Evidence for photoacclimation by the diatom Chaetoceros muelleri due to fluctuating light

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Abstract

The understanding of marine microalgae is important due to their significant contributions to the global environment. The standard balanced growth rates of two strains *Trichodesmium sp*. and *Chaetoceros muelleri* were found by exposing replicates of the strains to a standard "12 hour light, 12 hour dark" sinusoidal light treatment over a series of days. The growth was measured by fluorescence using an XE-PAM fluorometer. The standard growth is the typical growth of these two strains under optimal light circumstances. The next step was to expose the more successful algal strain to various fluctuating light regimes. Twelve replicates of *Chaetoceros muelleri* underwent four different light treatments (Figure 3): the basic 12 light, 12 dark sinusoidal light curve(light bank 2), step-function light program of 12 hours of exposed light with high and low light fluctuations every hour (light bank 3), step-function light program of 12 hours of exposed light with light fluctuations every 15 minutes (light bank 5), and a step-function light program of 12 hours of exposed light with fluctuations every 3 minutes (light bank 6). The variability of these light programs was to simulate the ocean's dynamic light environment. Three dilutions of all of the samples took place over the course of the experiment, and the average growth rate was calculated every day using the growth formula $N_t=N_o e^{\mu t}$. The grand growth rate mean (in relative units) across dilutions was as follows: LB2 .53±.02, LB3 .55±.02, LB5 .49±.03, and LB6 .50±.09. It was concluded that *Chaeotoceros muelleri* showed significant successful photoacclimation over the course of this experiment.

Article Outline

1.Introduction

Microalgae are commonly defined as aquatic photoautotrophic microbes. The understanding of these organisms is imperative because of their effect on the global environment, oxygen production, carbon cycling, and their central role in the marine food chain. Algae utilize available light resources (irradiance) to convert light into chemical energy through the process of photosynthesis. Photosynthesis is the biological conversion of light energy to chemical bond energy that is stored primarily in the form of organic carbon compounds (Falkowski et. al, 1997). Light, in the form of photons, is emitted from the sun which is then "harvested" by the photosynthetic pigment chlorophyll. Chlorophyll *a* is the only pigment that is able to convert absorbed light energy into high energy bonds of organic molecules (Graham and Wilcox 2000). The absorbed light is then transferred as excitation energy to the reaction center, where chemical reactions occur (Graham and Wilcox 2000). The energy must then be released by three different de-excitation pathways; heat, photosynthesis, and fluorescence. Photosynthesis plays a key role in the productivity of the microalgae, but in this experiment we are focused on the measurement of growth by way of fluorescence. When an electron is relaxed from a higher energy level to a lower energy level, there is a radiative emission of energy. Light is remitted when the relaxation to a lower energy state does not cause a change in the spin direction of the electron, or fluorescence is produced (Falkowski et al, 2007). The goal of this project was to analyze the average growth rates of the diatom *Chaeotoceros muelleri* under various light perturbation programs to help us better understand how these organisms maximize the use of solar energy. The exposure to various light treatments will show evidence of the species photoacclimating to their new environment. Photoacclimation is a term used when a photosynthetic organism is placed in an environment with a specific light regime and the organism acclimates to that within the limits of its genetic potential and environmental constraints to be successful (Falkowski et al. 2005)

2. Materials and methods

2.1. Culturing method

The prokaryotic cyanobacteria *Trichodesmium* and the eukaryotic diatom *Chaetoceros muelleri* were chosen for these experiments due to their perceived differences in growth habits. We followed the semi-continuous batch culture method for these experiments so a consistent growth rate could be calculated.

The semicontinuous batch culture approach involves the transfer of a small amount of exponentially growing cells to a container of medium as means of keeping the cells in exponential growth (Wood et al. 2005). This transfer guarantees that the samples are getting the sufficient amount of nutrients as well as keeps their medium fresh.

In the first experiment, 50 mLs of an incubated batch of collected *Trichodesmium* was placed into three autoclaved two-liter clear plastic bottles. The *Trichodesmium* was then diluted with 450 mLs of the low-nutrient media YBCII (Chen et al. 1996). *Trichodesmium* is typically found in the open-ocean, more nutrient-poor portions of the ocean, so a low-nutrient media is necessary. *Chaeotoceros* is more commonly found in high nutrient surface coastal waters, so the high-nutrient media F/2 is used for the diatoms (Guillard 1975). In the standard growth curve experiment, 50 mLs of *Chaeotoceros* were inoculated into 450 mLs of F/2. The rapid grow-ups in such a high-nutrient environment caused the decision to switch to a more dilute media, F/20, for the light bank experiment. The addition of 400 mLs

of filtered sea water to 50mLs of F/2 and 50 mLs of *Chaeotoceros* improved the length of growth. Dilutions were carried out when necessary for the sample bottles so that a fresh supply of nutrients remained steady.

2.2. Light Banks

The light banks may hold up to six two-liter plastic bottles in each tank. Light is provided to the samples from below the plastic aquarium. For the first experiment, the lights in all three banks were programmed to commence at 10:45, run the sinusoidal light curve for 12 hours, and terminate at 22:45. In the second experiment, the first two banks, light bank 2 and 3, came on at 10:45 and shut off 12 hours later, and the last two banks, light bank 5 and 6, began at 11:45 and shut off 12 hours later. The measurements must be taken with the strains being dark-acclimated. The cause for the delay in the lights for banks 5 and 6 was to provide a sufficient amount of time for the measurements of all of the sample bottles to be taken before the lights in their respected banks turned on.

2.3. XE-PAM Fluorometer

The pulse-amplitude-modulation (PAM) measuring principle is based on selective amplification of a fluorescence signal which is induced by very short and intense probe pulses of measuring light (Heinz 1996). The XE-Pam fluorometer is the machine used in these experiments to measure growth for our samples. The SAT (saturation) pulses used also are not strong enough to induce photosynthesis, but give off just enough intensity to provoke a fluorescence response of the sample. The fluorescence response of a sample is diagnostic of the cell's "health". The XE-Pam automatically records the measurements that it takes in the report tab of WinControl. Following the illumination of a dark-acclimated sample, the fluorescence emission increases to an initial level (0), or F_0 (Geider and Osborne 1992). F_0 is the term used when fluorescence emission is minimal and all of the reaction centers are open. A fluorescence peak, F_m, of the sample will then be reached. Opposite of F_0, F_m is the maximal fluorescence emission and all of the reaction centers are closed (Geider and Osborne 1992). The fluorescence yield of a culture in balanced growth is linearly correlated with cell number is detectable over a wide range of cell densities (Wood et al 2005). Both F_0 and F_m are of interest and are very important for the success of the analysis of growth patterns for these samples.

2.4. Standard growth experiment

The acclimated growth rate, or standard growth rate, of a culture is the growth rate after a population has made all physiological adjustments required to maintain the same rate of balanced growth through multiple serial transfers to a new media (Wood et al. 2005). Three samples of the *Trichodesmium* and three samples of the *Chaeotoceros* were prepared to be placed in the three utilized light banks. One of each of the samples were placed into the three separate banks to be exposed to the standard 12-light, 12-dark sinusoidal light curve. The three light banks all had the same light cycle so that the balanced growth curve that was found would have a low margin of error. Maximal (F_m) and minimal (F_0) fluorescence yields are only given when the samples are dark-acclimated, so the measurements must be taken before the lights in the light banks turn on. As previously mentioned, the light banks were

scheduled to come on at 10:45 every morning. To allow time to gather all of the measurements before the program began, the measurements were commonly started around 9:00.

To guarantee that the XE-Pam is measuring the fluorescence of the microalgae samples only, a filtered seawater blank must be ran through the fluorometer first. Sea water naturally has autofluoresent particles, so the subtraction of the F_m of the sea water from the F_m of the samples ensures that only the microalgae fluorescence is being measured.

First, the dark-acclimated *Trichodesmium* samples must be exposed to a light curve before it is measured with SAT pulses. The reason for this light curve is that *Trichodesmium* will provide a more accurate fluorescence measurement if they are light-acclimated. After a successful light curve treatment, three SAT pulses are taken from the same sample and the F_m and F_0 measurements are recorded in the report. This same procedure of exposing a light curve and taking SAT pulse measurements is done with the remaining two *Trichodesmium* samples from the last two light banks.

The diatoms do not need to go through a light curve to give accurate measurements, so the three SAT pulses are taken and recorded. The three SAT pulses are necessary such as having three light banks with the same light treatment, it decreases the margin of error. Immediately following the completion of the measurements, the samples are placed back into their respective light banks before the commencement of the lights. The tops of all of the bottles were loosened to allow gas exchange.

The PAM data were transferred to an Excel worksheet for analysis. As stated previously, the only information of our particular interest is the F_0 and F_m recordings. F_m-corrected must then be calculated by subtracting the blank. The F_m-corrected value is the point that is transferred onto the growth chart for that day. The three strains of *Trichodesmium* have a separate growth chart (Figure 1) from the *Chaetoceros* (Figure 2). The x-axis represents time elapsed in days, and the y-axis represents the fluorescence values in volts. The average growth between days is also calculated using the F_m-corrected values using the formula $N_t=N_oe^{\mu t}$. During exponential growth, the rate of increase in cells per unit of time is proportion to the number of cells present in the culture at the beginning of any unit of time, thus that formula is used (Wood et al. 2005). The first day, the day of the dilution, is day 0. The samples are incubated and consistently measured daily.

Dilutions of the samples are necessary when the collected F_m-corrected values and the exponential growth seem to cease and stabilize. This stabilization shows that the microalgae are utilizing all of their nutrient resources and will soon begin to die. To prevent a crash in population, removing the nutrient starving media and replacing it with fresh media is necessary. Diluting is also imperative if any growth curve is to be found. By nature, diatoms adapt easily to new environments and were thriving in their high-nutrient media. All of the *Chaetoceros* bottles seemed to be peaking every 2-3 days, so the decision to switch to the F/20 media was made. The *Trichodesmium* samples are less apt to adapt. The growth of the samples is easily affected by minor disruptions in nutrients, bacteria, light, and even temperature. Over the course of this 23-day experiment, the diatoms had 5 successful grow-ups; *Trichodesmium* did not have one consistent grow-up. The inconsistency of the cyanobacteria growth caused its removal from the next experiment.

Figure 1

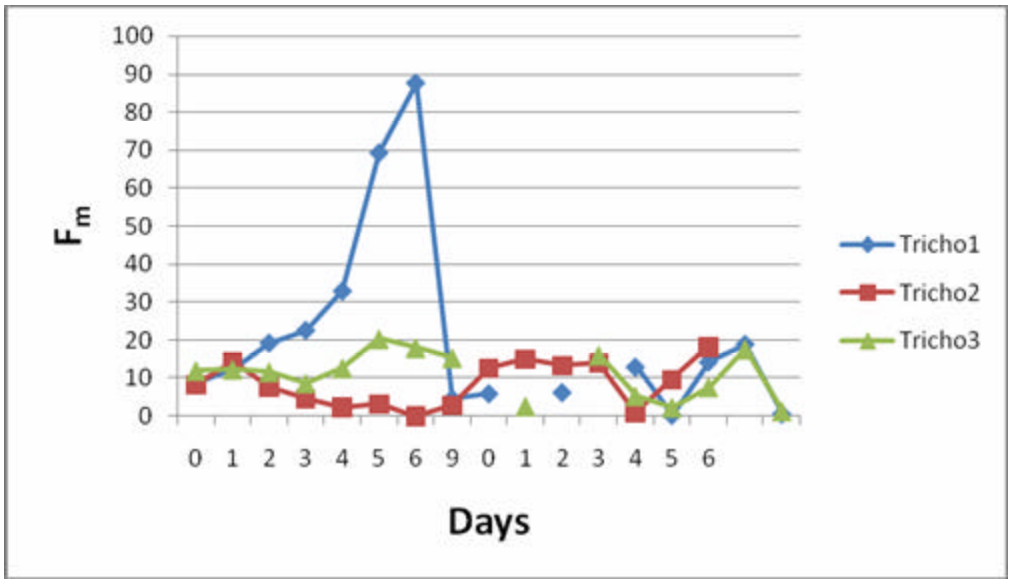


Figure 1. Flucuating growth of *Trichodesmium* over the course of standard growth experiment. Note inconsistency in grow-ups, thus being the cause of the cyanobacteria's removal from the proceeding experiment. F_m was measured in relative units.

Figure 2

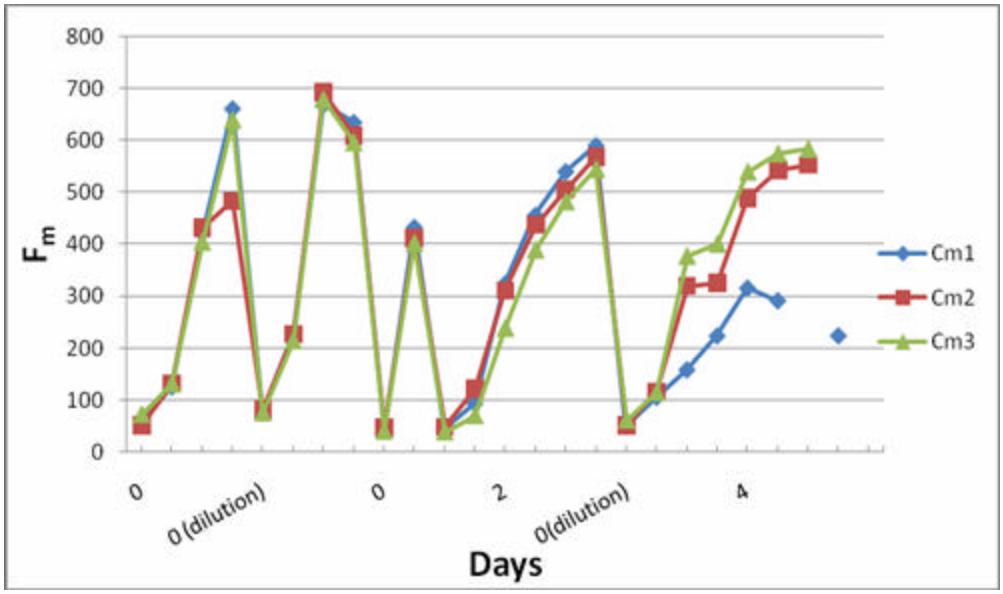


Figure 2. Consistent growth of *Chaotoceros* over the course of the experiment. F_m was measured in relative units. Note that *Chaetoceros*'s growth was peaking around 600 units above *Trichodesmium*.

2.5. Light bank experiment

To effectively study the growth of microalgae under varying light conditions, new light schedules

were added to the experiment. Instead of three light banks undergoing the standard sinusoidal light cycle, only one bank would continue with that program. The other three banks were switched to square-light functions with varying perturbations. Unlike the standard growth experiment which had its max light parameter set at 150 and its minimum at 0, the square function minimum would be 40 through its cycle. This was necessary to ensure that the other three light banks were being exposed to the same irradiance as the sinusoidal bank. A PAR-sensor was also used in all of the light-banks to make sure that the irradiance was equal. Light bank 3, during its 12-hr light cycle, would expose high light and low light at 1-hour intervals. Light bank 5 would expose high light and low light at 15 minute intervals, and light bank 6 would have 3 minute perturbations. The first two light banks began their cycles at 10:45, and the second two at 11:45. The square function banks also ran the sample bottles at the minimum light parameter for a period of time so the strains may become slightly acclimated before the high light was exposed. Similar to the first experiment, measurements with the XE-PAM are consistently taken, recorded, and transferred to an excel worksheet. The F_m -corrected value is then plotted on a growth chart. The chart represents each light bank with the three samples that were within (Figure 4). This experiment spanned 12-days, allowing three grow-ups.

Figure 3

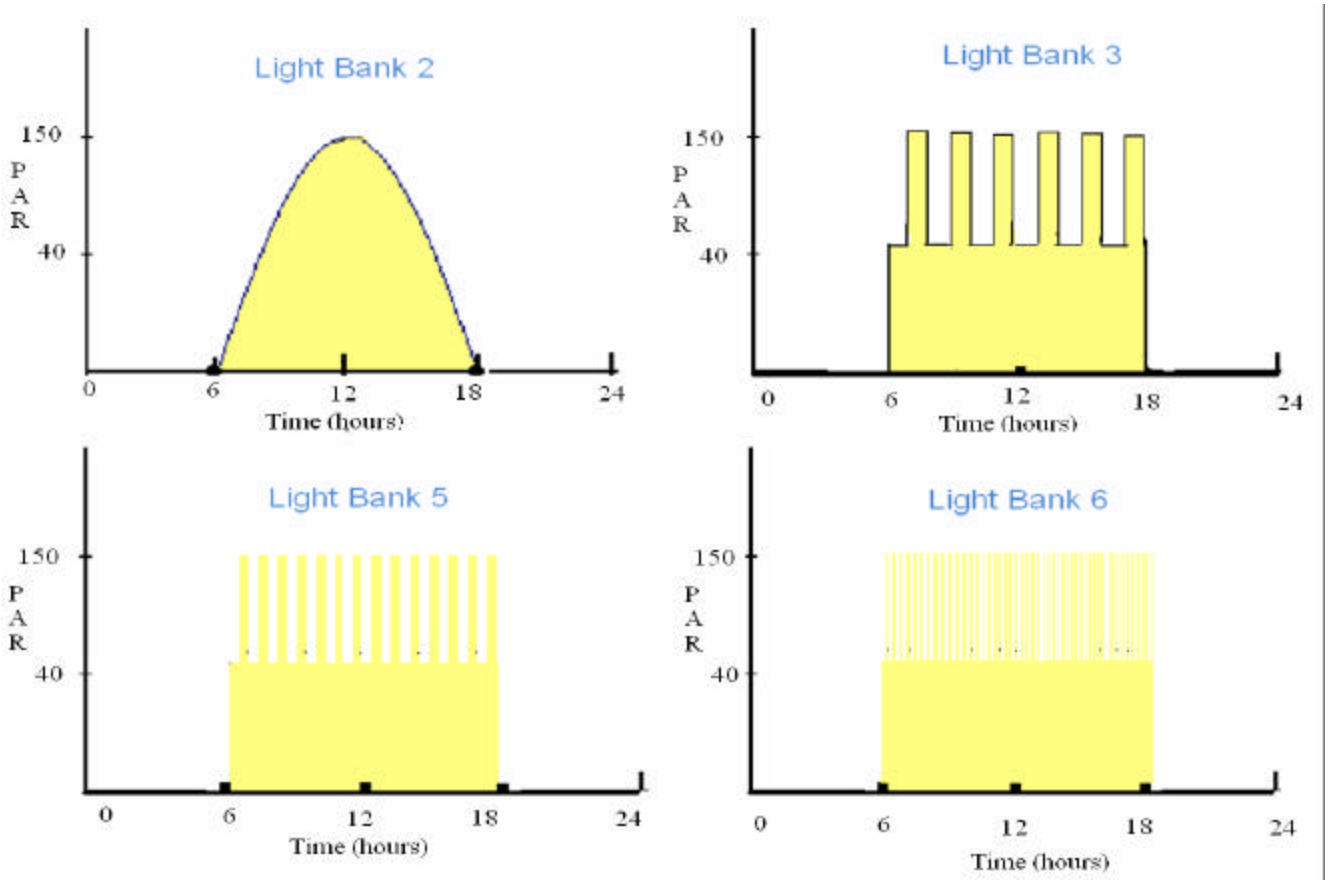
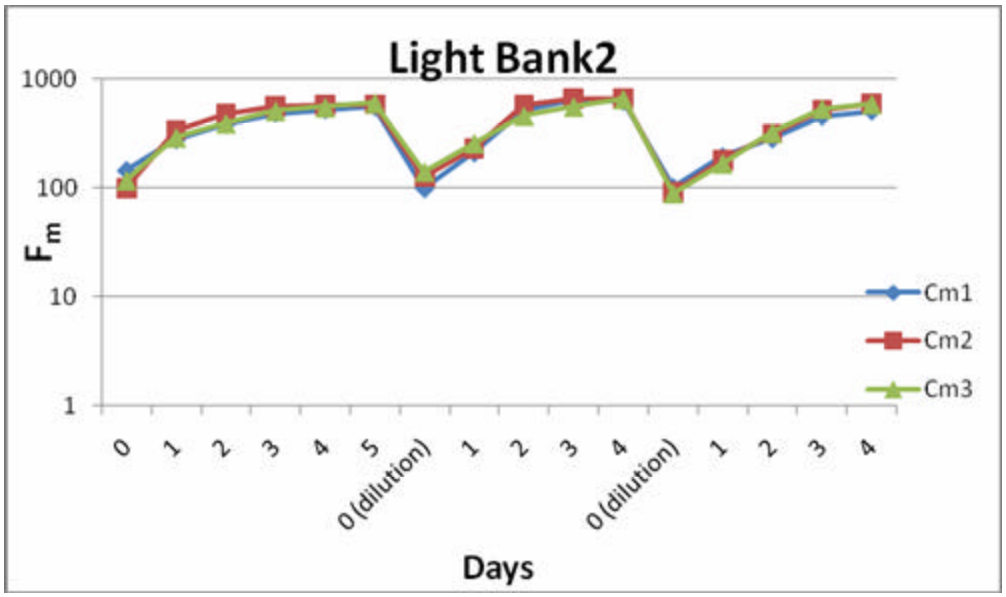


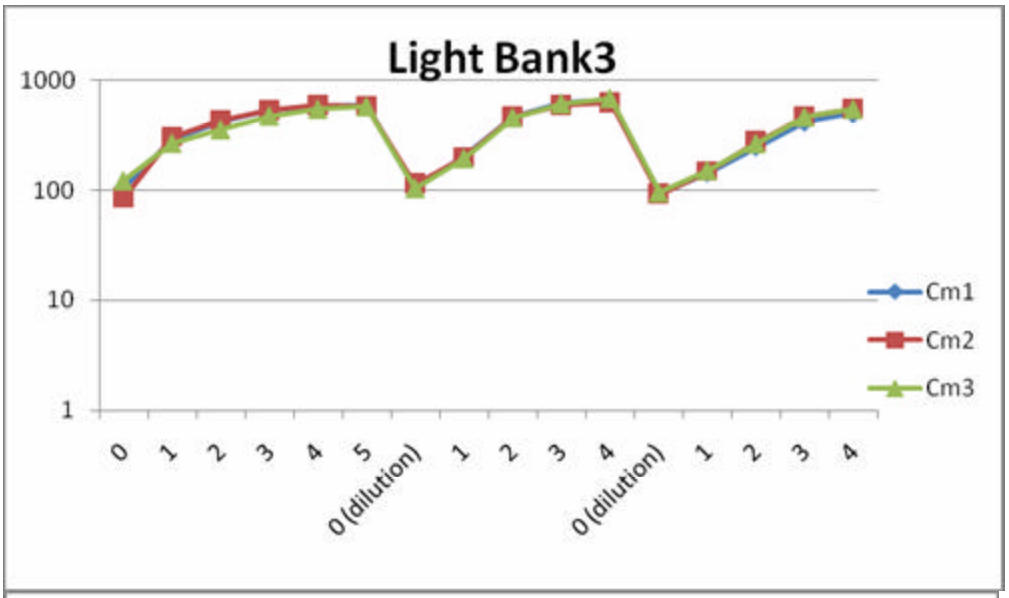
Figure 3. The four different light bank treatments. Light Bank 2 has the sinusoidal day curve. Light Bank 2 is the step-function with a 1-hour high light, 1-hour low light perturbation through the 12-hour light cycle. Light Bank 5 is the step-function with 15-minute high light, 15-minute low light. Light Bank 6 is the step-function with 3-minute high light, 3-minute low light perturbation. PAR (photosynthetic active radiation) is measured in mol photons m^{-2}.

Figure 4

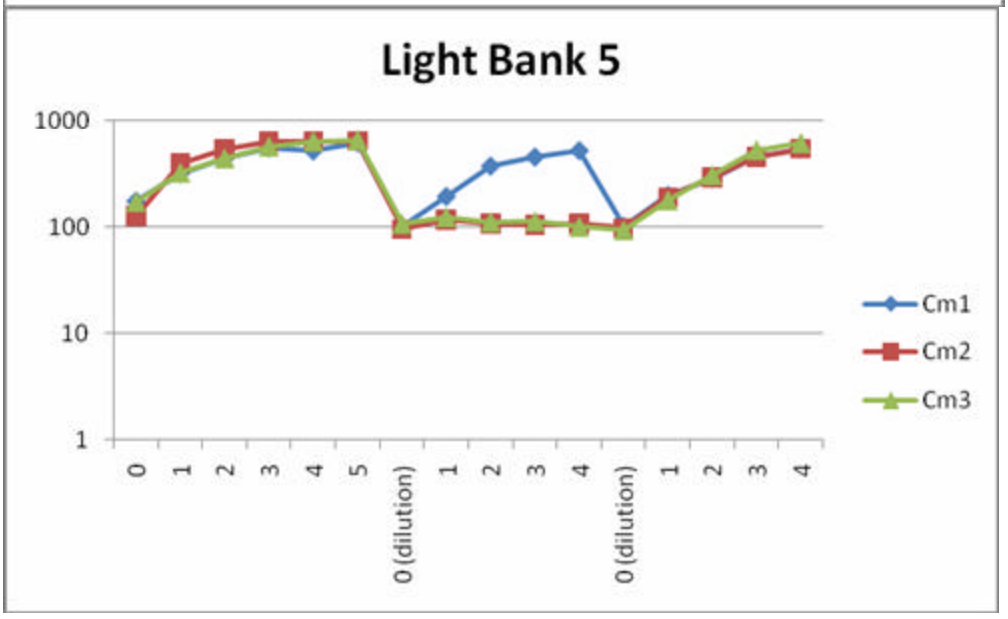
(a)


Light Bank2
1000
100
10
1
F_m
Cm1
Cm2
Cm3
0
1
2
3
4
5
0 (dilution)
1
2
3
4
0 (dilution)
1
2
3
4
Days

(b)


Light Bank3
1000
100
10
1
0
1
2
3
4
5
0 (dilution)
1
2
3
4
0 (dilution)
1
2
3
4
Cm1
Cm2
Cm3

(c)


Light Bank 5
1000
100
10
1
0
1
2
3
4
5
0 (dilution)
1
2
3
4
0 (dilution)
1
2
3
4
Cm1
Cm2
Cm3

(d)

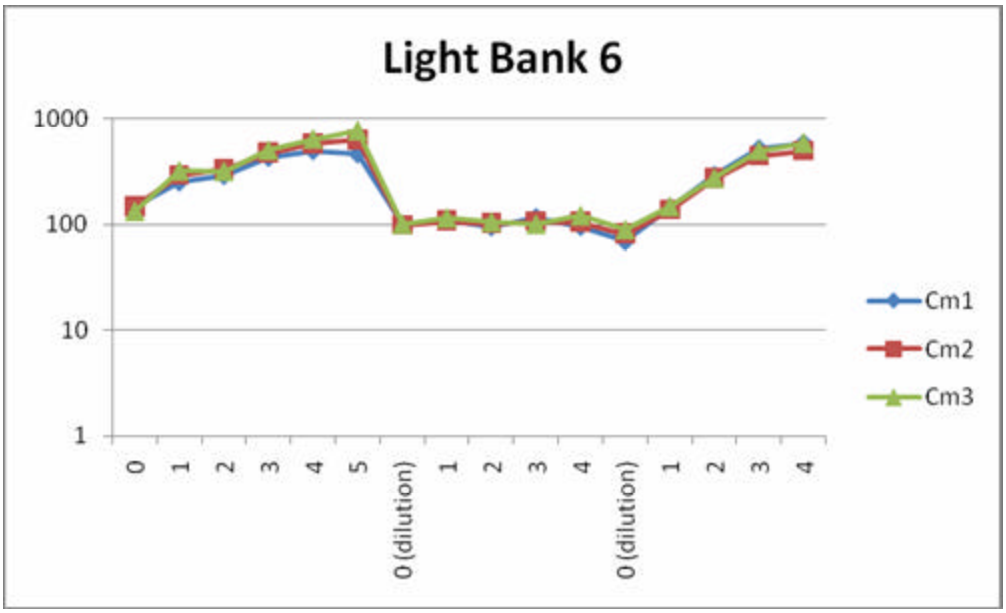


Figure 4. (a) Growth chart of 3 samples of *Chaotoceros* in light bank 2 (sinusoidal day curve) over course of experiment. (b) Growth chart of 3 samples in light bank 3 (step-function with 1 hour light pertubations). (c) Growth chart of 3 samples in light bank 5 (step-function with 15 minute light pertubations), note that Cm2 and Cm3 had no growth during the second dilution, this was caused by an inoculation mistake with the wrong media. (d) Growth chart of 3 samples in light bank 6 (step-function with 3 minute light pertubations), note that all three samples did not grow during the 2nd dilution for the same reason as the samples in light bank 5.

2.6. Results

Over the course of the experiment, the analyzed data showed that the average growth for the samples across the three dilutions were very similar. The similarity between the numbers shows evidence of photoacclimation in the diatoms.

To find the gross mean growth across the dilutions, the mean, variance, and standard deviation from the calculated growth rates from day 0 to day 3 for each sample must be found. Three data points are used to make sure that the sample is in exponential growth. The calculated statistics were found for the first 3 days across the 3 dilutions (Table 1). The three new statistical values were the numbers used to find the mean gross growth rate and the standard error value (Table 2 and Figure 5).

A single-factor analysis of variance (Zar 1999) was used to test the variance between the average mean growths of the samples. An F value of 0.532652102 was found from the collected data. The critical value, found in the F-distribution table, was 4.35. The F value is less than the critical value, so the null hypothesis stating that the mean growth rates were equal was not rejected. In conclusion, there was no variability between the average growth rates across the light banks.

Table 1

Dilution 1			Dilution 2			Dilution 3		
light bank	***mean***	***SE***	***light bank***	***mean***	***SE***	***light bank***	***mean***	***SE***
LB2	0.49	0.049	**LB2**	0.54	0.047	**LB2**	0.56	0.03
LB3	0.54	0.046	**LB3**	0.58	0.014	**LB3**	0.52	0.01
LB5	0.44	0.05	**LB5**	0.49		**LB5**	0.53	0.02
LB6	0.39	0.026	**LB6**			**LB6**	0.60	0.03
			Dilution 2					
			light bank	***mean***	***SE***			
			LB2	0.54	0.05			
			LB3	0.58	0.01			
			LB5	0.18	0.16			
			LB6	0.03	0.02			

Table 1. The mean for all of the samples represent the average of the three growth rates found for the first 3 days of that dilution. Note that the second chart for Dilution 2 that includes red values are values that were found with faulty data due to an incorrect inoculation on the last 5 sample bottles that were placed in Light Bank 5 and 6 during Dilution 2. This is also the explanation for the lack of mean and Standard Error values in the above Dilution 2 chart.

Table 2

	Grand mean across dilutions		
	mean	***se***	***Standard Dev***
LB2	0.53	0.02	0.04
LB3	0.55	0.02	0.03
LB5	0.49	0.03	0.05
LB6	0.49	0.09	0.15

Table 2. The mean for the light banks was found by averaging the 3 means across dilutions from Table 1. The standard error was calculated by using those 3 values as well.

Figure 5

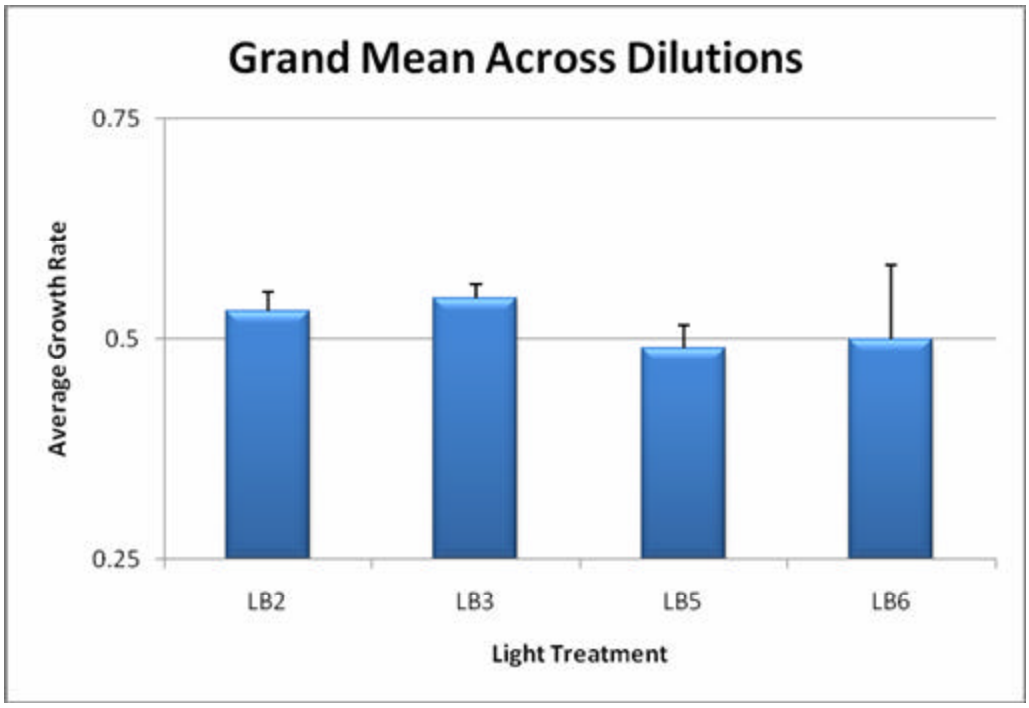


Figure 5. The average growth rate (in relative units) of *Chaetoceros* across light banks with standard error bars.

2.7. Discussion

The purpose of this experiment is to help in the understanding of photophysiological characteristics of microalgae that allow them to be successful under a variety of light circumstances. The experiment was modeled in such a way that the different light perturbations simulated the unpredictable and variable light environment of the ocean. The irradiance that microalgae are exposed to throughout the day can be affected by various factors such as water column mixing, cloud coverage , overhead objects, etc.. The purpose of the variety of light exposure was to simulate the most realistic environment possible for the samples. Doing this would give the most accurate and realistic measurements possible. The main result of the experiment showed that the growth rate did not in fact vary between the light treatments.

Trichodesmium is generally found in deeper water than *Chaetoceros* and is subject to less fluctuating light and slower mixing. *Chaetoceros* dwells in the more surface coastal waters which expose them to very rapid mixing and changes in light variability. We were unable to successfully grow *Trichodesmium* under a controlled and basic light program, but were quite capable of growing *Chaeotoceros* under more extreme and changing light circumstances. This evidence helps support the idea that *Chaetoceros's* acclimation to different light regimes may be an evolutionary adaption over time that has allowed them to thrive under a wide spectrum of environments.

If given the opportunity to continue this experiment again, or to do it over, I would not change

much. I would have liked to do one more dilution for error's sake, unfortunately time restraints did not allow it. A suggestion for a future experiment may to be design other light regimes that would be more realistic to the actual light and mixing that *Chaetoceros* would be exposed to and that *Trichodesmium* would be exposed to in their natural environment. Another suggestion would be to find another successful species that we can also analyze. Diatoms make up only a portion of the microalgae in the ocean so many more species are available to be analyzed. I also would like to see the effects salinity and changing temperatures may have on the growth process.

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