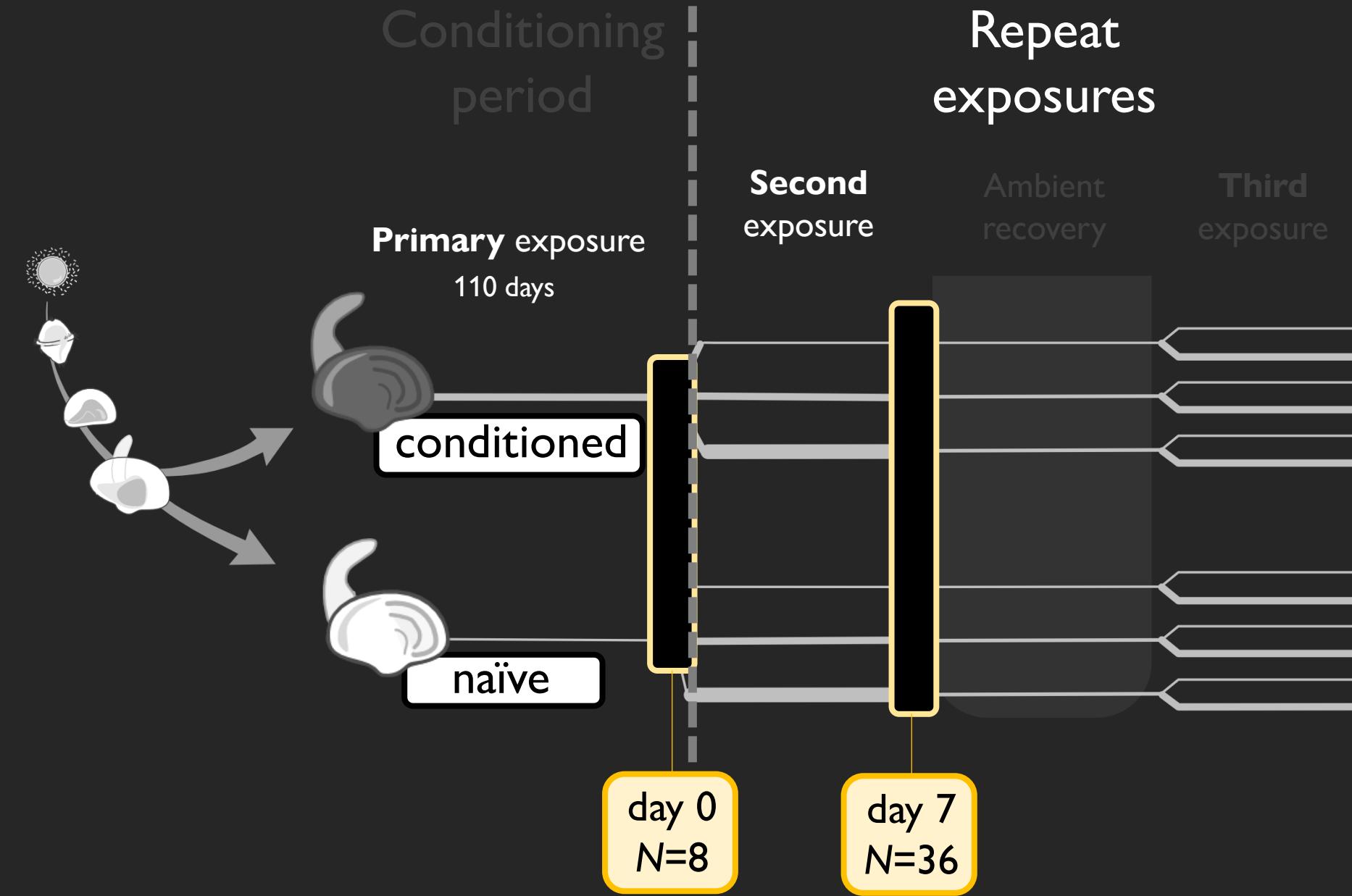
# Repeat exposures

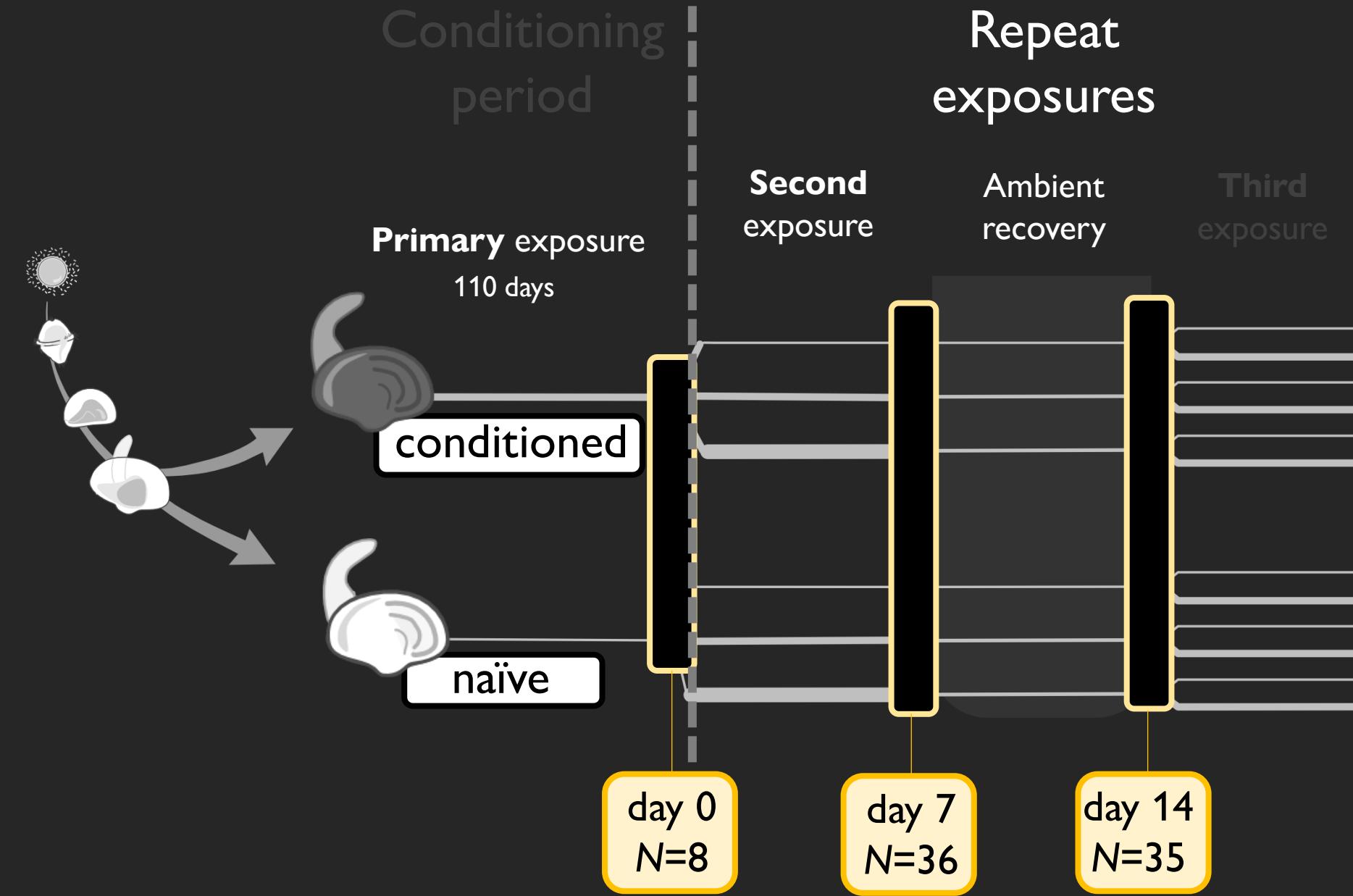
Second exposure

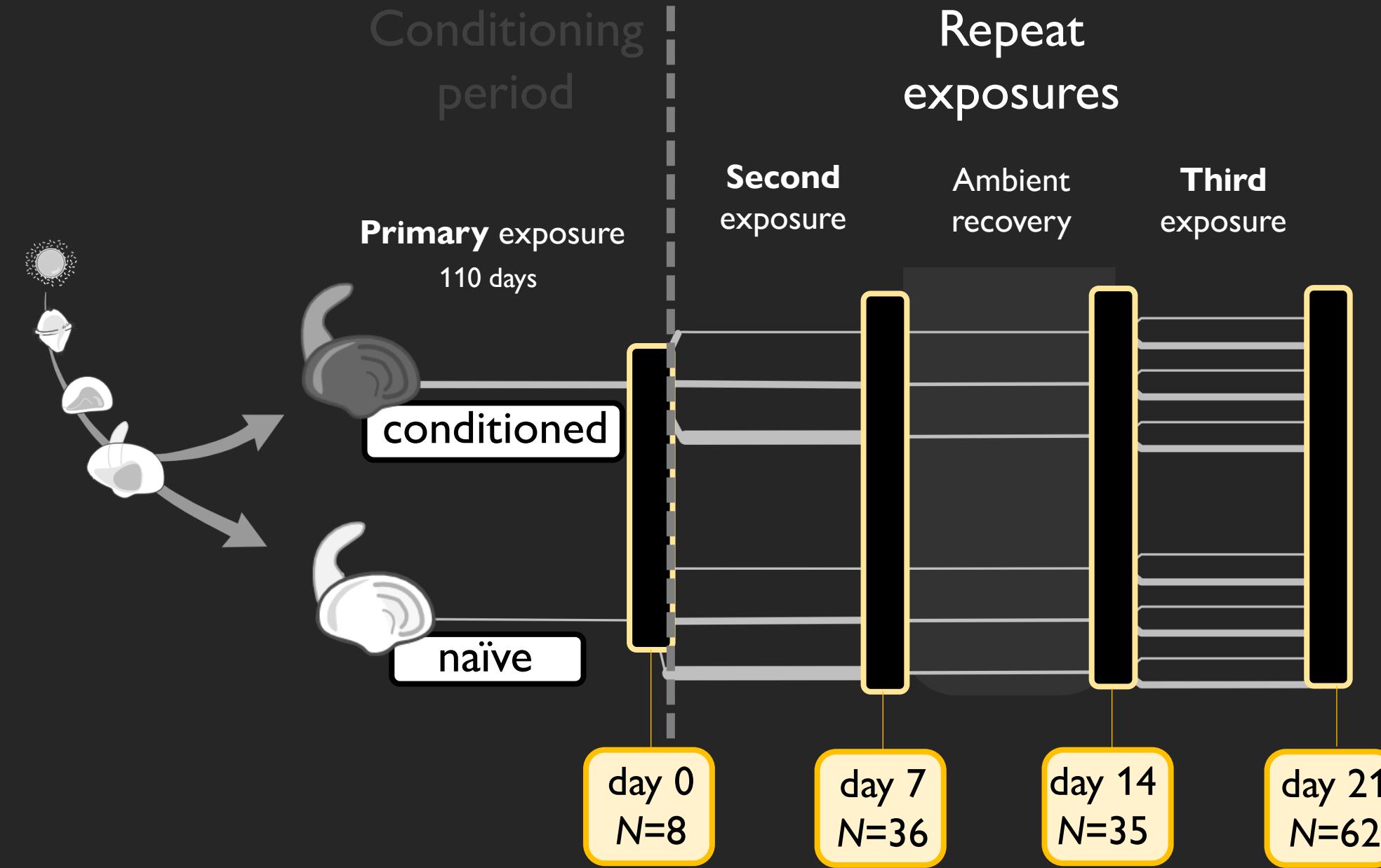
Ambient recovery

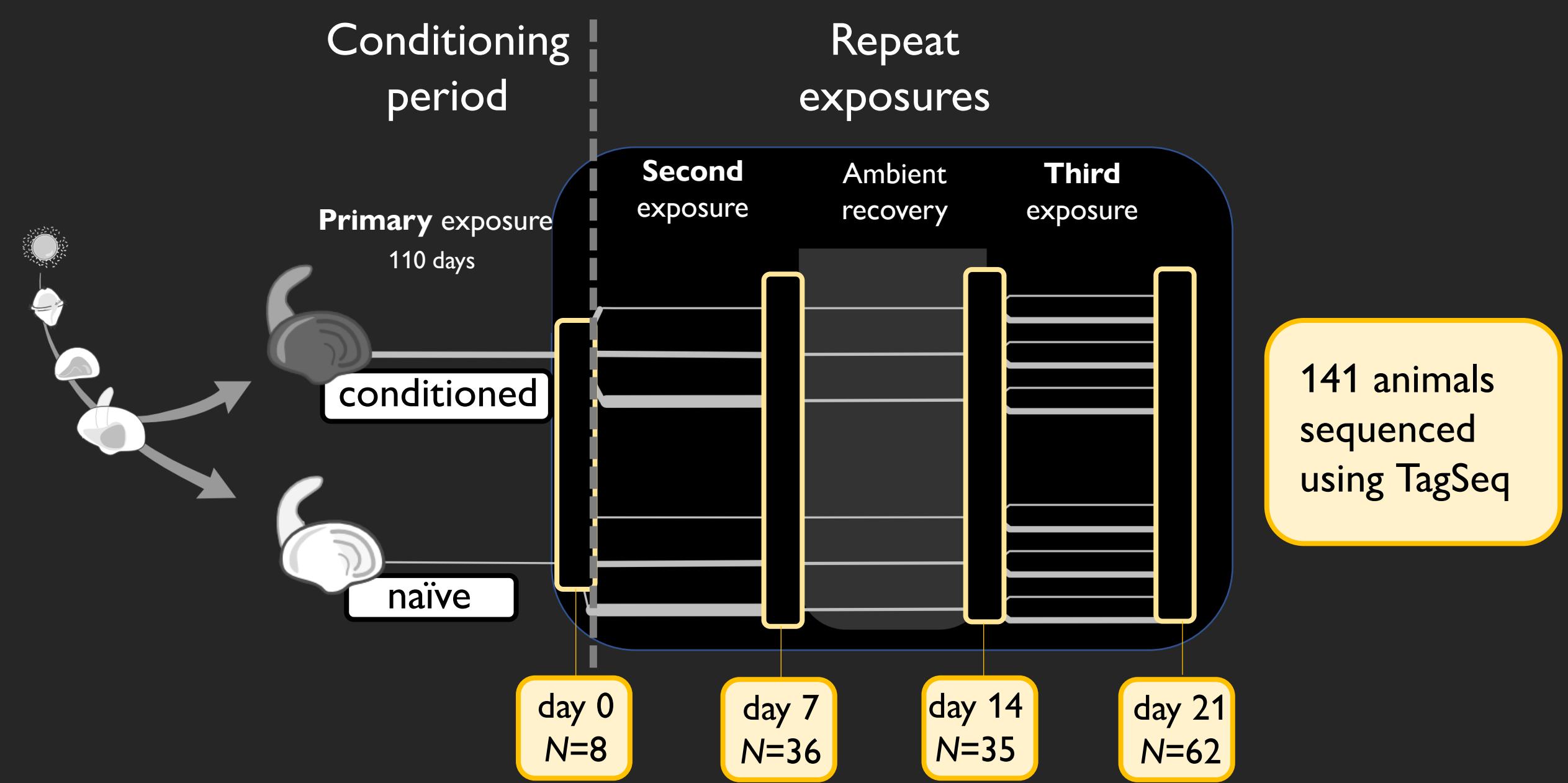
Third exposure







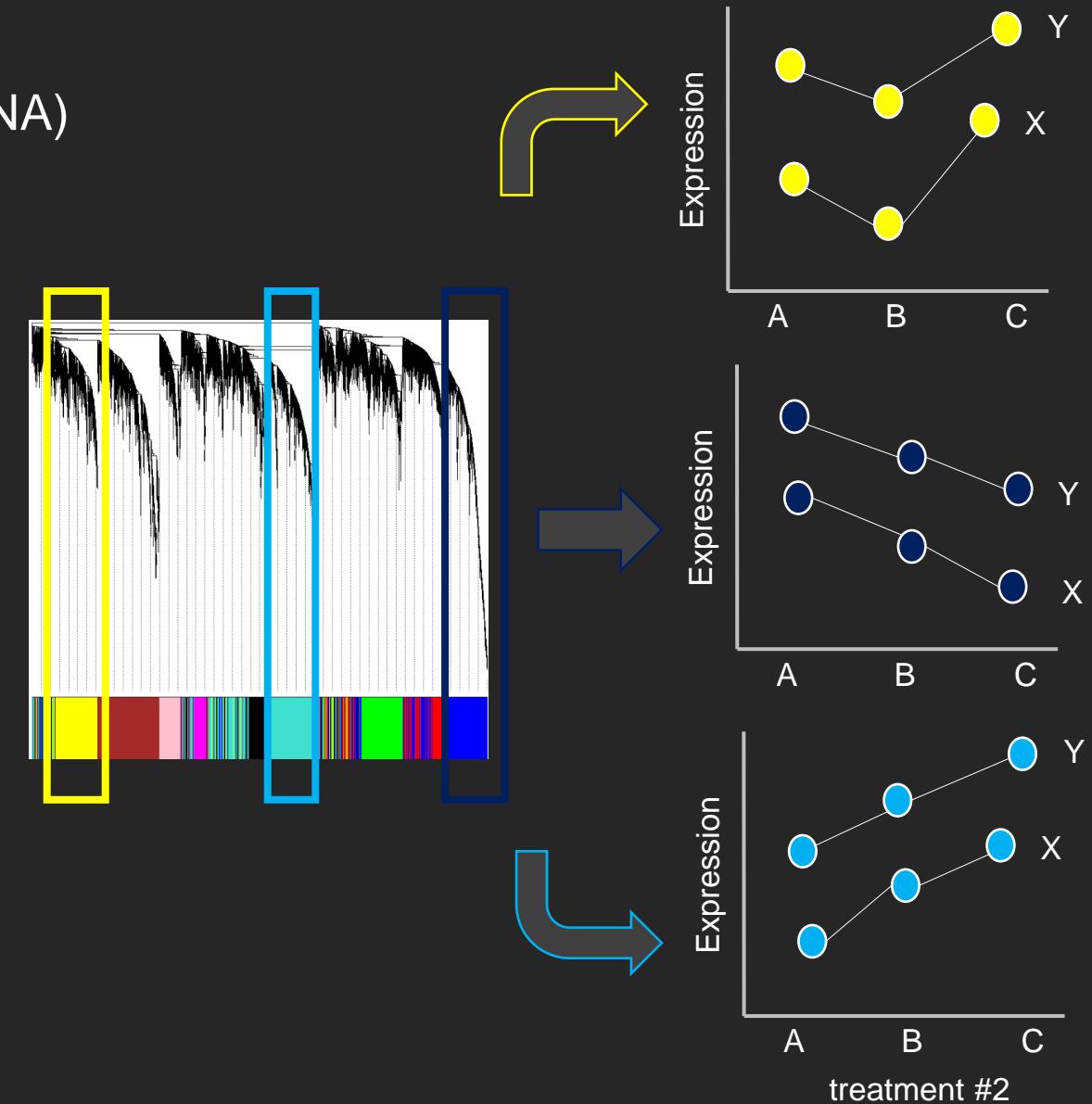




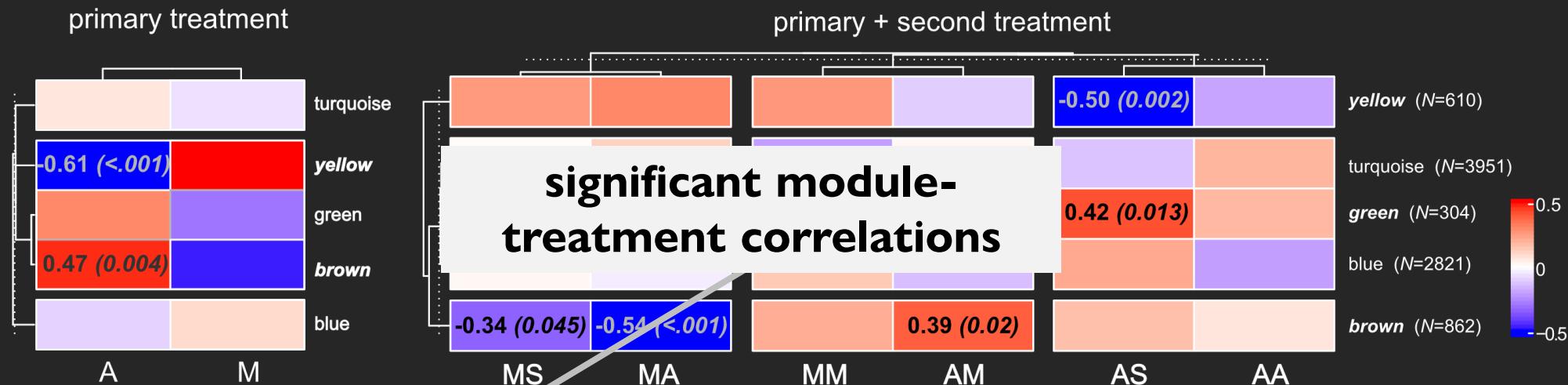
# Methods: Analysis

## Weighted Gene Correlation Network Analysis (WGCNA)

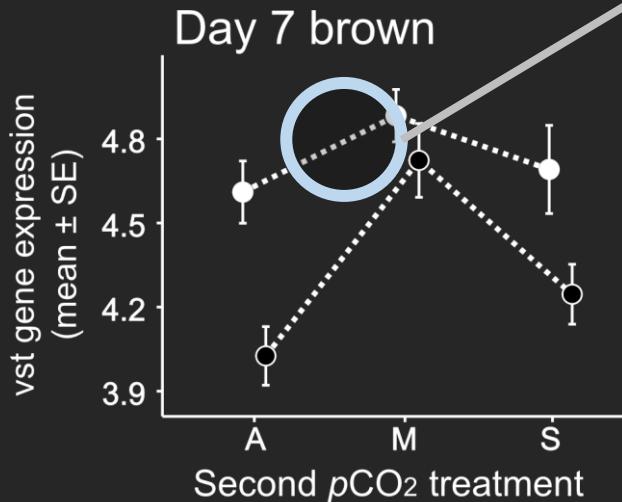
- expression-level directionality / patterns
- influence of compounding treatment history



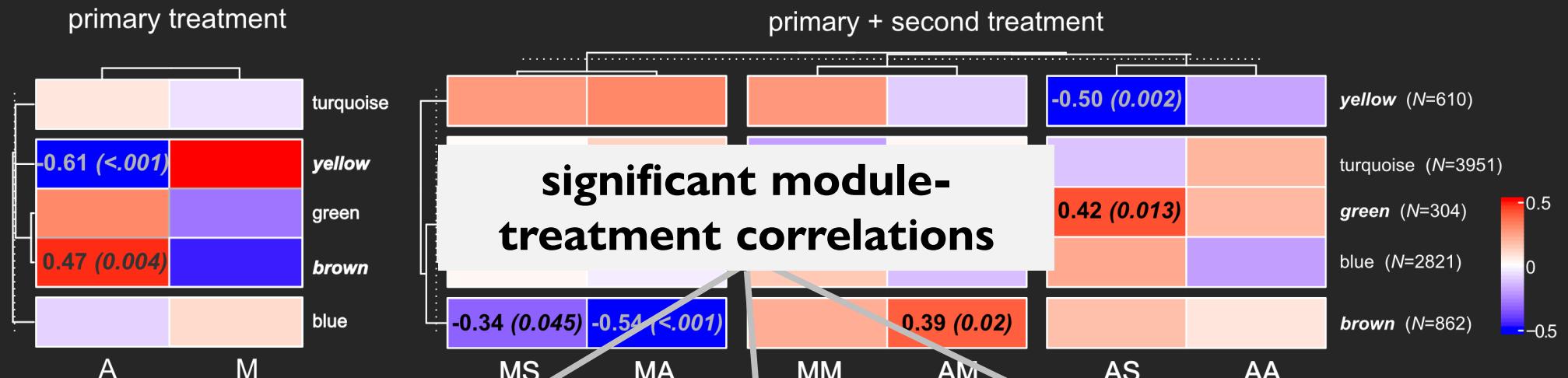
# Methods: WGCNA workflow



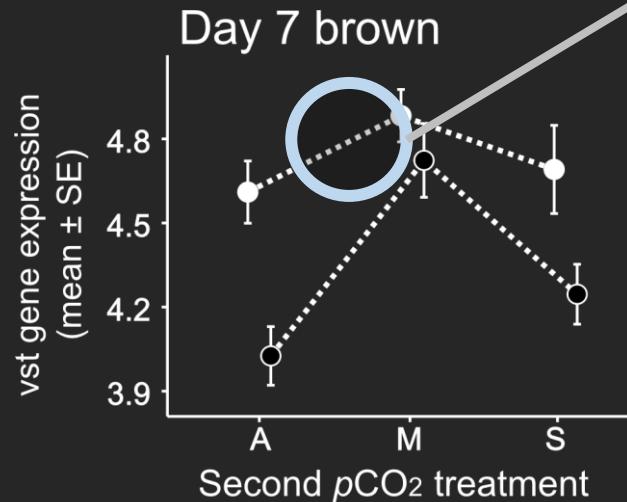
## expression patterns



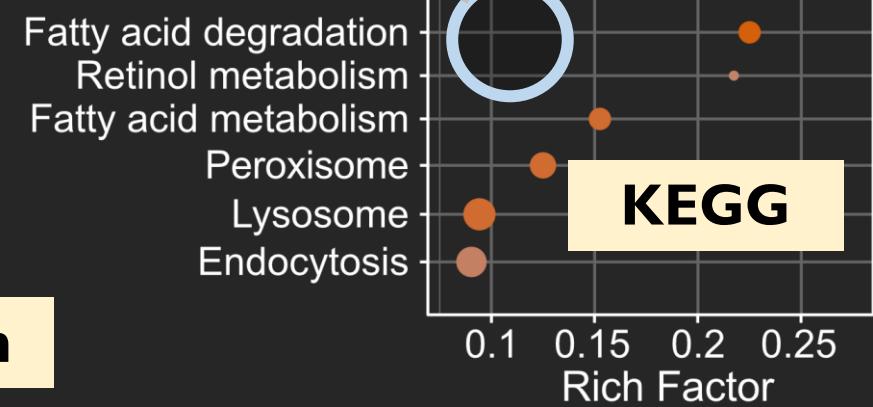
# Methods: WGCNA workflow



expression patterns

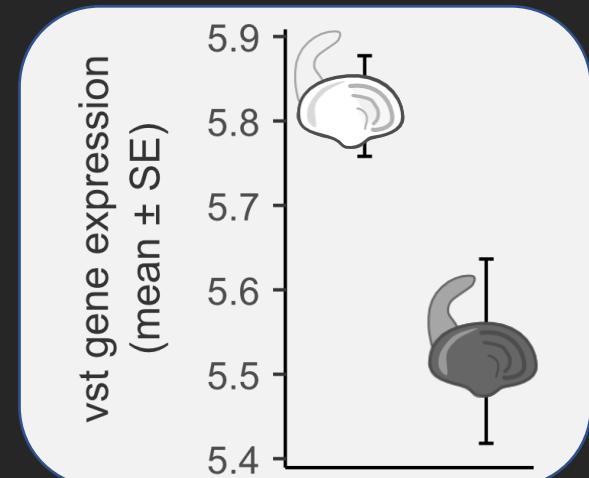
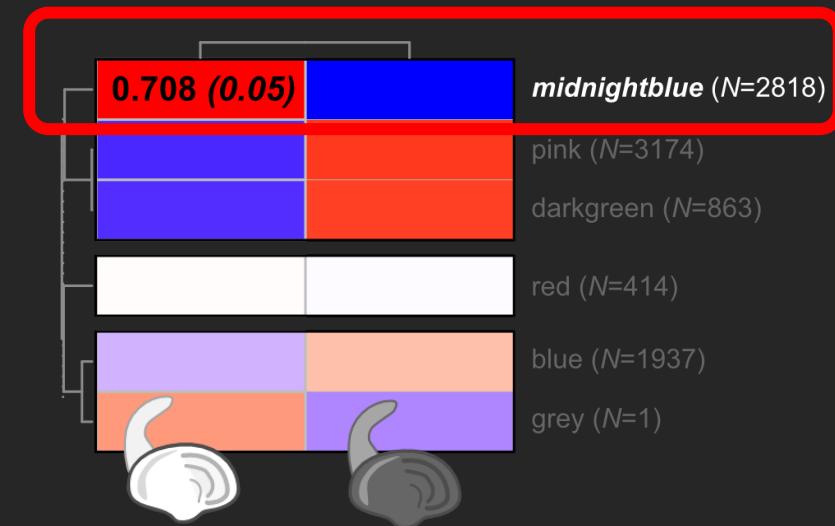
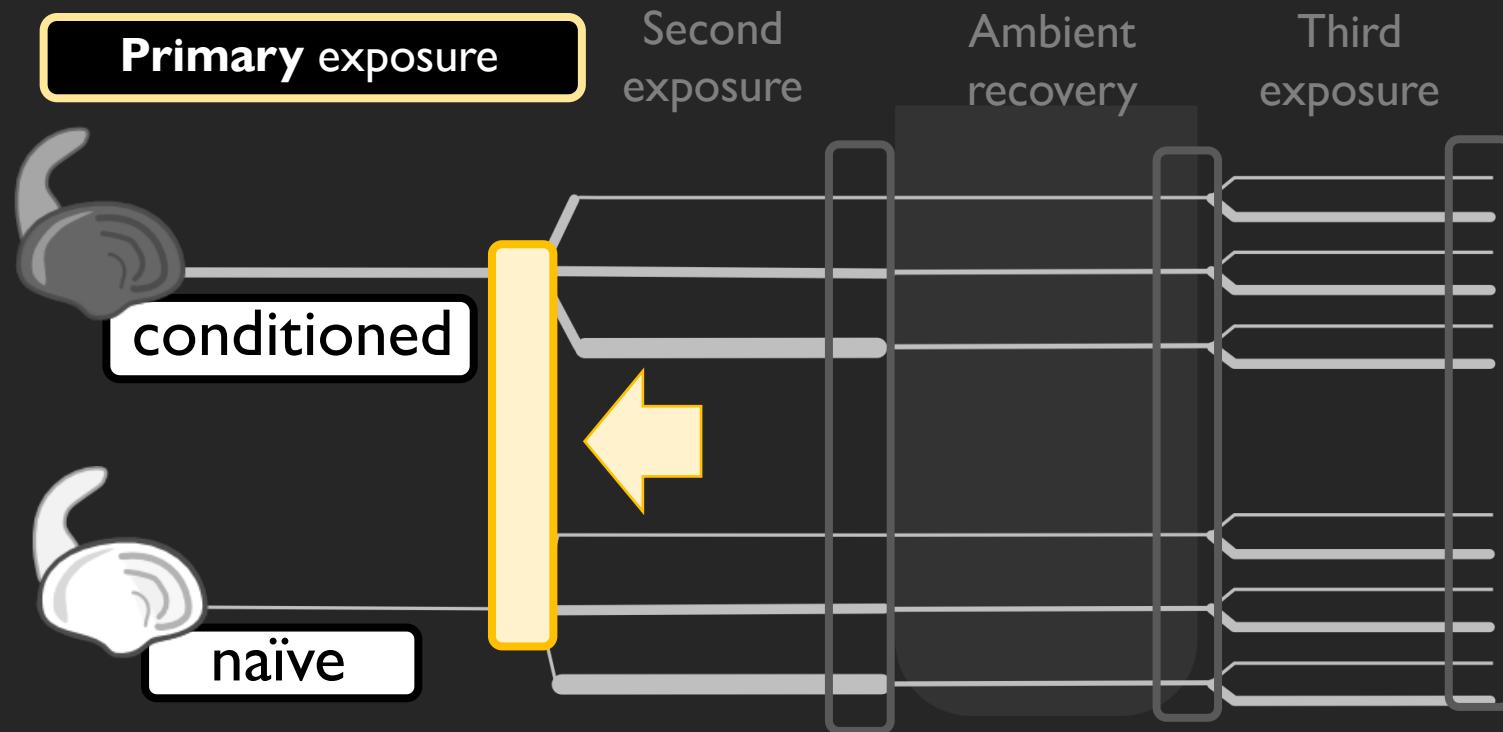


Function and pathway enrichment



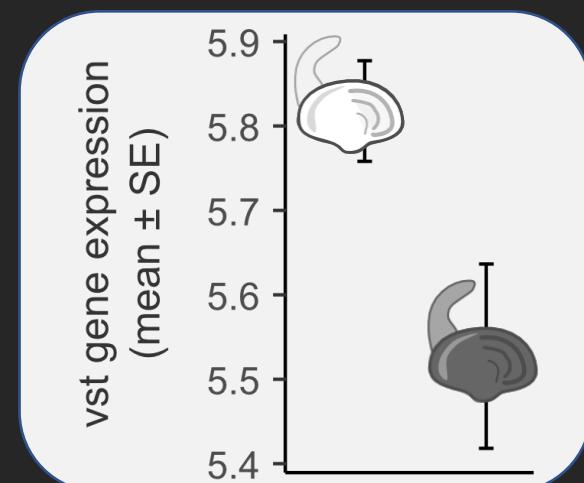
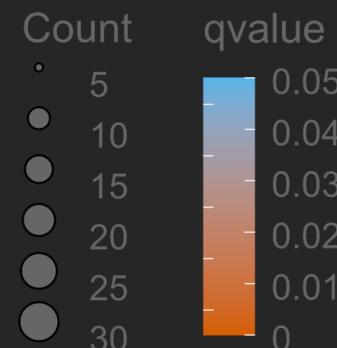
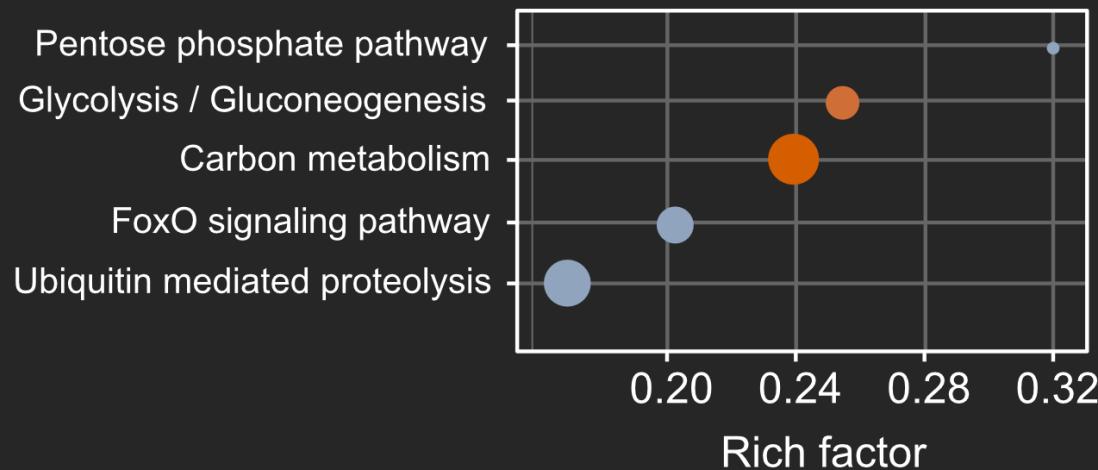
# Results: Day 0

one module showing higher gene expression  
by naïve animals



# Results: Day 0

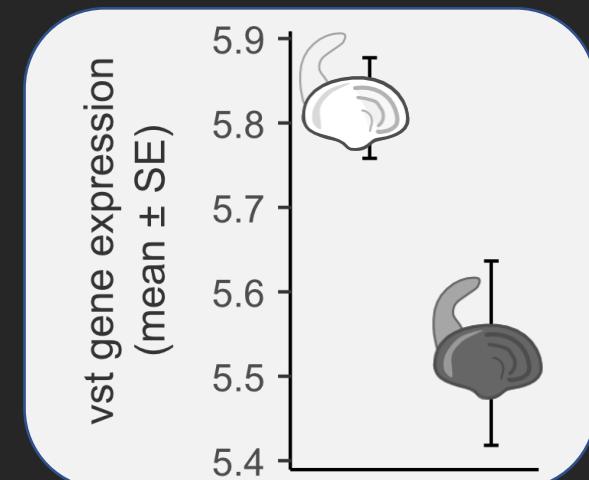
## KEGG pathway enrichment analysis...



# Results: Day 0

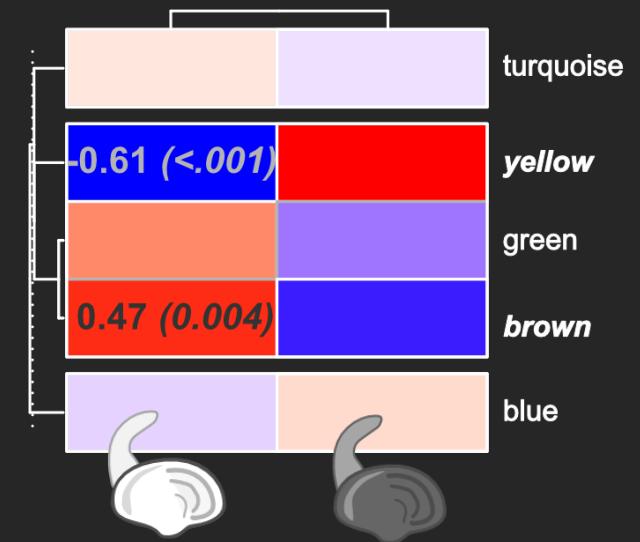
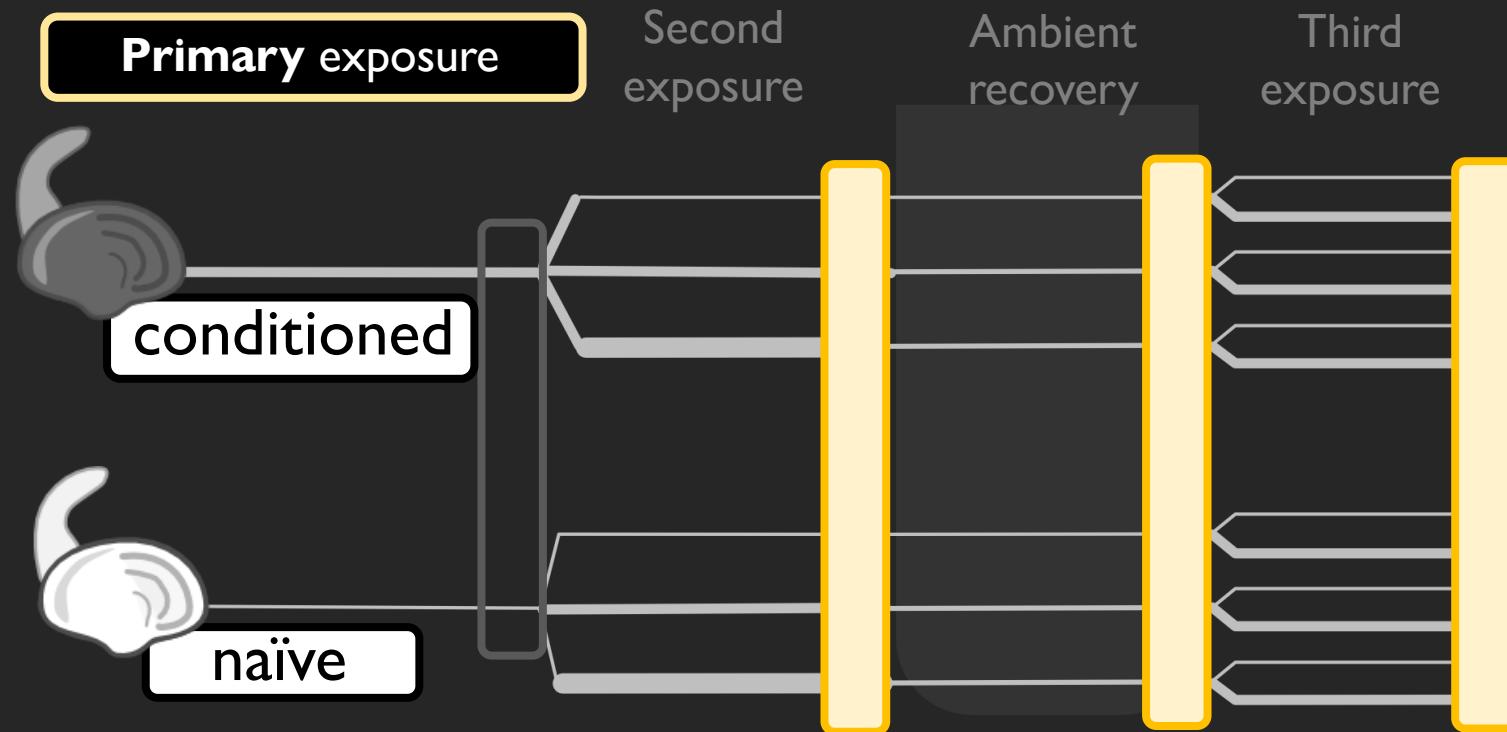
Before subsequent exposures...

- carbohydrate metabolism
- protein homeostasis
- biosynthetic processes
- signaling



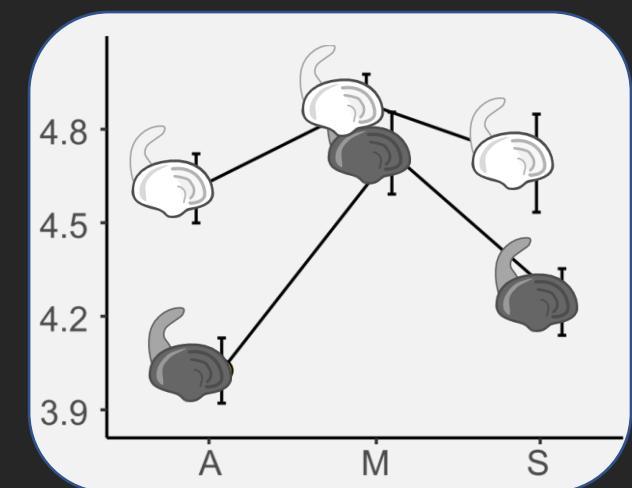
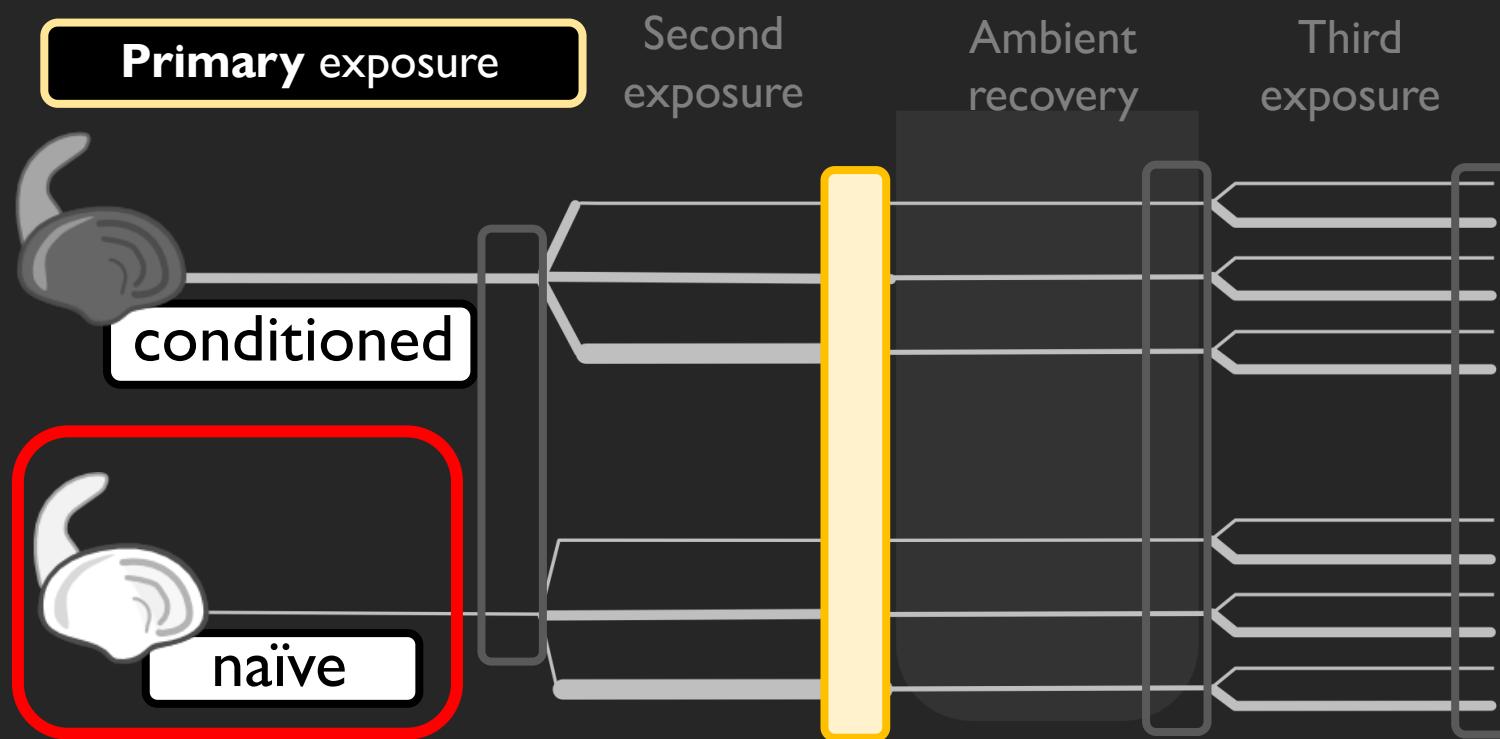
# Results: Primary-effect modules

modules affected by  
**primary exposure** on each sampling day



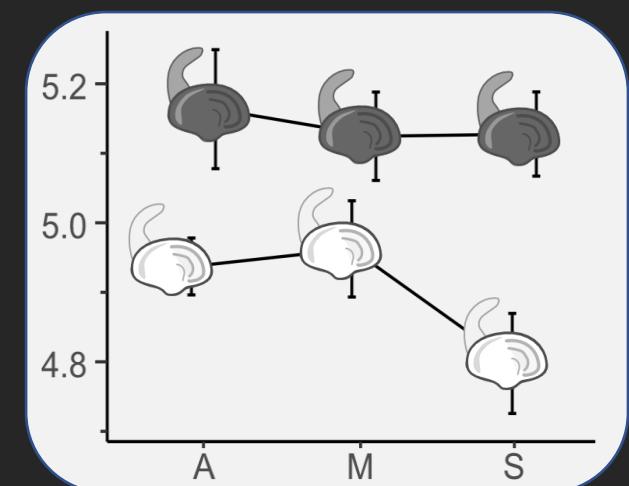
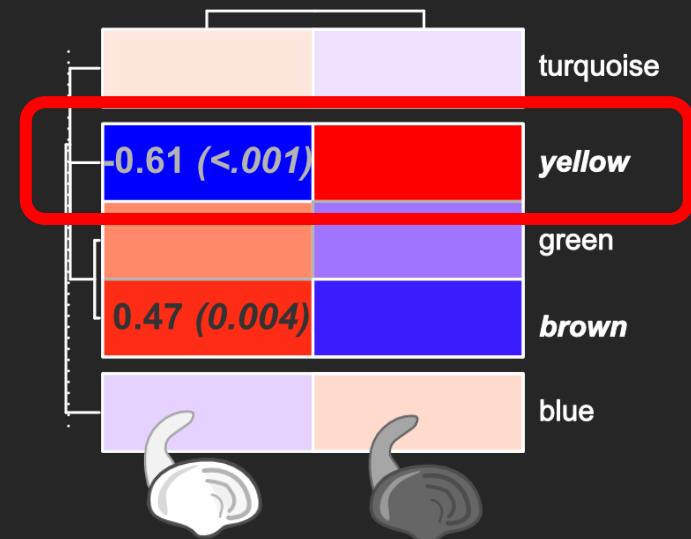
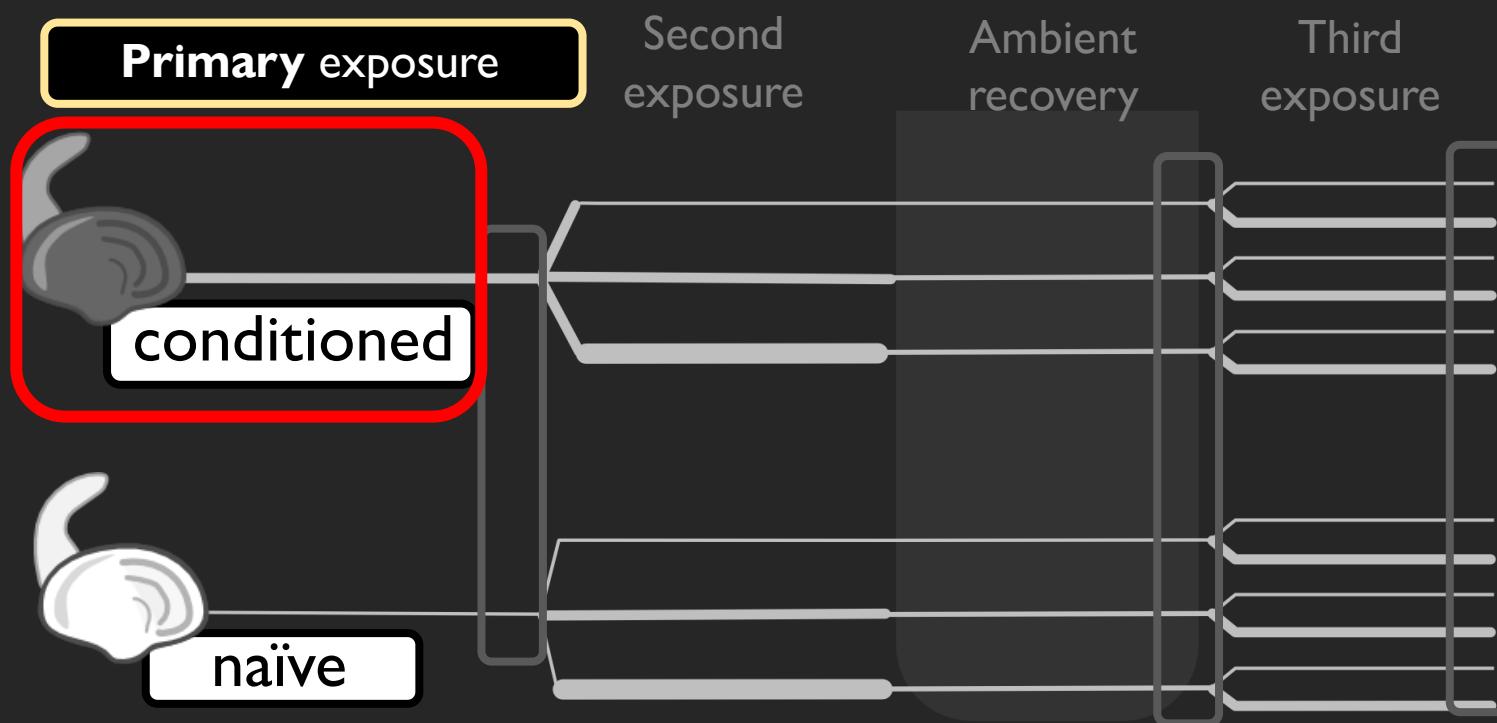
# Results: Naïve-effect modules

genes more abundantly expressed by naïve animals



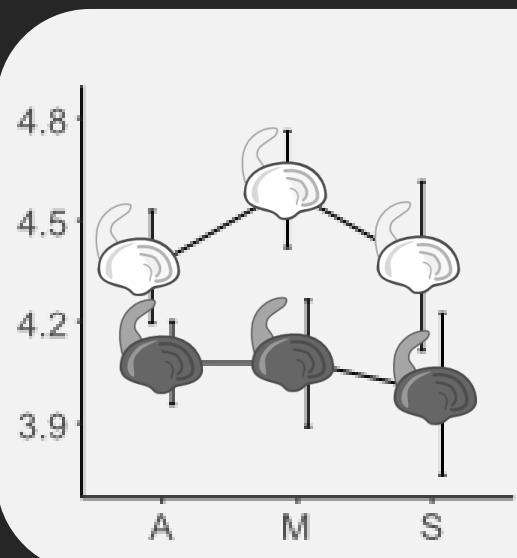
# Results: Conditioning-effect modules

genes more abundantly expressed by **conditioned animals**

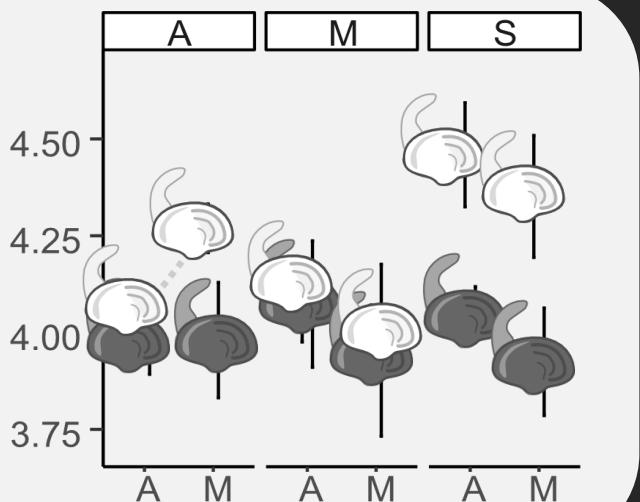


# Results: Primary-effect modules

naïve-effect modules

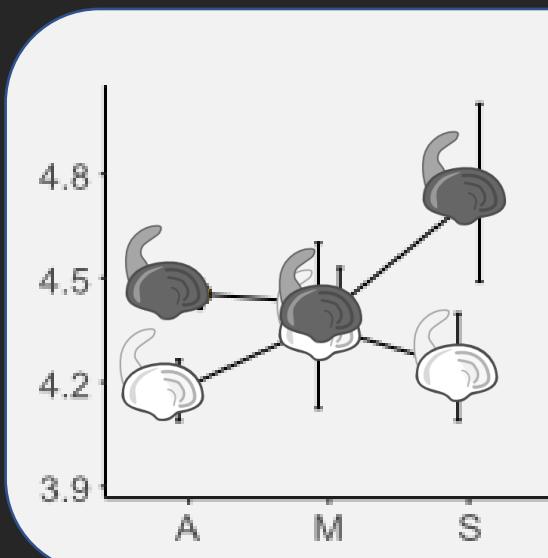


Day 14

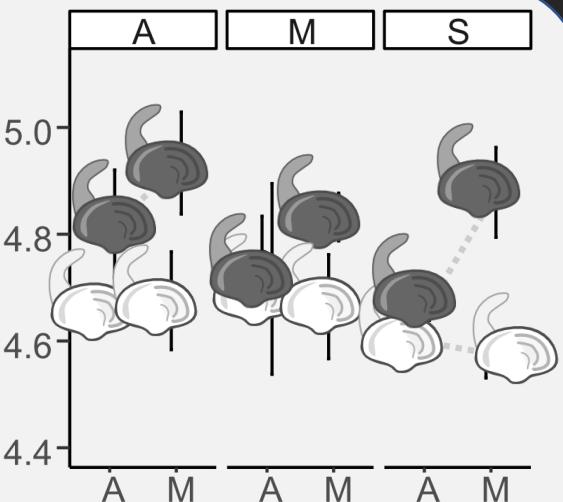


Day 21

conditioning-effect modules

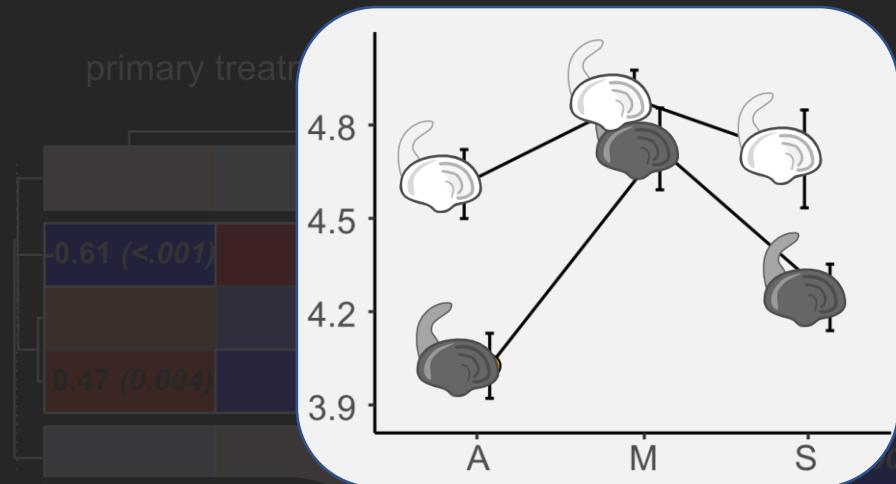


Day 14

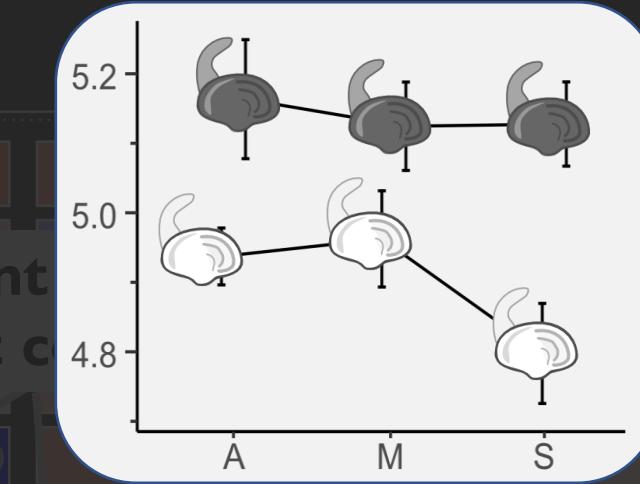


Day 21

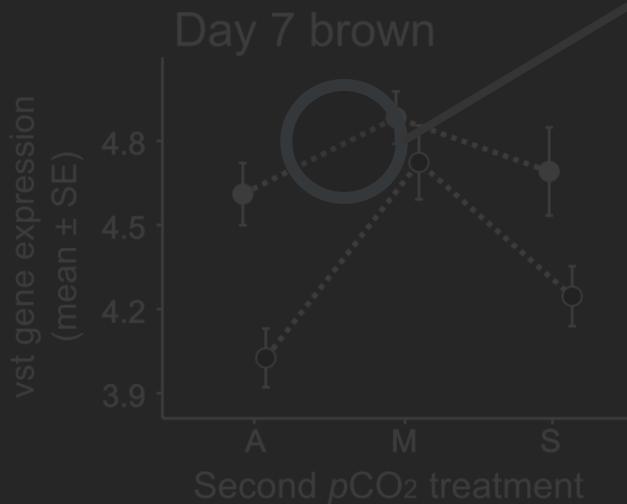
## naïve-effect modules



## conditioning-effect modules



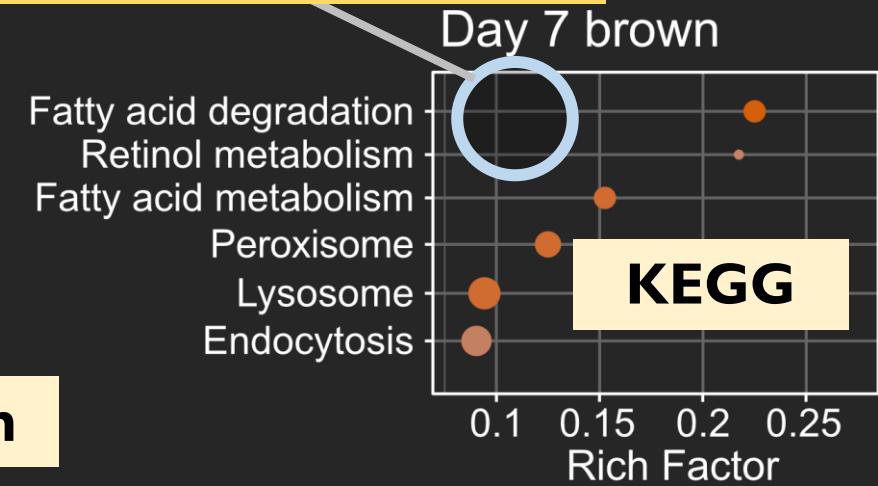
## expression patterns

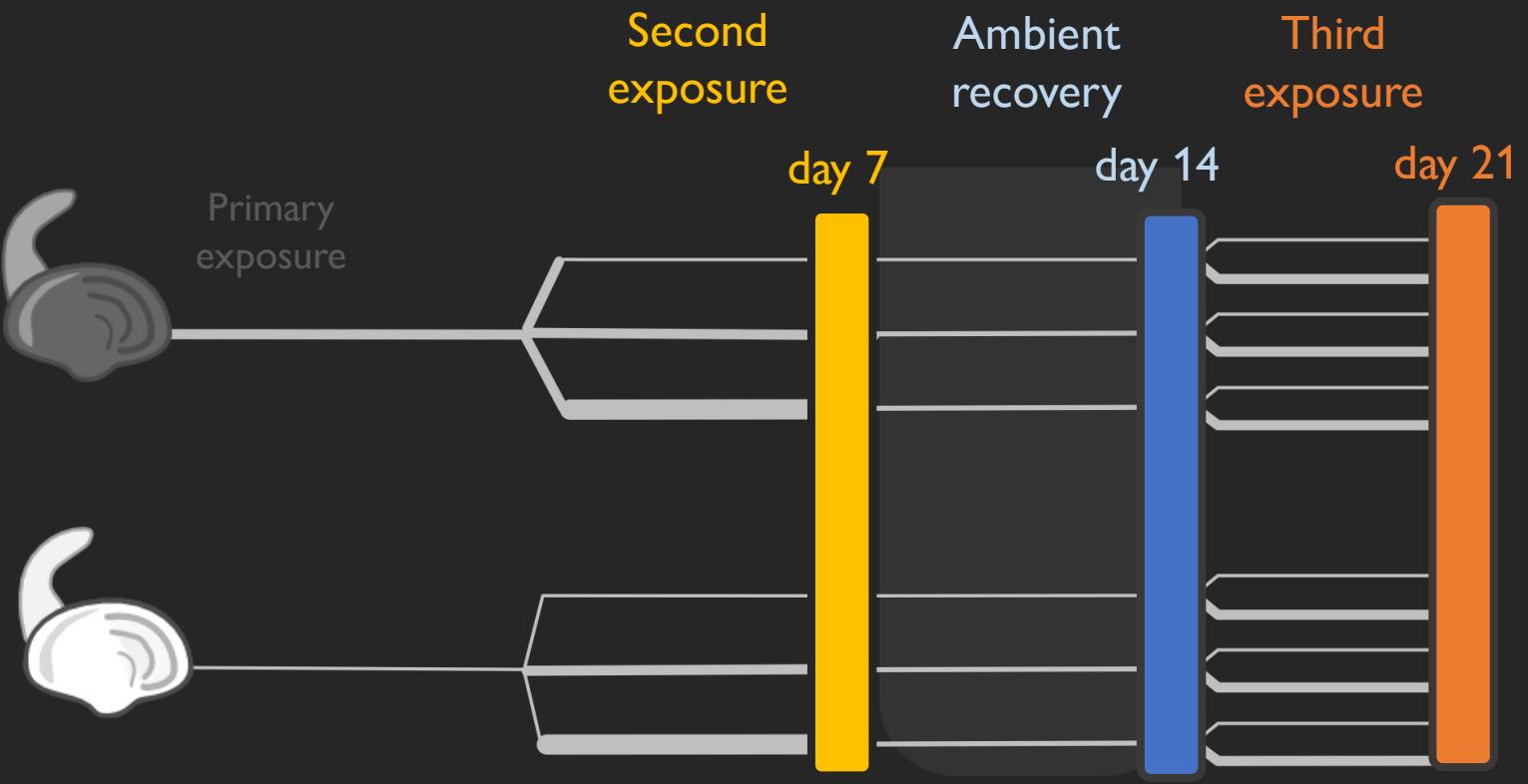


**distinct functions and pathways associated with naïve and conditioned clams?**



**goseq + goSlim**





# Results: Gene ontology analysis

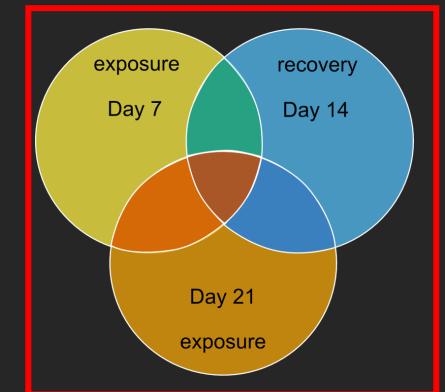
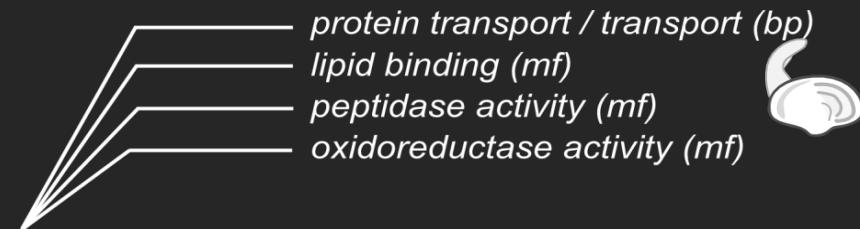
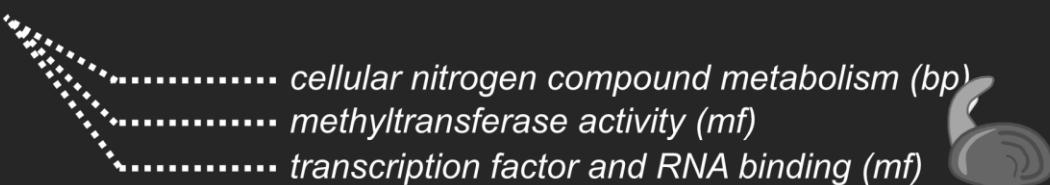


conditioning-effect  
modules



naïve-effect  
modules

continuously regulated



# Results: Gene ontology analysis



**conditioning-effect  
modules**



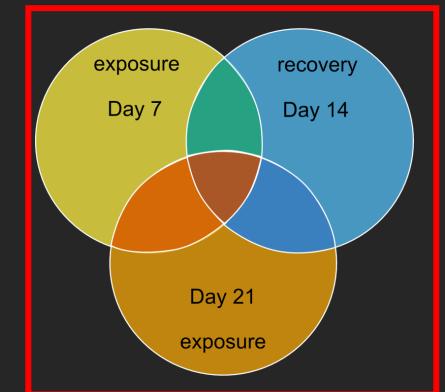
**naïve-effect  
modules**

continuously regulated

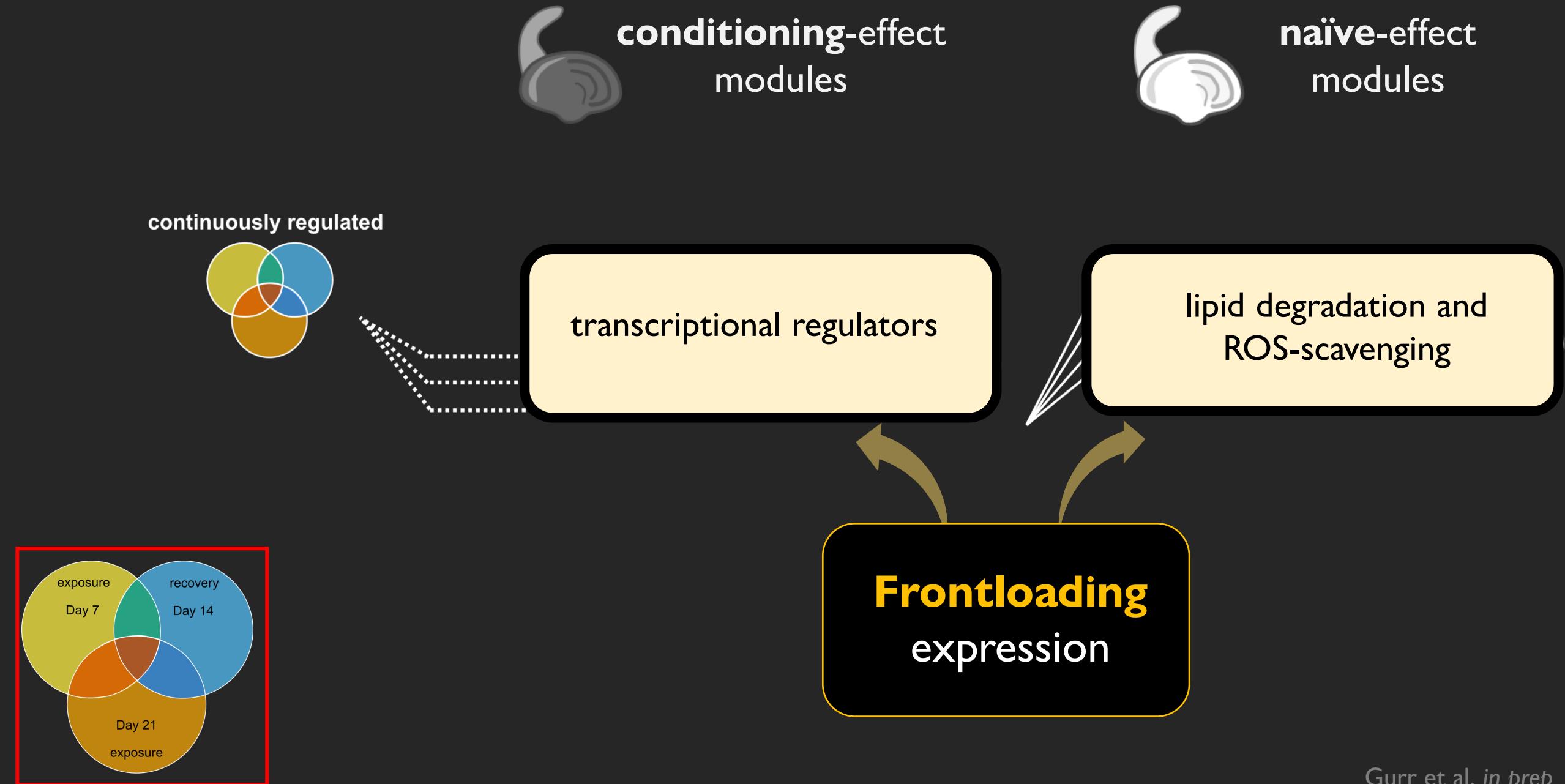


histone methyltransferases  
transcription factors  
(e.g. HIF-1, protein max)

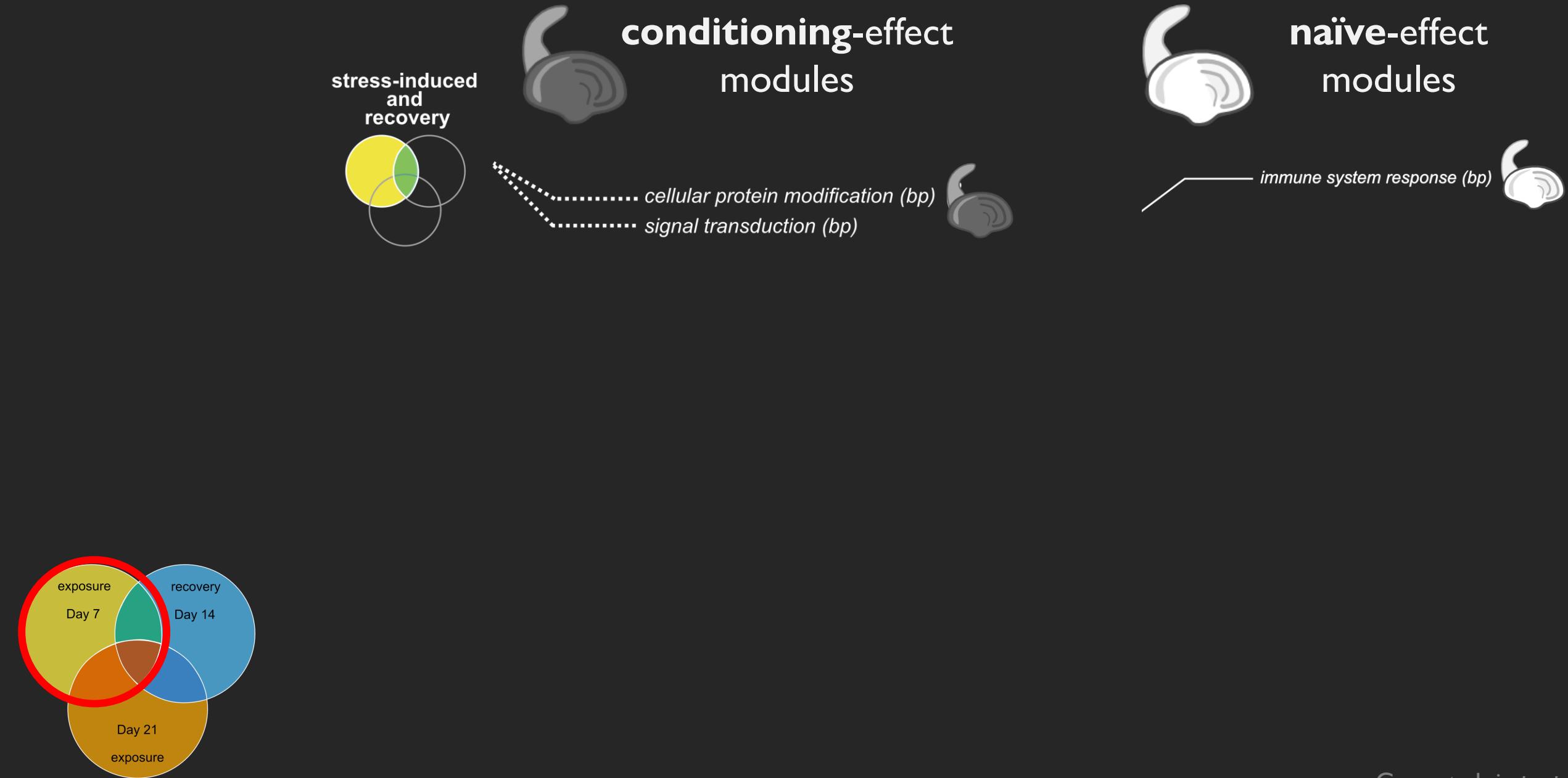
fatty-acid metabolism  
& bioremediation of  
free radicals



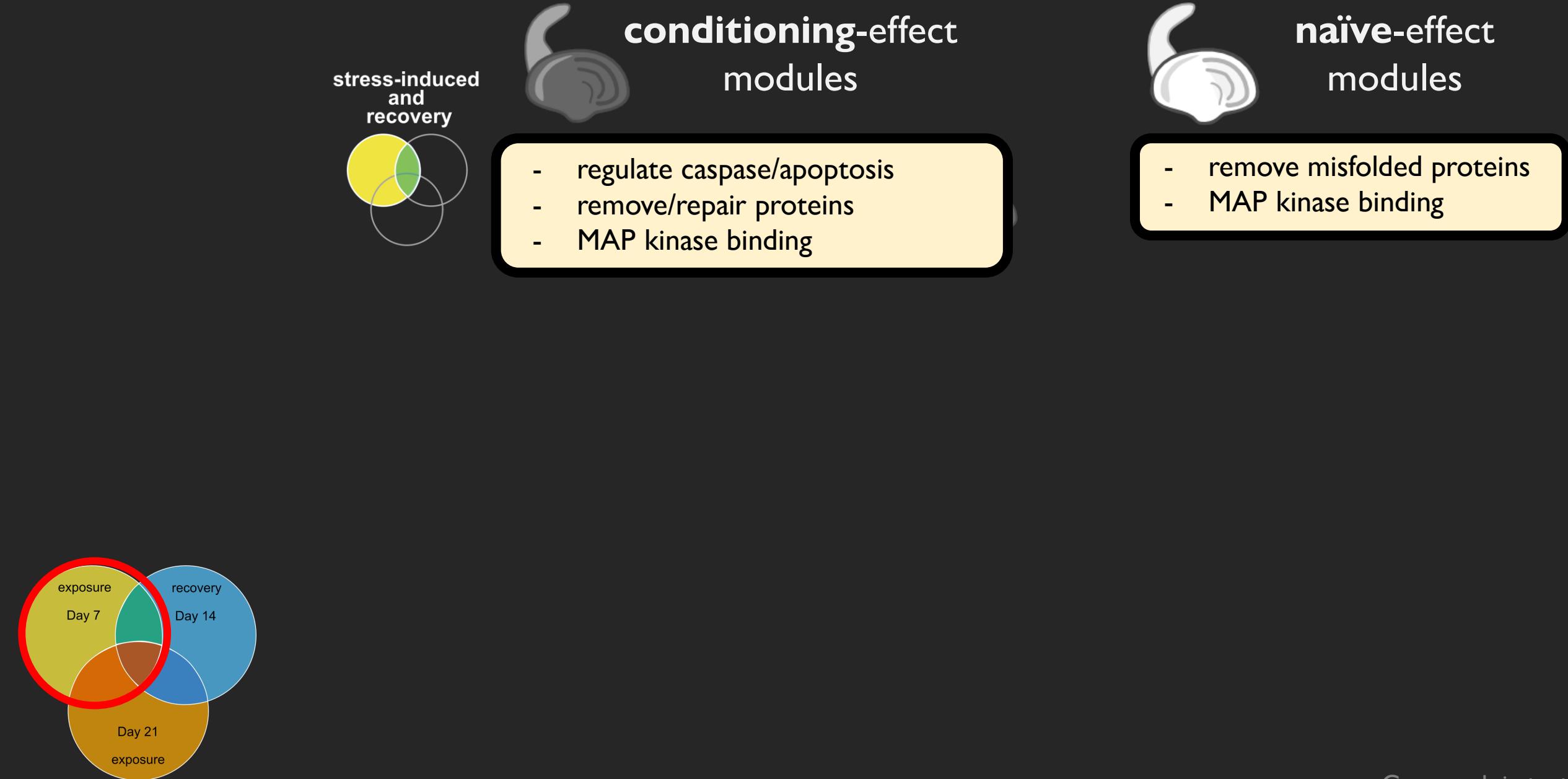
# Results: Gene ontology analysis



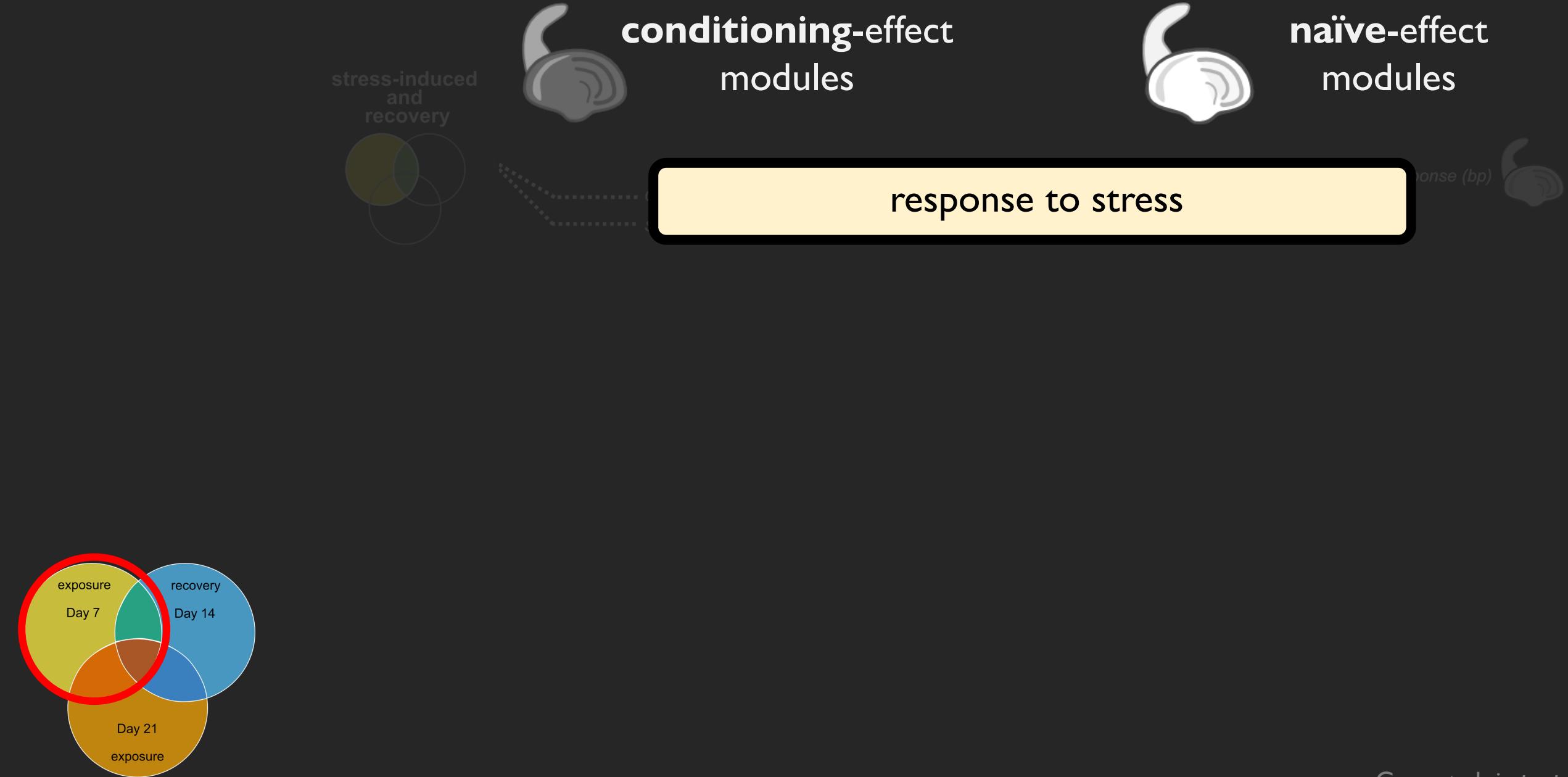
# Results: Gene ontology analysis



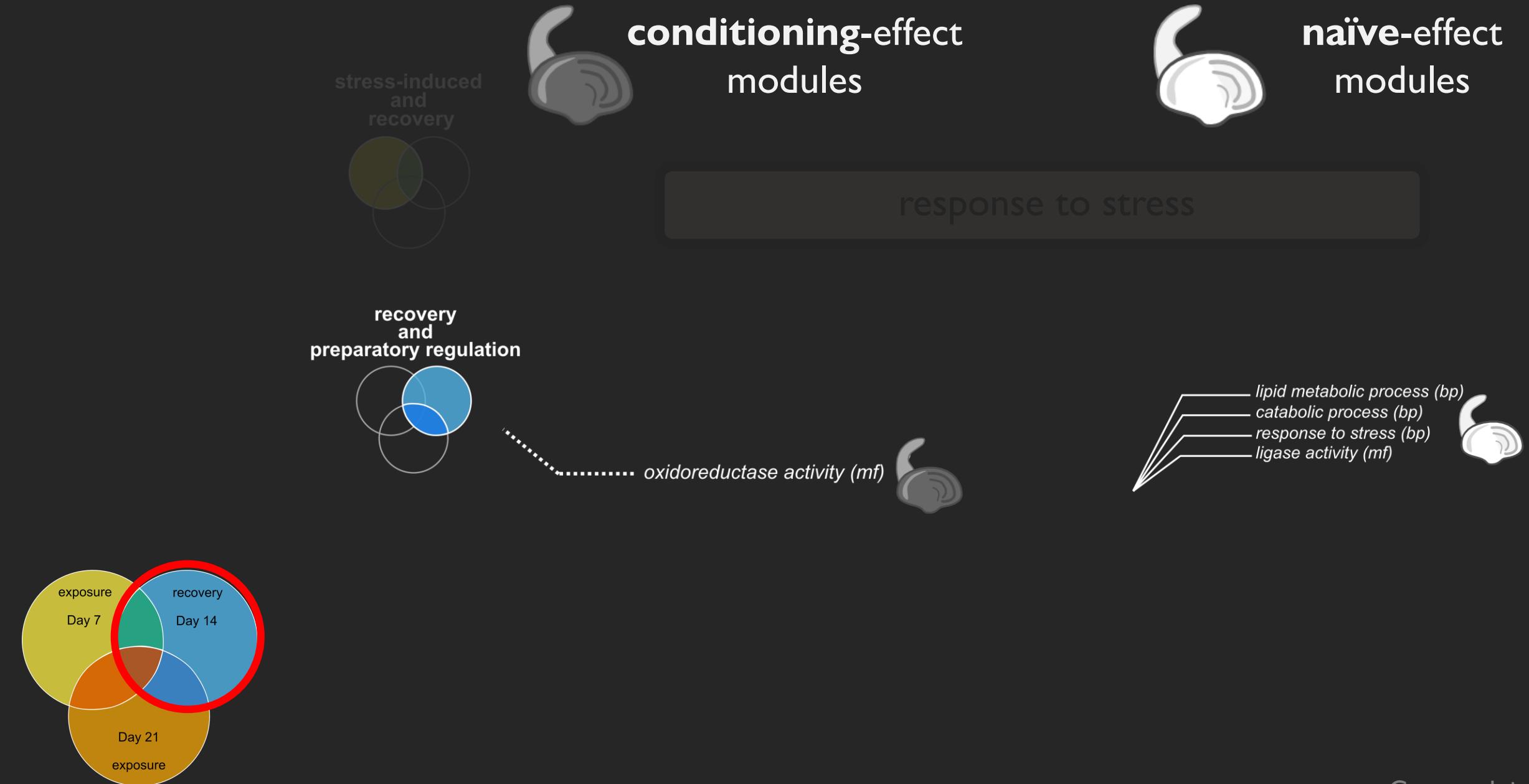
# Results: Gene ontology analysis



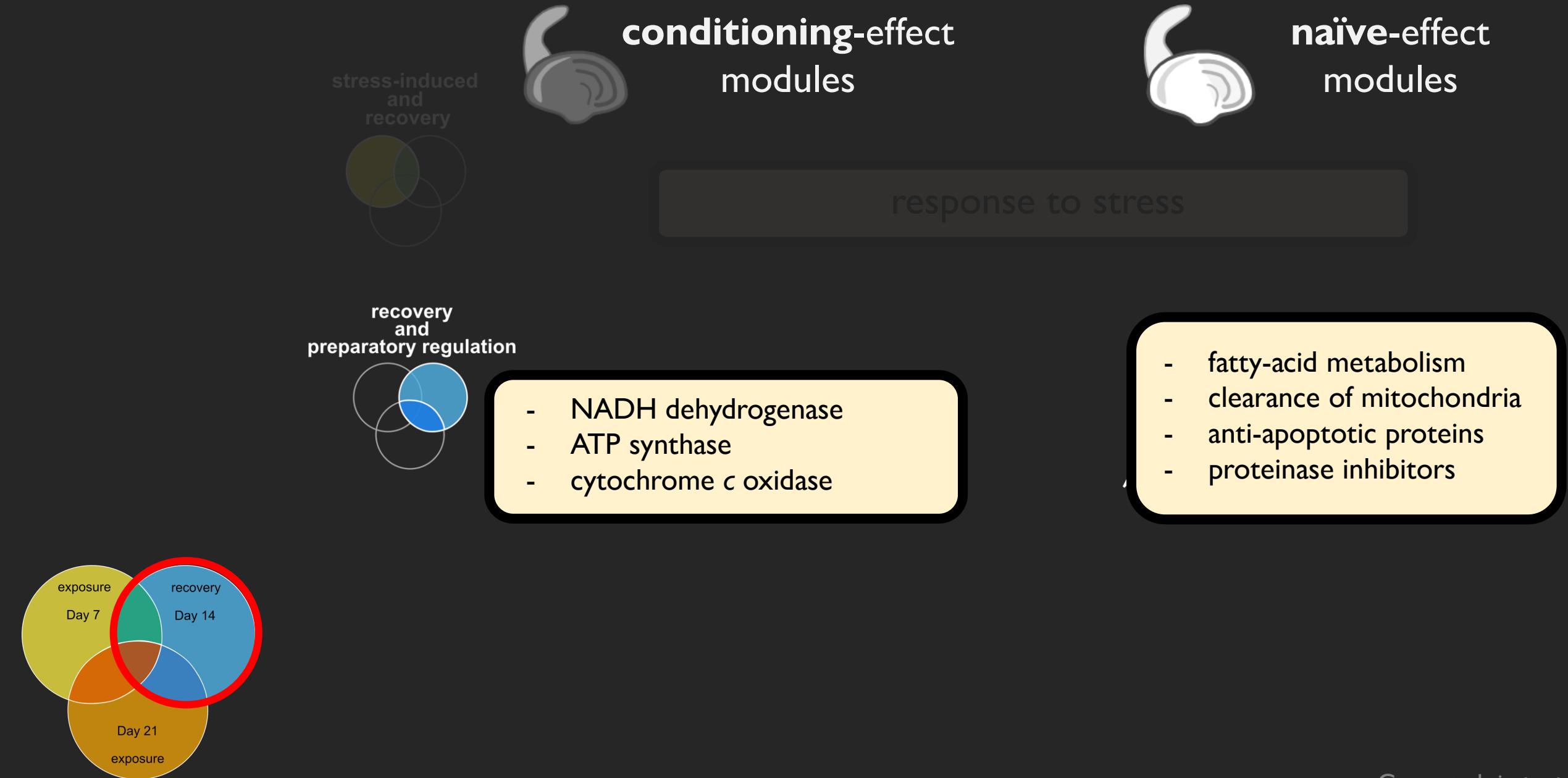
# Results: Gene ontology analysis



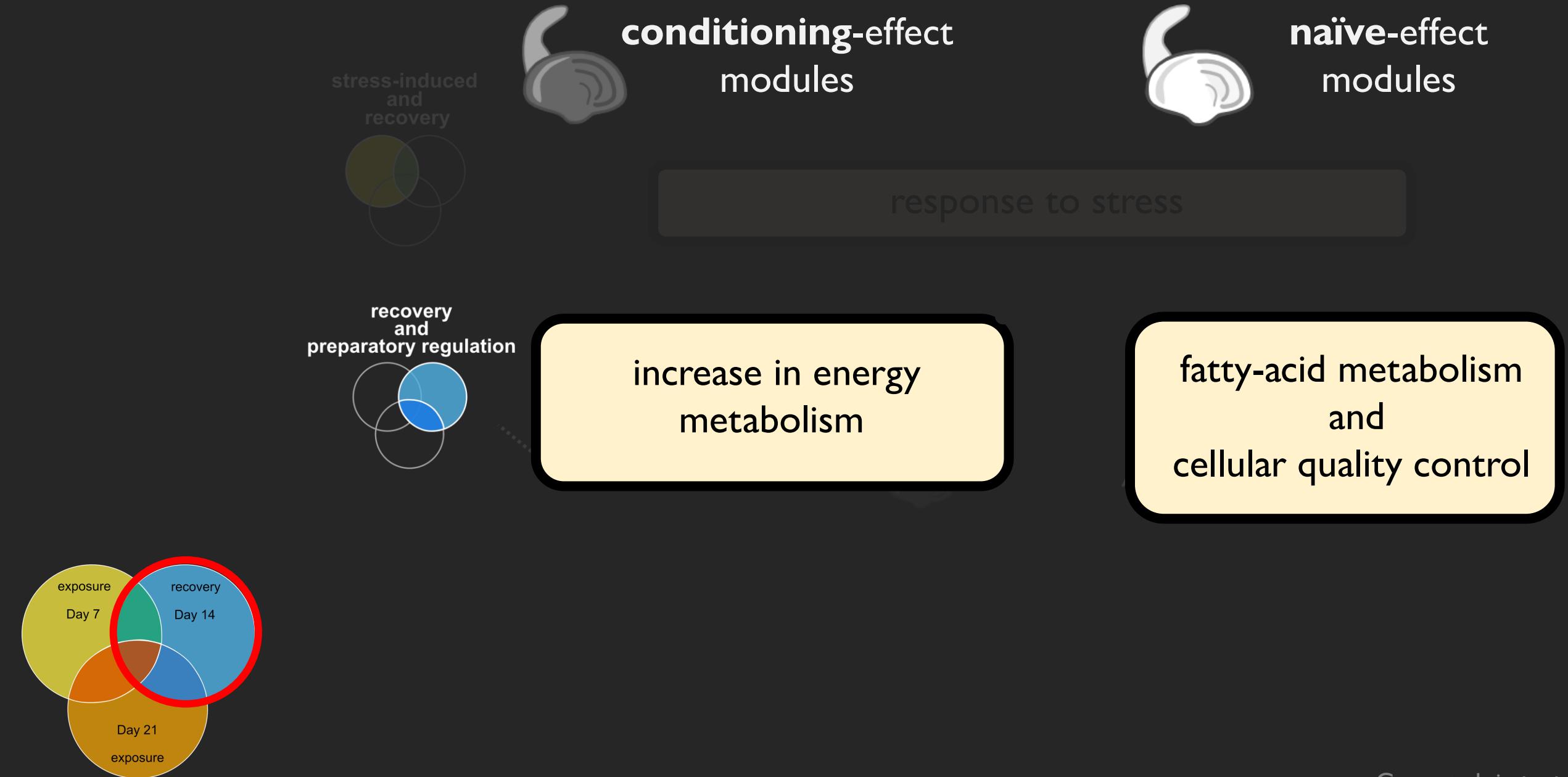
# Results: Gene ontology analysis



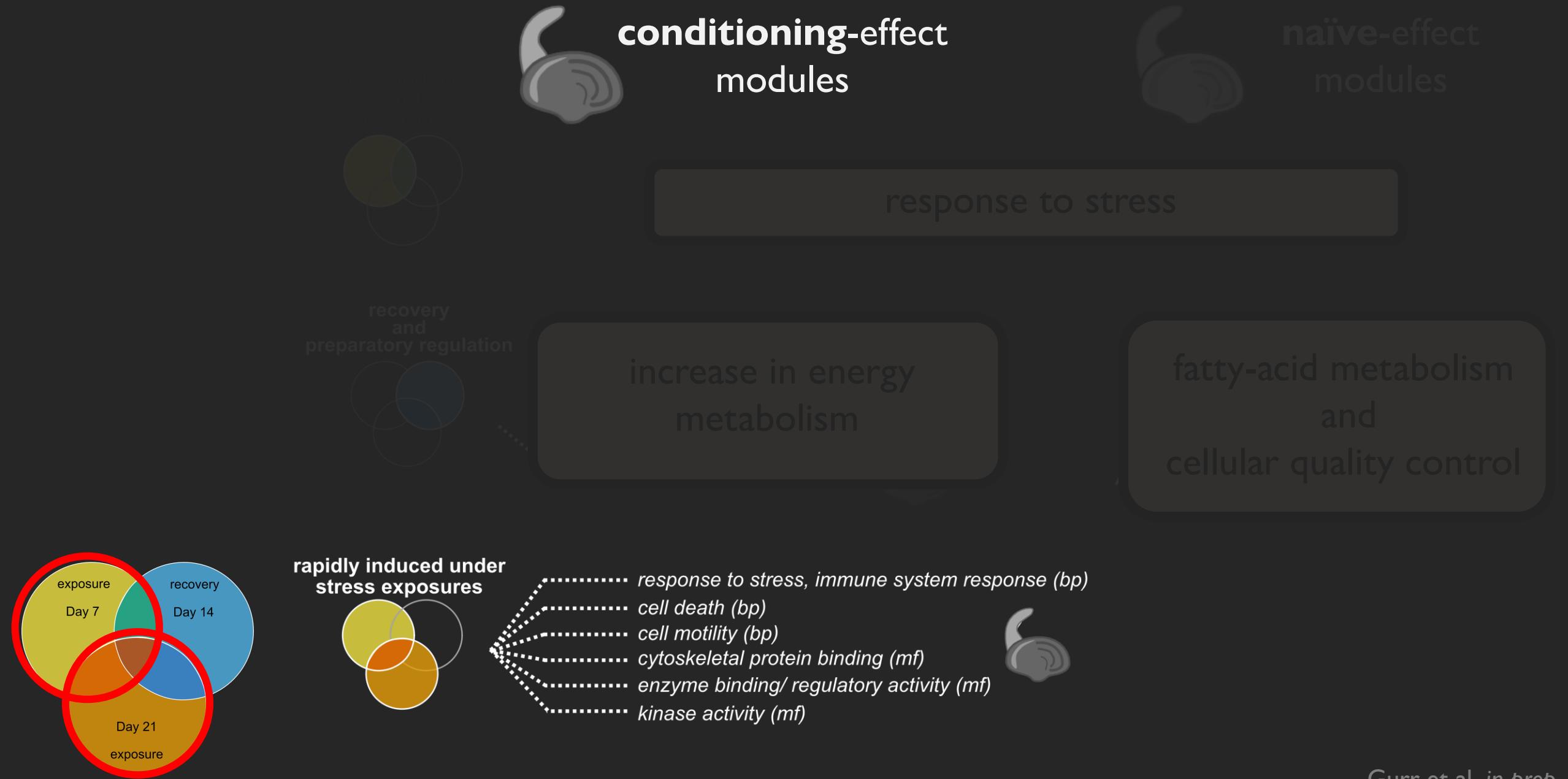
# Results: Gene ontology analysis



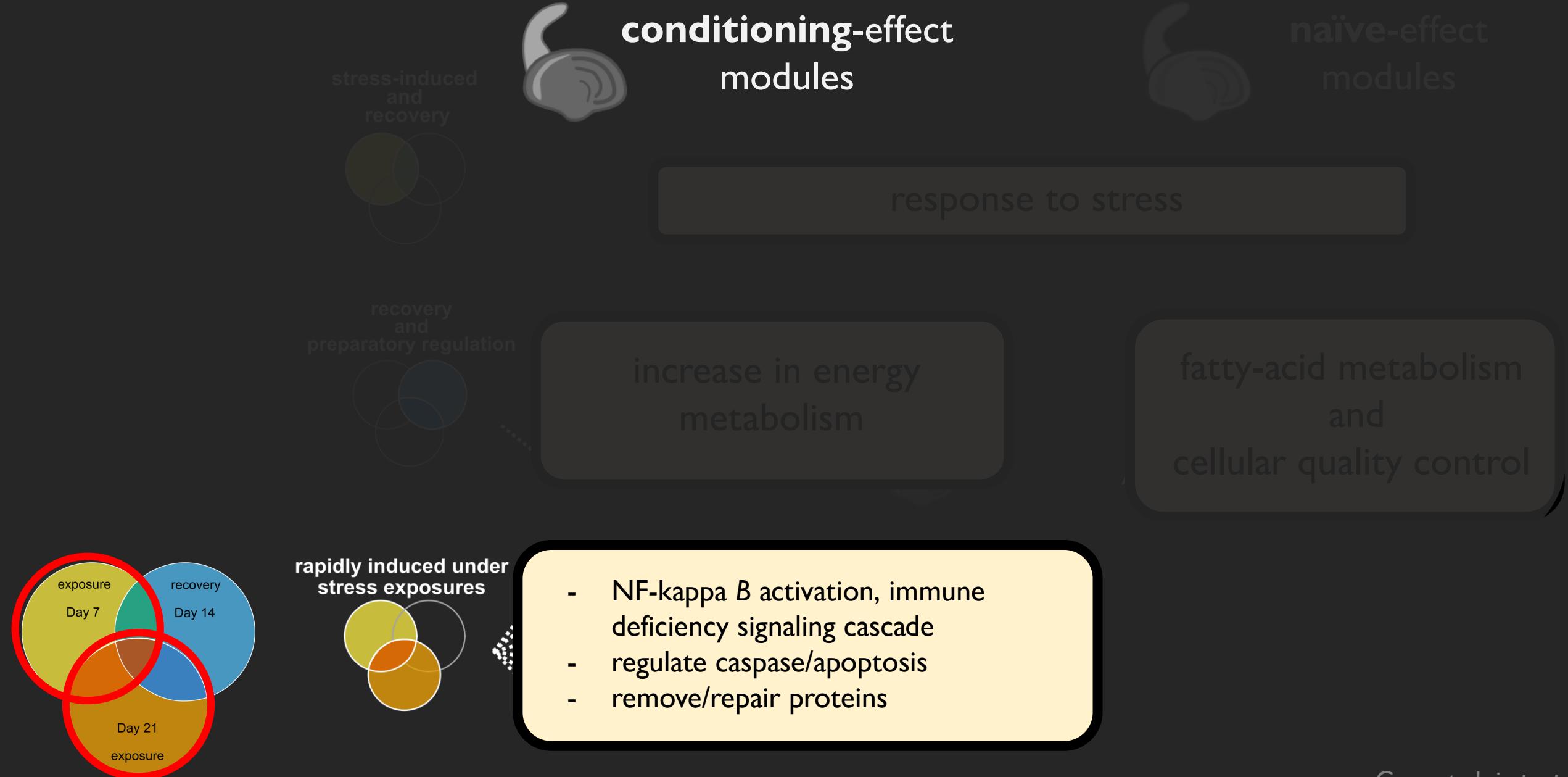
# Results: Gene ontology analysis



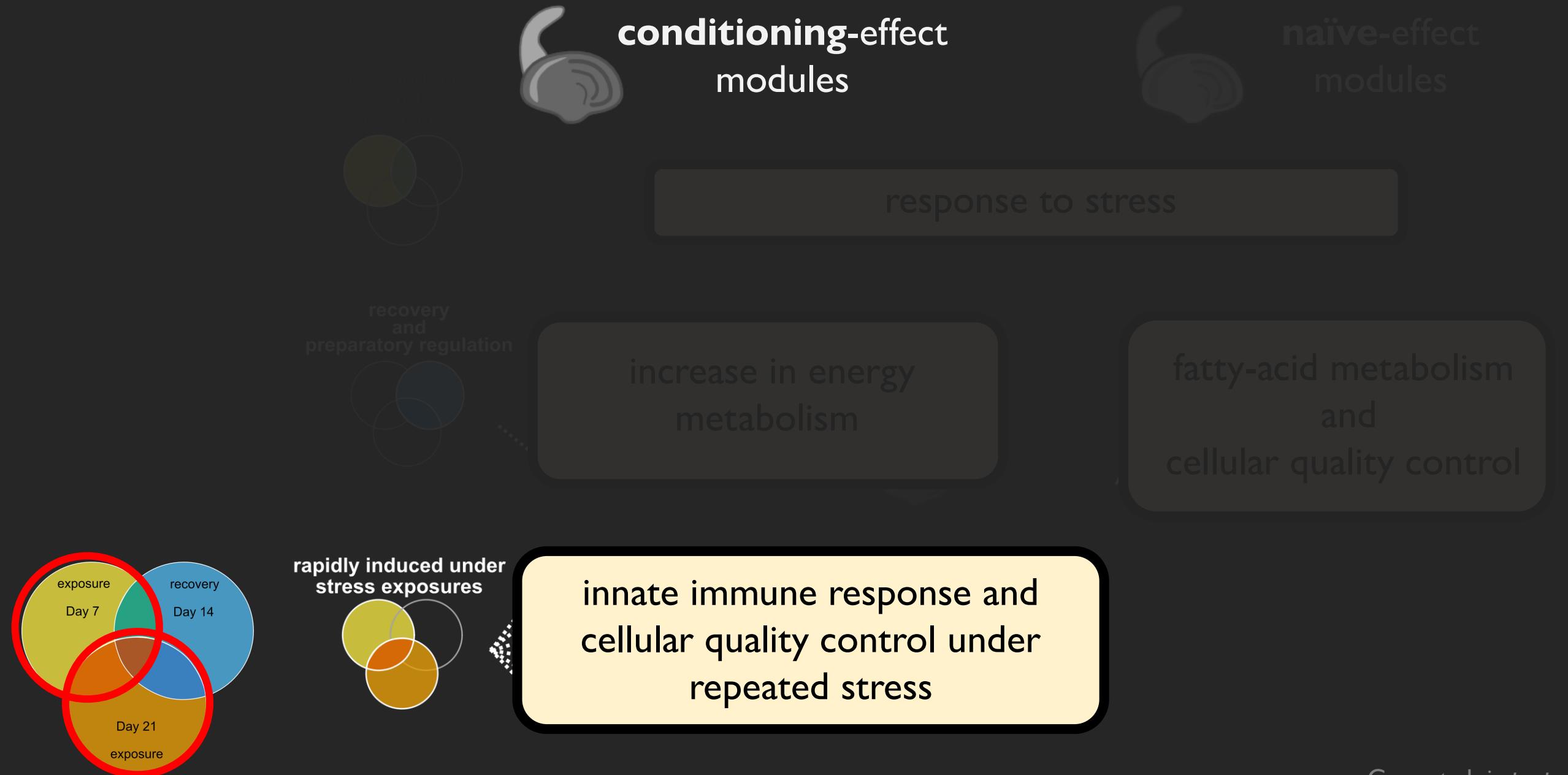
# Results: Gene ontology analysis



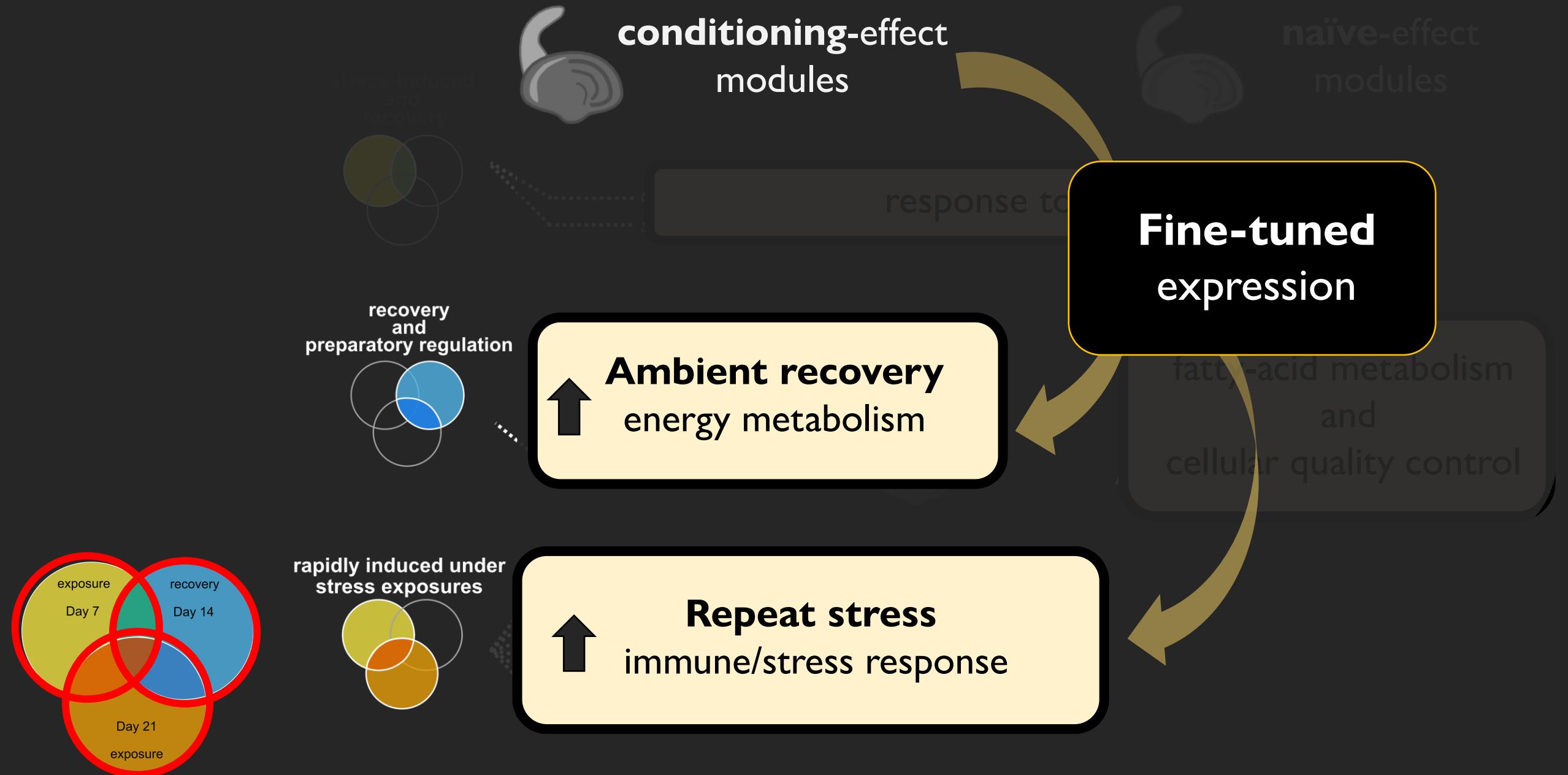
# Results: Gene ontology analysis



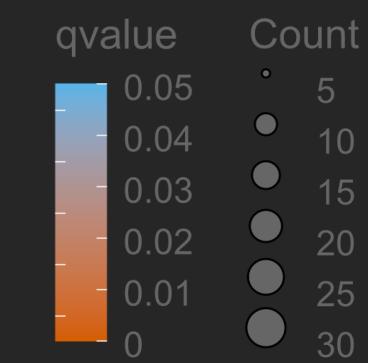
# Results: Gene ontology analysis



# Results: Gene ontology analysis



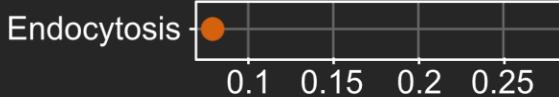
# Results: Pathway enrichment analysis



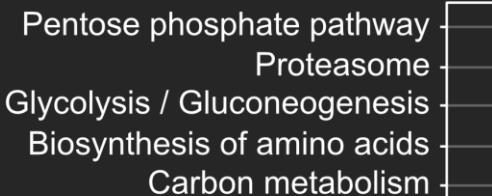
Day 7



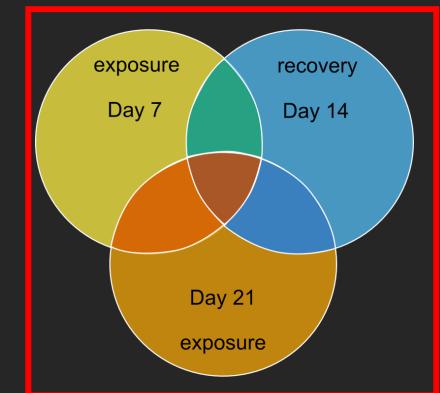
## conditioning-effect modules



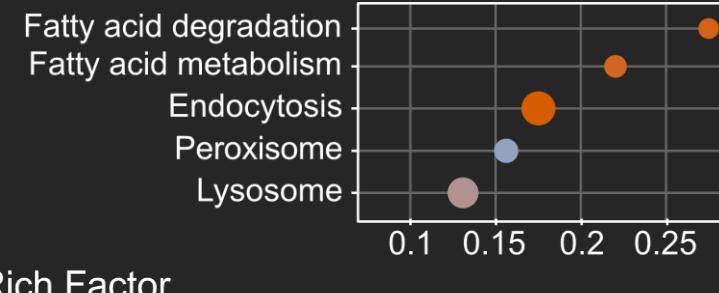
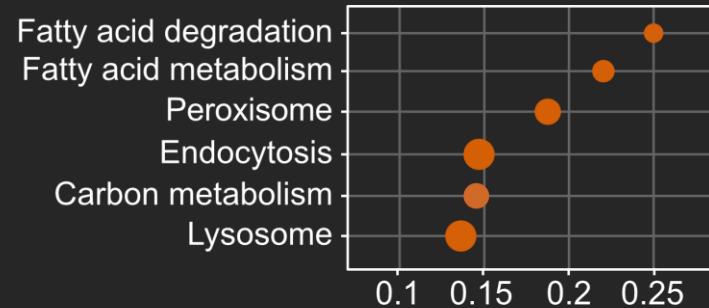
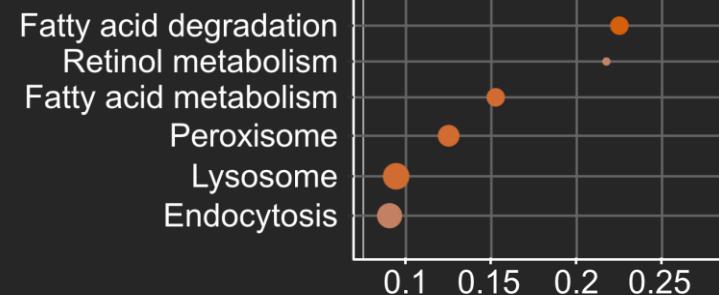
Day 14



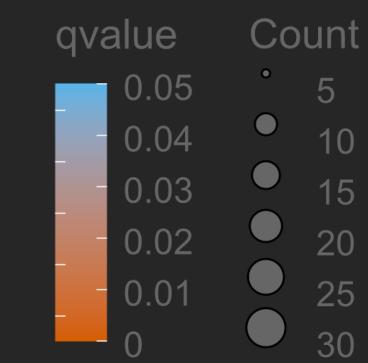
Day 21



## naïve-effect modules



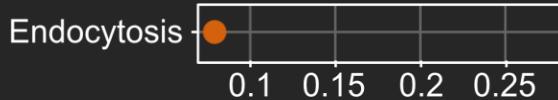
# Results: Pathway enrichment analysis



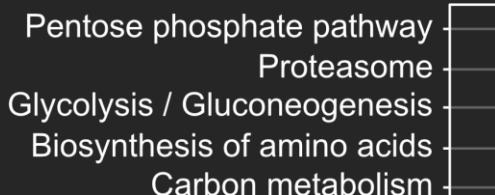
Day 7



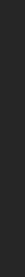
## conditioning-effect modules



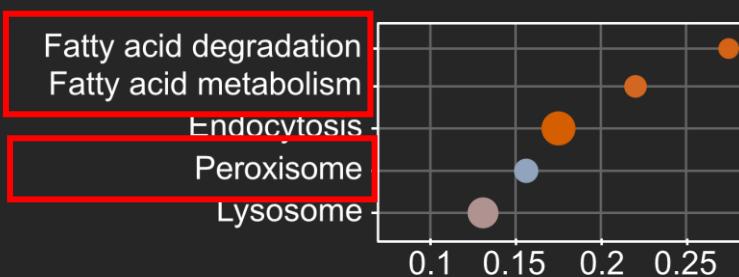
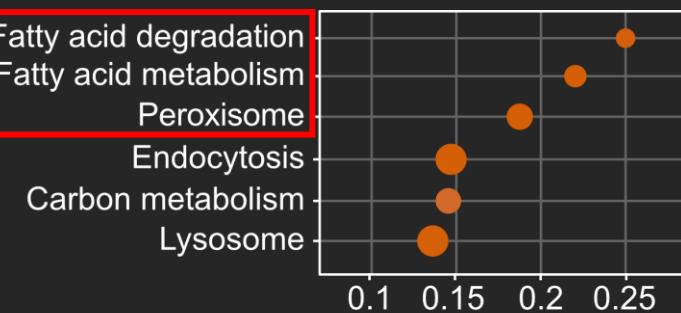
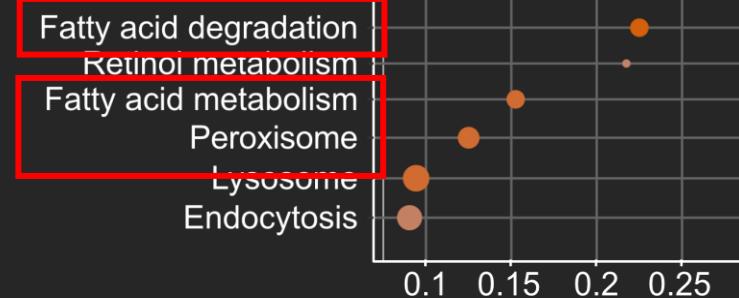
Day 14



Day 21

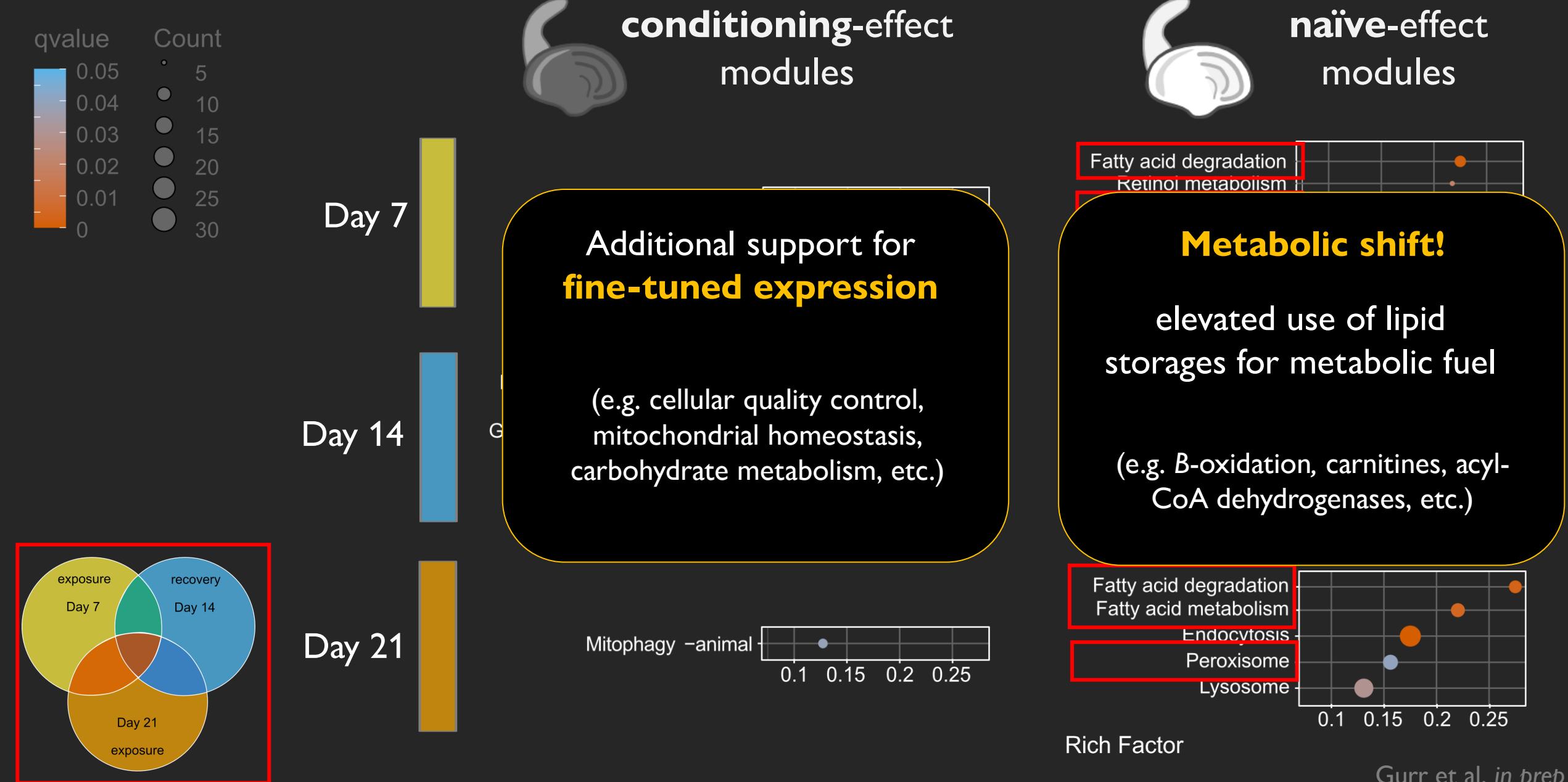


## naïve-effect modules



Rich Factor

# Results: Pathway enrichment analysis



# Chapter III: Conclusions

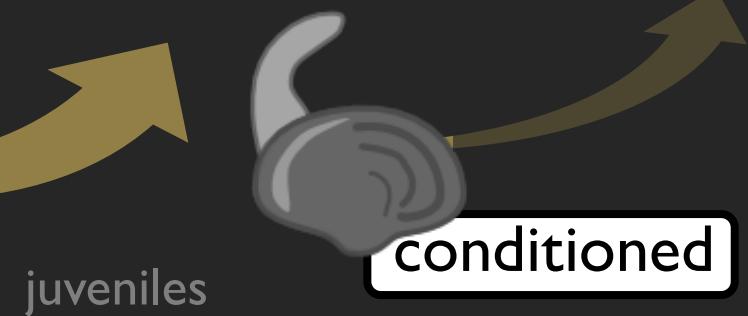
stress  
conditioning



pediveliger

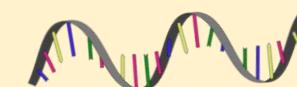


naïve



juveniles

Illuminate underlying  
mechanisms on the  
**molecular level**



# Chapter III: Conclusions

stress  
conditioning

pediveliger

Continuous metabolic shift  
(fatty-acid metabolism)  
and **oxidative stress**

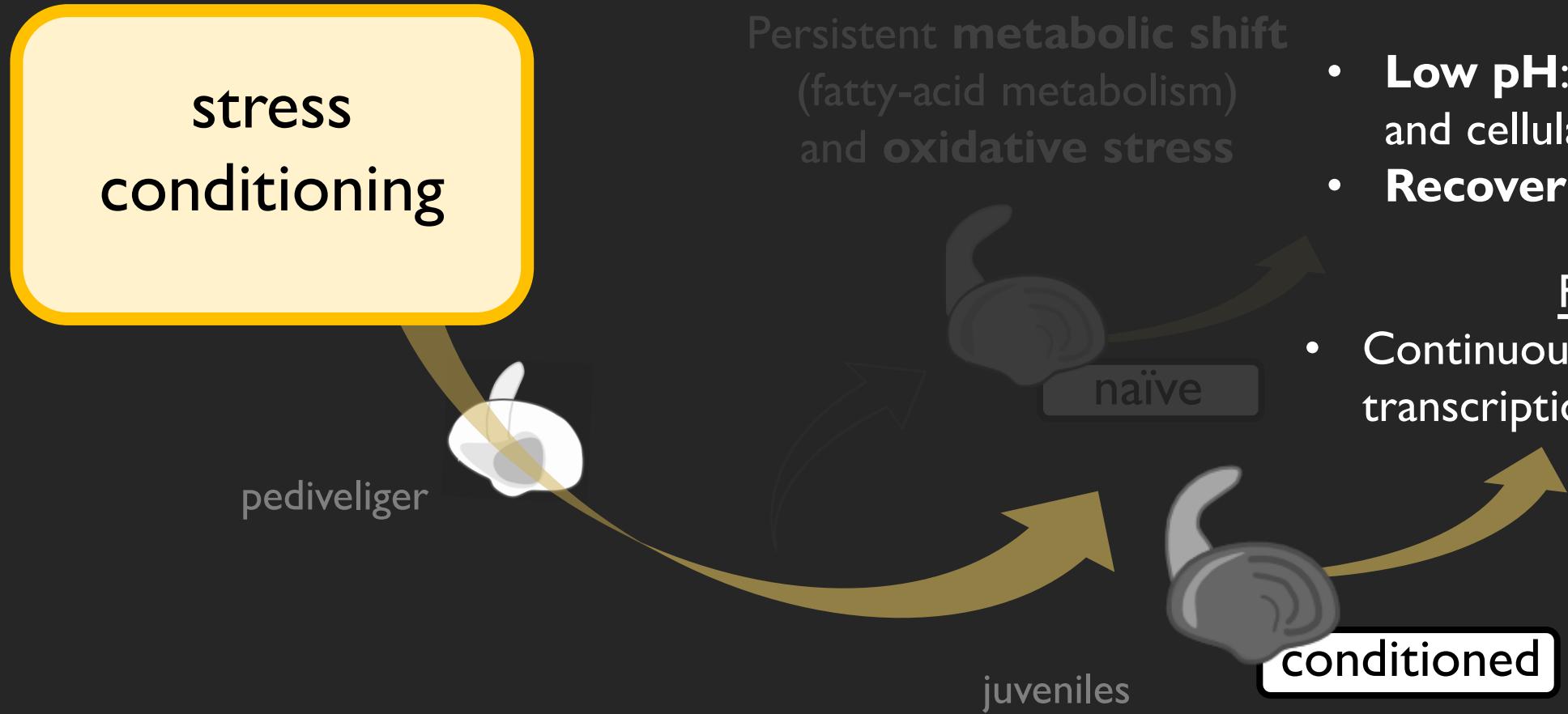
naïve

juveniles

conditioned



# Chapter III: Conclusions



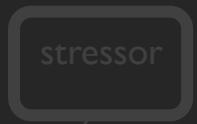
Fine-tuned

- **Low pH:** immune system and cellular quality control
- **Recovery:** energy metabolism

Frontloading

- Continuous expression of transcriptional regulators

# Chapter I



carryover

PHYSIOLOGY

## Hypothesis

Repeated stress exposure under elevated pCO<sub>2</sub> enhances intragenerational performance for Pacific geoduck.

# Chapter II

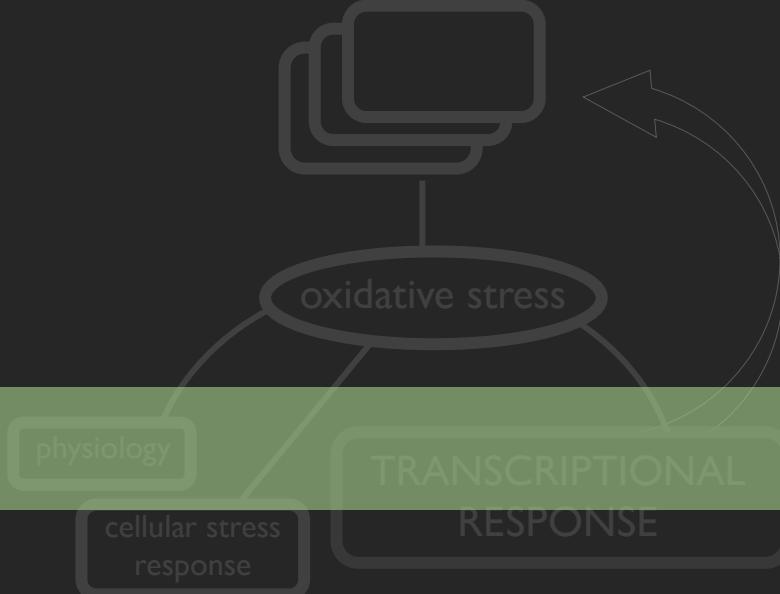


PHYSIOLOGY  
CELLULAR STRESS RESPONSE

## Hypothesis

Repeated stress exposure under elevated pCO<sub>2</sub> can enhance intragenerational performance for Pacific geoduck.

# Chapter III



physiology  
cellular stress response  
TRANSCRIPTIONAL RESPONSE

## Hypothesis

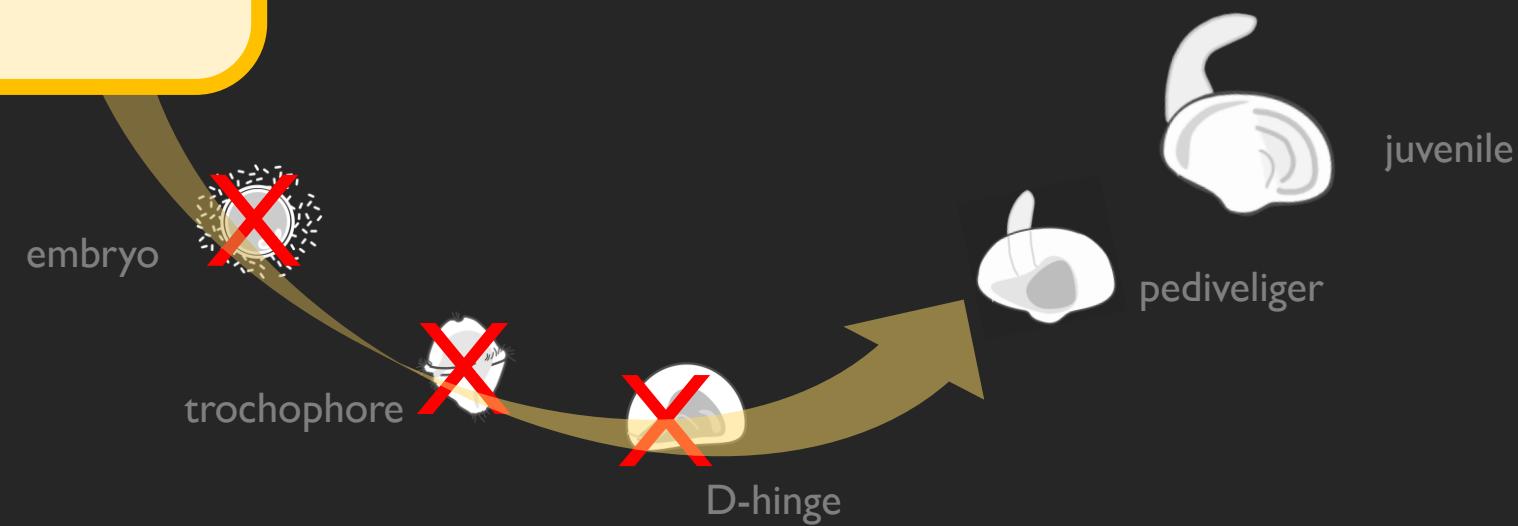
Early acclimation generates distinct functions and pathways underpinning divergent phenotypes

stress  
conditioning

What are the **mechanisms underlying a  
conditioned response**

Synthesis

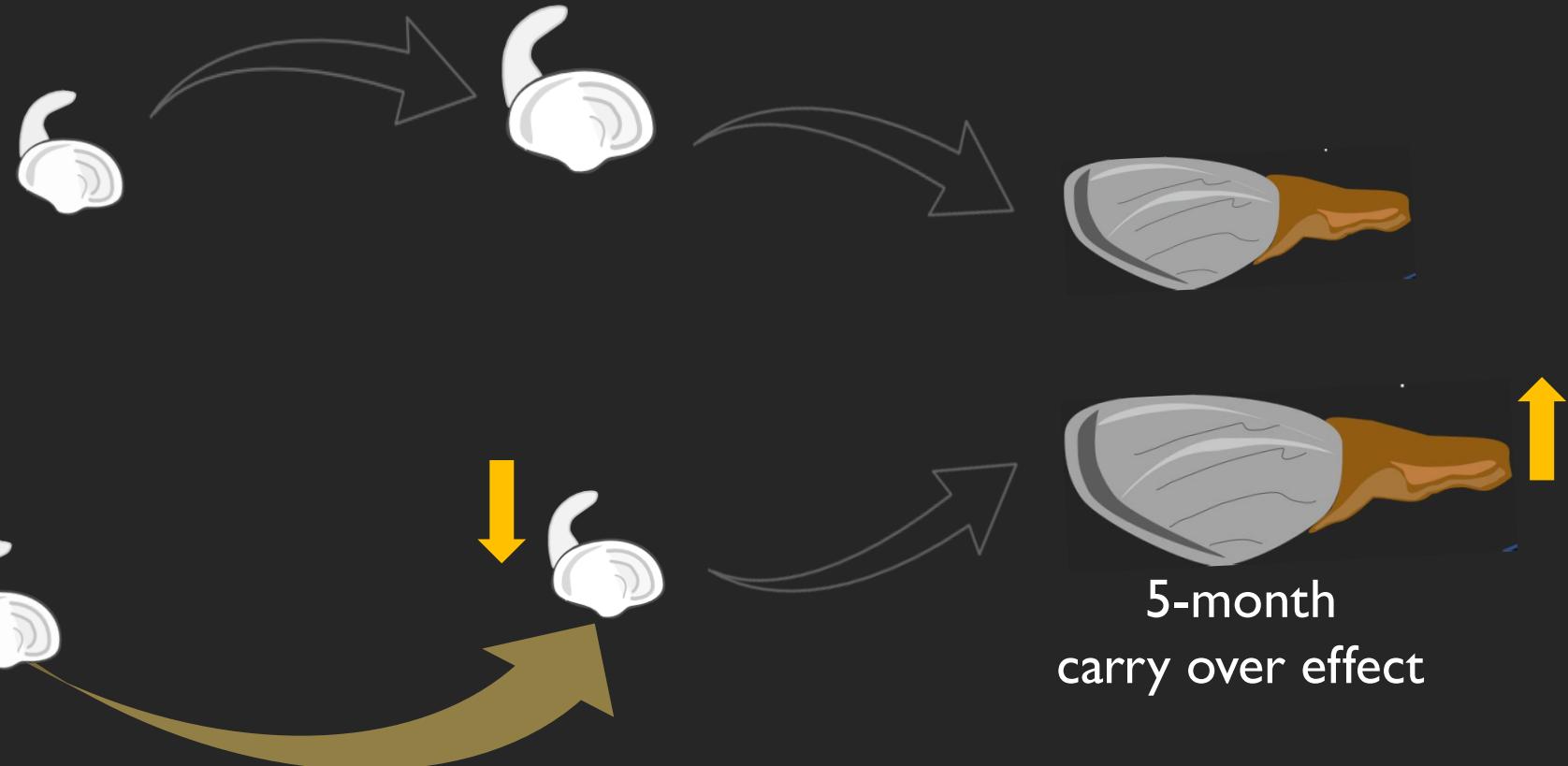
stress  
conditioning



Synthesis

Stress history (allow initially negative) preceded  
**growth compensation** and **metabolic recovery**

stress  
conditioning



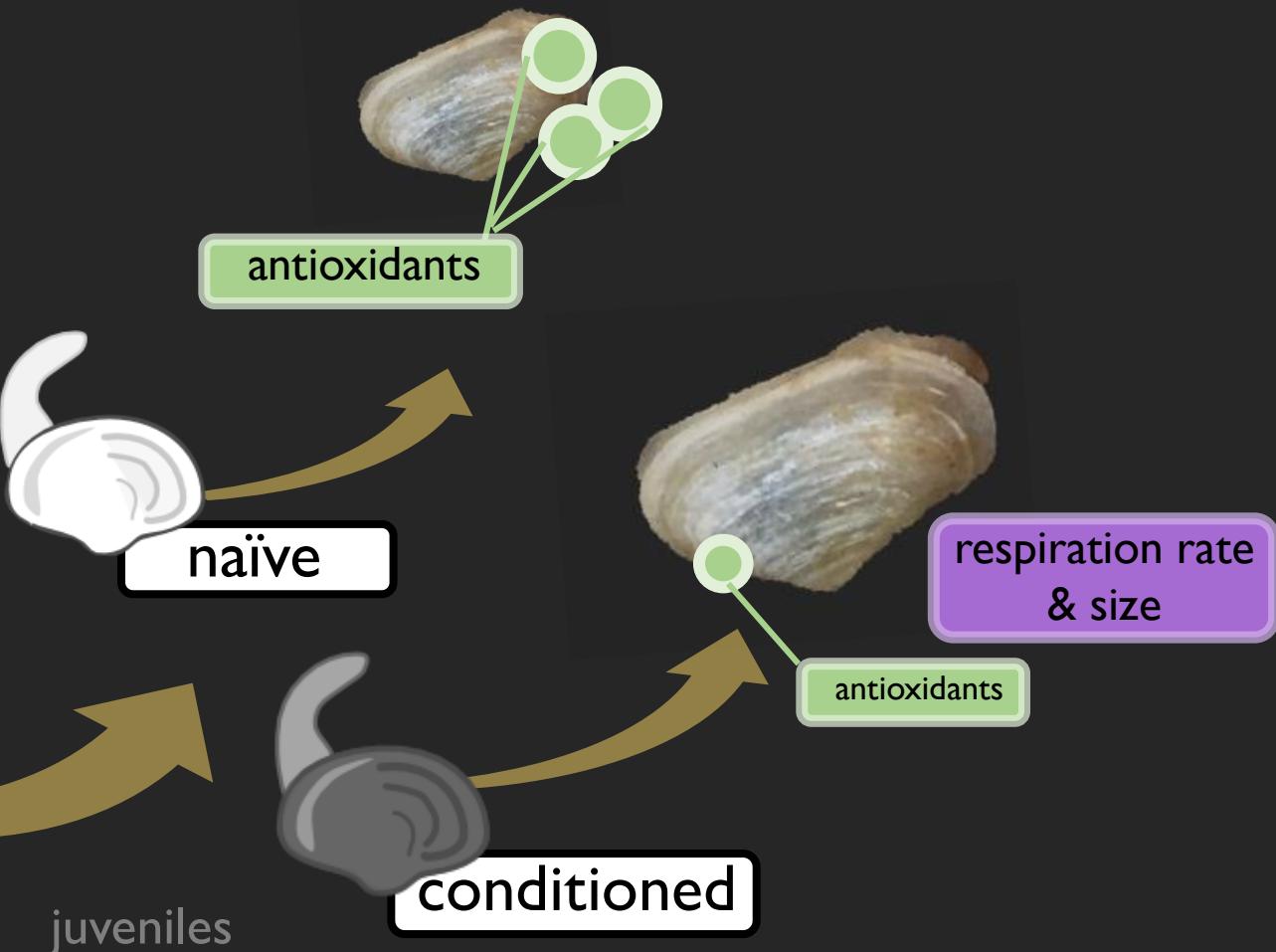
Synthesis

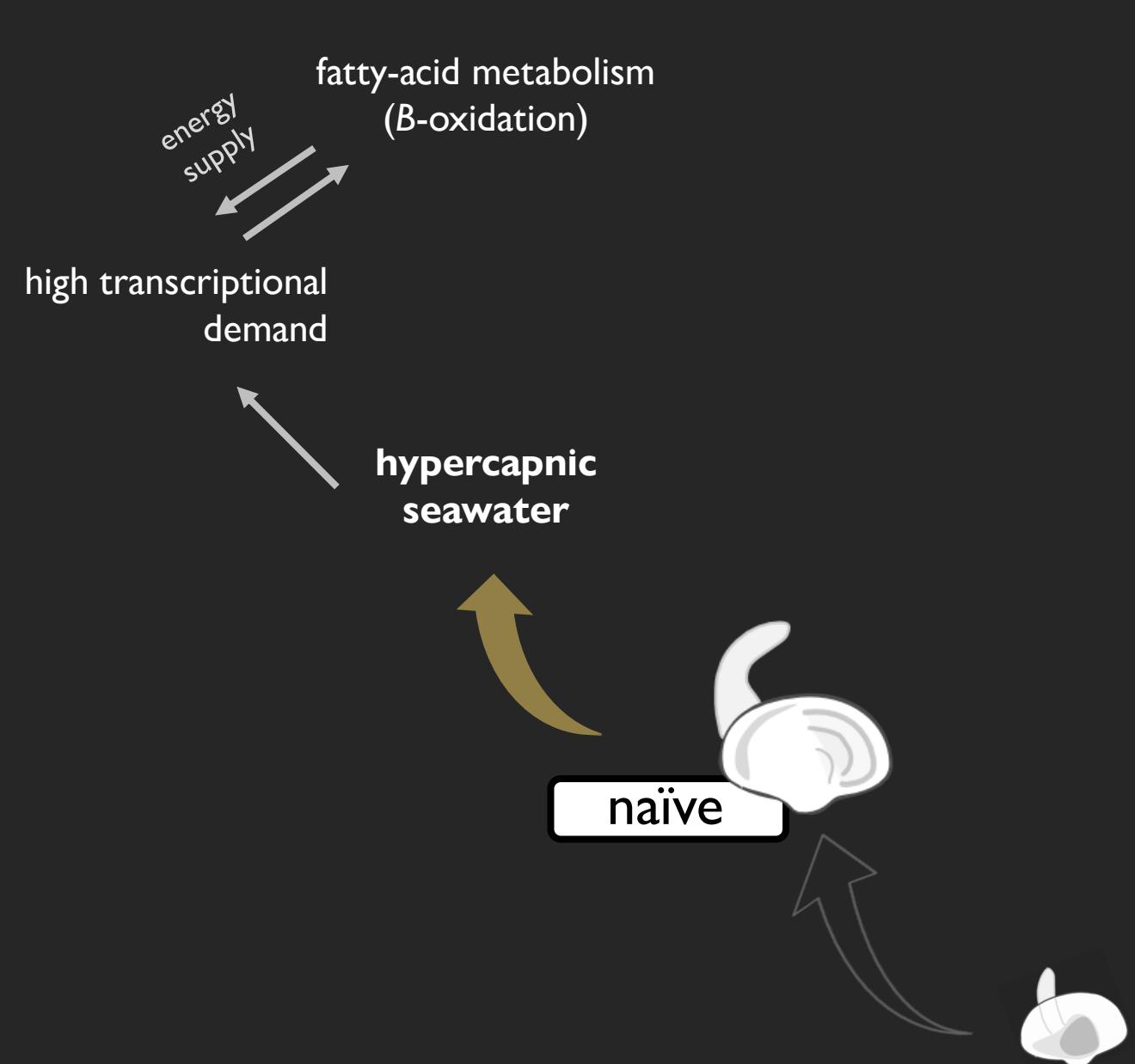
# stress conditioning



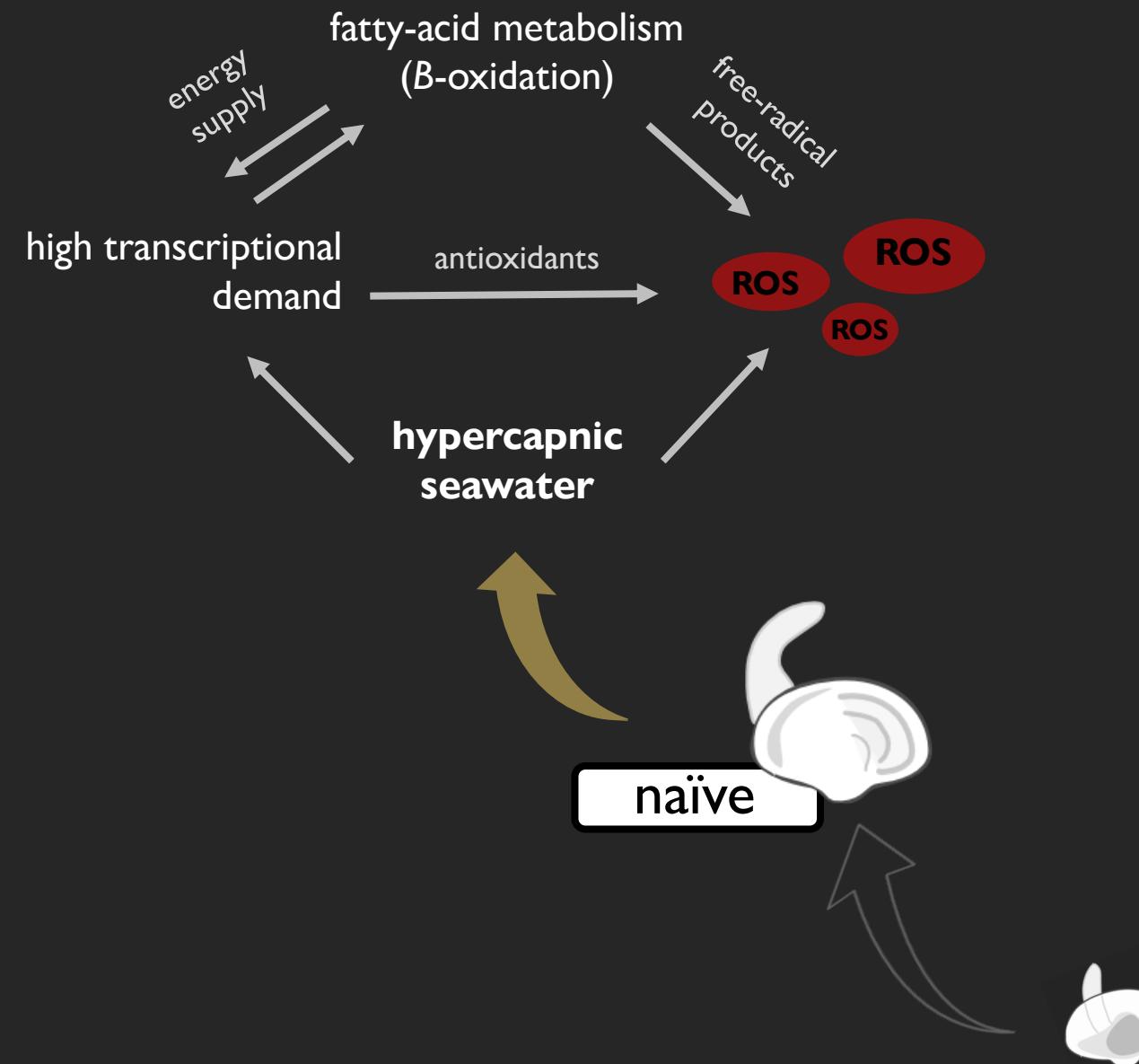
pediveliger

## Synthesis

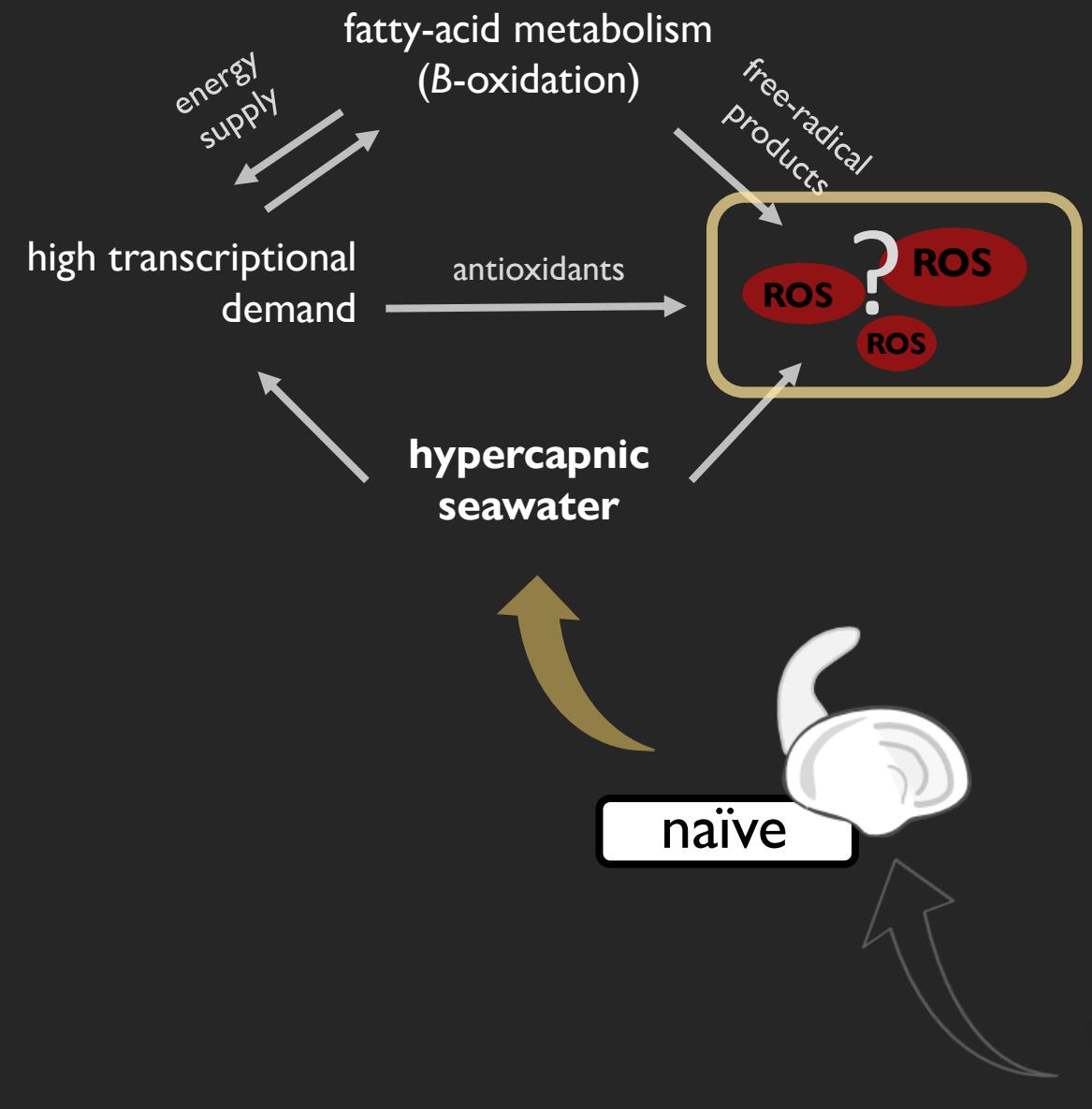




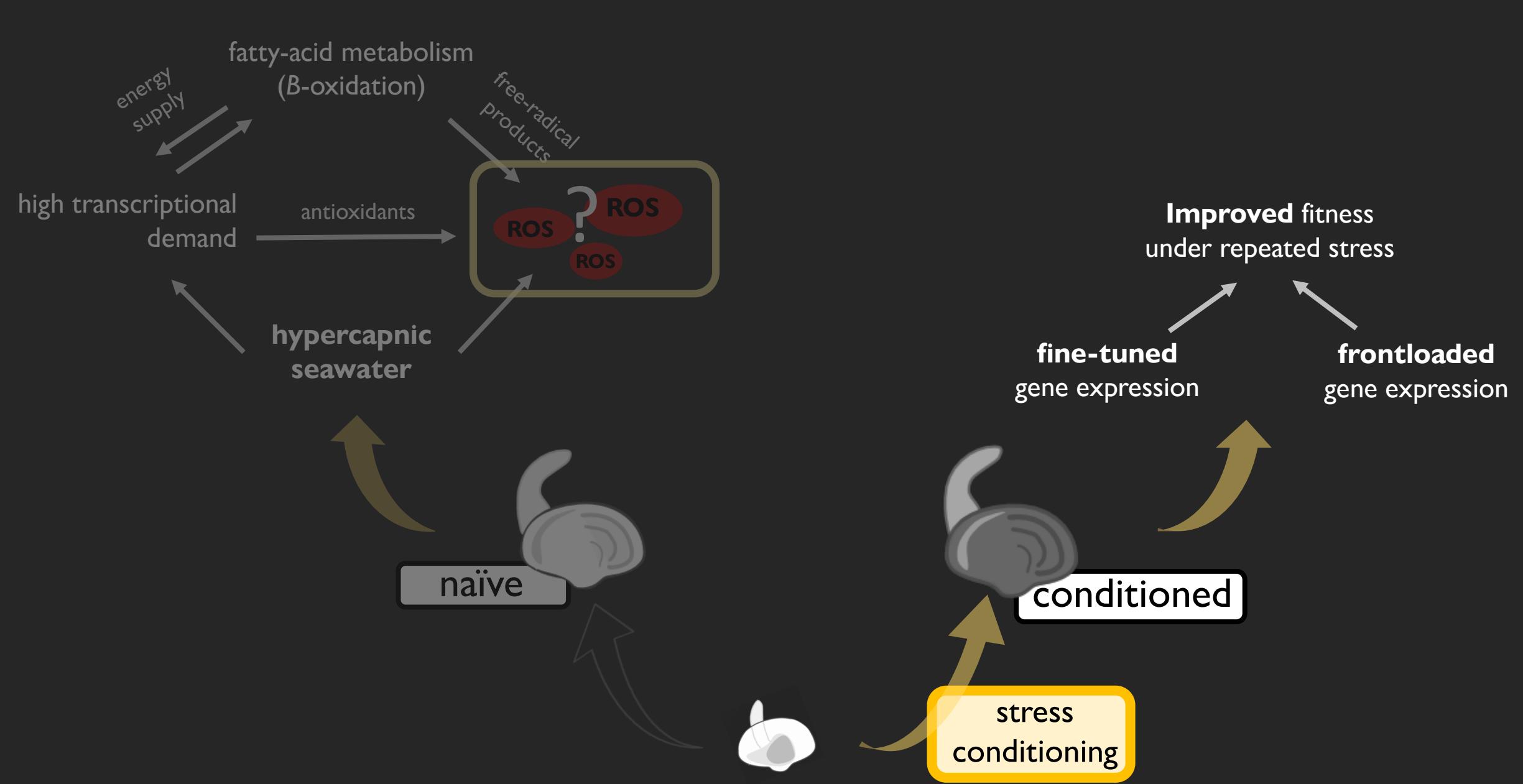
# Synthesis



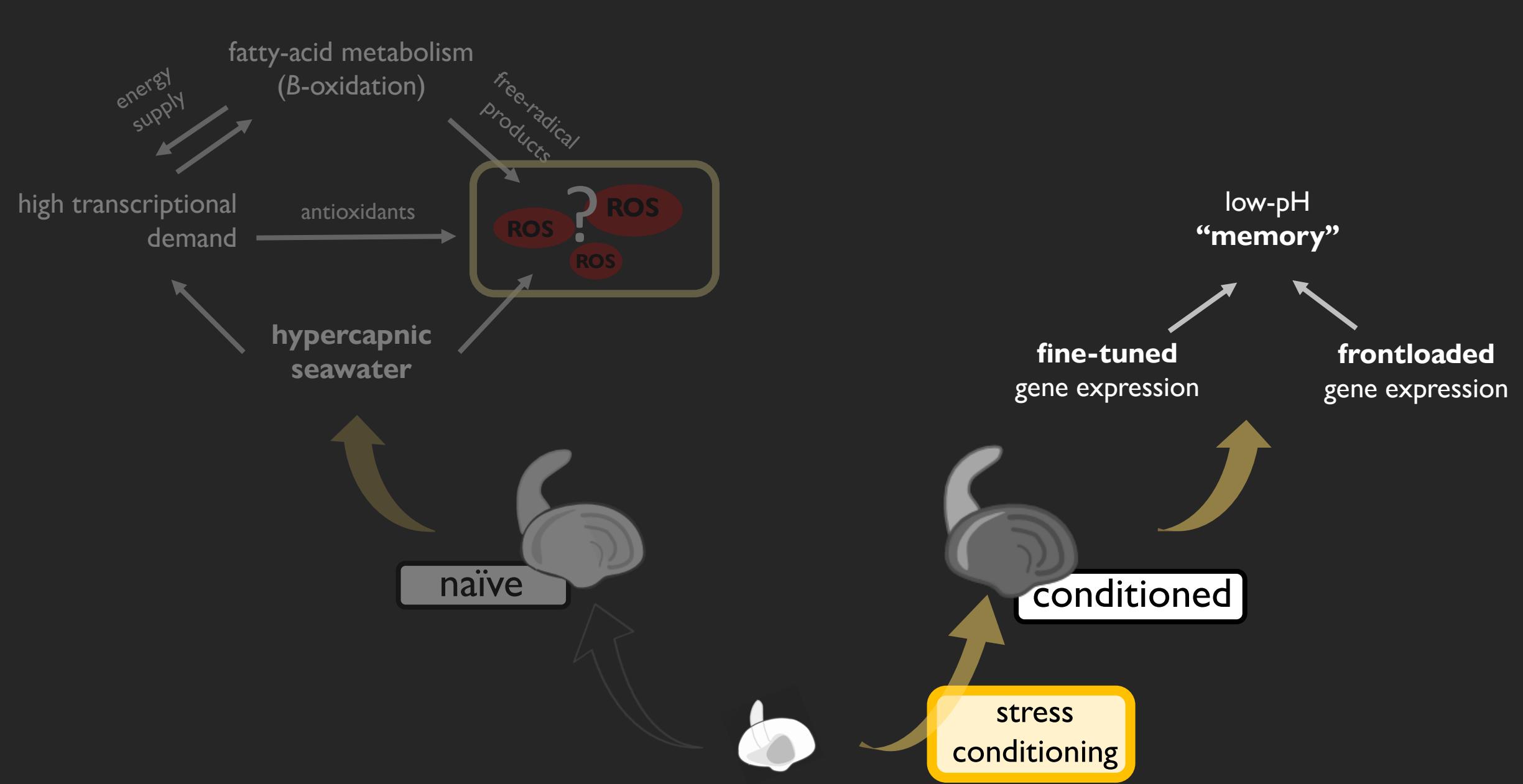
# Synthesis



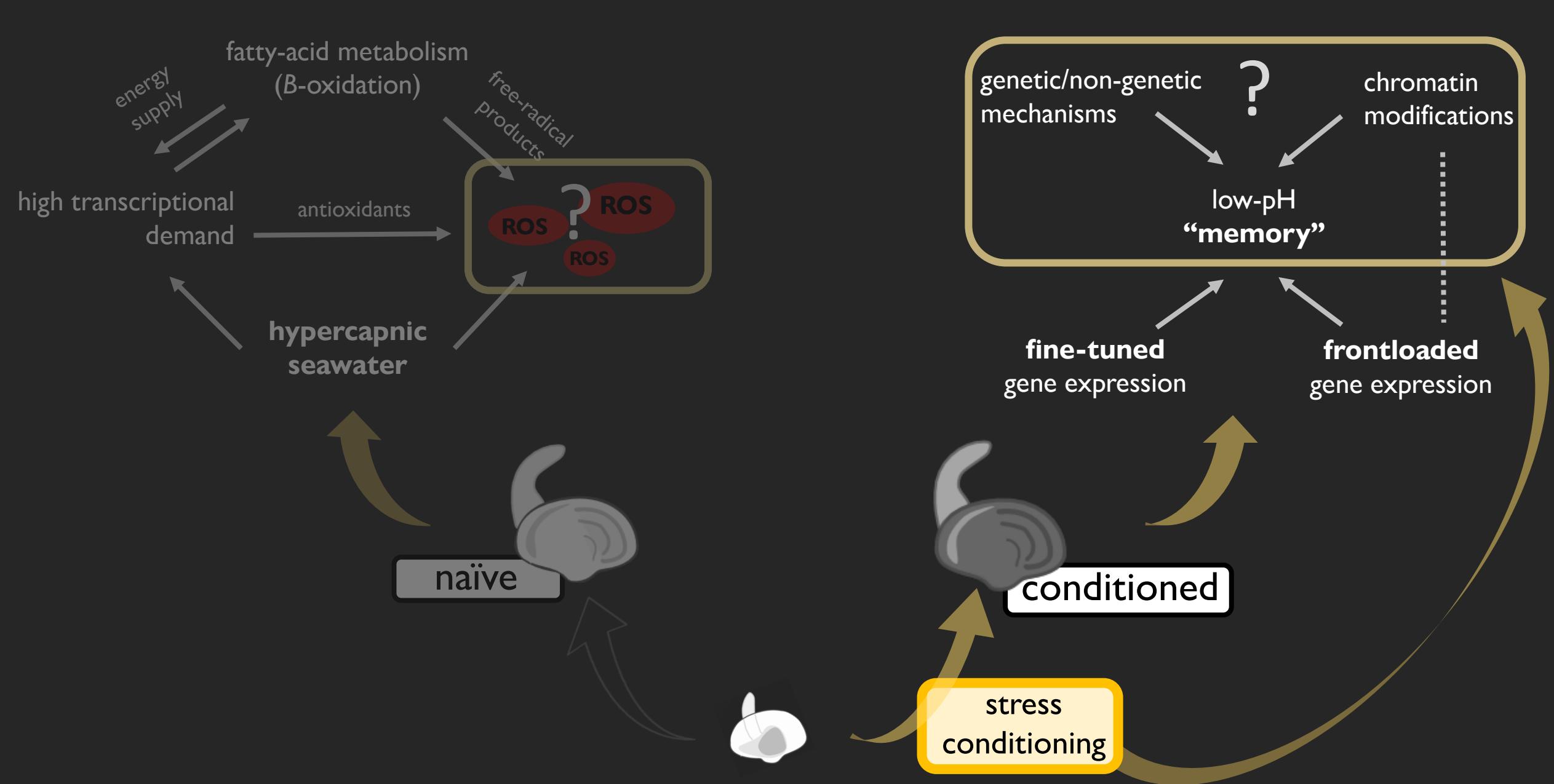
# Synthesis



# Synthesis



# Synthesis

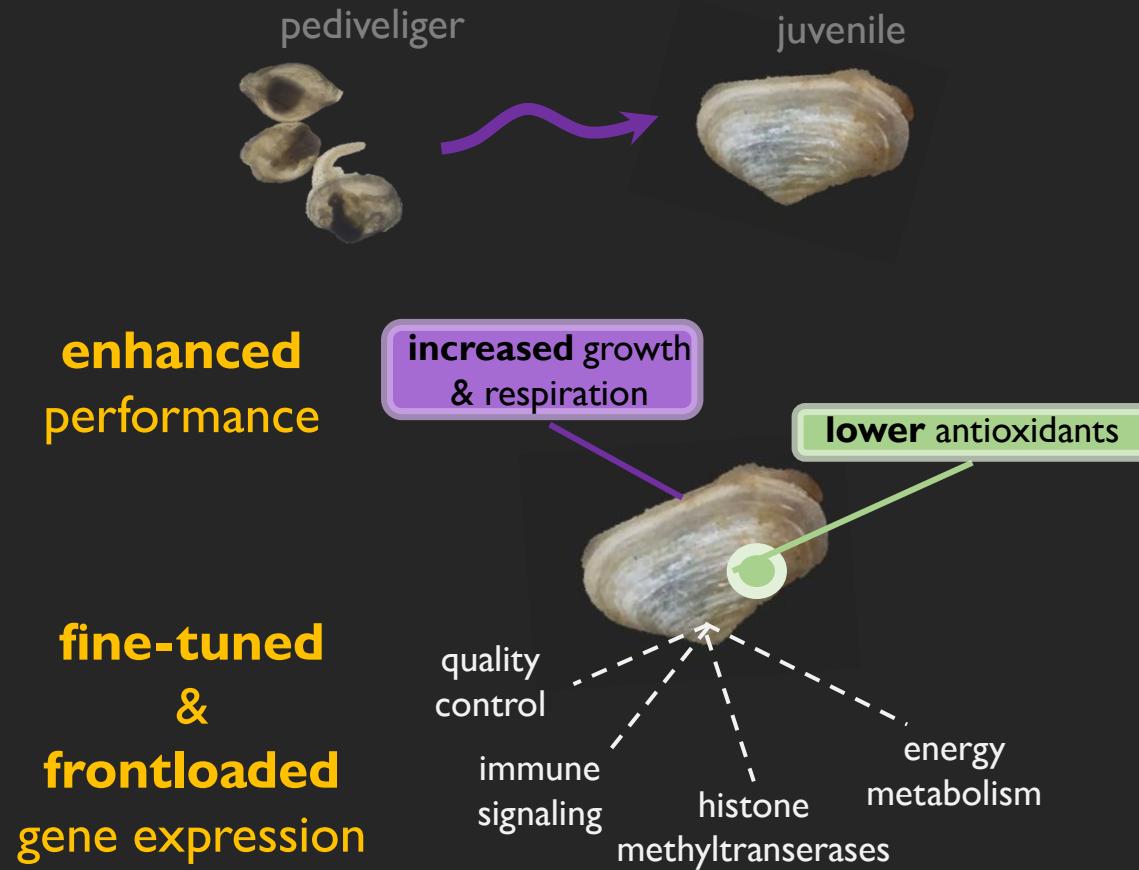


# Synthesis

# Takeaways and Future directions..

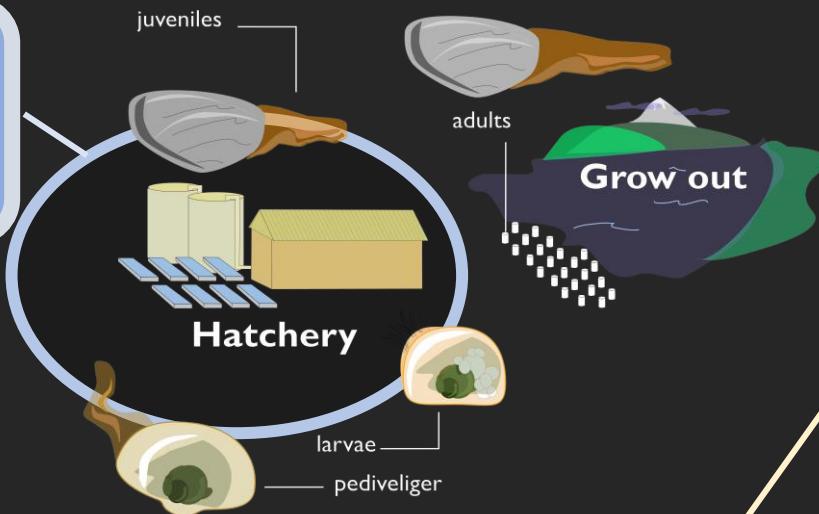
## Main conclusions

Pacific geoduck demonstrate **benefits of stress conditioning post-settlement**

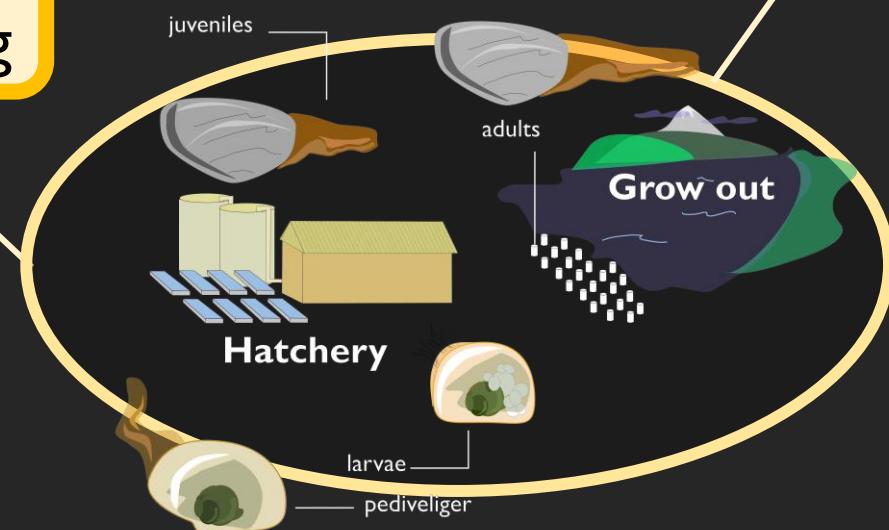


# Takeaways and Future directions..

short-term  
chemical remediation  
(e.g. sodium bicarbonate)



stress  
conditioning

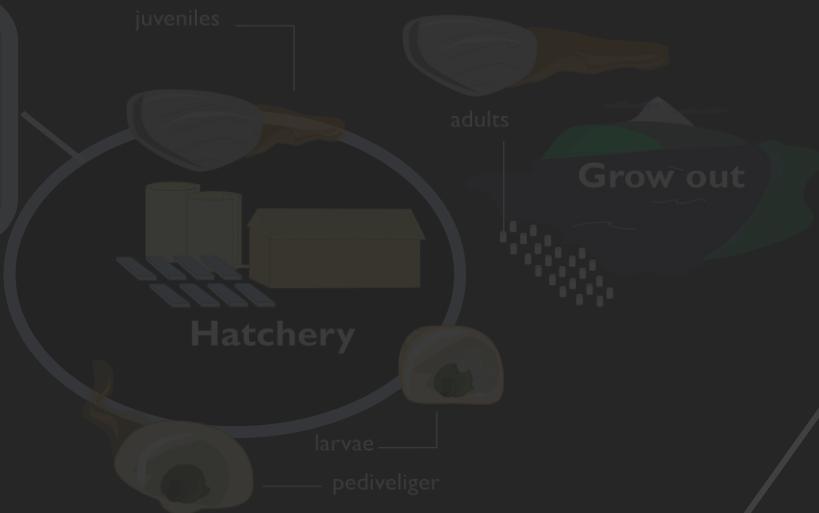


## Aquaculture industry and conservation

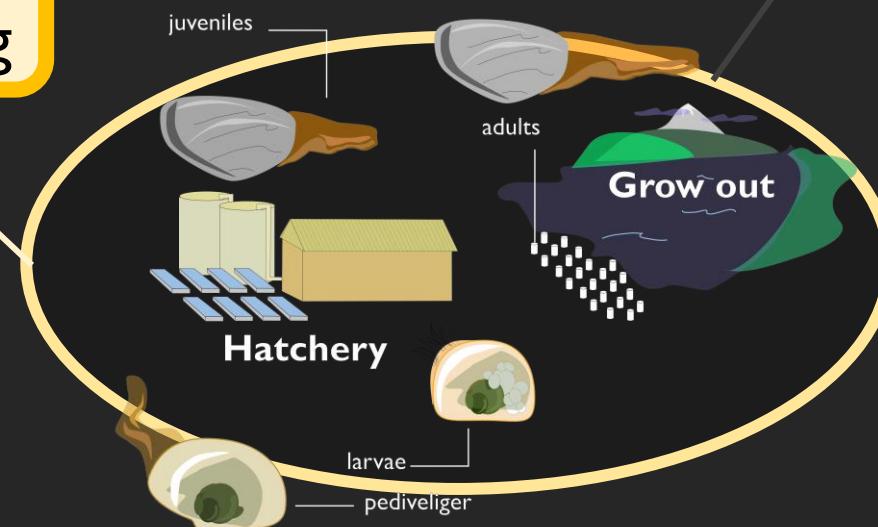
- an alternative strategy to **improve** shellfish production...

# Takeaways and Future directions..

short-term  
chemical remediation  
(e.g. sodium bicarbonate)



stress  
conditioning



## Aquaculture industry and conservation

- an alternative strategy to improve shellfish production...

## Future directions..

- long-term tracking of phenotypes
  - transient v. persistent responses
- transferrable mechanisms
  - genetic/non-genetic markers?
  - does fine-tuning occur across taxa?

## Jamestown S'Klallam shellfish hatchery

- Kurt Grinnell
- Matt Henderson
- Josh Valley

### PIs and Co-PIs

- Hollie Putnam
- Steven Roberts
- Brent Vadopalas
- Shelly Trigg

R  
W

### Committee members

- Jon Puritz
- Coleen Suckling
- Chair – Marta Gomez-Chiarri

### The Putnam Lab et al.!

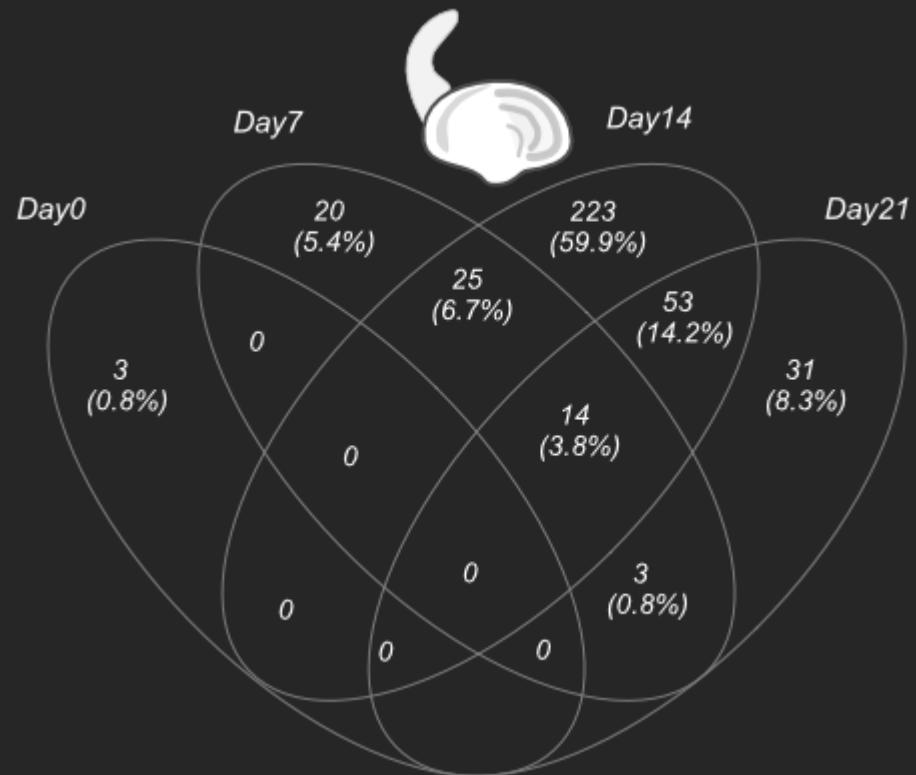
- Maggie Schedl
- Kevin Wong
- Emma Strand
- Erin Chile



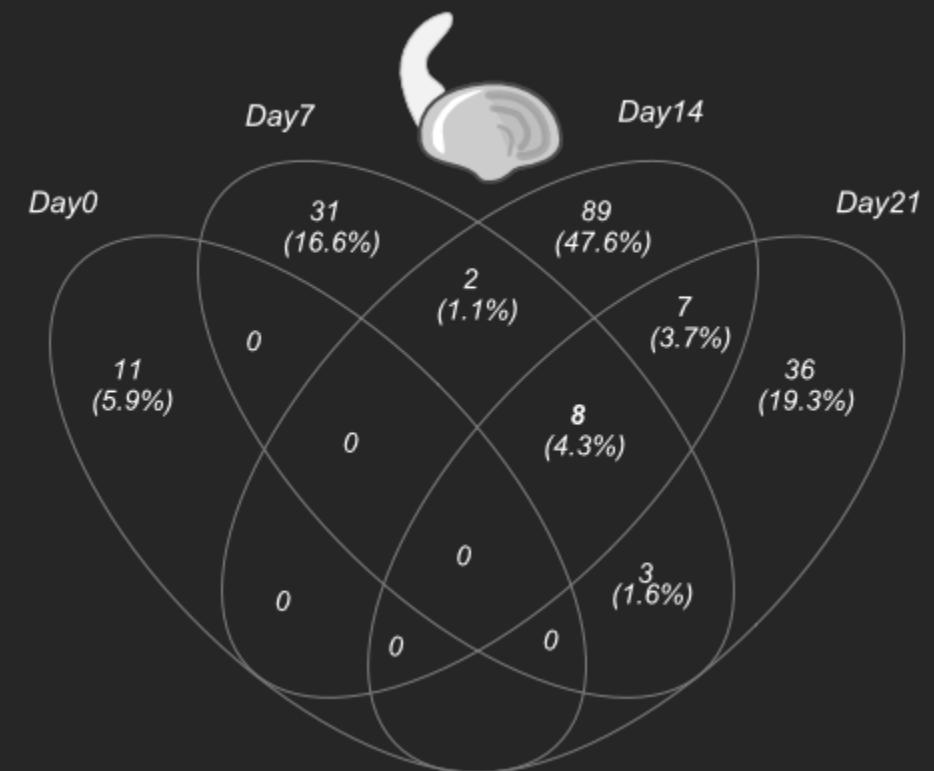
- National Shellfisheries Association
- Blount Shellfish Restoration Foundation
- Ruth D. Turner Foundation

# Results: DE Analysis

naïve



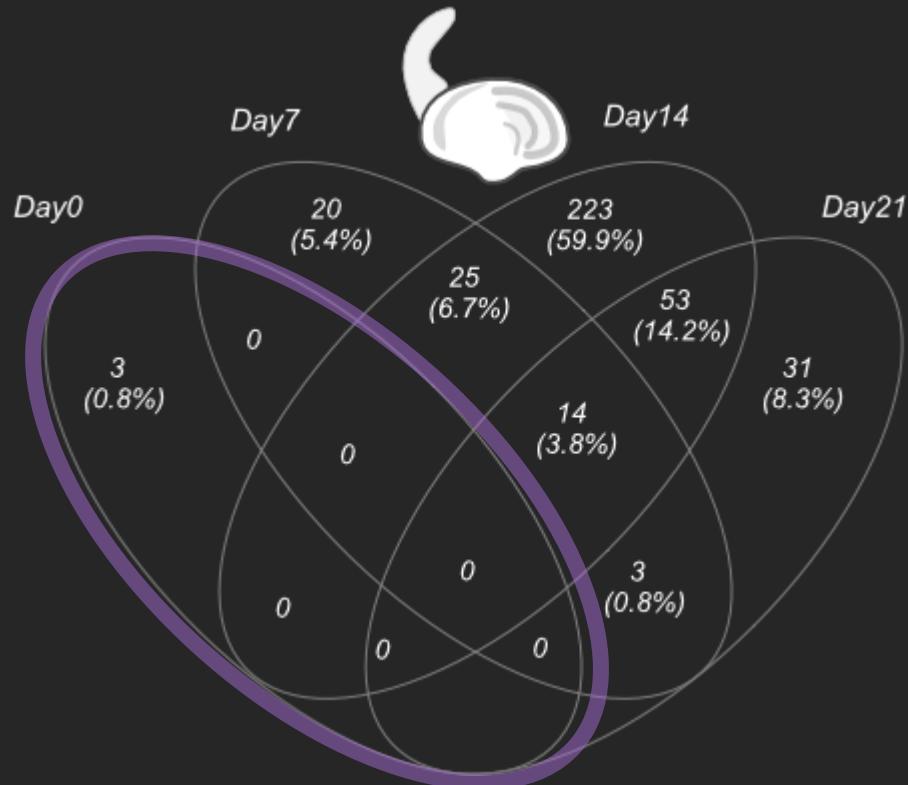
acclimated



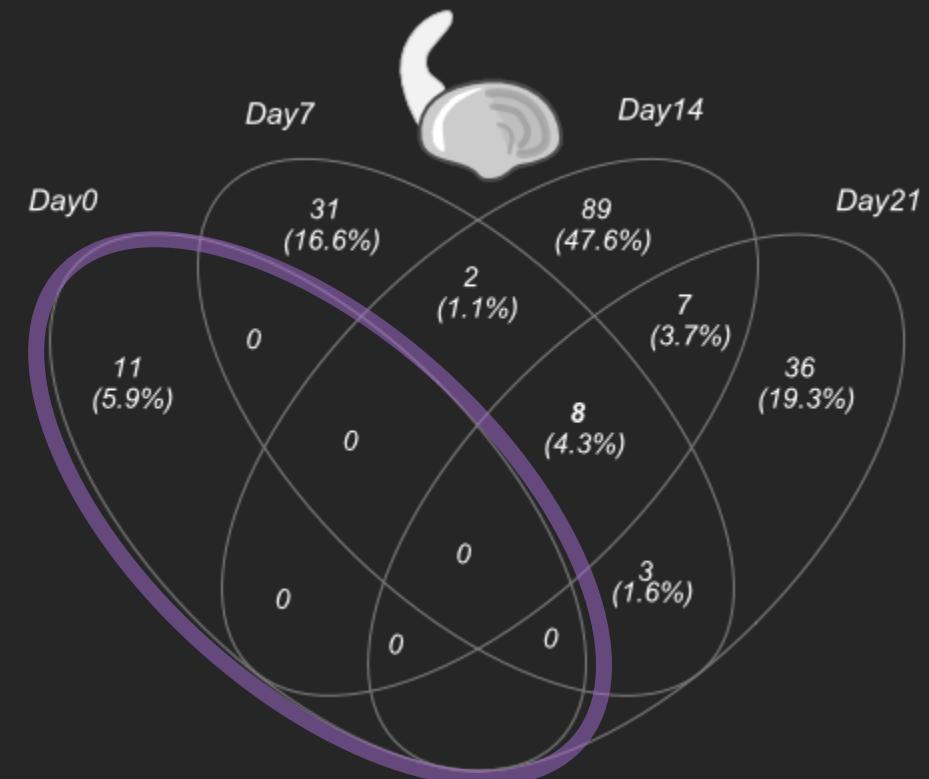
# Results: DE Analysis

*Minimal differential expression  
on day 0...*

naïve

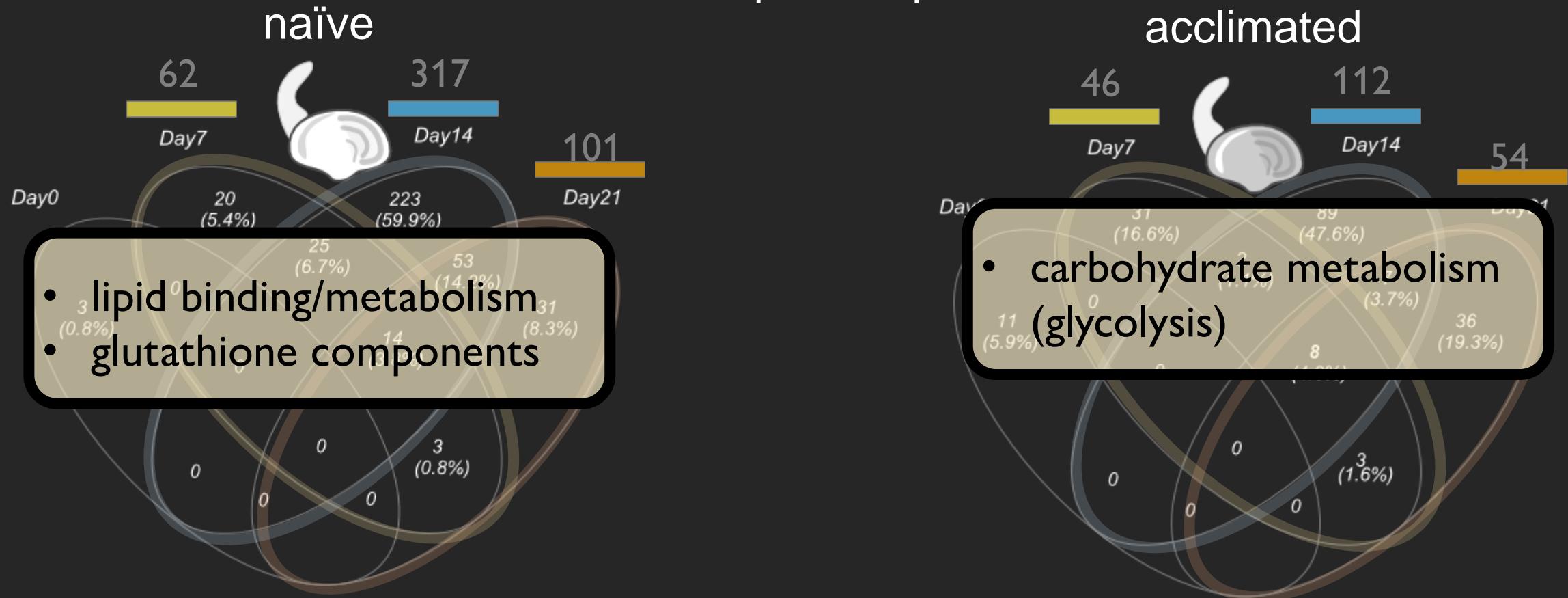


acclimated



# Results: DE Analysis

## *Effect of primary treatment* apparent under subsequent exposures



# Results: DE Analysis

*Effect of primary treatment* apparent  
under subsequent exposures



# Without acclimation

**decreased**  
shell size



**pH 7.5**



**delayed**  
settlement



**pH 7.1**



Ocean  
acidification

**Transcriptome profile**

**profiles shift over time**

**divergent metabolic needs**  
over development

**pH 7.5**

**pH 7.1**

# Results: DE Analysis

## Approach 2:

Differential expression analysis (DESeq2)



## HATCHERY

- Broodstock spawned
- Offspring reared for approx. **4-5 months**



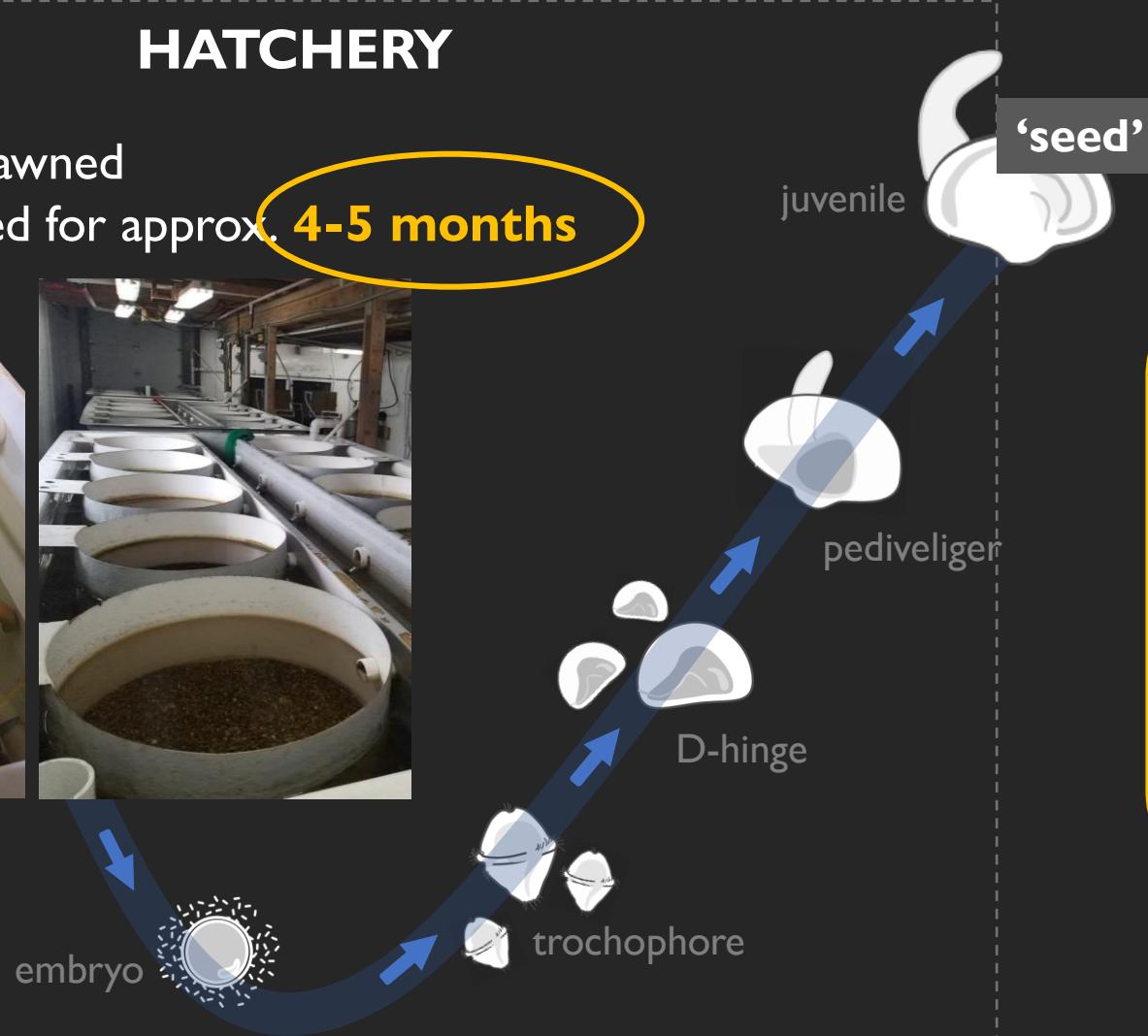
## OUTPLANT

Grown on mudflat for **5-7 years** until adults are harvested



## HATCHERY

- Broodstock spawned
- Offspring reared for approx. **4-5 months**



***Short window to improve production***

Environmental control

Culling

Breeding programs