TURN, TURN, TURN: THE EFFECTS OF LIGHT FLUCTUATION DUE TO TURBULENCE ON THE GROWTH RATE AND PHYSIOLOGICAL PROPERTIES OF RIVER PHYTOPLANKTON

BY

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ABSTRACT

Hydroelectric developments are a form of river management that can affect the turbulence of the river. Turbulence affects the position of phytoplankton cells in the water column through vertical cycling. This movement alters the daily light energy the phytoplankton cells receive as light penetration decreases with increasing water depth. In this experiment the steady state cell densities and chlorophyll content of two phytoplankton species were investigated in response to this light cycling stimulus. Six semi-continuous cultures of *Chlamydomonas reinhardtii* and *Nitzschia* sp. were grown under an initial 16-hour light, 8-hour dark diurnal light regime. These cultures were then subjected to a fluctuating light cycle superimposed on the diurnal light cycle, using a newly designed motor and timer apparatus which operated a Venetian blind positioned between the lights and the algal cultures to simulate the vertical cycling. Steady state cell densities decreased while chlorophyll concentrations increased in response to more limited light caused by the opening and closing of the blinds as compared to the diurnal control. The results suggest that under fluctuating light cycles which mimic vertical mixing, phytoplankton divert energy to chlorophyll production at the expense of quicker reproduction, and that the total amount of light energy is more critical than the pattern in which it is received.

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INTRODUCTION

PHYTOPLANKTON ECOLOGY

Phytoplankton are extremely important to the biosphere as they are the basis of aquatic food webs which support a large range of diverse organisms including commercially valuable species of fish and aquatic invertebrates. Through photosynthesis these organisms account for 70% to 80% of the oxygen production in the world (Reynolds, 1984). Phytoplankton also serve as important indicators of climatic change due to the greenhouse effect (Falkowski, 1994), ecosystem productivity (Loiselle *et al*., 2007), and water quality (Kelly *et al*., 1998). They are good indicators because they can respond rapidly to environmental changes (Spigel and Imberger, 1987) and because their generation times are relatively short (usually hours to days), so populations can be observed for possible trait selection influences over a relatively short period of time (Reynolds, 1993).

Traditionally, research on the effects of turbulent mixing and fluctuating light on phytoplankton has focused on lake phytoplankton (eg: Spigel and Imberger, 1987; Ibelings *et al*., 1994; Flöder *et al*., 2002), or marine phytoplankton (eg: Denman and Gargett, 1983; Quéguiner and Legendre, 1986; Estrada and Berdalet, 1997), with few studies on lotic phytoplankton (Bertrand *et al.*, 2001; Kalff, 2001; Reynolds 2000) sometimes called potamoplankton (Reynolds, 1984; and 2000; Wehr and Thorp, 1997). Several mathematical models have also been created to explore the different relationships between variables due to vertical mixing (Falkowski and Wirick, 1981; Spigel *et al*., 1986; Patterson, 1991; Huisman and Weissing, 1994; Weissing and Huisman, 1994; Diehl, 2002), however all of these deal with vertical mixing due to an environmental

condition such as wind induced mixing and/or Langmuir cells in lakes and oceans. There has been little study into the effects of turbulence within river bodies on phytoplankton (Reynolds, 1994; and Reynolds *et al*., 1994) and even less when it comes to turbulence caused by human management of rivers (Wehr and Thorp, 1997) especially run-of-the-river reservoirs, such as the Winnipeg River System, which Kalff states, " have received the least attention from limnologists" (2001).

There are numerous questions in potamoplankton ecology that are still unanswered such as: (1) how species maintain numbers in rivers when they are constantly being flushed out of the system (Reynolds, 1994; and 2000), (2) how numerous species of phytoplankton can coexist (Litchman, 1998; and Flöder *et al*., 2002) and (3) what processes cause community changes in the dominant phytoplankton composition throughout the year (Litchman, 2000). This thesis specifically looks at the responses of phytoplankton to light fluctuations, which may contribute to answering these questions that limnologists have posed. In nature, these light fluctuations are caused by vertical mixing which can be attributed to human management of rivers. These reaches of managed rivers would be characterized by constant mixing at a set rate for any given point along a river in which all other variables such as temperature and nutrients would be homogeneously distributed throughout the entire water body. Thus, the only limiting factor to phytoplankton growth would be light. This study is important as presently, very few large rivers in the world are not managed by humans and previous studies have not looked at light as a limiting factor on phytoplankton growth within these rivers. Also, previous studies have not looked at the effect that turbulence has on light intensity within these managed rivers.

Light penetration in water is highly variable: as depth increases in the water column, the amount of light penetration decreases as the light is absorbed (Denman and Gargett, 1983; Grobbelaar *et al*., 1992; Litchman 1998; and 2000; Havelková-Doušová *et al*., 2004). This causes a light gradient in the water column. Eventually a depth is reached where the rate of photosynthesis that can occur with the available light is equal to the rate of respiration; this is the compensation depth (Spigel and Imberger, 1987). Below the compensation depth primary production cannot occur. Within turbulent environments, such as managed rivers, phytoplankton are constantly moving up and down through this light gradient. Grobbelaar (1989) states that phytoplankton are subjected to light/dark cycles which can be common in turbid waters such as rivers and streams. Grobbelaar *et al*., (1992) take this farther by explaining that phytoplankton are transported through a light gradient by mixing, so that these shorter light/dark cycles become superimposed on diurnal light cycles. These light/dark cycles have been broken into three ranges by Grobbelaar (1989):

(1) high frequency fluctuations of 100 milliseconds or less

(2) medium frequency fluctuations of seconds to minutes, and

(3) low frequency cycles of hours to days and years.

This thesis looks at the effects of low to medium light fluctuations on phytoplankton.

Some of the first work on fluctuating light caused by turbulence was by Marra (1978a). He examined the effects of fluctuating light on the marine diatom *Lauderia borealis*, in comparison to a diurnal light cycle and a constant light cycle. It was found that rates of photosynthesis could possibly be enhanced by fluctuations in light levels. Marra (1978a) hypothesized that this enhanced outcome could be a result of suppression

of photoinhibition that could occur when phytoplankton were under a full and continuous light. He concluded that "the periodicity of environmental factors, such as light, on a time scale of phytoplankton growth and physiological processes should be recognized in conceptualizing models of the production ecology of lakes and oceans." After his work many people began to look at the different ways light became attenuated within the water column and how this affected phytoplankton.

The first work on fluctuating light due to turbulence was done *in vitro* by Marra (1978a), and as Sommer (1990) stated *in* Litchman (2000) "valuable insights can be gained from experiments even with simple laboratory systems," so too, this thesis uses an *in vitro* system to investigate light fluctuations caused by turbulence. Lab studies have also been done by Grobbelaar (1989; and 1994), Ibelings *et al*., (1994) and Litchman (1998; and 2000). Through their work and others, many different ways of modeling light attenuation *in vitro* have been described. Litchman (2000) admits that lab studies do not mimic natural light fluctuations. Her goal, which was also my goal, was to compare fluctuating light levels with a constant diurnal light regime in an effort to show that fluctuating light which can be caused by vertical cycling due to turbulence will have an effect on phytoplankton growth as measured by steady state cell densities and chlorophyll concentrations.

Numerous studies have also been completed *in situ* by Marra (1978b), Gervais *et al*., (1997), Köhler (1997), Diehl *et al*., (2002), and Köhler *et al*., (2002). As they were done in lakes or marine environments, they took into account other light attenuating sources such as turbidity of the water, cloud cover and shading from other phytoplankton. Diehl *et al*., (2002) point out that nutrients are recycled within an ecosystem but the light

energy received from the sun must be intercepted and used immediately. This has implications in relation to abiotic factors such as suspended sediments that can absorb light photons because this light then becomes lost for good.

OBJECTIVES

The objective of this experiment was to investigate the growth rate and physiological response of algal cells in a semi-continuous culture to varying levels of light intensity cycles that would be caused by medium to low frequency turbulence as seen in managed rivers. These light cycles would exist in certain managed rivers having a constant vertical mixing rate that would not be dependent on external environmental factors such as wind to be stimulated. In this situation, mixing would be constant, all other gradients in variables that are normally considered in lakes such as nutrients and temperature would not exist as the environment would be homogeneous. In my experiment, the only limiting factor to phytoplankton growth was light. This was compared to a control having a normal diurnal light cycle, which would be experienced in a calm water body where buoyant algal cells remain at the surface of the water.

Two common lotic phytoplankton species which are both good representatives of two of the main groups of river phytoplankton were used in these experiments. The species used were: *Nitzschia* sp. Hassall (Figure 1) and *Chlamydomonas reinhardtii* P.A. Dang (Figure 2) of the Bacillariophyceae (diatoms) and Chlorophyceae (green algae), respectively. These two species would represent many temperate rivers throughout North America (Reynolds, 1994; Lowe, 2003; and Nozaki, 2003).

My hypothesis was that the phytoplankton species would change their growth rates and physiological responses as measured by chlorophyll pigment concentrations due to the intermittent light that is associated with turbulence in a managed river.

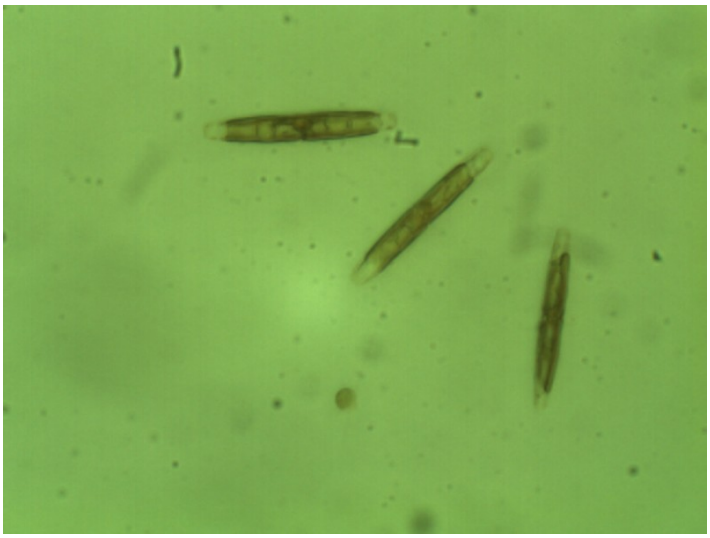

Figure 1: *Nitzschia* sp. used in this experiment viewed at 1000x magnification.

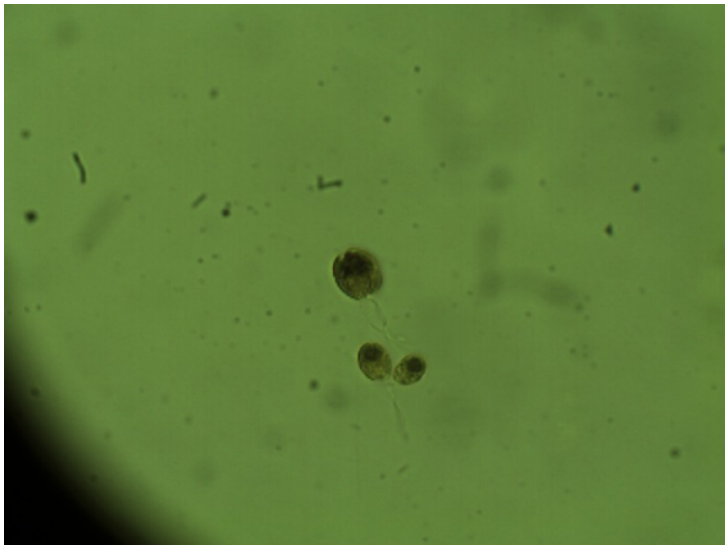

Figure 2: *Chlamydomonas reinhardtii* used in this experiment viewed at 1000x magnification.

MATERIALS AND METHODS

Nitzschia sp. stock cultures were collected and isolated from the Winnipeg River in August of 2006. *Chlamydomonas reinhardtii* stock cultures were obtained from Ward's Biological Supply Company (Rochester, New York). Stock cultures of both species were maintained in 250 mL. Erlenmeyer flasks in a WC' medium (Appendix I) at 18^{o}C, under approximately 60 µmol photons m^{-2} s^{-1} fluorescent light on a 16 hour light: 8 hour dark cycle. The length of the light cycle is comparable to southern Manitoba from the beginning of June to mid-July. All stock cultures were sub-cultured once every eight to ten days.

The *Nitzschia* sp. experiment was carried out in a wooden frame box, 148 cm wide by 80 cm high and 81 cm deep. The *Nitzschia* sp. chamber was housed in a small room in the animal complex at the University of Winnipeg. Due to poor ventilation, the room temperature fluctuated between 26 and 28°C. There were three sets of light banks stacked one on top of the other at the back of the *Nitzschia* sp. chamber. Light bank 1 (bottom) and light bank 2 (middle) each contained two high output, cool white fluorescent light bulbs (one each of Sylvania F48T12 CW HO and GE F48T12 CW HO). Light bank 3 (top) contained two cool white fluorescent bulbs (Sylvania F30T12 CW RS).

The *Chlamydomonas reinhardtii* experiment was housed in a walk-in environment chamber in the Department of Biology Animal Complex where temperature was controlled at 18°C ± 1°C. Light was provided by four single light banks which contained new Ecolux fluorescent bulbs (GE F32T8 SPX35 ECO). All light banks were controlled by programmable digital timers to turn on and off in a set sequence.

In each chamber six replicate, unialgal, semi-continuous cultures were set-up in such a way that all culture tubes received equivalent measured light levels. See Appendix II for a diagram (Figure 19) and photograph of the experimental setup (Figure 20).

In each chamber there were two media carboys each feeding three semi-continuous cultures. One air pump was used to pressurize both carboy heads. Two different air pumps were used; each aerated three of the semi-continuous cultures. In the *Nitzschia* sp. experiment chamber the carboys were stored on the same level as the light banks. To prevent growth of any algal contamination in the media carboys, they were wrapped in black, plastic garbage bags to limit their light exposure. In the *Chlamydomonas reinhardtii* experimental chamber, the carboys were placed on a shelf above the semi-continuous cultures so they were not exposed to light and therefore they were not wrapped in plastic.

Two similar diurnal light regimes, one in each chamber, were established for the experiment and acted as the control. Both consisted of 16 hours of light and eight hours of darkness. Tables 3 and 4 in Appendix III illustrate the programming used for the timers to turn the light banks on and off as well as the total photons experienced by the semi-continuous cultures throughout a 24-hour period. When a new light bank turned on or off, the increase or decrease was immediate, creating a stepwise irradiance pattern as shown by Figures 21 and 22 in Appendix IV.

The Venetian blind apparatus described here was inspired by the apparatus described by Kroon *et al*. (1992) and used by Ibelings *et al*., (1994) and Flameling and Kromkamp (1997). My apparatus consisted of a metal box measuring 13cm x 7cm x

10cm (Apparatus A; Apparatus B was 13cm x 7cm x 12cm) mounted on the arm of the Venetian blinds. The apparatus used in this experiment opened the Venetian blinds to a horizontal position and closed them in a vertical position. The blinds were hung in the back of the chambers between the light banks and the semi-continuous cultures. The metal box housed the timer and motor which controlled the arm of the blinds causing them to rotate 90° to open or close. Two dials on the outside of the box controlled the length of time that the blinds were open and the length of time that the blinds would be closed. There was also a switch to change the measurement of time used for the dials (sec, 10s sec, min, and hour). The apparatus is not able to create a smooth curve of light transmittance, as the blinds can only be controlled to open and close in a stepwise manner. Please refer to Appendix V for pictures of the blind apparatus.

Experimental runs consisted of superimposing the fluctuation of light do to opening and closing the blinds at varying cycles on top of the diurnal light cycle used for the control runs. The *Nitzschia* sp. cultures were run for 32 days; the first eight days were under a diurnal light regime with the blinds open, and the following seven days were run on a one hour open, one hour closed blind cycle. The next six days were run with the blinds on a half hour cycle followed by another six days of a diurnal light regime. Finally the last five days had the blinds closed all the time. The *C. reinhardtii* cultures were run for 22 days, the first eight days under diurnal light, the next seven with a blind cycle of a half hour, and the final seven with a blind cycle of two hours. Using the same cultures and modifying their light regimes allowed for the same cultures with the same media to be both the control and experimental groups, which helped eliminate many variables that could have altered the results. Figures 25 and 26 in Appendix VI

graphically represent the total light available to the cultures in the *Nitzschia* sp and *C. reinhardtii* cultures, respectively. It should be noted that approximately 20% of the light reaches the cultures when the blinds are closed. The maximum light intensity that the cultures were exposed to (180 μmoles of photons m^{-2} s^{-1}) is typical of many sites in the Winnipeg River at the surface level. Light at approximately 20% of the full light (when the blinds are closed) represents roughly a two to three metre depth in the Winnipeg River (Kent Simmons, personal communication). It should also be noted that if the cycle were to shift in time or be changed to a different time setting, as long as the length of time that the blinds are closed is equal to the time they are open, the total illumination per day remains constant. Thus, the total illumination per day during the diurnal control cycle has reduced to half when the blinds are opened and closed for equal periods of time.

Samples were collected from each replicate culture every day at approximately the same time after thorough mixing with the magnetic stir bar in the culture and subsequent resettling. Approximately 20% of each culture was removed (approximately 30mL) and 15 mLs was then sampled and kept for analysis. The semi-continuous cultures were then refilled with fresh media to their original levels as denoted by a fill line on the glass. Six cell counts of each of the six semi-continuous cultures were performed daily using a Neubaure Bright Line hemacytometer. If cell counts could not be completed the same day as sampling occurred, the samples were preserved using Lugol's iodine.

Cell count data were entered into a spreadsheet where cells per mL were calculated. An overall mean and standard deviation were calculated for an average cell

density from the six replicates for that particular sample day. These data were graphed to show the average cell density over time. Student's t-tests were performed on sequential days data to determine when steady states (growth rate equal to dilution rate of 20%) were reached.

Chlorophyll analysis was performed every third day starting on day seven for the *Nitzschia* sp. experiment. In the *C. reinhardtii* experiment, chlorophyll analysis was conducted twice during each light treatment. Ten milliliters of the daily sample were filtered using glass fiber filters and frozen in foil pouches until analyzed (up to three weeks in freezer). Analysis was conducted using the trichromatic approach as described in Wetzel and Likens (1991). Results were graphed and compared statistically using Student's t-test. F-tests were used as well to determine equal or unequal variances for the t-test.

Cell volumes were determined for every day that a chlorophyll sample was taken. Pictures of 20 cells from each tube were taken using a Moticam 1000 1.3 mega pixel USB 2.0 camera mounted on a Leitz Wetzlar (Wild) Dialux microscope at 1000x total magnification. Sample images for each species can be seen in Figures 1 and 2 and with measurements in Appendix VII Figures 27 and 28. Measurements were made with the Motic Images Plus 2.0 ML software after the microscope had been calibrated with a stage micrometer.

Formulae for cell volume calculations were taken from Wetzel and Likens (1991, see also Hillebrand *et al*., 1999; and Sun and Liu, 2003). In all previous literature, a spherical shape formula was recommended for *Chlamydomonas* sp. calculations (Wetzel and Likens, 1991; Hillebrand *et al*., 1999; Sun and Liu, 2003). However, it was found

that this design did not accurately reflect the shape of the *Chlamydomonas* cells and an ellipsoid (Wetzel and Likens, 1991) or prolate spheroid (Hillebrand *et al*., 1999; and Sun and Liu, 2003) formula was used instead. The ellipsoid formula used two measurements (height and diameter) rather than just diameter for the sphere.

$$\pi hd^2/6 = \text{cell volume for an ellipsoid}$$

In the formula for cell volume of an ellipsoid, h is the height and d is the diameter. The formula works for cells that are spherical as well, as height and diameter would then be equal (Figure 3).

Both Hillebrand *et al*., (1999) and Sun and Liu (2003) recommended a prism on a parallelogram base as the example shape for *Nitzschia* sp. cell volume calculations. This formula required three dimensional measurements which were not possible with the camera set-up. Instead a double-cone (Hillebrand *et al*., 1999; and Sun and Liu, 2003) or two cones (Wetzel and Likens, 1991) formula was used.

$$\pi hd^2/12 = \text{cell volume for a double cone}$$

In this formula, h was the height and d was the diameter at the widest middle point (Figure 4).

Average cell volume and standard deviation were calculated for each of the six tubes for each measured day. These data were used with the chlorophyll data to express chlorophyll concentrations per biomass rather than per cell, as this is a more accurate measure which takes cell dimensions into account.

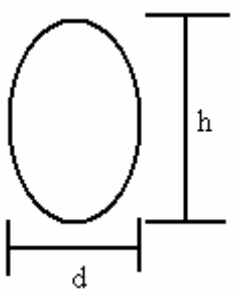

Figure 3: Diagram of an ellipsoid, used as model shape for *Chlamydomonas reinhardtii* cell volume measurements. Reproduced from Wetzel and Likens, 1991; Hillebrand *et al*., 1999; Sun and Liu, 2003.

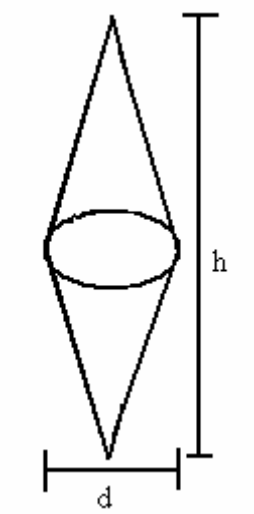

Figure 4: Diagram of double cone used as model shape for *Nitzschia* sp. cell volume measurements. Reproduced from Wetzel and Likens, 1991; Sun and Liu, 2003.

RESULTS

The analysis of these algal growth experiments was based on comparisons between the mean cell densities of the six replicate cultures once they have reached a "steady state" during the different light regimes. The concept of the "steady state" growth in the theory of continuous cultures, simply describes a culture whose cell densities remain constant from one sample period to the next. The method used in this study to determine the population levels of each culture, each day, are considered to be the most accurate, and the most labour intensive. However, these sampling and enumeration methods still generate population estimates that vary highly from culture to culture, and from day to day. This is a particular problem for certain species, such as the diatom *Nitzschia* sp. which tend to clump in culture. It should be noted that none of the comparisons of the mean cell densities between the light treatments showed any statistical significance. However, this could be due to the lack of resolution created by the sampling and enumeration issues. Bearing this in mind, it may be possible to look for trends in the data which might suggest areas of further study.

Cell densities for the *C. reinhardtii* cultures reached a steady state between days three and six under the initial diurnal light regime (Figure 5). The 50 percent decrease in light intensity due to the blinds opening and closing every half hour caused the steady state cell densities to establish at a lower level (Figure 5). When the periodicity of the light was changed so the blinds opened and closed every two hours, the steady state cell densities remained much the same as they were during the half hour blind cycling (Table 1 and Figure 6).

The cell densities of the *Nitzschia* sp. did not increase as fast as the *C. reinhardtii* cultures did (Table 2 and Figure 7). There was little difference between the steady state cell densities established on days six and seven and the steady state cell densities established after the initiation of the blinds opening and closing every hour. During the one hour light cycle run, the cell densities were still gradually increasing (Figure 8).

With the blinds open, on days 20 through 24 of the *Nitzschia* sp. experiment, the steady state cell densities rose, establishing the highest steady state cell density seen throughout the course of the experiment (Table 2 and Figure 9). An initial large decrease in cell density was seen on day 27 after the permanent closing of the blinds (Figure 10), then over the following three days the cell densities steadily increased with an average growth rate of $\mu = 0.27$ and appear to establish a new steady state at a level similar, but slightly less than, the steady state during the one hour blind cycle.

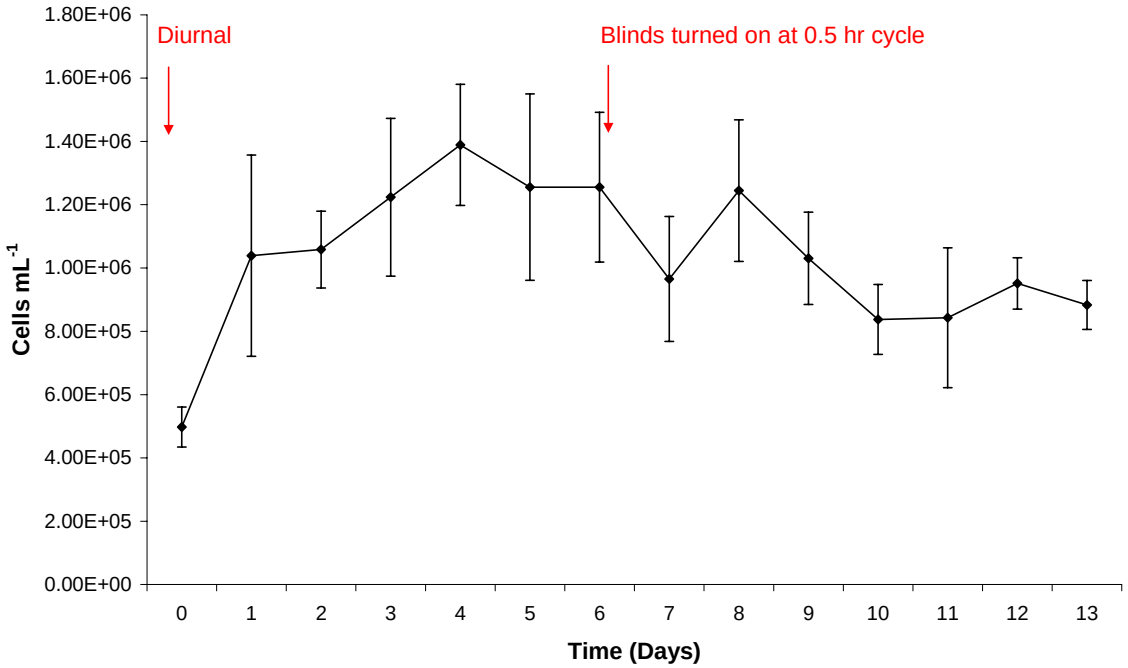

Figure 5: The average cells mL^{-1} of *Chlamydomonas reinhardtii* for the first 14 day sample period of exposure to diurnal light and a half hour blind cycle.

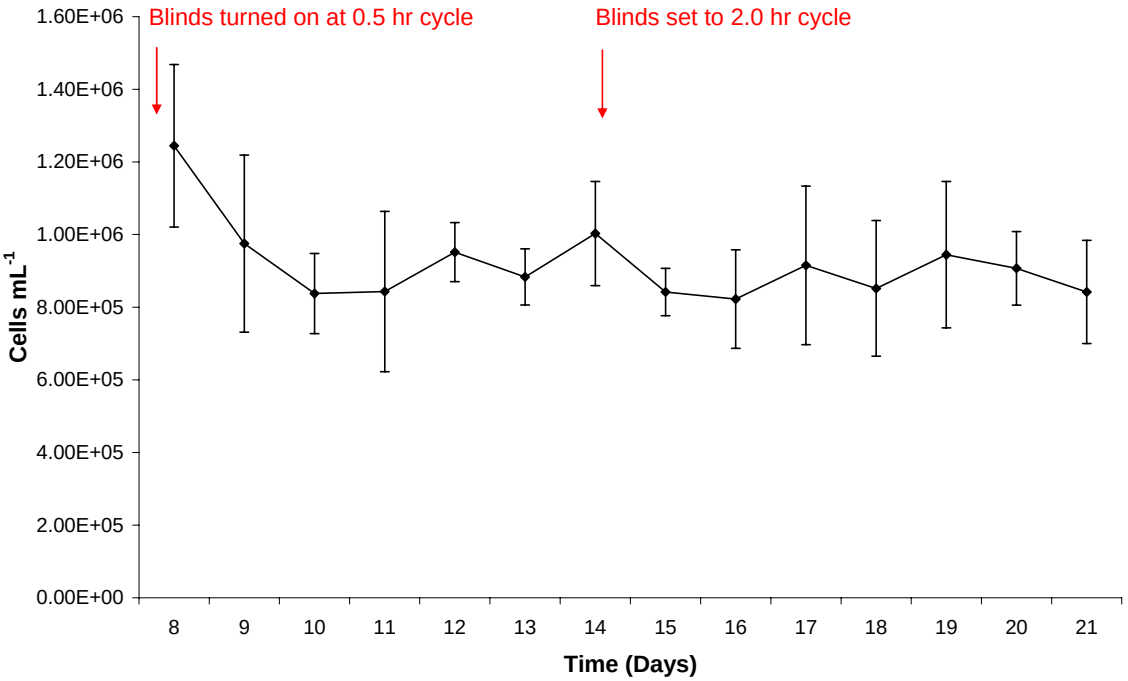

Figure 6: The average cells mL^{-1} of *Chlamydomonas reinhardtii* from days 8 – 21 of exposure to a half hour blind cycle followed by a two hour blind cycle.

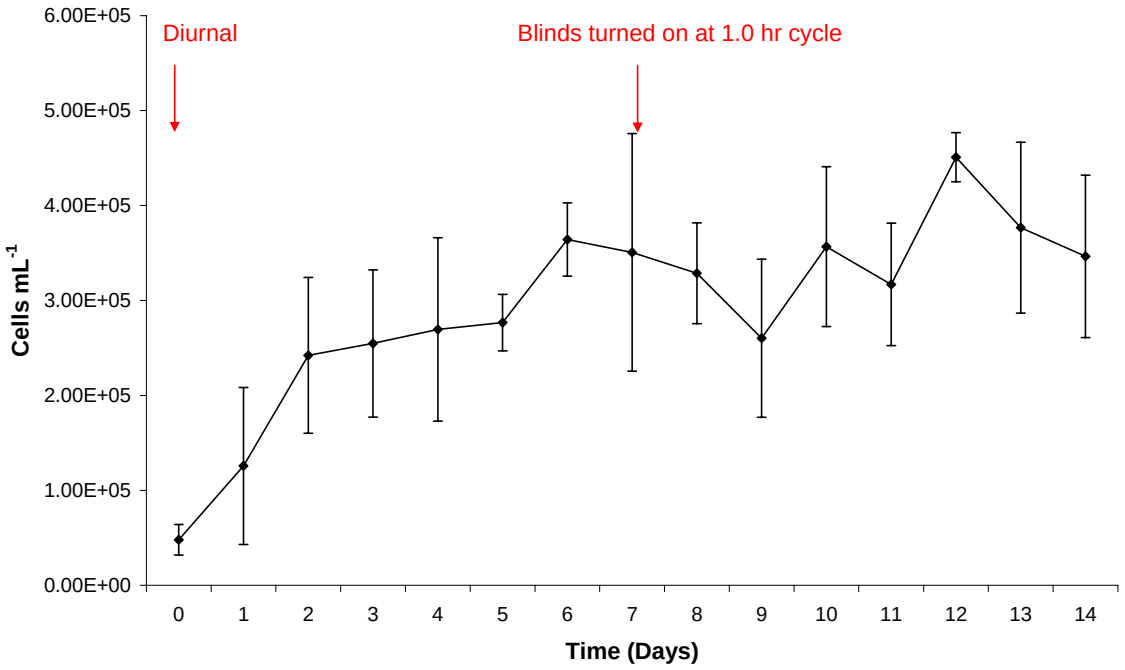

Figure 7: The average cells mL^{-1} of *Nitzschia* sp. for the first 14 day sample period of exposure to diurnal light and a one hour blind cycle.

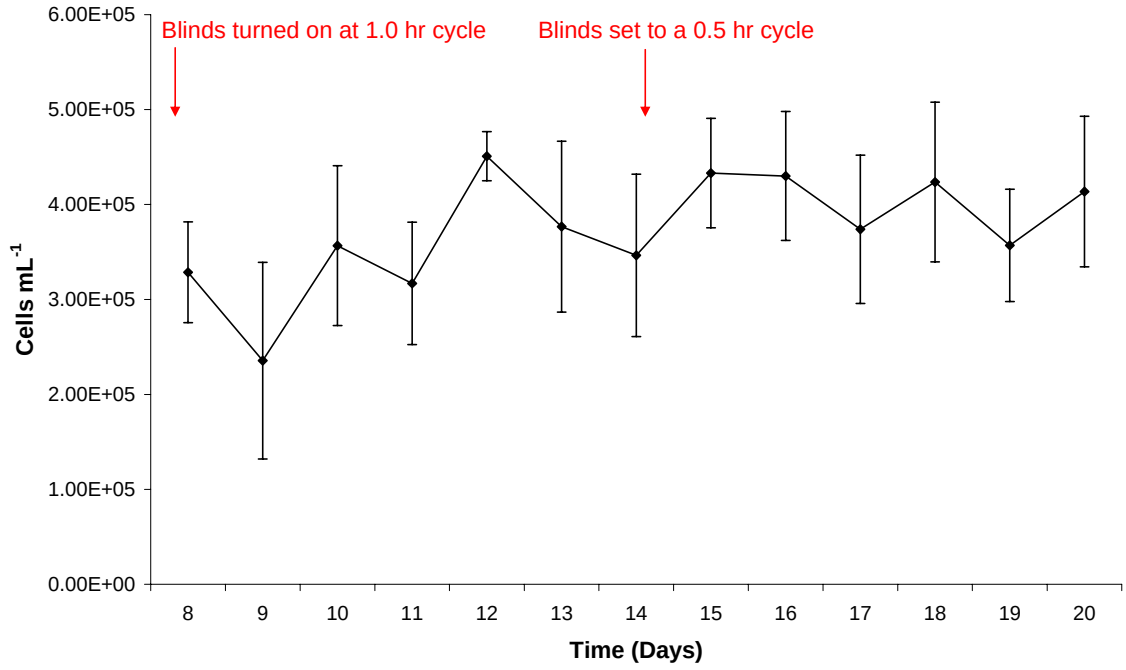

Figure 8: The average cells mL^{-1} of *Nitzschia* sp. from days 8 – 20 of exposure to a one hour blind cycle followed by a half hour blind cycle.

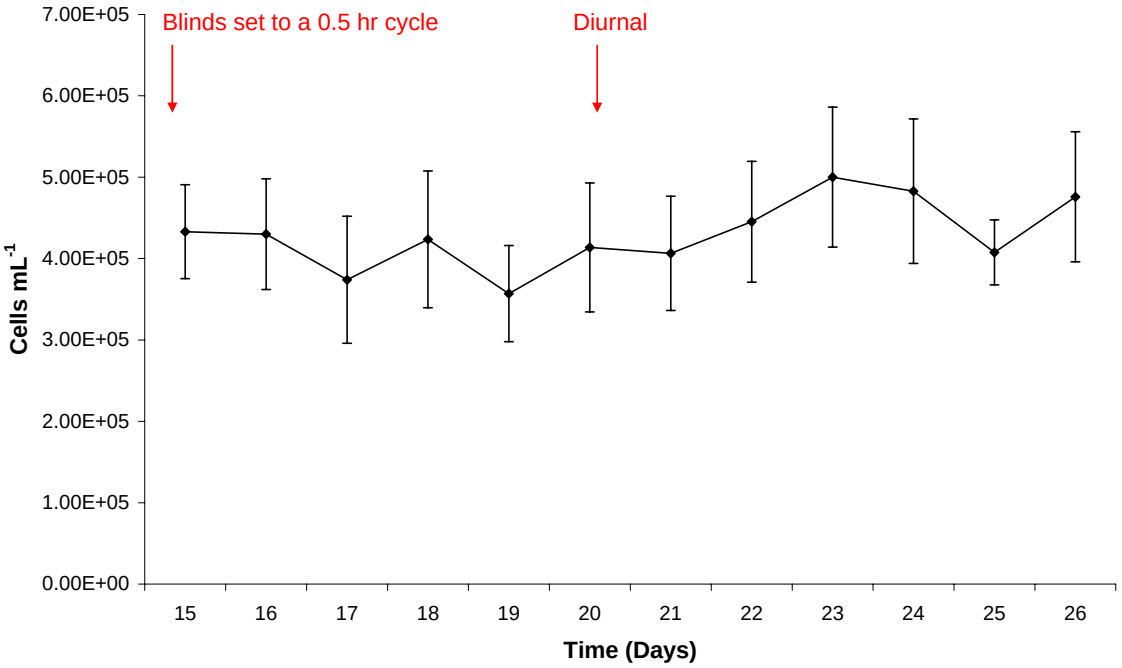

Figure 9: The average cells mL^{-1} of *Nitzschia* sp. from days 15 – 26 of exposure to a half hour blind cycle followed by a diurnal light cycle.

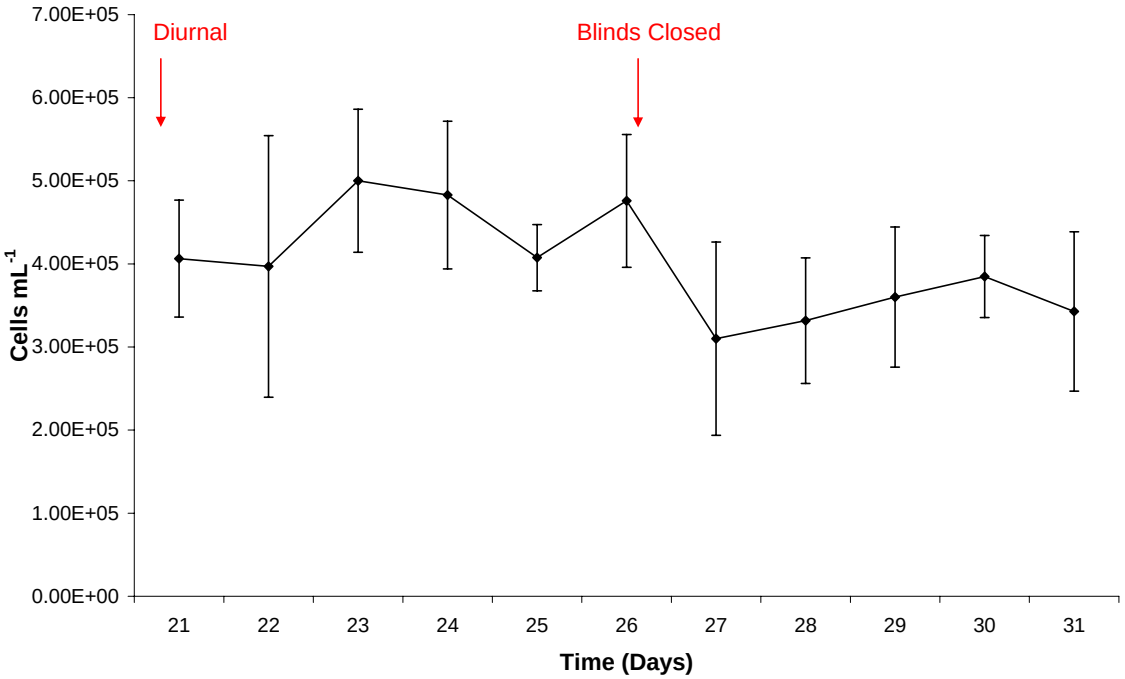

Figure 10: The average cells mL^{-1} of *Nitzschia* sp. from days 21 – 31 of exposure to a diurnal light cycle followed by a period where the blinds remained closed indefinitely.

Table 1: Comparison of steady state cell densities for different light regimes for *Chlamydomonas reinhardtii.*

Light Regime	Mean Cells mL^{-1} Steady State	SD
Diurnal (Days 3-6)	1.28E+06	7.35E+04
0.5 Hr Cycle (Days 10-13)	8.79E+05	5.25E+04
2.0 Hr Cycle (Days 15-21)	8.75E+05	4.65E+04

Table 2: Comparison of steady state cell densities for different light regimes for *Nitzschia* sp.

Light Regime	Mean Cells mL^{-1} Steady State	SD
Diurnal (1) (Days 6-7)	3.57E+05	9.62E+03
1.0 Hr Cycle (Days 8-14)	3.48E+05	5.85E+04
0.5 Hr Cycle (Days 15-20)	4.05E+05	3.20E+04
Diurnal (2) (Days 22-26)	4.62E+05	3.65E+04
Closed (Days 27-31)	3.46E+05	2.83E+04

In the *C. reinhardtii* experiment, the chlorophyll concentrations taken during the half hour light cycle increased over those taken during the diurnal light conditions (Figure 11). As well, the chlorophyll concentrations taken during the half hour cycle to the two hour cycle of opening and closing of the blinds also increased (Figure 12). By the two hour cycle the chlorophyll concentrations appear to have leveled off with the exception of the chlorophyll c concentrations which were slightly increased. The letters above the bars in the Figures denote trends of similarity.

Chlorophyll concentrations in the *Nitzschia* sp. experiment, measured during the one hour blind cycle, showed a slight decrease compared to the first diurnal regime, (Figure 13) however these three days remained similar to each other. A decrease in chlorophyll was seen for the day 16 sample (Figure 14) compared to the previous three samples. The chlorophyll levels seen on day 19 seem to have returned to the levels seen in the samples taken on the first three sample days, as it was similar to them.

As can be seen in Figure 15, the lowest chlorophyll levels for the *Nitzschia* sp. are seen on day 22, two days into the second diurnal light regime. By day 25, five days into this light regime, the chlorophyll concentrations have risen slightly, but are still at the third lowest concentrations seen throughout the experiment.

Figure 16 shows that the lowest chlorophyll concentrations were found at the highest light levels and the highest chlorophyll concentrations were seen at the lowest light levels. This change occurred within two days of the cells being subjected to the maximum light limitation possible and appears to be leveling off by day 31 with the exception of the chlorophyll a which was still increasing.

Visually, the *Nitzschia* cultures appeared more stressed when under fluctuating light conditions versus diurnal conditions. Under fluctuating light conditions the populations would be extremely clumped at the bottom of the culture tube.

Cell volumes were also measured, and were used to calculate chlorophyll per biomass. Overall no definite trends in cell size were observed with respect to light regimes as seen in Figures 17 and 18.

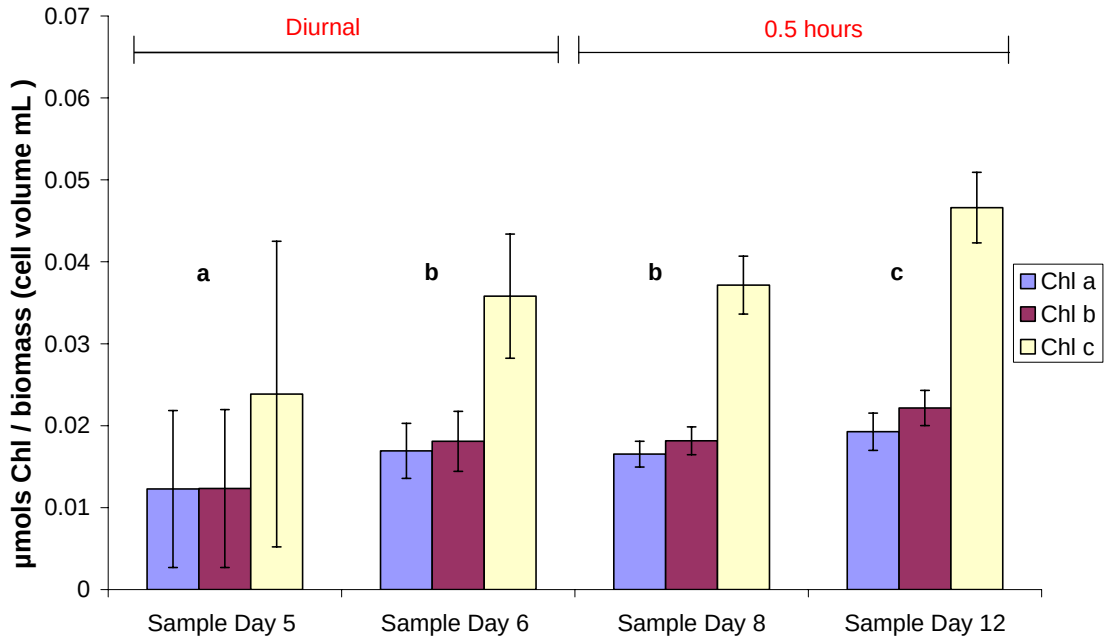

Figure 11: The effects of light cycles on chlorophyll levels over four sample days for *Chlamydomonas reinhardtii* exposed to a diurnal light cycle followed by a half hour blind cycle.

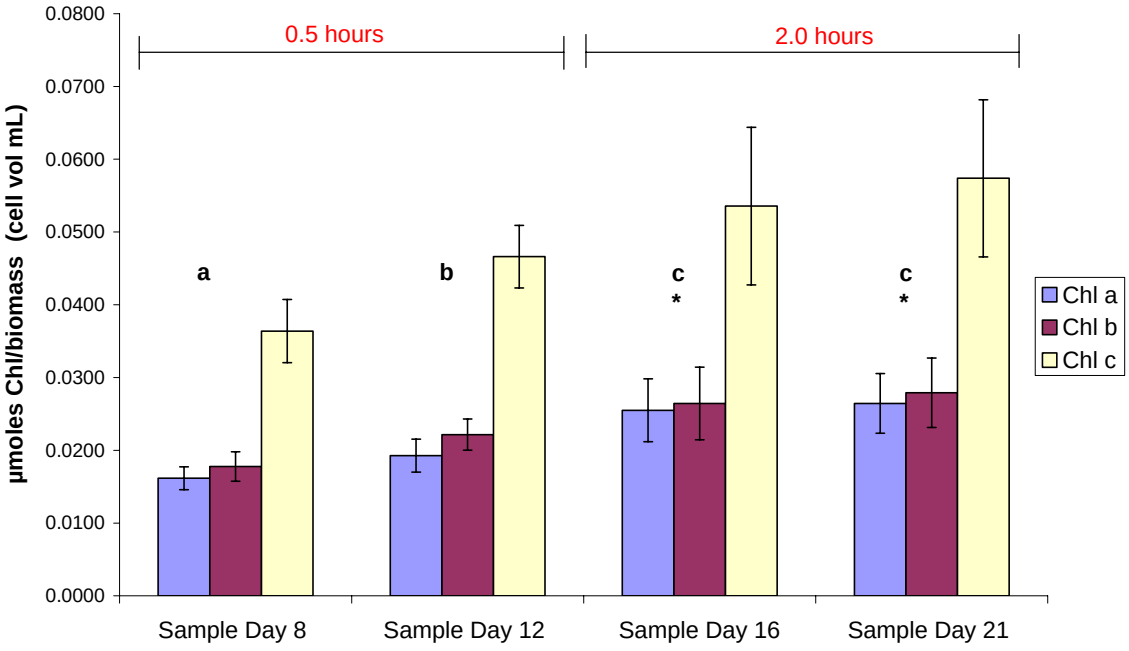

Figure 12: The effects of light cycles on chlorophyll levels over four sample days for *Chlamydomonas reinhardtii* exposed to a half hour blind cycle followed by a two hour blind cycle.
* While chlorophyll concentrations for chlorophyll a and b were similar for days 16 and 21, chlorophyll c was not ($P<0.60$).

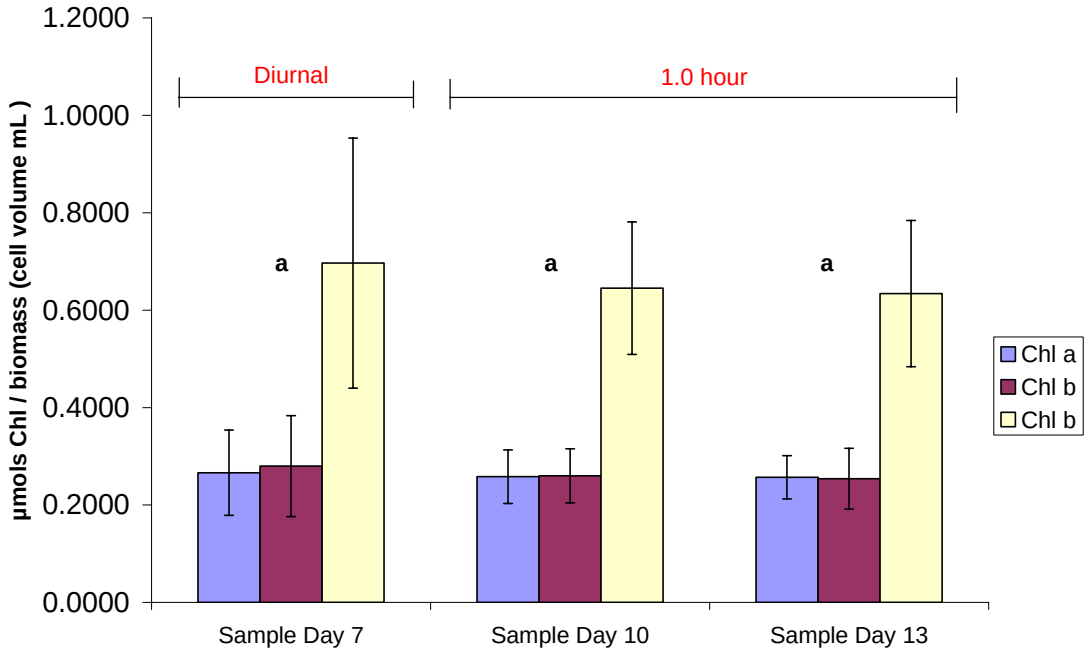

Figure 13: The effects of light cycles on chlorophyll levels over three sample days for *Nitzschia* sp. exposed to a diurnal light cycle followed by a one hour blind cycle.

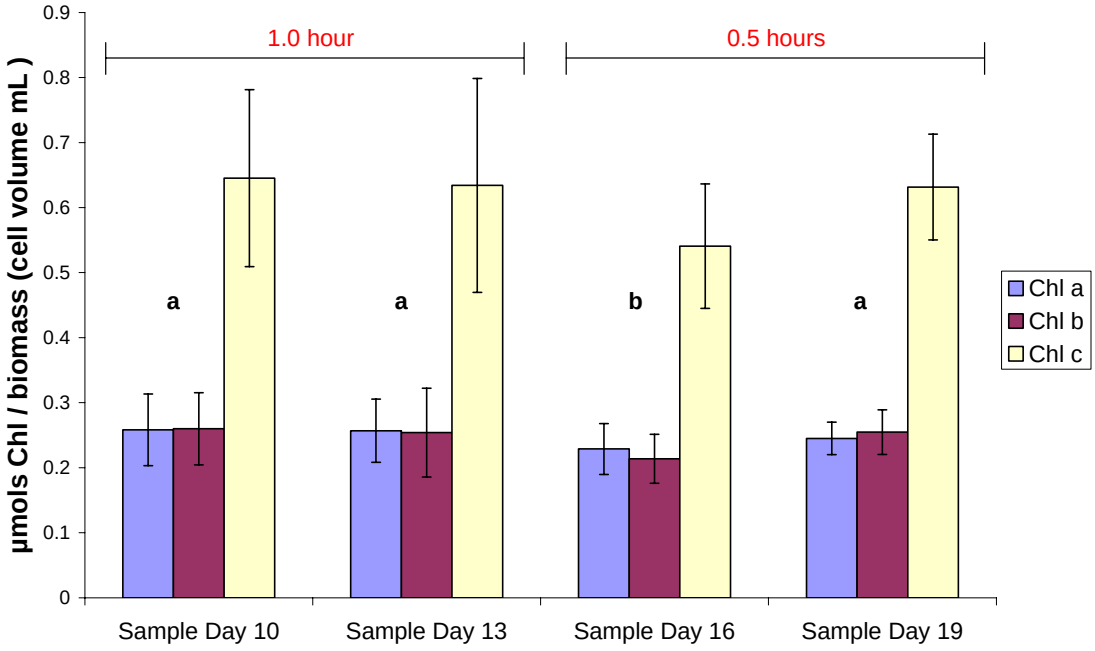

Figure 14: The effects of light cycles on chlorophyll levels over four sample days for *Nitzschia* sp. exposed to a one hour blind cycle followed by a half hour blind cycle.

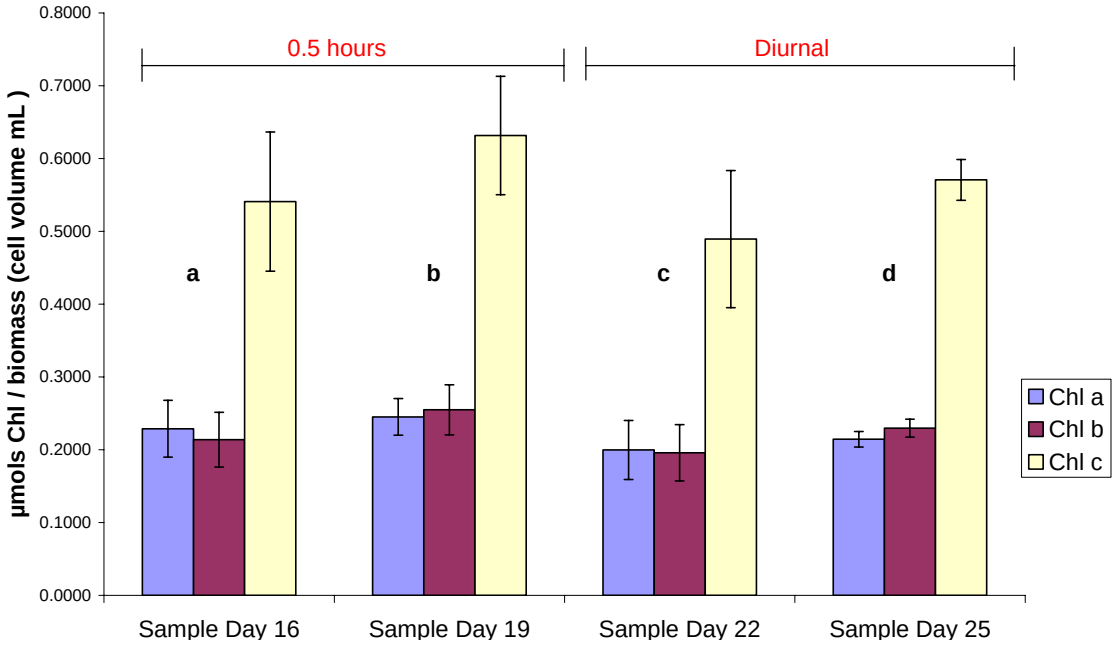

Figure 15: The effects of light cycles on chlorophyll levels over four sample days for *Nitzschia* sp. exposed to a half hour blind cycle followed by a diurnal light cycle.

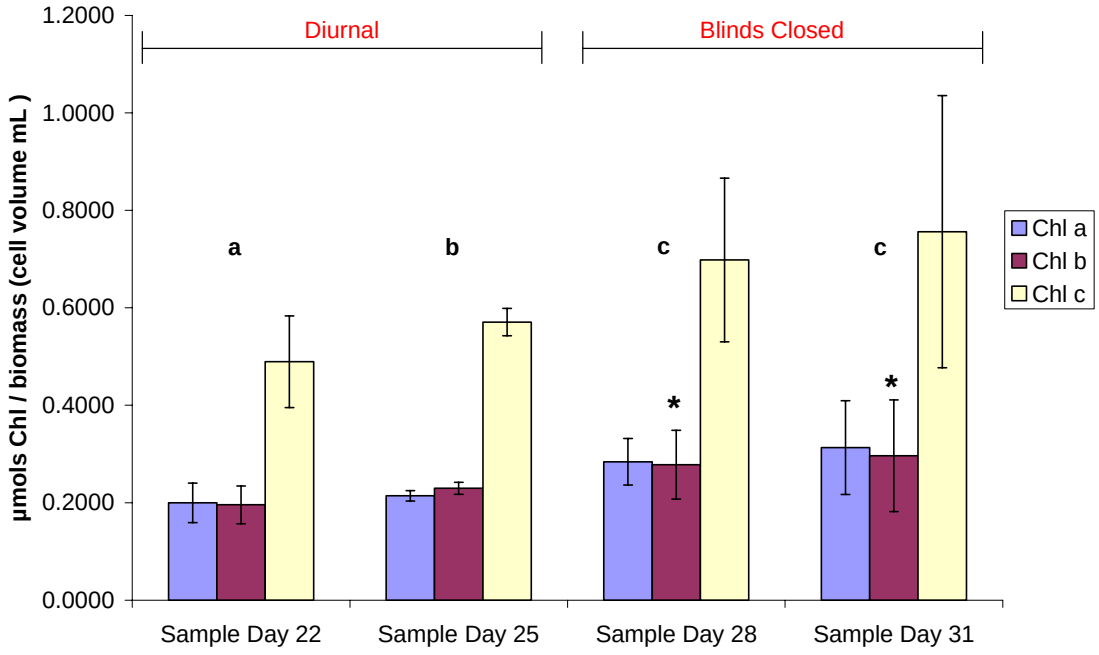

Figure 16: The effects of light cycles on chlorophyll levels over four sample days for *Nitzschia* sp. exposed to a diurnal light cycle followed by a period with the blinds closed. * While chlorophyll concentrations for chlorophyll b and c were similar for days 28 and 31, chlorophyll a was not.

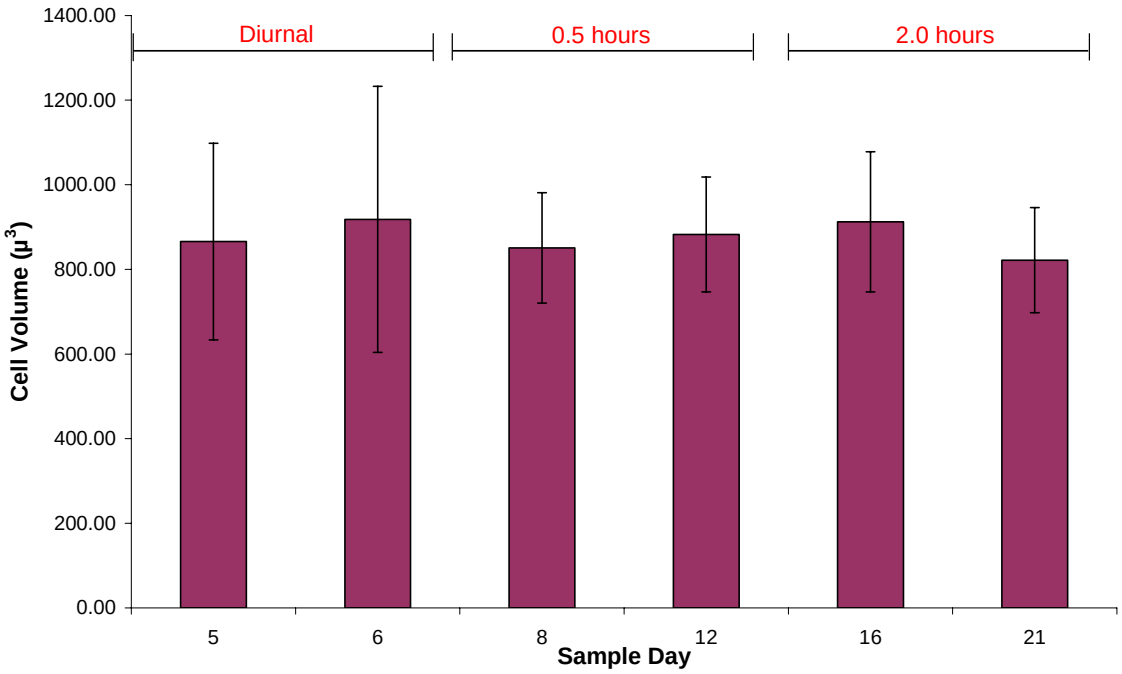

Figure 17: Average cell volumes of *Chlamydomonas reinhardtii* from six sample days over a 22 day semi-continuous culture run experiencing varying light levels.

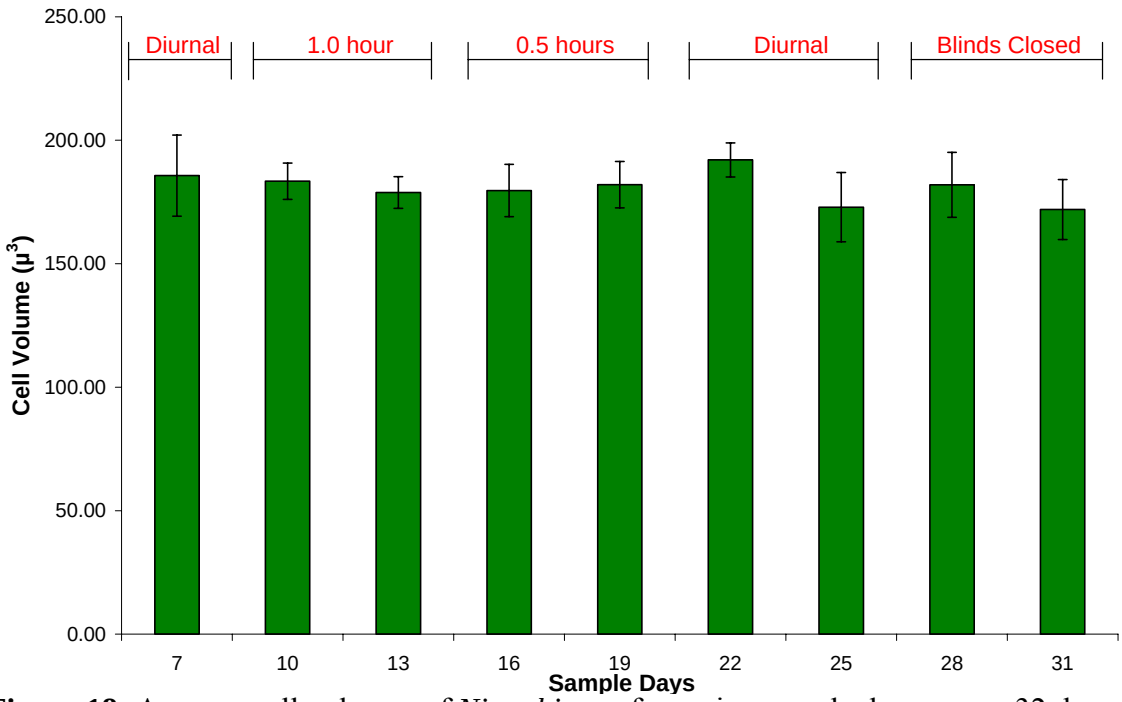

Figure 18: Average cell volumes of *Nitzschia* sp. from nine sample days over a 32 day semi-continuous culture run experiencing varying light levels.

DISCUSSION

The results show that generally the steady state cell densities in both species decreased while the chlorophyll concentrations increased as an effect of light fluctuation. The periodicity of these light fluctuations does not appear to be as important as the total amount of light received daily. This relationship was demonstrated by the unchanging steady state cell density of *C. reinhardtii* from the half hour blind cycle to the two hour blind cycle.

Based on these results, in turbulent environments where light becomes the limiting factor for growth, phytoplankton would invest more energy in chlorophyll production at the expense of growth rate. This chlorophyll-growth response was seen in both of the test species; however *C. reinhardtii* showed this trend to a greater degree than Nitzschia sp. This phenomenon of chlorophyll increase and decreased growth in numbers may have been obscured in the *Nitzschia* sp. because the *Nitzschia* sp. cell count data was much more variable. The variation in the *Nitzschia* sp. cell count data is likely due to the presence of clumps of cells encountered during enumeration. Such clumping of diatoms under duress has been reported by Reynolds (1994).

It is also possible that different taxonomic groups differ in their ability to alter their photosynthetic pigments (Richardson *et al*., 1983). Richardson *et al*., (1983) show that, different phytoplankton have a genetically controlled range of photon flux densities to which they are best adapted. Richardson *et al*., (1983) also gave many examples of phytoplankton that react to changes in photon flux by increasing chlorophyll concentrations. However, the magnitude of these photosynthetic pigment changes appeared to differ between different groups of algae (green algae, diatoms, and

dinoflagellates). Richardson *et al*., (1983) also showed that the largest shifts in photosynthetic pigment concentrations are seen in Chlorophyceaen algae such as *Chlamydomonas*.

Other studies have also noted this chlorophyll-growth response pattern. Quéguiner and Legendre (1986) studied the effects of high frequency light fluctuations on growth of the green alga *Dunaliella tertiolecta.* During two light fluctuation trials they showed that growth rate in numbers became decreased and chlorophyll increased as compared to steady illumination. They hypothesized that light fluctuations most likely control phytoplankton primary production in the natural environment. Ibelings *et al*., (1994) have also reported a decrease in growth rate in the green algae *Scenedesmus protuberans* and cyanobacterium *Microcystis aeroginosa* under a fluctuating light regime.

A different result was found by Flameling and Kromkamp (1997) who also grew *S. protuberans* in a fluctuating light pattern that simulated four to eight overturn cycles per day. They showed that while the number of photosynthetic units increased, the cellular chlorophyll content and photosynthetic unit size decreased. While the *S. protuberans* did not increase their chlorophyll concentrations as seen in the other studies, it did increase its maximum photosynthetic capacity as measured by photosynthetic oxygen evolution. This might suggest that in some species of algae, chlorophyll content alone may not be an adequate estimator of photosynthetic capacity.

Litchman (2000) examined the growth of the green algae *Sphaerocystis schroeteri* and cyanobacterium *Phormidium luripum*. These species showed a response similar to *C. reinhardtii* in my experiment, which had a decreased growth rate in numbers during exposure to fluctuating light levels compared to the initial diurnal treatment.

It is also quite likely that the photoadaptive abilities of certain groups of algae may be more complex. In this study when *Nitzschia* sp. was moved from a diurnal light cycle to a more light-limited one hour on, one hour off fluctuating cycle, its average steady state cell density continued to increase. Litchman (2000) also demonstrated that the rate of growth in numbers of *Nitzschia* sp. increased under low fluctuating light. Post *et al*., (1984) have suggested that diatoms may be able to rapidly increase their population growth rate when exposed to high light. This ability might account for the rapid *Nitzschia* sp. average steady state cell density increase, seen in my study, after day 21 when the blinds were opened and cultures returned to a diurnal light regime. The average steady state cell density of this second diurnal treatment (days 21 - 26) was significantly higher ($P = 0.002$) than the average steady state cell density achieved in the first diurnal cycle (days 2 to 6). As well, during this second diurnal cycle these *Nitzschia* sp. cultures showed a decline in chlorophyll concentration. It is possible that the *Nitzschia* sp. cells had been "conditioned" by the exposure to the fluctuating light levels (days 6 through 20). These "conditioned" *Nitzschia* sp. cultures were now apparently able to quickly shift energy into cell division and divert it away from chlorophyll synthesis, while the "unconditioned" cells in the first diurnal treatment were not. This "conditioning" may have two possible explanations. First, it is possible that the exposure to the fluctuating light may have induced a phenotypic plasticity in these cells, which provides them with an increased photosynthetic efficiency. Alternately it is possible that over the 14 days of fluctuating light exposure, the genetic makeup of these populations has changed. Given an average doubling time of approximately 30 to 35 hours during the 14 days of exposure to fluctuating light, this would translate into approximately 12 to 15 generations. While

this number of generations is small, it is possible that there was selection for a more photosynthetically efficient genotype. It would be interesting to compare the growth responses of these "conditioned" cells to those of the original stock culture.

There are likely other factors related to turbulence occurring in nature that could influence the growth and survival of river phytoplankton. Many other studies, both *in situ* and *in vitro*, have shown an increased growth rate with increasing light fluctuation compared to steady illumination (Marra, 1978b; Richardson *et al*., 1983; Ibelings *et* al., 1994; and Gocke and Lenz, 2004). These studies suggest this result is due to photoinhibition. They reason that the increase in growth rates and chlorophyll concentration when light begins to fluctuate are due to a reduction in the photo-oxidation of chlorophyll at extremely high light intensities (Richardson *et al*., 1983). As algal cells are circulated into the upper few centimeters of surface waters, they can experience extremely high photon flux densities, particularly on bright, cloudless days. Richardson *et al*., (1983) suggest that this photo-oxidation can occur at many different light levels, depending on the species sensitivity to light. The lowest threshold that they give for photoinhibition to occur is approximately 200 μmol photons $m^{-2}s^{-1}$. In this experiment maximum light intensities did not reach this magnitude, and therefore the process of photoinhibition was not considered important. For very good reviews of this process the reader should consult Richardson *et al*., (1983), Anderson *et* al., (1998), and Baroli and Melis (1998) for further information.

Grobbelaar (1989 and 1994) used two different *in vitro* methods to demonstrate that turbulence in a mixed water system not only facilitates the fluctuation of light, but it also increases the transfer rates of nutrients between the medium and the organism.

These studies suggest that both an increase in the transfer rates of nutrients, and the fluctuation of light, are both involved in the increase in phytoplankton productivity. However, Grobbelaar (1989 and 1994) reports that the majority of the increased productivity is due to the increase in nutrient exchange and not the light/dark frequencies. As cells are rapidly transported through the water column by turbulent mixing, the concentration gradients of nutrients due to absorption at the cell surface are greatly reduced. This should facilitate an increase in nutrient uptake rates and an increase in productivity. Grobbelaar (1994) created a situation where the rate of movement of the cells through the medium was variable and he was able to investigate the effect of varying nutrient diffusion rates on algal growth. In my experiments the cultures were aerated at a constant rate, and therefore cells circulated within the culture vessels at a constant rate. It is reasonable to assume that the cells were experiencing the same nutrient concentration gradients and would have therefore experienced similar nutrient uptake rates during all light treatments. It is not likely that turbulence induced nutrient uptake rates were a factor in my results.

There have been many different approaches to the experimental manipulation of light levels in both the field and in the lab. As stated previously the Venetian blind apparatus used in this study was inspired by the work of Kroon *et al*. (1992). Their apparatus was much larger than the two apparatuses designed and constructed by the University of Winnipeg Technical Services. Kroon *et al*. (1992) used an IBM personal computer to control a stepper motor to open and close their Venetian blinds in 100 small steps creating a photon flux density that resembled a sine curve. While the apparatus described here was not able to create a smooth curve of light transmittance, it is

significantly smaller as it is only a metal box mounted on the arm of the Venetian blinds. The apparatus used here was found to be very beneficial when space is limited. It is felt that the apparatus used in my study provides a good *in vitro* model for light limitation when used with semi-continuous cultures of phytoplankton. This system should prove beneficial in investigating the effects of light fluctuations on a host of other aquatic population and community level processes.

Several studies have investigated the effects of vertical mixing on light attenuation with respect to phytoplankton growth in the field (eg: Marra, 1978b; Gervais *et al*., 1997; Köhler, 1997; Diehl *et al*., 2002; and Köhler *et al*., 2002). Diehl *et al*., (2002) used cylindrical plastic bags at varying depths suspended from a raft in a lake to simulate experimental gradients that occurred with depth in the water column. Marra (1978b) suspended bottled phytoplankton samples at varying depths from a barge in a marine inlet, while Gervais *et al*., (1997) used a bottle lift to raise and lower their bottles in linear and circular motions in a lake. Köhler (1997) and Köhler *et al*., (2002) also used a lift to raise and lower dialysis chambers in a river. These *in situ* methods have proven useful in factoring in other variables affected by turbulence such as turbidity and algal sedimentation. The drawback of these field experiments is that, as with any ecosystem, the biotic and abiotic interactions are so complex, it is difficult to isolate the important variables that affect the phytoplankton and it is therefore difficult to draw conclusions concerning the effects seen. Köhler (1997) states that there is so little understanding of depth and velocity of mixing in water bodies, due to the complexities of temperature and wind velocities, that it is difficult to accurately model these systems. It could be useful for future studies to incorporate both lab and field work in a comparison approach to

study the effects of turbulence on phytoplankton. Such a study could use the Venetian blind apparatus to create a light gradient patterned on light measurements taken in the field. Lab cultures could then be compared with field cultures that are raised and lowered through different depths, which also varies light intensities. Comparison of the results using the two methods would allow identification of other variables such as temperature and nutrient gradients, cloud cover and turbidity that might be important in community productivity.

Regarding the three unanswered questions posed earlier, namely: "How species maintain numbers in rivers when they are constantly being flushed out of the system? How numerous species of phytoplankton can coexist? And what processes cause community changes in the dominant phytoplankton composition throughout the year?" it is my opinion that light fluctuations caused by turbulence in rivers are an important part in answering these questions and should be further investigated. Flöder *et al*., (2002) suggest that, "light fluctuations may impede competitive exclusion and sustain a higher diversity of phytoplankton species," which could be due to the fact that more niches are created by disturbance (Elliot *et al*., 2001). Litchman (1998) and Flöder and Burns (2005) found that fluctuating light maintained diverse communities of phytoplankton as compared to steady illumination which resulted in competitive exclusion by a few species. Light fluctuations apparently lead to environmental heterogeneity, which helps support significant biodiversity. The implications of these findings to river management practices suggest that changes in water flow could impact phytoplankton species composition by altering the conditions of competition. Changes in phytoplankton communities could resonate up the entire food web affecting the entire ecosystem.

Before any new management practices are imposed, researchers should consider the effects on phytoplankton species composition. Management practices should strive to mimic the natural conditions in the river as closely as possible to reduce any potential impact. The run-of-the-river reservoir system seen in the Winnipeg River may be a good example of this, since the control structures here do not create large stagnant reservoirs and little variation is seen in the phytoplankton populations above and below them (Simmons, personal communication).

Turbulent mixing may also be a possible solution for stagnating waters that have become eutrophic and experience toxic cyanobacterial blooms. Artificial mixing may be a possible management procedure that could be useful in creating more niches, so that other beneficial, competing phytoplankton species could be established. More study is required in this area though.

The method that has been described here was a good preliminary study of river phytoplankton ecology and the effects of fluctuating light on algal growth and chlorophyll production. This method has been useful for describing trends that occurred to phytoplankton steady state population numbers and chlorophyll concentrations as an effect of fluctuating light. However, this method has lacked statistical power to find significant effects from these light changes. For future studies, it is suggested that researchers run two concurrent experiments with the same phytoplankton in the same room. One run should be a control run which would experience diurnal light, while the other run would be an experimental run which would experience fluctuating light. To test for statistically significant differences between the control and experimental runs the researcher could use the Welch's t-test or a computer intensive Randomization technique

to assess environmental impacts in a BACI design (before-after, control-impact) as described in Krebs (1999). Preliminary results using this new method and statistical test have indicated significant differences between diurnal control groups and light fluctuating experimental groups in *Nitzschia* sp. (Kent Simmons, personal communication). Future research using these methods should yield more definitive results and contribute greatly to the knowledge of river phytoplankton ecology.

CONCLUSIONS

1) A chlorophyll-growth response was seen in both species. Steady state cell densities decreased while chlorophyll concentrations increased as an effect of light fluctuation which mimicked vertical mixing caused by turbulence.
2) Cell volume was not affected by the amount of light radiation received by a cell.
3) The total daily amount of light energy was more critical than the pattern in which it was received for *C. reinhardtii*.
4) The 14 days of light fluctuation "conditioned" the *Nitzschia* sp. cells to be able to quickly shift energy into cell division and divert it away from chlorophyll synthesis. This "conditioning" may have been the result of a phenotypic plasticity induced in the cells or a change in the genetic makeup in the population.
5) The apparatus used in this study provided a good *in vitro* model for light limitation when used with semi-continuous cultures of phytoplankton. This system is beneficial in investigating the effects of light fluctuations on a host of other aquatic population and community level processes.

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APPENDIX I: WC' Medium

Formula for WC' algal growth medium originally formulated as WC medium by Guillard and Lorenzen (1972). Modified to create a phosphate limited medium. 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	1.4	
	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.

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

‡A concentration stock solution of buffer should be prepared by adding 17.92 g of bicine to 1L of distilled water, then adjusting the pH of the stock solution to 7.0. Add 10 mL of stock solution to 1L 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 + .05 mL solution B + 99.0 mL of distilled water

Add 1.0 mL of solution C to 1 L of medium

APPENDIX II

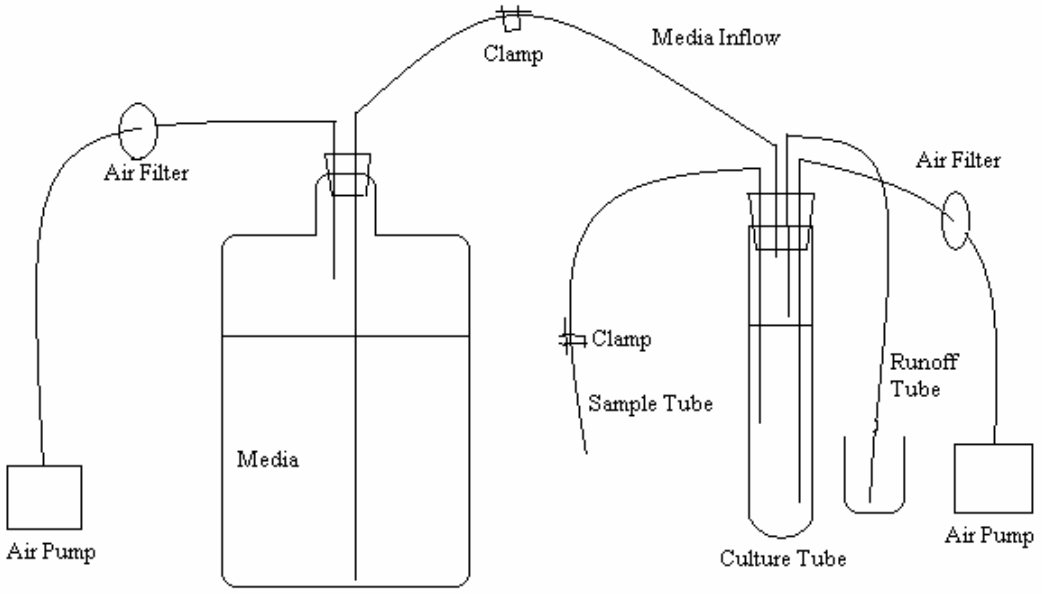

Figure 19: Diagram of one semi-continuous culture set-up illustrating all materials used and their connections.

Figure 20: Picture of the *Nitzschia* sp. chamber showing light banks 1, 2, and 3 (from bottom to top, 1 and 2 illuminated) as well as two carboys of media feeding the six semi-continuous culture tubes. Air lines and media inflow tubes were suspended above the culture tubes by wires.

APPENDIX III

Table 3: The sequence of light banks turning on and off in the *Nitzschia* sp. chamber over one 24 hour period with the total amount of photons the culture tubes experienced.

	Hours					
	0:00 - 6:00 22:00 - 24:00	**6:00 - 7:00 21:00 - 22:00**	**7:00 - 9:00 19:00 - 21:00**	**9:00 - 11:00 17:00 - 19:00**	**11:00 - 13:00 15:00 - 17:00**	**13:00 - 15:00**
Light Bank 1	OFF	OFF	ON	ON	ON	ON
Light Bank 2	OFF	OFF	OFF	OFF	ON	ON
Light Bank 3	OFF	ON	OFF	ON	OFF	ON
Total Photons ($\mu mol\ m^{-2}s^{-1}$)	0	30	60	100	140	180

Table 4: The sequence of light banks turning on and off in the *Chlamydomonas reinhardtii* chamber over one 24 hour period with the total amount of photons the culture tubes experienced.

	Hours				
	0:00 - 6:00 22:00 - 24:00	**6:00 - 8:00 20:00 - 22:00**	**8:00 - 10:00 18:00 - 20:00**	**10:00 - 12:00 16:00 - 18:00**	**12:00 - 16:00**
Light Bank 1	OFF	ON	ON	ON	ON
Light Bank 2	OFF	OFF	ON	ON	ON
Light Bank 3	OFF	OFF	OFF	ON	ON
Light Bank 4	OFF	OFF	OFF	OFF	ON
Total Photons ($\mu mol\ m^{-2}s^{-1}$)	0	30	85	125	180

APPENDIX IV

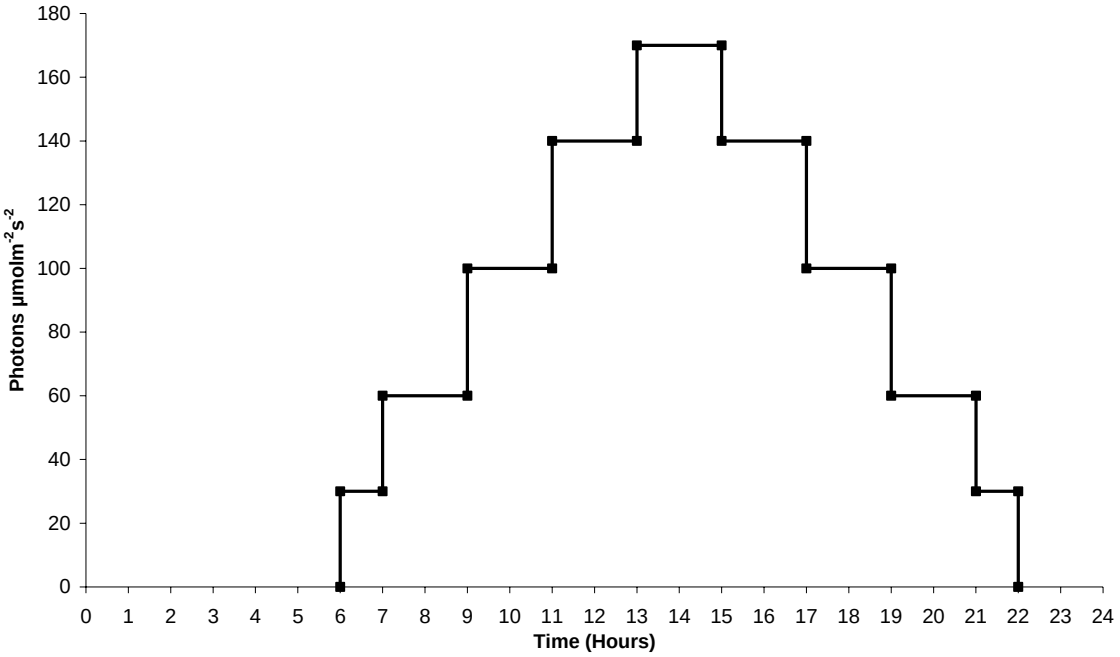

Figure 21: The total photons of light that were experienced by the *Nitzschia* sp. cultures on a diurnal light regime for one day. 16 hours of light accompanied by 8 hours of darkness.

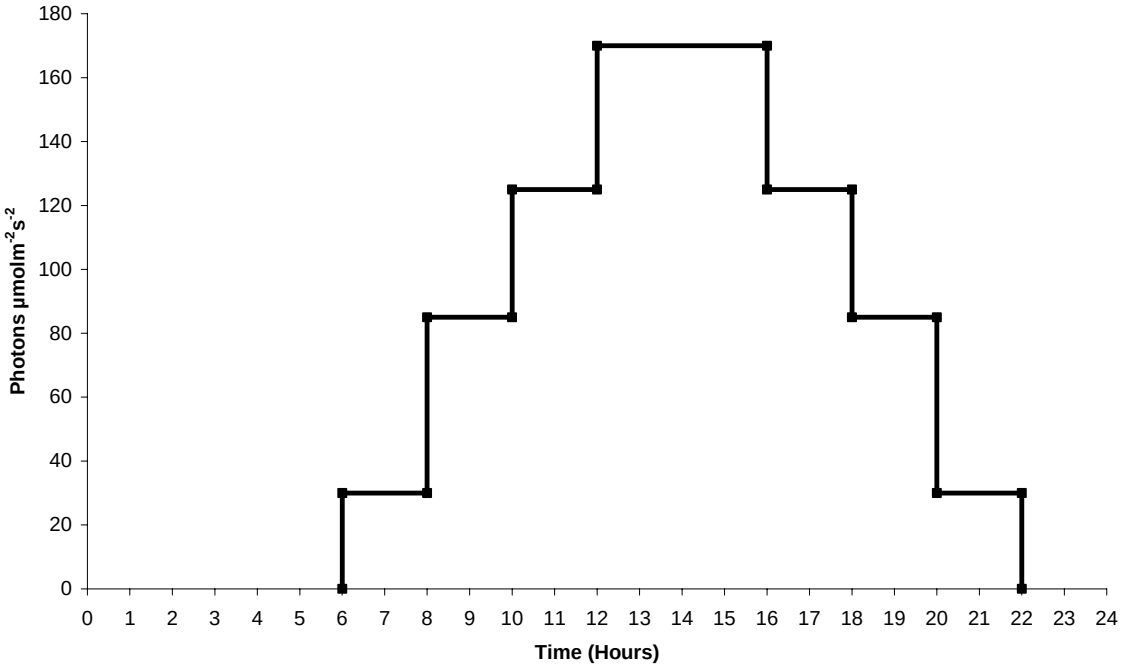

Figure 22: The total photons of light that were experienced by the *Chlamydomonas reinhardtii* cultures on a diurnal light regime for one day. 16 hours of light accompanied by 8 hours of darkness.

APPENDIX V

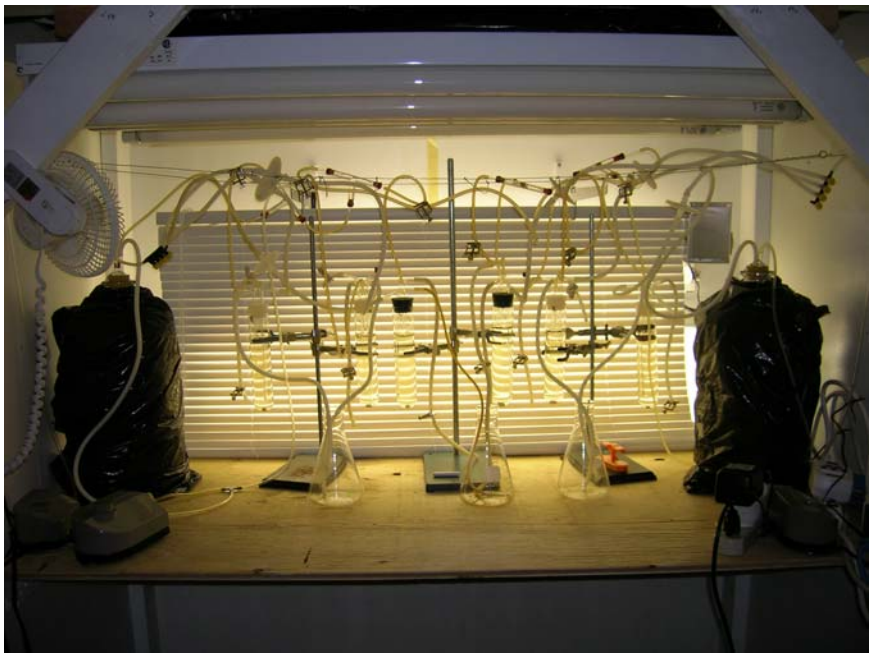

Figure 23: Picture of the *Nitzschia* sp. chamber as previously seen in Figure 20 with blind apparatus now installed between semi-continuous cultures and light banks. The metal box at the top right side of the blinds is the control bow for the apparatus.

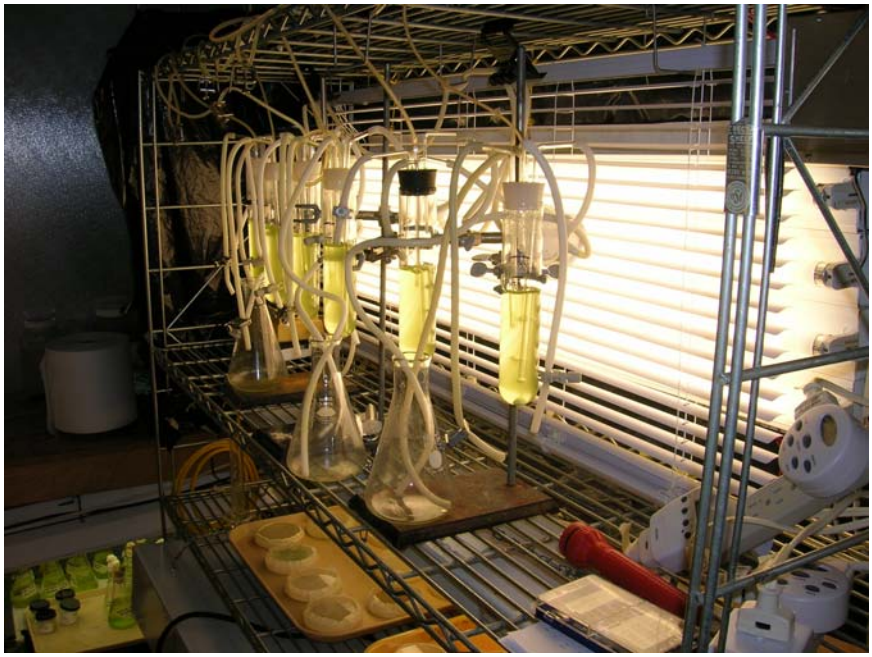

Figure 24: Picture of the *Chlamydomonas reinhardtii* chamber with the blind apparatus currently open allowing light through to the semi-continuous culture tubes.

APPENDIX VI

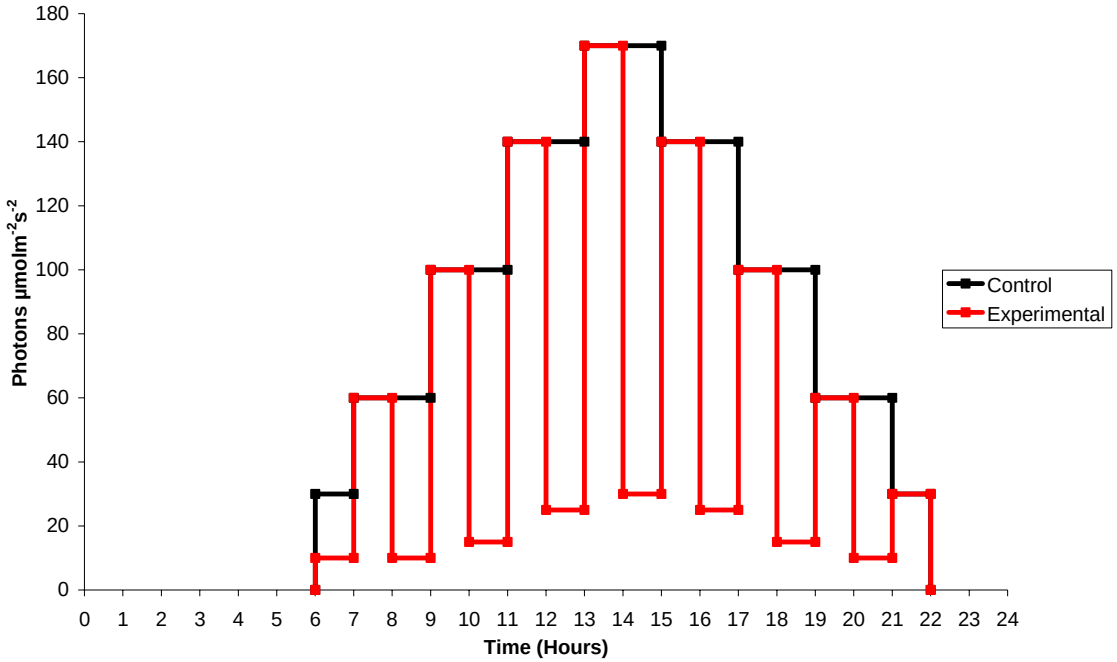

Figure 25: The total photons of light that will be experienced by the *Nitzschia* sp. cultures on an experimental light regime with a blind cycle of 1.0 hours for one day superimposed overtop the normal diurnal light cycle.

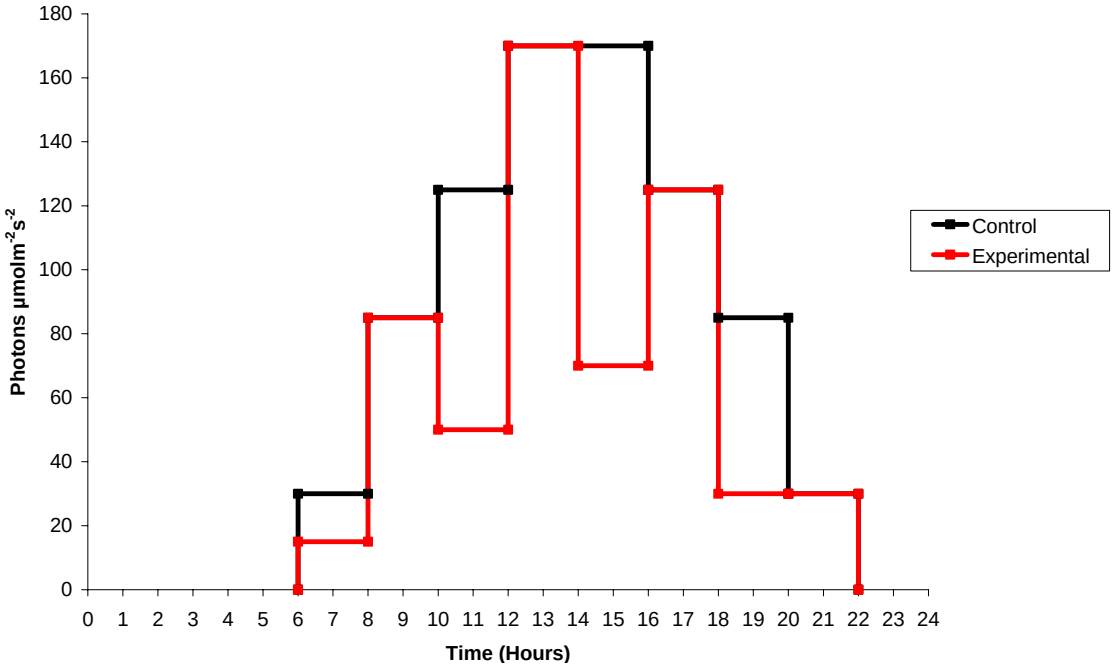

Figure 26: The total photons of light that will be experienced by the *Chlamydomonas reinhartii* cultures on an experimental light regime with a blind cycle of 2.0 hours for one day superimposed overtop the normal diurnal light cycle.

APPENDIX VII

Figure 27: Example picture of *Nitzschia* sp. viewed at 1000x with measurements for cell volume as described previously.

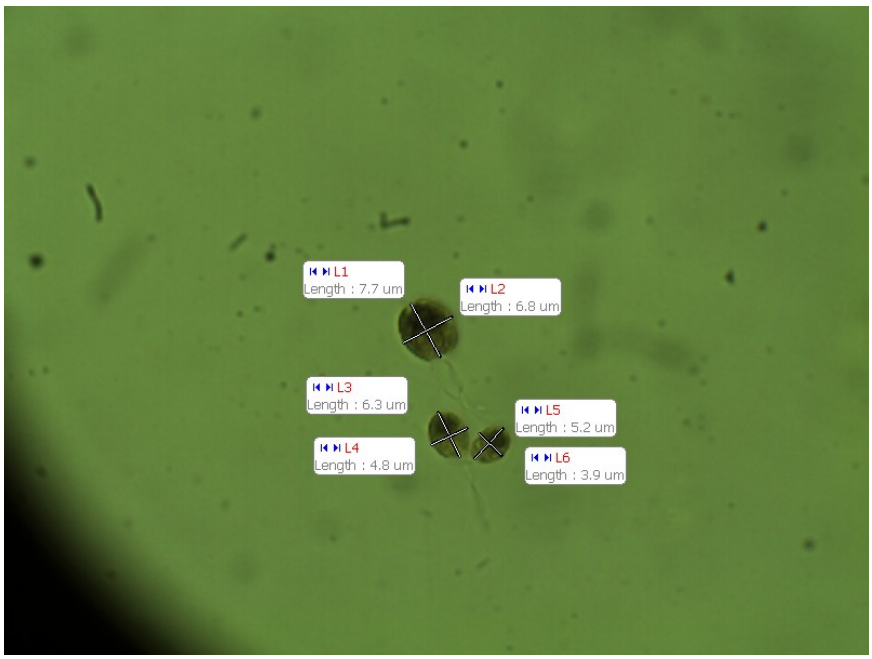

Figure 28: Example picture of *Chlamydomonas reinhardtii* viewed at 1000x with measurements for cell volume as described previously.