The Effect of Dark Period Duration on Synechococcus WH8102 Growth Rates

by
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A PROJECT

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in BioHealth Sciences
(Honors Associate)

Presented June 4, 2015
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Marius Ibuye Balola for the degree of Honors Baccalaureate of Science in BioHealth Sciences presented on June 4, 2015. Title: The Effect of Dark Period Duration on Synechococcus WH8102 Growth Rates.

Abstract approved:

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Kimberly Halsey

Synechococcus is one of the most abundant groups of primary producers in the marine environment. They play a crucial role in the food web by being the main source of alimentary energy to many marine organisms that contribute to human nutriments. The ability of Synechococcus to use light as a source of energy through photosynthesis makes them important in the marine environment. Understanding how these photosynthetic organisms respond and adapt to fluctuations in their environment is important to studies of phytoplankton physiology. To date, no studies have been done that measured the effect of dark period duration on the growth rate of Synechococcus. This sparked our curiosity to examine how the duration of a dark period impacts the growth rate of the ecologically important cyanobacteria, Synechococcus sp. WH8102. We found that exposure of Synechococcus WH8102 to a dark period did not enhance growth rate at any light intensity studied thus far. More research is needed to more finely resolve the potential effects of dark period on growth in this organism. These results will provide insights to understand the global success of this organism in marine environments.

Key Words: Cyanobacteria, Synechococcus, dark periods, growth rates

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Marius Ibuye Balola, Author

Introduction

Synechococcus is one of the most abundant groups of primary producers in the marine environment. As with many other cyanobacterial species, *Synechococcus* sp. plays a crucial role in the food web. They constitute the base of aquatic food web, thus providing nutrients to microorganisms as zooplankton, which in turn are consumed by larger organisms. [1] This food web, therefore, makes *Synechococcus* important for human food production since the fish stocks depend on the primary producers.

Cyanobacteria are responsible for the oxygenation of Earth's atmosphere. Cyanobacteria are known to be the first oxygen-producing organisms. [2] They contribute vastly to ecological oxygen cycle. As a component of this large and diverse group of phytoplankton, *Synechococcus* contribute 70 to 80 percent of the oxygen in the atmosphere and simultaneously, they fix around 25 megatons of carbon dioxide. [3] These bacteria reduce carbon dioxide in the oceans through their photosynthetic processes converting it into inorganic carbon.

Synechococcus is a prokaryotic unicellular cyanobacterium and thus carries out oxygenic photosynthesis and respiration activities within its plasma membrane. It is mostly found in well-lit surface of marine environment where light is non-limiting. During photosynthesis, *Synechococcus* uses light energy to make organic carbon, which is eventually used to make energy needed for growth.

Occasionally, *Synechococcus* will use hydrogen sulfide as electron donor, however water is the preferred electron source, causing oxygen to be created as a byproduct. [4] Through this process electrons become available for reduction of carbon dioxide into organic carbon. Photosynthetic electron transport also drives the formation of a proton gradient that is used to generate ATP. During these processes, different metabolic activities can be conducted together or separately during dark or light periods. Phycobilisomes and chlorophyll are used to harvest light to carry out photosynthesis during the day. In the dark (at night), the cells will use oxygen as a terminal electron acceptor, and through aerobic respiration, they breakdown their stored carbohydrate to fuel other metabolic processes. [5]

In contrast to eukaryotic algae, which can spatially separate carbon fixation and electron transport within chloroplasts and mitochondria, respectively, *Synechococcus* lacks membrane bound organelles and thus uses its plasma membrane for both activities. This difference in cellular organization led us to hypothesize that *Synechococcus* would benefit from a temporal separation of photosynthetic carbon fixation and respiration.

Synechococcus has adapted to various environments. Its successful adaptation is based on its ability to live in environments that fluctuate in availabilities of light and nutrients, such as oligotrophic areas that are characterized by extremely low

concentrations of nutrients. Such environments are typical for open sea spaces where these cells are specialized for obtaining necessary nutrients and trace metals. [6]

In addition to the responses of cyanobacteria to nutrient depletion, previous studies have examined how other factors such as light fluctuation, day length, and temperature affect the growth rate of cyanobacteria. Day length and temperature were observed to have separate and/or interactive impacts on cyanobacterial growth rate. In general, the results showed that cyanobacteria grew most efficiently with short daylengths and warm temperatures. [7]

We measured growth rate responses under different light intensities and light/dark periods. We hypothesized that for a given light intensity the growth rate would be faster when a dark period was provided. Our hypothesis also stated that there would be an optimal dark period duration where the enhancement of growth rate would be observed. The long-term goal is to understand how these photosynthetic organisms respond and adapt to fluctuations in their environment. In this case, light period was the variable we chose to study to learn how these cells optimize their growth in marine ecosystems. The results of this project will give clues to the physiological processes used by this abundant and ecologically important cyanobacterium.

Materials and Methods

Culture Conditions

Synechococcus WH8102 cultures were grown in artificial seawater, f/2 + Si media, at a constant temperature of 20 ºC and lit by fluorescent tubes. Nine glass flasks were inoculated with *Synechococcus* WH8102 to an initial concentration of about 10^4 cells per ml. Among these nine flasks, three were exposed to ~195-200 μmol photons m^{-2} s^{-1}, and six were exposed to lower light intensities that were achieved by screening the fluorescent tubes with neutral density screening. Thus, three flasks were exposed to ~ 45-50 μmol photons m^{-2} s^{-1} and three flasks were exposed to ~5-10 μmol photons m^{-2} s^{-1}. The intensity of light was measured using a PAR (photosynthetically active radiation) sensor. The experiment was conducted in three parts under a 24 hour cycle each. It should be noted that the light intensities remained the same throughout the experiment (10, 50, and 200 μmol photons m^{-2} s^{-1}).

In the first experiment, all nine flasks were gown with no dark period (24 hour light period). In the second experiment, flasks were exposed to 12 hours of light and 12 hours of dark time, and the third experiment was planned such that cultures would be exposed to light for 18 hours and 6 hours of dark period. A fourth experiment was planned such that cultures would be exposed to 6 hours of light and 18 hours of dark period. Note that time constraints did not allow completion of the third and fourth experiments.

Cell Density Measurements

Throughout the experiments the cell density of each flask was measured by flow cytometry to calculate the growth rate of *Synechococcus* in response to different light intensity and duration of dark period. One milliliter of each culture flask was transferred to a tube for cell counts by flow cytometry. For samples that were highly concentrated, 1/10 or 1/100 dilution was applied accordingly. Transferred samples were mixed with 1 µl 50% glyceraldehyde and incubated at room temperature for 15 minutes to fix the cells before running the samples in the flow cytometer.

The cell density (cells/mL) was obtained using the following formula:

(# events/time) X (flow rate)

where events are cells and the flow rate through the cytometer was 1 s/µl. The number of events was obtained from a delineated region known to capture the cell size and orange fluorescence signature of *Synechococcus*.

The average and standard deviation of the cell density was taken from the triplicate cultures grown at each light intensity, and results were plotted using Excel.

Results

Synechococcus WH8102 was grown in triplicate at each of three light intensities (10, 50, 200 µE) at a constant temperature (20ºC).

During the first experiment (Figure 1), cultures were grown under zero dark periods (constant light). This experiment lasted 20 days for cultures at 50 and 200 μE and 28 days under 10 μE. We observed the typical three phases of a growth curve for a bacterial batch culture (lag, exponential, stationary). However, variability in the apparent growth rate was observed in cells growing at 10 μE. From these growth curves, I calculated growth rates (Table 1) of cells grown at each light intensity. The fastest growth rate was observed for cells growing at 50 μE. Cells growing at 200 μE has a growth rate that was about half of cells grown at 50 µE. Cells growing at 10µE grew the slowest.

Light intensity	Growth Rate (division/day)
200 µE	0.57
50 µE	1.3
10 µE	0.15

Table 1: *Synechococcus* growth rate (division/time) at three different light intensities with zero dark periods

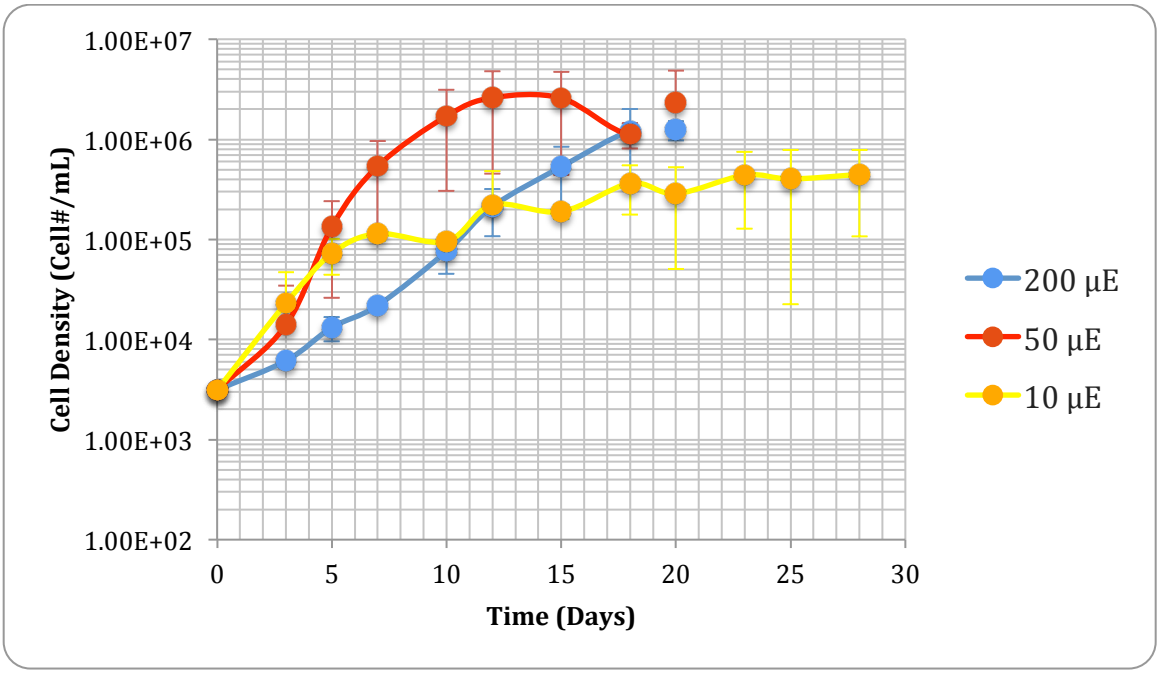

Figure 1: *Synechococcus* WH8102 growth at three different light intensities with zero dark periods (constant light).

In the second experiment (Figure 2), cultures were grown with a 12-hour dark and 12-hour light periods. This experiment lasted 24 days for cultures growing at 200 µE and 26 days for cultures growing at 10 and 50 µE. We observed a typical bacterium cell curve with 200 µE and curves with variability at 10 and 50 µE.

The growth rates calculated from Figure 2 are shown in Table 2. At 12-hour dark period the growth rates at 200 and 50 µE were almost the same. Negligible growth rate was observed at 10 µE due to insufficient light to sustain growth.

Light Intensity	Growth Rate (division/day)
200 µE	0.41
50 µE	0.44
10 µE	3.0 E-05 (negligible)

Table 2: *Synechococcus* WH8102 growth rates (division/day) at three different light intensities with a 12-hour dark period

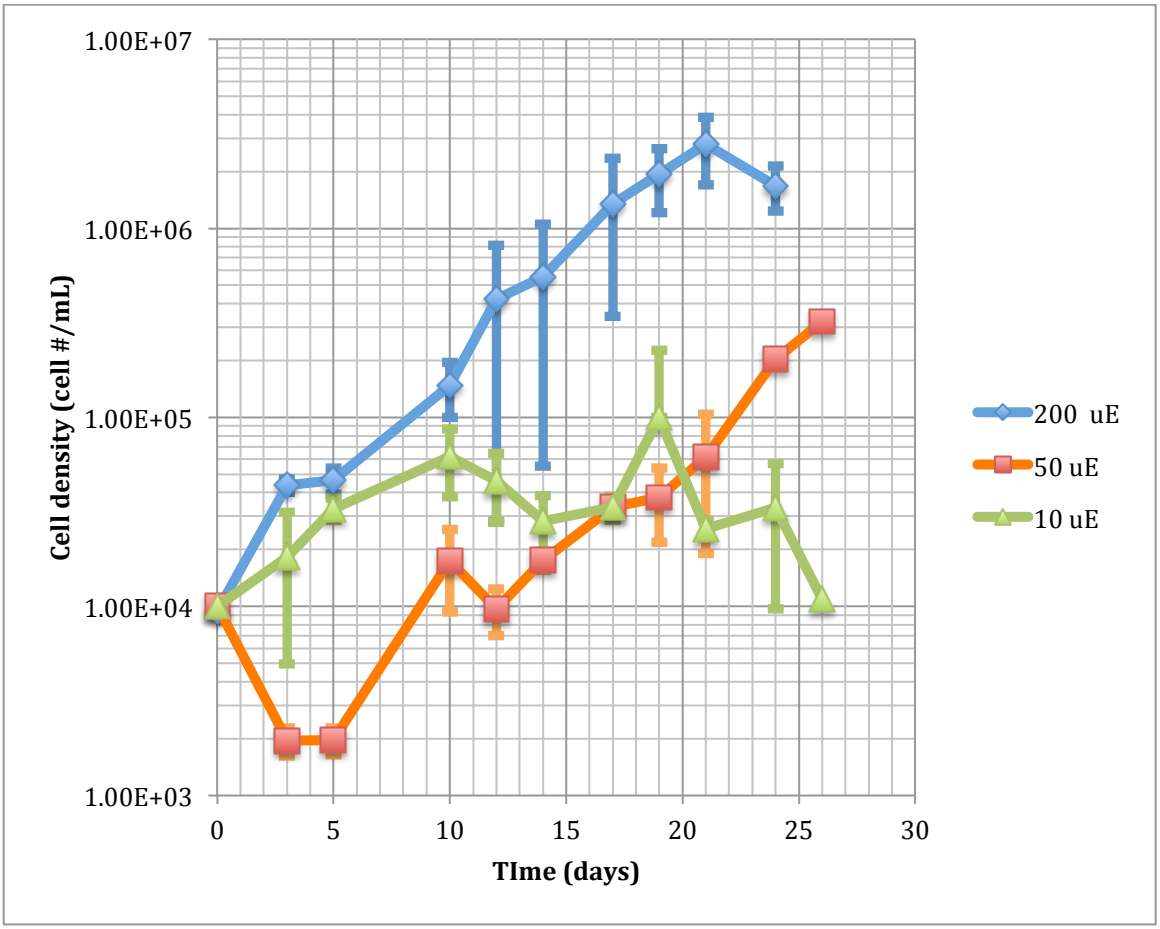

Figure 2: *Synechococcus* WH8102 growth at three different light intensities with a 12-hour dark period

Figure 3 provides a summary of the growth rates obtained from the 24 h light and 12 h light/12 h dark experiments. With 12 hours of darkness, the growth rate became maximal between 10 and 50 µE. With 12 hours of darkness the growth rate was 0.41 division/day (200 µE), 0.44 division/day (50uE), and negligible growth rate at 10uE compared to 1.32 division/day (200 µE), 0.58 (50 µE), and 0.15 (10 µE) at zero dark periods.

Growth rates were higher under zero dark periods in comparison to 12-hour dark periods. Growth rate changed between different light intensities, but the growth rates for 12-hour dark period periods did not change between 200 and 50 µE.

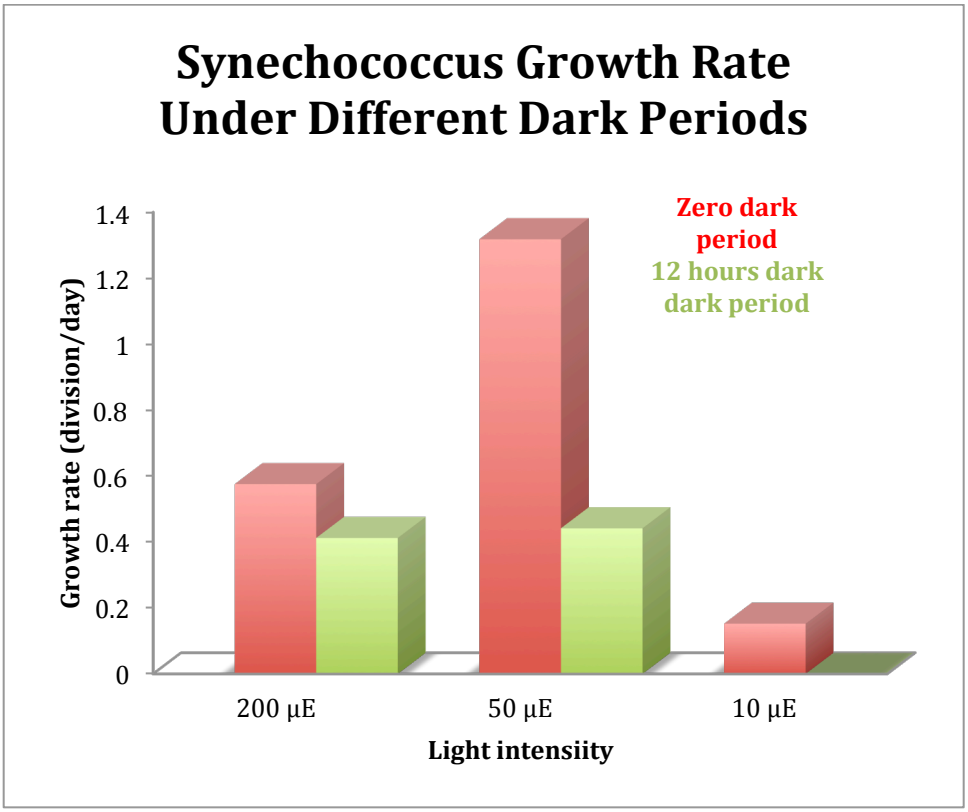

Figure 3: Summary table of different growth rates at different light intensities under zero and 12-hour dark periods.

Discussion

This research project measured the effect of dark period duration on the growth rate of *Synechococcus* WH8102. Exposure of the cells to a dark period had a significant effect on growth rate. The combination of light intensity and dark period gave additional information about how *Synechococcus* optimize growth under different light conditions. This study suggests that *Synechococcus* grows optimally when provide with a moderate light level and constant light.

The fastest growth rate was under 50 µE light intensity with no dark period. This result suggests that *Synechococcus* WH8102 has increased ability to convert inorganic carbon to organic carbon at this given condition (Constant light at 50µE). At this optimal condition, carbon was accumulated and used directly in respiration to make energy.

Growth rate was inhibited at light intensities higher and lower than 50 µE. It appeared that there was a minimum light intensity that is less or equal to 50 µE and

greater than 10 μE that results in the maximal growth rate observed at 50 and 200 μE.,

However, the presence of dark period caused a maximal growth rate that is about half of that observed in constant light. Interestingly, when provided 12-hour dark period, light intensities greater that 50 μE resulted in similar growth rates. A dark period does not appear to enhance growth rates at a given light intensity. More experiments are needed to determine the combined affects of light intensity and dark period, in particular to assess if shorter dark periods would lead to enhanced growth rates, particularly at higher (200 μE) light.

There are variable results observed between species when exposed to different periods of darkness. In a study of four different eukaryotic algae, two species decreased their growth rates and two species had growth rates that were unchanged when exposed to a 12 h dark period compared to constant light [8] At 50 μE and 12 h darkness, two species decreased growth rates and two increased growth rates compared to constant light. [8]

Similar to our results for *Synechococcus* WH8102, the growth rate of the Arctic cyanobacterium *Schizothirix calcicola,* did not respond positively to a dark period except when combined with warmer incubation temperatures. [7]

Conclusion

Synechococcus WH8102 grew optimally when provided a moderate light level and constant light. However, the presence of a dark period caused a maximal growth rate that is about half of that observed in constant light. Interestingly, when provided a 12-hour dark period, light intensity greater that 50 μE resulted in similar growth rates. At the resolution of the dark periods tested thus far, a dark period does not appear to enhance growth rates at a given light intensity. More experiments are needed to determine the combined affects of light intensity and dark period.

Future work will include measurements of chlorophyll content, carbon storage, and their changes with the day/night cycle.

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