A Pilot Study on Growth Rates of *Asterionellopsis glacialis*, *Thalassiosira sp.*, and *Chaetoceros sp.* for Potential Biofuel Production

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Abstract

The discovery that fossil fuels are not a sustainable fuel source has led to a search for alternatives. Many marine biologists hypothesize that the answer may lie in algae, more specifically in microalgae. The purpose of this pilot study is to provide baseline data for future research for biofuel production. Using cultures exposed to different temperatures and media containing different levels of nutrients, I found that *Asterionellopsis glacialis* was the best candidate among the algae tested from the Pacific Northwest to pursue for potential biofuel production in warmer climates. This was based on its tolerance to different nutrient levels and temperatures.

Keywords: *Thalassiosira*, *Chaetoceros*, lipid production, growth curve, specific growth rate, doubling time

Introduction

Since the realization that fossil fuels are not a sustainable energy source, there has been a widespread demand for suitable replacements. A leading candidate to fill this vacancy in the near future is biofuel produced from biomass (Demirbas 2010), in particular from microscopic photosynthetic algae called microalgae. Microalgae are theoretically able to produce large amounts of lipids, proteins, and carbohydrates in short periods of time, which then in turn can be transformed to biofuel (Demirbas 2011). The production of lipids is of special interest to scientists because it is a major fraction of the phytoplankton biomass and can be used in the creation of a sustainable biofuel (Lv et al., 2010). Diatoms may neutral lipids, including triacylglycerides (TAGs), which are considered to be the best substrate to create biofuels, and the production of these TAGs

can be manipulated with temperature, nutrient availability, and various exposures to light (Popovich et al., 2011).

The challenge of using microalgae as a source of biofuel is not whether it is possible to use microalgae as a source of renewable energy. Instead, can this process be economically viable and done at a large enough scale to meet demands? Currently, almost every detail of the production process needs to be optimized, i.e. cultivation, harvesting, extraction of lipids and the transesterification process of turning the lipids into alcohol esters (Pienkos et al., 2009). We must optimize the conditions for rapid growth and high production rates. Examining different species of diatoms, manipulating nutrients that are available, and changing the temperatures to which the cells are exposed. If enough is learned about the diatoms, it could lead to potential use in the field of biofuel production.

Pacific Northwest diatoms can grow in various conditions but tend to have high production levels in spring and summer months and little production in fall and winter (Wetz et al., 2004). Can a diatom isolated from our local cold waters be suitable for warm water aquaculture? The diatoms *Asterionellopsis glacialis, Thalassiosira sp.,* and *Chaetoceros sp.* all show specific traits suggesting that, if exposed to the correct conditions year round, their rapid growth rates would make them promising candidates for biofuel production (Wetz et al., 2004). The aims of this study are to establish a growth curve for *Asterionellopsis glacialis*, *Thalassiosira sp.*, and *Chaetoceros sp.* exposed to different temperatures and levels of nutrients and to suggest future research to optimize the production of algae for potential use as a biofuel.

These experiments were designed to test the hypothesis that: 1. *A. glacialis, Thalassiosira sp.,* and *Chaetoceros sp.* will have higher growth rates at 23°C than at 15°C and 2. These same species will have higher growth rates when in water rich in nutrients compared to water depleted in some nutrients.

Methods and Materials

Batch culture of *A. glacialis*, *Thalassiosira sp.* and *Chaetoceros sp.*

For the first experiment, isolated strains from the Friday Harbor Labs culture collection (O'Kelly unpublished) of *A. glacialis* (F055)*, Thalassiosira sp.* (F389) and *Chaetoceros sp.* (F085) were grown in f/2 medium as described by ccmp.bigelow.org (f/2 n.d). To test the effects of limiting nutrients, the second batch culture was grown in f/2 media with half the amount (0.5 mL) of NaH_2PO_4 and the third batch culture was grown in f/2 media with half the amount (0.5 mL) of $NaNO_3$. All three cultures were grown in 15°C and 23°C with a light cycle of 12 h:12 h light:dark and under a quantum irradiance of 40-μEm $^{-2}$ s^{-1}. Cultures were stirred once a day with gentle agitation and one milliliter of the culture was taken from the treatments each day for analysis and placed in a 1.5 mL tube with one drop of I_2KI as a preservative. The first culture was grown ahead of the second and third by a period of five days.

Cell counting methods

A. glacialis, Thalassiosira sp., and *Chaetoceros sp.* were pelleted in a microcentrifuge and then the supernatant was reduced from 1 mL to 300μL to concentrate the cells. After the suspension of the pellet, a drop of the sample was placed on a hemocytometer and the cells were counted using a compound microscope.

Specific growth rates and doubling times

Specific growth rates were found using the equation of Levasseur, μ=(ln m2/m1)/(t1-t2) where m2 and m1 are the number of units at certain times (1993). Doubling rates (T_d) were found using the slope of the line and calculating the denominator when the numerator was equal to two and then multiplying by 24 hours to get the correct units.

Results

Growth curves at variable temperatures

Each diatom grew at different rates when exposed to different temperatures. At 15°C, *A. glacialis* (T_d= 7.4 hrs) and *Thalassiosira sp.* (T_d=5.6) showed some measureable growth (fig. 1). In the 23°C culture, *A. glacialis* was the only species to show measureable growth (T_d= 2.4 hrs) (fig. 2). *Chaetoceros sp.* did not show any measureable growth in the experiments.

Growth curves at variable nutrient availability

Only *A. glacialis* was tested in varying nutrient levels at both 15°C and 23°C because of the results of the initial temperature experiments. *Thalassiosira sp.* was tested in varying nutrient levels at only 15°C. At 15°C *A. glacialis* grew similarly in f/2 ½ N, f/2 ½ P and replete f/2 (T_d=6.9 hrs, T_d= 9.6 hrs T_d=11.3 hrs respectively) (fig 3, 4). *A. glacialis* also grew similarly in the 23°C cultures with f/2 ½ N, f/2 ½ P and replete f/2 (T_d=2.5 hrs, T_d= 1.7 hrs, T_d=2.9 hrs respectively) (fig. 5). Specific growth rates were higher in the 23°C cultures compared to the 15°C cultures (fig. 6). *Thalassiosira sp.* showed higher growth rates in the culture that was nutrient rich (T_d=4.8 hrs) compared to

the growth rates when in cultures with reduced nutrients (f/2 ½ N and f/2 ½ P)(T_d=8.7 hrs and T_d=12 hrs respectively) (fig. 7).

Discussion

I hypothesized that exposure to warmer conditions would result in higher growth rates in diatoms in colder waters. This hypothesis was supported only by the growth characteristics of *A. glacialis*, which had a higher growth rate at 23°C than at 15°C. In contrast, *Thalassiosira sp.* did not grow well at this higher temperature, while *Chaetoceros sp.* did not grow in any of the experimental tests. *A. glacialis* also had the highest growth rates of both diatoms at all temperatures and various nutrient levels. Consistent with this hypothesis, it is distributed worldwide and grows in almost all conditions (Round 1990). As seen in figure 3 and 4, growth of *A. glacialis* was not limited by nutrients in the experiments, even in media with half the normal N and P levels. In these experiments I have seen *A. glacialis* grow very well in varying temperatures and nutrient conditions, thus, on the basis of its growth characteristics, it is the most promising candidate of the three for biofuel production.

I also hypothesized that diatoms exposed to lower levels of nutrients would grow more slowly than in replete culture. *Thalassiosira sp*. experienced limitation by the nutrients, showing a doubling time of about half when in a replete medium compared to when in a medium with depleted nutrients. *A. glacialis* showed similar growth rates in nutrient limited and replete conditions, doubling time did not vary when exposed to different media. Another possible interpretation is both *A. glacialis* and *Thalassiosira sp*. did not have sufficient growth time to reveal the effect of nutrient limitation. They were

only placed in reduced nutrient conditions for five days and this might not have been enough time to deplete all nutrients and see a slowing in their growth.

This study would have benefitted from longer growth periods. Five days is not enough time to see a measurable difference between cultures with depleted and replete nutrients, except what was seen in the case of *Thalassiosira sp*. Furthermore, nine days is not enough time to really make any definite conclusions about whether or not these diatoms are good biofuel candidates. The study would also benefit from a more accurate way to count cells, such as a flow cytometer or culture counter, and from preparing replicate cultures to produce more accurate results. But this pilot study does suggest some important next steps.

I would recommend continued studying of *A. glacialis* for potential use in the biofuels industry. It was easy to rule out *Thalassiosira* because it failed to grow at 23°C, meaning it could not grow closer to the equator where it is very easy to get a 12 h:12 h light:dark cycle. *Chaetoceros* also did not grow in this experiment. In contrast *A. glacialis* grew very quickly and was tolerant of variation in temperatures and nutrients, making it a candidate for the potential production of biofuels.

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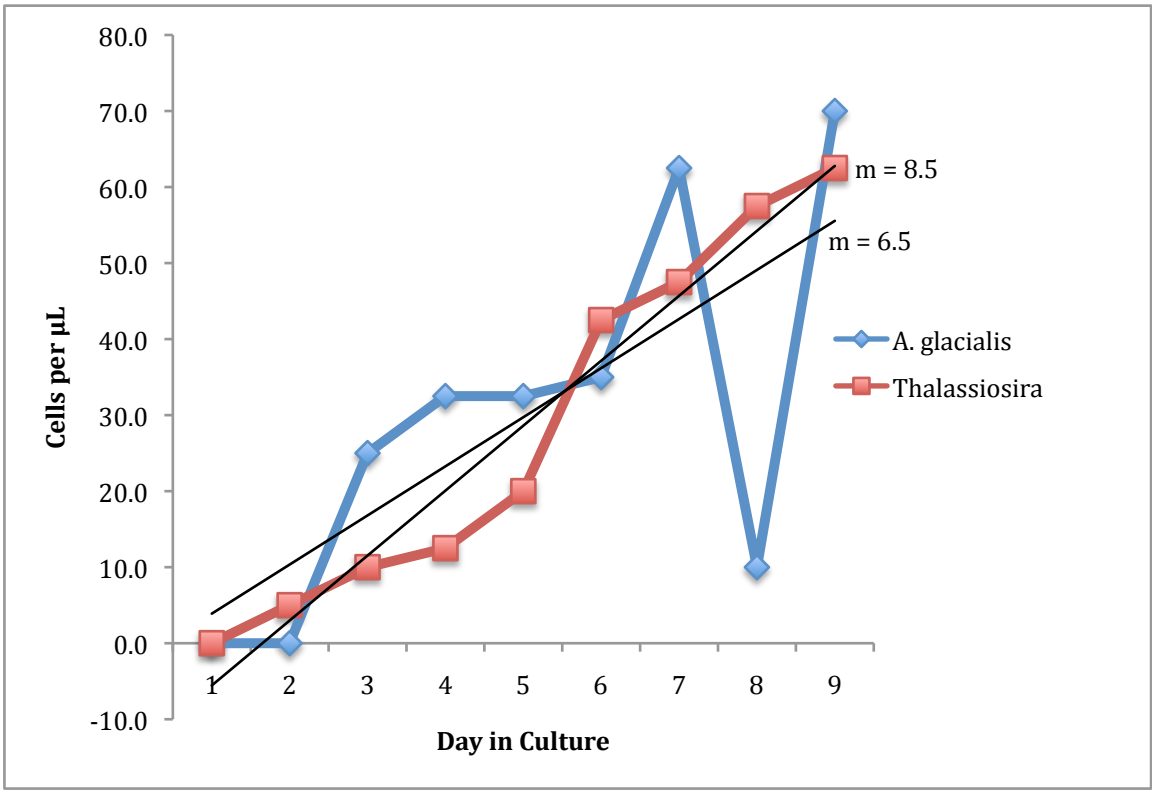

Figure 1 Growth curves for 15°C cultures. *A. glacialis* (m=6.5) and *Thalassiosira* (m=8.5) both show measurable growth in 15°C culture. *Chaetoceros* was omitted because there was no measurable growth. Measurable growth was defined by having a doubling rate in less than 12 hours or a slope of m=4 or greater.

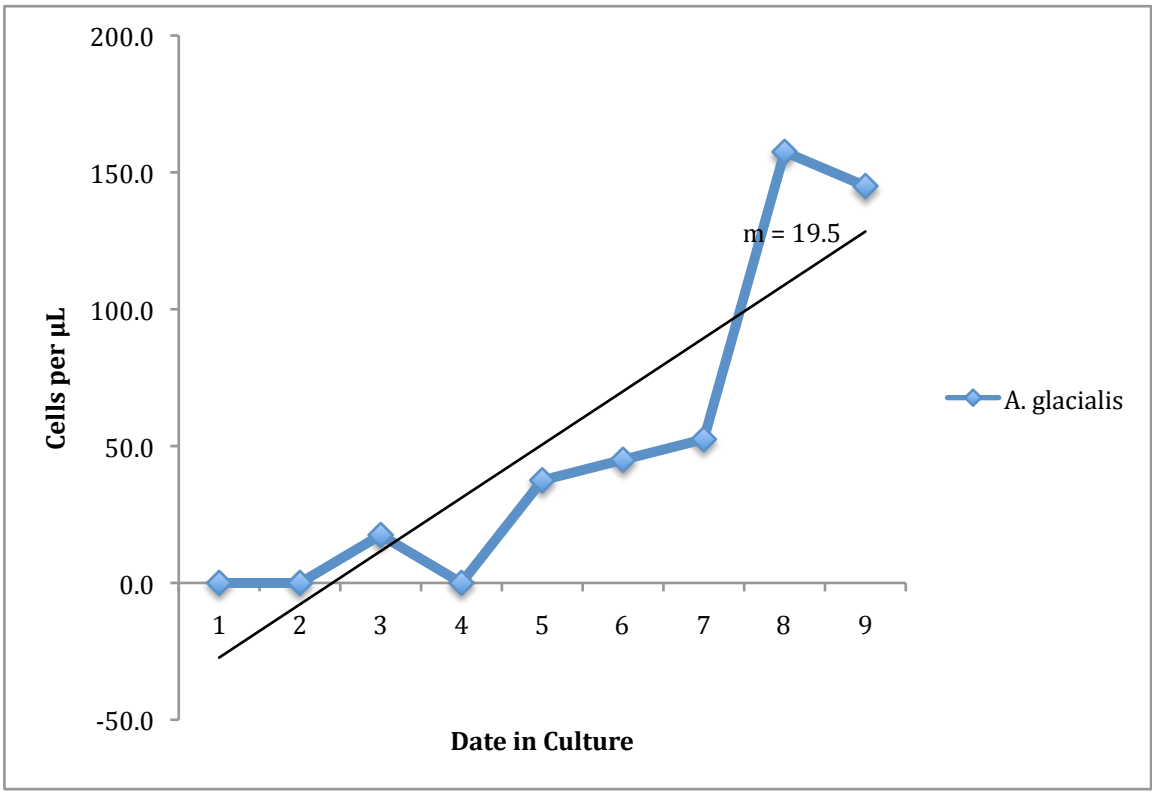

Figure 2 Growth curve for 23°C culture. *A. glacialis* (m=19.5) showed the only measurable growth in the 23°C water.

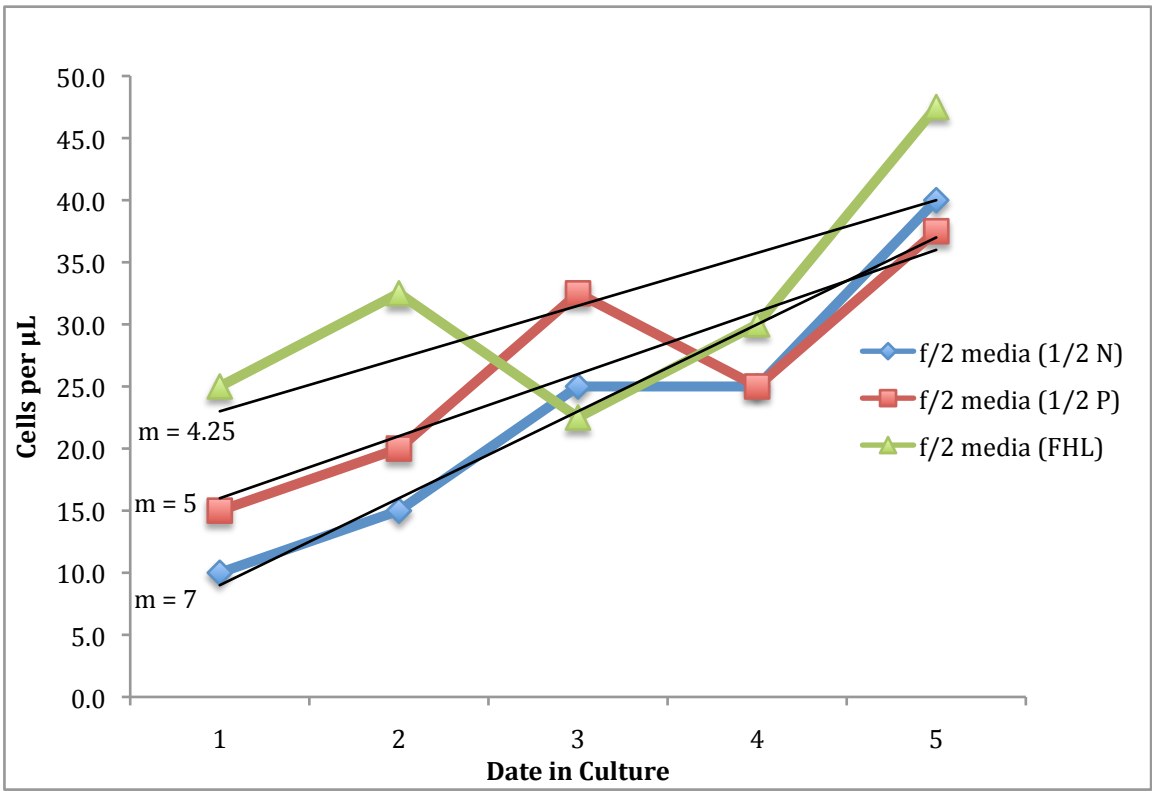

Figure 3 Growth curve for *A. glacialis* at 23°C in different nutrient levels. *A. glacialis* seemed to grow very similarly in all three conditions.

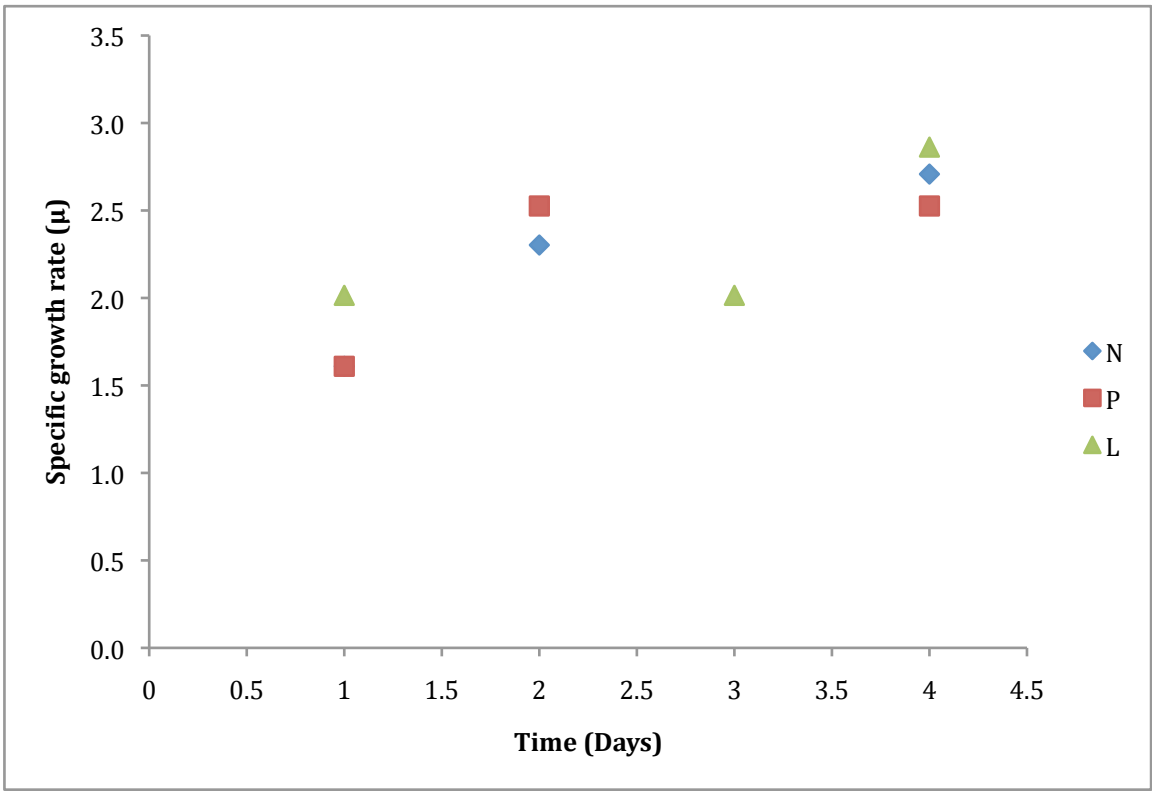

Figure 4 *A. glacialis* specific growth rates at 15°C in various nutrient levels. Specific growth rate was found using the equation μ=(ln m2/m1)/(t2-t1) where m2 and m1 are number of at a certain time and t1 and t2 times when cells were measured. N= media with ½ N. P= media with ½ P. L= f/2 media with no depletions.

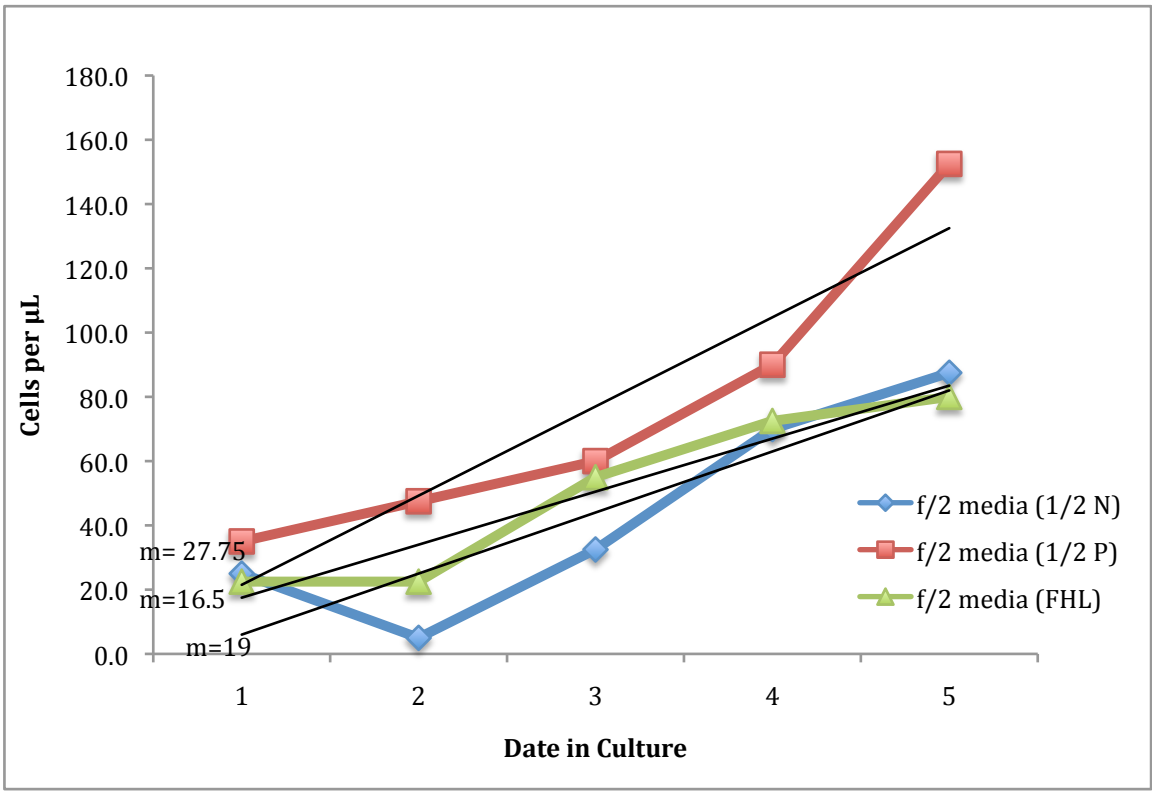

Figure 5 Growth curve for *A. glacialis* at 23°C in various nutrient levels.

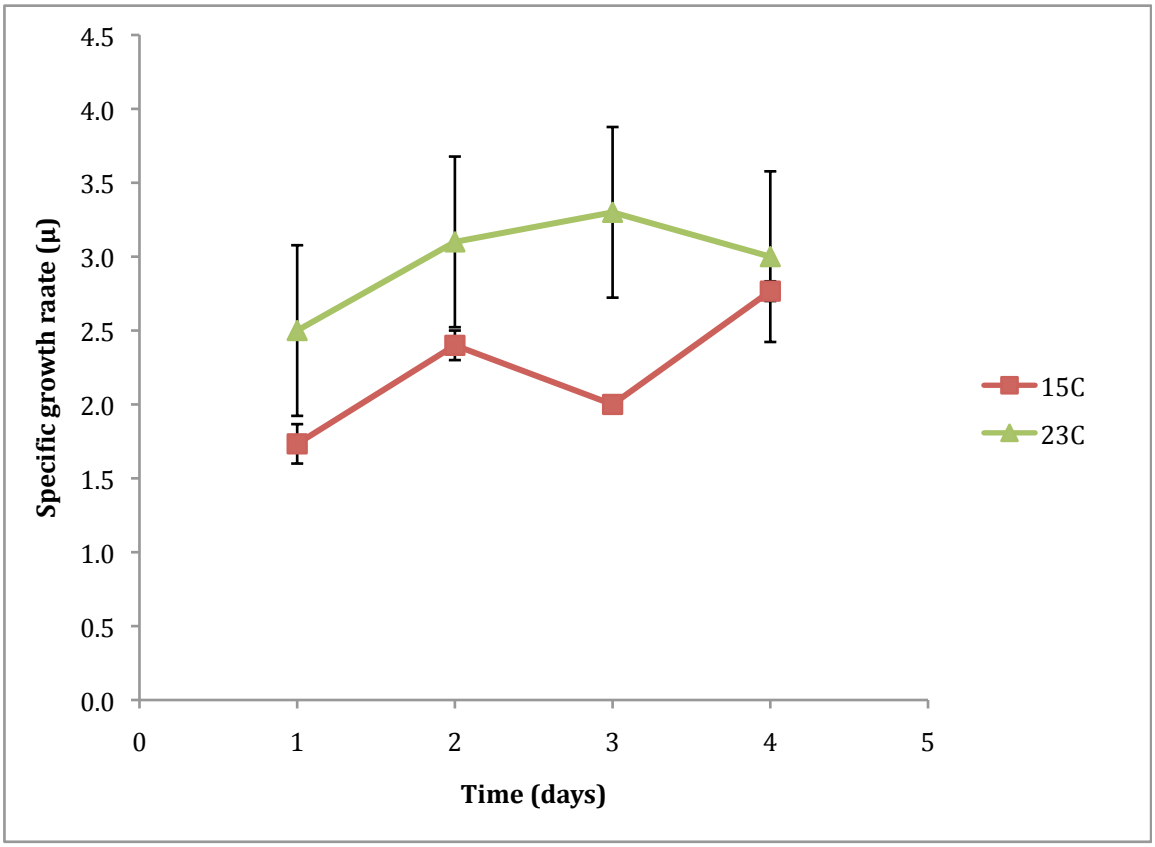

Figure 6 Specific growth rates of *A. glacialis* at 15°C and 23°C. Specific growth rate is found using the equation displayed in figure 4.

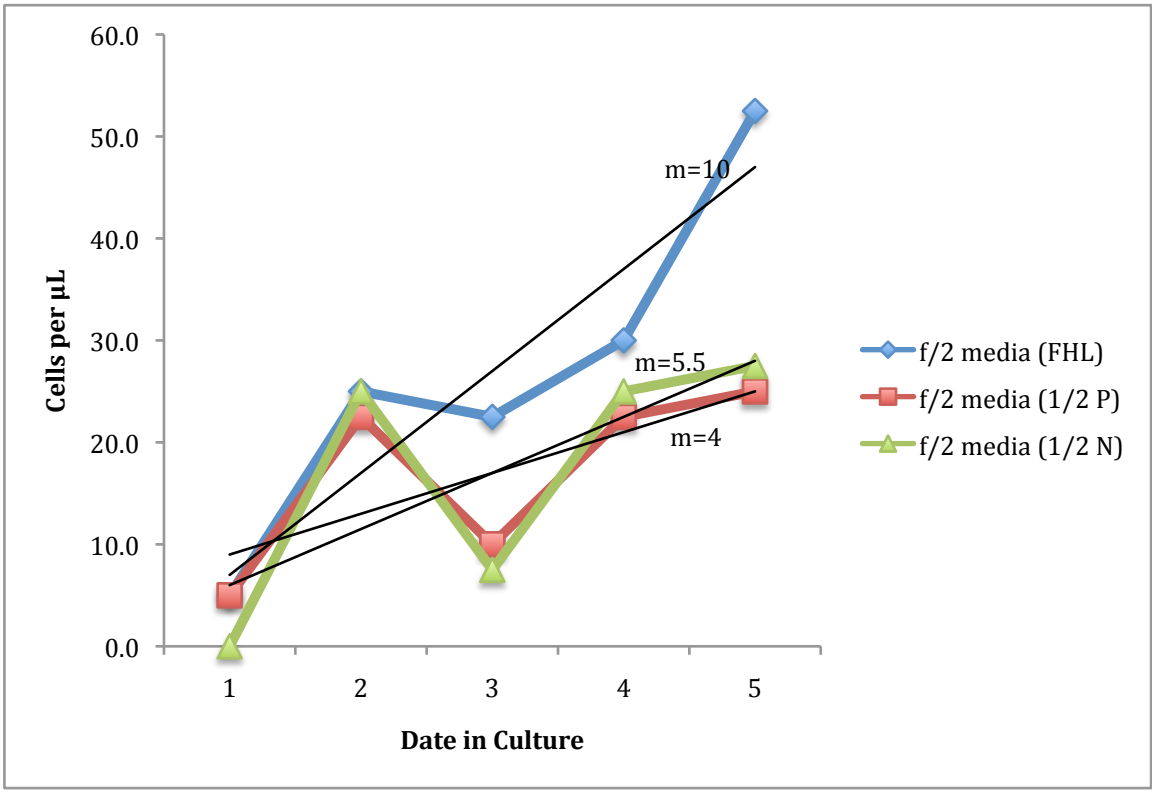

Figure 7 Growth curves for *Thalassiosira* at 15°C in various nutrient levels.

	A. glacialis	Thalassiosira	Chaetoceros
Temperature (°C) and Medium			
15°C f/2+ (P)	7.4 hrs	5.6 hrs	
23°C f/2 (P)	2.4 hrs	145.5 hrs	64 hrs
15°C f/2+ (1/2 N)	6.9 hrs	8.7 hrs	
15°C f/2+ (1/2 P)	9.6 hrs	12 hrs	
15°C f/2+ (FHL)	11.3 hrs	4.8 hrs	
23°C f/2+ (1/2 N)	2.5 hrs		
23°C f/2+ (1/2 P)	1.7 hrs		
23°C f/2+ (FHL)	2.9 hrs		

Table 1. Doubling times of each species in various temperatures and conditions. Doubling time was found using the equation $(2/m) \times 24\text{hrs} = T_d$. Blank spots were days where there was no measureable growth.