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The effects of intermittent exposure to low pH and oxygen conditions on survival and growth of juvenile red 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 5–10 mm). 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 h 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 repeated twice over a 15 day period, juveniles experienced higher mortality in the low oxygen treatments compared to control conditions, regardless of pH levels (pH 7.5 vs. 8.0). Growth rates were reduced significantly when juveniles were exposed to low pH or low oxygen treatments and the growth was lowest when low pH exposure was combined with low O_2. Furthermore, individual variation of growth rate increased when they were exposed 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 both pH and oxygen is a crucial factor for their growth. However, given the higher individual variation in growth rate, they may have an ability to adapt to extended exposure to upwelling conditions.

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).

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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 yr^{-1}) in upwelling-driven ecosystems such as those of the California Current and Peruvian upwelling 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. Nevertheless, frequent and prolonged upwelling or hypoxic events induced by climate change (Garcia-Reyes and Largier, 2010) may impact marine animals, particularly those that produce calcified structures (e.g. Kroeker et al., 2011). 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*. The red abalone, *Haliotis rufescens*, 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

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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. First, we studied the effect of short-term exposure (from 3–6 h) to low pH (pH 7.5) and low oxygen (5 mg L^{-1}) conditions that occur typically during coastal upwelling events (Booth et al., 2012). Second, to determine how abalone responded 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 and maintained at Hopkins Marine Station of Stanford University (HMS) in aquaria with replenishing water flow at ambient temperature (12–14 °C). They were fed 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 × 1, 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. 8 °C using two heaters and water pH was maintained at pH 8.0 (normal).

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2.2 Treatment protocol

Three treatments with two different levels of pH and oxygen were used: 1. low pH (7.5) and low O_2 (40 % saturation, 5 mg L^{-1}); 2. high pH (8.0) and low O_2; and 3. high pH (8.0) and high O_2 (100 % saturation, 12 mg L^{-1}), as a control treatment mimicking typical conditions. 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 regulated using an automated gas-regulated aquarium system (Barry, 2008). Water for each treatment was produced using this system in 3 separate treatment reservoir tanks. For each treatment, water was delivered at 42 mL min^{-1} to each jar from the treatment reservoir through a 3 cm diameter PVC manifold, and a 10 mm diameter hose. Seawater temperature for all treatments was maintained at 6 °C. Though this low temperature may be near the minimum level tolerable level for red abalone, the two to three degree difference between the holding tank and treatment water effectively mimicked the temperature reduction associated with upwelling in the central and northern Pacific coast of USA (e.g. Point St. George, CA, and Oregon). pH in each reservoir tank was monitored using Honeywell® pH electrodes. Oxygen and temperature were monitored using oxygen optodes (Aanderaa Inc., model 3835, www.aadi.no). pH and DO of seawater in each treatment were additionally checked in one randomly-selected jar at least twice for each exposure period, using a portable pH, DO meter (Thermo Scientific®: Orion 5 Star Series) (Table 1). This meter was regularly calibrated with pH values of Fisher Scientific® buffer salt solutions (pH 6.86 and pH 9.18, total scale).

2.3 Effects of short-term exposure

We tested the effects of repeated short-term exposure on juvenile mortality in a controlled laboratory experiment. Twenty juvenile abalone individuals were assigned to each of twelve 500 mL transparent glass jars with seawater. After 3 days of acclimation

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to control (O_2 100 %, pH 8.0) conditions in the holding tank, 4 randomly-selected jars were assigned to each of 3 treatments. Abalone were exposed to each treatment for the scheduled duration from 3 h to 6 h (see Supplement Fig. 1). After each exposure, abalone mortality was checked within each jar. All jars were then transferred to the holding tank again and maintained in control conditions for scheduled days. To check the mortality of juvenile abalone after exposure to each treatment, abalone 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. Juvenile mortality was recorded each day, and dead abalone, if any, were transferred to vials for later measurement. Because the short-term exposure experiment did not render enough time for abalone to grow, the growth rate was not measured at this time.

2.4 Effects of long-term exposure

To test the effects of long-term exposure, we used an experimental protocol similar to the short-term exposure experiment. Twelve abalone juveniles were allotted to each of 18 jars to increase the replication. 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 0.01 mm. The treatments were the same as the previous experiment except the exposure to treatment conditions (i.e. non-control waters) was extended from 3–6 h to 24 h twice (Supplement Fig. 1). Approximately forty-eight hours is the maximum consistent exposure duration to low pH and low DO water recorded in the Monterey Bay Area (Booth et al., 2012). However, we were more curious about the critical exposure time and frequency for abalone survival and growth rather than the effect of maximum duration exposure to low pH and DO events. After each exposure, the survival of each individual was checked and jars with remaining abalones were again immersed in control conditions in the holding tank for 1 week after the first exposure and for 17 days after the second exposure in the holding tank.

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The survival of each abalone was checked on the 1st, 2nd, 3rd, 5th, and 6th days 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

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

$$\frac{L_\mathrm{f} - L_\mathrm{i}}{L_\mathrm{i}} \times \frac{1}{D_\mathrm{if}}$$

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.

One-way ANOVA was then applied to assess differences in shell growth among treatments. 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.

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3 Results

3.1 Effects of short-term exposure

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 the cumulative mortality during short-term exposures ($F_{2,9} = 0.175$, $p = 0.841$).

3.2 Effects of long-term exposure

Repeated measures ANOVA indicated significant differences 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 high pH and low O_2 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 exposure, the rates of growth (proportional increase in length) did not differ among treatments (ANOVA: $F_{2,27} = 3.168$, $p = 0.0586$). After a second 24 h exposure to treatment conditions, however, growth rates differed significantly between treatments ($F_{2,147} = 11.231$, $p < 0.0001$, Fig. 2). Abalone in both high pH and low O_2 (Fisher's PLSD, $p = 0.04$) and low pH and low O_2 ($p < 0.0001$) had significantly lower growth rates than controls. In fact, shells in the low pH and low O_2 treatment showed negative growth, which was significantly lower from that in high pH and low

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O_2 treatment ($p = 0.006$). Variation in growth rate was significantly higher in low pH and low O_2 treatment than in control plots (F-test of variance: $F_{47,53} = 0.534$, $p = 0.02$) whereas all other comparisons among treatments were not significant.

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, prolonged exposure (24 h twice) to these conditions resulted in significant negative impacts on the survival of juveniles. Specifically, exposure to low oxygen significantly decreased survival and growth, and exposure to low pH 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 both lethal and growth-prohibiting 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 stronger effect on growth than low O_2. 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 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. Lower pH in association with high CO_2 reduces the

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calcite and aragonite saturation state of seawater less than 1 if constant alkalinity is assumed from surface waters to upwelling depth (~ 100 m), and should increase the energetic cost of shell formation for abalone composed of aragonite and calcite (Portner, 2008; Byrne et al., 2011). Low pH and hypoxia might have a differential influence on the growth of different life stages and different species. Interestingly, mortality did not differ between the control and low pH, O_2 treatments. Abalone may increase their tolerance to low O_2 events in the low pH conditions by unknown synergistic effects of oxygen and pH. Further information is required to understand the interactive effects of pH and O_2 on abalone performance.

Our results imply that abalone juveniles (*H. rufescens*) along this coast tolerate low oxygen, low pH conditions during typical, short-duration upwelling events. Prolonged events, however, appear to exceed a threshold for stressful conditions, resulting in depressed growth, survival, or both. 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 low O_2 ($4.6\,mg\,L^{-1}$), low pH (7.6) events in nearshore Monterey Bay environments is 2.4 ± 2.6 h (Booth, 2011). Juvenile abalone appear to have the capacity to cope with short immersion under these conditions. Prolonged upwelling conditions stressful to juvenile abalone may occur only rarely. Over the past 12 yr, the maximum observed duration of hypoxic 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., 2013).

Our results suggest that prolonged upwelling conditions can have a significant impact on the survival and growth of juvenile abalones, 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

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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.

On the other hand, greater level of individual variation in growth rate when they were exposed to low pH and low O_2 conditions is intriguing. This indicates that still a subset of the population of which growth rate was not influenced by extended exposure to low pH and low O_2 conditions and the red abalone population might have an ability to adapt to extended upwelling events by maintaining their calcification. This is in accordance 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). However, in terms of individual level, excessive energy should be allotted to maintain their physiology and calcification in response to hypercapnia (Wood et al., 2008) and hypoxia, and this should be verified in the future study.

Low O_2 and low pH events during upwelling should almost always occur simultaneously because these parameters are coupled to respiratory oxygen consumption and carbon dioxide release by the deep water biological community. Advection of deeper, low O_2, low pH waters toward the surface and inshore leads to exposure of coastal taxa to potential environmental stress (Bianucci et al., 2011; Nam et al., 2011). Increasing influx of CO_2 from the atmosphere in the future is expected to make the upwelled waters even more acidic (Feely et al., 2008, 2010), and will decouple, to some extent, the relationship between oxygen and carbon dioxide in upwelled waters. This study also did not include the factor of temperature that is accordingly changing with upwelling conditions. More studies on the effect of future changes in ocean physical and chemical factors involved in upwelling regimes on diverse nearshore species are needed to understand and predict ecological responses.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/10/3559/2013/bgd-10-3559-2013-supplement.pdf.

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Table 1. Mean (±SD) pH and DO of seawater in the jar in each treatment.

	high pH and low O_2	low pH and low O_2	high pH and high O_2 (control)
pH	8.01 (±0.18)	7.58 (±0.49)	8.09 (±0.18)
DO ($mg\,L^{-1}$)	5.99 (±0.91)	6.38 (±1.53)	11.68 (±2.86)

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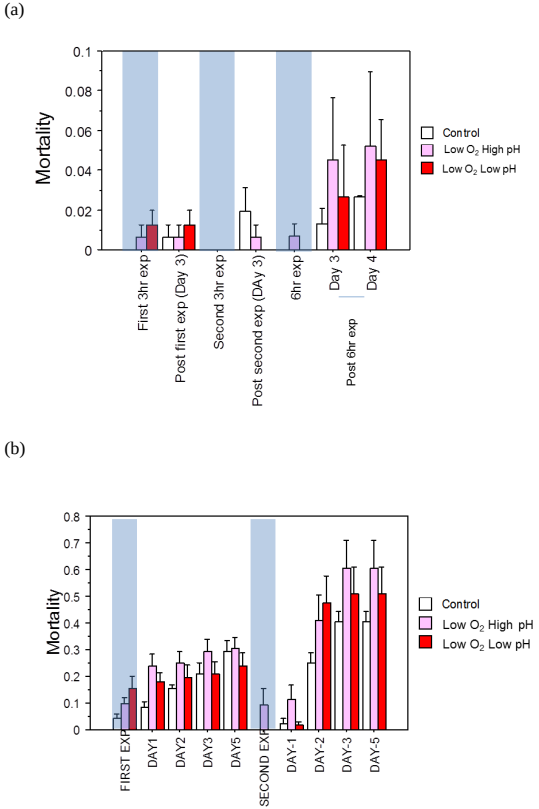

Fig. 1. *Haliotis rufescens*. Cumulative mortality (Mean ± s.e.) of juveniles **(a)** after each short-term exposure to seawater with three levels of oxygen and pH and **(b)** each 24 h-exposure to seawater with three levels of oxygen and pH. Shaded days represent exposure of abalones to different treatments. "Day number" represents the days after the exposure treatment.

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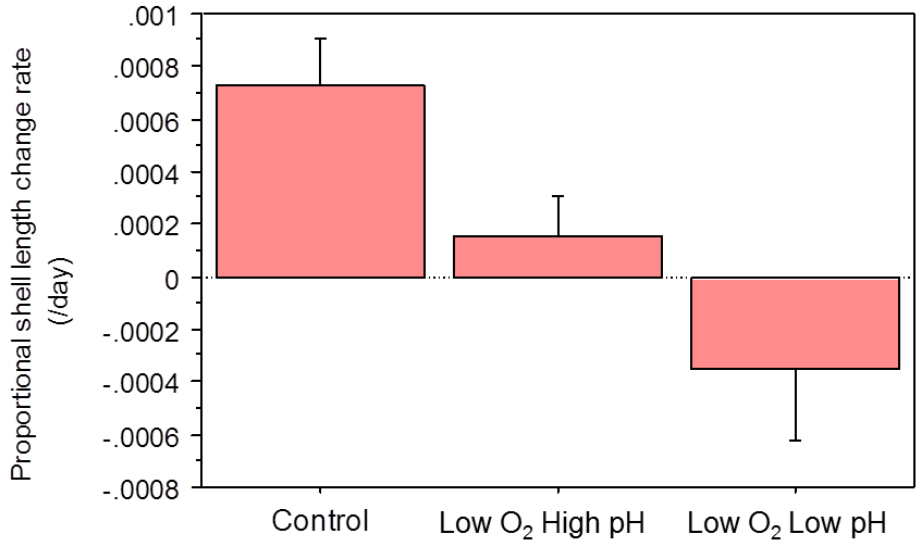

Fig. 2. *Haliotis rufescens*. Proportional shell length change rates (Mean ± s.e.) of juveniles with three levels of oxygen and pH.

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