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Measuring and Comparing Quantum Yield in Two Species of Marine Diatoms Subjected to Constant and Fluctuating Light Conditions

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MEASURING AND COMPARING QUANTUM YIELD IN TWO SPECIES OF MARINE DIATOMS SUBJECTED TO STATIC AND FLUCTUATING LIGHT CONDITIONS

by

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ABSTRACT

MEASURING AND COMPARING QUANTUM YIELD IN TWO SPECIES OF MARINE DIATOMS SUBJECTED TO STATIC AND FLUCTUATING LIGHT CONDITIONS

A small-scale study was conducted to determine the effects of light fluctuations on the photosynthetic efficiency of marine phytoplankton. Two species, *Phaeodactylum tricornutum* and *Chaetoceros gracile* were grown in specialized photobioreactors on a 12-hour:12-hour light:dark cycle. The cultures were diluted 50% daily to attain a specific growth rate of 0.70 d^{-1}. To simulate vertical mixing in high turbidity habitats under various wind conditions, dense cultures were subjected to fluctuating light treatments with frequencies ranging from 0.10 Hz to 2.00 Hz. Parallel experiments subjected the cultures to static light conditions with equal total daily light doses as those of the cultures in fluctuating light. Aside from the light parameters, all growth conditions remained the same for each paired experiment. Quantum yield was measured using two methods: ^{14}C fixation at the end of the light period to determine maximum quantum yield (Φ_{max}), and increase in depth-integrated particulate organic carbon during the day to determine daily averaged quantum yield (Φ_{ave}). Photosynthetic efficiency of Photosystem II photocenters was also determined using two types of variable fluorescence: FIRe (Φ_{FIRe}) and dual pulse amplitude modulated fluorescence (Φ_{PBR}). These analyses were performed under both nutrient-replete and nutrient-stressed conditions. Results have shown that, when subjected to fluctuating light, the Φ_{max} for *C. gracile* tended to increase for fluctuating light treatments up to a frequency of 2.00 Hz. However, no benefit of fluctuating light was evident in measures of Φ_{ave} for this strain. Results of Φ_{FIRe} did not appear to be different for the various light treatments for *C. gracile*, although the measurements of Φ_{PBR} were greater when acclimated to static light and to light fluctuating 0.50 Hz and 1.00 Hz than when acclimated to the other light treatments. Every quantum yield parameter determined for *P. tricornutum* when subjected to fluctuating light was lower, relative to static light values. These experiments help give insight into the

photosynthetic efficiency of these two strains and how they respond to various fluctuating light treatments. With this information, these, and other strains, can be manipulated to maximize their production and can be utilized on larger scales for pharmaceutical, biomedical, aquaculture, and biofuels applications.

DEDICATION

This work is dedicated to my loving family: Ma, Julisa, Chelcie, and Tina

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LIST OF ABBREVIATIONS

a*.....Spectrally Weighted Absorption Coefficient

.....Spectrally Weighted and Averaged Absorption Coefficient

α^B.....Chlorophyll *a* Normalized Photosynthetic Efficiency

β.....Photoinhibition Parameter

β^B.....Chlorophyll *a* Normalized Photoinhibition Parameter

d.....Wavelength Interval of Spectral Scan

DPM.....Disintegrations per Minute

E.....Irradiance

E_d.....Dark Measurement of Spectral Irradiance

E_k.....Irradiance Saturating for Photosynthesis

ΣCO_2.....Total Dissolved Carbon Dioxide

FIRe.....Fast Induction and Relaxation Fluorescence

F_m.....Maximum Fluorescence

F_0.....Initial Fluorescence

F_v.....Variable Fluorescence

F_v/F_m.....Photosynthetic Efficiency of Photosystem II

l.....Path Length

λ.....Wavelength

LEDs.....Light Emitting Diodes

OD.....Optical Density

OD_{chl}.....Difference in Optical Densities at 735 and 680 Nanometers

P^B.....Chlorophyll *a* Normalized Photosynthetic Rate

Φ.....Quantum Yield

Φ_{FIRe}.....Quantum Efficiency Measured by FIRe Fluorescence

Φ_{max}.....Maximum Quantum Yield Determined by ^{14}C Uptake

Φ_{PBR}.....Quantum Efficiency Measured by Photobioreactor System

POC.....Depth-Integrated Particulate Organic Carbon

P_{max}.....Maximum Photosynthetic Rate

PS.....Photosystem

P^S.....Potential Maximum, Light Saturated Photosynthetic Capacity

PSU.....Photosynthetic Unit

PUR.....Photosynthetically Utilized Radiation

T_L.....Time, in Milliseconds, Cells are Exposed to Light

T_D.....Time, in Milliseconds, Cells are Exposed to Dark

TDLD.....Total Daily Light Dose

.....Average Disintegrations per Minute of ^{14}C SPIKE

t.....Time

CHAPTER I

INTRODUCTION

Phytoplankton are subjected to a wide range of light fluctuations in natural oceanic waters (Denman and Gargett 1983; Flameling and Kromkamp 1997). Short term fluctuations of the light regime occur due to tidal cycles, Langmuir circulation, waves, and cloud cover (Dera and Gordon 1968; Walsh and Legendre 1983; Esposito *et al*. 2009). Turbidity and mixing depth also influence the light experienced by phytoplankton. In more turbid waters, the fluctuations tend to result in more time in the dark than the light, which has both positive and negative implications on algal photosystems (Cloern 1987; Ogbanna *et al*. 1995). Phytoplankton can respond to irradiance changes rapidly by changing their absorption characteristics and photosynthetic electron transport capacity (Falkowski *et al*. 1994; Geider *et al*. 1998). Irradiance fluctuations have been also shown to alter photosynthetic end products (Wallen and Geen 1971), productivity measurements (Walsh and Legendre 1983; Terry 1986; Ogbanna *et al*. 1995; Park *et al*. 2000), and light utilization efficiency (Prezelin 1976; Terry 1986).

Over the past 15-20 years, there has been increased interest in growing microalgae in mass culture systems to utilize their potential as a source of pigments, pharmaceuticals, a means of wastewater treatment, and CO_2 drawdown. Cells in high density cultures in large outdoor systems can experience fluctuating light environments. In large-scale outdoor pond raceway systems, such light fluctuations can be attained using a pump or airlift that generates a turbulent flow that keeps cells in suspension, typically a Reynolds number between 2000 and 20,000 (Huntley and Redalje 2007). In these systems with dense cultures, the light received by an individual cell changes due to vertical movement into and out of a light field that decreases exponentially from the surface with pond depth. Increasing the pumping speed increases the turbulence, and, hence, the rate at which cells move between high light and low light conditions. Due to this light modulation, the cells may respond with an increase in productivity (Terry 1986;

Bosca *et al*. 1991). Until the mid-1980s, however, vertical motion was normally a result of some random mixing created by circulating the culture. To increase productivity for a culture, however, a non-random mixing pattern is needed (Falkowski and Raven 2007). Laws *et al*. (1983) developed wing-shaped foils to create vortex circulation in an ordered pattern of mixing. It was reported that, due to this vertical turbulence, productivity improved in cultures of *Phaeodactylum* by a factor of 2. It is unknown, however, if these improvements were a function of the ordered light fluctuations alone, or due to other effects of mixing (i.e., increased nutrient uptake).

Grobbelaar (1994) concluded that in a well-mixed system, increased productivity was due mainly to increased mass transfer rates between the growth medium and the cultured organism. Thus, the light/dark fluctuations of ≤1 Hz did not influence productivity. Later work from the same author both reinforced these conclusions (Grobbelaar *et al*. 1995), and contradicted them (Grobbelaar 1996).

Lewis *et al*. (1984) and Litchman (2000) explained that there are two methods that can be applied by phytoplankton cells when exposed to fluctuating light. When the light fluctuations are faster than the physiological response times of the algae, the cells may integrate the irradiance over time. In such an instance, the population would be homogeneous throughout the water column and the rate of photosynthesis under fluctuating light would be equal to that under static light conditions with the same total daily light dose. If the fluctuations are slower than the physiological response time of the algae, the cells may be able to adjust their photosynthetic parameters to optimize their light absorption for carbon fixation based on the maximum light to which they are exposed. This involves not only the photon flux, but also the culture density, the optical path length, and both the ratio and frequency of alternating exposure to high and low light conditions within the culture (Terry 1986; Richmond *et al*. 1993). The objective of this project was to answer the question of whether light/dark fluctuations alone are responsible for increased quantum yield and under which frequencies of fluctuation such an increase could occur. This was

done with a small-scale system, using a flat-plate photobioreactor, subjecting cultures to static and fluctuating light regimes under the same constant turbulence, growth rate, and total daily light dose.

Background

The Influence of Light on Phytoplankton Cultures

Phytoplankton acclimate to the environment surrounding them by altering the structure and composition of their photosynthetic apparatus to optimize their light-harvesting ability (Falkowski 1981; Falkowski 1983; Li and Morris 1982; Berner and Sukenik 1998). Depending on their location, phytoplankton will experience extreme fluctuations in light as a function of waves, vertical mixing, and turbulence (Dera and Gordon, 1968; Denman and Gargett 1983; Gallegos and Platt 1982; Yoder and Bishop 1985). These changes in irradiance occur on various time scales, which can influence the potential of whether or not a cell will acclimate to the fluctuating light. If the cells are exposed to light fluctuations greater than the rate of acclimation, the population will become homogenous throughout the water column (Bailey 1997; Nedbal and Koblizek 2005). However, if the fluctuations are slower than the acclimation rate, there will be vertical structure (MacIntyre *et al.* 2000). Further work suggested that variations in mixing rates within isopycnal layers were connected to the vertical structure of phytoplankton communities, thus affecting fluorescence (Steinbeck *et al*. 2009).

Grobbelaar (1985) and Cloern (1987) explored turbidity as a control of phytoplankton productivity. In highly turbid waters, the time it takes for a cell to mix out of the photic zone is decreased due to increased light attenuation. These conditions yielded light:dark cycles ranging from a few seconds to a few minutes. Such effects are enhanced with greater wind stress. Mixing increases with increased wind stress, subjecting cells to higher light levels for shorter periods, while also increasing the frequency of exposure (Lohrenz *et al.* 2003). Such movements into and

out of the light field impact photosynthetic parameters, particularly with respect to photosynthesis-irradiance (P-E) parameters (Walsh and Legendre 1983).

The P-E relationship is determined by the incubation of water samples at several light levels during a fixed period. The resulting P-E curve parameters are then applied to productivity models. Most of these models are static because it is assumed that the P-E parameters are constant over time (e.g., Jassby and Platt 1976; Fasham and Platt 1983; Yoder and Bishop 1985). However, experimental evidence suggests that this static description is inappropriate (Marra 1978; Pahl-Wostl 1992; Esposito *et al.* 2009). The photosynthetic parameters are contingent upon light intensities experienced recently by the organisms. Phytoplankton cells may become photoinhibited under high light levels, but the relative strength of this depends on the exposure time of the cells to such high irradiance. Harris and Piccinin (1977) suggested that cells can maintain high rates of photosynthesis during the first few minutes after exposure to saturating irradiance before photoinhibition occurs. On the other hand, after light is switched off, production stops and recovery from photoinhibition takes longer (Belay 1981). Such static models and previous experimental results suggest that static P-E curves might lead to a significant underestimation of primary productivity (Macedo *et al.* 2002). Pahl-Wostl (1992) and MacIntyre *et al*. (2000) suggested that this is because the lag-time for photosynthetic response to increased irradiance is not accounted for in standard P-E approaches. Such hysteresis effects are superimposed on acclimation of the photosynthetic apparatus under fluctuating light given an incident level of photosynthetically available radiation.

Numerous experiments have been attempted to utilize this flashing light effect to increase productivity and quantum efficiency. Phillips and Myers (1954) reported that when *Chlorella pyrenoidosa* was grown under modulated light at frequencies between 1.5 and 144 Hz, the photochemical reactions were instantly saturated and the quantum yield for photosynthetic oxygen production and growth was higher than when grown under static light. This early study

began speculation that photosynthesis could be enhanced as a function of fluctuating light and/or agitation.

Marra (1978) reported increases in photosynthesis up to 87% by fluctuating light on the order of minutes to hours. Walsh and Legendre (1983) showed that fluctuating light at 2.5 Hz could increase productivity from that of a continuous light regime by up to 30%, depending upon the frequency of the fluctuation. Terry (1986) reported similar results working with *Pheaodactylum* cultures. Using a dense culture system, cells were subjected to vortex circulation to simulate light flashes on the order of 0.25 – 7.50 Hz. In this case, irradiance ranged from 250 – 1750 µmol photon m^{-2} s^{-1}. It was found that, while photosynthesis was enhanced, it was not dependent on the flash intensity.

Flameling and Kromkamp (1997) worked with a freshwater chlorophyte, subjecting it to maximum light intensities of 3.46-8.64 mol photon m^{-2} d^{-1}, with a constant total daily light dose (TDLD) of ~0.95 mol photon m^{-2} d^{-1}. They found that cells exposed to fluctuating light on the order of hours had a lower chlorophyll content and smaller photosynthetic units, but a larger number of photosynthetic units per cell, leading to higher maximum rates of gross photosynthesis.

The basis for the increased productivity due to fluctuating light is uncertain. Two processes are suggested: reduced enhancement of respiration following illumination, and disequilibrium between photosynthetic electron transport and the Calvin-Benson Cycle (Falkowski and Raven 2007). In the first instance, the enhancement of postillumination respiration is smaller in fluctuating light because the production of storage products during the light is reduced (Falkowski and Raven 2007). In the second instance, reductant and ATP are produced at a faster rate than they can be consumed. If a dark period is imposed between light periods, carbon fixation processes can "catch up" and consume the reductant and ATP generated by photosynthetic electron transport (Radmer and Kok 1977 p. 599).

Quantum Yield (Φ)

How are productivity and physiological health measured in phytoplankton cultures? A key assessment used to determine the physiological status of a cell is quantum yield, which is influenced by nutrient stress. It is calculated as the ratio of carbon fixed or oxygen evolved to photons absorbed by a culture. The theoretical limit for maximum quantum yield is 0.125 mol C mol photon^{-1}, assuming a minimum quantum requirement of 8 photons absorbed per mole of carbon fixed (Williams and Laurens 2010). Measurement of this parameter is typically done by dividing the initial slope of the P vs. E curve, normalized to chlorophyll *a* concentration, α^B, by the chlorophyll *a* normalized spectrally averaged and weighted optical absorption cross section, ^{B}. The quotient, after correcting for different irradiance and time units and converting milligrams of C to moles of C, yields final units of mol C per mol photon absorbed.

Measurement of Saturating Pulse Fluorescence (F_v/F_m)

The measured fluorescence at ambient temperature stems almost exclusively from chlorophyll associated with photosystem (PS) II. The fluorescence field of PS I is low unless measurements are done at low temperatures (Strasser and Butler 1977). This is due to non-radiative decay processes such as thermal emission and triplet formation (Hofstraat *et al.* 1994). Chlorophyll associated with the photochemical reaction centers represents only a small fraction of the chlorophyll content of the cell and has a low fluorescence quantum yield.

Photosystem II fluorescence yield, however, is variable, and is influenced strongly by the physiological state of the phytoplankton. Excited states are a result of light absorption. Deactivation of these excited states occurs via photosynthetic energy conversion, triplet formation, fluorescence dissipation, and/or radiative heat transfer. When the photochemical reaction centers are open (i.e., when they can use energy of an absorbed photon to drive an electron to a fluorescence quencher), the non-photochemical processes will be low, and photosynthetic energy conversion will be high. Thus, the fluorescence yield will vary inversely with the yield of the photochemistry. Fluorescence yield consists of a constant part and a variable part, which is

determined by the state of the photochemical reaction center (Butler 1972). The state of the reaction center is influenced by the environment (e.g., light history, pollution, nutrient availability).

The quantum yield of photochemistry for PSII, F_v/F_m, is the product of the probabilities of excitation transfer between antennae and PS II reaction centers, and *vice versa* (Parkhill *et al*. 2001). If non-radiative transfer in the reaction center is much smaller than the transfer back to the antennae, the yield of open PS II reaction center photochemistry is given by Equation 1:

— ———— , Equation 1

where F_m is maximal fluorescence after a saturating light pulse and F_o is the fluorescence intensity in dark-adapted phytoplankton, where all PS II reaction centers are open and photochemical quenching is maximal (Van Kooten and Snel 1990; Hofstraat *et al.* 1994).

Plants absorb light and can use energy in three ways. It will first be used in the photosynthetic process. If the light is too intense or the plant is unhealthy, it will be dissipated as fluorescence or as heat. The amount of fluorescence energy emitted infers the health of the plant. Higher values suggest a better physiological state while lower values mean that the plant is stressed.

Objectives

In their natural environment, phytoplankton persist under a wide range of irradiance fluctuation frequencies. To perform a comprehensive study of these effects would be very difficult. This project focused on potential frequencies that can be attained in a large scale outdoor raceway pond system. The objectives here were to determine the effects of irradiance fluctuations on photosynthetic parameters and quantum yield for two species of phytoplankton, *Phaeodactylum tricornutum* (Bohlin 1897) and *Chaetoceros gracile* (Lemmermann 1898), given equal total daily light dose, turbulence, growth rate, temperature, and nutrient concentrations. These experiments were designed to test the following hypotheses:

Research Hypotheses

Given that phytoplankton strains will undergo ordered light fluctuations, the following hypotheses were tested:

(H_1) Phytoplankton that have acclimated to using the fluctuating light effect will have greater quantum yields under fluctuating light regimes than under static light conditions when total daily light dose, nutrient concentration, and temperature are kept the same for both treatments.

(H_2) Each of the phytoplankton strains grown under a range of frequencies will yield the highest production at the highest frequency (2.00 Hz), because the culture will be able to better integrate the light fluctuation over the photoperiod than when the frequency is lower.

(H_3) The four methods for measuring quantum yield and F_v/F_m being used (Table 1) will yield similar trends to each other. The highest quantum yield values will be recorded under the highest light frequency (2.00 Hz) and the lowest values under the lowest frequency (0.10 Hz).

(H_{3a}). During nutrient-replete conditions, these values will be high. Under stressed conditions, they will be reduced.

(H_4) Effects of fluctuating light will be species-specific. *C. gracile* will have greater quantum yields than *P. tricornutum* under fluctuating light due to its ecological niche as a planktonic strain rather than an epibenthic strain, where light fluctuations are more likely to occur in nature.

CHAPTER II

METHODS AND MATERIALS

Phaeodactylum tricornutum and *Chaetoceros gracile* were selected for several reasons. First, it was important to use strains of similar taxa, but different photoadaptational patterns. *P. tricornutum* is a fast-growing diatom that sinks quickly in the water column when there is no turbulence. *C. gracile* is a planktonic strain that remains in suspension, even under little turbulence. Based on this, *P. tricornutum* should experience different light characteristics than *C. gracile* in the field. These strains are also conveniently available in the culture collection in the culture collection at The University of Southern Mississippi Department of Marine Science. The *C. gracile* culture was subjected to five frequencies of light fluctuation treatments (Table 1) in addition to a static light treatment.

Table 1

Light conditions per experiment. T_L is the time, in milliseconds, the cells were exposed to light and T_D is the time, in milliseconds, the cells were exposed to darkness. All cultures were exposed to a 12hr:12hr light:dark cycle. Irradiance is measured as μmol photon m^{-2} s^{-1} and the units for frequency are Hz.

Strain	Light Treatment	Irradiance	Frequency	T_L	T_D	$\frac{T_L}{(T_L+T_D)}$
Chaetoceros gracile	Static light	333	N/A	N/A	N/A	N/A
	Fluctuating Light	1000	0.10	3333	6667	1/3
			0.50	666	1334	
			0.67	500	1000	
			1.00	333	667	
			2.00	166	334	
Phaeodactylum tricornutum	Static light	333	N/A	N/A	N/A	N/A
	Fluctuating Light	1000	0.67	166	334	1/3

Each treatment received the same total daily light dose (TDLD). *P. tricornutum* was subjected to static light treatment and the fluctuating light treatment that yielded the highest chlorophyll *a* concentration per cell in the *C. gracile* culture. The cultures were subjected to the chosen light conditions using a 12hr:12hr light:dark cycle. To measure the response of the phytoplankton strains to the various light treatments, four diagnostics were used (Table 2).

Table 2

Description of diagnostics

Diagnostic	Units	Description
Φ_{max}	mol C (mol photons absorbed)$^{-1}$	Quantum Yield determined using the initial slope of the P-E curve (α^B) divided by the spectrally weighted optical absorption coefficient ( ).
Φ_{ave}	mol C (mol photons absorbed)$^{-1}$	Quantum Yield determined using the difference in total particulate carbon at the beginning and end of the day divided by the photosynthetically utilized radiation.
F_v/F_{mFIRe}	dimensionless	Quantum yield of photochemistry for PSII determined by variable fluorescence using a FIRe fluorometer.
F_v/F_{mPBR}	dimensionless	Quantum yield of photochemistry for PSII determined by variable fluorescence using fluorometer in photobioreactor.

Photosynthesis-irradiance curves and absorption spectra were constructed to determine instantaneous quantum yield (Φ_{max}). Additionally, depth-integrated total particulate carbon (POC) was measured and compared to photosynthetically utilized radiation (PUR) to ascertain a daily averaged calculation of quantum yield (Φ_{ave}). Readings of fast induction and relaxation (F_v/F_{mFIRe}) fluorescence and *in situ* Dual Pulse Amplitude Modulated (F_v/F_{mPBR}) fluorescence allowed for a quantified measure of the quantum yield of photochemistry for PSII.

Culture Conditions

Cultures were maintained in modified f/2 medium, with concentrations of 880 µM NO_3^-, 80 µM PO_4^-, 880 µM $Si(OH)_4$, f/20 metals, and f/2 vitamins (adapted from Guillard and Ryther

1962). Each of two photobioreactor (PBR) systems (described later) received equal rates of bubbling of a 2% CO_2 enriched air mixture via a GMS150 gas mixer (Photon Systems Instruments). Cultures received light treatments based on Table 1. *Chaetoceros gracile* was maintained at 30°C, *P. tricornutum* at 20°C. Experiments were diluted semi-continuously to maintain an average daily specific growth rate of 0.70 d^{-1}.

Photobioreactor System

The phytoplankton strains were grown in FMT150 photobioreactor systems developed by Photon Systems Instruments, Brno, Czech Republic (Nedbal *et al*. 2008; Figure 1). These were flat-plate photobioreactors with a capacity of 400 mL. Light, temperature, and gas composition could be oscillated at various frequencies and intensities. Light was provided by an array of 96 high power light-emitting diodes (LEDs), half in the red wavelength, half blue. These LEDs had the capacity to generate up to 2500 µmol photon m^{-2} s^{-1}. Growth was monitored by an integrated densitometer measuring optical density at 680 nm and 735 nm. A proxy measurement of chlorophyll *a* concentration was given as the difference between these two optical density values (OD_{chl}). Temperature was controlled by a thermal bridge at the bottom of the vessel using a Peltier thermocouple in the instrument base.

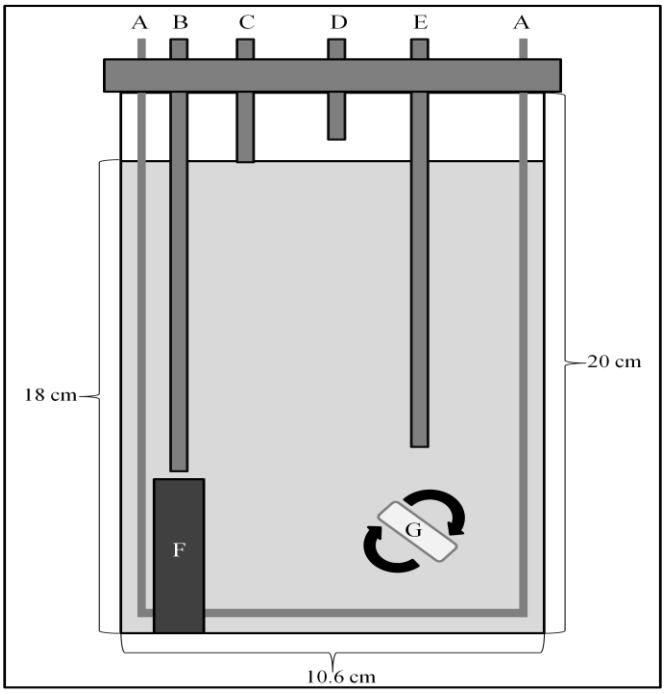

Figure 1. Diagram of the photobioreactor. A: Air/CO_2 inlet. B: Temperature probe. C: Overflow tube. D: Fresh medium inlet. E: Sampling port tube. F: Optical density sensor and fluorometer. G: Stirbar.

Experimental Design

The photobioreactors were inoculated with culture using a syringe (21G1½ gauge needle) through a rubber septum. The cultures were allowed to grow to an OD_{chl} of ~0.35 (e.g., Figure 2). At this point, semicontinuous dilutions occurred daily, with one-half of the culture being extracted just before the light was turned off via a specialized syringe design. The PBR was then filled to 400 mL with fresh medium.

During this phase of dilution, samples were taken three times per day. First, a sample was taken five minutes after lights were turned on in the morning. Second, a sample was taken just before the lights were turned off at the end of the day. When the lights switched off, the dilution took place. A third sample was taken after dilution. These were samples of ~10 mL that were used to measure chlorophyll *a* via the direct injection method (Johnson *et al*., 2007), *in vivo* fluorescence using a Turner Designs 10AU fluorometer, POC using an Costech Instruments ECS 4010 Elemental Combustion System, cell counts using a model Z2 Coulter Counter, and nutrients

measured using a SEAL AutoAnalyzer 3. These measurements were used to monitor growth rate and nutrient concentration.

When biomass measurements were consistent for three consecutive days (Figure 1), cultures were sampled for particulate absorption (Tassan and Ferrari, 1995; Mercado *et al*. 2004) and P-E curves (Lewis and Smith 1983), in addition to the aforementioned measurements. Quantum Yield was determined for each light condition from the P-E curves, coupled with spectral absorption (Φ_{max}), as well a measurement of net total particulate carbon gain divided by photosynthetically utilized radiation (Φ_{ave}). Additionally, the quantum yield of photochemistry in Photosystem II (Raven and Falkwoski 2007) was measured *in situ* via F_v/F_m calculations within the photobioreactors(F_v/F_{mPBR}). A second measure of F_v/F_m was measured using a sample taken from the growth chamber using the FIRe fluorometer (F_v/F_{mFIRe}).

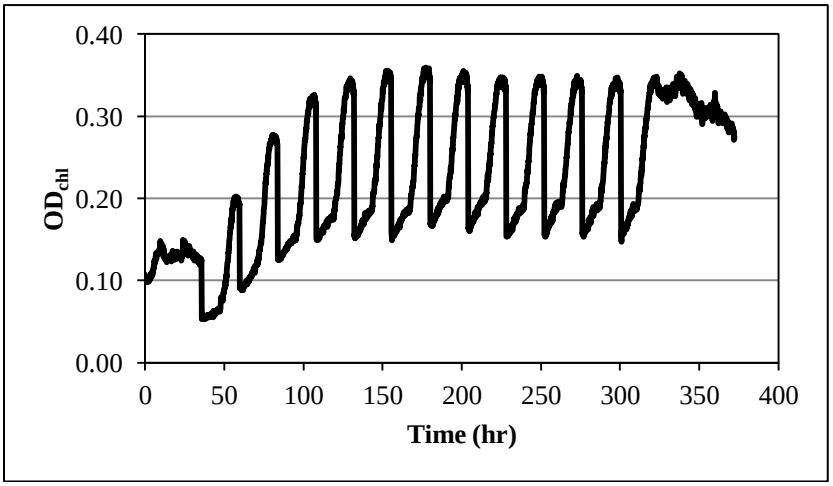

Figure 2. Optical density of Chlorophyll *a* content over time for the *C. gracile* culture exposed to 0.67 Hz fluctuating light. Nutrient replete assessments occurred when OD_{chl} was stable for 3 consecutive days (~hour 320). Cultures were then starved for 2 days for assessment when nutrient stressed (~hour 370).

Fresh medium was added to fill the PBRs and the culture was then run in a batch-type mode until the nutrients in the growth chamber had been depleted and the cultures became nutrient starved after 3 days. The same measurements as discussed above were taken under these stressed conditions.

Determination of Maximum Quantum Yield (Φ_{max})

Photosynthesis was determined using the ^{14}C uptake method developed by Steemann Nielsen (1952), modified to use small-volume, short term incubations in two photosynthetrons (Lewis and Smith 1983). Forty positions for 7 mL liquid scintillation (LS) vials were available in the two photosynthetrons, with each position subjecting a sample vial to a different light intensity (20 – 2030 µmol photon m^{-2} s^{-1}) provided by an ELH-type tungsten-halogen projection lamp (Sylvania) directed through a heat filter of circulating water and a 0.25 M solution of $CuSO_4$ to correct the light spectrum (Jitts *et al*. 1964). Quantum scalar irradiance was measured in each position with a Vernier 4π light sensor.

Two 30 mL samples were taken from the photobioreactor and inoculated with 30 µL of a 1 mCi mL^{-1} $NaH^{14}CO_3$ stock solution. Aliquots of 1 mL of the labeled sample were then placed into each of 23 glass scintillation vials in each of two photosynthetrons. Thus, duplicate curves were generated for each P-E experiment. One additional 1 mL aliquot of labeled sample per P-E curve was placed into an LS vial. At the moment the photosynthetron light was switched on, these samples received 500 µL 10% HCl in order to terminate photosynthesis immediately. The vial was placed in a dark area and was used to determine T_0. Two 50 µL subsamples of the labeled culture were placed into LS vials containing 50 µL of a 50:50 ethanol:ethanolamine mixture to determine the total ^{14}C addition ("SPIKE"). Incubations were stopped after 30 minutes with the addition of 10% HCl. Samples were then shaken on a VWR 3500 Standard Analog Shaker in a laboratory fume hood for at least 6 hours to drive off all volatized $^{14}CO_2$. Envirosafe liquid scintillation cocktail was then added to each vial. The vials were then shaken vigorously prior to determining algal assimilation of $^{14}CO_2$ using a WALLAC Winspectral α/β 1414 liquid scintillation counter.

Photosynthesis was calculated following equation 2 (adapted from Bailey 1997), then normalized to chlorophyll *a* concentration using equation 2:

Equation 2

$$\frac{\qquad\qquad\qquad\qquad\qquad\qquad}{},$$

where DPM_{cell} is the volume normalized disintegrations per minute for each sample in the photosynthetron, DPM_{To} is the volume normalized disintegrations per minute at time zero, 1.05 is the carbon isotope discrimination factor, 12011.2 is the conversion factor for total carbon dioxide ($\sum CO_2$) in meq L^{-1} to mg C m^{-3}, is the average volume normalized disintegrations per minute of two SPIKE samples, and t is the length of incubation in hours.

The P-E data were fitted to the empirically derived equation 3, described by Platt *et al.* (1980). This curve fitting allowed for an estimation of the initial slope of the P-E curve (α^B):

, Equation 3

where P^B is the chlorophyll *a* normalized photosynthetic capacity of the culture, P^S is the light saturated, potential maximum photosynthetic capacity, α^B is the chlorophyll *a* normalized photosynthetic efficiency in the initial, linear portion of the P-E curve, β^B is the chlorophyll *a* normalized photoinhibition parameter, and E is irradiance.

To determine the spectrally averaged optical absorption coefficient ( ), first a measure of the corrected optical density of the culture was determined. To do this, a 3 mL aliquot of culture was removed from the photobioreactor. This sample was then placed into a 1 mm path length optical glass cuvette. The cuvette was then placed into a Varian Cary 300Bio UV/Visible Spectrophotometer equipped with a diffuse reflectance accessory (integrating sphere). The cuvette was placed into a front-mounted cuvette holder that held the cuvette against the outside of the integrating sphere. Spectral absorbance was measured between wavelengths 800 nm and 350 nm. A sample of seawater filtered through a 0.2µm nitrocellulose filter and the filtrate was treated in the same manner as each culture sample. This was used as the instrument blank. A second aliquot of filtered seawater was treated similarly and used as a sample blank, as shown in Equation 4:

, Equation 4

where OD_{sample} is the optical density of the culture sample (dimensionless), OD_{blank} is the optical density of the 0.2µm filtered seawater sample (dimensionless), is the averaged optical density of the difference between the culture and the blank between 751nm and 800nm (dimensionless).

The calculation of the optical absorption coefficient (a*) was determined per Equation 5:

, Equation 5

where 2.303 is the conversion factor from between log and natural log, is the path length of light beam through culture (m), and [*Chl* a] is the concentration of chlorophyll *a* (mg chl *a* m^{-3}).

The spectrally averaged optical absorption coefficient ( ) was necessary to account for the output spectrum of the light emitting diodes used in the photobioreactor and was calculated using Equation 6:

, Equation 6

where is the incident spectral irradiance (µmol photons m^{-2} s^{-1}), is the dark measurement of spectral irradiance (µmol photons m^{-2} s^{-1}), and is the wavelength interval of the spectral scan (dimensionless).

Quantum yield was determined for both species at the various irradiance conditions by dividing the initial slope of the P-E curve normalized to chlorophyll *a* concentration (α^B), by the spectrally weighted optical absorption coefficient ( ). The result was corrected for different irradiance and time units to convert mg C m^{-3} hr^{-1} to mol C m^{-3} s^{-1}. Final quantum yield values are given in units of mol C (mol photons absorbed)$^{-1}$.

Determination of Daily Averaged Quantum Yield

To determine total particulate carbon, triplicate 2 mL samples were removed from the photobioreactor twice daily: once immediately after the lights were turned on and once just before

the lights were turned off 12 hours later. The samples were filtered on to 2.1 cm VWR 691 glass fiber filters (nominal porosity of 1.5 µm). Filters were placed into labeled, acid-washed petri dishes and into a drying oven set at 60^{o}C for 24 hours. The filters were then placed into a desiccator until they could be analyzed using a Costech Instruments ECS 4010 Elemental Combustion System. Total particulate carbon was measured as µmol C m^{-3}. The difference between total particulate carbon in the morning and the evening before dilution was considered the average daily carbon production (µmol C m^{-3} d^{-1}). Analogous to vertical integration in a water column, this value was divided by the depth of the growth chamber (0.02 m) in the photobioreactor to give units of µmol C m^{-2} d^{-1} (Figure 3).

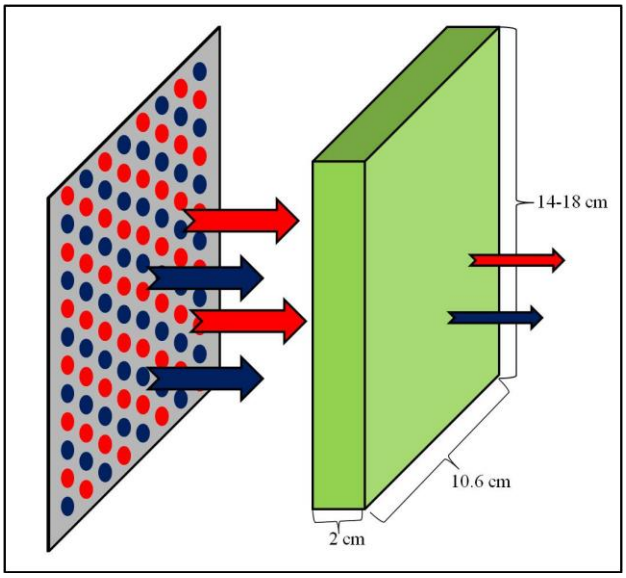

Figure 3. Illustration of measurement of PUR in the photobioreactor. A known photon flux was emitted through 48 red and 48 blue LEDs. The difference between the known available light (large arrows) and light measured on the front side of the growth chamber (small arrows) was determined to be the photosynthetically utilized radiation (PUR).

Prior to inoculation, triplicate measurements of irradiance for clear medium were made using a Biosciences LI-COR LI-250A Light Meter with a 10 second average, providing irradiance measurements in units of µmol photons m^{-2} s^{-1}. This allowed for an initial "blank" measurement of light available for absorption.

After cells were inoculated into the photobioreactor, subsequent measurements using the LI-COR sensor were made at "lights on" and just before "lights off." It was assumed that the difference between the average daily irradiance of medium with culture, less the average irradiance value of clear medium was absorbed by the culture. Thus, this value was the photosynthetically utilized radiation (PUR) of the culture (μmol photons absorbed m^{-2} s^{-1}). There was no account for scattering of light.

Daily averaged quantum yield (Φ_{ave}) was determined by dividing the total particulate carbon increase for a 12 hr light period by the photosynthetically utilized radiation, then correcting for different time units. Final Φ_{ave} values were given in units of mol C fixed (mol photons absorbed)$^{-1}$.

Determination of quantum yield of photochemistry for Photosystem II (F_v/F_{mFIRe} and F_v/F_{mPBR})

Quantum yield of photochemistry of Photosystem II (F_v/F_m) was determined two ways, both using variable fluorescence. First, *in situ* values of initial (F_t) and maximum fluorescence (F_m) were available from the photobioreactor software, which provided a calculation of variable fluorescence. Second, samples were removed from the photobioreactor to determine F_v/F_m using a Satlantic Fast Induction and Relaxation (FIRe) fluorometer.

These two instruments provide very similar information, but in a different way. The *in situ* measurement elicited chlorophyll fluorescence emission with a flash of blue light originating from the detector within the photobioreactor while the light itself was blocked by the detector filter. This measurement provided a "steady-state" emission yield, F_t. Following this, the cultures were exposed to simultaneous flash of all LED panels to saturate the photochemistry of PSII reaction centers, providing a maximum fluorescence emission, F_m (Nedbal *et al*. 2008). Quantum yield of PSII photochemistry (F_v/F_m) was then calculated using Equation 7:

Equation 7

where F_t is the initial measurement of fluorescence in the light, and F_m is the maximum fluorescence after a photosynthesis-saturating pulse of high light.

The FIRe fluorometer is an instrument that required a sample to be removed from the bioreactor cuvette and placed into a dark tube. The sample was then subjected to a short pulse of 80 μs duration to saturate PSII and measure the fluorescence induction from F_0 to F_m. A weak modulated light is then applied to determine the relaxation of the fluorescence yield on a time scale of 500 ms. Calculation of quantum yield of photochemistry for PSII was done as per Equation 7, substituting F_0 for F_t.

CHAPTER III

RESULTS

The objective of this project was to test the hypotheses that phytoplankton would respond to fluctuating light differently, as compared to their responses when subjected to static light. Results from these experiments suggest that there is, indeed, an altered response by two phytoplankton strains subjected to fluctuating light relative to when subjected to static light. There appeared to be a greater quantum yield for *C. gracile* when subjected to light fluctuating at 0.67 Hz and 1.00 Hz, as compared to when subjected to static light at an equal total daily light dose. However, when exposed to 0.10 Hz, 0.50 Hz, and 2.00 Hz, there appeared to be very small, if any, differences in quantum yield when nutrient replete. Additionally, *P. tricornutum* appeared to fix carbon less effectively per absorbed photon when exposed to light fluctuating at 0.67 Hz, relative to when exposed to static light. The quantum yield and quantum yield of photochemistry of PSII were both lower when this culture was subjected to fluctuating light.

Evaluation of Hypotheses

The first hypothesis was that two phytoplankton cultures would experience a greater quantum yield when acclimated to fluctuating light, relative to when acclimated to static light when nutrient replete. There were four diagnostics used to determine this (Tables 1 and 4).

The Φ_{max} for *C. gracile* subjected to static light was 0.030 mol C (mol photons)$^{-1}$. Maximum quantum yield values, and values of α^B tended to increase with increasing frequency of fluctuating light through 1.00 Hz, with a lower Φ_{max} at 2.00 Hz (Tables 3 and 4). Every fluctuating light treatment yielded a greater Φ_{max} than the static light treatment. The culture subjected to fluctuating light at 1.00 Hz yielded a Φ_{max} 2.16 times greater than that of static light, suggesting different responses to their respective light treatments (Figure 3).

Table 3

Photosynthesis-Irradiance parameters of experiments. Units for each parameter are as follows: cell counts: x10^6 cells mL^{-1}, chlorophyll a: µg mL^{-1}, Chl a per cell: ng chl a per cell; P^B_{max}: mg C mg Chl a^{-1} h^{-1}, α^B: mol C (mol photons absorbed)$^{-1}$, E_k: µmol photons m^{-2} s^{-1}, : m^2 mg chl a^{-1}, Depth-Integrated POC: mol C m^{-2} d^{-1}, and PUR: mol photon m^{-2} d^{-1}.

Light Condition	Cell Count	Chl *a*	Chl *a* per cell	P^B_{max}	α^B	E_k		Depth-Integrated POC	PUR
				Chaetoceros gracile					
Static	12.03	2876.8	239.1	3.639	0.014	253.726	0.0107	0.49	11.92
0.10 Hz	8.06	2825.9	350.6	4.001	0.015	266.673	0.0106	0.26	13.48
0.50 Hz	10.69	3403.4	318.4	5.558	0.018	301.080	0.0107	0.32	12.10
0.67 Hz	7.19	3849.9	535.5	5.266	0.022	241.249	0.00975	0.37	12.20
1.00 Hz	9.26	3462.8	373.9	8.189	0.026	318.559	0.00938	0.32	11.96
2.00 Hz	12.93	3284.2	254.0	3.767	0.016	231.860	0.0101	0.38	11.55
				Phaeodactylum tricornutum					
Static	5.34	2239.7	419.4	4.135	0.019	223.083	0.0094	0.30	8.46
0.67 Hz	2.27	1594.3	702.3	2.743	0.013	205.803	0.0086	0.09	6.91

However, the culture acclimated to light fluctuating at 0.10 Hz resulted in only a 7% greater Φ_{max} than that of the culture subjected to static light, implying that there was less of a benefit to the

culture when given this light treatment, relative to when subjected to higher frequencies (Figure 3). The *P. tricornutum* culture subjected to static light resulted in a Φ_{max} of 0.047 mol C (mol photons absorbed)$^{-1}$. This is 27% higher than 0.035 mol C (mol photons absorbed)$^{-1}$, the value given by the culture acclimated to light fluctuating at 0.67 Hz, suggesting that *P. tricornutum* fixed carbon more efficiently at the end of the day under static light.

Information regarding nitrogen concentration at the end of the light period is available in Table 4. The initial concentration of nitrate in the medium was 836.8 μM for the *C. gracile* cultures and 811.0 μM for the *P. tricornutum* cultures. The greatest values of PN were recorded for the nutrient replete *C. gracile* cultures. The lowest values were recorded for the nutrient stressed *P. tricornutum* cultures. These concentrations were roughly half of what was in the initial medium stock.

Table 4

Nitrogen concentrations of all experiments. NO_3^- concentration and particulate nitrogen (PN) are in units of μM.

Phytoplankton Strain (Initial Medium [NO_3^-])	Light Treatment	Nutrient Status	NO_3^-	PN
Chaetoceros gracile (836.8 μM)	Static	Replete	1.764	930.041
	0.10 Hz		2.608	841.882
	0.50 Hz		2.672	793.396
	0.67 Hz		2.349	853.949
	1.00 Hz		0.000	691.237
	2.00 Hz		2.898	787.163
	Static	Stressed	1.528	856.143
	0.10 Hz		1.796	695.919
	0.50 Hz		3.754	779.690
	0.67 Hz		1.861	760.379
	1.00 Hz		0.045	624.571
	2.00 Hz		1.014	706.619
Phaeodactylum tricornutum	Static	Replete	0.921	690.335
	0.67 Hz		3.901	476.504

(811.0 µM)	Static	Stressed	2.07	718.977
	0.67 Hz		3.701	487.116

The daily averaged quantum yield (Φ_{ave}) for each experiment was less than Φ_{max}, with the exception of the *C. gracile* culture exposed to static light (Figure 4, Table 5). However, the trends are not similar. Under no fluctuating light treatment was the Φ_{ave} of *C. gracile* higher than that of the culture subjected to static light, indicating that over the course of 12 hours, *C. gracile* tends to fix carbon better on the average under static light (Figure 4, Table 5). Average quantum yield did increase with increasing frequency, but there was a lower Φ_{ave} for the culture acclimated to 1.00 Hz than for those acclimated to 0.67 Hz and 2.00 Hz. There appeared to be a reduction in Φ_{ave} between 21% and 53% for *C. gracile* cultures acclimated to fluctuating light relative to the culture subjected to static light (Figure 3). *P. tricornutum* cultures acclimated to static light had a greater Φ_{ave} than those acclimated to light fluctuating at 0.67 Hz by more than a factor of two, suggesting that over the course of a 12 hour daylight period, *P. tricornutum* also tended to fix carbon more efficiently under static light.

Table 5

All quantum yield and quantum yield of PSII photochemistry measurements. Units for Φ_{max} and Φ_{ave} are in mol C (mol photons absorbed)$^{-1}$. There are no units for F_v/F_{mPBR} or F_v/F_{mFIRe}.

Phytoplankton Strain	Light Treatment	Φ_{max}	Φ_{ave}	F_v/F_{mPBR}	F_v/F_{mFIRe}
Chaetoceros gracile	Static	0.030	0.052	0.480	0.502
	Fluctuating at 0.10Hz	0.033	0.021	0.381	0.500
	Fluctuating at 0.50Hz	0.040	0.032	0.538	0.465
	Fluctuating at 0.67Hz	0.052	0.035	0.285	0.519
	Fluctuating at 1.00Hz	0.063	0.031	0.517	0.478
	Fluctuating at 2.00Hz	0.037	0.039	0.180	0.498
Phaeodactylum tricornutum	Static	0.046	0.039	0.511	0.457
	Fluctuating at 0.67Hz	0.036	0.011	0.357	0.239

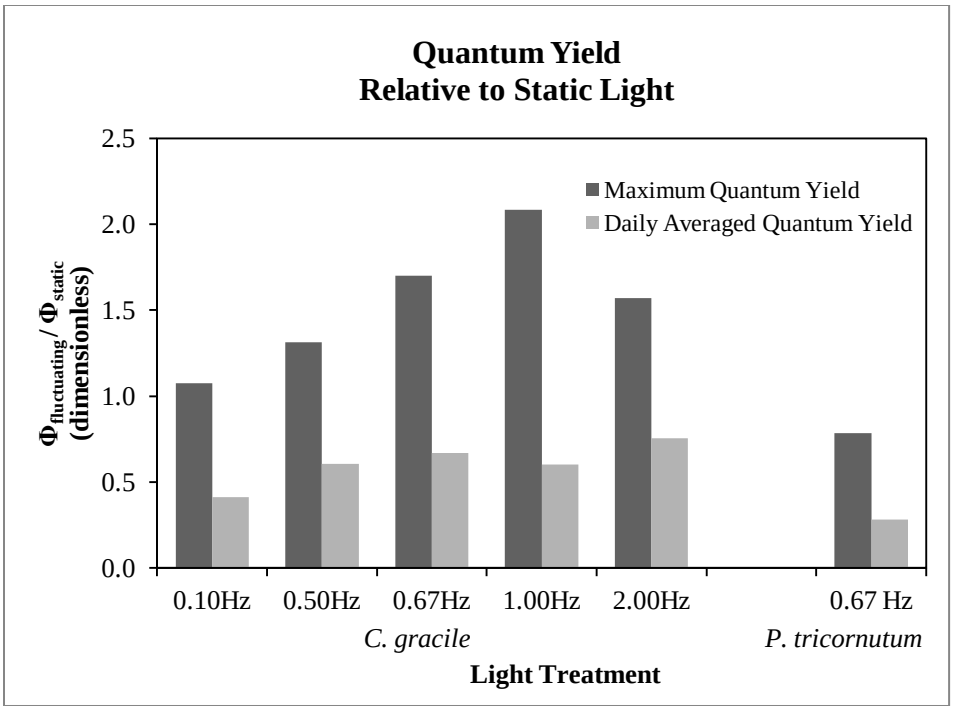

Figure 4. Ratios of fluctuating light relative to static light for Φ_{max} and Φ_{ave}.

The F_v/F_{mPBR} was greatest for nutrient replete *C. gracile* when grown in an environment of light fluctuating at 0.50 Hz (Table 3). However, this was only 12% greater than the F_v/F_{mPBR} for *C. gracile* acclimated to static light (Figure 5). The F_v/F_{mPBR} for *C. gracile* acclimated to 0.67 Hz was 41% lower than that of the culture acclimated to static light. There appeared to be a larger difference between the culture subjected to static light relative to that which was acclimated to light fluctuating at 2.00 Hz (62%), again indicating that the culture was not able use light energy as efficiently under this light treatment compared with the static light treatment. *Phaeodactylum tricornutum* had a 26% greater F_v/F_{mPBR} when subjected to static light than when subjected to light fluctuating at 0.67 Hz.

The F_v/F_{mFIRe} for nutrient replete *C. gracile* was very similar between light treatments, with values ranging from 0.465 for the culture acclimated to light fluctuating at 0.50 Hz to 0.519 when subjected to light fluctuating at 0.67 Hz (Table 5). For every fluctuating light treatment, there was less than a 7% difference in F_v/F_{mFIRe} relative to the culture acclimated to static light (Figure 5). The F_v/F_{mFIRe} of *P. tricornutum* subjected to static light was nearly twice that of

cultures subjected to fluctuating light, further implying that this strain acclimates better to static light than fluctuating light.

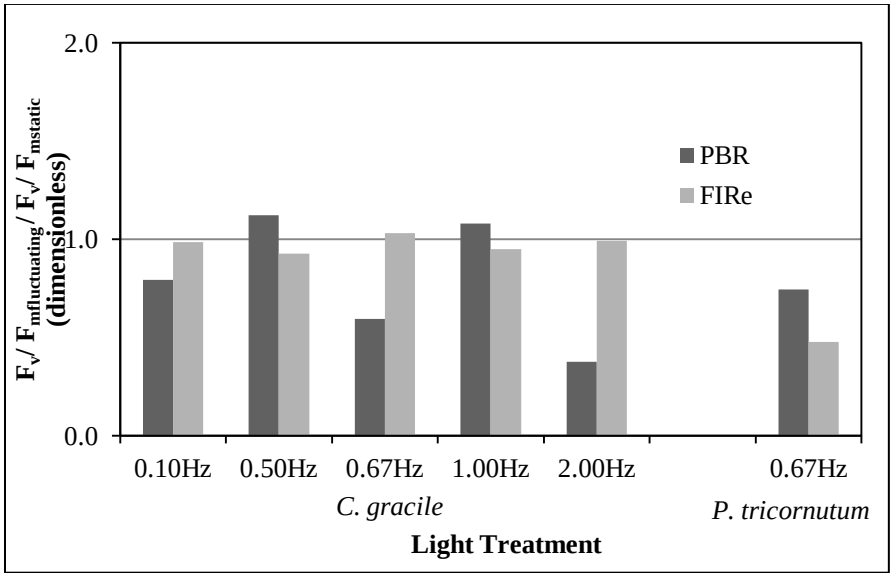

Figure 5. Ratios of fluctuating light relative to static light for F_v/F_{mPBR} and F_v/F_{mFIRe}.

The second hypothesis stated that quantum yield would be greater for the two phytoplankton cultures when nutrient replete than when nutrient stressed. This hypothesis was tested using four diagnostics (Table 1).

The Φ_{max} for every experiment for both strains was lower when the cultures were allowed to grow without nutrient replenishment compared to the cultures under nutrient replete conditions (Figure 6). The degree of reduction was variable, however. For the *C. gracile* culture acclimated to static light, the reduction was ~85%, from 0.030 mol C (mol photons absorbed)$^{-1}$ to 0.005 mol C (mol photons absorbed)$^{-1}$. When subjected to light fluctuating at 0.10 Hz, however, the Φ_{max} was only 36% of that when the culture was nutrient replete, from 0.033 mol C (mol photons absorbed)$^{-1}$ to 0.021 mol C (mol photons absorbed)$^{-1}$. Additionally, the pattern of Φ_{max} found for nutrient stressed cultures was not the same as that of nutrient replete cultures (Figure 6). There was not an apparent increase in Φ_{max} with increasing frequency of light fluctuation. When nutrient stressed, the *C. gracile* cultures acclimated to fluctuating light yielded a greater Φ_{max} than the culture acclimated to static light. However, the Φ_{max} of fluctuating light treatments between 0.10

Hz and 1.00 Hz had a range of values less than 0.007 mol C (mol photons absorbed)$^{-1}$ from each other. The Φ_{max} for *P. tricornutum* subjected to static light was ~22% lower than that subjected to fluctuating light when nutrient deficient, opposite that of when the cells were nutrient replete.

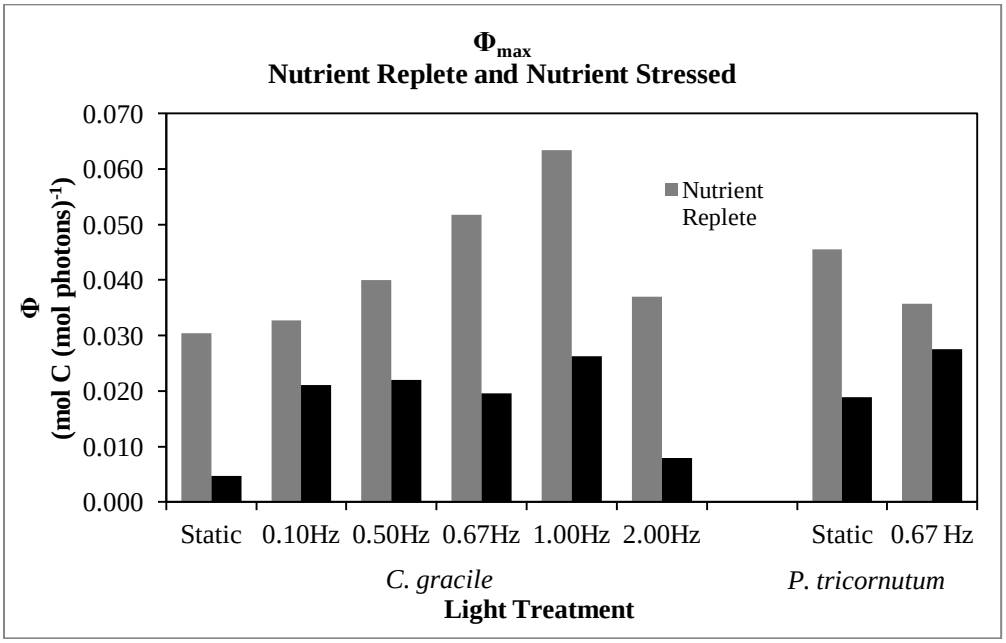

Figure 6. Maximum quantum yield for both diatom strains under nutrient replete and nutrient stressed conditions.

The Φ_{ave} was much lower for nutrient stressed cultures of *C. gracile* relative to when nutrient replete (Figure 7). The lowest Φ_{ave} was recorded for the culture subjected to light fluctuating at 0.50 Hz when nutrient stressed at 0.0014 mol C (mol photons absorbed)$^{-1}$, while the highest was recorded for the culture acclimated to light fluctuating at 0.67 Hz at 0.006 mol C (mol photons absorbed)$^{-1}$. The *P. tricornutum* cultures responded differently to nutrient stress. Under static light conditions, the Φ_{ave} decreased from 0.039 mol C (mol photons absorbed)$^{-1}$ when nutrient replete to 0.017 mol C (mol photons absorbed)$^{-1}$ when stressed. This is only a 56% reduction in Φ_{ave}. Every culture of *C. gracile*, by comparison, showed at least an 80% reduction in quantum yield when nutrient stressed, relative to when nutrient replete. The *P. tricornutum* culture exposed to light fluctuating at 0.67 Hz showed the least percent reduction of Φ_{ave} for any

culture, from 0.011 mol C (mol photons absorbed)$^{-1}$ when nutrient replete to 0.008 mol C (mol photons absorbed)$^{-1}$ when stressed (25% reduction).

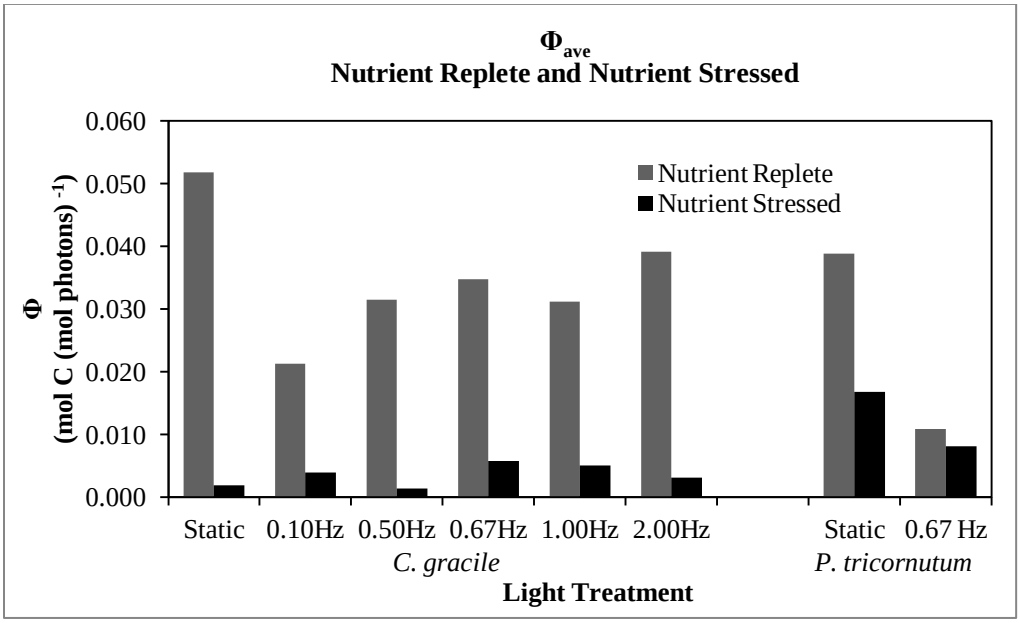

Figure 7. Daily averaged quantum yield for both diatom strains under nutrient replete and stressed conditions.

Reductions in F_v/F_{mPBR} were evident for all *C. gracile* cultures upon nutrient starvation (Figure 8). The *C. gracile* culture acclimated to static light showed a very large (91%) decrease in F_v/F_{mPBR} upon nutrient stress (from 0.48 to 0.05). The smallest reduction in F_v/F_{mPBR} upon nutrient stress was recorded for the *C. gracile* culture acclimated to light fluctuating at 1.00 Hz at 47% (from 0.52 to 0.28). The changes in F_v/F_{mFIRe} when nutrient stressed, however, were lower in magnitude, relative to nutrient stressed F_v/F_{mPBR} (Figure 8). There existed a 49% in decrease F_v/F_{mFIRe} upon nutrient starvation for the *C. gracile* culture acclimated to static light and a 51% reduction when the *C. gracile* culture was acclimated to light fluctuating at 2.00 Hz. However, the reductions were in the 14% - 16% range for the other fluctuating light conditions.

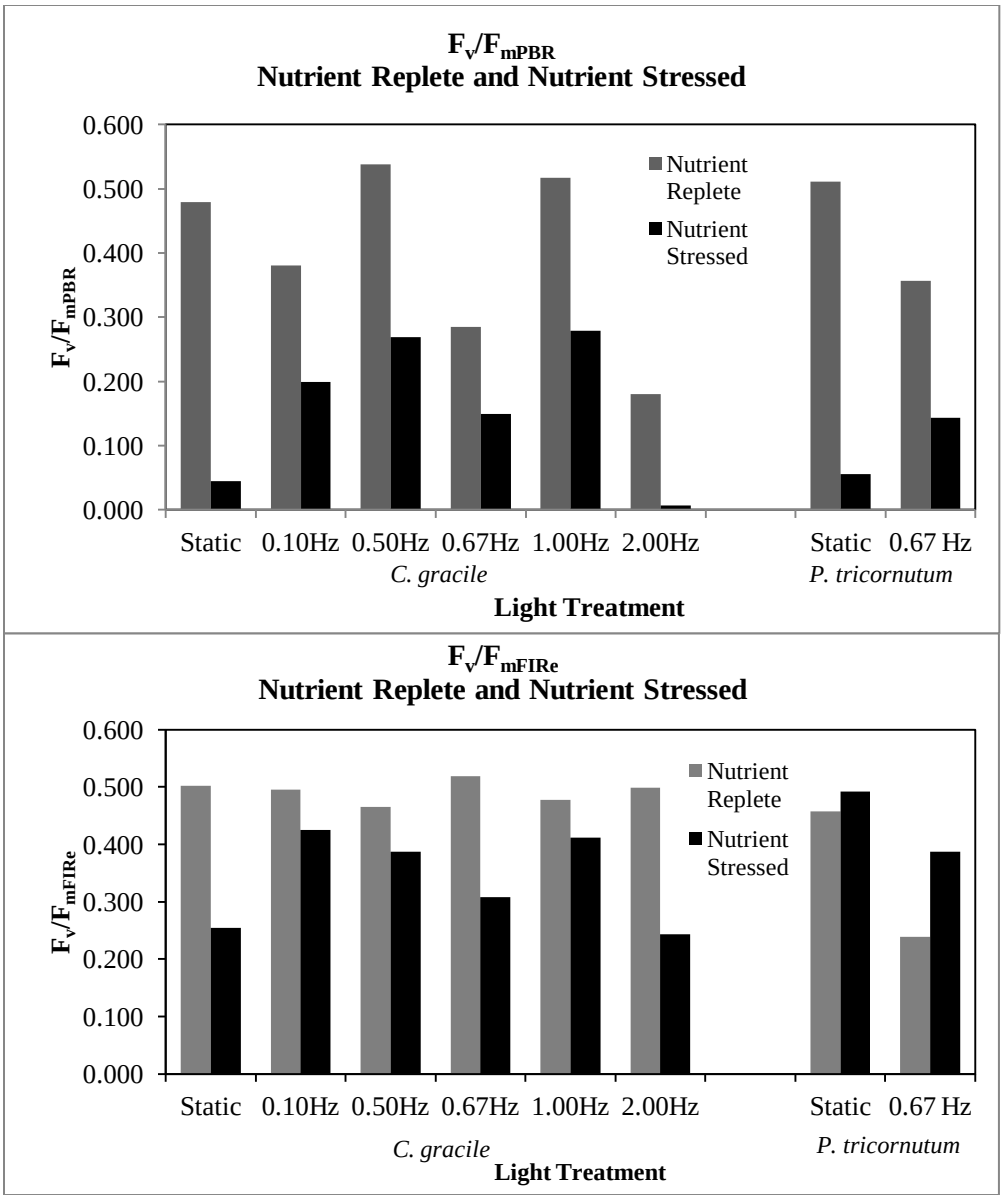

Figure 8. F_v/F_{mPBR} and F_v/F_{mFIRe} for both diatom strains under nutrient replete and stressed conditions.

The final hypothesis was that the effects of fluctuating light on phytoplankton cultures would be species-specific. This was tested using four diagnostics (Table 1). When sufficient nutrients were available, the Φ_{max} and F_v/F_{mFIRe} for *C. gracile* subjected to static light were lower than that when subjected to fluctuating light of any frequency. However, Φ_{ave} and F_v/F_{mPBR} showed lower values under fluctuating light compared to static light (Table 6). When nutrient stressed, all measures were greater for the fluctuating light treatments for this strain.

Opposite trends were evident with *P. tricornutum.* All measures of quantum yield were greater for this strain when subjected to static light compared to fluctuating light when nutrients were plentiful. Upon starvation, Φ_{max} and F_v/F_{mPBR} were lower for the static light treatment than for the fluctuating light treatment.

Table 6

Comparison of quantum yield measurements between diatom strains. Measurements of Φ_{max} and Φ_{ave} are given as mol C (mol photons)$^{-1}$. Values of F_v/F_{mPBR} and F_v/F_{mFIRe} are dimensionless.

Diatom Strain	Nutrient Status	Light Treatment	Φ_{max}	Φ_{ave}	F_v/F_{mPBR}	F_v/F_{mFIRe}
Chaetoceros gracile	Replete	Static	0.030	0.052	0.479	0.503
		0.67 Hz	0.052	0.035	0.285	0.519
	Stressed	Static	0.0047	0.0019	0.0451	0.255
		0.67 Hz	0.020	0.0058	0.149	0.308
Phaeodactylum tricornutum	Replete	Static	0.046	0.039	0.511	0.457
		0.67 Hz	0.036	0.011	0.357	0.239
	Stressed	Static	0.019	0.017	0.0562	0.492
		0.67 Hz	0.028	0.008	0.143	0.387

CHAPTER IV

DISCUSSION

The objective of this study was to investigate the responses of two phytoplankton cultures when subjected to light fluctuating on the order of 0.10 Hz to 2.00 Hz, and to compare those responses to the same cultures subjected to static light. This objective was met. The relationship between quantum yield calculated from ^{14}C assimilation at the end of a 12 hour daylight period (Φ_{max}) and daily averaged carbon gain over the same period (Φ_{ave}), along with end-of-day F_v/F_m measurements determined with the photobioreactor and FIRe fluorescence, were investigated as a function of short term light fluctuations for two strains of marine diatoms. The results demonstrated that, while there seemed to be greater measures of Φ_{max} and Φ_{ave} for some of the fluctuating light conditions relative to static light conditions, the same could not be stated for F_v/F_m. These findings are dependent upon several factors, including frequency of light fluctuation, phytoplankton strain, nutrient concentration, cell density, and turbulence. These factors are discussed throughout the evaluation of hypotheses.

Evaluation of Hypotheses

For clarity, hypotheses one, two, and four are discussed together. Hypothesis three is different from these in that it references nutrient stressed cultures whereas the others do not.

The first hypothesis was that fluctuating light rapidly in cultures of *C. gracile* and *P. tricornutum* would result in greater quantum yields than under static light conditions of the same TDLD. This hypothesis was supported by the data obtained for the *C. gracile* strain, but not the *P. tricornutum* strain. The second hypothesis was that each of the strains grown under a range of frequencies would yield the highest quantum yield at the highest fluctuation frequency of 2.00 Hz. This hypothesis was not supported by the results. The fourth hypothesis was that the effects of fluctuating light would be species-specific, and was supported by the data.

Given that the photon flux density of all experiments was equal and saturating (Geider et al. 1985; Table 3), this phenomenon cannot be attributed to differing average light exposure. An explanation for this was offered by Weller and Franck (1941) and Terry (1986), where it was

suggested that increased efficiency under fluctuating light is the result of integration of the light intensity experienced over tim, rather than the instantaneous light intensity. This integration increased with increased flash frequency. Data from this project suggested that there was a zone of frequency fluctuation (greater than 0.50 Hz and less than 2.00 Hz) that yielded peak performance for this *C. gracile* strain. These results relate to photoacclimation to lower light levels. The average light available to these cultures was 333 μmol photons m^{-2} s^{-1}.While the cultures were exposed to light levels three times that required to saturate the photosystems (Falkowksi and Raven 2007), there were extremely high cell densities (Table 3). Such high cell counts forced the cells into low-light acclimation, yielding very high chlorophyll *a* per cell values. The highest chlorophyll *a* per cell values are recorded with *C. gracile* cultures acclimated to light fluctuating at 0.67 Hz, and 1.00 Hz (Table 3). These two cultures, then, were more effective in absorbing light when exposed to surface irradiance. It is possible then that *C. gracile* was able to obtain a greater balance between photosynthesis and respiration at higher frequencies, allowing for increased quantum yield. The 0.10 Hz and 0.50 Hz fluctuating light treatments may not have been rapid enough to maintain this balance, leading to quantum yield results that are not evident of enhancement of the photosynthetic apparatus. While the light period may have allowed all of the PSII reaction centers in the cells to become filled under low frequency light fluctuations, there may have been greater respiration during the dark part of the fluctuation, which outweighed the gains during the light. This explanation is not satisfactory, however, for the *C. gracile* culture acclimated to 2.00 Hz fluctuating light. In this case, perhaps the dark period was not long enough for the reaction centers to "clear" before the next light period occurred. In this case, there may have been either non0use of the photons during excitation of PSII pigments which resulted in reduced efficiency, or there may have been back-reactions of electron transport from PSI back to PSII, thereby reducing the efficiency of electron transfer and, thus, reducing carbon fixation and

quantum yield. Another possible explanation could be simple dissipation of energy via heat or fluorescence.

The results from *P. tricornutum* were very different from those of *C. gracile*. The *P. tricornutum* culture subjected to static light yielded a higher Φ_{max}, Φ_{ave}, F_v/F_{mPBR}, and F_v/F_{mFIRE} than the culture subjected to light fluctuating at 0.67 Hz. This is in contrast to previous studies where positive effects were evident by subjecting the strain to fluctuating light (Terry 1986; Laws et al. 1983; Wagner et al. 2005). The culture acclimated to fluctuating light had an extremely high chl *a* concentration per cell (Table 3). Falkowski and Owens (1980) reported that *Dunaliella tertiolecta* and *Skeletonema costatum* responded to low light either by increasing chl *a* concentrations or the size of the photosynthetic unit. In previous studies, the cultures may have reached a cell density where low-light acclimation may have been occurring, or that there was a lower PAR than in this study. For *P. tricornutum* to yield a higher Φ_{max} than Φ_{ave} for the same experiment suggests that something is occurring between the time lights are switched on and switched off. The answer to this question may be found in the analysis of nutrients. The concentration of nitrogen in the medium for *P. tricornutum* was 811 μM. The particulate nitrogen determined at the time of analyses was ~700 μM, with nearly zero nitrogen left in dissolved form in the medium. The remaining nitrogen must have been removed from the system in particulate form, either by overflow, sticking to the sides of the photobioreactor cuvette, or by sinking. Resuspension of the culture was attempted prior to sampling by way of removing the cuvette from the photobioreactor housing and shaking it manually. However, keeping the cells in suspension throughout experimentation and during the sampling process was very difficult for this culture. Therefore, it is likely that sinking played a role in underestimating the calculation for Φ_{ave}. By reducing the true calculation of POC, Φ_{ave} was also reduced.

Each of the experiments yielded F_v/F_m values of ~0.50, suggesting that there was no difference in quantum yield of photochemistry of PSII for *C. gracile* when acclimated to the

various light treatments. Hartig *et al*. (1998) showed that a high linearity between F_v/F_m and Φ_{max} could only be observed up to values of 0.018 mol C (mol photons absorbed)$^{-1}$. Given that all Φ_{max} values for this project were greater than 0.030 mol C (mol photons absorbed)$^{-1}$, it is inappropriate to assume that there would be a linear increase in F_v/F_m with increasing frequency. Franklin and Badger (2001) and Kashino *et al*. (2002) suggested that the loss of correlation may be due to nonphotochemical quenching, especially given that the readings were taken immediately after removal from the system where no time was allotted for dark-acclimation.Hartig *et al*. (1998) and Wagner *et al*. (2005) suggested that this can be explained by different amounts of alternative electron cycling (i.e., Mehler reaction) or nitrogen reduction. This could also be explained by variations in the photosynthetic quotients (Falkowski *et al*. 1985; Carignan *et al*. 2000).

The third hypothesis was that quantum yield would be greater when cultures were nutrient replete, relative to when nutrient stressed. This hypothesis is supported by the data obtained. For each of the experiments, the Fv/Fm of stressed cultures were much lower than when nutrient replete. This result is not novel. Falkowski (1992) showed that by starving phytoplankton of nitrogen, there is a decline of reaction center proteins, leading to PSII reaction center inactivation and changes in chemical composition. Kolber (1988) and Parkhill *et al*. (2001) further showed that decreased nutrients resulted in a decrease in Fv/Fm, allowing the use of this parameter as a diagnostic of physiological stress. After the culture has used its stores and is in a state of nutrient starvation, the culture will show adverse physiological effects (Cleveland and Perry 1987; Falkowski and Raven 1997). These physiological changes upon nutrient starvation were evident in both cultures under all light treatments.

From this study, it was clear that phytoplankton species respond differently to fluctuating light, relative to static light. This investigation showed an enhancement of the photosynthetic performance for *C. gracile*, but a reduction in the quantum yield for *P. tricornutum* when acclimated to fluctuating light, relative to when acclimated to static light with an equal total daily

light dose. This can be explained y examining the photoadaptational strategies of each of these two species. Bailey (1997) reported that *S. costatum* exposed to fluctuating light altered its pigment composition, resulting in altered light absorption spectra relative to a static light condition with an equal total daily light dose. Falkowski and Owens (1980) worked with the diatom *S. costatum* and the chlorophyte *D. tertiolecta*, where it was found that the strategy used by each of these species results in effective light harvesting and transfer of light energy through the photosystems of the cells. The cultures that this current project worked with were extremely dense (Table 3) by the end of the day. Due to the nature of semicontinuous dilution, the light field continued to change throughout the day in the photobioreactors until maximum optical density was reached just prior to dilution. Given this fact, the cultures experienced a natural decrease in light availability due to "self-shading." It was suggested by Falkowski and Owens (1980) that *S. costatum* acclimated to low light by increasing the size of its photosynthetic units rather than the number of them. While changes in pigments and PSU suze were not investigated in this project, it may be assumed that *C. gracile* was able to either increase the average size or number of photosynthetic units more efficiently than *P. tricornutum*.

Conclusions

The quantum yield and the quantum yield of photochemistry of PSII under fluctuating light and static light conditions is dependent on a number of factors. First, according to this study, there appears to be a change in the response of phytoplankton according the frequency of the light fluctuation. There seems to be an optimal speed by which the cells were able to utilize the light most efficiently. In the case of this study, that zone ranged from 0.50 Hz to 1.00 Hz. Second, it was suggested that the ratio of time cells spend in the light relative to time spent in the dark during a cycle influences productivity (Terry 1986). This study was not an attempt to prove or disprove this, as the ratio was consistent for all experiments. A third factor of import is that of settling. The *P. tricornutum* culture in this study tended to settle out of suspension very quickly

during experimentation and sampling. This may have led to the introduction of two cultures within the photobioreactor cuvette: that which was still in suspension and physiologically sound, and that which had settled out. This could explain why there was an increase in quantum yield for this culture when nutrient stressed. As the cells became nutrient depleted, they settled out, leaving only non-nutrient deplete cells in suspension, which were the only ones that could be sampled using the technology available. Finally, a fourth factor is shading due to high cell density. This is applicable on a culture level and a cellular level. As a culture with a high cell density, there was increased potential for shading of the culture by itself, disallowing light to reach some cells and thereby reducing quantum yield. On a cellular level, there was increased potential of the "package effect," where cells respond to light changes by altering their size or shape, their chloroplast number, the degree of thylakoid membrane stacking, and/or the optical properties of the thylakoid membranes, leading to alterations in a* and, thus, quantum yield (Berner *et al*. 1989). This factor was also not tested in this project and should be considered for future work.

A large controversy exists involving experimentation regarding fluctuating light with phytoplankton photosynthesis. In large part, the experiments in the literature are difficult to compare to one another because of different growth rates, light availability, light quality, frequency of fluctuation, light:dark ratio, methodology for growth, and, most important, the nature of species speficity. A much larger scale project may be done in the future in order to determine how different species respond to the above parameters when set equally. Such a project would invole triplicate experiments with each of the several strains of phytoplankton, subjecting each of them to a wide range of light fluctuation frequencies. Additionally, some more work on the response of cultures to various light:dark ratios is important. While Terry (1986) made the assertion that phytoplankton production is most efficient with a light dark ratio of between 1:2 and 1:10, this was only true in this study with *P. tricornutum*. These types of studies should be expanded to include other diatom strains, and other genera of marine phytoplankton.

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