Effects of Environmental Variability on Metabolism in the Red Abalone, *Haliotis rufescens*

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ABSTRACT

Through fossil fuel emissions, the ocean chemistry is increasing to be more acidic and hypoxic in the deep and warmer at the surface. Through seasonal coastal upwelling from below the continental shelf and advection, acidic and hypoxic waters will be brought toward the coast, which may be stressful for the marine organisms. For the experiment, we tested how the juvenile red abalone, *Haliotis rufescens*’s metabolism will be affected by exposing them to the upwelling condition in the lab. Before the experiment, two sets of abalones were exposed to different environmental condition for five days. The first set was exposed to the upwelling condition and the other set was exposed to the constant condition, which takes the mean of all the environmental variables (7.595 pH; 5.55 mg L^{-1}; 15.1^{o}C). The metabolism rate from both sets of abalones did not show a significant difference, as it indicated that the exposure rate was short. However, during when oxygen rate increased in the upwelling cycle, there was a slight difference in metabolism, suggesting that the abalones from the upwelling exposure had adapted faster to become an oxyregulator when there is excess oxygen in the surrounding.

INTRODUCTION

Marine ecosystems are under threat from anthropogenic induced climate change such as ocean acidification (OA). This phenomenon is resulted through excess burden of fossil fuel CO_2 dissolving into the ocean (Hofmann *et al*. 2010). OA is known to affect marine populations through changes in individual metabolism, growth, and mortality due to their physiological responses (Bibby *et al*. 2008; Michaelidis *et al*. 2005; Lord *et al.* 2017). When CO_2 enters the ocean, it reduces the carbonate ion concentrations along with the saturation states for aragonite and calcite, which disrupts biological calcification processes and substrate binding rates (Booth 2011). It is a higher priority for science and policy making to understand how the effects of OA alone or with other natural and anthropogenic drivers on individual organism will emerge at the population level. Other than OA, nearshore environments are also influenced by low-pH as well as low-dissolved oxygen (DO) and temperature through coastal upwelling. This seasonal phenomenon occurs daily for few hours between Spring through Summer (Booth 2011). In upwelling-driven ecosystems such as the California Current Large Marine Ecosystems (CCLME), large-scale declines in pH, coupled with decreasing DO and shoaling of the oxygen minimum zone (OMZ) will likely impact benthic organisms in nearshore environments (Gilly *et al.* 2012).

The Eastern Pacific Ocean contains the world's largest OMZ and it is contributing to the severe hypoxia documented recently within the CCLME. These OMZs are driven by coastal eutrophication and strong thermal stratification; They are more likely to bear low oxygen levels at lower depth (Keller *et al.* 2017). OMZs from the deep waters can be positioned very close to shore, and can be transported onto the shelf through upwelling. The same coastal eutrophication process that creates OMZs also produce high levels of CO_2 and thus similarly creates low pH and low carbonate ion availability (Gobler and Baumann 2016).

The combination of global OA and deoxygenation with regional upwelling may be particularly stressful for marine organisms. In addition to more frequent and intensified upwelling events due to anthropogenic climate change, marine organisms may suffer in the futuristic environment in the ocean (Garcia-Reyes and Largier 2010). However, only few studies have been conducted on the individual joint effects of multiple stressors on marine species, especially for benthic calcifies within the coastal upwelling systems.

Benthic calcifies are ideal candidates for evaluating the impacts of OA and the interactive effects of DO and temperature (Kim *et al.* 2013). Also, they are unlikely to escape extreme exposure associated with upwelling events. A good model specie that resides in the CCLME is the red abalone (*Haliotis rufescens*). The red abalone is a gastropod mollusk that is a key recreational fishery and aquaculture resource in California (Micheli *et al.* 2008). Red abalones can be easily cultivated in labs and obtained from local abalone farms. A previous research shows that low pH and DO have synergistic negative effects on juvenile red abalone's growth (Kim *et al.* 2013). In particular, juveniles are ideal candidates because they are sensitive to environmental changes and may be highly vulnerable to these extremes, thus easier to observe the effect of the environmental stressors. There are currently series of projects going on to find out the growth and mortality of juvenile red abalones (Boch *et al*. 2017; Kim *et al.* 2013; Zippay and Hofmann 2010). However, there has been no previous research that focuses on the effects of near-future ocean condition in coastal upwelling system on metabolism of red abalone. Understanding the metabolism aspect of the organism provides a baseline to separate environmental sensitivities between species (Boyd *et al.* 2018). Unlike growth, metabolism looks at stress in cellular activity (Sokolova *et al.* 2012). Through metabolic rate, we can narrow the tolerance window of OA and hypoxia in near future upwelling scenario in marine animals.

To assess the sustainability of red abalone populations, their sensitivity and the ability to adapt to futuristic environmental condition should be identified. In this project, we examined how juvenile red abalone's metabolism can adapt by measuring their respiration in a near futuristic upwelling scenario. First, we had two sets of juvenile abalones going through different exposure conditions for five days. One set of abalones were exposed to near futuristic upwelling scenario while the other set of abalones were exposed to the mean environmental condition of the upwelling scenario. Second, we exposed both sets to the near futuristic upwelling scenario and measured the respiration of the abalone during when the environmental stressors were at its mean, low, and high in the upwelling cycle.

MATERIALS AND METHODS

ACQUISITION

Juvenile abalones were purchased from American Abalone Farm in Davenport, CA. When the abalones were brought to the lab, each was marked with a bee tag with an assigned number and color. Individual abalones were then placed in 1 L tub filled with control seawater (8.0 pH and 13^{o}C) while being fed *Macrocystis perifera*.

EXPOSURE

When the experiment started, juvenile abalones (shell length 30-40mm) in groups of three were exposed to either the variable or the constant environmental condition. The variable condition consisted of a cycle of 360 min of relaxation stage (7.7 pH; 7.2 mg/L^{-1}; 16.6^{o}C), 180 min of upwelling stage (7.42 pH; 2.8 mg L^{-1}; 12.6^{o}C), and 90 min of transition stage between the two stages. The constant condition took the mean values between the two stages in the variable condition (7.595 pH; 5.55 mg L^{-1}; 15.1^{o}C). Abalones in both exposures were acclimated in their designated environment for five days. The pH, DO, and the temperature in the two exposures were controlled through the gas-regulated aquarium system at the Monterey Bay Aquarium Research Institution (Barry *et al.,* 2008). This system modifies ambient seawater from the institution's ocean intake to produce water with system controlled environmental variables. Individual abalone were placed into 473 mL glass jar with two holes for consistent water flow. The water produced by the control system was delivered at 30 mL min^{-1} to each jar through a 3 cm diameter PVC manifold, and a 10 mm diameter hose. During the acclimation, abalones were not fed.

EXPRIMENT SETUP

After five days of exposure, the abalones were placed into a separate 473 mL glass jar with a magnetic mixer and an elevated plastic sheet. The lid of the jar had two three-way gates and a DO meter (Vernier Optical DO Probe®). One of the three-way valve was used to connect to the control system via the RVC manifold, the other valve was used to control the outflow of the water. The jar was placed in a temperature bath filled with the outflow seawater from the jar. The tub is made of a plastic container which is connected to a rubber tube to prevent overflow. For the magnetic mixer to function, we put the mixer underneath the respiration system.

SAMPLING

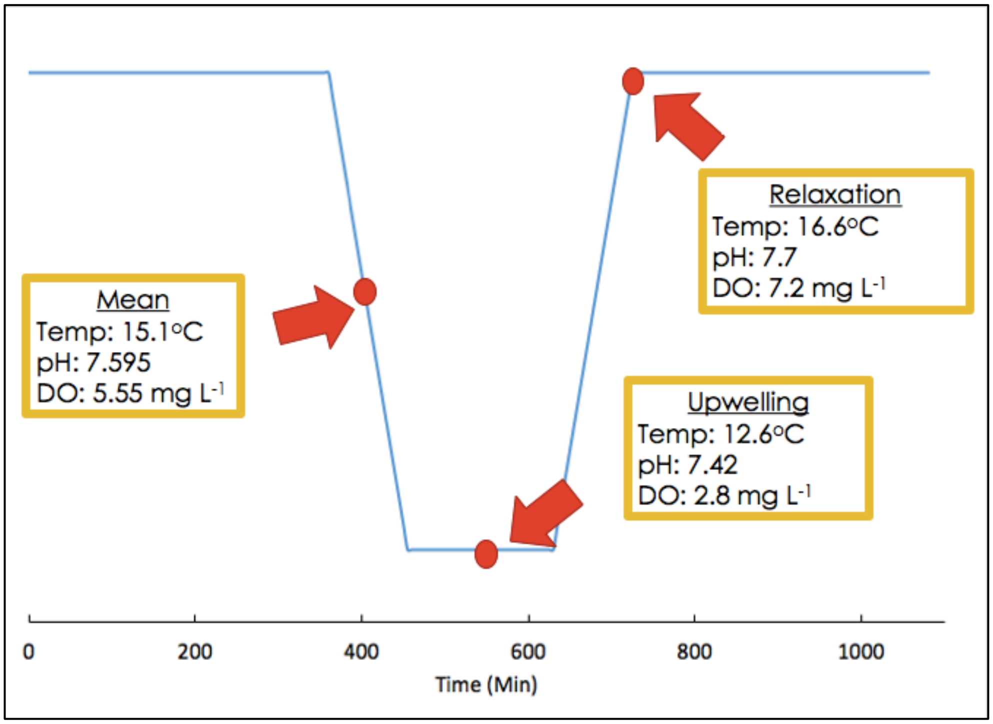

Figure 1. Sampling points and their environmental condition for each upwelling cycle.

Abalones from both exposures were placed under the variable condition. The experiment went through three upwelling cycles and the slope of the DO were measured at the cycle's high, mean, and low points (Fig 1). When the slopes were measured, the 3-way valve was used to stop the water flow. The sample's measuring period was approximately 1000 seconds. After the experiment, all six abalone's wet and buoyant weights were measured. We used the average slopes from the sampling point of each replicating cycles_to calculate the individual abalone's metabolic rate.

RESULTS

Two-way ANOVA indicated that no significant variation in metabolic rate between the individuals from the variable exposure and the constant exposure (ANOVA: F=2.824, p=0.115 Fig. 2). The two-way ANOVA did show a significant variation between the different points across the upwelling cycle (ANOVA: F=20.176, p=0.00).

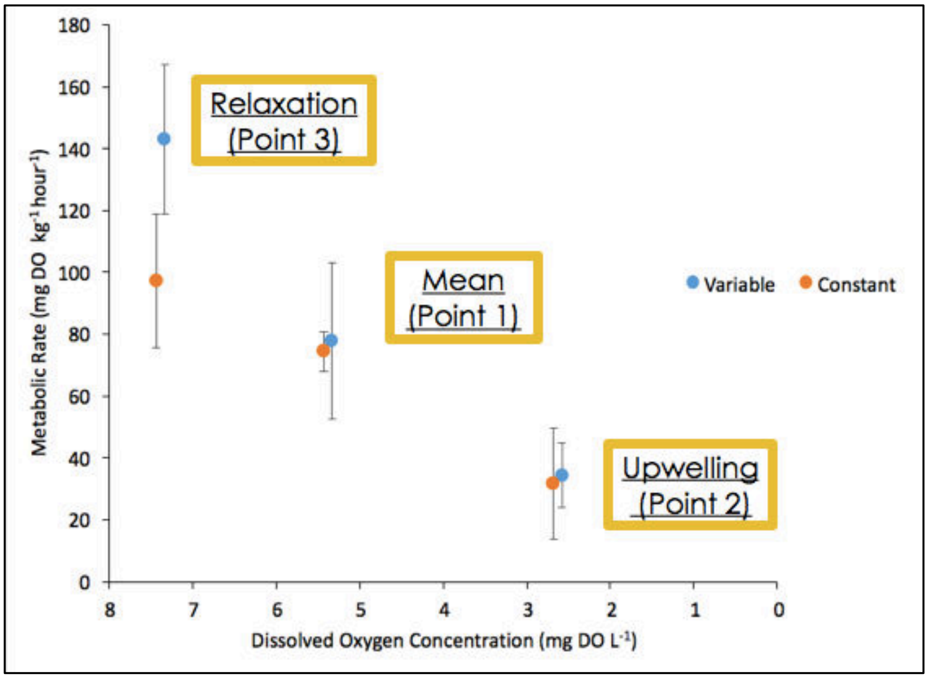

Figure 2. Metabolic respiration at different oxygen concentration. P<0.05 between different point when both conditions are pooled.

DISCUSSION

The experiment shows that short-term exposure of juvenile abalones during upwelling has no effect on their metabolism. However, there is a higher variability in metabolism for individuals who went through variable exposure at the mean condition. This result suggests that the abalones from the variable exposure were not adapted to the mean condition, since the abalones that went through constant exposure had a closer metabolic rate between the individuals. At relaxation, the metabolism rate of abalones from the variable exposure had a higher overall range. The differences in the range can be explained because the abalones from the constant exposure are lagging to be oxyregulators during relaxation. Similar to this experiment, there was a research that observed metabolic respiration of juvenile *H. laevigata*

(Greenlip abalones). Comparing the metabolic respiration to Fig. 2, the red abalone's metabolic rate from the variable exposure were similar to the greenlip abalone's metabolic rate when it was an oxyregulator (Harris *et al.* 1999). There were no significant differences across upwelling conditions, but the differences between the metabolic rate at the respiration condition shows that previous exposure may affect metabolic rate at higher DO.

CONCLUSION

The exposure history and the environmental variability may likely impact abalone metabolism. The experiment conducted with the greenlip abalones had an exposure time of 2-3 months. This is relatively a long exposure time compared to this experiment. However, even in the short amount of time, we saw that the abalones from the variable exposure had a faster reaction rate to become an oxyregulator when oxygen became abundant. To further understand the result of this experiment, it needs to have prolonged exposure time and multiple replication. For now, this project serves as a starter to learn more about how benthic organism's metabolism is affected by futuristic ocean condition. As a possible future direction from this project, juvenile red abalone's metabolic rate can be compared between current and future upwelling scenario. Also, another possibility for this project is to isolate environmental drivers and narrow down which specific drivers and which interactions has a major effect on juvenile abalone's metabolic rate. These studies will further enlighten our knowledge about the effects on benthic organism's metabolism by futuristic ocean condition.

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