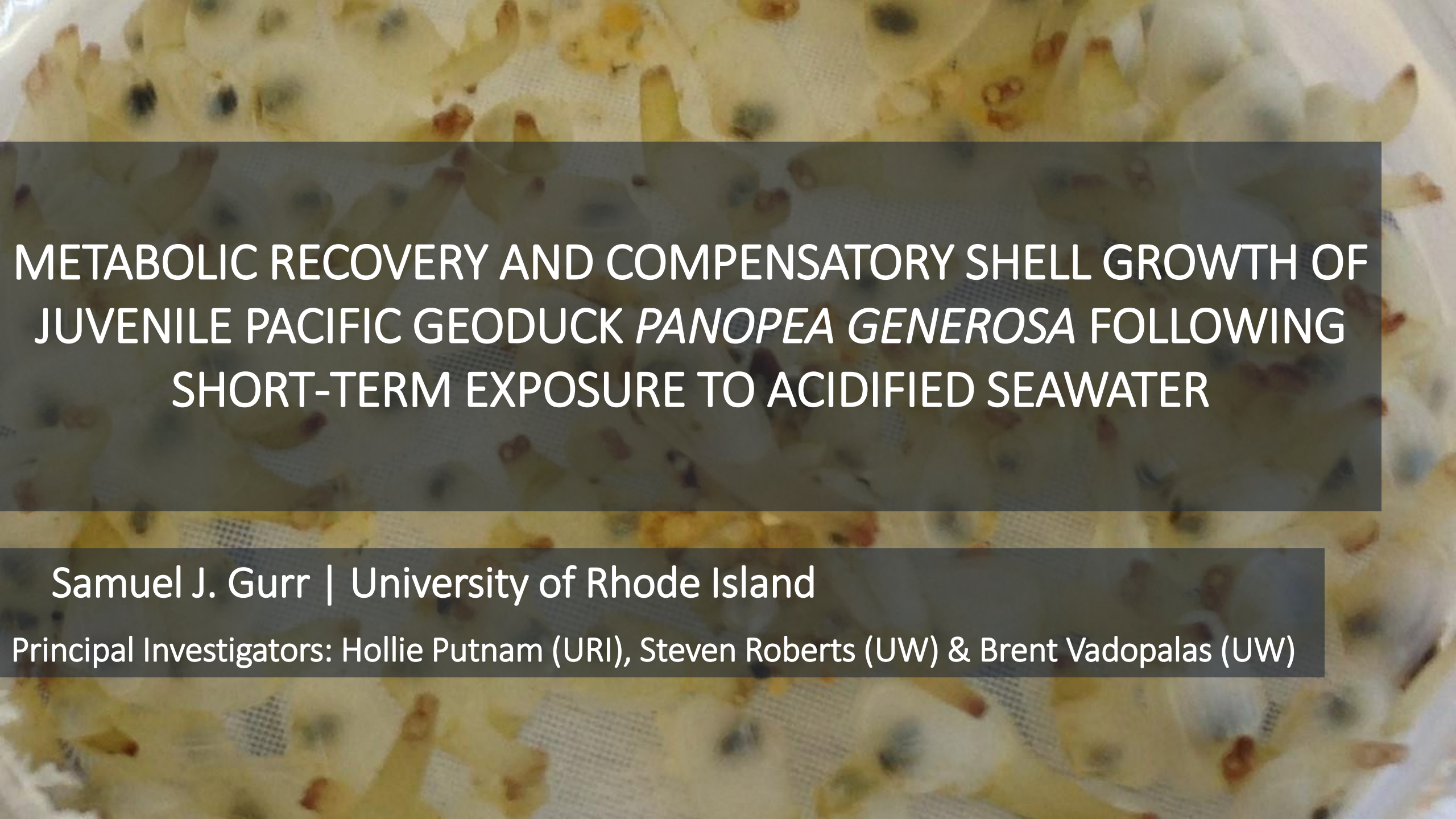


METABOLIC RECOVERY AND COMPENSATORY SHELL GROWTH OF JUVENILE PACIFIC GEODUCK *PANOPEA GENEROSA* FOLLOWING SHORT-TERM EXPOSURE TO ACIDIFIED SEAWATER

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 coastal communities of PNW

Photo credit: Mat Henderson

Sustainable production

Geoduck aquaculture

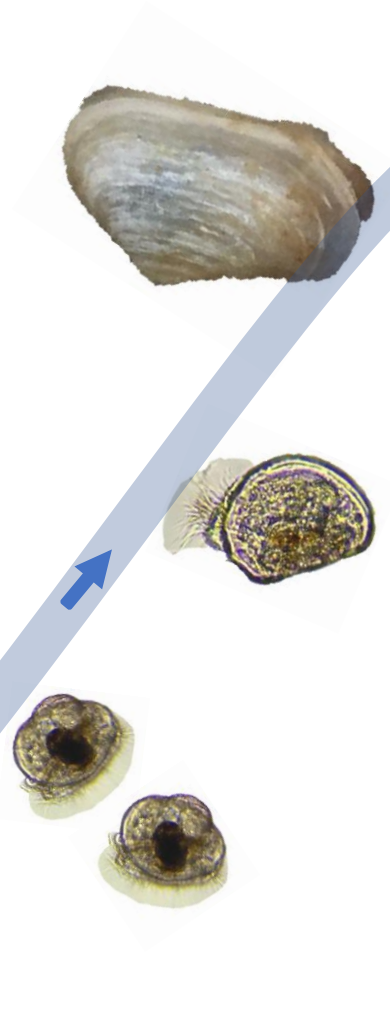
- Prevents overexploitation of wild populations
- Satisfy growing demand in recent decades for international trade
- approx. 90% global geoduck produced from WA state
  - annual revenue > 24 million USD year-1
  - $14 pound^{-1} (as of 2015)

Photo credit: Mat Henderson

Sustainable production

HATCHERY

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



OUTPLANT

- Grown on mudflat for ~4-5 years until adults are harvested

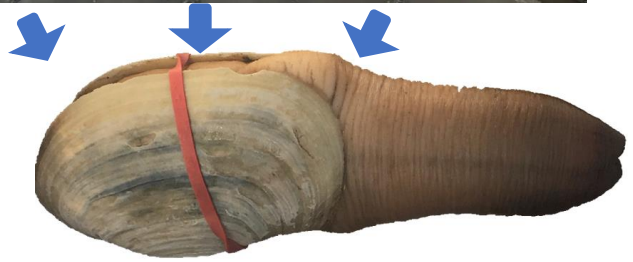


Photo credit: Steven Roberts & Kaitlyn Mitchell

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

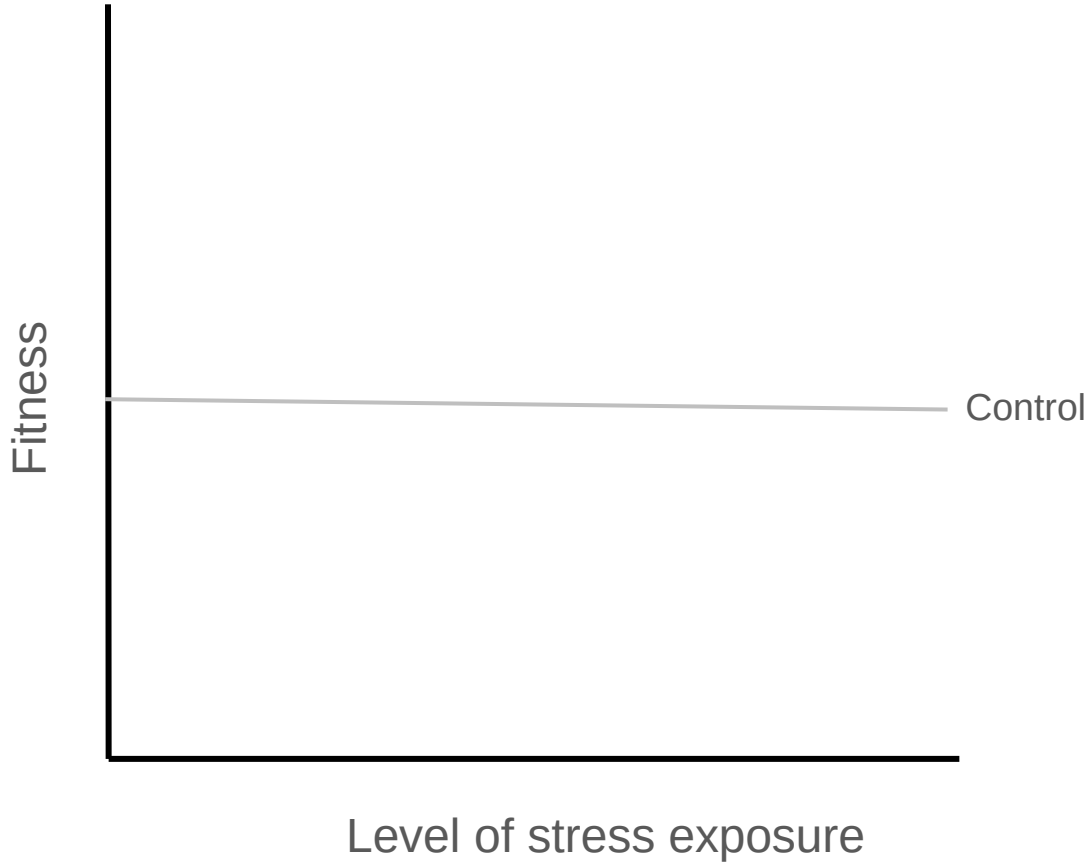
How can we enhance resilience and increase hatchery production?

Stress conditioning

What is “**stress conditioning**”?

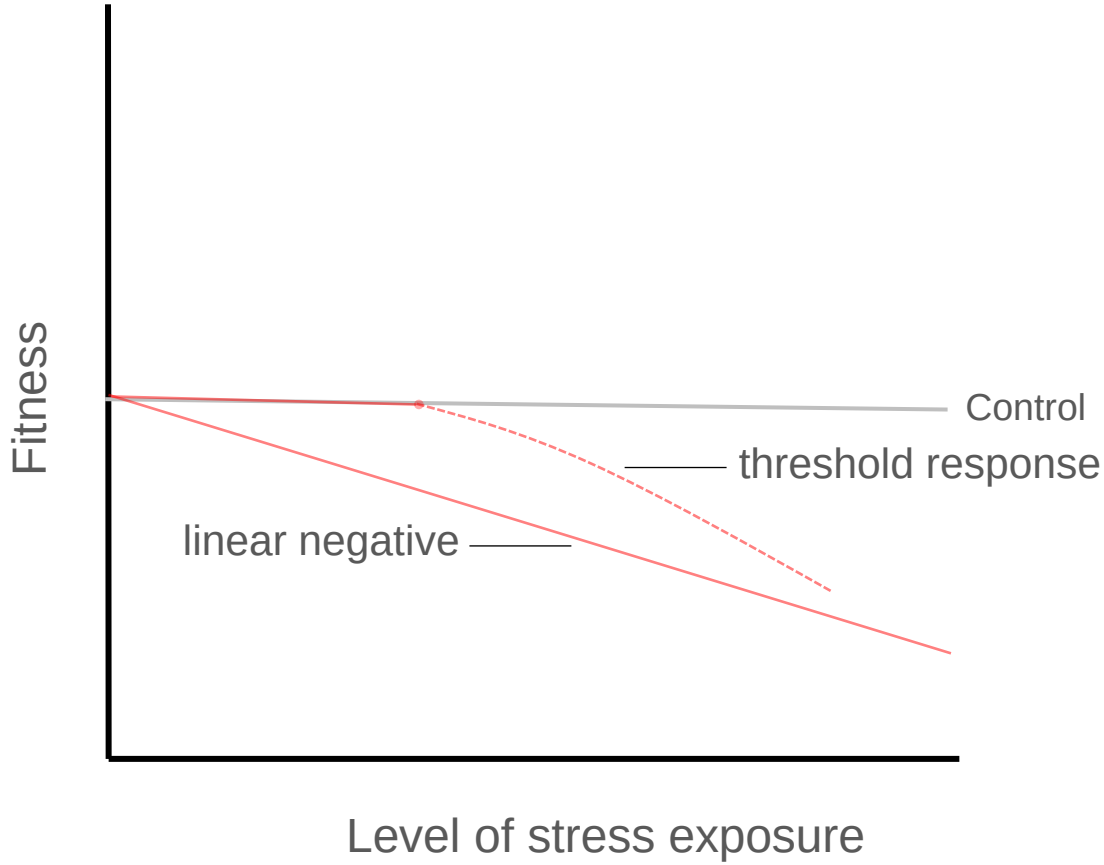
Priming organisms with sub-lethal exposure to increase stress-resilience and performance under a **subsequent encounter**

Stress conditioning



Stress conditioning

Response model under initial exposure…



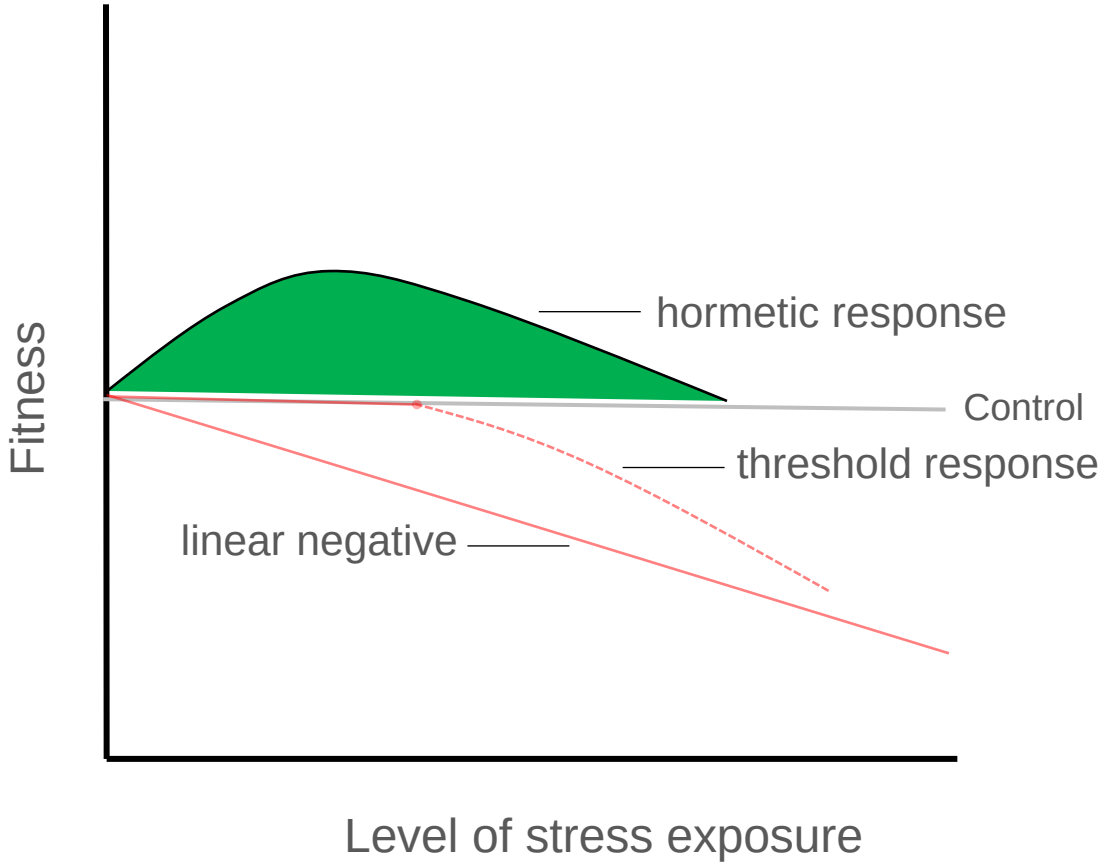
Negative linear or threshold response

Stress conditioning

Positive effect on performance

Negative linear or threshold response

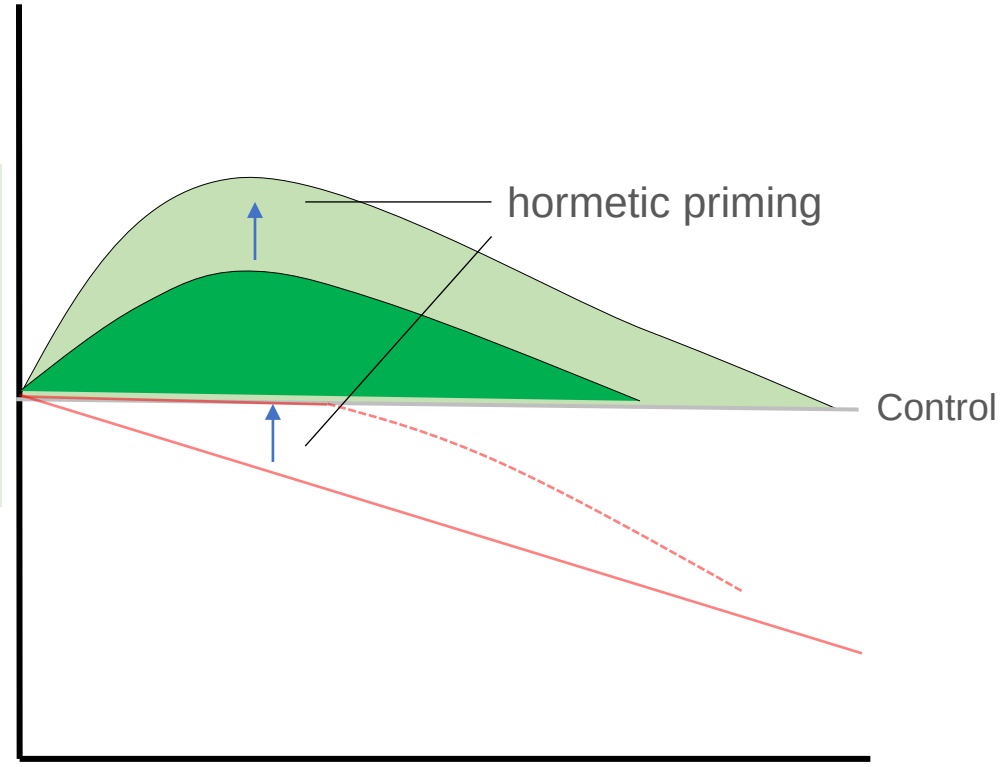
Response model under initial exposure…



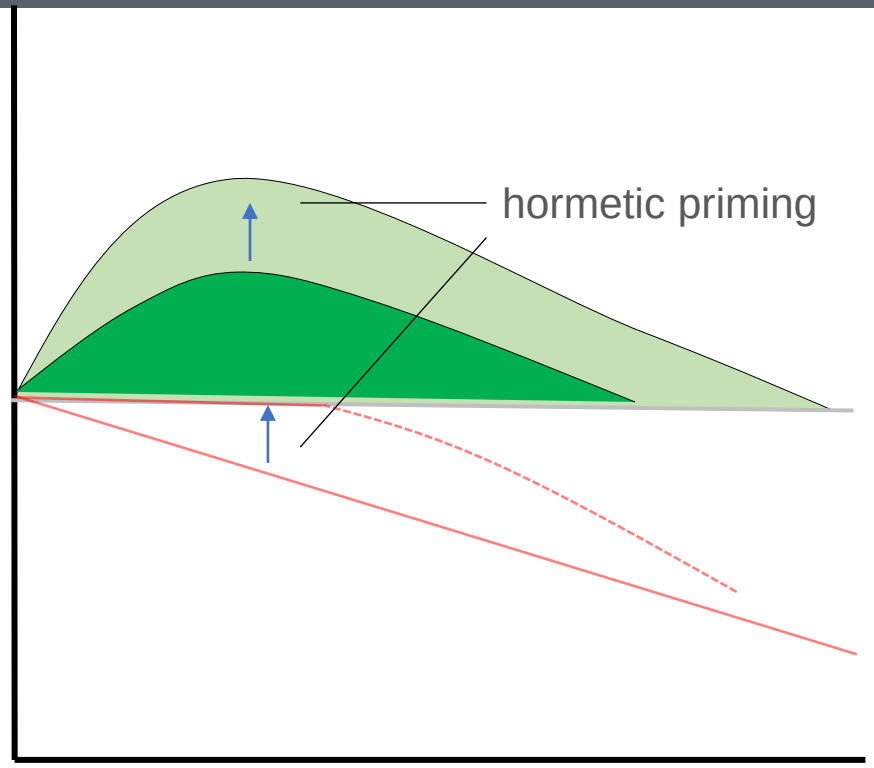
Stress conditioning

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

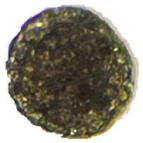
Ocean acidification & stress conditioning

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

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

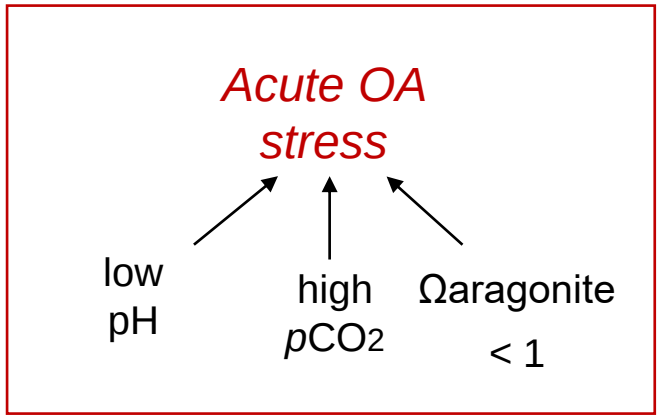
b). Long-term – costly and labor intensive; seasonal or future acidification scenarios

Ocean acidification & stress conditioning



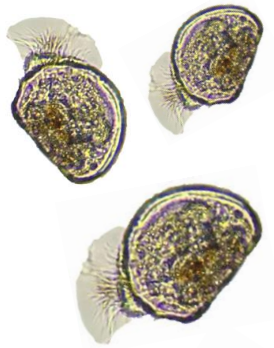
Intragenerational exposure – targets stress-acclimation **within** a generation

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

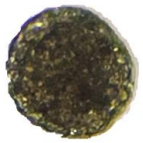


Common indicators of bivalve performance

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

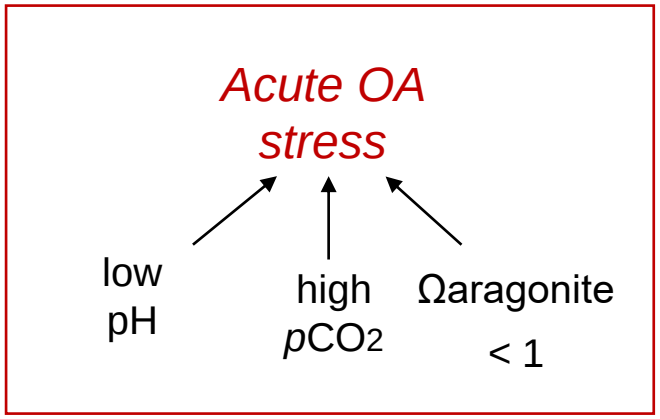


Ocean acidification & stress conditioning



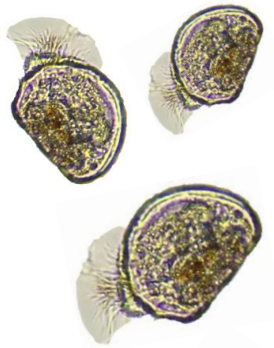
Intragenerational exposure – targets stress-acclimation **within** a generation

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



Common indicators of bivalve performance

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



Responses particularly relevant for commercial production

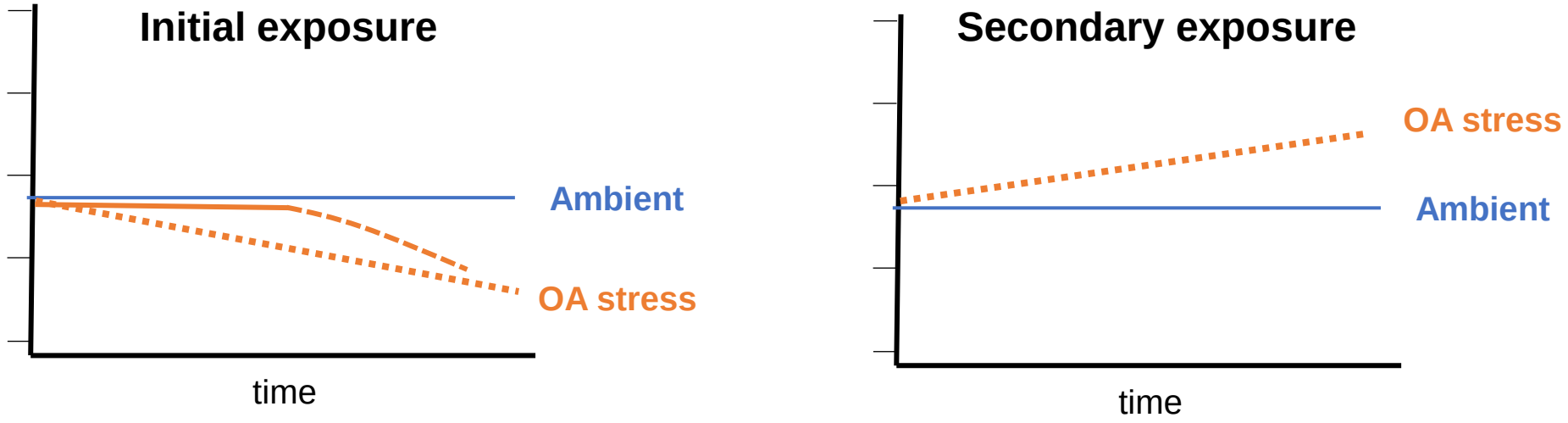
Stress conditioning in a commercial hatchery

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



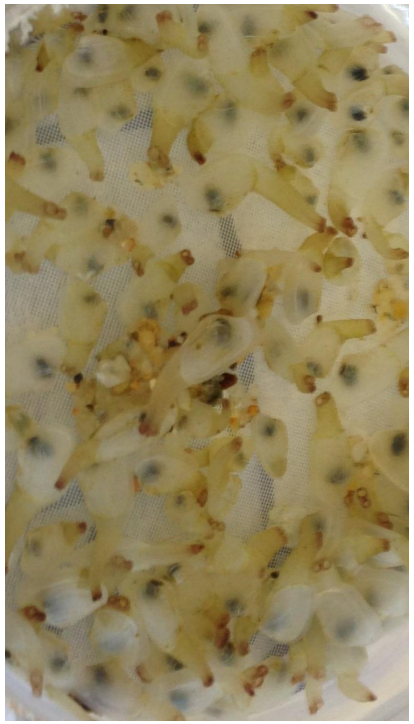
Animal collection and exposure treatments

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

Hatchery-reared juveniles

5 months post-spawn

5 mm shell length



Animal collection and exposure treatments

- 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

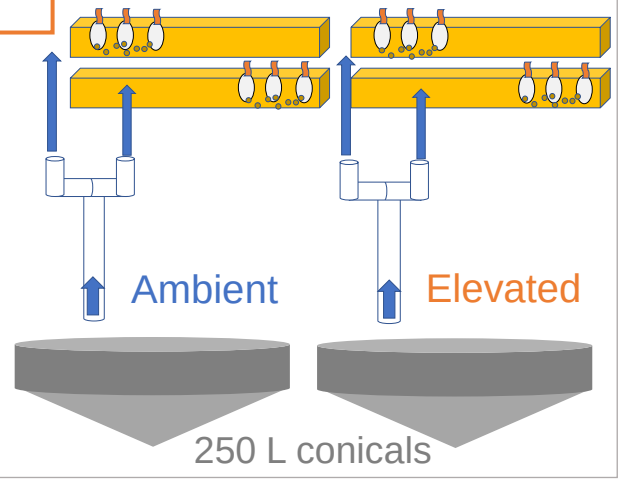
Ambient pCO_2

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

v.

Elevated pCO_2

pCO_2	=	**2400**
pH	=	7.3
$\Omega_{aragonite}$	=	0.4



Constants:
Total alkalinity: approx. 2050 µmol kg^{-1}
Diet: 5×10^7 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}

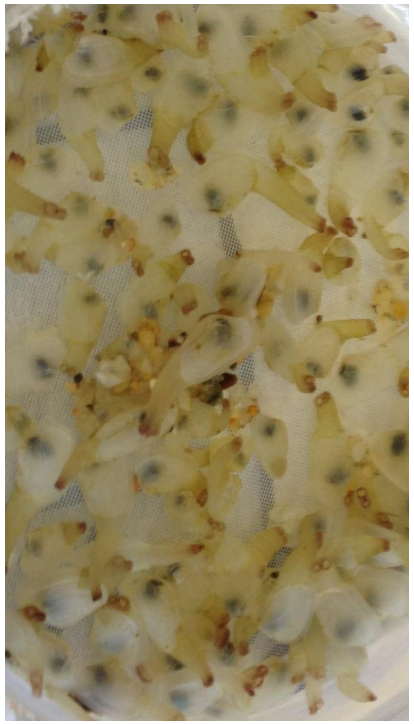
Animal collection and exposure treatments

- 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
$\Omega_{aragonite}$	=	0.4

***Constants*:**

Total alkalinity: approx. 2050 µmol kg^{-1}

Diet: 5×10^7 live algae cells d^{-1} ind^{-1}

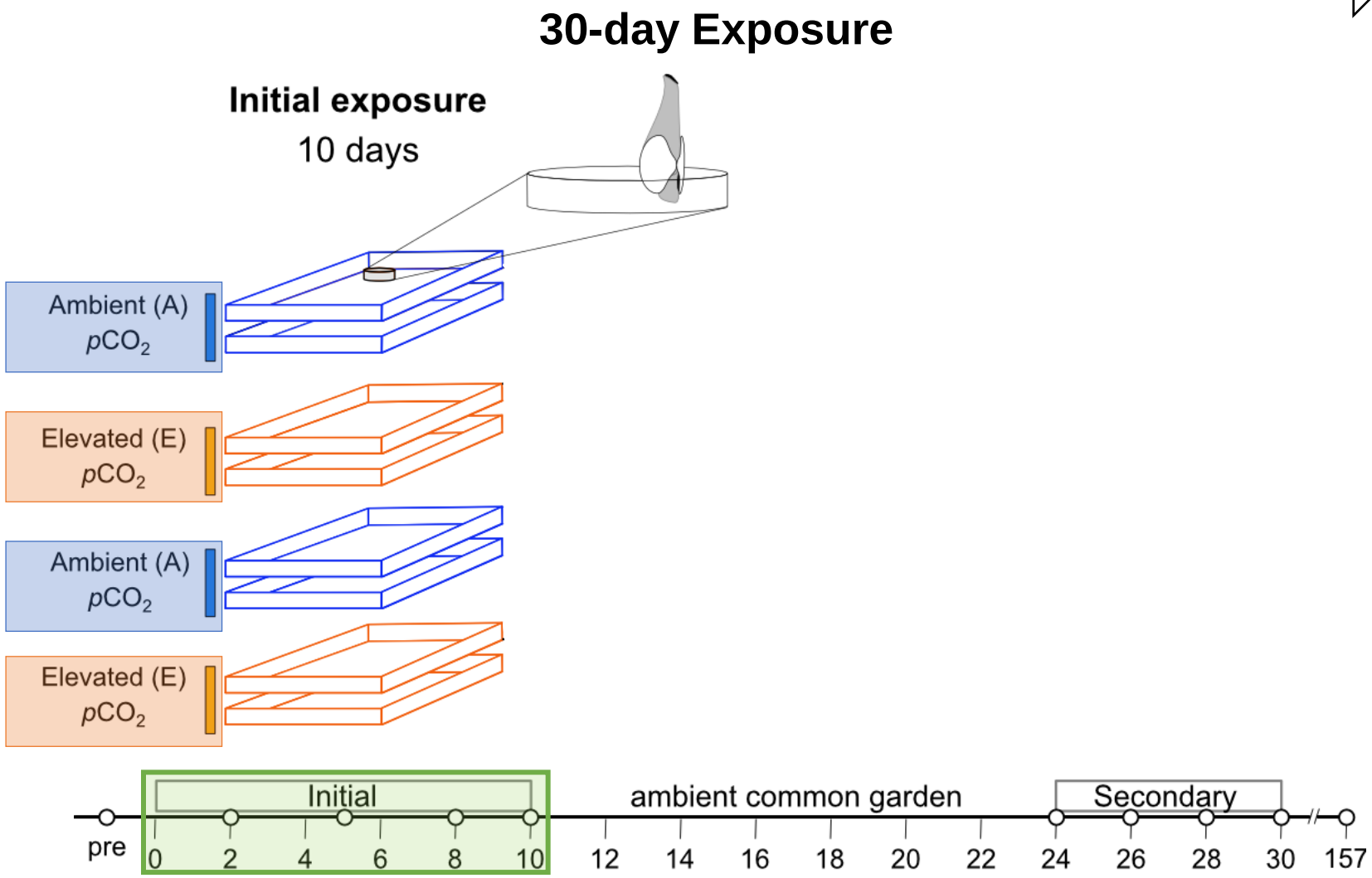
Temperature:15.4 ± 1.1°C

Salinity: 28.9 ± 0.2 psu

Flow rate: 480 mL min^{-1}

Animals in an isolated dish for physiological assessment

Experimental design & physiology data

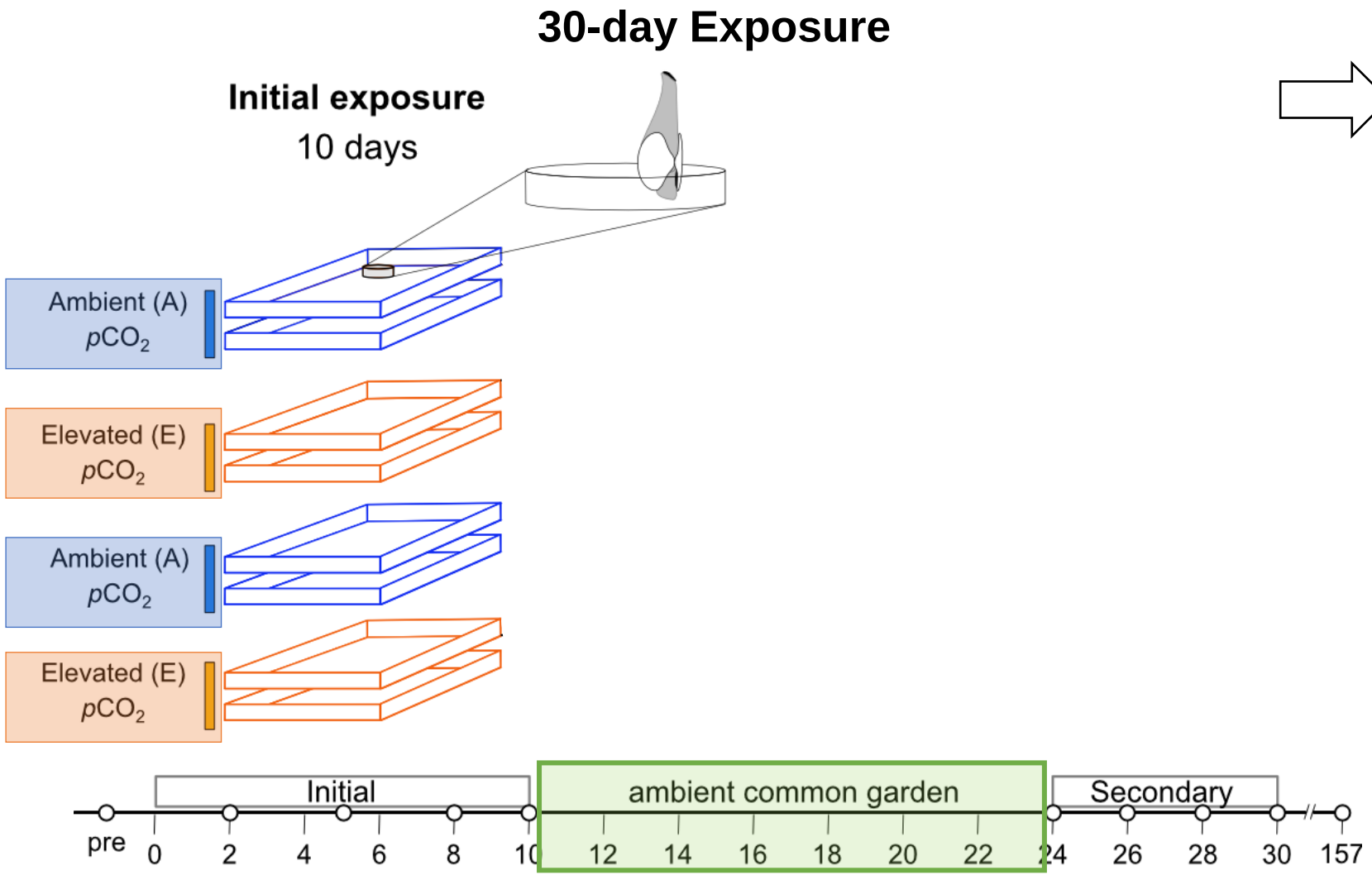


- Initial exposure (10 days)
  n = 4 trays treatment^{-1}
- Ambient common garden
- Secondary exposure (6 days)
  n = 2 trays treatment^{-1}
- 5 months post-exposure
  n = 2 trays treatment^{-1}

Ambient pCO_2
pH = 7.9

Elevated pCO_2
pH = 7.3

Experimental design & physiology data

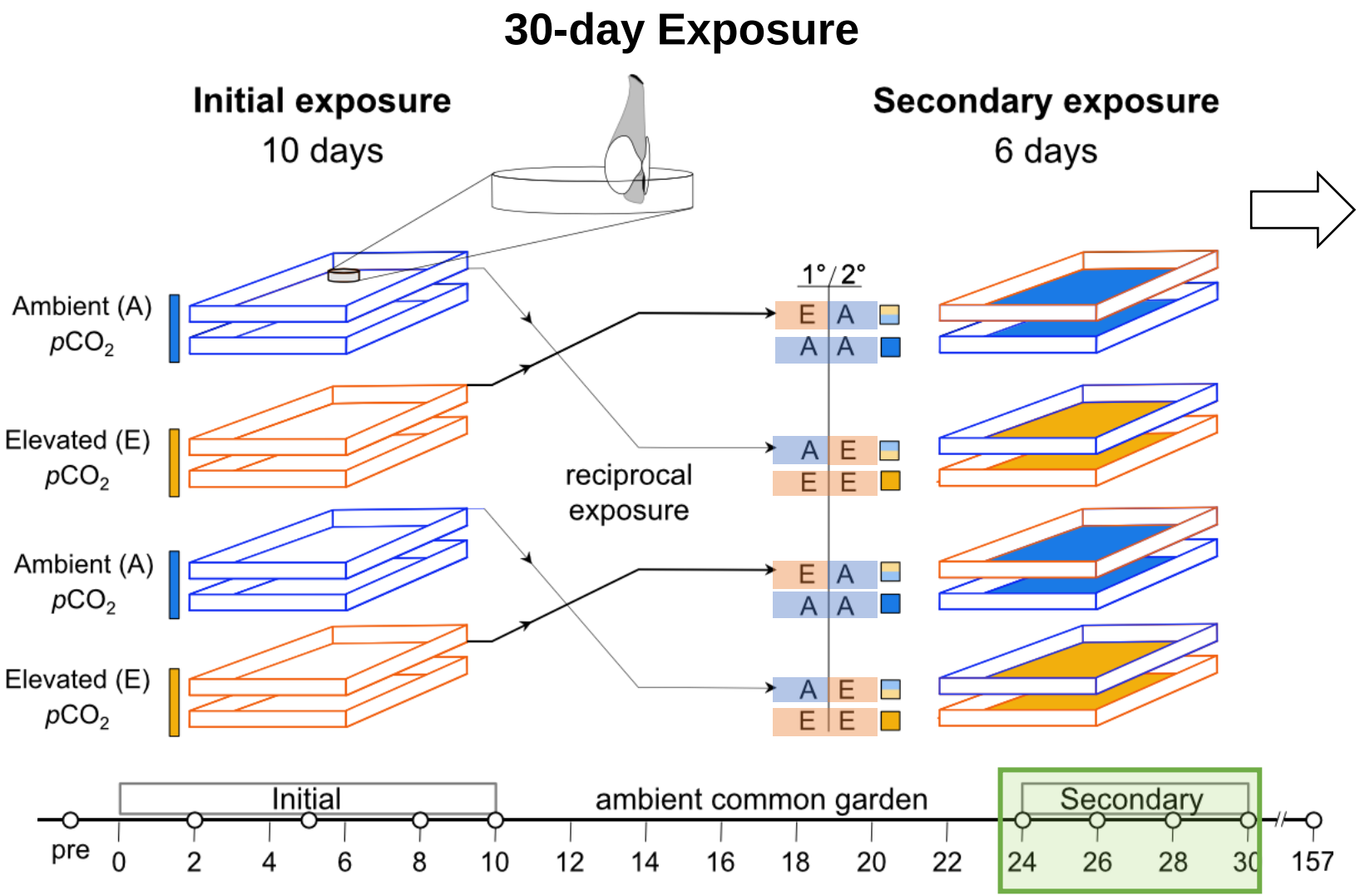


- Initial exposure (10 days)
  n = 4 trays treatment^{-1}
- Ambient common garden
- Secondary exposure (6 days)
  n = 2 trays treatment^{-1}
- 5 months post-exposure
  n = 2 trays treatment^{-1}

Ambient pCO_2
pH = 7.9

Elevated pCO_2
pH = 7.3

Experimental design & physiology data



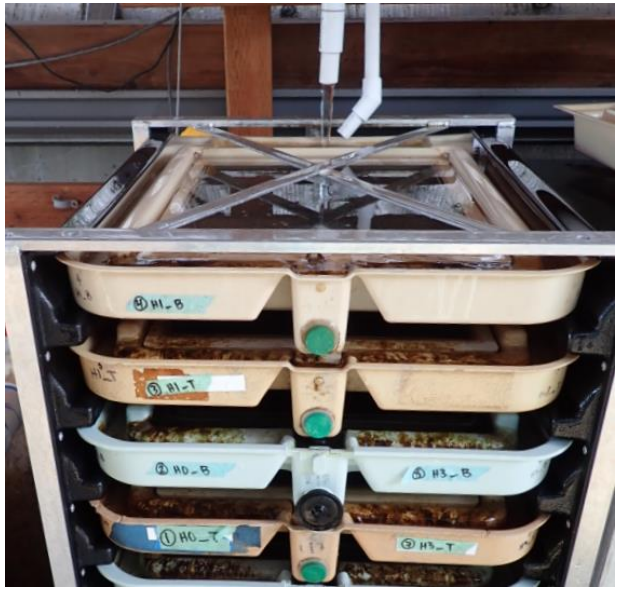
- Initial exposure (10 days)
  n = 4 trays treatment^{-1}
- Ambient common garden
- **Secondary exposure (6 days)**
  n = 2 trays treatment^{-1}
- 5 months post-exposure
  n = 2 trays treatment^{-1}

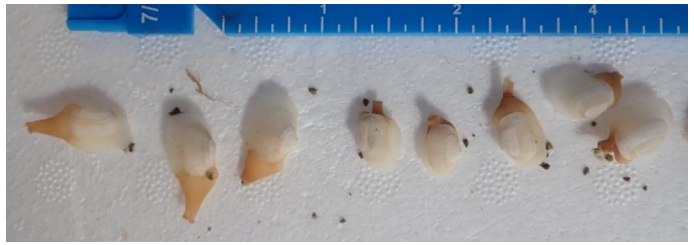
Ambient pCO_2
pH = 7.9

Elevated pCO_2
pH = 7.3

Experimental design & physiology data

~5 months post-exposure



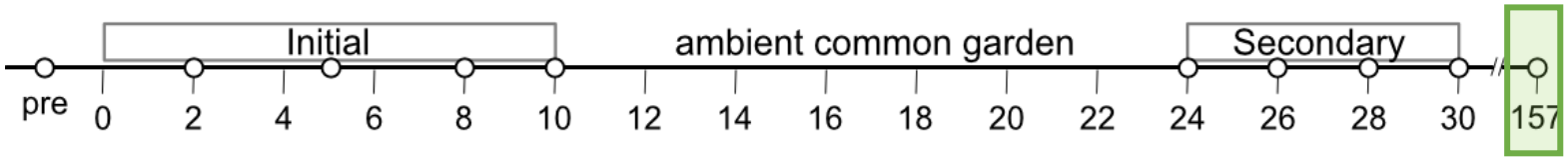


- Initial exposure (10 days)
  n = 4 trays treatment^{-1}
- Ambient common garden
- Secondary exposure (6 days)
  n = 2 trays treatment^{-1}

- 5 months post-exposure
  n = 2 trays treatment^{-1}

Ambient pCO_2
pH = 7.9

Elevated pCO_2
pH = 7.3

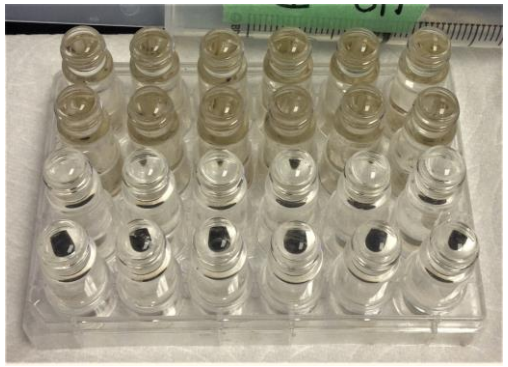


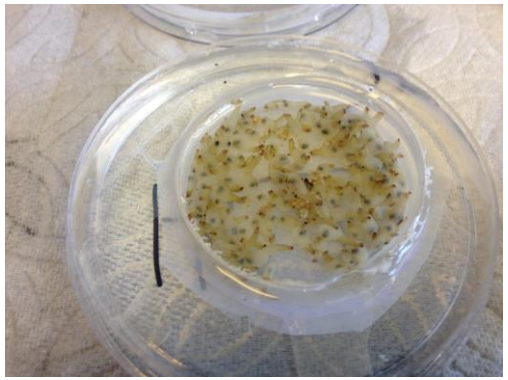
Experimental design & physiology data

Physiology

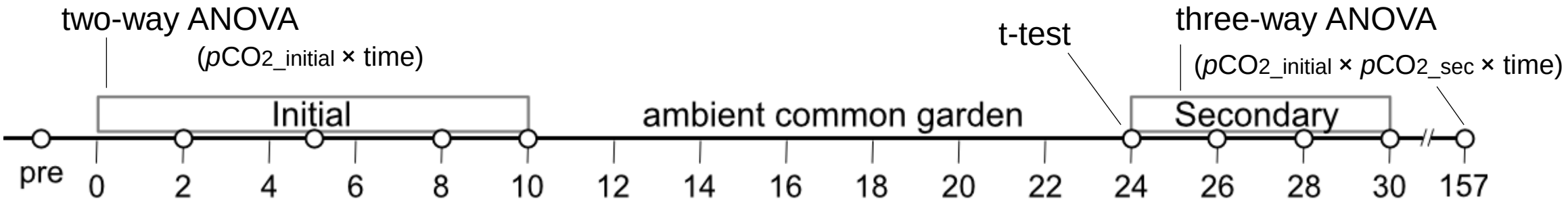
- Geoduck removed periodically during exposure to measure:

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

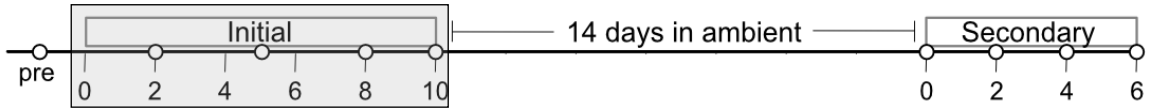




Statistical approach

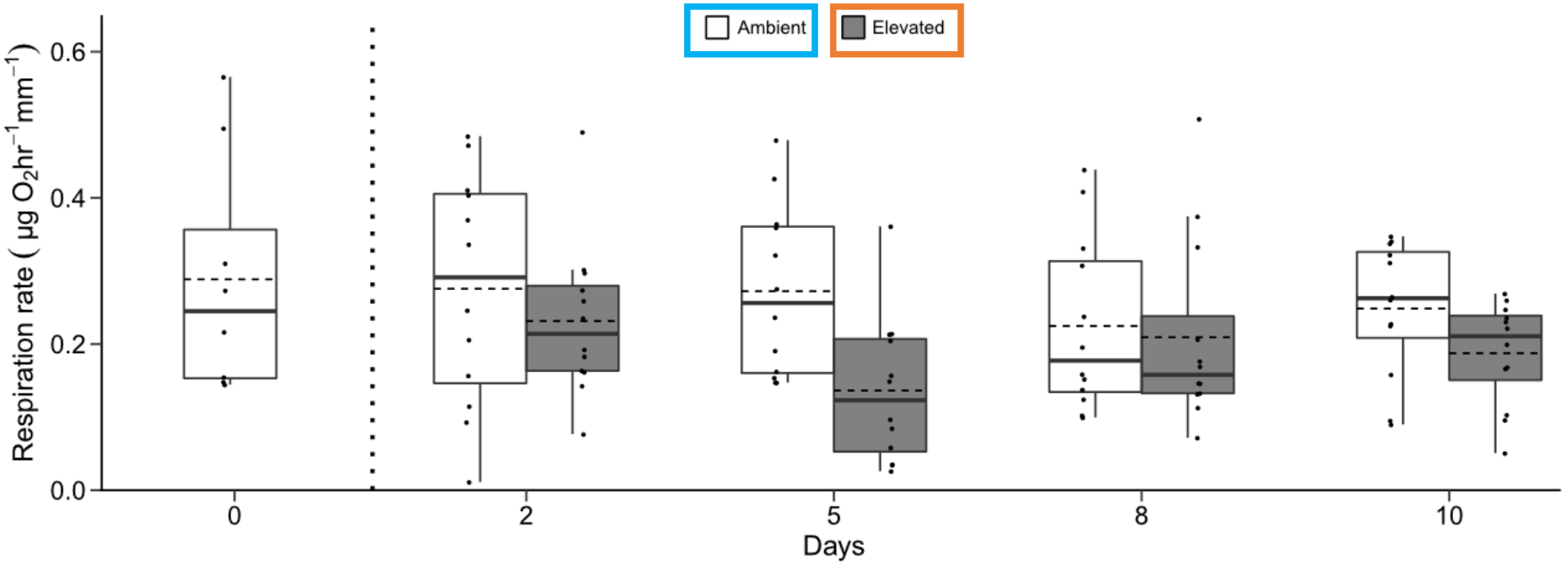


Results: Initial exposure

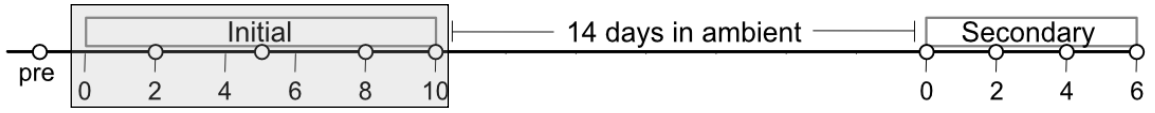


Metabolic rate:

			df	SS	MS	*F*	P
Initial exposure		*Two-way ANOVA*					
Respiration rate	time		3	0.0323	0.011	0.822	0.485
	pCO_2		1	0.0983	0.098	7.512	**0.007**
	pCO_2 × time		3	0.0475	0.016	1.210	0.311



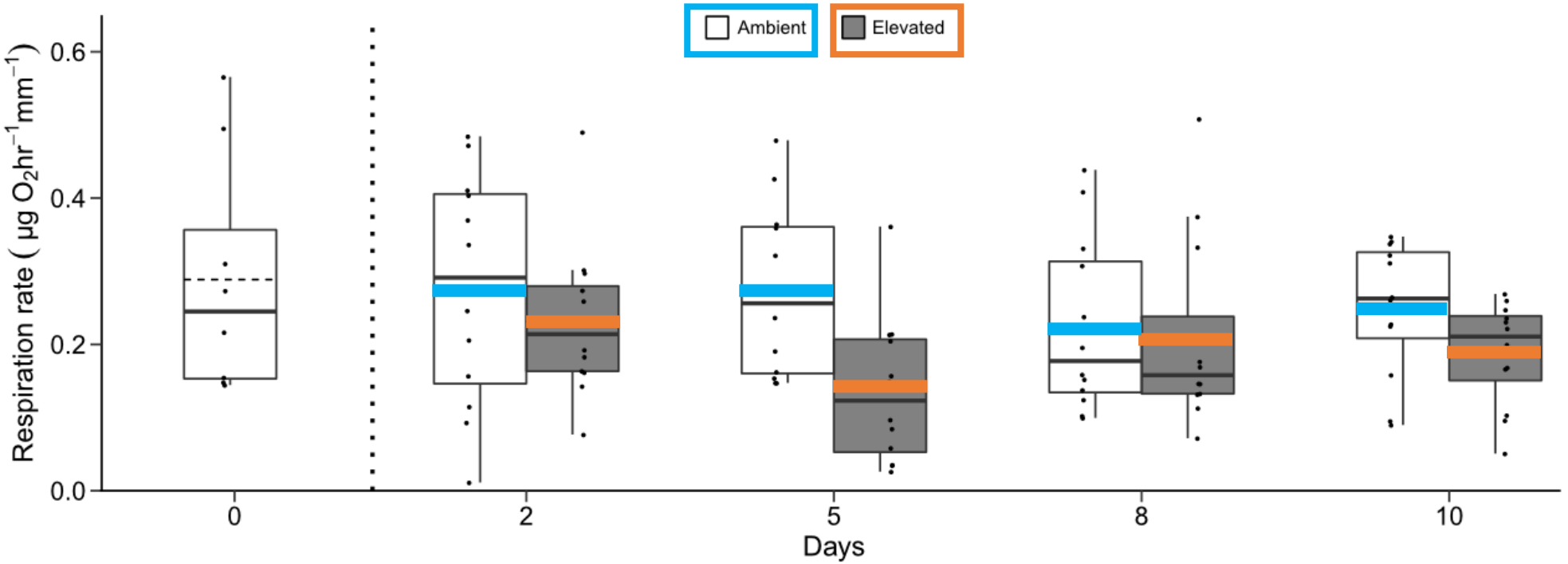
Results: Initial exposure



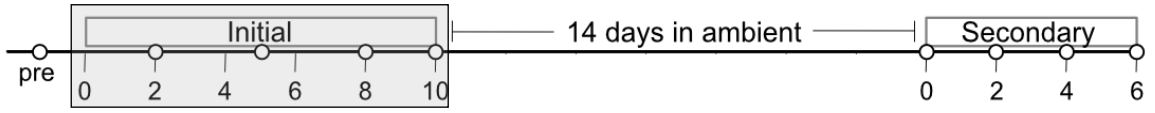
		df	SS	MS	*F*	P
Initial exposure	*Two-way ANOVA*					
Respiration rate	time	3	0.0323	0.011	0.822	0.485
	pCO_2	1	0.0983	0.098	7.512	**0.007**
	pCO_2 × time	3	0.0475	0.016	1.210	0.311

Metabolic rate:

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



Results: Initial exposure



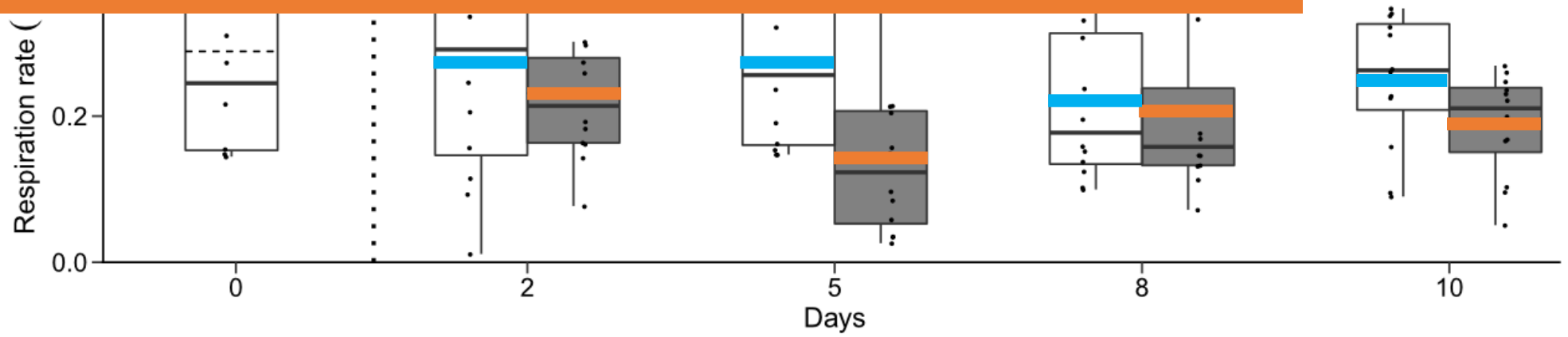
		df	SS	MS	*F*	P
Initial exposure	*Two-way ANOVA*					
Respiration rate	time	3	0.0323	0.011	0.822	0.485
	pCO_2	1	0.0983	0.098	7.512	**0.007**
	pCO_2 × time	3	0.0475	0.016	1.210	0.311

Metabolic rate:

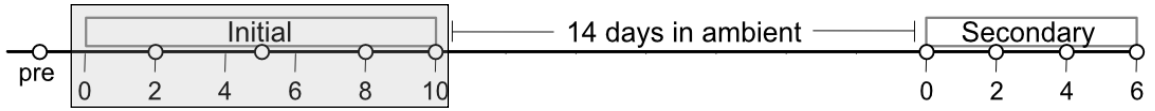
- **25% reduction** in respiration rate under **elevate**

INITIAL EXPOSURE: METABOLIC RATE

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



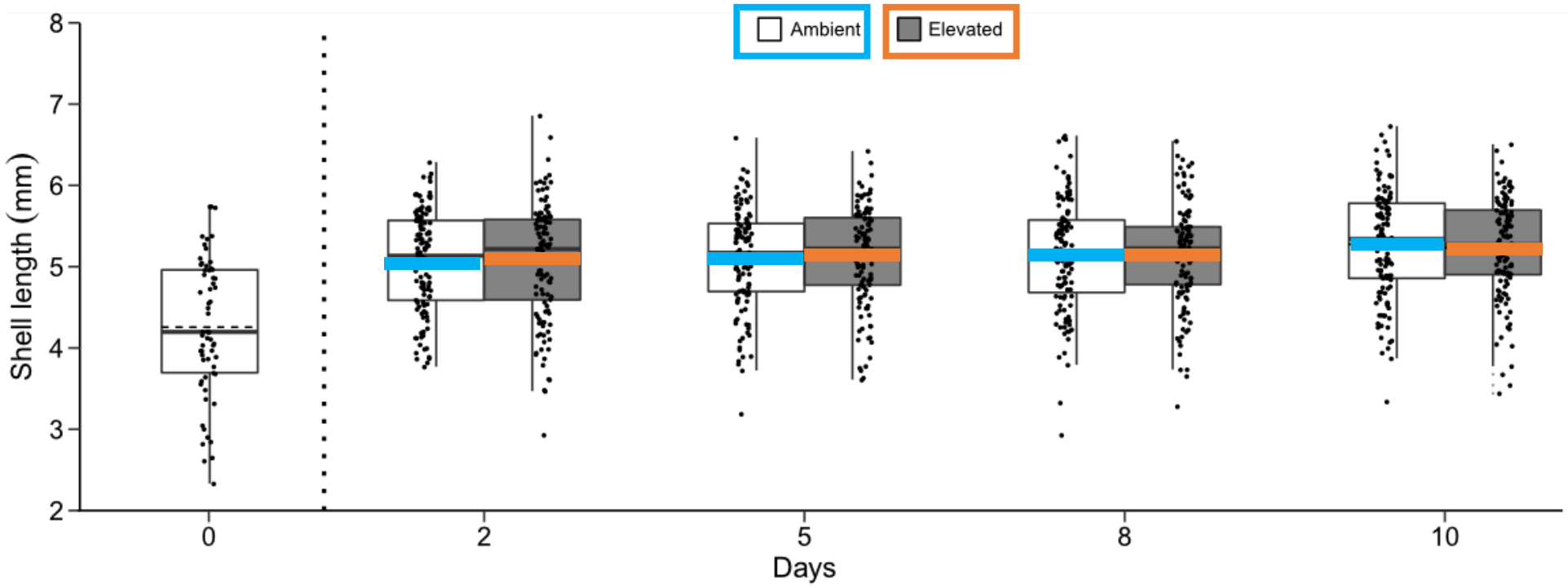
Results: Initial exposure



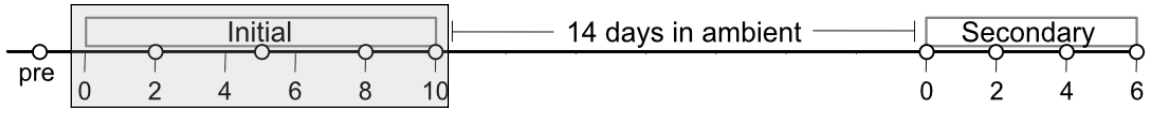
		df	SS	MS	*F*	P
Initial exposure	*Two-way ANOVA*					
Shell length	time	3	4.250	1.415	3.392	**0.018**
	pCO_2	1	0	0.0005	0.0012	0.973
	pCO_2 × time	3	0.170	0.058	0.138	0.937

Shell length:

- **No response** under elevated pCO_2



Results: Initial exposure



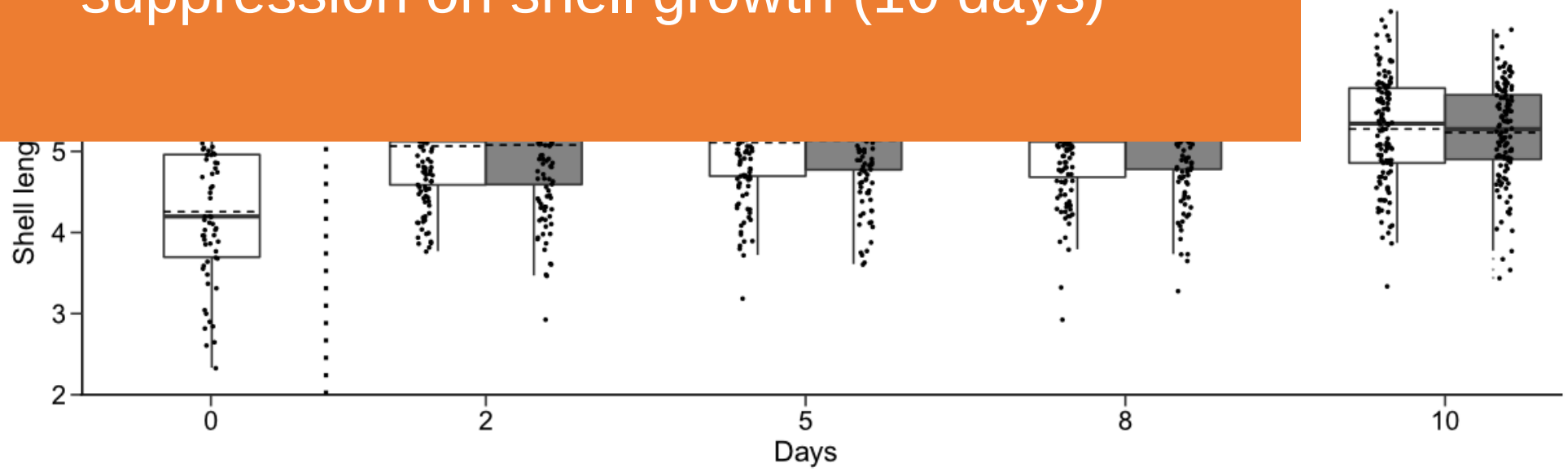
		df	SS	MS	*F*	P
Initial exposure	*Two-way ANOVA*					
Shell length	time	3	4.250	1.415	3.392	**0.018**
	pCO_2	1	0	0.0005	0.0012	0.973
	pCO_2 × time	3	0.170	0.058	0.138	0.937

Shell length:

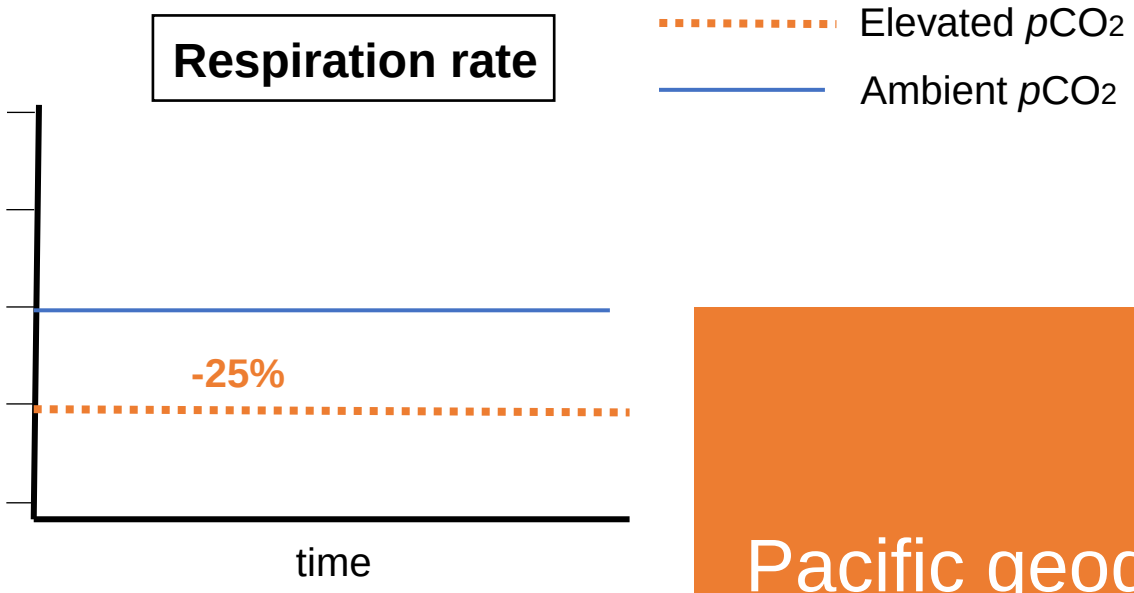
- **No response** under elevated pCO_2

INITIAL EXPOSURE: SHELL GROWTH

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



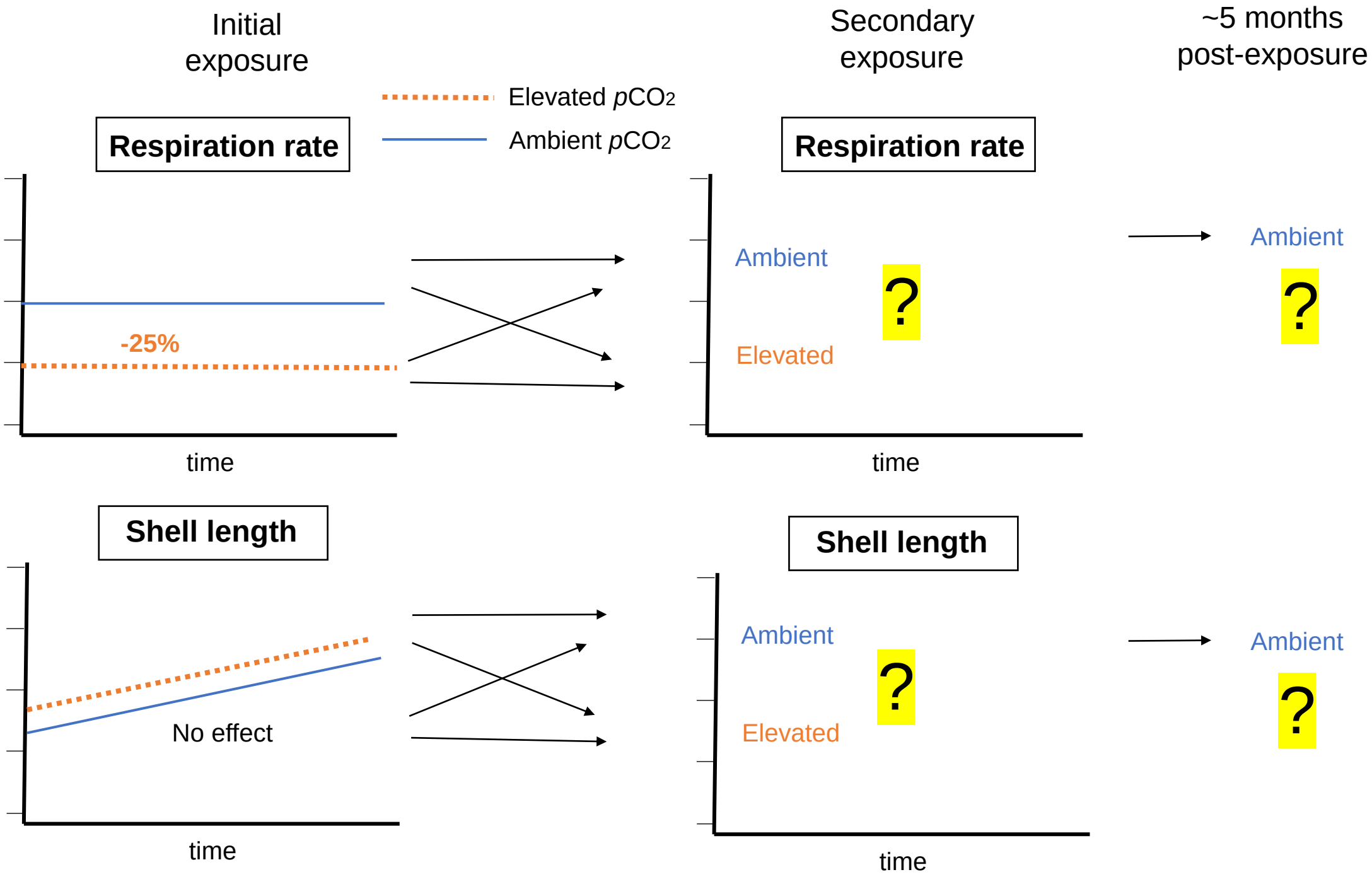
Initial exposure



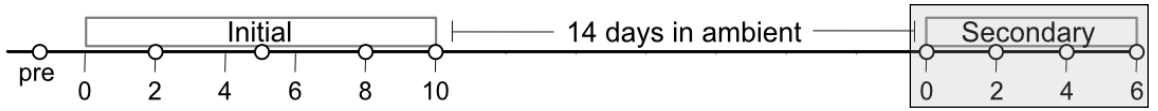
INITIAL EXPOSURE

Pacific geoduck under short-term acidification

- Suppressed metabolic activity
- Shell growth not affected


Initial exposure
Secondary exposure
~5 months post-exposure
Elevated pCO2
Ambient pCO2
Respiration rate
-25%
time
Respiration rate
Ambient
?
Elevated
time
Ambient
?
Shell length
No effect
time
Shell length
Ambient
?
Elevated
time
Ambient
?

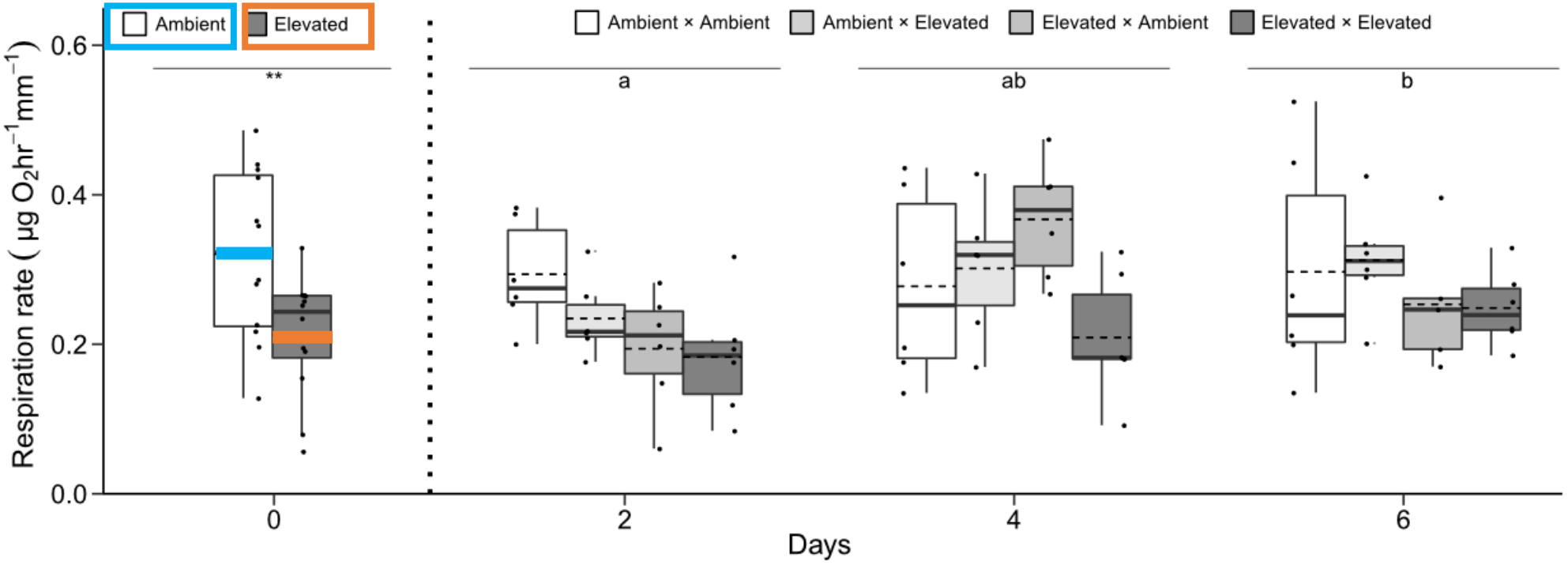
Results: Secondary exposure



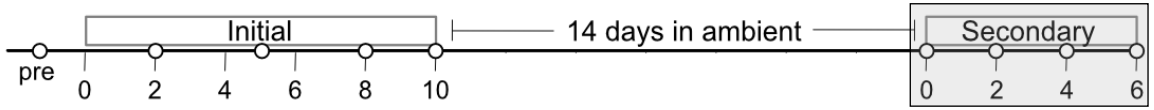
		df	t	P
Ambient common garden	*Welch Two Sample t-test*			
Respiration rate	pCO_2	19.833	2.673	**0.015**

Metabolic rate:

- Continued **metabolic suppression** prior to exposure



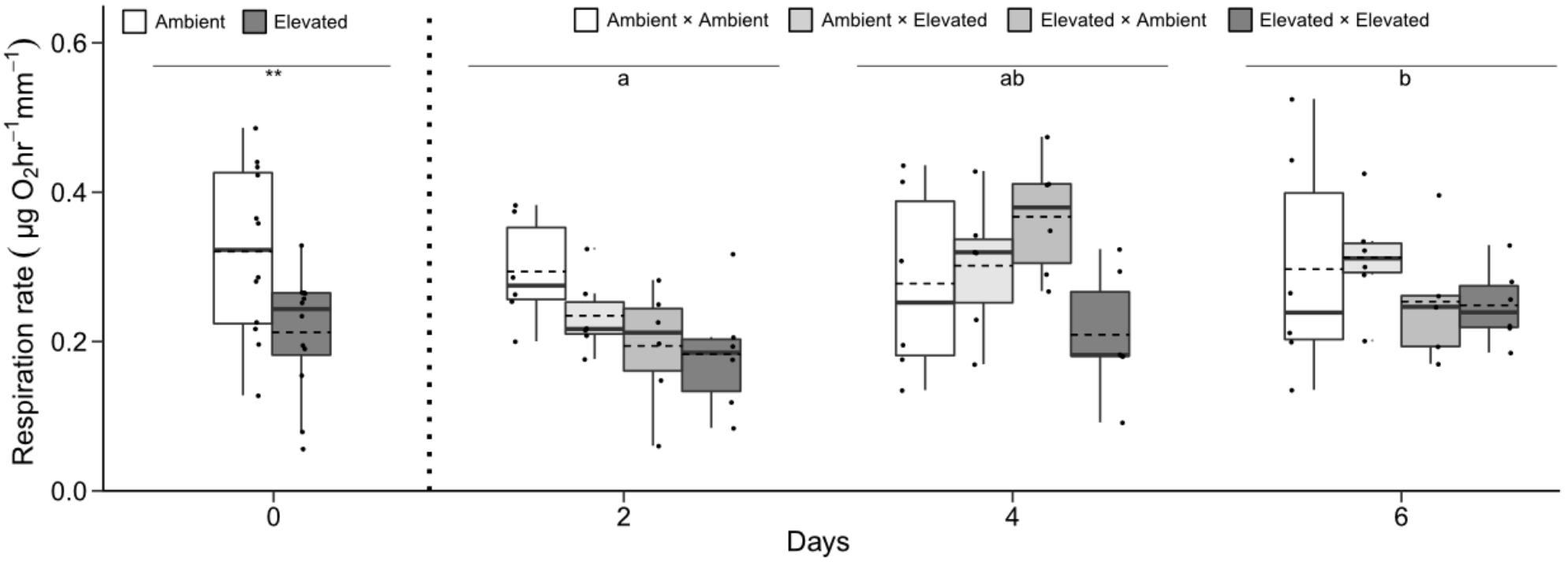
Results: Secondary exposure



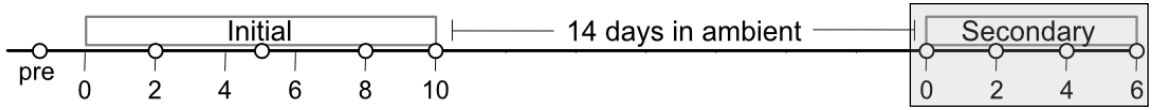
Metabolic rate:

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

		df	SS	MS	*F*	P
Secondary exposure	*Three-way ANOVA*					
Respiration rate	time	2	0.068	0.034	3.137	0.051
	$p\,CO_{2\ initial}$	1	0.021	0.021	1.916	0.171
	$p\,CO_{2\ secondary}$	1	0.032	0.032	2.926	0.092
	$p\,CO_{2\ initial} \times p\,CO_{2\ secondary}$	1	0.023	0.023	2.080	0.154
	$p\,CO_{2\ initial} \times$ time	2	0.016	0.008	0.724	0.489
	$p\,CO_{2\ secondary} \times$ time	2	0.002	0.001	0.103	0.903
	$p\,CO_{2\ initial} \times p\,CO_{2\ secondary} \times$ time	2	0.035	0.017	1.608	0.209



Results: Secondary exposure



Metabolic rate:

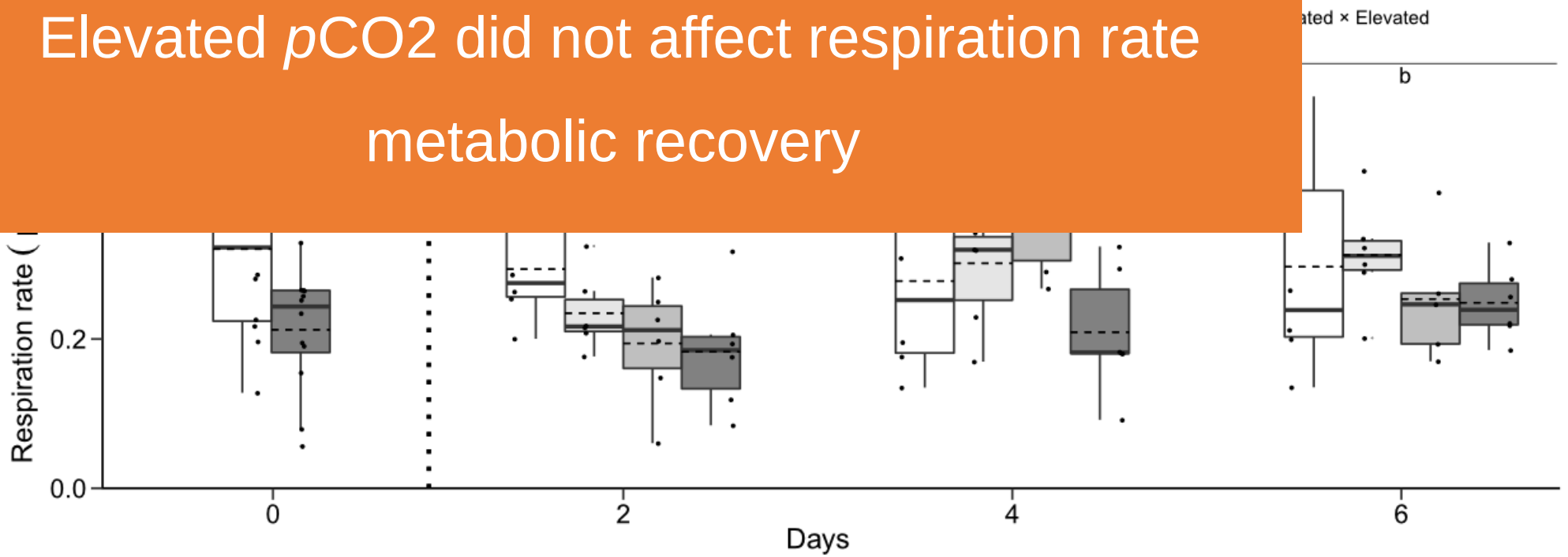
- No effect of treatment,
  metabolic rec

		df	SS	MS	*F*	P
Secondary exposure	*Three-way ANOVA*					
Respiration rate	time	2	0.068	0.034	3.137	0.051
	$p\,CO_{2\ initial}$	1	0.021	0.021	1.916	0.171
	$p\,CO_{2\ secondary}$	1	0.032	0.032	2.926	0.092
	$p\,CO_{2\ initial} \times p\,CO_{2\ secondary}$	1	0.023	0.023	2.080	0.154
	$p\,CO_{2\ initial}$ × time	2	0.016	0.008	0.724	0.489
	$p\,CO_{2\ secondary}$ × time	2	0.002	0.001	0.103	0.903
				0.017	1.608	0.209

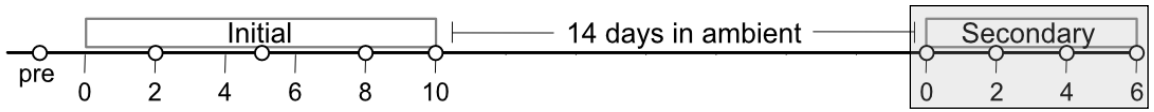
SECONDARY EXPOSURE

Elevated *p*CO2 did not affect respiration rate

metabolic recovery



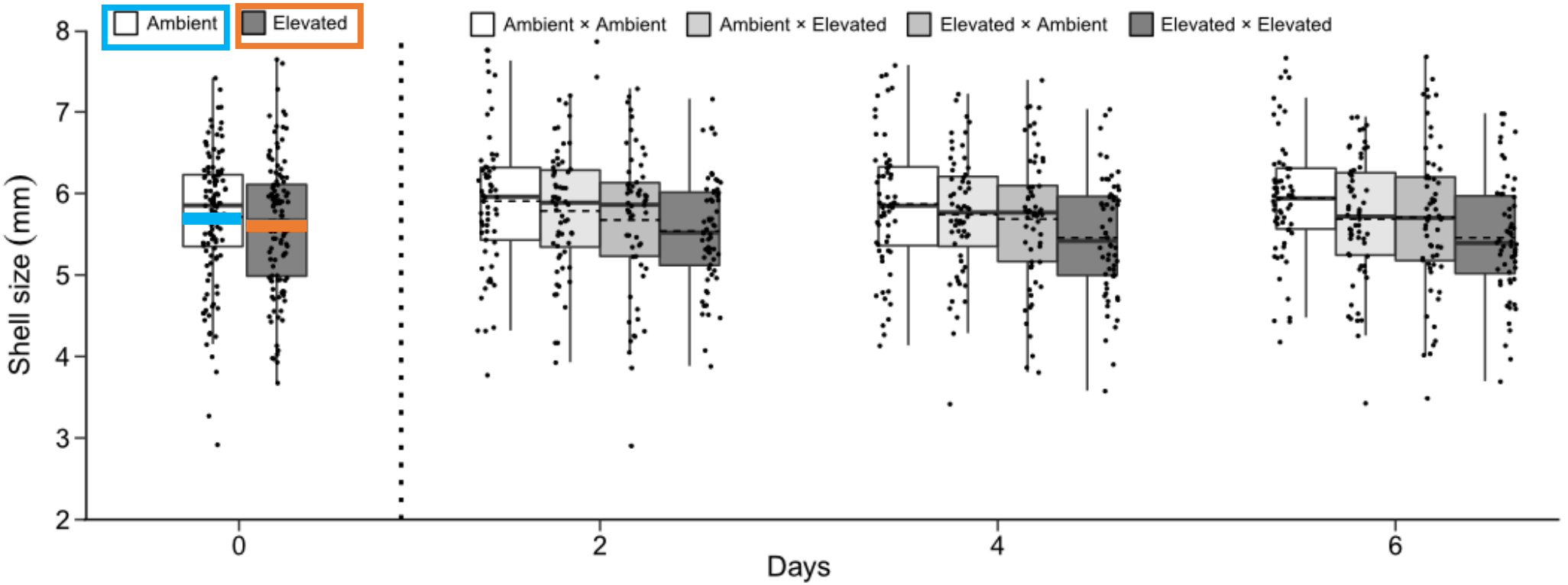
Results: Secondary exposure



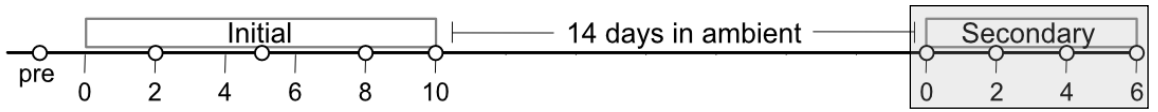
		df	t	P
Ambient common garden	*Welch Two Sample t-test*			
Shell length	pCO_2	1.146	236.680	0.253

Shell length:

- **No treatment effect** prior to exposure



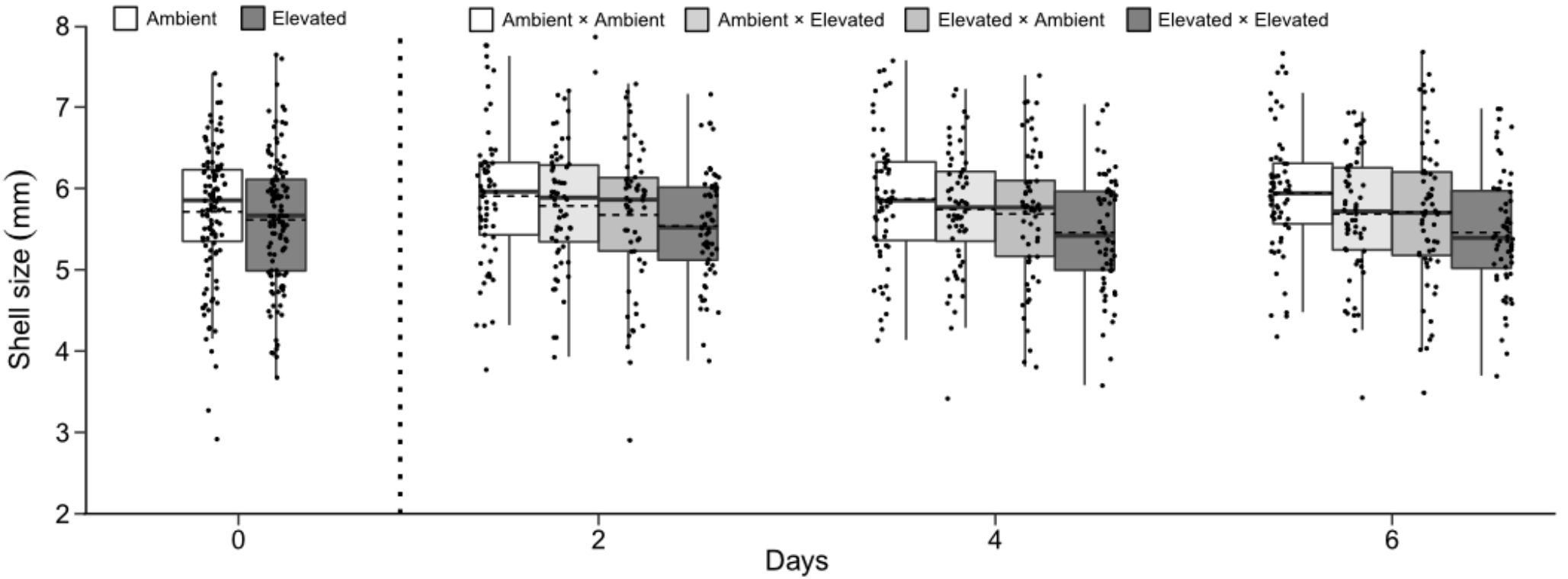
Results: Secondary exposure



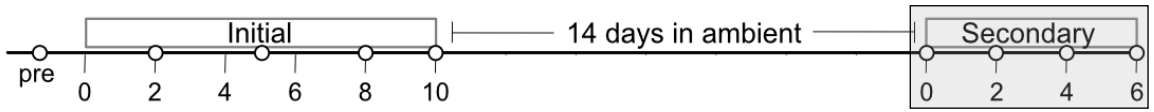
Shell length:

- Initial and secondary **treatment effects**

		df	SS	MS	*F*	P
Secondary exposure	*Three-way ANOVA*					
Shell length	time	2	0.190	0.095	0.152	0.859
	$pCO_{2\ initial}$	1	9.910	9.910	15.821	**<0.001**
	$pCO_{2\ secondary}$	1	6.210	6.212	9.917	**0.002**
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	0.060	0.063	0.100	0.752
	$pCO_{2\ initial} \times$ time	2	0	0.001	0.002	0.998
	$pCO_{2\ secondary} \times$ time	2	0.460	0.231	0.368	0.692
	$pCO_{2\ initial} \times pCO_{2\ secondary} \times$ time	2	0.100	0.048	0.076	0.927



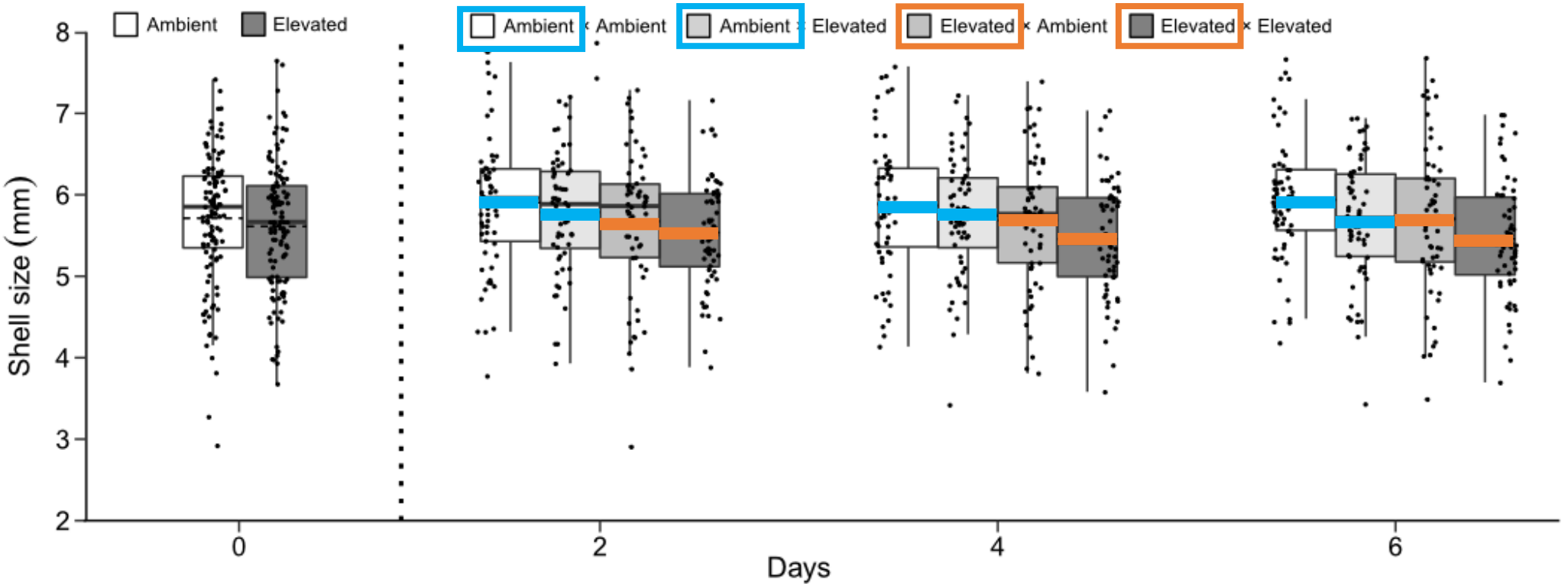
Results: Secondary exposure



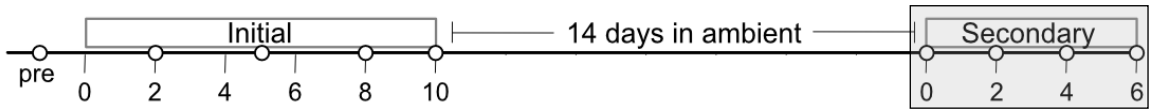
		df	SS	MS	*F*	P
Secondary exposure	*Three-way ANOVA*					
Shell length	time	2	0.190	0.095	0.152	0.859
	$pCO_{2\ initial}$	1	9.910	9.910	15.821	**<0.001**
	$pCO_{2\ secondary}$	1	6.210	6.212	9.917	**0.002**
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	0.060	0.063	0.100	0.752
	$pCO_{2\ initial} \times$ time	2	0	0.001	0.002	0.998
	$pCO_{2\ secondary} \times$ time	2	0.460	0.231	0.368	0.692
	$pCO_{2\ initial} \times pCO_{2\ secondary} \times$ time	2	0.100	0.048	0.076	0.927

Shell length:

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



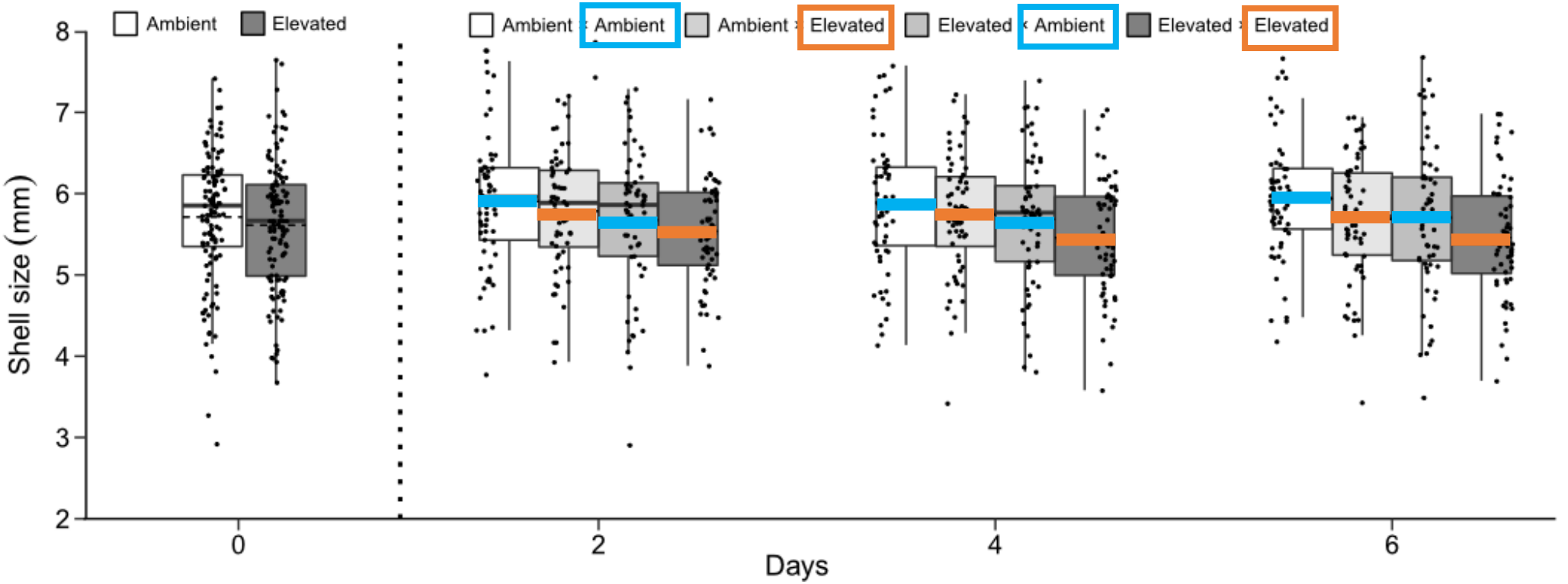
Results: Secondary exposure



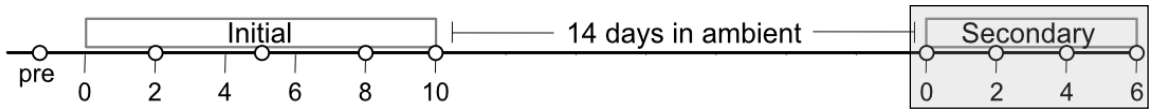
		df	SS	MS	*F*	P
Secondary exposure	*Three-way ANOVA*					
Shell length	time	2	0.190	0.095	0.152	0.859
	$pCO_{2\ initial}$	1	9.910	9.910	15.821	**<0.001**
	$pCO_{2\ secondary}$	1	6.210	6.212	9.917	**0.002**
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	0.060	0.063	0.100	0.752
	$pCO_{2\ initial} \times$ time	2	0	0.001	0.002	0.998
	$pCO_{2\ secondary} \times$ time	2	0.460	0.231	0.368	0.692
	$pCO_{2\ initial} \times pCO_{2\ secondary} \times$ time	2	0.100	0.048	0.076	0.927

Shell length:

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



Results: Secondary exposure



		df	SS	MS	*F*	P
Secondary exposure	*Three-way ANOVA*					
Shell length	time	2	0.190	0.095	0.152	0.859
	$pCO_{2\ initial}$	1	9.910	9.910	15.821	**<0.001**
	$pCO_{2\ secondary}$	1	6.210	6.212	9.917	**0.002**
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	0.060	0.063	0.100	0.752
	$pCO_{2\ initial} \times$ time	2	0	0.001	0.002	0.998
				0.231	0.368	0.692
				0.048	0.076	0.927

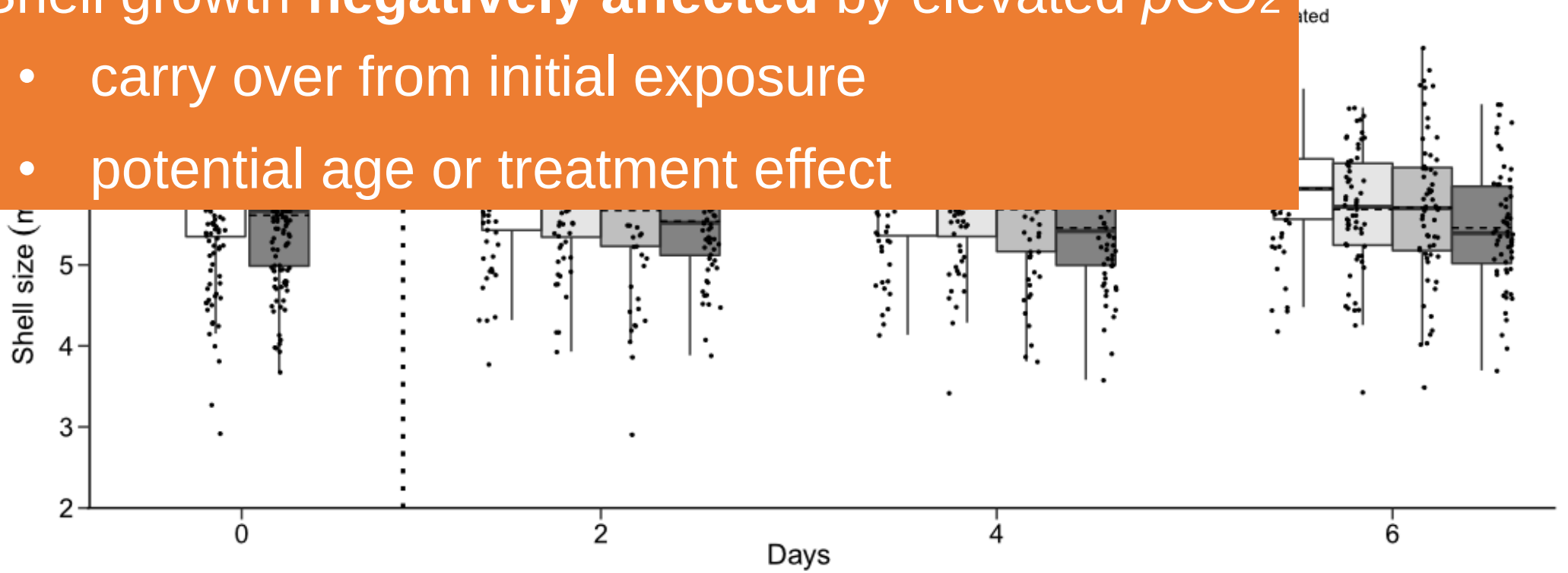
Shell length:

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

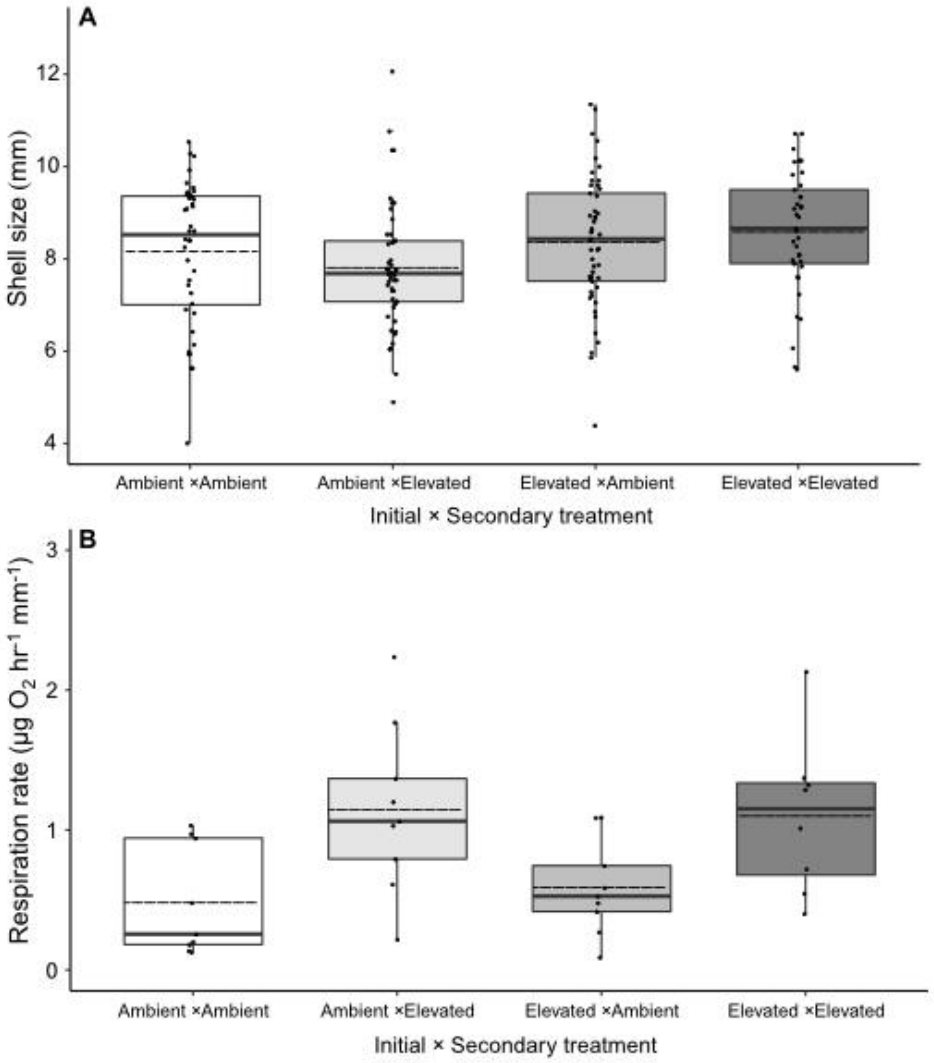
SECONDARY EXPOSURE

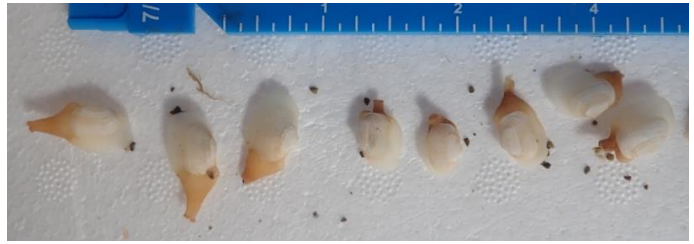
Shell growth **negatively affected** by elevated *p*CO_2

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

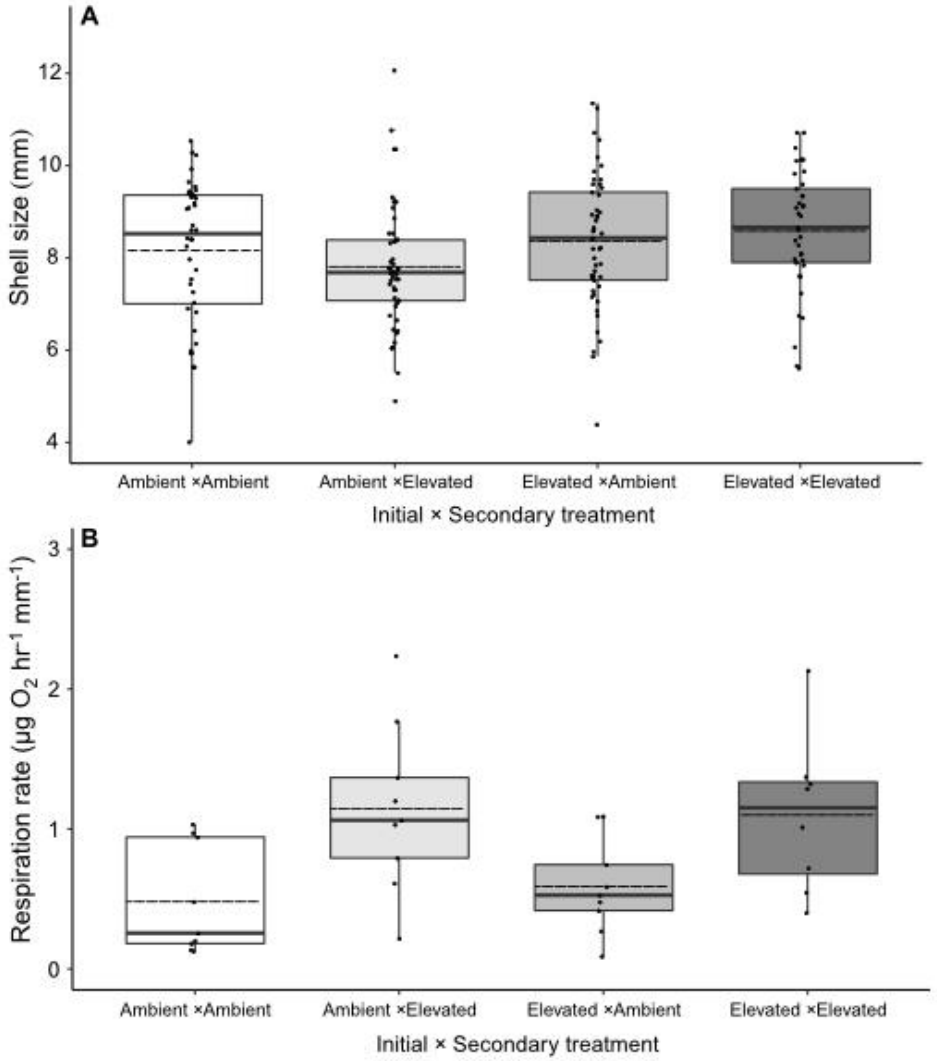


Results: ~5 months post-exposure





Results: ~5 months post-exposure



		df	SS	MS	*F*	P
157 days post	*Two-way ANOVA*					
Respiration rate	$pCO_{2\ initial}$	1	0.003	0.002	0.011	0.919
	$pCO_{2\ secondary}$	1	3.037	3.037	13.008	**0.001**
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	0.050	0.050	0.212	0.648
Shell length	$pCO_{2\ initial}$	1	10.600	10.597	5.228	**0.023**
	$pCO_{2\ secondary}$	1	0.210	0.214	0.105	0.746
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	3.510	3.507	1.730	0.190

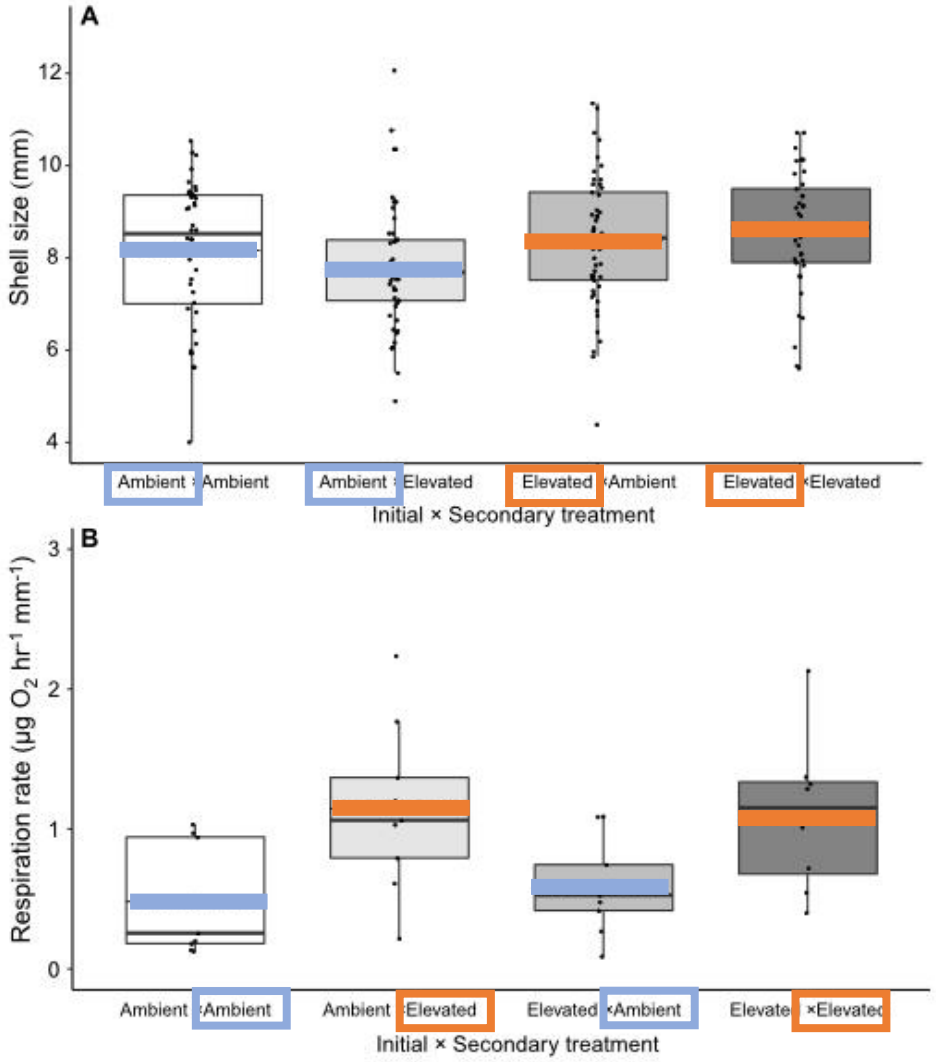
Shell length:

- Initial treatment:

Metabolic rate:

- Secondary treatment:

Results: ~5 months post-exposure



		df	SS	MS	*F*	P
157 days post	*Two-way ANOVA*					
Respiration rate	$pCO_{2\ initial}$	1	0.003	0.002	0.011	0.919
	$pCO_{2\ secondary}$	1	3.037	3.037	13.008	**0.001**
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	0.050	0.050	0.212	0.648
Shell length	$pCO_{2\ initial}$	1	10.600	10.597	5.228	**0.023**
	$pCO_{2\ secondary}$	1	0.210	0.214	0.105	0.746
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	3.510	3.507	1.730	0.190

Shell length:

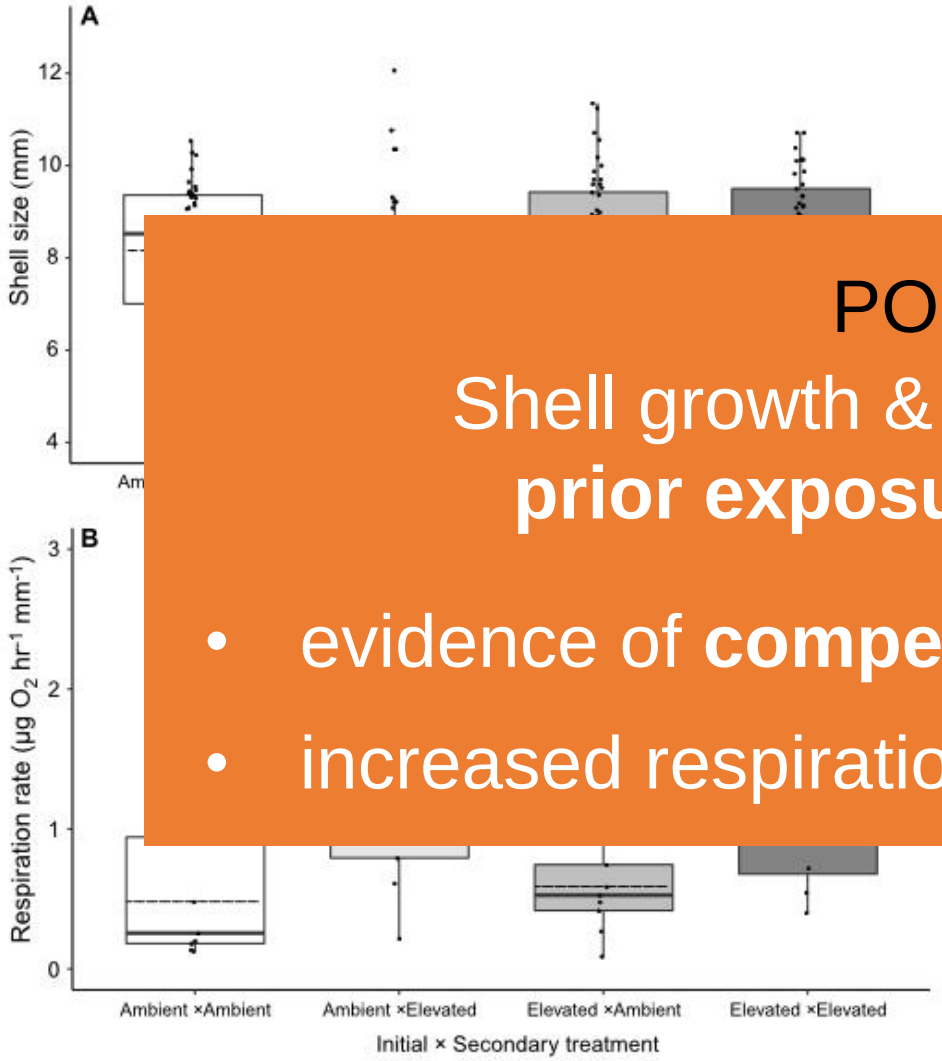
- Initial treatment:
  5.80% (mm length) **larger size** in animals with prior exposure to **elevated *p*CO2**

Metabolic rate:

- Secondary treatment:
  52.4% greater respiration rate in animals with prior exposure to **elevated *p*CO2**

Results: ~5 months post-exposure

		df	SS	MS	*F*	P
157 days post	*Two-way ANOVA*					
Respiration rate	$pCO_{2\ initial}$	1	0.003	0.002	0.011	0.919
	$pCO_{2\ secondary}$	1	3.037	3.037	13.008	**0.001**
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	0.050	0.050	0.212	0.648
Shell length	$pCO_{2\ initial}$	1	10.600	10.597	5.228	**0.023**
	$pCO_{2\ secondary}$	1	0.210	0.214	0.105	0.746
	$pCO_{2\ initial} \times pCO_{2\ secondary}$	1	3.510	3.507	1.730	0.190



POST-EXPOSURE

Shell growth & respiration affected by **prior exposure** to elevated *p*CO_2

- evidence of **compensatory growth**
- increased respiration rate by pre-conditioned clams

O_2

Secondary treatment:
52.4% increase in respiration rate
from prior exposure under elevated *p*CO_2

Conclusions

Metabolic resilience

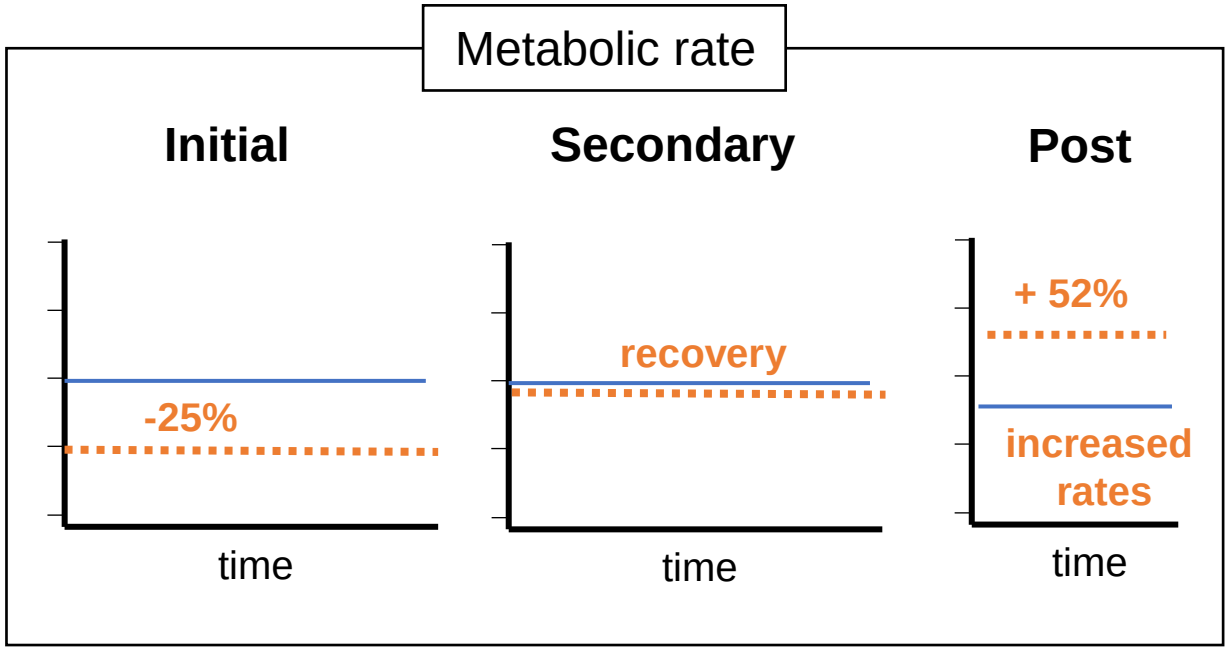
- Suppressed metabolic state
  under **initial exposure** to elevated pCO_2

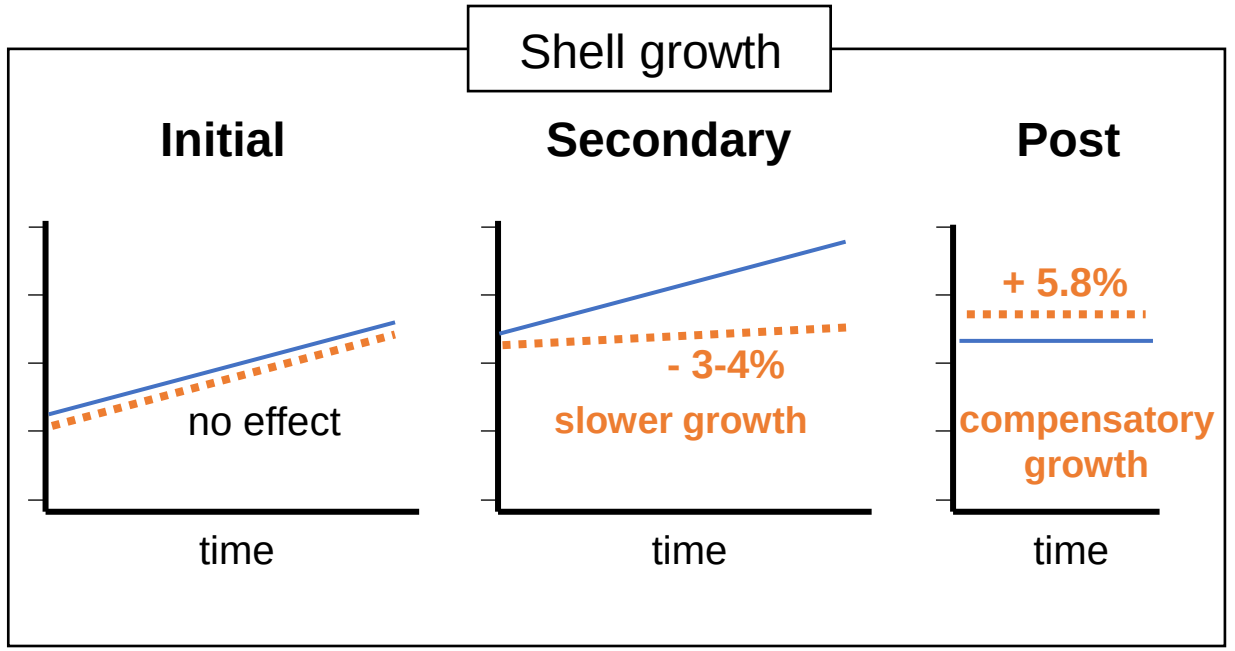
- Metabolic recovery & increased rates
  under **subsequent exposure** to elevated pCO_2
  & after **ambient grow-out**

Compensatory shell growth

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

- Compensatory response
  increased shell length after **ambient grow-out**





Conclusions

Metabolic resilience

- Suppressed metabolic state
  under initial exposure to elevated pCO_2
- Metabolic recovery & increased rates
  under subsequent exposure to elevated pCO_2
  & after ambient grow-out

Compensatory shell growth

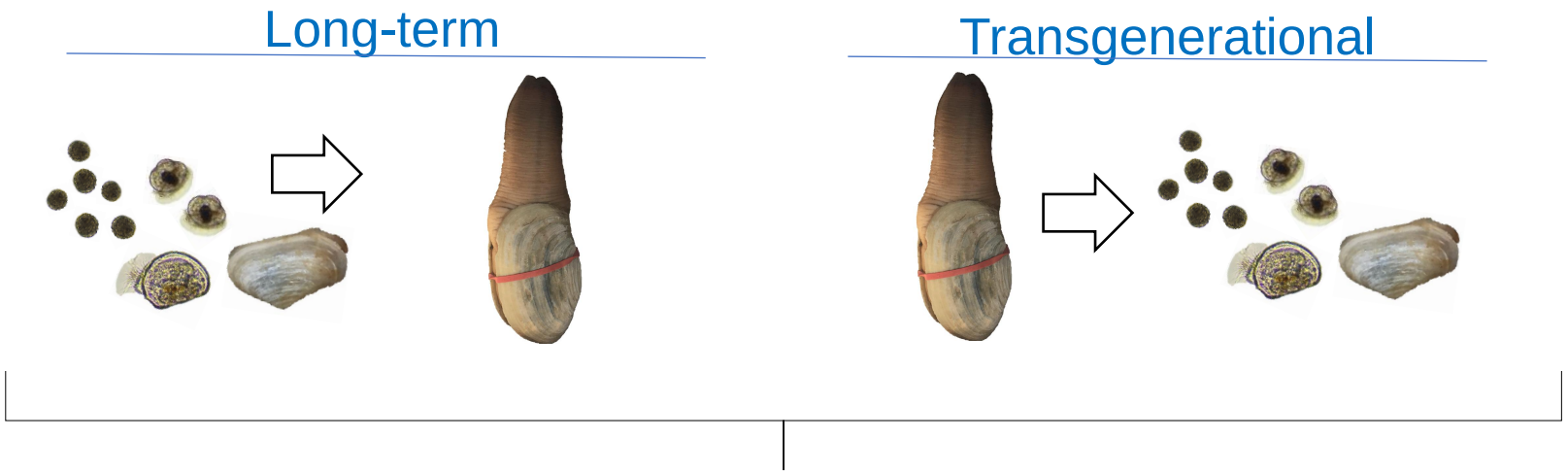
- Slowed shell growth
  under repeated short-term exposure elevated pCO_2
- Compensatory response
  increased shell length after ambient grow-out

Take-home message for Pacific geoduck production..

(1) Short-term exposure to moderate *p*CO2 stress may acclimatize juvenile geoduck and elicit benefits for performance

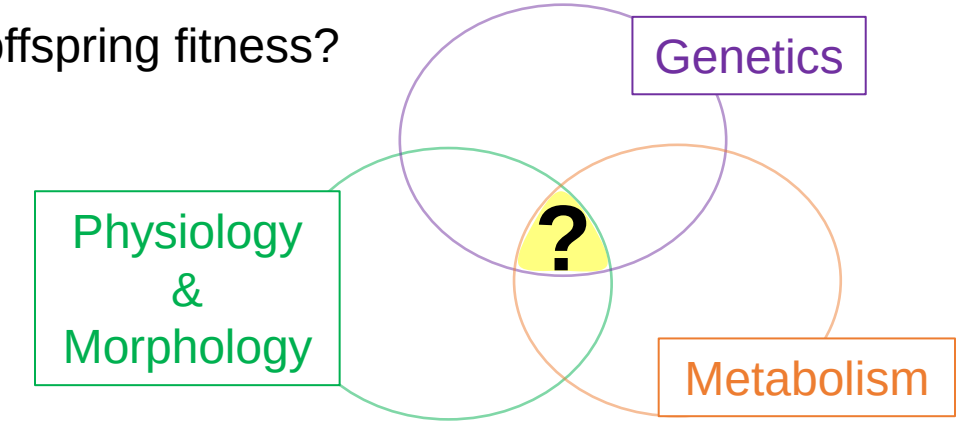
(2) Stress conditioning must account for:
- life-stage/age dependence
- sensitivity to stress intensity

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



Acknowledgements

Hollie Putnam, PhD (PI)
Emma Strand
Maddie Sherman

Steven Roberts, PhD (PI)
Brent Vadopalas, PhD (PI)
Kaitlyn Mitchell

Kurt Grinnell
Matt Henderson
Josh Valley
Clara Duncan
Jim Parsons, PhD

JAMESTOWN S'KLALLAM TRIBE

FFAR


Questions?

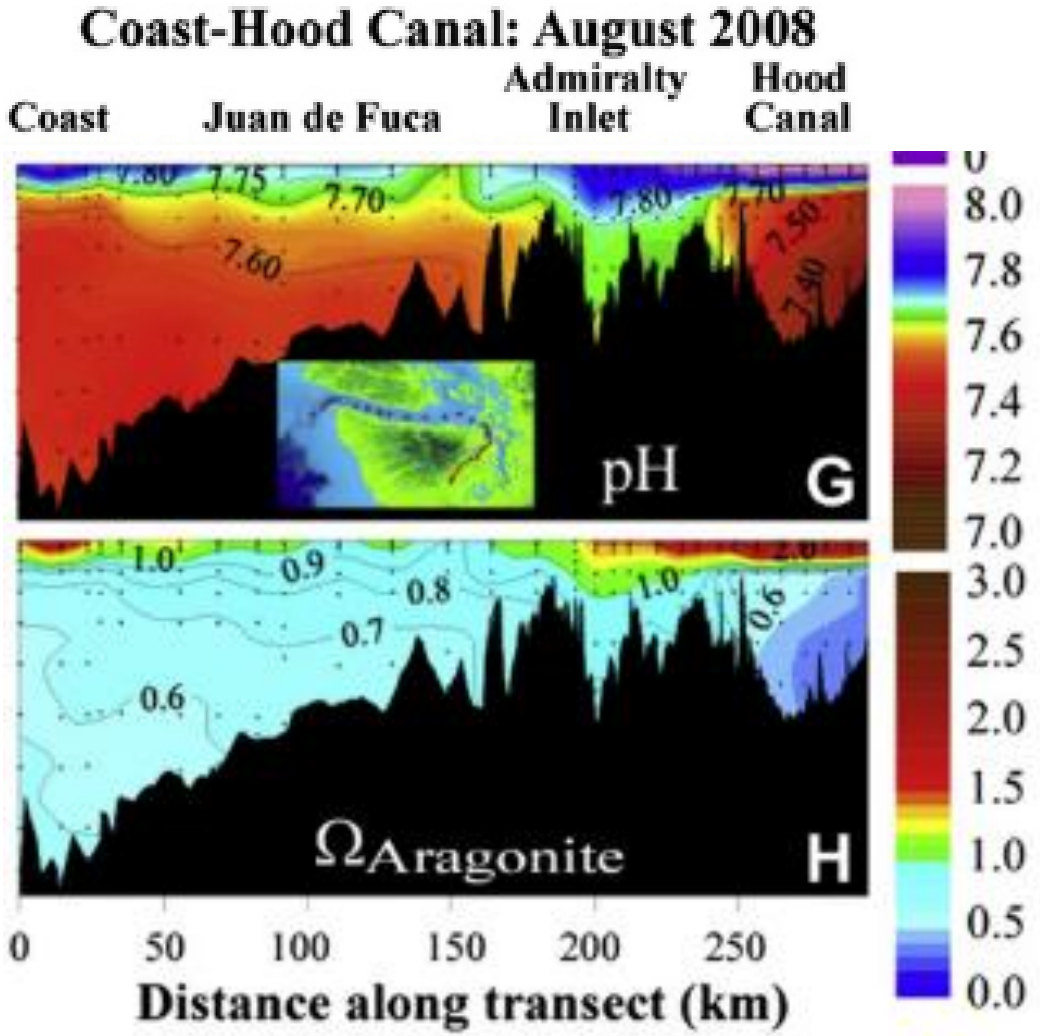
Carb chemistry tables

Initial exposure

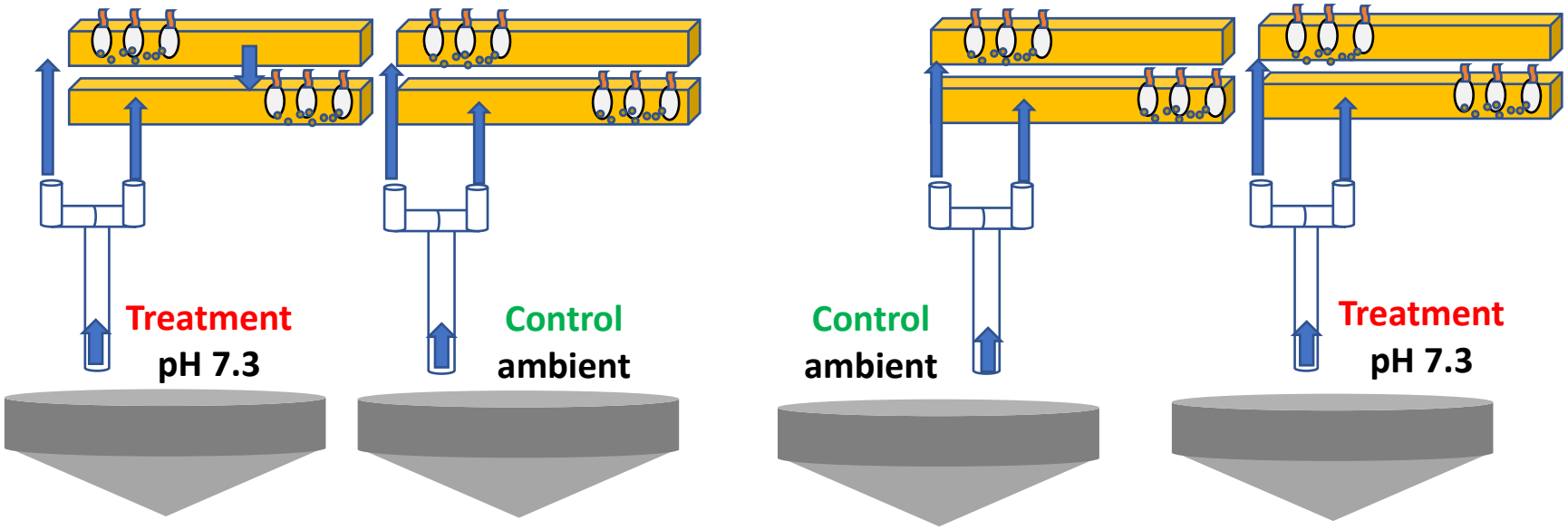
Treatment	Temperature	Salinity	Flow rate L min^{-1}	pH, Total Scale	CO_2 µmol kg^{-1}	pCO_2 µatm	HCO_3 µmol kg^{-1}	CO_3 µmol kg^{-1}	DIC µmol kg^{-1}	Total Alkalinity µmol kg^{-1}	Aragonite Saturation state
Ambient	14.82 ± 0.12	29 ± 0.03	496 ± 139	7.86 ± 0.01	24 ± 0.46	608 ± 11	1842 ± 4	86 ± 1	1952 ± 3	2056 ± 1	1.35 ± 0.02
Low	14.91 ± 0.12	29 ± 0.04	486 ± 153	7.31 ± 0.004	91 ± 1	2345 ± 20	1992 ± 1	26 ± 0.20	2108 ± 1	2056 ± 1	0.41 ± 0.003

Secondary exposure

Treatment	Temperature	Salinity	Flow rate L min^{-1}	pH, Total Scale	CO_2 µmol kg^{-1}	pCO_2 µatm	HCO_3 µmol kg^{-1}	CO_3 µmol kg^{-1}	DIC µmol kg^{-1}	Total Alkalinity µmol kg^{-1}	Aragonite Saturation state
Ambient	16.33 ± 0.22	28.67 ± 0.03	495 ± 143	7.93 ± 0.004	19 ± 0.3	506 ± 5	1781 ± 5	102 ± 1	1902 ± 4	2033 ± 2	1.60 ± 0.02
Low	16.40 ± 0.22	28.67 ± 0.04	472 ± 87	7.27 ± 0.007	95 ± 1	2551 ± 42	1972 ± 3	25 ± 0.3	2091 ± 3	2033 ± 3	0.39 ± 0.004



Feely et al. 2010


Treatment
pH 7.3
Control
ambient
Control
ambient
Treatment
pH 7.3