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Effects of Climate Change on the Larval Development of *Haliotis rufescens*

INTRODUCTION

Rising carbon dioxide levels in the Earth's atmosphere have been threatening global populations and will continue to threaten marine organisms in the near future (Doney et al., 2009; Kim et al., 2013). The ocean absorbs much of this atmospheric CO_2, and through a series of chemical reactions and other biological processes, shifts the balance of our oceans. This abundance of CO_2 causes a drop in the ocean's pH while atmospheric CO_2 traps heat and warms the surface layers. This results in a combination of increased acidity and decreased O_2 concentration in deeper areas which is then brought to near-shore ecosystems through a process known as upwelling (Ross et al., 2011; Barry et al., 2011). Marine organisms that reside in coastal habitats risk exposure to these potentially stressful conditions, which begs the question: Will larval growth of marine organisms be affected by these conditions as global climate change continues to worsen these environmental conditions? Concern about the stability and conservation of marine organisms has prompted studies in regions that are already experiencing this. Understanding how larvae will develop under climate change conditions may provide insight to how populations will fare in the future.

While many studies focus on one factor of climate change (i.e. temperature, oxygen concentration or pH), this novel study aims to understand how the combination of pH and O_2 concentration will affect developing larvae and hatching success. Choosing the commercially important Red Abalone, *Haliotis rufescens*, as a model organism will provide a better

understanding of how shell-forming, calcifying organisms will be impacted by factors related to upwelling and climate change.

METHODS

Acquisition

Gravid *H. rufescens* were acquired from American Abalone Farms in Davenport, CA two weeks before spawning. This experiment also aimed to answer the question of whether post-spawners in the lab would be able to spawn again with chemical inducement. Six post-spawners (3 of each gender) were introduced into the tubs along with eight newly-purchased *H. rufescens* (4 of each gender) – resulting in 14 broodstock total.

Spawning

After two weeks, gravid females were removed first from the tubs. Each female was weighed, measured for shell length and placed in a 6 L tub with one other abalone of the same gender. These tubs were filled with control seawater (8.0 pH and 190 µmol/kg O_2) at a starting temperature of 13ºC. Eight 6 L tubs total containing control seawater and aeration were stationed on a water table. Fresh water surrounded the tubs to serve as the temperature ramp to aid in spawning. The temperature of freshwater began at 13ºC and steadily increased to 17ºC by the time spawning occurred.

Thirty minutes after females were introduced to the spawning tubs, each tub received 40 mL of Tris buffer. Once females were exposed to the Tris buffer for15 minutes, 25 mL of H_2O_2 was added (Morse *et al.*, 1977). An hour and a half after females were separated into spawning tubs, males were removed from their stable environment and placed in the same conditions as the females. The same protocol for Tris buffer and H_2O_2 was followed for the male broodstock. By the end of the addition of chemical solutions, the temperature inside the 6 L tubs had arrived at

17ºC. Once the males were exposed to the solutions for the proper amount of time, the tubs were drained and refilled with control seawater. This marked the beginning of the spawning timeframe which lasted approximately two hours.

Collection

Once spawning occurred, sperm collection was prioritized because of the rapid dilution. Once collected from the tubs, sperm was placed in a clean 250 mL plastic beaker. Eggs were collected from the bottom of the tub with a turkey baster and placed in a clean 500 mL glass beaker. I calculated density for the gametes after the spawning process was finished. Density of eggs and sperm were approximately 1,200 eggs/mL and 10^6 sperm/mL, respectively.

Fertilization

Using the most successful density for fertilization, (10^6 for sperm and 10^5 for eggs), eggs were added to five 6 L tubs with the goal of creating a thin layer on the bottom for maximum fertilization success. I divided the amount of sperm between the 5 tubs and allowed gametes to fertilize in control seawater (7.9 pH and O_2 concentration of 190 μmol/kg) for 30 minutes.

Development

After this period of time, the total pool of developing embryos was divided into 9 experimental glass jars for the remainder of the experiment (Figure 1). Three conditions were used (n=3) to test which factor associated with climate change (pH or O_2) would impact hatching of *H. rufescens* embryos (Table 1). The control replicates common conditions seen in the Monterey Bay, which is a pH of 7.9 and an O_2 concentration of 190 μmol/kg. This control will be referred to as HH (High pH/High O_2). Two treatments were chosen to compare pH to oxygen. Three jars contained seawater with a pH of 7.2 and an O_2 concentration of 190 μmol/kg. This

will be denoted as LH (Low pH/High O_2). Three other jars contained seawater with the same pH of 7.2 but an O_2 concentration of 60 μmol/kg, which will be denoted at LL (Low pH/Low O_2).

I engineered the 1 ½ L experimental jars to have a small inlet tube and an outlet tube of the same diameter to minimize gas exchange. The inlet tube extended to the bottom of the jar to allow for a circular flow and to suspend embryos during development. My effort to minimize impingement was done by creating a circular filter wrapped in a 65 μm Nytex mesh and capped with Falcon tube lids on either end. This filter was connected to the outlet tube through a hole I drilled in the center of the cap.

Sampling

Sampling began directly after fertilized embryos were separated into the 9 experimental jars. I collected both live and fixed samples. Fixing was done by sieving 2-4 mL of embryos from each jar and transferring this sample to 20 mL scintillation vials and adding 8-16 mL of 10% Phosphate buffered formalin, respectively. The sampling schedule was: 0, 4, 8, 16, 22, 25 and 41 hours post fertilization (HPF). I chose this schedule based on speed of development and appearance of larval characteristics.

During each of these time points, 9 fixed samples were processed and stowed for later analysis while 9 live samples were photographed. Counts of each developmental stage seen were recorded during each sampling point. The stages of development that were of interest for this project were: 1 cell, 2 cell, 4 cell, 4+ cell/gastrula, pre-trochophore and trochophore (Figure 2). Defining 4+ cell and gastrula became difficult past 8 HPF and were grouped together to avoid confusion and/or error. Categorizing pre-trochophore vs. trochophore relied upon the vitelline envelope still encompassing the larvae. Larvae that were not encased in this envelope were considered successfully hatched.

RESULTS

The metric used to test success relied on hatching of *H. rufescens* larvae from the vitelline envelope. From this experiment alone, we were able to determine through ANOVA and running a post-hoc Tukey test, there is a significant difference between HH and LL; however, there is no significance between HH vs. LH and LH vs. LL (Table 2). For 25 HPF, the averages for larvae hatched is as follows: HH=25%, LH=56% and LL=34%. For 41 HPF, hatched larvae averages were HH=96%, LH=82% and LL=50%. Comparing 25 HPF to 41 HPF, there is a large difference for HH (Figure 3).

CONCLUSION

Findings suggest there is a significant difference between hatching success of *H. rufescens* larvae between the control and LL. Future experiments organized as fully factorial will provide better insight as to which factors (pH or O_2 concentration) have more impact. Further data analysis from this experiment may also provide insight for separate developmental stages as opposed to only hatching success. Comparing each sampling time point with the development stages seen for each treatment would create a better understanding of how each larval stage is impacted by these conditions. This information can be useful for conservation efforts, as well as providing a fundamental understanding of how stressful environmental conditions related to climate change affect developing embryos. Future experiments aim to understand how climate change conditions affect development of the full abalone life cycle, and applying this information to conservation efforts for populations of *H. rufescens* in the future.

APPENDICES

I. References

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II. Tables and Figures

	High O_2	Low O_2
High pH	X	N/A
Low pH	X	X

Table 1. Experimental treatment setup. I excluded high pH and low O_2 because this combination was not available to our lab.

Figure 1. During the separation phase. Embryos were suspended in experimental jars during development.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
HH	LH	34.333	15.128	.064	-2.684	71.351
	LL	47.417*	15.128	.020	10.399	84.434
LH	HH	-34.333	15.128	.064	-71.351	2.684
	LL	13.083	15.128	.420	-23.934	50.101
LL	HH	-47.417*	15.128	.020	-84.434	-10.399
	LH	-13.083	15.128	.420	-50.101	23.934

Table 2. Pairwise comparisons for HH (high pH/high O_2), LH (low pH/low O_2) and LL (low pH/low O_2). The mean difference is significant at the .05 level.

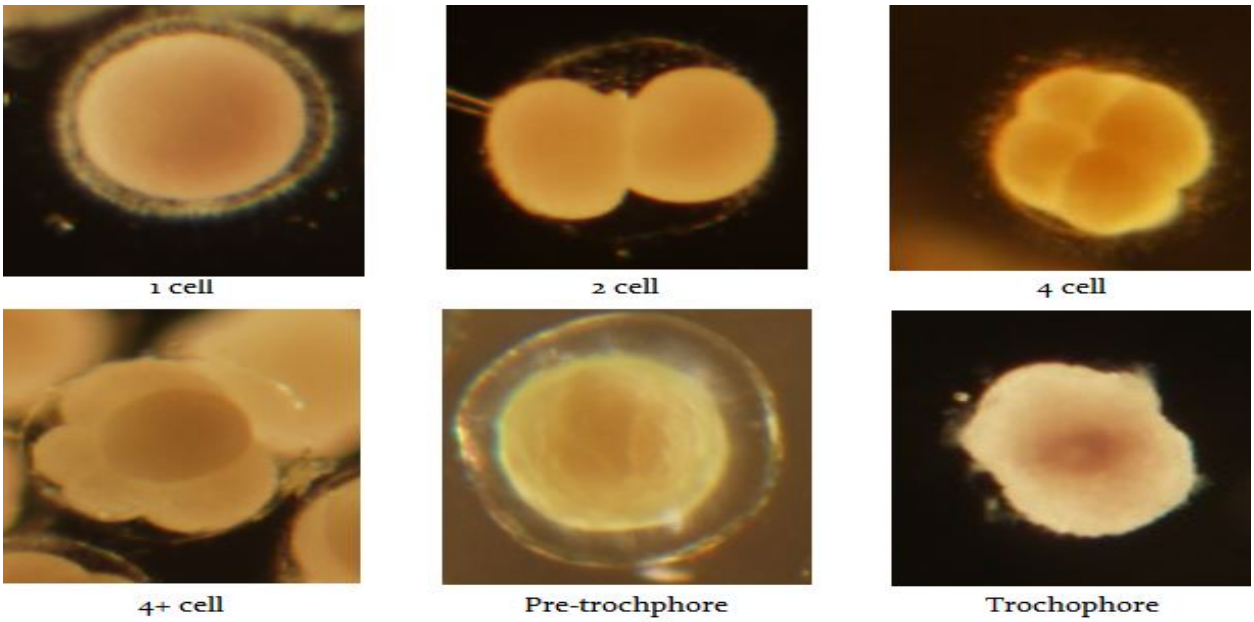

Figure 2. The above six stages were used to create a timeline for development of *H. rufescens* larvae. Sampling ended after the trochophore stage.

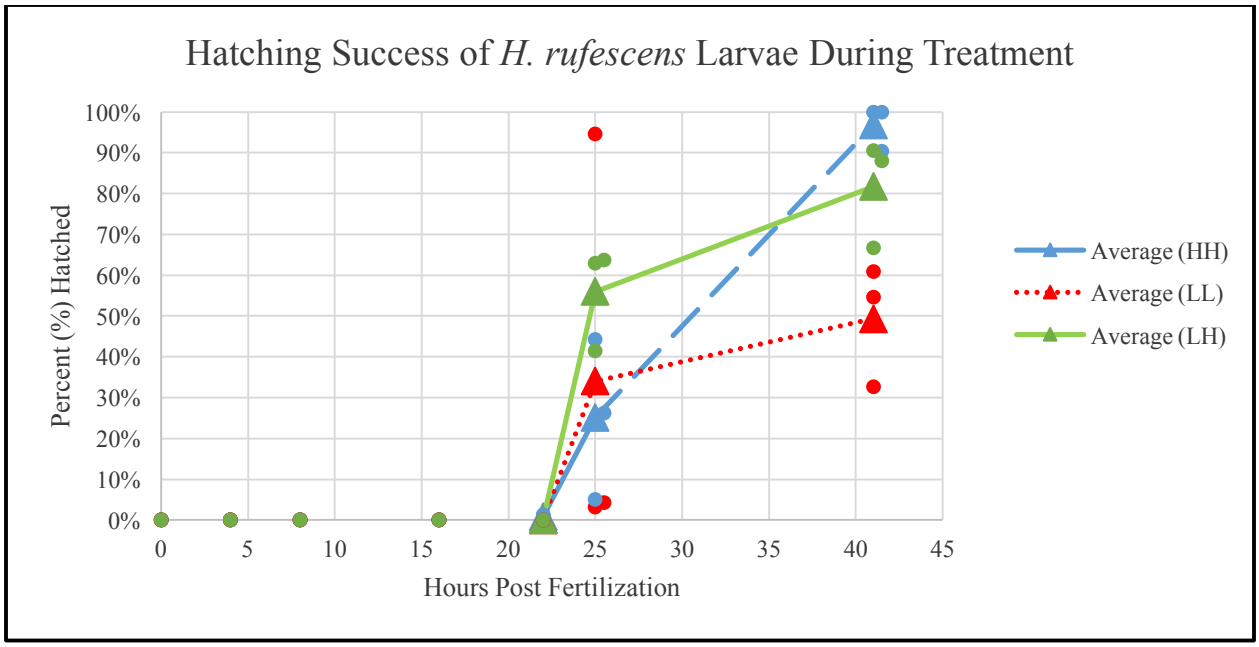

Figure 3. Hatching success of *H. rufescens* larvae for each condition. Long dashes line refers to the high pH/high O_2 control. The solid line refers to the low pH/high O_2 treatment. The dotted line refers to the low pH/low O_2 treatment. From the graph, there is visible variability in hatching success for 25 hours post fertilization and 41 hours post fertilization. Ultimately, the order of hatching success followed the hypothesis, with low pH/low O_2 having the most effect on hatching.