Effects of intragenerational pCO_2 conditioning on metabolism, oxidative stress response, and DNA methylation of juvenile Pacific geoduck *Panopea generosa*

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Introduction

Moderate or intermittent oxidative stress is known to induce positive carry over effects and is a theorized driver of stress memory and enhanced lifespan. Stress conditioning may increase resilient phenotypes and epigenotypes advantageous for sustainable aquaculture. However, the importance of pCO_2 stress intensity remains understudied for intragenerational adaptive capacity to cope with subsequent exposure(s). Transient phenotypic and -omic mechanisms elicited under moderate oxidative stress may provide novel hallmarks of pCO_2 stress conditioning in commercially and ecologically important bivalves. Life-stage and stress-level dependence of stress conditioning and phenotypic and epigenetic mechanisms of stress resilience require a holistic characterization of organism response (e.g. physiological, proteomic, and differential methylation) under long-term and hormetic dose-response experimental frameworks.

Hypothesis: Pacific geoduck exposed to elevated pCO_2 during early development are acclimatized, and thereby increase (a) performance and (b) oxidative status under subsequent acute stress encounters.

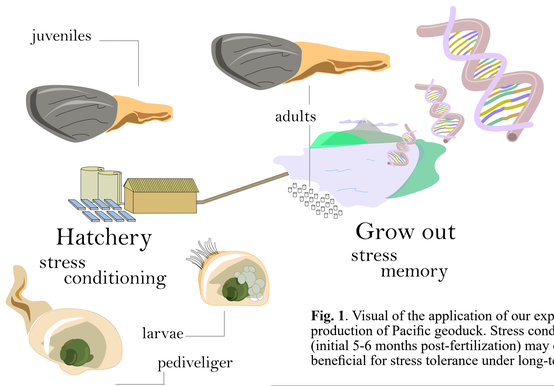


Fig. 1. Visual of the application of our experimental approach on commercial production of Pacific geoduck. Stress conditioning during hatchery production (initial 5-6 months post-fertilization) may elicit intragenerational markers beneficial for stress tolerance under long-term growing periods (~3-5 years).

Methods

To test whether pCO_2 stress elicits beneficial responses under subsequent encounters, we conditioned postlarval Pacific geoduck *Panopea generosa* for 120 days in ambient and elevated pCO_2 conditions (920 µatm and 2870 µatm, respectively) before subjecting juvenile clams (~5 months old) to reciprocal exposure periods over 21 days (n = 7 d exposure-1).

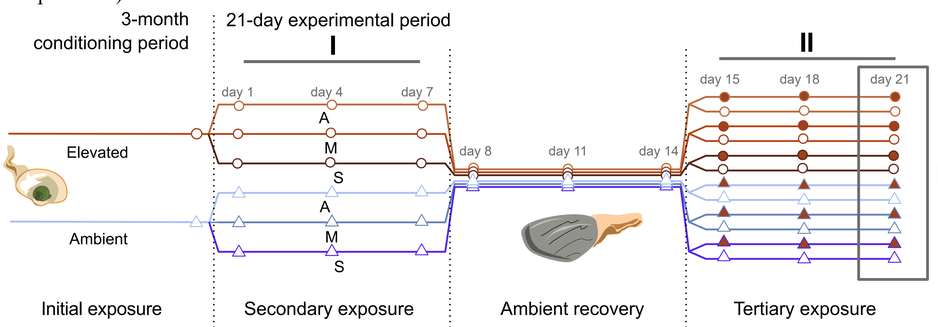


Fig. 2. Schematic showing all pCO_2 treatments during the conditioning and 21-day experiment. Points present samoling days when animals were used for physiological measurements and frozen for later analysis. Shading refers to the three pCO_2 intensities under the secondary exposure (days 1 - 7): ambient (A), moderate (M), and severe (S) pCO_2. Treatments converge during ambient recovery (days 8 - 14) to present the lack of differential pCO_2 exposure before subjected to ambient (open points) and moderate pCO_2 (closed points) under the tertiary exposure period (cumulatively as days 15 - 21). Roman numerals are referenced in the results (I-III; Figures 7 - 10).

Fig. 3. Juvenile geoduck reared in eight trays from postlarval (pediveliger) to juvenile stage (~5-7 mm shell length).

Fig. 4. 750 ml replicate tanks for reciprocal exposure periods. Six replicate tanks were used per treatment under secondary and tertiary exposures (n = 36 and 72 tanks, respectively).

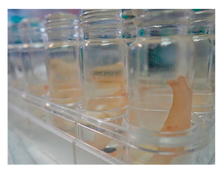


Fig. 5. Geoduck respiration rate measurements completed in 4-ml vials (PreSens).

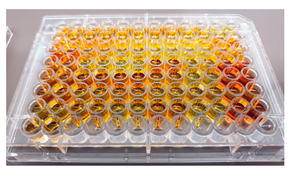
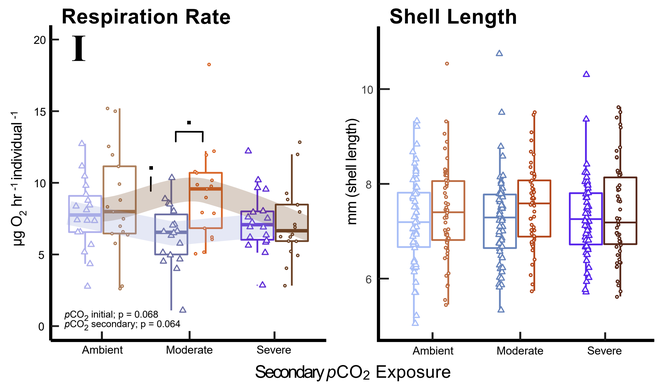


Fig. 6. Homogenized whole tissues were used for spectrophotometric assays of total protein and total antioxidant capacity.

We fixed whole tissue samples and measured respiration rate and shell length periodically under three conditions during secondary exposure: ambient (740 ± 40 µatm), moderate (2715 ± 70 µatm), and severe pCO_2 (4876 ± 101 µatm); this was followed by a 7-day period of ambient recovery (861 ± 45 µatm) before subsequent tertiary exposure under two conditions: ambient (939 ± 31 µatm) and moderate pCO_2 (2983 ± 79 µatm).

Results & Discussion

- **Respiration rate:** Under subsequent secondary exposure, the initial pCO_2 treatment had a marginal effect on metabolism (three-way ANOVA; pCO_2_initial, $F_{1,89}$=3.409, P=0.068) with respiration rates 12.4% greater in animals reared under elevated pCO_2 (Figure 7) and a marginal interaction with secondary pCO_2 treatment under moderate pCO_2 (three-way ANOVA; pCO_2_initial× pCO_2_secondary, $F_{2,89}$= 2.824, P=0.0647). Respiration rates under tertiary exposure were affected by initial×secondary pCO_2 treatment with _% greater respiration rates by stress-conditioned animals secondarily exposed to severe pCO_2 relative to those under ambient (Figure 7.). These results suggest intensity, duration, and life-stage specific effects of pCO_2 stress on aerobic metabolism



- **Total antioxidant capacity:** Stress conditioning decreased antioxidant capacity. There was a significant effect of initial pCO_2 conditioning on TAC on day 21 (three-way ANOVA; pCO_2_initial, F1,56=8.069, P=0.0063) with 22% greater antioxidant capacity by clams conditioned to ambient conditions.

- **Shell growth & tissue assimilation:** Shell growth was unaffected by pCO_2 treatment(s) until tertiary exposure in which growth has an interaction with pCO_2 treatments (initial×secondary×tertiary) driven by greatest mean shell growth of 0.56 ± 0.147 mm in conditioned animals subsequently exposed to severe×moderate pCO_2 in secondary×tertiary exposure periods (Figure 7).

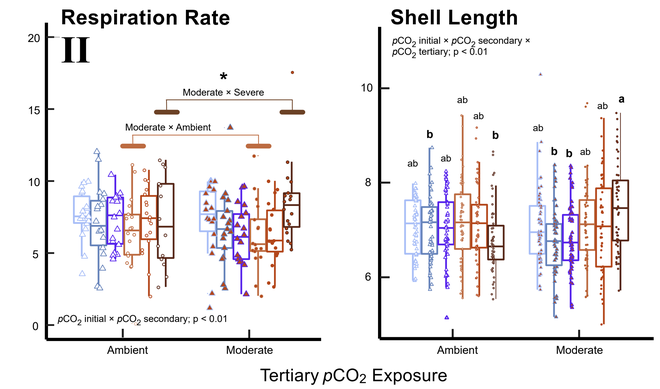


Fig. 7. Respiration rate and shell growth during the secondary (I) and tertiary (II) exposure periods. Color, shape, and fill are shown in Figure 2. *A posteriori* tests of three and four-way ANOVA models are shown for significant (P < 0.05; "*") and marginal (P < 0.1; ".") pairwise differences.

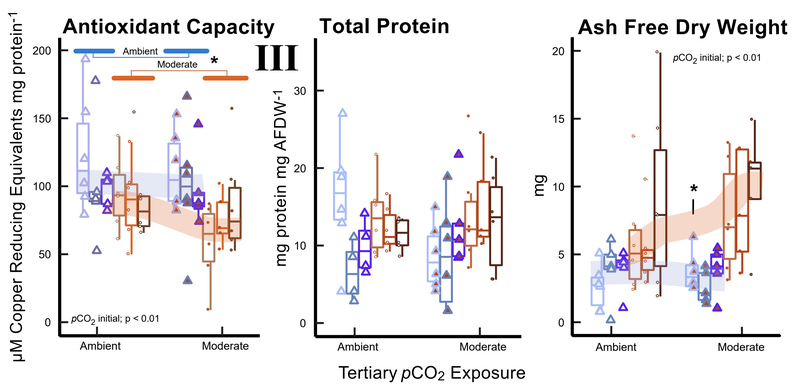


Fig. 8. Total antioxidant capacity, total protein, and ash free dry weight (AFDW) at the end of the exposure period (sumulatively as day 21). Color, shape, and fill are shown in the boxed region of Figure 6 (III). *A posteriori* tests of three and four-way ANOVA models are shown for significant (P < 0.05; "*") pairwise differences.

Future directions

- Our analysis of this experiment is ongoing; we intend to measure (1) oxidative damage and (2) sequence geoduck tissue to determine differential mRNA expression and DNA methylation in response to pCO_2 treatments.

- Alternative oxidase pathway (AOX) poses a promising direction to explore differential mitochondrial regulation of redox status and energy homeostasis due to stress acclimatization.

- Epigenetic translational regulation of mitochondrial capacity may play a critical role in (1) modification and maintenance of alternative mitochondrial pathways (i.e. AOX) and (2) shifting energy metabolism to enhance protein homoestasis and phenotypic plasticity to cope with subsequent intragenerational stress encounters.

Conclusions

- Long-term (3 months) pCO_2 conditioning of postlarval geoduck decreased total antioxidant capacity regardless of subsequent exposure(s) suggesting early-life stress acclimatization may have an important role on modifications of mitochondrial regulation of redox status (lower mETC production of ROS) and protein/energy homeostasis.

- Subsequent acute exposure to severe pCO_2 (>4500 atm; 7 days) in juvenile clams increased respiration rate and shell growth suggesting a stress intensity-dependent benefit of stress conditioning on juvenile geoduck performance.

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