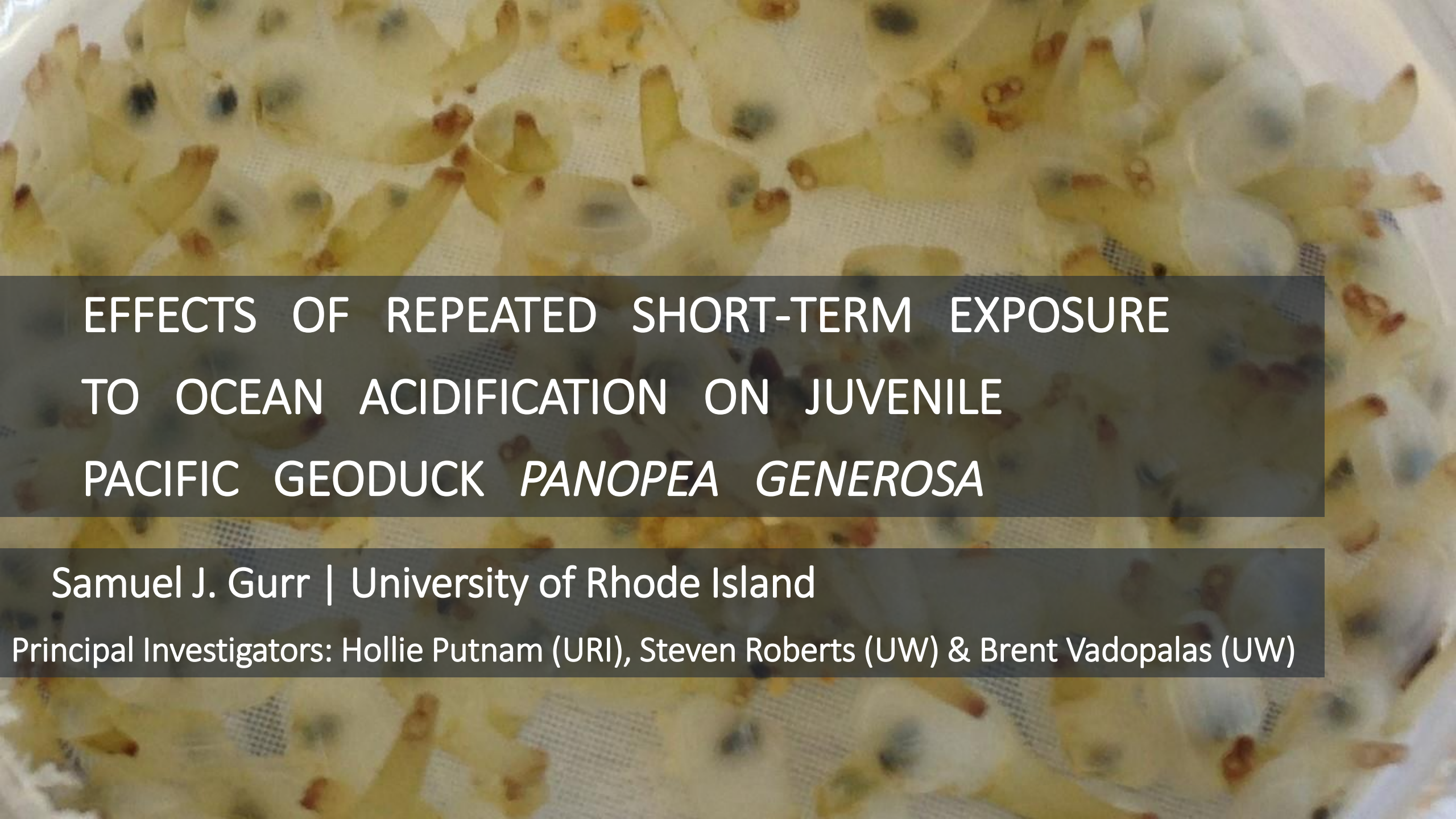


# EFFECTS OF REPEATED SHORT-TERM EXPOSURE TO OCEAN ACIDIFICATION ON JUVENILE PACIFIC GEODUCK *PANOPEA GENEROSA*

Samuel J. Gurr | University of Rhode Island

Principal Investigators: Hollie Putnam (URI), Steven Roberts (UW) & Brent Vadopalas (UW)

# Pacific geoduck *Panopea generosa*

- Large and long lived infauna clam
  - longest recorded as 168 years of age

- Native range from Alaska – Baja, California
  - intertidal to 100+ m depth

- Cultural and economic importance for tribal and local communities of PNW

Photo credit: Mat Henderson

# Sustainable production

**Geoduck aquaculture**

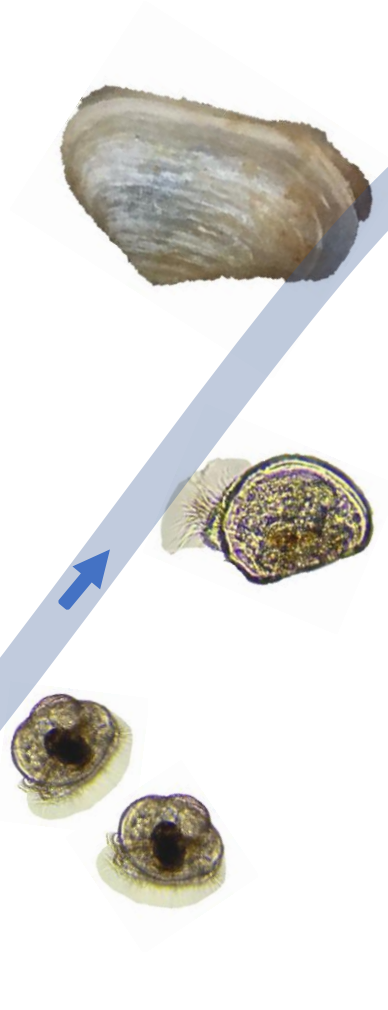
- Prevents overexploitation of wild populations
- Satisfy growing demand in recent decades for international trade
- approx. 90% global geoduck produced from WA state
  - annual revenue > 24 million USD year-1
  - $14 pound$^{-1}$ (as of 2015)


Photo credit: Mat Henderson

# Sustainable production

## HATCHERY

- Broodstock spawned
- Reared for approx. 4-5 months



## OUTPLANT

- Grown on mudflat for 2-3 years until adults are harvested

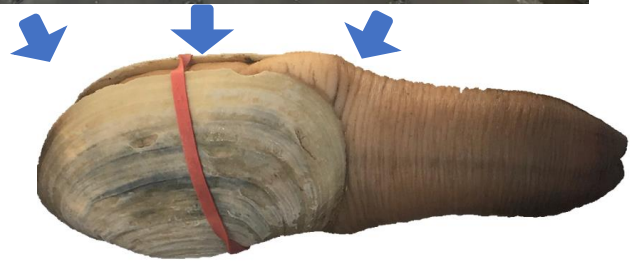


Photo credit: Steven Roberts & Kaitlyn Mitchell

# Bottleneck of hatchery rearing

**HATCHERY**

- Bottleneck for geoduck production



- Early-life stage bivalves are **highly susceptible** to stress

- Biotic and abiotic challenges limit hatchery production:
  - pathogens
  - diet
  - pH / Ωarag.
  - harmful algae
  - temperature
  - salinity

Threat to aquaculture
- records of **pH-induced mass mortality** at shellfish hatcheries (Barton et al. 2012)

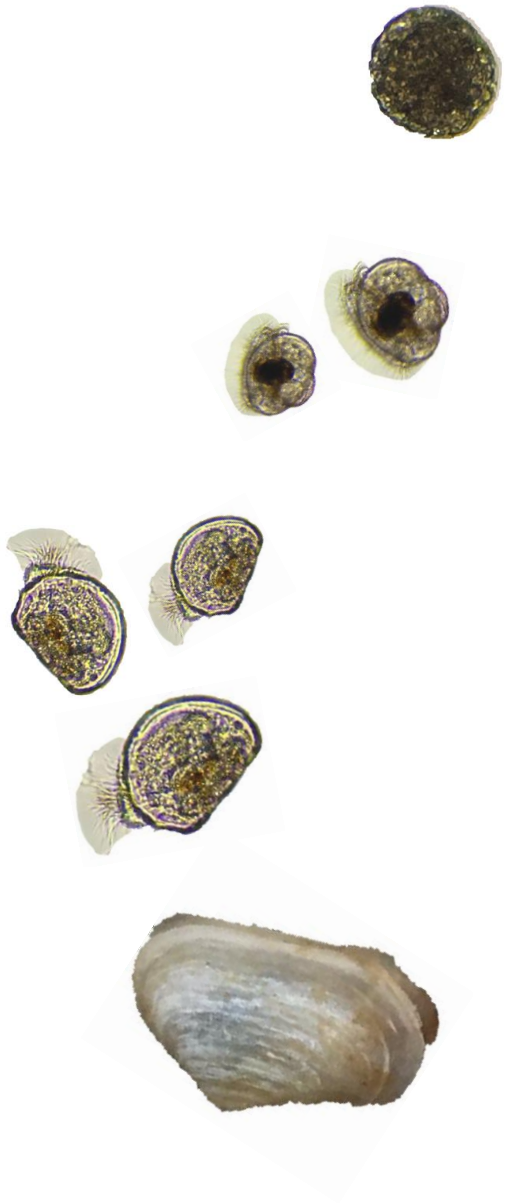
Undisputed **sub-lethal effects** important for commercial production:
- metabolism
- shell growth
- development

# How can we enhance resilience and increase hatchery production?

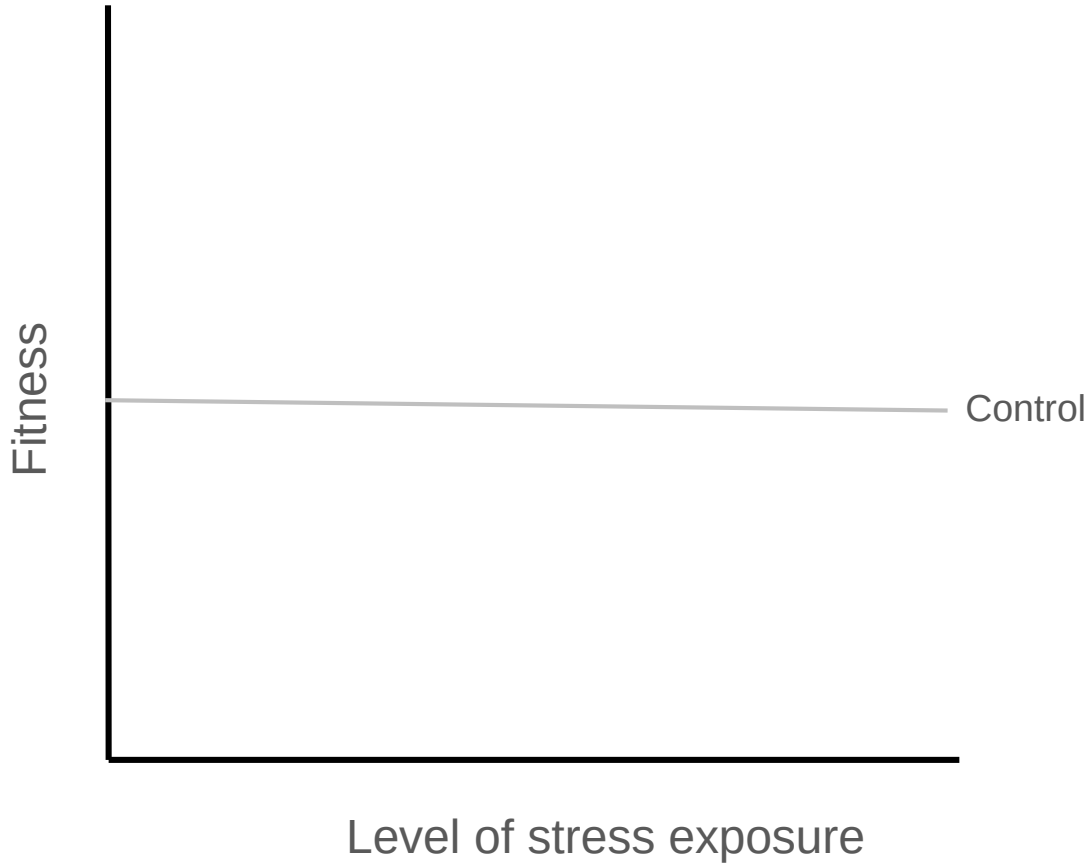
# Stress conditioning

What is “**stress conditioning**”?

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

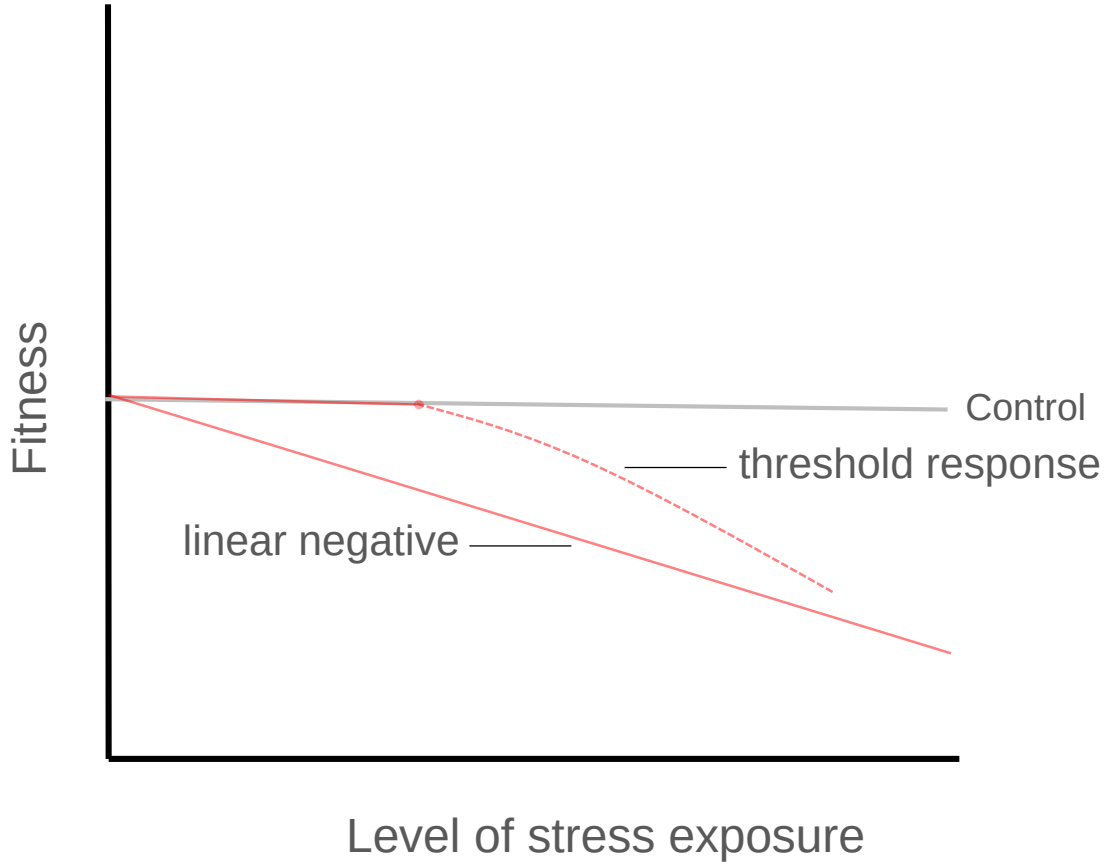


# Stress conditioning




# Stress conditioning

Response model under initial exposure…



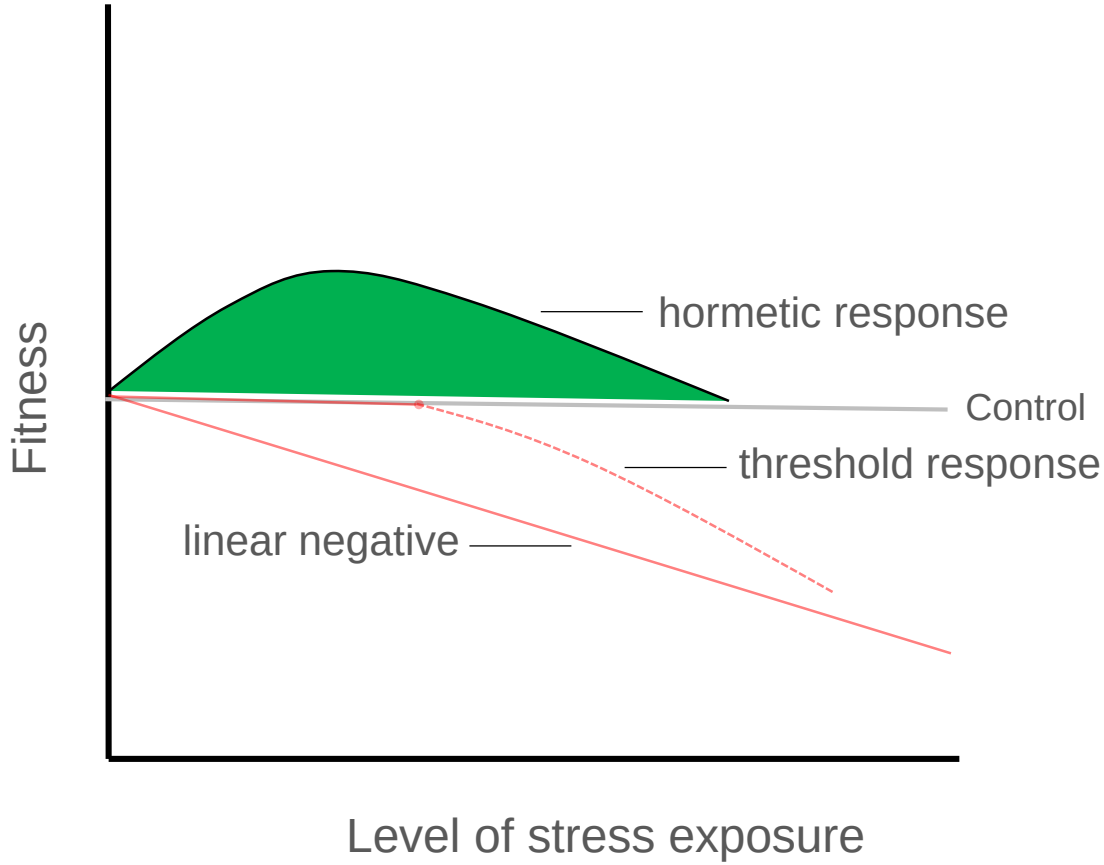

Negative linear or threshold response

# Stress conditioning

**Positive** effect on performance

Negative linear or threshold response

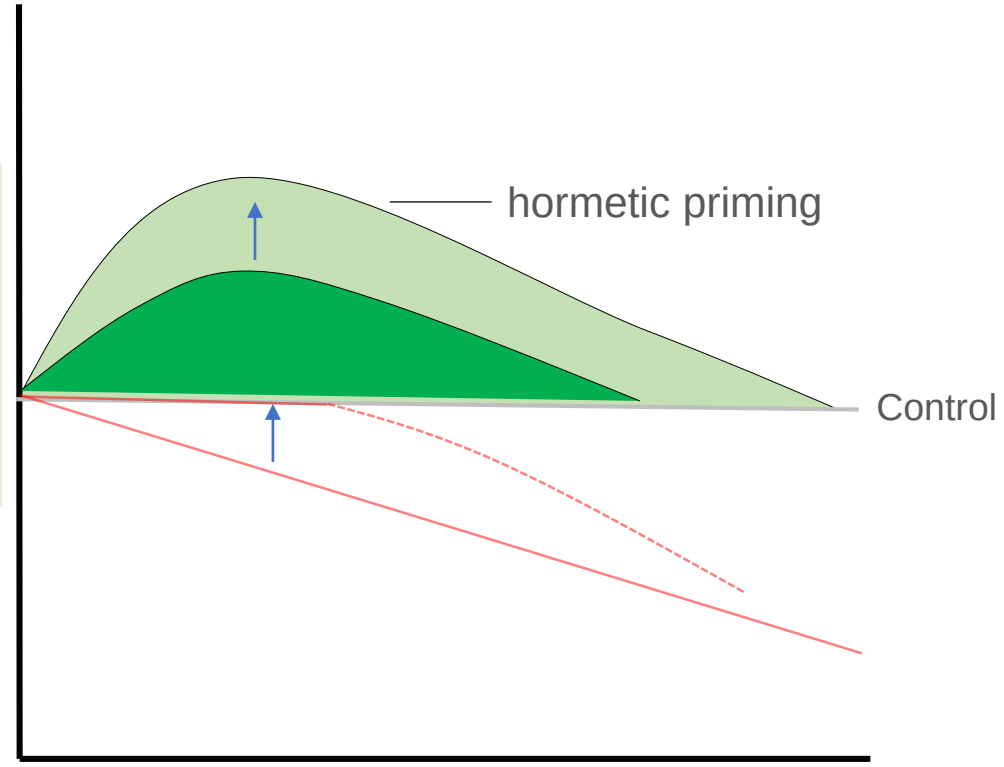
Response model under initial exposure…



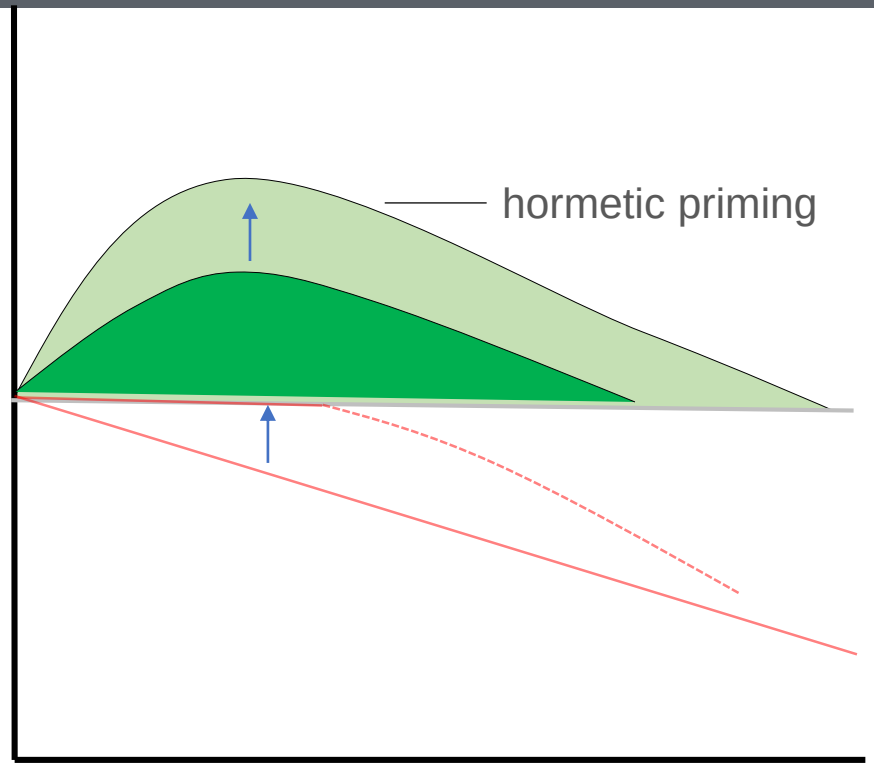

# Stress conditioning

Response model under **subsequent exposure**…

**“Hormetic priming”** prior stress exposure increases performance under a subsequent encounter




# Is hormetic priming a viable enhancement strategy for aquaculture?

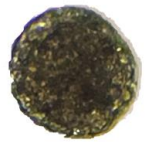


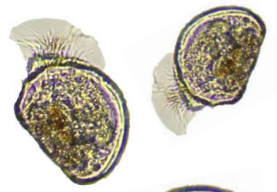
# Ocean acidification & stress conditioning

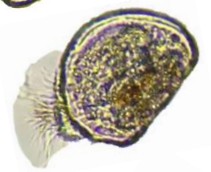
Intragenerational exposure – targets stress-acclimation **within** a generation

a.) Acute

b). Long-term







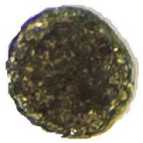
# Ocean acidification & stress conditioning

Intragenerational exposure – targets stress-acclimation **within** a generation

**a.) Acute** – relatively simple to integrate in hatchery practice; coastal/estuarine dynamics

b). Long-term – costly and labor intensive; seasonal or future acidification scenarios

# Ocean acidification & stress conditioning



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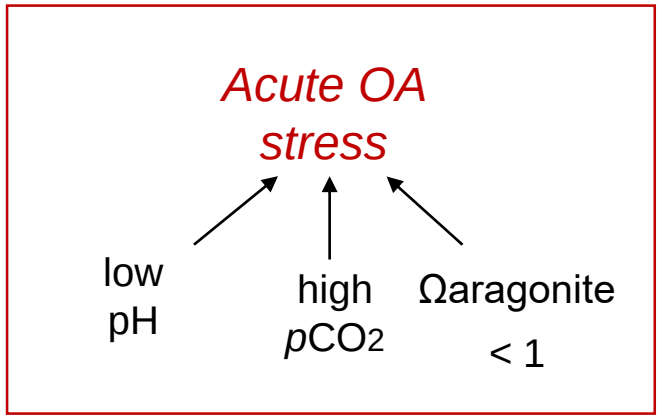
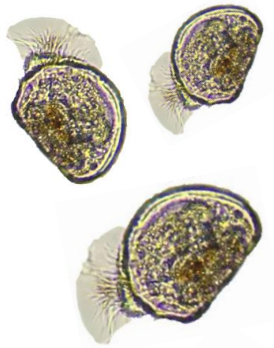



**Common indicators of bivalve performance**

- Cellular respiration
- Shell growth
- Acid-base status / ion regulation
- Development & morphology
- Ingestion rate
- Regulation of gene expression



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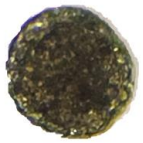

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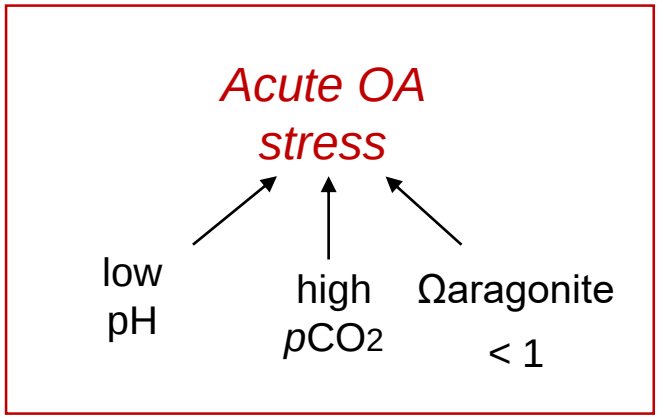


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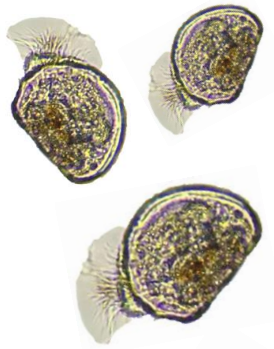


Responses particularly relevant for commercial production

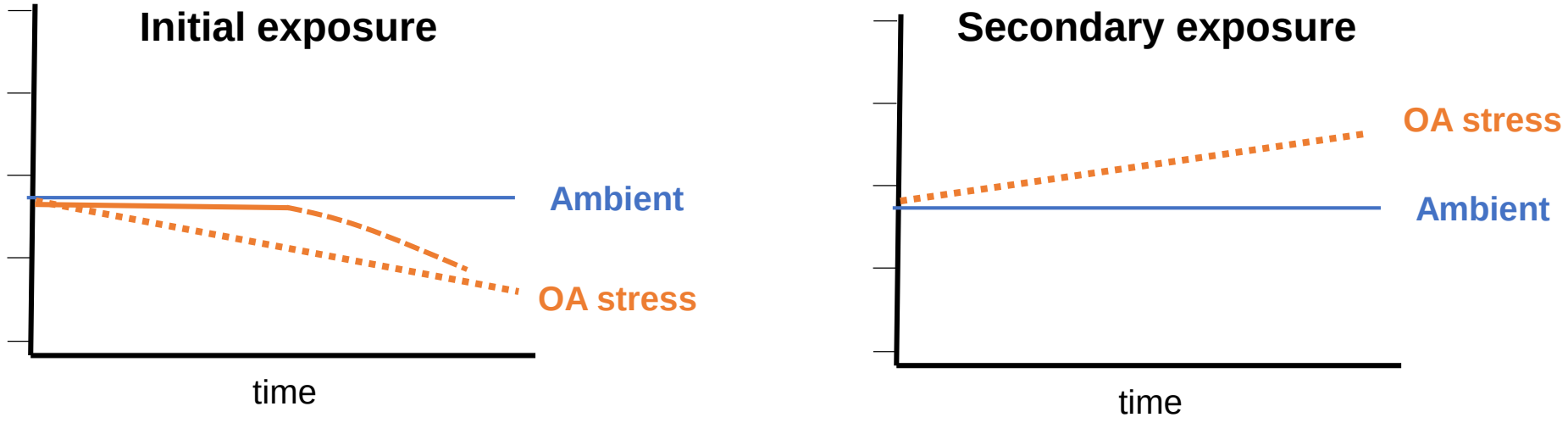
# Stress conditioning in a commercial hatchery

**Q1**: How do juvenile geoduck respond **metabolically** under repeated exposure to acidification?

**Q2**: How is **shell growth** affected by repeated encounters?

*Expected findings*

Respiration rate & growth



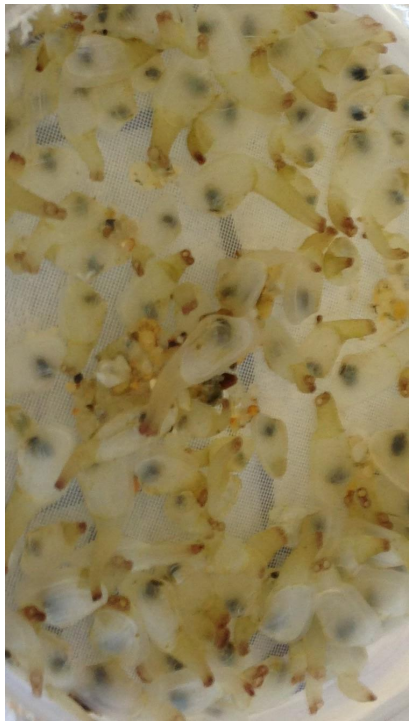
# Animal collection and exposure treatments

- Site: Jamestown Point Whitney Shellfish Hatchery - Brinnon, WA

**Hatchery-reared juveniles**

5 months post-spawn

5 mm shell length



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- Site: Jamestown Point Whitney Shellfish Hatchery - Brinnon, WA

## Hatchery-reared juveniles

5 months post-spawn
5 mm shell length

## Experimental approach

8 heath trays (n = 30 geoduck per tray)

### Target treatments

**Ambient $pCO_2$**

| | | |
|---|---|---|
| $pCO_2$ | = | **570** |
| pH | = | 7.9 |
| $\Omega_{aragonite}$ | = | 1.4 |

**v.**

**Elevated $pCO_2$**

| | | |
|---|---|---|
| $pCO_2$ | = | **2400** |
| pH | = | 7.3 |
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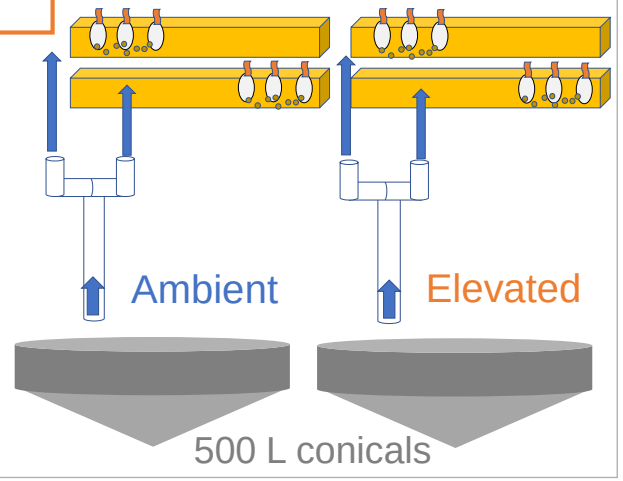


***Constants***:
Total alkalinity: approx. 2050 µmol $kg^{-1}$
Diet: $5 \times 10^7$ live algae cells $d^{-1}$ $ind^{-1}$
Temperature:15.4 ± 1.1°C
Salinity: 28.9 ± 0.2 psu
Flow rate: 480 mL $min^{-1}$

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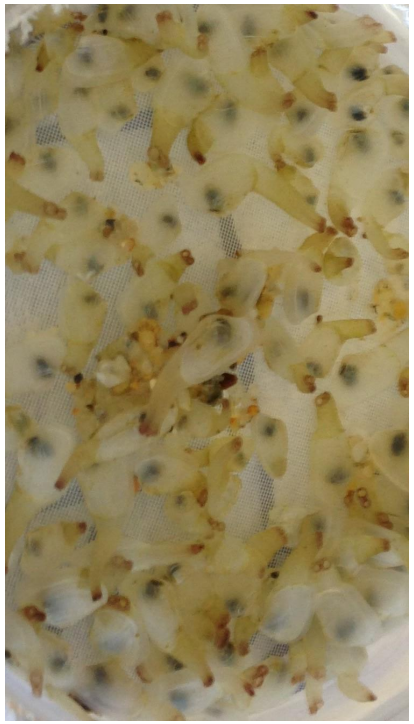

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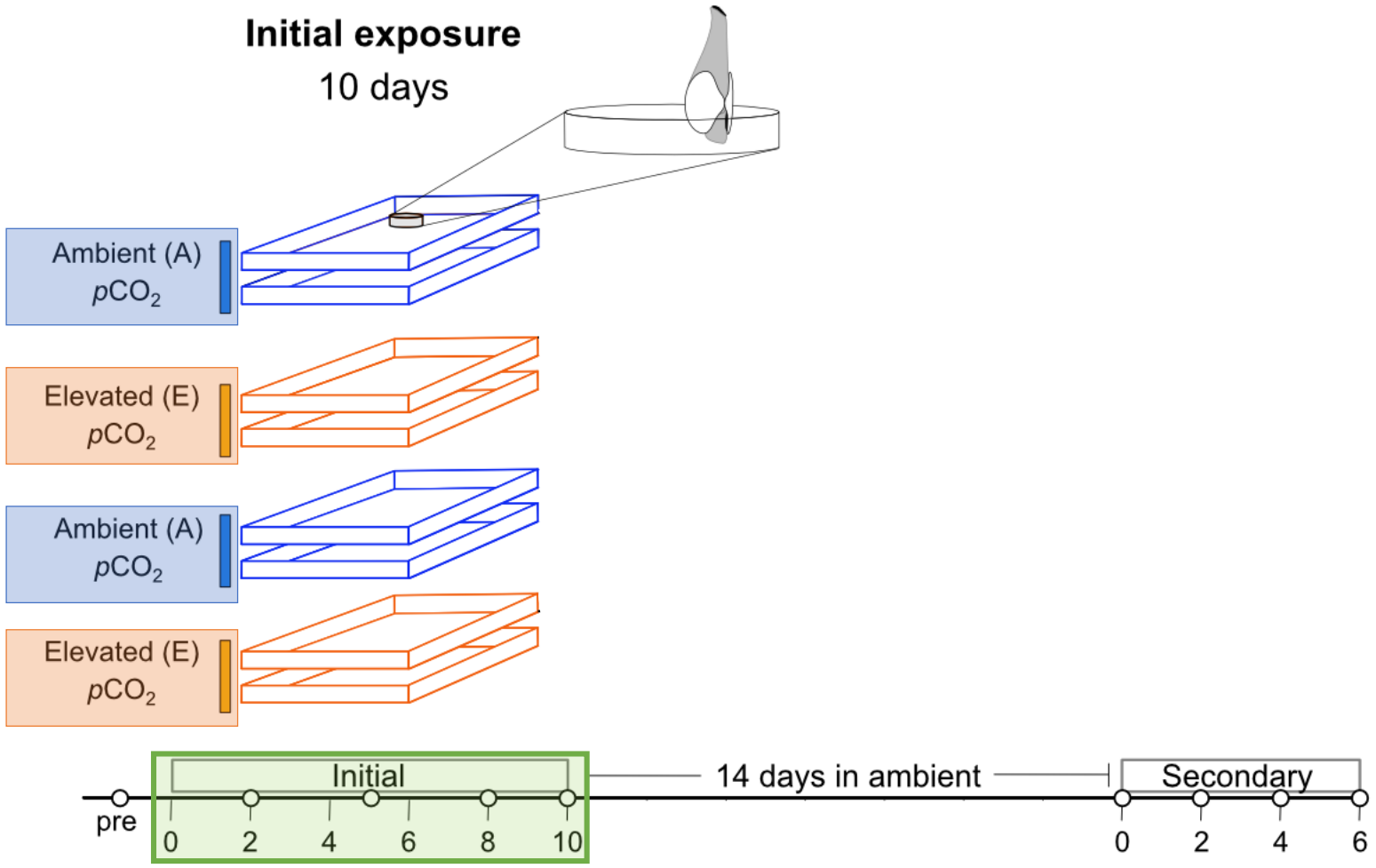
Salinity: 28.9 ± 0.2 psu

Flow rate: 480 mL $min^{-1}$

Animals in an isolated dish for physiological assessment

# Experimental design & physiology data

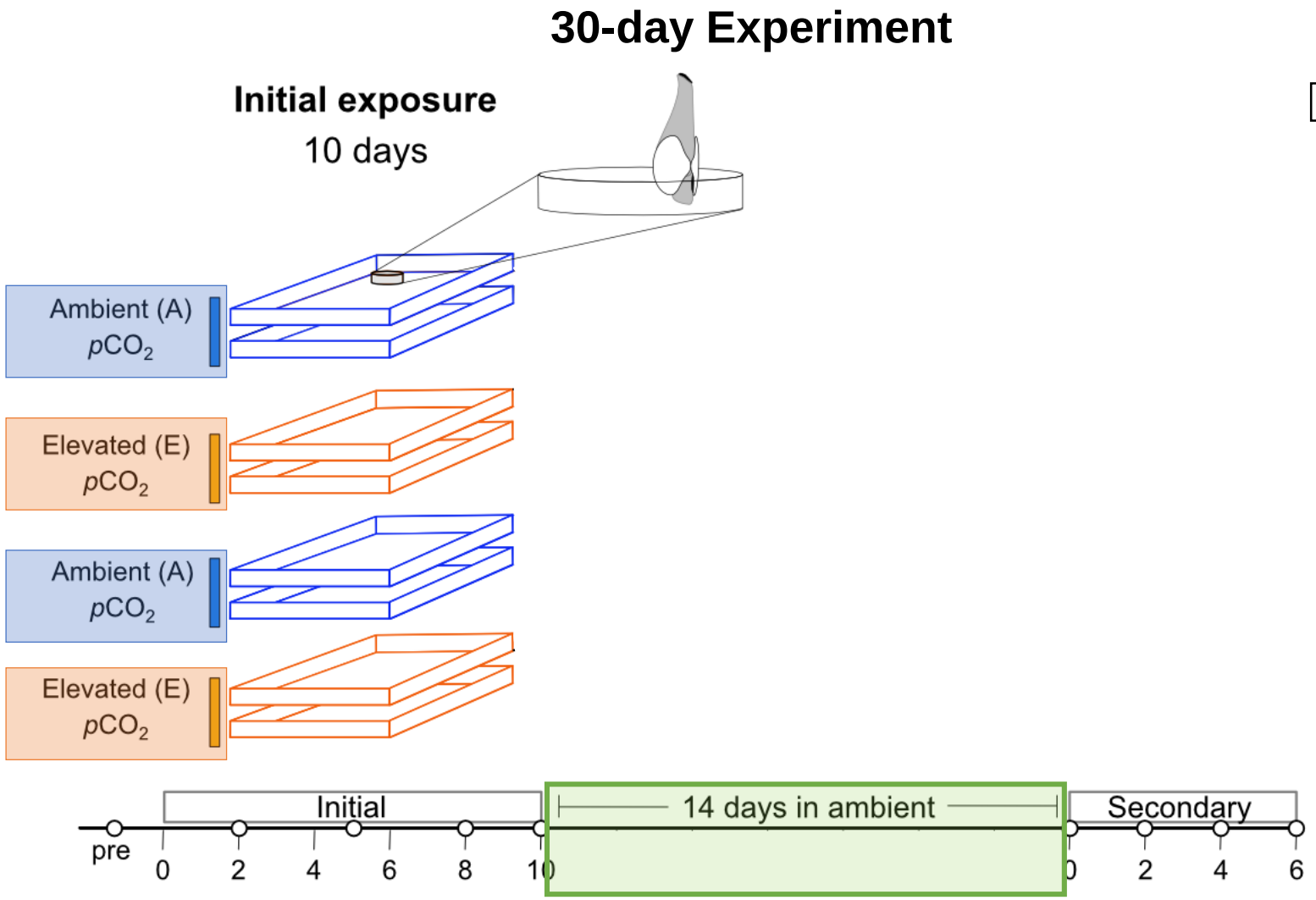



- Initial exposure (10 days)
  **n = 4 trays treatment$^{-1}$**
- Ambient common garden
- Secondary exposure (6 days)
  n = 2 trays treatment$^{-1}$

**Ambient $pCO_2$**
pH = 7.9

**Elevated $pCO_2$**
pH = 7.3

# Experimental design & physiology data

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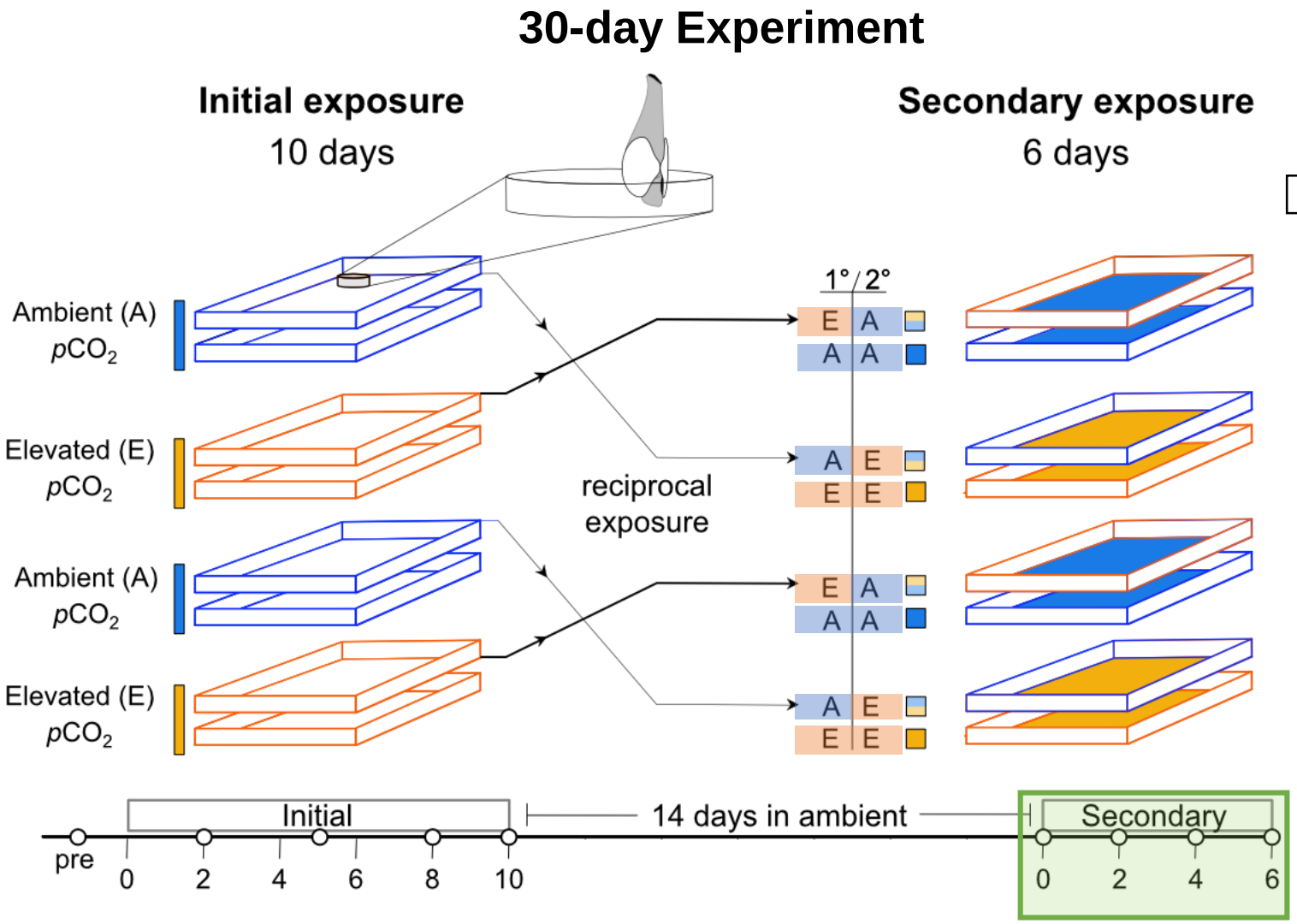
# Experimental design & physiology data




- Initial exposure (10 days)
  n = 4 trays treatment$^{-1}$
- Ambient common garden
- Secondary exposure (6 days)
  **n = 2 trays treatment$^{-1}$**
  (initial × secondary)

**Ambient $pCO_2$**
pH = 7.9

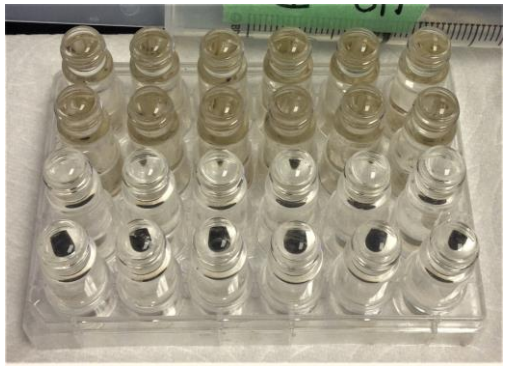
**Elevated $pCO_2$**
pH = 7.3

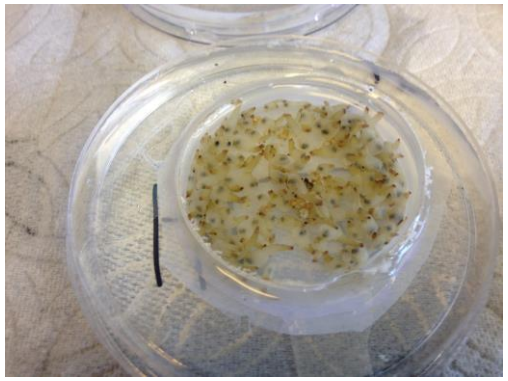
# Experimental design & physiology data

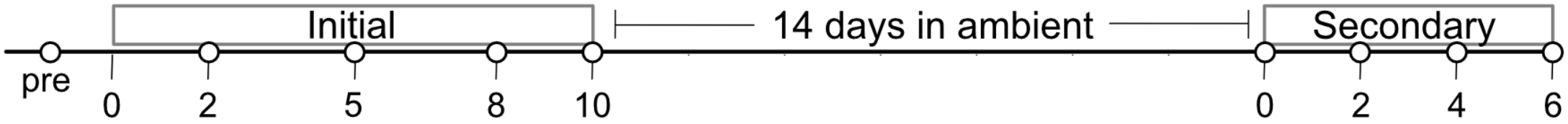
**Physiology**

- Geoduck removed periodically during exposure to measure:

- Metabolic rate: µg $hr^{-1}$ $mm^{-1}$
- Shell growth: mm length





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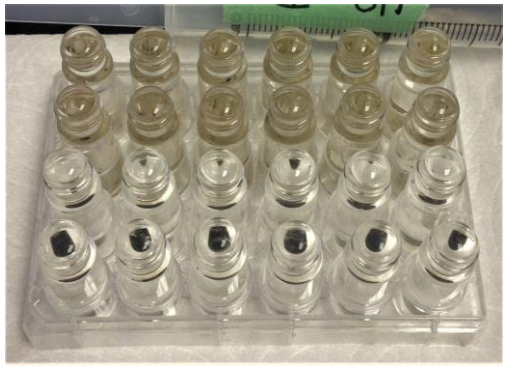
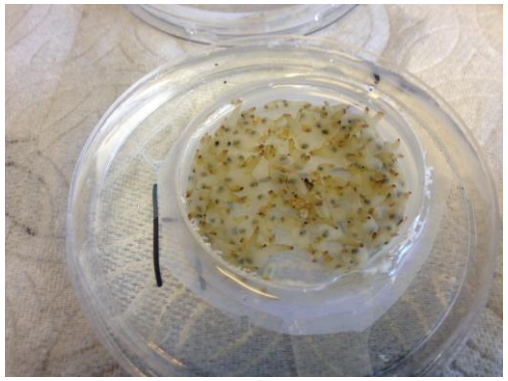
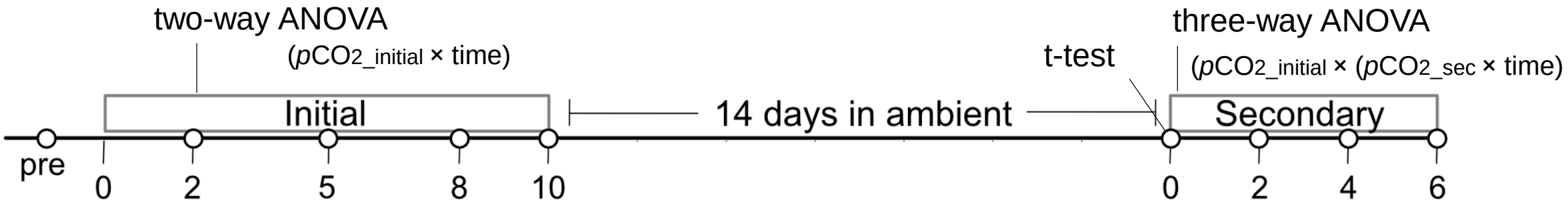
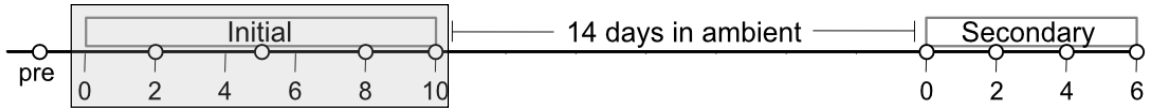





## Statistical approach

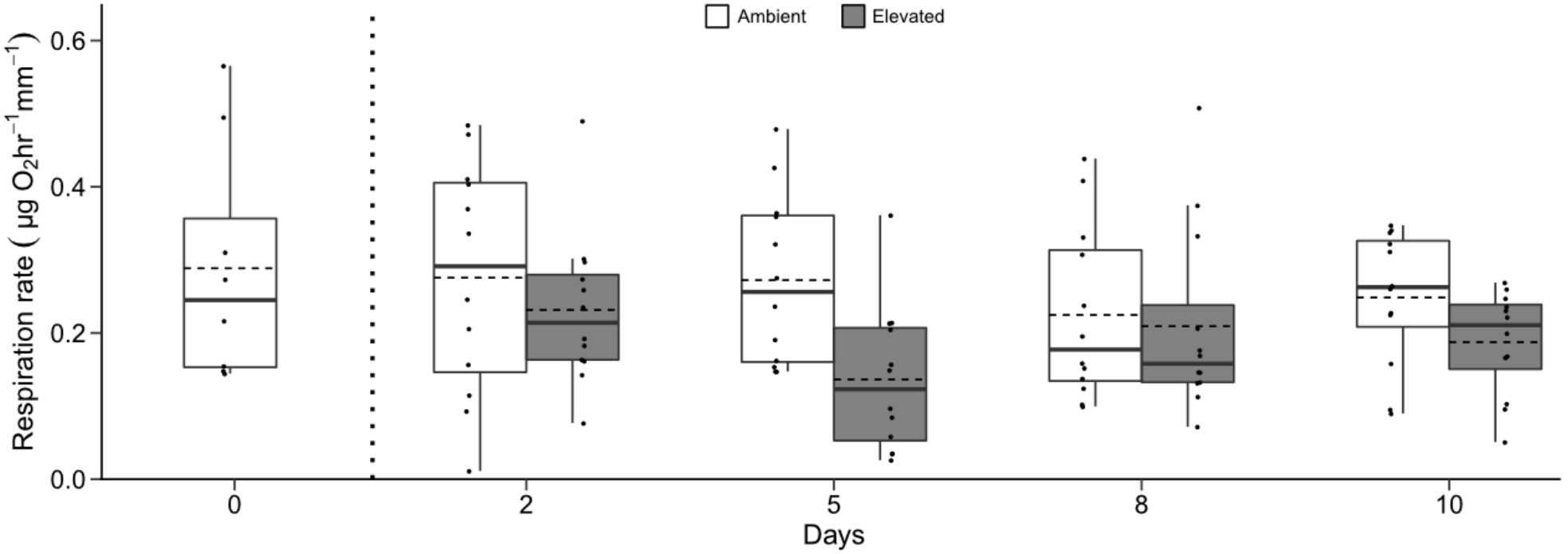


# Results: Initial exposure




**Metabolic rate:**

| | | DF | Sum Sq | Mean Sq | F value | p-value |
|---|---|---|---|---|---|---|
| ***Initial exposure*** | *Two-way ANOVA* | | | | | |
| Respiration rate | time | 1 | 0.03229 | 0.011 | 0.822 | 0.485 |
| | $p\,CO_2$ | 3 | 0.09834 | 0.098 | 7.512 | **0.007** |
| | $p\,CO_2$ × time | 3 | 0.04753 | 0.016 | 1.120 | 0.311 |



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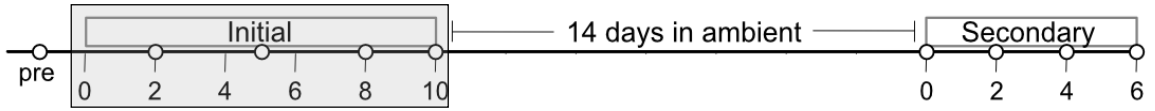


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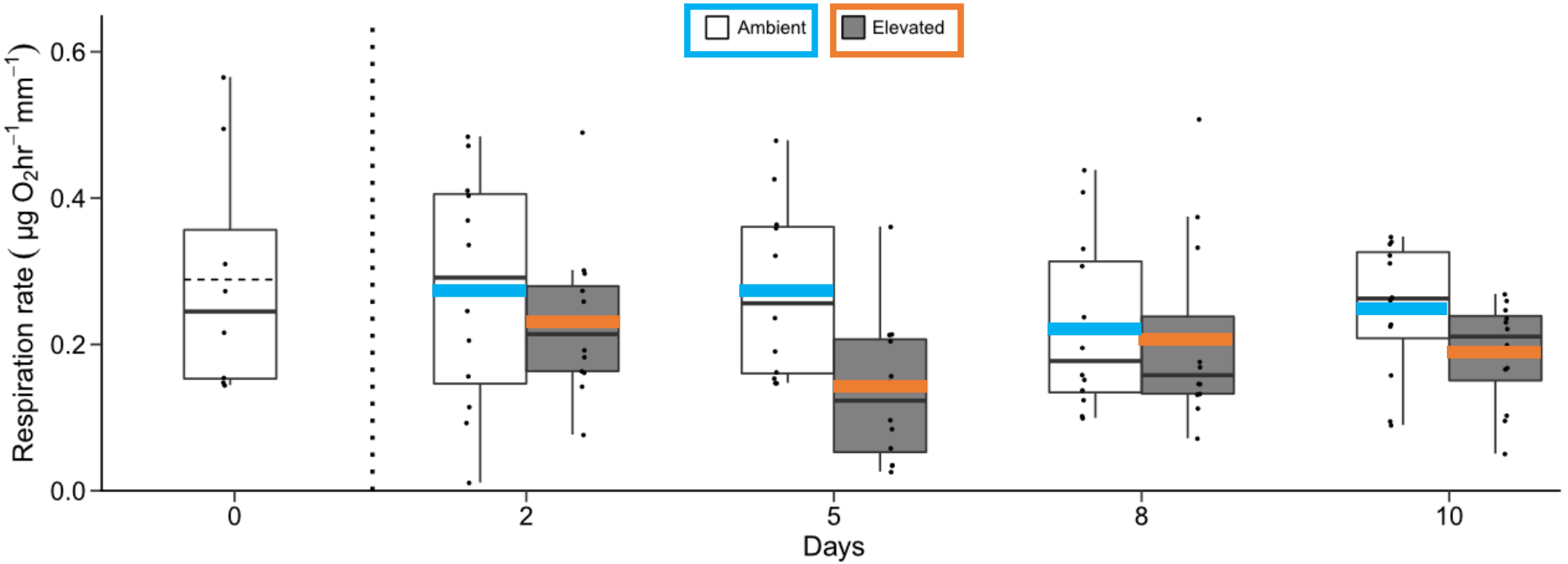
**Metabolic rate:**

- **25% reduction** in respiration rate under **elevated *p*CO2**



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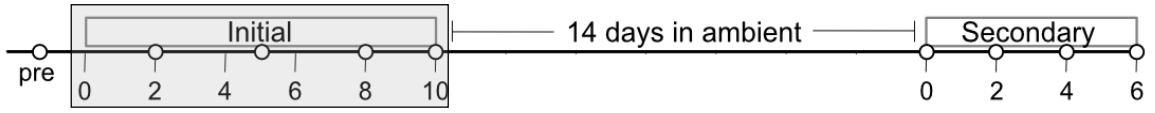


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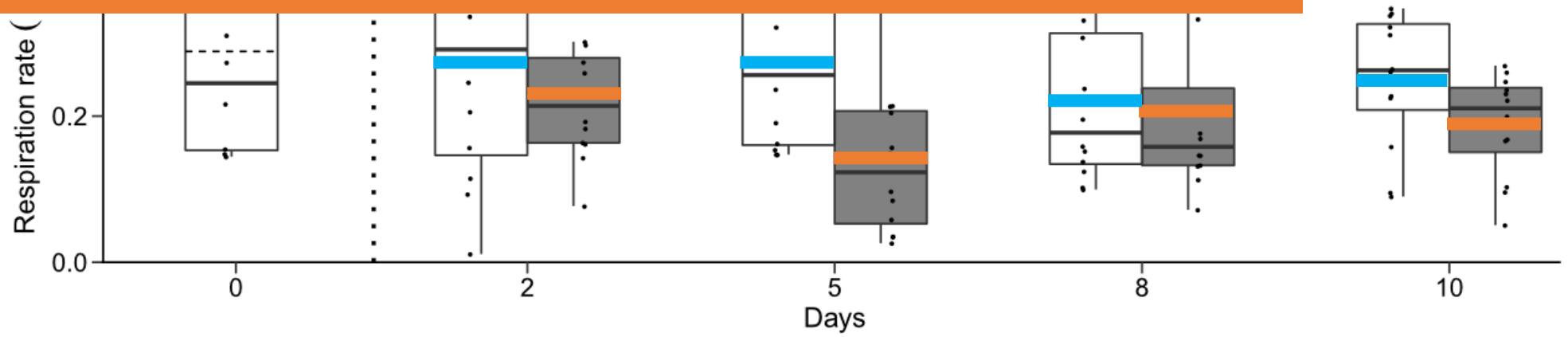
## INITIAL EXPOSURE: METABOLIC RATE

**Suppressed metabolic state** under a short-term period (10 days)



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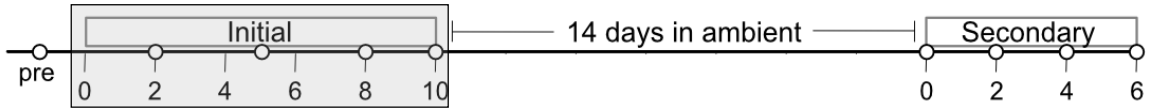
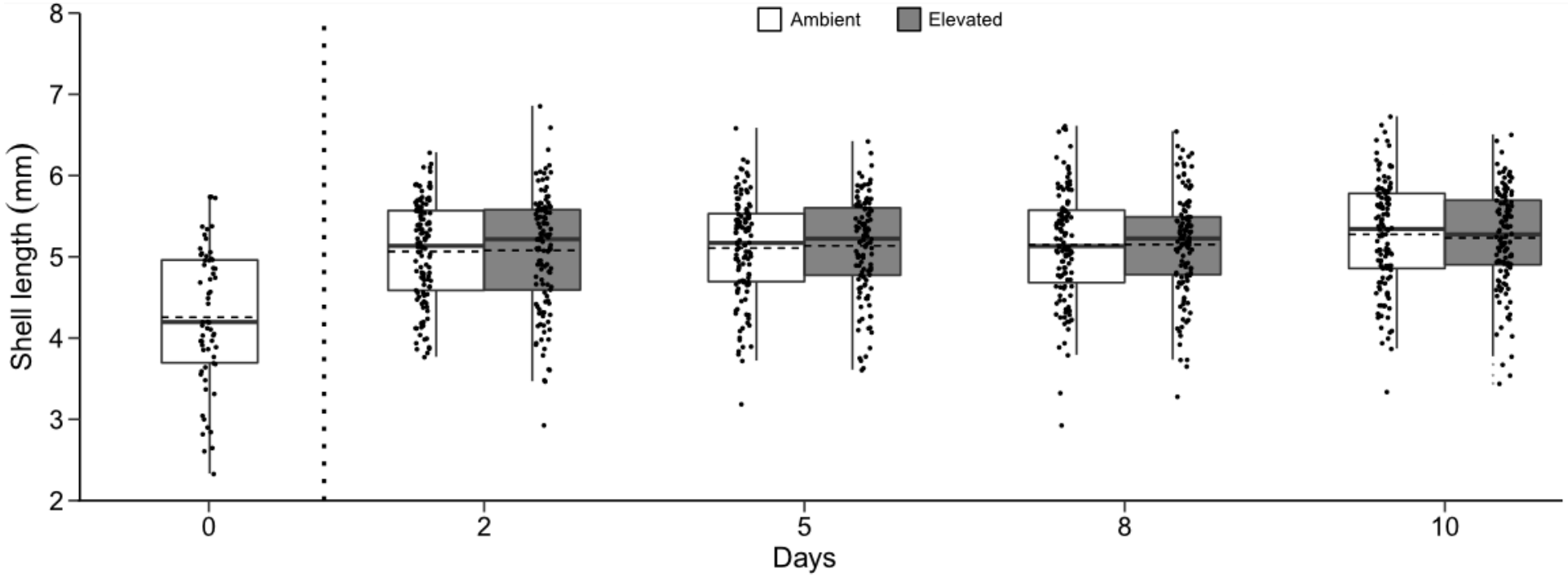



| | | DF | Sum Sq | Mean Sq | F value | p-value |
|---|---|---|---|---|---|---|
| ***Initial exposure*** | *Two-way ANOVA* | | | | | |
| Shell length | time | 3 | 4.250 | 1.415 | 3.392 | **0.018** |
| | $p\,CO_2$ | 1 | 0.000 | 0.000 | 0.001 | 0.973 |
| | $p\,CO_2$ × time | 3 | 0.170 | 0.058 | 0.138 | 0.937 |

**Growth:**

- **No response** under to elevated $pCO_2$



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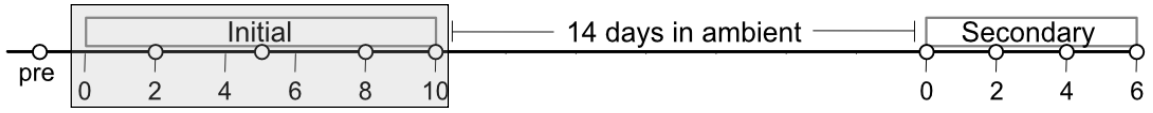


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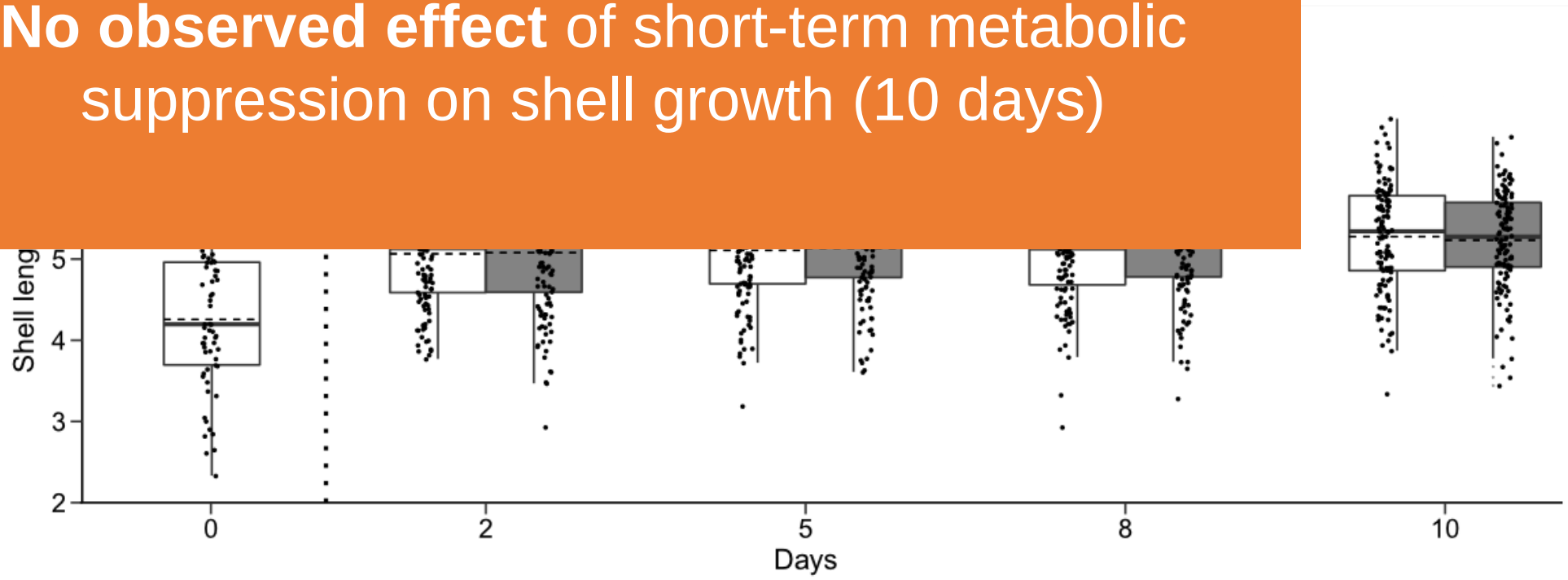
| Shell length | time | 3 | 4.250 | 1.415 | 3.392 | **0.018** |
| | $p\,CO_2$ | 1 | 0.000 | 0.000 | 0.001 | 0.973 |
| | $p\,CO_2$ × time | 3 | 0.170 | 0.058 | 0.138 | 0.937 |

**Growth:**

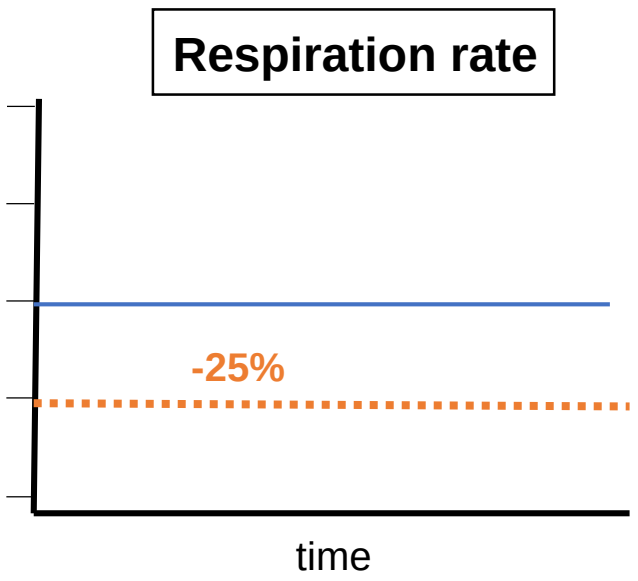
- **No response** under to elevated $pCO_2$

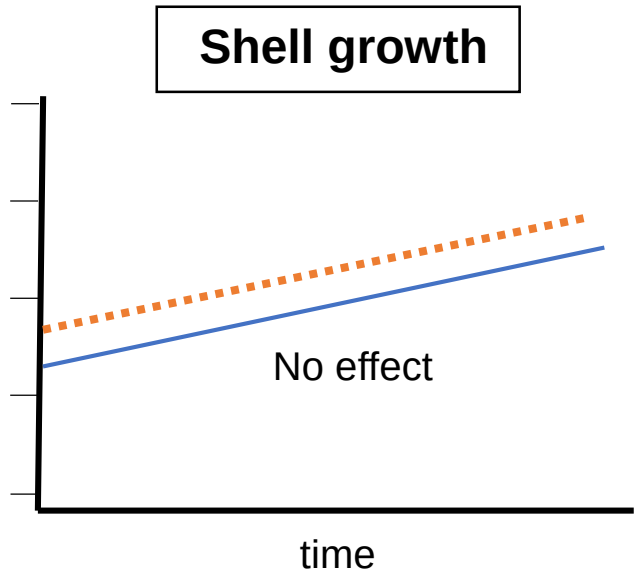
## INITIAL EXPOSURE: SHELL GROWTH

**No observed effect** of short-term metabolic suppression on shell growth (10 days)



INITIAL EXPOSURE




Elevated $pCO_2$

Ambient $pCO_2$

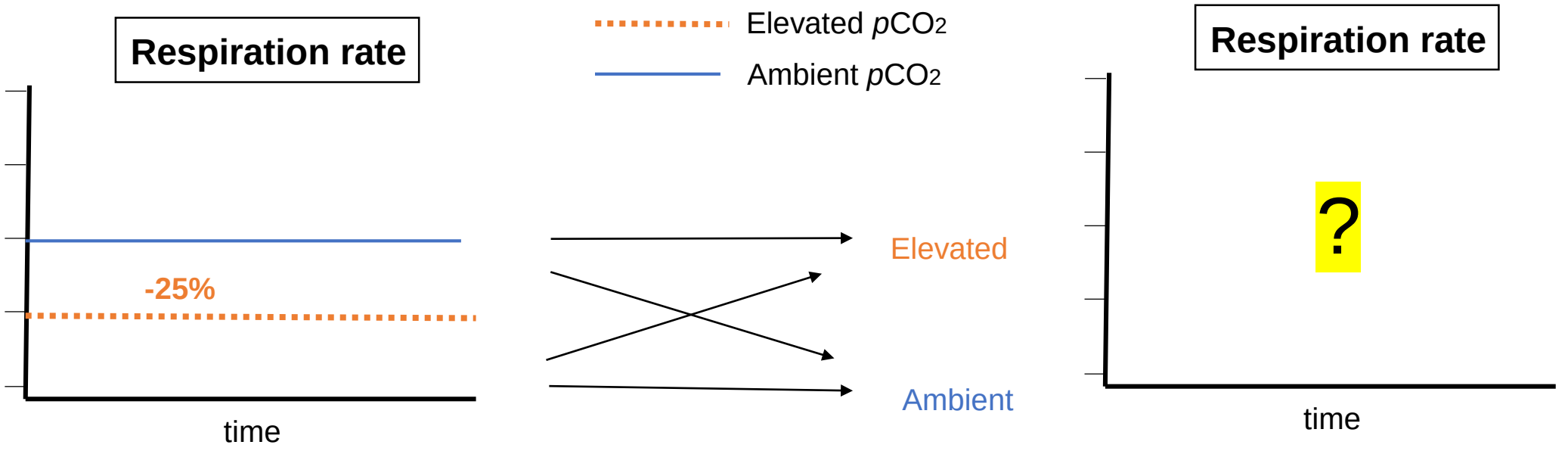
## INITIAL EXPOSURE

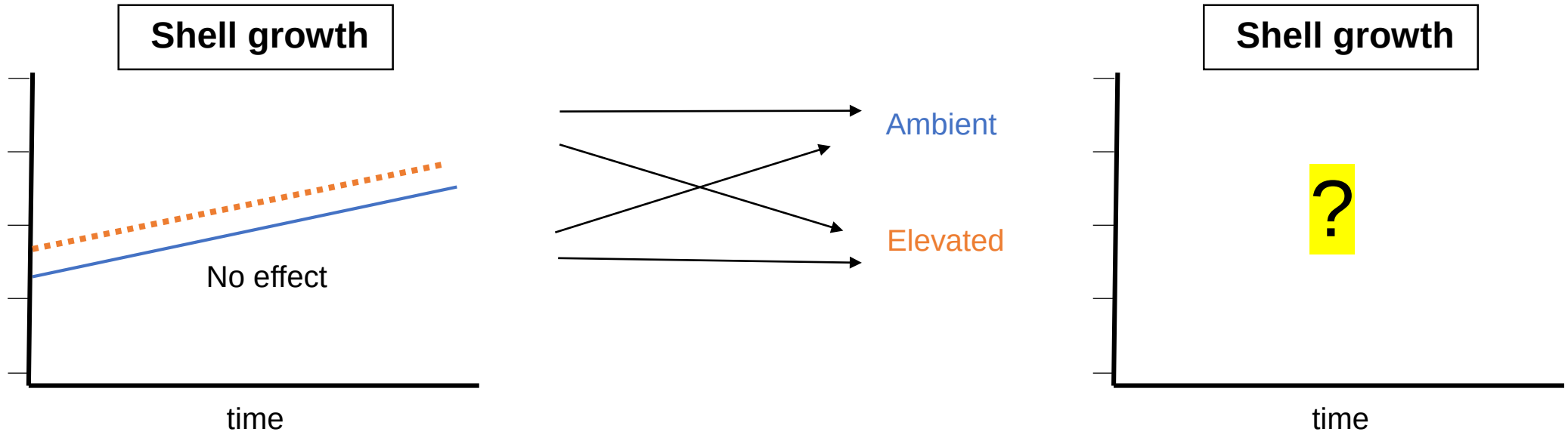
Pacific geoduck under short-term acidification

- Suppressed metabolic activity
- Shell growth not affected

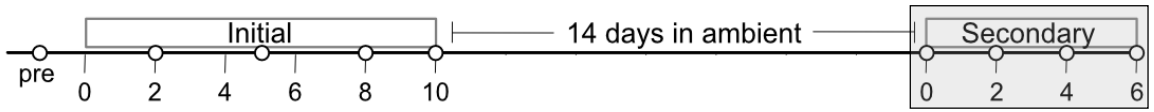
INITIAL EXPOSURE

SECONDARY EXPOSURE



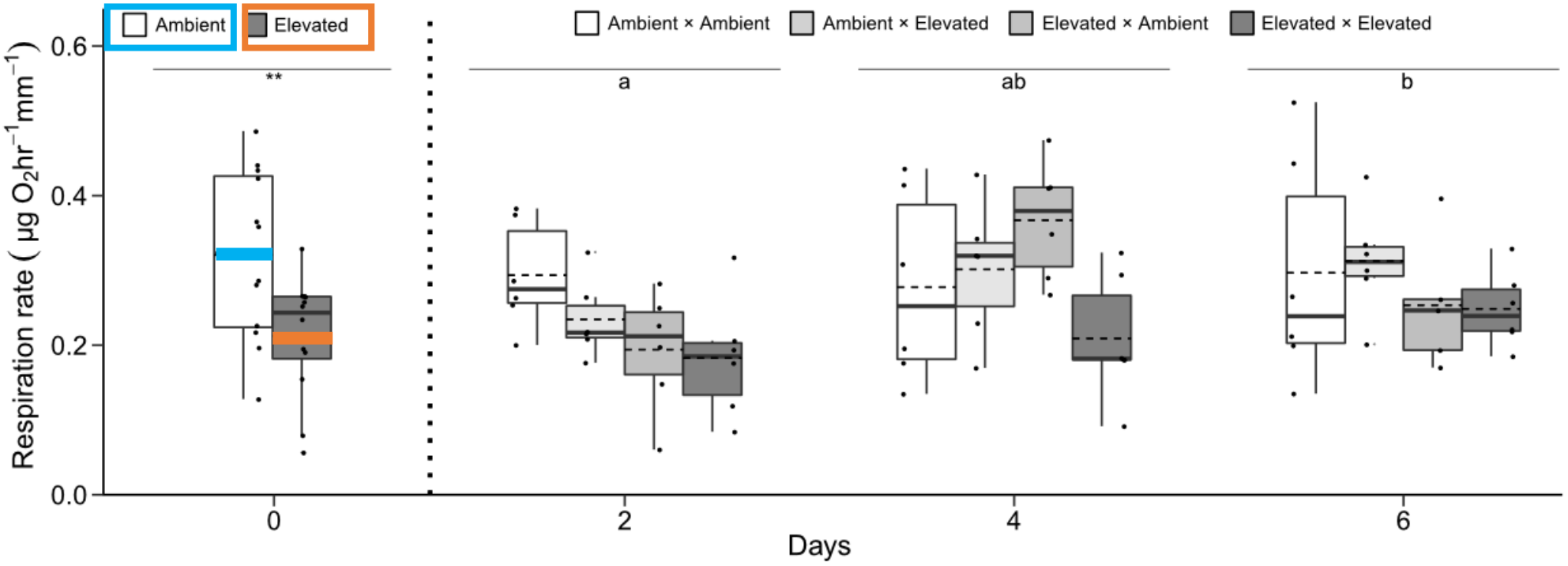
# Results: Secondary exposure



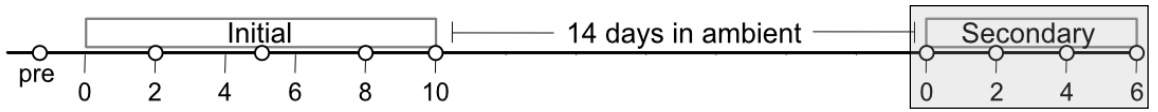

| | | DF | t | p-value |
|---|---|---|---|---|
| | *Welch Two Sample t-test* | | | |
| Respiration rate | $p\,CO_2$ | 19.833 | 2.673 | **0.015** |

**Metabolic rate:**

- Continued **metabolic suppression** prior to exposure



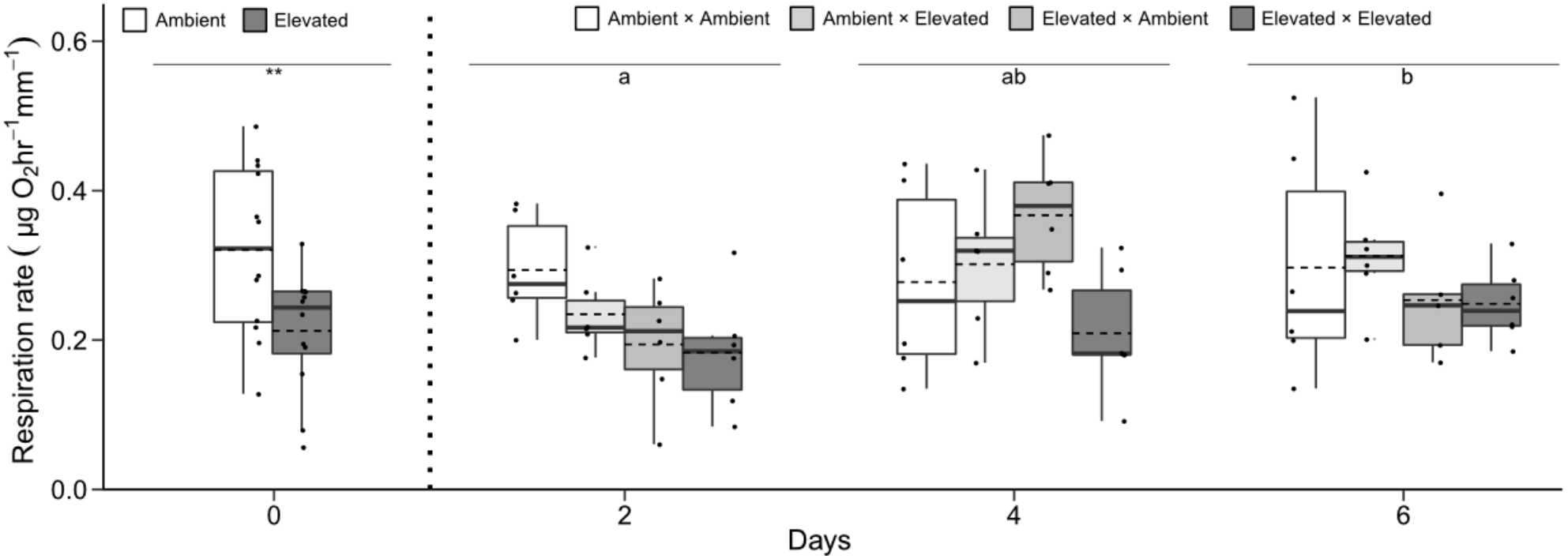
# Results: Secondary exposure



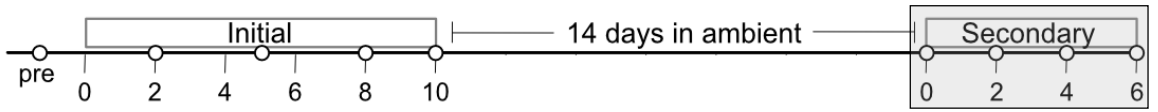

**Metabolic rate:**

- No effect of treatment,
  **metabolic <u>recovery</u> under subsequent encounter**

| | | DF | Sum Sq | Mean Sq | F value | p-value |
|---|---|---|---|---|---|---|
| ***Secondary exposure*** | *Three-way ANOVA* | | | | | |
| Respiration rate | time | 2 | 0.072 | 0.036 | 3.267 | **0.045** |
| | $p\,CO_{2\ initial}$ | 1 | 0.017 | 0.017 | 1.554 | 0.217 |
| | $p\,CO_{2\ secondary}$ | 1 | 0.037 | 0.037 | 3.339 | 0.073 |
| | $p\,CO_{2\ initial} \times p\,CO_{2\ secondary}$ | 1 | 0.027 | 0.027 | 2.435 | 0.124 |
| | $p\,CO_{2\ initial} \times$ time | 2 | 0.019 | 0.009 | 0.850 | 0.433 |
| | $p\,CO_{2\ secondary} \times$ time | 2 | 0.004 | 0.002 | 0.199 | 0.820 |
| | $p\,CO_{2\ initial} \times p\,CO_{2\ secondary} \times$ time | 2 | 0.040 | 0.020 | 1.842 | 0.167 |



# Results: Secondary exposure




| | | DF | Sum Sq | Mean Sq | F value | p-value |
|---|---|---|---|---|---|---|
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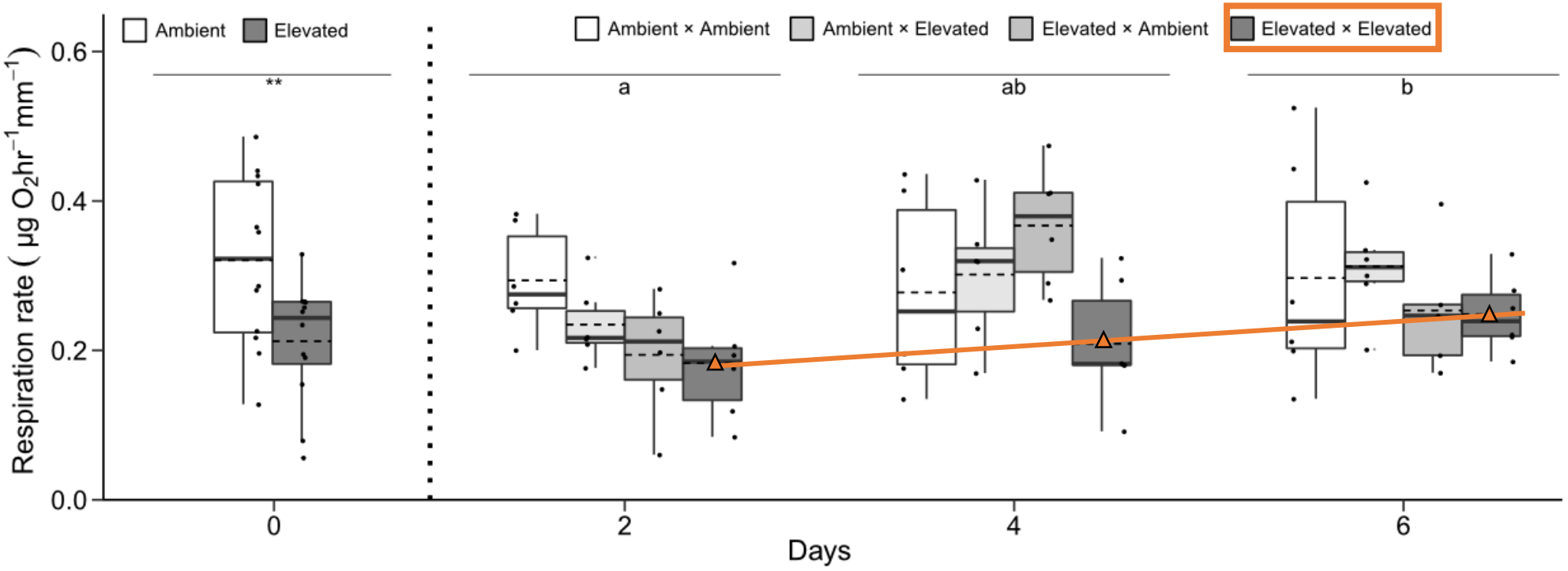
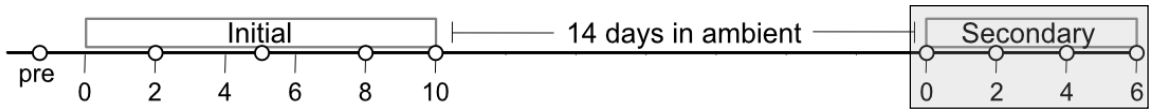
**Metabolic rate:**

- No effect of treatment,
  **metabolic <u>recovery</u> under subsequent encounter**



# Results: Secondary exposure




**Metabolic rate:**

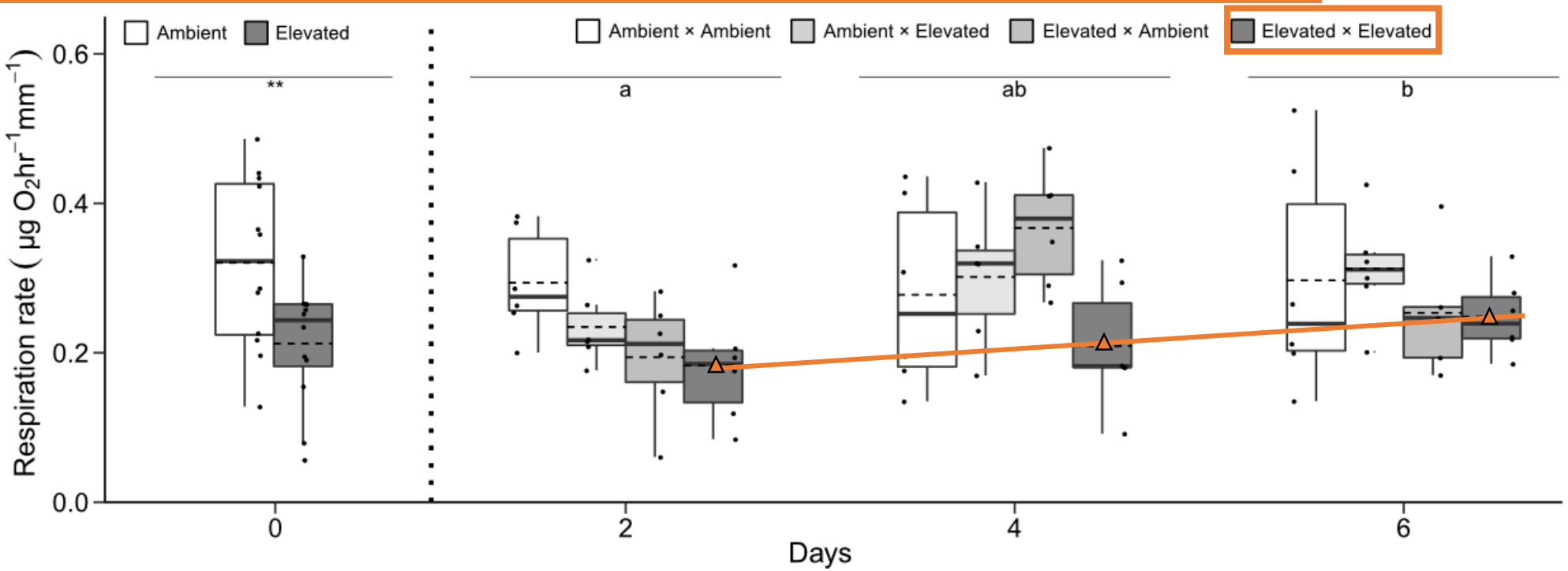
- No effect of trea
  **metabolic rec**

## SECONDARY EXPOSURE: METABOLIC RATE

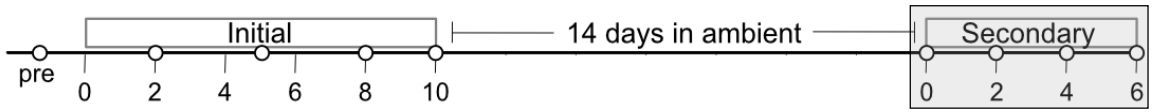
Elevated *p*CO2 did not affect respiration rate

Potential for metabolic recovery

| | Mean Sq | F value | p-value |
|---|---|---|---|
| | 0.036 | 3.267 | **0.045** |
| | 0.017 | 1.554 | 0.217 |
| | 0.037 | 3.339 | 0.073 |
| | 0.027 | 2.435 | 0.124 |
| | 0.009 | 0.850 | 0.433 |
| | 0.002 | 0.199 | 0.820 |
| | 0.020 | 1.842 | 0.167 |



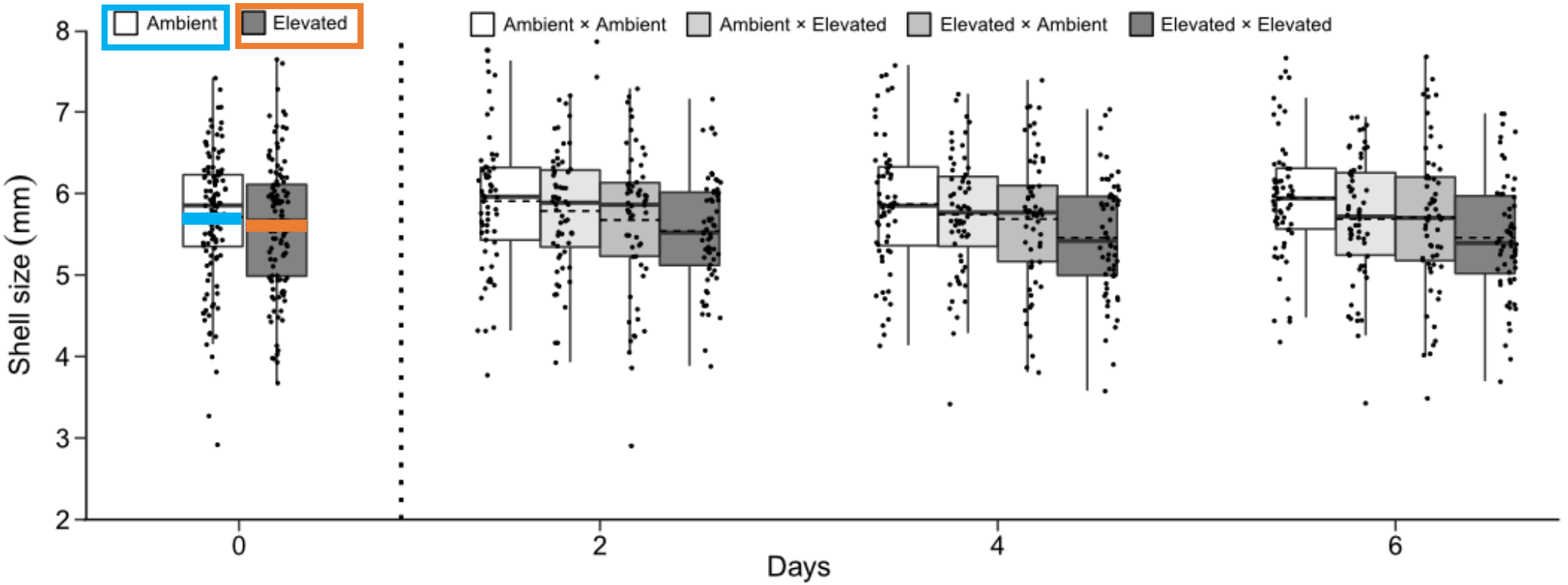
# Results: Secondary exposure



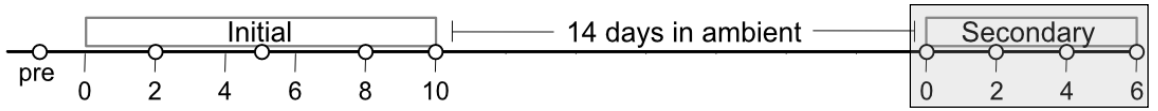

| | | DF | t | p-value |
|---|---|---|---|---|
| | *Welch Two Sample t-test* | | | |
| Shell length | $p\,CO_2$ | 1.146 | 236.680 | 0.253 |

**Growth:**

- **No treatment effect** prior to exposure



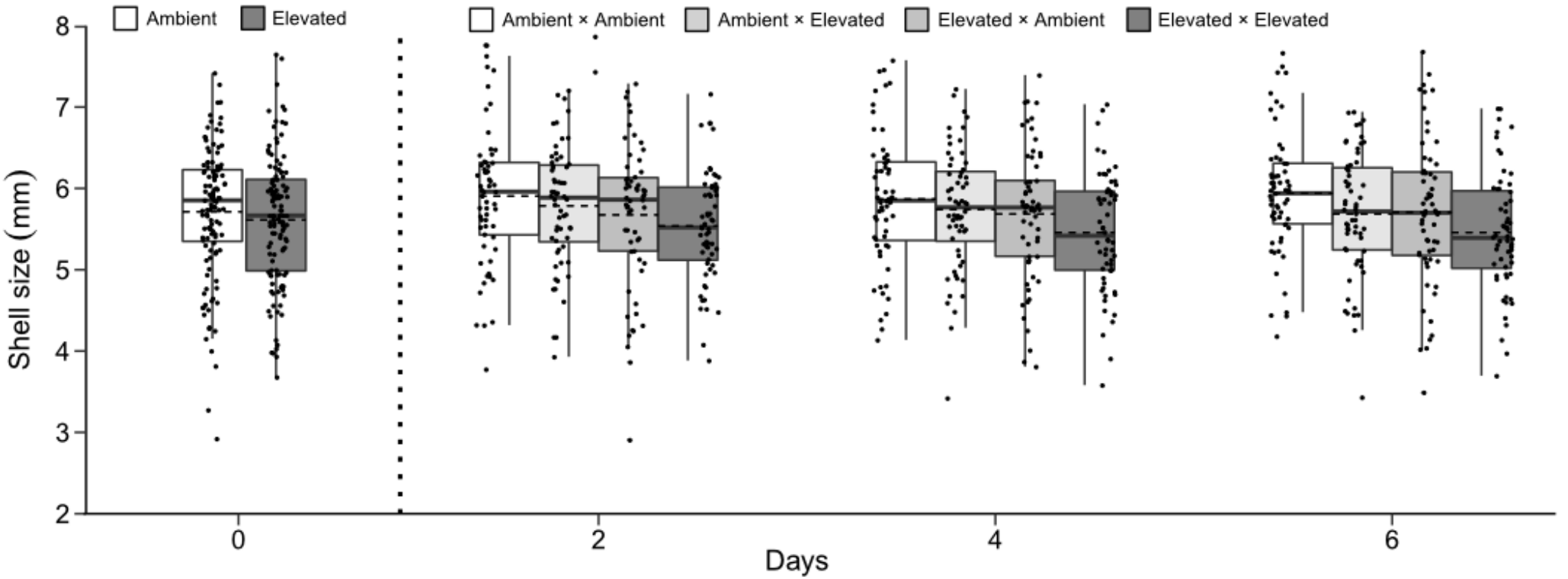
# Results: Secondary exposure



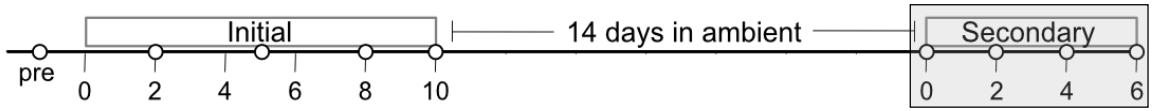

**Growth:**

- Initial and secondary **treatment effects**

| | | DF | Sum Sq | Mean Sq | F value | p-value |
|---|---|---|---|---|---|---|
| ***Secondary exposure*** | *Three-way ANOVA* | | | | | |
| Shell length | time | 2 | 0.190 | 0.095 | 0.152 | 0.859 |
| | $p\,CO_{2\ initial}$ | 1 | 9.910 | 9.910 | 15.821 | **<0.001** |
| | $p\,CO_{2\ secondary}$ | 1 | 6.210 | 6.212 | 9.917 | **0.002** |
| | $p\,CO_{2\ initial} \times p\,CO_{2\ secondary}$ | 1 | 0.060 | 0.063 | 0.100 | 0.752 |
| | $p\,CO_{2\ initial} \times$ time | 2 | 0.000 | 0.001 | 0.002 | 0.998 |
| | $p\,CO_{2\ secondary} \times$ time | 2 | 0.460 | 0.231 | 0.368 | 0.692 |
| | $p\,CO_{2\ initial} \times p\,CO_{2\ secondary} \times$ time | 2 | 0.100 | 0.048 | 0.076 | 0.927 |



# Results: Secondary exposure




| | | DF | Sum Sq | Mean Sq | F value | p-value |
|---|---|---|---|---|---|---|
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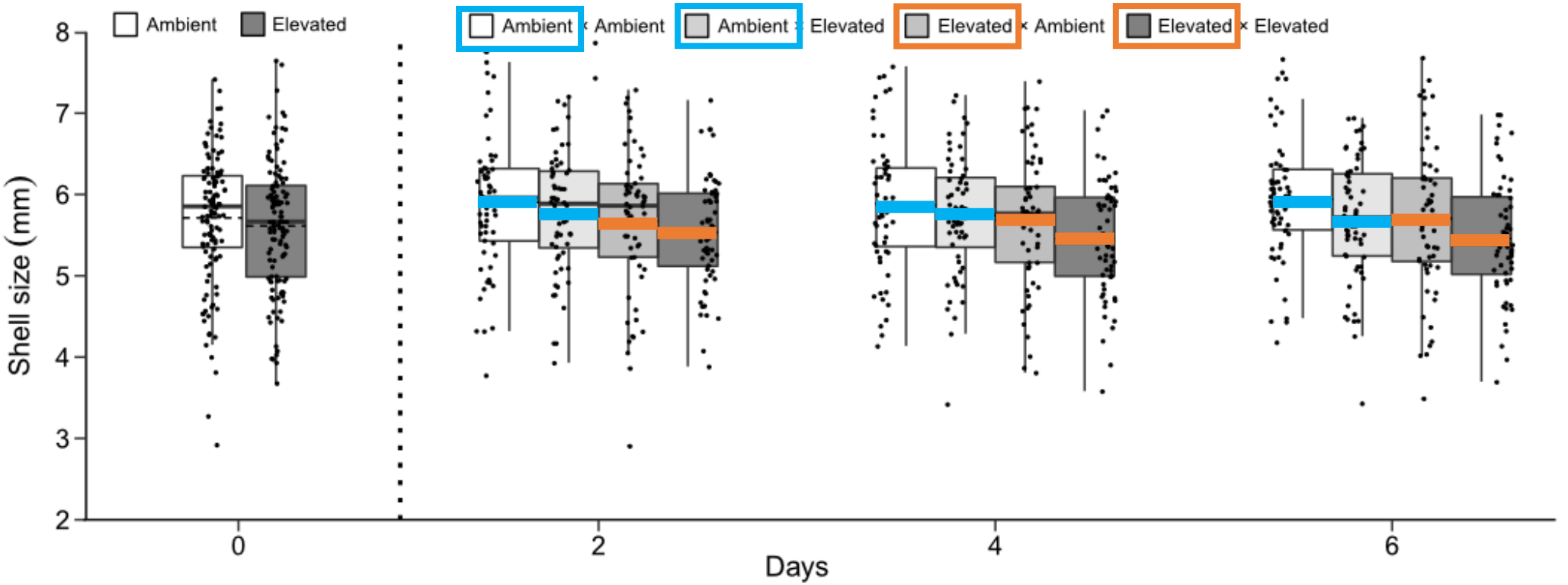
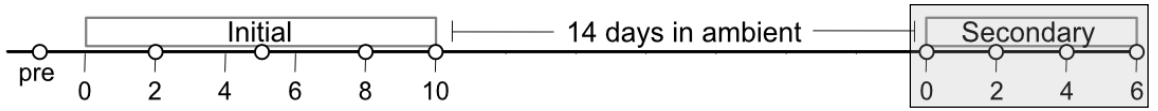
## Growth:

- Initial and secondary **treatment effects**
- Initial treatment:
  **4.02%** (mm length) **smaller shells** under **elevated**



# Results: Secondary exposure

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|---|---|---|---|---|---|---|
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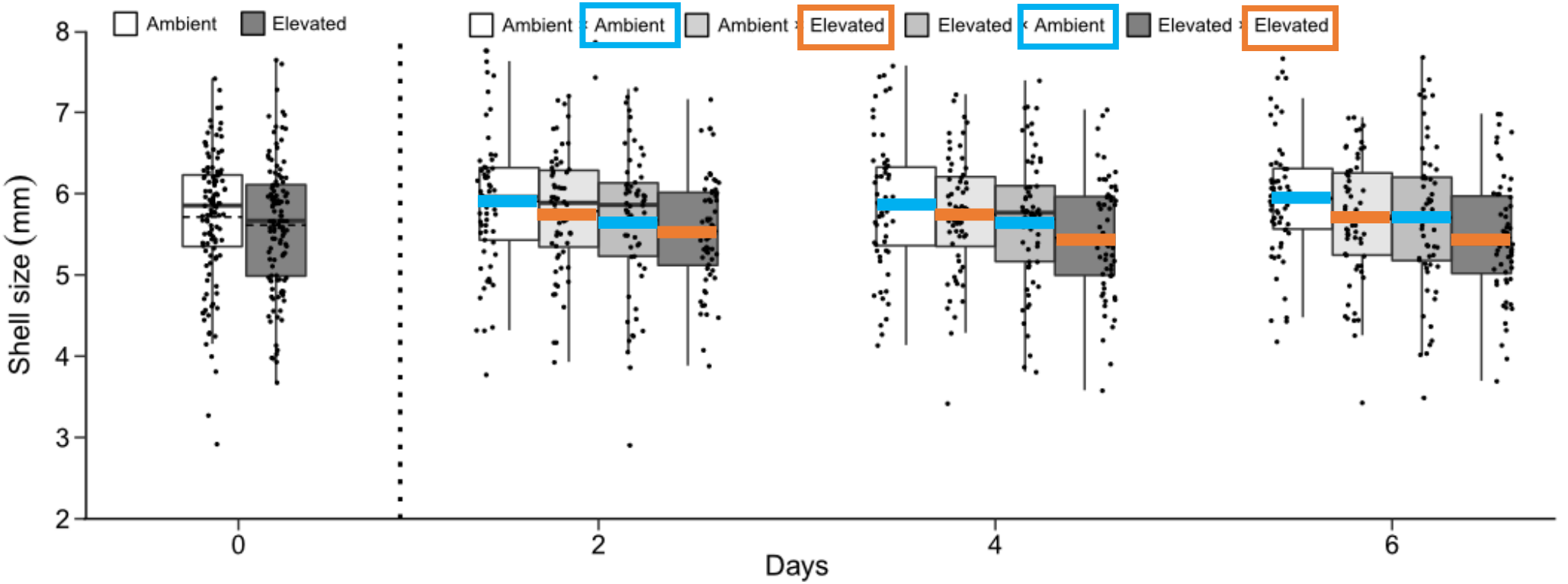
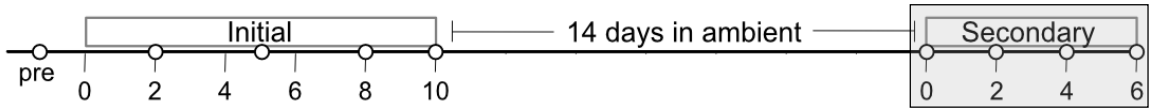
| | $p\,CO_{2\ initial} \times p\,CO_{2\ secondary} \times$ time | 2 | 0.100 | 0.048 | 0.076 | 0.927 |

## Growth:

- Initial and secondary **treatment effects**
- Second treatment:
  **3.20%** (mm length) **smaller shells** under **elevated**



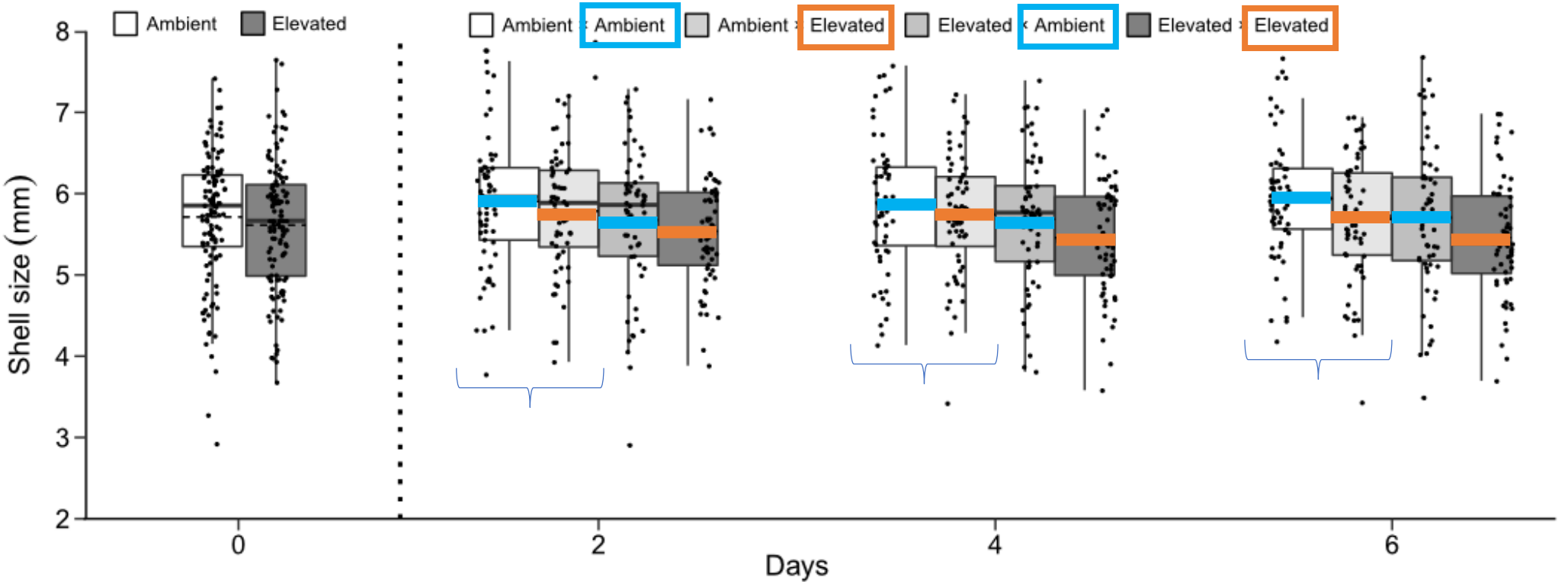
# Results: Secondary exposure



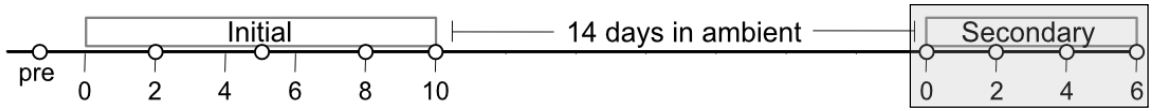

| | | DF | Sum Sq | Mean Sq | F value | p-value |
|---|---|---|---|---|---|---|
| ***Secondary exposure*** | *Three-way ANOVA* | | | | | |
| Shell length | time | 2 | 0.190 | 0.095 | 0.152 | 0.859 |
| | $p\,CO_{2\ initial}$ | 1 | 9.910 | 9.910 | 15.821 | **<0.001** |
| | $p\,CO_{2\ secondary}$ | 1 | 6.210 | 6.212 | 9.917 | **0.002** |
| | $p\,CO_{2\ initial} \times p\,CO_{2\ secondary}$ | 1 | 0.060 | 0.063 | 0.100 | 0.752 |
| | $p\,CO_{2\ initial} \times$ time | 2 | 0.000 | 0.001 | 0.002 | 0.998 |
| | $p\,CO_{2\ secondary} \times$ time | 2 | 0.460 | 0.231 | 0.368 | 0.692 |
| | $p\,CO_{2\ initial} \times p\,CO_{2\ secondary} \times$ time | 2 | 0.100 | 0.048 | 0.076 | 0.927 |

**Growth:**

- Initial and secondary **treatment effects**
- Second treatment:
  **3.20%** (mm length) **smaller shells** under **elevated**



# Results: Secondary exposure




**Growth:**

- Initial and secondary **treatment effects**
- Second treat
  **3.20%** (mm l

| | | DF | Sum Sq | Mean Sq | F value | p-value |
|---|---|---|---|---|---|---|
| ***Secondary exposure*** | *Three-way ANOVA* | | | | | |
| Shell length | time | 2 | 0.190 | 0.095 | 0.152 | 0.859 |
| | $p\,CO_{2\ initial}$ | 1 | 9.910 | 9.910 | 15.821 | **<0.001** |
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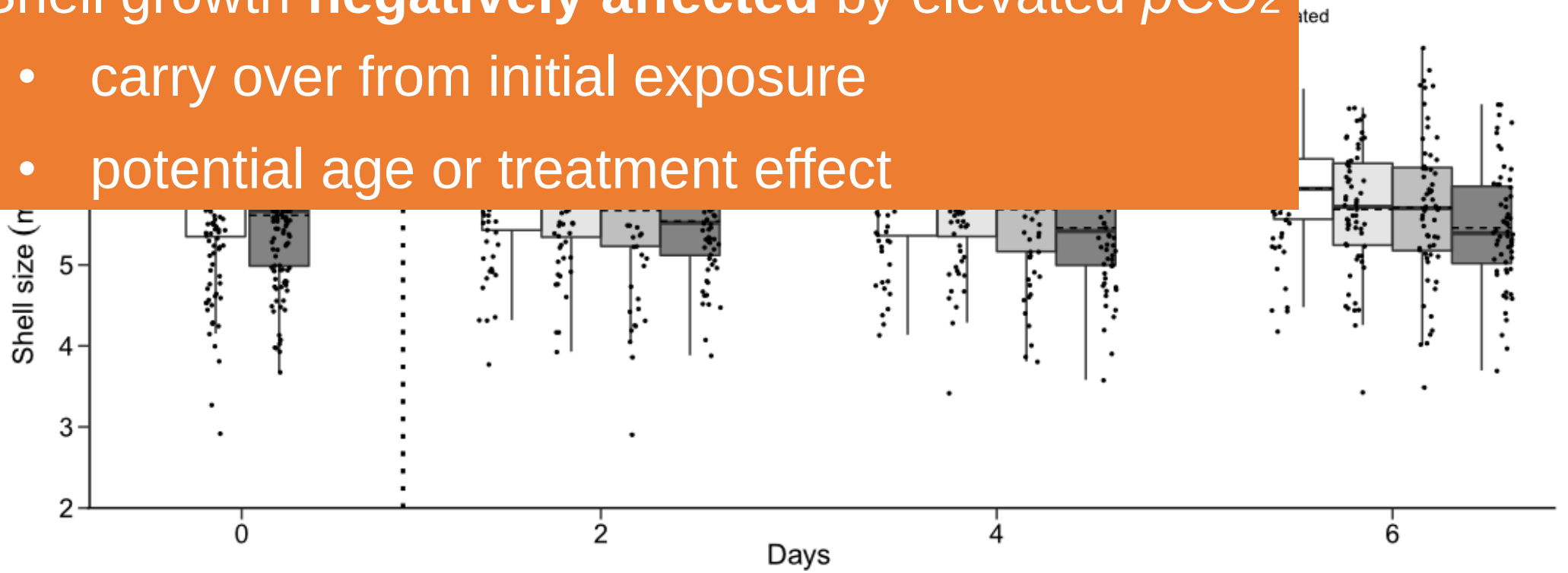
| | $p\,CO_{2\ initial}$ × time | 2 | 0.000 | 0.001 | 0.002 | 0.998 |
| | | | | 0.231 | 0.368 | 0.692 |
| | | | | 0.048 | 0.076 | 0.927 |

## SECONDARY EXPOSURE: SHELL GROWTH

Shell growth **negatively affected** by elevated $pCO_2$

- carry over from initial exposure
- potential age or treatment effect



# Conclusions

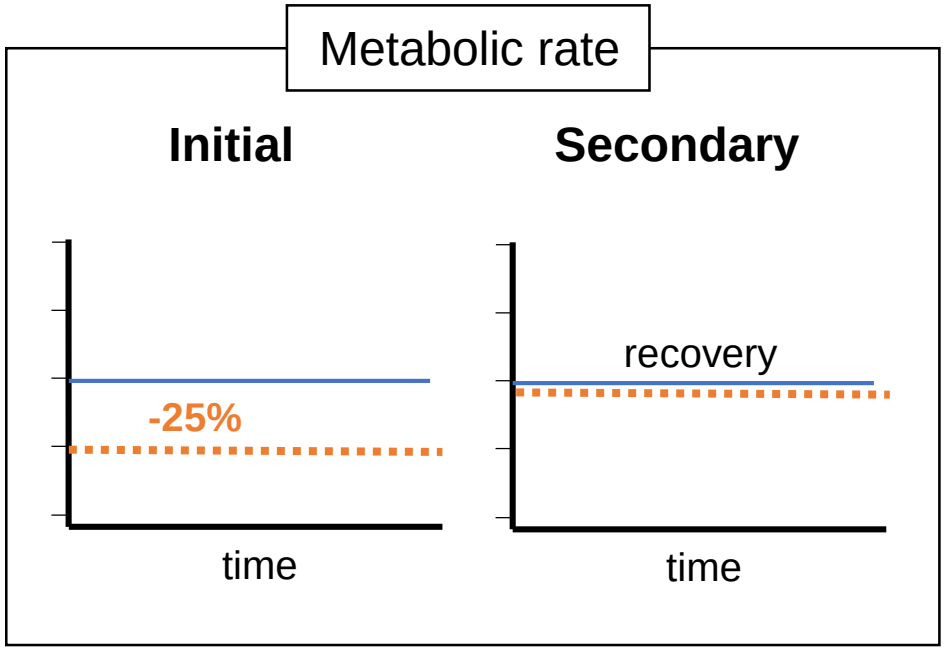
## Metabolic resilience

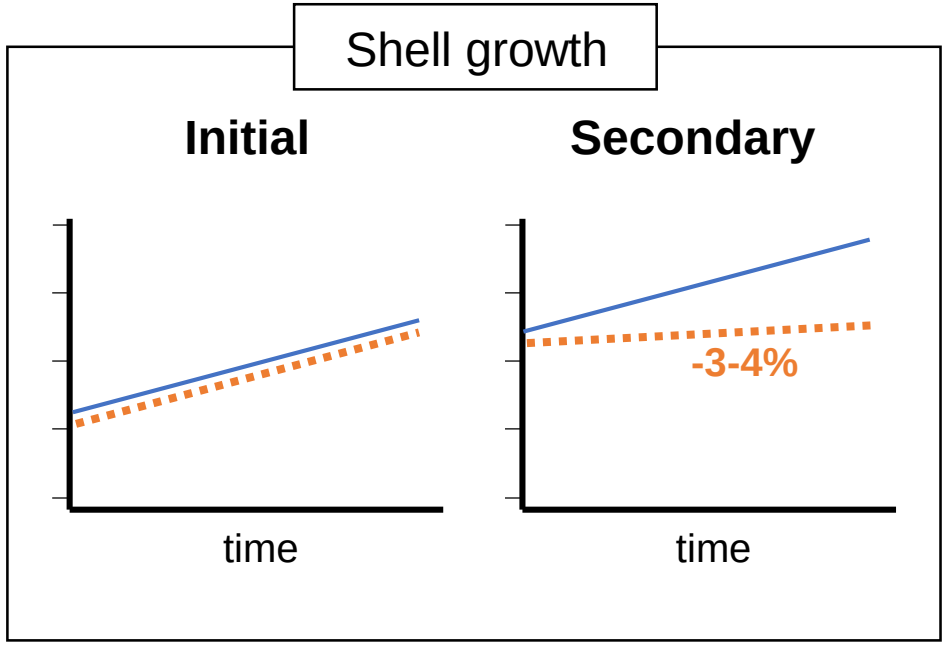
- Suppressed metabolic state
  under **initial exposure** to elevated $pCO_2$

- Metabolic recovery
  under **subsequent exposure** to elevated $pCO_2$

## Negative impact on shell growth

- Slowed shell growth
  under repeated short-term exposure elevated $pCO_2$



# Conclusions

**Metabolic resilience**

- Suppressed metabolic state
  under **initial exposure** to elevated $pCO_2$
- Metabolic recovery
  under **subsequent exposure** to elevated $pCO_2$

**Negative impact on shell growth**

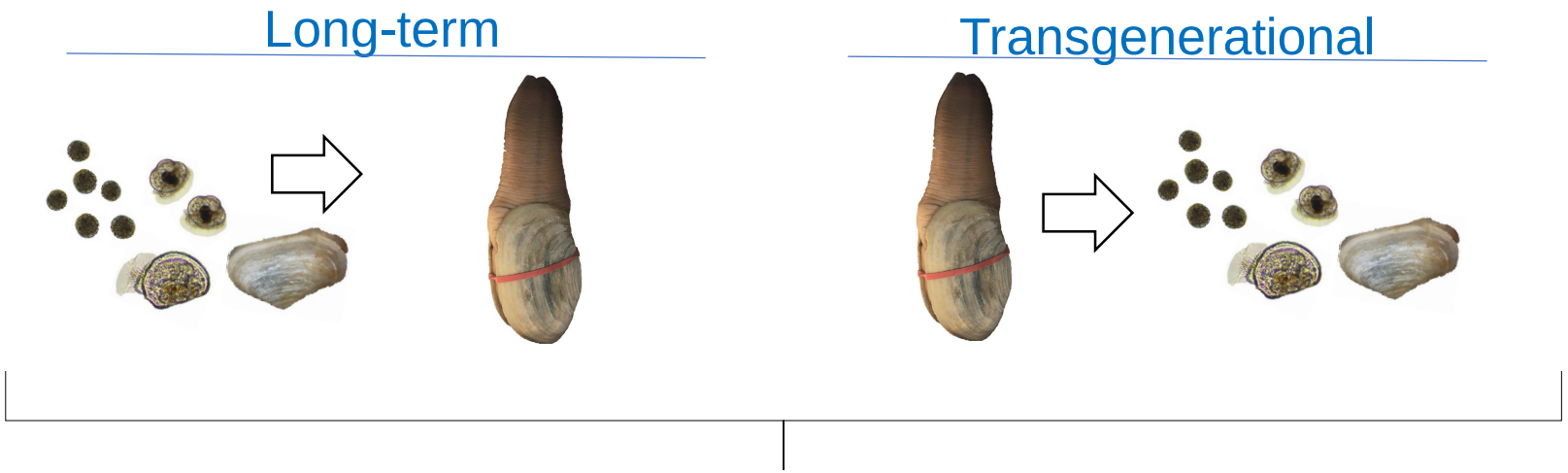
- Slowed shell growth
  under repeated short-term exposure elevated $pCO_2$

***Take-home message for Pacific geoduck production..***

(1) Metabolic trends demonstrate potential recovery and compensatory response under repeated exposure

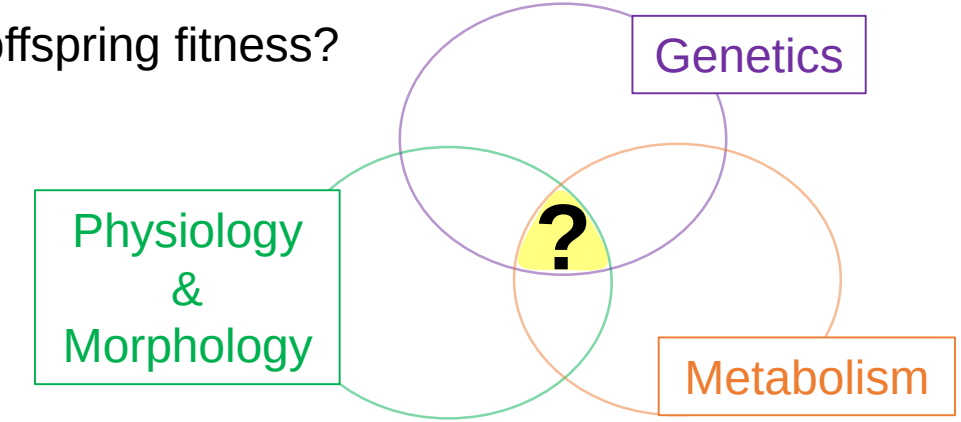
(2) Growth responses demonstrate susceptibility to acidification:
- impeded growth can carry over from prior stress
- potential age dependence and sensitivity to stress intensity

# Future research




- **Need a holistic baseline response** under acidification to determine life stages critical for environmental priming
- What are **costs and drivers** of metabolic alterations (i.e. suppression/recovery) under long-term acidification?
- Can **parental conditioning** enhance reproductive performance and offspring fitness?

Use complex network of stress responses



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Jim Parsons, PhD



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Questions?