Living near the coast helped Alyssa Medina develop an interest in studying marine biology, and her coursework encouraged her to pursue research into how the environment and climate change impact intertidal organisms. Her project gave her the opportunity to develop relationships with others working in her field, and she particularly appreciated the chance to work so closely with Professor Sorte and her other mentors. Alyssa credits her research experience with challenging her and improving her work ethic; she hopes to attend medical school after graduation, and she feels that these qualities have helped her prepare to meet that goal.

Increased environmental stresses, including thermal stress, resulting from anthropogenic climate change may have profound effects on the species of the intertidal zone. The effects on shellfish such as mussels are of particular interest, as they are a foundation species that support a diverse community of associated species in rocky intertidal habitats. In this study, we employed an environmental gradient approach, testing the effects of environmental stress on size at reproductive maturity of the California mussel, *Mytilus californianus*, across a natural gradient of temperature and feeding opportunity. Mussels were collected from low, mid, and high tide heights and length, width, depth, total weight, gonad weight, shell weight, and gonad stage were measured. We found that size at reproductive maturity differed across tide heights, with adults reaching maturity at smaller sizes at higher tide heights. This finding may represent metabolic limitations as gonadosomatic index (ratio of gonad to tissue weight) was correlated with thermal stress (90th percentile temperatures) and feeding opportunity (using submergence time as a proxy). This suggests that life history characteristics of mussels, and their ability to create habitat for species that depend on them, will be impacted by alterations in temperature and food availability resulting from climate change.

We now know from Alyssa Medina’s work that shellfish living just a few meters further away from the ocean reach adulthood at smaller sizes than their wetter brethren—and that individuals of the same size can be different ages depending on where they live on the coastline. This variation is likely driven by differences in temperatures and food. Thus, Alyssa’s findings tell us about physiological trade-offs and give us glimpses into what could happen as the climate warms and ocean food supply changes. These outcomes are above and beyond the benefits of undergraduate research, which include experience for the researchers and mentors at all levels.
Introduction

Anthropogenic climate change threatens marine systems, such as the intertidal zone, which is defined as the area between the low and high tide marks. The intertidal zone is a unique habitat where diverse communities of organisms of primarily marine origin must endure the stressors of both marine and terrestrial habitats. As a result of climate change, air and sea surface temperatures have increased by 0.65 to 1.06 °C in the past century, and further increases of 0.3 to 0.7 °C in global surface temperature are expected by 2035 (IPCC, 2014). As many organisms currently live at their thermal limits, these environmental changes may threaten population dynamics or even persistence of intertidal species (Somero, 2002). In the face of climatic changes, marine habitats, like the intertidal zone, are important to protect because they provide many economic and ecosystem services such as food, raw materials, disturbance regulation, and nutrient cycling (Costanza et al., 1997).

The California mussel, Mytilus californianus, is a competitively dominant shellfish species in rocky intertidal habitats of the North American West Coast, from Alaska to Baja California, whose persistence under climate change is of supreme importance as it increases intertidal diversity (Suchanek, 1992). Because M. californianus individuals grow in aggregate formations, or mussel beds, which provide critical resources, such as habitat and refuge from abiotic stressors, mussels are considered a foundation species in intertidal habitats (Suchanek, 1979; Dittman and Robles, 1991). Therefore, the conservation and management of M. californianus is of great importance for the species diversity and overall health of our rocky intertidal zones. To understand the effects climate change will have on the inhabitants of the intertidal zone, it is important to consider responses at the local, within-site scales at which organisms experience broad environmental variations that rival variations seen along greater latitudinal gradients (Denny et al., 2011; Connor and Robles, 2015).

Intertidal organisms experience different levels of feeding opportunity, desiccation, thermal stress, and wave exposure depending on their vertical position and aspect in the intertidal zone (Denny and Paine, 1998; Helmuth et al., 2002; Petes et al., 2008). Organisms living in the high intertidal zone experience greater abiotic stress, such as increased thermal stress, as well as reduced feeding opportunity because they spend less time submerged under water (Wethey, 1983; Denny and Paine, 1998; Harley and Helmuth, 2003). Organisms living in the low intertidal zone experience increased feeding opportunity and lower thermal stress, but they also experience increased levels of competition and predation, because they spend more time submerged under water (Paine, 1974; Menge, 1976). Climate change can alter these stresses and, therefore, the effects they have on intertidal organisms.

Because of differing thermal stress and feeding opportunity across the intertidal zone, organisms shift their resource allocation in order to persist in varying conditions. These metabolic trade-offs occur due to limitations in resources and energy expenditure, meaning one process occurs at the sacrifice of another (Roff, 1992; Stearns, 1992; Bertrand et al., 2005). Since many intertidal organisms are sessile, they are unable to move and seek refuge from abiotic stresses, and must instead rely on allocation of energy to costly physiological defenses for survival (Halpin et al., 2004; Petes et al., 2008). This results in a metabolic trade-off between survival, growth, and reproduction (Petes et al., 2008; Eschweiler and Christensen, 2011). For example, Petes et al. (2008) found that mussels in the high intertidal zone, where individuals experience longer exposures to stressful air temperatures and reduced feeding opportunity, tend to spawn early and invest less relative energy towards reproduction. Instead, they allocate that energy towards costly physiological defenses, such as carotenoid pigment assimilation to protect gametes from oxidative damage (Petes et al., 2008). Leslie et al. (2005) found that the intertidal barnacle Balanus glandula, a species which shares a life history similar to most marine invertebrates, exhibited greater reproductive output when individuals inhabited areas of higher phytoplankton abundance. Reproduction, the costliest of these life history trade-offs, exhibits a plastic response to increased thermal stress and altered feeding opportunity (Stearns, 1992; Leslie et al., 2005; Petes et al., 2008). Therefore, one way in which we can monitor the success of these species and their vulnerability to environmental stressors and climate change is by studying their reproductive characteristics and abilities.

Variation in reproductive characteristics, such as size at reproductive maturity, can alter population growth rate and size, so investigating variation in reproduction can provide useful life history information that can improve or direct management and conservation efforts of these species (Leslie et al., 2005). Because reproduction shows the effects of stressful conditions sooner than other biological processes (Petes et al., 2008), it provides us with a timely indicator of stress across intertidal habitats. Here, we assessed reproductive ability across an intertidal gradient to gain insight into how this key life history characteristic is influenced by increasing environmental stress similar to that predicted under future climate change.
The purpose of this study was to answer the following questions: (1) Does reproductive potential and size at reproductive maturity for *M. californianus* differ across tide heights? (2) Is reproductive potential correlated with temperature or food availability? By sampling vertically across the intertidal zone at Crystal Cove State Park (CCSP), CA, a natural gradient in temperature and feeding opportunity, we were able to test our hypotheses that: (1) reproductive potential and size at reproductive maturity differs across tide heights, and (2) reproductive potential is correlated with temperature and feeding opportunity.

**Materials and Methods**

**Study Site and Collection**

Field collections of *M. californianus* were performed in the rocky intertidal zone at Reef Point, Crystal Cove State Park (Newport Beach, CA, 33°33'57" N, 117°50'00" W) under California Department of Fish and Wildlife Scientific Collecting Permit #13026 and local permission from the State Park (Figure 1). To assess how the environment affects the reproductive potential of mussels and the size at which mussels become reproductive, mussels were collected at low (0.6 m), mid (1.2 m), and at high (1.8 m) tide heights within the mussel bed (Figure 2). Five 100 cm² quadrats were placed at 6 m intervals along a 30 m horizontal transect at each tide height (Figure 2). Each quadrat was visually surveyed for percent cover of general classes of species, and all *M. californianus* individuals were counted and their sizes recorded in 20 mm size class bins. Each quadrat was also photographed prior to and after removing all organisms from within the quadrat. The contents of each quadrat were stored in a labeled and sealed plastic bag within a cooler after collection and during transport to the lab. All collections took place within a three-day period in December 2015. All samples were processed in the lab within 6 h of collection.

**Metrics**

Reproductive potential and maturity of mussels was assessed using multiple indicators, including Gonad Index (GI), Gonadosomatic Index (GSI), and gonad stage (Suchanek, 1981; Dittman and Robles, 1991; Phillips, 2007; Petes et al., 2008). GI and GSI are quantitative indicators of gonad abundance, which represent the proportion of soft tissue allocated towards reproduction as the ratio of gonad weight to body length and the ratio of gonad weight to total tissue weight, respectively (Suchanek, 1981; Petes et al., 2008). Suchanek (1981) used GI to estimate the life stage and...
The reproductive status of a population of *M. californianus* on Tatoosh Island (Washington, USA) and found that individuals can become reproductive at a length of 25–30 mm, but usually become reproductive at a length of 35–40 mm. GI and GSI are continuous metrics which do not differentiate between individuals that have reached reproductive maturity and those that are unable to release gametes for reproduction.

In mussels, the majority of gonad tissue is stored within the mantle cavity, and released by a system of transport canals known as gonoducts (Pipe, 1987). Qualitative indicators such as gonad stage rely on visual estimates of gamete storage within the mantle cavity and development of these gonoducts to assess the stage of gamete maturity, allowing for clear demarcation of reproductive and non-reproductive individuals (Kautsky, 1982; Seed, 1969). All of these methods of determining reproductive status are invasive and require the harvesting of mussels. In order to use small sample sizes of harvested mussels to make predictions about larger populations of individuals, we assessed the ability of morphological metrics—such as length, width, and depth—to serve as proxies for reproductive maturity.

**Gonad Analyses**

*Determining Gonadosomatic Index (GSI).* To calculate GSI and examine variation in GSI among tide heights, we measured morphological characteristics of *M. californianus* individuals collected. After removing any fouling organisms attached to the mussel shells, we measured each mussel’s length, width, and depth (Figure 3) with Vernier calipers, as well as the total wet weight. Mussels were then dissected by severing the adductor muscle with a scalpel. We recorded the presence and ripeness of the gonoduct system (Phillips, 2007), based on visual determination of the development, or lack thereof, of gonad tissue extending around the digestive organs and the mesosoma. We then removed the gonad tissue and weighed it. The somatic tissue was removed, and the shells were weighed. Total soft tissue weight was calculated as the difference between total wet weight and shell weight. Using the weight of the gonad tissue and total soft tissue weight, we calculated the GSI using the following formula (Petes et al., 2008):

\[
GSI = \frac{\text{gonad tissue wt (g)}}{\text{total soft tissue wt (g)}} \times 100
\]

Half of the gonad tissue from each mussel was placed in a labeled centrifuge tube, fixed with ethanol, and frozen for possible future histological analysis. The other half was placed in a labeled six-well plate to be analyzed for reproductive stage.

*Determining Reproductive Gonad Stage.* To qualitatively determine the reproductive stage of *M. californianus*, we examined isolated, whole gonad tissue under a Zeiss Discovery V8 SteREO microscope at 20x magnification and classified the ripeness of the tissue using a scale from Phillips.

**Table 1**

Gonad stage descriptions from Phillips (2007) and photos taken under the microscope. Photos are of gonad tissue only, and are not indicators of gonoduct development.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>clear mantle tissue - juvenile</td>
</tr>
<tr>
<td>1</td>
<td>little development of reproductive tissue - juvenile</td>
</tr>
<tr>
<td>2</td>
<td>thickened tissue; some gonoduct development - adults</td>
</tr>
<tr>
<td>3</td>
<td>well developed and extensive gonoducts - adults</td>
</tr>
</tbody>
</table>

Figure 3
Length (umbo to shell edge), width (hinge to opening) and depth (valve to valve) were measured as above.
23

Alyssa D. Medina

(2007) (Table 1). Because there is little glycogen storage in the gonad tissue of *M. californianus*, visual indices have been used to successfully determine reproductive maturity (Dittman and Robles, 1991).

Mussels classified as stages 2 or 3 are considered adult mussels, which are reproductively mature and capable of spawning, while those in stages 0 or 1 are considered unable to reproduce and, therefore, juveniles (Phillips, 2007). After determining the stage of a mussel’s gonad tissue, we photographed each gonad tissue sample using a Zeiss Axiocam 105 color camera with Zen 2012 imaging software for visualization.

Temperature and Feeding Opportunity Analyses

Determining Temperature across the Intertidal Zone. To determine how thermal environment differed across tide height, we deployed iButton data loggers at each of the tide heights (low, mid, and high) which recorded the temperature at 12-minute intervals from December 2015 until February 2016. To hindcast temperatures experienced at each tide height prior to collection (September 2015 to November 2015), we quantified the linear relationship between nearby daily maximum aerial temperature data from John Wane (Orange County) Airport (NNDC Climate Data Online, http://www7.ncdc.noaa.gov/) and the daily maxima of logged data at each tide height, and used the linear relationship to hindcast temperatures across tide heights. We used hindcasted temperature data to calculate the average 90th percentile of daily max temperature for low, mid, and high tide heights, and used this as a proxy for thermal differences across tide heights in our analyses.

Determining Feeding Opportunity. Since the high intertidal zone is underwater for less time than the lower intertidal zone, and *M. californianus* is only able to feed when it is submerged, the time an organism is able to feed varies markedly across tide heights. We used submergence time as a proxy for feeding opportunity. Tide height data were downloaded at 2-min intervals for Balboa Pier, Newport Beach, CA during the year 2015 (WWW Tide and Current Predictor, tbone.biol.sc.edu/tide/). The proportion of predictions where tide level was higher than each of our three (low, mid, high) tide heights were calculated and used as submergence time indices. Total estimated submergence time for each tide height was used in analyses as an indicator of feeding opportunity.

Statistical Analyses

All analyses were performed using R statistical software v. 3.2.2. To compare reproductive maturity to size, we performed regression analyses with length, width, and depth as explanatory variables and GSI and gonad stage as response variables. We created scatterplot matrices to compare linear relationships between explanatory and response variables for each tide height, as well as a combination of all tide heights. We assessed the significance of these relationships at *P < 0.001*. To assess whether size at reproductive maturity differs across tide heights, we performed a non-parametric Kruskal-Wallis test to detect differences in size at stage 2 across tide heights. Generalized linear models were used for non-parametric ANCOVA analysis to determine whether reproductive maturity is correlated with the covarying factors of 90th percentile temperatures at each tide height and feeding opportunity.

Results

Indicators of Reproductive Maturity

When data from all tide heights were combined, all body size measurements (length, width and depth) were strongly related to both gonad stage and GSI (*R^2 > 0.8; P < 0.001*) (Figure 4). The proportion of variance explained by each of these body size measurements was similar between GSI and gonad stage, so only proportion of variance explained by gonad stage are shown (Table 2). These results were consistent across tide heights, although the correlations were weaker at the high tide height (*R^2 ≤ 0.478*). Overall, there was a slight increase in ability to predict reproductive maturity from width to length to depth.

<table>
<thead>
<tr>
<th>Tide Height</th>
<th>Body size measurement</th>
<th>R^2 value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Length</td>
<td>0.868</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>0.878</td>
</tr>
<tr>
<td></td>
<td>Depth</td>
<td>0.881</td>
</tr>
<tr>
<td>Mid</td>
<td>Length</td>
<td>0.808</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>0.779</td>
</tr>
<tr>
<td></td>
<td>Depth</td>
<td>0.806</td>
</tr>
<tr>
<td>High</td>
<td>Length</td>
<td>0.375</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>0.288</td>
</tr>
<tr>
<td></td>
<td>Depth</td>
<td>0.476</td>
</tr>
</tbody>
</table>

Reproductive Maturity across Tide Heights

The mean size at each stage differed across tide heights (Figure 5). The Kruskal-Wallis test detected a significant difference between mean lengths at reproductive maturity (individuals in stage 2) across tide heights (*P = 0.001325*) (Figure 6). Specifically, mussels at the higher tide height...
Figure 4
Relationships between gonad stage/GSI and body size measurements across all tide heights. $R^2$ values are based on linear regression analysis; $P < 0.001$ for all comparisons.

Figure 5
Distribution of lengths at each stage across tide heights. The first and third quartiles are at the ends of the box, the median is indicated with a vertical line in the interior of the box, the maximum and minimum are at the ends of the whiskers, and circles indicate outliers. The number of samples at each stage is indicated along the right.
Alyssa D. Medina

reached reproductive maturity at smaller lengths than muscles at the lower tide height.

Reproductive Maturity across an Environmental Stress Gradient

Our temperature estimates indicated that temperature varies across tide height (Figure 7). The 90th percentile maximum temperatures for low, mid, and high tide heights were calculated as 35.21 °C, 35.90 °C, and 42.68 °C, respectively.

As a proxy for feeding opportunity, we calculated the percentage of time mussels spent submerged (underwater) at each tide height. The percentage of time submerged for low, mid, and high tide heights was 68.24%, 22.76%, and 1.24%, respectively. Both 90th percentile temperature ($P = 1.33 \times 10^{-8}$) and submergence time ($P = 2.35 \times 10^{-16}$) were strongly related to GSI (Figure 8).

Discussion

This study was designed to assess reproductive ability across an intertidal gradient to gain insight into how this key life history characteristic is influenced by increasing environmental stress similar to that predicted under future climate change. Our results demonstrate that reproductive potential and size at reproductive maturity differ dramatically across tide heights. It is likely that temperature and feeding opportunity play a combined role in these differences, as they are correlated with reproductive maturity. We also determined that reproductive maturity could adequately be predicted by length, width, and depth as long as tide height is taken into account. In the high intertidal zone, body size measurements were less capable of predicting reproductive maturity. Though sample size was a factor, this may also indicate greater variability between mussels here. Though the top predictor varied by tide height, depth was generally more strongly correlated with reproductive maturity compared to length and width. This may be because reproductive maturity is dependent on growth rate, and other studies suggest

![Figure 6](image)

**Figure 6**

Average size (mean ± SE) at reproductive maturity (Stage 2) across tide heights. Kruskal-Wallis test: $P < 0.05$.

![Figure 7](image)

**Figure 7**

Daily maximum temperature at low, mid and high tide heights from iButton data loggers at Reef Point, CCSP, CA (December 2015 to February 2016) and hindcasted daily maximum temperature at low, mid and high tide heights (September 2015 to November 2015). Recorded data are represented by solid lines and hindcasted data are represented by dotted lines.
that depth of the mussel may be more strongly correlated to growth rate than other size indicators (Coe and Fox, 1944; Seed, 1969; Suchanek, 1981; Dittman and Robles, 1991). Ecologists have traditionally used length as an indicator of age or reproductive maturity in the field, but depth may prove more useful as it may be easier to measure the depth of the mussel than the length without disturbing nearby mussels. Because all size indicators were strongly correlated to reproductive maturity, they provide us with an alternative to invasive methods for future research in size and maturity related studies. Furthermore, the difference in top predictor across tide heights may likely be related to the differences in reproductive potential and size at reproductive maturity seen across tide heights.

Variation in reproduction can reveal a great deal about the effects of climate change on population dynamics. The average length at each gonad stage, compared to its respective stage at other tide heights, showed a decreasing trend as tide height increased. Only 4.4% of mussels at the high tide height were adults (stage ≥ 2). On the other hand, 29.2% of mussels at the low tide height and 57.5% of mussels at the mid tide height were adults, so perhaps in the high intertidal zone, mussels are too nutrient-limited to survive long enough to reach a critical age at which they become reproductively mature. This same pattern is seen with variation in the size at which mussels are able to spawn. Comparing the average length at stage 2 across tide heights confirmed our prediction that size at reproductive maturity significantly varies across tide heights. Mussels become sexually mature at shorter average lengths in the high intertidal zone than in the low intertidal zone. Interestingly, the minimum size at reproductive maturity was similar across tide heights (28.8–33.7 mm) and was comparable to that of a population of *M. californianus* on Tatoosh Island (Washington, USA) which reached maturity at minimum lengths of 25–30 mm (Suchanek, 1981). However, the differences in reproductive potential and size at reproductive maturity across tide heights were expected because environmental stresses vary across tide heights and have been shown to affect reproduction and growth of mussels (Coe and Fox, 1944; Blanchette et al., 2007; Leslie et al., 2005; Petes et al., 2008). The correlations between temperature and submergence time, and reproductive maturity seen here support this theory. The percent of time spent submerged in water increased notably as tide height decreased, meaning mussels at low tide heights are submerged for 67% more time than mussels at high tide heights. Though temperature and feeding opportunity are co-varying factors inherently tied to tide height and their effects on reproductive maturity cannot be separated in this study, it is evident that, much like Seed (1969) and Suchanek (1981) found, environmental conditions impact the size at which mussels reach reproductive maturity. Overall, these results seem to indicate that most of the reproductive potential in the intertidal zone comes from the low intertidal zone. As other studies have shown, mussels, and likely other sessile species in the high intertidal zone, exhibit a metabolic trade-off, exerting more energy towards survival than growth in response to limited feeding opportunity and greater thermal stress (Petes et al., 2008). With many intertidal species currently living at their thermal limits, it may be especially important to focus conservation efforts on protecting the persistence of species in the high intertidal zone as metabolic trade-offs may not be enough to combat the stress of further climate change. Furthermore, the low intertidal zone may serve as a primary source of important ecosystem services under climate change as the organisms there demonstrate greater reproductive potential.

In summary, mussels at higher tide heights generally become adults at smaller lengths than mussels at lower tide heights, likely as a response to the greater stress experienced at higher tide heights. Also, all the size measurements we tested were adequate in predicting reproductive maturity. This means that many different types of measurements can be used to investigate life stages of mussels rather than dissecting them. Though the intertidal only spans a few meters, the environment varies significantly and this has
a significant effect on the reproduction of these mussels. We can consider these differences in mussel reproductive maturity across tide heights as an indicator of how mussels, as well as the organisms that depend on them, may respond to increasing thermal stress and altered feeding opportunity caused by climate change.

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Works Cited


