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

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Lay summary: (50-word summary of the paper)
Commercial shellfish hatcheries provide essential food security, but often production can be hampered by sensitivity of shellfish at early life stages. Repeated short-term exposures can increase tolerance and performance of the geoduck clam with implications for sustainable aquaculture.

Author contributions:
SJG, BV, SBR, and HMP designed the experiments, SJG conducted the experiments, SJG, BV, SBR, and HMP drafted, revised, read and approved the final version of the manuscript.

Abstract

While acute stressors can be detrimental, environmental stress conditioning can improve performance. To test the hypothesis that physiological status is altered by stress conditioning, we subjected juvenile Pacific geoduck, *Panopea generosa*, to repeated exposures of elevated pCO_2 in a commercial hatchery setting followed by a period in ambient common garden. Metabolic rate and shell length were measured for juvenile geoduck periodically throughout short-term repeated reciprocal exposure periods in ambient (~550 µatm) or elevated (~2400 µatm) pCO_2 treatments and in common, ambient conditions, five months after exposure. Short-term exposure periods comprised an initial 10-day exposure followed by 14 days in ambient before a secondary 6-day reciprocal exposure. The initial exposure to elevated pCO_2 significantly reduced metabolic rate by 25% relative to ambient conditions, but no effect on shell growth was detected. Following 14 days in common, ambient conditions, reciprocal exposure to elevated or ambient pCO_2 did not alter juvenile metabolic rates, indicating ability for metabolic recovery under subsequent conditions. Shell growth was negatively affected during the reciprocal treatment in both exposure histories, however clams exposed to the initial elevated pCO_2 showed compensatory growth with 5.8% greater shell length (on average between the two secondary exposures) after five months in ambient conditions. Additionally, clams exposed to the secondary elevated pCO_2 showed 52.4% increase in respiration rate after five months in ambient conditions. Early exposure to low pH appears to trigger carry over effects suggesting bioenergetic re-allocation facilitates growth compensation. Life stage-specific exposures to stress can determine when it may be especially detrimental, or advantageous, to apply stress conditioning for commercial production of this long-lived burrowing clam.

1. Introduction

Sustainable food production minimizes overexploitation of wild populations and degradation of ecological health (Campbell *et al.*, 1998; Shumway *et al.*, 2003; Orensanz *et al.*, 2004; Zhang and Hand, 2006). Shellfish aquaculture has expanded worldwide in recent decades to satisfy international trade (FAO 2018). However, early larval and juvenile rearing pose a production bottleneck. For example, early life histories are highly sensitive to biotic (e.g. harmful algae, pathogens; Prado *et al.*, 2005; Rojas *et al.*, 2015) and abiotic stressors (Kroeker *et al.*, 2010; Gimenez *et al.*, 2018). These stressors are known to intensify in coastal marine systems (Cloern, 2001; Diaz and Rosenberg, 2001; Cai *et al.*, 2011; Wallace *et al.*, 2014) causing mass mortality for early-stage bivalves in wild or hatchery settings (Elston *et al.*, 2008; Barton *et al.*, 2015). Local and global anthropogenic stressors such as CO_2-induced changes in pH and carbonate mineral saturation states can reduce performance and normal shell development (White *et al.*, 2013; Waldbusser *et al.*, 2015; Kapsenberg *et al.*, 2018).

Ocean acidification, or the decrease of oceanic pH due to elevated atmospheric partial pressures (µatm pCO_2), poses a threat to aquaculture (Barton *et al.*, 2012; Froehlich *et al.*, 2018; Mangi *et al.*, 2018). Elevated pCO_2 and aragonite undersaturation ($\Omega_{aragonite} < 1$) generally have detrimental consequences for aerobic performance (Pörtner *et al.*, 2004; Portner and Farrell, 2008) and shell biomineralization in marine calcifiers (Shirayama, 2005; Talmage and Gobler, 2010; Waldbusser *et al.*, 2010, 2015; Gazeau *et al.*, 2013). Responses to acidification can be species (Ries *et al.*, 2009) and population specific (Lemasson *et al.*, 2018), but it is widely established to be impactful during early life stages for bivalves (Dupont and Thorndyke, 2009; Gazeau *et al.*, 2010; Kroeker *et al.*, 2010; Gimenez *et al.*, 2018). Experimental research is commonly focused on species with short generational times, (Parker *et al.*, 2011, 2015; Lohbeck *et al.*, 2012) limiting

91 evidence for effects of acidification on long-lived mollusks important for food and economic
92 security (Melzner *et al.*, 2009).

93 The Pacific geoduck *Panopea generosa* is a large and long-lived infaunal clam of cultural
94 and ecological importance (Dethier, 2006) with an increasing presence in sustainable shellfish
95 industry (Cubillo *et al.*, 2018). Geoduck production in Washington (USA) provides ~90% of
96 global supply (Shamshak and King, 2015) and alone constitutes 27% of the overall shellfish
97 revenue in the state valued at >$24 million yr^{-1} and >$14 $pound^{-1}$ as of 2015 (Washington Sea
98 Grant, 2015). Geoduck are known to live in dynamic CO_2-enriched low pH waters such as Hood
99 Canal in Puget Sound, WA where conditions in summer can reach $\Omega_{aragonite}$ 0.4 and pH 7.4 (Feely
100 *et al.*, 2010). Although *P. generosa* may be adapted and able to acclimatize to local stressors
101 (Putnam *et al.*, 2017; Spencer *et al.*, 2018), acidification has caused massive losses of larval
102 geoduck in hatcheries (Barton *et al.*, 2015), identifying a critical need for assessment of
103 physiological stress tolerance during early life stages.

104 Evidence of acclimatory mechanisms in response to acidification (Goncalves *et al.*, 2018)
105 and enhanced performance within and across generations (Parker *et al.*, 2011, 2015; Putnam and
106 Gates, 2015; Ross *et al.*, 2016; Thomsen *et al.*, 2017; Zhao *et al.*, 2017) support conditioning as a
107 viable strategy to mitigate the negative effects of stress exposure and enhance organismal
108 performance under high pCO_2 (Parker *et al.*, 2011; Dupont *et al.*, 2012; Suckling *et al.*, 2015; Foo
109 and Byrne, 2016). Hormesis is a biphasic low-dose-stimulatory response, as identified in
110 toxicological studies (Calabrese, 2008), and suggests beneficial carryover effects of moderate
111 stress exposure (Calabrese *et al.*, 2007; Costantini *et al.*, 2010; Costantini, 2014; Putnam *et al.*,
112 2018). Conditioning-hormesis can explain patterns of inter- and transgenerational plasticity for
113 organisms under environmental change (Calabrese and Mattson, 2011; Costantini *et al.*, 2012;

López-Martínez and Hahn, 2012; Putnam *et al.*, 2018; Visser *et al.*, 2018), but is understudied for stress resilience in bivalves likely due to generally negative physiological implications of acidification (Gazeau *et al.*, 2013). In one example of early-life stage conditioning in bivalves, Putnam et al. (2017) found *P. generosa* exhibit compensatory shell growth after an acute exposure under elevated pCO_2. This finding suggests acute exposures may present a strategy for stress-hardening and enhancement of sustainable geoduck production. We therefore tested the hypothesis that repeated stress exposure under elevated pCO_2 can enhance intragenerational performance for Pacific geoduck. To this end, we measured the standard metabolic rate and shell growth of juvenile geoduck in a commercial hatchery under repeated acute periods of elevated pCO_2 and aragonite undersaturation, and the longer term (~5 months) carry over effects.

2. Methods

2.1. Exposure of juveniles

Juvenile geoduck (n = 640; mean ± SEM initial size, 5.08 ± 0.66 mm shell length [measured parallel to hinge]) were provided by Jamestown Point Whitney Shellfish Hatchery and allocated into eight trays (Heath/Tecna water tray 10 L; n = 80 clams per tray) for the experiment (Fig. 1). Trays were filled with 5 mm depth of rinsed sand (35-45 µm grain size) that allowed geoduck to burrow and siphons could clearly be seen extended above the sediment throughout the experiments. To enable measurements of metabolic activity and shell growth, 30 geoduck were placed in an open circular dish (6.5 cm diameter and 3 cm height) with equal mesh size and sand depth submerged in each tray, the remaining 50 geoduck in each tray burrowed in the surrounding sediment (Fig. 1). Seawater at the Jamestown Point Whitney Shellfish Hatchery (Brinnon, WA, USA) was pumped from offshore (100 m) in Quilcene Bay (WA, USA), bag-filtered (5 µm), and UV sterilized before fed to 250-L conical tanks at rate of 1 L min^{-1}. Four conical tanks were used

as replicates for two treatments: elevated pCO_2 level of ~2000-3000 µatm and ~7.2-7.4 pH (total scale); and ambient hatchery conditions of ~480-730 µatm and ~7.8-8.0 pH (total scale). The elevated pCO_2 level was set with a pH-stat system (Neptune Apex Controller System; Putnam *et al.*, 2016) and gas solenoid valves for a target pH of 7.2. pH (NBS scale) and pH and temperature (°C) were measured every 10 seconds in conicals (Neptune Systems; accuracy: ± 0.01 pH units and ± 0.1°C, resolution: ± 0.1 pH units and ± 0.1°C). These treatments were delivered to replicate exposure trays, which were gravity fed seawater from conicals (Fig. 1; n = 4 per treatment). The experiment began with an initial exposure period of 10 days under elevated pCO_2 (2345 µatm) and ambient treatments (608 µatm; Table 1). Preliminary exposure was followed by 14 days in ambient common garden (557 ± 17 µatm; $pH_{t.s.}$ 7.9 ± 0.01; $\Omega_{aragonite}$ 1.46 ± 0.04, mean ± SEM) before secondary exposure for 6 days to reciprocal treatments of elevated pCO_2 (2552 µatm) and ambient treatments (506 µatm; Table 2). For the secondary exposure period, one tray was crossed to the opposite treatment to address both repeated and reciprocal exposure (n = 2 trays per initial×secondary pCO_2 treatment; Fig. 1). Following this the juveniles were exposed to ambient conditions for 157 days within the replicate trays.

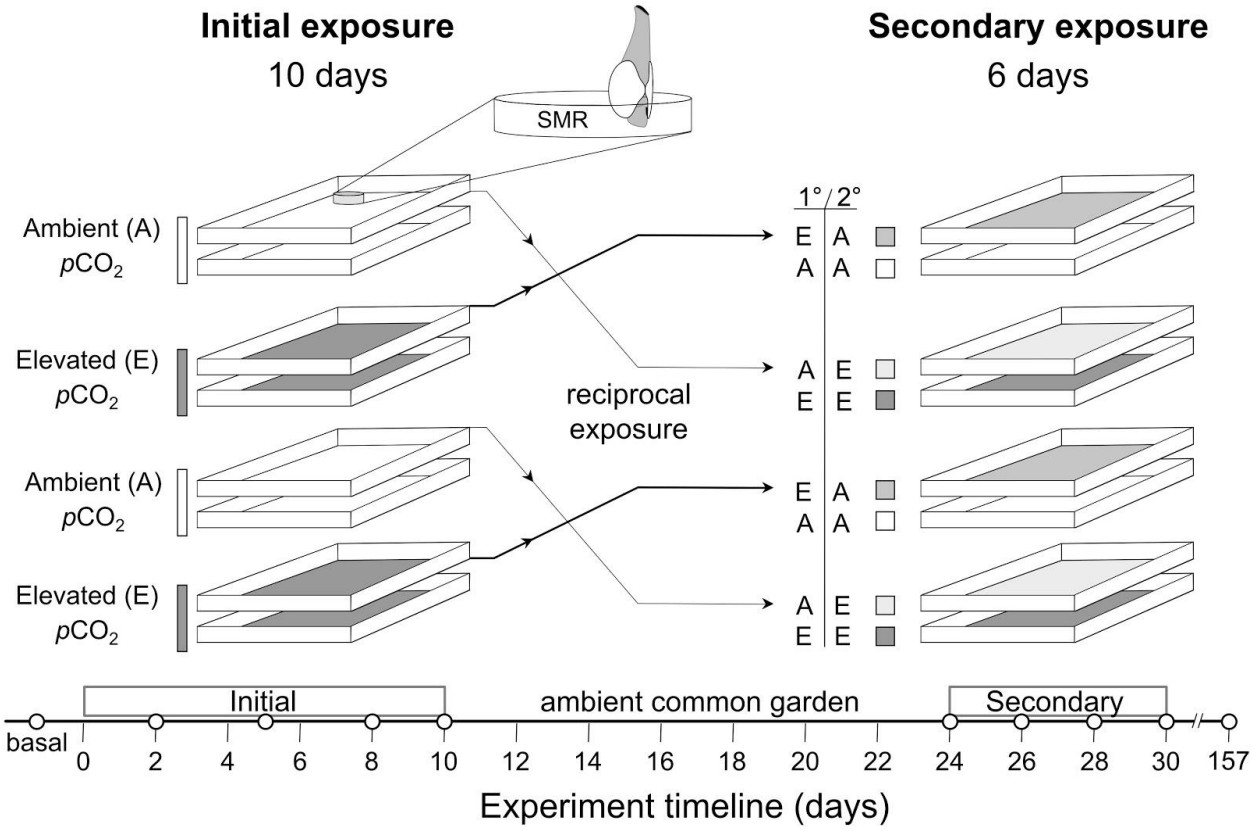

Figure 1. Schematic of the repeated exposure experimental design for two exposure trials, initial (10-day) and secondary (6-day), in ambient and elevated pCO_2 treatments. Timeline displays respiration and growth measurements as solid white circles.

Juvenile geoduck were fed semi-continuously with a mixed algae diet (30% *Isocrysis galbana*, 30% *Pavlova lutheri*, and 40% *Tetraselmis suecica*) throughout the 30-d experiment with a programmable dosing pump (Jebao DP-4 auto dosing pump). Large algae batch cultures were counted daily via bright-field image-based analysis (Nexcelom T4 Cellometer; Gurr *et al.*, 2018) to calculate a daily ration of 5×10^7 live algae cells d^{-1} $individual^{-1}$. Diet was calculated with an equation in Utting & Spencer (1991) catered for 5-mm clams:

$V = (S \times 0.4) \div (7 \times W \times C)$; this equation accounts for a feed ration of 0.4 mg dried algae mg live animal $weight^{-1}$ $week^{-1}$, the live animal weight (mg) of spat (S; estimated from regression of shell

length and weight of Manilla clams in Utting and Spencer 1991), weight (mg) of one million algal cells (W), and cell concentration of the culture (cells μl^{-1}) to calculate the total volume (V) of each species in a mixed-algae diet. Tray flow rates (mean flow rate, approx. 480 ± 9 ml^{-1} min^{-1}) and food delivery were measured and adjusted daily.

All geoduck survived the exposure periods. Half of the remaining juveniles burrowed in each tray were maintained at the hatchery, positioned in the same replicate trays. The juveniles were fed cultured algae *ad libitum* daily for 157 days before shell length and metabolic rates were measured.

2.2. Respirometry and shell length measurements

Juvenile geoduck were measured on days 2, 5, 8, and 10 of initial exposure, days 0, 2, 4, and 6 (cumulatively as day 24, 26, 28, and 30, respectively) of secondary exposure, and 157 days after the exposure period (cumulatively as day 187) to assess rates of oxygen consumption normalized to shell length. Calibrated optical sensor vials (PreSens, SensorVial SV-PSt5-4ml) were used to measure oxygen consumption on a 24-well plate sensor system (Presens SDR SensorDish). Juveniles in each treatment dish were divided into three sensor vials (10 individuals $vial^{-1}$ for exposure periods; 1 individual $vial^{-1}$ at 157-d post-exposure), each filled with 0.2 µm-filtered seawater from corresponding trays. Three blank vials per tray, filled only with 0.2 µm-filtered seawater, were used to account for potential microbial oxygen consumption. Respiratory runs occurred within an incubator at 15°C, with the vials and sensor placed on a rotator for mixing. Each set of measurements lasted ~30 minutes and trials ceased when oxygen concentration declined to ~70-80% saturation. Geoduck were subsequently photographed and shell length (parallel to hinge) was measured using Image J with a size standard (1 mm stage micrometer).

Rates of respiration (oxygen consumption) were estimated from repeated local linear regressions using the R package LoLinR (Olito *et al.*, 2017). An initial criterion of fixed constants (from the LoLin R package) for weighting method ($L_{\%}$) and observations (alpha = 0.2) was run individually for each respirometry measurement over the full 30-minute record as a "reference" dataset. These are considered to be the most robust parameters as suggested by the R package authors (Olito *et al.*, 2017). Diagnostic plots (from the LoLin R package) were individually observed and $L_{\%}$ and alpha were altered as necessary to best approximate the peak empirical distribution of local linear regressions (see https://github.com/SamGurr/Juvenile_geoduck_OA "version_20190620" for full details). To determine the optimal set of parameters, respiration data was calculated using three alpha values and data truncations (alpha = 0.2, 0.4, and 0.6; truncation = 10-20 minutes, 10-25 minutes, and no truncation; weighting method = $L_{\%}$) and each was compared to the initial reference dataset with two curve fitting steps (local polynomial regressions) to calculate unbiased and reproducible rates of oxygen consumption similar to the reference (10-day exposure, r^2=0.88; 6-day exposure, r^2=0.95). Final metabolic rates of juvenile geoduck were corrected for vial volume, blank values, and standardized by mean shell length (µg O_2 hr^{-1} mm^{-1}).

2.3. Seawater carbonate chemistry

Total alkalinity (TA; µmol kg^{-1} seawater) water samples were collected from trays and concials once daily during treatment periods, in combination with measurements of pH by handheld probe (Mettler Toledo pH probe; resolution: 1 mV, 0.01 pH ; accuracy: ± 1 mV, ± 0.01 pH; Thermo Scientific Orion Star A series A325), salinity (Orion 013010MD Conductivity Cell; range 1 µS/cm - 200 mS/cm; accuracy: ± 0.01 psu), and temperature (Fisherbrand Traceable Platinum Ultra-Accurate Digital Thermometer; resolution; 0.001°C; accuracy: ± 0.05 °C). Seawater chemistry was measured for three consecutive days during the 14 days of ambient

common garden between initial and secondary treatment periods. Quality control for pH data was assessed daily with Tris standard (Dickson Lab Tris Standard Batch T27) and handheld conductivity probes used for discrete measurements were calibrated every three days. TA was measured using an open cell titration (SOP 3b; Dickson *et al.*, 2007) with certified HCl titrant (~0.1 mol kg^{-1}, ~0.6 mol kg^{-1} NaCl; Dickson Lab) and TA measurements identified <1% error when compared against certified reference materials (Dickson Lab CO_2 CRM Batches 137 and 168). Seawater chemistry was completed following Guide to Best Practices (Dickson *et al.*, 2007); daily measurements were used to calculate carbonate chemistry, CO_2, pCO_2, HCO^{3-}, CO_3, and $\Omega_{aragonite}$, using the SEACARB package (Gattuso *et al.*, 2018) in R v3.5.1 (R Core Team, 2018).

2.4. Data Analysis

A two-way Analysis of Variance (ANOVA) was used to analyze the effect of time (fixed), pCO_2 treatment (fixed), and time×pCO_2 interaction for respiration and shell length during initial exposure. A t-test was used to test the effect of initial pCO_2 treatment on respiration rate and shell length prior to the secondary exposure (last day of ambient common garden, cumulatively day 24, day 0). For the secondary exposure period, a three-way ANOVA was used to test the effects of time (fixed), initial pCO_2 treatment (fixed), secondary pCO_2 treatment (fixed), and their interactions on respiration rate and shell length. Significant model effects were followed with pairwise comparisons with a Tukey's *a posteriori* HSD. We used a two-way ANOVA to analyze the effects of initial (fixed) and secondary (fixed) pCO_2 treatments on respiration and shell length after 157 days in ambient conditions. In all cases, model residuals were tested for normality assumptions with visual inspection of diagnostic plots (residual vs. fitted and normal Q-Q; Kozak and Piepho, 2018) and homogeneity of variance was tested with Levene's test. Model effects using raw data were robust to transformation(s) that resolved normality assumptions via Shapiro-Wilk

test. Statistical tests were completed using R (v3.5.1; R Core Team, 2018). All code is available (https://github.com/SamGurr/Juvenile_geoduck_OA released as "version_20190620") and a doi will be released upon acceptance for publication.

3. Results

3.1. Exposure 1

Elevated pCO_2 had a significant effect on respiration rate over the initial 10-day exposure (pCO_2 treatment, $F_{1,88}$ = 7.512; P < 0.01) with a 25% reduction (averaged across all days) in metabolic rate in elevated pCO_2 treatment relative to ambient (Fig. 2A). Juvenile geoduck grew significantly with time under the initial 10-d exposure (time, $F_{3,949}$ = 3.392; P = 0.018) with a 3.6% increase in shell length between days 2 and 10 (Fig. 2B), but there was no effect of pCO_2 treatment on shell length (Table 2). Significant differences in respiration rate from the initial pCO_2 treatment were still apparent after 14 days in ambient common garden and before the onset of the secondary exposure (Table 2 and Fig. 3A). In contrast, there was no significant change in shell length due to initial pCO_2 treatment after 14 days in ambient common garden (Table 2).

3.2. Exposure 2

There was no interaction between initial and secondary pCO_2 treatments nor between treatments and time on respiration rate or shell length (Table 2). There was a marginal effect of time on respiration rate (Table 2; time, $F_{2,60}$ = 3.137; P = 0.0506) with a 31% increase in average respiration rate between days 2 and 6. Initial pCO_2 treatment had a significant effect on shell length, with on average a ~4% reduction in shell size under high pCO_2 relative to ambient initial exposure (Fig. 3B; $pCO_{2\_initial}$, $F_{1,709}$ = 15.821; P < 0.001). This same trend was present under the secondary high pCO_2 exposure, (Fig. 3B; $pCO_{2\_secondary}$, $F_{1,709}$ = 9.917; P = 0.002) with 3.20% smaller shells for individuals exposed to elevated pCO_2 treatments. There were pairwise differences in shell size

between animals only exposed to ambient and animals repeatedly exposed to elevated pCO_2 (Fig. 3B; day 6, P = 0.0415; day 6 ambient - day 4 elevated, P = 0.0406).

3.3. Common garden after exposure periods

There was no interaction between initial and secondary pCO_2 treatments on respiration rate or shell length (Table 2). The initial exposure period had a significant stimulatory effect on shell length of juveniles previously exposed to high pCO_2, after 157 days in ambient common garden (Fig. 4A; $pCO_{2\_initial}$, $F_{1,170}$ = 5.228; P = 0.023), where average shell lengths were 5.8% larger in juveniles exposed to initial elevated pCO_2. Secondary 6-day exposure had a significant effect on respiration rates after 157 days in ambient common garden (Fig. 4B; $pCO_{2\_seccondary}$, $F_{1,31}$ =13.008; P = 0.001) with an average of 52.4% greater respiration rates in juveniles secondarily exposed to elevated pCO_2.

4. Discussion

Metabolic recovery and compensatory shell growth by juvenile *P. generosa* presents a novel application of hormetic framework for resilience of a mollusc to acidification. To date, within-generation carry over effects remain poorly understood for marine molluscs (Ross *et al.*, 2016) with few examples of either positive and negative responses after stress challenges (Hettinger *et al.*, 2012; Gobler and Talmage, 2013; Putnam *et al.*, 2017). Results of this study support conditioning-hormesis as a possible driver for physiological acclimation and phenotypic rescue under environmental change (Costantini, 2019).

4.1. Metabolic depression and compensatory response

Metabolic depression, such that was found under initial exposure of geoduck to elevated pCO_2, has been suggested as an adaptive mechanism to extend survival (Guppy and Withers, 1999). Stress-induced metabolic depression has been documented for a variety of marine

invertebrates in response to environmental stress. For example, in the New Zealand geoduck, *Panopea zelandica*, there was a 2-fold reduction in respiration rate under abiotic stress (Le *et al.*, 2016). Prior work has shown metabolic reductions up to 60-95% of basal performance at rest for marine molluscs (Guppy and Withers, 1999). Here, metabolic depression by juvenile geoduck to ~25% in comparison with metabolic rates under ambient conditions suggests *P. generosa* are relatively tolerant to short-term acidification and may have adaptive physiology to cope with environmental acidification and high pCO_2. Responsiveness to acidification is critical for pH-tolerant taxa to maintain buffering capacity and cope with acidosis (high intracellular pCO_2; (Melzner *et al.*, 2009). However, pH-induced metabolic depression to a similar degree found in this study has caused a permanent decrease in extracellular pH and increase in protein degradation and ammonia excretion in the Mediterranean mussel (*Mytilus galloprovincialis*) (Michaelidis *et al.*, 2005). Conversely, metabolic elevation is relatively common for early-life stage bivalves exposed to low pH and $\Omega_{aragonite}$ undersaturation and typically coincides with consequences for performance and survival (Michaelidis *et al.*, 2005; Beniash *et al.*, 2010; Thomsen and Melzner, 2010; Fernández-Reiriz *et al.*, 2011; Waldbusser *et al.*, 2015; Lemasson *et al.*, 2018). Whether depressed or elevated, stress-induced metabolic alterations are known to coincide with biochemical implications (i.e. intracellular hypercapnia and hemolymph acidosis; Pörtner *et al.*, 2004; Spicer *et al.*, 2011), increased ammonia excretion, and reduced growth for invertebrate fauna (Michaelidis *et al.*, 2005; Beniash *et al.*, 2010; Lannig *et al.*, 2010; Thomsen and Melzner, 2010; Gazeau *et al.*, 2013).

Juvenile geoduck repeatedly exposed to elevated pCO_2 showed possible stress "memory" with rebound from metabolic depression under subsequent stress and compensatory shell growth after long-term recovery. This hormetic-like response (Calabrese *et al.*, 2007; Costantini, 2014)

demonstrates a benefit of early stress-priming for later performance and the adaptive plasticity of *P. generosa* to elevated pCO_2. Use of hormesis to conceptualize carry over effects of mild stress exposure is largely confined to model insects, plants, and microorganisms (Lee *et al.*, 1987; Calabrese and Blain, 2009; López-Martínez and Hahn, 2012; Visser *et al.*, 2018). For example, Visser et al. (Visser *et al.*, 2018) found the Caribbean fruit fly, *Anastrepha suspensa*, exposed to oxidative stress early in life enhanced survivorship and investment in fertility and lipid synthesis under subsequent stress during adulthood. Further mechanistic molecular and biochemical assessments under different stress intensities (i.e. magnitude, duration, and frequency) are planned to determine the threshold between low-dose stimulation and high-dose inhibition from stress-conditioning.

4.2. Age and intensity dependence of shell growth

Metabolic recovery was coupled with reduced shell growth under a repeated stress encounter (Fig. 3) and compensatory shell growth after approximately five months in ambient conditions (Fig. 4). This could be explained by several hypotheses such as: carry over effect from metabolic depression under initial exposure to elevated pCO_2 (Fig. 2A), differing sensitivity to stress intensity (Table 1), and/or age dependence for environmental hardening, or the interaction with increasing temperature through the season (see Supplementary Figure 1.). Bivalves known to exhibit metabolic suppression under acute and long-term acidification are often attributed with increased ammonia excretion rates and decreased ingestion and clearance rates as possible contributors to protein degradation and reduced growth (Michaelidis *et al.*, 2005; Thomsen and Melzner, 2010; Fernández-Reiriz *et al.*, 2011; Navarro *et al.*, 2013). Therefore, decreased shell length under secondary exposure may be a carry over effect of metabolic depression during initial exposure. However, shell length was also reduced for clams initially exposed to the elevated

328 treatment in the second exposure period (Table 2, Fig. 3B) indicating potential age-dependence on
329 calcification and bioenergetic effects for juvenile *P. generosa.* This reduction however, could be
330 explained by the fact the secondary elevated pCO_2 treatment was on average ~0.04 pH units lower
331 than the initial exposure (Table 1) suggesting possible sensitivity to increased stress intensity. It is
332 likely that both temporal dynamics and stress thresholds influence intragenerational carry over
333 effects and further experimental efforts with repeated reciprocal design are needed.

334 Respiration rates and shell growth five months post-exposure show a latent enhancement
335 for animals repeatedly stressed or exposed to a stress event earlier in life, emphasizing the
336 importance of the severity, duration, and timing of intragenerational stress-conditioning. These
337 specific findings present a window in their life history where it may be advantageous to condition
338 Pacific geoduck for enhancement of sustainable aquaculture.

339 A growing body of literature describes the importance of designing environmentally
340 relevant stressor regimes to assess the physiology and survival of early-stage and adult
341 invertebrates (Suckling *et al.*, 2015; Cole *et al.*, 2016; Parker *et al.*, 2017; Scanes *et al.*, 2017;
342 Lemasson *et al.*, 2018). Fewer studies have, however, tested responses of benthic infauna to
343 realistic environmental regimes found within an organism's natural habitat (Green *et al.*, 2009;
344 Thomsen *et al.*, 2017; García *et al.*, 2018). Such experiments have shown coupled effects of
345 acidification alongside pathogens, food availability, and environmental chemistry (Sanders *et al.*,
346 2013; Thomsen *et al.*, 2013; Cao *et al.*, 2018; Stevens and Gobler, 2018). For example, Mackenzie
347 *et al*. (2014) found temperature outweighed the effects of elevated pCO_2 for decreased shell growth
348 of the adult blue mussel *Mytilus edulis*. Averaged temperatures in the present study increased ~1.5-
349 2°C between initial and secondary exposures (Table 1), therefore decreased shell length during
350 secondary exposure to elevated pCO_2 (Fig. 3B) could have been driven additively by temperature.

Further investigations of *P. generosa* must address coupled stressors of varied frequency and duration to determine the detrimental, advantageous, or neutral impacts and interactions of multiple stressors (Gunderson *et al.*, 2016).

4.3. Environmental applications of experimental findings

Although this study was primarily focused on production enhancement in a hatchery setting, effects on shell growth and metabolic activity have important applications to natural systems. Seawater carbonate chemistry targeted for stress treatments was more severe than levels commonly used in experimental research (Gazeau *et al.*, 2010; Navarro *et al.*, 2013; Diaz *et al.*, 2018), but relevant to summer subsurface conditions within the natural range of *P. generosa* (pH 7.41; $\Omega_{aragonite}$ 0.42 in Hood Canal, WA; Feely *et al.*, 2010). Thus, survival, metabolic recovery, and compensatory growth in *P. generosa* in this study demonstrates a resilience to short-term acidification in the water column. Enhanced growth rates during juvenile development can present benefits for burrowing behavior (Green *et al.*, 2009; Clements *et al.*, 2016; Meseck *et al.*, 2018) and survival due to decreased risk of predation and susceptibility to environmental stress (Przeslawski and Webb, 2009; Johnson and Smee, 2012). Specific to juvenile *P. generosa*, time to metamorphosis (to dissoconch), pre-burrowing time (time elapsed to anchor into substrate and obtain upright position), and burrowing depth are directly related to growth and survival (Goodwin and Pease, 1989; Tapia-Morales *et al.*, 2015). Thus, stress conditioning under CO_2-enrichment and low pH may enhance survivorship of juvenile geoduck in natural systems. Water column carbonate chemistry may be critical for sustainable production of infaunal clams, such as *P. generosa*, that are outplanted for several years *in-situ* on mudflats known to exhibit dynamic abiotic gradients (Green *et al.*, 1993; Burdige *et al.*, 2008; Feely *et al.*, 2010) adjacent to seasonally acidified and undersaturated water bodies (Feely *et al.*, 2010; Reum *et al.*, 2014).

Conclusion

Data in this present study provides evidence of capacity to cope with short-term acidification for an understudied infaunal clam of high economic importance. Survival of all individuals over the 30-d experiment demonstrates the resilience of this species to low pH and reduced carbonate saturation. Juvenile geoduck exposed to low pH for 10 days recovered from metabolic depression under subsequent stress exposure and conditioned animals showed a significant increase in both shell length and metabolic rate compared to controls after five months under ambient conditions, suggesting stress "memory" and compensatory growth as possible indicators of enhanced performance from intragenerational stress-conditioning. Our focus on industry enhancement must expand to test developmental morphology, physiology, and genetic and non-genetic markers over larval and juvenile stages in a multi-generational experiment to generate a more holistic assessment of stress hardening and the effects of exposure on cellular stress response (Costantini *et al.*, 2010; Foo and Byrne, 2016; Eirin-Lopez and Putnam, 2018) for advancement of sustainable aquaculture (Branch *et al.*, 2013). Parental conditioning for the benefit of production (Utting and Millican, 1997) has merit for economically important bivalves (Parker *et al.*, 2012; 2013; 2015). Advancements in genome sequencing demands further research to synthesize -omic profiling (i.e global DNA methylation and differential expression) with physiological responses throughout reproductive and offspring development under environmental stress (Gavery and Roberts, 2014; Li *et al.*, 2019) to determine if these mechanisms are transferable among species. Stress conditioning within a generation at critical life stages may yield beneficial responses for food production and provide a baseline for other long-lived burrowing bivalves of ecological and economic importance.

Supplementary Material:

Supplementary Figure 1. Continuous temp and pH data from APEX system

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Initial exposure

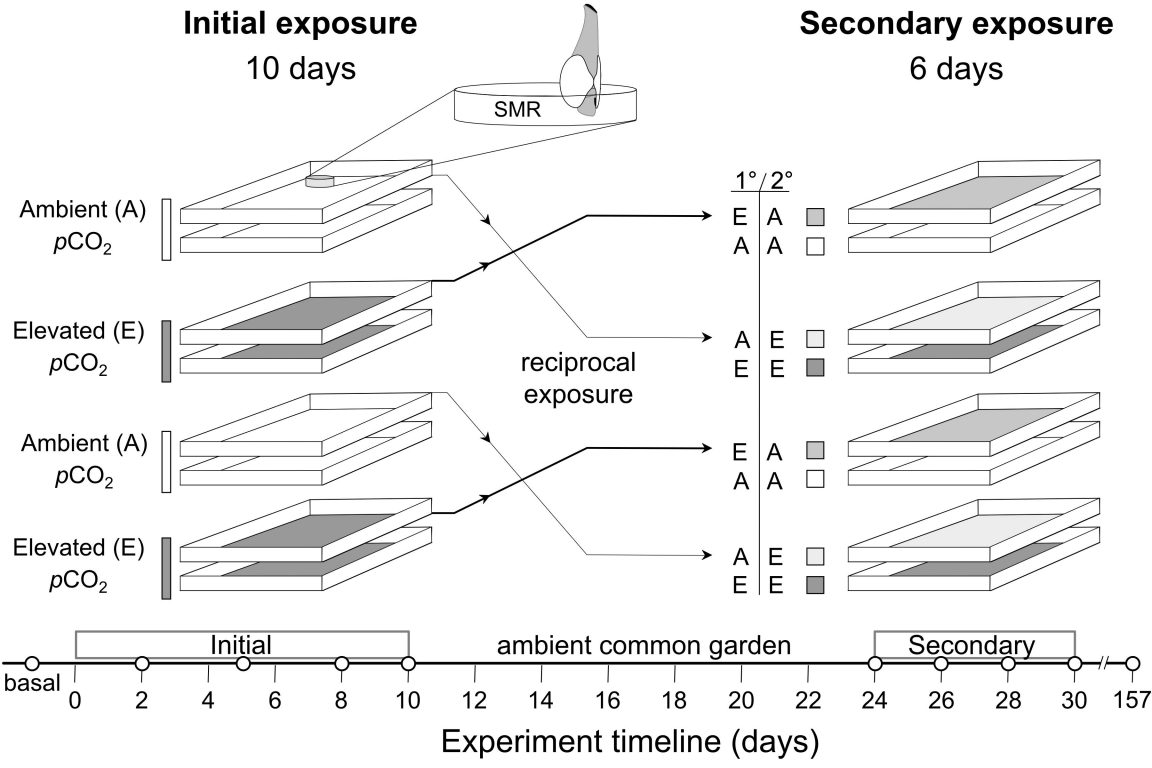
Treatment	Temperature	Salinity	Flow rate L min^{-1}	pH, Total Scale	CO_2 µmol kg^{-1}	pCO_2 µatm	HCO_3 µmol kg^{-1}	CO_3 µmol kg^{-1}	DIC µmol kg^{-1}	Total Alkalinity µmol kg^{-1}	Aragonite Saturation state
Ambient	14.82 ± 0.12	29 ± 0.03	496 ± 139	7.86 ± 0.007	24 ± 0.5	608 ± 11	1842 ± 4	86 ± 1.4	1952 ± 3	2056 ± 1	1.35 ± 0.02
Low	14.91 ± 0.12	29 ± 0.04	486 ± 153	7.31 ± 0.004	91 ± 0.7	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 ± 1.4	1902 ± 4	2033 ± 2	1.60 ± 0.02
Low	16.40 ± 0.22	28.67 ± 0.04	472 ± 87	7.27 ± 0.007	95 ± 1.3	2551 ± 42	1972 ± 3	25 ± 0.3	2091 ± 3	2033 ± 3	0.39 ± 0.005

		df	SS	MS	*F*	P
Initial exposure	*Two-way ANOVA*					
Respiration rate	time	3	0.0323	0.011	0.822	0.485
	$p\,CO_2$	1	0.0983	0.098	7.512	**0.007**
	$p\,CO_2$ × time	3	0.0475	0.016	1.210	0.311
Shell length	time	3	4.250	1.415	3.392	**0.018**
	$p\,CO_2$	1	0	0.0005	0.0012	0.973
	$p\,CO_2$ × time	3	0.170	0.058	0.138	0.937
Ambient common garden	*Welch Two Sample t-test*	df	t	P		
Respiration rate	$p\,CO_2$	19.833	2.673	**0.015**	-	-
Shell length	$p\,CO_2$	1.146	236.680	0.253	-	-
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
	$p\,CO_{2\ initial}$ × $p\,CO_{2\ secondary}$	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
	$p\,CO_{2\ initial}$ × $p\,CO_{2\ secondary}$ × time	2	0.035	0.017	1.608	0.209
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**
	$p\,CO_{2\ initial}$ × $p\,CO_{2\ secondary}$	1	0.060	0.063	0.100	0.752
	$p\,CO_{2\ initial}$ × time	2	0	0.001	0.002	0.998
	$p\,CO_{2\ secondary}$ × time	2	0.460	0.231	0.368	0.692
	$p\,CO_{2\ initial}$ × $p\,CO_{2\ secondary}$ × time	2	0.100	0.048	0.076	0.927
157 days post	*Two-way ANOVA*					
Respiration rate	$p\,CO_{2\ initial}$	1	0.003	0.002	0.011	0.919
	$p\,CO_{2\ secondary}$	1	3.037	3.037	13.008	**0.001**
	$p\,CO_{2\ initial}$ × $p\,CO_{2\ secondary}$	1	0.050	0.050	0.212	0.648
Shell length	$p\,CO_{2\ initial}$	1	10.600	10.597	5.228	**0.023**
	$p\,CO_{2\ secondary}$	1	0.210	0.214	0.105	0.746
	$p\,CO_{2\ initial}$ × $p\,CO_{2\ secondary}$	1	3.510	3.507	1.730	0.190

Significant P-values (< 0.05) are bolded.


Initial exposure
10 days
Secondary exposure
6 days
SMR
Ambient (A)
pCO2
Elevated (E)
pCO2
Ambient (A)
pCO2
Elevated (E)
pCO2
reciprocal
exposure
1°/2°
E A
A A
A E
E E
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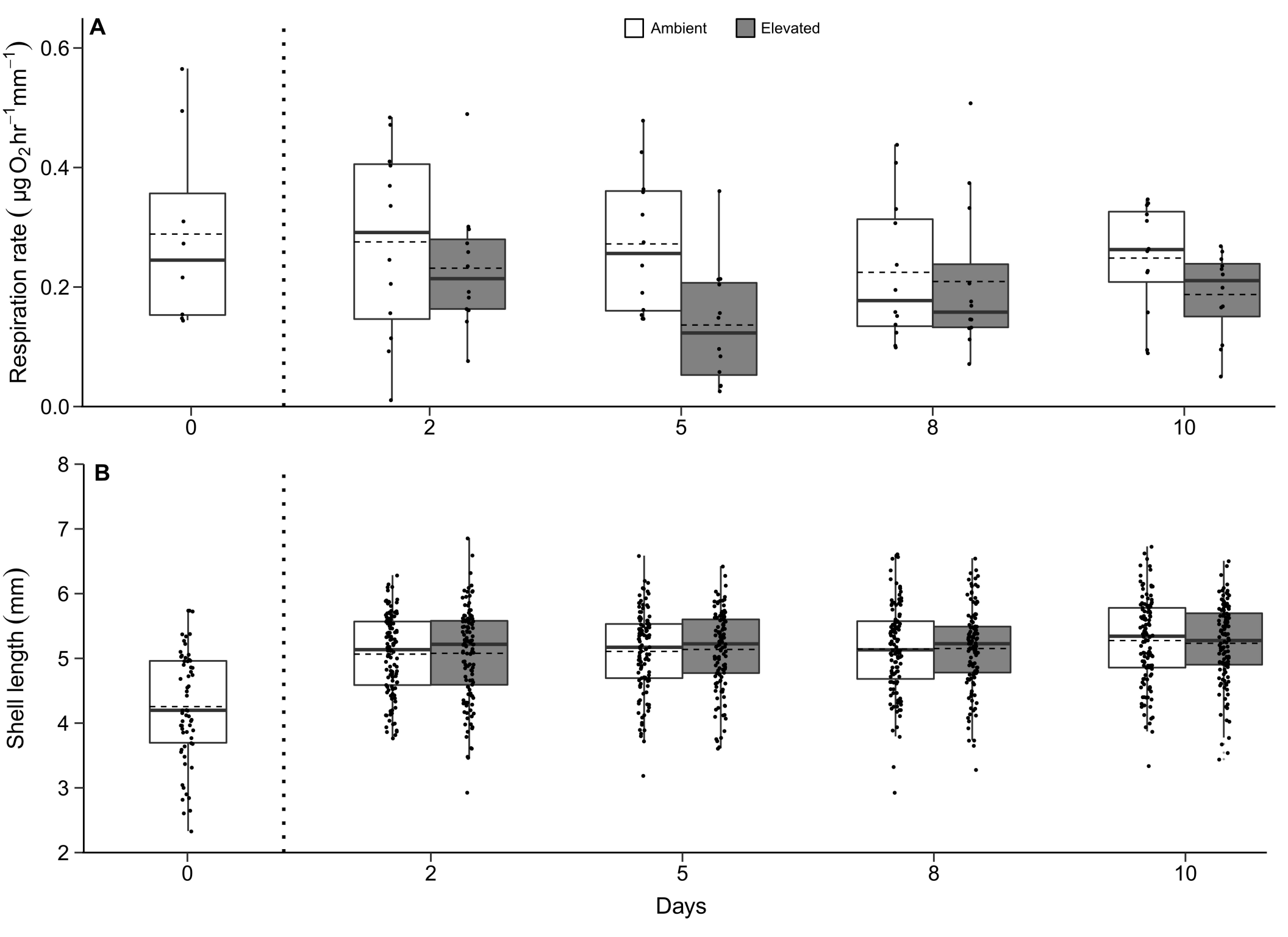
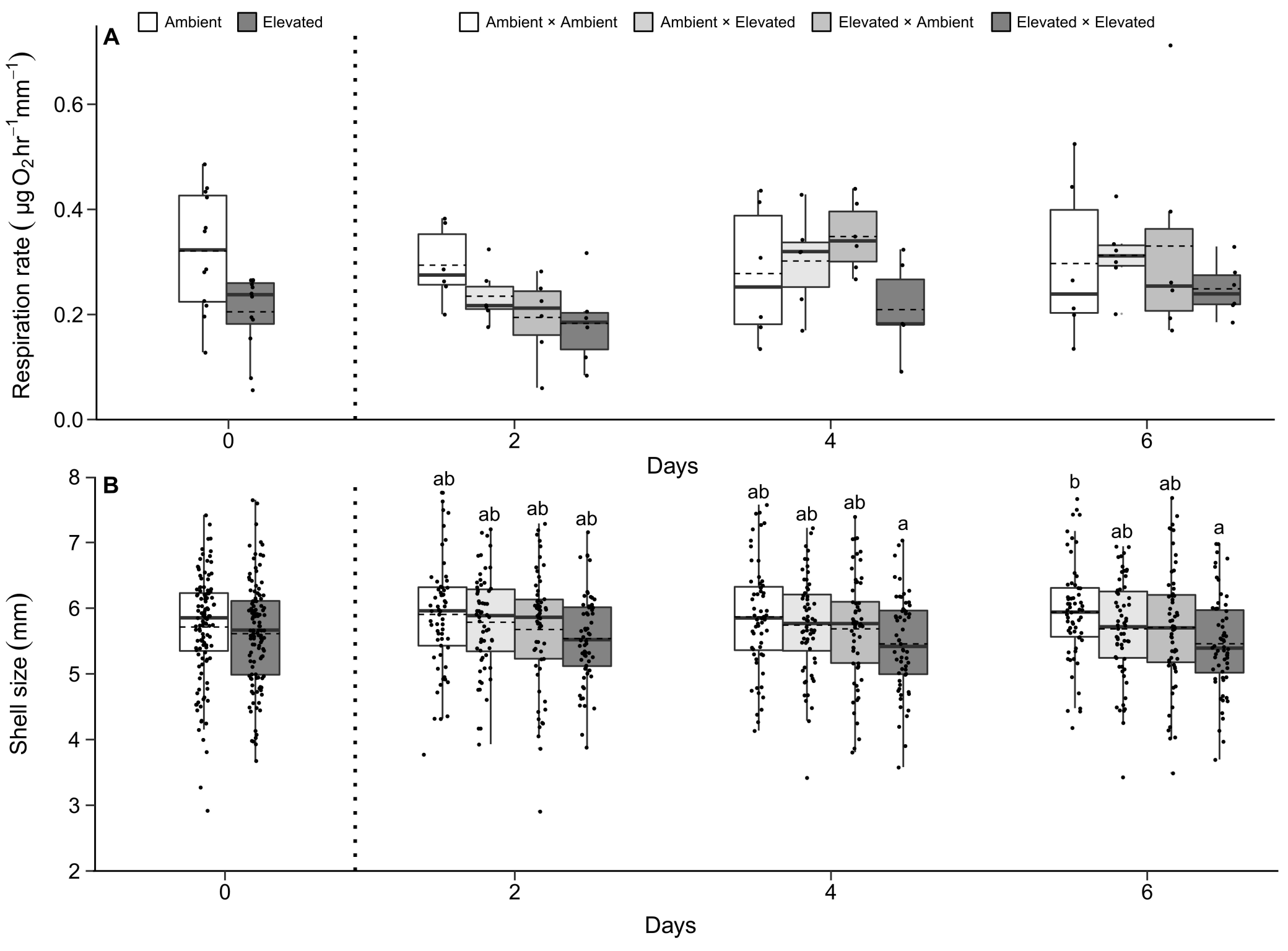
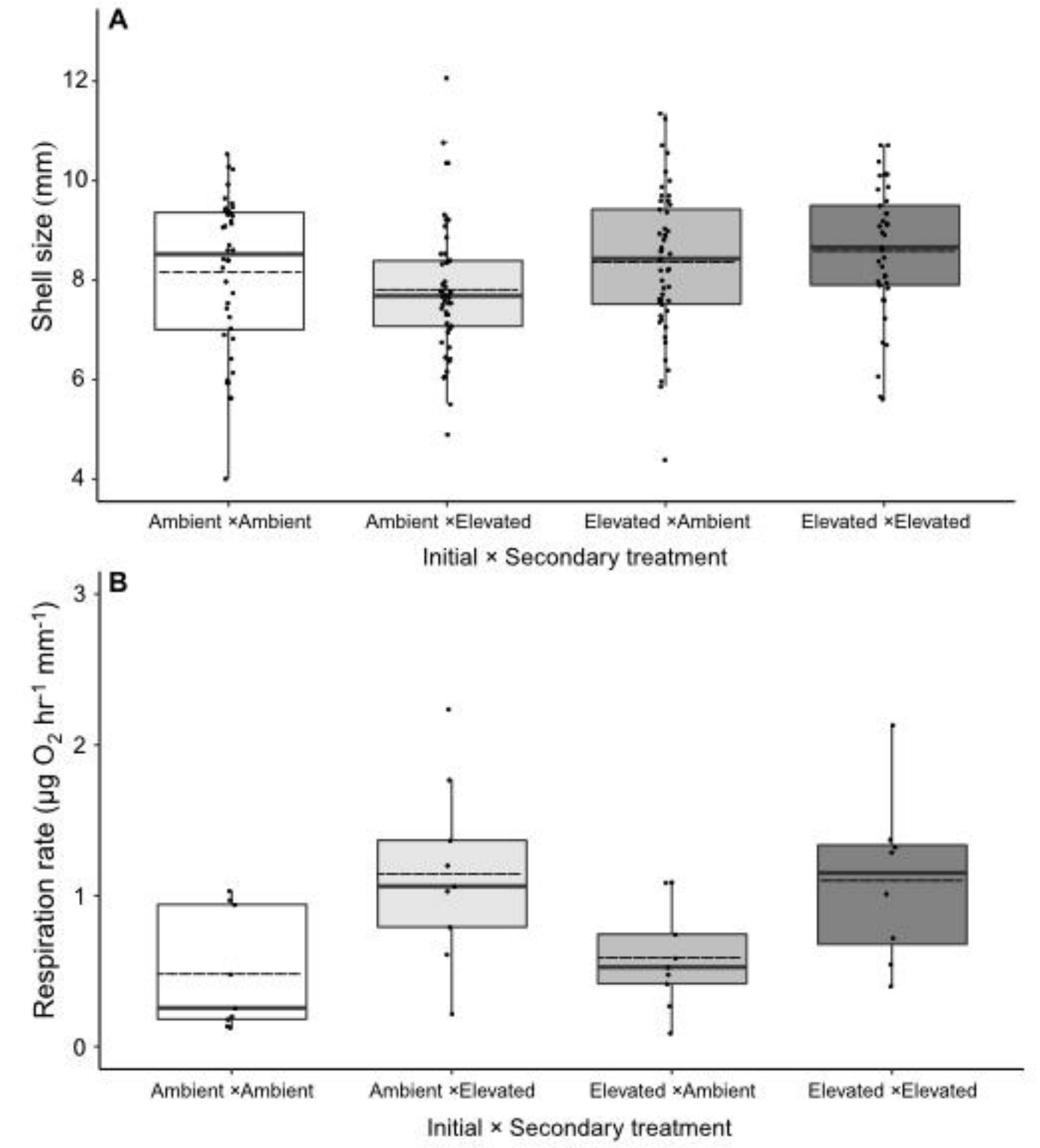
Initial
ambient common garden
Secondary
basal
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 157
Experiment timeline (days)


A
Ambient
Elevated
Respiration rate (μg O2 hr−1 mm−1)
0.0
0.2
0.4
0.6
0
2
5
8
10
B
Shell length (mm)
2
3
4
5
6
7
8
0
2
5
8
10
Days


Ambient
Elevated
Ambient × Ambient
Ambient × Elevated
Elevated × Ambient
Elevated × Elevated
A
Respiration rate (μg O2 hr−1 mm−1)
0.0
0.2
0.4
0.6
0
2
4
6
Days
B
Shell size (mm)
2
3
4
5
6
7
8
ab
ab
ab
ab
ab
ab
ab
a
b
ab
ab
a
0
2
4
6
Days


A
Shell size (mm)
12
10
8
6
4
Ambient ×Ambient
Ambient ×Elevated
Elevated ×Ambient
Elevated ×Elevated
Initial × Secondary treatment
B
Respiration rate (µg O2 hr-1 mm-1)
3
2
1
0
Ambient ×Ambient
Ambient ×Elevated
Elevated ×Ambient
Elevated ×Elevated
Initial × Secondary treatment