

## Evidence of Overwintering *Ulva* Propagule Bank in the San Francisco Bay Sediments

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### ABSTRACT

Blooms of the ephemeral macroalgae, *Ulva* spp., negatively affect coastal ecosystems and are typically observed seasonally in spring and summer. Studies have demonstrated that *Ulva* spp. germlings or propagules survive periods of colder temperatures and seasonal ice cover. These overwintering stages can promote populations of ephemeral species by buffering the negative effects of unfavorable environmental conditions and competition. This study is focused on the survival of overwintering propagules in the shallow sediments of the San Francisco Bay as a mechanism contributing to spring recruitment pulses. Sediment samples were collected from three central bay sites and incubated in enriched media for 8 weeks in the dark at mean winter temperature. Post incubation samples were aliquoted and placed into one of 4 temperature treatments (winter low 6°C, winter mean 11°C, summer mean 17°C, or summer high 25°C; n=3). Aliquots were cultured under a 12:12 light regime. Sediments cultured under all four conditions produced tubes and blades after 5 weeks in culture, but the seasonal temperature regime had a marked effect on recruitment rates. Winter treatments yielded few and mostly microscopic (<1mm) recruits. Summer treatments produced many macroscopic blades and tubes, with mean summer temperature treatments yielding the greatest amounts of recruits/cm<sup>2</sup>; indicating that propagules not only survive winter incubation periods but success of recruitment is influenced by seasonal increases in water temperature.

### KEYWORDS

microscopic propagules, macroalgal blooms, germlings, eutrophication, temperatures

## INTRODUCTION

Species in the genus *Ulva* are one of the most commonly-researched macroalgal species as it is a fast-growing species and frequently dominates large macroalgal blooms, commonly known as green tides (Rinehart et al. 2014). Blooms of these ephemeral macroalgae, *Ulva* spp., negatively affect coastal ecosystems and are typically observed seasonally during the spring and summer (Guidone and Thornber 2013). Ecological impacts such as eutrophication and waterborne toxic macromolecular substances are of most concern (Lotze et al. 2001, Ye et al. 2011). As more nutrients, particularly agricultural fertilizers, wash into the world's oceans, the presence of inorganic matter (predominantly nitrogen and phosphorus) causes an overgrowth of algae, creating algal mats that cover large areas of the water surface.

This algal blanketing prevents sunlight from passing through, causing marine plants underneath to die off. Moreover, during the decay of *Ulva* spp. at the end of its life cycle, decomposing bacteria consume a large amount of dissolved oxygen, which makes the waterbody environment anoxic, adversely affecting marine organisms who rely on oxygen to survive (Ye et al. 2011). Additionally, certain algal species can create harmful natural toxins -- most commonly, domoic acid and cyanobacteria. Both toxins are very harmful to marine organisms and humans and can result in severe poisoning or death (Power 2015). The increase in the severity and geographic range of algal blooms is a global phenomenon and the well-observed ecological effects of green tides have become an economic problem that is currently of interest to both scientists and governments (Rinehart et al. 2014). Current prevention and mitigation strategies, however, are not sufficiently developed, which is mainly due to the lack of understanding of the origin of green tide blooms (Ye et al. 2011) or, more generally, their 'seed origin'.

'Seed banks' refers to the natural storage of dormant but viable seeds in the environment, which serves as a well-known mechanism that has allowed many species to survive adverse conditions (Worm et al. 2001). The term first originated out of the research on terrestrial plants, where the mechanism is relatively well-understood. For many years scientists have studied the dispersal and dormancy of terrestrial plants, which are two capabilities that allow the creation of seed banks and help increase survival rates of terrestrial plants in variable environmental conditions. More concretely, dispersal decreases the risk of local extinction, while dormancy protects from harsh environments (Worm et al. 2001). Aquatic ecologists, however, have

comparatively only recently begun to realize the importance of propagule banks formed in marine habitats (Hairston and DeStasio 1988, Madhupratap et al. 1996). In particular, macroalgal propagules banks have rarely been investigated (Worm et al. 2001) and little attempt has been made at establishing if its microscopic stages actually function as survival banks (Hoffmann and Santelices 1991). In the last three decades some evidence of microscopic propagule survival has emerged and, in analogy to terrestrial seed banks, Chapman (1986) proposed the term ‘banks of microscopic stages’. These banks consist of micro recruits, settled spores, and other microscopic forms, which suspend growth when environmental conditions are unfavorable and propagate quickly once suitable environmental conditions return (Worm et al. 2001, Rinehart et al. 2014). Due to the recency of research in that area, little is known about the factors that affect them and, in particular, their origination and ‘seed source’.

This was restricted to the San Francisco Bay area and focused on the abundance and species composition of *Ulva* microscopic propagules in the sediment and its role in the formation of green tides. Building on the knowledge gained from previous studies and using the methods from Liu (2011), we aimed to find and confirm that *Ulva* spp. germlings survive periods of colder temperatures in the Bay intertidal areas. In this study we use an *in-vitro* experiment recreating the seasonal environmental conditions to investigate the overwintering stages of *Ulva*. We seek to identify the algal species that are prevalent, as propagules, in the overwintering sediments of San Francisco bay area. Then based on the morphological characteristics of species we can suggest a biogeographic origin of the free-floating *Ulva* (Ding et al., 2009). Our goal is to determine if *Ulva* spp. populations survive unfavorable environmental conditions by creating ‘seed banks’ of propagules in intertidal sediment.

## BACKGROUND

Climate change is a well-known phenomenon and its consequences, amongst many things, are adversely affecting our oceans. The constant increase in carbon dioxide in the atmosphere (CO<sub>2</sub>) is leading to the increase in the atmospheric temperatures. As a consequence, ocean surface water temperatures rise and seawater pH is reduced, resulting in an effect called acidification. Anthropogenic impacts, such as increase use of fertilizers, urbanization and the heavy use of coastal areas, also play an effect leading to the increase in the nutrient content of coastal waters

(Carpenter et al., 1998; Smith et al., 1999) negatively affecting marine community structure, diversity, and functioning (Lotze, and Worm 2002).

Eutrophication and bacterial respiration of algal biomass, occurring as a result of water that has been over-enriched with nutrients, is known to affect the already-susceptible to acidification coastal waters (Cai et al., 2011). Eutrophication occurs when inorganic nutrients -- usually nitrogen and phosphorus -- exceed the limit of a given system for a balanced flow and cycling (Fletcher 1996a, b). This process is a great threat to coastal ecosystems, and one of the main drivers of macroalgal blooms (Bricker et al., 2008). “Green tides” are an extraordinary blooms of macroalgal biomass, which have a substantial negative economic and ecological impacts (Smetacek and Zingone, 2013). According to Fletcher, *Ulva* is the dominant contributor to the majority of the green tide events. Its species are mainly distributed in the North Temperate Zone, with America, Europe and Asian-Pacific, (Figure 1). More concretely, *Ulva prolifera* is determined to be most widely distributed in the intertidal zones around the world (Gao et al. 2008; Fu et al. 2008; Zhang et al. 2010).



**Figure. 1. Worldwide green tide distribution during the last three decades (only the most frequently impacted sites are included).** The red circle marks the world's largest green tide, which occurred in the Yellow Sea, China, in both 2008 and 2009 (Ye et al., 2011).

Presently, research provides different origination and seed source of *Ulva* dominated algal blooms. Macroalgal blooms usually consist of a fast-growing algae that can rapidly utilize resources (Valiela et al., 1997, Guidone et al. 2013, Smetacek and Zingone 2013). According to Worm, propagule banks favor the reproduction of fast-growing species. Ideally, molecular analysis and infrageneric classification of green-tide-forming macroalgae are used to determine algal species.

## METHODS

### Sample collection and set up for incubation experiment

In November 2015 the lab team collected three sediment samples from three field sites in San Francisco Bay area (Figure, 2): Pt. Potrero (37.9041°N, 122.3664°W), Pt. Isabel (37.9009°N, 122.3247°W), and Tiburon (37.8735°N, 122.4566°W). Approximately 50g of coastal sediment samples were collected (into zip lock bags using a laboratory scoop that was rinsed with sea water before each collection) at low tide within one meter of the water edge at zero, 25-meter and 50-meter spacings (Figure, 3). After collection the samples were transported to the UC Berkeley laboratory within less than a hour. In the lab, the collected sediment samples were added to 2L Mason jars fully filled with F/2, where an ‘instant ocean’ product was used as a base medium for F/2. The sediment fluid in all jars was thoroughly stirred using a sterile glass rod and covered with parafilm and left undisturbed at 11°C in a cold chamber in 24-h dark regime for 8 weeks.



**Figure. 2. Locations of three field collection sites in SF Bay. Pt. Potrero, Pt. Isabel, and Tiburon.**

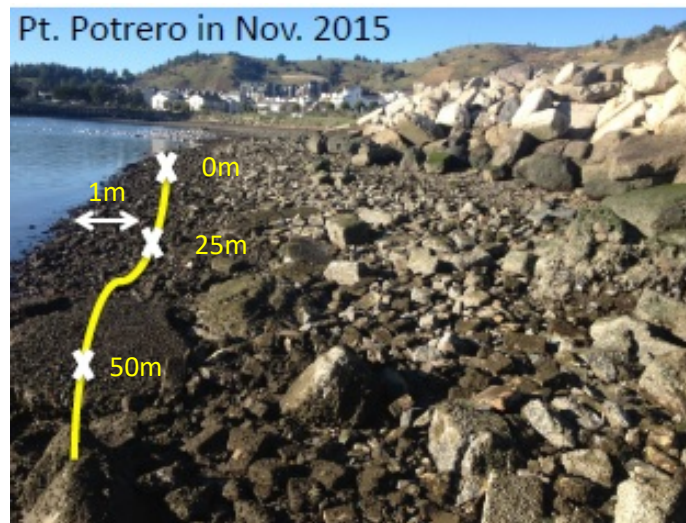


Figure. 3. Field site showing collection sample and spacing (0, 25, 50-meter) at Pt. Potrero.

### Screening process

Before the commencement of the experiment, the collected samples were tested for the presence of propagules in the sediment. To determine initial presence of *Ulva* propagules or microscopic stages in the sediment, three 50ml samples of sediment fluid were aliquoted from each 2L Mason jar and added to 75cm<sup>2</sup> culture flask (n=3, 3 sites x 3 samples/site x 3 aliquots/sample = 27 flasks). The culture flasks were maintained at a constant 17°C in a growth chamber. Light regime for the culture flasks were 12-h of light per day and the F/2 media was replaced weekly. After 20 days, visible propagules grew on the wall and bottom of the flasks (Figure, 4).

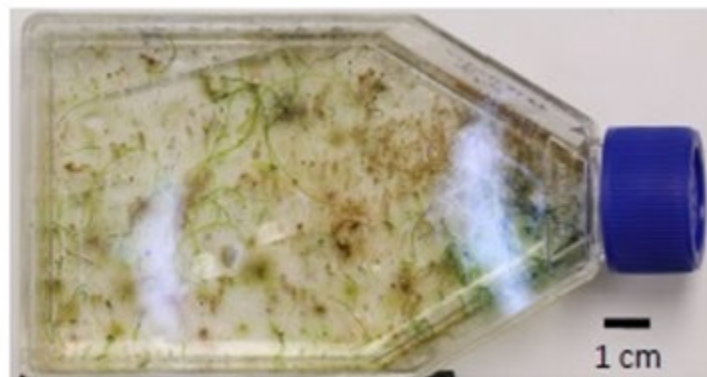
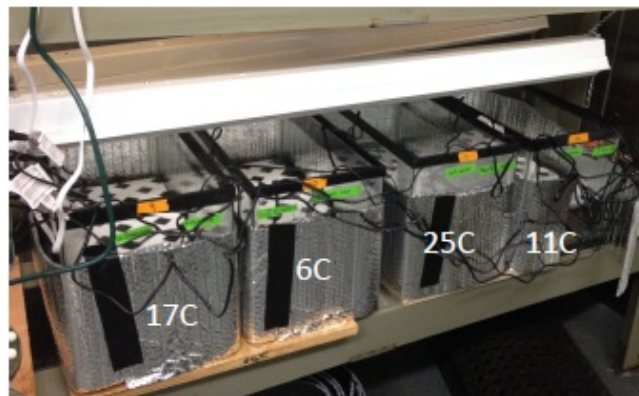


Figure. 4. Culture flask after 20 days, showing the growth of survived propagules.

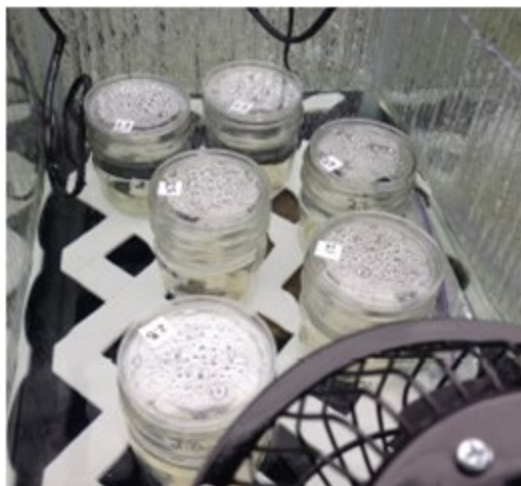
## Incubation and culture treatment

For the quantification of the *Ulva* propagules (above experiment) and the treatment in cold and dark conditions, the lab team followed the base procedure from Liu (2011) with minor adjustments. For the incubation experiment, three 2L mason jars with sediment fluid (described in the set up for incubation section) were maintained undisturbed at 11°C in 24-h dark regime for 8 weeks. This step was used to recreate dormancy condition that algal propagules naturally experience during the winter period in the SF Bay area. After the 8-week incubation (dormancy) period, equal amounts of 50ml of sediment fluid samples were aliquoted from each 2L jar into labeled 250ml Mason jars with a petri dish on the bottom. A total of 72 250ml samples were aliquoted and placed in 12 water-filled tanks with different temperature controllers. Each tank had six jars and each jar was filled with 200ml F/2 media and arranged randomly within each tank with at least 2 representatives of each site in each tank. Each tank was filled with 10-gal water bath and maintained at an assigned temperatures of 6°C (winter low), 11°C (winter mean), 17°C (summer mean), and 25°C (summer high) (Figures 5 and 6). Those temperatures were maintained using a cooling-heating system.



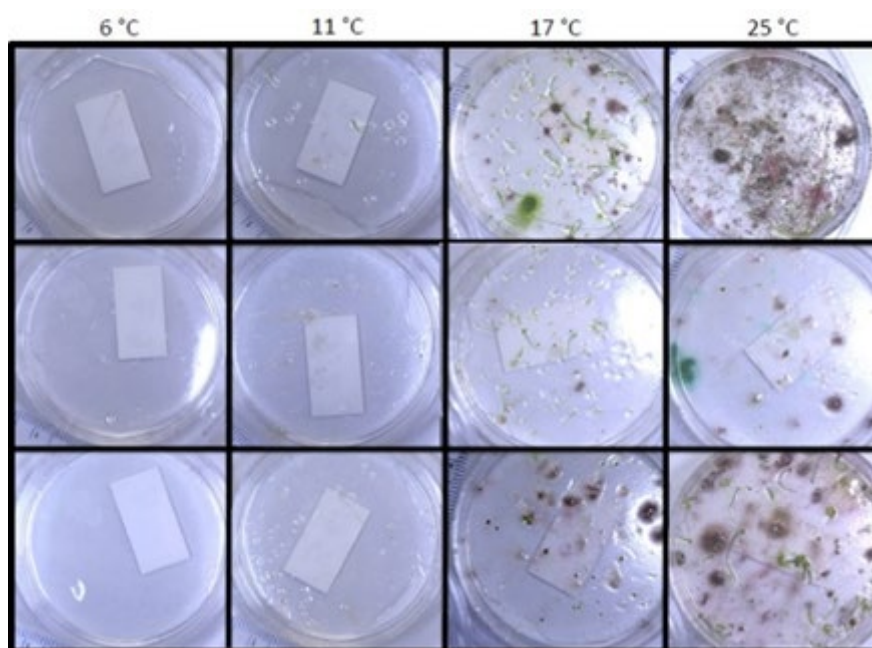
**Figure. 5. Four tanks with various temperatures set up.** Temperatures: 6°C winter low, 11°C winter average, 17°C, summer average, 25°C summer high.





**Figure. 6. Inside of each tank set up.** Each tank contained 6 mason jars, 2 from each site. Each mason jar had a petri dish on the bottom and was covered with a lid. Jars were suspended into 10-gal water-filled tanks with different temperature controllers.

After 4 weeks of culture treatment, we extracted petri dishes from the jars and photographed the samples (Figure 7).



**Figure. 7. Sample of photographed petri dishes from various temperatures showing various growth pattern.** Values on the left are the winter temperatures and values on the right are summer temperatures.



## Count of algal recruits and their morphology variations

We uploaded images of all samples to a custom Web applet built by Lucy M. Chang (University of California, Berkeley) and interpreted each photographed sample using built-in zoom tool. For this experiment we counted the number of all recruits present on each petri dish and identify their morphology (Figure 8) We identified seven morphologies present: *Ulva* blades, *Ulva* blades that arise from a shared holdfast, *Ulva* tubes, *Ulva* tubes arise from a shared holdfast, *Ulva* branched tubes, UTB (*Ulva* that looks like a blade near a holdfast but has a hollow tube shape near the apex), UBT (*Ulva* recruit that looks like a hollow tube near the holdfast but has a flattened blade shape near the apex), not including *Ulva* recruits with ambiguous morphologies. Ambiguous morphologies were assigned to the species that were too small to identify.



**Figure 8. Web applet screenshot.** Yellow dots represent the counted recruits. Recruits were counted in order of identified morphologies and counted results was projected on the screen overlaying petri dish image.

## Analysis

We used ANOVA and Duncan's new multiple range test to test for differences in recruits among temperatures levels. The average recruit count and standard error for each single temperature treatment were estimated from total recruit count in each applicable petri dish. The collected data was analyzed using one-way analysis of variance (ANOVA) as per Liu (Liu *et al.* 2012) using the R statistical environment. Additionally, Duncan's new multiple range test was

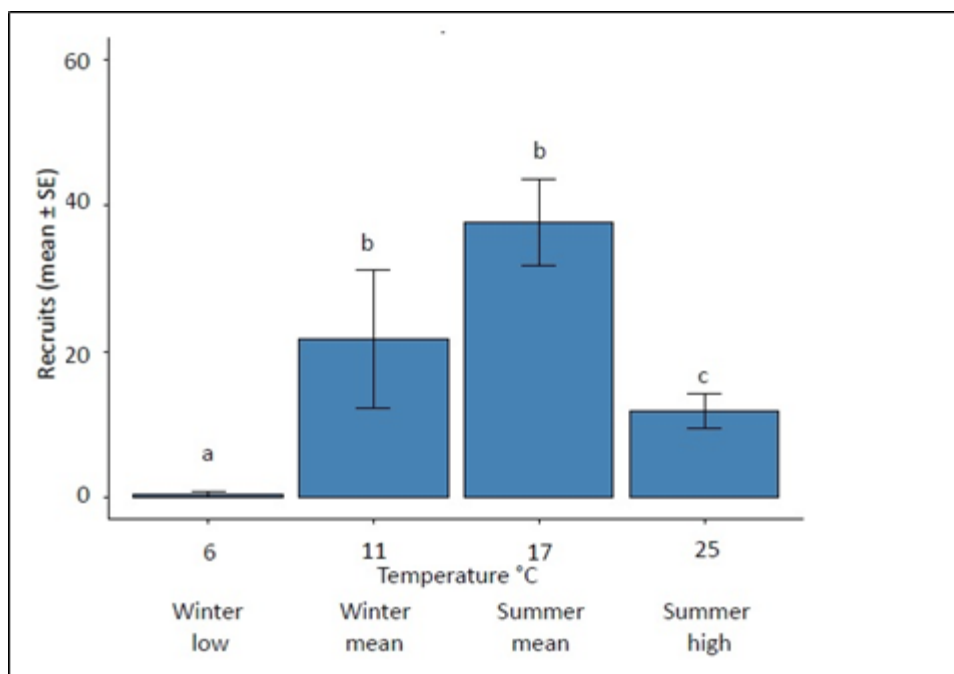
used to determine the statistical significance of the difference between the resulting means at  $p < 0.05$ .

## **RESULTS**

### **Abundance of recruits from propagule bank in various temperatures**

Sediments cultured under all four conditions produced growth after 4 weeks and the seasonal temperature regime has a marked effect on recruitment rates. In the present study, germination success increased with temperature from 6°C to 17°C. Winter treatments yielded few and mostly microscopic (<1mm) recruits, while summer treatments produced many macroscopic blades and tubes, with mean summer temperature treatments yielding the greatest amounts of recruits (Figures 9 and 10). Winter low temperature showed little to no recruitment. Yet the summer high temperatures decreased growth in number of species compare to mean summer and mean winter temperatures.

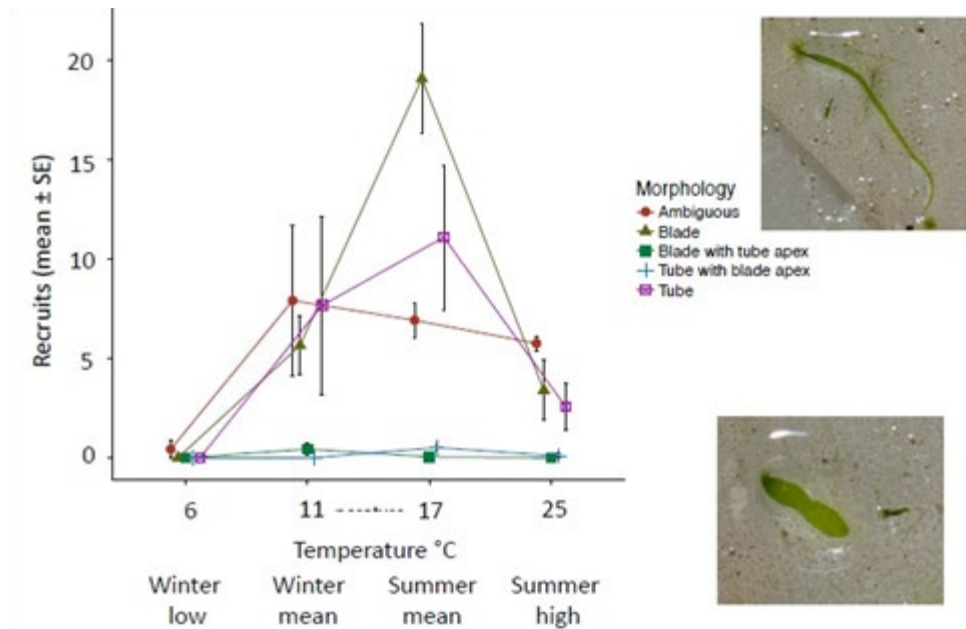
The multiple comparison test showed significant difference (at the  $p < 0.05$  level), between 6°C and 25°C. Temperatures of 11°C and 17°C are statistically different from the other two (Figure 9). There were no significant differences between mean winter and mean summer temperatures, and we were unable to reject the null hypothesis ( $p < 0.05$ ) when comparing the growth under the 11°C and 17°C conditions.



**Figure. 9.** Average abundance of recruits from propagule bank in various temperatures after a 4 weeks culture treatment in nutrient-enriched seawater. Means and SE are shown (n=3). Values with statistically significant differences ( $P < 0.05$ ) are indicated by different letters.

### Species composition across various temperatures

Species composition among various temperatures showed highest numbers of tubes and blades at the mean summer temperature (Figure 10) with the tubes recruits being higher than any other morphology. Except for the average summer temperature at 17°C, ambiguous morphology is the highest in number of recruits. The number of recruits of all morphologies at 25°C is relatively similar, as at 11°C; however, known morphologies are much lower than at 25°C. And, at winter low temperature, only ambiguous morphologies are detected due to small size of recruits.



**Figure. 10. Species composition across various temperatures.** Means and SE are shown (n=3). Most common morphologies are shown on the photograph to the right of the graph: Tube morphology(top), blade morphology (bottom).

## DISCUSSION

Our experiment provides evidence that *Ulva* spp. germlings survive periods of colder temperatures in San Francisco Bay sediments. An *in-vitro* experiment recreating the overwintering conditions determined that *Ulva* spp. populations survive the unfavorable environmental conditions by creating ‘seed banks’ of propagules in intertidal sediment. The quantitative analysis revealed the settlement and proportions of various *Ulva* morphologies at various temperatures whereas the morphological analysis showed that propagule banks, in this experiment, favor the reproduction of *Ulva* tube species. Unfortunately, taxonomy data was not sufficient to conclusively determine the connection and contribution of the collected algal species to macroalgal green tides and, thus, further investigation is required.

*Ulva* propagules survive unfavorable environmental conditions and its reproduction is accelerated by higher temperatures. Our study shows that germination and reproduction success increased significantly with the temperature rising from 6 °C to 17 °C, with the mean summer temperature (17 °C) treatments yielding the greatest amounts of recruits. The number of recruits at the higher summer temperature (25 °C), however, decreased. Previous laboratory culture

experiments from samples of Narragansett Bay, Rhode Island, US (Rinehart, 2014) and experiments sampled from the Yellow Sea, Jiansu province, China (Liu, 2012) both suggest the overwintering survival mechanism of *Ulva* spp.. And that higher temperatures enhance the growth of survived propagules. The field experiment in the Western Baltic Sea of Germany on manipulating propagule banks (Worm, 2001) also confirms the above predictions.

Furthermore, temperature experimental outcomes vary and appear to be location- and species-dependent. Specifically, the reproduction rates differ between *Ulva* spp. and maximum high temperature. According to Gao (2012), higher temperatures increase the settlement and stimulate the physiological performance of *Ulva*. *Ulva rigida* significantly enhanced its settlement and reproduction at the high temperature of 18°C. For *U. intestinalis*, germination rates increased at the maximum of 23°C (Christie and Shaw, 1968), for *U. fasciata* (Mantri et al., 2011) and *U. compressa* at 25°C (Callow et al., 1997). According to Liu (2011), however, the length of *Ulva* individuals continuously increased, even when it reached 25°C the highest used in the experiment.

Temperature and culture treatment outcomes may explain algal seasonal behavior. Under their experimental conditions, *Ulva* propagules are able to survive and germinate in cold temperatures (Hoffman 1991). However, too high summer temperatures could be a limiting factor for algae survival. Novaczek (1986) suggests that temperature requirements of gametophytes for sexual reproduction and temperature tolerance of sporophytes in culture experiments significantly varies from natural habitat and identified lowest and highest temperatures of settlement and reproduction are usually exaggerated in in-vitro experiments. Thus, these traits will likely not occur in the natural environment. Additional scientific data also suggests that temperature is vital for *Ulva* growth, especially for young *Ulva* recruits. Higher temperatures during the first few days of culture tend to increase the growth of both the young and adult plants (Kim and Lee, 1996; Mantri et al., 2011). However, the growth rate of the adult species at high temperatures diminishes just a few days later. This evidence suggests that at certain temperatures -- which varies by species -- the physiological performance of *Ulva* spp. is no longer stimulated by higher temperatures.

Additionally, studies of morphological taxonomy determined that the mean summer temperature has the highest numbers of tubes and blades morphologies, with the tubes recruits being the highest. However, errors of identification can occur while sampling small organisms with morphologies that are hard to distinguish. Although molecular analysis\* was originally planned for the purposes of taxonomic identification, it failed to identify the species tested. (This

is likely due to inaccurate testing procedure). Thus, the data collected for taxonomy analysis in this project, was insufficient to identify the prevalent species of *Ulva*. Moreover, according to Manoylov (2014) morphological and molecular analysis is commonly used to identified algae taxonomy. However, currently, morphological analysis is only used to confirm the molecular identification data (Manoylov 2014).

Other natural factors, which were not included in this experiment, can affect settlement and germination of propagules. According to Gao (2012), the effects of nitrate, temperature and CO<sub>2</sub> in its low and high values and their various combinations lead to several different outcomes on species survival and reproduction. The amount of nutrient present can alter the growth rates and abundance of algal species. For instance, nitrogen content can affect cellular lipid accumulation in macroalgae, where lower amounts of nitrogen tend to increase or decrease the production of lipids depending on the algal species (Roessler, 1990, Kumari and Reed). Other experiments have demonstrated that algal species abundance depends on competition and predation factors (Lubchenco 1980). Whereas grazers can prevent the spread of the fast-growing alga, nutrients can override and reverse the effect. Stewart's (1989) research suggests that the effects of these interactions vary based on the species involved and the particular habitat's characteristics. Her study on species along the Pacific coast of North America has found that competition plays a major role in the dominance of certain species. In the natural environment all the above factors can affect *Ulva* propagule settlement, germination and reproduction. Hence, we cannot conclude based on only morphological analysis and temperature variations that tubes morphology is the dominant dormant propagules in the natural San Francisco bay sediment.

Overall higher temperatures accelerate the onset and magnitude of gametes settlement and reproduction. However, for certain species and locations, higher temperature is not always beneficial. and, may, in fact, have a detrimental effect on reproduction. *Ulva* tubes morphology was identified<sup>1</sup> as the one with the highest survival and reproduction rates, suggesting the strong ability of *Ulva* tubes to invest into overwintering propagules and, as a result, recruit in high density at more favorable environmental conditions. Considering that macroalgal blooms usually consist of fast-growing algae, tubes could be the most obvious source of coastal green tides. However,

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<sup>1</sup> \*molecular analysis was not a part of this project and was only meant to be used as a support evidence for species identification. Hence, its procedure and analysis is not described in this paper.



additional research on molecular taxonomic identification and effects of various environmental factors on the algal species is required.

## **FUTURE WORK**

Our future work would include the size identification of ambiguous recruits. To identify the size of recruits we will measure its length (in mm) using ImageJ. This experiment is necessary to verify if ambiguous morphologies have passed their microscopic stage and are on their way to become an adult. If this turns out to be the case, they will be counted and compared to other samples and used in the statistical analysis. If not, they will be ignored due to insufficient growth length. The size (as determined by length) at which a recruit is considered an adult will be determined after further investigations. Additionally, the length data will be cross-referenced to the set of temperatures. The results will be compared to the Yellow Sea shores analysis performed by Liu (2012).

We further propose a percent cover experiment. To analyze the vegetation percent cover, ImageJ software will be used. Percent cover could be estimated by tracing the total area of the vegetation cover on the image and dividing it by the total area of the petri dish. This part of the experiment will show what part of petri dishes is covered by algae and what part is covered by other vegetation or empty space at various temperature treatments. The vegetation cover density will help us figure out if competition plays a role in the survival rates of overwintering propagules. Furthermore, based on the current results and future percent coverage experiment, we expect to be able to prove a significant difference between the growth at higher and lower temperatures. In particular, we anticipate seeing much higher growth of various communities at 25°C compared to 17°C. We expect that at a higher temperature the density and composition of vegetation will increase, while the recruitment rates of *Ulva* spp., specifically, will decrease.

## **CONCLUSION**

At our current level of understanding of the present problem, we lack mechanisms for effectively regulating the blooms of any green tide species and further research is necessary to find and mitigate the algal blooms at their origin. Although there are potential mitigation techniques,

such as using algal commercially as fertilizer, we are far from having alternative solutions to the mitigation or even the utilization the excessive amounts of alga. The most obvious solution of taking out the pollution out of the ocean will create a land pollution problem (Ye, 2011). Furthermore, although ecological and economic impacts of algal blooms are widely studied, the anthropogenic impacts and global climate change may exacerbate this to an even larger problem. Increasing nutrients in coastal environments are negatively affecting marine community structure, diversity, and functioning (Lotze, and Worm 2002). Hence, it is important to understand that human influences have the potential to cause negative effects on the environment and studies in that area are just as important (Ye et al. 2011). Advances in the effective management and mitigation of algal blooms can only help us better safeguard coastal ecosystems and local economies.

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