Growth Rates of Selected Pacific Northwest Diatoms and the Potential for Biofuel Production

Angela Little[1]

Under the direction of Dr. Charles J. O'Kelly, Ph.D[2]

University of Washington Friday Harbor Laboratories

BIOL 479 Zoobots Spring 2011

[1]Department of Biology, University of Washington, Seattle, WA 98195

[2]Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250

Keywords: Skeletonema, Fragilaria, Diatoms, Biofuels, Growth rates

Abstract

Algae may be an answer to the world's future oil crisis but a lot of groundwork needs to be done before algae can provide enough oil to keep up with human consumption. Environmental stresses can cause diatoms to produce higher lipid contents and grow at different than normal rates. Three species of Pacific Northwest diatoms were observed in four different nutrient media, including two nutrient-reduced media, and in two different temperatures for highest growth rates. One species of *Fragilaria sp.* (strain # F084) showed little or no growth, while another species of *Fragilaria sp.* (strain # F074) grew best when all essential nutrients were present. *Skeletonema sp. sp.* (strain # F164) grew well in nutrient reduced media (F/2+ 1/2 $NaNO_3$ and F/2+ 1/2 $NaNO_3$) , as well as pink F/2+, and another species of *Fragilaria sp.* (F074). grew best when all essential nutrients were present. This pilot study is the basis for future experiments into finding which Pacific Northwest diatom may be worthy of algal biofuel candidacy.

Introduction

Fuel from algae is not a new concept, however, algal biofuels have gained major attention in recent years, due to the discovery that microscopic photosynthetic algae, known as microalgae, can double their mass several times in a 24-hour period, with more than half of that mass potentially being lipids. They can also produce several types of lipids, hydrocarbons or other complex oils (Lv et al., 2010). The class of algae known as diatoms (*Bacillariophyceae*) make up the largest fraction of marine phytoplankton and are the largest biomass producers in the world (Demirbas, 2010). Stress conditions, such as nutrient depletion, can induce production and storage of neutral lipids and complex oils through a process of triacylglyceride (TAG) synthesis and accumulation late in the stationary growth phase. When cell division blockage occurs due to nutritional deficiency and photosynthetic assimilation continues carbohydrates are stored as lipids. Growth rates and lipid storage responses vary among diatom species. Some increase lipid content, others may retain current lipid content but decrease growth rate (Popovich et al., 2011). High neutral lipid storage combined with a high growth rate is the best combination to produce algal biofuels. Finding the ideal growing conditions with the right strain is vital to the future of the industry.

Skeletonema sp. is a genus containing several species making this genus widely distributed, easily culturable, and tolerant to ranges of salinities, temperatures, light intensity, and nutrient conditions (Sarno et al., 2005). A study done by Griffiths et al. (2009) report a doubling time of ten to fifteen hours with up to 30% dry weight in lipids under nutrient-replete and nitrogen deprived conditions for the tropical species *Skeletonema sp. costatum.* Montagnes et al. (2001) report a growth rate of 0.7 μ d^{-1} at 16℃ and 0.8 μ d^{-1} at 25℃. High growth rates of *Skeletonema sp. sp.* (F164) are expected under all nutrient and temperature conditions tested here.

The genus *Fragilaria sp.* is largely freshwater and is often abundant in lakes. There are over 86 known species found throughout the world. Two different marine species of Fragilaria, F074 and F084, have been chosen to measure growth rates. Identification of species without DNA sequencing is difficult but morphologies vary in each of the chosen species. F074 is a chain forming diatom with long valve plates (see figure 1), F084 was not observed in chain form and has a more round valve plate (figure 2). One study done by Descamps-Julien et al. (2005) on *Fragilaria sp. crotonensis* showed some growth at 24℃ but higher growth between 6 and 18℃. Based on this, expected growth for *Fragilaria sp. sp.* should be higher in 15℃ than in 23℃.

This study was designed to examine the growth rates of these three diatom species, native to Friday Harbor Washington, under selected growing conditions. Two temperatures, to simulate tropical and temperate water temperatures, and three nutrient media were selected to detect ideal growing conditions to ask which will perform better in which nutrient media and can our local species grow in warmer water. The specific species of diatoms were carefully selected for their abundance in the marine waters of Friday Harbor Washington as potential algal biofuel candidates.

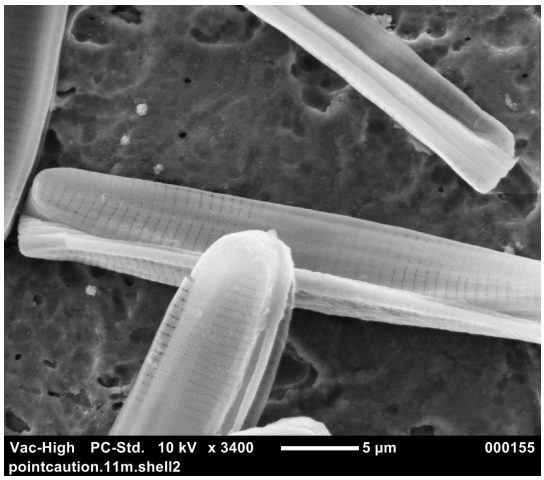

Figure 1. SEM of F074 *Fragilaria sp.*

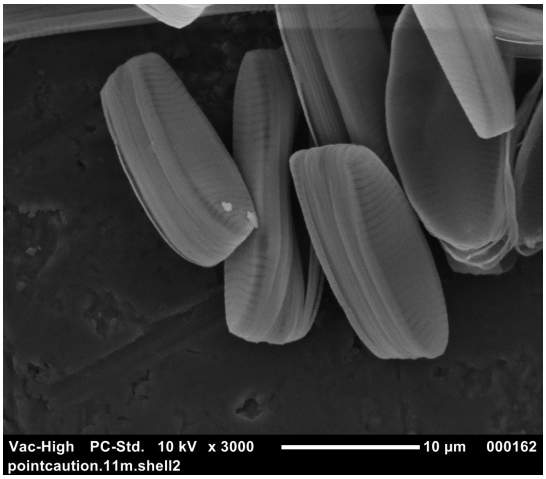

Figure 2. SEM of F084 *Fragilaria sp.*

Methods

Strains of *Skeletonema sp.* (F162). and *Fragilaria sp.*. (F074, F084) were obtained from Dr. Charles O'Kelly's collection at the University of Washington Friday Harbor Laboratory (FHL). Previously prepared f/2+ media (pink f/2+) was also obtained from Dr. O'Kelly's inventory to be used as a control and uses a different chelator than f/2+ media made at FHL (FHL f/2+). The diatom strains were allowed to grow to stock culture in pink F/2+ for nine days in 23℃ and 15℃ with 40 ± 10 µE/m^2/second of quantum irradiance from cool-white fluorescent lamps. Approximately 4 mL stock cultures were transferred into 75 mL of pink F/2+ media and allowed to cultivate for an additional five days in each temperature as the control before nutrient media were prepared.

Three media were prepared using Guillard's f/2 media protocol (1975), FHL F/2+ media, Phosphate reduced by 1/2 (f/2+ ½ NaH_2PO_4), and Nitrogen reduced by 1/2 (f/2+ ½ $NaNO_3$). 75mL were dispersed into three flasks for each temperature followed with 4 mL of each of the diatom stock cultures and grown in 23℃ and 15℃ with 40 ± 10 µE/m^2/second of quantum irradiance from cool-white fluorescent lamps.

On the first day following nutrient media preparation 1 mL of medium samples were placed into Eppendorf tubes with 0.04 mL potassium iodide to inhibit cell growth. Cells were counted using a hemocytometer. Cell counts were repeated each day for five days to determine a rate of growth per day.

Results

Comparing the pink F/2+ with the FHL F/2+ (Figure 3) shows a difference in the number of cells per µL for each water temperature. *Fragilaria sp.* F074 at 15℃ in the FHL F/2+ performed better than it did in the pink F/2+ and at 23℃ *Fragilaria sp.* F074 performed better in the pink F/2+. For *Skeletonema sp. sp*. the Friday Harbor prepared F/2+ media performed better at both temperatures (Figure 4) in the FHL F/2+.

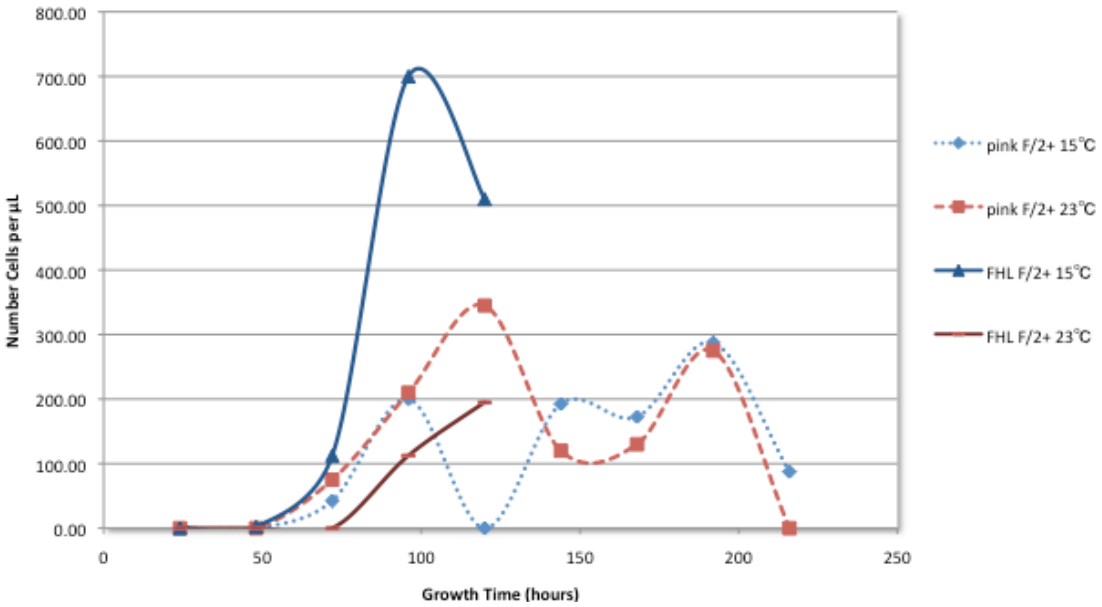

Figure 3. Growth rate of *Fragilaria* F074 comparing pink F/2+ and FHL F/2+ at 15℃ and 23℃.
Doubling times: no observed exponential growth in pink F/2+, FHL F/2+ at 15℃ = 22.0 hours, FHL F/2+ at 23℃ = 30.2.
Time constraints only allowed for 120 hours of data from FHL F/2+.

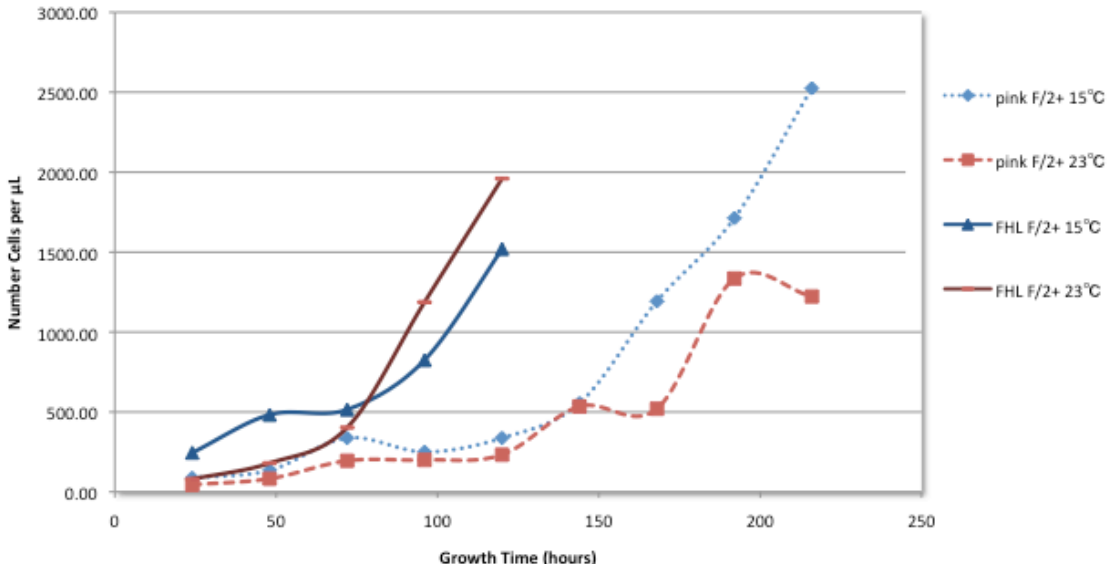

Figure 4. Growth rate of *Skeletonema* comparing pink F/2+ and FHL F/2+ at 15℃ and 23℃.
Doubling times: pink F/2+ at 15℃ = 33.0 hours, pink F/2+ at 23℃ = 38.9 hours, FHL F/2+ at 15℃ = 30.7 hours, FHL F/2+ at 23℃ = 21.0.
Time constraints only allowed for 120 hours of data from FHL F/2+.

Growth rates for *Fragilaria sp.* F074 at both temperatures in FHL f/2+, f/2+ ½ NaH_2PO_4, and F/2+ ½ $NaNO_3$ were calculated by the slope. Correlations between growth time and number of cells per µL at 15℃ (Figure 5) and growth rates were observed in FHL f/2+ ($y = 7.1563x – 250.25$, $R^2 = 0.7166$) and f/2+ ½ $NaNO_3$ ($y = 6.5417x – 187.5$, $R^2 = 0.71629$). At 23℃ (Figure 6) FHL f/2+ shows a higher correlation ($y = 2.0938x – 89.25$, $R^2 = 0.79479$) and F/2+ ½ $NaNO_3$ shows little correlation ($y = 1.1042x + 19.5$, $R^2 = 0.30706$).

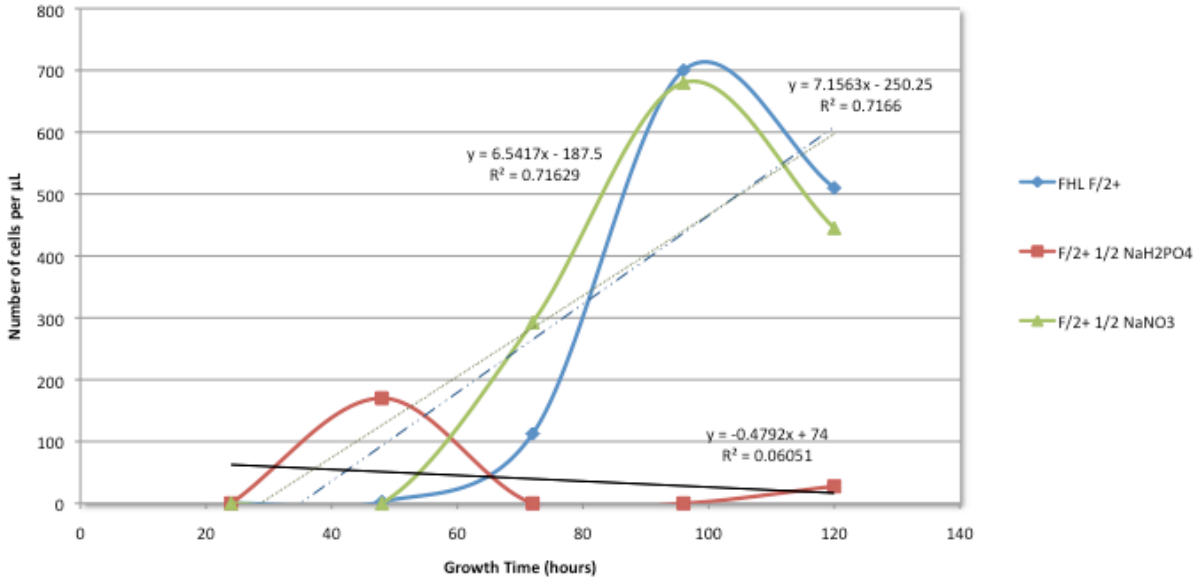

Figure 5. Growth curve for *Fragilaria* F074 at 15℃ in FHL F/2+ (Growth Rate = 7.1563, $R^2 = 0.7166$), F/2+ 1/2 NaH_2PO_4 (Growth Rate = -0.4792, R2 = 0.06051), and F/2+ 1/2 $NaNO_3$ (Growth Rate = 6.5417, $R^2 = 0.71629$).

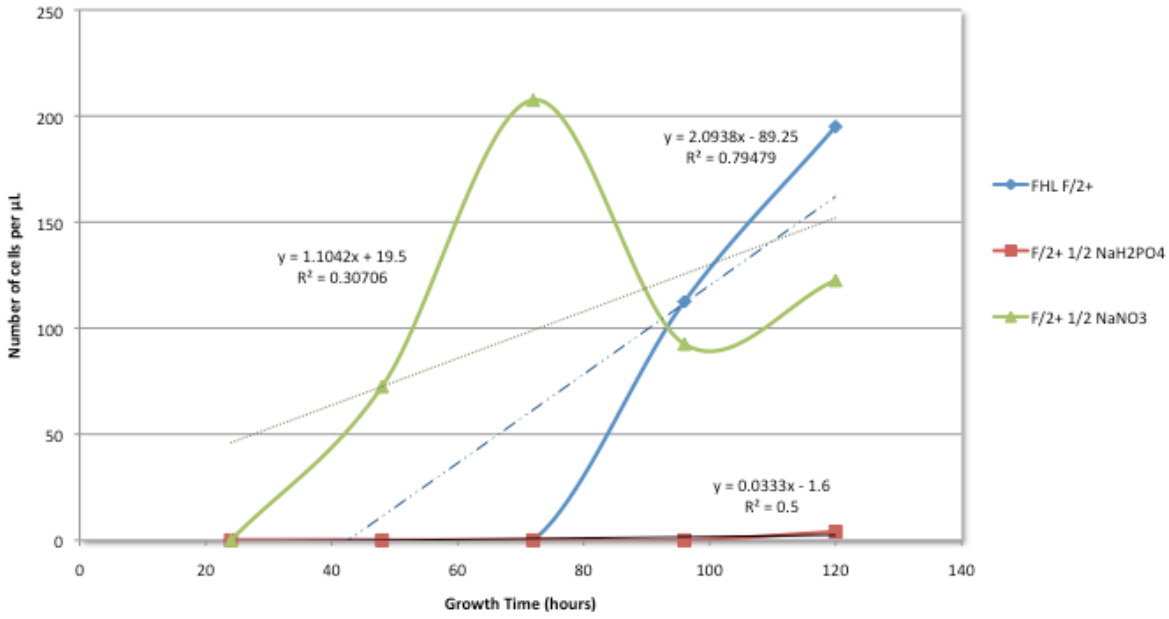

Figure 6. Growth curve for *Fragilaria* F074 at 23℃ in FHL F/2+ (Growth Rate = 2.0938, $R^2 = 0.79479$), F/2+ 1/2 NaH_2PO_4 (Growth Rate =0.333, R2 = -1.6), and F/2+ 1/2 $NaNO_3$ (Growth Rate =1.1042, $R^2 = 0.30706$).

Growth rates for *Skeletonema sp.* F164 at both temperatures in FHL F/2+, F/2+ ½ NaH_2PO_4, and F/2+ ½ $NaNO_3$ were calculated by the slope. Correlations between growth time and number of cells per µL at 15℃ (Figure 7) and growth rates were observed in FHL F/2+ ($y = 12.052x – 150.25$, $R^2 = 0.85806$), F/2+ ½ NaH_2PO_4 ($y = 19.865x – 668.25$, $R^2 = 0.9774$) and F/2+ ½ $NaNO_3$ ($y = 8.8229x + 364.25$, $R^2 = 0.9774$). At 23℃ (Figure 8) FHL F/2+ shows a lower correlation ($y = 2.0938x – 89.25$, $R^2 = 0.79479$), F/2+ ½ NaH_2PO_4 shows a higher correlation ($y = 1.6333x + 2.3$, $R^2 = 0.98088$) and F/2+ ½ $NaNO_3$ shows lower correlation ($y = 6.8542 + 222$, $R^2 = 0.88431$).

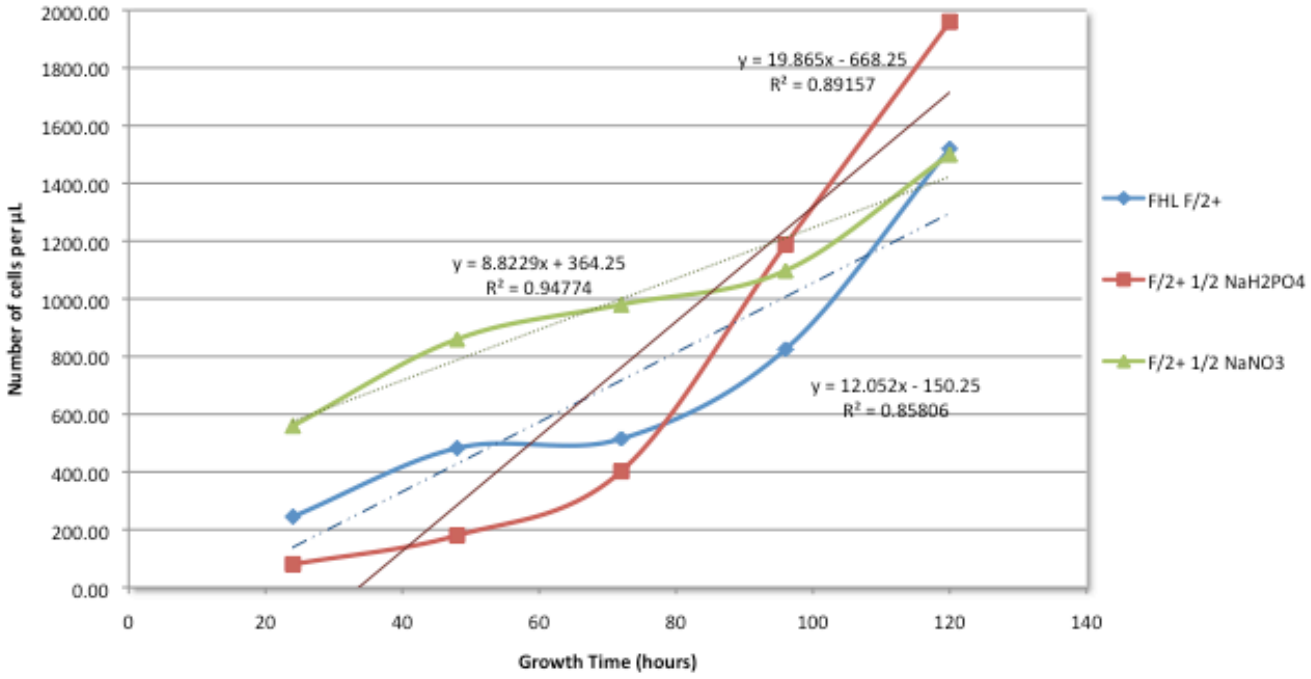

Figure 7. Growth curve for *Skeletonema* F164 at 15℃ in FHL F/2+ (Growth Rate = 12.052, $R^2 = 0.85806$), F/2+ 1/2 NaH_2PO_4 (Growth Rate = 19.865, $R^2 = 0.89157$), and F/2+ 1/2 $NaNO_3$ (Growth Rate = 8.8229, $R^2 = 0.94774$).

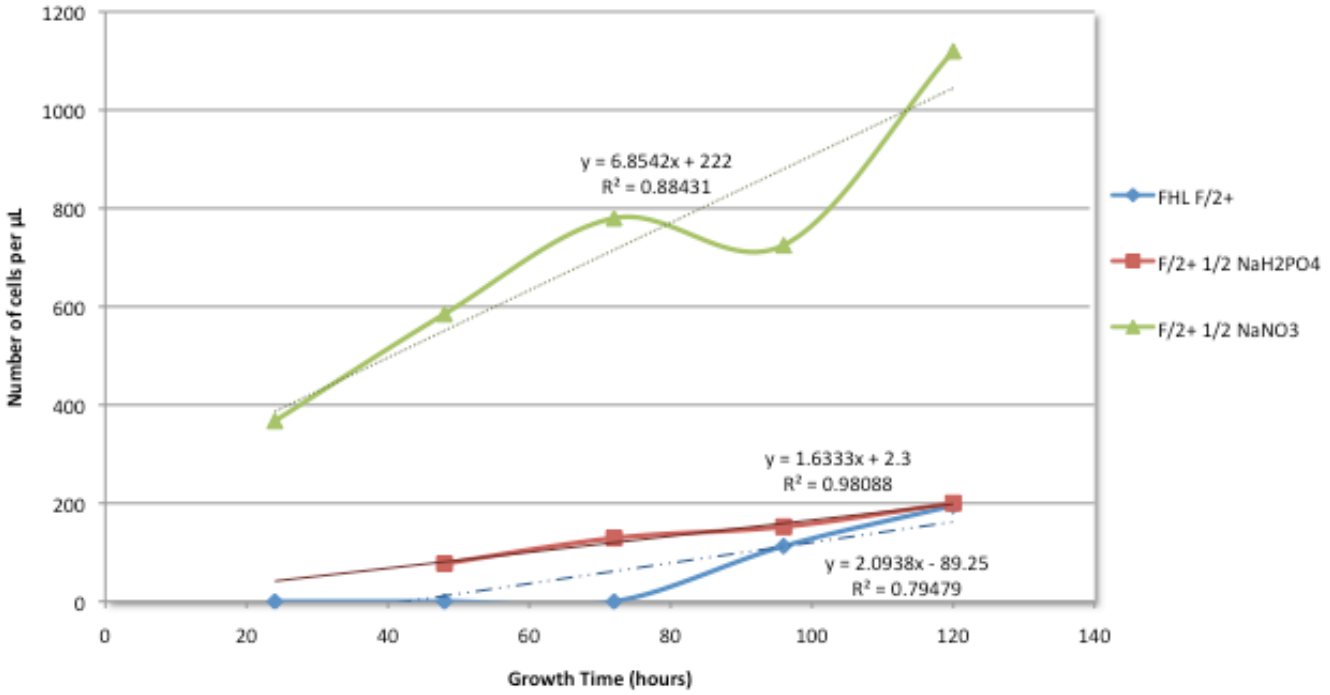

Figure 8. Growth curve for *Skeletonema* F164 at 23℃ in FHL F/2+ (Growth Rate = 2.0938, $R^2 = 0.79479$), F/2+ 1/2 NaH_2PO_4 (Growth Rate = 1.6333, $R^2 = 0.98088$), and F/2+ 1/2 $NaNO_3$ (Growth Rate = 6.8542, $R^2 = 0.88431$).

Average doubling times were calculated (Figure 9) for each species, combined nutrient media, and show Skeletonema sp. F164 with a doubling time under 10 hours. Fragilaria sp. F084 shows a doubling time above 60 hours.

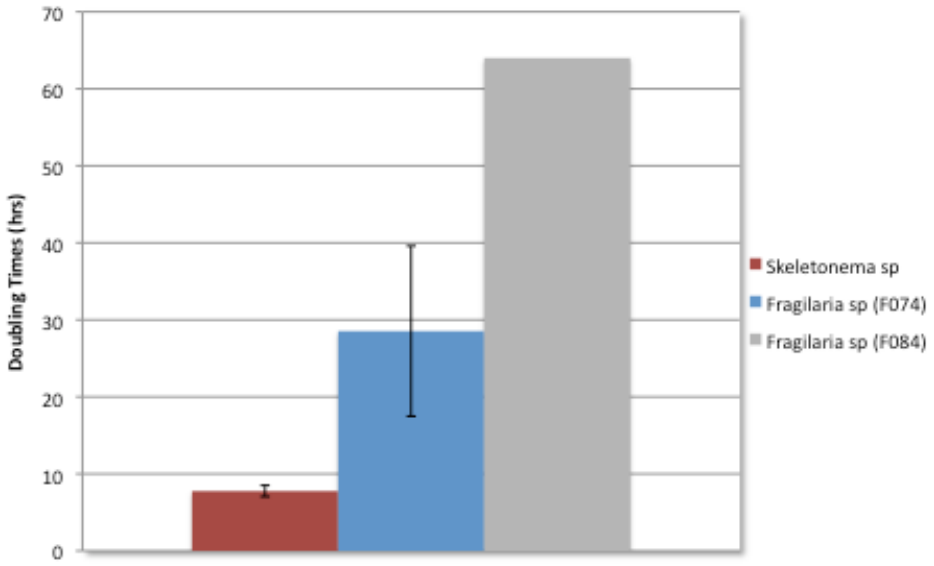

Figure 9. Average doubling times for *Skeletonema* F164, *Fragilaria* F074, and *Fragilaria* F084.

Discussion

The results showed that species of diatoms grow at very different rates in different media and temperatures; these data will help determine parameters for growth rate experiments on *Skeletonema sp.* F162 and *Fragilaria sp.* F074 and F084. Later experimental results could be strengthened for further studies by increasing number of 1.0 mL samples collected daily, increasing number of nutrient flasks for each species and by allowing more growth time. In a typical growth curve one would observe a lag phase, log phase (where exponential growth occurs), stationary phase and death phase. Stationary phase wasn't observed with any species, therefore, log phase might have continued. Correlations, however, between number of cells per μL and time of growth were shown for *Skeletonema sp.* F164 meaning that given more time cells would have reached a stationary phase. *Fragilaria sp.* F074, being a chain-forming diatom, may have shown better growth curves with longer growing times causing less overall fluctuations in the number of cells per μL. Because of this correlations tended to be lower than that observed with *Skeletonema sp.* F0162. *Fragilaria sp.* F074 shows little or no growth in phosphate-reduced media, which may indicate that phosphate is a limiting factor for this strain.

Trends in the data show that *Skeletonema sp.* F164 is the fastest growing species tested in nutrient reduced media regardless of temperature. The average doubling time is under 10 hours which may be indicate high growth rates suitable for biofuels production and further tests would be beneficial for this strain. I would recommend that this experiment be repeated with *Skeletonema* sp. F164 with a longer growth time since Skeletonema sp. F164 showed promising growth.

Fragilaria sp. F074 shows a faster growth rate than *Skeletonema sp.* F162 in both of the pink F/2+ media and a higher growth rate in the FHL F/2+. There seems to be a difference between the two control mediums (pink F/2+ and FHL F/2+), which may be due to the different chelators used. Further chelator experiments in diatom stock cultures may be beneficial to biofuel research. *Fragilaria sp.* F084 failed to yield anything that would perform. This may be due to a lag in the initial growth phase or due to stock culture conditions.

Both *Skeletonema sp.* F164 and *Fragilaria sp*. F074 showed growth in 23℃ indicating that Friday Harbor diatom species are capable of growing in warmer temperatures. It would be interesting to test this further with more local species to see if *Sekeltonema sp*. is unusual in this respect or if there are more temperature tolerant species local to Friday Harbor.

Algal biofuel research is ongoing and seems almost endless. Every experiment is one closer to finding the perfect combination. This pilot study is only one small piece of a large effort but indications of experimental success are evident with obtained results. Continuation of research with local species and strains may prove to be a worthy endeavor for all involved.

Acknowledgements

I would like to thank Dr. Charles O'Kelly, Ph.D for supplying diatom cultures and continued support through his passion for and vast knowledge of algae and algal biofuels and my research partner Jason Olmstead. I also thank Dr. Megan Dethier, Ph.D for her guidance through the research process and critical editing of report writing, Dr. Adam Summers, Ph.D for his inspirational talks and criticism, Hilary Hayford and Mike Nishizaki, The Mary Gates Foundation for help with funding, and lastly, Friday Harbor Laboratory and the many staff, without them none of this would have been possible.

References

Demirbas, Ayhan. "Use of Algae as Biofuel Sources." *Energy Conversion and Management* 51.12 (2010) : 2738-2749. Web. 11 Apr 2011.

Descamps-Julien, Blandine, and Andrew Gonzalez. "Stable Coexistence in a Fluctuating Environment: An Experimental Demonstration." *Ecology* 86.10 (2005) : 2815-2824. Print.

"F/2 Medium (Guillard and Ryther 1962, Guillard 1975)." *The Provasioli-Guillard National Center for Culture of Marine Phytoplankton*. Web. 24 May 2011.

Griffiths, Melinda J., and Susan T. L. Harrison. "Lipid Productivity as a Key Characteristic for Choosing Algal Species for Biodiesel Production." *Journal of Applied Phycology* 21.5 (2009) : 493-507. Web. 6 May 2011.

Lv, Xinxin, Li Zou, Baowei Sun, Jiangtao Wang, Ming-Yi Sun. "Variations in Lipid Yields and Compositions of Marine Microalgae During Cell Growth and Respiration, and Within Intracellular Structures." *Journal of Experimental Marine Biology and Ecology* 391.1-2 (2010) : 73-83. Web. 27 Apr 2011.

Montagnes, David J. S., and Daniel J. Franklin. "Effect of Temperature on Diatom Volume, Growth Rate, and Carbon and Nitrogen Content: Reconsidering Some Paradigms." *Limnology and Oceanography* 46.8 (2001) : 2008-2018. Print.

Popovich, Cecilia A., Cecilia Damiani, Diana Constenla, and Patricia Leonardi. "Lipid Quality of the Diatoms Skeletonema Costatum and Navicula Gregaria from the

South Atlantic Coast (Argentina): Evaluation of Its Suitability as Biodiesel Feedstock." *Journal of Applied Phycology* (2011) : n. pag. Web. 29 Apr 2011.

Sarno, Diana, Wiebe H. C. C. Kooistra, Linda Medlin, Isabella Percopo, Adriana Zingone. "Diversity in the Genus Skeletonema (Bacillariophyceae). II. An Assessment of the Taxonomy of S. Costatum-Like Species With the Description of Four New Species." *Journal of Phycology* 41.1 (2005) : 151-176. Web. 25 May 2011.