Competency time and settlement preferences of zoospores of *Ulva fenestrata* habiting the mid intertidal

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FHL 470 Research in Marine Biology: Metamorphosis in the Oceans and Across Kingdoms

Spring 2019

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Keywords: Ulva fenestrata, bacterial biofilms, settlement, competency, zoospores

Abstract

Macroalgae are increasingly being considered as an important factor in natural ecosystems and in economies around the world. *Ulva* is one such genus that provides various ecosystem services to humans and marine environments. Within marine ecosystems *Ulva* is a primary producer, providing direct nutrition for herbivores such as sea urchins and snails, and creates vital habitat structures for juvenile and larval organisms. Ulva can be both a cost and a prize to our economies as is both a significant component of the aquaculture industry and a major source of marine biofouling. Ulva zoospores have been known to settle due to bacterial cues and have an observed distribution primarily in the mid to low intertidal. This study attempted to quantify settlement of the quadriflagellate zoospores of *Ulva fenestrata* on bacterial biofilms from a variety of tidal heights. The goal was to ascertain if bacterial assemblages specific to tidal height were an impacting settlement factor. Competency time was also investigated to ascertain how long *Ulva fenestrata* zoospores remain motile. All bacterial biofilms were found to have equal recruitment, with no statistical differences in spore settlement. Zoospores were found to remain motile for up to three hours and 30 minutes with some variability. These findings indicate that observed distributions of *Ulva fenestrata* are due to some nonbacterial settlement cue or outside pressure and opens up further research questions into the nature of this cue.

Introduction

Ulva sp. (Chlorophyta, Ulvophyceaae) as well as other marine macroalgae provides, various ecosystem services to humans and marine environments. Within marine ecosystems macroalgae are large primary producers, providing direct nutrition for herbivores such as sea urchins and snails, and create vital habitat structures for juvenile and larval organisms. For instance, in Hood Canal, WA, up to 28% of net primary production was found to be from green macroalgae like *Ulva* sp. (Simenstad and Wissmar 1985). In New Jersey, areas with *Ulva* had a higher density of decapods and epibenthic fishes (Sogard and Able 1991). Detritus produced by macroalgae can also power other ecosystems by being a resource subsidy to deep water or terrestrial ecosystems (Liebowitz et al. 2016).

Macroalgae such as *Ulva* can be both a benefit and a cost to our economies. The benefits come largely from the aforementioned ecosystem services they provide as well as their being a significant component of the aquaculture industry. First, bioenergy and biochemicals can be obtained from seaweeds (Bruhn et al. 2011). In 2013 alone 26,978,000 tons of aquatic plants were cultivated around the world for these purposes as well as for direct and indirect human consumption (FAO 2016). *Ulva* in particular serves as a human food and livestock fodder and, is present in a variety of cuisines served around the world (Makkar et al. 2016). *Ulva* is consumed primarily in Japan as ao-nori and can be a dietary staple providing macronutrients such as protein (Fujiwara-Arasaki et al. 1984). However in addition to these diverse benefits, *Ulva* can have societal costs. For example, this same alga often causes marine biofouling on ships, increasing fuel costs and decreasing speed of marine vessels (Schumacher et al. 2007). Because *Ulva* has such diverse impacts, its settlement behaviors and time of competency are of interest.

Ulva sp. disperses its spores as plankton just as many animals do. The spores spend time drifting, before selecting a site appropriate for settlement based on a variety of biotic and abiotic factors. Recruitment is based both on the availability of an appropriate site and the length of time during which spores are competent to settle. *Ulva* sp. has distinct swimming behaviors that allow it to explore a variety of substrates by temporarily binding to them before using a glycoprotein to initiate final settlement (Heydt et al. 2012).

Settlement of *Ulva* has been shown to be linked to biofilm, which is the film of organic matter formed by microorganisms like bacteria (Park et al. 2011). It has specifically been linked by Joint et al. (2007) to the presence of bacteria producing N-acylhomoserine lactones. Presence of biofilm has been linked to accelerated settlement of green algae and high species richness (Park et al. 2011). Additionally, it has been shown that alga like *Ulva mutabilis* are dependent on regulatory factors that are excreted by bacteria for growth (Spoerner et al. 2012)

In this experiment, settlement of zoospores of *Ulva fenestrata* (Postels & Ruprecht) were tested on biofilms from a variety of tidal heights to ascertain if the tendency of this species to settle in the mid-low intertidal is a choice based on the biofilm of that tidal height. It was also a goal to quantify the competency period of *Ulva fenestrata*, something that has received much attention with invertebrate larvae, but is less present in the literature with regard to algal spores.

Methods

Intertidal biofilms were collected from the University of Washington's Friday Harbor Labs beach (GPS 48.545919, -123.011593). The biofilms were from low, mid and high intertidal heights and a subtidal floating dock. Subtidal biofilm was collected from the University of Washington's Friday Harbor Labs floating dock (GPS 48.545688, -123.012751). For the purposes of this study the high intertidal was between +1.5 to +0.8 m MLLW. Mid intertidal was +0.7 to -0.2 m and low intertidal was -0.1 to -1.5 m MLLW. Subtidal biofilms were collected at about 0.5 m deep off a tire on the side of a floating dock. Because Schumacher et al. (2007) have previously found roughness of the settlement substrate to impact settlement, all rocks were visually inspected and scored to control for roughness.

Ulva fenestrata was collected at low tide from the University of Washington's Friday Harbor Labs beach (GPS 48.545919, -123.011593) on May 23rd, 24th, 27th and 28th. Specimens were then inspected to ascertain if a zoosporophyte or gametophyte was obtained; only zoosporophytes were used in the current study. *Ulva fenestrata* zoosporophytes were placed in two paper towels and left to dry for 2 minutes, then placed in a beaker with 500 mL of filtered seawater. If spores were released by the alga within 30 min of collection, an initial spore density was calculated, and 3.9×10^4 spores were added to 12 finger bowls each containing 60 ml of filtered seawater (filtered with a 45 micron mesh) and bacterial biofilms from a variety of tidal heights.

Three controls were used; a bowl with an autoclaved rock, a control with no biofilm and a control with no biofilm and sterilized filtered sea water. Sea water was sterilized by being put in a flask and being brought to a gentle boil in a microwave. Two kinds of bacterial biofilms were collected or made for the treatments: natural and 'manmade'. Natural bacterial biofilms were isolated by chiseling a 2x2 cm piece of bedrock from each intertidal zone. The subtidal rock was produced by scraping a trash can off the side of the dock and introducing the bacterial biofilm to an autoclaved rock. 'Manmade' biofilms were produced by attaching petri dishes to rocks at different intertidal and a subtidal height using fishing line. After 7 days, petri dishes were brought into the lab and two 2x2 squares were obtained from each petri dish.

Over the course of 4 hours after spore addition to the treatment bowls, spore density was calculated every 20 minutes using a hemocytometer. One drop of liquid was transferred to the hemocytometer using a glass pipette; the average of 4 replicate spore counts was then used to calculate the spore density per ml in each treatment bowl at each sampling time The hemocytometer and pipette were cleaned using tap and RO water to prevent transfer of spores between samples and the process was repeated. Light was randomized by conducting the experiment at different times throughout the day in different weather conditions.

Finlay et al. (2008) found that *Ulva* sp. spores can exhibit variable growth responses to different colors. While no studies have been done on color and settlement response in *Ulva* zoospores, this result indicates color could affect other parts of the *Ulva* life history, therefore all bowls were placed on a laminated white sheet to limit color variation (Finlay et al. 2008). Bowls were kept in a sea table with a constant flow of ocean water around the bowls to maintain temperature, which was measured once every 60 minutes using a TITAN® Infrared Thermometer 55010 (temperature range -50 ºC – 450 ºC). A relatively constant temperature between about 10-12 ºC was maintained, which was important, as temperature can increase settlement responses in *Ulva* Sp. (Gao et al. 2017; Christie and Shaw 1968). Higher temperatures also reduce the attraction of *Ulva* zoospores to biofilm, so it was vital to control it as much as possible (Tait et al. 2005).

After all data was collected it was analyzed with linear regressions examining number of spores remaining in suspension over time. The slopes of the lines created by these linear regressions were then used to run an ANOVA in excel to see if there were any similarities in trends. To ascertain the competency period of the zoospores, the mean of all time periods within which a suspended spore concentration of 0 was hit was taken. Due to experimental constraints the subtidal biofilm and the sterilized sea water control were only run twice each and never on the same day.

Results

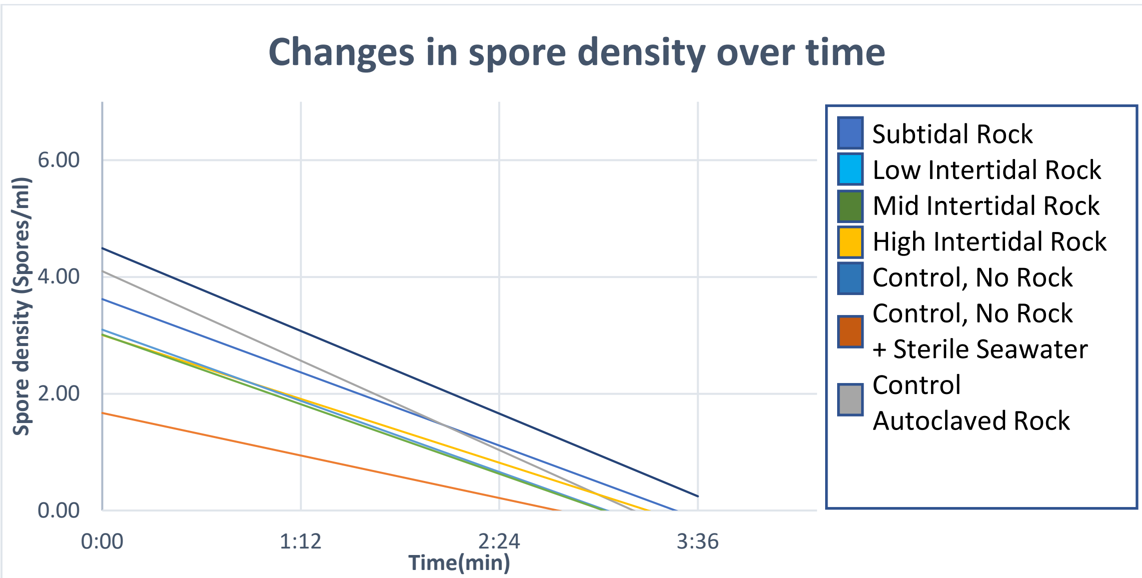

Figure 1 (above). Linear regression lines of changes of *Ulva* spore density over time

Table 1 (below). R^2 and P values for linear regression lines in figure 1

	R^2	P	Treatment repetitions
Subtidal Rock	0.4796	0.000177	2
Low Intertidal Rock	0.4294	4.33E-07	8
Mid Intertidal Rock	0.3802	3.07E-06	8
High Intertidal Rock	0.3997	1.44E-06	8
Control, No Rock	0.3139	3.46E-05	8
Control, No Rock+ Sterile Seawater	0.5477	3.57E-05	2
Control Autoclaved Rock	0.3602	6.51E-06	12

As seen in Figure 1, spore density changed evenly in all substrates with almost constant rates. As seen in Table 1, linear regression described the results well, and no statistically significant differences were found in the zoospore settlement rate (ANOVA, P=0.797533). Results were only obtained from the natural biofilm repetitions as the 'manmade' biofilms were lost to the ocean, thus no standard rate of spore settlement over time was obtained in these latter treatments. Zoospore density reached zero on average after 163 minutes from their release from an adult alga, with a standard deviation of 47.0 minutes. The competency period for *Ulva fenestrata* (i.e. the maximum time until the zoospore density reached zero in all of the treatments) was found to be 210 minutes after release. Despite this, there was high variability, with one sample being fully settled at only forty minutes after release.

Discussion

The results of this experiment are unexpected as Joint et al. (2007) found that the presence of bacterial biofilms enhanced settlement and that the number of bacteria in a biofilm was positively correlated to spore settlement. There has been much research to learn which bacterial biofilms attract or repel *Ulva* zoospores, so we know there is at least a capability to sense bacteria, but settlement behaviors with regards to tidal height seem to be unvaried. Future studies should investigate if zoospores are not using this sensing capability to discriminate which tidal heights are optimal for settlement at all.

The behaviors of zoospores are important because they can be vital both to the successful recruitment of new crop in aquaculture industries and the successful prevention of marine biofouling for cargo ships and military vessels. While no preferences for bacterial biofilms were found, *Ulva* found around the Friday Harbor Laboratories is regularly observed to grow primarily in the mid to low intertidal and the subtidal floating dock. Further research into the mechanisms used by zoospores to choose these preferred areas could be extremely valuable to improving our understanding of this important macroalga.

Future experiments should determine if the observed distribution of *Ulva fenestrata* is due to some other, nonbacterial, mechanism used as a settlement cue. The mid intertidal for example, is prone to desiccation and low salinity from freshwater input. This could affect settlement of zoospores. Herbivory could also be regulating from the top down at heights where spores are successfully growing into mature adults.

Naturally, results found here can be due to many factors and it is important to consider situations in which bacterial cues not employed in this experiment could still be a settlement factor. An uncontrolled variable was the age of biofilms. A study found that *Ulva fasciata* attached variably to biofilm that was 1, 3, 6 or 9 days old (Shin 2008). As the natural biofilm was collected directly from its environment its age is an unknown. As Shin (2008) found less attachment to older biofilm, the fact that the biofilm substrate had most likely been in the ocean for a significant period of time could be impacting the ability of *Ulva fenestrata* zoospores to attach. A future experiment should control the age of biofilms to rule out this variable.

Another observation during the experiment was that spore density went up and down over time and was not entirely a linear downward slope. This could be an example of surface testing, which is a process whereby zoospores temporarily attach to a substrate and then release if the surface is not optimal (Callow et al. 1997). A future experimental design should account for such behavior.

We also learned that our observed spore densities can be variable due to clumping; the hypothesis for this behavior is that zoospores could be attracted to other zoospores, creating a situation in which random pipette sampling could hit or miss a clump. This would explain a highly variable pattern of spore density. Further research into attraction between zoospores could further investigate this phenomenon as a potential factor in the timing of their settlement process.

As macroalgae becomes increasingly important to human populations, we realize the gaps in our knowledge are vast. This experiment attempted to learn about selection of zoospores with regards to tidal heights and attempted to qualify their competency period. What guides *Ulva* to its current distribution remains a question but hopefully the questions raised by this study can spark new research into a macroalga that holds importance for both marine ecosystems and the humans that increasingly depend on them.

Acknowledgements

I would like to thank Tom Mumford for his guidance, help and amazing phycology wisdom. Shannon Cefalu for being a huge support and writing down data when I was rushing to count zoospores. Mo Turner for being a statistics goddess. I would also like thank both Leland Wood for editing help and, both Leland and Morgan Thompson for supporting me from sunset to sun rise. Haley Mawson whose edits were both insightful and supportive. Ashley Cullen who not only is a great editor but has been a ray of sunshine throughout the process of writing. Jason Hodin without whose teachings on metamorphosis and larval settlement I wouldn't have been here and Karly Cohen for being awesomely supportive and helpful. Lastly I would like to thank everyone in the Zoobot program for all the sunset study breaks and just generally being awesome.

References

Bruhn A, Dahl J, Nielsen HB, et al (2011) Bioenergy potential of *Ulva lactuca*: Biomass yield, methane production and combustion. Bioresour Technol 102:2595–2604. doi: 10.1016/j.biortech.2010.10.010

Callow ME, Callow JA, Pickett-Heaps JD, Wetherbee R (1997) Ulvales) Propagules: Quantitative Settlement Studies and Video Microscopy'. J Phycol 33:938–947

Christie AO, Shaw M (1968) Settlement experiments with zoospores of *Enteromorpha intestinalis* (L.) link. Br Phycol Bull 3:529–534. doi: 10.1080/00071616800650141

Finlay JA, Fletcher BR, Callow ME, Callow JA (2008) Effect of background colour on growth and adhesion strength of *Ulva* sporelings. Biofouling 24:219–225. doi: 10.1080/08927010802040693

Food and Agriculture Organization (2016) The State of World Fisheries and Aquaculture (SOFIA) | FAO | Food and Agriculture Organization of the United Nations

Fujiwara-Arasaki T, Mino N, Kuroda M (1984) The protein value in human nutrition of edible marine algae in Japan. In: Bird CJ, Ragan MA (eds) Eleventh International Seaweed Symposium. Springer Netherlands, Dordrecht, pp 513–516

Gao G, Clare AS, Rose C, Caldwell GS (2017) Eutrophication and warming-driven green tides (*Ulva rigida*) are predicted to increase under future climate change scenarios. Mar Pollut Bull 114:439–447. doi: 10.1016/j.marpolbul.2016.10.003

Heydt M, Pettitt ME, Cao X, et al (2012) Settlement behavior of zoospores of *Ulva linza* during surface selection studied by digital holographic microscopy. Biointerphases 7:1–7. doi: 10.1007/s13758-012-0033-y

Joint I, Tait K, Wheeler G (2007) Cross-kingdom signalling: Exploitation of bacterial quorum sensing molecules by the green seaweed *Ulva*. Philos Trans R Soc B Biol Sci 362:1223–1233. doi: 10.1098/rstb.2007.2047

Liebowitz DM, Nielsen KJ, Dugan JE, et al (2016) Ecosystem connectivity and trophic subsidies of sandy beaches. Ecosphere 7:1–19. doi: 10.1002/ecs2.1503

Makkar HPS, Tran G, Heuzé V, et al (2016) Seaweeds for livestock diets: A review. Anim Feed Sci Technol 212:1–17. doi: 10.1016/j.anifeedsci.2015.09.018

Park SR, Kang YH, Choi CG (2011) Biofilm: A crucial factor affecting the settlement of seaweed on intertidal rocky surfaces. Estuar Coast Shelf Sci 91:163–167. doi: 10.1016/j.ecss.2010.10.013

Schumacher JF, Carman ML, Estes TG, et al (2007) Engineered antifouling microtopographies - Effect of feature size, geometry, and roughness on settlement of zoospores of the green alga *Ulva*. Biofouling 23:55–62. doi: 10.1080/08927010601136957

Shin HW (2008) Rapid attachment of spores of fouling alga *Ulva fasciata* on biofilms. J Environ Biol 29:613–619

Simenstad CA, Wissmar RC (1985) Carbon in Estuarine and Nearshore Food Webs. Mar Ecol 22:141–152

Spoerner M, Wichard T, Bachhuber T, Stratmann J, Oertel W (2012) Growth and thallus morphogenesis of *Ulva mutabilis* (Chlorophyta) depends on a combination of two bacterial species excreting regulatory factors. J Phycol 48: 1433–1447.

Tait K, Joint I, Daykin M, et al (2005) Disruption of quorum sensing in seawater abolishes attraction of zoospores of the green alga *Ulva* to bacterial biofilms. Environ Microbiol 7:229–240. doi: 10.1111/j.1462-2920.2004.00706.x