THE EFFECTS OF THE TRANSITION TO FLUCTUATING LIGHT ON THE GROWTH OF TWO RIVER PHYTOPLANKTON IN A PHOSPHATE RICH ENVIRONMENT

By

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ABSTRACT

A semi-continuous culture system was used to examine the effects of fluctuating light on two species of river algae: *Chlamydomonas reinhardtii* and *Nitzschia* sp.. Experimental replicates were exposed to a fluctuating light regime of a half-hour (half-hour light: half-hour dark) or one hour (one hour light: one hour dark) superimposed on a diurnal light cycle and compared to controls which were grown under a diurnal light regime only. The results show that for both the half-hour and full-hour light fluctuation experiments, the growth rate of *C. reinhardtii* decreased after the impact. This reduction in growth rate was accompanied by a decrease in cell volume and an increase in chlorophyll levels. From the results for both the half-hour and full-hour light fluctuation experiments on *Nitzschia* sp., it was shown this species did not exhibit a significant change in growth rate, cell volume or chlorophyll levels. These results could explain why, under eutrophic conditions, a diatom species such as *Nitzschia* tends to dominate the phytoplankton communities in turbulent environments such as rivers while faster growing green algae such as *Chlamydomonas* tends to dominate in more stable environments, such as in tributaries.

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INTRODUCTION

PHYTOPLANKTON BIOLOGY

Phytoplankton make up a large and diverse group of organisms that inhabit water and spend all or a portion of their lives as autotrophs. From this diverse group of organisms, Reynolds (1984) identifies three subgroups of freshwater phytoplankton: those that live in lakes (limnoplankton), those that live in ponds (heleoplankton), and those that live in rivers (potamnoplankton). Phytoplankters are an important part of all three freshwater environments as they are an important source of food for invertebrates and fish. Phytoplankton also account for about 70% - 80% of the world production of oxygen (Reynolds, 1984). The phytoplankton community also acts as an indicator of environmental problems including trophic status (Fore and Grafe, 2002), environmental change (McCormick and Cairns, 1994), water quality (Kelly *et al.,* 1998) and environmental disasters (Sabater, 2000).

In any river system, different parts of the river will have different levels of turbulence. Areas at the edges of the river will have lower levels of turbulence, while in the main river channel the turbulence is higher. The main river channel is a more dynamic environment compared to the edges (Reynolds, 2000). The level of turbulence in the river channel depends on a number of factors including bed structure (Sukholdolov *et al.*, 1998; Buffin-Bélanger and Roy, 1998; Roy *et al*., 2004), river flow (Babaeyan-Koopaei *et al*., 2002) and water temperature (Nikora *et al*., 1994). In many ways a river

can be compared to a shallow lake in the way turbulence affects the algae present (Reynolds *et al*., 1994)

The turbulence in rivers has the effect of mixing the algae vertically in the water column. When the algae go to the bottom of the water column they receive less light due to light attenuation (Moss, 1980). Turbulence reduces the light available for photosynthesis (Loiselle, 2007). Numerous mathematical equations exist which predict various aspect of algal behavior in turbulent environments. Spigel and Imberger (1987) looked at numerous factors that go into producing turbulence in lakes, as well as fluctuations that occur due to various natural processes and how these factors affect phytoplankton. Huissman and Weissing (1994) examined the effects of light limitation on growth based on numerous factors including light intensity, biomass density, depth, and nutrient level. Husiman *et al*., (1999) studied the effects of critical levels of depth and turbulence on the formation of algal blooms, and how above certain levels of turbulence and depth, blooms cannot occur. Ebert *et al*. (2001) looked at the effects of light and depth as factors in algal bloom formation and created mathematical equations based on these factors. The frequency of turbulent fluctuations can be broken down into three categories defined by Grobbelaar (1994):

1) High frequency fluctuations of 100 ms (10Hz) and less
2) Medium frequency fluctuations of seconds to minutes, and
3) Low frequency cycles of hours to days and years.

Due to different frequency light fluctuations, diverse types of algae dominate in waters with varying levels of turbulence (Peters, 2000). Previous studies have shown that

under turbulent conditions, where there are lower levels of light, diatoms tend to dominate. In situations where conditions are less turbulent and irradiance levels (measured as mol m^{-2} s^{-1}) are high, green algae tend to dominate (Litchman, 1998). Fluctuating light also has the effect of increasing the diversity of system (Floder *et al.,* 2002). Some species of algae are unable to adapt to certain frequencies of fluctuating light and will not be present in these environments. (Reynolds, 1994). The diatoms seem to be particularly well adapted to living in turbulent environments, especially when nutrient levels are high (Estrada, 1997). One method for phytoplankton to adapt to environments with fluctuating light is to alter its photosynthetic capability. The process by which algae alter their photosynthetic capability is known as photoadaptation and has been studied for example by Post *el al.* (1984), Cullen (1988), Berner *et al.* (1989), Lesser and Shick (1989) and Geider *et al*. (1996). Photoadaptation is the process by which photosynthetic organisms alter the way in which they absorb photosynthetically active radiation. Methods of photoadaptation have been examined in both plants and algae. Photoadaptation in algae can either be through mechanical means (i.e. swimming to more intense light) or through physiological means (i.e. the production of more chlorophyll units).

A previous study performed by Dickson (2007) examined the effects of light variation on algae in a phosphate limited environment. Dickson's study found that under nutrient limited conditions, *Chlamydomonas reinhardtii* and *Nitzschia* sp. both showed no significant change in growth rate due to decreased light levels. Since Dickson's study was performed at nutrient limited levels and no significant change was found, it was assumed that the nutrients were still the limiting factor to the growth of the algae. For

that reason, this study was performed under similar conditions, but with phosphate and all other nutrients in excess creating a eutrophic environment. Eutrophication is the process by which waterways are loaded with nutrients from various sources (Moss, 1980). The sources of eutrophication can be anything from sewage runoff, atmospheric deposition, groundwater flow, and agricultural runoff and flow (Anderson *et al*., 2002).

Studies on the ecology of algae have usually been performed in one of two ways. The first method of study is an *in vitro* study as exemplified by Marra (1978a), Grobbelaar (1989; 1994), Ibelinggs *et al.* (1994), Litchman (1998; 2000), and Dickson (2007). The other method for study algae is done *in situ*. Such studies have been performed by Marra (1978b), Carrick *et al.* (1993), Gervais *et al.* (1997), Köhler (1997), and Diehl *et al.* (2002). For each type of study there are advantages and drawbacks. In laboratory studies, the experimenter is able to control almost all aspects of the experiment, thereby simplifying the interpretation of the results. However, many ecologists point out that laboratory studies lack the ability to accurately replicate the real environment. On the other hand *in situ* studies performed in the field more accurately represent natural conditions but make it difficult to control many variables, which can make interpretation of the results more difficult. Therefore it is important to weigh the consequences of each type of study and choose the one that is best suited to the situation. For this experiment, it was decided to use an *in vitro* system, as the technology existed, and measurements were considered to be more accurate in the lab.

Leland (2003) characterized the algal composition of the San Joaquin River in California according to water depth, flow regime and water chemistry and found that water depth and flow regime had a greater influence algal abundance then did water

chemistry. A study by Kohler *et al.* (2002) examined the growth of phytoplankton based on light supply and mixing depth. In this study it was found that due to differences in light adaptation and sedimentation loss, chlorophytes appear to dominate in shallow rivers, while diatoms were dominant in deeper rivers. Bahnwart *et al.* (1999) also found that different algal species dominate depending on the water depth and flow velocity of the body of water. Engelhardt *et al.* (2004) found that stream geomorphology is an important factor in determining the abundance of algae.

Studies on the effects of regulated rivers on algae have tended to focus on either the drift of algae in these environments (Bertrand *et al*., 2001) or the effect at the community level (Wehr and Thorp, 1997). Studies like these are becoming more important as more rivers are being dammed to be used for hydroelectric power and water control projects.

OBJECTIVES

The objective of this study was to examine the changes in growth rate, cell volume and chlorophyll levels, in two species of phytoplankton common to waters in Manitoba as they are moved from a stable light environment to a light environment simulating that of a turbulent environment. The two genera used in this experiment were *Chlamydomonas reinhardtii* (Figure 1) and *Nitzschia* sp. (Figure 2), both of which are common lotic algal species found in Manitoba. These algae were grown under a diurnal light cycle until the cultures reach a steady state cell density. When the algal cultures reached steady state cell density, a set of timed blinds were turned on to simulate the vertical movement of the algae caused by turbulence. Cell density measurements, cell

volumes and chlorophyll levels were used to assess whether the impact has had any effect on the growth rates of the algal cultures.

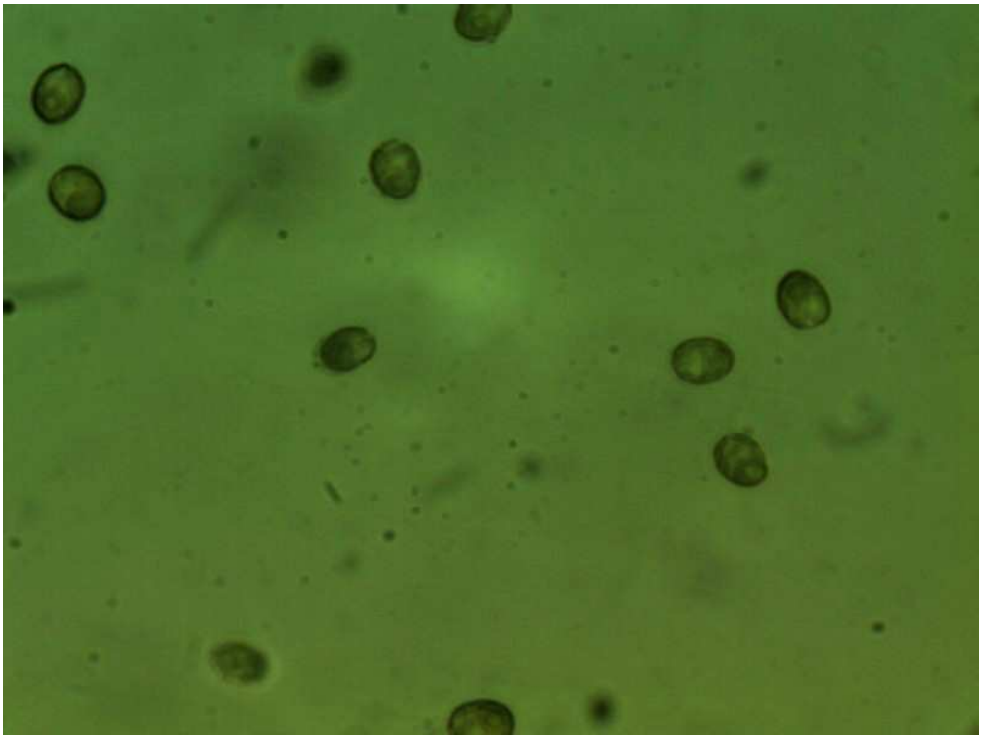

Figure 1: *Chlamydomonas reinhardtii* used in this experiment viewed at 1000 x magnification.

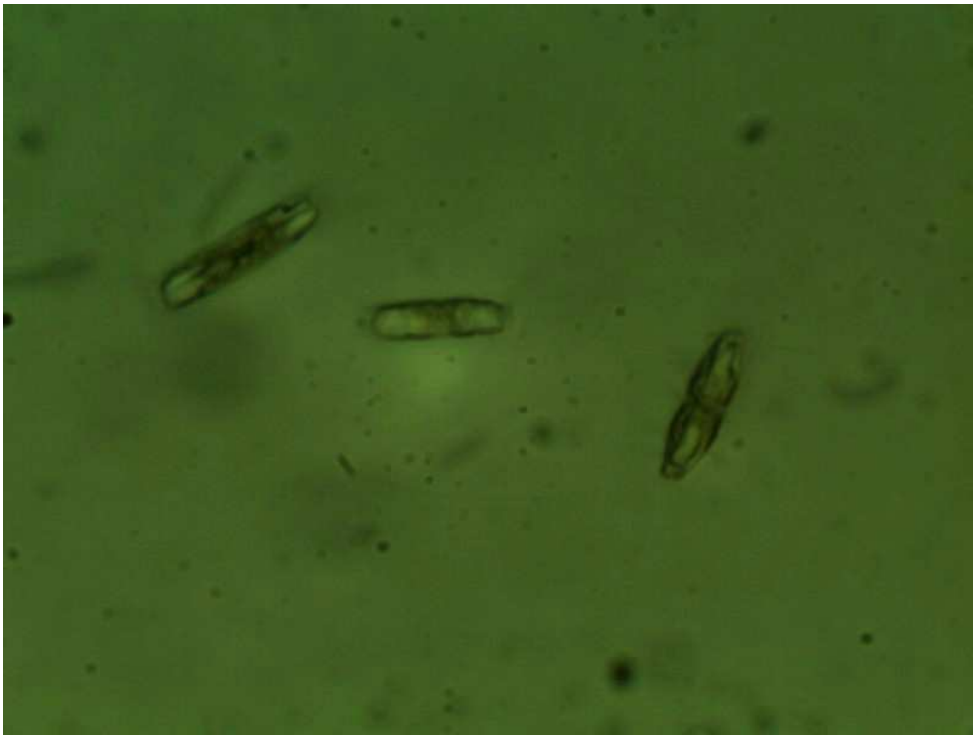

Figure 2: *Nitzchia* sp. used in this experiment viewed at 1000 x magnification.

The hypothesis for this project was that the algae would show a drop in growth rate (as measured by steady state cell density) and that cells would show an increase in total chlorophyll levels, as a result of moving from a diurnal light cycle to the decreased irradiance light cycle.

MATERIALS AND METHODS

The alga species *Chlamydomonas reinhardtii* and *Nitzschia* sp. were used in this study. These specimens were isolated from the Winnipeg River on August 6, 2006. Both specimens were maintained in stock cultures, in 250 mL Erlenmeyer flasks on a rotary shaker table, in WC media (Guillard and Lorenzen, 1972; see Appendix I), under a 16 hours light:8 hours dark light cycle at 17.5°C ± 1°C. The stock cultures were sub-cultured once every six to ten days.

During this study, algae were grown in a semi-continuous culture system, which consisted of 150 mL. culture tubes under constant aeration (Figure 3). The dilution rate was maintained at 20% per day (30mL.) WC phosphate enriched media (3.5 mg/L PO_4^{2-}) was used in throughout all experiments. The culture apparatus' were housed in

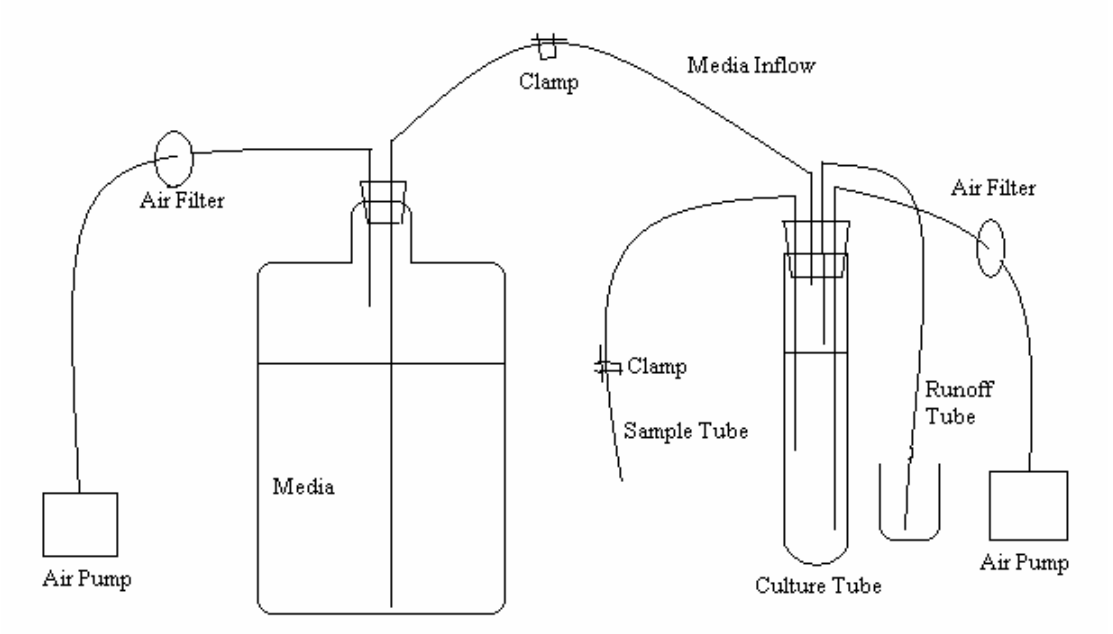

Figure 3: Diagram of a semi-continuous culture set-up illustrating all materials used and their connections.

a Conviron (C 609) walk-in environmental chamber throughout the duration of the experiments (Figure 4).

Figure 4: Connviron environment chamber with the continuous culture system.

The experimental design for this study consisted of 6 experimental semi-continuous culture replicates, and 6 control semi-continuous culture replicates. Both the experimental and the control cultures were illuminated by a series of four, 4 foot, tubular

fluorescent lights (Ecolux, GE F32T8 SPX35 ECO) and exposed to a diurnal light pattern. At 0600 h, the first of the fluorescent bulbs was turned on, two hours later the second bulb was turned on. Each additional bulb was turned on in sequence every two hours until at 1200 h all bulbs were illuminated. Then each bulb was turned off at two hour intervals starting at 1600 h until 2200 h all bulbs were off. This produced a diurnal light intensity pattern that is illustrated in Figure #5.

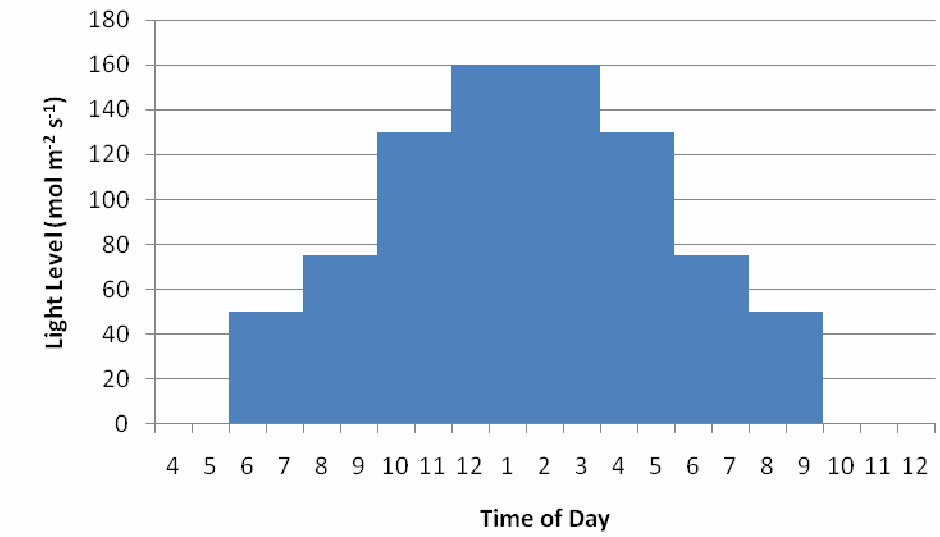

Figure 5: Graphical representation of a diurnal light cycle.

Each day a 30 mL sample was taken from each culture and used for cell density and chlorophyll estimates as well as for imaging and cell dimension measurements. All chlorophyll estimates were made using live cultures on the day of sampling, however cell counts were made on samples preserved with Lugol's iodine solution.

Cell density measurements were obtained by counting cells using a Neubauer Bright Line haemacytometer. Daily cell density values for each replicate were obtained by averaging the counts from 6 haemacytometer grids. Cell densities from each of the 6

replicate cultures were then averaged to generate the number of cells/mL described in (Figures 8-11). Chlorophyll levels were estimated using the Alkaline Acetone extraction and monochromatic and trichromatic estimation methods as described in Wetzel and Likens (1991). The average values for the chlorophyll values were obtained from a single sample analysis on each of the six samples. All chlorophyll values were then standardized to moles of chlorophyll per mL. of cell volume. On each day a chlorophyll measurement was made, a series of photographs of the cells were taken using a Motic Images Moticam 1000 digital camera mounted on a Leitz Wetzlar compound microscope, and analyzed using the Motic Images image analysis software. Figures 20 and 21 (Appendix II) show pictures of the *C. reinhardii* and *Nitzschia* with measurements from the Motic Images program and Figures 20 and 22 (Appendix III) shows the diagrammatic representations of these measurements. From these photographs, measurements of cell length and width were made, then used in following formulae to estimate the average cell volumes, for each replicate (Sun and Liu, 2003).

Chlamydomonas cell volume = $\pi hd^2/6$ = cell volume for an ellipsoid

Nitzschia cell volume = $\pi hd^2/12$ = cell volume for a double cone

In order to simulate the changing light levels that river algae experience in a turbulent river as they rise and fall in the water column, the 6 experimental replicate cultures were subjected to fluctuating levels of light (Figures 6 and 7), superimposed on the diurnal light pattern already described (Figure 5). Light fluctuations were produced by placing a venetian blind between the bank of lights and the cultures. The blinds were opened and closed by a programmable timer and motor system (See Figure 4).

In all experiments the control cultures were exposed to the diurnal light pattern with the blinds set in the open position for the entire experimental run. The experimental cultures were exposed to the diurnal light pattern with the blinds in the open position for about the first 5 days of the run, until the cultures reached a steady state cell density. Once steady state had been reached the blinds of the experimental cultures were engaged, and they opened and closed on a 30 minute open:30 minute closed cycle. In this way the experimental cultures were simulating algal cells that had initially grown in a calm non-turbulent environment, and then simulated those same cells having been swept out into a more turbulent river current, where they would experience rapid fluctuations in light levels. This experiment was repeated for both of the test species. The experiment was then repeated for both species exposing the experimental cultures to a less rapid light fluctuation pattern of 1 hour open: 1 hour closed cycle.

The differences in the average steady state cell densities of the experimental cultures and the control cultures were then calculated for each day. A Welch's t-test (Krebs, 1999) was used to determine if there was any significant difference in those differences during the period before the blinds were engaged (impact) and the period during which the experimental cultures were subjected to the fluctuating light pattern. A Student's t-test was used to examine if there were any significant differences between the control and experimental data for the cell volume and chlorophyll *a* data.

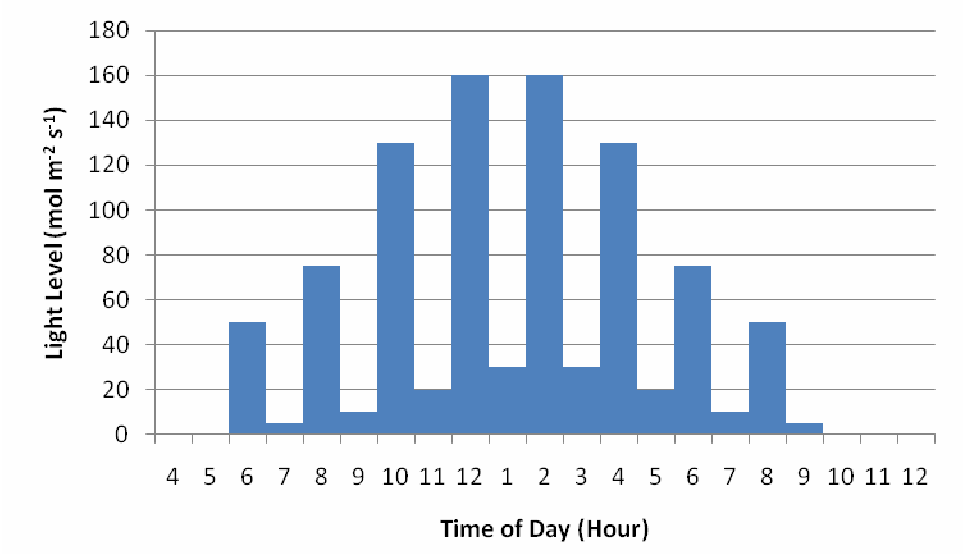

Figure 6: Graphical representationg of the one hour light fluctuations imposed on a diurnal light patten

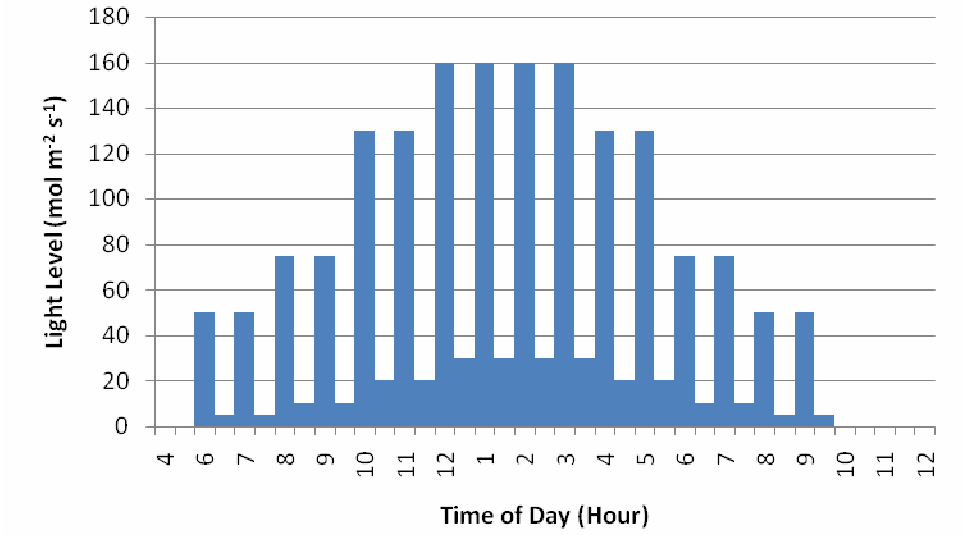

Figure 7: Graphical representationg of the half hour light fluctuations imposed on a diurnal light patten.

RESULTS

The cell densities of both the experimental and control replicates of the *C. reinhardtii* half hour light fluctuation experiment established steady state (≈ 1.50 x 10^6 cells/mL) on approximately day five under a diurnal light regime (Figure 8). The blinds were engaged on the experimental cultures on day fourteen. The imposed light fluctuation caused the average steady state cell densities to shift to a lower level in comparison with the average steady state cell densities before the impact. When the Welch's t-test was applied to this data, it was found that there was a statistically significant change in the differences between the steady state cell densities between of the experimental and the control replicates after the imposition of the light fluctuations, compared to the differences between the steady state cell densities of the experimental and the control replicates seen before the imposition of the light fluctuations (t_{calc} = -3.32; t_{crit} = 2.08; α = 0.05; d.f. = 21)

The cell densities of both the experimental and control replicates of the *C. reinhardtii* full hour light fluctuation experiment established steady state (≈ 1.25 x 10^6 cells/mL) on approximately day 4 under a diurnal light regime (Figure 9). The blinds were engaged on the experimental cultures on day nine. The light fluctuation pattern caused the experimental cultures to rapidly establish a new lower steady state cell density. When the Welch's t-test was applied to this data, it was found that there was a statistically significant change between the steady state cell densities between of the experimental and the control replicates after the imposition of the light fluctuations, compared to the steady state cell densities between of the experimental and the control

replicates seen before the imposition of the light fluctuations. (t_{calc} = -7.80; t_{crit} = 2.13; α = 0.05; d.f. = 15).

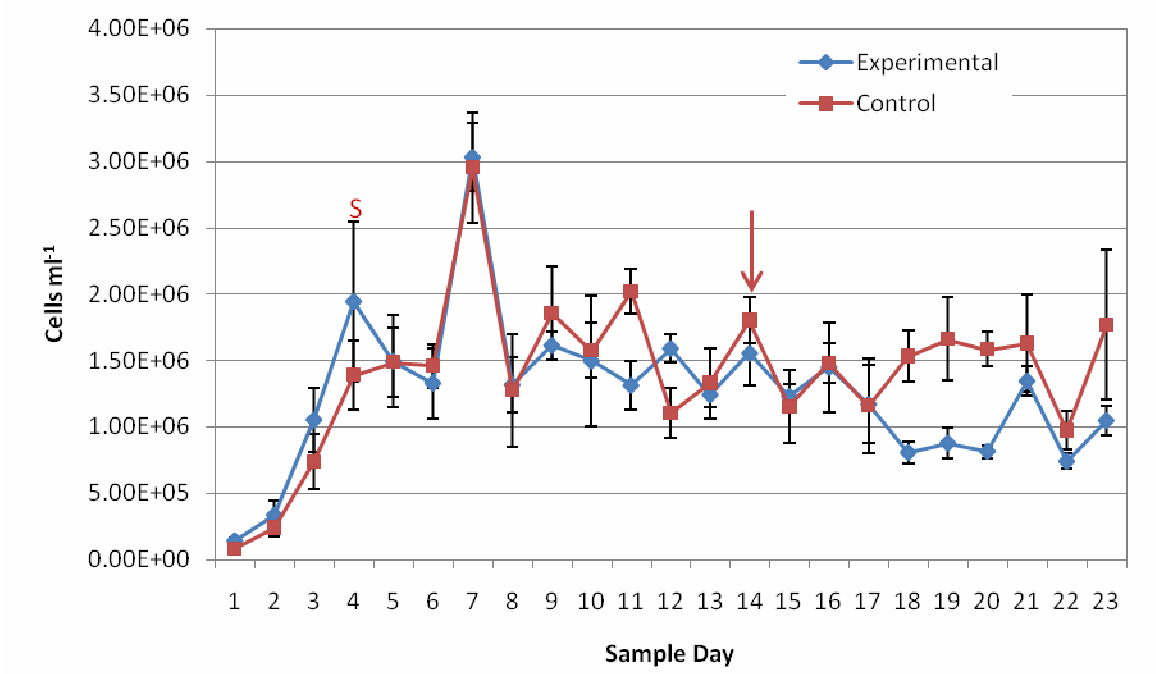

Figure 8: Steady state cell density for the half hour light fluctuation experiment of *Chlamydomonas reihardtii*. Each point represents the mean cell density of six replicates. Error bars represent standard deviation.

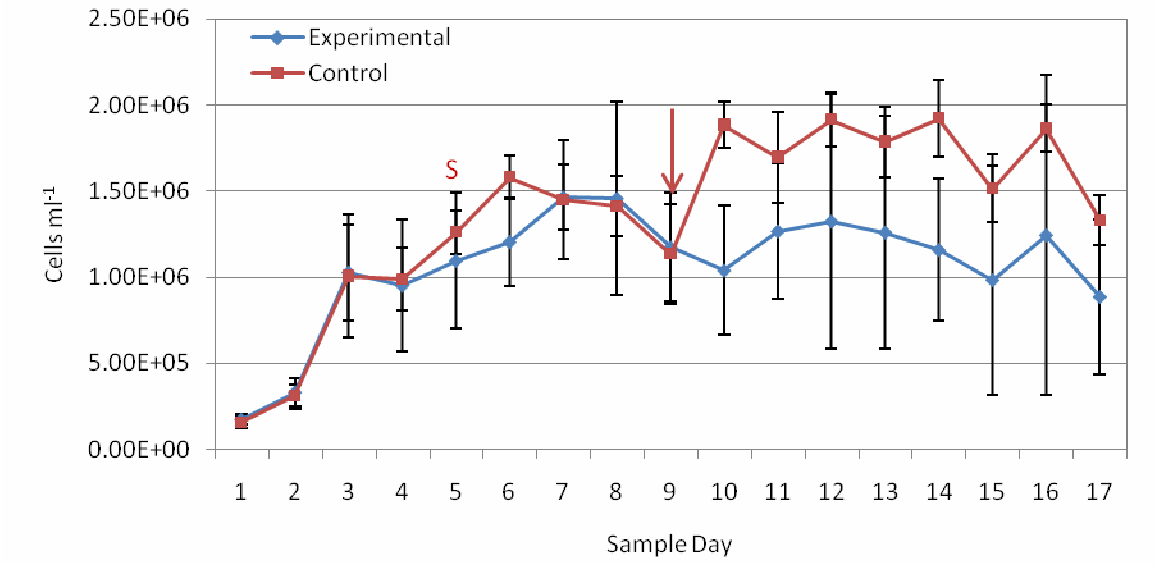

Figure 9: Steady state cell density for the full hour light fluctuation experiment of *Chlamydomonas reinhardtii*. Each point represents the mean cell density of six replicates. Error bars represent standard deviation.

In the half hour light fluctuation experiment with *Nitzchia* sp., the experimental cultures reached steady state cell density (≈ 2.00 x 10^5 cells/mL) on approximately day five (Figure 10). The experimental *Nitzschia* sp. cultures were exposed to the half hour fluctuating light cycle on day 12. The *Nitzschia* sp. cultures did not seem to decrease in cell density in response to the rapidly fluctuation light as did the *Chlamydomonas reinhardtii* cultures. When the Welch's t-test was applied to this data, it was found that there was no statistically significant change in the differences between the steady state cell densities between of the experimental and the control replicates after the imposition of the light fluctuations, compared to the differences between the steady state cell densities between of the experimental and the control replicates seen before the imposition of the light fluctuations ($t_{calc} = 0.74$; $t_{crit} = 1.76$; $\alpha = 0.05$; d.f. = 14).

In the full hour light fluctuation experiment with *Nitzschia* sp., the experimental cultures reached steady state cell density (≈ 2.50 x 10^5 cells/mL) on approximately day four (Figure 11). The experimental *Nitzschia* sp. cultures were exposed to the half hour fluctuating light cycle on day 9. Once again, the *Nitzschia* sp. cultures did not seem to respond to the less frequent light fluctuation compared with the *Chlamydomonas reinhardtii* cultures. When the Welch's t-test was applied to this data, it was found that there was no statistically significant change in the differences between the steady state cell densities between of the experimental and the control replicates after the imposition of the light fluctuations, compared to the differences between the steady state cell densities between of the experimental and the control replicates seen before the imposition of the light fluctuations. ($t_{calc} = -0.39$; $t_{crit} = 1.77$; $\alpha = 0.05$; d.f. = 13).

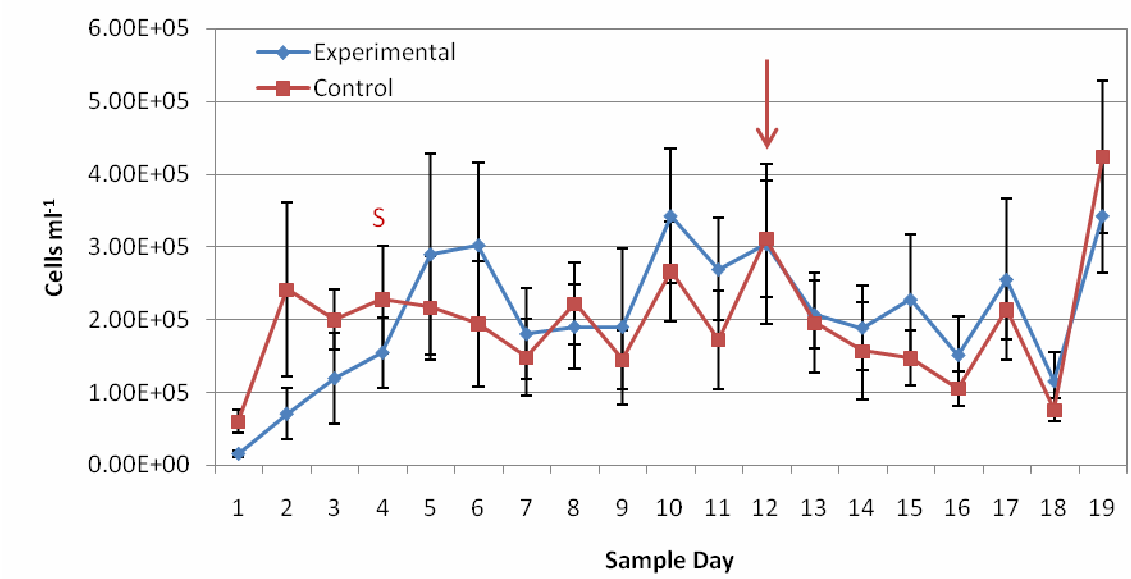

Figure 10: Steady state cell density for the half hour light fluctuation experiment of *Nitzschia* sp. Each point represents the mean cell density of six replicates. Error bars represent standard deviation.

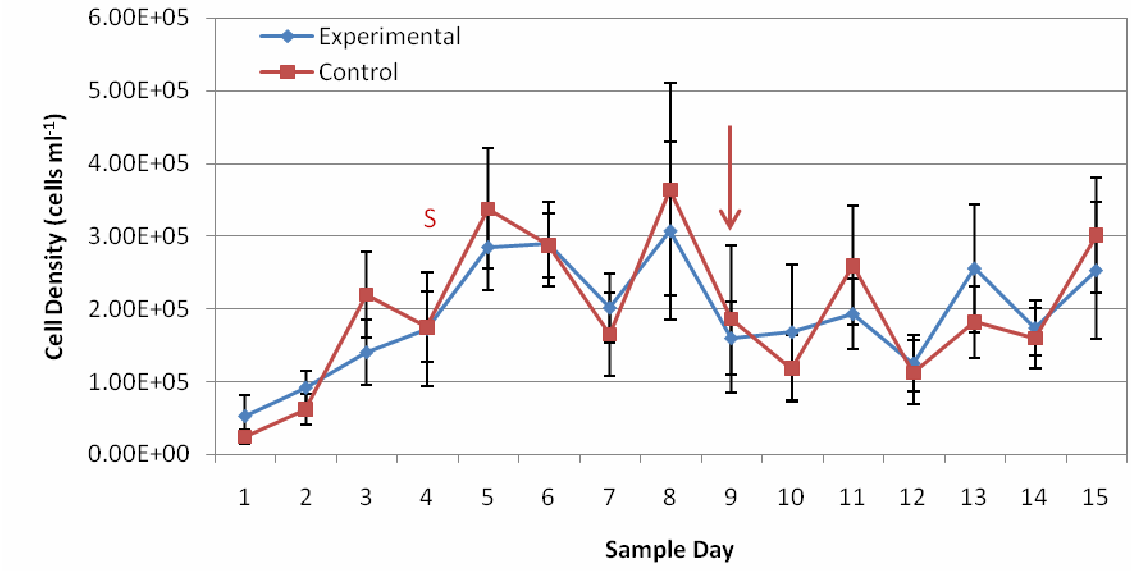

Figure 11: Steady state cell density for the full hour light fluctuation experiment of *Nitzschia* sp. Each point represents the mean cell density of six replicates. Error Bars represent standard deviation.

C. reinhardtii cell volumes observed in the half-hour light fluctuation experiment can be seen in Figure 12. By running a Student's t-test on this data it was found that control and experimental values all varied significantly after day six (P = 0.0008, 0.01, 0.03 and 0.03 respectively) while on day one there was no significant difference (P = 0.29). The experimental cultures show a decrease in cell volume after the imposition of the half hour fluctuating light regime.

Cell volumes for the full hour light fluctuation run can be seen in Figure 13. A Student's t-test has shown that for all days, the control and experimental cell volumes were significantly different from one another (P = 0.007, 0.002, 0.00007 and 0.0002 respectively). The experimental cell volumes did not show any directional change after the impositions of the fluctuating light regime.

Cell volumes for the half hour light fluctuation run of *Nitzschia* sp. can be seen in Figure 14. It was found that there were no significant differences between the experimental and control volumes on any of the days. The cell volume of the experimental cultures did not show a directional response after the imposition of the impact.

Cell volumes for the full hour light fluctuation run of *Nitzschia* sp. can be seen in Figure 15. It was found that the experimental and control cultures varied significantly only on Day 15 (P = 0.02). On all of the other days the experimental and control volumes were not significantly different.

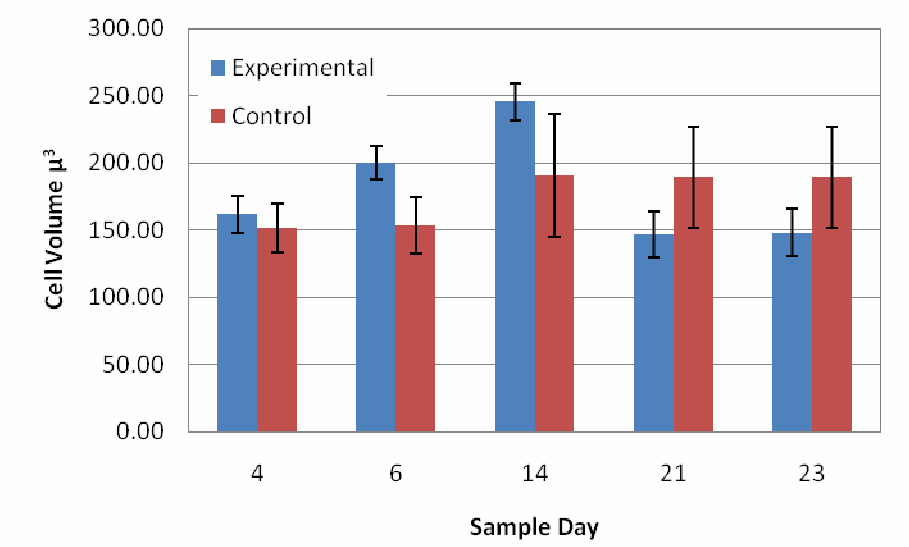

Figure 12: Cell volumes for the half hour light fluctuation run of *C. reinhardtii*. Error bars represent standard deviation. The impact was imposed on the cultures on day 14 after sampling.

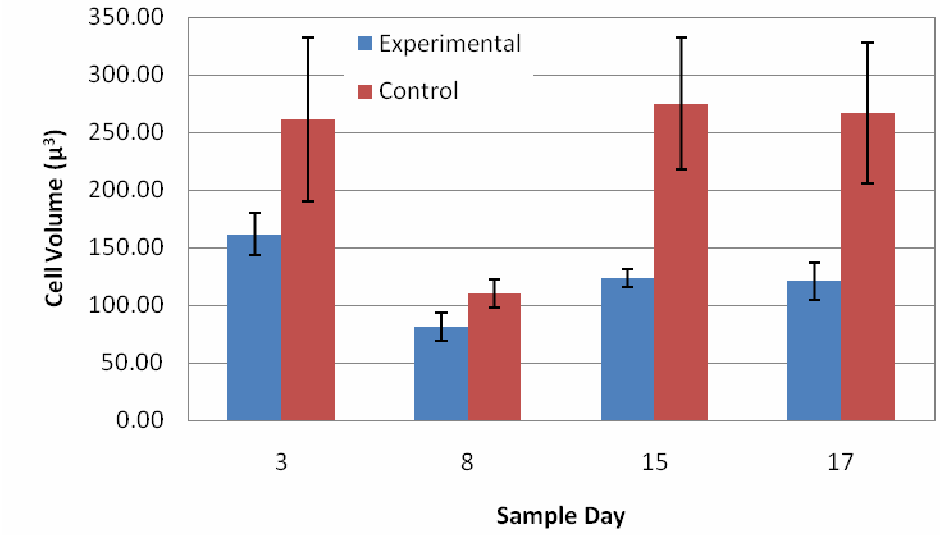

Figure 13: Cell volumes for the half hour light fluctuation run of *C. reinhardtii*. Error bars represent standard deviation. The impact was imposed on the cultures on day 9 after sampling.

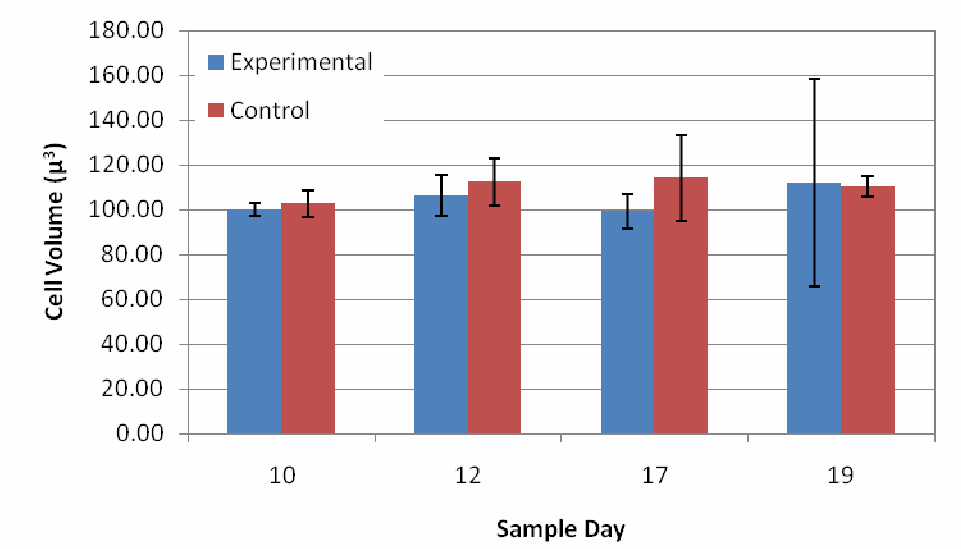

Figure 14: Cell volumes for the half hour light fluctuation run of *Nitzschia* sp. Error bars represent standard deviation. The impact was imposed on the cultures on day 12 after sampling.

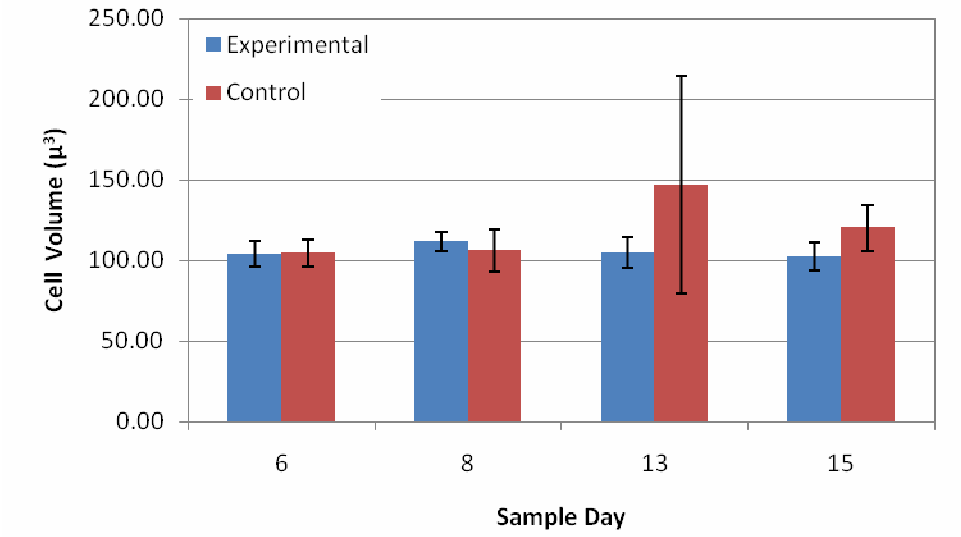

Figure 15: Cell volumes for the full hour light fluctuation run of *Nitzschia* sp. Error bars represent standard deviation. The impact was imposed on the cultures on day 9 after sampling.

C. reinhardtii chlorophyll *a* per unit biomass of the half hour light fluctuation run can be seen in Figure 16. Chlorophyll *a* values varied significantly between experimental and control on day 6 before the impact, and days 21 and 23 after the impact ($P = 0.02$, 0.01 and 0.001 respectively), while values on the two days before the impact did not show a significant difference. Experimental values were shown to have increased after the imposition of the impact, while control values remained relatively constant.

Figure 17 shows the amount of chlorophyll *a* per unit biomass of *C. reinhardtii* for the full hour light fluctuation run. It was found that for both of the days before the impact the experimental and control chlorophyll values were not significantly different ($P = 0.4$ and 0.2 respectively). For both of the days after the impact the control and experimental values became significantly different ($P < 0.001$ for both). It was also seen that after the impact the experimental values increase while the control values decrease.

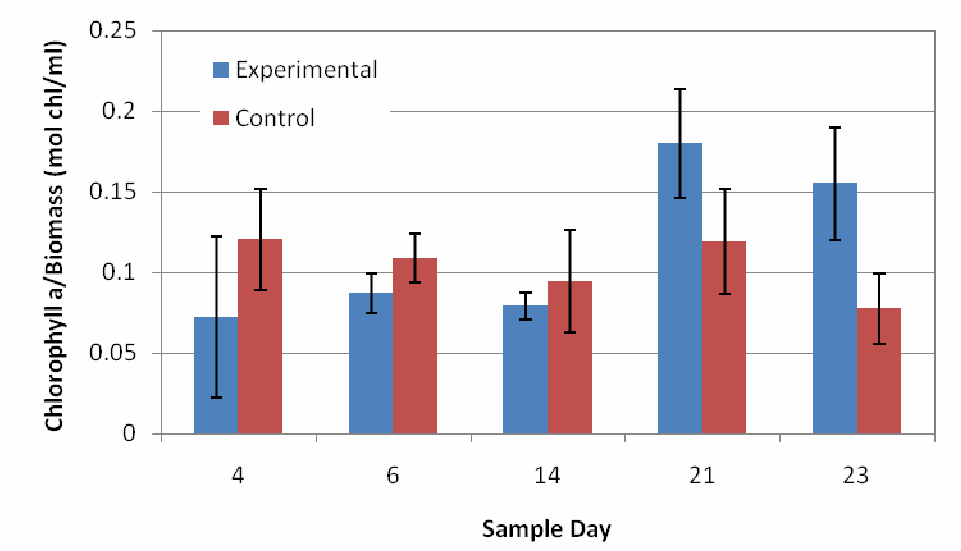

Figure 16: Moles of chlorophyll *a* per ml of cell biomass for the half hour light fluctuation run of *C. reinhardii*. Error bars represent standard deviation. The impact was imposed on the cultures on day 14 after sampling.

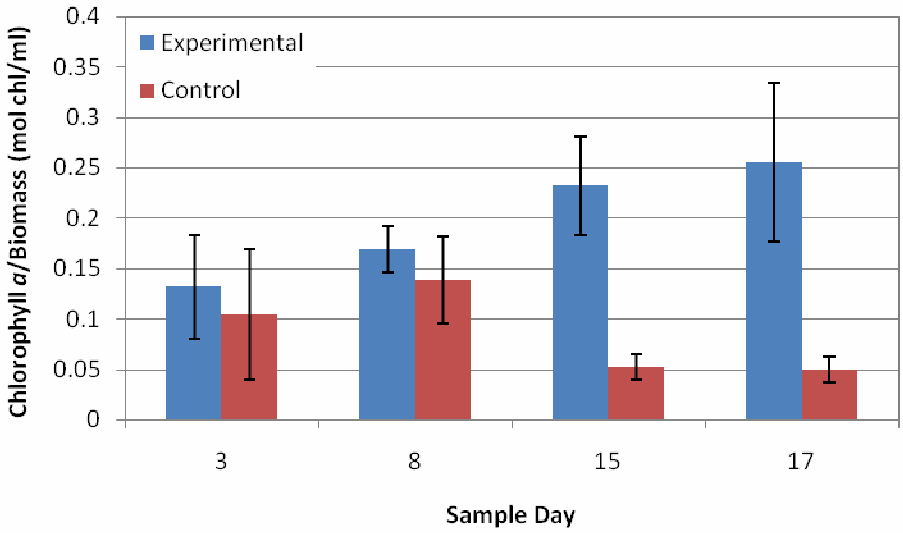

Figure 17: Moles of chlorophyll *a* per ml of cell biomass for the full hour light fluctuation run of *C. reinhardii*. Error bars represent standard deviation. The impact was imposed on the cultures on day 9 after sampling.

The chlorophyll *a* per biomass of the *Nitzschia* sp. half hour light fluctuation experiment can be seen in Figure 18. The experimental and control values were not significantly different on any of the sampling days and the imposition of the impact had no effect on experimental values.

The chlorophyll *a* values for the full hour light fluctuation experiment of *Nitzschia* sp. can be seen in Figure 19. The experimental and control values were shown not to be significantly different on any of the sampling days. The experimental cultures did show a non significant increase in their chlorophyll *a* values after the imposition of the impact.

Chlorophyll values for chlorophylls *a*, *b*, and *c* for both the experimental and control runs at half hour and full hour light fluctuations for *C. reinhardtii* and *Nitzschia* sp. can be found in Appendices IV through VII. It should be noted that the trends seen in the chlorophyll *c* values are indicative of chlorophylls *a* and *b* as well.

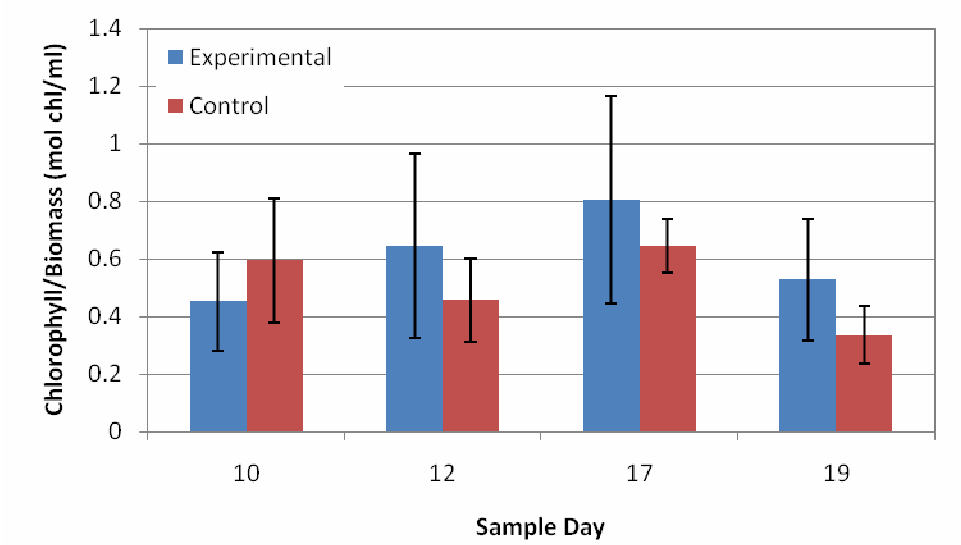

Figure 18: Moles of chlorophyll *a* per ml of cell biomass for the half hour light fluctuation run of *Nitzschia* sp. Error bars represent standard deviation. The impact was imposed on the cultures on day 12 after sampling.

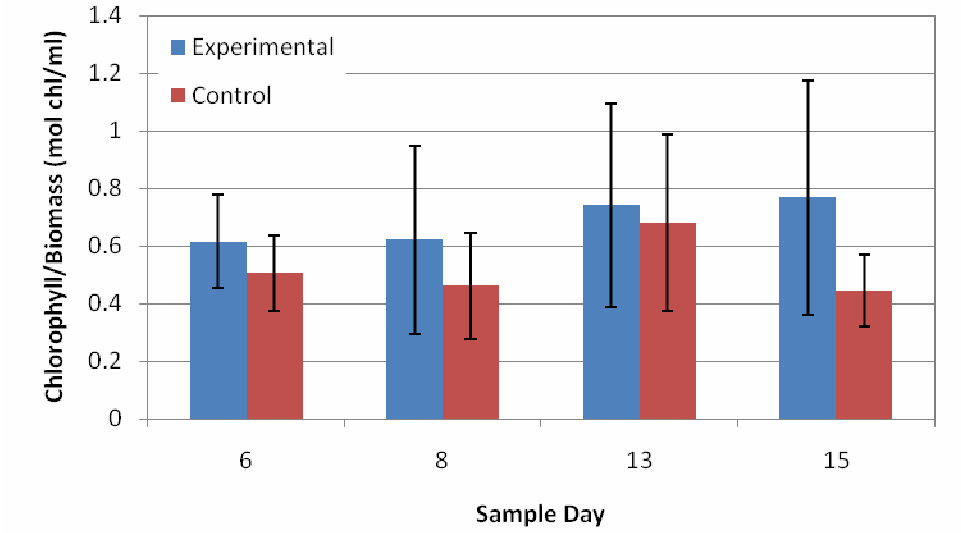

Figure 19: Moles of chlorophyll *a* per ml of cell biomass for the full hour light fluctuation run of *Nitzschia* sp. Error bars represent standard deviation. The impact was imposed on the cultures on day 9 after sampling.

DISCUSSION

A semi-continuous culture system like the one in this experiment offers a unique opportunity to investigate many different aspects of algal biology. By sampling a constant volume everyday and replacing the sample amount with fresh media, a constant dilution rate is established (20% in this experiment). When a constant dilution rate is maintained over a period of time under the same conditions, the culture will reach a steady state. By measuring the cell density everyday, one can determine when the steady state cell density has been established. In a continuous culture system, a steady state cell density can be equated with a certain growth rate, as the cells in the culture must grow back to steady state after every sample is removed. Therefore after an impact has been imposed on the culture, any change in steady state cell density can be assumed to be a change in growth rate (Fogg and Thake, 1965)

The results of this experiment show that the growth rate of the replicates of both the experiments on *Chlamydomonas* drop significantly after the imposition of the fluctuating light cycle compared with the control replicates. The results also show that the experimental replicates of the *Nitzschia* half-hour and full-hour light fluctuation experiments show no significant change in growth rate compared to the controls. From the results of these experiments, *Chlamydomonas* appears to be more sensitive to changing environmental light than does the diatom *Nitzschia* under elevated phosphate levels.

A previous study by Dickson (2007) found that for both full and half hour light fluctuation experiments, only small changes in growth rate as represented by steady state cell densities occurred, and it was concluded that phosphate was likely a growth limiting

resource. Since phosphate levels were increased in this study to near hypertrophic conditions, it appears that light became the major growth limiting factor for *Chlamydomonas*.

The results of this study are also consistent with the findings of others. Sciandra *et al*. (1997) found that there was a rapid decreased in growth rate in the green algae *Dunaliella tertiolecta* in response to being moved from a non-limiting irradiation level (430 μmol m^{-2} s^{-1}) to a limiting irradiation level (72 μmol m^{-2} s^{-1}) of diurnal light.

A study by Litchman (2000) found that at a low average irradiance level (25 - 50 μmol m^{-2} s^{-1}), fluctuating light caused an increase in the growth rate of *Nitzchia* sp. while it depressed the growth rate of the green algae *Sphaerocystis schroeteri*. At saturating irradiation levels (100 - 120 μmol m^{-2} s^{-1}), light fluctuations were not found to significantly affect the growth of either the diatom or the green algae. Even though Litchman (2000) does report the growth rates of the organisms she studied, she does point out that the importance of physiological factors such as chlorophyll levels should not be ignored.

Many ecologists recognize that there are two components that can affect the competitive abilities of algal species. Many species grow rapidly when resources are plentiful, but appear to be rather inefficient at obtaining resources when concentrations decrease. Species that are able to grow faster when resources are not limiting will then dominate in environments without limiting resources. Other species appear to grow very slowly when resources are plentiful, but when resources decline they continue to grow at slow but constant rate, because they are apparently able to maintain uptake of resources

when limited. When resources are limiting the fast growing species are unable to maintain their rapid growth because they are unable to acquire the resources they need for survival. Hence, under conditions of resource limitation, the slow-growing, more efficient species will be dominant (Molles, 2008). Nutrient uptake efficiency may explain my results with regard to *C. reinhardtii* and *Nitzschia* sp. in the current experiment. *Chlamydomonas reinhardtii* seems to be able to grow fastest when light is not limiting, but its growth slows as light becomes a limiting resource. *Nitzschia* sp., on the other hand, grows slowly both when light is not limiting, and when light becomes limiting.

Algal cells are known to respond to changes in their environment by adjusting cell volume. The present study found a decrease in cell volume for the *Chlamydomonas* in the half hour and full hour light fluctuation trials. Since both the growth rate and average cell volumes decreased, it is likely that the deceased energy budget (ie reduced irradiance levels) has resulted in a decrease in cell processes that require energy. The decrease in cell volume and decrease in growth rate were accompanied by an increase in the chlorophyll *a* per unit biomass in *Chlamydomonas reinhardtii* By expressing the chlorophyll *a* values in a standard units per biomass we are confident that the apparent chlorophyll *a* increase was real and not an artifact of a decrease in cell volume. It may be reasonable to speculate that the increase on chlorophyll *a* in *Chlamydomonas reinhardtii* cells, was an attempt to increase their light harvesting capacity, perhaps by increasing the number of light harvesting complexes. The fact that the growth rate and cell volumes of *Chlamydomonas reinhardtii* still declined might indicate that the increase in chlorophyll was less than successful.

Happey-Wodds (1988) reported that green algae grow rapidly under higher irradiances in comparison with other major algal groups such as diatoms. She also described how flagella and the stigma (eye spot) allow some green algae to adjust their position in the water column. This presumably would allow the alga to remain at a point in the water column that would maximize their photosynthetic production and growth rate. Mechanical adaptations work very well in calm waters, however they become less effective as turbulence increases. Algal adaptations such as those described above become useless in rapidly circulating water, as algal cells would rise and fall at the whim of turbulence. It would be reasonable to speculate that cells adapted to calm waters by the use of morphological features such as the flagella and stigma, would be less likely to be able to adjust their photosynthesis in rapidly circulating waters, as they are adapted to physically moving themselves to alter their photosynthetic capability.

The cell volumes of the *Nitzchia* sp. during the half hour and full hour light fluctuation trials remained constant. The lack of change is likely an artifact of the siliceous cell wall that encompasses these cells. Since the frustules separate and serve as a template for the formation of the inner (hypothecae) half of the diatom cell wall, cell size is dictated by the cell size of the previous generation. It is unlikely that the diatoms would be able to respond to a decrease in irradiance levels by a decrease in cell dimensions. For *Nitzschia* sp. the chlorophyll *a* level in the half hour light fluctuation trial did not show any significant difference on any day between experimental and control. Neither the full hour nor the half hour light fluctuation trials for *Nitzschia* sp. showed any significant changes in chlorophyll *a* values between the experimental and control replicates after the imposition of the fluctuating light regime.

Grobbelaar (1989) has suggested that all algae have the ability to adjust to fluctuating light in some manner. A study by Richardson *et al*. (1983) has shown that while all algae have the capability to alter their photosynthetic pigments to some extent, the green algae have the highest capability. Queguiner and Legandre (1986) examined the effects of high frequency light fluctuations on *Dunaliella tertiolecta* and found that chlorophyll increased in response to the lowered light levels. Havelkova-Dousova *et al*. (2004) also studied *Dunaliella tertiolecta* and found that chlorophyll levels were higher at lower irradiance levels. A study by Sciandra *et al.* (1997) also found an increase in chlorophyll *a* levels in *Dunaliella tertiolecta* after being transferred from the non limiting irradiance to limiting levels irradiance.

Dickson (2007) showed a similar increase in the chlorophyll concentrations for *Chlamydomonas*, as was shown in this study. The increase in concentration of chlorophyll pigments shown in the Dickson (2007) study for *Nitzschia* was not seen to the same degree in this present study. These results suggest that in Dickson's experiment, the *Chlamydomonas* did increase their chlorophyll concentrations in response to the decreased light levels, but were able to maintain their cell density. The growth of *Chlamydomonas* was most likely limited by the reduced phosphate levels that reduced important metabolic processes like protein synthesis. In the present study, the increase in chlorophyll concentration was accompanied by a decrease in growth rate. This might indicate that growth rates at higher phosphate levels could not be maintained when irradiance levels dropped. Therefore it might be inferred that light became the limiting factor for the growth of the algae, since all nutrient requirements were provided in excess.

The results of this study, combined with the results of other authors leads to the conclusion that reducing the total amount of light available for photosynthesis does decrease the growth rate and induce physiological adaptations to gather more light in some algae. While a reduction in the level of light seems to be important, the frequency of the light level reduction does not seem to be as important. In a study by Sciandra et. al. (1997), reduced irradiance levels over a diurnal light cycle caused the algal growth rate to decrease. In studies by Litchman (1998; 2000) growth rates changes occurred when a fluctuating light regime was imposed on the specimens, regardless of the frequency of the fluctuations. Similarly in this study a significant decline in growth rate was seen at both the full and half hour light fluctuation levels in *C. reinhardii*. It might then be inferred that reducing the total amount of light received by the algal specimens is a factor that influences their growth, and the manner in which they receive this reduced light does not seem to impact how they grow. This is a topic which should be studied under a wider array of light fluctuations than those studied here.

The method used for creating fluctuating light in this experiment is one area which may be problematic. A number of studies have been performed using a similar venetian blind system (van Leeuwe *et al.,* 2005; Veen *et al*., 1997; and Ibelings *et al*. 1994). These three experiments both used a venetian blind system that was hooked up to a step motor, and controlled by a computer, to simulate the fluctuating light regime. In these studies the light pattern achieved resembled very closely a bell shaped diurnal light pattern. In this experiment the blinds were attached to a timed motor which switched the blinds open and closed at predetermined intervals. In the present experiment, the light regime is more like a stair-step pattern but still follows the same pattern. The other

advantage of the studies mentioned above is the fact that when the fluctuating light pattern is imposed, the transition to reduced light levels is gradual. This adds the benefit of the transition from the top of the water column down to the bottom and is more realistic than the abrupt change presented in this experiment. The simple stair-step pattern presented in this experiment is the closest approximation that can be achieved with our equipment, and still resembles a diurnal light pattern.

An unusual increase in cell density occurred during the half hour experiment on day 7 as can be seen in Figure 8. When it was examined why there was an increase there was found to be no change in temperature in the environment chamber, or any increase in the amount of media being given to the cultures. Since this increase was seen both in the control and experimental replicates, it was assumed that something must have occurred to affect the entire system. After this anomaly, the cultures seemed to reestablish their original steady state cell density compared with day 6. It is this researchers opinion that this was simply an anomaly in the data, and should be overlooked when looking at the results overall.

The method used to determine cell volume may not be entirely accurate due to logistic constraints. The Motic images hardware and software offer an excellent way of accurately measuring both *Nitzschia* sp. and *Chlamydomonas reinhardii*, however measurements can only be made in two dimensions, which limits the types of mathematical models that can be used to estimate cell volumes. For *Chlamydomonas* this is not a problem since both models that can be used to estimate cell volume only require measurements in two dimensions. Two independent sources both recommend similar geometric formulas for the estimation of cell volume (Sun and Liu, 2003; Hillebrand *et*

al., 1999). Both sources state that for *C. reinhardii*, the optimal model to be used would be the spheroid. In this study, cell volumes were estimated using the formula for a prolate spheroid, as it was felt this model was a more accurate representation of *C. reinhardii*. For *Nitszchia* both of the above studies recommend using a shape known a prism on a trapezoid base, which requires three dimensional measurements to give an estimation of volume. Since three dimensional measurements were not possible in this study, a model known as the double cone was used to estimate cell volume. The double cone model requires measurements in two planes. The requirement to use this model decreases the accuracy of the cell volume (and later chlorophyll per unit biomass) measurements, which then makes comparing measurements between *C. reinhardtii* and *Nitzschia* impractical. Methods exist which allow for the automatic sizing of cells via computers (Gray *et al*., 2002) but these methods rely on complicated computer programs which may not deliver accurate results.

CONCLUSION

The results of the present study are consistent with what is seen in nature, in that *C. reinhardtii*, is the dominant species in low turbulence, stable environments and *Nitzschia* sp. is the dominant species in high turbulence rivers. These results are important for the management of algae in rivers which are regulated. In regulated rivers, the upstream reservoir portion of the river will tend to be calmer, but will also be deeper. The downstream portion will have variable turbulence levels controlled by the dam. It is important to note that the results from this study cannot be extrapolated across groups of algae. The two species used in this study have specific, unique adaptations and other

species have different adaptations and may not react in a similar fashion. Further study should be done to study the effects of fluctuating light on different species of algae.

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APPENDIX I: WC Media Series

Formula for the WC algal growth media series as originally formulated by Guillard and Lorenzen (1972). This growth medium can be modified to create a phosphate limited or a phosphate rich media by altering the concentration of KH_2PO_4. The final pH of the Medium should be adjusted to between 7.49 and 7.51 using HCl and NaOH.

	COMPOUND	FINAL CONCENTRATION	
***MAJOR ELEMENTS**	$NaNO_3$	17.0	(mg/L)
	KH_2PO_4	3.5†	
	KCl	3.0	
	$MgSO_4 \cdot 7H_2O$	37.0	
	$CaCl_2 \cdot 2H_2O$	36.8	
	$NaHCO_3$	12.6	
	$NaSiO_3 \cdot 9H_2O$	56.8	
‡TRACE ELEMENTS	Na_2EDTA	2.185	(mg/L)
	$FeCl_3 \cdot 6H_2O$	1.575	
	H_3BO_3	0.500	
	$MnCl_2 \cdot 4H_2O$	0.090	
	$Na_2MoO_4 \cdot 2H_2O$	0.003	
	$ZnSO_4 \cdot 7H_2O$	0.011	
	$CoCl_2 \cdot 6H_2O$	0.005	
	$CuSO_4 \cdot 5H_2O$	0.005	
§BUFFER	Bicine	179.2	(mg/L)
£VITAMINS	A: Cyanocobolamine (B_{12})	0.50	(µg/L)
	B: Biotin	0.50	
	C: Thiamine	100.00	

*Aqueous stocks of the major elements should be added to distilled water in the order given.

†Phosphate concentration can be modified to achieve either a phosphate limited, phosphate enriched, or average phosphate concentration media.

‡A concentration stock solution of trace elements should be first prepared by adding the trace elements in the order listed to 1 L of distilled water, then adding 2.5 mL of the stock solution to 1 L of medium.

§A concentration stock solution buffer should be prepared by adding 17.92 g of bicine to 1 L of distilled water, then adjusting the pH of the stock solution to 7.0. Add 10 mL of stock solution to 1 L of medium.

£Three separate solutions should be prepared:

A: 10.0 mg/100 mL of distilled water

B: 10.0 mg/100 mL of distilled water

C: 10.0 mg + 0.5 mL solution A + 0.05 mL solution B + 99.0 mL of distilled water

Add 1.0 mL of solution C to 1 L of medium.

APPENDIX II

Figure 20: Example picture of *Chlamydomonas reinhardtii* viewed at 1000 x with measurements for cell volume taken with Motic Images.

Figure 21: Example picture of *Nitzschia* sp. viewed at 1000 x with measurements for cell volume taken with Motic Images.

APPENDIX III

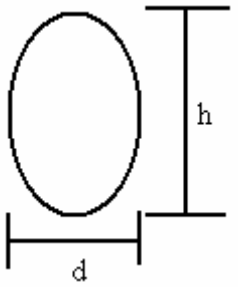

Figure 22: Diagram of an ellipsoid, used as model shape for *Chlamydomonas reinhardtii* cell volume measurements. Reproduced from Wetzel and Likens, 1991; Hillebrand *et al*., 1999; Sun and Liu, 2003.

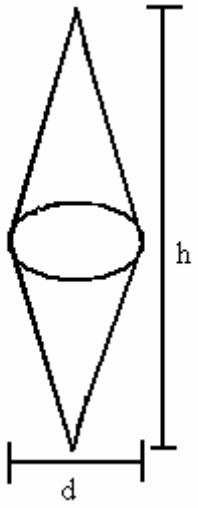

Figure 23: Diagram of double cone used as model shape for *Nitzschia* sp. cell volume measurements. Reproduced from Wetzel and Likens, 1991; Sun and Liu, 2003.

APPENDIX IV

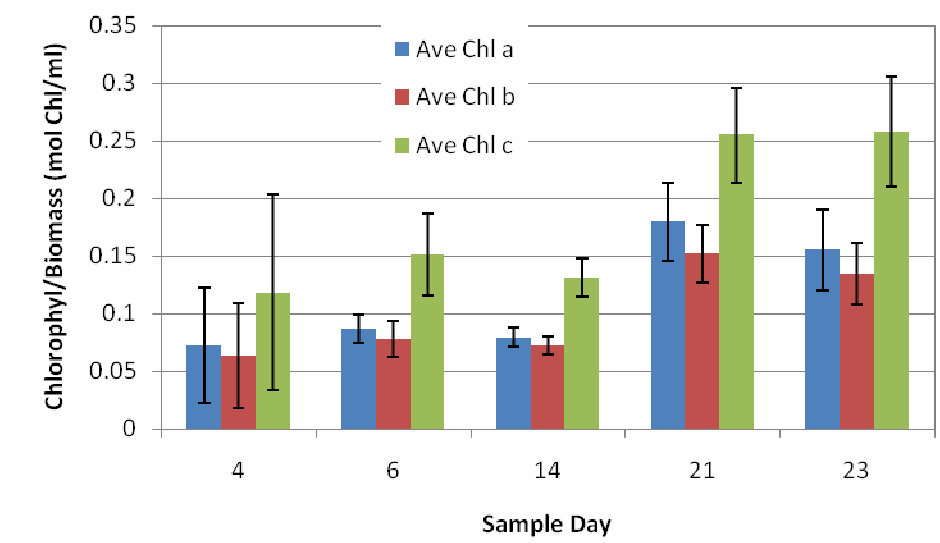

Figure 24: Average chlorophyll concentrations for the experimental trial of the half hour light fluctuation experiment of *Chlamydomonas reinhardtii*.

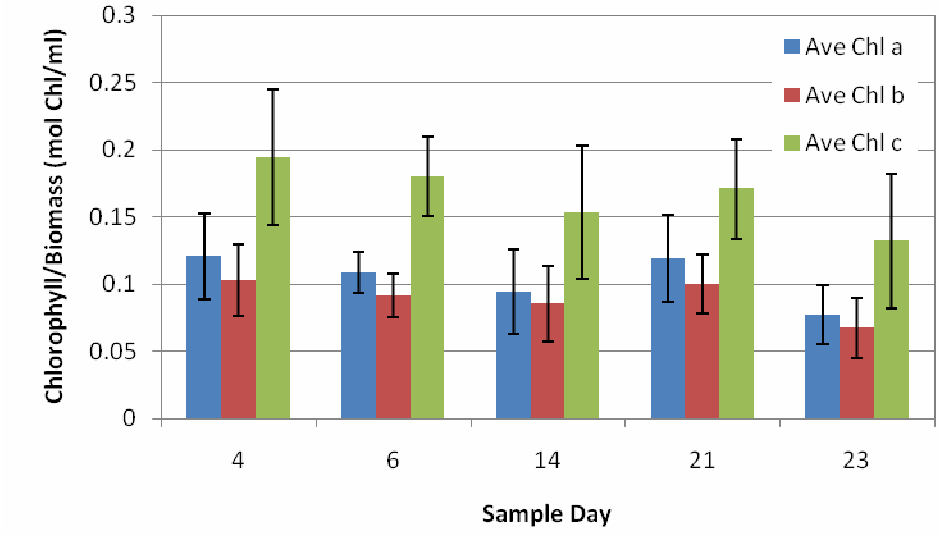

Figure 25: Average chlorophyll concentrations for the control trial of the half hour light fluctuation experiment of *Chlamydomonas reinhardtii*.

APPENDIX V

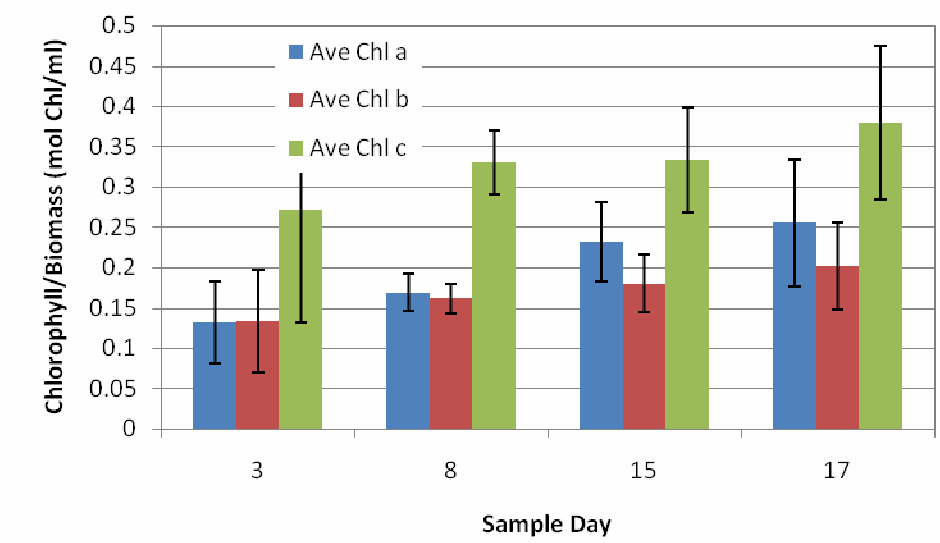

Figure 26: Average chlorophyll concentrations for the experimental trial of the full hour light fluctuation experiment of *Chlamydomonas reinhardtii*.

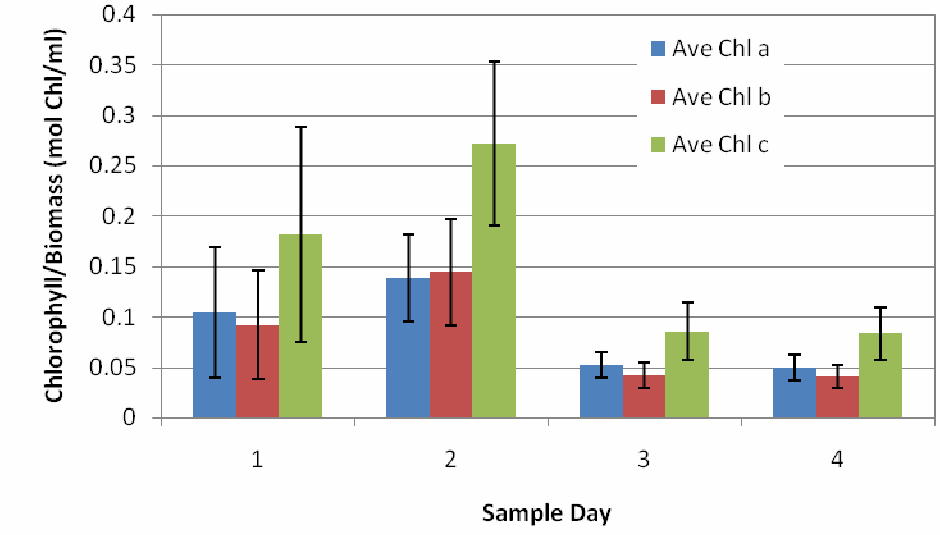

Figure 27: Average chlorophyll concentrations for the control trial of the full hour light fluctuation experiment of *Chlamydomonas reinhardtii*.

APPENDIX VI

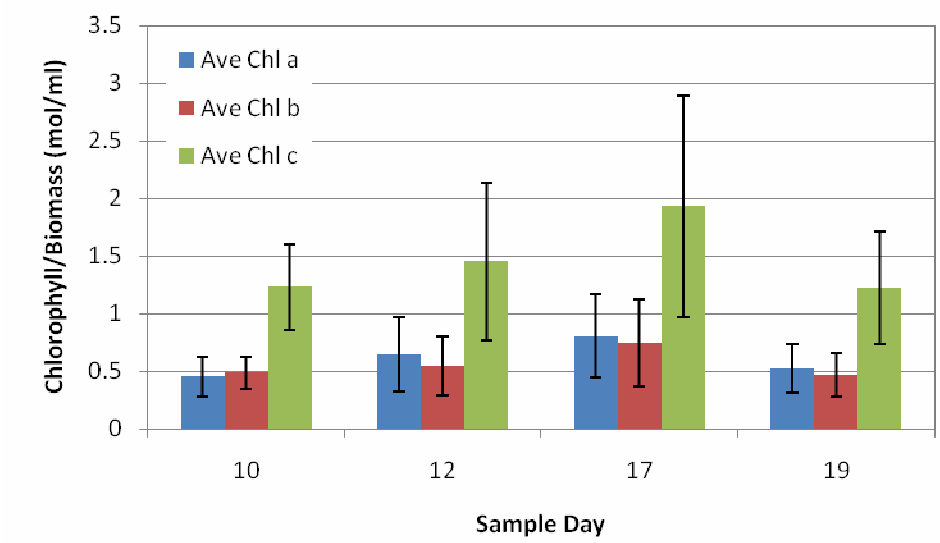

Figure 28: Average chlorophyll concentrations for the experimental trial of the half hour light fluctuation experiment of *Nitzschia* sp.

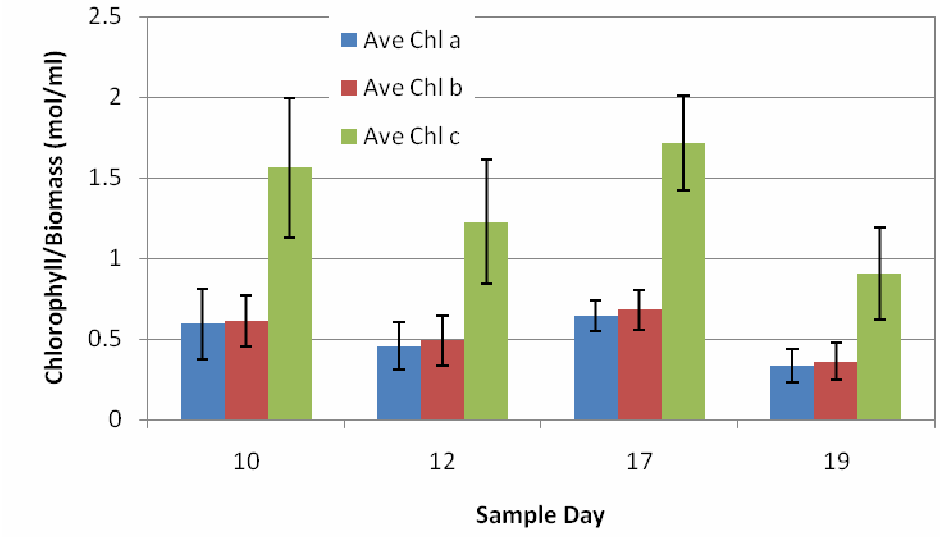

Figure 29: Average chlorophyll concentrations for the control trial of the half hour light fluctuation experiment of *Nitzschia* sp.

APPENDIX VII

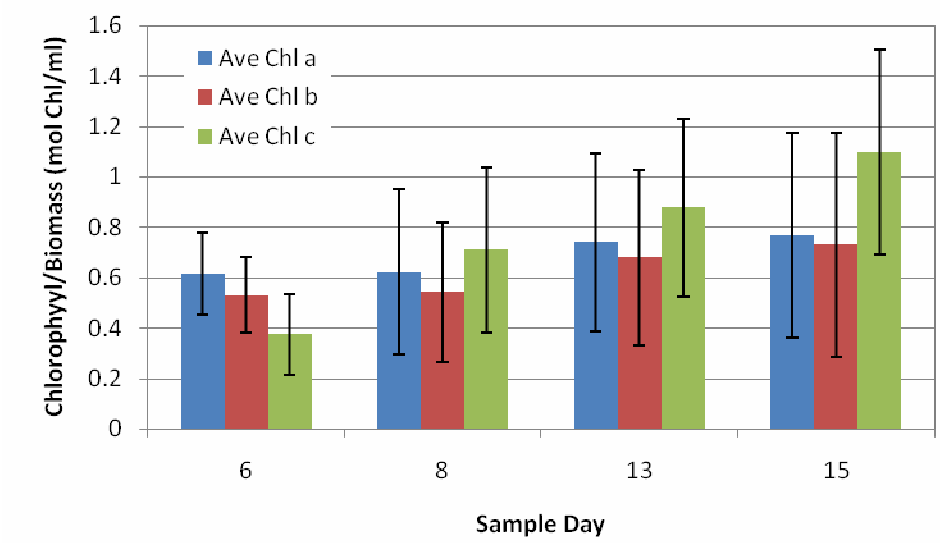

Figure 30: Average chlorophyll concentrations for the experimental trial of the full hour light fluctuation experiment of *Nitzschia* sp.

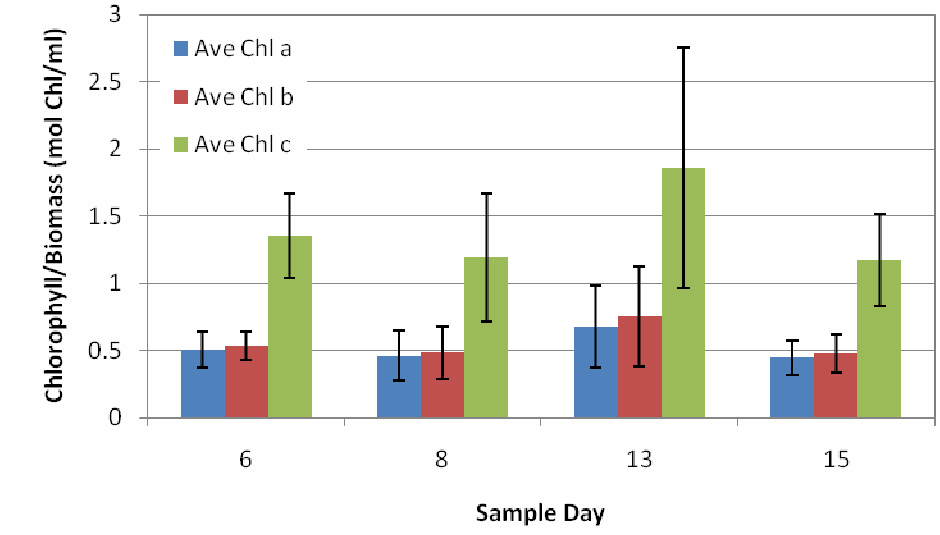

Figure 31: Average chlorophyll concentrations for the control trial of the full hour light fluctuation experiment of *Nitzschia* sp.