Meltdown in Algae Town:

Implications of Warming Oceans on San Juan Island *Ulva*

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ABSTRACT

The green alga, *Ulva* spp., represents a widely distributed and ecologically influential genus of macroalgae that occurs in subtidal and intertidal habitats worldwide. A majority of *Ulva* spp. have high nutrient uptake rates and rapid growth in nutrient-rich habitats, forming prolific algal blooms known as green tides. The increasing frequency and severity of green tide events has been attributed to coastal anthropogenic eutrophication, particularly as it relates to nitrogen. Previous studies have indicated that high-temperature conditions may affect nitrogen metabolism in *Ulva*, but further research is needed to understand the impacts and associations between temperature and eutrophication on *Ulva* growth. The aim of this study was to gain insight into the implications of climate change and consequent increasing ocean temperatures on the proliferation of *Ulva* and potential for harmful green tide events. Effects of temperature on *Ulva* were investigated by selecting one individual alga at each of three San Juan Island locations (Eagle Cove, False Bay, and Friday Harbor Laboratories). A total of 9 *Ulva* discs (314 mm^2) were taken from each individual representative to place into three temperature treatments (12ºC-14ºC, 17ºC-19ºC, and 23ºC-25ºC). Growth of discs was recorded three times during the experimental period. No significant difference in temperature-dependent growth was found based on site. However, the high temperature treatment was found to result in negative growth independent of site. Future experiments should include a larger sample size, investigation of additional abiotic factors and a longer experimental timeframe.

INTRODUCTION

Projections indicate that by year 2080, global sea surface temperatures will increase between 1.3°C and 2.7°C, accompanied by rising carbon dioxide (CO_2) levels and ocean acidification (Jewett and Romanou 2017). As temperatures increase in direct correlation with anthropogenic-induced climate change, warming oceans will generate cascading impacts to marine ecosystem functioning and the species that belong to this bionetwork. Marine flora in particular operate in a vital role as primary producers of the food web and have unique vulnerabilities to climatic changes (Jewett and Romanou 2017).

Ulva (Phylum Chlorophyta, Ulvophyceae, Ulvales), commonly known as “sea lettuce”, is a prevalent alga distributed among mid to low intertidal zones in the northwest Pacific Ocean (Kim et al. 2004). Seasonality determines *Ulva* abundance; generally, in the winter months, this genus can be found in the mid intertidal zone and during the warmer months, *Ulva* grows in low exposure areas within the lower intertidal zone to protect itself from higher temperatures (Harvey 1846–1851). Although a total of 85 *Ulva* species have been taxonomically accepted, morphological identification is difficult (Guiry 2021; Hughey et al. 2021). *Ulva* phenotypic plasticity obscures species classification and often leads to taxonomical errors, therefore further molecular analysis is necessary in order to fully comprehend the range of species (Kang 2019). *Ulva* thalli can be a distromatic sheet-like blade or composed of hollow cylinders. Thallus structure is reliant on surface bacteria production of the morphogenetic reducer, thallusin (Matsuo et al. 2005). Asexual reproduction occurs at the margins of the thallus, and every spring new blades grow from the perennial holdfast allowing for *Ulva* biomass to accumulate without the loss of adult tissue (Lüning et al. 2008; Graham 2016).

Ulva is an opportunistic macroalga capable of high nutrient uptake and storage and swift growth rates (Luo et al. 2012). Previous studies found a positive correlation between temperature and levels of metabolic activity, nutrient production and consequently growth in *Ulva* spp. Increasing temperatures also resulted in higher zoospore yields in *Ulva compressa* and germination rates increased in *Ulva fasciata* (Gao et al. 2017). In addition to increasing temperatures stimulating macroalgal growth, excess nutrient loading from human activities results in high nitrogen levels in the ocean (Gao et al. 2017). The intertidal macroalgal community has high assimilative capacity for nutrients and acts as nutrient sinks, allowing rapid algal growth (Tang and Gobbler 2011). In coastal regions, excess nutrient loading causes eutrophication, which results in macroalgae blooms or green tide events.

When this alga dominates coastal regions during these events, other species become smothered by the canopy of *Ulva* (Gao et al. 2017). These smothering *Ulva* mats can cause ecological harm to other species. For example, *Ulva lactuca* is found to be toxic to larval winter flounder and adult barnacles, *Balanus balanoides* (Sogard and Able 1991). Elevated nutrient supply by eutrophication favors the growth of bloom-forming annual algae with high nutrient-uptake such as *Ulva* and can result in the displacement of canopy-forming perennial algae, including fucoids and laminarians (Worm and Lotze 2006). This displacement can lead to disruptions in habitat formation and diminished ecosystem functions, such as carbon storage and nitrogen retention (Worm and Lotze 2006).

Through selective feeding on early life-history stages of *Ulva*, grazers such as littorinid snails, isopods, and amphipods may be able to reduce or even prevent algal blooms, thereby enabling higher biodiversity among coastal rocky shore communities (Worm and Lotze 2006). However, with excessive nutrient loads from increasing coastal eutrophication, studies such as

Worm and Lotze (2006) have indicated that the role of grazers as mitigators of algal blooms will be insufficient in overriding the effects of bloom-forming annual algae on ecosystem functions and community diversity. The presence of *Ulva* can be beneficial for some local fauna however, contributing to primary production used by higher trophic levels. In the northeastern Pacific (Puget Sound region), macroalgal grazers include snails, urchins and chitons (Nelson and Waaland 1997). Formation of Ulva mats provide habitation for shrimp, crabs, and small fish (Mackenzie 2000). These canopies provide shelter from predation for certain species and serve as a nursery habitat for fish species such as the blackfish, *Tautoga onitis* (Sogard and Able 1991). The green algal pigmentation also provides camouflage benefits for juvenile blackfish (Sogard and Able 1991).

The interactive effects of marine heatwaves (MHW) and eutrophication to the ecophysiology of intertidal macroalgae such as *Ulva* have been linked to more frequent green tide occurrences caused by extreme temperature, resulting in high nutrient accumulation in the water (Gouvêa et al. 2017). Long periods of extreme hot weather events can affect the growth and life cycles of macroalgae to the point where their survival is threatened. When threatened, it can lead to an irreversible change to the ecosystem by reducing primary productivity in coastal waters (Gao et al. 2019). However, short-term stress caused by increased temperature and high levels of photosynthetic active radiation (PAR) and ultraviolet radiation (UVR) does not affect some species of *Ulva* because of the high nutrient supply available to the alga (Figueroa et al. 2009). Kakinuma et al. (2006) state that as global temperature continues to rise, intertidal macroalgae such as *Ulva* will take advantage of the physiological and biochemical changes to help them acclimate to fluctuating temperatures and salinities that will lead to more frequent blooms. Therefore, further research investigating these factors is needed in order to adequately

address the implications of physiological responses of *Ulva* to these imminent climate events. Forecasting physiological changes to macroalgae as a result of rising temperatures may provide insight into potential community shifts and ecosystem resilience methods in preparation for extreme climate change. Natural resource managers could use these trends to inform decisions relevant to species dependence on these algae for food, desiccation refuge, and predator avoidance.

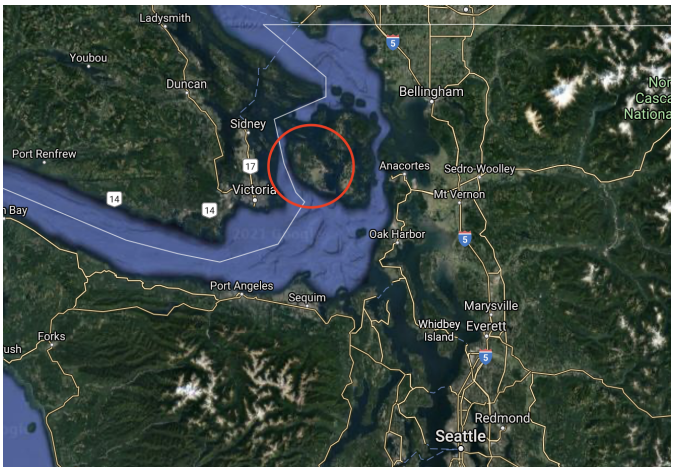
We hypothesized that increasing temperatures would result in favorable growth for *Ulva* sp. until it reached maximum growth rate (Gao et al. 2017). Based on the findings of Gao et al. (2017), which assessed the effects of two temperature regimes (14°C and 18°C) and found both to be within *Ulva*'s thermal niche, our study sought to expand on the results of their research by adding a third temperature regime (23ºC - 25ºC) much higher than their previously documented thermal niche. Based on these temperature regimes, we hypothesized that *Ulva* sp. collected from the subtidal site (FHL) would experience the least growth in higher temperature ranges compared to specimens collected from the intertidal sites (Eagle Cove and False Bay). This is because the intertidal sites experience a wider temperature range compared to the subtidal site.

MATERIALS & METHODS

Study Sites and Specimen Collection

We investigated how varying temperature regimes would impact the growth of *Ulva* from one subtidal and two intertidal environments in the San Juan Islands. The collection sites were chosen for their distinctive temperature differences, which could indicate unique growth conditions for the *Ulva* sp. that settle there. The three sites chosen were the subtidal habitat at

Friday Harbor Laboratories (FHL) and the intertidal habitats at False Bay and Eagle Cove, all of which are located on San Juan Island in the Pacific Northwest (Fig. 1, Table 1). *Ulva* collected from FHL was obtained on June 26th, 2021, from a dock tire on the unexposed (sheltered) side of the dock (Fig. 2A), facing the labs. In this location, *Ulva* is found in what is essentially a subtidal zone, which experiences a water temperature range from 12°C - 15°C and a salinity range from 26 - 31 ppt (Fig. 2C; Sato et al. 2020). False Bay is an intertidal environment which experiences extreme tidal transitions throughout the day with low tides exposing shallow tide pools, tidal channels, sandbars, and mud flats. *Ulva* was found growing on rocky substrates as well as freely floating throughout the mudflats, covering much of the exposed bay when the collections were made. Temperatures of the standing tide pools reach up to 30°C during the midday hours of low tide. During high tide, seawater floods the bay and temperatures can drop to 10°C (Fritz et al. 2000). We collected *Ulva* from False Bay during low tide and high exposure on June 26th, 2021 (Fig. 2C). Eagle Cove (12ºC - 15ºC) is located on the southwest shore of San Juan Island and consists of an expansive sandy beach with several regions of rocky intertidal habitats along the margins of the sandy shoreline. The rocky intertidal zones are composed of patches of sand and loose cobble, featuring a large rocky promontory and several sizable rocky substrates dispersed throughout the sand and cobble. At time of collection, *Ulva* was primarily found growing on rocky substrates in low to mid intertidal zones and was collected from this site during low tide on June 26th, 2021 (Fig. 2B). By morphological identification (Gabrielson and Lindstrom 2018) we identified all samples to match *Ulva lactuca*.

A. 

B. 

Figure 1. A. San Juan Island location in the Salish Sea. **B.** Collection sites on San Juan Island. The rectangle represents Friday Harbor Lab, the circle represents False Bay, and the hexagon represents Eagle Cove. (Screenshots taken from Google Maps)

A.

B.

C.

Figure 2. Specific site location information represented by red circles. **A.** Friday Harbor Lab. **B.** Eagle Cove. **C.** False Bay. (Screenshots taken from Google Maps)

Samples collected at all three sites were held inside Lab 4 in water tables supplied with ambient seawater from Friday Harbor for three days to acclimate prior to processing. Both temperature (Taylor®, Item # 9842FDA) and salinity (VeeGee®, Catalog # 43036) were taken on July 8th to reflect an accurate tidal range data as per Sato et al. (2020) since specimens were collected during an unusual heat wave at the San Juan Islands. The tidal height was -0.38 m (Friday Harbor Labs), -0.42 m (False Bay), and -0.11 m (Eagle Cove) at time of temperature and salinity data collection (Table 1).

Table 1. Collection Site Information (salinity and temperature at collection time). Please note that the same blade was used for each site.

Site	Latitude	Longitude	Air Temperature (ºC)	Water Temperature (ºC)	Salinity ‰	n
Friday Harbor Labs (FHL)	48°32'42.96"N	123° 0'43.20"W	17.2	11.5	30	3
False Bay	48°29'21.14"N	123° 4'4.27"W	13.9	17.8	30	3
Eagle Cove	48°27'40.00"N	123° 1'52.10"W	15.0	14.3	30	3

Experimental Set-Up

This experiment was performed in water tables F, C, and D between Lab 4 and Lab 5 at FHL. Two immersible aquarium water heaters (300W and 400W) per table were used to warm the seawater of the two water tables to median (17ºC - 19ºC) in tank D and high (23ºC - 25ºC) in tank C respectively. The third experimental water table F remained at ambient flow temperature (low, 12ºC - 14ºC).

The thalli of three *Ulva* (one per site) were cut into 2 cm diameter discs using a hollow hole punch tool. Discs were taken from the same blade for each site to determine plasticity

within an individual with varying temperature regimes. A voucher was prepared for specimens from each site after discs were cut (n=3; Fig. 3).

Figure 3. Vouchers for Friday Harbor Labs (left), False Bay (middle), and Eagle Cove (right). A represents hole and B represents disc.

Based on the methods of Wang et al. (2007), each disc was placed into an individual 50 mm petri dish (Sterilin™, Catalog # 502014-07P) and acclimated in ambient control conditions for 24 hours prior to experimental temperature treatments. A total of 12 holes (0.140 inch drill bit) were added on the lid and base of each petri dish to allow for sufficient water exchange (Fig. 4) during acclimation. Petri dishes were secured with one rubber band each to prevent loss of algal discs. A total of 27 acclimation chambers (n=9 per site) were placed into three separate water tables in Lab 4 for 24 hours prior to being exposed to the three temperature treatments.

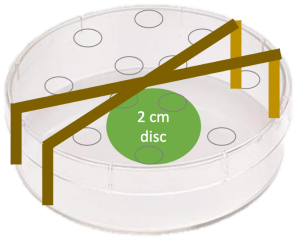


Figure 4. Acclimation chamber for each alga. Brown lines indicate a rubber band.

After the 24-hour acclimation period, each disc was placed inside an individual, open Tupperware® container (708 ml capacity) with one aquarium air stone (2.2 cm height x 1.5 cm diameter) for water circulation to keep each alga in suspension. A total of 700 ml of fresh seawater from the seawater system spigot was added to each container. Three containers for each site were placed into each of the three water tables. One temperature logger (HOBOware®, Part # UA-002-64) was placed into each of the three water tables past the heaters in front of the experimental Tupperware® container (700 ml) with an airstone to simulate the same environmental regime as experienced by the experimental containers for the duration of the experiment. A rock (rinsed in seawater) was added to each tupperware container to prevent containers from floating in the water baths. A total immersion alcohol thermometer (Enviro-Safe®, Item # 1017419) was placed in the mid-section of each tank to enable daily temperature checks every morning at 8:00 PST for the duration of the experiment. We used shade cloth and an E-Z UP® to cover the three water baths (light measured with LI-250A Model: Quantum) to control the amount of photosynthetically active radiation (PAR) in the water tables. Light levels were adjusted to about 300 $\mu mol/m^{-2}/sec^{-1}$ photons, about 70% of direct sunlight. In the event of water temperature deviation, we increased shade, heat, or replenished containers with fresh seawater that was either heated or unheated and supplied from the dockside at FHL (Fig. 5). Each water table contained a 5L carboy (Nalgene®**,** Item **#:** 73086) with the respective temperature to facilitate water changes.

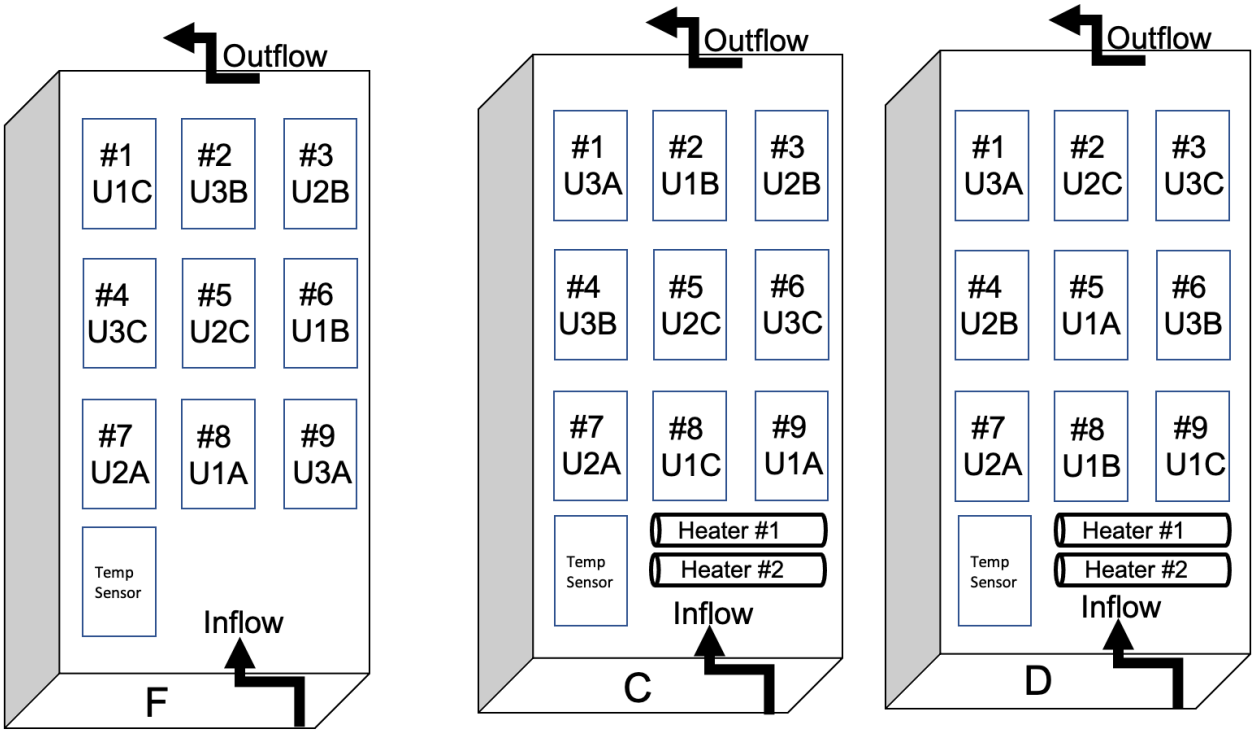


Figure 5. Basic water table set-up. Airstones not shown in diagram. U= Ulva, 1-3= Site ID, A-C= Disc ID

Data Collection

For the duration of the experiment, we measured temperature (Taylor®, Item # 9842FDA) and salinity (VeeGee®, Catalog # 43036) in every container at 17:00 PST every day. On July 2nd, 4th, and 6th, discs were removed from the water tables and placed on a HDPE sheet with a metric ruler and unique identifier next to each alga (n=27). *Ulva* disc photos were taken using an iPhone 12. Photos were imported into ImageJ (Schneider et al. 2012) and were converted into an 8-bit image. Growth was quantified in mm^2 using the "analyze particles" function. To increase contrast, any tissue loss was outlined in white using PowerPoint prior to being processed in ImageJ (Fig. 6). Before returning each *Ulva* replicate to its designated location, 350 ml of seawater was replaced with fresh seawater from the carboys in the water tables in every container including the three temperature logger containers. Salinity was

measured using a refractometer (VeeGee®, Catalog # 43036) every day for the entirety of the experiment which ran for a total of six days.

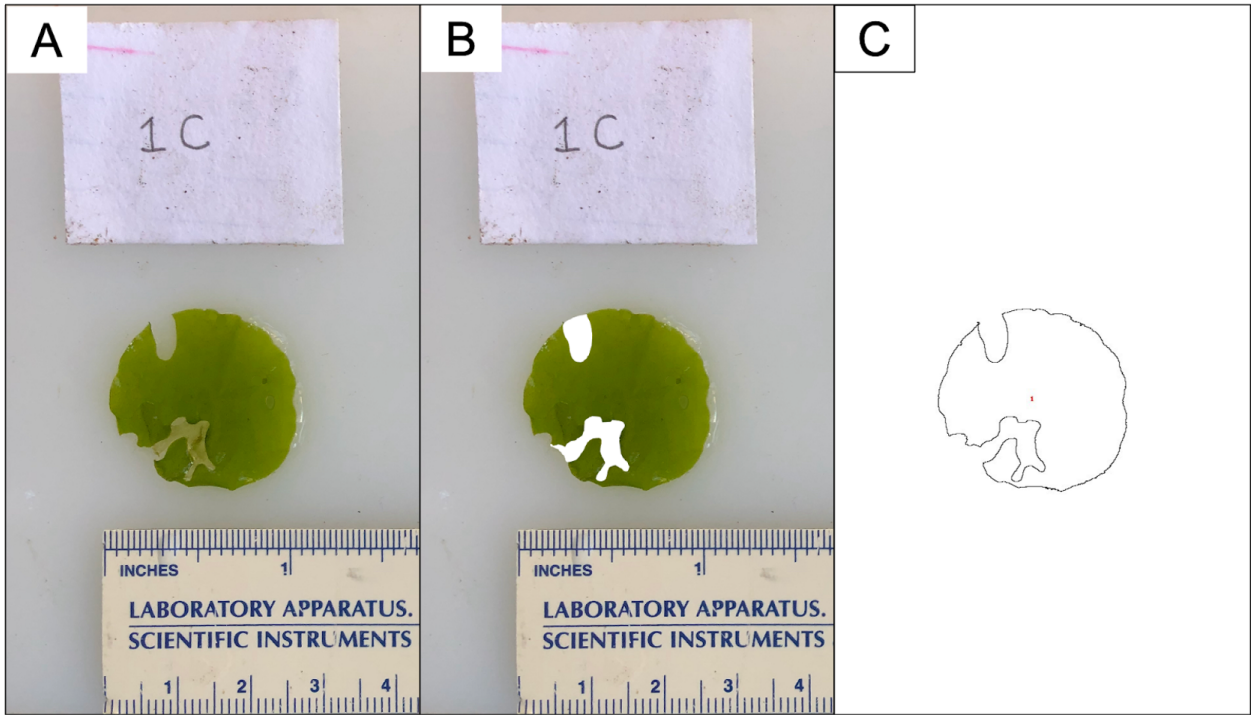


Figure 6. ImageJ processing of one sample. A= Original image, B= Modified image with higher contrast, C= Area calculation results in ImageJ.

Data Analyses

Growth rate as measured in change of thallus surface area (dependent variable) elucidated as to how climatic changes with respect to temperature and site (independent variables) could affect organisms in the genus *Ulva* on San Juan Island. Mean growth rates were compared for the three temperature regimes using a Two-way ANOVA in RStudio (R Core Team 2021). Before running a Two-way ANOVA, the data was tested for normality using a Q - Q plot and Shapiro-Wilk test (Shapiro and Wilk 1965) and for equality of variances using Levene's test (Levene 1960). After a significant Two-way ANOVA, multiple comparisons were assessed using a Tukey post-hoc test in order to determine which site-specific and temperature treatment groups had

significant effects on *Ulva* growth (Tukey 1949). Readouts from the temperature loggers (HOBOware®) and salinity (VeeGee®, Catalog # 43036) measurements were used to further identify any correlation between temperature and growth rates.

RESULTS

Effects of Temperature on Ulva Growth

Results showed that temperature treatment had no effect on growth per site (ANOVA, $F_{4, 72}$=0.715, p= 0.585; Fig. 9). However, temperature did show an overall effect on *Ulva* growth irrespective of site (ANOVA, $F_{2, 72}$=13.904, p= 7.84^{-06}; Fig. 11). The high temperature (23ºC - 25ºC) treatment had a statistically significant negative effect on growth across all sites (Fig. 9). Data were analyzed for normal distribution using a Q - Q plot, which showed an abnormal distribution at the tails of the quantiles. This result was confirmed using Shapiro-Wilk's test, which indicated that the sample distribution for temperature-dependent growth was statistically significantly different from the normal distribution (W=0.92132, p=9.957^{-05}). Homogeneity of variances was assessed using Levene's test, which found no statistically significant difference in variances between temperature treatment groups (Levene's test, $F_{8,72}$=1.3974, p=0.2124). Having met the assumption for equality of variances, Two-way ANOVA and Tukey post-hoc tests were interpreted. Throughout the experiment, salinity and temperature were held relatively constant

and temperature treatments remained different from one another (Fig. 7; Fig. 8).

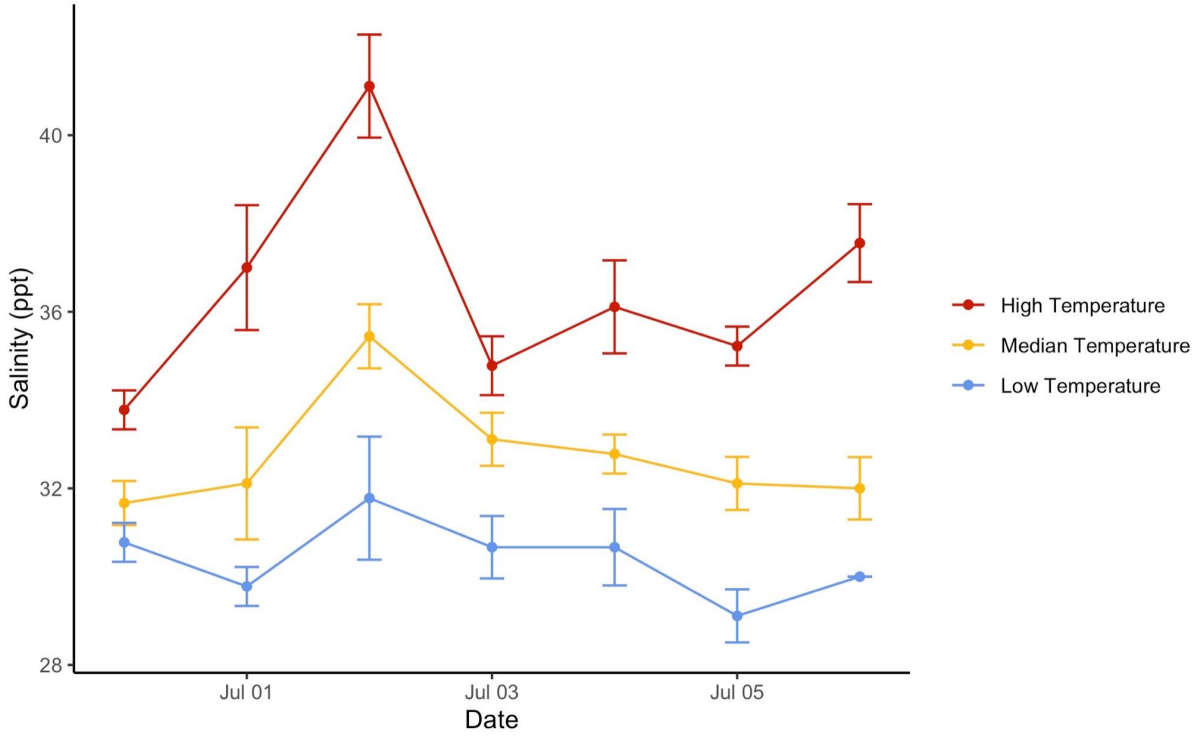


Figure 7. Average (±SD) salinity measurements per treatment over time (high temperature= 36.5 ± 2.4 ppt, median temperature= 32.7 ± 1.3 ppt , low temperature= 30.4 ± 0.9 ppt).

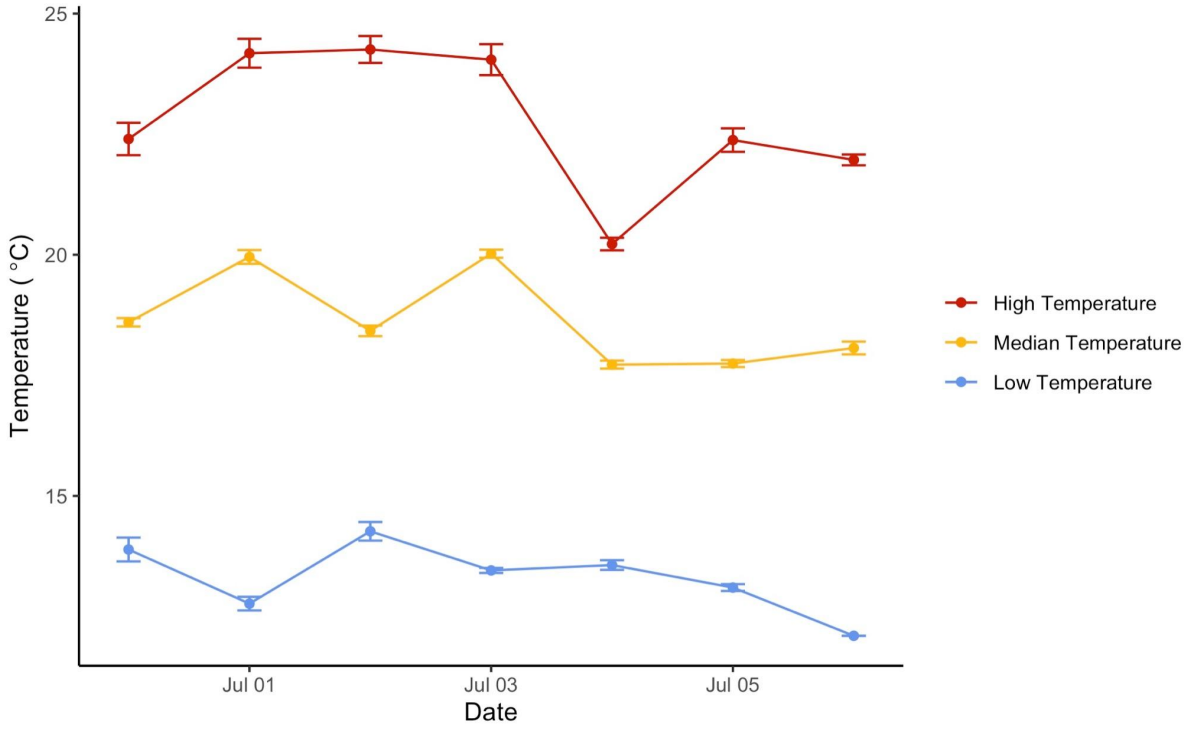


Figure 8. Average (±SD) temperature measurements per treatment over time (high temperature= 22.8 ± 1.5ºC, median temperature= 18.6 ± 1.0ºC, low temperature= 13.3 ± 0.7ºC).

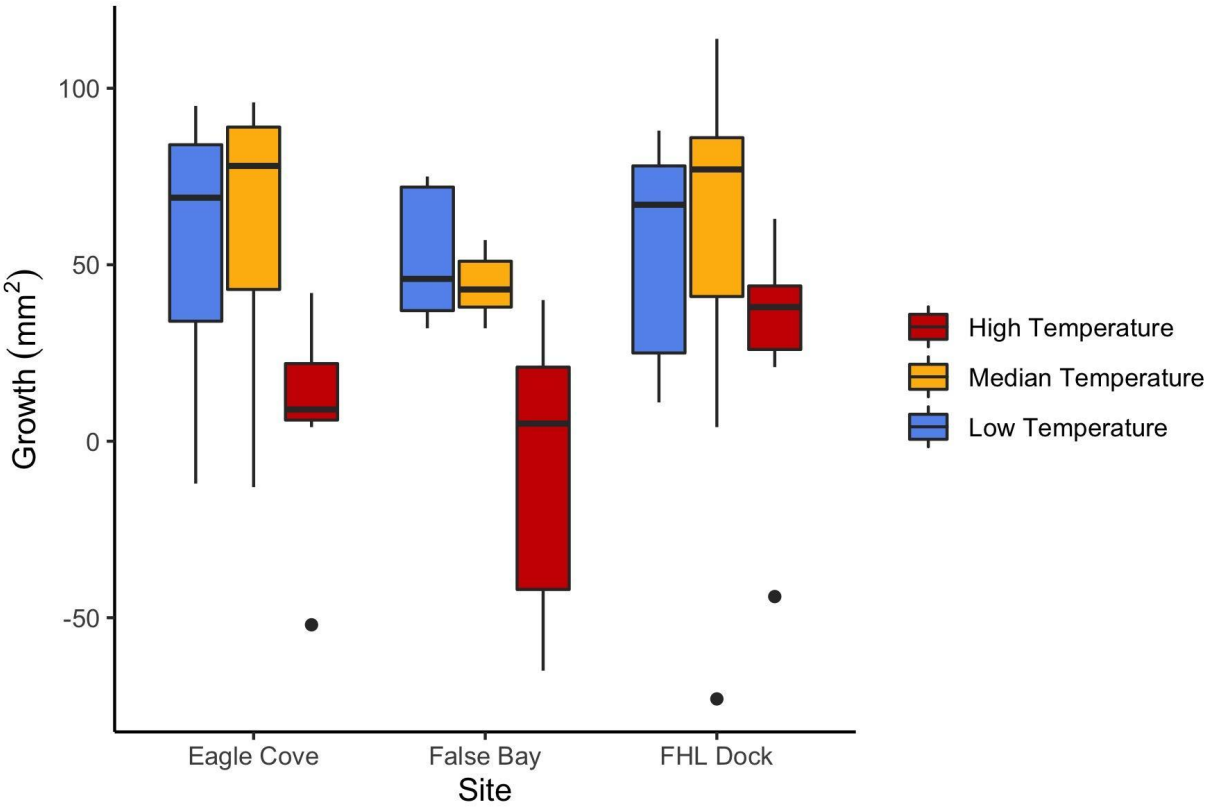


Figure 9. *Ulva* growth changes (over 3 sampling days) for high, median, and low temperatures (n=9 with 3 replicates per time point (n=3) and site). Boxes represent quartiles. The horizontal line in each box indicates the median of the site data. Black dots represent outliers.

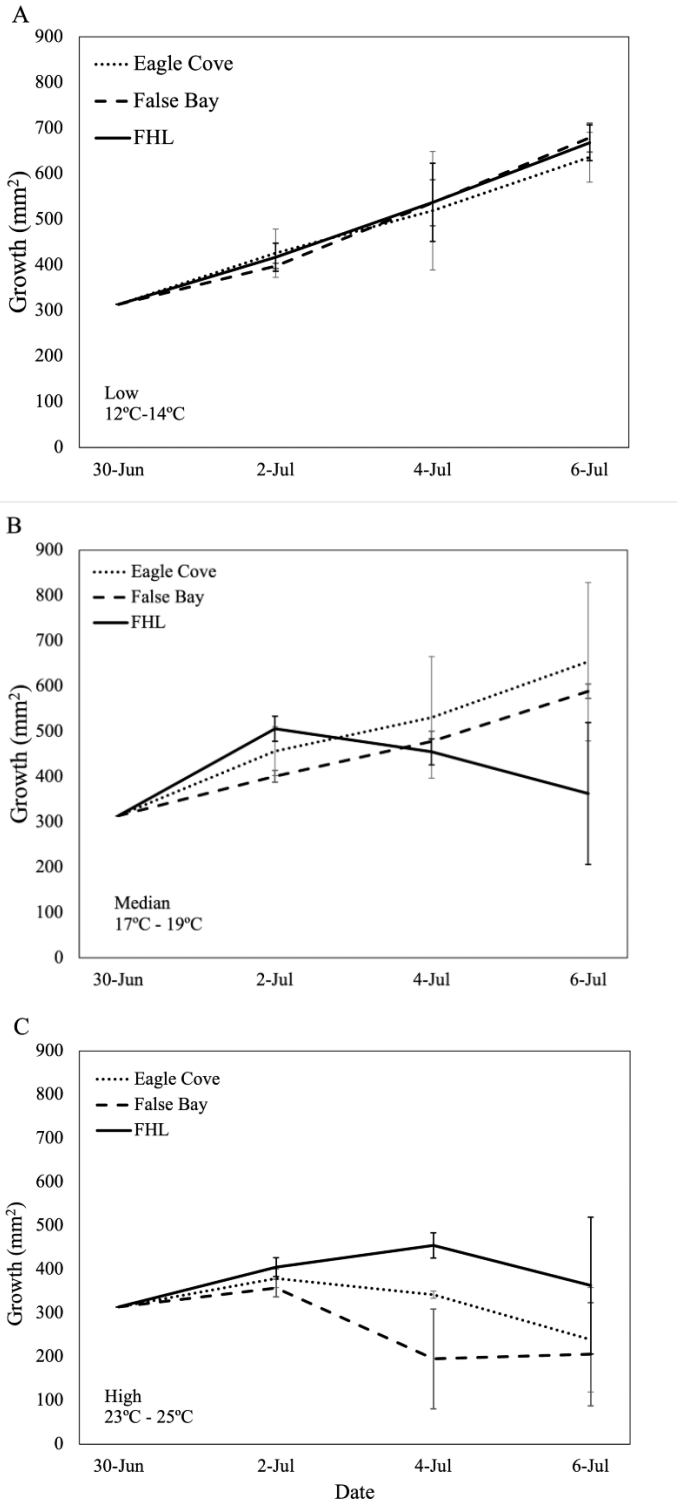


Figure 10. Site specific (n=3) growth responses for each temperature treatment (n=3) over time. A= low temperature (12ºC - 14ºC), B= median temperature (17ºC - 19ºC), and C= high temperature (23ºC - 25ºC). Bars represent standard error which was used to see how precisely the samples would represent the population.

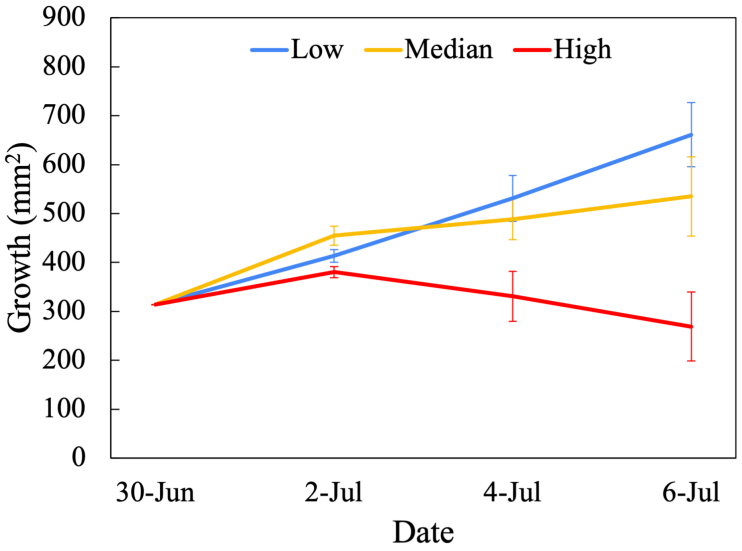


Figure 11. Growth responses as indicated by change in surface area of discs for all sites pooled in each temperature treatment over time. Low= 12ºC - 14ºC, Median= 17ºC - 19ºC, and High= 23ºC - 25ºC. Bars represent standard error which was used to see how precisely the pooled site data would represent the population.

DISCUSSION

The results of our analyses suggested that there was a statistically significant detriment to *Ulva* growth in the high temperature (23ºC - 25ºC) treatment relative to both the low (12ºC - 14ºC) and median temperature (17ºC - 19ºC) treatments across all collection sites. Evaluations comparing the effects of these temperatures on *Ulva* growth between the three collection sites revealed no statistically significant relationships. These findings indicate that while *Ulva* growth was affected by temperature beyond a certain threshold, the specific sites from which samples were collected had no apparent influence on growth. We hypothesized that increasing temperature would result in favorable growth for *Ulva* until a maximum growth rate was reached, which was inconsistent with our overall findings. Results indicated a trend of increasing

growth across all sites for the low temperature treatment (Fig. 10A). For the median temperature treatment, specimens from both Eagle Cove and False Bay continued to show this trend of increasing growth, which supported our hypothesis, while FHL demonstrated a negative trend in growth (Fig. 10B). For the high temperature treatment, specimens from each site showed an overall negative trend in growth except for FHL, which exhibited increasing growth until July 4 (Fig. 10C). The trends in growth were not found to be statistically significant between the low and median temperature treatment, however, the experiment was limited by only testing three temperature treatments (Fig. 11). With regards to temperature treatments and site-specific growth differences, we hypothesized that *Ulva* collected from the high-exposure intertidal site, False Bay, would show the greatest tolerance to high temperature and therefore exhibit the most growth, followed by medium-exposure intertidal site, Eagle Cove, with median growth, and the low-exposure subtidal FHL dock with the least growth.

Results of Q - Q plot analysis and subsequent Shapiro-Wilk's test showed that the distribution of the data violated the assumption of normality, suggesting that the statistically significant findings of the Two-way ANOVA and Tukey HSD post-hoc tests from the sample data could not reasonably be generalized to the population. We suggest that future experiments should be conducted with a larger sample size and for a longer period of time. Other factors that could have influenced these results may have been related to using the same thallus for the disc replicates for each site (Hurlbert 1984). Our hypotheses were inconsistent with our results, which determined the collection site to have no significant influence on temperature tolerances and growth of *Ulva* (Fig.9; Fig.10A,10B).

On account of finding no significant relationship between temperature tolerance and growth of *Ulva* among the three collection sites, possible weaknesses in our study have been

considered. The purpose of comparing temperature-dependent growth between three sites was to evaluate the potential for *Ulva* to exhibit different tolerances to environmental stressors, as they are considered to exhibit considerable phenotypic plasticity (Hayden and Waaland 2004). Previous studies suggest that oxygen saturation may negatively affect growth in green algae (Raso et al. 2012). Each of our experimental units had an airstone, which could have saturated the water and negatively affected *Ulva* growth. We suggest that any future data collection with respect to growth observations include oxygen concentration measurements.

Our data indicates *Ulva* has a negative growth rate when exposed to high temperatures (23°C - 25°C), which differs from some previous studies of temperature effects on *Ulva,* but may corroborate previous studies which indicate *Ulva* spp. have an optimal temperature growth rate. In the Hiraoka et al. (2020) study, *Ulva meridionalis* reached the highest (fourfold) growth rate when exposed to 30°C temperature conditions. Wu et al. (2018) found exposing *Ulva prolifera* cultures to temperature treatments of 18°C, 22°C, and 26°C resulted in negative growth rates for the highest temperatures, yet increasing light intensity resulted in positive growth. There are indications that tolerance to temperature is species-specific (Rautenberger and Bischof 2006). Given the morphological ambiguity of *Ulva*, DNA analysis is a vital requisite to determine how different *Ulva* spp. would react to increasing temperatures, and ultimately determine green tide patterns based on species spatial distribution.

Further, to determine precise thresholds of independent variables that contribute to *Ulva* growth and thus green tide events, additional experimental variables are required. In the Gao et al. (2017) study, temperature treatments were set at 14°C and 18°C concurrently with pH and nitrate availability controls. Our experiment was unable to implement similar variables due to equipment constraints. While this study does not replicate eutrophic conditions because we only

tested temperature effects on growth rate, it may serve as an indicator of additional environmental conditions necessary for a comprehensive examination of the threshold ranges under impending climate conditions.

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ATTRIBUTIONS

Experiment set-up & Execution & Document Edits	All
Abstract	Alex Pinney
Introduction	Paige Rushing, Alex Pinney, Frau Calumpiano
Materials/Methods/Diagrams & Acknowledgements	Gabriela Wood
R analysis	Madison Logan (Fig.7-9)
Analysis/Results	Madison Logan, Gabriela Wood (Fig. 10 and 11)
Discussion	Alex Pinney, Paige Rushing
References & Document Formatting	Frau Calumpiano

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