AN ABSTRACT OF THE THESIS OF

Amanda C. Amstutz for the degree of Honors Baccalaureate of Science in Zoology presented on June 2, 2009. Title: Effects of Hypoxia on Survival and Growth of Barnacle Larvae Off the Oregon Coast.

Abstract approved: ________________________________
Bruce Menge

In recent years off the United States Oregon coast severe hypoxic events have resulted in areas of mass vertebrate and invertebrate species mortality known as dead zones. Creation of dead zones and alteration of community species composition in response to inner shelf hypoxic conditions have been observed annually from 2002 to 2007. Near shore hypoxic events have already begun to redefine ecosystems off the Oregon Coast and other areas in the California Current Large Marine Ecosystem (CCLME). Barnacle species are of great importance in these communities. As a part of the life history of the barnacle, successful growth and survival of larval stages is essential to the success of sessile adults. We carried out experiments at Oregon State University's Hatfield Marine Science Center to determine the rate of nauplii (larvae) mortality in the barnacle species *Balanus glandula* at increasingly severe levels of hypoxia. We also investigated the effects of exposure to hypoxic conditions on *B. glandula* larval development. We found no difference in nauplii survival or development between exposure to normoxic (5.5 mg/L dissolved oxygen) and microxic (0.5 mg/L dissolved oxygen) conditions for 24 hours.

Key Words: *Balanus glandula*, hypoxia, dead zones, larval development
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Effects of Hypoxia on Survival and Growth of Barnacle Larvae Off the Oregon Coast

by

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Amanda C. Amstutz

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Introduction

Coastal marine ecosystems are some of the most productive ecosystems on the planet, providing a large percentage of the world's food and innumerable ecosystem services[1]. Within coastal marine ecosystems, hypoxia is a worldwide phenomenon of increasing concern. Hypoxia (dissolved oxygen levels of 1.43 ml/L or less) has the potential to cause large die-offs of marine organisms and severely stress marine ecosystems, threatening them with collapse[2]. In the last 50 years no other variable of ecological importance in near shore and estuarine marine ecosystems has changed so radically as dissolved oxygen levels[2]. The majority of this change has been brought on by eutrophication spurred by human activity[2], yet we have also begun to observe the introduction of hypoxia in systems where eutrophication is not a possible cause[3]. An example of non-runoff generated hypoxia occurs off the coast of Oregon, USA, which has recently experienced the unprecedented onset of seasonal near shore hypoxia. When introduced to naive ecosystems, hypoxia can have devastating consequences, a pattern which we have already seen off the coast of Oregon in the form of large dead zones devoid of marine life[4].

The purpose of this project is to investigate the effects of hypoxia on a single organism with great ecological importance. Specifically, we will address the issues of survival and development in the barnacle *Balanus glandula*. We hope to gain a better understanding of larger scale consequences of the new phenomenon of near shore hypoxia, and ideally be able to make predictions on how it will affect our ecosystem of interest. But first it is important to understand explicitly the causes and implications of hypoxia.

Background

As mentioned, hypoxia is found worldwide. Hypoxia as the result of natural causes is most common in estuaries and upwelling systems[2]. Wind driven upwelling in eastern boundary current systems has created some of the most productive marine ecosystems known. Eastern boundary current systems off of South America, Western Africa, India, Pakistan, and Western North America provide over 20 percent of international fishery yields each year[3]. This staggering productivity is fueled by the upwelling of nutrient rich water into shallow photic zones[3]. Upwelling not only supplies photic zones with nutrient-rich water, but also with oxygen-poor water. These systems are more vulnerable to the development of hypoxia because they are seasonally supplied with oxygen-poor waters[4,5]. The dissolved oxygen levels in oxygen-poor water delivered onto continental shelves can be further reduced by respiration, especially when the corresponding influx of nutrients produces population booms in phytoplankton[3,4]. These "plankton blooms," if large enough to overwhelm the capacity for consumers to process the increase in organic material, result in an excess of organic material which is decomposed by bacteria. The subsequent increase in oxygen demand during decomposition within the ecosystem can cause hypoxic and anoxic conditions, with larger amounts of excess organic material causing more severe hypoxia[3]. Decreased dissolved oxygen levels can then be further exacerbated by stratification of the water column due to differences in salinity and temperature and low physical energy in the form of tides, waves, or currents[2].

Hypoxia is a concern in marine ecosystems because it can alter fisheries and ecological and biogeochemical processes[3,4,6,7]. Seasonal development of hypoxia in coastal upwelling ecosystems (CUEs) has the potential to disrupt food webs, decrease species diversity, and create dead zones, areas of mass die-offs. These factors have adverse effects on fisheries and threaten CUE systems with collapse[2,3,8,9]. Survival, development, and growth of marine organisms have been shown to be negatively affected by hypoxia among a variety of both invertebrate and vertebrate organisms. Response to hypoxia varies depending on the organism, life stage, and developmental stage. Some benthic fauna are capable of surviving extended periods of hypoxia of approximately 0.5-1ml/L for days or weeks[10]. However, these organisms tend to be burrowing species that are regularly exposed to hypoxia

Studies of mussels, oysters, and estuarine fish have demonstrated the aforementioned negative effects of hypoxia and increased mortality. These mortalities are in addition to the already high levels of juvenile mortality common in benthic invertebrates[11]. *Crassotrea virginica* oyster larvae exposed to hypoxia and anoxia showed a significant reduction in larval settlement, growth, and survival compared to larvae exposed to "normoxic" (normal oxygen conditions) conditions[12]. Studies of planktonic scallop larva and juvenile fish species indicate decreasing probability of survival as dissolved oxygen concentration decreases[13,14]. However, the majority of studies addressing the effect of hypoxia on survival have involved estuarine and bay species. The majority of studies have been on adults, and few address larval stages. Even fewer studies investigate the effects of hypoxia on larval stages of coastal marine species, especially open water invertebrates.

Of the existing research on the effects of hypoxia on larval invertebrates, mostly addressing estuarine and bay species, the findings suggest overall negative impacts of hypoxia with a life stage specific component. Tolerance among *Crassotrea virginica* to increasing hypoxia varies with size and developmental stage from juvenile to adult. Adult and recently settled *C. virginica* reduce activity and metabolic rate to increase their hypoxia tolerance while larvae do not employ this technique[12]. In the bay scallop *Argopecten irradians*, hypoxia reduces developmental rates: below a certain oxygen level, bay scallop larvae will not progress to the next developmental stage[13]. Growth of mussel larvae *Mytilus edulis* also has been shown to be stunted by hypoxia with larval age specific responses. In terms of growth, larger larvae of the mussel *Mytilus edulis* grow (measured by increase in shell length) more slowly under hypoxic conditions than smaller larvae[15]. Mortality rates under hypoxic conditions are known to be lower in mussel larvae of larger sizes, with larger larvae having higher resistance to anoxic conditions than smaller larvae, and advanced developmental stages[15]. Again, the data are biased towards estuarine and bay species, and adult stages, giving us few clues as to how larvae of open coastal species could react to hypoxia. This bias may be due to the fact that open coastal marine ecosystems have historically not experienced hypoxia. In light of recent hypoxic events in coastal systems, investigating the effects on coastal species is important. This is of particular importance because early developmental stages play a key role in the life cycle of marine species of coastal communities. For example, in the rocky intertidal barnacle *Balanus glandula,* growth and development of the early life stages (nauplii) is essential to adult success as only cyprids of a minimum size will settle and metamorphose into adults[16].

California Current Large Marine Ecosystem

In the past, only two of the world's eastern boundary current systems have experienced hypoxic conditions: the Humboldt and Benguela systems. In the California Current System CUE, moderate hypoxia is normal and occurs seasonally (June-September) off the Oregon Coast as southward winds promote upwelling of cold, oxygen depleted waters to shallower depths[3]. Hypoxia is normal in a layer of water known as the Oxygen Minimum Zone (OMZ) that crosses the continental slope at depths greater than 600m[2,3] .

However, from 2002 to 2007 scientists have measured an unprecedented increase in severity and spread of inner continental shelf (depths <50m) hypoxic events[9]. The effects of hypoxia on fish and benthic invertebrate communities during these events were also measured. Remotely operated video surveys revealed dead fish or expiring invertebrates in areas where rockfish had, in previous years, averaged 12.8 per 100m [2]. Invertebrate die-offs had previously not been observed in this area[4]. A 75% mortality rate in crab pots (according to commercial fishery data) occurred in 2002 where previously the norm had been 0% mortality. Large numbers of dead fish and invertebrates were washed ashore and scuba divers reported observing large groupings of fish in shallow waters[4]. These observations point to altered survival and behavior due to low dissolved oxygen levels. As we can see from the often shocking consequences of hypoxia, our understanding of the effects of near shore hypoxia on biological communities is essential to successful management and conservation of the CCLME.

Barnacle species are of great importance in these communities. Adult barnacle species are central in shaping benthic communities in the CCLME. They play important roles in community succession, are important food sources, and promote species diversity[17,18]. As a part of the life history of the barnacle, successful growth and survival of larval stages is essential to the success of sessile adults. Little is known concerning the effects of hypoxia on larval stages of barnacles or any other benthic invertebrate in coastal upwelling ecosystems. Studies of planktonic scallop larva[13] and juvenile fish[14] species indicate decreasing probability of survival as dissolved oxygen concentration drops. Growth of mussel larvae has also been shown to be stunted by hypoxia[15]. As the same principles of oxygen use and metabolism apply across the animal kingdom, barnacle larvae exposed to hypoxia should experience similar increases in mortality and decrease in growth.

We've chosen barnacle larvae as model organisms to understand the effects of Oregon coast hypoxia on coastal marine invertebrate larvae. Our research goals are: 1) To determine the rate of mortality of nauplii (larvae) in the species *Balanus glandula* at increasingly severe levels of hypoxia and 2) To determine if exposure to hypoxic conditions adversely affects development of nauplii and if so, how this trend changes with severity of hypoxia.

Materials and methods

Egg masses from species *B. glandula* were collected from the field and reared in the lab to produce nauplii of the desired stages. Groups of nauplii were then exposed to a treatment of filtered sea water with dissolved oxygen levels of either 0.5 (microxia), 1.43 (hypoxia), or 1.8 (low oxygen water typical of the OMZ) ml/L for 24 hours. Nauplii were also exposed to dissolved oxygen levels of 5.5 (typical of near shore conditions, a.k.a. normoxia) for a 24 hour period to act as a control. Experimental treatments were conducted in replicates of three.

Differing levels of dissolved oxygen were created by infusing sea water with nitrogen gas. Four separate parallel carboys fed with sea water from a header tank were infused with different amounts of nitrogen gas. Each carboy in turn fed water into four separate and parallel one liter polypropylene containers through tubing inserted through a silicone stopper sealing the containers. Three of these containers contained nauplii while the fourth was used to measure oxygen level inside the one liter containers. The experimental setup allowed for continuous flow of seawater from the header tank through the one liter containers and out of the system.

Following treatments larval survival was measured for each of the three replicates per treatment exposure. After survival was quantified in each replicate, the surviving larvae from each replicate were reared for an additional seven days to monitor post treatment survival and development. The number of surviving larvae and the average percent of nauplii in each developmental stage for each treatment cohort was recorded.

Three 24 hour trials have been completed. For each of these three trials, only the microxic and normoxic oxygen carboys held oxygen levels stable enough to provide reliable data. ANOVA and MANOVA analysis were applied to the first two trials. Comparison of average mortality between normoxic and microxic treatments directly after 24 hour exposure was performed for each trial using a one-tailed t-test. A similar comparison was also performed on larvae seven days after the 24 hour exposure. The third trial (Sept.11) was separated from the analysis of the first two trials because changes were made in the system setup after the second trial to make sure the system was airtight.

Results

Survival

A significant difference was found for percent survival through a six day period after 24 hour exposures between microxic and normoxic treatments when MANOVA was applied to the first two trials (Aug. 21 and Aug. 27). Larvae exposed to microxia had a higher percent survival than larvae exposed to normoxia (p= 0.014, see fig. 1).

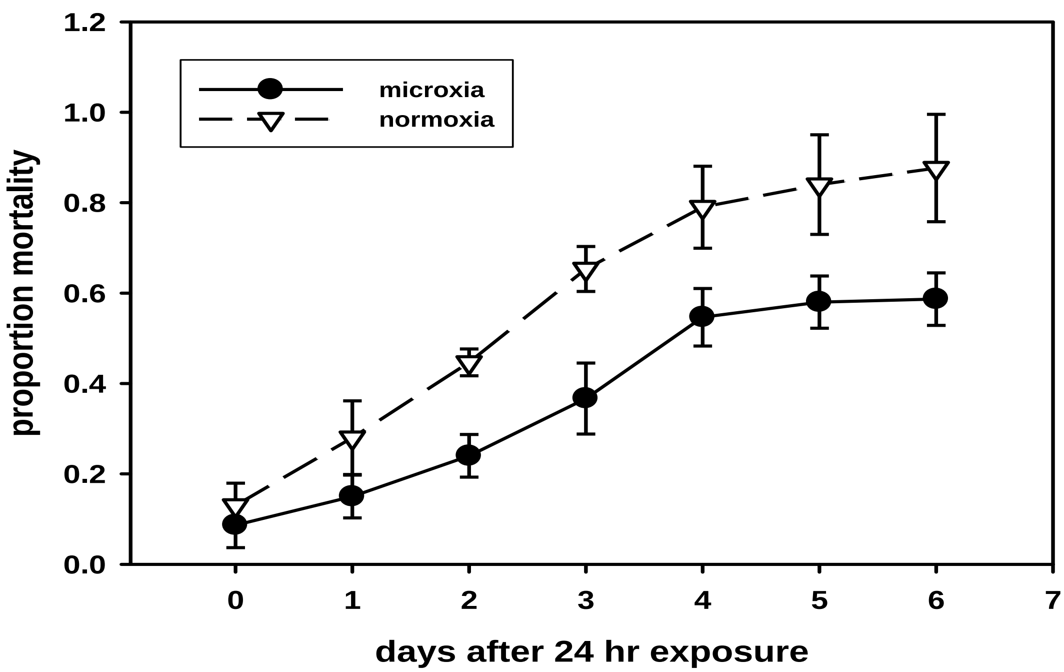

Figure 1. **Cumulative mortality of *Balanus glandula* larvae post 24 hour exposure.** Cumulative mortality of Balanus glandula mixed stage larvae through a six day period after exposure to microxia (0.5 mg/L dissolved oxygen) or normoxia (5.5 mg/L dissolved oxygen) for 24 hours. Data are from Aug.21 and Aug. 27 trials (MANOA Means±SD, normoxia n= 3, microxia n= 3, p= 0.014).

However, instantaneous analysis of percent survival between treatments directly after exposure showed no significant difference between survival in microxic and normoxic treatments in any of the three trials (p=0.192, 0.191, and 0.637 for Aug.21, Aug. 27, Sept. 11 respectively, see fig. 2). Similarly, instantaneous analysis of cohorts exposed to different treatments on day 7 after exposure did not show a difference in survival between treatments (p= 0.0811, 0.196, 0.579 for Aug. 21, Aug. 27, Sept. 11 respestively, see fig. 3).

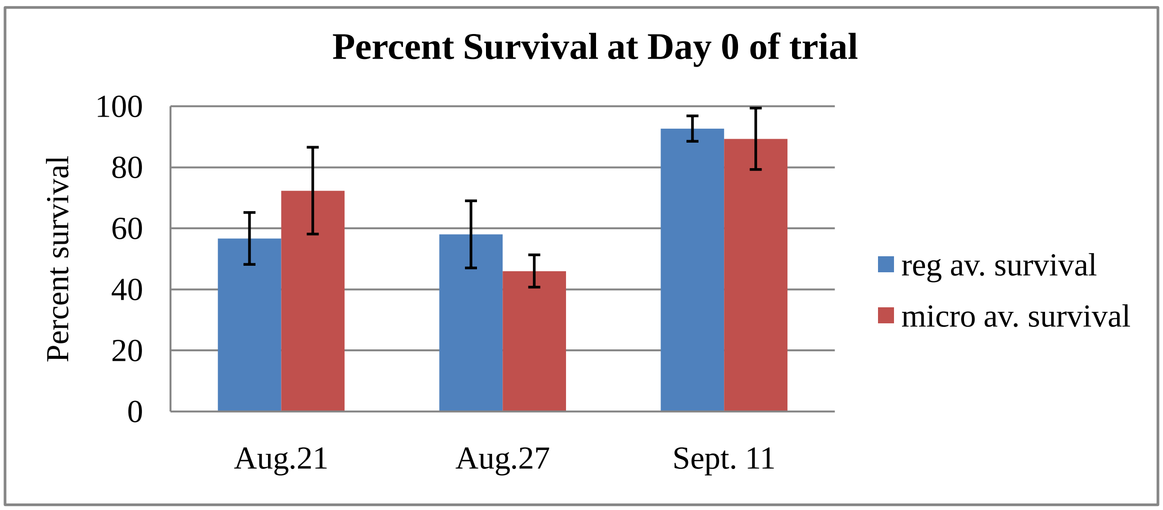

Figure 2. Percent survival of *Balanus glandula* larvae directly after 24 hour exposure . Percent survival of barnacle larvae for normoxic (5.5 mg/L dissolved oxygen) and microxic (0.5 mg/L dissolved oxygen) oxygen conditions directly after 24 hour exposure. Data for Aug.21, Aug.27, and Sept.11 trials is shown. There was no significant difference in percent survival between the two oxygen treatments for any of the trials (one tailed t-test: Aug 21: p= 0.192, Aug.27: p= 0.191, Sept. 11: p= 0.637).

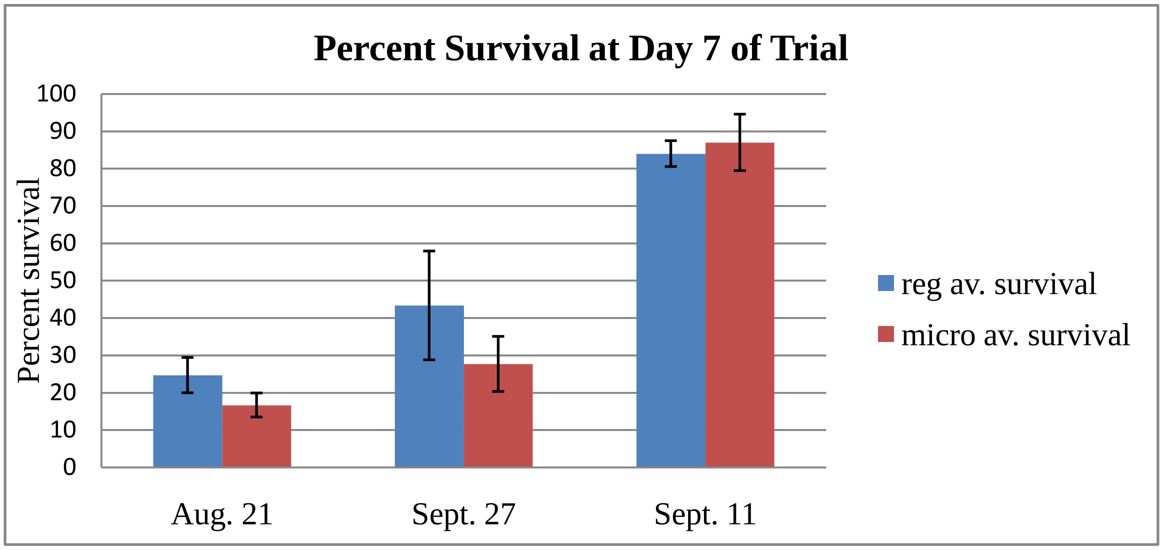

Figure 3. Percent survival of *Balanus glandula* larvae seven days post 24 hour exposure . Percent survival of barnacle larvae for normoxic (5.5 mg/L dissolved oxygen) and microxic (0.5 mg/L dissolved oxygen) oxygen conditions seven days after 24 hour exposure. Data for Aug. 21, Aug.27, and Sept.11 trials is shown. There was no significant difference in percent survival between the two oxygen treatments in any of the trials (One tailed t-test: Aug.21: $p = 0.0811$, Aug.27: $p = 0.196$, Sept.11: $p = 0.579$).

Development

Larval development was tracked for each replicate within each treatment for 7 days following exposures. On the final day of monitoring, analysis showed no difference in the average percent of larvae in the six stages between microxia and normoxic treatments ($p = 0.5$, see fig. 4). However, average percent of larvae in each stage was different enough that a trend of delayed development was apparent (see fig.4). On the final day of monitoring, larvae exposed to normoxia and reached stage VI while larvae in the microxia treatment reached stage V of development.

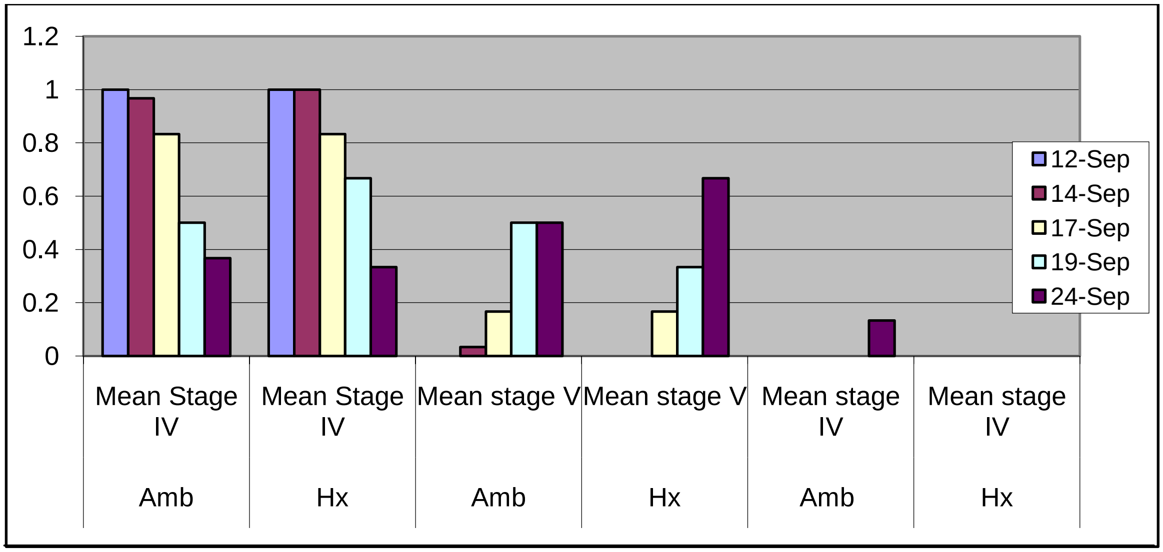

Figure 4. Developmental progression of *Balanus glandula* larvae post 24 hour exposure . Percent of larvae at developmental stages after 24 hour exposure to normoxic (5.5 mg/L dissolved oxygen) and microxic (0.5 mg/L dissolved oxygen) oxygen conditions. Number of larvae at each stage was calculated during a two week period after exposure to treatments. No significant difference in percent larvae in each stage was found between normoxic and microxic treatments (p= 0.500).

Discussion

Survival

Our results did not support the prediction that *B. glandula* larvae would experience increasing levels of mortality with exposure to decreasing dissolved oxygen levels. The majority of our analysis did not reveal a significant difference in nauplii survival between normoxic and microxic treatments. When a significant difference between treatments was present the nauplii responded opposite to what we expected, with nauplii in microxic treatments having a higher survival percentage than those in the normoxic treatment. Our results do not suggest an inverse relationship between survival and decreasing oxygen levels, but instead suggest that either hypoxia does not have an effect on survival of nauplii or that confounding factors other than dissolved oxygen levels are determining the observed survival rate. Our results do not clarify which of these two scenarios is the case as our hypoxic and OMZ oxygen levels were not consistent enough to provide data addressing nauplii survival under a variety of low oxygen conditions.

Given that our data addresses nauplii survival at only two dissolved oxygen levels and that we found contrasting results between different statistical analysis of treatments (i.e. between MANOVA and t-tests applied to Aug. 21 and 27 trials) we cannot provide a rate of survival expected under microxic or hypoxic conditions. Furthermore, it is still unclear if nauplii survive equally, better, or worse, between normoxic, hypoxic, and microxic conditions. More trials will need to be run and consistent oxygen levels achieved in all treatments before a conclusion can be made. Also, it is unclear whether our results in the first two trials run have been affected by errors in the carboy system which could introduce air into the system and expose the larvae to different oxygen conditions than were measured.

Development

Data on rates of development for larvae exposed to microxic and normoxic conditions did not show that oxygen level affected development significantly ($p = 0.50$). However, although there was no statistical difference between microxic and normoxic treatments, larvae exposed to normoxia would often progress to the following stage before the larvae that had been exposed to microxia. Based on our observations in the lab, we predict that future trials and further monitoring of development will reveal a decrease in developmental rate as oxygen levels decrease. Period of exposure to low oxygen levels may also be a factor affecting developmental rate, an issue we will be able to address as we progress to longer trials. If we were to discover a significant difference between developmental rates in different oxygen levels, it would allow us to make predictions regarding settlement rates of *B. glandula* and infer how hypoxic events might affect rocky intertidal ecosystem structure. Again, more trials and consistent oxygen levels across treatments are needed for further analysis.

Conclusion

The response of organisms and ecosystems to unprecedented hypoxic events is an exciting and timely area of research. The understanding of ecosystem responses to this form of hypoxia is limited at best, but researchers have begun to lay the foundations for future comprehension of biological and geochemical processes in affected systems. The inconclusive results from our preliminary analysis indicate that further work is needed in the form of longer more vigorous experiments. We look forward to continue investigating how *B. glandula* can contribute to the functioning of coastal ecosystems experiencing unprecedented hypoxia.

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