Site and Light: Looking at factors that affect the growth rate of *Saccharina sessilis*

Kristin Beem, Ariana Meltvedt (Snow), & Alexander Carsh

Department of Biology, Oregon State University

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Introduction

In the intertidal zone, interactions between and within species govern ecosystem function and stability (Kavanaugh 2005). Processes and dynamics in the basal species can affect interactions at higher trophic levels. With increasing anthropogenic pressure on coastal marine ecosystems, the intertidal zone has become an important model system for biological studies. An important basal group in the intertidal zone is macroalgae, including kelps such as *Saccharina sessilis* (lettuce kelp). Macroalgae can serve as a bioindicator of increased nutrient input, water quality, and shifts in the intertidal environment (Lyngby and Mortensen 2008). Optimal growth for kelp species often fluctuates with season, reflecting strong influence from day length, radiation intensity, and temperature (Lee and Brinkhuis 1988). Too much UV exposure, however, can inhibit growth in some macroalgae (Apprill and Lesser 2003).

These variations result in changes of reproduction timing and primary production levels (Broch and Slagstad 2012, Gévaert et al. 2002). Competition for light exists between macroalgae species and with other groups including phytoplankton (Broch and Slagstad 2012). Phytoplankton growth has shown to increase with higher nutrient input, often from anthropogenic sources, sedimentation, or coastal upwelling (Lyngby and Mortensen 2008). As the accessibility of light changes under these conditions, physiological structure and ability to adapt may lead to a decrease in primary production and growth of macroalgal species (Hanelt et al. 1997, Gerard 1988, Holzinger et al. 2011).

The dynamics of various sites may also play a role in how sensitive *S. sessilis* is to varying light conditions. For example, sites with broad continental shelves, such as Strawberry Hill at Cape Perpetua, have higher nutrient and microorganism retention rates than sites with narrower shelves, such as Boiler Bay at Cape Foulweather (Menge et al. 1997). Because of this shelf difference, the waters at Strawberry Hill have more phytoplankton bloom than those at Boiler Bay, lowering the light penetration into the water column (Menge et al. 1997). *S. sessilis* found at Strawberry Hill would likely to demonstrate phenotypic plasticity and adapt to shaded conditions than those from Boiler Bay (Kavanaugh 2005).

The purpose of this study is to present data on *S. sessilis* growth rates under shade and ambient light conditions and to record the primary production of *S. sessilis* after experimental light application in field and laboratory trials.

Field observations of *S. sessilis* morphology and distribution differences led to us questions about the resilience of macroalgae to environmental stress and fluctuations in abiotic conditions. We would expect that the growth rates would be higher for *S. sessilis* that is exposed to sunlight than for *S. sessilis* that has been covered by shade cloth. Site characteristics at Boiler Bay and Strawberry Hill may lead to different levels of growth in the laboratory experiment. Between algae populations and may also exhibit differences in site growth. Specifically our hypotheses were:

H_{0a}: There will be no difference in the growth rates between *S. sessilis* grown in ambient sunlight and *S. sessilis* grown in shaded conditions.

H_{1a}: There will be a difference in the growth rates between *S. sessilis* grown in ambient sunlight and *S. sessilis* grown in shaded conditions.

H_{0b}: There will be no difference in the growth rate between *S. sessilis* collected from Boiler Bay and Strawberry Hill study sites.

H_{1b}: There will be a difference in the growth rate between *S. sessilis* collected from Boiler Bay and Strawberry Hill study sites.

H_{0c}: There will be no difference in the growth rates between *S. sessilis* growing at SH and *S. sessilis* growing at BB.

H_{1c}: There will be a difference in the growth rates between *S. sessilis* growing at SH and *S. sessilis* growing at BB.

Materials and Methods

The lab experiment was conducted at the Hatfield Marine Science Center (44.6223, -124.0451) in Newport, OR. Algae growth trials were held in the blue outdoor tanks of the Hatfield facility connected to the salt water pump system. Field experiments were conducted at Strawberry Hill (44.2540, -124.1123) in the protected low zone and Boiler Bay (44.8331,-124.0628) in the protected low/mid zone (Fig. 1).

Laboratory

Saccharina sessilis was collected for this experiment at two sites: Boiler Bay (BB) and Strawberry Hill (SH). Collection at SH was on May 11, 2013 at low tide and collection at BB was on May 12, 2013 at low tide. SH samples had an acclimation period of nine full days in the BI450 laboratory. BB samples had eight full days of acclimation. The samples were moved to the outdoor tanks on May 20, 2013 and had two full days of acclimation prior to establishing growth markers.

Growth markers were established by cutting small horizontal slits in the blades 5cm from the holdfast, with only one growth marker per holdfast, to ensure independent growth rates. A cable tie was secured through each slit for future growth measurements. The cable ties indicate sites of origin, with green cable ties identifying BB samples and white cable ties identifying SH samples (Fig. 2). In total, 16 *Saccharina* specimens are used in this experiment. Each of the two study sites has four replicates, each with a shaded sample and sun-exposed sample. Eight outdoor tanks were used, four for each site with a shade and sun-exposed trial (Fig. 3).

We measured growth of each sample every two days by recording the progression of length from the holdfast to the cable tie marker using a metric ruler. We measured the biomass of each sample by using laboratory scales to record wet weight (OHAUS Scout-Pro, ±0.1g). These measurements were then run used in the Minitab program using two-way ANOVA tests to compare samples among site, light differences, and both site and light differences.

Light (μmol·s-1·m-2) and water temperature (°C) were collected May 30 to June 2 in order to get an accurate depiction of the normal conditions in the tanks for the laboratory experiment. The light was measured using a LI-COR Photometer, model LI-250A.

Field

At low tide on May 28, we used a hole-punch method to mark the baseline growth approx. 5cm above the holdfast of 50 *S. sessilis* specimens at each field study site (BB and SH) (Fig. 4). The area surrounding the samples will be marked with white zip ties. On June 2 and June 3, we returned to the field sites to measure the progression mean daily growth of marked *S. sessilis.* Minitab was used to run a two-way ANOVA test to compare the sites.

Results

Laboratory

Two-way ANOVA tests were used to compare samples among site, light differences, and both site and light differences. SH open samples had the highest mean growth rates for length; however, there were no statistical differences between any of the samples (Fig. 5). When comparing lengths in shaded versus open samples, there was no significant difference between the mean growth rate (p-value = 0.95). There was no significant difference between the mean growth rate of BB and SH samples (p-value = 0.425). No significant difference was found when site and light interactions were combined (p-value = 0.461). When taking the average growth rate for length and comparing site differences, SH growth rates fluctuated from day to day while BB showed more constant growth (Fig. 6). The average growth rate for length for the shaded samples peaked during day 10 while the open samples peaked for growth rate on day 4 (Fig. 7.) The average length growth for the BB open samples was 1.8mm/day (±0.5) and for the BB shaded samples it was 2.0mm/day (±0.3). The average length growth for the SH open samples was 2.3mm/day (±0.5) and for the SH shaded samples it was 2.0mm/day (±0.3).

When comparing biomass in shaded versus open samples, there was no significant difference between the means of the growth rate (p-value = 0.657) (Fig. 8). There was no significant difference between the means of growth rate of BB and SH samples (p-value = 0.814). No significant difference was found when site and light interactions were combined (p-value = 0.187). The average biomass growth for the BB open samples was 8.1g/day (±4.4) and for the BB shaded samples it was 6.7g/day (±0.3). The average biomass growth for the SH open samples was 10.2g/day (±4.3) and for the SH shaded samples it was 12.3g/day (±12.3).

After removing the interactions of site, light, and site*light, the P-values were still not significant among all of the laboratory sample groups.

For the tanks, the water temperature, ambient light and shaded light were recorded May 30 to June 2 2013. The first day of measuring, May 30, there were heavy rains and the ambient light was well below the ambient light of the rest of the days (Table 1).

Field

When the *S. sessilis* samples were measured in the field on June 2 and June 3, 40 samples were recovered and recorded at BB and 25 samples were recovered and recorded at SH. Using a two-way ANOVA test comparing BB and SH daily growth rate for length, there was a significant difference between the means of the length growth rate ($p < 0.0001$). BB showed a higher mean growth rate of 11.4mm/day (±1.0) while SH had a lower mean growth rate of 5.9 (±0.9) (Fig. 9).

Discussion

For the first hypothesis, that there will be a difference in the growth rates between *S. sessilis* grown in ambient sunlight and *S. sessilis* grown in shaded conditions, we failed to reject the null hypothesis. Our study didn't show statistical difference between the shaded samples and the open samples causing in a confounding result. Our study conducted in ten days and the treatments might not have been able to fully take effect in such a limited amount of time. If the light levels were too high in the open samples, this could limit photosynthetic rates and therefore inhibit growth in macroalgae (Apprill and Lesser 2003).

Since macroalgae can exhibit different morphologies and adapt to the conditions they are in (Kavanaugh 2005), we used the biomass data to account for the blade growing wider than in length. However, some of the samples were decaying and our measurements reflected this with fluctuating data points. For this reason, our biomass data does not accurately represent the mean growth. If this study was to be repeated, we would recommend a shorter lab acclamation time.

For our second hypothesis, that there will be a difference in the growth rate between *S. sessilis* collected from Boiler Bay and Strawberry Hill study sites, we failed to reject the null hypothesis. There was no statistical significance between the two sites in the laboratory setting. This could be due to a short study time, among other study variables, but it could also be attributed to the plasticity of plants (Hanelt et al. 1997). A study done by Bolton and Luening found that the origin of the plant had very little effect on growth. A wide variation of temperature was tested on several species of macroalgae from different sites and there was no difference among the sites (Bolton and Luening 1982). This suggests that the origin of the plants could have little effect on the samples once they are in the lab, producing the result we saw in our study.

For the third hypothesis, that there will be a difference in the growth rates between *S. sessilis* growing at SH and *S. sessilis* growing at BB, we rejected the null hypothesis. We did find statistical significance between growth rate at BB and at SH. The mean growth rate was much higher at BB than at SH. This could be attributed to the differences in continental shelf differences and nutrient availability (Menge et al. 1997). There is a higher abundance of macroalgae at BB then SH which would suggest that algae also grow better at BB as seen in our data (Kacanaugh 2005). It was sometimes difficult to differentiate between natural holes and our marked holes when measuring the field samples, however, the replicates helped minimize this unintentional variable. It would also be useful to have shaded and open plots in situ at both SH and BB to look at results in the field and record the environmental conditions along with the growth learn the optimal conditions and thresholds for this important macroalgae.

Macroalgae act as one of the bases of coastal food webs the main reasons for the highly productive coastal waters (Kavanaugh 2005). Because of their large contribution to the coastal ecosystem, processes and events that impact the survival of macroalga can have important effects for other species. These species could include ones of economic importance and could potentially have devastating bottom-up effect throughout other trophic levels In addition, because algae blooms are occurring more frequently off the coast of Oregon (Menge et al. 1997), it is important to study the results of shading on macroalgae like *Saccharina*.

Acknowledgements

I would first like to thank our colleagues, Ariana Meltvedt (Snow) and Alexander Carsh. I think we all worked really well together collecting data and bouncing ideas off one another. Next, I would like to thank all of our professors (Dr. Sally Hacker, Dr. Sarah Henkel, Dr. Scott Heppell, Dr. Bruce Menge, and Dr. Annette Olson) for teaching us different subtopics of the rocky intertidal zone. I would like to give a special thanks to Dr. Sally Hacker and Dr. Sarah Henkel for guidance in coming up with the project idea. I would also like to thank Reuben Biel and Sarah Close for helping us with statistics and equipment. Last but not least, I would like to thank Erin Jaco for lending us a hand at Strawberry Hill during data collection.

Literature Cited

Apprill, A. M. and M. P. Lesser. (2003) Effects of ultraviolet radiation on *Laminaria saccharina* in relation to depth and tidal height in the Gulf of Maine. Marine Ecology Progress Series **256**:75-85.

Bolton, J. J. and K. Lüning. (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. Marine Biology **66**: 89-94.

Broch, O. J. ,and D. Slagstad. (2012) Modelling seasonal growth and composition of the kelp *Saccharina latissima*. Journal of Applied Phycology **24**:759-776.

Gerard, V.A. (1988) Ecotypic differentiation in light-related traits of the kelp *Laminaria saccharina*. Marine Biology **97**: 25-36.

Gévaert, F., A. Creach, D. Davoult, A. C. Holl, L. Seuront, and Y. Lemoine. (2002) *Laminaria saccharina* photosynthesis measured in situ: photoinhibition and xanthophyll cycle during a tidal cycle. Plant, Cell, and Environment **25**:859-972.

Hanelt, D., C. Wiencke, U. Karsten, and W. Nultsch. (1997) Photoinhibition and recovery after high light stress in different developmental and life history stages of *Laminaria saccharina*. Journal of Phycology **33**:387-395.

Holzinger, A., L. Di Piazza, C. Lütz, M. Y. and Y. Roleda. (2011) Sporogenic and vegetative tissues of *Saccharina latissima* (Laminariales, phaeophyceae) exhibit distinctive sensitivity to experimentally enhanced ultraviolet radiation: photosynthetically active radiation ratio. Phycological Research **59**: 221-235.

Kavanaugh, M. T., B. A. Menge, K. J. Nielsen, L. M. and Goodrich. (2005) Phytoplankton Shading of Marine Benthic Macrophytes: Implications for Intertidal Community Structure. MS Thesis, Oregon State University, Corvallis, Oregon.

Lee, J. A. and B. H. Brinkhuis. (1988) Seasonal light and temperature interaction effects on development of *Laminaria saccharina* gametophytes and juvenile sporophytes. Journal of Phycology **24**:181-191.

Lyngby, J. E. and S. M. Mortensen. (2008) Effects of dredging activities on growth of *Laminaria saccharina*. Marine Ecology **17**:345-354**.**

Menge, B. A., B. A. Daley, P. A. Wheeler, E. Dahlhoff, E. Sanford, and P. T. Strub, (1997) Benthic-pelagic links and rocky intertidal communities: Bottom-up effects on top-down

control? Proceedings of the National Academy of Sciences of the United States of America 94: 14530-14535.

Place, S. P., B. A. Menge, and G. E. Hofmann. (2012) Transcriptome profiles link environmental variation and physiological response of *Mytilus californianus* between Pacific tides. Functional Ecology **26**:144-155.

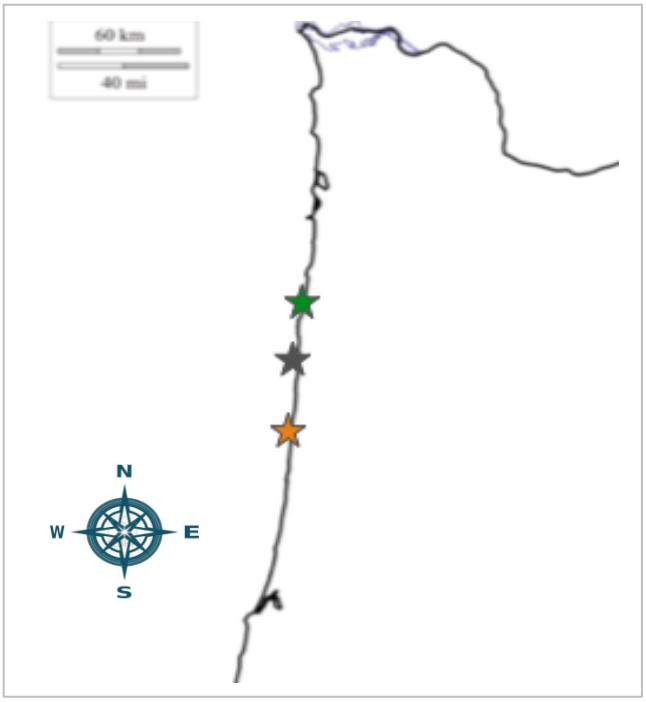

Fig. 1. Map of study sites Boiler Bay (green star), Hatfield Marine Science Center (gray star), and Strawberry Hill (orange star) in the central Oregon Coast. Field study and collection took place at Boiler Bay (44.8331,-124.0628) and Strawberry Hill (44.2540, -124.1123). Laboratory experiments took place at Hatfield Marine Science Center (44.6223, -124.0451). Experiments and observations conducted during May-June 2013.

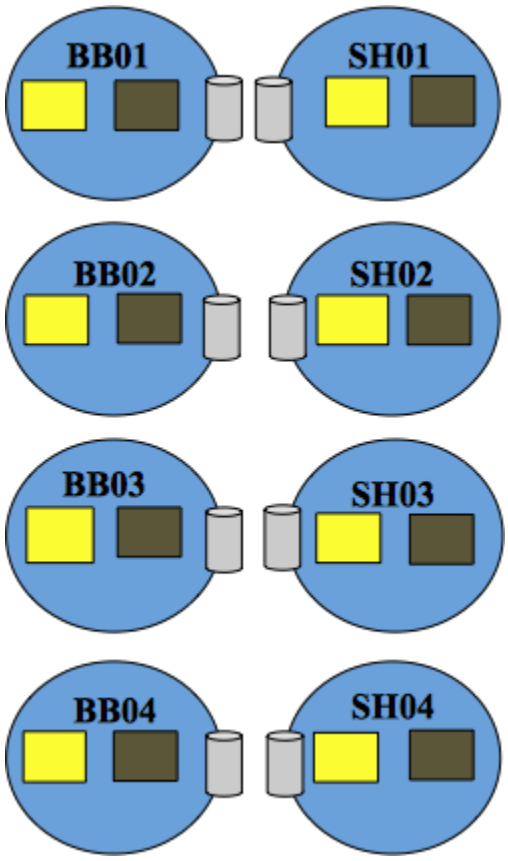

Fig. 2. Diagram of the *Saccharina sessilis* growth laboratory experiment. The blue circles represent the outdoor flow tanks, and each tank contains two colanders (squares). Each colander holds one *S. sessilis* sample, and the yellow represents the ambient light while the brown represents the samples covered by shade cloth. Strawberry Hill (SH) and Boiler Bay (BB) each had four replicates. The gray cylinders represent the salt-water pump. Length and biomass were measured over ten days in May-June 2013 at the Hatfield Marine Science Center in Newport, OR.

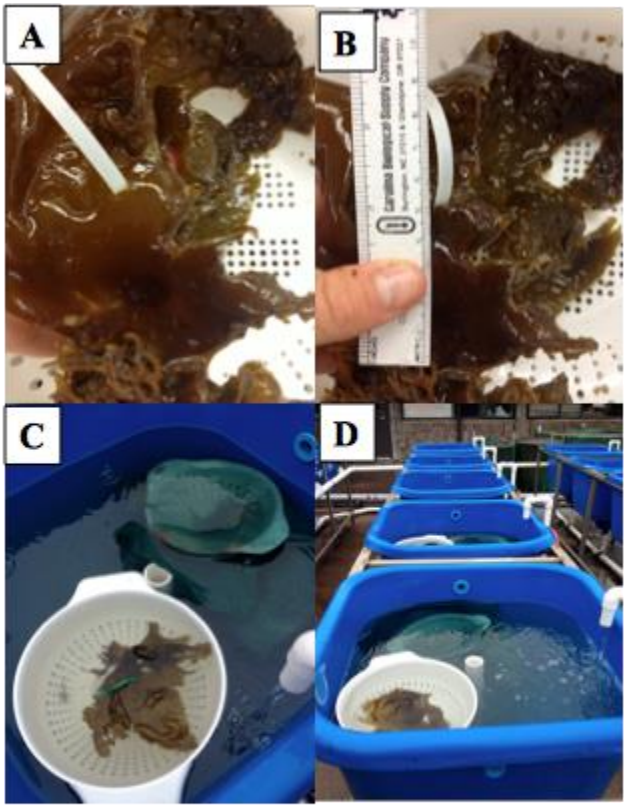

Fig. 3. Photos show securing cable tie as growth marker (A) and measuring growth from base of blade (B). Shaded and ambient light examples placed in outdoor saltwater tanks in colanders (C). Two sets of four tanks used for each study site trail (D). Growth measurement experiment conducted over ten days in May-June 2013 at the Hatfield Marine Science Center in Newport, OR.

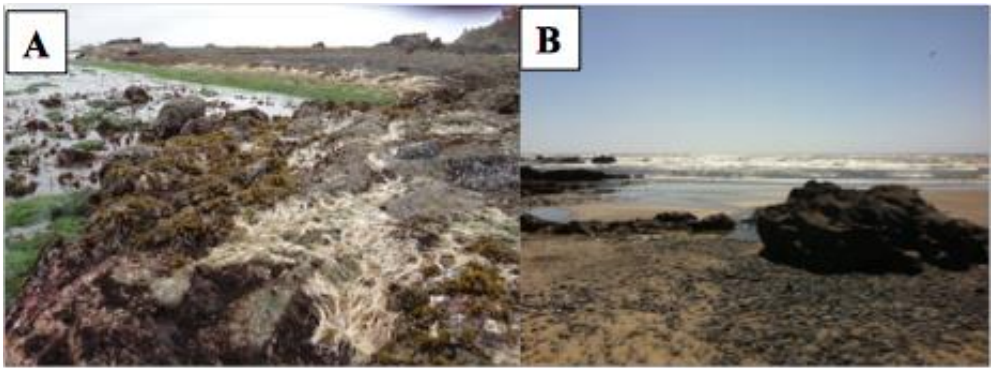

Fig. 4. Photos show field study site habitat topography at BB (A) and SH (B). Field growth documented over course of six days at SH and five days at BB in May-June 2013 on the Central Oregon Coast.

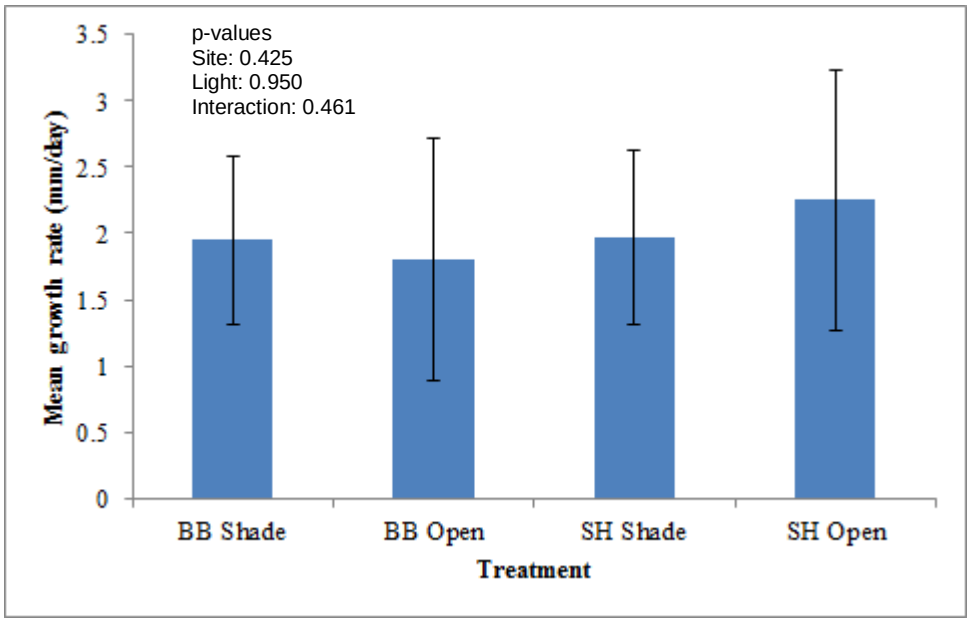

Fig. 5. Data presented is from laboratory data collected 10 days between May – June 2013. *Saccharina sessilis* samples were measured in length from the holdfast every two days. Error bars are represented by 95% Confidence Intervals

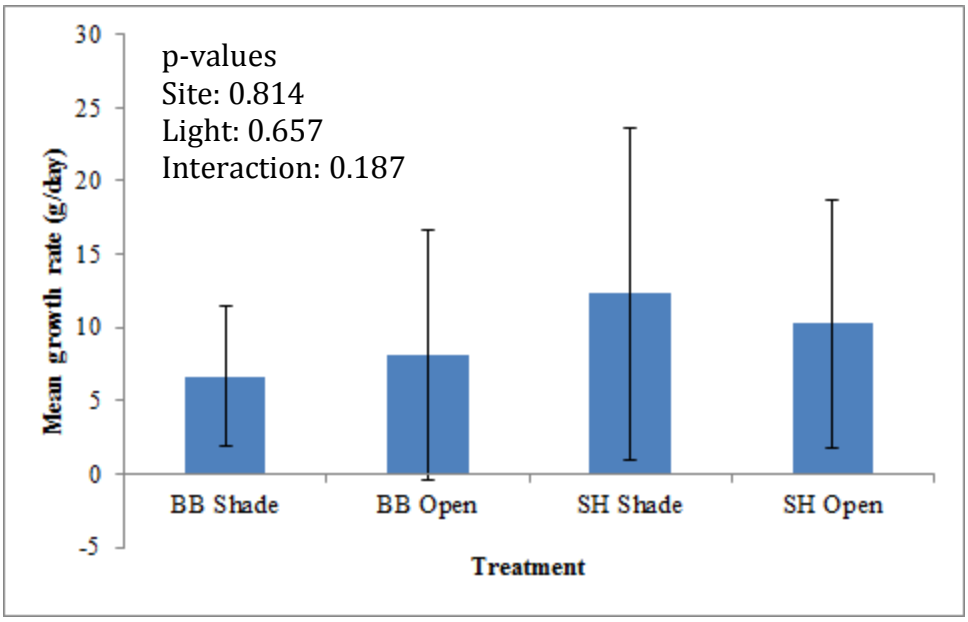

Fig. 6. Data presented is from laboratory data collected 10 days between May – June 2013. *Saccharina sessilis* samples were measured in biomass every two days. Error bars are represented by 95% Confidence Intervals

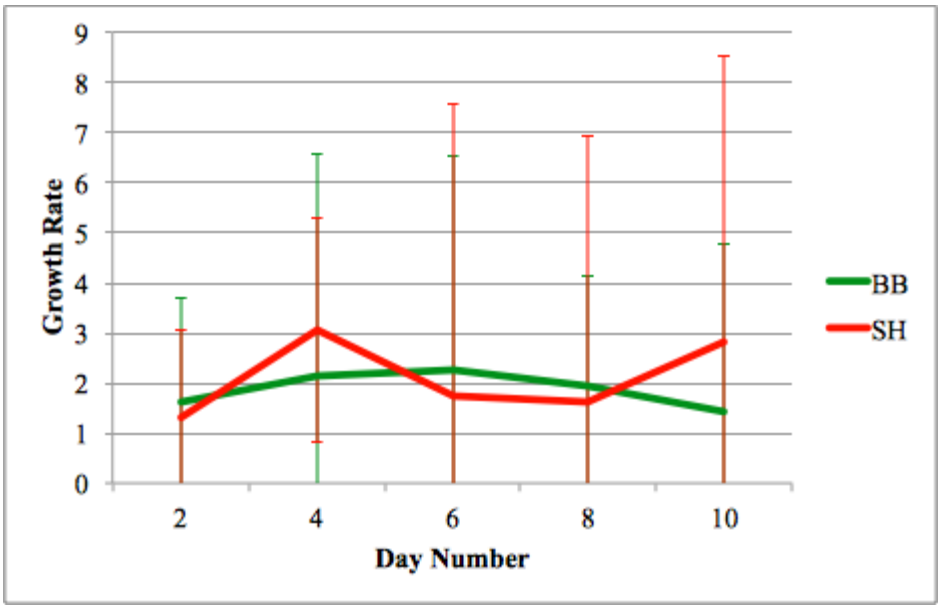

Fig. 7. Graph showing mean growth rate of BB (green) combined samples and SH (red) combined samples over ten-day period at Hatfield Marine Science Center in Newport, OR. Error bars show 95% confidence interval. Growth measured in mm/day.

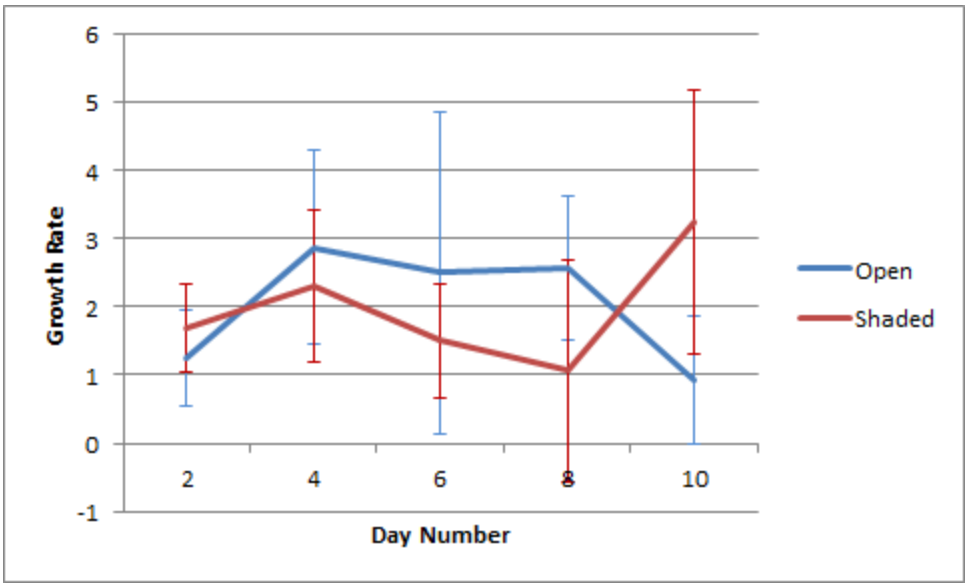

Fig. 8. Graph showing mean growth rate for length of shaded (red) combined samples and open (blue) combined samples over ten-day period at Hatfield Marine Science Center in Newport, OR. Error bars show 95% confidence interval for the individual days. Growth measured in mm/day.

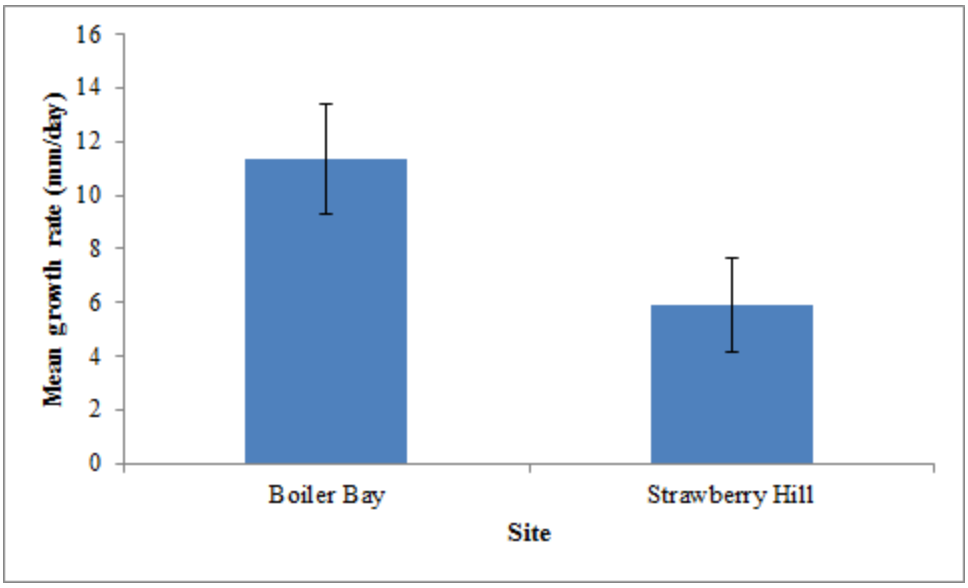

Fig. 9. Graph showing mean growth rate from field data at Boiler Bay (44.8331,-124.0628) and Strawberry Hill (44.2540, -124.1123). *Saccharina sessilis* samples were marked on May 28 2013 and were re-measured on June 2 and June 3. Error bars show 95% confidence interval for the individual days. Growth measured in mm/day.

Date	Time	Ambient Light (PAR units)	Shaded Light (PAR units)	Temperature (°C)
05/30/2013	12:00	764.2	392.5	11.1
05/31/2013	12:00	1286.5	392.5	10.9
06/01/2013	1:00	1342.7	401.7	10.9
06/02/2013	1:10	1297.4	396.4	11.0

Table 1. Data collected from the outdoor tanks at Hatfield Marine Science Center, Newport, OR over four days between May and June 2013. The ambient light was measured slightly submerged in the open colanders and shaded light was measured slightly submerged and under the shade cloth, mimicking the conditions of the *Saccharina sessilis* samples. Temperature was collected from HMSC tank data at the same time the light was collected.