# Quantifying the Effects of Changing Temperature, Dissolved Oxygen, and pH Associated with Upwelling on the Growth and Survivorship of Juvenile *Haliotis rufescens*


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**ABSTRACT**


Climate-related changes in ocean conditions, including warming, reduced dissolved oxygen (DO), increased upwelling, and ocean acidification (OA), are impacting marine ecosystems. Although understanding of these effects is emerging, knowledge of their combined effects on marine life in the California Current Large Marine Ecosystem (CCLME) is limited. To gain a better understanding, we aim to quantify the effects of three environmental drivers: temperature, DO, and pH on the growth and survivorship of the relatively sedentary nearshore species, *Haliotis rufescens* (red abalone). Species residing in shallow coastal waters are subject to upwelling events which can deliver these potentially stressful conditions by bringing up cold, low DO, low pH waters, which are then advected into nearshore habitats. Red abalone in three size classes (10-20, 20-30, 30-40mm) were exposed to one of six treatments representing the varying exposure of temperature, DO, and pH associated with current and future upwelling scenarios. Mortality and growth, measured as change in buoyant mass, somatic mass, shell size and mass, and mortality was obtained after four weeks. Size classes 20-30mm and 30-40mm had a trend of showing highest percent

growth overall within moderate upwelling conditions. All size classes had a trend of showing low percent growth overall within the most futuristic upwelling conditions. Mortalities were only observed in the smallest size class, with those exposed to moderate upwelling conditions having the lowest mortality and those exposed to the most futuristic upwelling conditions experiencing the highest mortality. Knowledge gained from this experiment can provide insight on how marine population dynamics and associated ecosystem services, such as commercial and recreational fishing, might respond to climate change. This information can also help the management and sustainability of upwelling driven marine ecosystems beyond the CCLME into the future.

## INTRODUCTION

Climate change is contributing to warming temperatures, decreasing dissolved oxygen (DO) levels, and increasing ocean acidification (OA) effects such as falling pH levels, in the ocean, consequently affecting marine ecosystems (Somero et al. 2015). Mollusks are particularly vulnerable to OA as they are organisms which require carbonate ions to calcify. Calcification is aided by a high pH and high carbonate ion concentrations (Hansson and Gattuso 2011) and OA inhibits shell deposition and the calcifying process by reducing the availability of carbonate ions in the ocean. By impacting larval development, settlement, and calcification of mollusks, OA can ultimately which can negatively impact habitat structure and food web interactions (Parker et al. 2013). Coastal upwelling conditions can intensify the effects of climate change by bringing up waters increasingly low in DO and pH, affecting near-shore benthic organisms (Somero et al. 2015). Although upwelling is a natural phenomenon which provides nutrients that fuel productive coastal ecosystems like the California Current Large Marine Ecosystem (CCLME), climate change could be delivering water with conditions that are potentially stressful to nearshore organisms. Upwelling is contributing to deoxygenation trends in the ocean which can negatively affect aerobic marine organisms. Low DO and low pH conditions can occur diurnally in coastal ecosystems depending on respiration rates and hydrodynamics (Gobler et al. 2017), and low DO events can make it difficult for aerobic organisms to produce ATP or metabolize (Somero et al. 2015) Lastly, temperature is a significant environmental driver which can affect almost all physiological processes of marine organisms, especially the building structures of calcifying organisms (Somero et al. 2015).

A combination of these environmental drivers could negatively impact species such as *H. rufescens*. Red abalone is an ideal experimental model species because they live in near-shore habitats and are relatively sedentary organisms which cannot avoid exposure to stressful conditions associated with upwelling (NSF Award #1416877) The implications of these conditions are that low DO levels have been linked to reduced growth rates in abalone (Harris et al. 1999), while another study (Lord et al. 2017), found that abalone shells grew 40% less under high carbon dioxide conditions. Moreover, low DO and low pH levels have been found to have synergistic negative effects on juvenile abalone (Kim et al. 2013).


**Figure 1.** Map of the California Current Large Marine Ecosystem

The focus area for this study is the CCLME, shown in Figure 1. The effects of variability in pH, DO, and temperature associated with upwelling on marine organisms are not well understood. Observing these environmental driver's co-occurrence with upwelling conditions is crucial because the CCLME experiences seasonal upwelling events in the spring (Bograd et al. 2009) Thus, the question of interest is how does upwelling associated variability in pH, DO, and temperature levels affect the growth and survivorship of juvenile *H. rufescens*? In this experiment, variability of these three drivers will be assessed in association with upwelling under current and potential future climate scenarios within the CCLME. Understanding how low pH, low DO and temperature impact species on the individual level, can provide insight on population level and ecosystem services effects such as fishery output. Two hypothesis will be tested to answer this question. 1) Size classes exposed to current conditions will experience lower mortality rates than those exposed to treatments representing future climate change scenarios 2) Size classes exposed to current conditions will experience higher growth rates than those exposed to futuristic conditions.

## MATERIALS AND METHODS

### EXPERIMENTAL DESIGN

Red abalone from three different size classes (10-20mm, 20-30mm, 30-40mm) were exposed to one of six treatments of varying levels of pH, DO, and temperature (Table 1). These treatments were scenario-based and designed to gradually project future climate conditions associated with upwelling. The individuals were placed in jars, or experimental tanks (ETs), which were hooked up to conditioning tanks (CTs) which delivered varying levels of pH, dissolved oxygen (DO) in mg/L and temperature (C°) into the ETs. The system was designed so that each size class went through each of the six treatments for four weeks. The upwelling system was controlled by a custom-designed computer software which allowed for each treatment of pH, DO, and temperature to be delivered to their corresponding CTs throughout the four weeks of the experiment.

**Table 1.** The distribution of size classes within treatments in each experimental tank.

| Size class (mm) | ETs per size class | # of abalone per ET | Total # of abalone per tank | Total # of abalone per treatment | Total # of abalone needed |
|---|---|---|---|---|---|
| 10 – 20< | 1 | 20 | 20 | 40 | 240 |
| 20 – 30< | 1 | 10 | 10 | 20 | 120 |
| 30 – 40< | 1 | 5 | 5 | 10 | 60 |
| Total | **3** | | | | **420** |

The six treatments delivered specific levels of three environmental drivers: temperature, DO, and pH (Figure 2). Treatment 1 was the control and it represented the current conditions associated with upwelling within the CCLME. Treatment 6 represented the most stressful possible future scenario. There were two fluctuations in temperature, DO, and pH across a 24-hour period per treatment to simulate the semi-diurnal fluctuations associated with seasonal upwelling and the tidal cycle (Gobler et al. 2017). These fluctuations occurred simultaneously across the three variables. Treatments started at relaxation for 360 minutes, then transitioned to upwelling conditions within 90 minutes, stayed at upwelling conditions for 180 minutes, and transitioned back to relaxation within 90 minutes. This occurred twice daily for four weeks. For temperature, relaxation conditions started at 13 °C in treatment 1 and rose to 22 °C by treatment 6 while upwelling conditions went from 9 °C in treatment 1 to 18 °C by treatment 6. DO stayed at 10 mg/L because it always rose back up during relaxation, but dropped during upwelling conditions from 4 mg/L to 1mg/L. Similarity, pH relaxation went from ~8.0 to 7.5 and during upwelling went from 7.6 to ~7.2 (Figure 2).

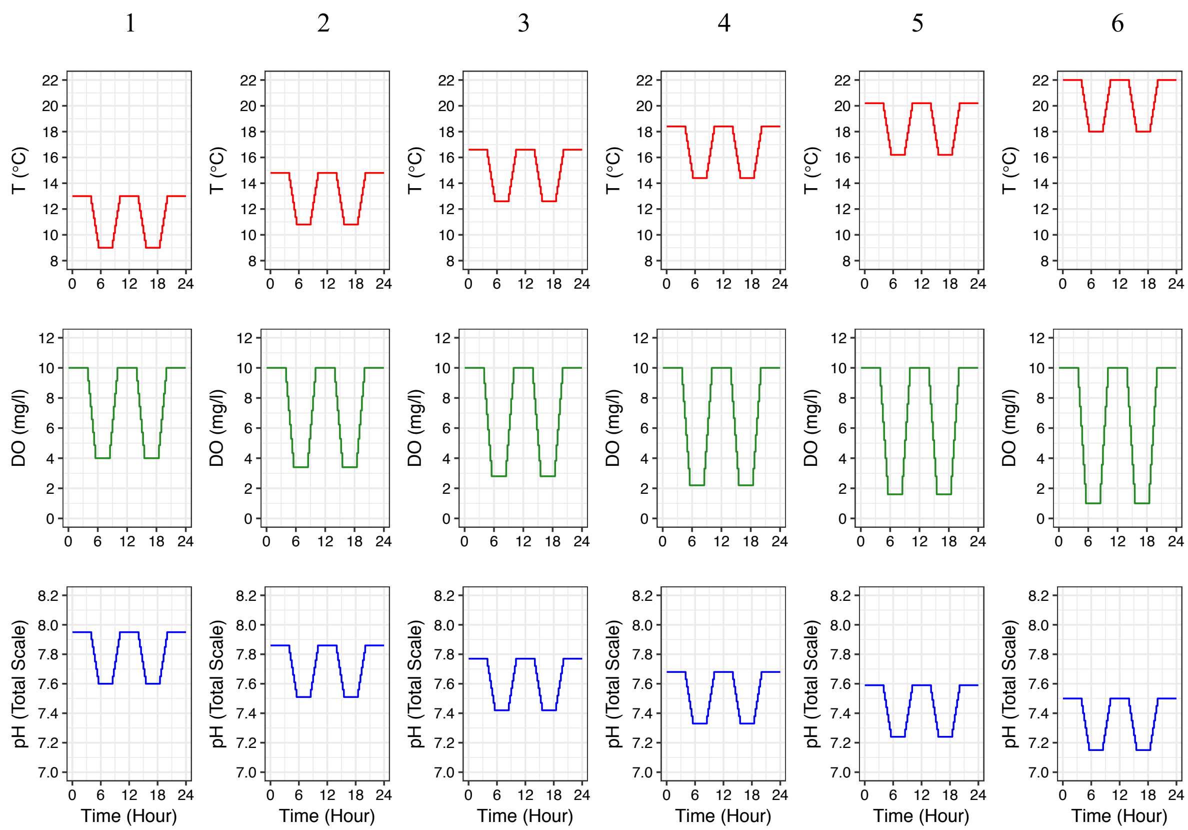


**Figure 2.** The six treatments for the juvenile abalone experiment multifaceted by varying levels of temperature (C°), dissolved oxygen (mg/L) and pH based on current conditions to possible future scenarios.

## DATA COLLECTION AND ANALYSIS

At the beginning of the experiment, each abalone was tagged using a color and number system and had their measurements taken including buoyant weight and wet weight along with an initial digital image. After four weeks, only 78 of the 420 abalone were sacrificed to acquire "mid-experiment" results and data was attained from three size classes (10-20, 20-30, 30-40mm). Thirty individuals were sacrificed from size classes 10-20mm and 20-30mm each and 18 from size class 30-40mm. For each abalone sacrificed, buoyant weight and wet weight, somatic wet weight, and dry shell weight were measured and a final digital image taken. The initial and final images were analyzed using the computer software ImageJ to acquire shell area and ferret diameter (measure of length) for comparison. Initial somatic wet weight was estimated by subtracting final somatic wet weight from initial buoyant weight, which is a proxy for shell weight. Initial shell weight was estimated by multiplying initial buoyant weight by a regression equation calculated by plotting final shell weight versus final buoyant weight. Survivorship was assessed using a time series graph and percent changes in measures of growth were analyzing using a 1-way ANOVA for each size class.

## RESULTS

### SURVIVORSHIP

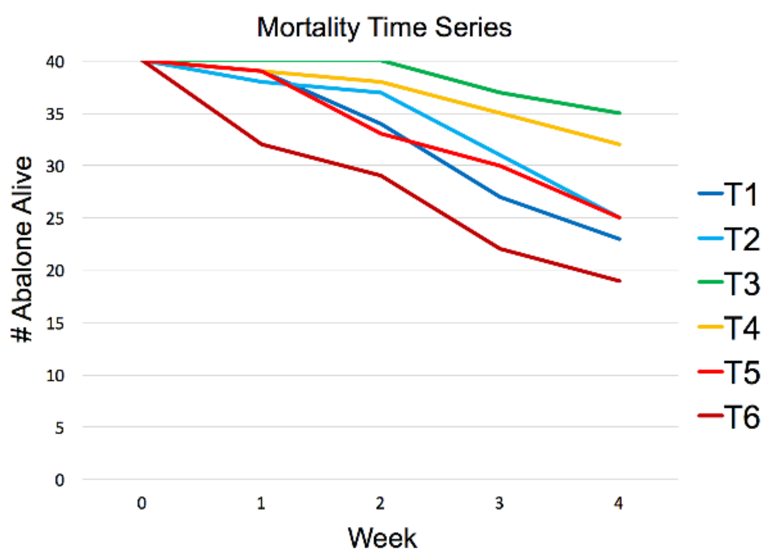


**Figure 3.** Mortality Time Series graph representing the 10-20mm size class over four weeks

Survivorship results, shown as a mortality time series graph (figure 3) only represents mortalities within size class 10-20mm because mortalities were not observed in the other size classes. Each treatment started with 40 abalone and treatment 6 performed the worst and lost 21 individuals. Treatment 3 performed the best and only lost 5 individuals. Treatment 1 lost 17 individuals while treatments 2, 4, and 5 experienced moderate mortality rates compared to the others.

PERCENT CHANGE IN WET WEIGHT

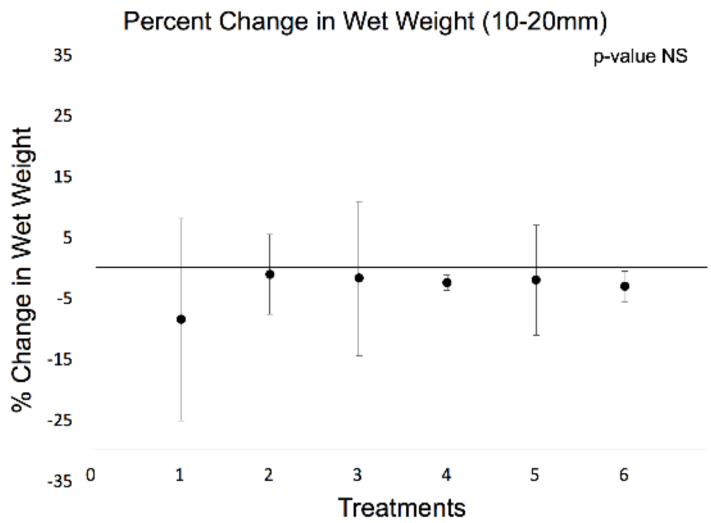


**Figure 4.** Percent change in wet weight within size class 10-20mm across treatments

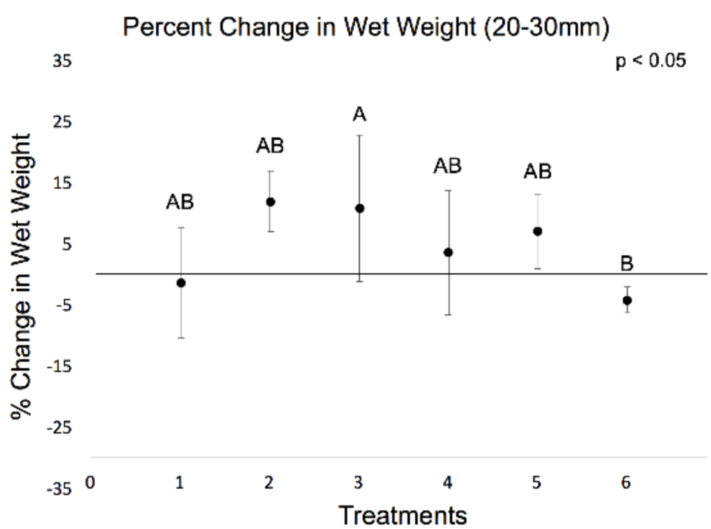


**Figure 5.** Percent change in wet weight within size class 20-30mm across treatments

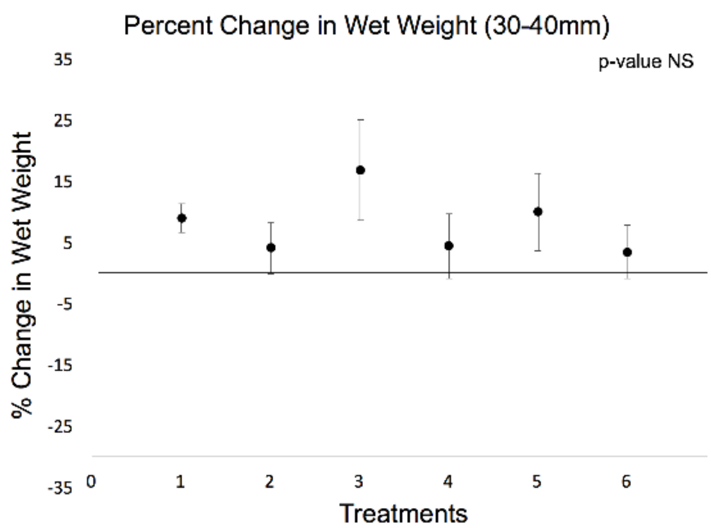


**Figure 6.** Percent change in wet weight within size class 30-40mm across treatments

The 1-way ANOVA results for percent change in wet weight were not statistically significant within size class 10-20mm (figure 4) and 30-40mm (figure 6) but they were found to be significant between treatments 3 and 6 within size class 20-30mm ($P < 0.05$) (figure 5). Individuals in the 20-30mm and 30-40mm size classes within treatment 3 were among those with the highest percent change in total wet weight, while treatment 6 was among those which experienced the lowest percent change across all size classes. Size class 10-20mm also experienced negative percent growth in total wet weight across all treatments.

PERCENT CHANGE IN SHELL AREA

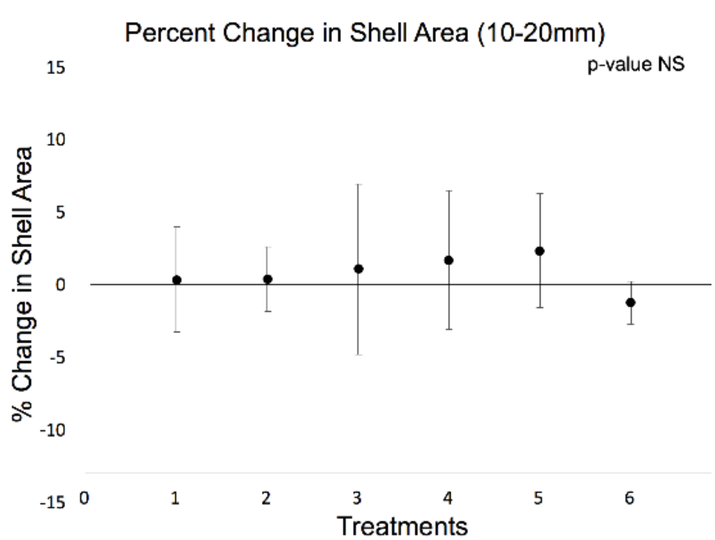

**Figure 7.** Percent change in shell area within size class 10-20mm across treatments

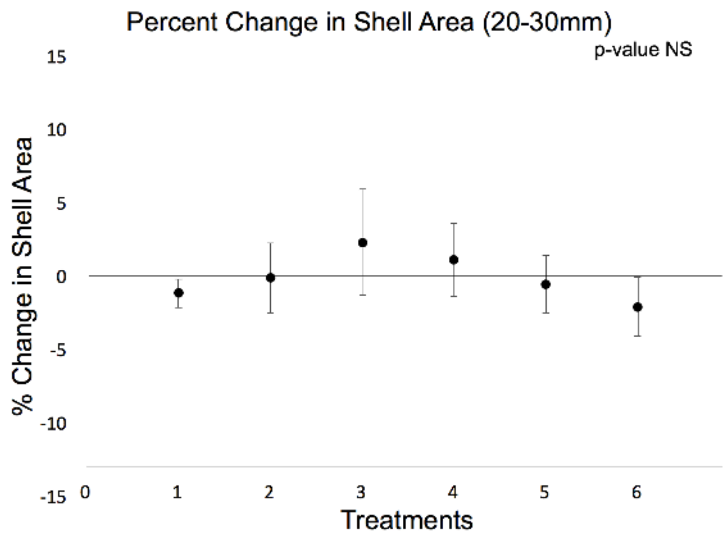


**Figure 8.** Percent change in shell area within size class 20-30mm across treatments

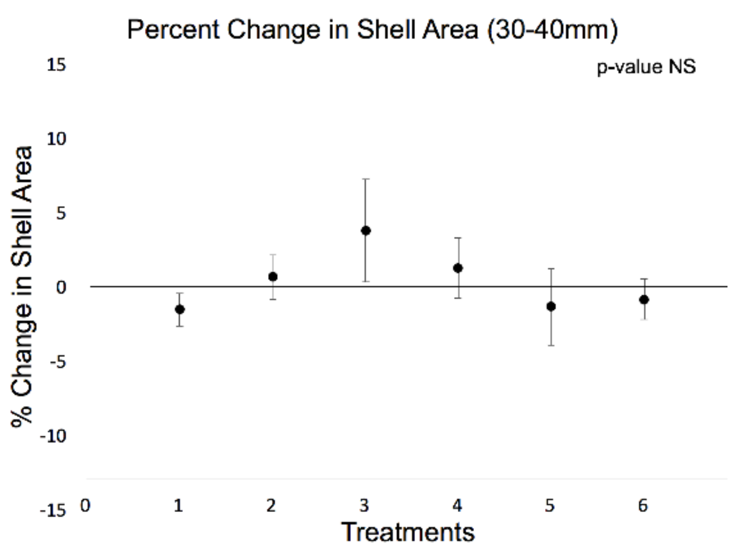


**Figure 9.** Percent change in shell area within size class 30-40mm across treatments

The 1-way ANOVA results for percent change in shell area were not statistically significant within any of the size classes. However, similar trends in high percent change in shell area was observed in individuals within treatment 3 in size classes 20-30mm (figure 8) and 30-40mm (figure 9) compared to other treatments. Abalone in all three size classes from treatments 1 and 6 showed trends of having the lowest or even negative percent changes in shell area (figures 7, 8, and 9).

PERCENT CHANGE IN SHELL WEIGHT

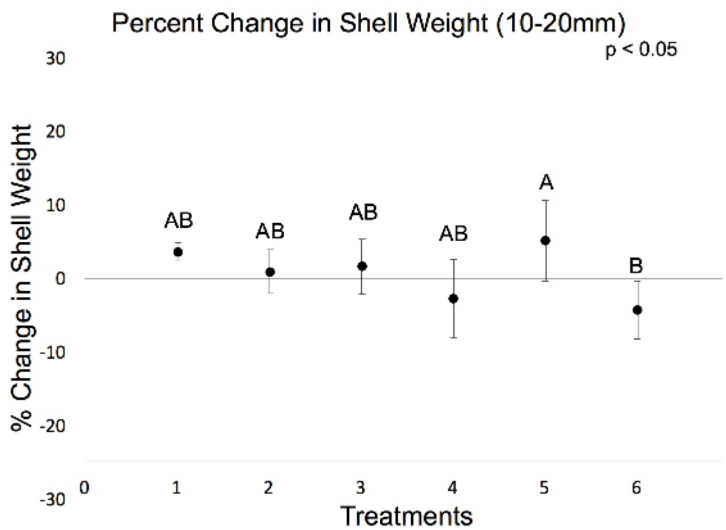


**Figure 10.** Percent change in shell weight within size class 10-20mm across treatments

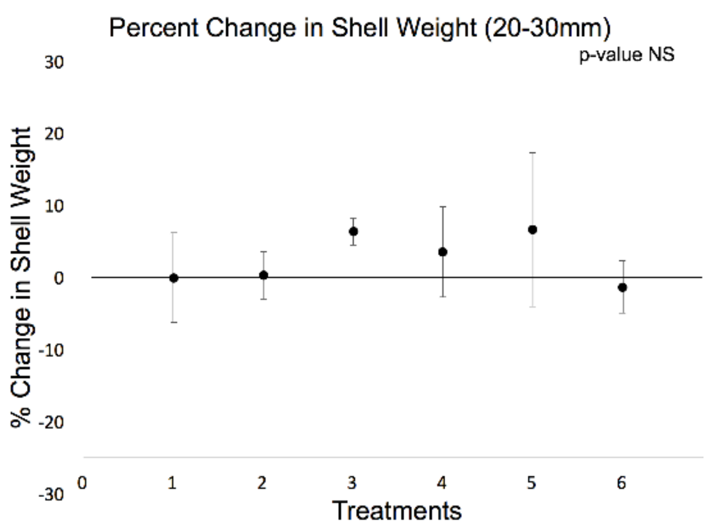


**Figure 11.** Percent change in shell weight within size class 20-30mm across treatments

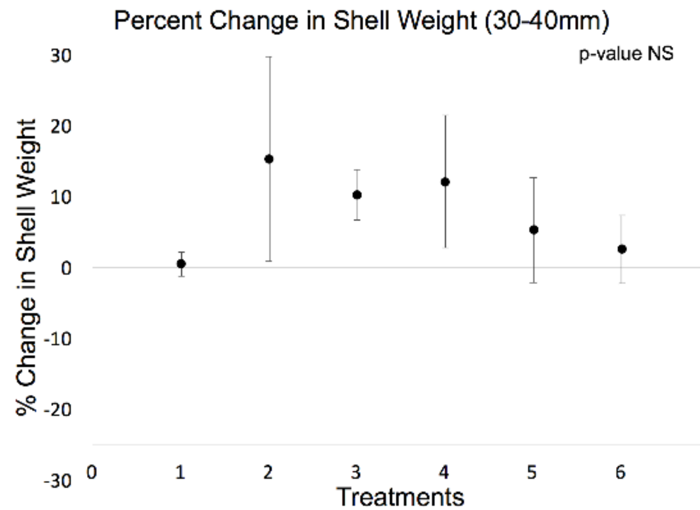


**Figure 12.** Percent change in shell weight within size class 20-30mm across treatments

The 1-way ANOVA results for percent change in shell weight were not statistically significant within size classes 20-30mm (figure 11) and 30-40mm (figure 12) but they were significant between treatments 5 and 6 among the 10-20mm size class ($P < 0.05$) (figure 10). Treatment 6 was observed to have the lowest and even negative percent change in shell weight across all three size classes.

PERCENT CHANGE IN SOMATIC WET WEIGHT

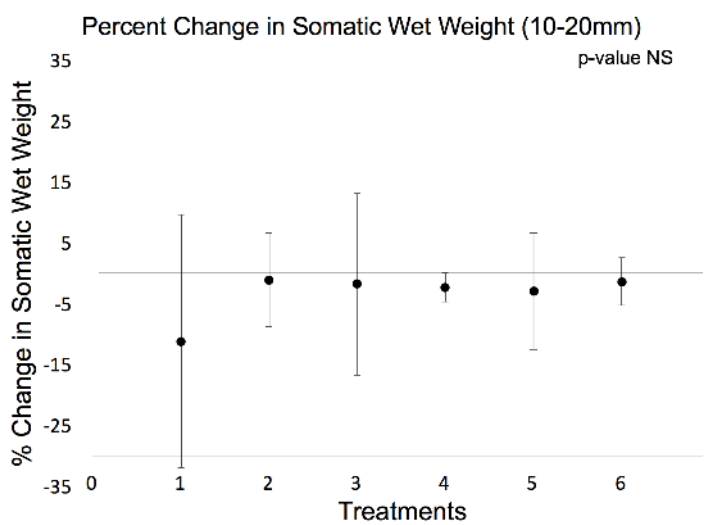


**Figure 13.** Percent change in somatic wet weight within size class 10-20mm across treatments

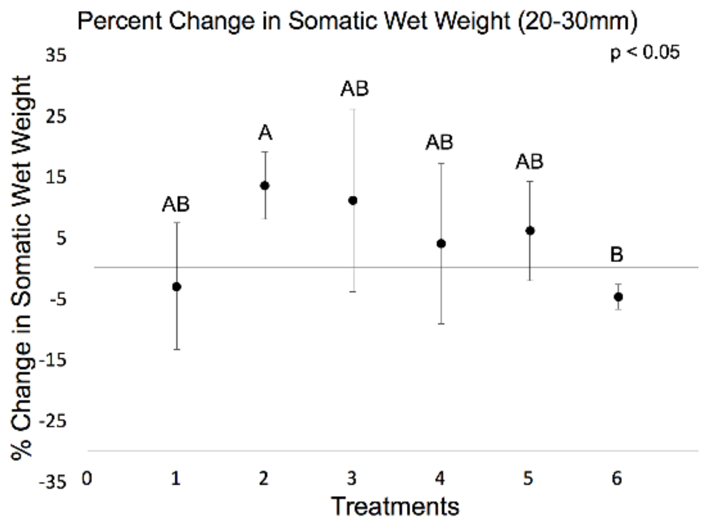


**Figure 14.** Percent change in somatic wet weight within size class 20-30mm across treatments

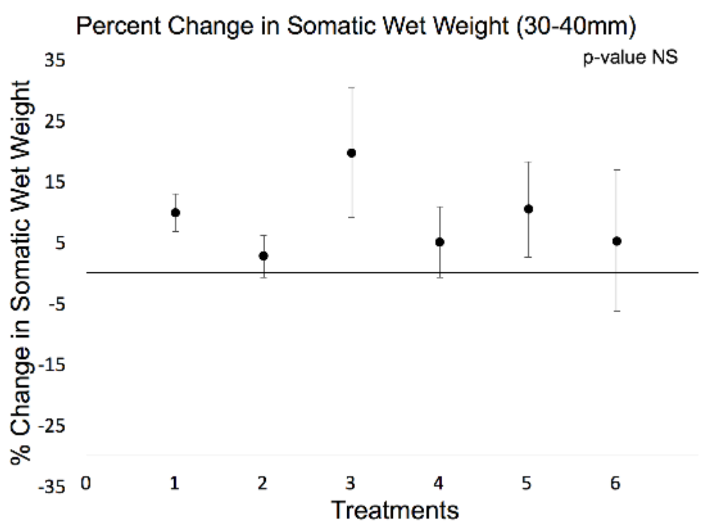


**Figure 15.** Percent change in somatic wet weight within size class 30-40mm across treatments

The 1-way ANOVA results for percent change in somatic wet weight were not significant within the 10-20mm (figure 13) or 30-40mm (figure 15) size classes but were found to be significant between treatments 2 and 6 among the 20-30mm size class ($P < 0.05$) (figure 14). Similar trends in size classes 20-30mm and 30-40mm were observed within treatment 3 having among the highest percent change and treatment 6 having among the lowest percent change in somatic wet weight. The 10-20mm size class experienced negative percent growth across all treatments, similar to their percent change in total wet weight.

## DISCUSSION

The survivorship results contradicted my first hypothesis stating that size classes exposed to current conditions would experience lower mortality rates than those exposed to treatments representing future climate change scenarios. The results showed that 10-20mm individuals within treatment 3, which had moderate levels of temperature, DO and pH, experienced the lowest mortality rates across all treatments. Interestingly, 10-20mm individuals in treatment 1 (current conditions) had almost as many mortalities as treatment 6 did (figure 3), which had high temperatures, low DO, and low pH. These high mortality results were not expected from treatment 1.

The results of percent change in growth also contradicted my second hypothesis stating that size classes exposed to current conditions will experience higher growth rates than those exposed to the futuristic climate change scenarios. Size classes in treatment 1, the current upwelling conditions, experienced the lowest and even negative percent growth. Meanwhile, size classes in treatment 3, the moderate upwelling scenario, were among those that had the highest percent change in growth. Results from percent change in shell weight were not as conclusive as results from percent change in total wet weight and shell area, however, treatment 6 still had the lowest percent growth across the board. This was expected because it aligned with other studies showing low growth rates as a result of these conditions (Harris et al. 1999, Lord et al. 2017) However, treatment 1 was not expected to have as high mortality and low percent change in growth as it did, and treatment 3 was not expected to have the lowest mortality and highest percent change in growth. Treatments 2, 4, and 5 showed a mix of positive and negative responses in terms of growth and mortality, therefore, more data must be acquired to obtain more definitive results.

## CONCLUSIONS/RECOMMENDATIONS

The results of this study produced high standard deviations and statistically insignificant differences within percent change in growth among each size class mainly due to the small sample size. Only 78 abalone were sacrificed after four weeks out of the 420 and they were categorized by treatment and size class, which made the sample size of each measure of growth even smaller. Data from a full experiment sacrifice after eight weeks must be acquired to obtain more conclusive results. For future studies, looking at the combined effects of DO and pH separately from temperature could be useful within multiple life stages, as these two drivers are known to have synergistic negative effects on juvenile abalone (Kim et al. 2013) while the effects of temperature on organisms may vary. Studying the effects of the drivers separately can provide insight on which drivers have what effects on abalone. Furthermore, looking at different combinations of the drivers may be more indicative of what results in low growth rates and high mortality and vice versa. By understanding how these multiple drivers affect the growth and survivorship of marine species subject to upwelling conditions, we can model how a population or ecosystem will respond to such changes. This, in turn, can help the management of not only the CCLME, but all marine ecosystems facing the detrimental effects of climate change in the future.

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