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Assessing the Significance of Substrate Color and Temperature on Balanus glandula Growth and Survivorship

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Assessing the Significance of Substrate Color and Temperature on *Balanus glandula* Growth and Survivorship

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ABSTRACT

The body temperatures of intertidal species are strongly dependent on the temperature of their external environment. This study sought to understand the impact of a substrate's color and subsequent temperature on *Balanus glandula* in order to predict the potential effects of warming temperatures from climate change on intertidal species. Barnacles were allowed to settle and grow on three differently colored plates and were photographically monitored over the course of eleven weeks. Settlement and survivorship were recorded on-site, while growth was tracked utilizing digital imaging software. It was hypothesized that barnacles on peach plates would perform the best since the ambient temperature most closely matched their natural substrate's. The results supported this, finding that ambient, peach tiles had significantly higher growth rates compared to cool, white and warm, green tiles ($F < 0.0001$, $p=0<0.0001$, $df=2,301.1$) over a five-week subperiod. However, barnacle survivorship showed no significant difference between treatments ($F=2.17$ $p=0.143$ $df=2,18$) due to high mortality for all tiles. Overall, the study found that substrate temperature had significant effects on barnacle growth and survival over a short-term period, but is less important in the long term. Considering the other impacts of climate change unaccounted for in this study, the combined effects of these variations in addition to temperature could threaten the survival of thermosensitive intertidal species as global temperatures continue to rise.

Key words: *Barnacles · Balanus glandula · Temperature · Intertidal zone · Climate change · Growth · Survival*

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INTRODUCTION

The past decades have experienced heightened changes in climate at the regional and global level due to the exponential rise of atmospheric greenhouse gases. Abiotic changes caused by climate change, particularly warming temperatures, can have complex and harmful effects on marine ecosystems (Harley et al., 2006). As a result, temperature is one of the primary factors in determining a species' distribution, abundance, and biodiversity. With the exponential growth of climate change threatening their survival, it is integral that we learn to predict the effects of warming temperatures on marine species.

At the convergence of land and ocean, the intertidal zone is a region that experiences varying water levels throughout the day (National Geographic Society, 2019). The zone is inundated with water during high tide and exposed to air during low tide. When exposed to air, referred to as emersion, intertidal species are more susceptible to greater changes in abiotic factors such as temperature, desiccation, salinity, oxygen concentration, and pH level. As a result, the species living in the intertidal zone became evolutionarily adapted in order to survive the fluctuating conditions of this ecosystem. This distribution based on environmental tolerance is reflected in their vertical zonation patterns (Miller & Stillman, 2012).

In particular, intertidal communities regularly exposed to low tide and air will be the most sensitive to climatic changes in temperatures. Overheating and water loss are the largest abiotic threats to these species because of their vulnerability during emersion (Ober et al., 2019). Many have a thermal performance curve at which they operate best under certain temperatures (Miller & Stillman, 2012), but decline in performance at thermal extremes. As a result, heat stress is the primary abiotic factor in determining species distribution and mortality in intertidal zones (Helmuth & Hofmann, 2001). Therefore, when examining the impacts of warming temperatures from climate change, intertidal species would be a strong indicator of how organisms will react to changes in thermal stress.

The acorn barnacle, known as *Balanus glandula (B. glandula)*, is one of the most widespread barnacle species along the Northwestern Pacific coast in the U.S. They are distributed throughout Alaska and the Aleutian Islands down to Bahia de San Quintin, Baja California (Cowles, 2005). Due to its abundance and accessibility, *B. glandula* was chosen as the study species for this experiment.

The effects of physical and environmental factors on intertidal species like barnacles has been studied extensively (Maggi et. al., 2015; Dias et al., 2018), but we still know very little about how climate change will influence those processes. In order to focus solely on the effects of temperature on intertidal species, this study examines how they significantly impact barnacle growth and survival. Previous studies have focused on regional and local factors such as phytoplankton abundance (Sanford & Menge, 2001) or abiotic conditions (Jenewein & Gosselin, 2013) and how they affect growth and survival in addition to temperature. However, both suggested that biotic, trophic relationships overshadowed the effects of physical environment alone. Robinson (2017) examined the effect of substrate temperature on barnacle growth and mortality during their early life-history. The study found temperature had no significant impact on the early life processes of *B. glandula.* However, the experiment did exhibit significant differences between the substrates themselves; this leaves potential for future research to reexamine the relationship beyond the initial life processes to encompass lifelong growth and survival.

We tested the effects of warming and cooling temperatures on the growth rate and survivorship of *B. glandula* by manipulating the substrate color where the barnacles grew.

Different colored tiles absorbed varying amounts of heat and correlated with varying temperatures: with warmest being green tiles, followed by ambient peach, and then cool white (Gilman, n.d.). The tile temperature itself would then directly affect the barnacles' body temperature. Thus, the logic of our experiment emphasizes that tile color impacts barnacle body temperature, which in turn impacts barnacle growth and survival. In doing so, we sought to replicate the impacts of climate change on intertidal species and examine the potential consequences of higher global temperatures. This study is a continuation of the previous studies conducted by Iafe (2008) and Wong (2012) and incorporates all of the data collected from those experiments. Mortality and settlement data were recorded over an eleven-week period, and individual growth was later analyzed utilizing imaging software. While more limited in data and time scope, both of the previous studies showcased higher rates of growth and survivorship for barnacles on peach tiles. Average maximum diameters of barnacle opercula were highest in the peach treatment after 13 and 23 days (Iafe), indicating higher growth. Peach, along with white, also had better overall survivorship than green tiles (Wong).

Considering these previous results and the effects of thermal stress, I hypothesized that the *B. glandula* growing on the peach tiles would exhibit higher growth rates and improved survivorship, since peach is the closest to their natural substrate temperature. Being darker, green tiles experienced higher temperatures compared to the white tiles (S. Gilman, n.d.). For intertidal species like barnacles, it is likely that heat stress and water loss is more detrimental compared to the effects of cooler temperatures. Thus, green tiles should have lower survivorship and growth.

METHODS AND MATERIALS

Study site. From May to August of 2007, the field experiment was conducted at Colin's Cove in the Friday Harbor Laboratory Marine Reserve in Colin's Cove on San Juan Island, Washington (48° 32' N, 123° 04' W). The intertidal zone had rocky conditions. The ten sites ranged in height from 0.5 to 1.2 meters, above Mean Lower Low Water (+MLLW), and were on flat or gently sloped terrain.

Plate design and structure. The growth plates were modified from a design used by Sanford and Menge (2001). They consisted of ceramic tiles, measuring 10.16 x 10.16 centimeters in length. They were specially designed with a matrix of 6x6 circular shallow pits, with rows (1-6) and columns (A-F) totaling 32 pits when excluding the four center pits which held the bolt anchor. The pits were 1 millimeter in diameter and 0.5 millimeter in depth, which were spaced at a distance of approximately 18 millimeters apart. This allowed for barnacle settlement and growth. Ten sets of each plate color (green, peach, white) were prepared for each site, totaling 30 plates (now interchangeably referred to as 'tiles'). They were set to lay on flat areas on the coast (Figure 1).

Rocky intertidal zones where acorn barnacles inhabit are typically tan and gray, which peach is most similar to among our three colors. This means that the temperatures on the peach tiles are closest to the temperatures of the natural rock; thus, barnacles would naturally be better adapted to peach tiles and that temperature range.

Before placing them on the sites, each plate was hung under a shaded dock for a few months to allow for natural larval settlement. The area received no direct sunlight, removing the effect of tile color on temperature during the settlement period. The plates were then in sets of 3 at each study site on 23rd May, 2007.

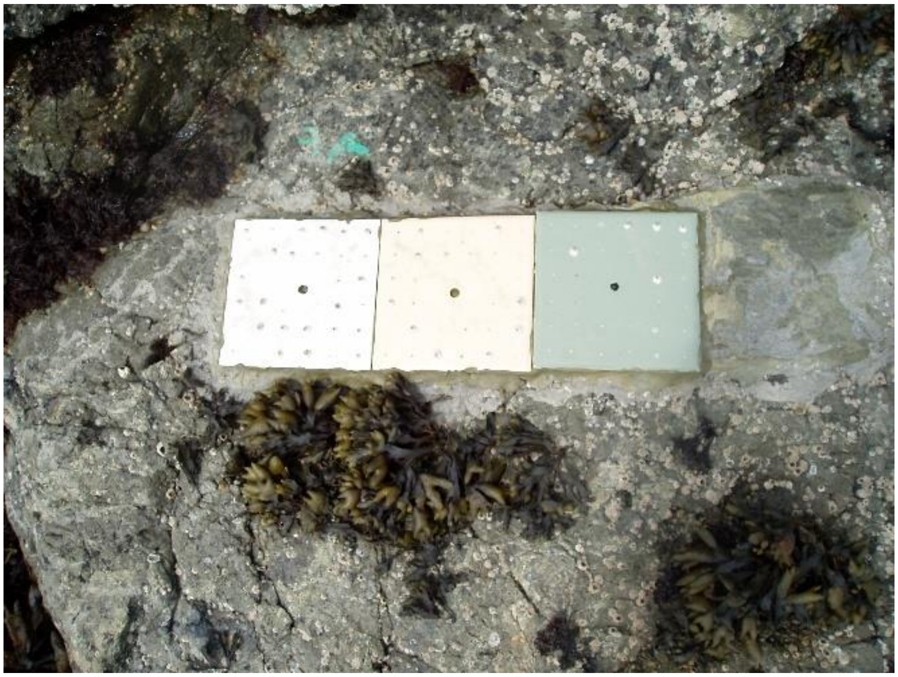

Figure 1: Intertidal zone along the coast of Colin's Cove where the study was conducted. Depicted are three tiles, from left to right: white, peach, and green.

Photographic monitoring. Digital photographs were taken of each tile on May 23rd, May 28th, June 10th, June 20th, July 4th, July 29th, and August 12th, 2007. We used May 28th as the initial census date to exclude mortality that might have resulted from the stress of transplanting. Records of barnacle mortality, settlement, and death were similarly taken in a field notebook on each of the following dates. A segment of a ruler, measuring at least 2 centimeters in length, was placed in the center region of each tile.

Digital image analysis. Images were transferred to a computer and analyzed using the software ImageJ (Rasband, U. S. National Institutes of Health). Pixels were scaled to centimeters using the ruler in the images as a reference. The barnacles' longest operculum diameter (Figure 2) was measured and saved along with tile number, color, and position.

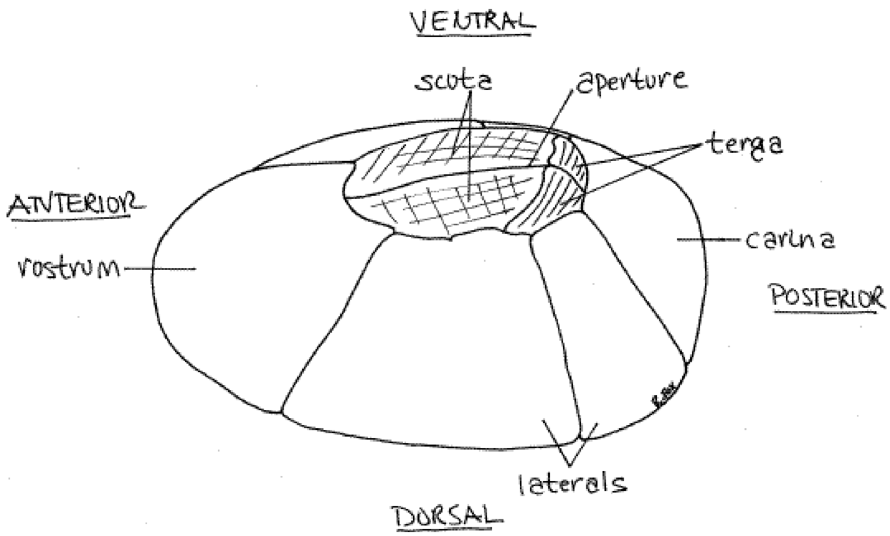

Figure 2: Anatomy of *Balanus glandula* depicting operculum, aperture, and major plates (Fox, 2006).

Only barnacles marked as alive (X) in the field notebook were recorded. Those marked with a question mark (?), indicating an ambiguous mortality status, were clarified through consultation with the research advisor. Those marked as newly settled (S) or dead (D) were not included in the measurements. Recordings were saved and transferred to Excel for each photograph.

Data compilation. Data from May 28th, June 10th, June 20th, July 4th, and July 16th, 2007 were taken from the previous studies (Iafe, 2008, Wong, 2012). The final two sets of data on July 29th and August 12th were measured concurrently during the process of this paper. To compare measurement consistency across researchers, two random tiles (15 barnacles from tile 21P and 6 barnacles from tile 9AG) from each of the two previous studies were remeasured. The Iafe data had a maximum 11.11% difference in barnacle size and the Wong data had a maximum difference of 15.13%. Results displayed an acceptable margin of error, indicating reliable data across all researchers.

A master sheet of data was compiled for each date, recording tile number, color, barnacle position, file label, and length for each of the barnacles. New barnacles that grew from larvae that settled after May 28th were also recorded. Those which appeared in the same position as previously dead ones were compared to confirm that they were new settlers and not the prior barnacle. Due to the increased mortality over time, I only used the subset of size data from May 28th to July 4th for data analysis on barnacle growth.

Statistical analysis. All statistical analyses were conducted in JMP v.14 (SAS Institute Inc., Cary, NC). We analyzed the relationship between substrate color and barnacle survivorship for the eleven-week period between May 28th to August 12th, 2007 using a two-factor mixed model ANOVA. Similarly, a two-factor mixed model ANOVA was conducted for substrate color versus barnacle growth, from May 28th to July 4th 2007, determine the statistical significance of the relationship.

RESULTS

Survivorship. The total number of barnacles on the tiles declined over time, with the exception of August 12th which showed an increase (Figure 4). This decrease in barnacles actually underestimates the mortality rate, as Figure 4 includes the barnacles gained from new settlements; the uptick on August 12th results from higher settlement than mortality. Warm (green) tiles consistently had the lowest numbers compared to cool (white) and ambient (peach). Between June 10th and July 16th, cool tiles had the highest total surviving barnacles. For May 28th, July 24th, and August 12th, ambient tiles had the highest total alive barnacles.

The fraction of barnacles surviving the entire 11-week period ranged from a high of 0.173 on the ambient tile to a low of 0.0924 on the warm tiles. Survival for all barnacles for the entire eleven-week study period was greatest on the ambient tiles, followed by cool and then warm (Figure 3). However, the ANOVA showed that the differences were not significant (F=2.17 p=0.143 df=2,18) due to high mortality rates for all treatments in later dates. The statistical analysis also showed that 72.44% of the variance between tiles was due to the subgroups; in our case, subgroups refer to different areas along the coast where the tiles were placed. This indicates that tile location may be an important factor of influence for barnacle survival.

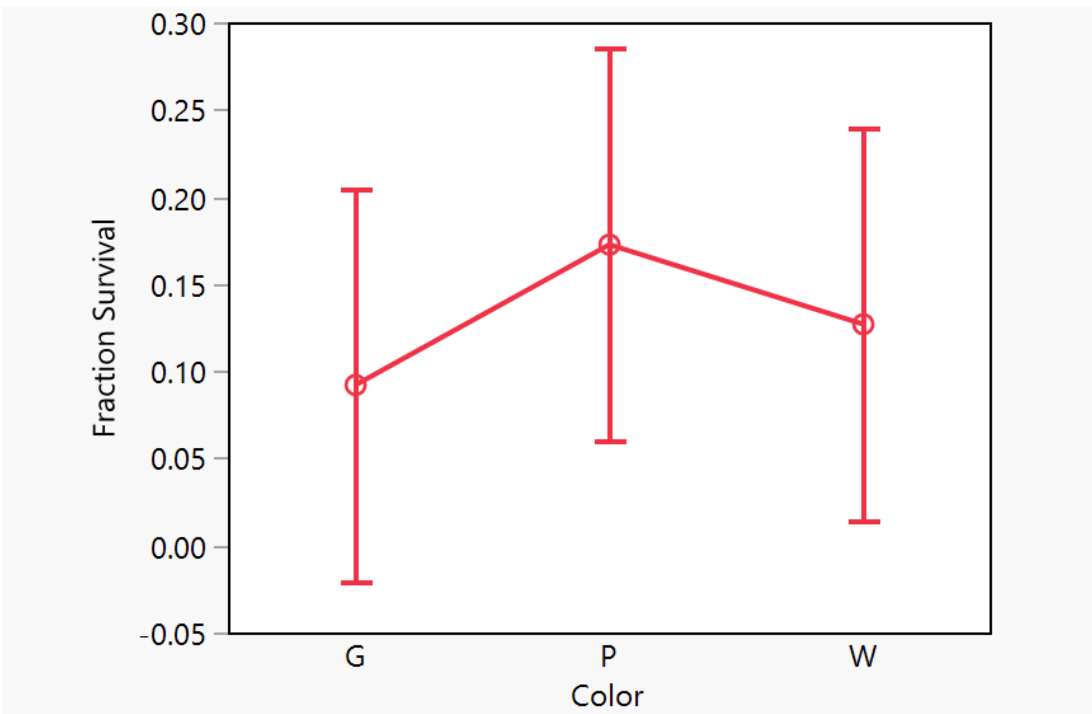

Figure 3: Least squared mean survivorship of *B. glandula* from August 12th to May 28th for each tile color. Peach (P) displayed the highest fraction survival, followed by white (W) and green (G), but not to a significant degree.

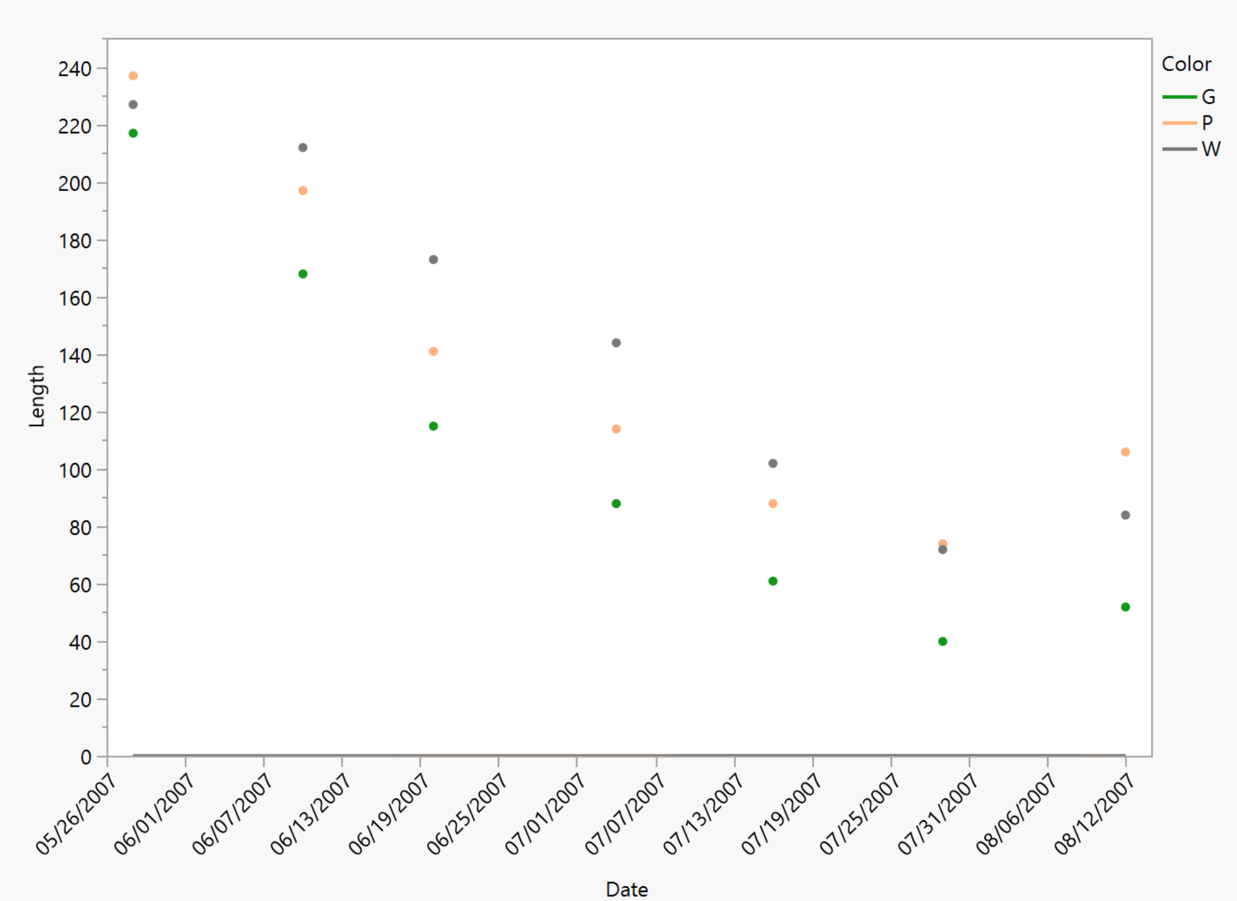

Figure 4: The number of barnacles present at each timepoint plotted against tile color, showing declining mortality over time until the last date, August 12th. Green (G) displayed lowest total number of barnacles, while peach (P) and white (W) alternated in having the highest number.

Barnacle growth. Due to the increasing mortality across time (Figure 4), a subset of the dataset – dates May 28th to July 4th – was used to preserve an appropriate sampling size. There were 310 individual barnacles analyzed for the ANOVA. Highest growth was observed on ambient tiles at 0.0852 centimeters, followed by 0.0685 cm on cool tiles and 0.0525 cm on warm tiles. Barnacle growth from May 28th to July 4th was significantly higher on ambient tiles ($F < 0.0001$, $p=0$, df=2,301.1), than cool and warm tiles (Figure 5). Cool tiles also displayed significantly higher growth compared to warm tiles, which had the lowest.

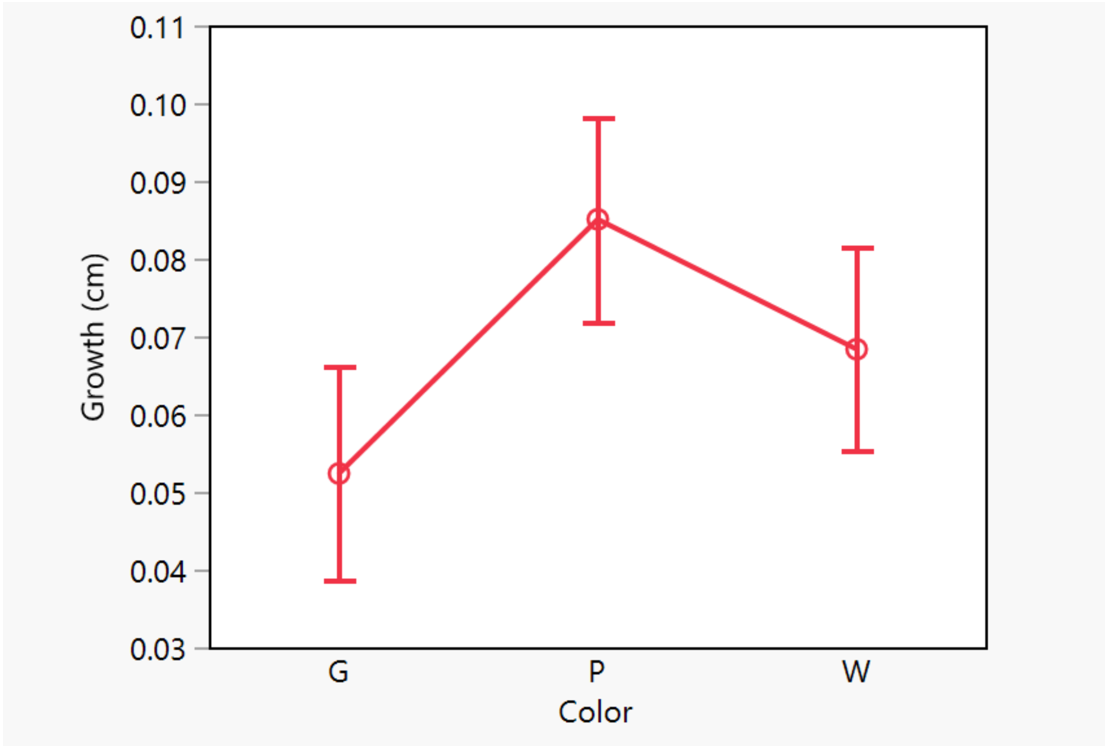

Figure 5: Least squared mean growth of *B. glandula* from July 4th to May 28th for each tile color, with peach (P) tiles showing significantly higher growth than white (W) and green (G). White tiles also displayed significantly higher growth than green.

DISCUSSION

The purpose of this study was to determine the impact of temperature on the growth and survival of barnacles. Ambient, peach tiles which most closely matched the barnacles' natural substrate in temperature was predicted to perform the best, as the species would be more adapted to those conditions. Warm, green tiles were expected to perform worse compared to the cool, white tiles based on the detrimental effects of heat stress on intertidal species. Results from both growth and survival supported this rationale, but varied in significance depending on the time span examined. This suggests the overriding influence of other factors beyond temperature.

Substrate temperature did not significantly impact survivorship throughout the entire eleven-week period, as high mortality rates were observed for all treatments. Previous analyses of survivorship data displayed the significant effect of tile color after 13 days (Iafe, 2008), but not at 50 days (Wong, 2012). This indicates that substrate temperature has a significant effect in the short-term, but not over a longer period of time due to high mortality across treatments.

Substrate temperature significantly influenced barnacle growth over a short-term period, with ambient being the most favorable condition followed by cool then warm. However, while a subset was created to maintain sampling size, the pattern of higher growth on ambient tiles was still observable in the later dates. This aligned with my hypothesis and the rationale that barnacles would fare best on substrate temperatures similar to that of their natural habitats. Cool tile barnacles performing better than warm ones similarly matches with my hypothesis, indicating that heat stress is the main component differentiating these treatments (Miller & Stillman, 2012). Barnacles showed a significant difference in growth after 13 and 50 days (Iafe, 2008; Wong, 2012). Similarly, my results were only applicable for 37 days. It is likely that temperature, while impactful in determining both short-term growth and long-term, cannot become statistically significant in the long-term due to continuing mortality.

Sanford & Menge (2001) have similarly reported high post-settlement mortality as a result of external factors. Predation, in particular, has been shown to play a stronger role in barnacle survival compared to abiotic factors like temperature (Jenewein & Gosselin, 2013). Our experiment did not exclude the presence of predators, so it is possible for them to have influenced our barnacle mortality. Barnacle behavior and internal adaptations to heat stress may have similarly affected survival since they are more directly impactful on body temperature than substrate color (Wong, 2012). Despite this, it is still important to consider substrate and temperature due to the compounding effects it can cause with other factors. Particularly, in the context of increasing global climate change.

The results of this study support the importance of substrate color and temperature on barnacle growth over a short-term period. The different treatments of substrate color were meant to replicate a range of temperatures, including the increase in temperatures deriving from climate change. I found that in the warmest treatment, barnacle growth was significantly lower compared to the other temperatures. While substrate alone may not be a defining factor in overall barnacle survival, it can compound with additional factors to cause critical ramifications for intertidal species like *B. glandula.* Such changes may include water temperature, nutrient and food supply, declined biodiversity, and habitat degradation, all of which were not accounted for in this experiment. Climate change will exacerbate and complicate the abiotic and biotic processes that affect these species (Harley et al., 2006), so it is crucial that we study its potential impacts now in order to develop a comprehensive and effective conservation strategy. By simulating the effects of climate change by manipulating substrate temperature, we can observe its potential effects on the thermosensitive intertidal species and safeguard them from future threats.

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