Nereocystis and diatoms, going with the flow

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ABSTRACT

The purpose of this experiment was to observe the effect of water flow on fertilization success of *Nereocystis* gametophytes and growth rates of young sporophytes. My null hypothesis was that there would be no differences between the flows. However, I found that in my experimental setup, benthic diatoms outcompeted the *Nereocystis* sporophytes and grew over them. I thus analyzed differences in diatom growth under different water flow treatments, and found that there was a clear trend of more diatom chlorophyll and organic biomass in high flow conditions.

INTRODUCTION

Macroalgae are vital contributors to coastal food webs with many species dependent on them. However, much is still unknown of their life histories; from settlement of their zoospores to what ultimately happens to their biomass. The biomass of macroalgae may enter the food web as particulate organic matter or dissolved organic carbon (Duggins & Eckman 1994). Observational and experimental studies have demonstrated that organic carbon derived from kelp is found throughout the food web, regardless of consumer feeding mode (Dunton & Schell 1987).

Few studies have been done to investigate the important processes involved in macroalgal propagation, particularly the events that happen after zoospore settlement. Reed (1987) found that fecundity in *Macrocystis* is largely determined by total vegetative biomass. For the successful recruitment of kelp, there must be completion of an alternate sexual generation, the microscopic gametophyte stage (Reed 1990). The biology of this gametophyte generation has been extensively studied; however, little is understood about its ecology. After the release of

motile zoospores from the adult sporophyte, they settle and germinate into either male or female gametophytes (Reed 1990). Gametophytes produce eggs or sperm, and when sexually mature they produce a visible sporophyte (Reed 1990). However, any events occurring shortly after fertilization may greatly influence sporophyte growth. In the same way as in land plants, various environmental factors determine whether a seed will germinate and how the surviving seedlings will be distributed in the soil (Reed 1990).

A number of factors affect the growth and production of macroalgae including light, nutrients, temperature, rates of herbivory, competition for space, and water motion (Hurd 2000). Water motion can influence all the other factors important to macroalgal growth. For example, rates of nutrient uptake by macroalgae are dependent on the velocity of the sea water (Wheeler 1988). Water motion also affects how far spores are carried from their origins. The importance of spore transport is crucial to understanding kelp interactions with surrounding species (Gaylord et al. 2011). Recruitment can depend strongly on competition for space and light, and on the ability of settled spores to adhere under rapid flows (Gaylord et al. 2011).

The hydrodynamic environment in which macroalgae grow also determines their ability to acquire and utilize essential nutrients, and other physiological response. Whitford and Schumacher (1964) found that for the filamentous green alga, *Oedogonium kurzii*, phosphorus uptake was over 10 times greater and respiration was over 70% greater in a current of 18 cm/sec than in still water. Wheeler (1980) demonstrated that macroalgal production in situ may be frequently limited by slow moving water; rates of photosynthesis and inorganic nutrient uptake increase with increasing mainstream velocities. Another experiment by Parker (1981) confirmed that macroalgal growth rates are reduced under still or very slow water movement compared with those in cultures that are stirred or aerated.

These studies imply that rates of macroalgal production should be higher in moderately wave-exposed environments than at sites with low energy. However, Gerard and Mann (1979) found that the annual production of *Laminaria saccharina* was greater in a wave-sheltered site than a wave-exposed site, and this was explained by greater nutrient availability at the wave-sheltered site.

Studies of factors affecting macroalgal recruitment and growth are difficult to conduct because so many parameters need to be controlled. The purpose of my experiment was to observe the effect of water flow on fertilization success of *Nereocystis* gametophytes and on growth rates of young sporophytes. My null hypothesis was that there will be no differences between the flows. Alternatively one could predict that fertilization of gametophytes and faster growth of kelp sporophytes will occur in fast flow because rates of photosynthesis and inorganic nutrient uptake increase with increasing water flow.

METHODS

My study was done at University of Washington Friday Harbor Laboratories in a large seawater table. The water table that also was connected to the seawater system held an array of clear water pipes. All pipes were submerged in the water table (flow through) to maintain ambient temperature. The table was surrounded by a large black plastic tarp to prevent any light exposure from the outside. Water temperature was controlled by all experimental pipes sitting in seawater pumped directly from the sea. Light for all treatments was provided by 5 bright overhead lights. Light measurements were taken by a Licor light meter once during the whole experiment.

Sori of *Nereocystis luetkeana* were collected near San Juan Island from four different blades of one *Nereocystis* frond. On that same day, each sorus was cut into thirds and placed on top of a salt-water dampened paper towel and layered with another salt-water dampened paper towel, then another sorus; this process was repeated for all blades. Layers of paper towel and sori were left overnight at three different temperatures; 4^{o}C, 10^{o}C, and room temperature. The next day, all sori were taken out of the paper towels and placed into one bucket of sea water filtered to 4 microns. The bucket was kept in the cold room at 10^{o}C. 6 hours later the sori solution was poured into a dish containing 44 small plastic discs of ~7cm^2 with rough surface facing up and smooth surface facing down. This dish was left in the cold room overnight. The next day each disc with an equal exposure to *Nereocystis* zoospores on it was carefully screwed on to plastic pvc strips (6 discs per strip), which was then screwed in place inside a clear plastic tube.

All plastic tubes were placed into the seawater table (Figure 1). In this way we prepared 3 pipes for high flow, 3 pipes for slow flow and 1 pipe for no flow. The water flows were adjusted so that highs are ~0.5m/s and lows are ~0.025m/s. Flow rates were measured every other day by using a stopwatch to time how fast fluorescent dye would flow through a meter of pipe length. It was difficult to use fluorescent dye for the low flows because of so much dissipation, so we timed particles moving in the tubes along 10 centimeters. To control for effects of flow always being in one direction, the pipes were flipped every other day.

After a month, each disc was taken out of the pipes. The discs became so covered with colonial benthic diatoms that few kelp sporophytes were visible enough to measure. I thus

changed my sample design to quantify the abundances of diatoms in the different flow treatments.

Three discs each from the high and low flow pipes and from the no flow pipe were used to collect ash free dry weight of accumulated algae; this was done by scraping all algae off each discs onto pre weighed aluminum weigh boats and placing into a dry oven at 50^{o}C for 24 hours. Samples were weighed again and then placed into a muffle furnace at 550^{o}C for 5 hours and were again weighed. From each pipe the remaining 3 discs in addition to 3 clean plastic discs for control purposes were used to measure chlorophyll absorbance. This was done by cutting each disc into half and placed into 50mL plastic centrifuge tubes filled with 25mL pure methanol. The tubes were placed in a refrigerator overnight and the next day were centrifuged at 10000 rpm for 10 minutes. The supernatant was then used for measurements in the spectrophotometer and the absorbance was read at 665 nm. The null hypotheses for this experiment were that there will be no differences in biomass or chlorophyll concentration with flow.

RESULTS

Flow rates were highly significantly different in the high and low flow pipes. The high flow had an average of 0.46m/s and low flow an average of 0.03m/s (Fig. 2). Standard errors for both treatments were very low (Fig. 2). Differences in flows are significant at $p < 0.001$, thus I rejected the null hypothesis that there are no differences in flows.

Light measurements were very similar among all the pipes. The high flow pipes averaged 104.2 µmoles/m2/s and low flow pipes 98.3 µmoles/m2/s. These amounts were not significantly different (Table 2; $p > 0.05$)

An abundance of diatoms were observed growing on the discs, appearing different among flow treatments. The no flow treatment had a thin layer of growth, while both high and low flows had thick layers of growth. The high flow had darker and longer strands of diatoms than the low flow which had lighter and shorter, fuzzier strands of diatoms.

Algal dry weight on the discs clearly varied with flow treatments (Fig. 4). These differences were significant overall (Table 4). Pairwise comparisons showed that only high and no flow treatments were significantly different (Table 4).

The chlorophyll absorbance for each treatment is shown in Figure 6. As with the dry weight data, chlorophyll was significantly different among treatments (Table 5). The comparisons between low and no flow were not statistically significant (Table 6; $p > 0.05$), but were significantly different when comparing high with low and no flow.

DISCUSSION AND CONCLUSIONS

The initial focus for this study was growth and fertilization of *Nereocystis* sporophytes in different flows. However, as the experiment proceeded and after one month had gone by, no sporophytes were visible and instead abundant growths of diatoms were seen. This led to a new question of whether there was a difference in diatom growth in the different flow treatments. .

Growth of diatoms was clearly greater in pipes under high flow than under low flow or no flow. This result was seen both in the diatom biomass (measured as dry weight) and in the amount of chlorophyll (measured as absorbance). All pairwise comparisons among treatments probably would have been significant if there had been more samples.

It was interesting to see that the disc from no flow had the least diatom growth, since it was the pipe that should have been receiving the most amount of light, but nutrients from no flow could have been a limiting factor. It was especially interesting to see that there were actually inorganic materials in the high flow because one would assume that sediment or other inorganics would be unable to settle onto the high flow pipe (figure 5). However, this may be due to the disruptions of growth and boundary layer present in the pipes.

In regards to the *Nereocystis* sporophytes, a few were seen under the microscope but were too low (and too small) to take measurements on. They were buried under many diatoms, which may have outcompeted them. Competition between diatoms and sporophytes could have been for light and/or nutrients.

Competitive mechanisms could be tested further by first settling the sporophytes under clean cultures until they become visible before placing them into the water pipe treatments. This would make it impossible to test the effect of flow on fertilization success of settling sporophytes, but the question of flow rates affecting growth could still be tested. A different possible approach to improve this experiment could be placing the settling sporophytes in the pipe treatments under low light to prevent the diatoms from growing. Once visible sporophytes can be seen brighter control light can be used.

In the process of this experiment, various sources of potential error arose. For example: scraping off diatoms into weigh boats, cleaning off equipment during absorbance measurements, cutting the discs in half, or carrying the weigh boats to and from different labs. In the future a larger sample size is recommended, as well as more than a one month time period for growth.

In conclusion, this experiment (in spite of setbacks regarding the original question) demonstrated significant differences in growth of benthic diatoms among different flow treatments. The same effects could be possible for *Nereocystis* growth and I believe a new, improved experiment on that topic would be interesting.

ACKNOWLEDGEMENTS

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Figure 1: Picture of experimental setup. The entire table was covered by a large black plastic tarp to prevent any light exposure from the outside.

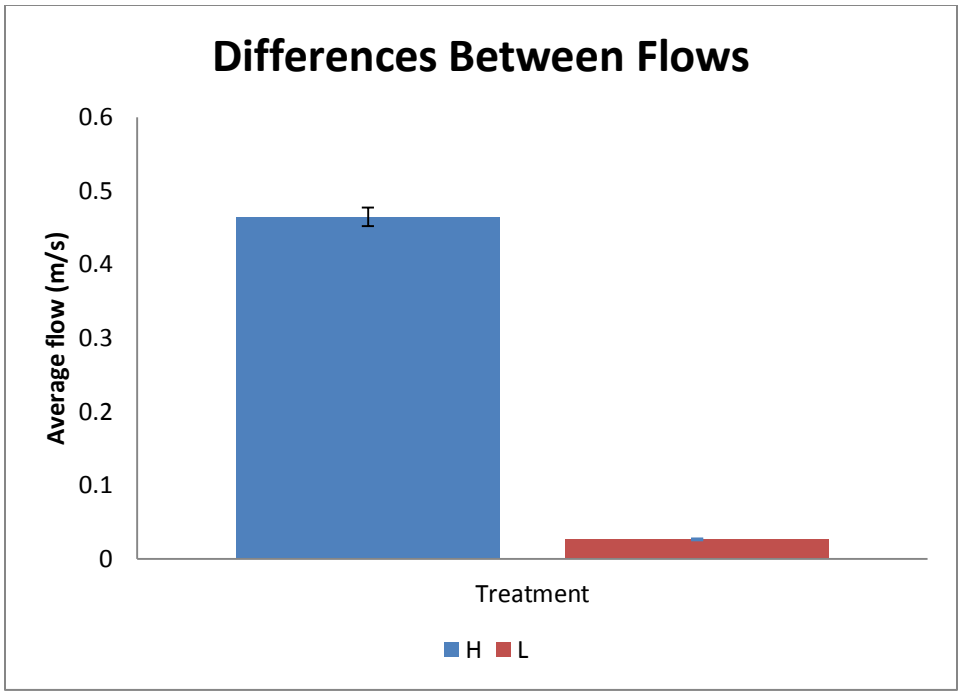

Figure 2: Bar graph showing the differences between H (high flow) and L (low flow).

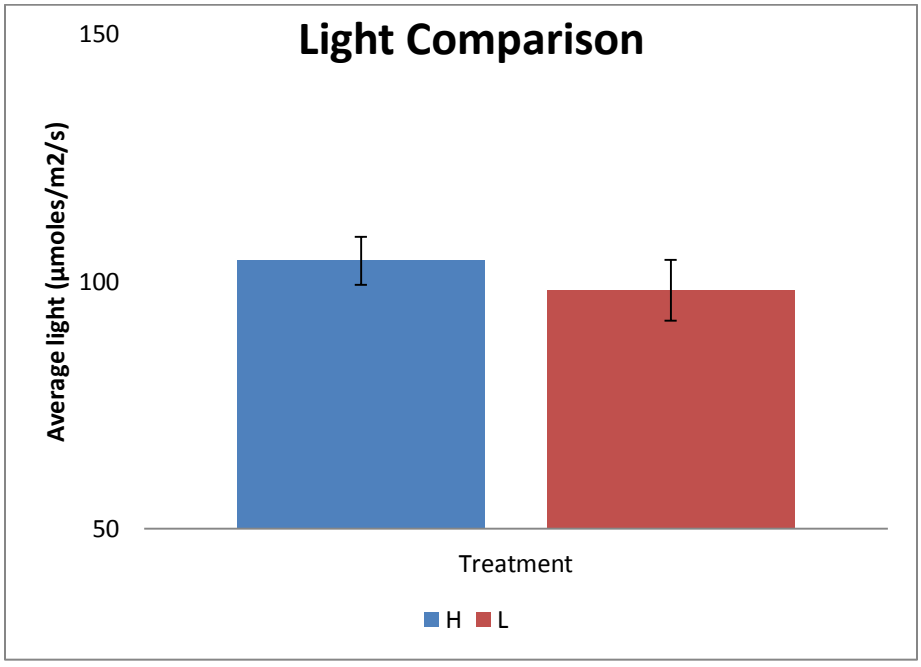

Figure 3: Compares the average amount of light that each treatment (high and low) receives.

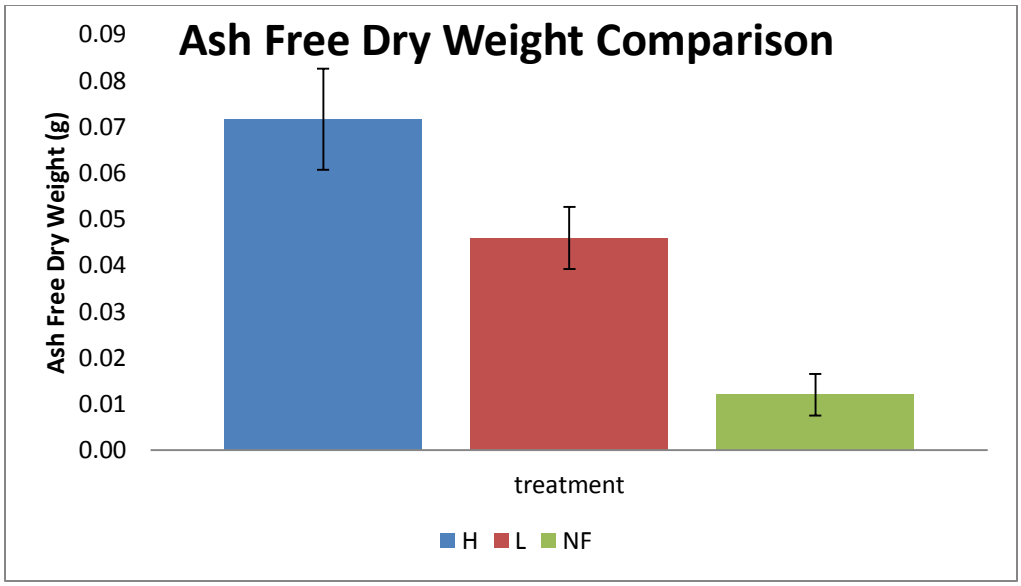

Figure 4: Compares the average ash free dry weight of algal growth on discs from H (high flow), L (low flow) and NF (no flow) pipes.

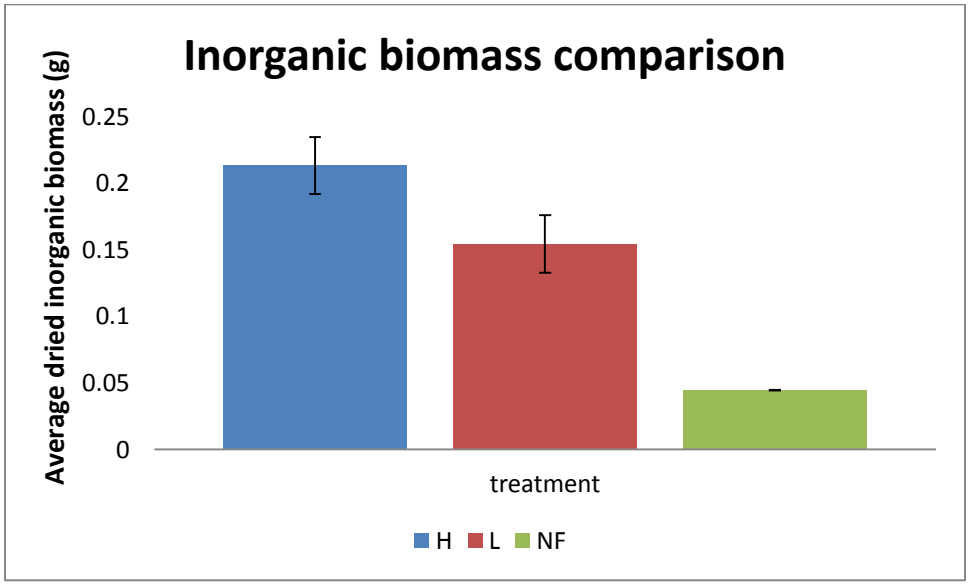

Figure 5: Compares the average inorganic biomass of algal growth on discs from H (high flow), L (low flow) and NF (no flow) pipes.

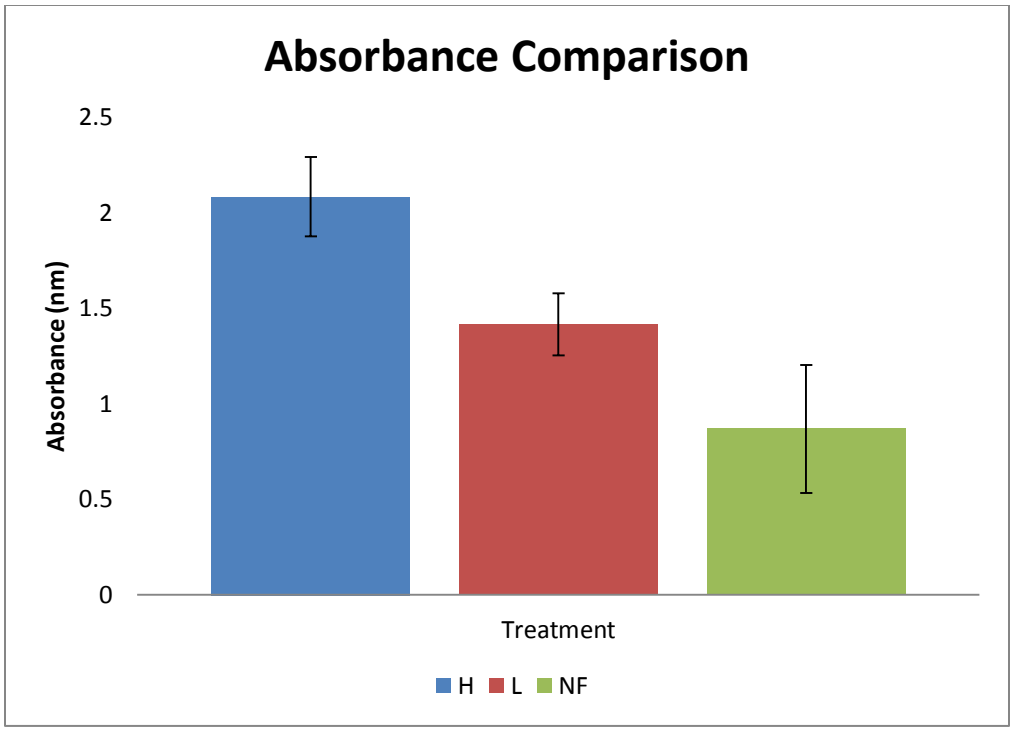

Figure 6: Comparison of chlorophyll absorbance among the growth on discs from H (high flow), L (low flow) and NF (no flow) pipes.

Table 1: Showing the differences between high and low flows.

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
TREATMENTS	12.959	1	12.959	1,247.42	0.000
Error	2.784	268	0.01		

Table 2: Compares the average amount of light that each treatment (high and low) receives.

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
TREATMENTS	369.291	1	369.291	0.573	0.453
Error	25,771.32	40	644.283		

Table 3: Compares the average ash free dry weight of algal growth on discs from H (high flow), L (low flow) and NF (no flow) pipes.

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
TREATMENTS	0.009	2	0.004	6.509	0.007
Error	0.012	18	0.001		

Table 4: Pairwise comparisons of the ash free dry weight data.

Tukeys's Honestly-Significant-Difference Test					
TREATMENT$(i)	**TREATMENT$(j)**	**Difference**	**p-Value**	**95% Confidence Interval**	
				Lower	**Upper**
H	L	0.026	0.113	-0.005	0.057
H	NF	0.06	0.007	0.016	0.103
L	NF	0.034	0.147	-0.01	0.078

Table 5: Comparison of chlorophyll absorbance among the growth on discs from H (high flow), L (low flow), and NF (no flow) pipes.

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
TREATMENTS	4.007	2	2.004	6.356	0.008
Error	5.674	18	0.315		

Table 6: Pairwise comparisons of chlorophyll absorbance data.

Tukeys's Honestly-Significant-Difference Test					
TREATMENT$(i)	TREATMENT$(j)	Difference	p-Value	95% Confidence Interval	
				Lower	Upper
H	L	0.668	0.053	-0.007	1.344
H	NF	1.215	0.012	0.26	2.171
L	NF	0.547	0.332	-0.408	1.502