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The effects of intermittent exposure to low pH and oxygen conditions on survival and growth of juvenile red abalone

Running head: Low O_2 and pH effects on abalone

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ABSTRACT

Exposure of nearshore animals to hypoxic, low pH waters upwelled from below the continental shelf and advected near the coast may be stressful to marine organisms and lead to impaired physiological performance. We mimicked upwelling conditions in the laboratory and tested the effect of fluctuating exposure to water with low pH and/or low oxygen levels on the mortality and growth of juvenile red abalone (*Haliotis rufescens*, shell length 5-10mm). Mortality rates of juvenile abalone exposed to low pH (7.5, total scale) and low O_2 (40% saturation, 5 mg l^{-1}) conditions for periods of 3 to 6 hours every 3-5 days over 2 weeks did not differ from those exposed to control conditions (O_2: 100% saturation, 12 mg l^{-1}; pH 8.0). However, when exposure was extended to 24 h, twice over a 15 day period, juveniles experienced 5-20 % higher mortality in the low oxygen treatments compared to control conditions. Growth rates were reduced significantly when juveniles were exposed to low oxygen and low pH treatments. Furthermore, individual variation of growth rate increased when juveniles were exposed simultaneously to low pH and low O_2 conditions. These results indicate that prolonged exposure to low oxygen levels is detrimental for the survival of red abalone, whereas pH is a crucial factor for their growth. However, the high individual variation in growth rate under low levels of both pH and oxygen suggests that cryptic phenotypic plasticity may promote resistance to prolonged upwelling conditions by a portion of the population.

Keywords: abalone, hypoxia, growth, mortality, ocean acidification, upwelling

1. INTRODUCTION

Marine ecosystems are under threat from ocean acidification as the excess burden of fossil fuel CO_2 dissolves into the ocean (Orr et al., 2005;Hofmann et al., 2010). Many studies of the biological effects of ocean acidification have focused on the predicted change of mean pH of ocean surface waters derived from IPCC climate scenarios (e.g., Bibby et al., 2007;Orr et al., 2005;Dupont et al., 2010;Byrne et al., 2011). However, nearshore environments are also influenced by low pH, low oxygen waters upwelled from below the continental shelf and advected to shallow nearshore environments (Grantham et al., 2004;Feely et al., 2008;Hauri et al., 2009). Even though the low-pH, low-oxygen conditions generally persist in the nearshore environment for only a few hours, this can happen routinely (50- 200 times / yr) in upwelling-driven ecosystems such as the California Current and Humboldt Current large marine ecosystems (Garcia-Reyes and Largier, 2010;Booth, 2011;Micheli et al., 2012).

It is assumed that many benthic animals living in nearshore marine environments are either adapted to the local natural fluctuations of pH and dissolved oxygen (DO), or tolerate occasional short-lived exposure to potentially stressful conditions (Alenius and Munguia, 2012;Vaquer-Sunyer and Duarte, 2008). Nevertheless, more frequent and prolonged upwelling or hypoxic events induced by climate change (Garcia-Reyes and Largier, 2010) may impact marine animals. Local extremes in several factors, associated with upwelling (e.g. oxygen, pH, and temperature), could be stressful for the population. In particular, juveniles and other sensitive life stages may be highly vulnerable to these extremes. To date, however, few experimental studies have evaluated the influence of upwelling-related exposure to low pH and O_2 events on the mortality or growth of nearshore marine animals.

Individual variation in response to elevated environmental CO_2 and decreased O_2 is another concern when investigating the response of organisms to upwelling events. Even when environmental change causes significant negative impacts on most individuals' performance,

tolerance by a subset of the population may promote adaptation for population persistence (Sih et al., 2012;Charmantier et al., 2008). High variation among individuals in response to elevated CO_2 has been shown to represent genetic diversity in populations of some marine taxa (Sunday et al., 2011;Langer et al., 2006;Pistevos et al., 2011) and this high individual variation could benefit the population and species as a whole.

Here we examine the effect of fluctuating exposure to low pH and low oxygen water on juvenile stages of the red abalone, *Haliotis rufescens*. It is a large gastropod mollusk inhabiting lower intertidal to shallow subtidal (to 30 m depth) environments from Oregon to Baja California (Micheli et al., 2008;Rogers-Bennett et al., 2007). As a key recreational fishery and aquaculture resource in California, it is legally harvested north of San Francisco and also cultured in abalone farms. To assess the sustainability of red abalone populations, their sensitivity to current environmental stressors should be identified. We investigated how fluctuating exposure to low pH and low oxygen waters affects the mortality and growth of juvenile abalone using a pulse exposure experiment mimicking upwelling conditions. Booth et al. (2012) reported that the mean duration of upwelling nearshore water (17 m in depth) with pH 7.6 and DO 4.6 mg/L off Monterey CA is 2.4 hrs, with a maximum duration of 40 hrs. Moreover, pH can drop dramatically by ca. 0.4 units within an hour. First, we studied the effects of short-term (3 – 6 h) exposure to low pH (pH 7.5) and low oxygen (5 mg/ L) conditions that occur typically during coastal upwelling events (Booth et al., 2012). Second, to determine how abalone respond to prolonged low pH and hypoxic events, we extended the exposure to "upwelling" conditions to 24 h twice within a 2 week period – an uncommon, but occasional event in upwelling regions (Booth et al., 2012;Micheli et al., 2012).

2. MATERIALS AND METHODS

2.1. Study species

Juvenile abalone (*Haliotis rufescens*, shell length 4-12 mm, 4-5 months of age) were acquired from the Abalone Farm Co. located in Cayucos, California, where many individuals collected over several years from Southern California abalone populations are reared at ambient temperature (Mean ± SD: 11.9 ± 0.76). Juveniles in this study were maintained at Hopkins Marine Station of Stanford University (HMS) in aquaria with replenishing water flow at ambient temperature (Mean ± SD: 12.6 ± 3.4, 11-13.8 °C) and pH (Mean ± SD: 7.79 ± 0.16,Ranges: 7.61-7.98). They were fed *ad libitum* with small portions of ground Hikari ® algae wafers every other day. The opening of each jar was covered by a 12×12 cm nylon mesh (1×l mm mesh size), secured with a rubber band. For each experiment, jars with juvenile abalone were maintained at HMS for 3 days. Jars were then transferred to the Monterey Bay Aquarium Research Institute (MBARI), Moss Landing, California, and immersed in a transparent plastic holding tank (60×30×35 cm) with flow-through circulation in a chilled environmental chamber for at least 3 days for acclimation. The water temperature of the holding tank was maintained at approx. 9 °C using two heaters and water pH_T (total scale) was maintained at pH 8.0 (normal).

2.2. Treatment protocol

Three treatments with two different levels of pH and oxygen were used: 1. Low pH (7.5) & Low O_2 (40% saturation, 5 mg l^{-1}) =‘ upwelling’’; 2. High pH (8.0) & Low O_2 = ‘ low oxygen’ and 3. High pH (8.0) & High O_2 (100% saturation, 12 mg l^{-1}) as a ‘control’ (Table 1). A low pH and high DO treatment was not included in this experiment due to space, and because the primary focus of the experiment concerned the effects of upwelling conditions rather than acidification alone.

Seawater pH, oxygen, and temperature levels were controlled using a gas-regulated aquarium system (Barry et al., 2008). This system is capable of modifying ambient seawater

110 from MBARI's ocean intake to produce water with specified pH, pO_2, and temperature. For
111 each treatment, water produced by the control system was delivered at approx. 30 ml/min to
112 each jar through a 3 cm diameter PVC manifold, and a 10 mm diameter hose. Consequently,
113 when experimental animals in the holding tanks were transitioned to treatment conditions
114 (ΔpH, ΔpO_2), inflowing treatment waters would mix with normal water in each jar causing
115 the pH and pO_2 to approach specified treatment levels over ca. 15-20 minutes as treatment
116 waters were flushed from the jar.

117 Seawater temperature for all treatments was maintained at 6 °C. Though this low
118 temperature may be near the minimum level tolerable level for red abalone, the two to three
119 degree difference between the holding tank and treatment water effectively mimicked the
120 temperature reduction associated with upwelling in the central and northern Pacific coast of
121 USA (e.g. Point St. George, CA, and Oregon).

122 The pH and DO of seawater of treatment waters produced by the aquarium control system
123 were monitored continuously. In addition, conditions were measured in one randomly-
124 selected jar at least twice for each exposure period, using a portable pH, DO meter (Thermo
125 Scientific®: Orion 5 Star Series) (Table 1). Continuous monitoring of pH in control waters in
126 the aquarium system was not available for the experimental period. Measurements of the
127 ambient pH water (nominal pH 8.0) at MBARI performed before (Oct 26, Nov 22, Dec 16
128 2010) and after the experiment (Mar 30, 2011) using the spectrophotometric method of
129 Dickson et al. (2007) were pH 7.99 ± 0.04 (SD), and were very similar to values measured
130 for the high pH & low O_2 (pH 8.01 ± 0.18) treatment and control (high pH & high O_2)
131 treatment (pH 8.09 ± 0.18) during the experiment using a portable pH meter. Therefore, we
132 assumed that there was little deviation from pH 8.0 both in the high pH treatment water and
133 in normal water in the holding tank at MBARI. However, aquaria at HMS during the
134 recovery period for experiment II experienced rather low pH (See the supplementary Figure

1a). pH meters were regularly calibrated with pH values of Fisher Scientific® buffer salt solutions (pH 6.86 and pH 9.18, total scale) measured by a UV spectrophotometer (UV-1601 Shimadzu®). To determine the calcite and aragonite saturation states of treatment waters, samples were collected from all treatments three times after the experiment. pH of the samples was measured using the spectrophotometric method (Dickson et al., 2007) and DIC (Dissolved Inorganic Carbon) was measured by non-dispersive infrared analysis (LI-COR model 6262), as detailed by Friederich et al. (2002)

2.3. Experiment I

We tested the effects of repeated short-term exposure on the mortality of juvenile abalone in a controlled laboratory experiment. Twenty juvenile abalone individuals (Mean ± SD shell length: 7.36 ± 0.76 mm) were assigned to each of twelve 500ml transparent glass jars with seawater. After 3 days of acclimation to natural (high O_2 (100% sat.), high pH_T (8.0)) conditions in the holding tank, 4 randomly-selected jars were assigned to each of 3 treatments (control, upwelling, and low oxygen treatments). At the completion of the acclimation period, abalone were exposed to their assigned treatment for 3 h, the returned to the holding tank for 3 days. This 3h treatment: 3d control cycle was then repeated once. Abalone were then exposed to treatment conditions for 6h, followed by a 2 week recovery period (See SM Fig. 1).

The mortality of juvenile abalone in each jar was checked immediately after exposure to treatment conditions and daily during the 3 day and 2 week recovery periods. To determine the live / dead status of juvenile abalone, they were overturned and then pricked using a dissecting pin. Individuals that did not respond to the pin were considered dead, and were transferred to vials containing 70% ethanol solution. Due to the short duration of this experiment, growth rates of abalone were not measured.

2.4. Experiment II

To test the effects of more prolonged periods of exposure to upwelling waters, we used an experimental protocol similar to the Experiment I, but with extended periods of exposure to treatment waters. Twelve abalone juveniles (Mean ± SD, shell length: 7.42 ± 1.23 mm) were allotted to each of 18 jars. Each individual was marked with a combination of color dots on the shell using paint markers, which were then coated with instant glue to preserve the color coding. The shell length and width of each individual was measured using a digital caliper to the nearest 0.01mm. The control and treatment waters were the same used in the previous experiment. After an acclimation period of 3 days under control conditions, abalone were exposed to their assigned treatment conditions for 24h, followed by immersion in water in the holding tank for 6 days. Abalone were then returned for 24 h to treatment waters, and then immersed in the holding tank for 6 days again and then returned to the aquarium at HMS for recovery and immersed for 11 days (SM Fig. 1). The survival of each abalone was checked on the 1^{st}, 2^{nd}, 3^{rd}, 5^{th}, and 6^{th} day after each exposure. The shell length and width of each individual were measured after its death or at the end of the experiment, for both exposures.

2.5. Statistical analyses

Mortality was calculated by dividing the number of dead abalone by the number of abalone initially placed in each jar. We calculated the cumulative mortality of all abalone in each jar after each exposure time point and used repeated measures, one-way ANOVA for each exposure, including post-exposure periods. Repeated-measures ANOVA was then applied to the entire experimental period with the same abalone group. In all repeated-measures analyses, the assumption of equal between-group correlations and group variances ("sphericity") was not violated (Mauchly's test, all $p > 0.05$). When significant differences

were detected among treatments, Tukey tests were applied for post-hoc comparisons.

The daily proportional rate of change in shell length (growth) was calculated as:

$$\frac{L_f - L_i}{L_i} \times \frac{1}{D_{if}}$$

L_f is the final shell length at the time of death or in the end of the experiment, L_i is the initial shell length measured before the exposure experiment, D_{if} is the interval (days) between measurements of initial shell length and final shell length.

We assumed linear growth for juvenile abalone because their shell lengths were similar at the beginning of the experiments (for both experiments) and the experimental period was short. To evaluate the relationship between size and growth, we plotted daily proportional growth rate versus initial shell size and found no significant slope ($F_{1,149} = 1.80$, $p = 0.1818$), indicating that, at least in the range of sizes used here, growth rate is unrelated to initial size.

To verify if both treatment level and live/dead status at the end of the experiment influence the proportional shell growth, we applied a two-way ANOVA. All growth data were transformed by arcsine square root prior to analysis. Fisher's PLSD tests were applied for post-hoc comparisons. An *F*-Test of equality for variances was used to determine differences in the variance of growth rate between treatments.

3. RESULTS

3.1. Experiment I

Abalone mortality did not differ among treatments during both 3 h exposure periods (1st exposure: $F_{2,9} = 1.286$, $p = 0.322$; 2nd exposure: no mortality in any treatments) or during the 3 days after those exposures (1st exposure: $F_{2,9} = 0.300$, $p = 0.7479$, 2nd exposure: $F_{2,9} = 1.544$, $p = 0.265$, Fig. 1a). There was also no significant difference in mortality between treatments during the 6 h-exposure ($F_{2,9} = 1.000$, $p = 0.405$) or post-exposure periods ($F_{2,9} =$

0.459, p = 0.645, Fig. 1a). Overall, there was no significant difference in cumulative mortality among treatments during Experiment I ($F_{2,9}$ = 0.175, p = 0.841).

3.2. Experiment II

Repeated measures ANOVA indicated significant variation in mortality among treatments following 24 h exposures to treatment levels, for up to 3 days after exposure (2 days: $F_{2,15}$ = 4.059, p = 0.039, 3 days: $F_{2,15}$ = 3.966, p = 0.041, Fig. 1b). Mortality was significantly higher in the low oxygen treatment than the control (Tukey post-hoc test, p = 0.035) but all other comparisons among treatments were not significant. Cumulative mortality over 4 days after exposure to control or treatment conditions did not vary significantly, indicating that the effects of exposure may not persist beyond 4 days.

After the first 24 h exposure, the daily proportional growth rate did not differ among treatments (ANOVA: $F_{2, 27}$ = 3.168, p = 0.0586). By the end of the second 24 h exposure to treatment conditions, growth rates differed significantly between treatments ($F_{2, 149}$ = 16.509, p < 0.0001, Fig. 2). In fact, shells in the upwelling treatment (low pH and low O_2) showed negative growth, significantly lower than for animals from control or low-oxygen conditions (p < 0.0001 for both). Variation in growth rate was significantly different between treatments (F-test of variance: $F_{2, 149}$ = 16.509, p < 0.0001). In particular, it was higher in the upwelling treatment than in control (F-test of variance: $F_{53, 50}$ = 0.411, p < 0.0001) and low-oxygen treatments ($F_{49, 52}$ = 0.411).

Whether abalone individuals died during the experiment was not related to differences in growth. Comparison of "alive" or "dead" status or its interaction with treatment did not influence the growth rate (Two-way ANOVA: Alive/dead status: $F_{1, 146}$ = 0.116, p = 0.7343, Alive/dead status × treatment : $F_{1, 146}$ = 1.011, p = 0.3665).

4. DISCUSSION

These experiments indicate that repeated short-term (3-6 h) exposure of abalone juveniles to conditions documented during upwelling (Booth, 2011) has no detectable immediate effect on their survival. In contrast, periodic, prolonged exposure (24 h twice) to low oxygen significantly decreased survival and exposure to upwelling treatment significantly reduced the shell growth. These results suggest that abalone populations may be adapted to fluctuations in pH and O_2 over time scales typically occurring along the central California coast, but not to more prolonged duration of low pH and O_2, similar to those documented off the coast of Mexico (Micheli et al., 2012). Low O_2 may always be stressful, but tolerable for short periods. Extended periods of hypoxia and/or hypercapnia may lead to accumulation of impacts and eventually reduced growth or death.

Because extended exposure to low oxygen *per se* has lethal effects on juvenile abalone, oxygen concentration is likely to be a crucial factor influencing the persistence of abalone populations. However, seawater pH should be also considered as an important factor influencing abalone populations because low pH has a deleterious effect on growth. Growth can influence reproductive success and susceptibility to predation (Rossetto et al., 2012). Shell length of individuals exposed to the low pH, low O_2 treatment simulating upwelled waters decreased, suggesting that shells were dissolving in the low pH water. Given that ocean acidification did not change the expression of shell growth genes in the larval stage in a previous experiment (Zippay and Hofmann, 2010), epigenetic processes or differential physiological investment might have influenced the shell growth. The aragonite and calcite saturation states of the upwelling treatment waters were less than 1 (Table 1), which should increase the energetic cost of shell formation for abalone (Portner, 2008;Byrne et al., 2011).

The effects of low pH and low oxygen may have different effects on the growth of different life stages and different species. Interestingly, mortality did not differ between the

control and upwelling treatment with low pH and low O_2. Our expectation was that exposure to low pH waters during upwelling, coupled with low oxygen levels, would be the most stressful, leading to reduced survival or growth or both. Our results suggest otherwise, and further information is required to understand the combined effects of pH and O2 on abalone performance.

Although very few data are available concerning variation in the carbonate chemistry of nearshore waters in Monterey Bay, time-series measurements of conditions at the intake pipe for the Monterey Bay Aquarium indicate that the average duration of upwelling conditions (~4.6 mg $.L^{-1}$ O_2, pH ~7.6) in nearshore Monterey Bay environments is 2.4 ± 2.6 h (Booth, 2011). Juvenile abalone appear to have the capacity to cope with short exposure to these conditions. Prolonged upwelling conditions stressful to juvenile abalone may occur only rarely. Over the past 12 years, the maximum observed duration of low oxygen and low pH events nearshore Monterey Bay was 40 h (Booth, 2011), similar to recent measurements off Baja California (Micheli et al., 2012). A trend towards prolonged upwelling conditions associated with recent climatic changes (Garcia-Reyes and Largier, 2010) is expected to increase the frequency and duration of low-oxygen, low-pH events (Feely et al., 2008;Nam et al., 2011;Melzner et al., 2012), possibly leading to more frequent prolonged exposure.

Prolonged upwelling conditions can have a significant impact on the growth of juvenile abalone, and possibly other calcifying, marine invertebrates (e.g., Vaquer-Sunyer and Duarte, 2008;Kroeker et al., 2010). Changes in individual performance (growth, survival, reproduction) of abalone in response to future ocean conditions will likely have significant, but poorly known effects on the demographic dynamics of California abalone populations and the ecology of nearshore ecosystems.

Our observation of increased individual variation in growth rate after exposure to upwelling conditions is intriguing. Such variation among individuals suggests that phenotypic

plasticity or genetic variation or both promote a range of performance within the population in response to shifts in pH and oxygen. While a large portion of the population may experience reduced growth or survival, some individuals may acclimate to extended upwelling events by maintaining their calcification, thereby promoting adaptation through enhanced survival and fitness by this subpopulation. This is in agreement with results of other studies that have shown high individual variation of marine animals in response to low pH conditions (Sunday et al., 2011;Schlegel et al., 2012;Pistevos et al., 2011). Phenotypic plasticity of abalone populations may explain higher individual variation in growth rate in response to low pH and low oxygen. Cryptic genetic variation might also be expressed when juveniles are exposed to the novel environments (Ghalambor et al., 2007). Some part of the reaction norm can be adaptive to new conditions. If such adaptive variation is heritable, red abalone may be able to adapt to extended exposure to upwelling conditions. Indeed, recent genetic information on red abalone suggests that genes for biomineralization and resistance to hypoxia are under selection in upwelling regions (de Wit and Palumbi, 2013). Gene expression studies of abalone responses to upwelling conditions are likely to shed light on this issue.

Low O_2 and low pH events during upwelling periods are largely coupled because these parameters are regulated by respiratory oxygen consumption and carbon dioxide release by the deep water biological community. Advection of deeper, low O2, low pH waters toward the surface and inshore leads to exposure of coastal taxa to potentially stressful environmental conditions (Bianucci et al., 2011; Nam et al., 2011). Future increases in the influx of CO_2 from the atmosphere to the ocean will further reduce the pH of upwelled waters (Feely et al., 2008;Feely et al., 2010), and will decouple, to some extent, the linkage between oxygen and carbon dioxide in these waters. This study did not assess the influence of increasing temperature, which is also changing with upwelling, pH, and oxygen. Further

study of the effects of projected changes in ocean conditions in upwelling regimes on diverse nearshore species are needed to understand and predict future shifts in the structure and function of coastal ecosystems.

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433 Table 1. Carbonate system and other physical parameters for experimental treatments
434 measuring the response of juvenile abalone to upwelling conditions (Mean ± SD).
435
436

Variable	Treatments		
	High pH & Low O_2 (low-oxygen)	Low pH & Low O_2 (upwelling)	High pH & High O_2 (control)
Measured pH (total scale)	8.00 ± 0.05	7.51 ± 0.08	7.93 ± 0.03
DO (mg l^{-1})	5.99 ±0.91	6.38 ±1.53	11.68 ±2.86
TCO_2 (µmol kg^{-1})	2116.10±56.74	2233.33±54.29	2196.72 ± 141.23
Salinity (ppt)	33.0±0.1	33.0 ±0.1	33.0 ±0.1
Temperature (°C)	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
PCO_2 (µatm)	434.36±47.33	1424.36±224.91	535.48 ± 23.65
Alkalinity (µEq kg^{-1})	2249.75 ± 80.59	2217.70 ± 73.34	2310.09 ± 152.68
Calcite saturation	2.43 ± 0.38	0.85 ± 0.19	2.23 ± 0.25
Aragonite saturation	1.53 ± 0.23	0.54 ± 0.12	1.41 ± 0.16
HCO_3^- (µmole · kg^{-1})	1993.14 ± 43.57	2125.44 ± 56.19	2076.88 ± 130.79
CO_3^{2-}(µmole · kg^{-1})	100.86 ± 15.67	35.40 ± 7.80	92.58 ± 10.55

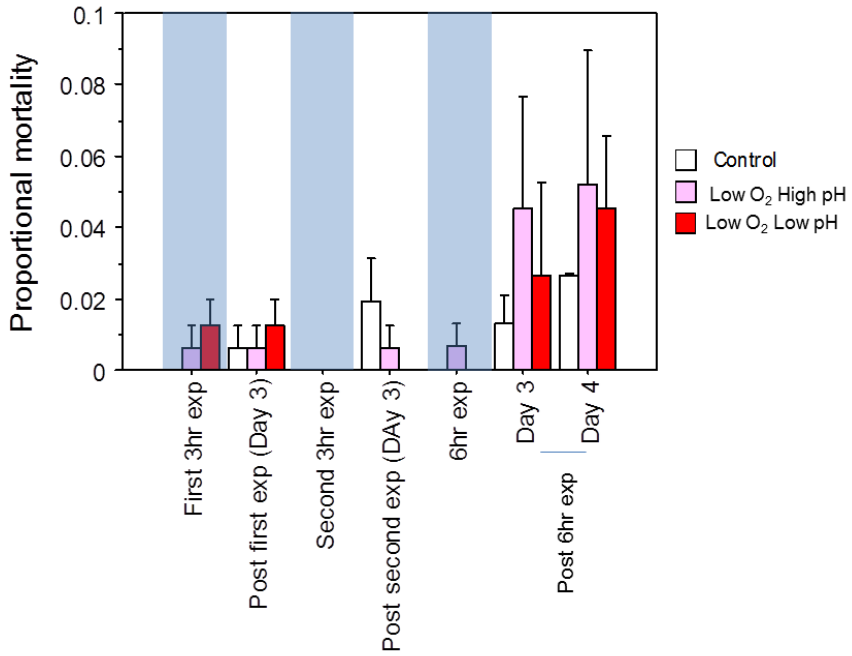
437

438 The parameters were calculated with CO2sys (Pierrot et al., 2006) using the pH and TCO_2
439 values with dissociation constants from Dickson & Millero (1987) and KSO_4 using Dickson

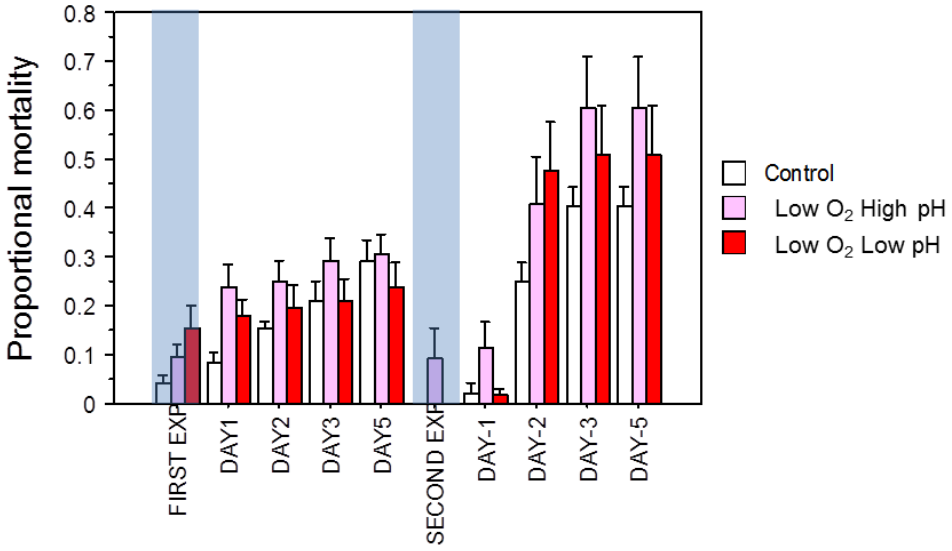
(1990).

Figure 1.

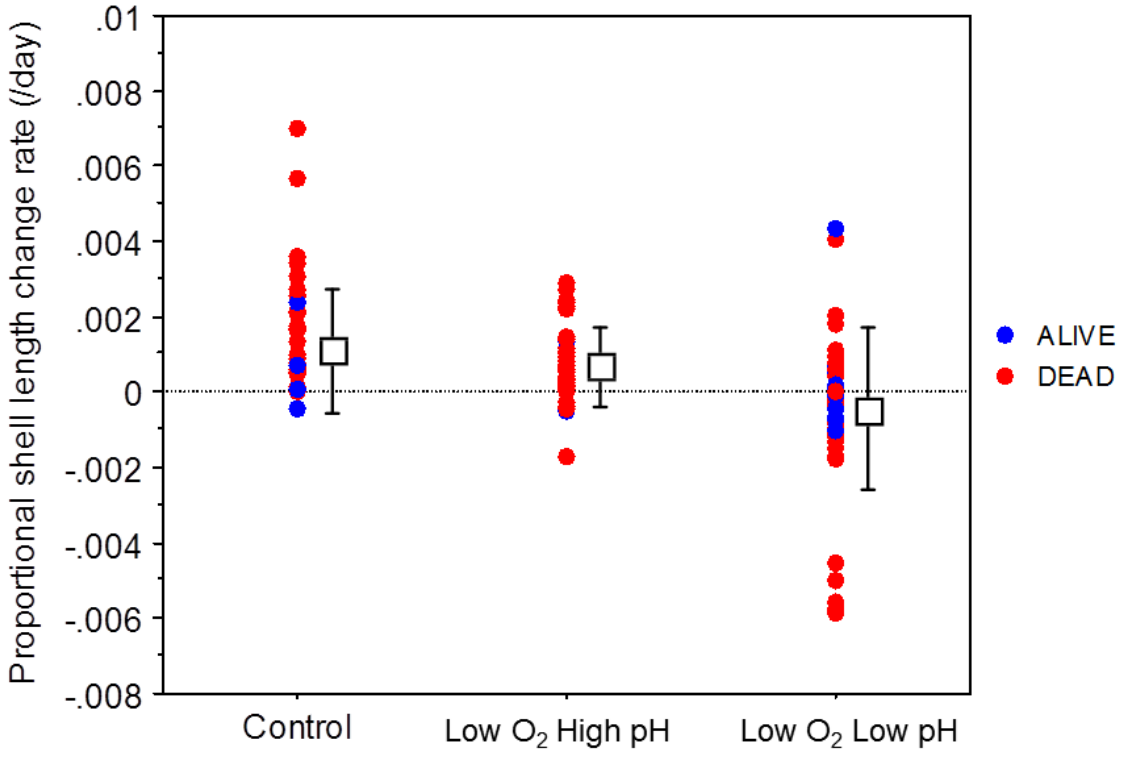
(a)


Proportional mortality
0.1
0.08
0.06
0.04
0.02
0
First 3hr exp
Post first exp (Day 3)
Second 3hr exp
Post second exp (DAy 3)
6hr exp
Day 3
Day 4
Post 6hr exp
Control
Low O2 High pH
Low O2 Low pH

(b)


Proportional mortality
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
FIRST EXP
DAY1
DAY2
DAY3
DAY5
SECOND EXP
DAY-1
DAY-2
DAY-3
DAY-5
Control
Low O2 High pH
Low O2 Low pH

446 Figure 2.

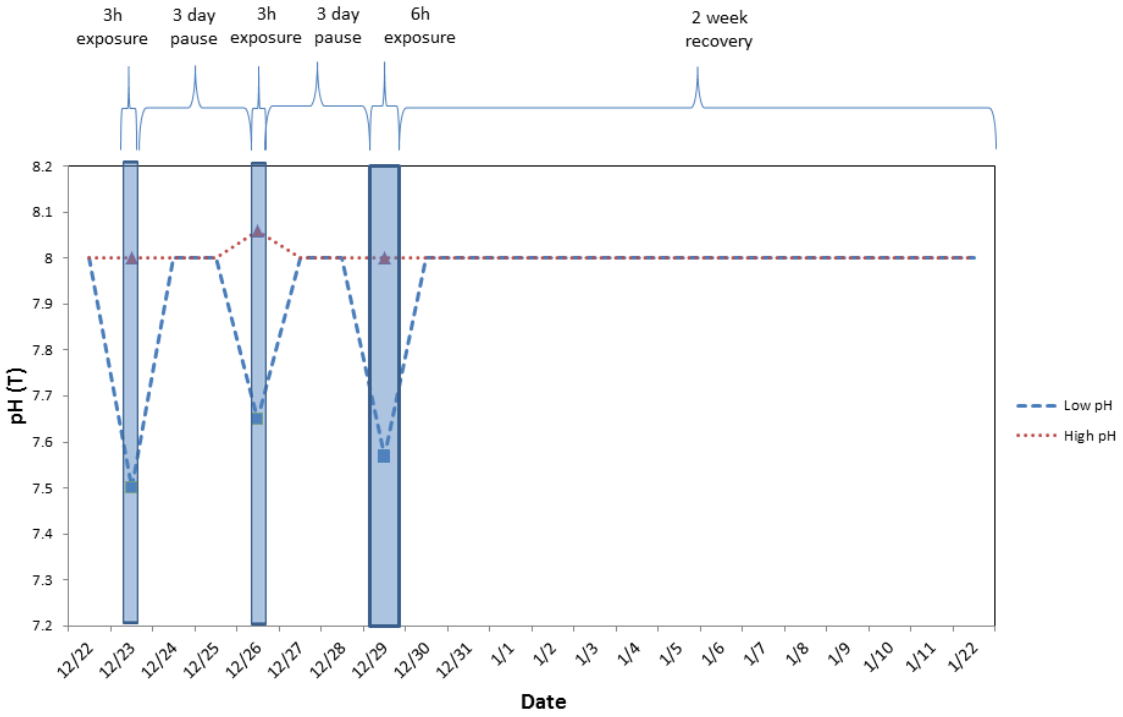


447

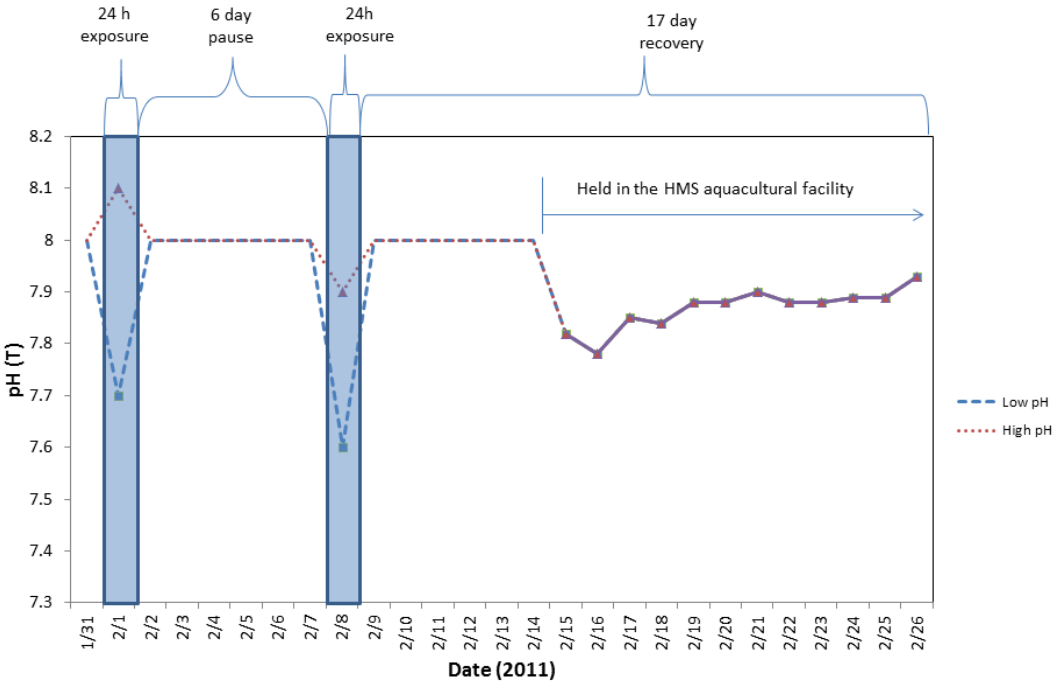
448

SM Figure 1

(a)



(b)



454 Figure legends

455

456 Fig. 1. *Haliotis rufescens*. Cumulative mortality (Mean ± s.e.) of juveniles abalone after
457 each exposure to control, upwelling, and low-oxygen treatments. (a) Experiment I. (b)
458 Experiment II. Shaded days represent exposure of abalones to different treatments. "Day
459 number" represents the days after the exposure treatment.

460

461 Fig. 2. *Haliotis rufescens*. Daily proportional change in shell length of juveniles among
462 control, upwelling, or low-oxygen treatments. Square dot and line represent mean ± s.e.

463

464 SM Fig. 1. Diagram describing treatment exposure and recovery schedule and pH values
465 changing for (a) Experiment I and (b) Experiment II. Red triangle (high pH) and blue square
466 (low pH) dots represent the pH values measured using a pH meter and dashed line represent
467 the assumed pH value throughout the experiment.

468

469 Short tile: Low pH and O_2 effects on abalone