THE INTERACTIVE EFFECTS OF OCEAN ACIDIFICATION, FOOD AVAILABILITY, AND SOURCE LOCATION ON THE GROWTH AND PHYSIOLOGY OF THE CALIFORNIA MUSSEL

By

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A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

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May 2018

ABSTRACT

THE INTERACTIVE EFFECTS OF FOOD AVAILABILITY AND OCEAN ACIDIFICATION ON THE GROWTH AND PHYSIOLOGY OF THE CALIFORNIA MUSSEL

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Research shows ocean acidification (OA) can have largely negative impacts on marine organisms and ecosystems. Prior laboratory studies show that shelled marine invertebrates (e.g., molluscs) exhibit reduced growth rates and weaker shells when experiencing OA-related stress. However, populations of the critical intertidal mussel species, *Mytilus californianus*, which experience naturally acidic water due to upwelling in certain parts of Northern California have been observed to have relatively stronger and thicker shells and higher growth rates than those that experience less frequent exposure to upwelling. To address the discrepancies between negative effects of OA exposure in the laboratory and seemingly positive effects off OA exposure in the field we collected juvenile mussels from four separate locations on the northern California coast that vary in exposure to upwelling-driven OA and raised them under ambient, constantly acidified, or intermittently acidified seawater conditions. Half of the mussels in each of the experimental treatments were given access to either ambient or elevated food concentrations. Although higher food availability increased shell and overall mussel growth, variation in mussel life-history traits among locations appears to be driven

primarily by inherent differences (i.e. genetics or epigenetics). In particular, overall growth, soft tissue mass, and shell dissolution in mussels were associated with source-specific upwelling strength while adductor muscle mass along with shell growth and strength of mussels were associated with source-specific levels of predation risk. Oxygen consumption of mussels did not significantly vary among food, pH or source location treatments, suggesting that differences in growth rates were not due to differences in differences in metabolic or energetic efficiencies between individuals. Although not statistically significant, mussels from areas of high crab predation risk tended to survive crab attacks in the lab better than mussels from other areas. My data suggests that the adaptive potential of *M. californianus* to respond to future OA conditions is dependent on local environmental factors such as upwelling strength, food availability, and predation risk. My study addresses a significant gap in our understanding of the mechanism behind conflicting observations of increased growth in the field associated with low pH and previous laboratory results, demonstrating the importance of environmental context in shaping the organismal response to current and future OA conditions

ACKNOWLEDGEMENTS

I would first like to thank Dr. Paul Bourdeau for his help and guidance in the planning, execution, and synthesis of my thesis. He gave me the freedom to work independently and grow as a scientist but was always available to provide academic and emotional support when I needed it.

I would also like to thank my committee members for their helpful comments and expertise on my thesis. Their input improved the quality of my thesis by orders of magnitude and greatly contributed to my professional development.

I would also like to acknowledge Corianna Flannery and Roxanne Robertson for teaching me how to conduct closed volume respirometry and how to extract and analyze chlorophyll-a, respectively. The use of their equipment, along with instructions contributed to major sections of my thesis.

I would also like to thank my lab mates in PEB lab for their time, aid, and individual expertise. Particularly, I would like to thank Jessica Coming for her contribution to the shell dissolution experiment.

I would like to thank my funding sources: the CSU COAST Graduate Student Research Award, the Humboldt MSCI Graduate Student Research Award, the California Sea Grant, the Oliphant Scholarship in Marine Science, and private loans from Eric Man and Letine Ly.

Finally, I would like to thank Dad, Mom, Brother, Cousins, Aunts and Uncles for their unwavering support and love.

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INTRODUCTION

Since the industrial revolution, humans have increased the concentration of carbon dioxide in the atmosphere. Roughly a third of that carbon dioxide is absorbed by the ocean, which causes a series of chemical reactions that reduce the pH of seawater, resulting in the phenomenon referred to as ocean acidification (OA) (Brewer et al. 1997; Feely et al. 2009). Research shows OA to have largely negative impacts on marine organisms and ecosystems (Bibby et al. 2007; Somero et al. 2016; Melatunan et al. 2013; Hale et al. 2011). Prior work investigating OA effects emphasizes the inherent susceptibility of calcifying organisms to reductions in environmental pH (Gazeau et al. 2013; Gaylord et al. 2011; Sanford et al. 2014). Studies have linked OA to adverse effects on fitness and life history traits of many marine bivalves ranging from weakened shells and byssal threads to reduced tissue mass and slower larval development (Gaylord et al. 2011; O'Donnell et al. 2013; Gobler et al. 2014; Parker et al. 2012; Sunday et al. 2011). Negative effects of OA stress on bivalves are not only an ecological concern but also an economic one as well, as roughly $270 million USD are annually brought in via commercial shellfish farms along the west coast of the United States alone (Barton et al. 2015; Washington State Blue Ribbon Panel on Ocean Acidification, 2012).

Many studies on the effects of OA on the genus *Mytilus* have shown reductions in somatic growth, byssal thread strength, and shell deposition when subjected to reduced pH conditions (O'Donnell et al. 2013; Duarte et al. 2013; Gaylord et al. 2011). The magnitudes in responses to OA stress vary between species and can also be dependent on

other environmental factors such as food availability and prior exposure to low pH conditions (Vargas et al. 2017; Thomsen et al. 2013; Duarte et al. 2014). Kroeker at al. 2016 observed differences in the growth of *Mytilus californianus* along the California Current Large Marine Ecosystem (CCLME) and suggested that dynamic environments with low pH and consistent food can increase individual performance and growth.

Along the California coast, upwelling brings seawater that are nutrient rich and low in pH to the surface. A gradient of upwelling intensities occurs along the CCLME and form local hotspots of intense upwelling that are persistent year to year (Feely et al. 2008; Chan et al. 2017). Throughout the CCLME, variations in local wind stress conditions and shelf slope bathymetry cause some nearshore habitats (e.g., near the HSU marine lab in Trinidad, CA) to be exposed to intense levels of upwelling which can expose rocky intertidal habitats to water with a pH as low as, and sometimes lower than, 7.6 during strong upwelling events (Jacox & Edwards 2011; Jacox & Edwards 2012; Appendix A).

Comparisons among populations of the intertidal mussel, *Mytilus californianus*, inside and out of upwelling zones found greater shell thickness and strength in upwelling zones (Bourdeau, unpublished), similar to results found by Kroeker et al. (2016). Increased physiological performance associated with low pH conditions conflicts with previous lab experiments that repeatedly show reduced growth and structural integrity in shelled molluscs exposed to low pH (Gazeau et al. 2013; Melzner et al. 2011; Kroeker at al. 2014; Gaylord et al. 2011). One possible explanation for this surprising result is that upwelling along the CCLME is not only associated with the delivery of acidic waters to

nearshore habitats, it also leads to increased food availability for suspension feeding organisms like mussels; a factor that has been shown to ameliorate the effects of temperature and OA stress in *M. californianus* and other bivalves (Fitzgerald-Dehoog et al. 2012; Melzner et al. 2011; Hettinger at al. 2013; Thomsen et al. 2013). Responding to OA stress (e.g., maintaining pH balance, building and maintaining calcified structures) may incur energetic costs, which must be balanced against the cost of somatic, shell, and reproductive growth (Pörtner 2008; Ries 2011; Pan et al. 2015). Mussels in upwelling zones in the CCLME, although exposed to acidic waters, may be less negatively impacted by low pH water because they take in more energy from nutrient-rich (and consequently phytoplankton-rich) upwelled waters. Mussels from areas of intense upwelling may also be locally adapted to low pH conditions through evolved genetic differences or via epigenetics or other physiological acclimatization that allow them to take advantage of environments high in food availability that would be otherwise stressful to mussels without prior exposure to those conditions.

For my thesis, I experimentally examined the separate and interactive effects of exposure to acidic water, food availability, and source location on *M. californianus* growth to examine the contribution of local adaptation to pH stress, enhanced energy availability, or a combination of both factors that allows mussel to build thicker and stronger shells in upwelling zones. I tested whether *M. californianus* from areas regularly exposed to upwelling-driven OA (as low as predicted for global oceans in 50-100 years) (Calderia & Wickett 2003), differ from mussels in areas with higher and more stable oceanic pH levels in terms of their allocation to soft tissue growth and shell development

when exposed to varying, combinations of pH and food. By investigating the responses of different populations of key a foundation species that have different prior experience with OA stress, we will be better able to predict the susceptibility of these critical species to future climate change.

METHODS

Collection and Maintenance of Study Organisms

In late June of 2017, I collected juvenile mussels (mean shell mass = 1.96g +/- 0.77 SD) from four source locations, each characterized by similar sea surface temperature climatology, different coastal upwelling regimes, and seasonal and periodic exposure to acidic seawater and nutrients (Appendix B, C, D; Bourdeau, unpublished; Feely et al. 2008; Chan et al. 2017). Two sites were partially wave-exposed boulder fields that are exposed to lower pH and more nutrient rich water (Point. St. George [41°47'02.8"N 124°15'17.2"W], near Crescent City, CA, and Baker Beach [41°02'57.4"N 124°07'39.9"W], near Trinidad, CA) (Appendix B, D). The two other sites were moderately wave-protected rock benches that experience less intense upwelling, higher pH, and less nutrient availability (Mussel Rock [40°20'51.0"N 124°21'52.2"W], near Cape Mendocino, CA and Belinda Point [39°23'54.9"N 123°49'11.0"W], near Fort Bragg, CA) (Appendix B, D). Collected mussels were cleaned of epibionts, individually labeled using plastic tags (Queen Marking Kit; The Bee Works, Orillia, ON, Canada), weighed in air and water to calculate overall soft tissue mass, and shell mass non-destructively (as described in Palmer, 1982), and acclimated to lab conditions for 48 hours before being randomly assigned to experimental treatments. To measure overall weights in air, individuals were forced shut while submerged and dried using a paper towel to remove error in overall weight due to excess water.

Laboratory Growth Experiment

I conducted a split-unit laboratory experiment that manipulated both pH and food availability as whole units and mussel source location as the split-unit. I had 6 treatment combinations made up of 3 pH treatments: constant ambient (7.82+/- 0.08 SD), constant low pH (7.61 +/- 0.09 SD) and variable pH (alternating between a pH of 7.82 and 7.60 every 10 days which is roughly equivalent to the average duration of low pH upwelling in the field); and 2 food concentration treatments: ambient (1.64 µg chl *a*/L +/- 0.77 SD) and enhanced (5.95 µg chl *a*/L +/- 2.37 SD). Six header tanks (45L) were assigned randomly to each treatment combination, with each header tank feeding into eleven spatially and haphazardly interspersed replicate containers (473mL), which served as whole units, and where the mussels were held for 112 days (Figure 1). Each replicate container was haphazardly assigned 16 mussels total; four mussels from each of the four sites. Replicate containers were placed in one of two sea tables (114.3 cm wide x 33.02 cm deep x 15.24 cm tall) and partially submerged (~5 cm) in flow-through seawater to maintain consistent temperatures among containers (daily flow-through seawater temperature $\bar{x}$ = 13.0˚C +/- 0.28 SD). To minimize the effects of pseudoreplication, replicate containers were spatially shuffled and header tanks were cleaned and reassigned to different treatment combinations every 20 days.

The pH of the header tanks was regulated using a low-cost, but precise CO_2 dosing system as described by Wilcox-Freeburg et al. (2013). Double junction laboratory grade pH probes (Bulk Reef Supply; Golden Valley, MN) and a Digital Aquatics

aquarium controller (Digital Aquatics; Woodinville, WA) were used to continuously measure the pH of seawater in each header tank and automatically dose CO_2 using a gas solenoid (AZOO; New Taipei City, Taiwan). CO_2 was incorporated into the seawater in each header tank using an ISTA Max Mix CO_2 reactor powered with a Taam RIO+ 400 powerhead to insure the efficient use of CO_2, along with even mixing of seawater within header tanks. Samples of seawater from each header tank were taken daily and pH was measured using a temperature corrected benchtop pH meter (Oakton pH 700 Benchtop Meter). pH probes in header tanks and bench top meters were calibrated every 20 and 7 days, respectively, using the same two-point calibration standard (pH 7.00 and 10.00, Fisher Chemicals Buffer Solution)

The concentration of food was manipulated by dosing diluted (1:50) microalgae concentrate (*Isochrysis*, *Pavlova*, *Tetraselmis*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii* and *Thalassiosira pseudonana*; Shellfish Diet; Reed Mariculture, Campbell, CA) every 2 hours into each header tank using automated peristaltic pumps (JEBAO DP-4 Dosing Pump). Chlorophyll samples were taken from the header tank and vacuum filtered through 47mm GF/F filters. The filters were placed in borosilicate tubes along with 4ml of 90% acetone and stored in a spark-free freezer for 18-24 hours. Fluorometric measurements were then taken before and after the addition of 10% HCl and compared against a solid standard to calculate chlorophyll-a concentration. Samples were taken from varying intervals after microalgae dosing to determine the average chlorophyll-a concentration in each treatment.

At the end of the experiment, I re-measured the same mussel shell metrics as those at the beginning of the experiment, along with mussel mass in both air and water. Each mussel was then sacrificed and stored in a -80◦C freezer before being shucked, dissected, and having their shell valves desiccated. During the dissection of mussels, anterior adductor muscles were removed and placed on wax paper-lined tins, baked at 85°C for at least 36 hours, and then weighed.

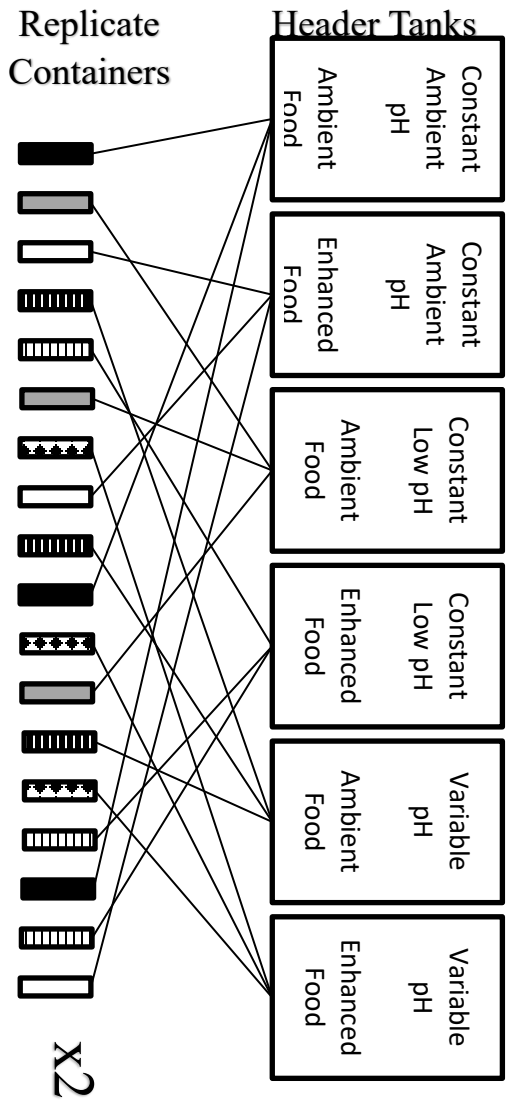

Figure 1: Schematic of laboratory growth experiment where header tanks were assigned unique pH/food treatment combinations. Header tanks fed replicate containers. The color/pattern combination of each replicate container indicates the experimental treatment combination delivered from each header tank. KEY: Constant ambient pH/ ambient food: solid black fill; constant ambient pH/ enhanced food: solid white; constant low pH/ ambient food: solid grey fill; constant low pH/ enhanced food: white fill with black lines; variable pH/ ambient food: black fill with white lines; variable pH and enhanced food: solid white with black diamonds.

Respiration Assays

Respirometry assays were run during the laboratory growth experiment to measure mass-specific oxygen consumption rates as a proxy for metabolism. Randomly selected containers were assayed 2-3 times during the experiment. Oxygen consumption was measured in 128 ml glass respirometry chambers (Loligo Systems Inc.) using fiber-optic oxygen sensors (PreSens, Regensberg, Germany). Due to the small size of individuals, the four mussels from each source location within a replicate container were grouped and measured together in each chamber. Four chambers were placed in an insulated cooler (189.27 L) partially filled with continuously UV-filtered seawater manipulated to match the pH treatment of the mussels. The seawater was manipulated by manually dosing CO_2 and monitoring the pH using an APEX® aquarium controller. Each chamber was connected to two pumps, which either recirculated seawater through the chamber during measurement periods or flushed the chamber with seawater from the cooler between measurement periods. Chambers were flushed for 6 minutes, recirculated for 100 seconds, then oxygen concentrations were measured every second for 15 minutes. Respiration rate was measured as the change in oxygen concentration in the respiration chamber per wet mass of mussel per hour. To determine the volume of water in the chamber due to the displacement of the mussels, the mussels were measured using a hydrostatic suspension technique (Hughes, 2005) (Figure 2).

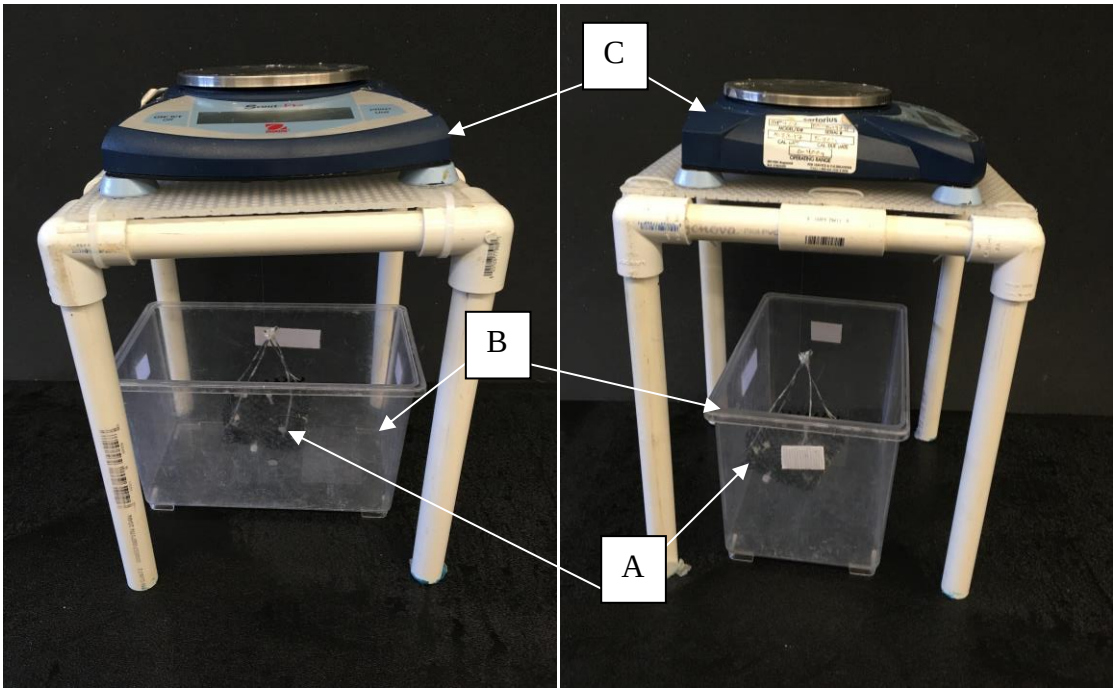

Figure 2: Front (Left Panel) and side (Right Panel) view of the hydrostatic suspension measurement apparatus. A mesh basket (A) was submerged in a seawater filled plastic container (B) and attached to a digital scale (C). The mass of submerged mussels can be used to determine the volume of water displaced by the mussel and in turn, the volume of the mussel.

Shell Strength

Left valves of mussels from the laboratory growth experiment were desiccated for 24 hours by placing individual valves in resealable plastic bags with perforated silica gel packets (Pillow Pak 2g Silica Gel Packets). The valves were then weighed and placed in an Instron® materials testing machine. The maximum force required for catastrophic valve failure was measured for each individual and scaled using the mass of the desiccated valve.

Crab Predation Assay

In early October of 2017, 80 mussels (mean shell length = 42.1mm +/- 4.5 SD) from each of the same four source locations as those used in the laboratory growth experiment were collected, measured, and tagged using nail polish before being acclimated to laboratory conditions for one week. Predatory crabs, (brown rock crab, *Romaleon antennarium)* were trap-collected from the Trinidad pier (41°03'20.0"N 124°08'49.5"W) in Trinidad, California, USA and hand-collected from Fields Landing (40°43'35.8"N 124°13'14.7"W) in Eureka, California, USA. Twelve crabs were placed in individual plastic tubs (5L) held in one of two sea-tables (53.34 cm wide x 114.3 cm deep x 20.32 cm tall) at Humboldt State University's Trinidad Marine Lab. Each tub independently received seawater from a flow-through system and was spaced apart to avoid receiving potential cues from adjacent tubs. Crabs were fed thawed capelin (*Mallotus villosus*) *ad libitum* before being starved 24 hours prior to the beginning of the

feeding assay. Each crab was given 20 mussels (5 from each source location), tubs were covered, and crabs were allowed to feed for 16 hours before ending the experiment and counting surviving mussels.

Shell Dissolution Experiment

In early October of 2017, 70 juvenile mussels (~2.5cm shell length) were collected from each of the same four source location as those used in the laboratory manipulation experiment and sacrificed by freezing. Their valves were then separated, cleaned, and labelled before being desiccated for 24 hours in a glass desiccation chamber with Drierite desiccant. Each valve was then weighed before randomly assigning one set of four right valves (one from each source location) to each of 66 replicate containers (473mL). Six large header tanks (45L) were assigned randomly to three pH treatments: ambient pH (7.81), low pH (7.59), or variable (alternating between a pH of 7.81 and 7.59 every 10 days), for a total of 2 header tanks per treatment. The pH of the header tanks was regulated using the same CO_2 dosing system as described above. Each header tank fed into eleven replicate containers that were spatially interspersed in one of two sea tables (114.3 cm wide x 33.02 cm deep x 15.24 cm tall); providing 22 replicate containers per treatment. To avoid pseudoreplication, replicate header tanks were cleaned, spatially re-arranged, and reassigned to different treatment combinations every 5 days. The experiment ran for 20 days.

Statistical Analysis

Overall growth (Shell + Soft Tissue) growth, soft tissue growth, shell growth, shell strength, final adductor: soft tissue ratio, and oxygen consumption rates were analyzed using separate, split-unit ANOVAs. The measurements taken on individuals were averaged among source location within a replicate container, thus pH and food treatments were applied to whole units (replicate containers) and source location was applied to split units within the whole units. Shell dissolution was analyzed using a similar split-plot ANOVA except with just pH treatment applied to whole units (replicate containers) and source location applied to split units within the whole units. Mussel survival during the crab predation assay was analyzed using a Generalized Linear Model with mussel source location as a fixed factor, mussel length as a covariate, and mussel survival as the response variable. All statistical analyses were done using R (R Core Team 2013) and RStudio Version 1.0.143 (RStudio, Inc.).

RESULTS

Overall (Shell + Soft Tissue) Growth

The overall (shell + soft tissue) growth of mussels was significantly affected by source location, food availability, and pH but without any significant interactions among the factors (Split-unit ANOVA: Source, $F_{3,62} = 76.3$, $P < 0.001$, [Fig.3 (top)]; Food, $F_{1,62} = 10.1$, $P < 0.001$, [Fig.3 (middle)]; pH, $F_{2,62} = 3.29$, $P < 0.05$, [Fig.3 (bottom)]; All interactions, $P > 0.25$, [Fig. 4]). Mussels collected from sites experiencing lower upwelling (Mussel Rock, $\bar{x}$ =1.62%, SE = 0.23%; Belinda Point, $\bar{x}$ =1.89%, SE = 0.34%) grew significantly less than mussels from sites that experience more intense upwelling (Point St. George, $\bar{x}$ = 8.07%, SE = 0.35%; Bakers Beach, $\bar{x}$ = 4.67%, SE = 0.34%; Tukey's HSD, $P < 0.001$). However, of the mussels from high upwelling sites, those from Point St. George grew significantly more than those from Baker's Beach (Tukey's HSD, $P < 0.001$). Increased food availability yielded a higher percent change in total growth (Enhanced, $\bar{x}$ = 4.60%, SE = 0.28%; Ambient, $\bar{x}$ =3.52%, SE = 0.22%). Although pH was significant in our model, the Tukey's HSD test failed to show any significant pairwise differences between overall growth in ambient ($\bar{x}$ = 4.34%, SE = 0.35%), variable ($\bar{x}$ = 4.40%, SE = 0.31%), and low ($\bar{x}$ =3.44%, SE = 0.29%) treatments (P >0.15). The lack of significance of the post hoc comparisons is due to the conservative nature of the Tukey's HSD test, which corrects for experiment-wise Type I error rate.

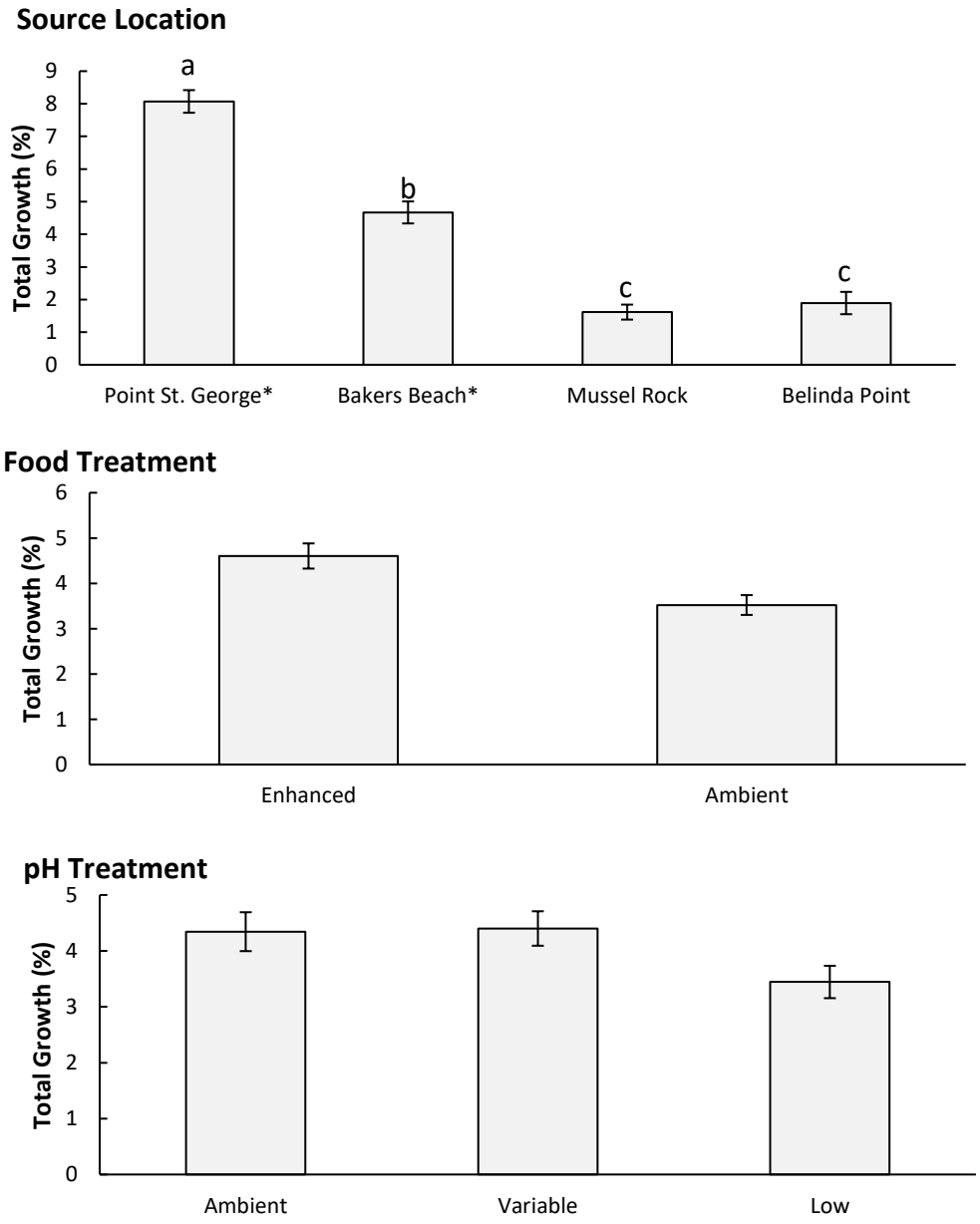

Figure 3: Total percent change ($\bar{x}$ +/- SE) in dried in-air mass of mussels after 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling) (top), food treatment (middle), and pH treatments (bottom). Different letters indicate significant differences among treatment groups at the $\alpha = 0.05$ level with Tukey's HSD test.

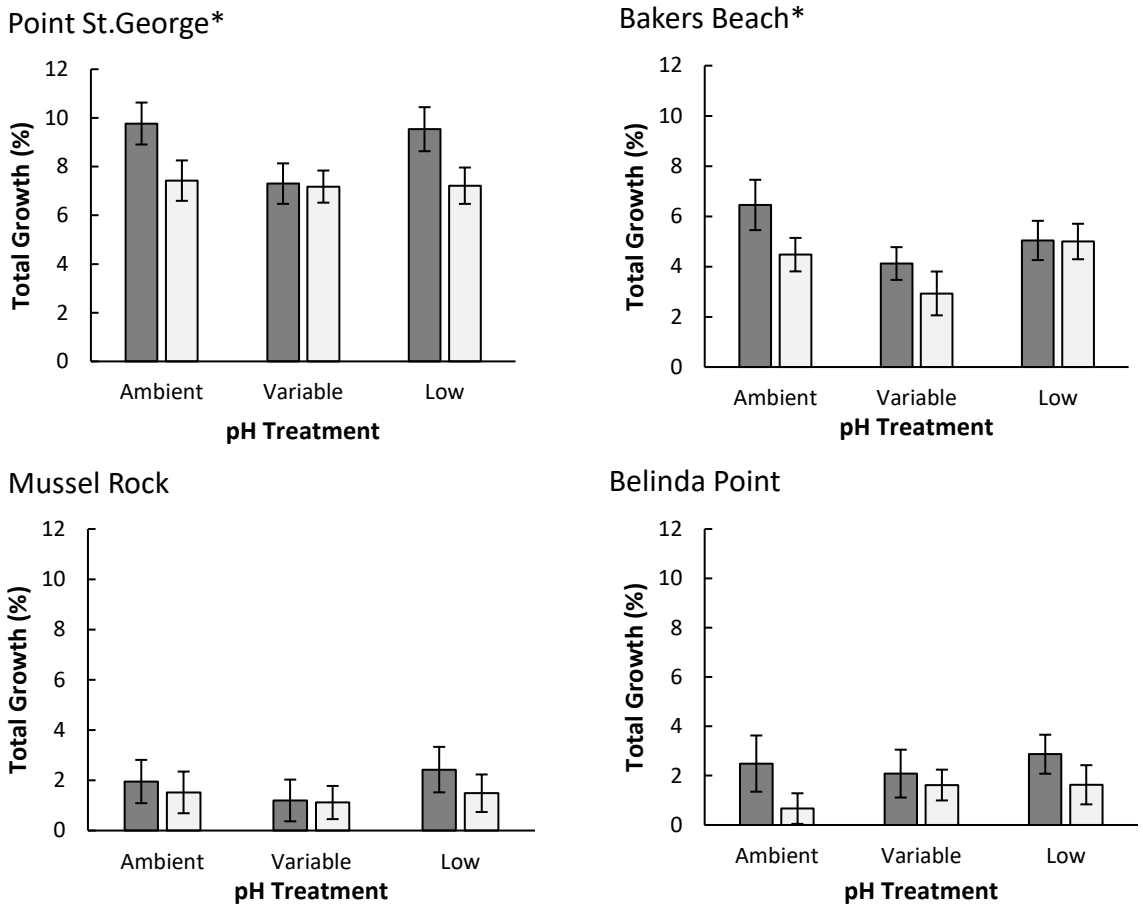

Figure 4: Total percent change ($\bar{x}$ +/- SE) in dried in-air mass of mussels after 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling), pH treatment, and food treatments (Enhanced [Dark grey bars]; Ambient [Light grey bars]).

Soft Tissue Growth

The soft tissue growth of mussels was significantly influenced by source location, but not by food, pH, or the interactions among factors (Split-unit ANOVA: Source, $F_{3,59}$ = 76.3, $P < 0.001$, [Fig.5 (top)]; Food, $F_{1,59} = 0.74$, $P = 0.40$, [Fig.5 (middle)]; pH, $F_{2,59} = 0.67$, $P = 0.52$, [Fig.5 (bottom)]; All interactions, $P > 0.25$, [Fig.6]). Mussels from sites experiencing more intense upwelling (Point St. George, $\bar{x}$ =19.11%, SE = 1.67%; Baker's Beach, $\bar{x}$ =20.13%, SE = 1.62%) had significantly more soft-tissue growth than mussels from sites with lower exposure to upwelling (Mussel Rock, $\bar{x}$ = 6.10%, SE = 1.08%; Belinda Point, 7.88%, SE = 1.04%; Tukey's HSD, $P < 0.001$).

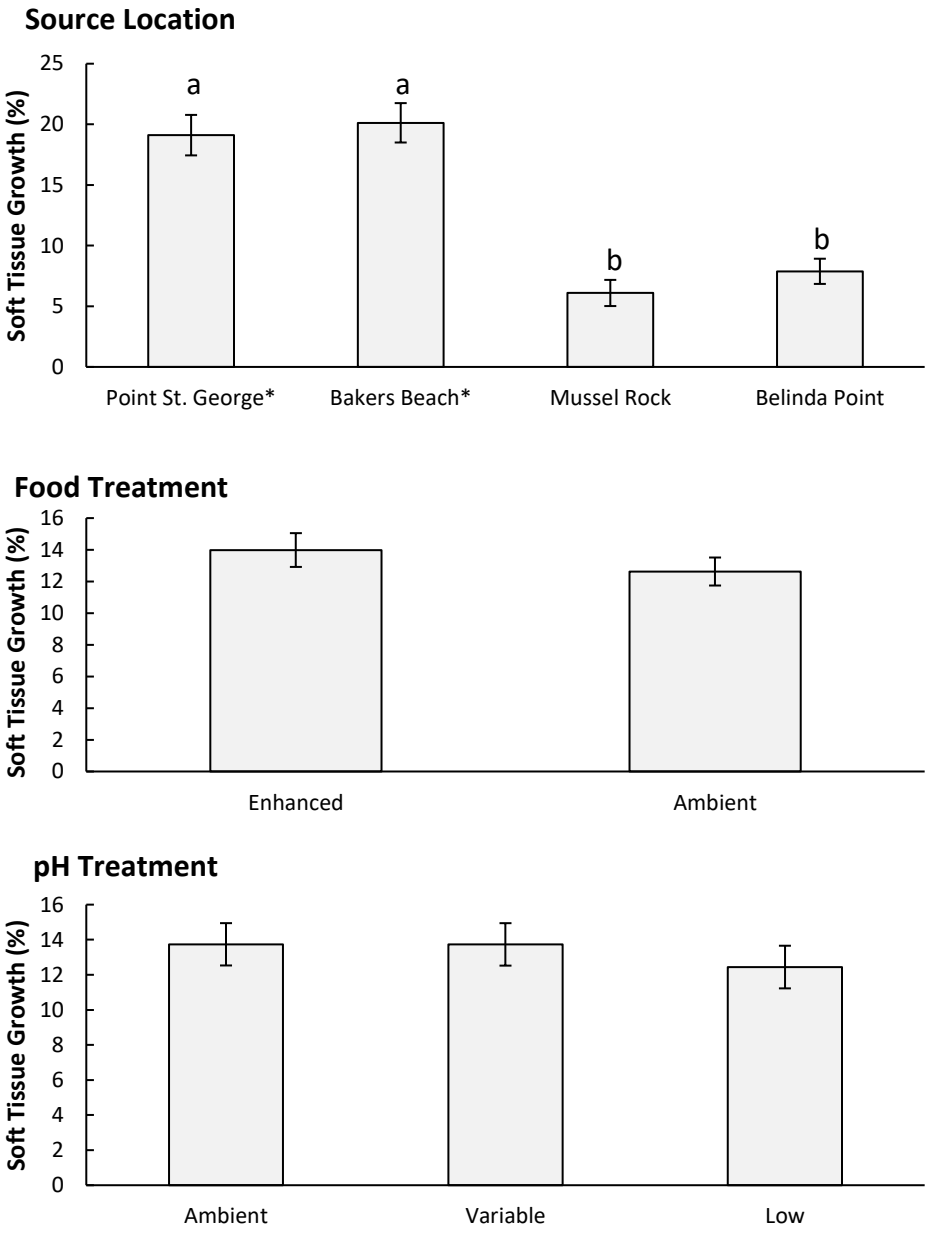

Figure 5: Soft tissue growth percent change ($\bar{x}$ +/- SE) of mussels after 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling) (top), food treatment (middle), and pH treatments (bottom). Different letters indicate significant differences among treatment groups at the $\alpha = 0.05$ level with Tukey's HSD test.

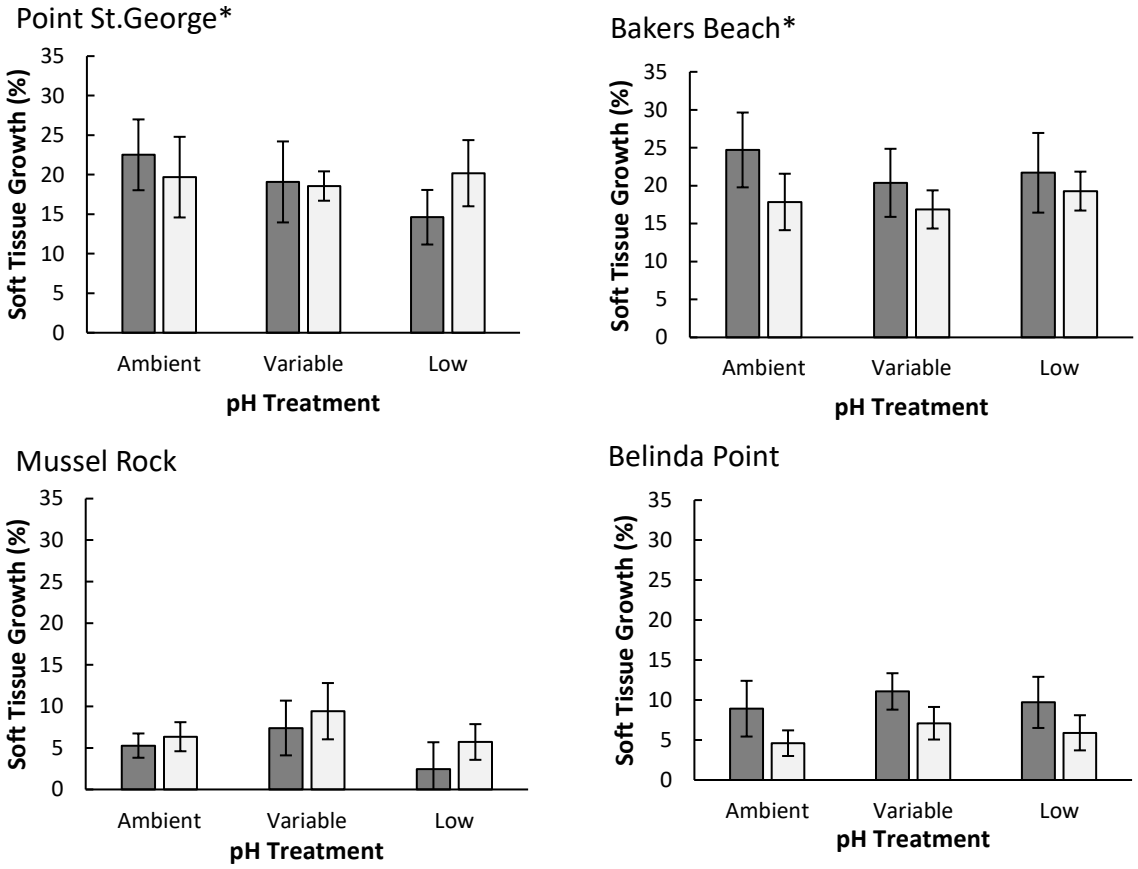

Figure 6: Soft tissue growth percent change ($\bar{x}$ +/- SE) of mussels after 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling), pH treatment, and food treatments (Enhanced [Dark grey bars]; Ambient [Light grey bars]).

Shell Growth

Shell mass change was significantly influenced by source location and food treatment, but not by pH, or the interactions among factors (Split-unit ANOVA: Source, $F_{3,61} = 35.34$, $P < 0.001$, [Fig.7 (top)]; Food, $F_{1,61} = 5.24$, $P < 0.05$, [Fig.7 (middle)]; pH, $F_{2,61} = 0.45$, $P = 0.64$, [Fig.7 (bottom]; All interactions, $P > 0.25$, [Fig.8]). Mussels from Point St. George ($\bar{x}$ = 4.73%, SE = 0.63%) and Mussel Rock ($\bar{x}$ =0.28%, SE = 0.38%) had significantly different changes in shell mass from all other sites (Tukey's HSD, $P < 0.001$) while Bakers Beach ($\bar{x}$ = -3.01%, SE = 0.48%) and Belinda Point ($\bar{x}$ = -1.69%, SE = 0.36%) were not significantly different and lost shell mass (Tukey's HSD, $P = 0.13$). On average, mussels exposed to enhanced food concentrations ($\bar{x}$ = 0.73%, SE = 0.38%) gained more shell mass than those under ambient food conditions ($\bar{x}$ = -0.58%, SE = 0.33%.)

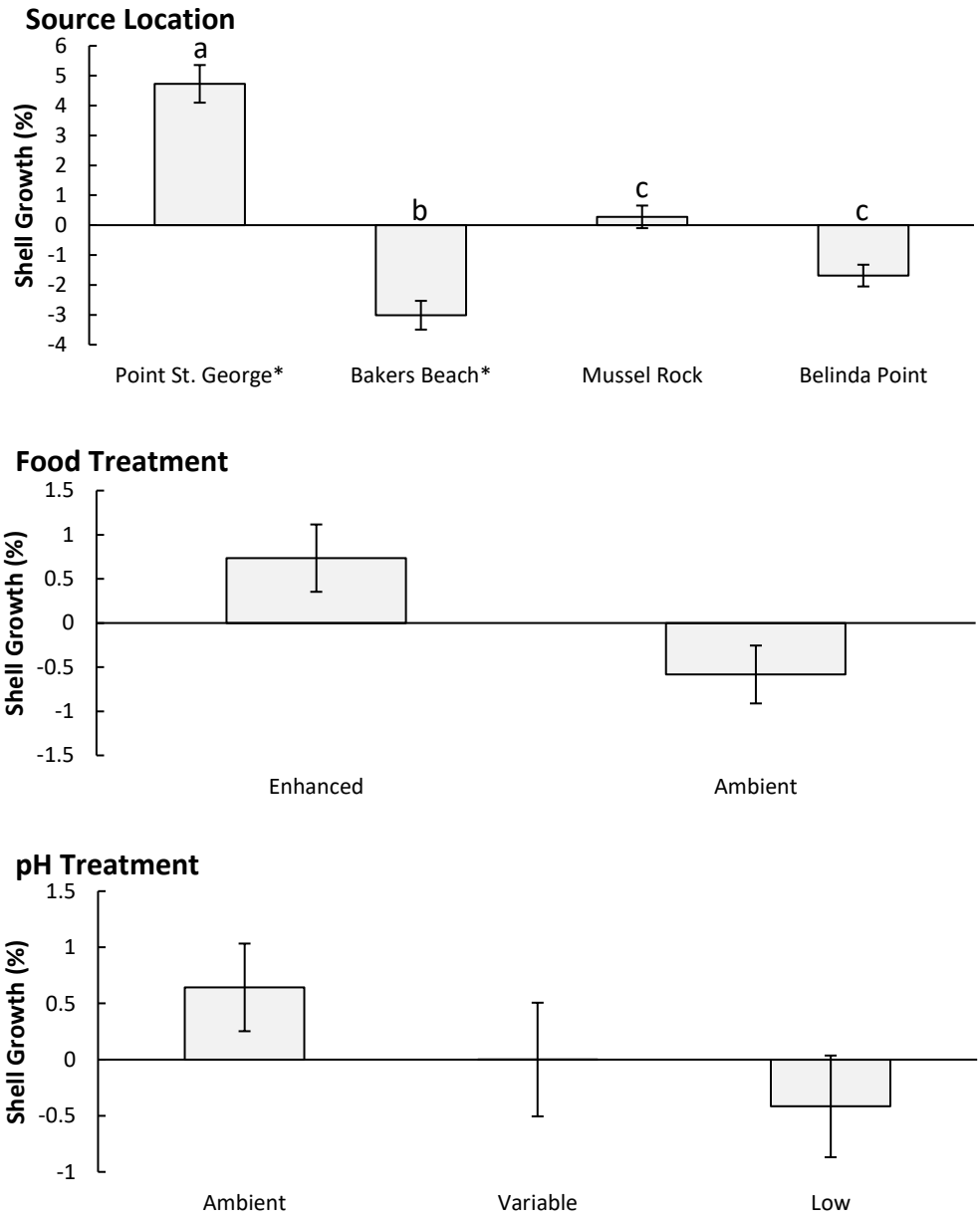

Figure 7: Shell growth percent change ($\bar{x}$ +/- SE) of mussels after 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling) (top), food treatment (middle), and pH treatments (bottom). Different letters indicate significant differences among treatment groups at the $\alpha = 0.05$ level with Tukey's HSD test.

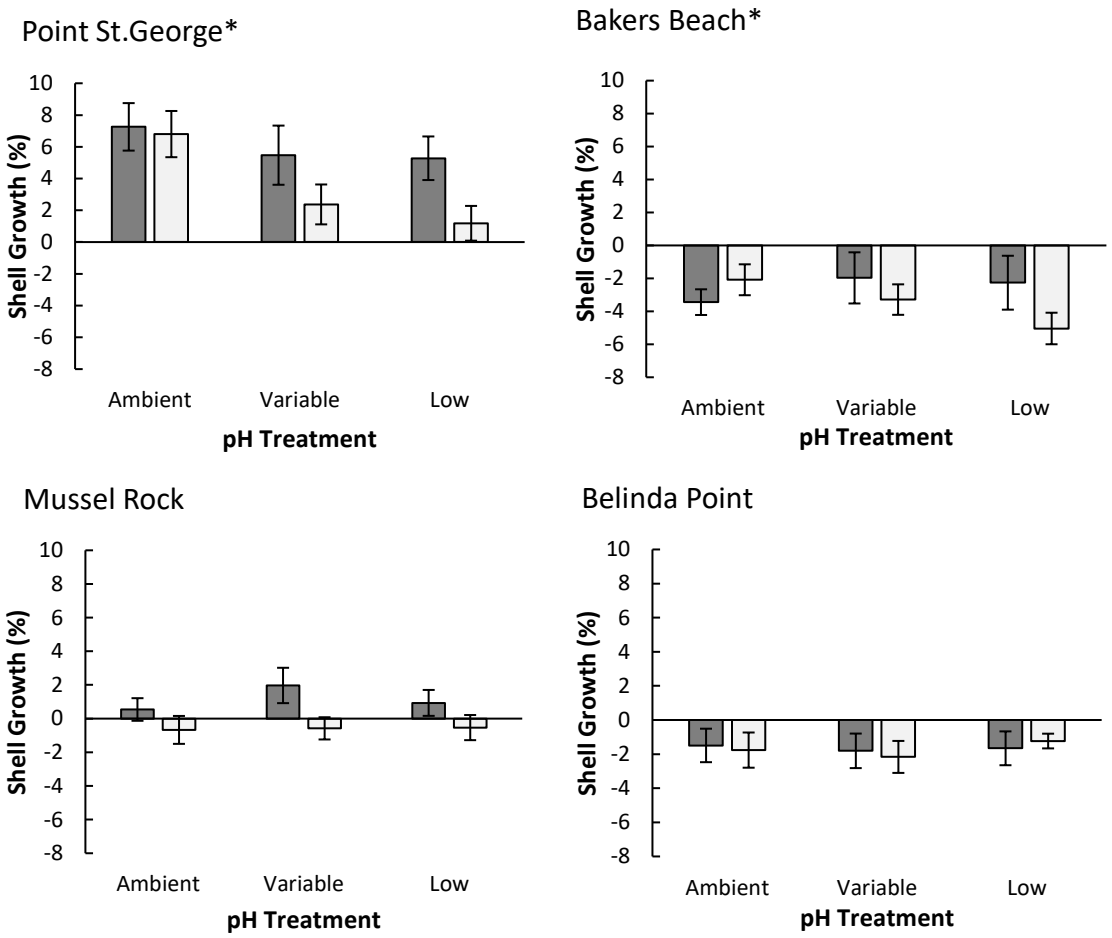

Figure 8: Shell growth percent change ($\bar{x}$ +/- SE) of mussels after 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling), pH treatment, and food treatments (Enhanced [Dark grey bars]; Ambient [Light grey bars]).

Shell Strength

Source location had significant effects on the strength of shells while food, pH, and treatment interactions did not (Split-unit ANOVA: Source, $F_{3,57} = 15.2$, $P < 0.001$, [Fig.9 (top)]; Food, $F_{1,57} = 0.53$, $P = 0.47$, [Fig.9 (middle)]; pH, $F_{2,57} = 1.70$, $P = 0.19$ [Fig.9 (bottom)]; All interactions, $P > 0.25$, [Fig.10]). Shells from mussels from Point St. George (106.64 N/g, SE = 3.54) and Mussel Rock ($\bar{x}$ = 104.48 N/g, SE = 4.18) are stronger than shells from Bakers Beach ($\bar{x}$ = 86.80 N/g, SE = 3.26) and Belinda Point ($\bar{x}$ = 74.18 N/g, SE = 2.74; Tukey's HSD, $P < 0.01$).

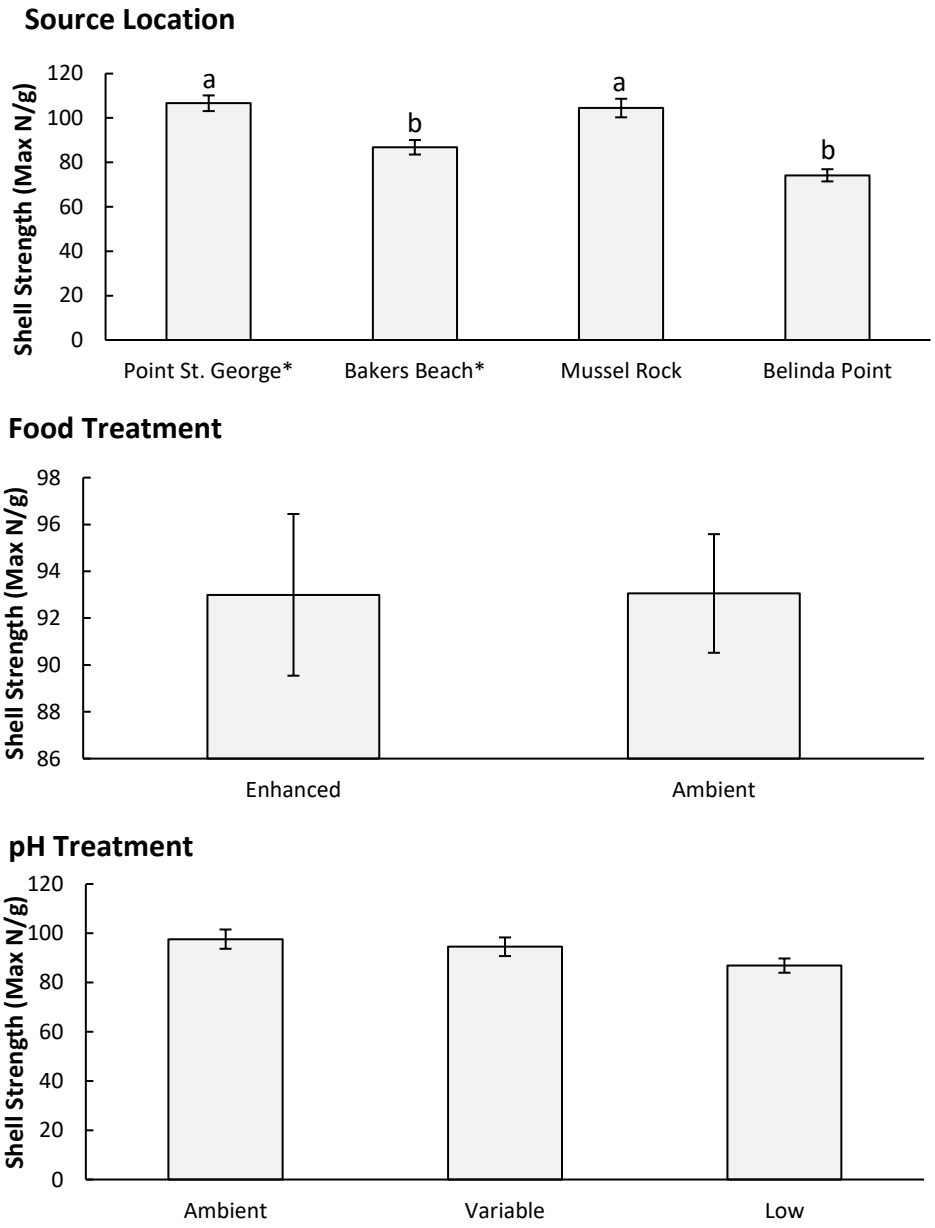

Figure 9: Strength of shell ($\bar{x}$ +/- SE) from 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling) (top), food treatment (middle), and pH treatments (bottom). Different letters indicate significant differences among treatment groups at the $\alpha = 0.05$ level with Tukey's HSD test.

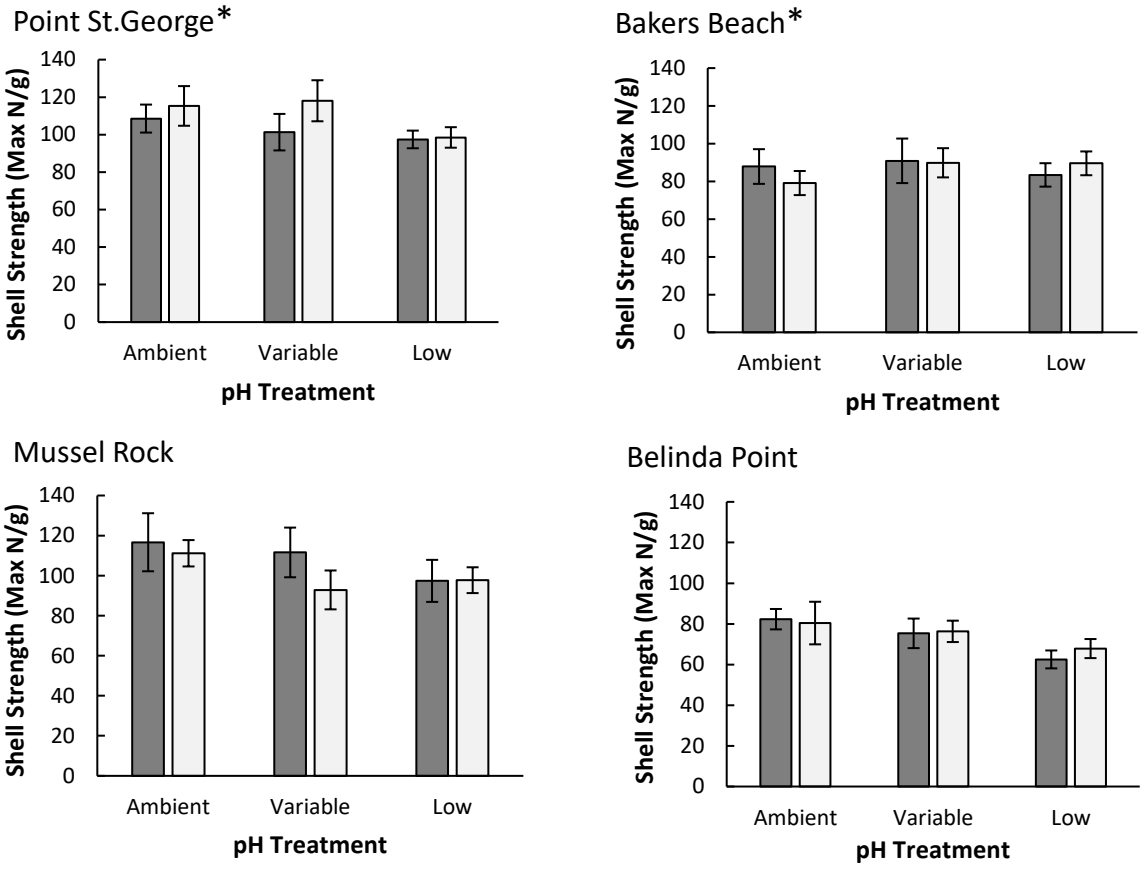

Figure 10: Strength of shell ($\bar{x}$ +/- SE) from 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling), pH treatment, and food treatments (Enhanced [Dark grey bars]; Ambient [Light grey bars]).

Adductor Muscle Mass: Soft Tissue Mass Ratio

Source location had a significant effect on the ratio of adductor mass to soft tissue mass in experimental mussels, but food, pH, and treatment interactions did not (Split-unit ANOVA: Site, $F_{3,59} = 63.3$, $P < 0.001$, [Fig.11 (top)]; Food, $F_{1,59} = 1.04$, $P = 0.31$, [Fig.11 (middle)]; pH, $F_{2,59} = 1.968$, $P = 0.15$, [Fig.11 (bottom)]; All interactions, $P > 0.25$, [Fig.12]). Mussels from Point St. George ($\bar{x} = 14.15$, SE= 0.35) had the highest ratio of adductor mussel to soft tissue mass. Individuals from Mussel Rock ($\bar{x} = 11.77$, SE= 0.35) and Bakers Beach ($\bar{x} = 10.80$, SE= 0.24) were not significantly different from one another but had significantly greater ratios than Belinda Point ($\bar{x} = 7.70$, SE= 0.21; Tukey's HSD, $P < 0.001$).

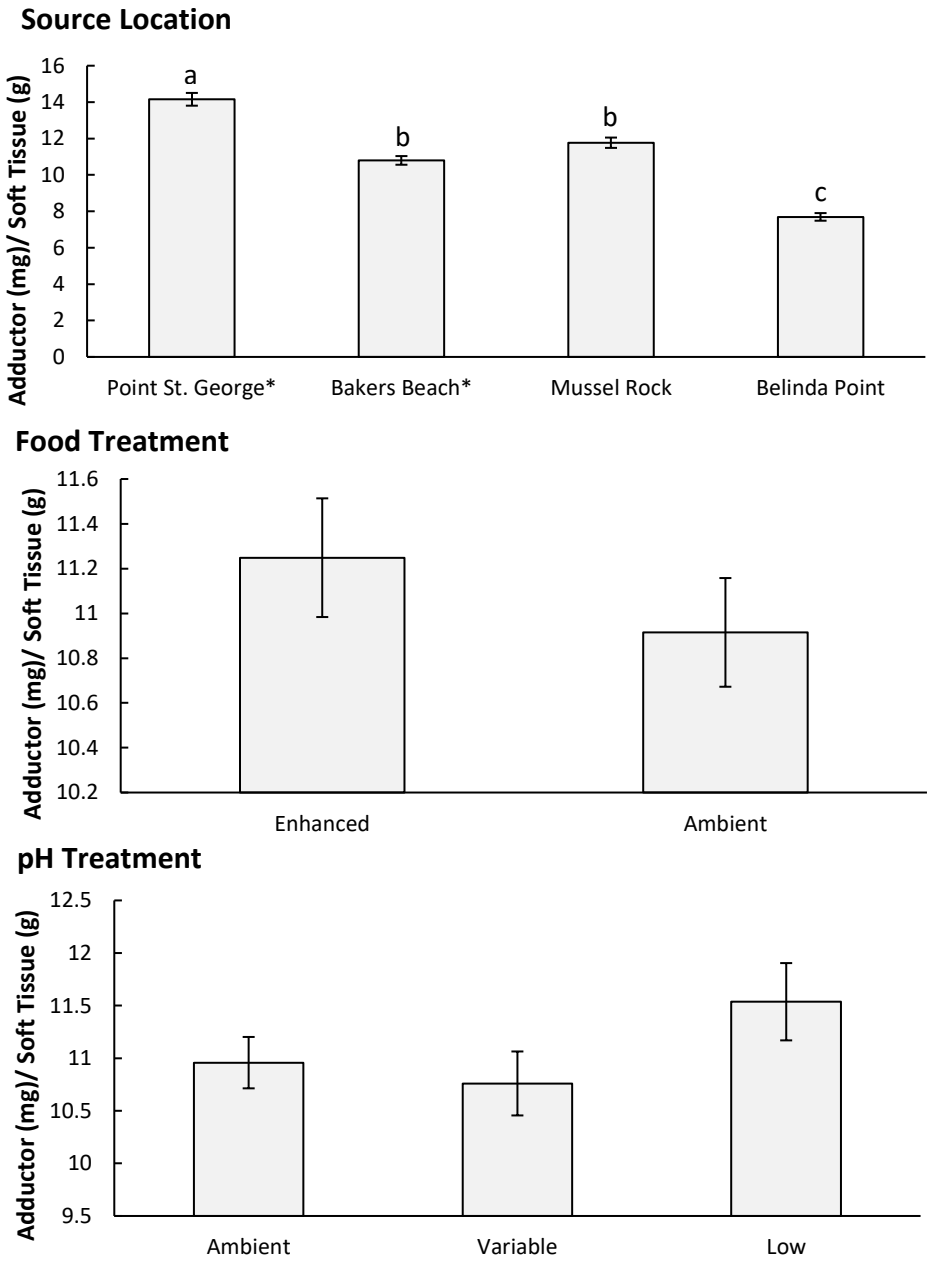

Figure 11: Final dried adductor mass to soft-tissue mass ratio ($\bar{x}$ +/- SE) of mussels after 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling) (top), food treatment (middle), and pH treatments (bottom). Different letters indicate significant differences among treatment groups at the $\alpha = 0.05$ level with Tukey's HSD test.

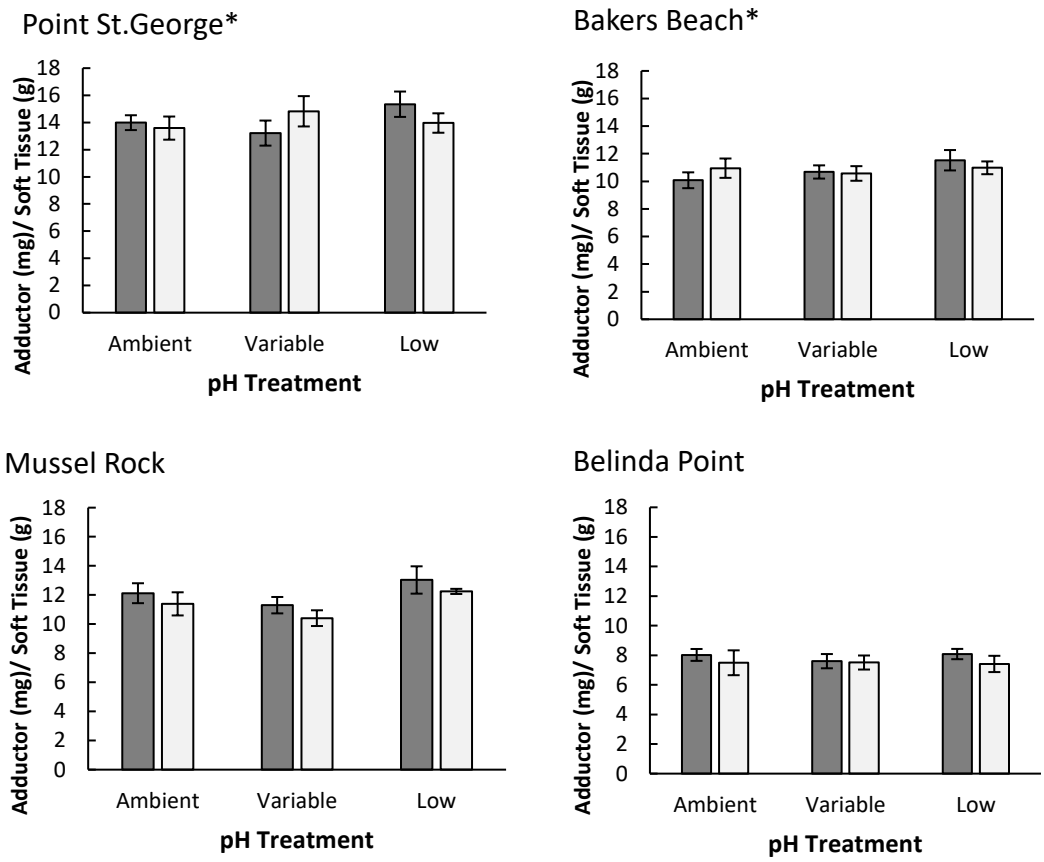

Figure 12: Final dried adductor mass to soft-tissue mass ratio ($\bar{x}$ +/- SE) of mussels after 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling), pH treatment, and food treatments (Enhanced [Dark grey bars]; Ambient [Light grey bars]).

Shell Dissolution

Source location of mussels had a significant effect on the dissolution of empty mussel shells, but pH and their interactions did not (Split-unit ANOVA: Source, $F_{3,60}$ = 48.9, $P < 0.001$, [Fig.13 (top)]; pH, $F_{2,60} = 0.060$, [Fig.13 (bottom)], $P = 0.94$; All interactions, $P > 0.25$, [Fig.14]). Shells from Point St. George ($\bar{x}$ = -1.48%, SE = 0.07) dissolved the most, followed by Bakers Beach ($\bar{x}$ = -0.82%, SE= 0.07), then Belinda Point ($\bar{x}$ = -0.52%, SE= 0.05). Each site was significantly different from the others, except for Mussel Rock ($\bar{x}$ = -0.62%, SE= 0.05), which was only significantly less than Point St. George (Tukey's HSD, $P < 0.001$).

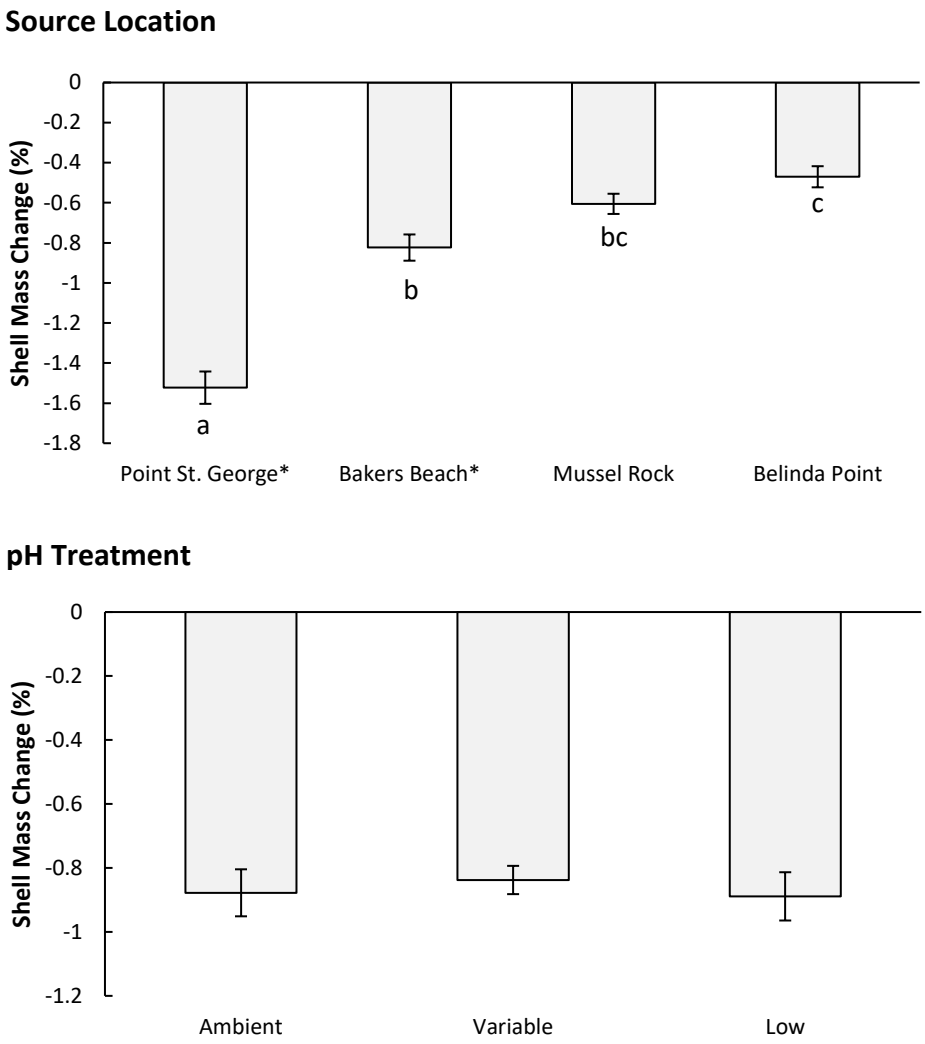

Figure 13: Percent mass change ($\bar{x}$ +/- SE) of desiccated mussels shells after a 20-day laboratory shell dissolution experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling) (top) and pH treatments (bottom). Different letters indicate significant differences among treatment groups at the $\alpha = 0.05$ level with Tukey's HSD test.

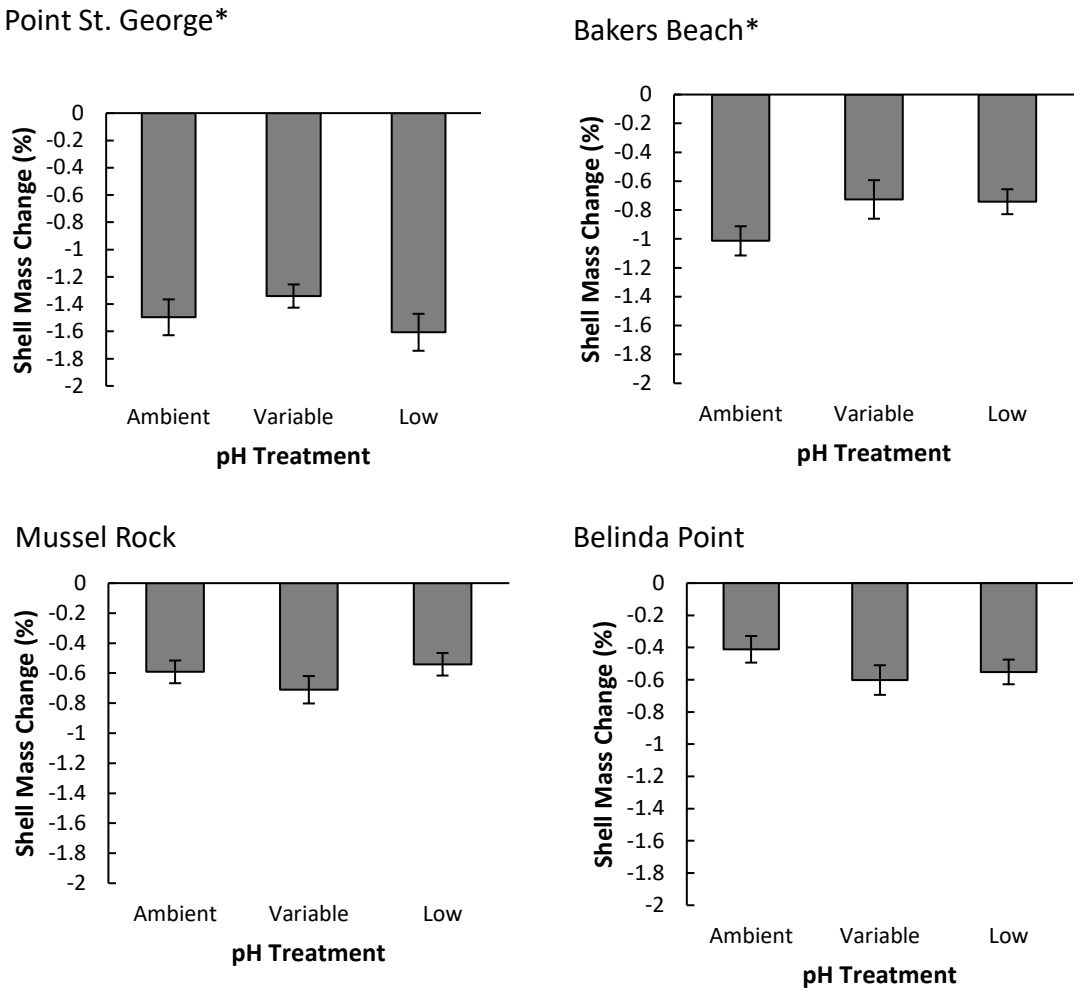

Figure 14: Percent mass change ($\bar{x}$ +/- SE) of desiccated mussels shells after a 20-day laboratory shell dissolution experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling) and pH treatment.

Crab Predation Assay

There were no significant differences in survival rates among mussels from different source locations (GLM; Belinda Point, $Z_4 = 0.72$, $P = 0.47$; Bakers Beach, $Z_4 = -0.82$, $P = 0.93$; Mussel Rock, $Z_4 = 0.59$, $P = 0.56$; Point St. George, $Z_4 = 1.67$, $P = 0.10$; Mussel Length, $Z_4 = 0.25$, $P = 0.80$, [Fig. 15]). There is a non-significant trend reduced susceptibility to crab predation in mussels collected from Point St. George.

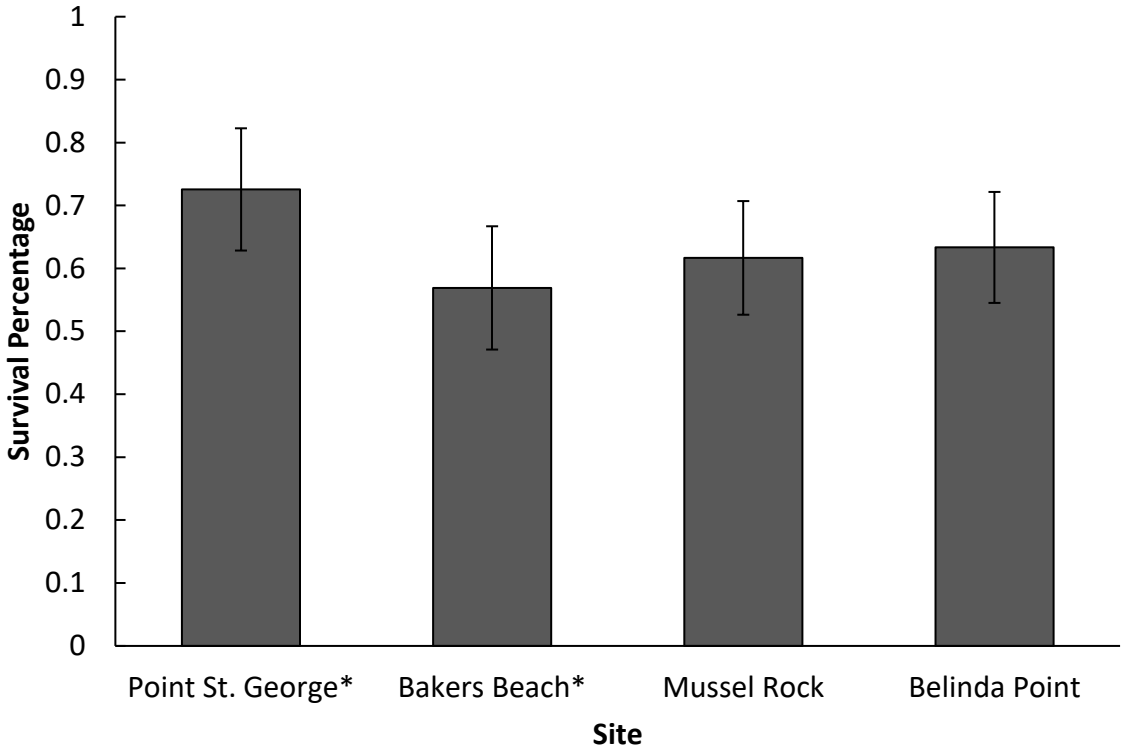

Figure 15: Percent survival ($\bar{x}$ +/- SE) of field-collected mussels supplied to *Romaleon antennarium* during a 16-hour crab predation assay. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling).

Respiration Assays

There were no significant differences in respiration rates between source locations, pH, food treatments, or their interactions (Split-unit ANOVA: Source, $F_{3,22} = 0.34$, $P = 0.20$, [Fig.16 (top)]; pH, $F_{2,22} = 1.61$, $P = 0.22$, [Fig.16 (middle)]; Food, $F_{1,22} = 0.02$, $P = 0.89$, [Fig.16 (bottom)]; All interactions, $P > 0.25$, [Fig.17]).

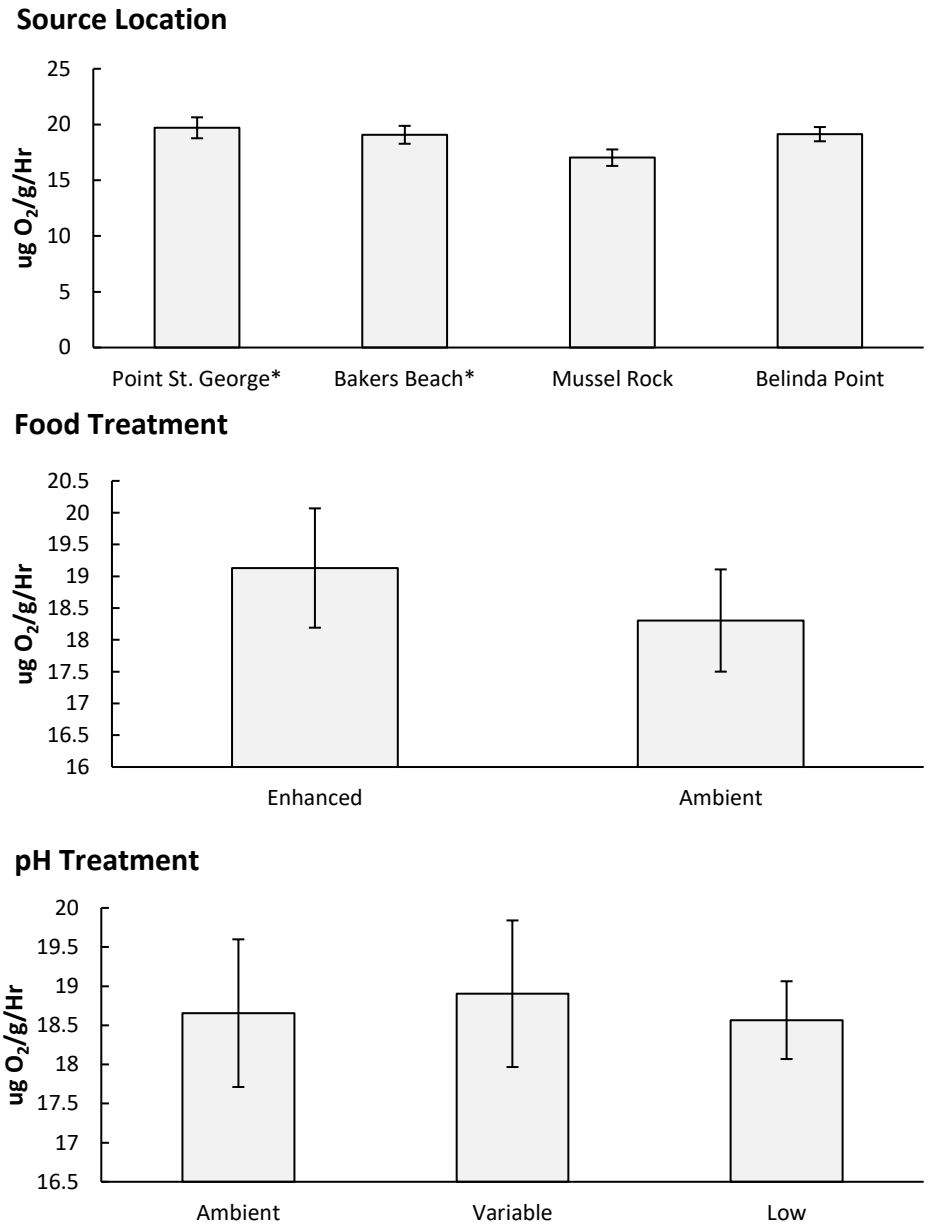

Figure 16: Mass specific oxygen consumption rate ($\bar{x}$ +/- SE) of mussels during 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling) (top), food treatment (middle), and pH treatments (bottom). Different letters indicate significant differences among treatment groups at the $\alpha = 0.05$ level with Tukey's HSD test.

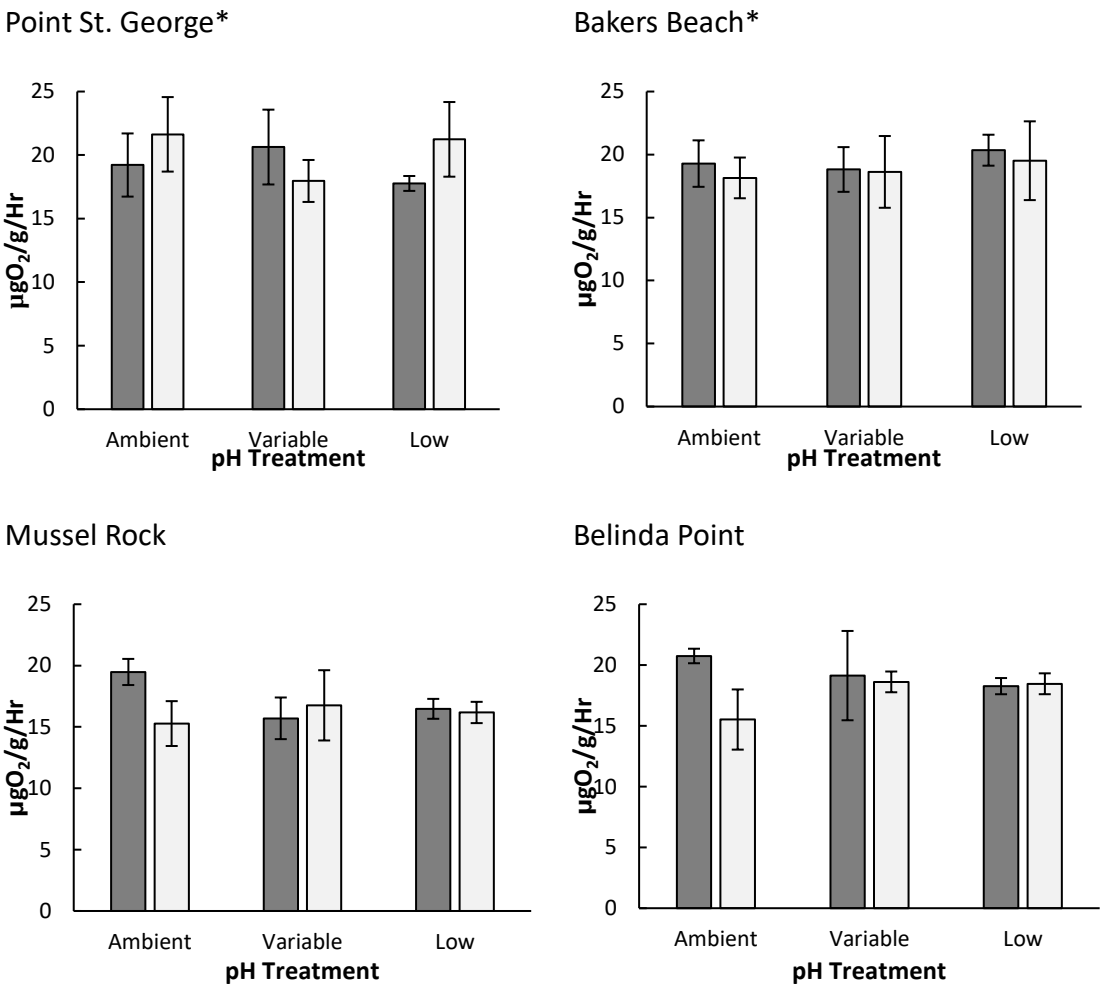

Figure 17: Mass specific oxygen consumption rate ($\bar{x}$ +/- SE) of mussels during 112-day laboratory growth experiment. Mussels varied with respect to source location (Locations annotated with * indicate areas that regularly experience intense upwelling), pH treatment, and food treatments (Enhanced [Dark grey bars]; Ambient [Light grey bars]).

DISCUSSION

The key factor in determining the overall (shell + soft tissue) growth rates of mussels in my experiment was their source location. Mussels from areas with intense upwelling (Point St. George and Bakers Beach) grew faster than those that experience less intense upwelling (Mussel Rock and Belinda Point). Enhanced food availability also had a significant effect on mussels by increasing their overall growth regardless of site or pH treatment. It should be noted that food concentrations supplied to mussels during the laboratory growth experiment were within the range of naturally occurring concentrations in the field. Differences in overall growth might have become more pronounced had we reduced food concentrations below natural conditions found in the field, but we wanted to isolate the potential effects of site-level differences in upwelling-driven primary production in our laboratory experiment. The greatest average difference of overall growth among source locations was 6-times larger than the average difference in overall growth between food treatments. The larger effect of source location relative to food availability on mussel growth suggests that mussels from areas exposed to intense upwelling regions can better convert food, at the levels available in our experiment, to growth, than mussels from areas exposed to less intense upwelling. Alternatively, mussels from less intense upwelling sites may convert food just as well as those from intense upwelling sites but allocate a larger proportion of energy towards autoregulatory processes associated with maintaining pH balance. Differences in clearance rates and abilities to capture food among mussels from different source locations could have also

contributed to the observed differences in growth rate among mussels from different source locations. These latter two hypotheses could be tested with simple laboratory experiments examining differences in clearance rates and food assimilation rates among mussels from my four source locations.

The effects of pH were only significant in influencing the total growth of mussels in my experiment. The lack of a detectable pH effect on mussel soft tissue growth and shell growth may be due to the relatively small amount of mussel growth in the experiment. Compared to *Mytilus edulis*, which has been shown to be very responsive to changes in pH, *M. californianus* is significantly longer-lived and slower growing (Gazeau et al. 2013; Suchanek 1981); therefore, significant responses to OA stress may take longer to manifest in *M. californianus* than in other mussel species. The minimal response of mussels across pH treatments in my laboratory growth experiment could also be due, in part, to the "ambient" pH treatment being more acidic than actual ambient conditions in the field where upwelling is less intense (e.g., Mussel Rock and Belinda Point). In theory, less low pH adapted mussels from areas of less intense upwelling should have the largest response to differences in pH. Sea water at the Telonicher Marine Lab, where the experiment took place, is sourced within an area that regularly experiences intense upwelling. Although the "ambient" seawater (7.82+/- 0.08 SD) was not as acidic as sea water measured during periods of intense upwelling, it was still more acidic than is typical (Feely et al. 2008; Chan et al. 2017; Bourdeau, unpublished data) of water at Mussel Rock or Belinda Point (Appendix D). Indeed, my results suggest that the common garden conditions of the experiment were stressful for mussels from areas of

less intense upwelling (i.e., Mussel Rock and Belinda Point) and muted the effect of the different pH treatments in the experiment.

Whereas overall (shell + soft tissue) and soft tissue growth were both greater in mussels from source locations of intense upwelling; shell dissolution, and shell strength deviated from these patterns. Mussels from Mussel Rock (low upwelling) gained more shell mass and withstood more crushing force than those from Bakers Beach (more intense upwelling). The deviations in shell growth and strength from the patterns in overall growth could be explained by varying selection pressures imposed by crushing predators, like crabs. Crab predation intensity among my four source locations has been estimated using the attack frequencies of crabs on wax mussel replicas in field, a robust proxy for crab attack frequency on living molluscs (Tyler et al. 2015), and crabs are more abundant at Mussel Rock than at Baker's Beach. It is important to note that the wax mussel arrays in this study were not placed at Mussel Rock, but at a neighboring site "Devils Gate" instead. The distance between both locations is roughly 7 km and is thought to have roughly similar levels of predation risk (Bourdeau, unpublished data). Mussels that exhibited higher shell growth and stronger shells (traits that influence survival from crushing predators, like crabs) in my experiment came from source locations (Point St. George and Mussel Rock) where average daily attack frequencies on mussel replicas were highest (Appendix E, F). Further, although not statistically significant, mussels from Point St. George, where crab attacks are most common among the four sites, tended to survive crab attacks in the lab better than mussels from the other sites (Figure 15). Thus, it may be that local adaptation to crab predation risk (selection on

shell deposition and strength) could be more important in driving the among-treatment patterns observed in my experiment.

The shell mass lost during my shell dissolution experiment was the greatest in mussels from source locations that also grew the most overall during my laboratory growth experiment. Mussels collected from nutrient rich upwelling regions (e.g., Point St. George, Bakers Beach) may tradeoff shells composed of more dissolution-tolerant materials (i.e., calcite) for shells made up of stronger, but more dissolution-susceptible materials (i.e., aragonite) (Morse et al. 2007). Differences in shell dissolution rates between individuals from different source locations have been seen in *Mytilus chilensis*, except with lower dissolution rates in sites that experience lower pH (Duarte et al. 2014). Because aragonite is costlier to produce and maintain than calcite, especially in acidic waters (Day et al. 2000; Mucci 1983), mussels in my experiment could justify the enhanced energetic cost of producing aragonite if there is enhanced and consistent food in the environment or if the risk of mortality due to shell fracture (e.g. predation from crabs) is greater than the risk of mortality due to dissolution. Shell deposition was able to compensate for the shell dissolution in Point St. George and Mussel Rock where predation from crab is high while dissolution was greater than dissolution at Bakers Beach and Belinda Point where crab predation is low (Appendix E). This suggests that selection pressure by predation from crabs may influence the shell deposition quantity while selective pressure from pH and food availability associated with upwelling strength may influence the quality of shell deposited. Evidence for shell growth being energy-

dependent can be seen in Figure 7 (middle), where individuals given enhanced food concentrations gained more shell mass than those under ambient food conditions.

Although soft tissue growth was greater in mussels from areas of more intense upwelling, increased food availability did not increase this growth. *M. californianus* has been shown to allocate a higher fraction of energy to somatic mass (relative to shell mass) than other bivalve species, presumably in response to prolonged periods of starvation (Matzelle et al. 2014). Since mussels in my experiment were submerged and provided with consistent food throughout the duration of the laboratory growth experiment, differences in soft tissue growth between food treatments may not have been observed since mussels never underwent prolonged periods of time without food. Another explanation for the non-significant effect of food on soft tissue growth, could be due to shell dissolution experienced during the laboratory growth experiment. Since live mussels either gained shell mass (e.g., Point St. George) or lost less shell mass than the shells alone in the dissolution trial, excess energy could have been allocated toward the maintenance of shell mass instead of soft tissue growth in the relatively low pH environments in my experiment.

Adductor muscle mass to soft tissue mass ratios were greatest in mussels from Point St. George, smallest in mussels from Belinda Point, and intermediate in mussels from Bakers Beach and Mussel Rock. It is difficult to devise a convincing hypothesis for how these among-site differences in adductor muscle mass would reflect local adaptation to upwelling regimes. However, the observed variation could be explained by local adaptation, either via fixed genetic differences resulting from selection from, or predator-

induced plasticity in response to predation pressure from sea stars (Freeman 2005). Across the four source locations from which I collected my experimental mussels, there also exists a gradient of *Pisaster ochaceus* density (Appendix G) and the trends in adductor muscle size that I observed in my experiment match sea star abundances in the field. Further, other bivalves have been shown to plastically increase their adductor muscle mass relative to body size in response to sea star predation risk (Reimer & Tedengren 1996). The larger adductor muscles presumably make it more difficult for the sea stars to pry apart the mussel's valves. Mussels in my experiment therefore may be investing more energy into larger adductor muscles in response to increased predation risk from sea stars at their collection sites.

CONCLUSIONS

The goal of my experiment was to expose mussels from sites that differ in their exposure to upwelling-driven OA to pH and food conditions naturally found in the field to address the mechanism explaining previously observed differences in growth and condition in the field. While my results are consistent with others that highlight the importance of food availability in ameliorating the potential negative impacts of climate change (Fitzgerald-Dehoog et al. 2012; Melzner et al. 2011; Hettinger at al. 2013; Thomsen et al. 2013), they also seem to fit in a growing body of work that suggest that organismal responses to climate change may be shaped by local adaptation and/or phenotypic plasticity (Stapp et al. 2017; Vargas et al. 2017). While *M. californianus* has been shown to be genetically homogenous across their spatial distribution (Addison et al. 2008), they have also been shown to modify their life history traits across geographic and oceanographic environments (Kroeker et al. 2016; Smith et al. 2009). Mussels in my experiment are sourced across a relatively small 270 km stretch of northern California but showed variable growth and physiological responses to pH stress. Since source-specific responses were associated with local environmental drivers such as pH stress, food availability, and predation risk, it is possible for sub-populations of mussels from areas that currently experience minimal pH stress to become adapted, either through the evolution of constitutive genetic traits or physiological acclimatization to low pH stress if environmental conditions change. Populations of mussels already exposed to low pH from intense upwelling may also be ‘pre-adapted’ to persist in future OA conditions.

Future studies should investigate whether the differences in growth among mussels from different source locations measured in my experiment are due to differences in competitive ability, acclimatization though plastic physiological responses, epigenetic modifications, or evolved genetic differences. The mechanism through which potential genetic differences are selected for should also be investigated, whether by pre-settlement selection of early life history stages or differential reproduction. The pH ranges used in my experiment were meant to explore how current pH gradients influence the growth and physiology of mussels. While this was effective for investigating the effects of current upwelling-driven OA on mussels, other studies should consider exposing individuals to more extreme pH conditions to better predict how future climate change scenarios will influence this important foundation species. Nevertheless, my results suggest that future studies need to take into account the adaptive potential of populations when making predictions of organismal level responses to future climate change scenarios.

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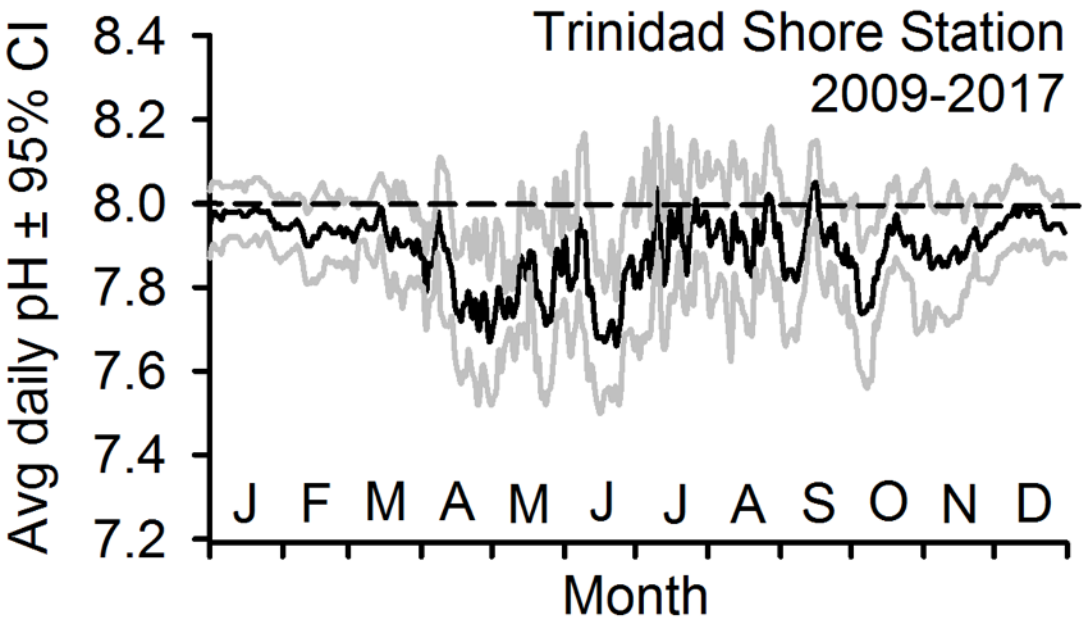
APPENDICES

APPENDIX A

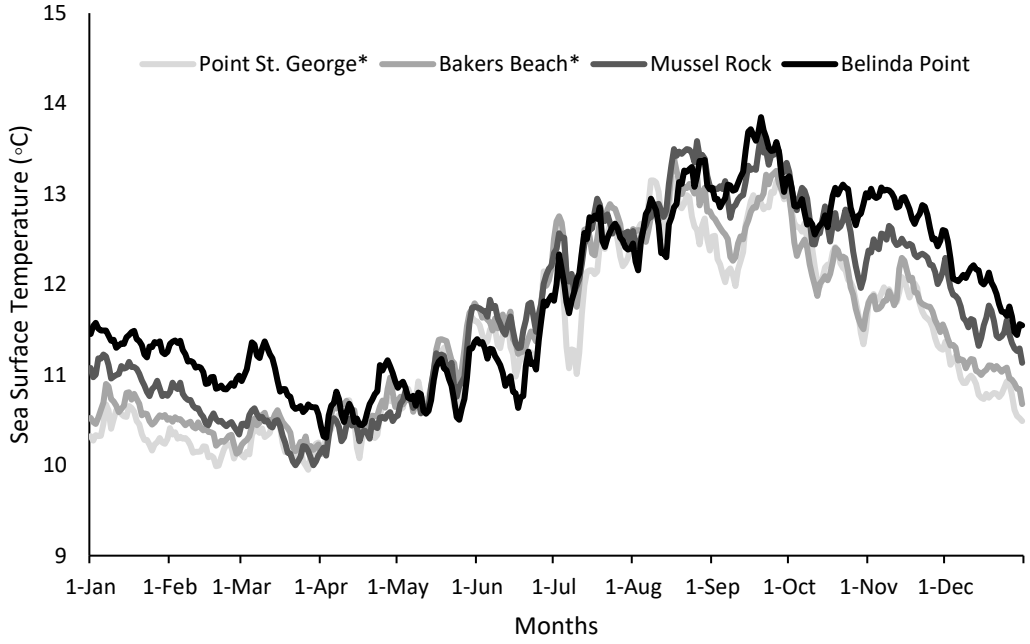


Appendix A: Ocean chemistry recorded at Trinidad Pier from 2009- 2017 showing consistent fluctuations in pH across nice years, with the intertidal zone near the HSU Marine Lab regularly experiencing low pH conditions during the spring and summer. Black line represents mean daily pH, grey lines denote 95% confidence interval; dashed line represents pH = 8.0. Data from the Central and Northern California Ocean Observing System.

APPENDIX B

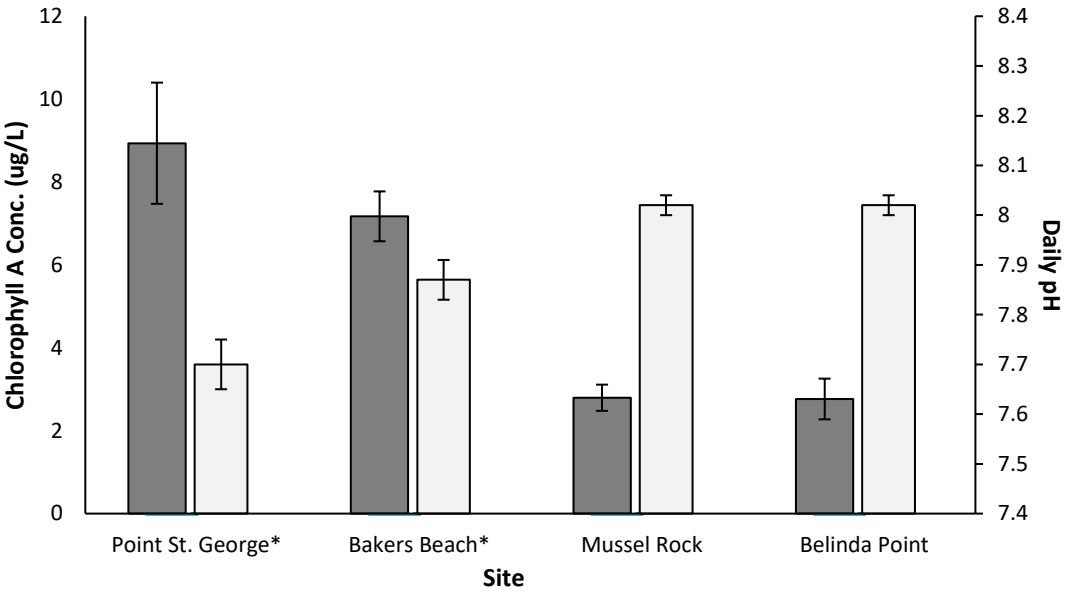
Appendix B: Map of Northern California with the collection locations marked with arrows. Locations annotated with * indicate areas that regularly experience intense upwelling.

APPENDIX C



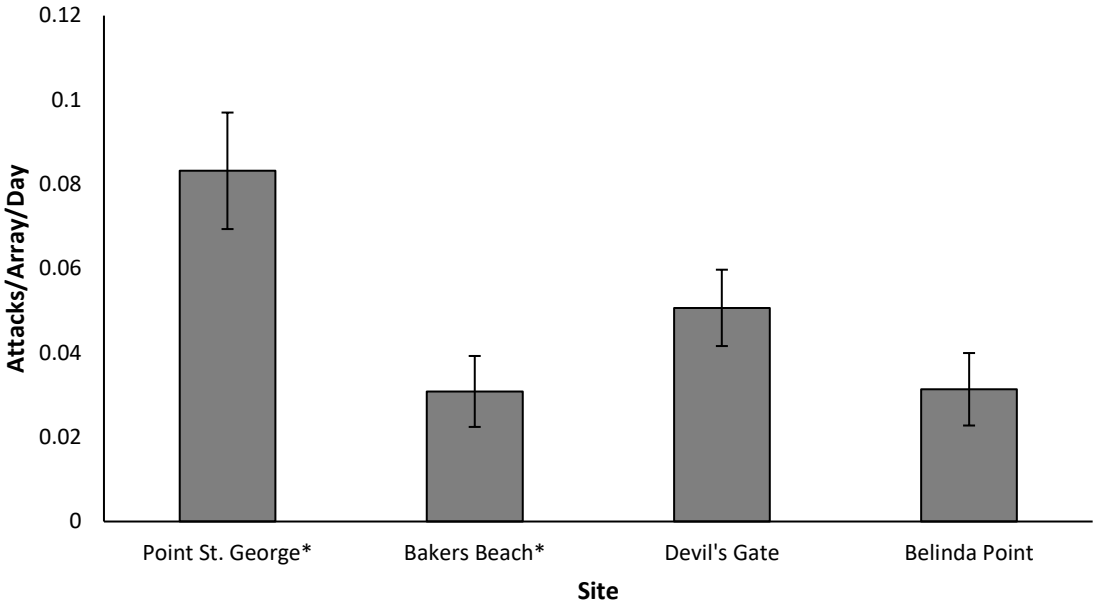
Appendix C: Average sea surface temperatures from 2004 - 2013. Anomalous years (2014-2016) were excluded. Data were extracted from NASA's MUR SST database (https://mur.jpl.nasa.gov/), courtesy of Dr. Eric Bjorkstedt.

APPENDIX D



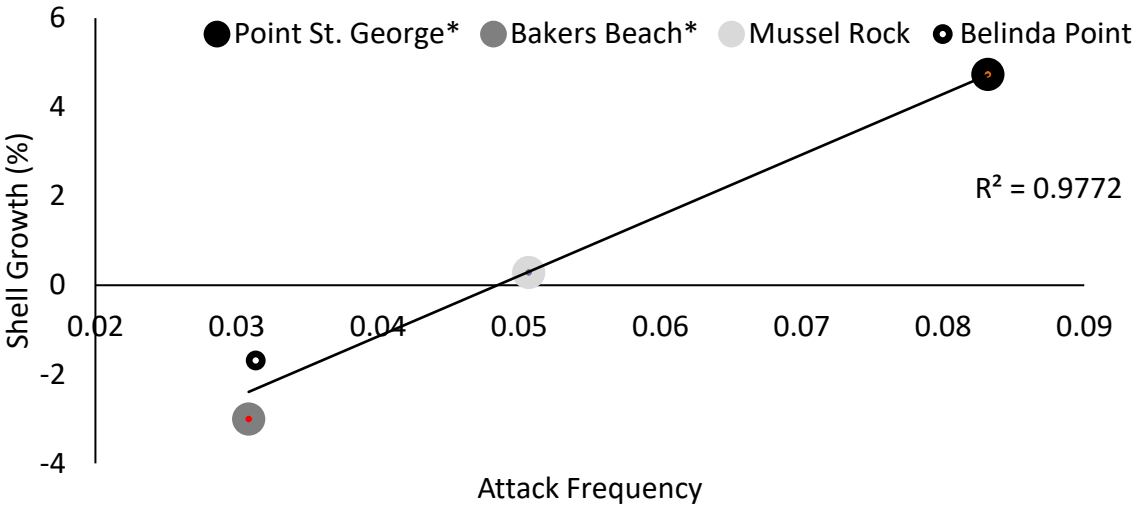
Appendix D: Chlorophyll A concentrations (Dark grey bars; $\bar{x}$ +/- SE) and pH (Light grey bars: $\bar{x}$ +/- SE) sampled during four low tide series during the summer of 2017 (May – July; Bourdeau, unpublished data). Locations annotated with * indicate areas that regularly experience intense upwelling.

APPENDIX E



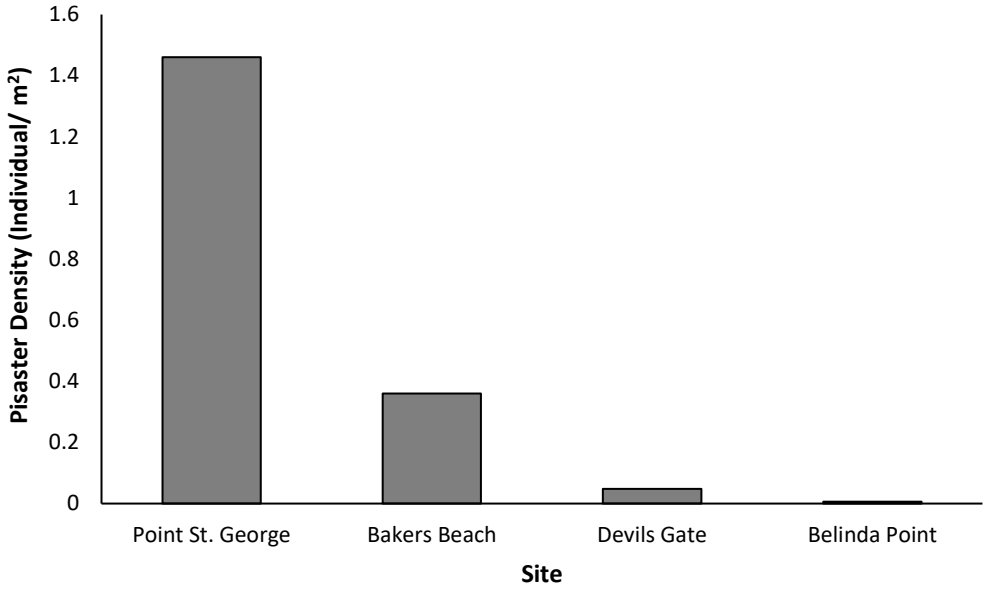
Appendix E: Frequency of daily attack by crabs ($\bar{x}$ +/- SE) on wax mussel replicas during the summer of 2017 (May – July; Bourdeau, unpublished data). Locations annotated with * indicate areas that regularly experience intense upwelling.

APPENDIX F



Appendix F: The relationship between the mean daily crab attack frequencies on wax mussel replicas by crabs at each of the four source locations in 2017 (May – July; Bourdeau, unpublished data) and mean percent shell growth of mussels from each of the four source locations after 112-day laboratory growth experiment.

APPENDIX G



Appendix G: Total densities of *Pisaster ochraceous* (new recruits, juveniles, and adults) along 30m x 2m transects at each of my four collection sites during the spring of 2017 (Bourdeau, unpublished data).