# EFFECTS OF ELEVATED WATER TEMPERATURES ON DIFFERENT POPULATIONS OF *Zostera marina*

by

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# ABSTRACT

## Effects of elevated water temperatures on different populations of *Zostera marina*

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I tested the effects of elevated water temperature, in the upper threshold limit, on the ecological performance of the native eelgrass *Zostera marina* in a mesocosm experiment to identify the most resilient population. Five populations (Cherry Point, Elliott Bay, Fidalgo Bay, Nisqually Reach, and Willapa Bay) spanning the Washington coast and Puget Sound were exposed to four constant treatment levels (14, 17, 20, 23 °C) during a 6-week hydroponic experiment. Changes in eelgrass performance were evaluated by measuring morphological changes and photosynthetic efficiency. The Fidalgo Bay population was more tolerant to thermal stress than Cherry Point, Elliott Bay, Nisqually Reach, and Willapa Bay populations. By the end of the experiment, Fidalgo Bay overall had the lowest percentage loss of shoot length (14, 17 and 20°C treatments), lowest blade loss (14, 17 and 20°C), most growth (14, 17 and 20°C) and highest photosynthetic yield (14°C). The initial morphological measurements indicated that morphology was possibly connected to eelgrass performance as Fidalgo Bay had the longest blade length, sheath width, and sheath length. Fidalgo Bay outperformed all populations even though it has the coolest daily mean water temperature. Higher temperatures significantly affected growth and survival in treatment levels 17°C and higher for all five populations. Changes in eelgrass performance occurred noticeably during week two in all four performance measurements. This indicated that long-duration thermal stress has negative consequences for eelgrass productivity and resilience.

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# Acknowledgements

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## Literature Review

Over the last few years, there have been numerous studies that looked at eelgrass loss due to human activity and natural induced causes (Duarte, 2002; Orth et al., 2006; Short and Neckles, 1999). The goal of this literature review is to highlight some of the most relevant articles that pertain to the project. Many factors will negatively affect eelgrass but with this review, I will focus on elevated water temperatures. First, we will look at some of the stressors that will affect eelgrass, followed by how these stressors will impact eelgrass. Thirdly, we will look at how climate change might contribute to these stressors and then look at results from stressor studies done on eelgrass. Lastly, I will discuss the Department of Natural Resources' ANeMoNe Network.

### *A global crisis for eelgrass ecosystems*

Orth has written compressive reviews on the value of eelgrass, what makes it unique, and the threats that it faces. Orth et al. (2006) review the evolutionary history of eelgrass and describe the characteristics which allow a vascular plant to live underwater. The writer points out that the rapid shift in eelgrass distribution is a result of human activity. Orth writes that eelgrass is an indicator of ecosystem health and implies that a loss of eelgrass is a loss of ecosystem services like nursery grounds, trophic transfer facilitation, current speed reduction which traps and stores nutrients, carbon sequestration, and enhanced biodiversity. They identified multiple stressors that led to eelgrass decline including water quality, shifting sediment, increased nutrients, and increased temperatures. Other emerging threats to eelgrass are aquaculture activities and invasive species. Lastly, the writer touched on the issues of restoration, conservation, management, and monitoring. Eelgrass is good indicator species, and their loss is usually the symptom of a larger problem. To effectively conserve eelgrass, resource managers must identify and address problems that affect coastal systems like water quality and land-use practices. Restoration efforts should then consider the natural capacity of eelgrass to recover.

### ***Possible effects of climate change on eelgrass***

Short and Neckles (1999) evaluated how climate change might affect eelgrass productivity and distribution by applying current eelgrass biology knowledge and how various taxa respond to the environment. Two of the major environmental forcing factors that are likely to affect *Zostera marina's* productivity and distribution are rising sea levels and increasing water temperatures. Depending on the status of the ecosystem and the location of the interaction, these forcing factors can have compounding effects and may act in a variety of ways.

The first factor, rising sea level, will affect the amount of light that the eelgrass receives as the amount of light that travels through the water column decreases at an exponential rate the deeper it must penetrate. The change in water depth will cause a shift in the eelgrass habitat location as it moves to areas with higher light levels that are better suited for photosynthesis. Beds that currently exist in the maximum depth distribution area will die off as the light availability decreases. A coastal squeeze can occur when the beds are prevented from moving shoreward while losing habitat in the maximum depth distribution area. Previously shallower areas might be colonized by eelgrass, but it may also be hampered by human modification of the shoreline. Light availability could also be affected by other factors such as tidal range change, increased epiphytes, and increased turbidity which will exacerbate any negative effects.

The second important factor is increasing water temperatures, eelgrass respiration rate increases faster than the photosynthetic rate which causes a decrease in the photosynthesis-to-respiration ratio. Therefore, the eelgrass has a seasonal growth optimum with decreased productivity when temperatures go above this optimum. The growth of epiphytes, dinoflagellates, and diatoms are stimulated by higher water temperatures leading to lower light availability. Increasing water temperatures are predicted to be detrimental to eelgrass.

### ***Eelgrass response to climate change***

Duarte (2002) forecast how eelgrass ecosystems will respond for the next 20 years from the date published. They mainly focused on the effects of climate change and the pressure of an increasing human population. It also covered the basic environmental requirements like sediment type, redox potential, light level, and salinity.

Duarte talked about the effect of human impact on eelgrass ecosystems. The pressure on eelgrass that stood out to me the most was the cultural eutrophication of coastal waters. This is an issue that we have been struggling with at MAVEN (DNR's research facility) where we are currently growing eelgrass in large tanks outside. The tanks have been plagued with excess algae growth which I hypothesize is due to the nutrient loading from the LOTT treatment plant in Budd Inlet. The increasing nutrients in the water column stimulate macroalgae and phytoplankton growth. As primary producers, they are well suited to take advantage of the constant nutrient supply. The productivity of eelgrass is reduced when these primary producers receive less light, by either covering the eelgrass or by filling the water column.

Duarte further listed more threats to eelgrass that are linked to the increasing human population: increased physical disturbances due to human activity; increased nutrient loads, even with the decline in wastewater treatment; changes in eelgrass coverage and productivity due to climate change; increase in coastal aquaculture. Present global eelgrass decline is predicted to worsen which leads to a decrease in biodiversity and modification of food webs.

More knowledge of eelgrass ecosystems is required to better manage and respond to threats effectively. Duarte highlighted the need for increased monitoring and an early indicator of decline. Pressures on eelgrass need to be managed with the easiest being mechanical disturbances as it is a direct effect and easily identifiable. Indirect effects, such as nutrient inputs from watersheds, are harder to manage and should be coupled with public education and awareness of eelgrass ecosystems.

### ***Effects of elevated temperature on growth dynamics of eelgrass***

Lee et al. (2007) found that the optimal temperature range for *Zostera marina* growth has an average of 15.3$^{o}$C ± 1.6$^{o}$C and the optimal temperature for eelgrass photosynthesis being 23.3$^{o}$C ± 2.5$^{o}$C which can vary by location and species. Lee concludes that photosynthesis and growth may be inhibited with high summer temperatures and may ultimately be detrimental to the success of eelgrass.

Lee found that eelgrass exhibits optimal growth at an intermediate temperature in its range of tolerance while production is almost nonexistent during winter. As light decreases during winter, the optimal temperature range for photosynthesis also decreases. Again, location determines the extent to which the eelgrass goes dormant. Lower latitudes will have a shorter period of dormancy or may be completely absent in warmer areas. Shoot growth decreases towards the end of fall when ambient temperatures drop below the optimal range of 15°C -17°C and during summer when ambient temperatures rise above the optimal range. The optimum temperature for photosynthesis depends on light availability and ranges from 16°C to 30°C. Above this range, eelgrass shows a decrease in productivity.

### *Puget Sound water temperatures*

The Acidification Nearshore Monitoring Network (ANeMoNe) is a Department of Natural Resources (DNR) water monitoring network that spans the Puget Sound and Pacific coast. ANeMoNe is a network of sensors that continuously record water chemistry parameters like pH, temperature, salinity, dissolved oxygen, and chlorophyll. This data can be used to evaluate site-specific variability in pH, assess impacts on marine organisms, and identify potential sites that may be more exposed or buffered to changes in marine chemistry. All the ANeMoNe sites are located within eelgrass beds. Each site has two sensors with a sensor placed both inside and outside of the eelgrass bed.

Eelgrass for this project was collected from five of the ten ANeMoNe sites. This ANeMoNe report provided me with the temperature ranges of all five sites. Figure 2 shows the mean high temperature at 12.4 °C and the low mean temperature at 7.4 °C. The mean temperature was lowest in Fidalgo Bay at 7.4 °C with a variation of 5.3 °C followed by Cherry Point at 10.3°C with a variation of 13 °C, Elliott Bay at 10.6 °C with a variation of 6.3 °C, and Nisqually Reach at 11.3 °C with a variation of 13.3 °C. Willapa Bay had both the highest mean temperature at 12.4°C and the highest variation of 13.9 °C.

# Introduction

Globally eelgrass is in decline due to threats from climate change, declining water quality, and sustained pressure from coastal development (Hemminga and Duarte 2000; Orth et al., 2006). This has resulted in a reduction of fish habitat, changes in coastal productivity, and increases in erosion (Boese et al*.,* 2008). Eelgrass has been shown to maintain healthy fish populations (Zeller and Pauly, 2014), provide shoreline protection (Spalding et al*.,* 2014), and contribute to recreational activities (Barbier*,* 2010).

Eelgrass beds provide ecosystem services that rate higher than most other ecosystems on earth, calculated to be US\$19,002 $ha^{-1}$ $yr^{-1}$ (Costanza et al., 1997). Found in shallow waters along much of Puget Sound's shoreline, eelgrass acts as an ecosystem engineer by stabilizing sediment, taking up nutrients, sequestering carbon, and providing habitat for a vast array of species including waterfowl, shellfish, shrimp, herring, crab, and salmonids (Heck et al*.,* 2003). Most eelgrass species inhabit temperate waters of the northern hemisphere (den Hartog, 1970) and is limited at the equator due to elevated temperatures.

The global average temperature is projected to warm between 2-4°C by 2100, mostly due to human activity (IPCC 2014) with similar increases projected for marine systems (Sheppard and Rioja-Nieto, 2005). These temperature changes can result in a slower growth rate, altered metabolism, shift in distribution, and changes in patterns of sexual reproduction and changes in their carbon balance (Short et al., 2001; Short and Neckles, 1999).

Temperature is among the most important factors determining eelgrass performance and distribution. Regional experimental work found that native *Z. marina* was healthiest at 5–8 °C and temperatures above 15 °C plants exhibit physiological stress (Thom et al., 2003). Another study observed a reduction in growth rate and increase respiration rate at higher temperatures

(Coles et al., 2004). The reduced productivity for individual species from elevated temperatures higher than the threshold will cause them to die off. Another study on the temperate *Zostera marina* has also shown that a 5°C increase in normal seawater temperature led to a significant loss in shoot density (Ehlers et al., 2008). However, they also determined that the genetic diversity of the species indicates that it might be able to recover from extreme temperatures. Increased water temperature has also been found to affect eelgrass seed germination and flowering, altering abundance and distribution (Phillips et al., 1983). Additionally, the growth of competitive epiphytes and algae might increase due to elevated water temperatures which can reduce light availability hindering their growth (Beer et al., 1996).

Eelgrass is a highly productive photosynthetic marine species that fix large amounts of carbon (Poppe, 2018). This growth fuels the nearshore food web. They are only found in shallow waters ranging from 1 meter to 10 meters as they need high light levels to grow and reproduce. It is therefore totally dependent on the nearshore environment (Mumford, 2007). It requires a well-defined set of physical conditions such as high ambient light and low water turbidity to allow it to absorb as much light as possible. There is only a narrow band of shallow nearshore area where adequate light is available, and the proper sandy substrate and sediment type exists. However, this shallow band of habitability also means that eelgrass communities are prone to fluctuating water levels that can lead to large and sometimes rapid changes in water temperature (Lartigue et al., 2003). Therefore, most eelgrass populations must be able to tolerate these temporary changes. It is known, however, that constant exposure to higher water temperature levels can be lethal (Lee et al., 2007; Phillips, 1983).

There is an increasing understanding that climate change and other anthropogenic factors could have a negative impact on eelgrass habitats. Indicators of resistance and recovery are needed, to better understand and predict ecosystem response to environmental change. Understanding how environmental stressors due to climate change might impact eelgrass populations can help resource managers develop effective mitigation and restoration strategies.

This study aimed to experimentally evaluate how different populations of *Z. marina* found across the Puget Sound and Washington coast respond to elevated water temperatures in the upper threshold that could potentially limit survival and growth. 70 Eelgrass shoots from 5 populations were exposed to four levels of water temperatures for 6 weeks where shoot length, blade count, growth rate, and photosynthetic efficiency were measured as response parameters.

## Materials and Methods

### *Sample sites*

The five sample sites Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB) were chosen from DNR's well-established program ANeMoNe (Acidification Nearshore Monitoring Network). This network was established in 2015 to study ocean acidification and climate change in Puget Sound nearshore environments. All ten ANeMoNe sites are located within eelgrass beds. The five sites chosen represent some of the main oceanographic regions recognized in the Puget Sound: Coastal (Willapa Bay), Northern Puget Sound (Cherry Point, Fidalgo Bay), Central Puget Sound (Elliott Bay), and South Puget Sound (Nisqually Reach). Locations include areas with the lowest mean water temperature (Fidalgo Bay) and the highest mean water temperature (Willapa Bay).

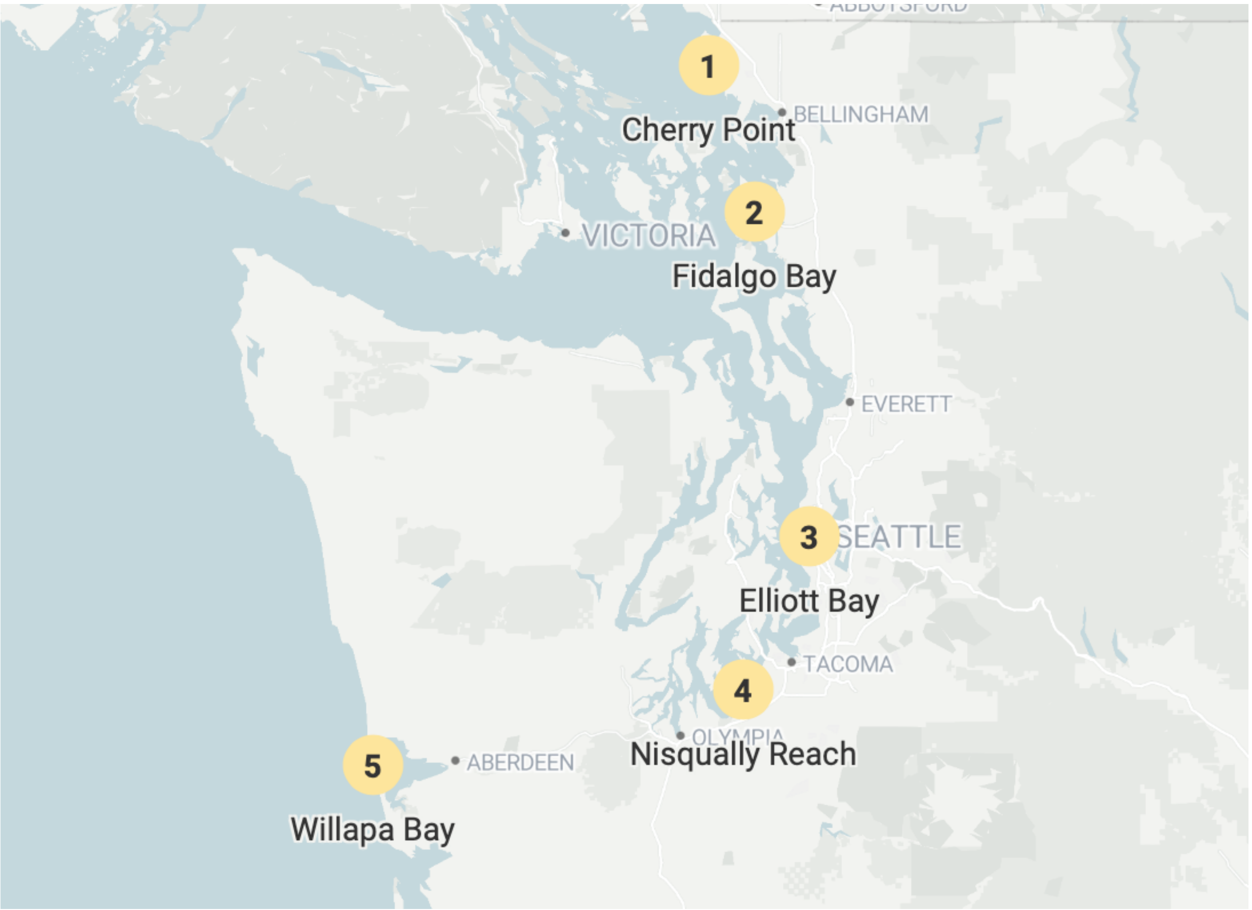

*Figure 1:*
*A map of Puget Sound and the Washington coast indicates the location of the five sample sites: Cherry Point, Fidalgo Bay, Elliott Bay, Nisqually Reach, and Willapa Bay.*

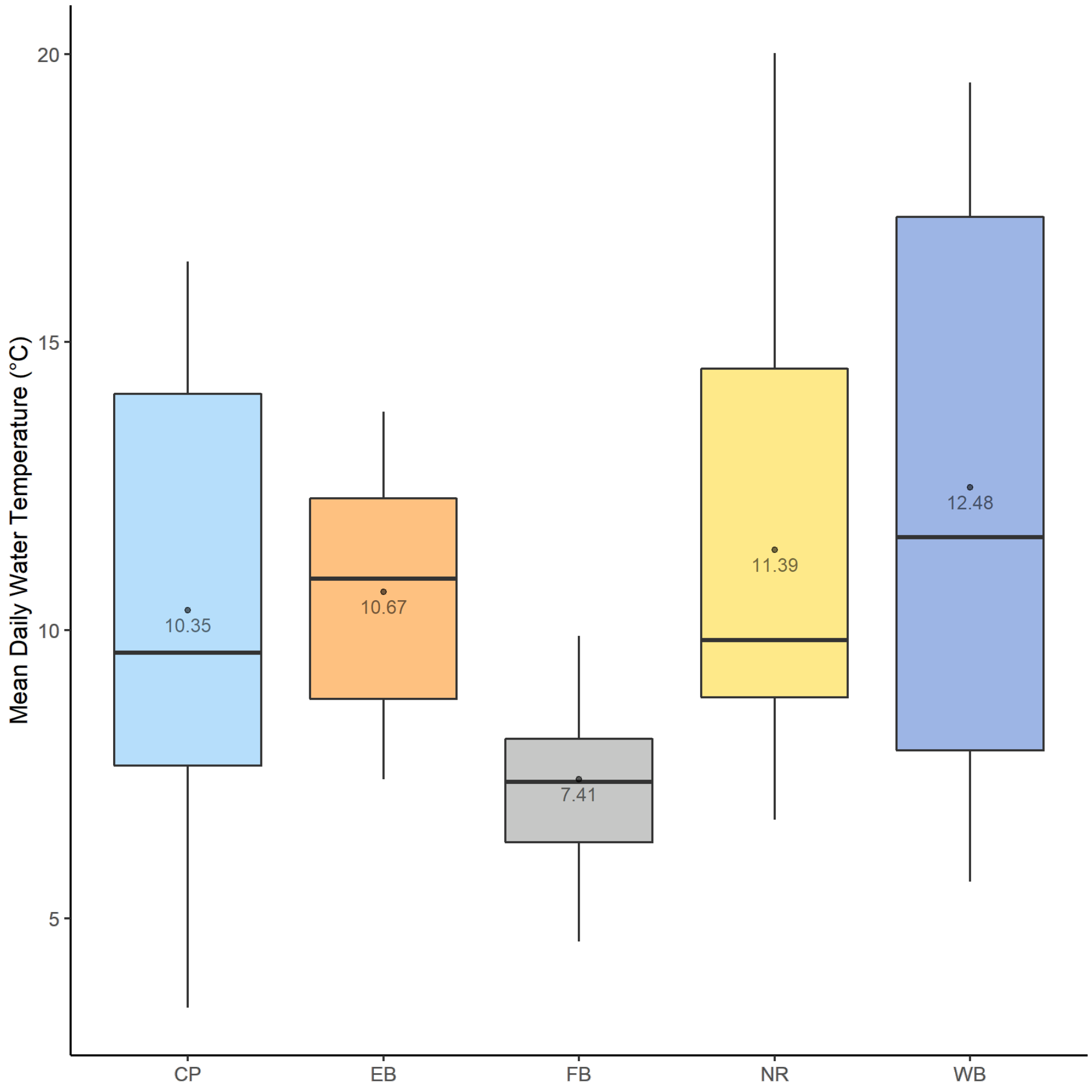

*Figure 2:*
*Annual mean daily water temperature variation across all sample sites over the period of an entire year (2018). The five sample sites: Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB).*

### ***Experimental design***

The experiment was conducted for six weeks during May 2021 to determine response to elevated water temperatures by evaluating eelgrass performance considering variations in morphological features among different populations. Each mesocosm contained five cages anchoring five eelgrass shoots from each population. The eelgrass was subjected to four constant water temperatures (14, 17, 20, and 23 °C) with three replicates for each temperature.

### *System setup*

A flow-through system was used to ensure sufficient levels of nutrients and inorganic carbon were maintained. Bay water was filtered through a sand filter and UV filter (80w) before being cooled down to 12°C. The chilled water was supplied to a treatment tank (20gal) where the temperature was raised to the required level by a heater (300w) and circulated into both the mesocosm (100gal) and treatment tank by a pump (100w). Water temperatures in mesocosm were maintained by a (100w) heater and water was circulated with a wavemaker (65w). Both heaters were monitored and adjusted to maintain the required temperature by an Apex Controller. Standpipes were standardized in length to control the water level of each tank. Water overflowed through the standpipe removing debris and algae growing on the surface. The Apex continuously recorded the temperature of each mesocosm. Even light distribution across the 4ft long tank was provided by fluorescent grow lights (55w). Apex light meter adjusted the light intensity at the surface to 120 μmol photons $m^{-2}$ $s^{-1}$ PAR which is close to the saturating level of eelgrass (Marsh et al., 1986; Olesen and Sand-Jensen, 1993) on a 12-hour photoperiod. There were six Apex instruments, each controlling 2 tanks.

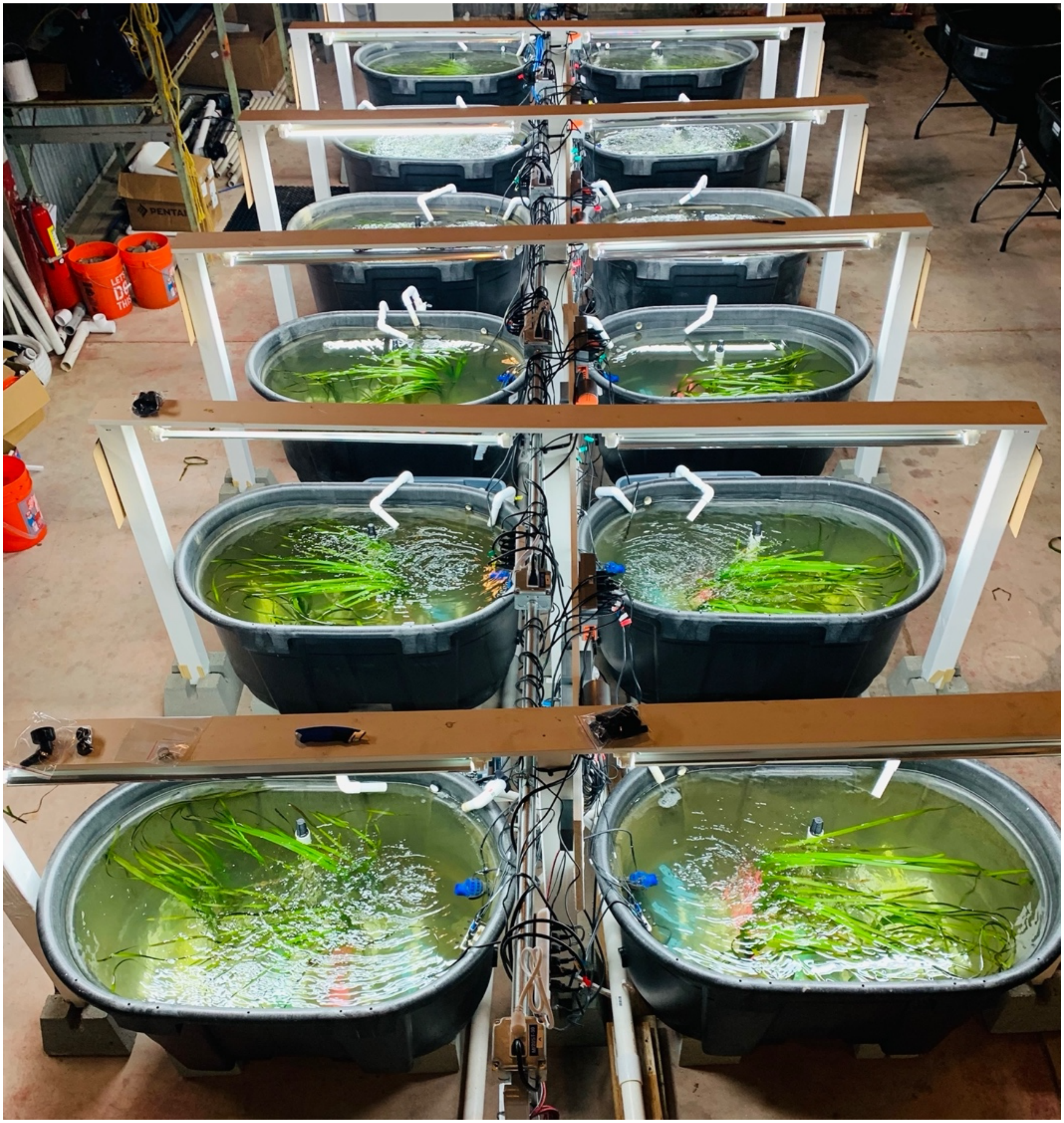

*Figure 3:*
*Experimental setup depicting all 12 mesocosms and the life support system.*

*Figure 4:*
*Close-up view of one of the mesocosms depicting the five cages of eelgrass at the bottom of the tank and arrangement of equipment.*

There were 12 mesocosms, each labeled with an associated Apex controller letter (A-F) and a tank number (1-12). There were four treatment levels (14, 17, 20, and 23°C) and three-level replicates for each treatment level. Each shoot was tagged with the following labeling scheme: Site acronym, Apex letter (A-F), tank number (1-12), and shoot number (1-5) (Fig 6). Tanks A1, A2, and B3 were treatment level 14°C. Tanks B4, C5, and C6 were treatment level 17°C. Tanks D7, D8, and E9 were treatment level 20°C. Tanks E10, F11, and F12 were treatment level 23°C.

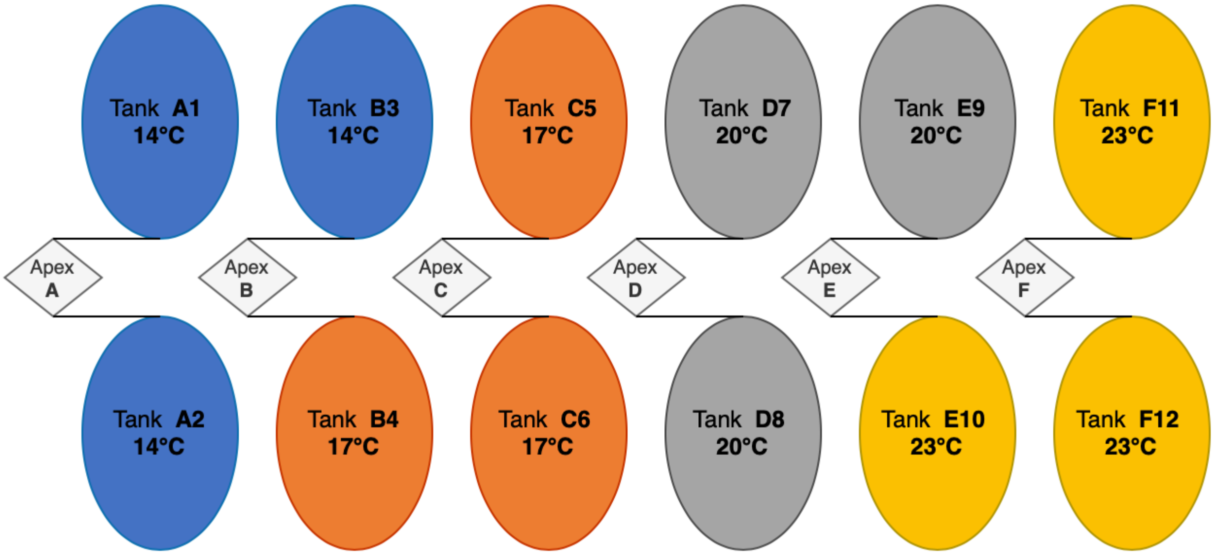

*Figure 5:*
*System layout indicating Apex controller and tank labeling as well as temperature treatments.*

### ***Eelgrass specimen collection, pre-treatment, and treatment***

Established shoots with well-developed rhizomes were collected from each of the five sites during low tide between depth range of -1.5 and – 2.0 m in late April. Plants were carefully removed from sediment by hand to ensure an intact rhizome system. Adult shoots bearing a healthy-looking rhizome, defined as 5-7 internodes, were sampled. Eelgrass was transported in cooler boxes filled with enough water from the sampling site to submerge all the eelgrass. A wet cloth was placed on the surface to prevent eelgrass from drying out. On-site eelgrass was kept at 15°C and under saturated light conditions until used in the experiment (24 hrs maximum). Senescent leaves were removed before transplantation to mesocosms. Shoots were further standardized by cutting older internodes to leave only four healthy rhizome internodes. No sediment was used as eelgrass had to be removed every week to be measured. Eelgrass was attached to plastic mesh which was zip-tied to a cage constructed out of PVC pipe (Fig 6). Holes were drilled in the pipe to allow air to escape once submerged. To prevent detritus buildup in and around the rhizomes, the cages were designed with a one-inch gap at the bottom to allow a flow-through of water. Each cage was anchored by a porcelain tile that was zip-tied to the cage.

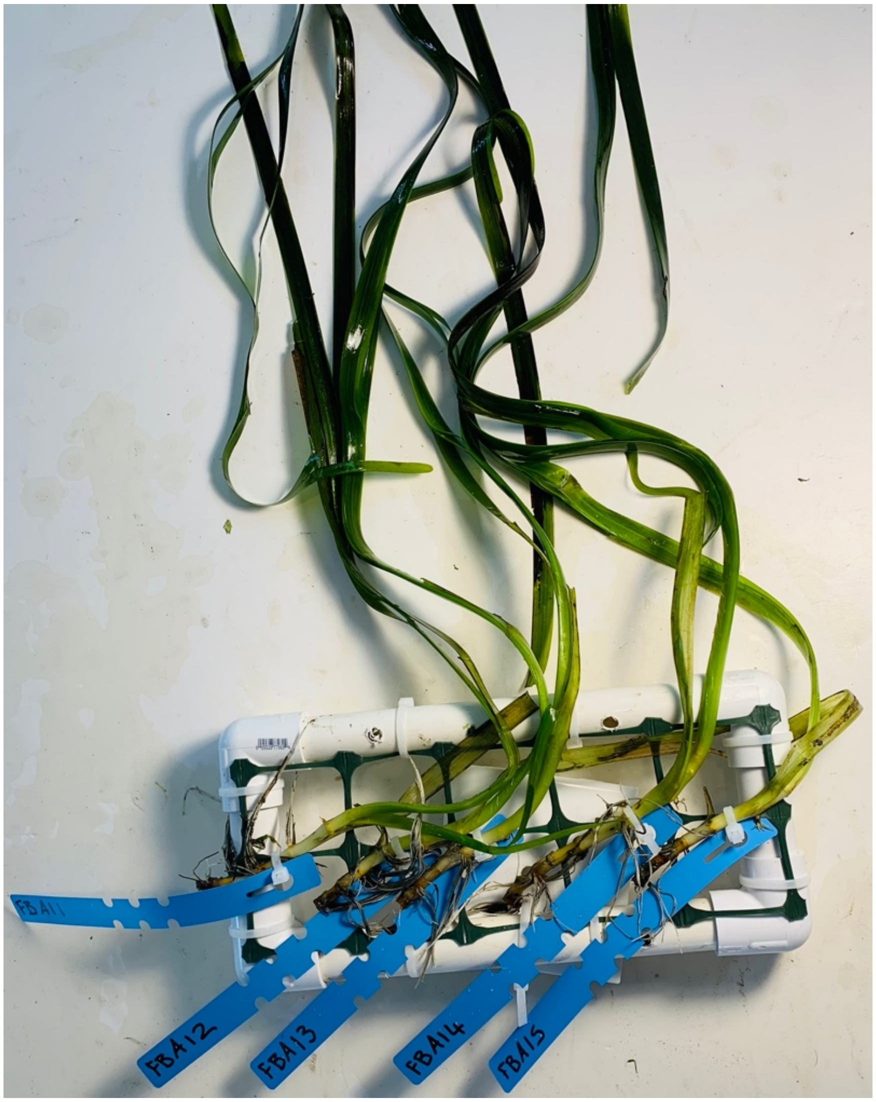

*Figure 6:*
*Eelgrass attachment to PVC cage and labeling.*

Each week all the samples were removed, one population at a time, to be cleaned and measured.  Once removed, it was carefully placed in a tub with seawater of the same temperature to keep eelgrass submerged while it is being cleaned. Epiphytes were scrubbed using a brush and by running individual blades through fingertips. Initial morphology and PAM fluorometry were measured at beginning of the experiment and once a week thereafter as described below.

### *Eelgrass morphology*

To measure changes in eelgrass morphology characteristics related to elevated temperatures, eelgrasses were closely monitored and measured once a week (days 0, 7, 14, 21, 28, 35, 42, and 49) for the duration of the experiment using the protocol outlined by (Short and Duarte, 2011). Three different random shoots from each population in each tank were chosen weekly for measurements. We recorded shoot count, shoot length (measuring tape in cm), sheath length (calipers in mm), sheath width (calipers in mm), and the total number of leaves. Reproductivity was measured from asexual lateral branching. The growth rate was assessed by using the pin-prick method as outlined by (Short and Duarte, 2001) to determine the total leaf growth per shoot relative to the days since last pricked (new leaf extension cm/day). New growth was measured from the pinprick below the sheath to the pinprick on the newest inner leaf. Raw values for shoot length, blade growth, and blade counts were used for statistical analysis.

### *Chlorophyll fluorescence (PAM: Pulse Amplitude Modulation)*

Samples were tested for photosynthetic efficiency ($F_v/F_m$) by evaluating the chlorophyll fluorescence of the leaf shoot adjacent to the meristem (youngest leaf) using a Diving PAM-II Fluorometer (Heinz Walz, Effeltrich, Germany). A different shoot from each population in each tank was selected so that no leaf was ever measured twice. Plants were tested one hour before the photoperiod started while it was dark-adapted, and cellular respiration occurred. $F_v/F_m$ readings on the PAM were used for statistical analysis.

## Results

Eelgrass performance was assessed using morphology (shoot length and the total number of leaves), growth (new leaf extension), and photosynthetic efficiency ($F_v/F_m$) to decern performance differences between populations. Means summaries were calculated for all treatment level replicates. The data for shoot length and blade count was normalized as these are absolute values. Through this loss, percentages were calculated from the initial measurement/count for each week. A one-tailed T-test was used to see if there was a significant difference between all the possible pairs of the five different populations. All calculations and analyses were done in Microsoft Office Excel.

### *Initial measurements*

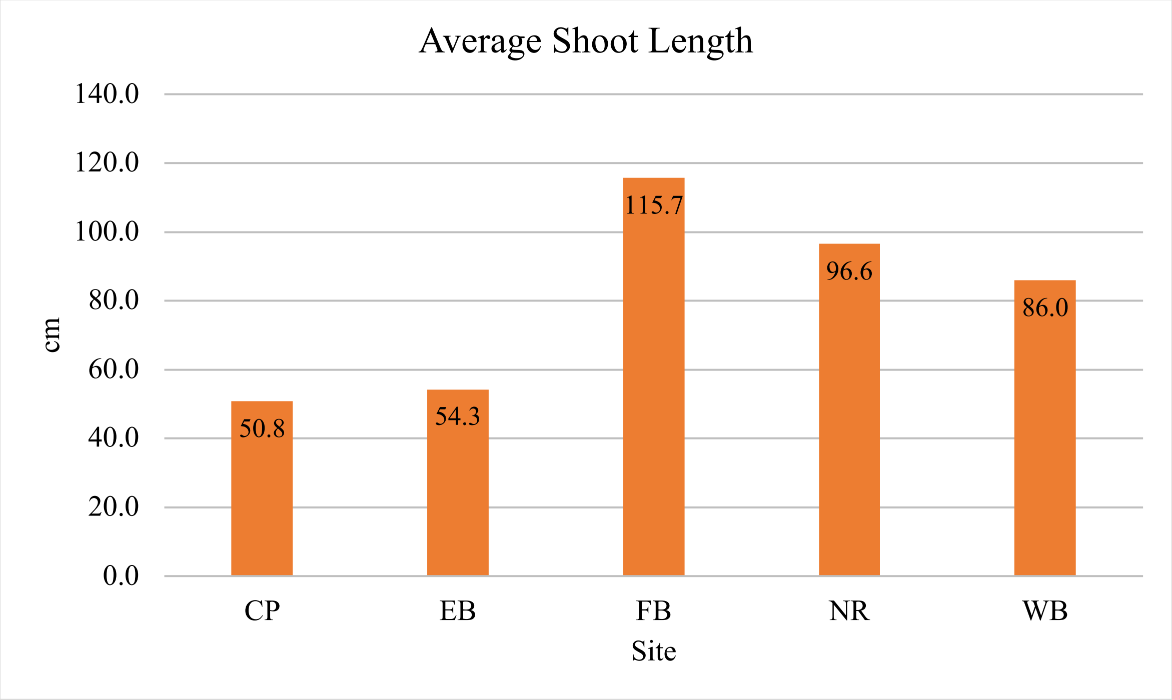

*Figure 7:*
*Mean averages for initial measurement of shoot length for each population. The five sample sites: Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB).*

All measurements were taken on day one to establish a baseline. Fidalgo Bay had the longest average shoot length at 115.7cm followed by Nisqually Reach at 96.6cm, Willapa Bay at 86cm, Elliott Bay at 54.3cm, and the shortest being Cherry Point at 50.8cm.

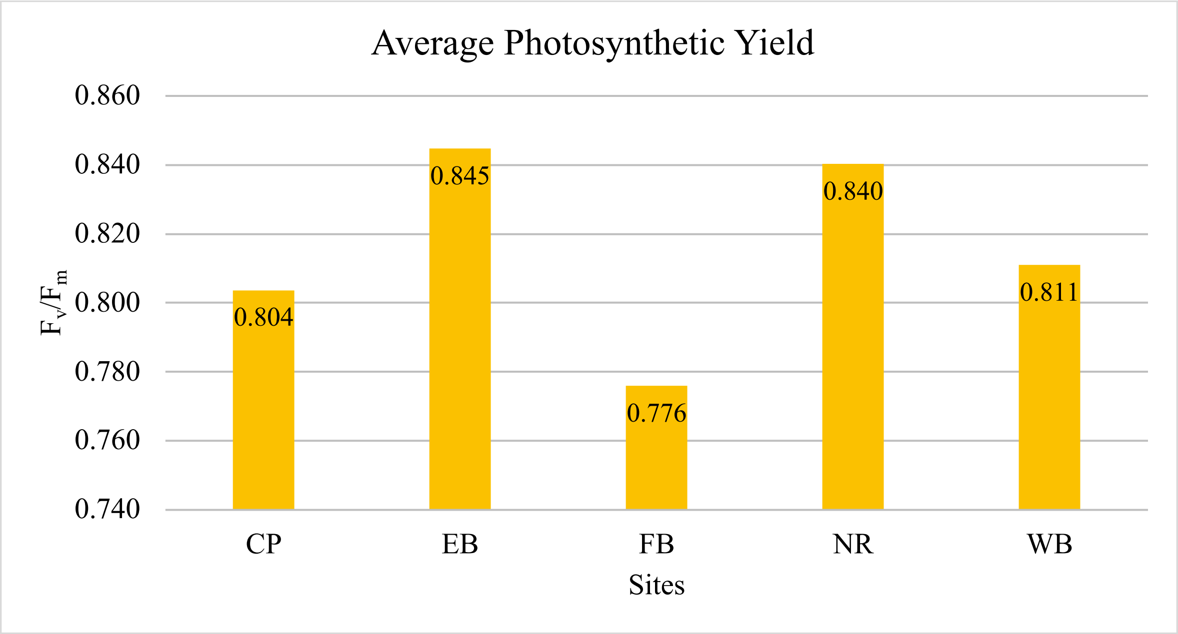

*Figure 8:*
*Mean averages for initial measurement of photosynthetic yield for each population. The five sample sites: Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB).*

Elliott Bay had the highest photosynthetic yield at 0.845 $F_v/F_m$ followed by Nisqually Reach at 0.84 $F_v/F_m$, Willapa Bay at 0.811 $F_v/F_m$, Cherry Point at 0.804 $F_v/F_m$, and the lowest being Fidalgo Bay at 0.776 $F_v/F_m$.

*Table 1:*
*The relative standard deviation of initial measurements for shoot length and photosynthetic yield.*

| | **CP** | **EB** | **FB** | **NR** | **WB** |
|---|---|---|---|---|---|
| **Shoot Length** | 15.1% | 13.9% | 16.8% | 16.1% | 12.2% |
| **Photosynthetic Yield** | 7.4% | 7.1% | 7.7% | 4.7% | 6.1% |

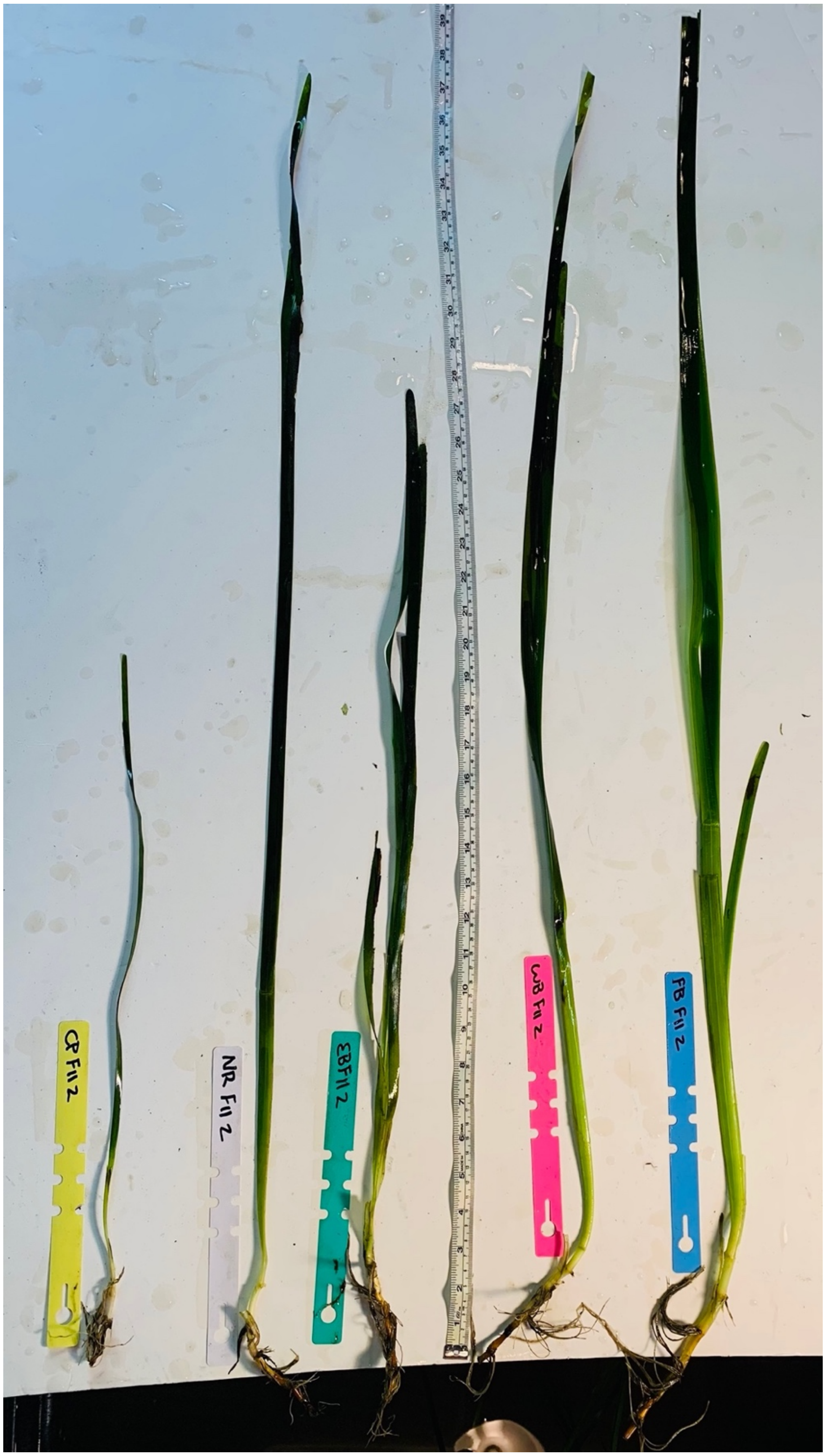

*Figure 9:*
*Morphological differences between the five sample sites: Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB).*

***Tank temperatures***

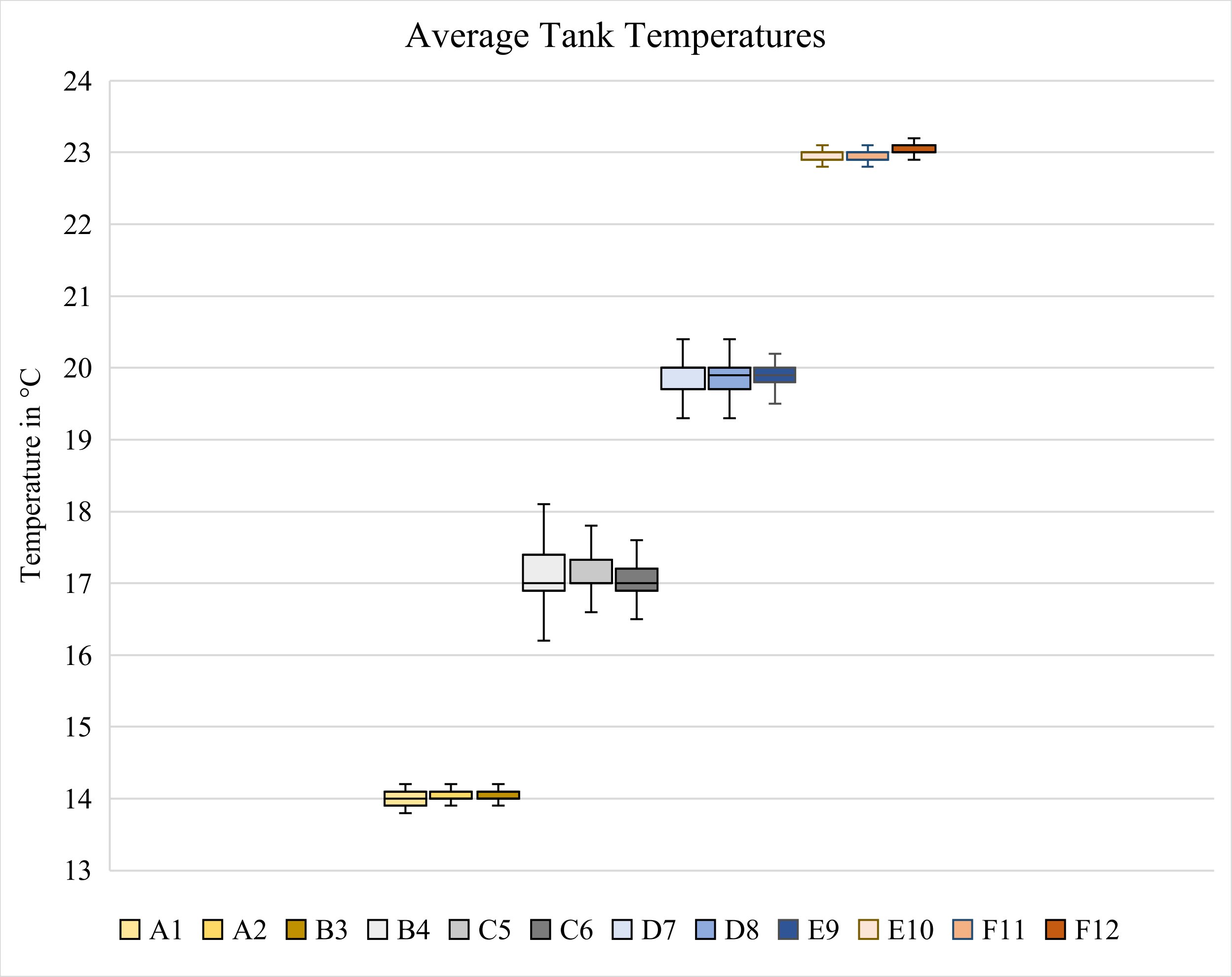

*Figure 10:*
*Average tank temperatures over the course of the experiment.*

*Table 2:*
*The standard deviation of the tank temperatures is in °C.*

| **A1** | **A2** | **B3** | **B4** | **C5** | **C6** | **D7** | **D8** | **E9** | **E10** | **F11** | **F12** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 | 0.1 | 0.2 | 0.6 | 0.7 | 0.6 | 0.8 | 0.7 | 0.7 | 0.1 | 0.2 | 0.2 |

Tank temperature readings were logged every ten minutes over the course of the experiment. The average temperature for each tank was: A1=13.99°C, A2=14.02°C, B3=14.05°C, B4=17.22°C, C5=17.31°C, C6=17.07°C, D7=19.74°C, D8=19.67°C, E9=19.7°C, E10=22.95°C, F11=22.95°C, F12=23.03°C. The average for the three replicates in treatment level 14°C was 14.02°C, for treatment level 17°C it was 17.2°C, for treatment level 20°C it was 19.7°C and for treatment level 23°C it was 22.99°C. On days 18, 19, and 20 there was a spike in temperature due to extremely hot days during which tanks B4, C5, and C6 had more than a degree increase for six hours.

***Shoot length***

*Table 3:*
*P-values for shoot length from one-tailed T-test for each treatment level. Significance was determined with a cutoff of p < 0.05. All significant p-values under that threshold are highlighted in orange.*

| **14°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
|---|---|---|---|---|---|---|
| | **CP** | | | | | |
| | **EB** | 0.019 | | | | |
| | **FB** | 0.056 | 0.459 | | | |
| | **NR** | 0.071 | 0.010 | 0.085 | | |
| | **WB** | 0.040 | 0.465 | 0.485 | 0.033 | |
| **17°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.026 | | | | |
| | **FB** | 0.012 | 0.101 | | | |
| | **NR** | 0.048 | 0.032 | 0.033 | | |
| | **WB** | 0.005 | 0.303 | 0.119 | 0.016 | |
| **20°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.060 | | | | |
| | **FB** | 0.018 | 0.202 | | | |
| | **NR** | 0.067 | 0.079 | 0.034 | | |
| | **WB** | 0.081 | 0.360 | 0.235 | 0.109 | |
| **23°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.178 | | | | |
| | **FB** | 0.178 | 0.178 | | | |
| | **NR** | 0.178 | 0.178 | 0.178 | | |
| | **WB** | 0.178 | 0.178 | 0.178 | 0.178 | |

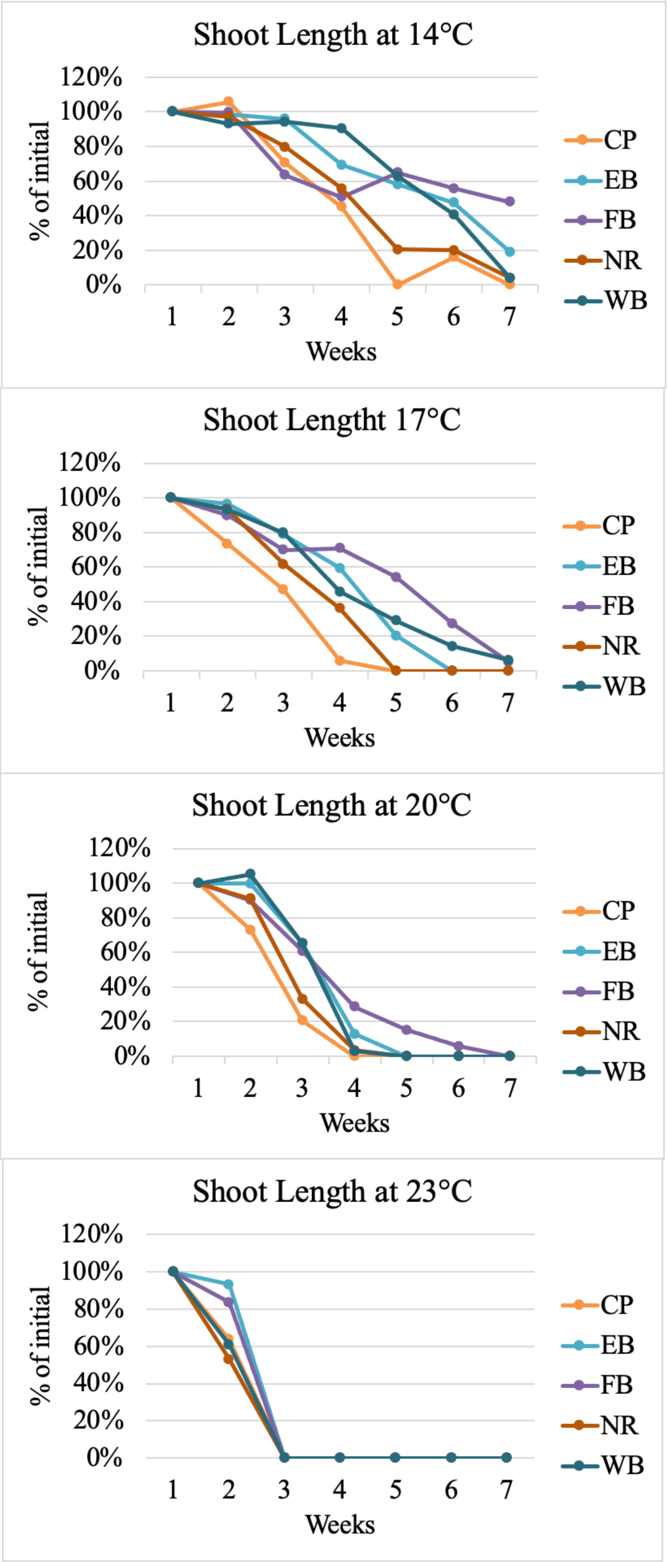

*Figure 11:*
*Comparable changes in shoot length for the different populations at each treatment level over the course of the experiment. The five sample sites: Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB).*

The shoot length of all populations decreased in all treatment levels over the course of the experiment. There was substantial blade loss up to the sheath amongst all populations by week 2 in the 23°C treatment level. Fidalgo Bay had the least percentage loss for treatment level 14°C, and overall slowest blade loss at 17°C and 20°C. There was a significant difference between Elliott Bay and Cherry Point in treatment levels 14°C, 20°C, and 17°C. Cherry Point and Nisqually Reach were significantly different from every other population at 17°C treatment level. There was no significant difference between the populations at 23°C.

**_Blade count_**

*Table 4:*
*P-values for blade count from one-tailed T-test for each treatment level. Significance was determined with a cutoff of p < 0.05. All significant p-values under that threshold are highlighted in orange.*

| | | CP | EB | FB | NR | WB |
|---|---|---|---|---|---|---|
| **14°C** | **CP** | | | | | |
| | **EB** | 0.018 | | | | |
| | **FB** | 0.024 | 0.324 | | | |
| | **NR** | 0.011 | 0.208 | 0.239 | | |
| | **WB** | 0.054 | 0.319 | 0.266 | 0.429 | |
| **17°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.047 | | | | |
| | **FB** | 0.010 | 0.003 | | | |
| | **NR** | 0.085 | 0.037 | 0.007 | | |
| | **WB** | 0.090 | 0.143 | 0.005 | 0.249 | |
| **20°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.364 | | | | |
| | **FB** | 0.288 | 0.235 | | | |
| | **NR** | 0.156 | 0.047 | 0.052 | | |
| | **WB** | 0.393 | 0.496 | 0.377 | 0.160 | |
| **23°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.178 | | | | |
| | **FB** | 0.178 | 0.178 | | | |
| | **NR** | 0.178 | 0.178 | 0.178 | | |
| | **WB** | 0.178 | 0.178 | 0.178 | 0.178 | |

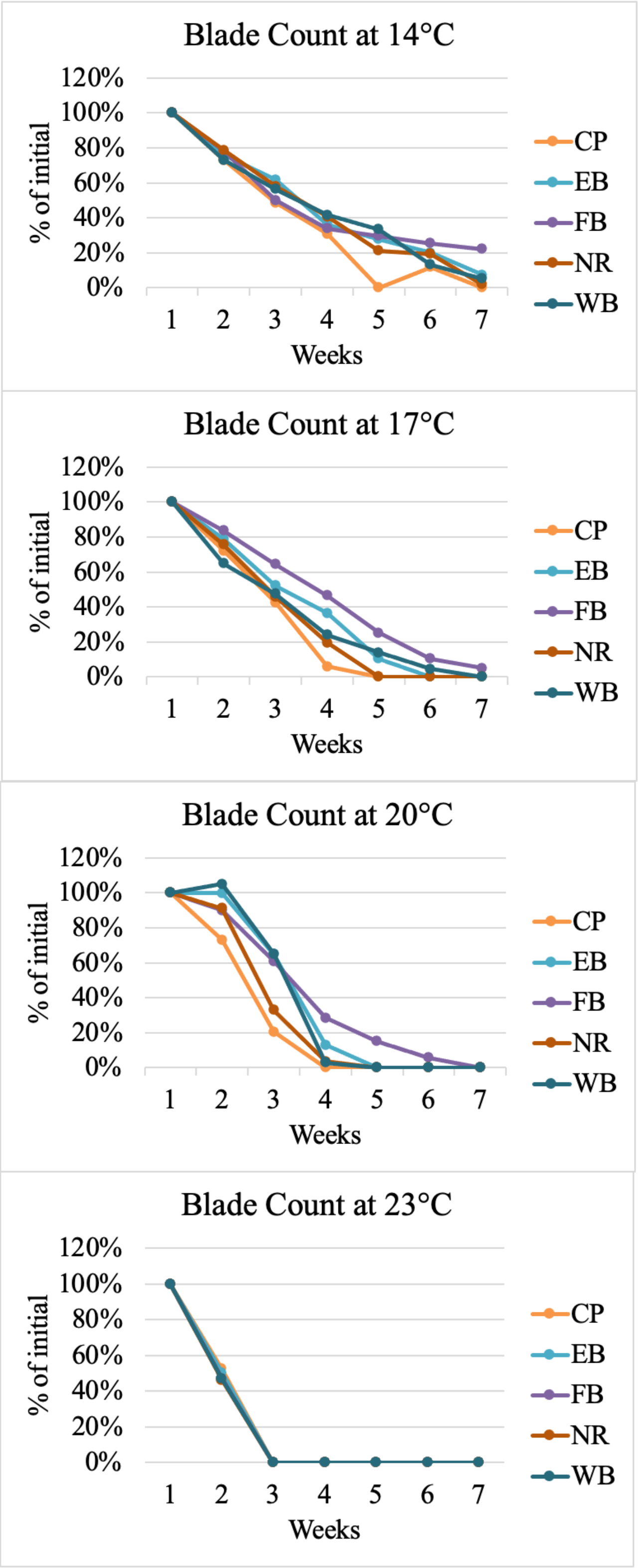

*Figure 12:*
*Comparable changes in blade count for the different populations at each treatment level over the course of the experiment. The five sample sites: Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB).*

Fidalgo Bay had the lowest percentage loss for treatment level 14°C, and overall slowest blade loss at 17°C and 20°C. There was a significant difference between Elliott Bay and Cherry Point in treatment levels 14°C and 17°C. Fidalgo Bay was significantly different from every other population at 17°C treatment level. There was no significant difference between the populations at 23°C.

***Growth***

*Table 5:*
*P-values for growth from one-tailed T-test for each treatment level. Significance was determined with a cutoff of p < 0.05. All significant p-values under that threshold are highlighted in orange.*

| **14°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
|---|---|---|---|---|---|---|
| | **CP** | | | | | |
| | **EB** | 0.009 | | | | |
| | **FB** | 0.000 | 0.001 | | | |
| | **NR** | 0.004 | 0.084 | 0.000 | | |
| | **WB** | 0.016 | 0.076 | 0.067 | 0.156 | |
| **17°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.020 | | | | |
| | **FB** | 0.005 | 0.003 | | | |
| | **NR** | 0.065 | 0.141 | 0.026 | | |
| | **WB** | 0.033 | 0.070 | 0.028 | 0.323 | |
| **20°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.053 | | | | |
| | **FB** | 0.028 | 0.032 | | | |
| | **NR** | 0.112 | 0.279 | 0.023 | | |
| | **WB** | 0.101 | 0.142 | 0.041 | 0.098 | |
| **23°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.182 | | | | |
| | **FB** | 0.182 | 0.182 | | | |
| | **NR** | 0.182 | 0.182 | 0.182 | | |
| | **WB** | 0.053 | 0.182 | 0.182 | 0.182 | |

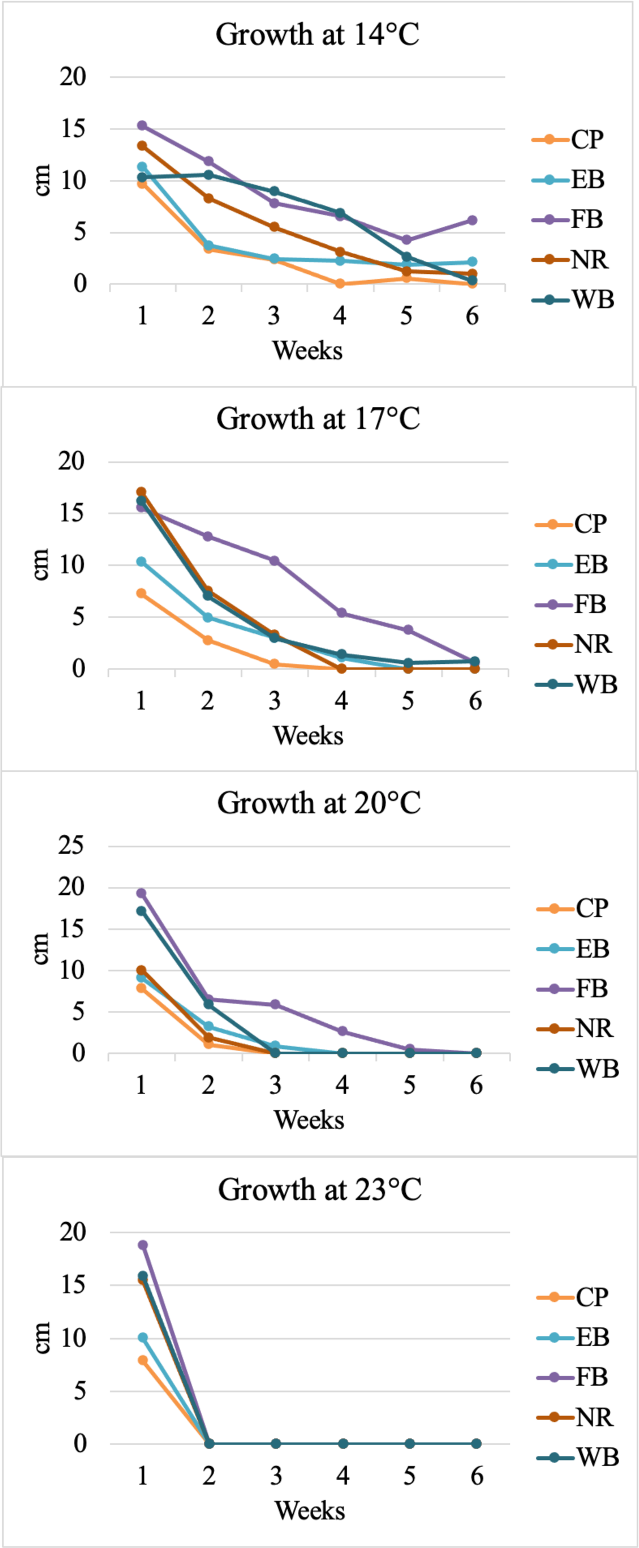

*Figure 13:*
*Comparable changes in growth rate for the different populations at each treatment level over the course of the experiment. The five sample sites: Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB).*

Fidalgo Bay had the most growth for treatment level 14°C whereas Willapa Bay had the most stable growth. Fidalgo Bay was significantly different from every other population at 17°C treatment level. There was no significant difference between the populations at 23°C.

***Photosynthetic yield***

*Table 6:*
*P-values for photosynthetic yield from one-tailed T-test for each treatment level. Significance was determined with a cutoff of $p < 0.05$. All significant p-values under that threshold are highlighted in orange.*

| 14°C | | **CP** | **EB** | **FB** | **NR** | **WB** |
|---|---|---|---|---|---|---|
| | **CP** | | | | | |
| | **EB** | 0.024 | | | | |
| | **FB** | 0.063 | 0.309 | | | |
| | **NR** | 0.275 | 0.026 | 0.065 | | |
| | **WB** | 0.020 | 0.028 | 0.416 | 0.014 | |
| **17°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.003 | | | | |
| | **FB** | 0.010 | 0.165 | | | |
| | **NR** | 0.049 | 0.045 | 0.050 | | |
| | **WB** | 0.011 | 0.137 | 0.430 | 0.020 | |
| **20°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.054 | | | | |
| | **FB** | 0.020 | 0.052 | | | |
| | **NR** | 0.042 | 0.163 | 0.029 | | |
| | **WB** | 0.046 | 0.176 | 0.140 | 0.107 | |
| **23°C** | | **CP** | **EB** | **FB** | **NR** | **WB** |
| | **CP** | | | | | |
| | **EB** | 0.182 | | | | |
| | **FB** | 0.105 | 0.111 | | | |
| | **NR** | 0.182 | 0.182 | 0.096 | | |
| | **WB** | 0.099 | 0.106 | 0.200 | 0.088 | |

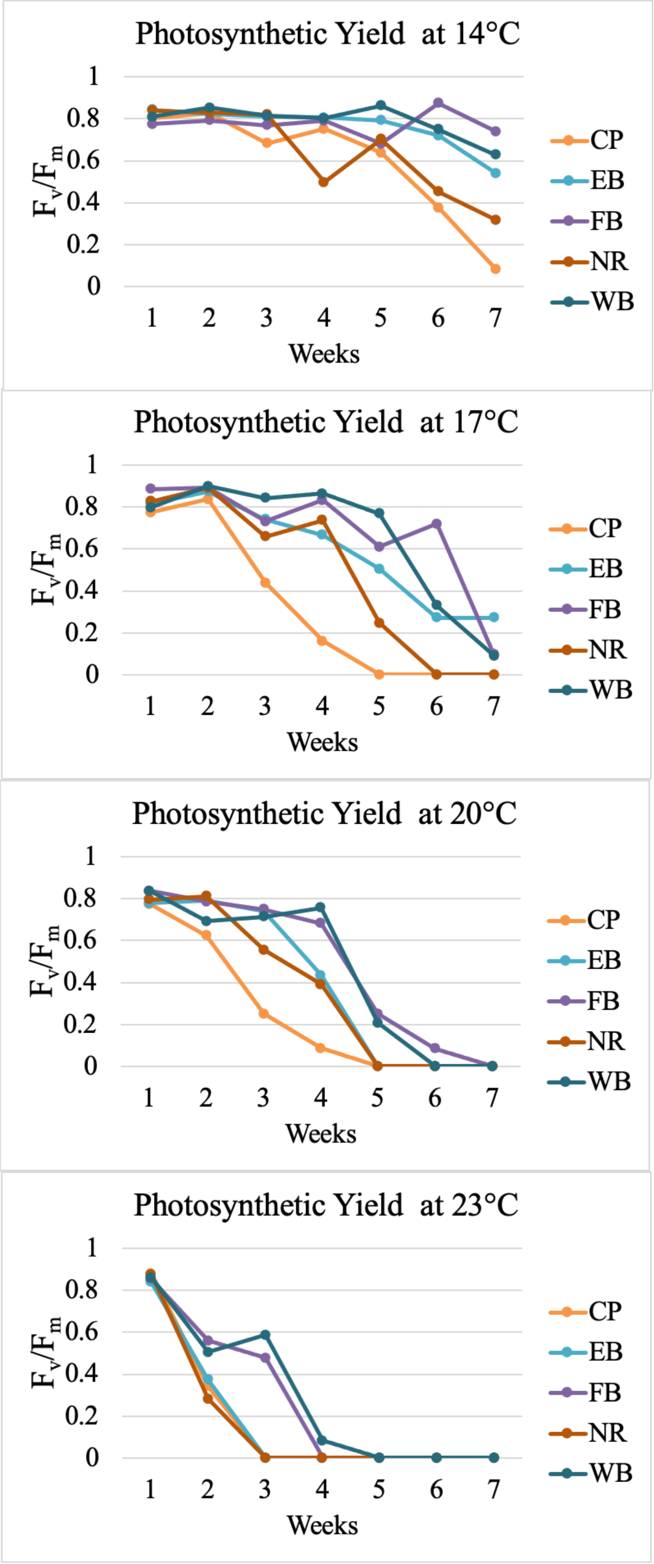

*Figure 14:*
*Comparable changes in photosynthetic yield for the different populations at each treatment level over the course of the experiment. The five sample sites: Cherry Point (CP), Elliott Bay (EB), Fidalgo Bay (FB), Nisqually Reach (NR), and Willapa Bay (WB).*

Fidalgo Bay had the highest photosynthetic yield in treatment level 14°C. Cherry Point and Nisqually Reach were significantly different from every other population in treatment level and 17°C. Cherry Point was significantly different from three other populations in the 20°C treatment level. Fidalgo Bay and Willapa Bay performed better than the other three populations for the first two weeks in treatment level 23°C. There was no significant difference between the populations at 23°C.

## Discussion

This single factor temperature experiment considered four water temperatures spanning the natural upper threshold range of *Z. marina* (Phillips, 1984; Thayer et al., 1984). The goal was to identify the most resilient population. Previous work has shown the optimal temperature range lies between 15° and 23°C, and that temperatures above this were lethal (Lee et al., 2007; Phillips, 1984). In our mesocosms, we exposed five different eelgrass populations to four treatment levels. In general, the highest temperature level (23°C) was lethal to all populations when compared to the lowest treatment level (14°C).

The key finding of this study was that the Fidalgo Bay population was more tolerant to thermal stress than Cherry Point, Elliott Bay, Nisqually Reach, and Willapa Bay. By the end of the experiment, Fidalgo Bay overall had the lowest percentage loss of shoot length (14, 17, 20°C), lowest blade loss (14, 17 and 20°C), most growth (14, 17 and 20°C), and highest photosynthetic yield (14°C). The initial morphological measurements indicate that morphology was possibly connected to eelgrass performance as Fidalgo Bay had the longest blade length, sheath width, and sheath length. Fidalgo Bay outperformed all populations even though it has the coolest daily mean water temperature at 7.41°C (Figure 2) which is 2.94°C cooler than the second coolest site (Cherry Point) and 5.07°C cooler than the hottest site (Willapa Bay).

Willapa Bay performed second-best overall, in the first two weeks, it had a lower percentage loss of shoot length than Fidalgo Bay in 14, 17 and 20°C, slower percentage blade loss than Fidalgo bay in 14°C, more growth than Fidalgo Bay in the first three weeks of 14°C, and higher photosynthetic yield than Fidalgo Bay for the first four weeks in 14°C and 17°C. Cherry Point performed worst in every measurement in all treatment levels.

The photosynthetic yield was more stable for longer when compared to the other performance measurements in 14, 17, and 20°C with Fidalgo Bay and Willapa Bay outperforming the other populations in 23°C for the first two weeks. While photosynthetic yield was stable, other performance measurements were declining.

The study also showed that higher temperatures will significantly affect growth and survival in treatment levels 17°C and higher for all five populations. Changes in eelgrass performance occurred noticeably during week two in all four performance measurements. This indicates that long-duration thermal stress has negative consequences for eelgrass productivity and resilience.

The limitations of this project were that not all natural conditions could be simulated in this artificial environment. The eelgrass was also placed under immense stress once removed from the sediment and keeping the rhizomes exposed during the experiment contributed to the mortality and slow growth. Studies focusing on sediment presence suggest that eelgrass produces more blades and has increased growth rates when rooted in sediment (Biber, 2006). It is therefore suggested that a future study could be done with all shoots grown in sediment. Further studies are required to identify other similar or more resilient populations; therefore, it is also suggested that the same study is done with populations that were not included in this experiment i.e., from the Strait of Georgia, the Canadian coast, and the remaining ANeMoNe sites. Future studies should evaluate light availability and pH or a combination of two factors like light turbidity and temperature. Lastly, it is suggested that a lower temperature threshold be selected as 23°C has shown to be lethal to all populations.

This study corroborates with a study done by Sylvia Yang (personal communication, 2020) that exposed eelgrass from Cherry Point and Fidalgo Bay to three different water temperatures (10°C,13°C, and 21°C). The higher treatment level showed a decrease in performance in evaluating the morphological parameters (shoot length, sheath length, sheath width, total number of leaves, and dry weight) as well as growth rate and mortality. Fidalgo Bay outperformed Cherry Point in every response parameter.

Eelgrass communities will face considerable stresses over the next several decades. As shown by other studies, elevated water temperature is one of the most important negative effects of climate change. When comparing the four treatment levels, the higher temperatures had notable higher mortality which indicates that a change to a higher climate regime will cause higher mortality. However, certain populations appear better adapted to handle higher temperatures. By identifying which populations are more resilient to these increase water temperatures, it can assist resource managers to develop effective mitigation and restoration strategies. One such way would be to use the most resilient population as a donor site for future restoration purposes. This also prevents the disruption of less resilient population sites that are more vulnerable and sensitive. There are ethical questions that arise from using one population to restore another as each population is genetically different and could have irreparable effects if not conducted correctly. This dilemma might also be overlooked due to the urgency of the problem and the survival of the species. Implications of the loss of species have ramifications for the organisms that depend on it.

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