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THE EFFECT OF CONSTANT RELATIVE GROWTH RATE VERSUS CONSTANT

SPECIFIC GROWTH RATE ON *OOCYSTIS* SP

by

Rachel Kriner

A Thesis
Submitted to the Graduate School,
the College of Arts and Sciences
and the School of Ocean Science and Engineering
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

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ABSTRACT

In this study, a strain of *Oocystis* sp. (S002) was grown using two different growth strategies at three different temperatures (15°C, 20°C, 25°C). One strategy (GS1) kept a constant specific growth rate (μ) and the other (GS2) kept a constant relative growth rate (μ/μ_{max}). In both strategies, the cultures were grown following the protocols of a hybrid growth system. The Dilution Phase is an initial semi-continuous growth phase that involves daily dilutions until set growth criteria are met. Followed by a batch culture growth protocol where the cultures were diluted at the onset of the first day and left to grow for two days (Harvest Phase). Six replicates were grown with samples taken at Dilution and Harvest Phase to study any effects on the physiology of S002. In order to compare the biomass composition, the following analyses were done: CN content, the extracted chl *a* concentration, lipid concentration using Nile Red stain, and the ash free dry weight. The results of this study indicated that the cultures grown most likely did not undergo nutrient stress except during GS1 at 20°C. Given the results under these conditions, neither GS1 nor GS2 is better than the other at keeping the biomass composition steady across the temperatures or for increasing the biomass and lipid during Harvest Phase of 25°C. However, given the results of this study, GS1 is as effective as GS2 when used in the optimal temperature range of the algal species used for experiments under these conditions.

ACKNOWLEDGMENTS

DEDICATION

For all who supported me, and those who want to expand their knowledge.

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LIST OF ABBREVIATIONS

USM	The University of Southern Mississippi
MAGIC	The Marine Algae Industrialization Consortium
ASP	The Aquatic Species Program
PBR	Photobioreactor
S002	Oocystis sp.
AFDW	Ash Free Dry Weight
chl a	Chlorophyll a
GS1	Growth Strategy 1
GS2	Growth Strategy 2
DMS	Department of Marine Science
MAD	Median Absolute Deviation
GF/F	Glass Fiber Filter
SOP	Standard Operating Procedure
DMSO	Dimethyl sulfoxide
CDLC	Chemically Defined Lipid Concentrate
POC	Particulate Organic Carbon
PN	Particulate Organic Nitrogen

CHAPTER I - INTRODUCTION

The idea that microalgae can be used for fuel has been around since before the

1970's (Sheehan et al., 1998). In 1978, the United States created the Aquatic Species

Program (ASP) in order to research the development of diesel fuel from microalgae

(Sheehan et al., 1998). During its funding period of almost twenty years, there was a

considerable increase in the knowledge of microalgal outdoor production, microalgae

physiology, a database of algae species, and the resources needed for mass production

(Sheehan, et al., 1998). After funding of the ASP ceased, the research in biodiesel

production declined until around 2006. Research on microalgal biofuel production to

reduce the use of fossil fuels then started increasing due to a push towards a reduction in

CO_2 production and utilizing biofuels as a renewable energy source. Additionally,

scientists began to search for better and more efficient biofuels for mass production

(Chen et al., 2015; Huntley and Redalje, 2006). Algae can grow quickly and produce a

high concentration of lipids that can be used to make biodiesel fuel (Sharma et al., 2012;

Jiang et al., 2012). The lipids produced by algae can easily be used for biodiesel

production and algae remove CO_2 from the atmosphere, making production of algal

biofuels an attractive option to consider (Huntley and Redalje, 2006; Sarkar and Shimizu,

2015; Sharma et al., 2012). However, the cost of production was too high to produce

biofuels at a cost that was feasible and comparable to that of fossil fuels (Chen et al.,

2015; Rogers et al., 2014). More research was needed in order to make the production of

biofuels more cost effective and one aspect of that is better and more consistent

production throughout the year.

Algal physiology is important to understanding why algae grow the way they do. Under changing environmental conditions, the physiology of a cell can acclimate to grow more efficiently (Shuter, 1979). The physiology of algae is very important when considering the growth and the production of algal biomass (Chen et al., 2015; Sarkar and Shimizu, 2015). To get consistent results, the growth conditions should remain constant throughout the growth cycle (Sarkar and Shimizu, 2015). In balanced growth of a culture, the lipid and protein content should increase throughout the life cycle until it reaches an optimal amount (Shuter, 1979). However, changes in the environment can increase or decrease the proportions of lipids and proteins in a cell during its life cycle. Stressed culture growth can result in more lipid synthesis in the alga, which is a helpful application in algal biofuel research (Sharma et al., 2012). There are many ways to induce stress - the main method is to simulate environmental stressors such as nutrient depletion, temperature changes, pH changes, and irradiance changes (Sharma et al., 2012; Jiang et al., 2012). Once the physiology of a species of microalga is understood, then modifying the growth conditions to optimize the lipid yield is a possibility (Shuter, 1979; Sharma et al., 2012).

There are three ways of growing algae cultures: batch growth, semi-continuous growth, and continuous growth (MacIntyre and Cullen, 2005). Batch growth is when there is a set amount of medium, which will be used to grow an alga and no new medium will be added; instead the nutrients will deplete as the culture grows. Semi-continuous growth is when the medium is diluted on a regular schedule. This prevents the medium from becoming nutrient limited, and the cell concentration from getting so high the cells start self-shading (MacIntyre and Cullen, 2005). It will also result in the culture growing

at a constant average daily specific growth rate given consistent daily dilution. In a continuous culture, fresh medium is pumped into a constant volume growth chamber at a set rate. That set dilution rate will determine the specific growth rate of the algae in the continuous culture system. As fresh medium is pumped into the growth chamber, spent medium and cells flow out of the growth chamber at the same rate that the new medium is introduced (MacIntyre and Cullen, 2005).

There are two main ways to grow algae commercially: closed photobioreactors (PBRs) and open raceway ponds (Sarkar and Shimizu, 2015; Li et al., 2015; Chen et al., 2015; Hannon et al., 2010). PBRs provide growth in a very highly controlled environment which allows the culture to be grown under ideal growth conditions for whichever species is used (Sarkar and Shimizu, 2015; Li et al., 2015; Chen et al., 2015; Hannon et al., 2010). PBRs can maintain optimal growth conditions, which can lead to higher biomass production and greater culture density and self-shading if the culture is not sufficiently mixed (Sarkar and Shimizu, 2015; Chen et al., 2015). Any of the three ways to grow algae can be used in a PBR, however semi-continuous and continuous are the most commonly used (Sarkar and Shimizu, 2015; Li et al., 2015; Hannon et al., 2010). The upkeep for these can be expensive and can lead to them being ineffective as an option for a more affordable renewable fuel source if the harvest does not lead to an overall profit. Open raceway ponds are open to the elements and utilize natural lighting and thus require no artificial light to be used, making them a less expensive option than the PBR (Sarkar and Shimizu, 2015; Rogers et al., 20144; Li et al., 2015; Chen et al., 2015; Hannon et al., 2010). Open raceway ponds are typically used for semi-continuous growth and due to the nature of the system, do not have very controlled growth

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conditions (Sarkar and Shimizu, 2015; Rogers et al., 20144; Li et al., 2015; Chen et al., 2015; Hannon et al., 2010; Laws et al., 1983). Being open to the environment also leads to greater water loss due to evaporation and can be an additional cost to overcome in a system which runs at a continuous harvest (Rogers et al., 2014; Sarkar and Shimizu, 2015; Chen et al., 2015). There can be airborne or waterborne contamination due to protozoa introduced into the system, which can lead to decreased output unless the algae is able to outcompete the invasive species (Sarkar and Shimizu, 2015; Hannon et al., 2010). Open raceway ponds typically have a lower culture density and biomass productivity than PBRs (Li et al., 2015; Chen et al., 2015; Kumar et al., 2015). A greater understanding of the growth physiology of microalgae can lead to changes in these systems to bolster the harvest, such as adding in turbulence in the open raceway pond to increase the light availability to all cells and increasing growth (Laws et al., 1983). While both of these systems have their drawbacks, using the PBR and open raceway pond together can increase the overall harvest and reduce some of the problems associated with each system.

The Marine Algae Industrialization Consortium (MAGIC) at the Duke Lab in Beaufort, North Carolina is using a hybrid system to grow their algae which utilizes both open ponds and closed PBRs and has the highest growth and lipid production (Huntley and Redalje, 2006; Huntley et al., 2015). Growing cultures in the PBR first allows a controlled growth environment that will accumulate a dense growth and prevent contamination (Borowitzka, 1999; Huntly and Redalje, 2006; Ugwu et al., 2008; Sakar and Shimizu, 2015). A portion of the culture is then transferred to an open raceway pond for stressing, lipid accumulation, and then harvesting (Huntly and Redalje, 2006; Ugwu

et al., 2008; Sakar and Shimizu, 2015). This strategy employs two different ways of growth, the first portion in the PBR using semi-continuous growth, and the second using batch growth. By inoculating the open raceway pond with dense culture from the PBR, the stress and harvest phases of algae take place in the elements for a short time and there is a smaller chance for water loss due to evaporation and for contamination to occur (Li et al., 2015; Huntley et al., 2015). There are two ways to operate the PBR in a system like this: use of a constant daily dilution over the natural seasonal variability in temperatures causing variable growth (GS1) or to employ a variable dilution to maintain a constant relative growth rate (GS2) over the seasonal variations in temperature. There are questions as to which method of operation is more effective for the growth of algae for biofuels that will also provide the most consistent quality of algal product over seasonally varying ambient temperatures.

This experiment focused on three major points. The physiological acclimation to growth under a constant dilution rate (GS1) at the temperature range expected for Beaufort, North Carolina over the seasons and growth at a constant relative (GS2) growth rate ($= \mu/\mu_{max}$, where μ is the specific growth rate and μ_{max} is the maximum specific growth rate expected for each growth temperature), how these changes affect the composition, and which is better for the growth of the algal culture.

Relative growth rate for microalgal cultures has not been studied in detail for the past several decades, and it is unknown whether relative growth rate or specific growth rate is more important for a semi-continuous culture. While specific growth rate is species specific, the relative growth rate allows the relative changes in cellular physiology to be normalized and therefore it is easier to compare differences in growth

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(Goldman, 1980; Goldman et al., 1979). The changes in the cellular physiology are influenced by the relative growth rate and are used in the Droop model which allows optimal allocation of nutrients to predict algae growth (Goldman, 1980; Pahlow and Oschlies, 2013). Many things affect growth rate, such as: light, nutrient availability, temperature, and the light: dark cycle (Geider et al., 1997). As an environment changes, each species will respond to those changes. When looking at the specific growth rate or at the maximum growth rate, it can be difficult to tell if the species has a similar response to the environmental changes. However, when using the relative growth rate it is possible to determine if the physiological response is the same (Goldman, 1980). In a paper by Laws et al. (1987), field sampling was conducted to study the plankton rate process in oligotrophic oceans that compared the absolute (specific) growth rate, relative growth rate, and vertically integrated photosynthetic rate as well as comparing these to historical values for the North Pacific Central Gyre. The values calculated were higher than the average historical values, but were similar to the greatest values and showed that while the oligotrophic ocean may be nutrient limited with low biomass as a result, it is not always so (Laws et al., 1987). A paper by Donaghay et al. (1978), discussed the discrepancies in the relationship between the specific growth rate and the N:C ratio between two studies of *Thalassiosira pseudonana* and how when the results are normalized to the maximum growth rate then the resulting relative growth rates of both studies are actually very similar. This shows how the relative growth rate can be used to compare responses to cellular changes.

This experiment will determine which growth strategy is better for mass culture. In order for a comparison between growth strategies that look at growth under a constant

dilution rate (GS1) and a strategy that grows the microalgae at a constant relative growth rate (GS2), it is important to determine what the maximum growth rates are for algae grown at the temperatures of interest in a study. In this manner, the results from both strategies could be compared and a best practice strategy could be developed based on expected temperatures for the location of an outdoor growth facility.

1.1 Approach for this study

After reviewing the results from prior studies in the literature there are questions about what might be the best growth strategy to employ in a large scale outdoor algal growth facility. Outdoor growth facilities will experience a seasonal range of temperatures and that variation in temperature will lead to varying specific growth rates and varying relative growth rates. In order to address this question, experiments have been designed to compare a growth strategy keeping a constant dilution rate over a range of environmental temperatures for Beaufort, North Carolina and thus variable culture specific growth rates (GS1) with a growth strategy that will involve varying the dilution rate to maintain a constant relative growth rate (GS2) over the course of seasonal temperature variations. The following hypotheses will be tested to determine which growth strategy will provide the best algal production for desired growth and composition.

1.2 Hypotheses

1: *Oocystis* sp. will have a more constant biomass composition under GS1 than

GS2 due to the constant dilution rate.

2: Lipid production will be significantly higher during the summer temperatures

than winter temperature.

s.   2. A: In both GS1 and GS2, the highest lipid production and lowest

protein production will be during Harvest Phase in the summer.

2. B: The added stress of GS1 will cause there to be higher lipid

production under GS1 than GS2.

3: The AFDW will be highest in the summer under the GS2 growth conditions.

CHAPTER II – METHODS

  All samples were grown from cultures obtained from the DMS Phytoplankton

Culture Lab. For all experiments, the algae were grown in a modified F-medium with

$1/10^{th}$ the trace metals, twice the sodium carbonate, and full vitamins for all the cultures;

all were grown under a 12:12 light and dark cycle at three different temperatures (15°,

20°, 25°C) (Guillard and Ryther, 1962). These temperatures were chosen based on the

seasonal growth temperature range of Beaufort, NC where the Duke Lab is located. Batch

culture was used for experiment 1 to determine the maximum growth rate of *Oocystis* sp.

(S002) at each growth temperature. For experiment 2a (GS1) and 2b (GS2), semi-

continuous culture growth was used with the culture undergoing a set daily dilution at the

same time every day so that the average daily specific growth rate would be constant.

Cultures were grown in 600 mL polystyrene culture flasks, filled to a volume of 500 mL.

There are two phases to each growth strategy. The first phase is the Dilution Phase,

where the cultures underwent dilution once every day once log phase growth was

achieved. When the population held steady for three days (indicating constant growth at

the predetermined growth rate or relative growth rate) then the Dilution Phase ended with

a final dilution and the Harvest Phase began. Harvest Phase was where the culture

underwent a final dilution, was allowed to grow with no further sampling for two days as

the culture was stressed, and then was finally sampled. Cell abundance, ash free dry

weight (AFDW), and chlorophyll *a* (chl *a*) are important measures of biomass for

microalgal cultures. The cell abundance gives the density of the alga in the culture, chl *a*

is a common metric for estimating algal biomass, and AFDW gives the mass of the

organic matter for the culture. Whether or not the parameters measured were similar or

different was calculated based on the median absolute deviation (MAD) and the Kruskal-Wallis (one way ANOVA on Ranks) which used the posthoc Tukey Test to see which values were significantly different.

2.1 Experimental Design

In this thesis project, the difference in biomass, lipids, and protein yields in cells grown under two different growth strategies, Growth Strategy 1 (GS1) and Growth Strategy 2 (GS2), at three different temperatures was explored. These were chosen because they represent the seasonal range of temperatures at the Duke Lab where the algae can be grown outdoors. This was conducted by completing the following experiments. First, the maximum growth rate of S002 was determined in experiment 1 - the slope of the linear increase in cell numbers was calculated to determine the maximum growth rate. The calculated maximum growth rates at each temperature were then used to determine the specific growth rates used in GS1, and relative growth rates used in GS2. To determine the composition of algae during GS1 and GS2, the lipid and protein content was analyzed and determined.

2.1.1 Experiment 1

In experiment 1 of this study, a strain of *Oocystis* sp. (S002) was grown at three different temperatures (15°, 20°, 25°C) and the max growth rate calculated at each temperature. This preliminary experiment developed the growth curve for S002. Three 600 mL flasks of S002 were grown at each temperature with daily *in vivo* fluorescence recorded. A 5 mL sample was taken and the cell count measured using a Beckman Coulter Z2 Coulter Particle Count and Size Analyzer. The same sample was used to determine the chl *a* concentration using a Turner Designs 10-AU Fluorometer. These

readings were used to calculate when the sample is emerging from lag phase growth,

when it is at exponential growth and at stationary phase, and used to develop a growth

curve at each temperature. Once a culture reached exponential growth, it was sampled for

dissolved inorganic carbon (DIC) concentration in the medium by filtering out all the

algae from a 30 mL sample through a 0.2 micron Millipore filter. After stationary phase

was reached, the culture was again sampled for DIC concentration, and then the

experiment was terminated (Figure A.1, A.2). These samples were then analyzed using a

UIC Coulometer following the procedures in Dickson (2007) SOP 2. Approximately 25

mL of each sample was acidified with 4 mL 1 M phosphoric acid and purged with an

inert gas. The evolved CO_2 was titrated by coulometry and the pH was determined by the

transmittance of the thymolphthalein indicator at 610 nm. These CO_2 concentrations were

compared to that of the original medium, and it was determined the culture growth had

not reduced the DIC concentration during the exponential growth phase of the experiment

to levels where it could potentially induce carbon limitation of growth (2-7 mg/L)

(Yuvraj and Padmanabhan, 2017). This experiment allowed S002 to go through its

growth cycle undisturbed except for daily readings. These growth curves were then used

to determine the maximum growth rate for each temperature. The relative growth rate

was calculated using the maximum growth rate 25°C with the specific growth rate of

0.693 d^{-1}. The resulting relative growth rate was held constant across the temperature

range. The difference specific growth rates at each temperature were then used to

calculate the dilution rate for each temperature. Relative growth rate is defined as

follows:

$$Relative\ growth\ rate = \frac{Specific\ growth\ rate\ (\mu)}{Maximum\ growth\ rate\ (\hat{\mu})} \qquad (Goldman, 1980).$$

Maximum growth rate is a function of growth temperature and will vary with growth temperatures used in experiments 2a and 2b.

2.1.2 Experiment 2a

Seven 50 mL test tubes with 40 mL of modified F-medium (Guillard and Ryther, 1962) were inoculated with approximately 0.05 mL of S002 from the DMS Culture Laboratory and left to grow for two weeks at 20°C under a 12:12 hour light and dark cycle. These cultures were used to inoculate eighteen 600 mL flasks filled with fresh 485 mL of medium with 15 mL of culture.

Experiment 2a tested GS1. In GS1, the physiology of S002 growing at a constant dilution rate over a range of growth temperatures was analyzed. Six flasks each of S002 were grown at the temperatures 15°C, 20°C, and 25°C under a daily dilution with a target of 1 division per day (50% dilution by volume of the medium). This should result in the cultures growing at a specific growth rate of 0.7 d^{-1} or 1 cell division d^{-1}. Six hours after the light cycle started, the flasks were sampled. A 5 mL sample was taken to determine the *in vivo* fluorescence and cell abundance, which was used to determine when the samples reached exponential growth. Once there was sufficient growth, determined by the daily cell count and fluorescence readings, the culture flasks underwent a daily 50% dilution (Figure A.1). The constant dilution of 50% d^{-1} forced the sample to grow at one doubling per day, which allowed it to repopulate the new medium that was added after the flask was sampled. During dilutions, every day the 250 mL of medium that was removed from the culture flask was used for testing the cell abundance and fluorescence and 250 mL of fresh medium was added to the remaining 250 mL sample (Figure A.3). Once the cell counts showed the culture had stabilized at a division rate of one doubling

per day ($\mu = 0.7$ d^{-1}), indicated by the cell count reaching a constant density three days in a row, the culture underwent one more dilution and was left undiluted for 48 hours. During the first day the culture grew under the same growth conditions that it had been acclimated to, which should deplete the nutrients in the medium. The second day the culture was not sampled and underwent nutrient starvation, stressing the algae and causing the algae to form lipids (Sharma et al., 2012). At the mid-point of the second day of Harvest Phase, the culture was harvested, and the following analyses were run: particulate nitrogen (PN) content, the extracted chl *a* concentration, lipid concentration using Nile Red to stain the neutral lipids, and the ash free dry weight (Figure A.1). Chl *a*, AFDW, lipids, and protein content are good measures of biomass of a culture.

2.1.3 Experiment 2b

Experiment 2b tested growth strategy 2 (GS2), where the physiology of S002 growing at a constant relative growth rate was studied (Goldman, 1980). Each temperature had a different dilution rate to keep the relative growth rate constant. For this experiment, the target relative growth rate of S002 was approximately 0.8 for all temperatures. To keep this constant at each temperature, the dilution rate was different for each temperature and depended on the calculated maximum growth rate from experiment 1.

The maximum growth rate at 25°C was calculated to be 0.8 d^{-1}. Using a specific growth rate of 0.7 d^{-1}, a relative growth rate of 0.8 was calculated. For 15°C, the dilution was 46% dilution, for 20°C the dilution was 48% dilution, and for 25°C the dilution was 50% dilution. As with the constant dilution, the experimental cultures were inoculated with 15 mL of sample culture from the DMS Phytoplankton Culture Laboratory made by

inoculating seven 50 mL tubes with 40 mL of modified F medium with approximately 0.05 mL of S002. Once the sample cultures grew, they were used to inoculate eighteen 600 mL flasks. Six flasks of S002 were grown at each of the temperatures: 15°C, 20°C, and 25°C. Six hours after the light cycle started the flasks had daily 5 mL samples taken to determine *in vivo* fluorescence and cell abundance, which were used to establish when the samples reached log phase growth. Once log phase was reached, the six flasks grown at each temperature underwent their predetermined daily dilution (Dilution Phase), as in the example above, until the cultures had established the desired constant daily growth rate (Figure A.4). Once the cultures grew to the same population density three days in a row, they underwent one more dilution, then were not sampled for 48 hours to undergo nutrient depletion and stress (Harvest Phase). After 48 hours, the cultures were sampled using the same methods as the samples for GS1 (Figure A.1).

2.2 Ash Free Dry Weight

The ash free dry weight (AFDW) was determined using the standard lab procedures from the Redalje Lab Group. Samples were filtered through 47 mm VWR 691 glass fiber filters that had been pre-combusted in a muffle furnace at 450°C for 6 hours. Once the samples were filtered and the filter rinsed with ammonium bicarbonate to remove salt, the filters were left in the drying oven overnight at 60°C (Zhu and Lee, 1997). After they were dried, they were weighed until the difference in weights for three consecutive times was less than 0.00005 g. Then the filters were placed into the muffle furnace for 6 hours at 450°C to combust all organic matter leaving only inorganic ash. Once the furnace completed its cycle, samples were placed in a desiccator for no less than

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two hours to cool, then weighed as described above. The dry weight and ash free dry weight were calculated as follows: dry weight – ash weight = AFDW.

2.3 Fluorescence

The chl *a* fluorescence was measured using a Turner Designs 10-AU Fluorometer with the Welschmeyer filter set (Welschmeyer, 1994) using two different methods. A 5 mL sample was used for *in vivo* fluorescence measurements every day; this same sample was used for daily cell counts following the fluorescence measurement. *In vivo* fluorescence is a way to measure the biomass of the sample. It is assumed that a culture in balanced growth has a fluorescence yield that is linearly related to the cell number (Wood et al., 2005). For determination of extracted chl *a* concentrations, a 100 µL sample was taken during Dilution Phase and during Harvest Phase, then injected into 6 mL of methanol and left for 24 hours in a dark box in a freezer to extract the chl *a* (Welschmeyer, 1994). The test tube was then vortexed for a few seconds and the fluorescence was measured using the fluorometer. The ch *a* concentration was calculated using the equation (Welschmeyer, 1994):

$$\text{Chl} \left(\frac{\mu g}{L}\right) = \frac{\left[(\text{F}_{sample} - \text{F}_{blank}) * \frac{6.1}{801.95}\right]}{0.0001}$$

2.4 Lipid Concentration

Nile Red was used to stain the neutral lipids in the samples taken and analyzed using the method described in Johnson et al. (2017). The Nile Red stock solution was made from a 1mg: 1mL mixture of Nile Red and dimethyl sulfoxide (DMSO). A 5% concentration solution of chemically defined lipid concentrate (CDLC) was used as the standard by which samples were compared. Using a SpectraMax M3 from Dr. Robert

Griffitt's lab at GCRL, 1 mL of sample was mixed with 6 μL of DMSO and another 1 mL sample was mixed with 6 μL of the Nile Red and DMSO solution. The same was done for all the samples, and a blank of fresh medium, and a standard of CDLC. The Nile Red fluorescence of the samples were measured in triplicate using 300 μL from each of the mixtures and were put into a 96-well plate for analysis. The fluorescence was read with the settings established by Johnson et al. (2017) every 5 minutes for 30 minutes, and recorded.

2.5 CN Analysis

A CN analysis determines the elemental composition of a sample giving the concentration of the particulate organic carbon (POC) and particulate nitrogen (PN). This measurement is a good indicator of stored carbon (Huntley et al., 2015). Since lipids serve as carbon storage, this is one of the measures used to determine the concentration of lipids (Sharma et al., 2012). A 5 mL sample was taken at Dilution Phase and Harvest Phase for each culture. It was then filtered through a pre-combusted (450°C for 6 hours) 21 mm VWR 691 glass fiber filter. The 5 mL samples were dried overnight in a drying oven at 60°C, then were folded into small tin boats and were analyzed using a COSTECH Elemental Combustion System 1040 with carbon and nitrogen detectors. Analysis of samples followed the standard procedure of the Costech analysis method.

2.5.1 Protein Concentration

The particulate nitrogen given by the CN analysis was used to calculate the protein content of the samples through a conversion factor that was based on analyses of microalgal protein and PN (Templeton and Laurens, 2015). This is an easier and more cost-effective way of calculating the protein content in microalgal biomass (Marcó et al.,

2002; Templeton and Laurens, 2015). The conversion factor used is 4.08 (mass protein = 4.08 x mass of PN) which is more conservative than the previous 6.25 (Templeton and Laurens, 2015). The older standard conversion factor of 6.25 (mass protein = 6.25 x mass of PN) was first used in 1839 and was based on animal protein, an unacceptable approach to estimate algal protein (Templeton and Laurens, 2015). There is some error in using a conversion factor since this is an estimate that was calculated using multiple strains of algae grown under a range of conditions and growth strategies. Some natural species specific variability in both protein and PN can be expected.

2.6 Cell Abundance

The cell abundance was determined using a Beckman-Coulter Z2 Particle Count and Size Analyzer. Seawater filtered through a 0.2 µm membrane filter was used to dilute samples. A 5 mL sample was diluted as necessary (100 µL of algal culture in 20 mL of filtered seawater) as the culture density increased to maintain accuracy and sampled using the procedures in the Beckman-Coulter Z2 series user manual 4591-D. This was used to calculate the daily growth rate and determined when to sample at Dilution Phase and Harvest Phase.

2.7 Total Carbon dioxide

For experiment 1, the total dissolved inorganic carbon was calculated using a CO_2 Coulometer. Samples of 30 mL were taken during Dilution Phase and Harvest Phase for S002 and were processed by Dr. Hayes's lab at USM. These samples were filtered through a 0.2 µm Millipore filter to remove all algae in the sample and stored in an airtight glass Erlenmeyer flask until analyzed. This was used to determine if samples were undergoing CO_2 stress over the course of the incubation.

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2.8 Statistics

Due to the small number of samples, nonparametric statistical tests were used to analyze the results. Since the sample size was so small it was assumed that the data would not have a normal distribution and so parametric statistical tests could not be used when analyzing the data. The median absolute deviation (MAD) was used to compare variability about the medians of the results, as there are only six subsamples for each sample. The MAD is a robust way to calculate the variability of data because it is less affected by outliers. The MAD is calculated by finding the median, subtracting the median from all values, and determining the median of the absolute values of the difference between the values and median of the sample set. This median of the absolute differences is the MAD. The median $\pm$ MAD was used to calculate the error bars. Another way statistical significance was tested was using the Kruskal-Wallis H test, (non-parametric one-way analysis of variance) in SigmaPlot 11 to determine if there was a statistically significant difference in the data; however it does not tell which data are different. The posthoc Tukey Test (following the Kruskal-Wallis H test, in SigmaPlot 11) was used to determine the difference between groups. This was used to compare results obtained from cultures grown at the three different temperatures under six different growth conditions. A significant difference was observed when the error bars do not overlap or when the Tukey Test has calculated $P < 0.05$. There was no significant difference when the error bars over lapped or when $P > 0.05$.

CHAPTER III - RESULTS

The major objective of this project was to conduct research to address the question of what is the best way to operate an algal mass culture project that follows the hybrid growth approach. First, employing a photobioreactor using semi-continuous growth to provide algal biomass of consistent physiological state to a standard raceway pond system undergoing batch growth. Then in the open raceway pond the algae are stressed to promote the synthesis of lipids that can be used for the production of biofuels. The options explored by this research compares cultures grown in a photobioreactor at a constant daily dilution rate over a range of expected environmental temperatures (GS1) to cultures in the photobioreactor that are grown with variable dilution rates (GS2) determined for each growth temperature such that at all temperatures the organism are grown at the same relative growth rate (μ/μ_{max}). In order to accomplish this objective, it was necessary to determine the maximum growth rate for *Oocystis* sp. at each of the temperatures selected and to determine what the set daily dilution rate for GS2 should be and to determine the variable dilution rate necessary to maintain a constant relative growth rate for GS2.

We found that neither GS1 nor GS2 is noticeably better in keeping a more constant biomass composition. The chl *a* concentration was similar between GS1 and GS2 and had the highest values at 25°C during Harvest Phase. The AFDW was greatest during 20°C at Harvest Phase. Lipid per volume production was greatest during 25°C Harvest Phase in both GS1 and GS2. Protein was greatest in GS1 during 20°C Harvest Phase and 25°C Dilution Phase. The protein per volume was greater than the lipid per

volume, however, the lipid per cell was greater than the protein per cell in both GS1 and GS2. Overall, there was not a significant difference between GS1 and GS2.

3.1 Establishment of Major Growth Conditions for GS1 and GS2

Experiment 1 was used to establish the maximum growth rate of S002 at each growth temperature. The maximum growth rate was calculated using cell counts taken each day to measure the population growth over the range of temperatures of the experiment. Each day the daily growth rate was calculated and once the population's growth started to decline then the experiment was ended for that temperature. The growth rates calculated from experiment 1 were used to calculate the growth rates for GS1 and GS2.

3.2 Maximum Growth Rates

Below (Figure 3.1) is a graph of the growth curves from experiment 1 where the yellow dots indicate the beginning and ending days used to calculate the maximum growth rate. The beginning and end points were chosen based on the greatest linear increase during log phase growth, even if that included multiple days of growth as was the case at 20°C. The maximum growth rate calculated at 15°C was 0.78 d^{-1}, at 20°C was 0.81 d^{-1}, and for 25°C was 0.84 d^{-1}. The maximum growth rate at 25°C was used to calculate the relative growth rate that was held constant over the temperatures for GS2. That relative growth rate was 0.83 and the chosen dilution rates were 46%, 48%, and 50% for 15°C, 20°C, and 25°C respectively so that the constant relative growth rate could be maintained over the range of temperatures employed. The growth rates during the final days of each experiment are shown below in Figure 3.2.

Maximum Growth Rate Curve
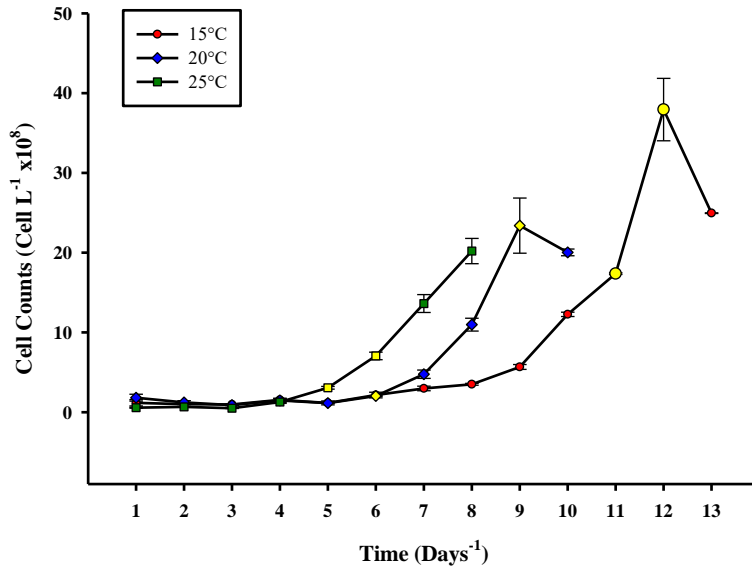
Figure 3.1 *Maximum Growth Curve*

A graph which shows the growth curve of S002 at three temperatures. The yellow dots depict the beginning and end points used for each temperature to calculate the maximum growth rate used for GS2.
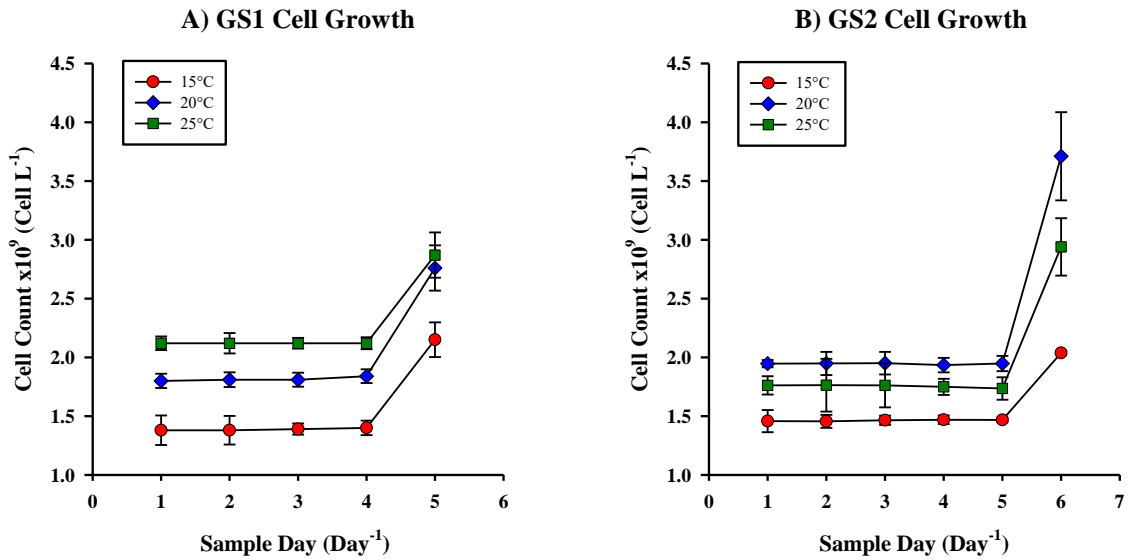
Figure 3.2 *GS1 and GS2 Cell Growth Curve*

The growth curve for GS1 (A) which shows the last dilution day on day three and the last day of stress on day five. B) the cell growth curve for GS2 which shows the last dilution day on day four and the last day of stress on day six.

3.3 Biomass Measurements

3.3.1 Chl *a*

The concentration of chl *a* was determined as a measure of biomass in GS1 and GS2. The concentration of chl *a* was greater in Harvest Phase than Dilution Phase for both GS1 and GS2, which was as expected (Figure 3.3A and Figure 3.4A). However, the opposite trend was seen for chl *a* cell^{-1} where the values for Harvest Phase were less than that of the Dilution Phase for both GS1 and GS2 as expected (Figure 3.3B and Figure 3.4B).

Comparing GS1 and GS2 chl *a* L^{-1} there was no significant difference in the concentrations except at 20°C Dilution Phase and at 25°C Dilution phase (P_{est}=0.036, P_{exact}=0.031). In both cases the concentration was greater during GS1 than GS2. The only significant difference between GS1 and GS2 chl *a* cell^{-1} was at 20°C Dilution Phase (P_{est}=0.036, P_{exact}=0.031).

During GS1 the chl *a* C^{-1} was significantly greater during the Dilution Phase than the Harvest Phase (Figure 3.5A). This means the carbon increased more than chl *a* resulting in the ratio decreasing from Dilution to Harvest Phase (Figure 3.5) as was expected. During GS2 the opposite trend was seen for growth at 15°C and 25°C, though there was not a significant difference between the Dilution Phase and Harvest Phase at 15°C, there was between the Phases at 25°C. Comparing GS1 and GS2 for chl *a* C^{-1}, the values for GS1 were significantly greater at all temperatures and phases except 15°C Dilution Phase and 25°C Harvest Phase than was the case for GS2. Overall the chl *a* concentration was similar between GS1 and GS2.

A) GS1 Chl *a* (ug L^{-1})

B) GS1 Chl *a* (ng Cell^{-1})
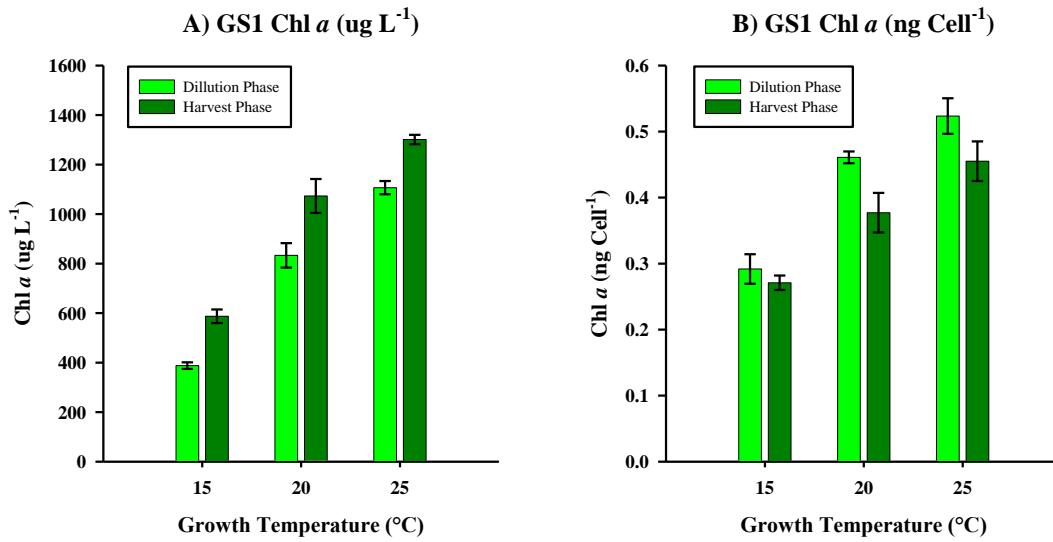
Figure 3.3 *GS1 Chlorophyll Concentrations*

Graph A) shows the chl *a* concentration in µg L^{-1} at each temperature in Dilution Phase and Harvest Phase for GS1. Graph B) shows the mass of chl *a* cell^{-1} at each temperature at Dilution Phase and Harvest Phase. In both graphs the error bars show the median absolute deviation. The greatest values of chl *a* are seen in the summer temperature.

A) GS2 Chl *a* (ug L^{-1})

B) GS2 Chl *a* (ng Cell^{-1})
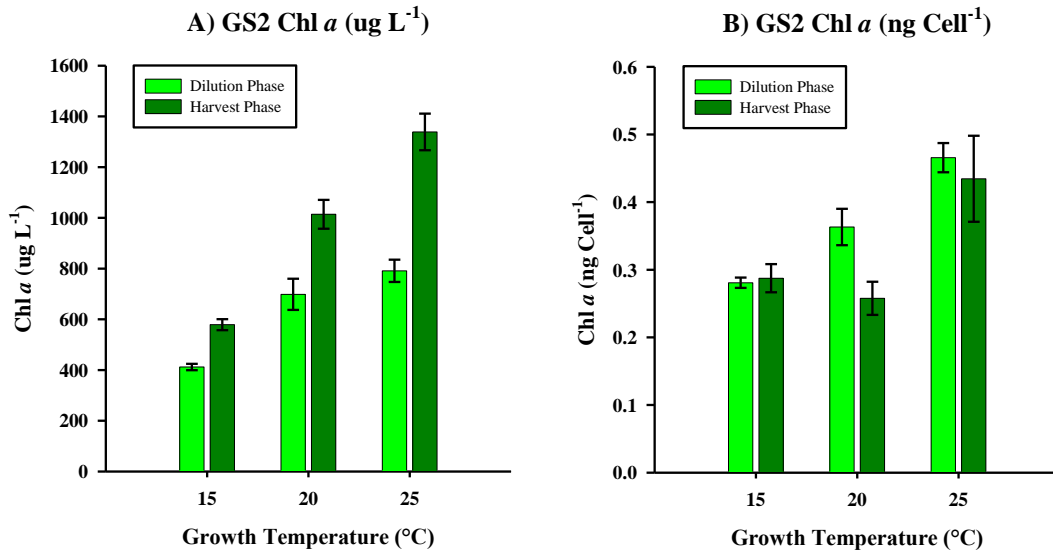
Figure 3.4 *GS2 Chlorophyll Concentrations*

Graph A) shows the Chl *a* L^{-1} at each temperature with a significant increase at Harvest Phase. Graph B) shows the mass cell^{-1} at each temperature with the only significant difference between the Phases at 20°C. In both graphs the error bars show the median absolute deviation.

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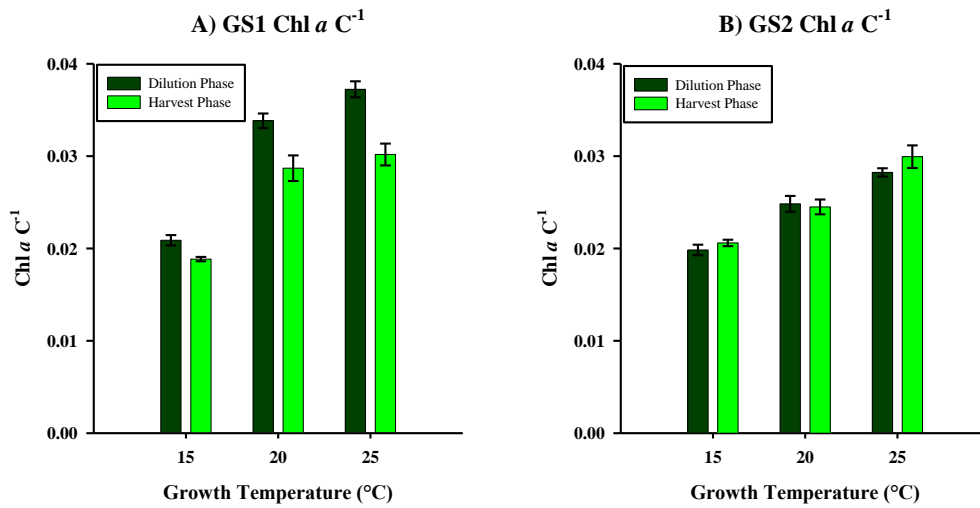
Figure 3.5 *GS1 and GS2 Chl a C^{-1}*

Graph A) which depicts the concentration of chl *a* in µg L^{-1} at each temperature during Dilution Phase and Harvest Phase. Graph B) shows the mass cell^{-1} at each temperature. In both graphs the error bars show the median absolute deviation. The greatest chl a concentrations were seen at the summer temperature
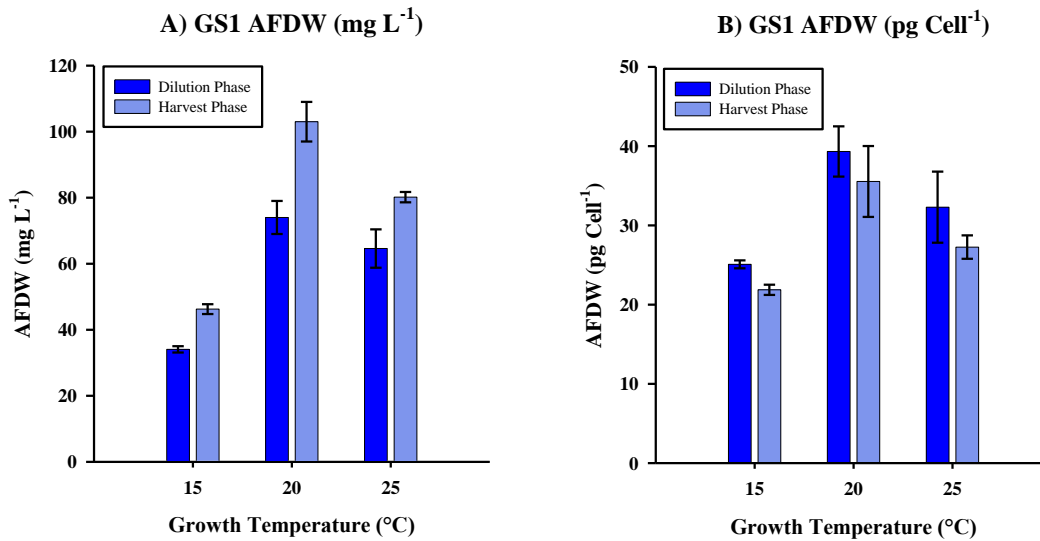
Figure 3.6 *GS1 AFDW Concentrations*

Graph A) shows the AFDW L^{-1} for GS1 at each temperature. Graph B) shows the AFDW cell^{-1} for GS1. In both graphs the error bars show the median absolute deviation. The greatest AFDW concentrations are at 20°C.

3.3.2 Ash Free Dry weight

Ash free dry weight was measured as another way to quantify the biomass of the cultures. The greatest AFDW L^{-1} occurred during Harvest Phase at 20°C (Figure 3.6A) and the greatest AFDW $cell^{-1}$ was during Dilution Phase at 20°C during GS1 (Figure 3.6B), which was unexpected. The AFDW L^{-1} significantly increased (P_{est}=0.036, P_{exact}=0.031) from Dilution to Harvest Phase from 15° to 25°C during both GS1 and GS2 as expected (Figure 3.7). During GS2, the AFDW $cell^{-1}$ was constant across the all three temperatures at Dilution Phase and Harvest Phase, except at 20°C, however the difference was not significant (P_{est}=0.093, P_{exact}=0.094). There was a significant difference in AFDW (mg L^{-1}) between the values obtained for GS1 and GS2 at 20°C Dilution and Harvest Phase (P_{est}=0.036, P_{exact}=0.031), where GS1 had greater AFDW L^{-1}.

The highest AFDW C^{-1} (Figure 3.8A) was during GS1 at 20°C. The AFDW C^{-1} was significantly greater during the Dilution Phase than the Harvest Phase at each temperature except 20°C during GS1 ($P = 0.031$, $P = 0.219$). It was expected for the carbon to increase at Harvest Phase, which would decrease the ratio, and so the results of GS1 were expected. During GS2 the AFDW C^{-1} was similar across all temperatures (Figure 3.8B). This was an unexpected result.

3.3 Nutrient Depletion

During the course of the experiment it was expected that the nutrients would be taken up during the Dilution Phase and consequently depleted in the 24-hour period before the next dilution of fresh medium. Then going from the Dilution Phase to the Harvest Phase the nutrients from the fresh medium would be depleted during the Harvest Phase of growth during both GS1 and GS2.
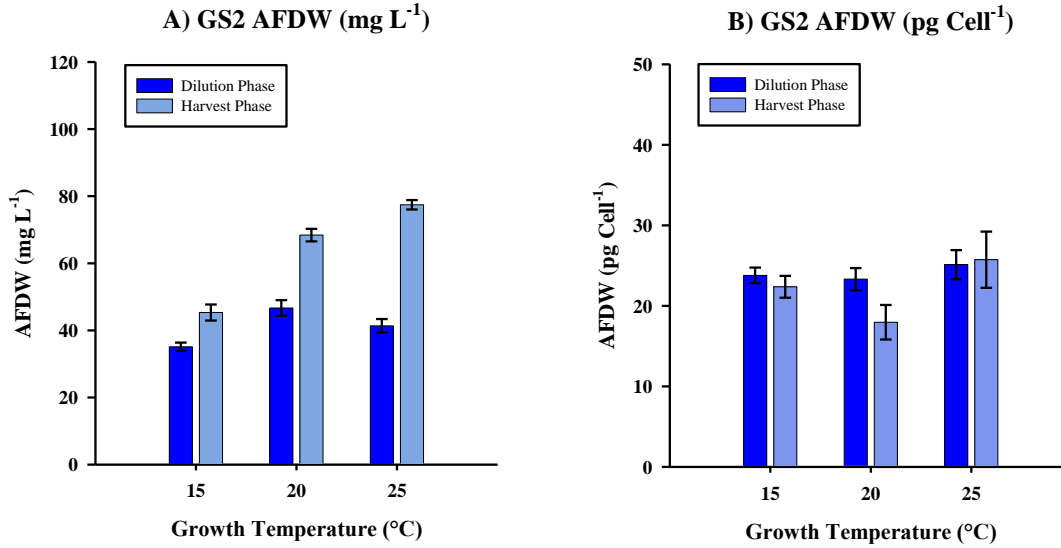
Figure 3.7 *GS2 AFDW Concentrations*

Graph A) shows the AFDW L^{-1} during GS2 at each temperature and graph B) shows the AFDW $cell^{-1}$ during GS2 at each temperature. In both graphs the error bars show the median absolute deviation. The greatest concentration of AFDW L^{-1} is during summer while the AFDW $cell^{-1}$ is relatively similar across all temperatures.
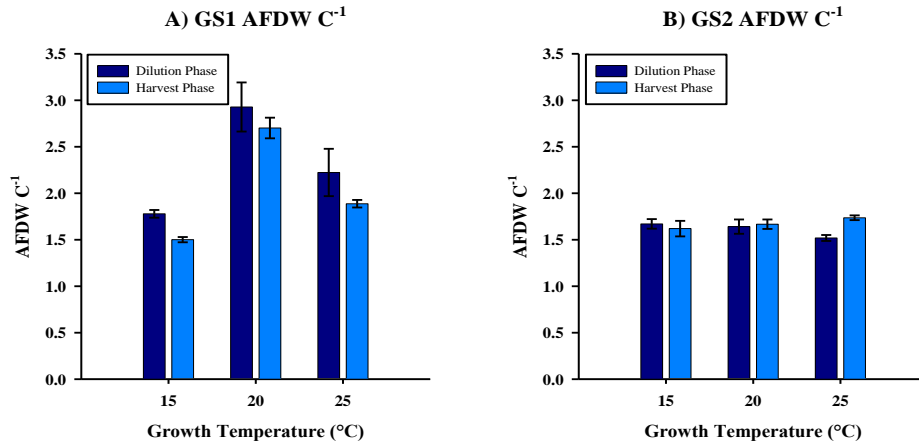
Figure 3.8 *GS1 and GS2 AFDW C^{-1}*

Graph A) shows the AFDW/C at each growth temperature during GS1. Graph B) shows the AFDW/C at each growth temperature during GS2. The error bars show the median absolute deviation. The highest AFDW/C was at 20°C during GS1. During GS2 the AFDW/C was similar across all temperatures.

A) GS1 Carbon (mg L^{-1})
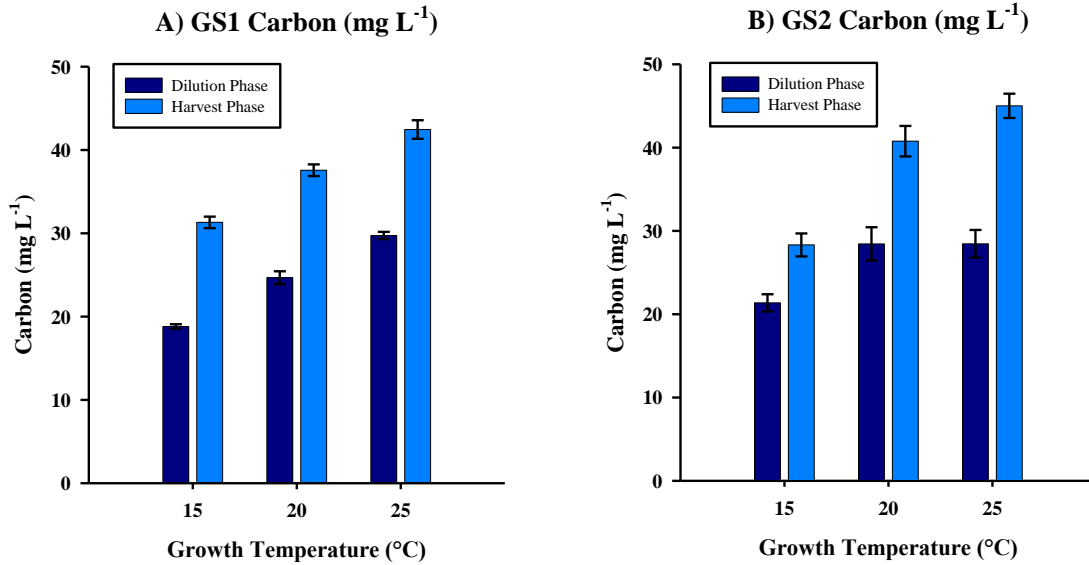
B) GS2 Carbon (mg L^{-1})

Figure 3.9 *GS1 and GS2 Carbon (mg L^{-1})*

Graph (A) shows the carbon L^{-1} at Dilution Phase and Harvest Phase at all three temperatures. Graph (B) shows the carbon L^{-1} for GS2 at all three temperatures. The error bars show the median absolute deviation. There is a significant increase from the Dilution Phase to the Harvest Phase at each temperature for both GS1 and GS2.

A) GS1 PN (mg L^{-1})

B) GS2 PN (mg L^{-1})
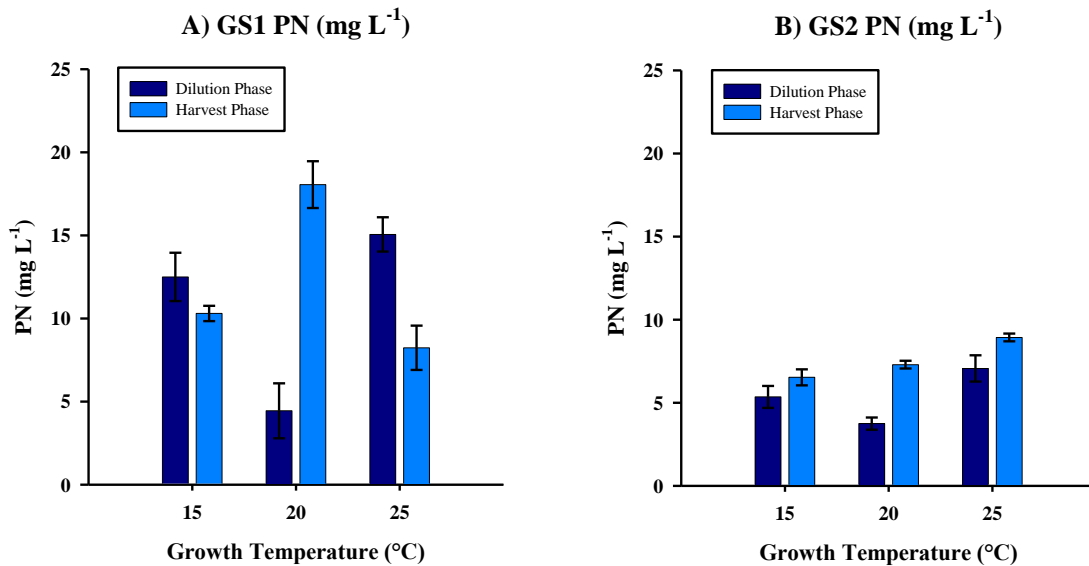
Figure 3.10 *GS1 and GS2 PN Content*

A) Shows the PN for GS1 in mg L^{-1} for all growth temperatures. B) Shows the PN for GS2 in mg L^{-1} for all growth temperatures. The error bars depict the median absolute deviation (MAD).

Table 3.1 *Expected and Actual Particulate Nitrogen Values in GS1 and GS2*

		15°C Harvest		20°C Harvest		25°C Harvest	
Particulate Nitrogen (umol/L)							
	Flask Number	**Actual**	**Expected**	**Actual**	**Expected**	**Actual**	**Expected**
GS1	1	583.0	1346.8	1360.2	1162.4	713.3	1460.2
	2	751.5	1387.8	1159.0	1214.5	399.9	1452.9
	3	685.9	1310.1	1009.1	1038.7	830.8	1300.8
	4	791.9	1448.0	1713.5	969.2	593.0	1410.4
	5	725.5	1209.2	1265.4	1042.8	583.3	1303.2
	6	745.9	1283.8	1313.9	994.1	523.3	1429.0
GS2	1	510.5	1063.9	484.6	1049.6	631.3	1109.9
	2	490.9	1036.8	533.9	1033.8	645.2	1224.3
	3	409.1	1021.3	521.6	992.9	570.0	1125.7
	4	455.2	1084.0	520.0	1008.9	643.9	1142.8
	5	373.1	1136.7	463.9	1022.5	612.0	1101.1
	6	477.5	1082.3	540.6	1007.7	710.7	1166.3

A table showing the calculated PN of Harvest Phase given the PN values at the time of the Dilution Phase. The blue highlighted section shows the only sampling time when the actual values are greater than or approximately equal to the expected values, indicating the cells have taken up the available nitrogen in the medium.

Complete nutrient samples were not measured, however the PN data shows the PN that resulted from the uptake of nutrients from Dilution Phase to Harvest Phase at each temperature (Figure 3.10). It was expected that all of the N in the nutrient medium would have been taken up and converted to PN each day in Dilution Phase and after the first day in the Harvest Phase, making PN reflect the complete assimilation of N in each case. The PN for GS1 follows the expected pattern at 15°C and 25°C but not at 20°C (Figure 3.11A). The PN for GS2 does not follow the expected pattern at any of the growth temperatures (Figure 3.10B). The values for PN in μmol L^{-1} at the time of Harvest are given in Table 3.1. The expected Harvest Phase PN was calculated using half the PN in μmol from Dilution Phase and the dissolved nitrogen which was in the medium which was added to the culture during the final dilution before Harvest Phase sampling. This was then normalized to volume and compared to the PN results at the Harvest Phase. The highlighted section of Table 3.1 showed the actual PN was greater than the calculated PN during GS1 at 20°C. This indicates that all the nitrogen was taken up at that point. At all other points of sampling, the actual PN was less than the calculated PN (Table 3.1).

3.4 Chemical Composition

3.4.1 Lipid

In both GS1 and GS2, it was expected that the cells grown in Dilution Phase would have lower lipid content than those grown in the Harvest Phase due to the impacts of nutrient stress. During GS1 at 20°C it was determined there was an outlier in the data set. When the Dilution Phase Lipid L^{-1} MAD was calculated it was 0.063 which was an order of magnitude larger than most other MAD values. Examining the data further, the first subsample had a low blank corrected value which led to a lower calculated lipid

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concentration. The data point was removed from the median and MAD calculation for testing the significance of data but was still graphed. During GS1 the lipid L^{-1} increased significantly at 15°C (P = 0.011), 20°C (P = 0.034) and 25°C (P = 0.045) during Harvest Phase. GS1 lipid cell^{-1} decreased from Dilution Phase to Harvest Phase at each temperature except 25°C (Figure 3.11A). In GS2 the lipid L^{-1} followed the same trend as in GS1, with a significant increase from Dilution Phase to Harvest Phase (P = 0.031) (except at 20°C where the increase was not significant (P = 0.563). Lipid cell^{-1} decreased significantly from Dilution Phase to Harvest Phase (P = 0.031), which was not expected (Figure 3.12B). The lipid L^{-1} was similar at each temperature during GS1 and GS2, with only a few significant differences. The Lipid L^{-1} was significantly greater in GS2 at 20°C Dilution Phase (P = 0.026) and 20°C harvest phase was greater (P = 0.026) during GS1 than GS2. The lipid cell^{-1} was significantly lower in GS1 at 15°C Harvest Phase (P = 0.026) and 25°C Dilution Phase (P = 0.026) than during GS2.

The Lipid C^{-1} was similar across all temperatures during GS1 (Figure 3.13A) but during GS2 there was significantly lower Lipid C^{-1} during 20°C Harvest Phase (P<0.05) than all other temperatures and phases (Figure 3.13B). There was a significant decrease from Dilution Phase to Harvest Phase at all temperatures during both GS1 and GS2 (P = 0.031), except GS1 at 20° and 25°C (P = 0.563).

Looking at the Lipid AFDW^{-1} during GS1, the only significant difference was at 15°C between Dilution and Harvest Phase (Figure 3.14A). In GS2 (Figure 3.14B), the only temperature without a significant difference between Dilution Phase and Harvest Phase was 15°C. The general trend was that the ratio was greater during Dilution Phase

than Harvest Phase. It is important to note that Lipid AFDW^{-1} and Lipid C^{-1} followed similar patterns for both GS1 and GS2 at all temperatures except GS1 25°C.

3.4.2 Protein

Protein concentration was calculated as a function of the PN measured during both GS1 and GS2 (see Methods section 2.4.1). There was no clear pattern in the protein content at GS1, except the concentrations at each temperature from Dilution to Harvest Phase kept the same pattern expressed both as L^{-1} and cell^{-1} (Figure 3.15). At 15°C and 25°C the concentration was greater during Dilution Phase than Harvest Phase which was the same for protein cell^{-1}. It was expected for the protein concentration to be lower at Harvest Phase than the Dilution Phase when expressed on a cell^{-1} basis, which makes the results of 20°C unexpected. However, given the values of PN in GS1 at 20°C (Figure 3.10) the protein values were as expected. The protein concentrations during GS2 do not have the same pattern as GS1 (Figure 3.16). The protein L^{-1} increased from Dilution Phase to Harvest Phase with the only significant difference at 20°C (P = 0.031). The protein cell^{-1} had no discernable pattern during GS2 (Figure 3.16B). However, there was a significant decrease cell^{-1} from the Dilution to Harvest Phase at 25°C as was expected (P = 0.031). The Protein C^{-1} ratio (Figure 3.17) was expected to decrease from Dilution Phase to Harvest Phase as was seen in GS1 15°C and 25°C, but not 20°C. During GS2, the same pattern was shown, however, there was no significant difference between the Phases at 15°C (P = 0.313).

The Lipid Protein^{-1} during GS1, exhibited a significant difference between the Dilution Phase and Harvest Phase for each temperature, but 20°C had the opposite trend than the other temperatures (Figure 3.18).

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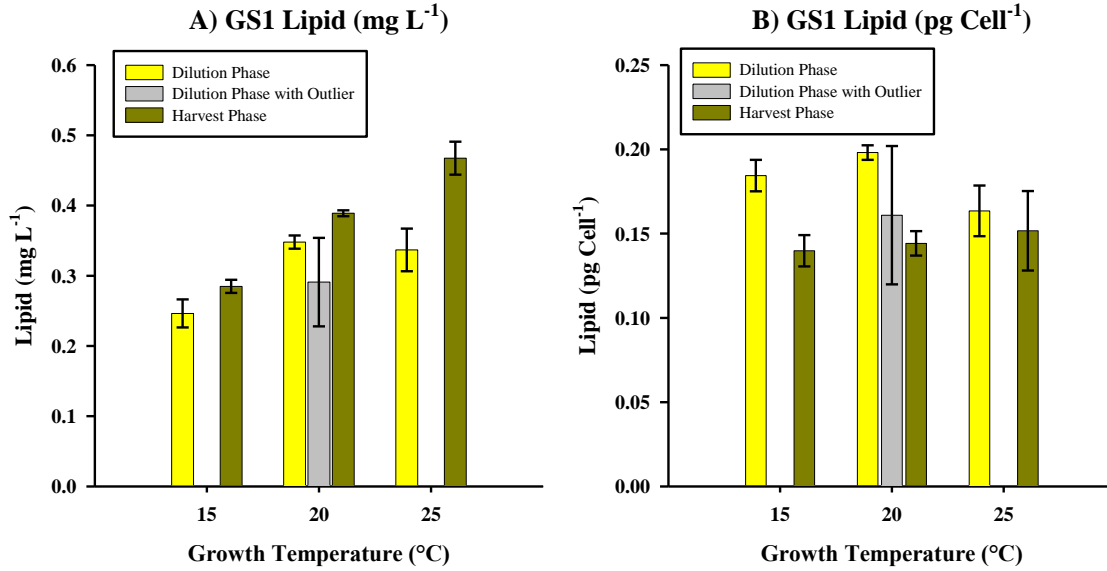
Figure 3.11 *GS1 Lipid Concentrations*

A figure showing the median lipid content across the growth temperatures in mg L^{-1} (A) and in pg cell^{-1} on the right (B). The error bars show the median absolute deviation. The grey bar includes one outlier from the 6 subsamples. The blank corrected value for that sample was low and caused the calculated sample to be much lower. The MAD was 0.063 and by removing that one point the MAD decreased to 0.009, which is similar to the other values. The greatest concentration were during the summer temperature per volume. The concentrations were similar across all temperatures for lipid cell^{-1}.
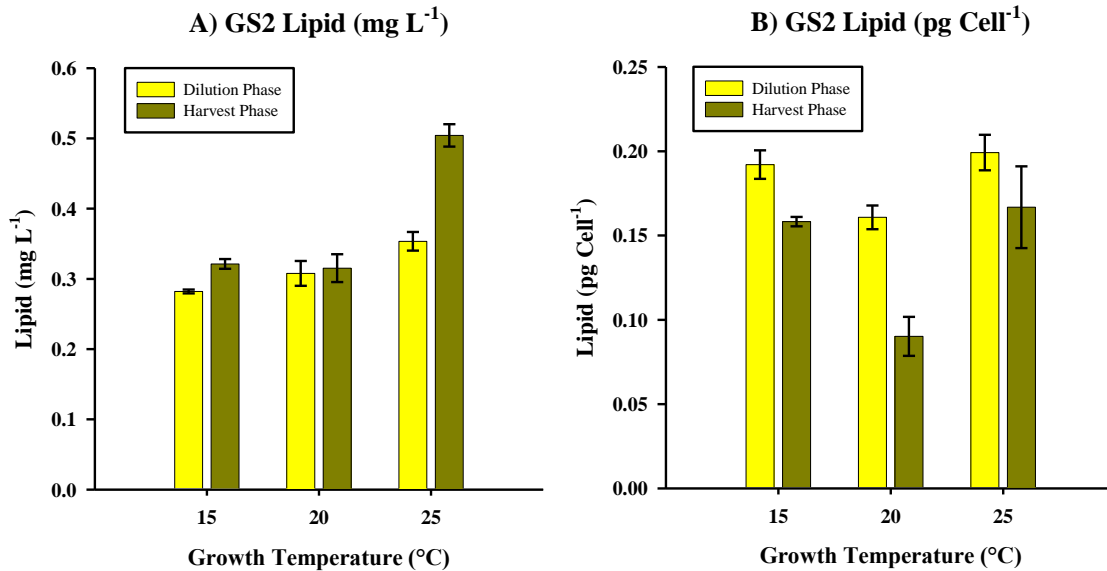
Figure 3.12 *GS2 Lipid Concentrations*

Graph A) shows the lipid concentration in mg L^{-1} and graph B) shows the lipid content in pg cell^{-1}. The error bars show the median absolute deviation. The greatest concentration of lipid L^{-1} is at the summer temperature.

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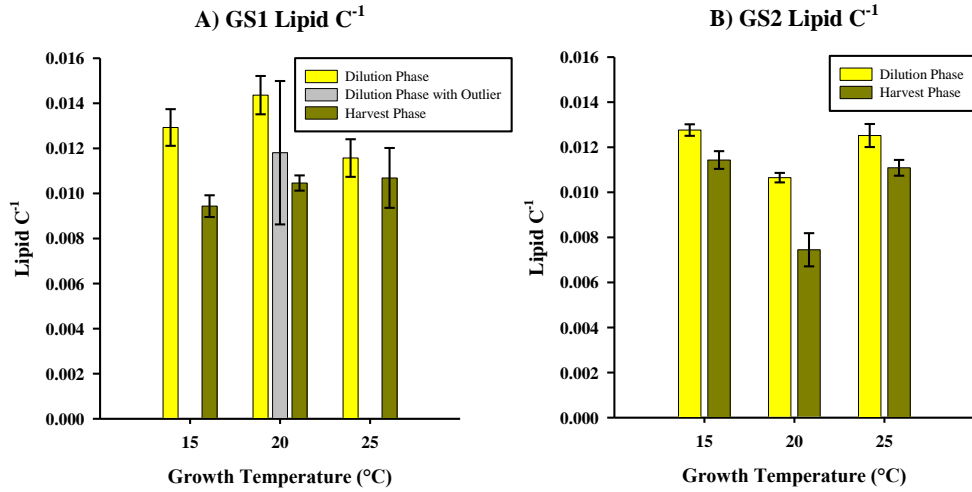
Figure 3.13 *GS1 and GS2 Lipid per Carbon*

Graph A) shows the Lipid C^{-1} during GS1 at each temperature. The grey bar is the Lipid C^{-1} with the outlier from the Lipid value included. Graph B) shows the Lipid C^{-1} during GS2 at each temperature. The error bars show the median absolute deviation. The outlier was determined as described in Figure 3.11. The Lipid C^{-1} is similar across all temperatures during GS1 but during GS2 there was a significantly lower Lipid C^{-1} ratio during 20°C.
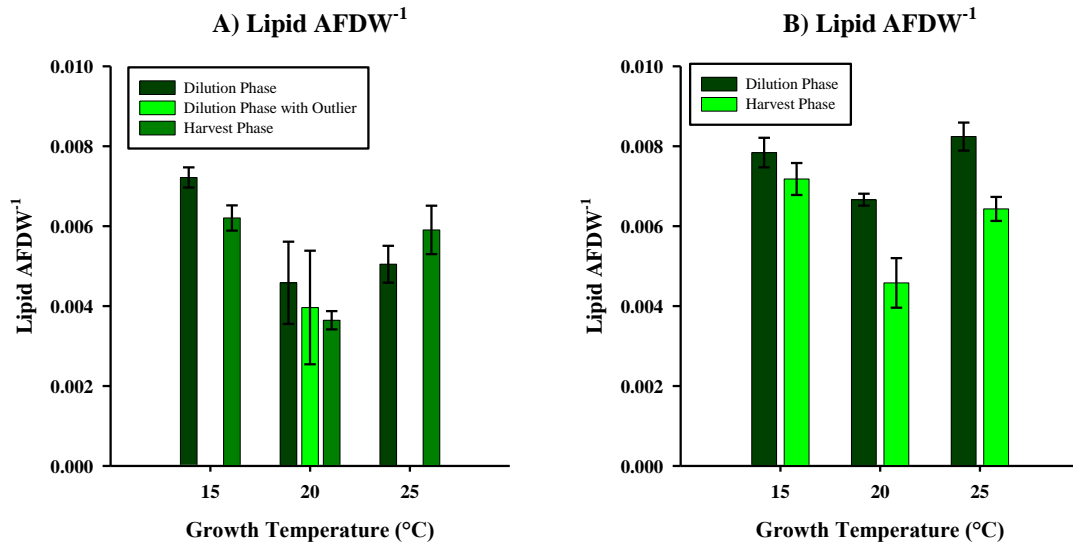
Figure 3.14 *GS1 and GS2 Lipid AFDW^{-1}*

Graph A) depicts the Lipid AFDW^{-1} during GS1 at all temperatures where the ratio decreased at 15°C and 20°C from Dilution Phase to Harvest Phase. The light green bar includes an outlier lipid value which is described in Figure 3.11. Graph B) depicts the Lipid AFDW^{-1} during GS2 at all growth temperatures which also had a general decrease from Dilution Phase to Harvest Phase. The error bars show the median absolute deviation.

It was expected for the ratio to be greater in Harvest Phase. In GS2, there was only a

significant difference between Dilution and Harvest Phase at 20°C and 25°C, and 20°C

had the opposite unexpected trend just like in GS1.

The concentrations of the biomass measures and chemical composition all

increased from the Dilution Phase to the Harvest Phase when looking at the concentration

L^{-1}. However, when looking at the same measures $cell^{-1}$ they decreased from Dilution

Phase to Harvest Phase, which was unexpected for the lipid and chl *a*. The protein

concentrations showed different trends from all other data which was unexpected. In

general the protein L^{-1} was greater than the lipid L^{-1}, with GS1 having the greatest values.

However, the lipid $cell^{-1}$ was greater than the protein $cell^{-1}$ with GS1 and GS2 having

similar values at all temperatures.
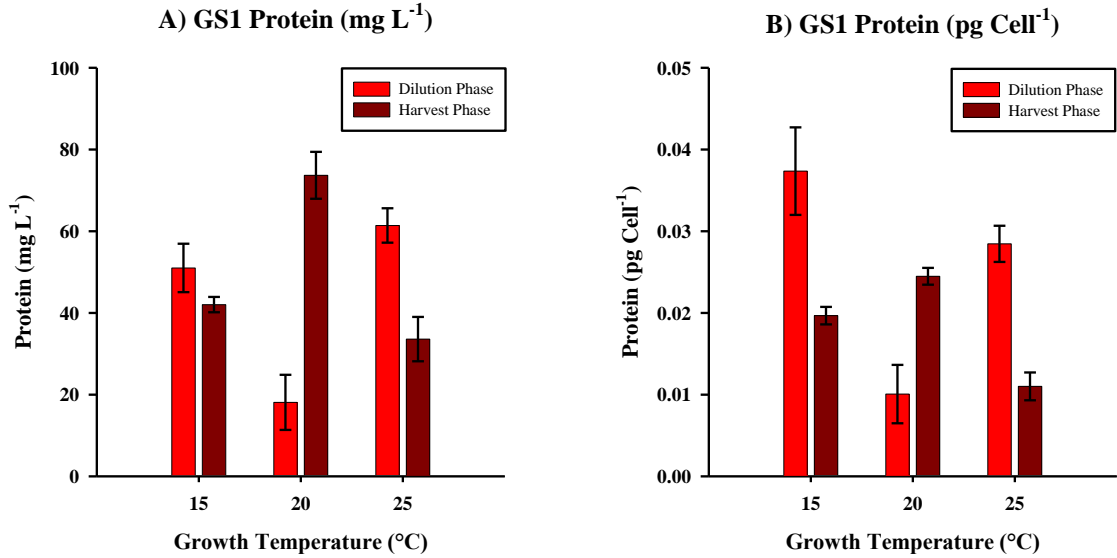
Figure 3.15 *GS1 Protein Concentrations*

A graph showing the protein L^{-1} (A) at all three temperatures during Dilution Phase and Harvest Phase. A graph (B) showing the protein $cell^{-1}$ at Dilution and Harvest Phase at all temperatures. The error bars on both graphs show the median absolute deviation. There was a significant difference between the Dilution and Harvest Phase across all temperatures with an unexpected result at 20°C.
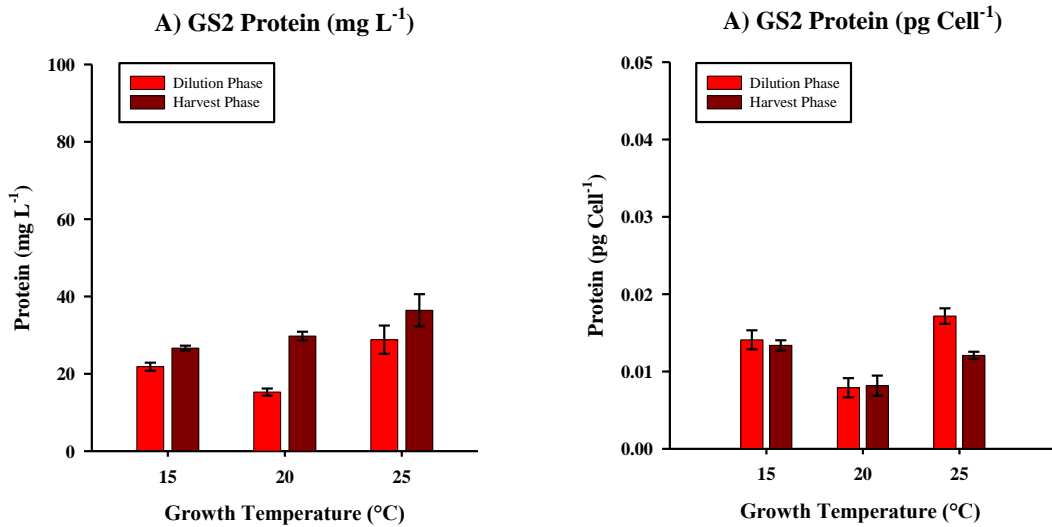
Figure 3.16 *GS2 Protein Concentrations*

Graph A) showing the protein L^{-1} at all three temperatures during Dilution and Harvest Phase. Graph B) showing the protein $cell^{-1}$ at Dilution and Harvest Phase at all three temperatures. The error bars on both graphs show the median absolute deviation.

A) GS1 Protein C^{-1}
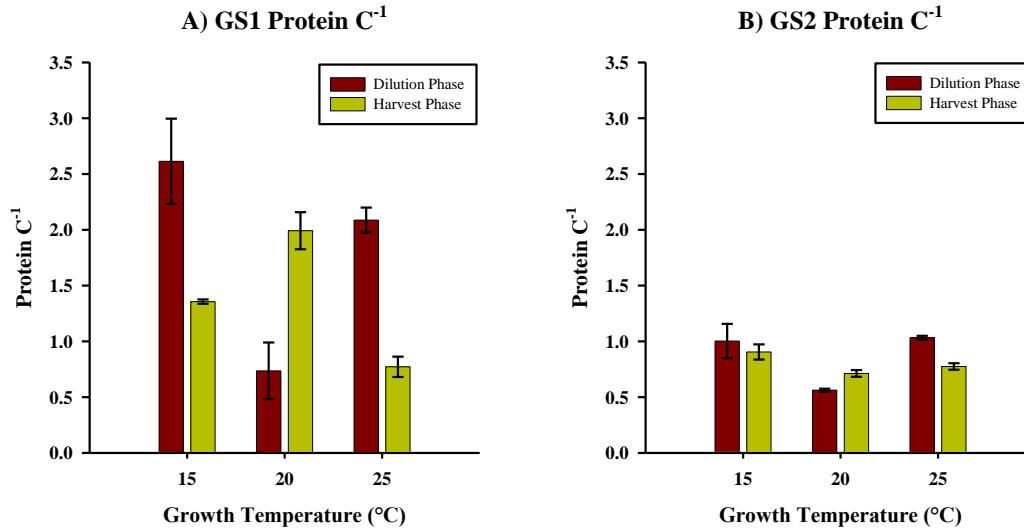
B) GS2 Protein C^{-1}

Figure 3.17 *GS1 and GS2 Protein C^{-1}*

Graph A) depicts the Protein C^{-1} during GS1 across all temperatures. There was a significant difference between Dilution Phase and Harvest Phase at each temperature had a decrease at 15°C and 25°C and an increase at 20°C. Graph B) shows the Protein C^{-1} during GS2 with a significant difference at 20°C and 25°C. The error bars show the median absolute deviation.

A) GS1 Lipid Protein^{-1}

B) GS2 Lipid Protein^{-1}
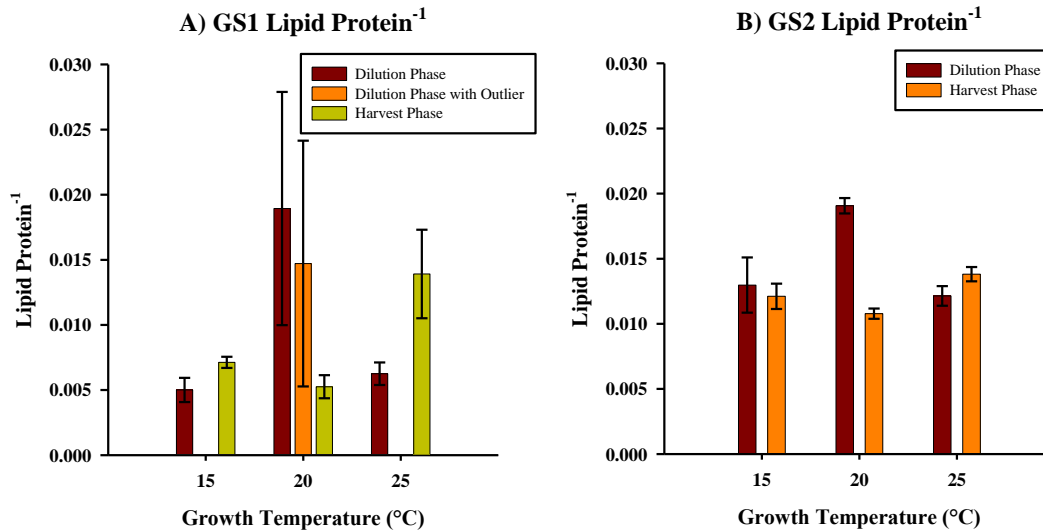
Figure 3.18 *GS1 and GS2 Lipid Protein^{-1}*

Graph A) is the Lipid Protein^{-1} for GS1 at all temperatures which shows a significant increase from Dilution Phase to Harvest Phase at 15°C and 20°C. The orange bar includes an outlier which was determined as described in Figure 3.11 Graph B) shows the Lipid Protein^{-1} for GS2 at all temperatures where 20°C Dilution Phase is significantly different from all other temperatures and phases. The error bars show the median absolute deviation.

CHAPTER IV – DISCUSSION

In this experiment there were three major hypotheses to be studied. First, *Oocystis* sp. (strain S002) will have a more constant biomass composition over the range of temperatures used under GS1 than GS2 due to the constant dilution rate across all temperatures. Second, the AFDW will be highest at 25°C under GS2. Lastly, the lipid production will be significantly higher at 25°C than at 15°C. The first part stated the highest lipid production in both GS1 and GS2 will be during the Harvest Phase at 25°C, and this is when the protein will be the lowest. The second part stated the added stress of GS1 will cause there to be higher lipid production under GS1 than GS2.

One of the challenges in comparing results that is common in biofuels is every experiment has a different methodology. Due to the small scale and highly regulated set up of this experiment, direct comparisons to other experiments are difficult to make. This experiment was done under constant light, employing a 12 hour: 12 hour light-dark cycle, and constant temperature conditions with regular dilutions which added a consistent amount of nutrients to the culture each day. This semi-continuous growth strategy will have different results than continuous and batch culture growth and this should be taken into consideration when comparing these results with those from the literature (MacIntyre and Cullen, 2005). The two part set up used in this experiment (Dilution Phase and Harvest Phase) was consistent with a hybrid growth system which combines a PBR and open raceway pond (Huntley and Redalje, 2006; Huntley et al., 2015).

4.1 Constant Biomass Composition

The first hypothesis that S002 would have a more constant biomass composition under GS1 than GS2 was not supported. The biomass composition differed across all

temperatures during both GS1 and GS2 (Figure 3.3, 3.4, 3.6, 3.7). There was no obvious strategy that did a better job at keeping a constant biomass composition between all temperatures.

4.1.1 Cell Abundance

During both GS1 and GS2 the cell counts increased from the Dilution Phase to the Harvest Phase (Figure 3.2). This was unexpected because cell division should slow down with nutrient limitation that was expected to occur during the Harvest Phase (Sharma et al., 2012, Jiang et al., 2012). One of the assumptions of this experiment was once the cultures reached steady state (coming back to the same population level each day) the algae would have depleted all the nutrients in the 24-hour period before the next dilution. If this held true the population cell abundance would not almost double during the phase when undergoing stress, as was seen in Figure 3.2. Since there was a large increase in cell abundances, it could suggest that nutrient depletion did not occur and that any nutrient limitation that might have occurred did not reduce the rate of cell division (Sharma et al., 2012, Jian et al., 2012). The cell count was lower than expected when compared to other growth experiments, although nutrient concentrations in the growth medium and environmental conditions of temperature and light would contribute to differences in cell abundance (Stephenson et al., 2010, Tan and Lin, 2011). The population density of this experiment is less than that in the Stephenson et al (2010) throughout the entirety of their experiment while compared to Tan and Lin (2011) the Harvest Phase is close in density to the inoculum of their experiment (Figure 3.2).

During GS1 the cell count increased as the temperature increased, which was expected as algal growth rates increase with increased temperatures up to their optimal

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growth temperature after which growth rates begin to decline due to thermal stress, leading to a greater abundance of cells (Figure 3.2A). During GS2, the cell count did not follow the expected pattern of increased cell count with increased temperature. Instead, growth at 20°C consistently resulted in the highest cell count throughout the Dilution Phase and Harvest Phase instead of 25°C. It is unclear why the cell count was higher at 20°C than at 25°C. It had been observed that *Oocystis* sp. grew well at both 25°C and 30°C (Redalje, unpublished data). There was some cell settling and sticking to the flasks at 25°C that could contribute to a lower cell count, since the agitation did not loosen the cells into the sample completely for testing. However, the cell settling did not seem to have this same effect during GS1 (Figure 3.2). Overall, the cell counts were more similar in values across the temperatures during GS2 than during GS1 and the increase in cells was greater at Harvest Phase during GS2 than GS1 (Figure 3.2). This could mean that GS2 could have a more consistent population size over a range of temperatures than GS1.

4.1.2 Ash Free Dry Weight

The AFDW in GS2 was the most consistent across all temperatures (Figure 3.7). There were no significant differences in the values of AFDW cell[-1] between the temperatures or between Dilution and Harvest Phases during GS2 (Figure 3.7B). Since there were few significant differences between these values, they were considered to be the most consistent across all temperatures.

In an experiment similar to this one done by Huntley et al. (2015), two types of algae were grown in a hybrid system (PBR to open raceway pond) with naturally variable temperature and light. Once culture was transferred to the pond from the PBRs, there were three days of Harvest Phase growth with samples taken each day (Huntley et al.,

2015). In the Huntley et al. (2015) experiment, there was an increase in the AFDW during their Harvest Phase, which was much greater than the one seen in this experiment. However, going into their Harvest Phase (Day 1) there were similar values to those for the Dilution Phase of this experiment. The AFDW L^{-1} results of GS1 and GS2 at 20°C and 25°C most closely resemble those of Huntley et al. (2015) in their experiments with *Desmodesmus* sp. in a low nitrogen pond. The Huntley et al. (2015) experiment does not give their results per cell so that comparison cannot be made. Also, the exact temperature the cultures were grown at during the time of Harvest was not reported in their paper and could affect the results.

4.1.3 Chl a

The chl *a* L^{-1} and chl *a* cell^{-1} was not statistically different between all temperatures but it was statistically different for some. For both GS1 and GS2 the values of chl *a* L^{-1} and chl *a* cell^{-1} between 15°C and 25°C were significant ($p<0.05$) at both Dilution and Harvest Phase (Figures 3.3, 3.4). This means the chl *a* was not held constant over the tested temperature range. It should be noted that nitrogen limitation should result in decreased chl *a* concentrations in a culture (Benavente-Valdés et al., 2016). Thus, nutrient limitation could be the reason that the chl *a* cell^{-1} decreases were observed at Harvest Phase during both GS1 and GS2 at all temperatures except 15° C (Figures 3.3B, 3.4B). In the Huntley et al. (2015) experiment, there was an increase in the chl *a* content during their Harvest Phase, but there was no information on whether or not this was the case for chl *a* cell^{-1}. The results in Figures 3.3A and 3.4A, 20°C and 25°C (Table A.3, Appendix A) are similar to the results of the *Desmodesmus* sp. high nitrogen and low nitrogen treatment, day 1 to day 2 values of Harvest Phase in the Huntley et al. (2015)

experiment. In this experiment, the temperature was controlled throughout the entire experiment, but in Huntley et al. (2015) all experiments were conducted in outdoor culture facilities. During their 3 day Harvest period the cultures were grown in open raceway ponds with temperatures that ranged from 18°C to >30°C and under daily variable light as well. This could explain why the values of this experiment are lower than theirs as well as there being differences due to the growth of different algal species.

Overall, the biomass measures were not consistently significantly different between each temperature during either GS1 or GS2. While in some instances GS2 was more constant across the temperature ranges there was no clear evidence that one growth strategy was any better than the other under the experimental conditions tested here. So long as S002, or any future algal strain is grown within their optimal temperature range, GS1 may well be the best growth strategy to employ.

4.2 Ash Free Dry Weight

The hypothesis that the highest AFDW concentration will occur at the summer temperature under GS2 was not supported. The greatest value for AFDW L^{-1} occurred during GS1 at 20°C Harvest Phase (Figure 3.6A). It was significantly greater than only 15°C when using the Kruskal-Wallis test ($P < 0.05$) but significant from 25°C as well at 15°C when using the MAD (Figure 3.6A). The dry weight and AFDW calculated in this experiment was lower than expected when compared to results from the literature (Stephenson et al., 2010; Tin and Lin, 2011; Lin and Lin, 2011; Mandal and Mallick, 2009). Some of this can be attributed to the difference in algal species and the design of the experiment. In Tin and Lin (2011), their cultures were left to deplete the nutrients over a period of 15 days as a batch culture with three different concentrations of nitrogen

and three different concentrations of phosphorous. Mandal and Mallick (2009) grew their cultures in a batch culture for 30 days with several different treatments. The starting dry mass of Mandal and Mallick (2009) was similar to that of this experiment (Figures 3.6, 3.7). However, by Day 5 their values quickly exceeded those of this experiment. An experiment done by Lin and Lin (2011) grew a batch culture for seventeen days and had initial AFDW values that were like those seen during the Harvest Phase of this study (Figures 3.6, 3.7), however, they also had a large increase in their values which was not seen in this experiment.

The next greatest value for AFDW cell^{-1} was the Dilution Phase of GS1 at 20°C (Figure 3.6B). It was unclear why the AFDW was so much higher during 20°C than 25°C as was expected. The overall cell count was greater at 25°C than 20°C, which is expected but does not explain why the AFDW was greater at 20°C than that at 25°C (Figures 3.2, 3.6). There was greater settling and sticking to the growth container of the culture at 25°C than at any other growth temperature and this could contribute to the lower concentration of AFDW observed in this part of the study. However, this did not seem to effect the cultures of GS2 in the same way. The lipid and chl a were both greatest at 25°C as expected (Figures 3.3, 3.4, 3.11, 3.12). During GS1 at 20°C the protein L^{-1} also showed some unexpected values. The PN values during this time showed there was some level of nutrient depletion (Figure 3.10, Table 3.1), which could be the driving force behind the high value of AFDW L^{-1}. This is the only time there seems to be nutrient depletion and could be why the values seem inconsistent compared to those for the rest of this study.

4.3 Lipid Production

The second hypothesis that lipid production will be greater for cultures grown under summer temperatures was supported for lipid L^{-1}, but not for lipid cell^{-1} (Figures 3.11, 3.12). The greater increase in lipids L^{-1} at a higher temperature that was not observed for lipid mass cell^{-1} could be attributed to the increase in the abundance of cells in the Harvest Phase (Figure 3.2). The first part of this hypothesis stated the greatest lipid production would occur during the Harvest Phase of the summer temperature for both GS1 and GS2, which was supported by these results (Figures 3.11, 3.12). The second part of this hypothesis stated the expectation that the added stress of GS1 would cause there to be higher lipid production during the Harvest Phase at all temperatures was not fully supported. The lipid L^{-1} did have a significant increase from Dilution Phase to Harvest Phase during both GS1 and GS2 for all temperatures except GS2 20°C which supported the hypothesis. However, the lipid cell^{-1} did not have the same significant increase from Dilution Phase to Harvest Phase during GS1 and GS2. This disparity could be attributed to the unexpected increase in the cell density during Harvest Phase. The increase in cells could be attributed to the increase in lipid L^{-1} but still result in the lipid cell^{-1} decrease, as was seen in the results (Figures 3.11, 3.12).

The lowest protein production was expected to occur during the same growth conditions that resulted in the highest lipid content, which was not supported by the results of this study. The lowest protein mass was observed at 20°C during the Dilution Phase for both GS1 and GS2 (Figures 3.15, 3.16). This was unexpected not only because it was at 20°C and not 25°C, but also because this occurred during Dilution Phase instead of Harvest Phase. It is expected for the protein content to be greater when there was no

nutrient limitation occurring rather than when there was supposed to be nutrient

limitation occurring (Shuter, 1979; Sharma et al., 2012). When examining the PN during

GS1 20°C, the actual values were greater than the expected values meaning that the only

time nutrient depletion occurred was during GS1 Harvest Phase (Figure 3.10, Table 3.1).

Since this was the only time the nutrient content of the medium was completely taken up

and assimilated into PN, it therefore makes sense the protein would be highest during this

time than at other temperatures and Phases.

It was expected that during Harvest Phase the lipid production would increase and

the protein production would decrease or remain the same. As mentioned above, there

was a decrease in lipid cell^{-1} from the Dilution Phase to the Harvest Phase at each

temperature which was unexpected. This suggests either that the cultures were not

stressed sufficiently or there was something else affecting the growth that was controlled

under the experimental conditions tested in this study. The lipid content was much lower

than expected as well compared to other chlorophytes (Sharma et al., 2012; Huntley et

al., 2015; Jiang et al., 2012; Mandal and Mallick, 2009; Stephenson et al., 2010; Tan and

Lin, 2011; Lin and Lin, 2011). In Sharma et al (2012), the unstressed algae that were

studied had about a 5-20% lipid AFDW^{-1} content, whereas the stressed algae had 20-

50%. The lipid AFDW^{-1} in this experiment was as high as 8% when it was stressed. This

difference could be caused by the difference in the measurement of lipids. In this study

Nile Red was used to stain the cells for neutral lipids which only accounts for about half

of the lipid content in cells, but does represent the majority of lipids that can be used for

biofuels (Johnson et al., 2017; Sharma et al., 2012). Several of these other studies took

more complicated measurements, such as lipid extraction, which accounted for more than

just the neutral lipids and therefore would lead to a greater concentration in lipid content than what was calculated in this study (Lin and Lin, 2011; Mandal and Mallick, 2009; Tan and Lin, 2011; Stephenson et al., 2010). In Sharma et al (2012), there was no mention of the tests that were run to calculate the lipid content for each species studied, however, it was shown that some measured more lipid forms than just the neutral lipids. Nile Red has its limitations because it strongly correlates with the staining of the neutral lipids, but also the fluorescence signal changes over time based on the physiology of the sample (Johnson et al., 2017). There is a lag between the initial injection of the dye and the peak fluorescence and while sampling was done as efficiently as possible, the lag could affect the results of this experiment (Johnson et al., 2017). There is also a difference in the efficiency between different species that could also suggest that the Nile Red might not be the best stain to use for S002 (Johnson et al., 2017). To go along with this, the values in the Huntley et al. (2015) experiment were much greater than those in this experiment, although it is recognized that there will be differences in lipid production between different algal species. The only time the lipid values were similar to those of this study was for Day 1 Harvest Phase of the diatom *Staurosira* sp. at low nitrogen growth conditions (Huntley et al., 2015). There was still a greater increase from their Day 1 to Day 2, than during any phase or temperature from this experiment (Figures 3.10, 3.11; Huntley et al., 2015).

Protein content should have an inverse relationship with the lipid content in microalgae grown under the conditions of this experiment. As the culture becomes more stressed through N depletion, the energy used should go towards producing lipids rather than cell division and growth (Shuter, 1979; Sharma et al., 2012). In Figure 3.18 the lipid

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protein^{-1} for GS1 increased at 15°C and 25°C, meaning there was a larger increase in lipid than protein during that time, which was expected. However, at 20°C there was a decrease of the ratio in both GS1 and GS2, meaning there was a greater increase in protein than lipid during this time as seen in Figure 3.15 and Figure 3.16. During GS1 the protein L^{-1} and cell^{-1} decreased from Dilution to Harvest Phase, as expected for 15°C and 25°C (Figure 3.15). The protein values of this experiment were lower than those of the Huntley et al. (2015) experiment, but there was a difference in the calculations for the protein. In this experiment a more conservative ratio was used for the conversion of nitrogen to protein (See Methods 2.4.1), but the Huntley et al. (2015) experiment used the Kjeldahl ratio of 6.25, a model that was first suggested in 1839 and was based on animal protein (Templeton and Laurens, 2015). This would result in their greater values of protein compared to the values calculated in this experiment.

An assumption of both of these experiments was that the nutrients were completely taken up over the Harvest Phase (Huntley et al., 2015). With that in mind, the PN calculated in this experiment (Figure 3.10, Table 3.1) should be greater than what was seen. Since only GS1 20°C was greater PN than the concentration of the original medium, it could be considered that for the other treatment of this study, the culture did not deplete the nutrients and go into nutrient stress and therefore the results do not reflect a stressed culture. Since the culture did not seem to be stressed or have as high a cell density as some of the experiments in literature, leading to lower biomass values, there could have been something else preventing the cells from taking up as much nutrient as needed to reach the high biomass values expected. This could be due to light limitation since there was likely self-shading in these dense cultures, or perhaps a pH increase (not

monitored in this study) led to CO_2 limited growth. Although DIC was monitored in

Experiment 1 which determined limitation was not likely, Experiment 1 was a batch

culture growth and would have been more likely to experience limitation than during

GS1 or GS2 due to the semi-continuous dilution. Once a culture has reached a sustainable

cell density for a set level of irradiance and nutrient availability, the cell growth rate will

decrease, which decreases the biomass output (Chrismadha and Borowitzka, 1994). In

order to increase the growth rate again, the irradiance and nutrient input would need to be

increased (Chrismadha and Borowitzka, 1994). The starting pH of the medium was

approximately 7.5 and went unmonitored throughout the growth experiments and so

would have increased as the dissolved carbon dioxide was utilized. If the pH increased

past S002s ability to grow then the growth rate would have decreased (Goldman et al.,

1982). This could have led to the lower biomass yields seen in this experiment since this

is highly dependent on the species biochemical response to pH levels (Khalil et al.,

2010).

4.4 Recommendations For Future Research

In the future, initial experiments to determine the optimal growth conditions for a

specific species should be more extensive, including a greater number of replicates. A

greater range of temperatures should be used to determine what the optimal growth

temperature is for the alga and what temperatures it cannot grow at with a daily dilution.

From there, changes to the medium could be tested to see what concentration of nutrients

in a medium is too much or too little. This experiment used F medium (Guillard and

Ryther, 1962) that had a high concentration of nutrients that did not seem to be

completely utilized during the duration of this experiment. In this study it was assumed

that the N in the medium, when assimilated, would be incorporated into PN, which was measured as seen in Figure 3.10. The observed PN did not equal the N in the medium during most of the samples taken, meaning not all the N in the medium was taken up and assimilated into the PN that was measured (Table 3.1). Given the small scale of this experiment, it was likely the starting concentration of nutrients was too high to deplete in the short time during the Harvest Phase. To prevent this in future research the initial nutrients could be lowered to F/2 medium concentrations or the final dilution before the Harvest Phase could be replaced with filtered seawater instead of fresh medium to encourage stress (Guillard, 1975). One other suggestion could be to extend the Harvest Phase for another day. To test and see which of these suggestions would be best to use in future experiments more nutrient testing could be done. Differing concentrations of nitrogen and phosphorus affect the lipid yield (Tan and Lin, 2011). Additional medium tests should be done to see if a different combination of starting concentrations of nitrogen and phosphorous will result in a greater lipid yield. Start the nutrient testing with the initial fresh medium used and continue to test every day before the dilution to see if nutrients in the medium had been taken up fully or if there were still nutrients left in the medium. This would show if there was a complete uptake and assimilation of the dissolved nutrients that were added from the dilution process or if there were nutrients remaining unutilized after 24 hours, the time for the next dilution event. It was assumed in this experiment that there was complete uptake of the nutrients introduced in the daily dilutions. Adjustments to the medium nutrient concentration could be made. Continued testing during the Harvest Phase would determine if the uptake of nutrients was complete or if the Harvest Phase should last longer than 24-hours. In some experiments the nutrient

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depletion phase lasts for days to study how the physiology changes over time (Jiang et al., 2012, Huntley et al., 2015). This extended Harvest Phase highlights the different rates of nutrient uptake between the different species of algae as seen in Jiang et al. (2012), where the chlorophyte alga continued to grow well for several days after being moved to a nitrogen depleted pond, whereas the diatom declined in growth immediately. It should be determined how much nutrient can be taken up by the algal species being grown and then grow it with no more than that amount so the nutrient can then be depleted in the defined growth period, under each set of growth conditions. A study such as this should be repeated using an algal species where the concentration of limiting nutrient in the medium should be repeated with each medium tested until the nutrients are able to be depleted in the allotted time.

In this experiment the cultures were mixed gently and manually twice a day. This did lead to some culture settling and sticking to the culture flask inner walls that could not be re-suspended and could have affected the results of this experiment. One way to prevent this in future studies would be to use the flasks for only one day, so at the time of dilution the culture removed would be placed in a new culture flask with fresh medium. Another way would be to use a magnetic stir bar during growth, which would cause constant agitation and prevent cells settling. While it did not seem the culture was carbon limited, this could be prevented in future experiments by bubbling CO_2 enriched air during the experiments growth and adding some turbulence to the culture. Turbulence would be expected to provide more uniform access to light for the cells as well as mixing the nutrient medium more efficiently, perhaps increasing access to nutrients for the cultures, and decreasing self-shading. Daily testing of the pH could prove helpful in

preventing the pH from increasing to inhabitable levels, and is a good way to control how much CO_2 should be bubbled into the culture.

Different lipid testing strategies should be tested to determine which fits best with the algal species used and for the type of experiment conducted. The quick throughput testing with Nile Red was excellent for the number of samples that were run during this experiment, but perhaps more testing of other stains could have provided insight to whether this dye was the best to use for *Oocystis* sp.

Given all the data provided by this experiment it was determined that neither GS1 nor GS2 was better than the other at keeping the biomass composition steady across the temperatures or for increasing the biomass and lipid during the highest temperature it was grown at. The results seem to demonstrate that the cultures did not deplete the nitrogen, which should be the limiting nutrient (Figures 3.2, 3.10, Table 3.1) and could be the reason why the results are inconclusive as to which growth strategy would work better at a larger scale. However, given the results that are understood, GS1 is as effective as GS2 when used in the optimal temperature range of the algal species used for experiments.

APPENDIX A – Experimental Set Up

Figure A.1 *Schedule Of Sampling*

A chart showing what analyses will be taken in each experiment and when during the growth phase it will be sampled for.

Table A.1 *Modified F-Medium Concentrations*

Component	Stock Solution	Quantity	Molar Contentration in Final Medium
NaNo$_3$	75 g/L dH$_2$O	2 mL	1.667 x 10^{-3} M
NaH$_2$PO$_4$ H$_2$O	5 g/L dH$_2$O	2 mL	7.24 x 10^{-5} M
Trace Metal Solution		0.2 mL	
Vitamin Solution		2 mL	
Sodium Bicarbonate		0.4 g	4.76 x 10^{-3} M

The chemicals and their amounts listed in the modified F-Medium. These volumes are for each liter of seawater used.

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Figure A.2 Experiment 1 Diagram of Growth
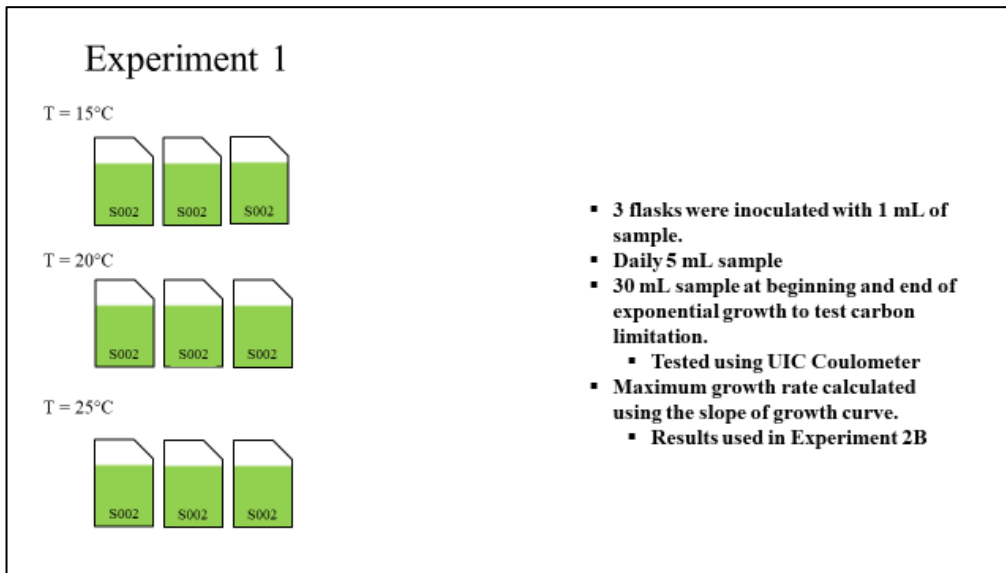
A chart showing how the cultures were grown for experiment one and what analyses were done.

Figure A.3 *Experiment 2A Diagram of Experimental Design*
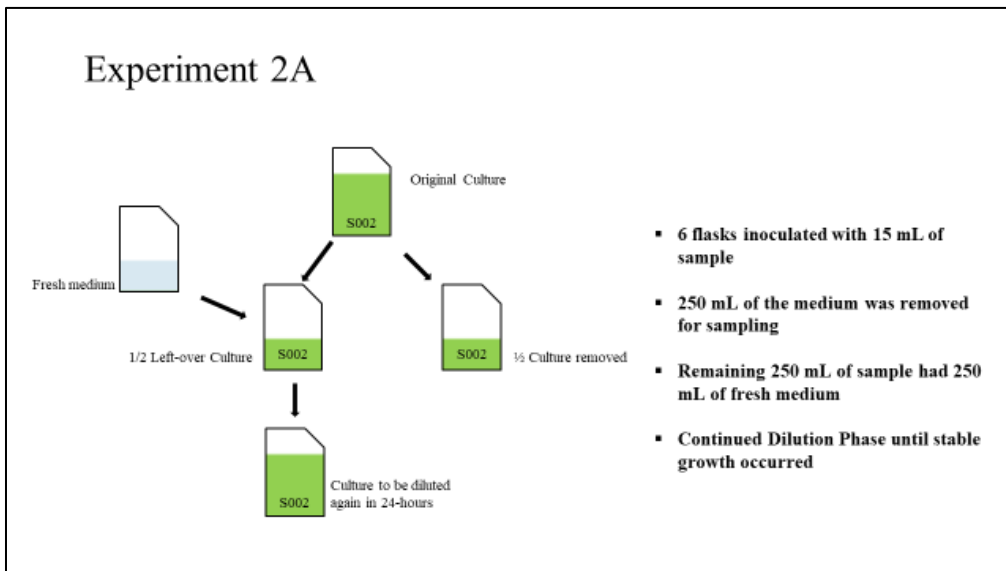
A diagram showing how the culture is divided during Experiment 2A.

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Figure A.4 Experiment 2B Diagram of Experimental Design
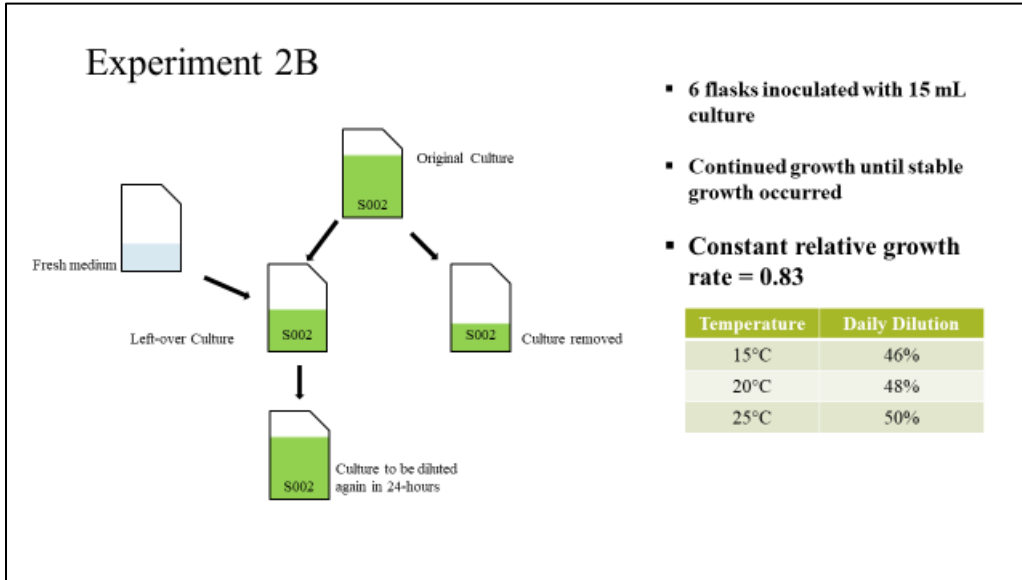
A chart showing how the culture is divided and used during the Dilution Phase of GS2 and the dilutions at each growth temperature.

Table A.2 *Coulometer Data*

Sample	Initial Weight (g)	Filled Weight (g)	Seawater Weight (g)	Reported Conc. (mgC/L)	Reported Conc. (ugC)	Corrected Conc. (mgC/L)
Blank					1.8	
Std1	34.5001	59.4437	24.9436	21.95		22.5
Std2	34.5327	59.5886	25.0559	23.51		24.0
Std3	34.5906	59.1143	24.5237	23.01		24.0
Std4	34.5228	59.0402	24.5174	23.38		24.4
Med	34.5303	59.9953	25.465	79.68		80.0
25C-D	34.5049	59.8189	25.314		218.8	8.8
20C-D	34.4942	59.9573	25.4631		283.6	11.4
15C-D	34.4996	59.716	25.2164	23.02		23.3
25C-H	34.497	59.7714	25.2744		49.2	2.0
20C-H	34.4997	59.9763	25.4766		25.2	1.0
15C-H	34.5047	59.9439	25.4392		106	4.3

A table the output from the UIC Coulometer. 'Std' = standard, 'Med' = medium blank, samples are listed as temperature-phase. 'D' = Dilution Phase, 'H' = Harvest Phase

Table B.1 *GS1 and GS2 Lipid Content Median and MAD Values*

	Flask Number	15°C Dilution Phase	15°C Harvest Phase	20°C Dilution Phase	20°C Harvest Phase	25°C Dilution Phase	25°C Harvest Phase
Lipid (mg/L)							
Growth Strategy 1	1	0.28	0.28	0.14 *	0.34	0.44	0.39
	2	0.26	0.30	0.23	0.41	0.29	0.55
	3	0.24	0.29	0.19	0.39	0.32	0.39
	4	0.22	0.28	0.35	0.39	0.35	0.47
	5	0.24	0.27	0.35	0.39	0.37	0.47
	6	0.29	0.31	0.36	0.39	0.31	0.49
	GS1 Median ± MAD	**0.25 ± 0.02**	**0.28 ± 0.01**	**0.29 ± 0.06**	**0.39 ± 0.00**	**0.34 ± 0.03**	**0.47 ± 0.02**
Growth Strategy 2	1	0.28	0.32	0.34	0.30	0.33	0.48
	2	0.28	0.32	0.32	0.32	0.35	0.51
	3	0.29	0.33	0.31	0.31	0.33	0.47
	4	0.28	0.31	0.28	0.25	0.37	0.51
	5	0.28	0.32	0.31	0.35	0.36	0.59
	6	0.26	0.33	0.27	0.34	0.37	0.50
	GS2 Median ± MAD	**0.28 ± 0.00**	**0.32 ± 0.01**	**0.31 ± 0.02**	**0.032 ± 0.02**	**0.35 ± 0.01**	**0.50 ± 0.02**
Lipid (pg/Cell)							
Growth Strategy 1	1	0.18	0.11	0.07 *	0.11	0.21	0.13
	2	0.19	0.14	0.12	0.15	0.14	0.23
	3	0.19	0.15	0.10	0.15	0.18	0.14
	4	0.15	0.14	0.20	0.10	0.16	0.09
	5	0.17	0.12	0.20	0.14	0.17	0.16
	6	0.20	0.15	0.20	0.15	0.15	0.17
	GS1 Median ± MAD	**0.18 ± 0.01**	**0.14 ± 0.01**	**0.16 ± 0.04**	**0.14 ± 0.01**	**0.16 ± 0.02**	**0.15 ± 0.02**
Growth Strategy 2	1	0.18	0.16	0.16	0.10	0.19	0.16
	2	0.20	0.16	0.17	0.07	0.19	0.11
	3	0.20	0.14	0.16	0.10	0.20	0.17
	4	0.19	0.16	0.15	0.07	0.22	0.19
	5	0.19	0.16	0.16	0.09	0.23	0.20
	6	0.18	0.16	0.11	0.09	0.20	0.14
	GS2 Median ± MAD	**0.19 ± 0.01**	**0.16 ± 0.00**	**0.16 ± 0.01**	**0.09 ± 0.01**	**0.20 ± 0.01**	**0.17 ± 0.02**

A table showing the six replicates at each temperature and phase of the calculated Lipid (mg/L) and the Lipid (pg/Cell). The * denotes that this value is an outlier and has been removed from some of the graphs above but the median and MAD values include this data point.

Table B.2 *GS1 and GS2 AFDW Content Median and MAD Values*

	Flask Number	15°C Dilution Phase	15°C Harvest Phase	20°C Dilution Phase	20°C Harvest Phase	25°C Dilution Phase	25°C Harvest Phase
AFDW (mg/L)							
Growth Strategy 1	1	38.9	52.6	78.0	100	57.6	83.8
	2	34.9	48.4	70.0	104	60.0	82.1
	3	31.3	42.9	92.0	102	61.2	81.0
	4	33.0	45.4	68.0	116	68.0	79.3
	5	33.4	45.8	62.0	92.0	86.0	79.0
	6	34.8	46.7	78.0	112	92.0	77.1
	GS1 Median ± MAD	34.1 ± 0.94	46.3 ± 1.50	74.0 ± 5.00	103.0 ± 6.00	64.6 ± 5.80	80.2 ± 1.56
Growth Strategy 2	1	38.5	48.4	47.8	74.0	41.6	74.8
	2	35.5	52.8	54.1	69.3	38.3	85.6
	3	39.7	45.1	47.5	68.5	40.4	79.2
	4	34.7	42.7	43.1	68.4	44.6	78.4
	5	34.0	45.6	45.9	65.5	41.2	76.3
	6	33.8	43.2	40.1	61.2	48.2	76.5
	GS2 Median ± MAD	35.13 ± 1.23	45.33 ± 2.38	46.69 ± 2.33	68.41 ± 1.87	41.36 ± 2.03	77.44 ± 1.40
AFDW (mg/Cell)							
Growth Strategy 1	1	25.5	21.4	39.6	30.7	27.4	27.1
	2	25.6	21.6	37.0	38.1	28.3	35.3
	3	25.5	22.1	49.7	39.6	33.7	30.0
	4	23.1	23.5	39.1	29.3	30.9	15.1
	5	24.7	20.5	35.3	32.9	38.5	27.0
	6	24.0	22.7	44.2	43.7	43.3	27.4
	GS1 Median ± MAD	25.1 ± 0.50	21.9 ± 0.64	39.3 ± 3.17	35.5 ± 4.47	32.3 ± 4.49	27.3 ± 1.47
Growth Strategy 2	1	23.8	23.7	22.9	25.4	23.3	24.9
	2	25.0	26.0	29.3	15.7	21.0	19.1
	3	28.5	18.2	25.0	21.9	23.6	29.6
	4	23.8	22.2	22.3	19.0	27.3	28.9
	5	23.0	22.6	23.8	16.9	26.9	26.6
	6	22.6	21.0	16.6	16.0	26.6	21.2
	GS2 Median ± MAD	23.78 ± 0.97	22.36 ± 1.35	23.32 ± 1.38	17.97 ± 2.14	25.12 ± 1.81	25.73 ± 3.49

A table showing the six replicates at each temperature and phase of the calculated AFDW (mg/L) and AFDW (mg/Cell).

Table B.3 *GS1 and GS2 Chl a Content Median and MAD Values*

	Flask Number	15°C Dilution Phase	15°C Harvest Phase	20°C Dilution Phase	20°C Harvest Phase	25°C Dilution Phase	25°C Harvest Phase
Chl-a (µg/L)							
Growth Strategy 1	1	486	628	932	1153	1092	1301
	2	433	599	879	1130	1152	1354
	3	367	534	864	1092	1145	1301
	4	382	541	757	978	1069	1217
	5	383	575	803	1054	1092	1324
	6	392	602	780	963	1122	1286
	GS1 Median ± MAD	388 ± 13.3	587 ± 27.6	833 ± 49.4	1073 ± 68.5	1107 ± 26.6	1301 ± 19.0
Growth Strategy 2	1	458	667	800	1098	733	1346
	2	432	651	785	1060	856	1529
	3	407	565	733	1067	765	1331
	4	392	553	645	968	818	1354
	5	408	562	664	953	749	1210
	6	416	593	629	869	837	1095
	GS2 Median ± MAD	412 ± 12.6	579 ± 21.7	698 ± 61.6	1014 ± 57.0	791 ± 43.7	1339 ± 72.3
Chl-a (ng/Cell)							
Growth Strategy 1	1	0.32	0.26	0.47	0.35	0.52	0.42
	2	0.32	0.27	0.46	0.41	0.54	0.58
	3	0.30	0.27	0.47	0.42	0.63	0.48
	4	0.27	0.28	0.44	0.25	0.49	0.23
	5	0.28	0.26	0.46	0.38	0.49	0.45
	6	0.27	0.29	0.44	0.38	0.53	0.46
	GS1 Median ± MAD	0.29 ± 0.02	0.27 ± 0.01	0.46 ± 0.01	0.38 ± 0.03	0.52 ± 0.03	0.45 ± 0.03
Growth Strategy 2	1	0.28	0.33	0.38	0.38	0.41	0.45
	2	0.30	0.32	0.42	0.24	0.47	0.34
	3	0.29	0.23	0.39	0.34	0.45	0.50
	4	0.27	0.29	0.33	0.27	0.50	0.50
	5	0.28	0.28	0.34	0.25	0.49	0.42
	6	0.28	0.29	0.26	0.23	0.46	0.30
	GS2 Median ± MAD	0.28 ± 0.01	0.29 ± 0.02	0.36 ± 0.03	0.26 ± 0.02	0.47 ± 0.02	0.43 ± 0.06

A table showing the six replicates at each temperature and phase of the calculated Chl *a* (µg/L) and Chl *a* (ng/Cell).

Table B.4 *GS1 and GS2 Protein Content Median and MAD Values*

	Flask Number	15°C Dilution Phase	15°C Harvest Phase	20°C Dilution Phase	20°C Harvest Phase	25°C Dilution Phase	25°C Harvest Phase
		Protein (mg/L)					
Growth Strategy 1	1	53.1	33.3	32.0	77.7	66.1	40.7
	2	57.8	42.9	38.0	66.2	65.2	22.8
	3	48.9	39.2	17.9	57.6	47.8	47.5
	4	64.7	45.2	10.0	97.9	60.4	33.9
	5	37.4	41.4	18.4	72.3	48.1	33.3
	6	45.9	42.6	12.8	75.0	62.5	29.9
	GS1 Median ± MAD	51.0 ± 5.9	42.0 ± 1.9	18.1 ± 6.7	73.7 ± 5.7	61.4 ± 4.2	33.6 ± 5.4
Growth Strategy 2	1	20.8	29.2	19.1	27.7	26.0	36.1
	2	17.7	28.0	17.3	30.5	39.1	36.9
	3	15.9	23.4	12.7	29.8	27.8	32.6
	4	23.1	26.0	14.5	29.7	29.8	36.8
	5	29.1	21.3	16.1	26.5	25.0	35.0
	6	22.9	27.3	14.4	30.9	32.5	40.6
	GS2 Median ± MAD	21.8 ± 1.0	26.6 ± 0.6	15.3 ± 0.9	29.7 ± 1.1	28.8 ± 3.7	36.4 ± 4.2
		Protein (pg/Cell)					
Growth Strategy 1	1	0.035	0.014	0.016	0.024	0.031	0.013
	2	0.042	0.019	0.020	0.024	0.031	0.010
	3	0.040	0.020	0.010	0.022	0.026	0.018
	4	0.045	0.023	0.006	0.025	0.027	0.006
	5	0.028	0.019	0.010	0.026	0.022	0.011
	6	0.032	0.021	0.007	0.029	0.029	0.011
	GS1 Median ± MAD	0.037 ± 0.005	0.020 ± 0.001	0.010 ± 0.004	0.024 ± 0.001	0.028 ± 0.002	0.011 ± 0.002
Growth Strategy 2	1	0.013	0.014	0.009	0.010	0.015	0.012
	2	0.012	0.014	0.009	0.007	0.021	0.008
	3	0.011	0.009	0.007	0.010	0.016	0.012
	4	0.016	0.013	0.007	0.008	0.018	0.014
	5	0.020	0.011	0.008	0.007	0.016	0.012
	6	0.015	0.013	0.006	0.008	0.018	0.011
	GS2 Median ± MAD	0.014 ± 0.002	0.013 ± 0.001	0.008 ± 0.001	0.008 ± 0.001	0.017 ± 0.001	0.012 ± 0.000

A table showing the six replicate values at each temperature and phase for the calculated Protein (mg/L) and Protein (ng/Cell) during GS1 and GS2.

Table B.5 *Biomass and Biochemical Measures in Reference to Carbon*

	Flask Number	15°C Dilution Phase	15°C Harvest Phase	20°C Dilution Phase	20°C Harvest Phase	25°C Dilution Phase	25°C Harvest Phase
				Chl-a/C			
Growth Strategy 1	1	0.022	0.019	0.036	0.031	0.037	0.029
	2	0.023	0.019	0.035	0.030	0.038	0.032
	3	0.021	0.019	0.034	0.028	0.040	0.031
	4	0.020	0.018	0.032	0.027	0.037	0.030
	5	0.020	0.018	0.033	0.029	0.036	0.028
	6	0.021	0.019	0.033	0.026	0.038	0.031
	GS1 Median ± MAD	**0.021 ± 0.001**	**0.019 ± 0.000**	**0.034 ± 0.001**	**0.029 ± 0.001**	**0.037 ± 0.001**	**0.030 ± 0.001**
Growth Strategy 2	1	0.020	0.021	0.025	0.026	0.029	0.031
	2	0.020	0.023	0.026	0.025	0.029	0.031
	3	0.021	0.020	0.026	0.026	0.029	0.031
	4	0.019	0.021	0.025	0.024	0.028	0.029
	5	0.018	0.021	0.023	0.024	0.027	0.026
	6	0.020	0.020	0.024	0.022	0.027	0.025
	GS2 Median ± MAD	**0.020 ± 0.001**	**0.021 ± 0.000**	**0.025 ± 0.001**	**0.024 ± 0.001**	**0.028 ± 0.000**	**0.030 ± 0.001**
				AFDW/C			
Growth Strategy 1	1	1.72	1.60	2.99	2.67	1.94	1.84
	2	1.88	1.54	2.77	2.74	2.00	1.93
	3	1.79	1.50	3.67	2.65	2.11	1.92
	4	1.77	1.50	2.86	3.20	2.33	1.93
	5	1.75	1.47	2.56	2.53	2.80	1.69
	6	1.84	1.48	3.32	2.97	3.09	1.86
	GS1 Median ± MAD	**1.78 ± 0.04**	**1.50 ± 0.03**	**2.93 ± 0.26**	**2.70 ± 0.11**	**2.22 ± 0.25**	**1.89 ± 0.04**
Growth Strategy 2	1	1.70	1.50	1.48	1.74	1.62	1.75
	2	1.64	1.84	1.77	1.60	1.28	1.73
	3	2.05	1.62	1.66	1.65	1.52	1.82
	4	1.70	1.62	1.66	1.71	1.52	1.70
	5	1.51	1.66	1.62	1.68	1.49	1.64
	6	1.60	1.43	1.51	1.57	1.56	1.74
	GS2 Median ± MAD	**1.67 ± 0.05**	**1.62 ± 0.08**	**1.64 ± 0.08**	**1.67 ± 0.05**	**1.52 ± 0.03**	**1.74 ± 0.03**
				Lipid/C			
Growth Strategy 1	1	0.012	0.008	0.005 *	0.009	0.015	0.009
	2	0.014	0.010	0.009	0.011	0.010	0.013
	3	0.014	0.010	0.007	0.010	0.011	0.009
	4	0.012	0.009	0.015	0.011	0.012	0.011
	5	0.012	0.009	0.014	0.011	0.012	0.010
	6	0.015	0.010	0.015	0.010	0.010	0.012
	GS1 Median ± MAD	**0.013 ± 0.001**	**0.009 ± 0.000**	**0.012 ± 0.003**	**0.010 ± 0.000**	**0.012 ± 0.001**	**0.011 ± 0.001**
Growth Strategy 2	1	0.013	0.010	0.011	0.007	0.013	0.011
	2	0.013	0.011	0.010	0.007	0.012	0.010
	3	0.015	0.012	0.011	0.007	0.013	0.011
	4	0.014	0.012	0.011	0.006	0.012	0.011
	5	0.012	0.012	0.011	0.009	0.013	0.013
	6	0.013	0.011	0.010	0.009	0.012	0.011
	GS2 Median ± MAD	**0.013 ± 0.000**	**0.011 ± 0.000**	**0.011 ± 0.000**	**0.007 ± 0.001**	**0.013 ± 0.001**	**0.011 ± 0.000**
				Protein/C			
Growth Strategy 1	1	2.35	1.01	1.23	2.07	2.22	0.90
	2	3.12	1.37	1.50	1.74	2.17	0.54
	3	2.80	1.37	0.71	1.50	1.65	1.12
	4	3.46	1.49	0.42	2.70	2.07	0.82
	5	1.96	1.33	0.76	1.99	1.57	0.71
	6	2.43	1.35	0.55	1.99	2.10	0.72
	GS1 Median ± MAD	**2.61 ± 0.38**	**1.36 ± 0.02**	**0.74 ± 0.25**	**1.99 ± 0.17**	**2.09 ± 0.11**	**0.77 ± 0.09**
Growth Strategy 2	1	0.92	0.90	0.59	0.65	1.02	0.84
	2	0.82	0.97	0.57	0.71	1.31	0.74
	3	0.82	0.84	0.44	0.72	1.05	0.75
	4	1.13	0.99	0.56	0.74	1.02	0.80
	5	1.29	0.78	0.57	0.68	0.91	0.75
	6	1.08	0.90	0.54	0.79	1.05	0.93
	GS2 Median ± MAD	**1.00 ± 0.15**	**0.90 ± 0.07**	**0.56 ± 0.01**	**0.71 ± 0.03**	**1.03 ± 0.02**	**0.77 ± 0.03**

A table showing the six replicate values of the biomass and biochemical measures per Carbon at each temperature and Phase during GS1 and GS2. The outlier during GS1 20°C Flask 1 was not included in the median and MAD values.

Table B.6 *Raw Particular Organic Carbon Data for GS1 and GS2*

		Carbon (mg/L)					
	Flask Number	**15°C Dilution Phase**	**15°C Harvest Phase**	**20°C Dilution Phase**	**20°C Harvest Phase**	**25°C Dilution Phase**	**25°C Harvest Phase**
Growth Strategy 1	**1**	22.5	32.9	26.1	37.5	29.7	45.5
	2	18.5	31.4	25.3	38.0	30.0	42.6
	3	17.5	28.6	25.1	38.5	29.0	42.3
	4	18.7	30.3	23.7	36.3	29.1	41.2
	5	19.1	31.2	24.2	36.3	30.7	46.7
	6	18.9	31.7	23.5	37.7	29.7	41.5
	GS1 Median ± MAD	**18.8 ± 0.3**	**31.3 ± 0.7**	**24.7 ± 0.8**	**37.6 ± 0.7**	**29.7 ± 0.4**	**42.5 ± 1.1**
Growth Strategy 2	**1**	22.6	32.3	32.2	42.6	25.6	42.8
	2	21.6	28.8	30.6	43.2	29.9	49.6
	3	19.4	27.9	28.5	41.4	26.6	43.6
	4	20.5	26.4	26.0	40.1	29.4	46.1
	5	22.5	27.4	28.4	39.0	27.5	46.5
	6	21.1	30.1	26.6	38.9	30.9	43.9
	GS2 Median ± MAD	**21.4 ± 1.0**	**28.3 ± 1.4**	**28.4 ± 2.0**	**40.8 ± 1.8**	**28.4 ± 1.7**	**45.0 ± 1.5**

A table of POC (mg/L) values for the six replicates at all temperatures and phases during GS1 and GS2.

Table B.7 *Raw Particulate Nitrogen for GS1 and GS2*

		Particulate Nitrogen (mg/L)					
	Flask Number	**15°C Dilution Phase**	**15°C Harvest Phase**	**20°C Dilution Phase**	**20°C Harvest Phase**	**25°C Dilution Phase**	**25°C Harvest Phase**
Growth Strategy 1	**1**	13.0	8.2	7.9	19.0	16.2	10.0
	2	14.2	10.5	9.3	16.2	16.0	5.6
	3	12.0	9.6	4.4	14.1	11.7	11.6
	4	15.8	11.1	2.4	24.0	14.8	8.3
	5	9.2	10.2	4.5	17.7	11.8	8.2
	6	11.3	10.4	3.1	18.4	15.3	7.3
	GS1 Median ± MAD	**12.5 ± 1.5**	**10.3 ± 0.5**	**4.4 ± 1.7**	**18.1 ± 1.4**	**15.1 ± 1.0**	**8.2 ± 1.3**
Growth Strategy 2	**1**	5.09	7.15	4.69	6.78	6.38	8.84
	2	4.34	6.87	4.25	7.47	9.58	9.03
	3	3.90	5.73	3.10	7.30	6.82	7.98
	4	5.66	6.37	3.55	7.28	7.30	9.02
	5	7.13	5.22	3.93	6.49	6.14	8.57
	6	5.61	6.68	3.52	7.57	7.96	9.95
	GS2 Median ± MAD	**5.35 ± 0.66**	**6.53 ± 0.48**	**3.74 ± 0.37**	**7.29 ± 0.23**	**7.06 ± 0.79**	**8.93 ± 0.23**

A table of PN (mg/L) values for the six replicates at all temperatures and phases during GS1 and GS2.

Table B.8 *Lipid/AFDW for GS1 and GS2*

		Lipid/AFDW					
	Flask Number	**15°C Dilution Phase**	**15°C Harvest Phase**	**20°C Dilution Phase**	**20°C Harvest Phase**	**25°C Dilution Phase**	**25°C Harvest Phase**
Growth Strategy 1	**1**	0.007	0.005	0.002	0.003	0.008	0.005
	2	0.007	0.006	0.003	0.004	0.005	0.007
	3	0.008	0.007	0.002	0.004	0.005	0.005
	4	0.007	0.006	0.005	0.003	0.005	0.006
	5	0.007	0.006	0.006	0.004	0.004	0.006
	6	0.008	0.007	0.005	0.003	0.003	0.006
	GS1 Median ± MAD	**0.007 ± 0.000**	**0.006 ± 0.000**	**0.005 ± 0.001**	**0.004 ± 0.000**	**0.005 ± 0.000**	**0.006 ± 0.001**
Growth Strategy 2	**1**	0.007	0.007	0.007	0.004	0.008	0.006
	2	0.008	0.006	0.006	0.005	0.009	0.006
	3	0.007	0.007	0.006	0.005	0.008	0.006
	4	0.008	0.007	0.007	0.004	0.008	0.006
	5	0.008	0.007	0.007	0.005	0.009	0.008
	6	0.008	0.008	0.007	0.006	0.008	0.007
	GS2 Median ± MAD	**0.008 ± 0.000**	**0.007 ± 0.000**	**0.007 ± 0.000**	**0.005 ± 0.001**	**0.008 ± 0.000**	**0.006 ± 0.000**

A table of Lipid/AFDW values for all six replicates at all temperatures and phases during GS1 and GS2. The outlier during GS1 20°C Flask 1 was not included in the median and MAD values.

Table B.9 *Lipid/Protein for GS1 and GS2*

	Lipid/Protein						
	Flask Number	**15°C Dilution Phase**	**15°C Harvest Phase**	**20°C Dilution Phase**	**20°C Harvest Phase**	**25°C Dilution Phase**	**25°C Harvest Phase**
Growth Strategy 1	**1**	0.005	0.008	0.004	0.004	0.007	0.010
	2	0.004	0.007	0.006	0.006	0.005	0.024
	3	0.005	0.007	0.010	0.007	0.007	0.008
	4	0.003	0.006	0.035	0.004	0.006	0.014
	5	0.006	0.007	0.019	0.005	0.008	0.014
	6	0.006	0.007	0.028	0.005	0.005	0.016
	GS1 Median ± MAD	**0.005 ± 0.001**	**0.007 ± 0.000**	**0.019 ± 0.009**	**0.005 ± 0.001**	**0.006 ± 0.001**	**0.014 ± 0.003**
Growth Strategy 2	**1**	0.014	0.011	0.018	0.011	0.013	0.013
	2	0.016	0.011	0.018	0.011	0.009	0.014
	3	0.018	0.014	0.024	0.010	0.012	0.014
	4	0.012	0.012	0.020	0.008	0.012	0.014
	5	0.010	0.015	0.019	0.013	0.014	0.017
	6	0.012	0.012	0.019	0.011	0.011	0.012
	GS2 Median ± MAD	**0.013 ± 0.002**	**0.012 ± 0.001**	**0.019 ± 0.001**	**0.011 ± 0.000**	**0.012 ± 0.001**	**0.014 ± 0.001**

A table for Lipid/Protein values for all six replicates at each temperature and phase during GS1 and GS2. The outlier during GS1 20°C Flask 1 was not included in the median and MAD values.

APPENDIX C Daily Cell Count Raw Data

Table C.1 *Daily Cell Counts at 15°C during GS1*

Culture	10/11/ 2018	10/12/ 2018	10/13/ 2018	10/14/ 2018	10/15/ 2018	10/16/ 2018	10/17/ 2018	10/18/ 2018	10/19/ 2018	10/20/ 2018	10/21/ 2018	10/22/ 2018	10/23/ 2018	10/24/ 2018	10/25/ 2018	10/26/ 2018
GS1-15-F1	163	112	489	350	473	773	682	717	774	978	1908	1390	1668	2047	2234	2065
GS1-15-F2	246	410	249	412	358	561	519	598	634	738	1169	1459	1199	1504	1904	1399
GS1-15-F3	297	134	192	599	463	440	654	552	615	764	1281	1019	1200	1157	1609	1566
GS1-15-F4	290	102	213	347	357	482	515	498	549	643	1136	680	1054	1105	1156	1221
GS1-15-F5	260	136	359	805	336	317	425	974	390	708	606	618	696	574	704	1137
GS1-15-F6	243	146	260	423	310	356	454	991	865	629	597	684	759	749	962	959
Culture	10/27/ 2018	10/28/ 2018	10/29/ 2018	10/30/ 2018	10/31/ 2018	11/01/ 2018	11/02/ 2018	11/03/ 2018	11/04/ 2018	11/05/ 2018	11/06/ 2018	11/07/ 2018	11/08/ 2018	11/09/ 2018	11/10/ 2018	11/11/ 2018
GS1-15-F1	2627	2915	3309	3298	3658	3395	3472	3652	3938	3386	3099	3328	4929	3309	4675	4168
GS1-15-F2	2234	1992	1897	2154	2204	2317	2386	2672	2671	2644	2303	2452	2465	2752	2789	2798
GS1-15-F3	1813	1983	2906	3357	2526	2545	2726	2674	2897	2629	2392	3125	2645	2648	3134	2861
GS1-15-F4	2017	1476	2254	2047	2210	2928	2265	2364	3057	2845	2143	2262	2262	3686	3380	2580
GS1-15-F5	1046	924	1304	1013	1486	1237	1792	1334	1608	1278	1318	1780	1829	1641	1750	2034
GS1-15-F6	1588	1139	2270	1460	1555	1759	1974	1835	2065	1820	1839	1936	2136	2665	2398	3239
Culture	11/12/ 2018	11/13/ 2018	11/14/ 2018	11/15/ 2018	11/16/ 2018	11/17/ 2018	11/18/ 2018	11/19/ 2018	11/20/ 2018	11/21/ 2018	11/22/ 2018	11/23/ 2018	11/24/ 2018	11/25/ 2018	11/26/ 2018	11/27/ 2018
GS1-15-F1	4293	3581	3795	3679	3654	3753	4421	3921	4001	4483	4045	4649	4163	3988	4483	4081
GS1-15-F2	3235	3121	3193	3158	3131	4075	3712	3339	3662	3918	3657	3715	4188	3462	3778	4031
GS1-15-F3	3005	2897	3071	2920	2967	3236	3042	3002	4095	3760	3222	3286	3245	3508	3355	3571
GS1-15-F4	2608	2821	3121	3479	2804	2828	3246	3097	3808	3413	3426	3887	3236	4079	4298	4667
GS1-15-F5	2563	2023	2298	2476	2504	2661	2945	2997	3420	3373	3376	3449	3325	3444	3441	3309
GS1-15-F6	2769	2803	3288	2880	2987	3180	3773	3933	3781	3648	3620	3878	3439	3619	3650	4593
Culture	11/28/ 2018	11/29/ 2018	11/30/ 2018	12/01/ 2018	12/02/ 2018	12/03/ 2018	12/04/ 2018	12/05/ 2018	12/06/ 2018	12/07/ 2018	12/08/ 2018	12/09/ 2018	12/10/ 2018	12/11/ 2018	12/12/ 2018	12/13/ 2018
GS1-15-F1	4425	4382	4123	4493	4629	3930	4045	4168	3935	4084	3763	3887	3571	3798	3742	6117
GS1-15-F2	3550	5157	3364	3931	4200	3139	3431	3677	3651	3154	3280	3107	3452	3384	3359	5569
GS1-15-F3	4417	3755	3196	3872	3082	2995	3159	3336	3237	2988	3123	3576	2965	3047	3109	4834
GS1-15-F4	3482	4577	3521	3498	4169	3339	3017	3105	3174	3216	3046	3776	2854	3550	3663	4817
GS1-15-F5	3712	3772	3266	4117	3447	2907	3041	2965	2950	2937	3409	3111	4682	3361	3503	5565
GS1-15-F6	3388	3629	3275	3129	3336	3653	3109	3332	3135	4316	4074	3266	3434	3595	3468	5129

The daily cell counts given by the Beckman Coulter Counter. cell mL^{-1} = (value x 1000)/(0.1/20.1))/500

Table C.2 *Daily Cell Counts at 20°C during GS1*

Culture	10/11/ 2018	10/12/ 2018	10/13/ 2018	10/14/ 2018	10/15/ 2018	10/16/ 2018	10/17/ 2018	10/18/ 2018	10/19/ 2018	10/20/ 2018	10/21/ 2018	10/22/ 2018	10/23/ 2018	10/24/ 2018
GS1-20-F1	233	153	367	717	730	958	1315	1859	1880	2594	2733	2939	2982	5229
GS1-20-F2	174	145	428	622	776	1331	1362	1596	1982	3315	2633	2980	2912	4361
GS1-20-F3	216	173	195	591	1052	942	1728	2272	1943	2823	3159	3564	3342	4194
GS1-20-F4	367	174	306	654	1025	898	1684	1902	2162	2961	3640	3322	3803	3866
GS1-20-F5	327	125	333	540	631	907	1384	2012	2170	2824	2920	4320	3131	4151
GS1-20-F6	179	168	229	672	649	807	1364	1670	2679	2593	3050	3567	3723	4307
Culture	10/25/ 2018	10/26/ 2018	10/27/ 2018	10/28/ 2018	10/29/ 2018	10/30/ 2018	10/31/ 2018	11/01/ 2018	11/02/ 2018	11/03/ 2018	11/04/ 2018	11/05/ 2018	11/06/ 2018	11/07/ 2018
GS1-20-F1	3748	3776	4085	5214	6820	5785	5173	4224	5582	4650	4586	3934	3861	4957
GS1-20-F2	5322	3883	4125	5029	4884	3975	4132	3987	4263	4279	4372	3828	3490	3889
GS1-20-F3	4054	5748	4447	4803	4916	4963	6041	4710	5425	5673	4762	4334	3809	4359
GS1-20-F4	4431	5067	4204	6123	5245	4562	4534	5516	4649	4765	4343	4097	3809	4258
GS1-20-F5	4644	4044	4265	5959	5182	4451	5927	5511	4807	5023	4281	4048	3704	4116
GS1-20-F6	4366	4437	4633	5738	5566	5358	5017	5953	6204	4854	5264	4157	4021	4688
Culture	11/08/ 2018	11/09/ 2018	11/10/ 2018	11/11/ 2018	11/12/ 2018	11/13/ 2018	11/14/ 2018	11/15/ 2018	11/16/ 2018	11/17/ 2018	11/18/ 2018	11/19/ 2018	11/20/ 2018	
GS1-20-F1	5337	4748	4600	5196	4381	5044	5155	5255	5021	4899	4905	5037	8111	
GS1-20-F2	3915	4876	4337	6836	4174	4459	4478	5254	4447	4611	4705	4570	6787	
GS1-20-F3	4455	4420	4477	6850	4494	4580	4514	5206	5031	4613	4603	4588	6405	
GS1-20-F4	4104	4068	4184	3934	4354	4227	4124	4579	4296	4244	4328	4299	9857	
GS1-20-F5	4130	4097	4107	4189	4140	4096	4386	4241	4338	4301	4369	4591	6947	
GS1-20-F6	4243	4745	4180	4305	4403	5404	4471	4384	4490	4388	4392	4230	6372	

The daily cell counts given by the Beckman Coulter Counter during GS1 at 20°C. cell mL^{-1} = (value x 1000)/(0.1/20.1))/500

Table C.3 *Daily Cell Counts at 25°C during GS1*

Culture	10/11/2018	10/12/2018	10/13/2018	10/14/2018	10/15/2018	10/16/2018	10/17/2018	10/18/2018	10/19/2018	10/20/2018	10/21/2018	10/22/2018	10/23/2018	10/24/2018	10/25/2018
GS1-25-F1	343	154	279	717	1014	1566	2031	2804	3849	4796	4581	4746	4569	5920	5166
GS1-25-F2	460	168	283	1434	1081	1496	2164	3331	3967	3794	4552	5874	4582	5190	6131
GS1-25-F3	606	189	245	1281	1075	1433	1854	2627	3622	3602	4261	5059	4513	6150	6562
GS1-25-F4	362	166	264	816	1066	1987	2360	2839	3820	4195	4518	4567	4601	5411	5352
GS1-25-F5	310	163	233	689	1077	1523	2247	3803	3552	4565	4676	4628	5251	4892	6722
GS1-25-F6	420	129	262	746	1409	1566	2332	3905	3614	3601	4404	5071	5157	4920	6492

Culture	10/26/2018	10/27/2018	10/28/2018	10/29/2018	10/30/2018	10/31/2018	11/01/2018	11/02/2018	11/03/2018	11/04/2018	11/05/2018	11/06/2018	11/07/2018	11/08/2018	11/09/2018
GS1-25-F1	5223	5641	5344	5936	5277	6935	6068	5242	6237	5113	6057	5178	5116	4981	4966
GS1-25-F2	5842	5194	5420	6137	5995	5368	5556	5301	5343	6566	4410	4460	4518	5306	4535
GS1-25-F3	5807	5295	6194	5771	5556	5519	5431	5319	5224	5507	4304	4817	4635	5384	4569
GS1-25-F4	5434	5294	5420	5733	5539	5349	5030	5059	5802	5170	4639	4374	4398	4546	5139
GS1-25-F5	6295	9732	5508	6342	5656	5370	5353	5452	6936	6481	4292	4463	4479	4669	4642
GS1-25-F6	5540	5530	5834	6017	5743	5622	5320	5028	5738	5897	4201	4434	4473	4682	4592

Culture	11/10/2018	11/11/2018	11/12/2018	11/13/2018	11/14/2018	11/15/2018	11/16/2018	11/17/2018	11/18/2018	11/19/2018	11/20/2018	11/21/2018	11/22/2018	11/23/2018	
GS1-25-F1	4825	5864	5308	5255	5303	6574	4599	5123	5797	5585	5506	5238	5265	7677	
GS1-25-F2	6152	4855	4970	4623	5284	4749	5271	5600	4841	5189	5690	5279	5541	5789	
GS1-25-F3	5768	4723	4706	4560	5253	4848	4653	5246	4728	5398	5074	4524	5306	6717	
GS1-25-F4	4878	4650	6087	4690	4745	4791	5059	5284	5099	5360	5332	5466	5177	13057	
GS1-25-F5	6287	4841	4978	5543	5105	4782	4780	4677	4805	5111	5217	5550	5458	7265	
GS1-25-F6	4806	5606	4892	5262	4824	4551	5160	4455	5507	5054	4978	5281	5142	7009	

The daily cell counts given by the Beckman Coulter Counter during GS1 at 25°C. cell mL^{-1} = (value x 1000)/(0.1/20.1))/500

Table C.4 *Daily Cell Counts at 15°C during GS2*

Culture	2/5/2019	2/6/2019	2/7/2019	2/8/2019	2/9/2019	2/10/2019	2/11/2019	2/12/2019	2/13/2019	2/14/2019	2/15/2019	2/16/2019
GS2-15-F1	244	295	336	868	903	1144	1374	2036	2987	2782	2761	3013
GS2-15-F2	245	287	356	817	852	961	1151	1859	1809	2652	2382	2982
GS2-15-F3	246	275	328	698	781	977	1198	1582	1706	2608	2180	2774
GS2-15-F4	448	274	414	687	766	940	1068	1268	1816	2465	1972	2221
GS2-15-F5	230	271	534	729	681	786	783	1061	1559	1970	1535	1870
GS2-15-F6	251	325	366	720	617	831	971	1132	1332	2050	1932	1953

Culture	2/17/2019	2/18/2019	2/19/2019	2/20/2019	2/21/2019	2/22/2019	2/23/2019	2/24/2019	2/25/2019	2/26/2019	2/27/2019	2/28/2019
GS2-15-F1	3437	3697	3798	4325	4180	4305	4341	4128	4499	4072	4674	3990
GS2-15-F2	2999	3387	3588	3682	4386	3880	3600	3630	3801	3710	3767	3540
GS2-15-F3	3227	3121	3239	4031	3551	3500	3571	4015	3963	4607	4571	3741
GS2-15-F4	2929	3427	3062	2906	3334	3256	3114	3285	3857	3856	3663	3248
GS2-15-F5	2036	2198	2382	2475	2716	3152	4005	3614	4134	4219	3403	3601
GS2-15-F6	2384	2487	2738	3440	3194	3237	3290	3494	3361	3458	3837	3432

Culture	3/1/2019	3/2/2019	3/3/2019	3/4/2019	3/5/2019	3/6/2019	3/7/2019	3/8/2019	3/9/2019	3/10/2019	3/11/2019	
GS2-15-F1	4482	3858	3580	3449	4508	3604	3468	4038	4021	3625	5075	
GS2-15-F2	3665	4425	3509	3556	3698	3367	3718	3678	3534	3604	5061	
GS2-15-F3	3536	3708	3973	4579	4159	3896	3394	3506	3469	3674	6144	
GS2-15-F4	4108	4046	4166	4682	3399	4422	3525	3270	3633	3700	4796	
GS2-15-F5	4281	4512	4369	3473	4418	3649	4331	3699	3675	3588	5025	
GS2-15-F6	3738	3573	3485	4082	3378	3419	3741	3609	3716	3780	5116	

The daily cell counts given by the Beckman Coulter Counter during GS2 at 15°C. cell mL^{-1} = (value x 1000)/(0.1/20.1))/500

Table C.5 *Daily Cell Counts at 20°C during GS2*

Culture	2/5/2019	2/6/2019	2/7/2019	2/8/2019	2/9/2019	2/10/2019	2/11/2019	2/12/2019	2/13/2019
GS2-20-F1	395	852	670	1317	1991	2911	1371	3154	4220
GS2-20-F2	373	448	660	1511	1833	2168	1178	3313	4020
GS2-20-F3	362	737	676	1328	2362	2001	1212	3442	3963
GS2-20-F4	367	456	586	1424	1647	1911	1350	2770	3714
GS2-20-F5	377	403	632	1449	1520	1979	907	2823	3722
GS2-20-F6	285	805	586	1230	1589	2087	959	2400	3408
Culture	2/14/2019	2/15/2019	2/16/2019	2/17/2019	2/18/2019	2/19/2019	2/20/2019	2/21/2019	2/22/2019
GS2-20-F1	5379	4628	4982	4735	4921	5265	5198	5240	7237
GS2-20-F2	5955	4652	4668	4957	4771	4830	4602	5040	10999
GS2-20-F3	5277	4495	4830	4822	4513	4875	4716	4720	7785
GS2-20-F4	4416	4805	4904	4459	4557	4606	4817	4719	8928
GS2-20-F5	4241	5026	9183	4863	5351	4622	4807	4526	9651
GS2-20-F6	4600	6499	4081	4890	5047	5276	6021	4969	9532

The daily cell counts given by the Beckman Coulter Counter during GS2 at 20°C. cell mL^{-1} = (value x 1000)/(0.1/20.1))/500

Table C.6 *Daily Cell Counts at 25°C during GS2*

Culture	2/5/2019	2/6/2019	2/7/2019	2/8/2019	2/9/2019	2/10/2019	2/11/2019	2/12/2019	2/13/2019	2/14/2019	2/15/2019
GS2-25-F1	290	362	548	1405	2246	2572	2987	3958	4176	5305	4680
GS2-25-F2	351	281	543	1428	1855	2265	2849	3717	3630	5035	4379
GS2-25-F3	254	286	568	1319	2237	2026	2722	3676	3511	5427	4310
GS2-25-F4	319	339	504	1378	2170	2533	2990	3889	4451	5368	4534
GS2-25-F5	380	441	520	1480	1787	2165	2832	3637	4465	5477	4496
GS2-25-F6	465	353	579	1329	1891	2376	2904	3884	4623	4681	4640
Culture	2/16/2019	2/17/2019	2/18/2019	2/19/2019	2/20/2019	2/21/2019	2/22/2019	2/23/2019	2/24/2019	2/25/2019	
GS2-25-F1	4902	4450	4932	5124	4710	5487	5183	5151	5081	5734	
GS2-25-F2	4540	5423	6068	4782	4650	4773	4454	5096	4948	5145	
GS2-25-F3	4284	4186	4598	4470	4617	4560	4558	4979	4477	4408	
GS2-25-F4	4876	4755	5149	4645	4605	4374	5288	4575	4948	4627	
GS2-25-F5	5139	4424	4852	4714	4701	4539	4791	4558	5845	4844	
GS2-25-F6	4639	4582	4929	4931	4665	4750	4899	4712	4692	4219	
Culture	2/26/2019	2/27/2019	2/28/2019	3/1/2019	3/2/2019	3/3/2019	3/4/2019	3/5/2019	3/6/2019	3/7/2019	
GS2-25-F1	4683	4882	4795	4534	4313	3828	3878	4443	4016	7474	
GS2-25-F2	4171	4114	4355	4572	4117	4258	3954	4544	4141	11177	
GS2-25-F3	5145	5159	5246	5185	5383	4996	5612	4259	4250	6657	
GS2-25-F4	4415	3932	4386	5011	4450	3826	5036	4059	4383	6754	
GS2-25-F5	4548	4888	4887	4504	5711	5410	4671	3806	5136	7151	
GS2-25-F6	4369	4237	4423	4662	4257	4514	4092	4497	5289	8998	

The daily cell counts given by the Beckman Coulter Counter during GS2 at 25°C. cell mL^{-1} = (value x 1000)/(0.1/20.1))/500

Table D.1 *Daily in vivo Fluorescence at 15°C during GS1*

Culture	10/12/2018	10/13/2018	10/14/2018	10/15/2018	10/16/2018	10/17/2018	10/18/2018	10/19/2018	10/20/2018	10/21/2018	10/22/2018	10/23/2018	10/24/2018	10/25/2018	10/26/2018	10/27/2018
GS1-15-F1	1.52	1.77	3.34	3.65	4.58	5.86	6.57	7.57	9.22	11.2	12.6	14.7	16	18.7	21.8	28.3
GS1-15-F2	1.52	1.78	3.08	3.25	3.91	4.64	5.45	6.06	7.27	7.73	9.3	10.6	11.3	13.2	14	18.1
GS1-15-F3	1.53	1.76	2.99	3.46	3.86	4.54	5.33	6.02	7.2	8.6	9.39	10.7	11.3	13.8	15.3	17.6
GS1-15-F4	1.49	1.74	2.93	3.14	3.54	4.22	4.74	5.28	5.95	7.36	7.65	9.11	9.51	11	12	15.1
GS1-15-F5	1.48	1.72	2.77	2.69	2.93	3.38	3.67	3.84	4.08	4.81	4.59	5.51	6.4	6.82	7.68	9.56
GS1-15-F6	1.49	1.73	2.91	3.05	3.25	3.75	4.29	4.55	4.91	5.39	5.8	7	7.8	9	10	12.1
Blank	0.091	0.086	0.076	0.079	0.079	0.086	0.081	0.083	0.083	0.086	0.085	0.087	0.088	0.084	0.084	0.088

Culture	10/28/2018	10/29/2018	10/30/2018	10/31/2018	11/01/2018	11/02/2018	11/03/2018	11/04/2018	11/05/2018	11/06/2018	11/07/2018	11/08/2018	11/09/2018	11/10/2018	11/11/2018	11/12/2018
GS1-15-F1	27.2	30.3	33.6	33.8	32.9	33	33.2	37.1	37.2	36.5	37.3	38.4	38.6	40.5	39.4	40.1
GS1-15-F2	17.4	19.3	22.3	22.3	25	24.6	25.6	28.8	27.4	28.1	29.4	30.7	31.8	32	32.7	33.1
GS1-15-F3	17.2	18.8	21.8	18.2	23.8	23.9	25.3	28	27.1	29.3	28.8	29.4	29.5	29.8	32	29.6
GS1-15-F4	14.9	17	17.8	19.9	20.4	21	23.1	26	26.4	26.7	27.7	27.8	27.9	28.6	28.7	29.4
GS1-15-F5	9.14	8.67	10.7	11.2	12.4	12.1	12.7	14.3	14.7	15.6	17	17.5	18.6	19.3	20.5	22.1
GS1-15-F6	11.9	14.3	14.2	17.1	17.4	17.8	17.7	20.9	21.3	20.8	23.6	25.1	27.2	27.8	27.6	29.4
Blank	0.083	0.089	0.083	0.078	0.075	0.085	0.086	0.084	0.083	0.111	0.088	0.08	0.11	0.085	0.085	0.08

Culture	11/13/2018	11/14/2018	11/15/2018	11/16/2018	11/17/2018	11/18/2018	11/19/2018	11/20/2018	11/21/2018	11/22/2018	11/23/2018	11/24/2018	11/25/2018	11/26/2018	11/27/2018	11/28/2018
GS1-15-F1	38.7	39.2	38.7	39.3	38.5	41.1	37.5	36.4	38.8	39.6	37.8	37.5	32.7	37.4	38.8	36.3
GS1-15-F2	35.3	35.7	35.4	36.1	34.7	32.2	35.3	35.4	35.4	34.8	43.4	35.4	31.9	38	37.2	36
GS1-15-F3	31.1	30	30.2	29.8	30.7	31.6	30.7	29.8	29.7	30.4	33.9	29.9	28.1	30.6	28	32.8
GS1-15-F4	29.8	30.7	32.2	31	29.6	32.7	32.5	32.6	31.4	32.2	36.7	31.5	30.7	30.7	32.6	31.6
GS1-15-F5	23.3	24.6	25.7	26.2	27.7	30.6	29.7	31.1	30.8	31.6	35.2	33	29.3	34.5	32.8	33.2
GS1-15-F6	30.6	31.1	31.5	34.4	34.3	34.9	34.4	35.6	32.9	33.5	37	31.9	33.2	35.2	32.9	33.3
Blank	0.08	0.081	0.08	0.078	0.072	0.124	0.096	0.079	0.075	0.077	0.082	0.074	0.077	0.074	0.076	0.08

Culture	11/29/2018	11/30/2018	12/01/2018	12/02/2018	12/03/2018	12/04/2018	12/05/2018	12/06/2018	12/07/2018	12/08/2018	12/09/2018	12/10/2018	12/11/2018	12/12/2018	12/13/2018	
GS1-15-F1	39.3	39.5	37.6	38.2	37.8	37.9	36.3	35.5	37.2	34.4	37.1	37.6	39.4	37.9	59.5	
GS1-15-F2	35.1	36.1	34.6	34.9	33.8	33.7	32.5	31.3	32.2	34.3	33.4	36.2	37.8	36.4	60.9	
GS1-15-F3	30.2	29.7	29.2	28.9	29.2	29.7	30.7	29.2	29.2	30.4	29.7	29.4	28.6	31	54.1	
GS1-15-F4	31.5	30.5	30	30.1	27.4	29	29.2	31.2	31.6	32.1	31.5	31.4	30.9	31.9	55.8	
GS1-15-F5	31.5	32.5	29.1	28.8	30.7	29.7	30.1	29.2	31.3	31.5	29.1	31.4	31.5	33.3	54	
GS1-15-F6	33	30.6	31.6	32	31.8	30.8	33.3	30.6	33.2	32.2	33.4	34	32.1	36.5	60.6	
Blank	0.085	0.08	0.08	0.078	0.077	0.074	0.072	0.073	0.073	0.073	0.079	0.074	0.075	0.077	0.07	

Output from the Turner Designs 10-AU Fluorometer for the daily *in vivo* fluorescence during GS1 at 15°C.

Table D.2 *Daily in vivo Fluorescence for 20°C during GS1*

Culture	10/12/2018	10/13/2018	10/14/2018	10/15/2018	10/16/2018	10/17/2018	10/18/2018	10/19/2018	10/20/2018	10/21/2018	10/22/2018	10/23/2018	10/24/2018	10/25/2018
GS1-20-F1	1.57	2.08	5.5	8.3	12.5	17.2	21.4	25.8	31	36.2	36.3	42.2	46.7	45.1
GS1-20-F2	1.65	2.14	5.37	7.83	11.4	16.3	20.9	26.3	30.1	41.2	42	43.2	43.2	43.9
GS1-20-F3	1.62	2.11	5.3	8.16	11.5	16.1	21.4	26.9	31.3	37	42.9	42.2	48.2	47
GS1-20-F4	1.57	2.06	5.37	7.93	11.9	16.9	21.1	27.6	31.3	34.6	41.3	43.5	45.5	45.1
GS1-20-F5	1.58	2.03	5.21	7.67	12.8	16.8	21.7	26.9	30.6	36	37.7	40	45	45.1
GS1-20-F6	1.64	2.02	4.64	6.99	11.5	15.3	19.7	25.4	29.9	38.2	38.1	39.6	45.2	47.1
Blank	**0.091**	**0.086**	**0.076**	**0.079**	**0.079**	**0.086**	**0.081**	**0.085**	**0.086**	**0.086**	**0.085**	**0.087**	**0.088**	**0.084**

Culture	10/26/2018	10/27/2018	10/28/2018	10/29/2018	10/30/2018	10/31/2018	11/01/2018	11/02/2018	11/03/2018	11/04/2018	11/05/2018	11/06/2018	11/07/2018	11/08/2018
GS1-20-F1	46	46.7	48.4	52.9	60.4	53.6	54.1	52	50.1	56.5	51.3	54.3	59.9	56.8
GS1-20-F2	45.9	62.6	47.9	56	57	50.5	60.4	48.8	48.4	53.5	49.4	49.2	54.9	53
GS1-20-F3	50.1	52.6	48.1	57	62.2	58.8	59.3	53	53.4	56.4	52.1	53.5	56.7	55.6
GS1-20-F4	48	64.7	46.9	51.3	60.4	68	62.8	50.7	50.4	54.9	48.9	50.7	52.3	50.9
GS1-20-F5	46.3	47.4	47.4	54.1	59.3	56.8	62.2	51.9	50.7	52.8	49	49.3	52.8	50.4
GS1-20-F6	45.6	45	46.2	52	50.8	54.4	52.4	53.3	52.9	56.3	52.2	54.1	53.7	50.3
Blank	**0.084**	**0.088**	**0.083**	**0.089**	**0.083**	**0.078**	**0.075**	**0.085**	**0.086**	**0.084**	**0.083**	**0.111**	**0.088**	**0.08**

Culture	11/09/2018	11/10/2018	11/11/2018	11/12/2018	11/13/2018	11/14/2018	11/15/2018	11/16/2018	11/17/2018	11/18/2018	11/19/2018	11/20/2018		
GS1-20-F1	56.6	57	57.1	60.5	60.5	57.66	58	55.7	56.3	56	57.6	74.5		
GS1-20-F2	52.4	54.2	55.2	54	53.9	54.4	56.6	54	54.4	53.9	54.8	70.9		
GS1-20-F3	53.9	54.9	55.1	55.4	54.1	54.8	56.8	54.2	53.3	52.3	53.7	68.2		
GS1-20-F4	48.6	47.8	47.2	47.5	48	47.5	48.8	47.7	46.7	47.7	49.6	60.5		
GS1-20-F5	50.8	48	49.5	49	49.7	50	48.3	49.5	48.6	48.9	50.2	59.6		
GS1-20-F6	50.5	49.9	51.2	50.8	50.5	50.6	49.6	49.7	47.3	47.8	47.1	60.7		
Blank	**0.11**	**0.085**	**0.085**	**0.08**	**0.08**	**0.081**	**0.08**	**0.078**	**0.072**	**0.124**	**0.096**	**0.079**		

Output from the Turner Designs 10-AU Fluorometer for the daily *in vivo* fluorescence during GS1 at 20°C.

Table D.3 *Daily in vivo Fluorescence at 25°C during GS1*

Culture	10/12/2018	10/13/2018	10/14/2018	10/15/2018	10/16/2018	10/17/2018	10/18/2018	10/19/2018	10/20/2018	10/21/2018	10/22/2018	10/23/2018	10/24/2018	10/25/2018	10/26/2018
GS1-25-F1	1.6	2.24	7	11.6	17.6	26.9	32.3	41.7	44	46.9	49.9	53	54..4	54.8	54.3
GS1-25-F2	1.67	2.33	7.25	12.3	18.2	27.7	34.7	40.8	43	46.9	49.4	52.6	53.5	55.3	54.2
GS1-25-F3	1.67	2.29	7.01	11.7	16.4	25.8	31.9	38.6	41.8	44.1	46.5	51.8	53.4	53.6	54.3
GS1-25-F4	1.72	2.35	7.43	12.8	20.4	28.2	34.4	30.9	41.2	43	47.3	50.2	51.6	52.9	51.8
GS1-25-F5	1.66	2.37	7.03	12.7	17.9	26.3	32.7	36.6	41	43.5	45.2	49	52	52.5	53.5
GS1-25-F6	1.64	2.29	7.03	12.2	18.6	26.5	32.9	37.7	41.6	45	49.1	52	54.2	54.6	55
Blank	0.091	0.086	0.076	0.079	0.079	0.086	0.081	0.085	0.086	0.086	0.085	0.087	0.088	0.084	0.084
Culture	10/27/2018	10/28/2018	10/29/2018	10/30/2018	10/31/2018	11/01/2018	11/02/2018	11/03/2018	11/04/2018	11/05/2018	11/06/2018	11/07/2018	11/08/2018	11/09/2018	11/10/2018
GS1-25-F1	56.6	55.6	56.8	58	59	59.8	57.5	55	58.2	56.1	60.4	55.3	55.9	54.7	54.6
GS1-25-F2	57	52.3	55.1	57.5	56.6	54.3	57.1	54.3	55.9	53.3	54.6	55.8	55.7	56.5	57
GS1-25-F3	53.8	54.3	54	63.5	57	58.1	56.8	53	56.9	51.9	51.9	53.6	55.8	54.6	56.4
GS1-25-F4	56.8	50.1	51	56.6	55.1	51.6	52.8	51.2	52.1	52.1	52.1	51.3	51.7	52.1	53.2
GS1-25-F5	53.6	51.8	53.7	64.6	55.4	56.2	56.1	55	52.7	53.9	53.9	52.9	54	53.6	56.1
GS1-25-F6	54.2	53.5	56	63.1	59	55.8	55.3	55.7	54.9	53.1	53.1	54.2	55.3	58.2	58
Blank	0.088	0.083	0.089	0.083	0.078	0.075	0.085	0.086	0.084	0.083	0.111	0.088	0.08	0.11	0.085
Culture	11/11/2018	11/12/2018	11/13/2018	11/14/2018	11/15/2018	11/16/2018	11/17/2018	11/18/2018	11/19/2018	11/20/2018	11/21/2018	11/22/2018	11/23/2018		
GS1-25-F1	51.2	56.5	55.2	54.1	53.8	53.1	57.1	54.5	555.5	57.5	57.3	57.1	79.3		
GS1-25-F2	55.2	58.2	57.3	58.3	56.3	57.4	58.2	59.8	59.9	61.3	59.3	58.8	80.1		
GS1-25-F3	54.7	56	55.6	56	57.1	56.6	56.6	55.5	57.8	56.4	58.5	57.4	80.5		
GS1-25-F4	52.8	55.6	55.1	54	552.5	53.6	60.6	57	57.8	57.4	55.5	55.2	78		
GS1-25-F5	57.3	56.1	57.3	58	56.2	57.2	59.8	58.4	59.8	58.4	57.5	56.1	80.5		
GS1-25-F6	56	56.2	59.3	57.6	56.7	55.9	55.8	59.8	59.7	59.8	56.8	55.2	80.5		
Blank	0.085	0.08	0.08	0.081	0.08	0.078	0.072	0.124	0.096	0.079	0.075	0.077	0.082		

Output from the Turner Designs 10-AU Fluorometer for the daily *in vivo* fluorescence during GS1 at 25°C.

Table D.4 *Daily in vivo Fluorescence at 15°C during GS2*

Culture	2/6/2019	2/7/2019	2/8/2019	2/9/2019	2/10/2019	2/11/2019	2/12/2019	2/13/2019	2/14/2019	2/15/2019	2/16/2019	2/17/2019
GS2-15-F1	2.6	3.64	7.3	8.53	11	13.6	16.6	19.4	23.7	26.7	29.6	33.1
GS2-15-F2	2.59	3.5	6.82	7.99	9.99	11.4	13.9	16.7	19.3	22.3	26.7	27.8
GS2-15-F3	2.65	3.54	6.45	7.51	9.04	10.4	12.6	15.4	17.9	30.7	23.3	24.4
GS2-15-F4	2.59	3.59	6.4	7.27	8.68	10.4	11.7	14.4	16.2	18.9	21.6	22.2
GS2-15-F5	2.57	3.46	5.61	6.26	7.13	8.1	9.28	10.7	12.4	14.2	16.6	17.9
GS2-15-F6	2.52	3.48	5.96	6.54	8.05	9.46	10.5	12.9	14.8	16.6	19	21.5
Blank	0.12	0.132	0.108	0.101	0.099	0.103	0.108	0.125	0.114	0.118	0.113	0.12
Culture	2/18/2019	2/19/2019	2/20/2019	2/21/2019	2/22/2019	2/23/2019	2/24/2019	2/25/2019	2/26/2019	2/27/2019	2/28/2019	3/1/2019
GS2-15-F1	35.1	35.4	36.7	39	38.1	39.5	38.8	40.1	40.5	43.1	40.2	46.5
GS2-15-F2	31.5	32.5	35.6	36.5	35.5	35.2	36.3	36.7	35	36.6	40.7	41.7
GS2-15-F3	28.5	29.2	32.2	31.2	30.6	32.1	32	31.1	32.2	34.4	344	35.8
GS2-15-F4	26.2	26.7	29.7	28.8	29.5	29.7	30.7	29.7	29.9	30.2	31.2	32.7
GS2-15-F5	21.1	21.8	25.3	24.4	26.4	27.9	28.6	28.5	29.5	31.5	34.3	33.5
GS2-15-F6	23.9	25	27.7	29.2	28.7	33	32.4	31	33	34.9	33.3	39.3
Blank	0.123	0.125	0.123	0.125	0.133	0.124	0.125	0.126	0.123	0.126	0.122	0.124
Culture	3/2/2019	3/3/2019	3/4/2019	3/5/2019	3/6/2019	3/7/2019	3/8/2019	3/9/2019	3/10/2019	3/11/2019		
GS2-15-F1	47.8	39.7	41.9	41.6	42.3	45.7	48.1	45.1	46.3	70.4		
GS2-15-F2	44.2	40	40.1	38.1	39.2	42	44.6	41.3	41.7	68.2		
GS2-15-F3	35.7	35.2	35.4	35.7	35.2	37.4	38.1	41.7	37.9	62.2		
GS2-15-F4	34.2	32.3	32.6	32	32.2	34.6	35.9	39.1	34.9	64.4		
GS2-15-F5	35.8	35.8	33.4	32.1	32.2	38.7	38.2	41.1	34.7	62		
GS2-15-F6	37.8	36.3	38.4	36.1	36.5	38.9	40	40.4	38.9	66		
Blank	0.122	0.125	0.123	0.126	0.121	0.123	0.124	0.121	0.125	0.121		

Output from the Turner Designs 10-AU Fluorometer for the daily *in vivo* fluorescence during GS2 at 15°C.

Table D.5 *Daily in vivo Fluorescence at 20°C during GS2*

Culture	2/6/2019	2/7/2019	2/8/2019	2/9/2019	2/10/2019	2/11/2019	2/12/2019	2/13/2019	2/14/2019
GS2-20-F1	5.59	7.39	16.4	21.3	27	33	42	45.2	47.5
GS2-20-F2	5.31	7.3	15.7	20.8	26.8	34.2	39.7	41.7	46.4
GS2-20-F3	5.55	7.22	15.9	20.8	26.6	33.7	40.3	42.9	46.1
GS2-20-F4	5.37	6.74	14.2	17.5	22.2	28.1	33.3	36.8	38.7
GS2-20-F5	5.47	6.92	14.2	17.8	23.3	29.8	34.7	37.1	40.9
GS2-20-F6	5.5	6.75	13.9	17.7	23.7	25.1	30.6	34.6	38.6
Blank	0.12	0.132	0.108	0.101	0.099	0.103	0.108	0.125	0.114
Culture	2/15/2019	2/16/2019	2/17/2019	2/18/2019	2/19/2019	2/20/2019	2/21/2019	2/22/2019	
GS2-20-F1	49.5	51.7	54.2	54.2	54.5	51.5	51	69.6	
GS2-20-F2	49.3	49.4	54.8	54.8	52.8	52	50.1	68.7	
GS2-20-F3	47.5	50.3	51.4	51.4	50.1	48.3	48.9	71.2	
GS2-20-F4	41.6	42.9	46.7	46.7	44.4	44.3	43.1	63	
GS2-20-F5	41.7	45.8	49.5	49.5	46.5	46.2	47.3	65.6	
GS2-20-F6	40.2	42.8	46.3	46.3	44.5	41.9	44	60.2	
Blank	0.118	0.113	0.12	0.123	0.125	0.123	0.126	0.133	

Output from the Turner Designs 10-AU Fluorometer for the daily *in vivo* fluorescence during GS2 at 20°C.

Table D.6 *Daily in vivo Fluorescence at 25°C during GS2*

Culture	2/6/2019	2/7/2019	2/8/2019	2/9/2019	2/10/2019	2/11/2019	2/12/2019	2/13/2019	2/14/2019	2/15/2019
GS2-25-F1	4.12	5.81	14.7	21.1	26.7	32.2	39.7	42.6	43.4	45
GS2-25-F2	4.09	5.89	15.1	20.3	26.6	311.3	38.4	39.6	43.2	46
GS2-25-F3	4.04	5.83	14.4	19.6	24	29.4	35.7	41.1	40.7	43.6
GS2-25-F4	4.01	5.72	14.2	20.2	26.9	32.7	38.8	40.8	42.6	46.2
GS2-25-F5	3.99	5.51	13.6	18.7	25	30.4	35.5	40.8	39.4	46.8
GS2-25-F6	3.86	5.52	13.6	19.1	24.8	30.7	34.9	40	41.5	44.4
Blank	0.12	0.132	0.108	0.101	0.099	0.103	0.108	0.125	0.114	0.118
Culture	2/16/2019	2/17/2019	2/18/2019	2/19/2019	2/20/2019	2/21/2019	2/22/2019	2/23/2019	2/24/2019	2/25/2019
GS2-25-F1	45.8	47.2	47.9	48.3	49.2	49.3	49.7	48.1	51.7	52.6
GS2-25-F2	47.5	46.7	46.9	49.3	50.2	49.3	49.8	48.1	51.3	52.2
GS2-25-F3	45.3	44	46	46	48.2	45.3	51.9	47.1	48.7	49.8
GS2-25-F4	48.7	47.2	50.1	47	48.7	47	50.8	50.9	53.6	52.9
GS2-25-F5	48.9	47.5	48.4	49.1	48.5	47	50.2	53.6	52.2	53
GS2-25-F6	46.2	46.3	48.5	48.9	49.7	50.7	50.3	56.5	53.6	57.3
Blank	0.113	0.12	0.123	0.125	0.123	0.126	0.133	0.124	0.125	0.126
Culture	2/26/2019	2/27/2019	2/28/2019	3/1/2019	3/2/2019	3/3/2019	3/4/2019	3/5/2019	3/6/2019	3/7/2019
GS2-25-F1	52.6	53.6	52	52.9	53.1	49.9	48.6	47.1	51.1	81.3
GS2-25-F2	50.9	522.8	54.1	54	53.8	55.2	51.3	50.3	58	99.5
GS2-25-F3	48.2	46.5	44.9	52.6	47.5	57.3	52.1	46.4	58.5	90
GS2-25-F4	53.7	47.8	53.8	53.5	48.3	53.1	51.2	50	60.5	84.9
GS2-25-F5	48.1	44	45.2	56.4	46.3	51.6	45.3	44.9	67.5	79.5
GS2-25-F6	52.3	51.6	52.6	48.5	50	48.5	53.4	50	68.5	79.3
Blank	0.123	0.126	0.122	0.124	0.122	0.125	0.123	0.126	0.121	0.123

Output from the Turner Designs 10-AU Fluorometer for the daily *in vivo* fluorescence during GS2 at 25°C.

APPENDIX E Extracted chl *a* Data

Table E.1 *Extracted chl a at 15°C during GS1*

Dilution Culture	Reading	Blank Corrected	Chl *a* (µg/L)	Harvest Culture	Reading	Blank Corrected	Chl *a* (µg/L)
GS1-15-F1.1	6.83	6.58	500	GS1-15-F1.1	8.43	8.25	628
GS1-15-F1.2	6.64	6.39	486	GS1-15-F1.2	8.44	8.26	629
GS1-15-F1.3	6.43	6.18	470	GS1-15-F1.3	8.29	8.11	617
GS1-15-F2.1	6.03	5.78	440	GS1-15-F2.1	7.99	7.81	594
GS1-15-F2.2	5.9	5.65	430	GS1-15-F2.2	8.12	7.94	604
GS1-15-F2.3	5.95	5.70	433	GS1-15-F2.3	8.29	8.11	617
GS1-15-F3.1	5.29	5.04	383	GS1-15-F3.1	7.17	6.99	532
GS1-15-F3.2	5.03	4.78	364	GS1-15-F3.2	7.28	7.10	540
GS1-15-F3.3	5.08	4.83	367	GS1-15-F3.3	7.2	7.02	534
GS1-15-F4.1	5.3	5.05	384	GS1-15-F4.1	7.28	7.10	540
GS1-15-F4.2	5.27	5.02	382	GS1-15-F4.2	7.69	7.51	571
GS1-15-F4.3	5.23	4.98	379	GS1-15-F4.3	7.29	7.11	541
GS1-15-F5.1	5.55	5.30	403	GS1-15-F5.1	7.74	7.56	575
GS1-15-F5.2	5.29	5.04	383	GS1-15-F5.2	7.69	7.51	571
GS1-15-F5.3	5.25	5.00	380	GS1-15-F5.3	7.77	7.59	578
GS1-15-F6.1	5.41	5.16	392	GS1-15-F6.1	8.09	7.91	602
GS1-15-F6.2	5.48	5.23	398	GS1-15-F6.2	7.86	7.68	584
GS1-15-F6.3	5.4	5.15	392	GS1-15-F6.3	8.18	8.00	609
SWB1	0.296			SWB1	0.175		
SWB2	0.219			SWB2	0.175		
SWB3	0.238			SWB3	0.180		
SWB Avg	**0.251**			**SWB Avg**	**0.177**		

Turner Designs 10-AU Fluorometer extracted chl *a* readings from GS1 at 15°C. Chl *a* was calculated using the equation in Methods Section 2.3.

Table E.2 *Extracted chl a at 20°C during GS1*

Dilution Culture	Reading	Blank Corrected	Chl *a* (µg/L)	Harvest Culture	Reading	Blank Corrected	Chl *a* (µg/L)
GS1-20-F1.1	12.2	12.1	917	GS1-20-F1.1	15.3	15.2	1153
GS1-20-F1.2	12.4	12.3	932	GS1-20-F1.2	15.3	15.2	1153
GS1-20-F1.3	12.4	12.3	932	GS1-20-F1.3	15.7	15.6	1183
GS1-20-F2.1	12	11.9	902	GS1-20-F2.1	15	14.9	1130
GS1-20-F2.2	11.6	11.5	871	GS1-20-F2.2	15.1	15.0	1138
GS1-20-F2.3	11.7	11.6	879	GS1-20-F2.3	14.9	14.8	1123
GS1-20-F3.1	11.5	11.4	864	GS1-20-F3.1	14.2	14.1	1069
GS1-20-F3.2	11.5	11.4	864	GS1-20-F3.2	14.5	14.4	1092
GS1-20-F3.3	11.6	11.5	871	GS1-20-F3.3	14.6	14.5	1100
GS1-20-F4.1	10.1	10.0	757	GS1-20-F4.1	13	12.9	978
GS1-20-F4.2	10.1	10.0	757	GS1-20-F4.2	12.7	12.6	955
GS1-20-F4.3	10.2	10.1	765	GS1-20-F4.3	13	12.9	978
GS1-20-F5.1	10.8	10.7	810	GS1-20-F5.1	13.7	13.6	1031
GS1-20-F5.2	10.7	10.6	803	GS1-20-F5.2	14.1	14.0	1062
GS1-20-F5.3	10.7	10.6	803	GS1-20-F5.3	14	13.9	1054
GS1-20-F6.1	10.3	10.2	772	GS1-20-F6.1	12.9	12.8	970
GS1-20-F6.2	10.4	10.3	780	GS1-20-F6.2	12.8	12.7	963
GS1-20-F6.3	10.4	10.3	780	GS1-20-F6.3	12.8	12.7	963
SWB1	0.146			SWB1	0.143		
SWB2	0.145			SWB2	0.14		
SWB3	0.146			SWB3	0.143		
SWB Avg	**0.146**			**SWB Avg**	**0.142**		

Turner Designs 10-AU Fluorometer extracted chl *a* readings from GS1 at 20°C. Chl *a* was calculated using the equation in Methods Section 2.3.

Table E.3 *Extracted chl a at 25°C during GS1*

Dilution Culture	Reading	Blank Corrected	Chl *a* (µg/L)	Harvest Culture	Reading	Blank Corrected	Chl *a* (µg/L)
GS1-25-F1.1	14.4	14.2	1084	GS1-25-F1.1	17.1	16.9	1286
GS1-25-F1.2	14.6	14.4	1099	GS1-25-F1.2	17.7	17.5	1332
GS1-25-F1.3	14.5	14.3	1092	GS1-25-F1.3	17.3	17.1	1301
GS1-25-F2.1	15.3	15.1	1152	GS1-25-F2.1	17.3	17.1	1301
GS1-25-F2.2	15.3	15.1	1152	GS1-25-F2.2	18	17.8	1354
GS1-25-F2.3	15.6	15.4	1175	GS1-25-F2.3	18.7	18.5	1408
GS1-25-F3.1	15.2	15.0	1145	GS1-25-F3.1	17.3	17.1	1301
GS1-25-F3.2	15.1	14.9	1137	GS1-25-F3.2	17	16.8	1278
GS1-25-F3.3	15.3	15.1	1152	GS1-25-F3.3	17.3	17.1	1301
GS1-25-F4.1	14.1	13.9	1061	GS1-25-F4.1	16.1	15.9	1210
GS1-25-F4.2	14.2	14.0	1069	GS1-25-F4.2	16.2	16.0	1217
GS1-25-F4.3	14.4	14.2	1084	GS1-25-F4.3	16.5	16.3	1240
GS1-25-F5.1	14.4	14.2	1084	GS1-25-F5.1	17.3	17.1	1301
GS1-25-F5.2	14.5	14.3	1092	GS1-25-F5.2	17.8	17.6	1339
GS1-25-F5.3	15	14.8	1130	GS1-25-F5.3	17.6	17.4	1324
GS1-25-F6.1	14.6	14.4	1099	GS1-25-F6.1	17	16.8	1278
GS1-25-F6.2	15.1	14.9	1137	GS1-25-F6.2	17.1	16.9	1286
GS1-25-F6.3	14.9	14.7	1122	GS1-25-F6.3	17.9	17.7	1347
SWB1	0.152			SWB1	0.192		
SWB2	0.148			SWB2	0.201		
SWB3	0.151			SWB3	0.190		
SWB AVG	**0.150**			**SWB AVG**	**0.194**		

Turner Designs 10-AU Fluorometer extracted chl *a* readings from GS1 at 25°C. Chl *a* was calculated using the equation in Methods Section 2.3.

Table E.4 *Extracted chl a at 15°C during GS2*

Dilution Culture	Reading	Blank Corrected	Chl *a* (µg/L)	Harvest Culture	Reading	Blank Corrected	Chl *a* (µg/L)
GS2-15-F1.1	6.03	5.87	446	GS2-15-F1.1	8.96	8.79	668
GS2-15-F1.2	6.34	6.18	470	GS2-15-F1.2	8.95	8.78	667
GS2-15-F1.3	6.18	6.02	458	GS2-15-F1.3	8.92	8.75	665
GS2-15-F2.1	5.84	5.68	432	GS2-15-F2.1	8.73	8.56	651
GS2-15-F2.2	5.91	5.75	437	GS2-15-F2.2	8.77	8.60	654
GS2-15-F2.3	5.82	5.66	430	GS2-15-F2.3	8.54	8.37	636
GS2-15-F3.1	5.51	5.35	407	GS2-15-F3.1	7.61	7.44	566
GS2-15-F3.2	5.47	5.31	404	GS2-15-F3.2	7.55	7.38	561
GS2-15-F3.3	5.51	5.35	407	GS2-15-F3.3	7.6	7.43	565
GS2-15-F4.1	5.31	5.15	392	GS2-15-F4.1	7.24	7.07	537
GS2-15-F4.2	5.2	5.04	383	GS2-15-F4.2	7.45	7.28	553
GS2-15-F4.3	5.31	5.15	392	GS2-15-F4.3	7.44	7.27	553
GS2-15-F5.1	5.59	5.43	413	GS2-15-F5.1	7.56	7.39	562
GS2-15-F5.2	5.45	5.29	402	GS2-15-F5.2	7.45	7.28	553
GS2-15-F5.3	5.53	5.37	408	GS2-15-F5.3	7.94	7.77	591
GS2-15-F6.1	5.56	5.40	411	GS2-15-F6.1	7.97	7.80	593
GS2-15-F6.2	5.63	5.47	416	GS2-15-F6.2	8.08	7.91	601
GS2-15-F6.3	5.67	5.51	419	GS2-15-F6.3	7.97	7.80	593
SWB1	0.16			SWB1	0.175		
SWB2	0.163			SWB2	0.183		
SWB3	0.167			SWB3	0.17		
SWB AVG	**0.163**			**SWB AVG**	**0.175**		

Turner Designs 10-AU Fluorometer extracted chl *a* readings from GS2 at 15°C. Chl *a* was calculated using the equation in Methods Section 2.3.

Table E.5 *Extracted chl a at 20°C during GS2*

Dilution Culture	Reading	Blank Corrected	Chl *a* (µg/L)	Harvest Culture	Reading	Blank Corrected	Chl *a* (µg/L)
GS2-20-F1.1	10.7	10.5	800	GS2-20-F1.1	14.6	14.4	1098
GS2-20-F1.2	10.6	10.4	792	GS2-20-F1.2	14.3	14.1	1075
GS2-20-F1.3	10.8	10.6	808	GS2-20-F1.3	14.6	14.4	1098
GS2-20-F2.1	10.7	10.5	800	GS2-20-F2.1	14.1	13.9	1060
GS2-20-F2.2	10.5	10.3	785	GS2-20-F2.2	14.2	14.0	1067
GS2-20-F2.3	10.4	10.2	777	GS2-20-F2.3	13.9	13.7	1044
GS2-20-F3.1	9.79	9.61	731	GS2-20-F3.1	14.4	14.2	1082
GS2-20-F3.2	9.82	9.64	733	GS2-20-F3.2	14.2	14.0	1067
GS2-20-F3.3	9.99	9.81	746	GS2-20-F3.3	13.9	13.7	1044
GS2-20-F4.1	8.63	8.45	643	GS2-20-F4.1	12.9	12.7	968
GS2-20-F4.2	8.66	8.48	645	GS2-20-F4.2	12.9	12.7	968
GS2-20-F4.3	8.72	8.54	649	GS2-20-F4.3	13	12.8	976
GS2-20-F5.1	8.91	8.73	664	GS2-20-F5.1	12.9	12.7	968
GS2-20-F5.2	8.82	8.64	657	GS2-20-F5.2	12.2	12.0	915
GS2-20-F5.3	9.12	8.94	680	GS2-20-F5.3	12.7	12.5	953
GS2-20-F6.1	9.3	9.12	693	GS2-20-F6.1	11.6	11.4	869
GS2-20-F6.2	8.45	8.27	629	GS2-20-F6.2	11.9	11.7	892
GS2-20-F6.3	8.28	8.10	616	GS2-20-F6.3	11.6	11.4	869
SWB1	0.183			SWB1	0.169		
SWB2	0.186			SWB2	0.16		
SWB3	0.166			SWB3	0.170		
SWB AVG	**0.183**			**SWB AVG**	**0.169**		

Turner Designs 10-AU Fluorometer extracted chl *a* readings from GS2 at 20°C. Chl *a* was calculated using the equation in Methods Section 2.3.

Table E.6 *Extracted chl a at 25°C during GS2*

Dilution Culture	Reading	Blank Corrected	Chl *a* (µg/L)	Harvest Culture	Reading	Blank Corrected	Chl *a* (µg/L)
GS2-25-F1.1	9.89	9.64	733	GS2-25-F1.1	16.5	16.3	1240
GS2-25-F1.2	10.1	9.85	749	GS2-25-F1.2	17.9	17.7	1346
GS2-25-F1.3	9.75	9.50	723	GS2-25-F1.3	18	17.8	1354
GS2-25-F2.1	11.3	11.1	841	GS2-25-F2.1	19.7	19.5	1483
GS2-25-F2.2	11.8	11.6	879	GS2-25-F2.2	21	20.8	1582
GS2-25-F2.3	11.5	11.3	856	GS2-25-F2.3	20.3	20.1	1529
GS2-25-F3.1	10.3	10.1	765	GS2-25-F3.1	17.3	17.1	1301
GS2-25-F3.2	10.6	10.4	787	GS2-25-F3.2	17.7	17.5	1331
GS2-25-F3.3	10.3	10.1	765	GS2-25-F3.3	18	17.8	1354
GS2-25-F4.1	11	10.8	818	GS2-25-F4.1	17.5	17.3	1316
GS2-25-F4.2	11.4	11.2	848	GS2-25-F4.2	18	17.8	1354
GS2-25-F4.3	10.8	10.6	803	GS2-25-F4.3	18.2	18.0	1369
GS2-25-F5.1	10	9.75	742	GS2-25-F5.1	16.1	15.9	1210
GS2-25-F5.2	10.3	10.1	765	GS2-25-F5.2	16.6	16.4	1248
GS2-25-F5.3	10.1	9.85	749	GS2-25-F5.3	15.6	15.4	1172
GS2-25-F6.1	11.2	11.0	833	GS2-25-F6.1	14.6	14.4	1095
GS2-25-F6.2	11.3	11.1	841	GS2-25-F6.2	14.5	14.3	1088
GS2-25-F6.3	11.1	10.9	825	GS2-25-F6.3	14.8	14.6	1111
SWB1	0.262			SWB1	0.199		
SWB2	0.249			SWB2	0.197		
SWB3	0.24			SWB3	0.198		
SWB AVG	**0.249**			**SWB AVG**	**0.198**		

Turner Designs 10-AU Fluorometer extracted chl *a* readings from GS2 at 25°C. Chl *a* was calculated using the equation in Methods Section 2.3.

APPENDIX F Ash Free Dry Weight Raw Data

Table F.1 *Filter Weights for 15°C AFDW during GS1*

Sample	Filter #	Volume Filtered (mL)	Original Filter Weight			Avg Filter Weight	Dry Weight			Avg Dry Weight	Ashed Weight			Avg Ashed Weight
GS1 Dilution Phase at 15°C														
F1.1	111	70	0.0934	0.0935	0.0934	0.0934	0.0966	0.0966	0.0966	0.0966	0.0938	0.0938	0.0938	0.0938
F1.2	113	80	0.0939	0.0939	0.0939	0.0939	0.0966	0.0966	0.0966	0.0966	0.0936	0.0936	0.0936	0.0936
F1.3	114	80	0.0949	0.0949	0.0949	0.0949	0.0980	0.0980	0.0980	0.0980	0.0949	0.0949	0.0949	0.0949
F2.1	115	90	0.0937	0.0937	0.0937	0.0937	0.0971	0.0971	0.0971	0.0971	0.0940	0.0940	0.0940	0.0940
F2.2	108	80	0.0950	0.0950	0.0950	0.0950	0.0978	0.0978	0.0978	0.0978	0.0951	0.0951	0.0951	0.0951
F2.3	122	70	0.0940	0.0940	0.0940	0.0940	0.0964	0.0964	0.0964	0.0964	0.0939	0.0939	0.0939	0.0939
F3.1	121	80	0.0941	0.0941	0.0941	0.0941	0.0980	0.0980	0.0980	0.0980	0.0950	0.0950	0.0950	0.0950
F3.2	120	80	0.0941	0.0941	0.0941	0.0941	0.0966	0.0966	0.0966	0.0966	0.0941	0.0941	0.0941	0.0941
F3.3	117	70	0.0930	0.0930	0.0930	0.0930	0.0951	0.0951	0.0951	0.0951	0.0929	0.0929	0.0929	0.0929
F4.1	104	80	0.9362	0.0936	0.0936	0.0936	0.0962	0.0962	0.0962	0.0962	0.0935	0.0935	0.0935	0.0935
F4.2	116	80	0.0949	0.0949	0.0949	0.0949	0.0976	0.0968	0.0977	0.0976	0.0950	0.0950	0.0950	0.0950
F4.3	106	80	0.0938	0.0938	0.0938	0.0938	0.0963	0.0963	0.0963	0.0963	0.0938	0.0938	0.0938	0.0938
F5.1	105	80	0.0951	0.0951	0.0951	0.0951	0.0983	0.0983	0.0983	0.0983	0.0955	0.0956	0.0955	0.0955
F5.2	119	80	0.0916	0.0916	0.0916	0.0916	0.0945	0.0945	0.0945	0.0945	0.0918	0.0918	0.0919	0.0918
F5.3	112	82	0.0959	0.0959	0.0959	0.0959	0.0986	0.0986	0.0986	0.0986	0.0959	0.0959	0.0959	0.0959
F6.1	107	80	0.0958	0.0958	0.0958	0.0958	0.0991	0.0991	0.0991	0.0991	0.0964	0.0963	0.0964	0.0964
F6.2	118	80	0.0941	0.0941	0.0941	0.0941	0.0968	0.0968	0.0968	0.0968	0.0942	0.0942	0.0942	0.0942
F6.3	109	82	0.0952	0.0952	0.0952	0.0952	0.0981	0.0981	0.0981	0.0981	0.0953	0.0953	0.0953	0.0953
GS1 Harvest Phase at 15°C														
F1.1	136	110	0.0944	0.0944	0.0944	0.0944	0.0999	0.0999	0.0999	0.0999	0.0947	0.0947	0.0947	0.0947
F1.2	138	110	0.0941	0.0941	0.0941	0.0941	0.1005	0.1005	0.1005	0.1005	0.0942	0.0942	0.0942	0.0942
F1.3	133	120	0.0940	0.0940	0.0940	0.0940	0.1004	0.1004	0.1004	0.1004	0.0941	0.0941	0.0941	0.0941
F2.1	135	120	0.0934	0.0934	0.0934	0.0934	0.0994	0.0994	0.0994	0.0994	0.0934	0.0934	0.0934	0.0934
F2.2	139	140	0.0923	0.0923	0.0923	0.0923	0.0996	0.0996	0.0996	0.0996	0.0928	0.0928	0.0928	0.0928
F2.3	140	130	0.0926	0.0926	0.0926	0.0926	0.0987	0.0987	0.0987	0.0987	0.0927	0.0927	0.0927	0.0927
F3.1	127	100	0.0936	0.0936	0.0936	0.0936	0.0979	0.0979	0.0979	0.0979	0.0935	0.0935	0.0935	0.0935
F3.2	137	140	0.0943	0.0943	0.0943	0.0943	0.1000	0.1000	0.1000	0.1000	0.0944	0.0944	0.0944	0.0944
F3.3	126	130	0.0934	0.0934	0.0934	0.0934	0.0989	0.0989	0.0989	0.0989	0.0933	0.0933	0.0933	0.0933
F4.1	134	140	0.0960	0.0960	0.0960	0.0960	0.1025	0.1025	0.1025	0.1025	0.0962	0.0962	0.0962	0.0962
F4.2	132	140	0.0947	0.0947	0.0947	0.0947	0.1008	0.1008	0.1008	0.1008	0.0950	0.0950	0.0950	0.0950
F4.3	131	110	0.0947	0.0947	0.0947	0.0947	0.1008	0.1008	0.1008	0.1008	0.0951	0.0951	0.0951	0.0951
F5.1	129	120	0.0929	0.0929	0.0928	0.0929	0.0984	0.0984	0.0983	0.0984	0.0928	0.0928	0.0928	0.0928
F5.2	130	140	0.0933	0.0933	0.0933	0.0933	0.1000	0.1000	0.1000	0.1000	0.0936	0.0936	0.0936	0.0936
F5.3	125	150	0.0965	0.0965	0.0965	0.0965	0.1031	0.1031	0.1031	0.1031	0.0964	0.0964	0.0964	0.0964
F6.1	128	150	0.0945	0.0945	0.0945	0.0945	0.1015	0.1015	0.1015	0.1015	0.0945	0.0945	0.0945	0.0945
F6.2	124	150	0.0941	0.0941	0.0941	0.0941	0.1019	0.1019	0.1019	0.1019	0.0942	0.0942	0.0942	0.0942
F6.3	123	150	0.0936	0.0936	0.0936	0.0936	0.1007	0.1007	0.1007	0.1007	0.0937	0.0937	0.0937	0.0937

Filter weights used to calculate the AFDW during GS1 at 15°C. Samples are labeled as Flask number then subsample number (Flask 1 subsample 1 = F1.1).

Table F.2 *Filter Weights for 20°C AFDW during GS1*

GS1 Dilution Phase at 20°C														
Sample	Filter #	Volume Filtered (mL)	Original Filter Weight			Avg Filter Weight	Dry Weight			Avg Dry Weight	Ashed Weight			Avg Ashed Weight
F1.1	15	5	0.0974	0.0973	0.0973	0.0973	0.0976	0.0976	0.0976	0.0976	0.0972	0.0972	0.0972	0.0972
F1.2	27	5	0.0961	0.0961	0.0961	0.0961	0.0964	0.0964	0.0964	0.0964	0.0960	0.0960	0.0960	0.0960
F1.3	10	5	0.0968	0.0968	0.0968	0.0968	0.0970	0.0970	0.0970	0.0970	0.0965	0.0966	0.0966	0.0966
F2.1	52	5	0.0966	0.0966	0.0966	0.0966	0.0968	0.0968	0.0968	0.0968	0.0965	0.0964	0.0964	0.0964
F2.2	54	5	0.0965	0.0965	0.0965	0.0965	0.0967	0.0967	0.0967	0.0967	0.0964	0.0964	0.0964	0.0964
F2.3	20	5	0.0969	0.0969	0.0969	0.0969	0.0973	0.0973	0.0973	0.0973	0.0969	0.0969	0.0969	0.0969
F3.1	3	5	0.0967	0.0967	0.0967	0.0967	0.0969	0.0969	0.0969	0.0969	0.0965	0.0965	0.0965	0.0965
F3.2	28	5	0.0973	0.0973	0.0973	0.0973	0.0976	0.0976	0.0976	0.0976	0.0970	0.0970	0.0970	0.0970
F3.3	9	5	0.0967	0.0967	0.0967	0.0967	0.0968	0.0968	0.0968	0.0968	0.0965	0.0965	0.0965	0.0965
F4.1	7	5	0.0971	0.0972	0.0972	0.0972	0.0972	0.0972	0.0972	0.0972	0.0969	0.0969	0.0969	0.0969
F4.2	19	5	0.0974	0.0974	0.0974	0.0974	0.0976	0.0976	0.0976	0.0976	0.0973	0.0973	0.0973	0.0973
F4.3	25	5	0.0972	0.0972	0.0972	0.0972	0.0973	0.0973	0.0973	0.0973	0.0969	0.0969	0.0969	0.0969
F5.1	26	5	0.0969	0.0969	0.0969	0.0969	0.0970	0.0970	0.0970	0.0970	0.0967	0.0967	0.0967	0.0967
F5.2	14	5	0.0972	0.0972	0.0972	0.0972	0.0974	0.0974	0.0974	0.0974	0.0971	0.0971	0.0971	0.0971
F5.3	2	5	0.0979	0.0979	0.0979	0.0979	0.0979	0.0979	0.0979	0.0979	0.0974	0.0974	0.0974	0.0974
F6.1	21	5	0.0971	0.0971	0.0971	0.0971	0.0973	0.0972	0.0972	0.0972	0.0969	0.0968	0.0969	0.0969
F6.2	4	5	0.0970	0.0970	0.0970	0.0970	0.0972	0.0972	0.0972	0.0972	0.0969	0.0969	0.0969	0.0969
F6.3	5	5	0.0987	0.0987	0.0987	0.0987	0.0988	0.0988	0.0988	0.0988	0.0984	0.0984	0.0984	0.0984
GS1 Harvest Phase at 20°C														
Sample	Filter #	Volume Filtered (mL)	Original Filter Weight			Avg Filter Weight	Dry Weight			Avg Dry Weight	Ashed Weight			Avg Ashed Weight
F1.1	5A	5	0.0957	0.0957	0.0957	0.0957	0.0963	0.0962	0.0962	0.0962	0.0957	0.0957	0.0957	0.0957
F1.2	29	5	0.0965	0.0965	0.0965	0.0965	0.0968	0.0968	0.0968	0.0968	0.0963	0.0964	0.0963	0.0963
F1.3	8	5	0.0968	0.0968	0.0968	0.0968	0.0971	0.0971	0.0971	0.0971	0.0966	0.0966	0.0966	0.0966
F2.1	12	5	0.0978	0.0978	0.0978	0.0978	0.0969	0.0969	0.0969	0.0969	0.0963	0.0963	0.0963	0.0963
F2.2	14A	5	0.0961	0.0961	0.0961	0.0961	0.0964	0.0964	0.0964	0.0964	0.0959	0.0959	0.0959	0.0959
F2.3	18	5	0.1009	0.1010	0.1009	0.1009	0.1011	0.1011	0.1011	0.1011	0.1006	0.1006	0.1006	0.1006
F3.1	12A	5	0.0966	0.0966	0.0966	0.0966	0.0980	0.0980	0.0980	0.0980	0.0976	0.0976	0.0976	0.0976
F3.2	1A	5	0.0970	0.0970	0.0970	0.0970	0.0972	0.0972	0.0972	0.0972	0.0967	0.0967	0.0967	0.0967
F3.3	30	5	0.0989	0.0989	0.0989	0.0989	0.0990	0.0991	0.0990	0.0990	0.0985	0.0985	0.0985	0.0985
F4.1	1	5	0.0979	0.0979	0.0979	0.0979	0.0982	0.0982	0.0982	0.0982	0.0978	0.0977	0.0977	0.0977
F4.2	23	5	0.0982	0.0982	0.0982	0.0982	0.0985	0.0985	0.0985	0.0985	0.0979	0.0979	0.0979	0.0979
F4.3	13	5	0.0968	0.0968	0.0968	0.0968	0.0971	0.0971	0.0971	0.0971	0.0965	0.0965	0.0965	0.0965
F5.1	6	5	0.0984	0.0984	0.0984	0.0984	0.0986	0.0986	0.0985	0.0986	0.0981	0.0981	0.0981	0.0981
F5.2	24	5	0.0975	0.0975	0.0975	0.0975	0.0976	0.0976	0.0976	0.0976	0.0971	0.0971	0.0971	0.0971
F5.3	11	5	0.0981	0.0981	0.0981	0.0981	0.0983	0.0983	0.0983	0.0983	0.0978	0.0978	0.0978	0.0978
F6.1	17	5	0.0984	0.0984	0.0984	0.0984	0.0987	0.0987	0.0987	0.0987	0.0981	0.0981	0.0981	0.0981
F6.2	22	5	0.0975	0.0975	0.0974	0.0975	0.0977	0.0977	0.0977	0.0977	0.0972	0.0973	0.0973	0.0973
F6.3	53	5	0.0978	0.0978	0.0978	0.0978	0.0982	0.0982	0.0982	0.0982	0.0977	0.0977	0.0977	0.0977

Filter weights used to calculate the AFDW during GS1 at 20°C. Samples are labeled as Flask number then subsample number (Flask 1 subsample 1 = F1.1).

Table F.3 *Filter Weights for 25°C AFDW during GS1*

			GS1 Dilution Phase at 25°C										
Sample	Filter #	Volume Filtered (mL)	Original Filter Weight			Avg Filter Weight	Dry Weight			Avg Dry Weight	Ashed Weight			Avg Ashed Weight
F1.1	76	75	0.0968	0.0968	0.0968	0.0968	0.1013	0.1013	0.1013	0.1013	0.0970	0.0970	0.0970	0.0970
F1.2	85	75	0.0962	0.0962	0.0962	0.0962	0.1005	0.1006	0.1005	0.1005	0.0964	0.0964	0.0964	0.0964
F1.3	79	70	0.0975	0.0975	0.0975	0.0975	0.1018	0.1018	0.1018	0.1018	0.0976	0.0976	0.0976	0.0976
F2.1	84	75	0.0973	0.0973	0.0973	0.0973	0.1029	0.1028	0.1029	0.1029	0.0981	0.0981	0.0981	0.0981
F2.2	2A	75	0.0956	0.0956	0.0956	0.0956	0.1009	0.1009	0.1009	0.1009	0.0964	0.0964	0.0964	0.0964
F2.3	21A	70	0.1016	0.1016	0.1016	0.1016	0.1066	0.1066	0.1066	0.1066	0.1024	0.1024	0.1024	0.1024
F3.1	83	75	0.0960	0.0960	0.0960	0.0960	0.1005	0.1005	0.1005	0.1005	0.0959	0.0959	0.0960	0.0959
F3.2	77	75	0.0975	0.0975	0.0975	0.0975	0.1028	0.1028	0.1028	0.1028	0.0978	0.0978	0.0978	0.0978
F3.3	75	75	0.0970	0.0970	0.0970	0.0970	0.1016	0.1016	0.1016	0.1016	0.0970	0.0970	0.0970	0.0970
F4.1	13A	5	0.0962	0.0963	0.0962	0.0962	0.0965	0.0965	0.0965	0.0965	0.0962	0.0962	0.0962	0.0962
F4.2	16	5	0.0963	0.0963	0.0963	0.0963	0.0965	0.0965	0.0965	0.0965	0.0962	0.0962	0.0962	0.0962
F4.3	6A	5	0.0995	0.0995	0.0995	0.0995	0.0996	0.0996	0.0996	0.0996	0.0993	0.0993	0.0993	0.0993
F5.1	82	5	0.0969	0.0969	0.0969	0.0969	0.0972	0.0972	0.0972	0.0972	0.0968	0.0968	0.0968	0.0968
F5.2	86	5	0.0976	0.0976	0.0976	0.0976	0.0979	0.0979	0.0979	0.0979	0.0975	0.0974	0.0975	0.0975
F5.3	80	5	0.0982	0.0982	0.0982	0.0982	0.0984	0.0984	0.0984	0.0984	0.0980	0.9797	0.0980	0.0980
F6.1	7A	5	0.0961	0.0961	0.0961	0.0961	0.0964	0.0964	0.0964	0.0964	0.0960	0.0960	0.0960	0.0960
F6.2	78	5	0.0964	0.0974	0.0974	0.0974	0.0976	0.0976	0.0976	0.0976	0.0971	0.0971	0.0971	0.0971
F6.3	81	5	0.0978	0.0978	0.0978	0.0978	0.0980	0.0980	0.0980	0.0980	0.0975	0.0975	0.0975	0.0975
			GS1 Harvest Phase at 25°C										
Sample	Filter #	Volume Filtered (mL)	Original Filter Weight			Avg Filter Weight	Dry Weight			Avg Dry Weight	Ashed Weight			Avg Ashed Weight
F1.1	101	80	0.0971	0.0971	0.0972	0.0971	0.1043	0.1043	0.1043	0.1043	0.0976	0.0976	0.0976	0.0976
F1.2	91	70	0.0967	0.0967	0.0967	0.0967	0.1027	0.1027	0.1027	0.1027	0.0970	0.0970	0.0970	0.0970
F1.3	96	80	0.0974	0.0974	0.0974	0.0974	0.1053	0.1053	0.1053	0.1053	0.0982	0.0982	0.0982	0.0982
F2.1	88	80	0.0975	0.0975	0.0975	0.0975	0.1045	0.1045	0.1045	0.1045	0.0979	0.0980	0.0980	0.0980
F2.2	94	70	0.0968	0.0968	0.0968	0.0968	0.1028	0.1028	0.1028	0.1028	0.0969	0.0969	0.0693	0.0969
F2.3	92	60	0.0966	0.0966	0.0966	0.0966	0.1014	0.1014	0.1014	0.1014	0.0967	0.0967	0.0967	0.0967
F3.1	99	80	0.0957	0.0956	0.0956	0.0956	0.1027	0.1027	0.1027	0.1027	0.0962	0.0962	0.0962	0.0962
F3.2	100	80	0.0974	0.0974	0.0974	0.0974	0.1045	0.1045	0.1045	0.1045	0.0980	0.0980	0.0981	0.0980
F3.3	98	80	0.0965	0.0965	0.0965	0.0965	0.1044	0.1044	0.1044	0.1044	0.0976	0.0976	0.0976	0.0976
F4.1	103	90	0.0967	0.0967	0.0967	0.0967	0.1045	0.1045	0.1045	0.1045	0.0973	0.0973	0.0973	0.0973
F4.2	102	90	0.0976	0.0976	0.0976	0.0976	0.1046	0.1046	0.1046	0.1046	0.0980	0.0980	0.0980	0.0980
F4.3	110	70	0.0968	0.0968	0.0968	0.0968	0.1030	0.1030	0.1030	0.1030	0.0972	0.0972	0.0972	0.0972
F5.1	95	90	0.0956	0.0956	0.0956	0.0956	0.1034	0.1034	0.1034	0.1034	0.0959	0.0959	0.0959	0.0959
F5.2	93	90	0.0960	0.0960	0.0960	0.0960	0.1029	0.1029	0.1029	0.1029	0.0962	0.0962	0.0962	0.0962
F5.3	97	90	0.0975	0.0975	0.0975	0.0975	0.1051	0.1051	0.1051	0.1051	0.0980	0.0980	0.0980	0.0980
F6.1	90	80	0.0964	0.0965	0.0965	0.0965	0.1035	0.1035	0.1035	0.1035	0.0965	0.0965	0.0965	0.0965
F6.2	89	90	0.0972	0.0972	0.0972	0.0972	0.1043	0.1043	0.1043	0.1043	0.0973	0.0973	0.0973	0.0973
F6.3	87	80	0.0966	0.0966	0.0966	0.0966	0.1029	0.1029	0.1029	0.1029	0.0968	0.0968	0.0968	0.0968

Filter weights used to calculate the AFDW during GS1 at 25°C. Samples are labeled as Flask number then subsample number (Flask 1 subsample 1 = F1.1).

Table F.4 *Filter Weights for 15°C AFDW during GS2*

			GS2 Dilution Phase at 15°C											
Sample	Filter #	Volume Filtered (mL)	Original Filter Weight			Avg Filter Weight	Dry Weight			Avg Dry Weight	Ashed Weight			Avg Ashed Weight
F1.1	287	77	0.1004	0.1005	0.1005	0.1005	0.1046	0.1046	0.1046	0.1046	0.1011	0.1011	0.1011	0.1011
F1.2	284	71	0.0998	0.0998	0.0998	0.0998	0.1027	0.1027	0.1027	0.1027	0.1000	0.1000	0.1000	0.1000
F1.3	286	67	0.1011	0.1011	0.1011	0.1011	0.1036	0.1036	0.1036	0.1036	0.1010	0.1010	0.1010	0.1010
F2.1	280	70	0.0927	0.0927	0.0927	0.0927	0.0966	0.0966	0.0966	0.0966	0.0937	0.0937	0.0937	0.0937
F2.2	283	67	0.0958	0.0958	0.0958	0.0958	0.0981	0.0981	0.0981	0.0981	0.0957	0.0957	0.0957	0.0957
F2.3	281	79	0.0914	0.0914	0.0914	0.0914	0.0943	0.0943	0.0943	0.0943	0.0915	0.0915	0.0915	0.0915
F3.1	275	76	0.0925	0.0925	0.0925	0.0925	0.0961	0.0961	0.0961	0.0961	0.0931	0.0931	0.0931	0.0931
F3.2	273	71	0.0919	0.0920	0.0920	0.0920	0.0948	0.0948	0.0948	0.0948	0.0922	0.0922	0.0922	0.0922
F3.3	276	72	0.0929	0.0929	0.0929	0.0929	0.0967	0.0967	0.0967	0.0967	0.0939	0.0939	0.0939	0.0939
F4.1	279	74	0.0935	0.0935	0.0935	0.0935	0.0965	0.0965	0.0965	0.0965	0.0939	0.0939	0.0939	0.0939
F4.2	289	74	0.1007	0.1006	0.1007	0.1007	0.1030	0.1030	0.1030	0.1030	0.1005	0.1005	0.1005	0.1005
F4.3	282	74	0.0918	0.0918	0.0918	0.0918	0.0944	0.0944	0.0944	0.0944	0.0918	0.0918	0.0918	0.0918
F5.1	272	75	0.0918	0.0918	0.0918	0.0918	0.0952	0.0952	0.0952	0.0952	0.0924	0.0924	0.0924	0.0924
F5.2	288	73	0.1014	0.1014	0.1014	0.1014	0.1036	0.1036	0.1036	0.1036	0.1012	0.1012	0.1012	0.1012
F5.3	278	70	0.0931	0.0931	0.0931	0.0931	0.0954	0.0954	0.0954	0.0954	0.0931	0.0931	0.0931	0.0931
F6.1	285	77	0.1004	0.1004	0.1004	0.1004	0.1044	0.1044	0.1044	0.1044	0.1014	0.1014	0.1014	0.1014
F6.2	277	74	0.0929	0.0929	0.0929	0.0929	0.0954	0.0954	0.0954	0.0954	0.0929	0.0929	0.0929	0.0929
F6.3	274	68	0.0919	0.0919	0.0920	0.0919	0.0935	0.0935	0.0935	0.0935	0.0913	0.0913	0.0913	0.0913
			GS2 Harvest Phase at 15°C											
Sample	Filter #	Volume Filtered (mL)	Original Filter Weight			Avg Filter Weight	Dry Weight			Avg Dry Weight	Ashed Weight			Avg Ashed Weight
F1.1	305	140	0.1003	0.1004	0.1003	0.1003	0.1072	0.1072	0.1072	0.1072	0.1004	0.1004	0.1004	0.1004
F1.2	307	147	0.0999	0.0998	0.0998	0.0998	0.1069	0.1069	0.1069	0.1069	0.0999	0.0999	0.0999	0.0999
F1.3	304	140	0.1006	0.1006	0.1006	0.1006	0.1074	0.1074	0.1074	0.1074	0.1007	0.1006	0.1007	0.1007
F2.1	303	154	0.1002	0.1002	0.1002	0.1002	0.1077	0.1077	0.1077	0.1077	0.1004	0.1004	0.1004	0.1004
F2.2	302	120	0.0996	0.0996	0.0996	0.0996	0.1067	0.1067	0.1067	0.1067	0.0994	0.0994	0.0994	0.0994
F2.3	299	131	0.1014	0.1014	0.1014	0.1014	0.1084	0.1084	0.1084	0.1084	0.1015	0.1015	0.1015	0.1015
F3.1	297	142	0.1002	0.1002	0.1002	0.1002	0.1074	0.1074	0.1074	0.1074	0.1004	0.1004	0.1004	0.1004
F3.2	306	152	0.1004	0.1004	0.1004	0.1004	0.1073	0.1073	0.1073	0.1073	0.1005	0.1048	0.1005	0.1005
F3.3	295	150	0.0998	0.0998	0.0998	0.0998	0.1066	0.1066	0.1066	0.1066	0.0999	0.0998	0.0998	0.0998
F4.1	294	149	0.0999	0.0999	0.0999	0.0999	0.1064	0.1064	0.1064	0.1064	0.0999	0.1000	0.1000	0.1000
F4.2	293	174	0.1001	0.1001	0.1001	0.1001	0.1069	0.1069	0.1069	0.1069	0.1002	0.1002	0.1002	0.1002
F4.3	301	151	0.1003	0.1003	0.1003	0.1003	0.1067	0.1067	0.1067	0.1067	0.1002	0.1002	0.1002	0.1002
F5.1	296	164	0.1000	0.1000	0.1000	0.1000	0.1072	0.1072	0.1072	0.1072	0.1001	0.1001	0.1001	0.1001
F5.2	300	150	0.1004	0.1004	0.1004	0.1004	0.1073	0.1073	0.1073	0.1073	0.1005	0.1005	0.1005	0.1005
F5.3	298	147	0.1004	0.1004	0.1004	0.1004	0.1074	0.1074	0.1074	0.1074	0.1005	0.1005	0.1005	0.1005
F6.1	291	156	0.1002	0.1002	0.1002	0.1002	0.1070	0.1070	0.1070	0.1070	0.1003	0.1003	0.1003	0.1003
F6.2	292	170	0.1004	0.1004	0.1005	0.1004	0.1082	0.1082	0.1082	0.1082	0.1004	0.1004	0.1004	0.1004
F6.3	290	160	0.0996	0.0996	0.0996	0.0996	0.1066	0.1066	0.1066	0.1066	0.0997	0.0997	0.0997	0.0997

Filter weights used to calculate the AFDW during GS2 at 15°C. Samples are labeled as Flask number then subsample number (Flask 1 subsample 1 = F1.1).

Table F.5 *Filter Weights for 20°C AFDW during GS2*

Sample	Filter #	Volume Filtered (mL)	Original Filter Weight			Avg Filter Weight	Dry Weight			Avg Dry Weight	Ashed Weight			Avg Ashed Weight
GS2 Dilution Phase at 20°C														
F1.1	211	100	0.0944	0.0944	0.0944	0.0944	0.1006	0.1006	0.1006	0.1006	0.0958	0.0958	0.0958	0.0958
F1.2	206	80	0.0943	0.0943	0.0943	0.0943	0.0999	0.0999	0.0999	0.0999	0.0956	0.0956	0.0956	0.0956
F1.3	208	80	0.0938	0.0938	0.0938	0.0938	0.0986	0.0986	0.0986	0.0986	0.0948	0.0948	0.0948	0.0948
F2.1	202	90	0.0943	0.0943	0.0943	0.0943	0.1006	0.1006	0.1006	0.1006	0.0957	0.0957	0.0957	0.0957
F2.2	216	70	0.0951	0.0951	0.0951	0.0951	0.0991	0.0991	0.0991	0.0991	0.0953	0.0953	0.0953	0.0953
F2.3	207	70	0.0935	0.0935	0.0935	0.0935	0.0973	0.0973	0.0973	0.0973	0.0937	0.0937	0.0937	0.0937
F3.1	215	71	0.0943	0.0943	0.0943	0.0943	0.0979	0.0979	0.0979	0.0979	0.0946	0.0946	0.0946	0.0946
F3.2	205	73	0.0950	0.0949	0.0949	0.0949	0.0983	0.0983	0.0983	0.0983	0.0950	0.0950	0.0950	0.0950
F3.3	203	82	0.0949	0.0949	0.0949	0.0949	0.0989	0.0989	0.0989	0.0989	0.0948	0.0948	0.0948	0.0948
F4.1	200	70	0.0934	0.0934	0.0934	0.0934	0.0971	0.0971	0.0971	0.0971	0.0941	0.0941	0.0941	0.0941
F4.2	209	74	0.0945	0.0944	0.0944	0.0944	0.0977	0.0977	0.0977	0.0977	0.0946	0.0946	0.0946	0.0946
F4.3	212	82	0.0933	0.0933	0.0933	0.0933	0.9669	0.0967	0.0967	0.0967	0.0931	0.0931	0.0931	0.0931
F5.1	210	74	0.0942	0.0942	0.0942	0.0942	0.0983	0.0983	0.0983	0.0983	0.0949	0.0949	0.0949	0.0949
F5.2	201	76	0.0938	0.0938	0.0938	0.0938	0.0971	0.0971	0.0971	0.0971	0.0936	0.0936	0.0936	0.0936
F5.3	214	80	0.0939	0.0939	0.0939	0.0939	0.0976	0.0976	0.0976	0.0976	0.0941	0.0941	0.0941	0.0941
F6.1	213	74	0.0940	0.0941	0.0940	0.0940	0.0996	0.0996	0.0996	0.0996	0.0956	0.0956	0.0956	0.0956
F6.2	204	69	0.0941	0.0941	0.0941	0.0941	0.0968	0.0968	0.0968	0.0968	0.0941	0.0941	0.0941	0.0941
F6.3	218	75	0.0939	0.0939	0.0939	0.0939	0.0967	0.0967	0.0967	0.0967	0.0937	0.0937	0.0937	0.0937
GS2 Harvest Phase at 20°C														
F1.1	226	109	0.0932	0.0933	0.0932	0.0932	0.1014	0.1014	0.1014	0.1014	0.0934	0.0934	0.0934	0.0934
F1.2	232	102	0.0935	0.0935	0.0935	0.0935	0.1013	0.1013	0.1013	0.1013	0.0937	0.0937	0.0937	0.0937
F1.3	230	111	0.0935	0.0935	0.0935	0.0935	0.1024	0.1024	0.1024	0.1024	0.0942	0.0942	0.0942	0.0942
F2.1	233	110	0.0958	0.0958	0.0958	0.0958	0.1038	0.1038	0.1038	0.1038	0.0959	0.0959	0.0959	0.0959
F2.2	234	100	0.0944	0.0944	0.0944	0.0944	0.1014	0.1014	0.1014	0.1014	0.0945	0.0945	0.0945	0.0945
F2.3	235	121	0.0943	0.0943	0.0943	0.0943	0.1031	0.1031	0.1031	0.1031	0.0947	0.0947	0.0947	0.0947
F3.1	222	132	0.0932	0.0932	0.0932	0.0932	0.1016	0.1016	0.1016	0.1016	0.0933	0.0933	0.0933	0.0933
F3.2	231	130	0.0931	0.0931	0.0932	0.0931	0.1025	0.1025	0.1025	0.1025	0.0934	0.0934	0.0934	0.0934
F3.3	229	143	0.0931	0.0931	0.0931	0.0931	0.1029	0.1029	0.1029	0.1029	0.0932	0.0931	0.0931	0.0931
F4.1	217	130	0.0953	0.0953	0.0953	0.0953	0.1045	0.1045	0.1045	0.1045	0.0953	0.0953	0.0953	0.0953
F4.2	220	130	0.0928	0.0928	0.0928	0.0928	0.1018	0.1018	0.1018	0.1018	0.0929	0.0929	0.0929	0.0929
F4.3	223	140	0.0951	0.0951	0.0951	0.0951	0.1050	0.1050	0.1050	0.1050	0.0954	0.0954	0.0954	0.0954
F5.1	225	142	0.0933	0.0933	0.0934	0.0933	0.1023	0.1023	0.1023	0.1023	0.0937	0.0937	0.0937	0.0937
F5.2	228	141	0.0952	0.0916	0.0952	0.0952	0.1047	0.1047	0.1047	0.1047	0.0952	0.0952	0.0952	0.0952
F5.3	219	154	0.0945	0.0945	0.0945	0.0945	0.1047	0.1047	0.1047	0.1047	0.0946	0.0946	0.0946	0.0946
F6.1	224	158	0.0953	0.0953	0.0953	0.0953	0.1046	0.1046	0.1046	0.1046	0.0956	0.0956	0.0956	0.0956
F6.2	227	137	0.0944	0.0944	0.0944	0.0944	0.1028	0.1028	0.1028	0.1028	0.0944	0.0944	0.0944	0.0944
F6.3	221	163	0.0936	0.0936	0.0936	0.0936	0.1041	0.1041	0.1041	0.1041	0.0939	0.0939	0.0939	0.0939

Filter weights used to calculate the AFDW during GS2 at 20°C. Samples are labeled as Flask number then subsample number (Flask 1 subsample 1 = F1.1).

Table F.6 *Filter Weights for 25°C AFDW during GS2*

GS2 Dilution Phase at 25°C															
Sample	**Filter #**	**Volume Filtered (mL)**	**Original Filter Weight**			**Avg Filter Weight**	**Dry Weight**			**Avg Dry Weight**	**Ashed Weight**			**Avg Ashed Weight**
F1.1	250	72	0.0961	0.0961	0.0961	0.0961	0.0991	0.0991	0.0991	0.0991	0.0964	0.0964	0.0964	0.0964
F1.2	242	70	0.0929	0.0929	0.0929	0.0929	0.0961	0.0961	0.0961	0.0961	0.0931	0.0932	0.0931	0.0931
F1.3	253	93	0.0969	0.0969	0.0969	0.0969	0.1030	0.1030	0.1030	0.1030	0.0982	0.0982	0.0982	0.0982
F2.1	245	76	0.0941	0.0941	0.0941	0.0941	0.0970	0.0970	0.0970	0.0970	0.0941	0.0941	0.0941	0.0941
F2.2	241	75	0.0940	0.0940	0.0940	0.0940	0.0968	0.0968	0.0968	0.0968	0.0940	0.0939	0.0939	0.0939
F2.3	236	88	0.0927	0.0927	0.0927	0.0927	0.0979	0.0979	0.0979	0.0979	0.0937	0.0937	0.0937	0.0937
F3.1	249	78	0.0932	0.0932	0.0932	0.0932	0.0964	0.0964	0.0964	0.0964	0.0932	0.0932	0.0932	0.0932
F3.2	252	79	0.0969	0.0969	0.0969	0.0969	0.1000	0.1000	0.1000	0.1000	0.0968	0.0968	0.0968	0.0968
F3.3	240	83	0.0933	0.0093	0.0933	0.0933	0.0975	0.0975	0.0975	0.0975	0.0941	0.0941	0.0941	0.0941
F4.1	244	85	0.0942	0.0942	0.0942	0.0942	0.0980	0.0980	0.0980	0.0980	0.0944	0.0944	0.0944	0.0944
F4.2	238	74	0.0950	0.0950	0.0950	0.0950	0.0984	0.0984	0.0984	0.0984	0.0951	0.0951	0.0951	0.0951
F4.3	237	82	0.0941	0.0941	0.0941	0.0941	0.0972	0.0982	0.0982	0.0982	0.0946	0.0946	0.0946	0.0946
F5.1	239	78	0.0944	0.0944	0.0944	0.0944	0.0973	0.0973	0.0973	0.0973	0.0941	0.0941	0.0941	0.0941
F5.2	248	82	0.0932	0.0932	0.0932	0.0932	0.0965	0.0965	0.0965	0.0965	0.0929	0.0929	0.0929	0.0929
F5.3	251	78	0.0953	0.0953	0.0953	0.0953	0.0989	0.0989	0.0990	0.0989	0.0959	0.0959	0.0959	0.0959
F6.1	246	82	0.0954	0.0954	0.0954	0.0954	0.0993	0.0992	0.0992	0.0992	0.0953	0.0953	0.0953	0.0953
F6.2	247	79	0.0946	0.0946	0.0946	0.0946	0.0985	0.0985	0.0985	0.0985	0.0946	0.0946	0.0946	0.0946
F6.3	243	78	0.0942	0.0942	0.0942	0.0942	0.9803	0.0980	0.0980	0.0980	0.0946	0.0946	0.0946	0.0946
GS2 Harvest Phase at 25°C															
Sample	**Filter #**	**Volume Filtered (mL)**	**Original Filter Weight**			**Avg Filter Weight**	**Dry Weight**			**Avg Dry Weight**	**Ashed Weight**			**Avg Ashed Weight**
F1.1	267	124	0.0920	0.0919	0.0919	0.0919	0.1015	0.1015	0.1015	0.1015	0.0923	0.0923	0.0923	0.0923
F1.2	271	135	0.0923	0.0923	0.0923	0.0923	0.1027	0.1027	0.1026	0.1027	0.0924	0.0924	0.0924	0.0924
F1.3	261	137	0.0918	0.0918	0.0918	0.0918	0.1024	0.1024	0.1024	0.1024	0.0921	0.0921	0.0922	0.0921
F2.1	268	113	0.0917	0.0917	0.0917	0.0917	0.1014	0.1014	0.1014	0.1014	0.0921	0.0921	0.0921	0.0921
F2.2	265	126	0.0935	0.0935	0.0935	0.0935	0.1044	0.1045	0.1044	0.1044	0.0937	0.0937	0.0937	0.0937
F2.3	262	116	0.0935	0.0935	0.0935	0.0935	0.1042	0.1042	0.1042	0.1042	0.0940	0.0940	0.0940	0.0940
F3.1	263	118	0.0919	0.0919	0.0919	0.0919	0.1016	0.1016	0.1016	0.1016	0.0922	0.0922	0.0922	0.0922
F3.2	270	99	0.0938	0.0938	0.0938	0.0938	0.1032	0.1032	0.1032	0.1032	0.0942	0.0942	0.0942	0.0942
F3.3	266	114	0.0922	0.0922	0.0922	0.0922	0.1011	0.1011	0.1011	0.1011	0.0925	0.0925	0.0925	0.0925
F4.1	264	120	0.0923	0.0923	0.0923	0.0923	0.1023	0.1023	0.1023	0.1023	0.0927	0.0927	0.0927	0.0927
F4.2	260	129	0.0934	0.0933	0.0934	0.0934	0.1038	0.1037	0.1037	0.1037	0.0936	0.0936	0.0936	0.0936
F4.3	258	136	0.0918	0.0918	0.0918	0.0918	0.1012	0.1012	0.1012	0.1012	0.0923	0.0923	0.0923	0.0923
F5.1	269	134	0.0919	0.0919	0.0919	0.0919	0.1025	0.1025	0.1025	0.1025	0.0923	0.0923	0.0923	0.0923
F5.2	255	145	0.0921	0.0921	0.0921	0.0921	0.1044	0.1044	0.1044	0.1044	0.0927	0.0927	0.0927	0.0927
F5.3	259	144	0.0927	0.0926	0.0926	0.0926	0.1038	0.1038	0.1038	0.1038	0.0931	0.0931	0.0931	0.0931
F6.1	256	142	0.0925	0.0925	0.0925	0.0925	0.1043	0.1043	0.1043	0.1043	0.0932	0.0932	0.0932	0.0932
F6.2	257	162	0.0935	0.0935	0.0935	0.0935	0.1051	0.1051	0.1051	0.1051	0.0941	0.0941	0.0941	0.0941
F6.3	254	126	0.0923	0.0923	0.0923	0.0923	0.1024	0.1024	0.1024	0.1024	0.0928	0.0928	0.0928	0.0928

Filter weights used to calculate the AFDFW during GS2 at 25°C. Samples are labeled as Flask number then subsample number (Flask 1 subsample 1 = F1.1).

APPENDIX G Lipid Fluorescence Raw Data

Table G.1 *Fluorescence Values Plate 1*

Plate Set-Up									0 Minutes								
0%R	0%R	0%R	medR	medR	medR	5%R	5%R	5%R	7.57	7.245	7.395	7.555	7.522	7.159	379.838	403.714	488.072
0%	0%	0%	med	med	med	5%	5%	5%	4.418	3.691	3.437	3.907	4.555	3.575	11.119	9.042	30.463
5 Minutes									10 Minutes								
7.555	6.871	7.053	7.721	7.812	7.516	337.961	355.374	357.932	7.108	7.004	6.984	7.364	7.611	7.25	326.4	343.067	342.909
4.281	3.77	3.369	3.978	4.35	3.616	13.13	8.519	29.586	4.304	3.667	3.323	3.824	4.347	3.691	14.325	8.437	27.906
15 Minutes									20 Minutes								
6.879	6.573	6.67	7.377	7.583	6.741	323.37	338.063	336.182	6.564	6.336	6.169	7.511	7.479	7.063	321.158	333.216	331.093
4.335	3.851	3.581	4.031	4.381	3.505	17.342	8.576	29.638	4.277	3.68	3.408	3.787	4.34	3.574	16.769	8.67	25.337
25 Minutes									30 Minutes								
6.878	6.14	5.87	7.263	7.152	6.992	320.573	330.656	328.217	6.806	6.121	5.932	7.221	6.994	6.86	317.401	330.787	323.772
4.45	3.826	3.147	3.806	4.309	3.748	17.947	9.018	24.848	4.217	3.884	3.395	3.988	4.37	3.7	18.744	8.635	24.919

SpectraMax Fluorescence values for Plate 1. R = stained with Nile Red. Med = medium blank. The CDLC standards are listed as a percent.

Table G.2 *Fluorescence Values Plate 2*

Plate Set-Up												0 Minutes											
0%R	0%R	0%R	2.5%R	2.5%R	2.5%R	5%R	5%R	5%R	7.5%R	7.5%R	7.5%R	7.377	6.78	6.623	154.738	142.092	129.697	174.116	188.466	208.171	705.722	714.894	Sat
10%R	10%R	10%R	medR	medR	medR	G1.12.11R	G1.12.11R	G1.12.11R	W6.3.9R	W6.3.9R	W6.3.9R	539.701	531.763	598.177	6.73	7.053	6.308	18.057	17.081	17.254	16.214	16.863	16.83
Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	4.325	3.469	2.976	3.295	3.565	3.277	3.899	4.085	3.571	3.227	3.473	6.774
0%	0%	0%	2.50%	2.50%	2.50%	5%	5%	5%	7.50%	7.50%	7.50%	2.619	3.723	2.951	5.345	6.023	5.089	6.121	9.457	7.075	6.944	12.54	6.328
10%	10%	10%	med	med	med	G1.12.11	G1.12.11	G1.12.11	W6.3.9	W6.3.9	W6.3.9	16.966	11.224	17.645	2.804	3.275	4.016	5.997	6.335	5.401	5.274	5.55	6.59
5 Minutes												10 Minutes											
7.326	6.788	6.195	125.152	123.994	120.347	287.837	332.927	369.78	677.025	683.356	670.726	7.185	6.521	5.891	124.553	121.716	120.193	403.071	450.524	473.754	560.25	562.812	546.419
650.057	629.625	sat	7.091	6.737	6.221	17.83	16.612	17.12	18.909	18.515	19.559	sat	sat	sat	6.83	6.754	6.071	19.254	18.031	18.418	22.426	22.008	23.353
4.558	3.781	2.991	3.278	3.82	3.361	3.962	4.137	3.285	3.444	3.549	6.764	4.254	3.496	3.091	3.287	3.61	3.283	3.934	4.085	3.495	3.182	3.352	6.513
2.834	3.646	2.896	5.647	6.062	5.263	6.967	8.837	6.969	7.104	11.701	5.9	2.818	3.574	2.807	5.233	6.134	5.325	6.688	7.936	7.121	6.829	11.947	6.571
24.341	19.888	27.165	2.872	3.167	3.901	6.112	6.716	5.769	5.354	5.455	5.983	18.356	15.551	22.476	2.799	3.299	4.063	5.724	6.454	5.72	4.882	5.212	5.746
15 Minutes												20 Minutes											
6.719	6.58	5.576	125.836	123.14	121.31	467.552	469.772	461.175	499.453	505.578	494.233	6.519	6.498	5.505	124.031	122.763	120.52	462.162	422.142	404.882	469.386	480.286	469.288
sat	sat	sat	6.905	6.637	5.813	21.103	19.886	20.611	26.471	26.389	27.068	sat	sat	sat	6.735	6.269	5.544	24.721	22.018	22.997	28.549	27.867	29.415
4.539	3.777	3.143	3.409	3.715	3.302	3.884	4.288	3.42	3.363	3.614	6.962	4.551	3.605	3.122	3.315	3.724	3.296	3.999	4.148	3.345	3.286	3.448	6.904
2.901	3.732	2.816	6.29	6.827	5.534	7.198	7.813	7.578	7.345	11.536	6.377	2.752	3.575	2.902	5.97	6.58	5.331	7.347	7.674	7.343	7.046	11.438	6.566
21.517	19.337	25.357	2.888	3.214	4.006	6.56	6.939	6.024	5.399	6.063	6.662	20.655	17.855	22.689	2.862	3.478	3.834	6.356	6.604	6.089	6.26	5.653	6.483
25 Minutes												30 Minutes											
6.512	6.743	5.258	125.335	122.386	121.498	428.939	377.217	358.742	461.64	472.132	459.352	6.711	6.442	5.348	125.009	123.205	120.965	389.662	344.122	330.492	459.203	467.627	455.924
sat	sat	sat	6.469	6.099	5.339	27.179	24.241	25.511	27.963	27.713	28.798	sat	sat	sat	6.41	5.823	5.294	29.582	27.149	27.833	25.716	25.633	27.204
4.245	3.652	2.963	3.385	3.559	3.291	4.038	4.057	3.389	3.241	3.309	6.951	4.368	3.635	2.89	3.325	3.697	3.27	3.805	4.265	3.304	3.121	3.39	6.658
2.854	3.694	2.852	6.003	6.379	5.616	7.303	7.398	7.45	6.995	7.381	6.651	2.78	3.656	2.818	5.841	6.76	5.592	7.308	7.135	7.206	6.88	7.504	6.768
16.958	16.327	22.086	2.895	3.237	4.021	6.596	6.736	5.972	6.691	5.908	7.199	17.159	14.838	20.309	2.743	3.259	3.97	6.8	7.184	6.416	6.654	6.47	8.501

SpectraMax Fluorescence values for Plate 2. R = stained with Nile Red. Med = medium blank. The CDLC standards are listed as a percent. Samples are listed as Growth Strategy and Growth Temperature, Flask Number, Date Sampled. G1.12.11 = GS1 at 15°C, Flask 1, Sampled 12/11. All samples were run in triplicate.

Table G.3 *Fluorescence Values Plate 3*

Plate Set-Up												0 Minutes											
medR	medR	medR	5%R	5%R	5%R	G2.12.11R	G2.12.11R	G2.12.11R	G3.12.11R	G3.12.11R	G3.12.11R	7.439	7.461	6.985	302.991	333.056	371.477	17.316	16.319	16.196	15.152	15.334	15.967
G4.12.11R	G4.12.11R	G4.12.11R	G5.12.11R	G5.12.11R	G5.12.11R	G6.12.11R	G6.12.11R	G6.12.11R	G1.12.13R	G1.12.13R	G1.12.13R	15.325	14.134	13.749	15.391	15.912	15.606	17.193	16.824	15.169	19.907	19.355	20.813
G2.12.13R	G2.12.13R	G2.12.13R	G3.12.13R	G3.12.13R	G3.12.13R	G4.12.13R	G4.12.13R	G4.12.13R	G5.12.13R	G5.12.13R	G5.12.13R	19.936	20.572	20.238	17.231	13.388	18.364	19.362	19.594	19.225	18.519	18.806	18.634
G6.12.13R	G6.12.13R	G6.12.13R	M1.11.18R	M1.11.18R	M1.11.18R	M2.11.18R	M2.11.18R	M2.11.18R	M3.11.18R	M3.11.18R	M3.11.18R	19.997	20.327	19.536	24.815	25.37	25.602	21.274	21.465	20.739	24.964	25.87	26.204
med	med	med	5%	5%	5%	G2.12.11	G2.12.11	G2.12.11	G3.12.11	G3.12.11	G3.12.11	3.376	3.294	2.908	36.635	9.46	11.473	5.76	6.363	5.416	5.239	5.25	5.515
G4.12.11	G4.12.11	G4.12.11	G5.12.11	G5.12.11	G5.12.11	G6.12.11	G6.12.11	G6.12.11	G1.12.13	G1.12.13	G1.12.13	5.802	6.155	5.03	5.822	5.8	5.778	5.667	5.93	5.246	6.638	6.72	6.808
G2.12.13	G2.12.13	G2.12.13	G3.12.13	G3.12.13	G3.12.13	G4.12.13	G4.12.13	G4.12.13	G5.12.13	G5.12.13	G5.12.13	7.064	6.261	6.781	6.664	6.864	6.465	6.479	6.82	6.454	6.199	6.403	6.074
G6.12.13	G6.12.13	G6.12.13	M1.11.18	M1.11.18	M1.11.18	M2.11.18	M2.11.18	M2.11.18	M3.11.18	M3.11.18	M3.11.18	7.175	7.163	7.159	5.933	6.118	5.932	6.527	6.452	6.51	6.51	6.04	5.86
5 Minutes												10 Minutes											
7.446	7.379	6.679	381.853	422.546	465.896	17.181	17.048	16.67	15.662	15.124	16.718	7.773	7.233	6.641	445.672	483.801	497.992	19.478	18.276	17.719	16.67	16.643	17.359
15.175	14.727	13.438	15.773	16.425	15.781	22.727	21.483	20.053	20.558	19.981	20.966	16.381	15.74	14.811	16.642	17.363	16.657	28.859	27.978	25.48	22.084	23.347	22.471
20.247	20.762	20.35	17.781	13.996	19.186	18.982	20.653	19.502	18.835	19.019	18.904	22.591	22.851	22.727	19.663	14.373	21.063	20.74	21.534	20.358	20.207	20.758	20.759
20.214	20.604	19.25	23.594	23.897	24.184	20.734	20.878	20.725	24.285	24.296	24.225	22.92	22.546	21.103	24.165	23.559	23.884	21.227	20.965	20.891	23.691	23.703	23.842
3.489	3.504	3.069	50.043	10.261	12.729	5.274	6.225	5.267	5.049	5.252	5.706	3.511	3.564	3.047	47.989	9.533	12.273	5.303	6.156	5.256	5.323	4.985	5.581
5.752	6.221	4.995	5.548	5.626	5.984	5.793	5.96	5.161	6.97	7.08	7.075	5.699	6.389	4.998	5.368	5.82	6.177	5.547	5.721	5.167	6.935	6.975	7.267
6.845	6.45	6.557	6.566	6.674	6.423	6.67	6.95	6.665	6.599	6.91	6.308	7.615	6.454	7.17	6.829	7.421	6.869	7.145	7.491	6.875	6.838	7.281	6.487
7.436	7.253	7.225	5.693	6.347	5.87	7.329	6.37	7.115	7.174	6.337	6.435	8.242	8.104	7.746	8.837	6.31	6.468	9.407	6.476	7.909	8.244	6.521	7.824
15 Minutes												20 Minutes											
7.388	7.106	6.428	484.962	501.677	473.901	20.696	20.387	19.344	17.842	18.273	18.492	7.713	7.06	6.577	497.98	487.109	431.263	23.113	22.73	20.43	18.923	19.676	20.118
17.074	16.113	15.888	18.286	18.186	17.895	30.424	30.889	27.059	24.113	23.343	24.731	18.66	17.791	17.086	20.049	20.168	19.637	29.651	30.152	26.707	26.404	25.392	26.847
24.497	24.083	24.767	22.614	16.082	23.287	22.894	22.903	22.226	22.678	22.483	22.499	27.91	26.923	26.878	25.738	17.997	26.168	25.791	25.373	24.394	25.026	25.436	24.851
25.612	24.586	23.635	25.106	24.216	23.81	21.91	22.657	22.339	24.903	25.197	25.286	29.612	28.262	26.343	26.248	25.142	26.543	24.01	23.864	23.288	25.683	26.507	26.239
3.359	3.379	3.072	49.94	9.453	12.413	5.486	6.407	5.375	5.171	4.957	5.556	3.402	3.53	3.029	51.516	9.358	11.639	5.99	6.723	5.464	5.219	5.145	5.859
6.039	6.517	4.915	5.199	5.817	6.085	5.505	5.445	4.963	7.05	7.403	7.312	5.932	6.534	4.859	5.919	6.129	6.153	5.476	5.605	5.099	7.44	7.801	8.006
7.525	6.579	7.339	7.017	7.597	7.111	7.322	7.984	7.169	7.291	7.318	6.745	8.47	7.157	7.971	7.397	7.876	7.598	7.795	7.819	7.856	7.388	7.819	7.112
8.094	8.414	8.024	9.913	6.865	9.084	10.018	6.46	8.618	16.08	7.508	10.122	8.869	8.707	9.539	15.75	8.78	15.082	11.536	6.72	9.386	11.442	8.278	8.388
25 Minutes												30 Minutes											
7.321	6.832	6.422	491.217	456.478	387.841	25.395	24.449	22.438	20.23	20.361	22.223	7.061	6.99	6.348	474.859	422.461	358.801	27.337	26.227	23.98	22.525	21.867	23.67
20.323	19.657	18.384	22.459	22.001	21.644	28.41	28.535	25.953	28.079	27.82	29.479	22.125	21.154	19.557	24.022	23.807	23.91	27.466	28.122	25.248	30.822	29.857	31.613
31.639	30.398	30.203	29.106	20.802	29.601	29.477	28.145	27.139	27.642	27.729	27.158	35.043	33.461	33.342	33.012	22.514	32.563	31.961	30.644	28.994	30.72	29.998	29.893
33.575	31.488	29.203	28.411	26.042	26.481	26.245	25.418	25.137	27.086	27.661	27.615	36.643	35.701	33.043	29.814	27.856	27.151	29.926	29.037	28.034	29.009	29.681	29.076
3.322	3.492	3.128	46.819	8.439	10.617	5.874	6.849	5.752	5.154	5.056	5.766	3.265	3.498	2.931	50.081	8.722	11.15	5.872	6.838	6.045	5.411	5.138	5.812
5.826	6.93	5.147	5.919	5.852	6.167	5.689	5.628	5.312	7.836	7.764	7.835	5.959	6.977	5.09	5.5	5.873	6.324	5.547	5.706	5.42	8.015	8.108	7.944
8.052	6.951	7.969	8.088	7.805	7.599	8.052	8.121	8.097	7.896	7.738	7.36	8.013	7.455	8.028	8.014	7.89	7.844	7.772	8.417	8.434	7.628	8.225	7.656
8.863	8.917	8.533	24.574	7.705	9.402	12.156	6.957	10.113	11.505	9.467	9.833	8.976	8.732	8.449	20.617	10.484	17.444	12.39	6.6	10.391	18.235	11.671	10.579

SpectraMax Fluorescence values for Plate 3. R = stained with Nile Red. Med = medium blank. The CDLC standards are listed as a percent. Samples are listed as Growth Strategy and Growth Temperature, Flask Number, Date Sampled. G2.12.11 = GS1 at 15°C, Flask 2, Sampled 12/11. M = GS1 at 20°C All samples were run in triplicate.

Table G.4 *Fluorescence Values Plate 4*

Plate Set-Up												0 Minutes											
medR	medR	medR	5%R	5%R	5%R	M4.11.18R	M4.11.18R	M4.11.18R	M5.11.18R	M5.11.18R	M5.11.18R	7.253	6.338	6.36	207.116	226.616	242.176	31.737	32.069	32.575	28.249	28.799	29.537
M6.11.18R	M6.11.18R	M6.11.18R	M1.11.20R	M1.11.20R	M1.11.20R	M2.11.20R	M2.11.20R	M2.11.20R	M3.11.20R	M3.11.20R	M3.11.20R	25.415	25.396	26.373	28.611	30.536	28.988	34.856	32.87	32.141	29.974	29.557	31.121
M4.11.20R	M4.11.20R	M4.11.20R	M5.11.20R	M5.11.20R	M5.11.20R	M6.11.20R	M6.11.20R	M6.11.20R	Y1.11.21R	Y1.11.21R	Y1.11.21R	30.65	28.303	30.502	31.684	31.222	31.559	30.648	30.523	31.565	37.442	37.043	36.972
Y2.11.21R	Y2.11.21R	Y2.11.21R	Y3.11.21R	Y3.11.21R	Y3.11.21R	Y4.11.21R	Y4.11.21R	Y4.11.21R	Y5.11.21R	Y5.11.21R	Y5.11.21R	27.395	26.922	26.416	27.2	27.575	27.249	28.315	28.295	29.236	35.981	36.01	34.392
med	med	med	5%	5%	5%	M4.11.18	M4.11.19	M4.11.20	M5.11.18	M5.11.19	M5.11.20	3.286	3.513	2.998	9.866	9.195	7.011	6.402	7.315	6.306	6.122	5.752	6.483
M6.11.18	M6.11.18	M6.11.28	M1.11.20	M1.11.20	M1.11.20	M2.11.20	M2.11.20	M2.11.20	M3.11.20	M3.11.20	M3.11.20	7.195	6.656	5.614	9.738	9.708	9.359	9.293	9.409	9.173	9.115	8.596	8.433
M4.11.20	M4.11.20	M4.11.20	M5.11.20	M5.11.20	M5.11.20	M6.11.20	M6.11.20	M6.11.20	Y1.11.21	Y1.11.21	Y1.11.21	8.523	8.234	7.753	8.666	8.686	8.074	9.29	9.59	8.95	7.017	7.153	7.001
Y2.11.21	Y2.11.21	Y2.11.21	Y3.11.21	Y3.11.21	Y3.11.21	Y4.11.21	Y4.11.21	Y4.11.21	Y5.11.21	Y5.11.21	Y5.11.21	7.426	7.331	7.076	6.479	6.655	5.692	7.336	6.344	6.645	8.035	6.964	6.913

5 Minutes												10 Minutes											
7.086	6.202	6.067	311.196	349.11	373.353	37.144	37.449	38.124	32.102	32.173	33.522	6.933	6.094	6.225	415.103	447.803	465.836	40.041	40.052	39.874	35.723	36.411	37.832
27.242	27.015	28.615	28.894	29.951	28.988	38.325	25.908	25.946	30.632	31.189	32.402	30.598	30.097	33.147	31.373	31.698	30.172	44.348	40.625	41.362	33.627	34.578	35.574
33.022	29.582	31.445	32.444	31.941	32.946	31.289	31.211	31.172	45.008	44.765	44.195	39.175	32.321	33.7	35.621	34.745	36.25	35.462	33.454	33.671	50.473	51.045	50.29
26.678	26.206	26.554	26.93	28.433	28.886	29.138	29.289	29.164	42.464	41.572	38.728	28.652	28.52	28.34	30.623	30.57	32.353	30.565	30.571	32.272	46.039	45.657	42.621
3.362	3.594	2.879	11.908	7.775	7.007	6.121	6.889	6.786	6.081	6.088	6.598	3.162	3.505	2.956	10.671	7.838	7.187	6.783	7.072	6.872	6.789	6.39	6.915
6.396	6.656	5.496	9.434	10.219	8.548	9.429	9.397	8.845	9.249	8.607	9.382	6.419	6.747	5.366	10.028	10.915	9.238	9.84	10.292	9.373	9.911	10.48	10.125
8.668	8.046	7.972	9.062	9.294	8.45	9.677	9.376	9.766	7.287	7.351	7.183	8.87	8.663	8.499	10.084	9.578	8.843	9.985	9.662	10.179	7.788	7.871	7.644
7.321	7.499	7.248	6.824	6.736	5.863	7.738	6.459	7.216	8.633	7.197	7.451	8.53	8.883	7.983	7.904	7.445	6.273	9.459	7.132	8.213	8.884	7.585	7.915

15 Minutes												20 Minutes											
7.144	6.176	6.308	474.017	488.598	484.815	40.354	39.903	39.94	38.209	39.084	39.82	7.119	6.109	6.157	479.266	481.193	457.934	39.796	38.983	39.225	38.949	39.144	40.529
34.738	34.135	36.074	33.484	33.548	32.349	48.122	45.285	45.779	37.673	38.958	40.278	38	36.462	38.235	36.45	36.611	34.021	50.706	47.732	49.183	42.343	43.586	44.036
44.619	35.519	38.043	40.575	39.009	39.796	10.681	38.685	38.78	53.216	53.004	51.804	48.705	41.197	43.499	43.832	44.119	43.924	45.718	42.988	42.664	51.397	52.28	50.62
31.799	31.245	30.939	33.776	34.104	36.72	34.077	33.981	35.228	46.214	45.424	43.036	34.884	34.525	34.244	37.343	38.006	41.3	37.764	38.39	39.471	44.581	44.025	40.853
3.492	3.476	2.963	10.944	7.459	7.039	6.883	7.036	7.898	7.507	6.651	7.027	3.171	3.444	2.732	11.582	7.334	7.169	6.771	6.891	7.659	7.757	6.614	7.704
6.828	6.405	5.789	10.474	10.663	10.003	10.311	10.433	9.863	10.082	10.381	10.095	6.808	6.816	5.699	10.813	11	10.224	10.332	10.692	10.36	11.138	11.626	10.872
9.802	8.483	9.105	9.726	10.153	9.533	10.509	10.228	10.188	8.335	8.186	8.022	11.654	9.352	8.999	9.962	10.009	9.477	10.746	10.928	10.987	8.535	8.537	8.096
9.588	11.133	9.144	11.123	7.611	16.292	10.915	6.817	8.33	9.346	8.192	8.186	10.212	12.829	9.156	12.107	8.454	10.027	11.745	6.527	9.164	9.808	8.185	8.424

25 Minutes												30 Minutes											
7.103	6.12	5.936	453.015	442.596	412.533	40.191	37.925	38.909	38.346	39.029	39.85	7.15	5.764	6.102	421.438	404.757	376.091	38.768	37.298	37.887	38.059	38.485	40.147
39.383	38.293	39.071	39.475	39.247	37.309	51.101	48.319	51.022	45.125	46.435	47.258	39.226	38.817	40.475	43.12	42.452	40.978	51.337	49.01	51.423	47.52	48.137	49.082
50.167	44.621	46.349	45.214	45.873	46.064	47.454	46.269	46.181	49.572	49.171	48.132	48.784	47.57	48.618	47.256	47.987	47.45	49.13	48.41	47.771	47.869	47.714	46.549
38.858	38.94	37.507	42.218	41.864	43.793	41.82	41.216	42.363	42.597	42.54	39.036	41.151	41.98	41.225	44.115	44.663	44.067	44.833	45.041	45.135	41.552	41.604	38.963
3.274	3.561	2.869	10.766	7.313	7.257	6.873	7.064	7.7	7.667	7.043	7.819	3.366	3.423	2.756	10.52	7.282	6.891	6.802	7.19	8.05	8.572	7.807	8.493
7.132	6.883	6.048	11.191	11.381	10.553	11.074	10.867	10.215	11.32	10.376	10.808	7.275	6.991	6.288	11.727	11.729	11.541	11.152	11.232	10.644	11.328	10.886	10.94
10.686	9.35	10.355	10.775	10.225	9.522	10.911	11.086	11.694	8.981	8.862	8.383	12.192	10.021	11.234	11.112	10.226	10.26	11.437	11.183	11.853	9.183	9.08	8.699
10.901	13.726	9.772	10.705	8.969	9.857	11.767	6.302	9.563	10.206	8.436	8.69	11.545	17.096	10.329	36.845	9.423	10.286	12.512	9.376	10.175	11.193	8.546	8.745

SpectraMax Fluorescence values for Plate 3. R = stained with Nile Red. Med = medium blank. The CDLC standards are listed as a percent. Samples are listed as Growth Strategy and Growth Temperature, Flask Number, Date Sampled. M4.11.18 = GS1 at 20°C, Flask 4, Sampled 11/18. Y = GS1 at 25°C All samples were run in triplicate.

Table G.5 *Fluorescence Values Plate 5*

Plate Set-Up

medR	medR	medR	2.5%R	2.5%R	2.5%R	Y6.11.21R	Y6.11.21R	Y6.11.21R	Y1.11.23R	Y1.11.23R	Y1.11.23R
Y2.11.23R	Y2.11.23R	Y2.11.23R	Y3.11.23R	Y3.11.23R	Y3.11.23R	Y4.11.23R	Y4.11.23R	Y4.11.23R	Y5.11.23R	Y5.11.23R	Y5.11.23R
Y6.11.23R	Y6.11.23R	Y6.11.23R	W2.3.9R	W2.3.9R	W2.3.9R	W3.3.9R	W3.3.9R	W3.3.9R	W4.3.9R	W4.3.9R	W4.3.9R
W5.3.9R	W5.3.9R	W5.3.9R	W1.3.9R	W1.3.9R	W1.3.9R	W1.3.11R	W1.3.11R	W1.3.11R	W2.3.11R	W2.3.11R	W2.3.11R
med	med	med	2.50%	2.50%	2.50%	Y6.11.21	Y6.11.21	Y6.11.21	Y1.11.23	Y1.11.23	Y1.11.23
Y2.11.23	Y2.11.23	Y2.11.23	Y3.11.23	Y3.11.23	Y3.11.23	Y4.11.23	Y4.11.23	Y4.11.23	Y5.11.23	Y5.11.23	Y5.11.23
Y6.11.23	Y6.11.23	Y6.11.23	W2.3.9	W2.3.9	W2.3.9	W3.3.9	W3.3.9	W3.3.9	W4.3.9	W4.3.9	W4.3.9
W5.3.9	W5.3.9	W5.3.9	W1.3.9	W1.3.9	W1.3.9	W1.3.11	W1.3.11	W1.3.11	W2.3.11	W2.3.11	W2.3.11

0 Minutes

7.116	6.959	6.966	109.049	88.459	136.547	26.917	26.719	26.959	29.328	29.792	30.902
35.008	35.75	33.823	32.694	32.765	31.713	30.835	30.162	30.501	38.033	38.305	36.282
41.536	41.8	41.222	15.563	18.37	18.134	18.802	18.92	18.9	18.117	18.206	18.119
10.553	21.248	20.189	18.683	19.776	19.563	27.241	26.919	26.932	25.457	26.237	26.4
3.373	3.496	2.933	6.514	6.978	7.044	6.995	7.905	6.377	8.467	7.84	8.343
9.7	9.034	8.242	7.877	8.55	8.611	8.732	8.342	8.266	8.517	7.522	8.248
8.893	8.233	8.073	5.359	5.62	5.866	5.336	6.106	5.279	5.241	5.403	4.93
5.363	5.41	5.373	5.807	5.675	5.394	7.609	7.177	7.28	7.077	6.705	6.682

5 Minutes

7.165	6.672	6.52	201.906	187.029	201.308	26.599	26.619	27.552	31.613	32.424	32.984
41.264	39.497	37.674	33.192	33.144	32.25	33.365	33.736	32.825	39.378	40.26	39.611
43.489	43.146	42.719	15.581	17.94	17.458	18.567	17.923	18.516	17.807	18.193	18.119
9.423	18.59	17.679	17.935	18.483	18.133	23.834	23.667	23.691	21.807	22.302	22.488
3.291	3.425	2.884	6.049	6.518	6.479	7.103	7.915	6.417	8.721	8.736	8.514
10.17	9.14	8.224	8.032	8.541	9.007	8.326	8.117	8.108	8.522	7.794	8.217
8.507	8.665	7.989	4.997	5.661	5.531	5.08	6.094	5.317	5.05	5.006	4.821
4.974	5.132	5.037	5.584	5.682	5.068	7.136	7.193	7.206	6.946	6.575	6.35

10 Minutes

6.963	6.59	6.363	182.539	196.564	157.388	28.063	27.042	27.922	34.684	35.929	37.063
47.671	44.835	43.506	35.029	35.245	33.575	38.983	38.169	38.061	42.698	42.222	43.25
48.01	46.968	47.773	18.084	20.032	19.409	20.287	20.114	20.206	18.903	19.232	19.922
10.109	19.337	18.918	18.873	19.877	19.476	25.069	24.806	25.306	23.259	24.288	24.533
3.301	3.505	3.01	6.355	6.245	6.684	7.679	8.703	7.106	9.426	8.881	8.61
10.132	9.401	8.626	8.464	9.024	9.217	8.6	8.226	8.5	8.764	7.778	8.15
9.206	8.695	8.379	5.121	5.52	5.567	5.241	5.868	5.161	5.23	5.427	4.931
5.426	5.229	5.326	5.618	5.289	5.157	7.679	7.327	7.187	7.068	6.668	6.876

15 Minutes

6.726	6.305	6.268	150.938	161.456	136.482	30.472	28.775	29.899	39.381	39.899	40.645
54.457	50.854	48.885	37.474	37.975	35.733	46.039	44.307	44.56	46.822	46.519	46.66
52.081	51.088	51.388	20.317	22.793	22.113	23.006	22.587	23.152	22.623	21.873	24.146
11.063	22.21	21.241	21.696	23.125	22.323	29.337	28.906	29.679	26.749	27.488	27.424
3.26	3.552	3.072	6.446	6.992	6.684	8.008	9.216	7.257	9.737	9.357	9.34
10.213	10.15	9.52	9.239	9.491	9.685	10	9.708	9.471	9.961	8.69	9.273
9.797	9.602	9.532	6.714	6.692	6.588	5.945	6.656	6.305	5.721	6.201	5.562
7.309	6.691	6.914	7.612	6.928	6.713	9.363	8.404	8.348	8.295	7.6	7.451

20 Minutes

6.836	6.099	6.119	138.866	144.075	132.919	31.753	30.485	31.267	43.282	44.394	44.692
60.641	57.778	55.21	40.789	41.062	39.396	53.98	52.383	52.026	51.199	50.941	50.635
55.947	56.011	55.137	23.003	25.539	24.696	25.854	25.899	25.984	24.439	24.753	25.941
12.808	25.253	23.744	24.629	26.148	25.538	32.498	32.921	32.368	30.233	30.707	30.534
3.385	3.387	2.952	6.503	6.633	6.314	8.31	9.16	7.728	9.492	9.102	9.279
10.41	9.574	9.309	9.03	9.491	9.695	9.714	9.192	9.981	9.256	8.222	9.05
9.816	9.4	9.445	5.362	5.269	5.994	5.712	6.355	5.464	4.915	5.188	5.3
5.924	5.487	5.878	5.876	6.01	5.585	9.01	8.279	8.474	8.231	7.54	7.48

25 Minutes

6.84	6.096	5.781	135.746	138.228	130.574	34.096	31.431	33.32	45.888	46.912	47.361
65.269	63.222	60.409	44.813	44.217	42.876	57.934	56.52	55.839	54.98	54.251	54.246
59.622	58.823	58.833	25.578	28.069	27.049	28.254	28.247	28.522	27.236	26.804	28.414
13.88	27.834	26.615	27.871	29.105	28.205	35.859	35.741	35.37	33.364	34.731	34.539
3.408	3.478	2.866	6.988	6.929	6.49	8.728	9.917	7.948	10.016	9.393	9.274
10.655	9.89	9.305	9.314	9.623	10.242	9.96	9.2	10.179	9.495	8.413	8.952
10.144	9.733	9.298	5.312	5.591	5.656	5.752	5.994	5.187	5.213	5.125	5.28
5.86	5.658	5.798	5.68	5.791	5.434	9.641	8.955	8.858	8.33	7.808	7.344

30 Minutes

6.577	5.992	5.781	131.173	136.548	128.67	36.919	33.078	34.531	45.551	47.746	47.81
68.817	66.91	62.859	47.226	47.532	45.045	56.672	56.749	55.578	57.464	56.857	45.138
60.823	60.867	60.348	27.011	29.59	28.783	29.721	30.324	30.252	28.193	28.802	29.567
17.656	30.464	28.569	29.424	31.208	30.664	37.907	38.003	36.918	36.105	36.602	36.03
3.312	3.406	2.778	6.148	6.713	6.191	9.008	9.815	8.17	10.066	9.821	9.621
10.818	9.979	9.214	9.282	9.927	10.372	9.777	9.082	9.985	9.445	8.402	9.332
9.649	9.612	9.604	5.798	5.787	5.553	5.649	6.067	5.411	5.382	5.213	5.192
5.959	5.553	5.659	5.879	5.827	5.667	9.767	8.917	8.901	8.725	7.868	7.802

SpectraMax Fluorescence values for Plate 3. R = stained with Nile Red. Med = medium blank. The CDLC standards are listed as a percent. Samples are listed as Growth Strategy and Growth Temperature, Flask Number, Date Sampled. Y6.11.21 = GS1 at 25°C, Flask 6, Sampled 11/21. W = GS2 at 15°C All samples were run in triplicate.

Table G.6 *Fluorescence Values Plate 6*

Plate Set-Up												0 Minutes											
medR	medR	medR	2.5%R	2.5%R	2.5%R	W3.3.11R	W3.3.11R	W3.3.11R	W4.3.11R	W4.3.11R	W4.3.11R	8.094	6.938	6.642	169.893	118.388	158.331	19.026	19.724	19.905	20.504	20.814	21.608
W5.3.11R	W5.3.11R	W5.3.11R	W6.3.11R	W6.3.11R	W6.3.11R	B1.2.20R	B1.2.20R	B1.2.20R	B2.2.20R	B2.2.20R	B2.2.20R	19.882	19.64	19.619	19.384	19.919	19.754	24.155	23.051	23.595	22.734	22.799	22.687
B3.2.20R	B3.2.20R	B3.2.20R	B4.2.20R	B4.2.20R	B4.2.20R	B5.2.20R	B5.2.20R	B5.2.20R	B6.2.20R	B6.2.20R	B6.2.20R	22.739	22.137	13.438	23.971	23.456	22.683	22.468	22.082	21.624	19.813	19.156	19.698
B1.2.22R	B1.2.22R	B1.2.22R	B2.2.22R	B2.2.22R	B2.2.22R	B3.2.22R	B3.2.22R	B3.2.22R	B4.2.22R	B4.2.22R	B4.2.22R	36.946	34.508	34.267	36.012	35.544	32.858	33.532	33.253	31.917	25.957	26.977	26.006
med	med	med	2.5%	2.5%	2.5%	W3.3.11	W3.3.11	W3.3.11	W4.3.11	W4.3.11	W4.3.11	3.392	3.313	2.763	6.419	6.092	5.233	6.663	6.719	6.032	6.067	6.357	6.503
W5.3.11	W5.3.11	W5.3.11	W6.3.11	W6.3.11	W6.3.11	B1.2.20	B1.2.20	B1.2.20	B2.2.20	B2.2.20	B2.2.20	6.234	6.308	6.315	6.617	6.767	6.534	6.885	6.632	6.259	6.531	6.578	6.342
B3.2.20	B3.2.20	B3.2.20	B4.2.20	B4.2.20	B4.2.20	B5.2.20	B5.2.20	B5.2.20	B6.2.20	B6.2.20	B6.2.20	6.233	6.433	6.41	6.278	6.275	6.034	6.393	6.175	6.283	5.989	5.674	5.259
B1.2.22	B1.2.22	B1.2.22	B2.2.22	B2.2.22	B2.2.22	B3.2.22	B3.2.22	B3.2.22	B4.2.22	B4.2.22	B4.2.22	8.563	7.79	7.823	7.998	8.363	7.329	8.021	7.6	8.042	6.71	8.467	6.782

5 Minutes												10 Minutes											
8.289	6.985	6.738	210.116	216.829	222.614	22.917	22.886	23.123	22.258	22.158	23.158	8.53	6.872	6.659	154.93	196.309	169.803	30.818	31.6012	31.415	24.276	24.773	25.159
21.296	21.091	20.614	21.726	21.995	21.502	24.48	24.111	24.781	23.103	23.097	23.773	24.622	24.128	24.223	24.285	25.324	24.518	27.089	26.061	26.834	25.143	25.996	27.126
22.541	21.986	13.662	22.527	21.78	21.207	21.079	21.783	21.094	18.413	18.36	18.746	25.131	24.635	15.886	24.091	23.254	22.824	23.144	23.412	23.639	20.168	19.943	20.479
29.517	28.174	28.359	29.186	29.084	26.642	26.92	27.083	25.0462	21.031	20.657	20.685	28.733	27.483	27.471	27.956	28.302	26.14	26.226	27.158	25.999	21.293	20.826	20.99
3.193	3.331	2.973	8.666	6.146	6.388	6.641	7.072	6.161	6.327	6.599	6.87	3.332	3.278	3.029	8.681	6.627	6.584	7.084	7.187	6.351	6.429	6.558	6.554
6.191	6.323	5.779	6.497	6.137	6.388	6.955	6.484	6.568	6.61	6.682	6.59	6.522	6.207	6.126	6.621	6.091	6.377	7.688	6.673	6.552	6.961	6.788	6.654
6.24	6.23	6.293	6.407	5.875	6.026	6.084	5.978	5.919	6.088	5.906	5.105	6.64	6.581	6.671	6.893	6.246	6.114	6.068	6.082	6.087	6.196	6.01	5.387
8.655	7.623	7.251	8.144	7.888	6.866	8.301	7.74	8.003	6.243	8.467	7.07	9.051	8.189	8.104	8.627	8.362	7.48	9.282	7.864	8.303	6.682	8.741	7.332

15 Minutes												20 Minutes											
8.873	6.643	6.861	134.138	153.781	140.095	39.121	39.704	39.985	27.811	27.322	27.854	8.305	6.706	6.472	131.785	140.89	137.425	38.889	40.298	39.505	30.253	30.377	31.59
28.609	27.627	27.508	28.133	29.454	29.705	13.182	29.356	29.947	27.901	28.819	28.825	32.081	32.203	32.3	33.752	35.281	34.286	34.604	32.565	33.263	30.732	31.43	31.762
27.579	27.55	17.954	26.079	25.725	25.115	26.52	26.685	26.468	22.702	22.746	22.935	30.717	30.175	23.031	29.029	28.083	27.382	29.396	29.594	29.485	25.289	24.473	25.398
29.88	28.363	28.32	28.614	29.2	26.559	29	29.095	27.242	22.277	21.915	21.889	32.13	30.032	30.952	30.06	32.019	28.393	31.186	30.986	29.75	25.497	23.862	24.346
3.189	3.408	2.944	8.965	6.925	6.764	7.204	7.755	6.392	6.759	6.931	6.745	3.222	3.479	2.992	8.554	7.082	6.869	7.382	7.89	6.908	6.997	7.053	7.165
7.045	6.64	6.697	6.707	6.465	6.959	9.061	6.928	6.746	7.259	7.452	7.072	7.509	6.916	6.815	7.223	7.014	7.251	8.781	7.534	7.059	7.677	7.645	7.282
6.886	7.162	7.416	7.537	7.097	6.579	5.939	6.052	5.992	6.45	6.427	5.756	7.517	7.359	7.522	7.984	7.146	6.97	6.64	6.604	6.413	7.021	6.779	6.231
9.48	8.715	8.39	9.096	8.637	7.572	9.811	8.454	8.892	6.706	9.54	7.893	9.81	9.187	8.954	9.869	9.72	7.893	10.635	8.68	9.222	7.172	9.788	8.244

25 Minutes												30 Minutes											
8.108	6.467	6.349	131.209	135.335	134.491	36.533	37.369	36.603	34.105	33.396	34.617	8.622	6.461	6.195	130.426	133.822	132.891	34.133	35.023	35.096	36.075	36.011	36.664
35.401	36.145	36.521	38.504	39.526	38.781	39.288	35.604	36.764	34.161	34.474	35.075	37.768	38.453	37.799	40.005	39.792	39.468	41.655	38.382	39.448	36.674	37.256	38.106
33.644	33.702	26.258	31.925	30.938	30.006	33.002	32.636	32.541	27.853	27.583	27.675	36.323	36.056	26.685	34.462	34.088	33.551	35.445	35.787	35.537	30.467	29.897	30.648
34.323	32.788	33.001	33.671	35.364	31.329	34.648	35.089	34.483	28.455	26.73	27.042	38.466	36.137	35.962	38.32	40.296	35.782	38.978	39.7	39.379	31.425	30.595	31.179
3.123	3.523	2.96	9.82	7.309	7.338	7.647	8.391	7.032	7.452	7.36	7.595	3.179	3.424	2.984	9.311	7.518	7.216	7.834	8.38	7.255	7.821	7.687	7.476
7.85	7.241	7.381	7.607	7.535	7.239	8.715	7.652	7.627	7.953	7.568	7.734	8.145	7.38	7.789	8.147	7.715	7.591	8.96	8.083	7.84	8.112	8.08	7.968
7.921	7.636	7.963	8.525	7.396	7.017	6.957	6.72	6.788	7.034	6.718	6.367	8.049	8.036	8.083	9.109	7.835	7.438	7.268	7.262	7.284	7.07	6.873	6.615
10.299	9.599	8.912	9.639	9.834	8.221	10.819	8.99	9.478	7.353	10.276	8.221	10.471	9.689	9.304	10.134	10.112	8.232	11.161	9.416	9.968	7.854	10.449	8.634

SpectraMax Fluorescence values for Plate 3. R = stained with Nile Red. Med = medium blank. The CDLC standards are listed as a percent. Samples are listed as Growth Strategy and Growth Temperature, Flask Number, Date Sampled. W3.3.11 = GS2 at 15°C, Flask 3, Sampled 3/11. B = GS2 at 20°C All samples were run in triplicate.

Table G.7 *Fluorescence Values Plate 7*

Plate Set-Up												0 Minutes											
medR	medR	medR	2.5%R	2.5%R	2.5%R	B5.2.22R	B5.2.22R	B5.2.22R	B6.2.22R	B6.2.22R	B6.2.22R	7.595	6.966	6.917	151.698	163.286	164.103	28.886	27.561	28.346	23.733	23.632	24.26
R1.3.5R	R1.3.5R	R1.3.5R	R3.3.5R	R3.3.5R	R3.3.5R	R2.3.5R	R2.3.5R	R2.3.5R	R4.3.5R	R4.3.5R	R4.3.5R	23.37	22.687	23.631	23.328	23.998	23.582	24.688	24.96	25.168	24.703	25.759	26.055
R5.3.5R	R5.3.5R	R5.3.5R	R6.3.5R	R6.3.5R	R6.3.5R	R1.3.7R	R1.3.7R	R1.3.7R	R2.3.7R	R2.3.7R	R2.3.7R	23.812	24.527	24.355	18.328	23.572	25.973	37.958	36.032	36.099	40.37	38.18	39.511
R3.3.7R	R3.3.7R	R3.3.7R	R4.3.7R	R4.3.7R	R4.3.7R	R5.3.7R	R5.3.7R	R5.3.7R	R6.3.7R	R6.3.7R	R6.3.7R	40.346	39.57	39.754	42.566	42.844	41.284	48.908	47.555	47.758	49.919	49.759	49.639
med	med	med	2.5%	2.5%	2.5%	B5.2.22	B5.2.22	B5.2.22	B6.2.22	B6.2.22	B6.2.22	3.339	3.419	2.697	6.596	6.393	5.444	7.331	8.276	6.452	7.379	6.925	7.374
R1.3.5	R1.3.5	R1.3.5	R2.3.5	R2.3.5	R2.3.5	R3.3.5	R3.3.5	R3.3.5	R4.3.5	R4.3.5	R4.3.5	5.647	6.436	5.501	6.311	6.746	6.704	6.359	204.806	5.488	6.284	6.463	6.052
R5.3.5	R5.3.5	R5.3.5	R6.3.5	R6.3.5	R6.3.5	R1.3.7	R1.3.7	R1.3.7	R2.3.7	R2.3.7	R2.3.7	6.236	6.335	6.102	6.409	6.549	6.312	9.124	8.64	8.439	8.855	8.631	7.619
R3.3.7	R3.3.7	R3.3.7	R4.3.7	R4.3.7	R4.3.7	R5.3.7	R5.3.7	R5.3.7	R6.3.7	R6.3.7	R6.3.7	9.23	8.714	8.819	7.723	8.994	8.176	8.368	8.479	8.018	7.596	7.807	7.163

5 Minutes												10 Minutes											
7.508	6.624	6.63	200.959	200.833	205.352	27.536	26.918	27.649	24.061	24.505	25.255	7.511	6.741	6.74	156.568	154.281	159.748	29.124	28.115	29.691	25.606	26.065	26.778
28.391	27.487	27.882	27.143	27.56	27.583	28.434	29.558	29.072	28.759	29.332	29.99	32.828	31.776	31.649	31.241	32.897	31.264	33.11	33.819	33.22	34.336	34.669	34.884
27.042	27.534	27.518	20.338	25.533	28.836	26.969	38.084	36.659	41.036	40.183	41.321	31.532	31.988	31.679	23.378	29.571	33.954	42.636	43.235	41.74	47.207	46.331	46.08
36.487	36.974	36.939	37.858	38.653	38.896	40.346	39.799	38.984	39.729	40.151	38.899	41.153	41.347	41.034	43.233	46.713	44.095	43.675	43.15	73.793	38.623	41.021	39.636
3.305	3.501	2.856	9.065	6.551	5.005	7.812	8.637	7.141	7.793	6.977	7.845	3.261	3.54	2.69	8.198	6.444	5.065	8.313	8.61	7.333	8.187	7.593	7.985
5.349	5.792	5.285	6.085	6.724	6.59	6.165	196.892	5.48	5.975	6.379	6.367	5.608	5.803	5.053	6.441	8.03	6.405	6.315	204.936	5.51	6.037	6.415	6.097
5.949	5.992	5.681	6.283	6.567	6.532	9.526	8.803	7.988	9.354	8.921	8.306	5.883	6.126	5.445	6.765	7.186	6.602	9.528	9.319	8.821	9.523	9.492	8.475
9.543	8.688	8.573	7.775	9.31	8.271	8.193	8.234	8.111	7.225	7.819	6.817	10.436	9.567	9.408	8.89	9.9	8.818	8.212	8.632	7.887	7.536	7.896	7.018

15 Minutes												20 Minutes											
7.303	6.603	6.493	137.958	135.967	142.206	30.686	30.129	31.819	28.77	28.651	28.761	7.241	6.67	6.616	133.278	131.34	135.554	34.349	33.198	34.062	30.998	32.121	32.444
35.555	34.357	35.249	35.094	36.97	35.411	36.795	36.634	37.304	38.025	38.713	39.137	36.639	36.211	37.051	36.517	38.133	37.039	38.374	38.499	37.618	39.966	41.064	42.297
35.504	36.695	36.453	26.375	33.347	39.014	48.982	49.046	47.836	53.966	52.524	54.166	38.356	39.207	38.7	28.504	36.689	41.538	55.291	54.747	52.749	59.367	58.976	59.331
46.632	50.51	47.7	50.206	50.024	50.484	53.339	52.246	53.266	41.913	45.251	42.472	52.122	51.63	52.564	56.458	56.015	57.932	64.265	63.654	64.631	46.932	49.831	47
3.307	3.343	2.759	9.25	6.869	5.361	8.317	9.065	7.37	8.528	7.59	7.986	3.187	3.474	2.885	8.479	6.579	5.724	8.609	9.42	7.752	8.781	8.27	8.4
5.401	6.127	5.168	6.248	7.197	6.708	6.205	197.106	5.42	6.277	6.566	6.209	5.795	6.273	5.139	6.787	7.592	6.666	6.304	201.039	5.358	6.607	6.454	6.131
6.261	6.124	6.096	7.1	7.31	6.673	9.654	8.919	9.507	9.726	8.714	7.284	6.982	6.567	6.46	7.773	7.723	7.203	9.956	9.687	9.131	10.127	9.847	9.197
9.989	9.606	9.408	8.79	9.92	9.062	8.746	8.642	8.389	7.704	7.971	7.284	10.416	9.548	9.836	6.066	10.297	9.243	9.147	9.212	9.209	7.751	8.286	7.117

25 Minutes												30 Minutes											
7.123	6.239	6.369	131.38	129.906	134.609	39.714	36.813	38.258	35.083	36.4	36.301	7.028	6.389	6.194	130.574	129.512	133.855	42.594	40.562	42.057	39.041	41.461	40.373
36.456	36.235	37.064	37.343	38.899	36.389	38.985	38.114	38.82	41.458	40.401	41.938	36.041	34.673	36.203	37.056	37.647	36.349	37.913	38.07	38.021	40.629	40.411	41.08
40.335	40.702	41.241	29.175	37.136	43.36	57.951	59.981	56.089	62.694	62.576	61.963	40.288	41.354	40.934	29.409	37.217	43.455	59.532	59.036	57.492	64.219	62.916	63.137
55.095	56.167	56.633	60.831	60.706	61.303	70.047	68.126	68.208	52.054	55.213	52.558	57.676	59.43	58.405	63.96	62.718	64.188	68.641	69.244	67.761	56.945	60.86	57.978
3.244	3.305	2.966	8.396	6.734	5.567	8.737	9.842	7.445	9.308	8.622	8.175	3.312	3.515	2.896	8.574	6.775	5.618	8.755	9.572	7.763	9.547	8.498	8.738
6.037	6.439	5.269	7.407	8.433	6.704	6.29	234.46	5.501	7.205	6.78	6.471	5.979	6.567	5.107	7.251	7.871	7.16	6.535	222.322	5.711	7.556	7.112	6.474
7.477	6.379	6.446	8.392	7.872	7.568	10.472	10.067	9.825	10.295	10.086	9.459	7.54	6.433	7.094	8.535	8.167	7.352	10.553	9.544	9.508	10.339	10.59	8.298
11.082	10.224	10.077	9.754	10.47	9.629	9.655	9.658	8.894	8.043	8.392	7.419	11.384	10.275	10.186	9.654	10.83	9.423	9.783	9.765	9.205	8.029	8.319	7.58

SpectraMax Fluorescence values for Plate 3. R = stained with Nile Red. Med = medium blank. The CDLC standards are listed as a percent. Samples are listed as Growth Strategy and Growth Temperature, Flask Number, Date Sampled. B5.2.22 = GS2 at 20°C, Flask 5, Sampled 2/22. R = GS2 at 25°C All samples were run in triplicate. The shaded boxes were not included in the triplicate values as these were considered to be a technical or mechanical error.

APPENDIX H Raw CN Data

Table H.1 *CN Data for GS1 at 15°C*

Dilution Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (μg)	C (μg)	Harvest Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (μg)	C (μg)
GS1-15-F1.1	1	315.367	1561.707	304.064	1136.391	13.01345	20.94862	GS1-15-F1.1	1	220.629	2094.345	209.326	1669.029	8.16218	30.9959
GS1-15-F1.2	1	283.592	1646.4	272.289	1221.084	11.38634	22.54568	GS1-15-F1.2	1	218.374	2295.573	207.071	1870.257	8.046708	34.79372
GS1-15-F1.3	1	425.167	1790.401	413.864	1365.085	18.636	25.26158	GS1-15-F1.3	1	254.259	2194.916	242.956	1769.6	9.884278	32.89386
GS1-15-F2.1	1	325.811	1360.334	314.508	935.0177	13.54825	17.15208	GS1-15-F2.1	1	298.202	2117.535	286.899	1692.219	12.13448	31.43351
GS1-15-F2.2	1	337.829	1433.737	326.526	1008.421	14.16366	18.53584	GS1-15-F2.2	1	266.704	1979.062	255.401	1553.746	10.52155	28.82063
GS1-15-F2.3	1	358.083	1510.163	346.78	1084.847	15.20081	19.97674	GS1-15-F2.3	1	251.119	2129.684	239.816	1704.368	9.723487	31.66278
GS1-15-F3.1	1	289.228	1376.624	277.925	951.3077	11.67494	17.45916	GS1-15-F3.1	1	317.884	1912.385	306.581	1487.069	13.14234	27.56267
GS1-15-F3.2	1	295.313	1358.674	284.01	933.3577	11.98654	17.12079	GS1-15-F3.2	1	248.753	1995.938	237.45	1570.622	9.602331	29.13904
GS1-15-F3.3	1	296.453	1558.47	285.15	1133.154	12.04491	20.88758	GS1-15-F3.3	1	218.993	1968.24	207.69	1542.924	8.078405	28.61645
GS1-15-F4.1	1	281.63	1483.276	270.327	1057.96	11.28587	19.4698	GS1-15-F4.1	1	327.243	2055.413	315.94	1630.097	13.62158	30.26125
GS1-15-F4.2	1	370.695	1441.523	359.392	1016.207	15.84664	18.68262	GS1-15-F4.2	1	277.734	2007.622	266.431	1582.306	11.08637	29.35949
GS1-15-F4.3	1	393.761	1411.174	382.458	985.8577	17.02778	18.11047	GS1-15-F4.3	1	237.695	2301.858	226.392	1876.542	9.036082	34.91236
GS1-15-F5.1	1	411.636	1572.198	400.333	1146.882	17.94311	21.14644	GS1-15-F5.1	1	259.597	2103.54	248.294	1678.224	10.15762	31.16941
GS1-15-F5.2	1	240.143	1462.893	228.84	1037.577	9.161437	19.08551	GS1-15-F5.2	1	294.209	1958.625	282.906	1533.309	11.93	28.43504
GS1-15-F5.3	1	198.942	1267.07	187.639	841.7537	7.05165	15.39412	GS1-15-F5.3	1	194.809	2138.637	183.506	1713.321	6.840011	31.83174
GS1-15-F6.1	1	281.629	1385.83	270.326	960.5137	11.28582	17.6327	GS1-15-F6.1	1	344.51	2142.003	333.207	1716.687	14.50578	31.89526
GS1-15-F6.2	1	280.96	1470.458	269.657	1045.142	11.25156	19.22814	GS1-15-F6.2	1	265.167	2103.079	253.864	1677.763	10.44285	31.16071
GS1-15-F6.3	1	251.164	1454.022	239.861	1028.706	9.725792	18.91826	GS1-15-F6.3	1	263.602	2129.313	252.299	1703.997	10.36271	31.65578

Output from the Costech CN Analyzer. Nitrogen data was used to calculate the protein content. Samples for GS1 at 15°C.

Table H.2 *CN Data for GS1 at 20°C*

Dilution Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (µg)	C (µg)	Harvest Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (µg)	C (µg)
GS1-20-F1	1	214.562	1833.944	203.259	1408.628	7.851506	26.08292	GS1-20-F1	1	433.121	2437.991	421.818	2012.675	19.0433	37.48227
GS1-20-F2	1	243.048	1792.394	231.745	1367.078	9.310194	25.29917	GS1-20-F2	1	378.107	2466.506	366.804	2041.19	16.22619	38.02064
GS1-20-F3	1	146.891	1781.067	135.588	1355.751	4.386265	25.08552	GS1-20-F3	1	337.133	2492.553	325.83	2067.237	14.12802	38.51244
GS1-20-F4	1	108.937	1709.762	97.634	1284.446	2.442747	23.74064	GS1-20-F4	1	529.714	2374.875	518.411	1949.559	23.98955	36.29071
GS1-20-F5	1	149.158	1735.356	137.855	1310.04	4.502352	24.22335	GS1-20-F5	1	407.199	2376.552	395.896	1951.236	17.7159	36.32237
GS1-20-F6	1	122.544	1696.41	111.241	1271.094	3.139524	23.48882	GS1-20-F6	1	420.445	2447.09	409.142	2021.774	18.3942	37.65406

Output from the Costech CN Analyzer. Nitrogen data was used to calculate the protein content. Samples for GS1 at 20°C.

Table H.3 *CN Data for GS1 at 25°C*

Dilution Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (μg)	C (μg)	Harvest Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (μg)	C (μg)
GS1-25-F1	1	377.419	2026.538	366.116	1601.222	16.19095	29.71641	GS1-25-F1.1	1	233.207	2886.049	221.904	2460.733	8.806264	45.94427
GS1-25-F2	1	373.399	2042.055	362.096	1616.739	15.9851	30.0092	GS1-25-F1.2	1	256.251	2860.889	244.948	2435.573	9.986283	45.46895
GS1-25-F3	1	290.214	1986.941	278.911	1561.625	11.72543	28.96929	GS1-25-F1.3	1	262.284	2812.129	250.981	2386.813	10.29522	44.54784
GS1-25-F4	1	350.158	1996.161	338.855	1570.845	14.795	29.14325	GS1-25-F2.1	1	205.908	2709.857	194.605	2284.541	7.408359	42.61606
GS1-25-F5	1	291.557	2080.495	280.254	1655.179	11.7942	30.73454	GS1-25-F2.2	1	154.585	2842.854	143.282	2417.538	4.780253	45.12825
GS1-25-F6	1	360.345	2027.813	349.042	1602.497	15.31664	29.74047	GS1-25-F2.3	1	170.553	2676.835	159.25	2251.519	5.597929	41.99239
								GS1-25-F3.1	1	288.383	2692.393	277.08	2267.077	11.63167	42.28622
								GS1-25-F3.2	1	347.645	2603.576	336.342	2178.26	14.66631	40.60886
								GS1-25-F3.3	1	189.485	2830.728	178.182	2405.412	6.567384	44.89918
								GS1-25-F4.1	1	223.373	2633.064	212.07	2207.748	8.302693	41.16574
								GS1-25-F4.2	1	212.282	2732.407	200.979	2307.091	7.734754	43.04198
								GS1-25-F4.3	1	256.093	2569.361	244.79	2144.045	9.978192	39.96275
								GS1-25-F5.1	1	195.971	2799.462	184.668	2374.146	6.899514	44.30856
								GS1-25-F5.2	1	229.883	2923.807	218.58	2498.491	8.636052	46.65761
								GS1-25-F5.3	1	220.702	2948.025	209.399	2522.709	8.165918	47.11517
								GS1-25-F6.1	1	232.278	2744.546	220.975	2319.23	8.758693	43.27126
								GS1-25-F6.2	1	204.301	2647.889	192.998	2222.573	7.326069	41.44571
								GS1-25-F6.3	1	202.132	2650.927	190.829	2225.611	7.215001	41.50309

Output from the Costech CN Analyzer. Nitrogen data was used to calculate the protein content. Samples for GS1 at 25°C.

Table H.4 *CN Data for GS2 at 15°C*

Dilution Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (µg)	C (µg)	Harvest Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (µg)	C (µg)
GS2-15-F1.1	1	167.807	1806.759	143.326	1331.139	5.629078	22.567	GS2-15-F1.1	1	205.153	2526.338	180.672	2050.718	7.147055	33.2288
GS2-15-F1.2	1	154.649	1740.181	130.168	1264.561	5.094255	21.581	GS2-15-F1.2	1	190.414	2345.385	165.933	1869.765	6.547969	30.54591
GS2-15-F1.3	1	150.383	1839.214	125.902	1363.594	4.920858	23.047	GS2-15-F1.3	1	322.575	2461.897	298.094	1986.277	11.91982	32.27324
GS2-15-F2.1	1	135.978	1851.295	111.497	1375.675	4.335348	23.226	GS2-15-F2.1	1	191.241	2225.567	166.76	1749.947	6.581583	28.77004
GS2-15-F2.2	1	133.107	1605.577	108.626	1129.957	4.218652	19.589	GS2-15-F2.2	1	200.741	2315.956	176.26	1840.336	6.967723	30.10968
GS2-15-F2.3	1	170.265	1743.391	145.784	1267.771	5.728987	21.628	GS2-15-F2.3	1	198.396	2225.36	173.915	1749.74	6.872408	28.76697
GS2-15-F3.1	1	193.373	1760.555	168.892	1284.935	6.668241	21.883	GS2-15-F3.1	1	170.221	2164.529	145.74	1688.909	5.727199	27.86556
GS2-15-F3.2	1	125.285	1591.543	100.804	1115.923	3.900717	19.381	GS2-15-F3.2	1	151.746	2159.194	127.265	1683.574	4.976259	27.78651
GS2-15-F3.3	1	122.891	1315.813	98.41	840.1933	3.80341	15.302	GS2-15-F3.3	1	175.445	2259.811	150.964	1784.191	5.939535	29.27753
GS2-15-F4.1	1	183.183	1691.072	158.702	1215.452	6.254056	20.854	GS2-15-F4.1	1	220.772	2025.054	196.291	1549.434	7.781909	25.79925
GS2-15-F4.2	1	143.107	1664.394	118.626	1188.774	4.625115	20.459	GS2-15-F4.2	1	186.115	2062.552	161.634	1586.932	6.373231	26.35472
GS2-15-F4.3	1	168.482	1628.776	144.001	1153.156	5.656515	19.932	GS2-15-F4.3	1	178.755	2166.563	154.274	1690.943	6.074074	27.8957
GS2-15-F5.1	1	178.004	1734.891	153.523	1259.271	6.043549	21.503	GS2-15-F5.1	1	154.073	2121.912	129.592	1646.292	5.070842	27.23412
GS2-15-F5.2	1	204.741	1802.903	180.26	1327.283	7.130308	22.509	GS2-15-F5.2	1	157.832	2132.384	133.351	1656.764	5.223632	27.38928
GS2-15-F5.3	1	233.243	1829.784	208.762	1354.164	8.288809	22.907	GS2-15-F5.3	1	194.581	2202.255	170.1	1726.635	6.717342	28.42458
GS2-15-F6.1	1	167.281	1748.799	142.8	1273.179	5.607699	21.709	GS2-15-F6.1	1	193.78	2273.709	169.299	1798.089	6.684784	29.48351
GS2-15-F6.2	1	160.002	1561.422	135.521	1085.802	5.311834	18.935	GS2-15-F6.2	1	187.677	2320.975	163.196	1845.355	6.43672	30.18408
GS2-15-F6.3	1	168.754	1707.688	144.273	1232.068	5.667571	21.1	GS2-15-F6.3	1	226.439	2318.151	201.958	1842.531	8.012251	30.14222

Output from the Costech CN Analyzer. Nitrogen data was used to calculate the protein content. Samples for GS2 at 15°C.

Table H.5 *CN Data for GS2 at 20°C*

Dilution Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (µg)	C (µg)	Harvest Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (µg)	C (µg)
GS2-20-F1.1	1	138.108	2416.136	113.627	1940.516	4.421925	31.595	GS2-20-F1.1	1	201.227	3123.329	176.746	2647.709	6.987477	42.08794
GS2-20-F1.2	1	156.694	2569.304	132.213	2093.684	5.177376	33.866	GS2-20-F1.2	1	196.224	3159.578	171.743	2683.958	6.784124	42.62626
GS2-20-F1.3	1	144.776	2458.436	120.295	1982.816	4.692954	32.222	GS2-20-F1.3	1	186.989	3173.029	162.508	2697.409	6.408755	42.82602
GS2-20-F2.1	1	135.729	2289.383	111.248	1813.763	4.325227	29.716	GS2-20-F2.1	1	213.202	3163.418	188.721	2687.798	7.474217	42.68328
GS2-20-F2.2	1	129.917	2347.457	105.436	1871.837	4.088991	30.577	GS2-20-F2.2	1	215.076	3228.29	190.595	2752.67	7.550388	43.64678
GS2-20-F2.3	1	133.854	2406.041	109.373	1930.421	4.249015	31.445	GS2-20-F2.3	1	197.123	3197.719	172.642	2722.099	6.820665	43.19271
GS2-20-F3.1	1	119.381	2242.312	94.9	1766.692	3.660742	29.018	GS2-20-F3.1	1	218.726	3161.81	194.245	2686.19	7.698747	42.6594
GS2-20-F3.2	1	105.268	2209.16	80.787	1733.54	3.087101	28.527	GS2-20-F3.2	1	200.03	3080.182	175.549	2604.562	6.938824	41.44725
GS2-20-F3.3	1	105.702	2083.923	81.221	1608.303	3.104741	26.671	GS2-20-F3.3	1	208.985	3054.258	184.504	2578.638	7.302811	41.06234
GS2-20-F4.1	1	116.701	2036.416	92.22	1560.796	3.55181	25.968	GS2-20-F4.1	1	208.419	2939.142	183.938	2463.522	7.279805	39.35338
GS2-20-F4.2	1	134.041	2170.593	109.56	1694.973	4.256616	27.955	GS2-20-F4.2	1	227.64	3048.438	203.159	2572.818	8.061068	40.97592
GS2-20-F4.3	1	109.416	1854.858	84.935	1379.238	3.255701	23.279	GS2-20-F4.3	1	194.869	2988.721	170.388	2513.101	6.729048	40.08935
GS2-20-F5.1	1	132.471	2244.141	107.99	1768.521	4.192801	29.045	GS2-20-F5.1	1	182.959	2913.133	158.478	2437.513	6.244951	38.96733
GS2-20-F5.2	1	126.118	2166.575	101.637	1690.955	3.934576	27.896	GS2-20-F5.2	1	208.513	2910.938	184.032	2435.318	7.283626	38.93475
GS2-20-F5.3	1	116.071	2197.793	91.59	1722.173	3.526202	28.358	GS2-20-F5.3	1	189.11	2934.947	164.629	2459.327	6.494966	39.29111
GS2-20-F6.1	1	116.808	1963.835	92.327	1488.215	3.556159	24.893	GS2-20-F6.1	1	217.534	2838.623	193.053	2363.003	7.650296	37.8615
GS2-20-F6.2	1	103.93	2280.27	79.449	1804.65	3.032716	29.581	GS2-20-F6.2	1	215.527	2909.357	191.046	2433.737	7.568719	38.91128
GS2-20-F6.3	1	115.878	2077.771	91.397	1602.151	3.518358	26.58	GS2-20-F6.3	1	187.006	2962.803	162.525	2487.183	6.409446	39.7046

Output from the Costech CN Analyzer. Nitrogen data was used to calculate the protein content. Samples for GS2 at 20°C.

Table H.6 *CN Data for GS2 at 25°C*

Dilution Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (μg)	C (μg)	Harvest Phase Sample	Volume Filtered (mL)	N Area	C Area	N-Blank	C-Blank	N (μg)	C (μg)
GS2-25-F1.1	1	294.547	2011.571	270.066	1535.951	10.78059	25.6	GS2-25-F1.1	1	232.701	3169.027	208.22	2693.407	8.266778	42.76658
GS2-25-F1.2	1	186.279	2193.505	161.798	1717.885	6.379897	28.295	GS2-25-F1.2	1	246.743	3168.633	222.262	2693.013	8.837533	42.76073
GS2-25-F1.3	1	178.776	2013.742	154.295	1538.122	6.074928	25.632	GS2-25-F1.3	1	258.054	3197.139	233.573	2721.519	9.297284	43.1841
GS2-25-F2.1	1	265.087	2454.926	240.606	1979.306	9.583149	32.17	GS2-25-F2.1	1	274.289	3626.995	249.808	3151.375	9.957176	49.57157
GS2-25-F2.2	1	267.948	2222.487	243.467	1746.867	9.699438	28.724	GS2-25-F2.2	1	251.548	3644.275	227.067	3168.655	9.032839	49.82848
GS2-25-F2.3	1	203.716	2302.972	179.235	1827.352	7.088646	29.917	GS2-25-F2.3	1	240.909	3503.061	216.428	3027.441	8.600403	47.72932
GS2-25-F3.1	1	208.858	2070.78	184.377	1595.16	7.297649	26.477	GS2-25-F3.1	1	225.651	3225.082	201.17	2749.462	7.980222	43.59913
GS2-25-F3.2	1	197.183	2078.78	172.702	1603.16	6.823104	26.595	GS2-25-F3.2	1	244.242	3188.238	219.761	2712.618	8.735877	43.0519
GS2-25-F3.3	1	157.3	2095.59	132.819	1619.97	5.202008	26.844	GS2-25-F3.3	1	222.24	3396.303	197.759	2920.683	7.841578	46.14281
GS2-25-F4.1	1	313.865	2265.09	289.384	1789.47	11.56579	29.356	GS2-25-F4.1	1	251.112	3632.339	226.631	3156.719	9.015117	49.65102
GS2-25-F4.2	1	209.004	2272.588	184.523	1796.968	7.303583	29.467	GS2-25-F4.2	1	243.743	3305.784	219.262	2830.164	8.715595	44.79792
GS2-25-F4.3	1	182.158	2180.482	157.677	1704.862	6.212393	28.102	GS2-25-F4.3	1	300.083	3396.176	275.602	2920.556	11.00561	46.14092
GS2-25-F5.1	1	193.209	2159.245	168.728	1683.625	6.661575	27.787	GS2-25-F5.1	1	228.075	3309.969	203.594	2834.349	8.078749	44.8601
GS2-25-F5.2	1	180.266	2092.424	155.785	1616.804	6.135491	26.797	GS2-25-F5.2	1	263.851	3423.37	239.37	2947.75	9.53291	46.54501
GS2-25-F5.3	1	175.195	2142.525	150.714	1666.905	5.929373	27.54	GS2-25-F5.3	1	240.121	3421.933	215.64	2946.313	8.568374	46.52366
GS2-25-F6.1	1	214.9	2241.419	190.419	1765.799	7.543234	29.005	GS2-25-F6.1	1	275.739	3263.17	251.258	2787.55	10.01611	44.16488
GS2-25-F6.2	1	225.168	2369.85	200.687	1894.23	7.96059	30.909	GS2-25-F6.2	1	243.486	3180.013	219.005	2704.393	8.705149	42.92974
GS2-25-F6.3	1	273.987	2499.102	249.506	2023.482	9.944901	32.825	GS2-25-F6.3	1	274.094	3243.35	249.613	2767.73	9.94925	43.87047

Output from the Costech CN Analyzer. Nitrogen data was used to calculate the protein content. Samples for GS2 at 25°C.

REFERENCES

Benavente-Valdés, J., Aguilar C., Contreras-Esquivel J., Méndez-Zavala A., Montañez J. 2016. Strategies to enhance the production of photosynthetic pigments and lipids in chlorophycae species. Biotechnology Reports, 10:117-125.

Borowitzka, M.A. 1999. Commercial production of microalgae: ponds, tanks, tubes, and fermenters. Journal of biotechnology, 70: 313-321.

Chen, H., Qiu, T., Rong, J., He, C., Wang, Q. 2015. Microalgal biofuel revisited: an informatics-based analysis of developments to date and future prospects. Applied Energy, 155:585-598.

Chrismadha, T. and Borowitzka, M.A. 1994. Effect of cell density and irradiance on growth, proximate composition and eicosapentaenoic acid production of Phaeodactylum tricornutum grown in a tubular photobioreactor. Journal of Applied Phycology, 6(1):67-74.

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to Best Practices for Ocean CO2 Measurements. PICES Special Publication 3, 191 pp.

Geider, R.J., MacIntyre H.L., and Kana, T.M. 1997. Dynamic model of phytoplankton growth and acclimation: response of the balanced growth rate and the chlorophyll a: carbon ratio to light, nutrient-limitation and temperature. Marine Ecology Progress Series, 148(1/3):187-200.

Goldman J.C. 1980. Physiological Processes, Nutrient Availability, and the Concept of Relative Growth Rate in Marine Phytoplankton Ecology. In: Falkowski P.G. (eds) Primary Productivity in the Sea. Environmental Science Research, vol 19. Springer, Boston, MA.

Goldman, J.C., Azov, Y., Riley, C.B., and Dennett, M.R. 1982. The effect of pH in intensive microalgal cultures. I. Biomass Regulation. Journal of Experimental Marine Biology and Ecology, 57(1):1-13.

Goldman, J.C., McCarthy J.J., and Peavey D.G. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature, 279:210-215.

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. Pp 26-60. *In* Smith W.L. and Chanley M.H (Eds.) Culture of Marine Invertebrate Animals. Plenum Press, New York, USA.

Guillard, R. R. L. and Ryther, J. H. 1962. Studies of marine planktonic diatoms. II. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleave. Canadian Journal of Microbiology. 8:229-239.

Hannon, M., Gimpel J., Tran, M., Rasala, B., and Mayfield, S. Biofuels from algae: challenges and potential. Biofuels, 1(5):763-784.

Huntley, M.E., Redalje, D.G. 2006. CO_2 Mitigation and renewable oil form photosynthetic microbes: a new appraisal. Mitigation and Adaption Strategies for Global Change, 12:573-608.

Huntley, M.E., Johnson, Z.I., Brown, S.L., Sills, D.L., Gerber, L., Archibald, I., Machesky, S.C., Granados, J., Beal, C., Greene, C.H. 2015. Demonstrated large-scale production of marine microalgae for fuels and feed. Algal Research, 10:249-265.

Jiang, Y., Yoshida T., Quigg, A. 2012. Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. Plant Physiology and Biochemistry, 54:70-77.

Johnson, Z.I., Bidigare, R.R., Blinebry, S.K., Brown, S.L., Cullen, J.J., Loftus, S.E., Redalje, D.G., Swink, C., Van Mooy, B.A.S. 2017. Screening for lipids form marine microalgae using nile red. Handbook of Hydrocarbon and Lipid Microbiology.

Khalil, Z.I., Asker, M.M.S., El-Sayed, S., and Kobbia, I.A. 2010. Effect of pH on growth and biochemical responses of Bunaleilla bardawil and Chlorella ellipsoidea. World Journal of Microbiology and Biotechnology, 26:1225-1231.

Kumar K., Mishra, S.K., Shrivastav, A., Park, M.S., Yang, J. 2015. Recent trends in the mass cultivation of algae in raceway ponds. Renewable and Sustainable Energy Reviews, 51:875-885.

Laws, E.A., DiTullio, G.R., Redalje, D.G. 1987. High phytoplankton growth and production rates in the North Pacific subtropical gyre. Limnology and Oceanography, 32(4): 905-918.

Laws, E.A., Terry, K.L., Wickman, J., and Chalup, M.S. 1983. A simple algal production system designed to utilize the flashing light effect. Biotechnology and Bioengineering, 25:2319-2335.

Li, J., Stamato, M., and Velliou, E. 2015. Design and characterization of a scalable airlift flat panel photobioreactor for microalgae cultivation. Journal of Applied Phycology, 27:75-86.

Lin, Q. and Lin, J. 2011. Effects of nitrogen source and concentration on biomass and oil production of a Scenedesmus rubescens like microalga. Bioresource Technology, 102(2):1615-1621.

MacIntyre, H.L., and Cullen, J.J. 2005. Using cultures to investigate the physiological ecology of microalgae. Ch 19, Algal Culturing Techniques. pp 287-326.

Mandal, S. and Mallick, N. 2009. Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. Applied Microbiology and Biotechnology, 84:281-291.

Marcó, A., Rubio, R., Compañó, R., Casals, I. 2002. Comparison of the Kjeldahl method and a combustion method for total nitrogen determination in animal feed. Talanta, 57:1019-1026.

Pahlow, M. and Oschlies, A. 2013. Optimal allocation back Droop's cell-quota model. Marine Ecology Progress Series, 473:1-5.

Redalje, D.G. Report of cellana throughput screening study. (unpublished)

Rogers, J.N., Rosenberg, J.N., Guzman, B.J., Oh, V.H., Mimbela, L.E., Ghassemi, A., Betenbaugh, M.J., Oyler, G.A., Donohue, M.D. 2014. A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. Algal Research, 4:76-88.

Sarkar, D., and Shimizu, K. 2015. An overview on biofuel and biochemical production by photosynthetic microorganisms with understanding of the metabolism and by metabolic engineering together with efficient cultivation and downstream processing. Bioresources and Bioprocessing, 2(17): 1-19.

Sharma, K.K., Schuhmann, H., Schenk, P.M. 2012. High lipid induction in microalgae for biodiesel production. Energies, 5: 1532-1553.

Sheehan, John & Dunahay, Terri & Benemann, John & Roessler, Paul. (2009). A look back at the U.S. department of energy's aquatic species program: Biodiesel from algae.

Shuter, B. 1979. A model of physiological adaptation in unicellular algae. Journal of
Theoretical Biology, 78: 519-552.

Stephenson, A.L., Dennis, J.S., Howe, C.J., and Smith, A.G. 2010. Influence of nitrogen-
limitation regime of the production by Chlorella vulgaris of lipids for biodiesel
feedstocks. Biofuels, 1(1):47-58.

Tan, Y. and Lin, J. 2011. Biomass production and fatty acid profile of a Scenedesmus
rubescens-like microalga. Bioresource Technology, 102(21):10131-10135.

Templeton, D.W., Laurens, L. M. L. 2015. Nitrogen-to-protein conversion factors
revisited for applications of microalgal biomass conversion to food, feed and fuel.
Algal Research, 11: 359-367.

Ugwu, C.U., Aoyagi, H., Uchiyama, H. 2008. Photobioreactors for mass cultivation of
algae. Bioresource Technology, 99: 4021-4028.

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll a in the presence of
chlorophyll b and pheopigments. Limnology and Oceanography, 39(8):1985-
1992.

Yuvraj and Padmanabhan, P. 2017. Technical insight on the requirements for CO_2-
saturated growth of microalgae in photobioreactors. 3 Biotech, 7(2):119.

Zhu, C.J. and Lee, Y.K. 1997. Determination of biomass dry weight of marine
microalgae. Journal of Applied Phycology, 9: 189-194.