Responses to Light Intensity and Regimes by an Arctic strain of the picophytoplankton *Micromonas* CCMP2099

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ABSTRACT

While genetic differences within the genus *Micromonas* have revealed multiple clades and strains within the lineage, the physiological differences these impart are not well understood. We focused on *Micromonas* CCMP2099, an arctic strain. A growth-light curve was established, finding they are well adapted for low light intensities but can maintain stable growth rates when grown under higher light levels. Algae acclimated to higher light intensities had different pigment ratios than did those acclimated to lower light intensities and likely indicated an increase in concentration of photoprotective accessory pigments. Photostress was observed at the higher light intensities via reduction in photosynthetic efficiency, and major reductions in the functional absorption cross section of PSII. Photobioreactors were utilized to study photochemistry of the algae under different photoperiods where the light intensity mimicked natural dawn, noon and dusk conditions vs. a steady light regime. Growing the strain under different light regimes revealed a lack of endogenous rhythm in photosynthetic efficiency that *Micromonas* RCC299, a temperate strain, and *Ostreococcus tauri* posses. We propose that *Micromonas* CCMP2099 has lost this endogenous rhythm as an adaptation to the polar night and day of the Arctic.

INTRODUCTION

Phytoplankton spread throughout the world's ocean are responsible for approximately 50% of the world's carbon uptake (Field et al. 1998). Picophytoplankton, unicellular algae less than 2μm in diameter, are a taxonomically diverse group of organisms that fall across the tree of life (Worden et al. 2004). Together they hold an important ecological role as primary producers in the oceans (Worden et al. 2004).

While the majority of picophytoplankton cells are marine cyanobacteria, especially the genera *Synechococcus* and *Prochlorococcus*, less abundant photosynthetic picoeukaryotes account for a large proportion of primary productivity in oceanic and coastal waters (Worden et al. 2004). This is especially true in the Arctic where there are few cyanobacteria in the water (Vincent 2000). The relatively high growth rates and biomass found within Picoeukaryotic algal strains are important factors which need to be taken into account when forming better models of carbon uptake in the world's oceans.

Micromonas, a picoeukaryotic green algae in the Prasinophyceae family, is ubiquitous in oceanic and coastal waters (Worden et al 2009). Although long thought to consist of a single species, recent studies have confirmed that *Micromonas* is comprised of seven genetically distinct cladal lineages, each with multiple species (Worden et al. 2009). However, little is known about the physiological differences that these genetic distinctions impart.

Micromonas CCMP2099, an Arctic strain, is a member of the E2 *Micromonas* clade (Simons et al. 2015). While a growth curve for *Micromonas* CCMP2099 grown in different temperatures has been published (Lovejoy 2007), there is no similar growth curve for the strain grown under different light levels. Establishing a such a growth curve would allow for the calculation of the E_K value for CCMP2099. This would be a helpful baseline for future experimental studies of the strain.

In most experimental studies, algae have been grown under light-dark cycles where the lights are switched on and off. This is in contrast to natural conditions in which light intensity slowly increases and decreases during the day, following the rotation of the earth. The immediate onslaught of high levels of light could impact transcriptional analyses and physiology of the algae. Growing algae under a sinusoidal light curve, along

with the customary square wave, can help tease out any influence this change from the natural regime might have on the physiology.

The photobioreactors in the Worden Lab offer a unique opportunity to examine the differences in photochemistry caused by the varying light regimes. Connected to fast repetition rate fluorometer, the photobioreactors allow continuous monitoring of photochemistry through the FRR-Lift method (Pieruschka et al. 2010).

Understanding the physiology of *Micromoas* CCMP2099 is especially important as the Arctic region is highly sensitive. The Arctic is warming at approximately twice the rate of the rest of the planet, and the loss of sea ice projects further intensification of the warming with ongoing climate change (Richter-Menge et al. 2016). Furthermore, the northern seas are projected to acidify at a faster rate than the rest of the world's oceans (Richter-Menge et al. 2016).

MATERIALS AND METHODS

INCUBATOR CULTURES

To establish the growth-light curve of *Micromonas* CCMP2099, cultures were grown in an 8ºC incubator with 14 hours of white fluorescent light. Initially, three liters of media was prepared according to L_1 protocols, with a lower salinity artificial seawater. This media was autoclaved in half liter batches to ensure sterility for its use throughout the experiment.

Areas in the incubator with light intensities of 10 μE m^{-2} s^{-1}, 20 μE m^{-2} s^{-1}, 40 μE m^{-2} s^{-1}, 80 μE m^{-2} s^{-1}, 120 μE m^{-2} s^{-1}, 160 μE m^{-2} s^{-1}, and 200 μE m^{-2} s^{-1}were prepared with a combination of shade cloth, cardboard, and positioning. A 4 pi PAR sensor (Walz, Inc.) inserted into a 40 mL culture vessel filled with *Milli-Q* water was used to test the light intensity at each position. This testing was repeated periodically throughout the experiment to ensure that light intensities remained at the desired amounts.

Throughout the experiment, axenic CCMP2099 cultures were maintained at a density between 2-8 million cells ml^{-1} within 40mL rectangular culture vessel. Three biological replicates were utilized for each of the seven light levels. These cultures were sampled daily at 9:15am for 18 days. To sample the cultures, they were moved from the

incubator to a clean hood, and 500 μL was transferred from each culture vessel into a 1.7mL microtube with a sterile pipet.

The samples were run on an a flow cytometer (BD Accuri C6) every day. To prepare the samples for the flow cytometer, 100 μL of the sample was combined with 880 μL of filtered artificial sea water, 10 μL red beads and 10 μL yellow beads. A sample from each of the twenty-one cultures was run on the flow cytometer in this manner every morning. When forward scatter was plotted against red fluorescence, a gate was drawn around the algae population to separate it from the beads and noise in the mixture. Cell density, forward scatter, and fluorescence were collected from the flow cytometer.

The beads that were run with each sample were used to standardize fluorescence and forward scatter values. This was performed by dividing the values from the algae by the values from the beads. The resulting data on density, forward scatter, and fluorescence were analyzed with Excel and R.

At the end of the experiment, 7mL samples were taken from all the cultures. From this 2mL was used to generate rapid light curves with a Fast Repetition Rate Fluorometer, 2mL was used for multispectral analyses with the same fluorometer, and 2mL was used to generate an absorption spectrum with an integrating sphere. The data from all photochemical tests were analyzed in R.

When cultures reached a density of seven – eight million cells per mL, they were either diluted (e.g. increased from 18 mL to 40 mL) or transferred to a new vessel and diluted if there was too much liquid already in the vessel.

PHOTOBIOREACTORS

To probe the question of the impact of light regime on the photochemistry of *Micromonas* CCMP2099, the algae were grown in four photobioreactors that allow for a highly controlled and stable environment. They were grown in media prepared according to L_1 protocols, with a lower salinity artificial seawater held at 8ºC. Densities of about 3.5 million cells per mL were maintained by adjusting the speed of media inflow and outflow.

The algae were grown under three light conditions: the first to mimic the natural environment gradually increased and decreased throughout the day; the second, the more

common laboratory condition, simply turned on and off at “sunrise” and “sunset”, maintaining a constant intensity throughout the day; and the third held a continuous light to probe a baseline response to constant light. All three regimes maintained the same daily photon dose for the algae.

They were sampled daily, by removing 10 mL with a syringe, to track growth rates throughout the experiment. This sample was run through the flow cytometer under the same protocols as above. A fast repetition rate fluorometer was connected to the photobioreactors to allow for constant monitoring of photochemistry. The cultures were also monitored with pH probes in the photobioreactors

High resolution sampling was performed for the sine and square wave light regimes. The photobioreactors were sampled at 0500, 0900, 1200, 1500, 1900, 2100, 0000, 0300, and 0500 on July 18-19, 2017 for the sine wave light regime and July 22-23, 2017 for the square wave light regime. Samples were preserved for RNA analysis and later flow cytometery with the Influx (BD Influx Flow Cytometer).

RESULTS

GROWTH-LIGHT CULTURES

A growth-light curve for *Micromonas* CCMP2099 was established from the growth rates of the cultures grown in the incubator. Growth rate under the lowest light intensity was very low. It increased as light intensity increased and then leveled off around 0.4 with light intensities above 120 μE m^{-2} s^{-1} (Fig. 1). E_K was found to be 35 μE m^{-2} s^{-1}.

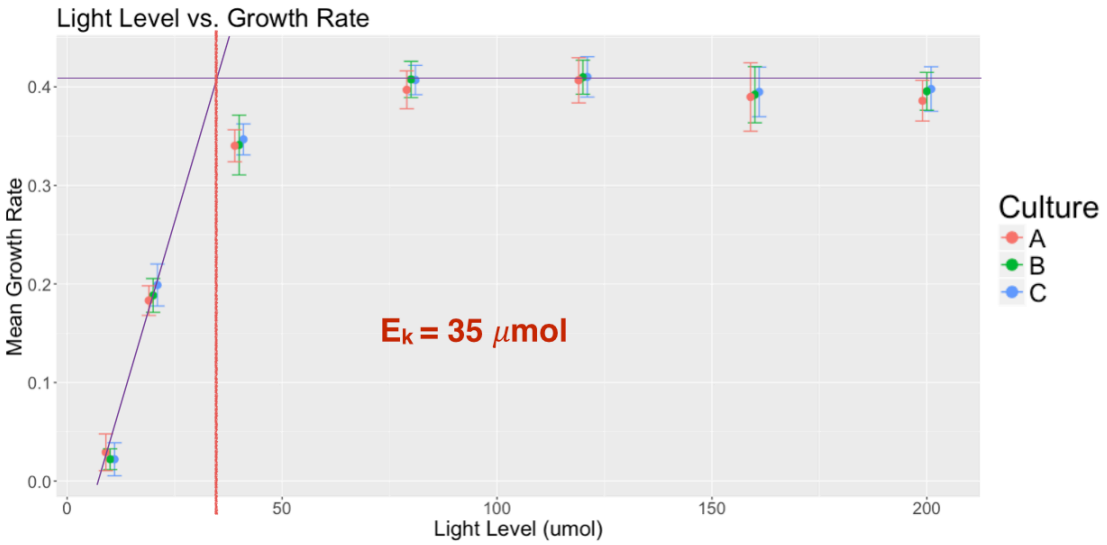

Figure 1. Average growth rate for each of three *Micromonas* CCMO2099 cultures (labeled A, B, and C) grown under each intensity of cool white fluorescent light (10, 20, 40, 80, 120, 160, and 200 µE m^{-2} s^{-1}). Bars are standard error. The growth rate increases from 0.02 under 10 µE m^{-2} s^{-1} to 0.4 under 80 µE m^{-2} s^{-1} where it levels off. E_K, the optimal light for growth found at the intersection of the initial slope of increase and the maximum growth rate, is 35 µE m^{-2} s^{-1}.

Fluorescence, measured daily with the flow cytometer, decreases as light intensity increases. Standardized fluorescence was highest under the lowest light intensity. It decreased, approximately exponentially, as light intensity increased (Fig. 2).

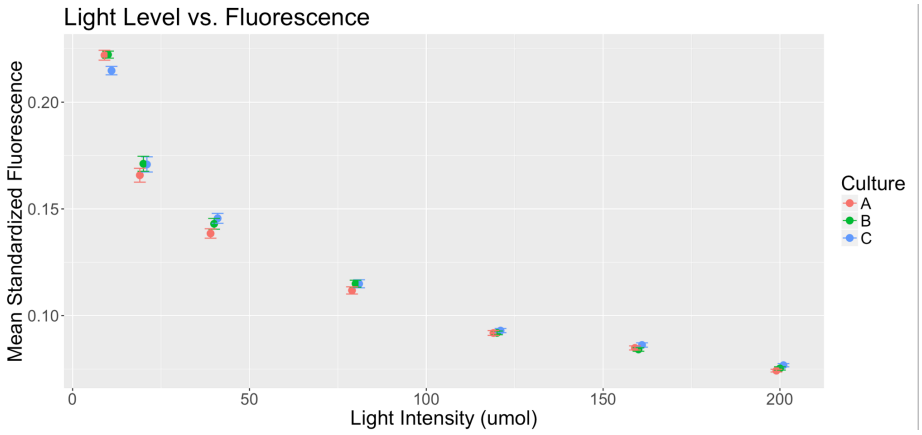

Figure 2. Average standardized fluorescence for each of three *Micromonas* CCMO2099 cultures (labeled A, B, and C) is plotted against the light intensity of cool white fluorescent light (10, 20, 40, 80, 120, 160, and 200 µE m^{-2} s^{-1}) each was grown under. Fluorescence decreases as light intensity increases.

Absorbance spectra normalized to density show changes in magnitude across the range of light intensities. The highest magnitude of absorbance spectra is from the cultures grown under the lowest light intensities, and it decreases with increasing light intensity (Fig. 3).

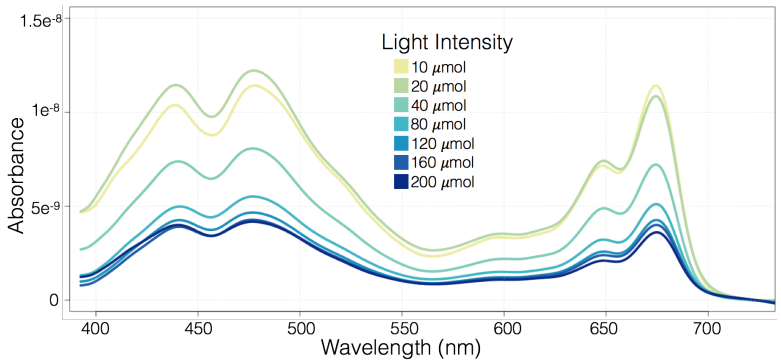

Figure 3. Absorbance spectra were averaged across the three cultures grown under each light intensity. Light intensity is shown with the colors of the spectra. Overall magnitude decreases as light intensity increases.

Absorbance spectra were normalized to the chlorophyll a peak at approximately 650 nm. This displays the different ratios between the peaks, corresponding to pigments in the cells. The chlorophyll b peak, around 650 nm, is higher for the cultures grown under the lowest light intensity, 10 μE m^{-2} s^{-1}, than for the cultures grown under the highest light intensity, 200 μE m^{-2} s^{-1}. However, the carotenoid peaks, around 440 and 475 nm, are higher for the cultures grown under the highest light intensity than the those grown under the lowest light intensity.

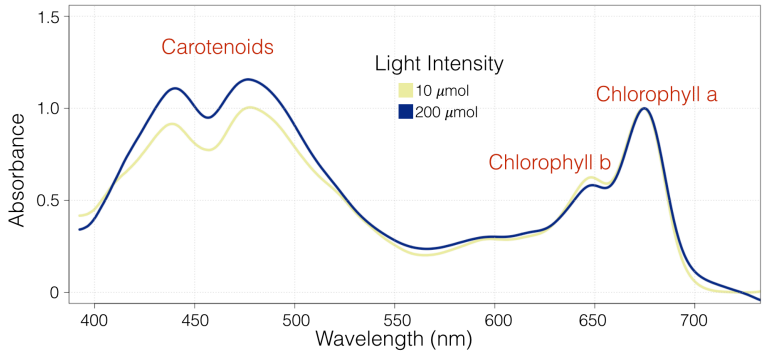

Figure 4. Absorbance spectra from cultures grown under light intensities of 10 μE m^{-2} s^{-1} and 200 μE m^{-2} s^{-1} were normalized to the chlorophyll a peak. The cultures grown under 10 μE m^{-2} s^{-1} have a higher chlorophyll b peak while those grown under 200 μE m^{-2} s^{-1} have higher absorbance values for the carotenoid peaks.

Rapid-light curves for dark acclimated samples were generated from fast repetition rate fluorometry. A measure of photosynthetic efficiency was calculated by dividing the variation in fluorescence (maximum fluorescence F_m – minimum fluorescence $F_0 = F_v$) by the maximum fluorescence F_m to get F_v/F_m. Photosynthetic efficiency slightly decreased with increasing light intensity from the lowest light intensity. It stabilized around 0.59 and then decreased sharply to 0.5 between 120 and 160 μE m^{-2} s^{-1}.

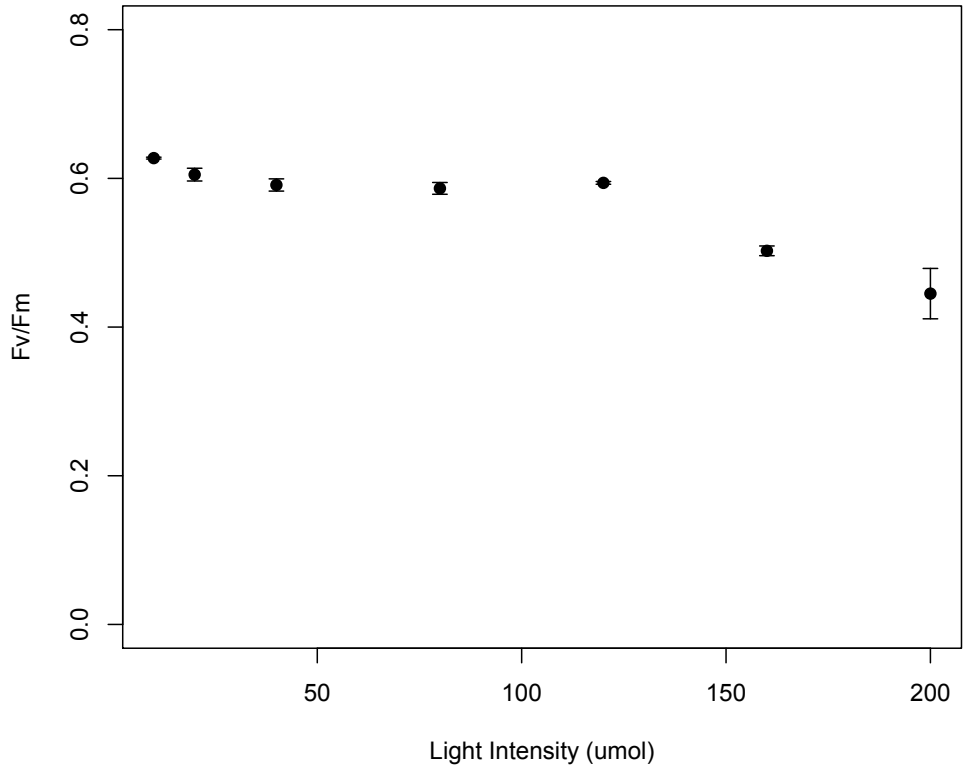

Figure 5. Rapid-light curves were generated with a fast repetition rate fluorometer for dark acclimated samples. Plot shows average photosynthetic efficiency, calculated by dividing variation in fluorescence by maximum fluorescence for cultures of *Micromonas* CCMP 2099 grown under light intensities of 10, 20, 40, 80, 120, 160, and 200 μE m^{-2} s^{-1}. Error bars are standard error.

The slope of the initial rise in fluorescence gives a measure of the functional absorption cross section of photosystem II, known as sigma. Sigma for dark acclimated cultures slightly increased between the lowest light intensities and then stabilized around

3000. It then sharply decreased between 120 and 160 μE m^{-2} s^{-1} and continued to decrease to a low of 1700 for the cultures grown under 200 μE m^{-2} s^{-1}.

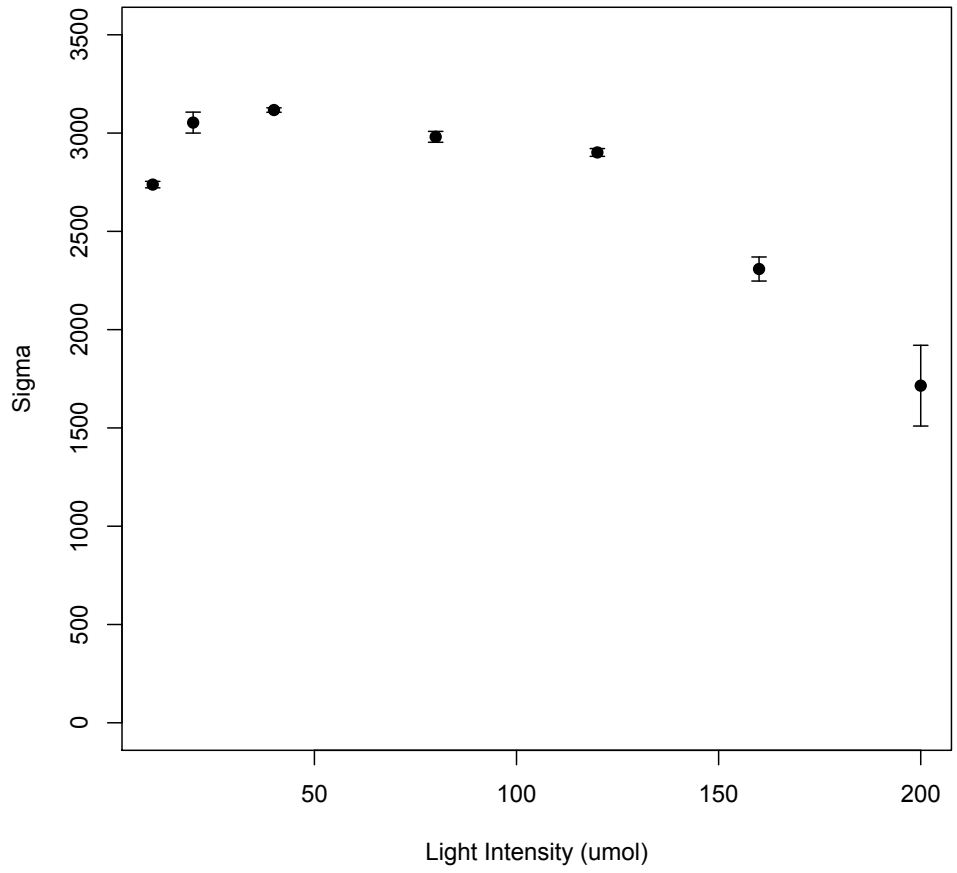

Figure 6. Rapid-light curves were generated with a fast repetition rate fluorometer for dark acclimated samples from cultures of *Micromonas* CCMP 2099 grown under light intensities of 10, 20, 40, 80, 120, 160, and 200 μE m^{-2} s^{-1}. Plot shows average functional absorption cross section of photosystem II, referred to as sigma, calculated from the slope of the increase in fluorescence. Error bars are standard error.

The reoxidation rate constant for the Q_a site is calculated from the FRR curve. Reoxidation sharply decreases from the cultures grown under 20 μE m^{-2} s^{-1} to those grown under 40 μE m^{-2} s^{-1} where it stabilizes around 1000. It decreases once more between 120 and 160 μE m^{-2} s^{-1} and continues to a low of 7100 under 200 μE m^{-2} s^{-1}.

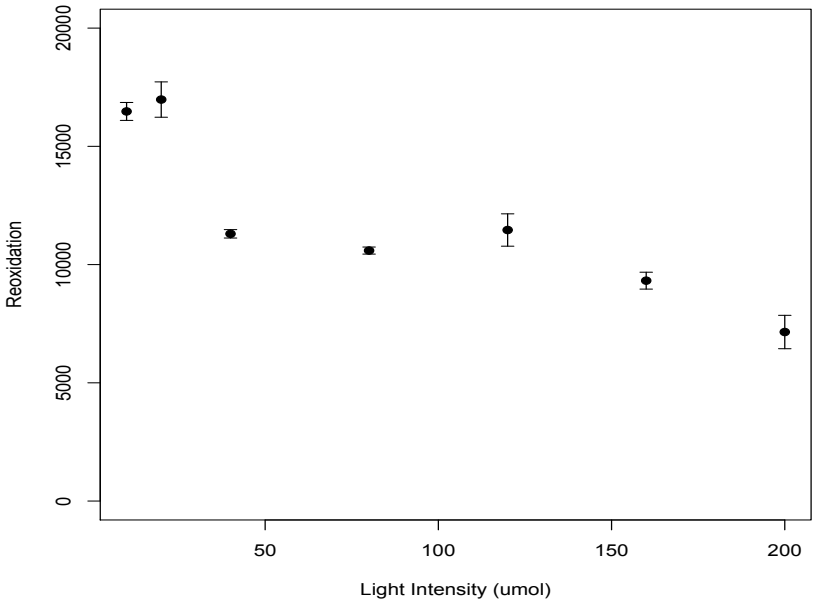

Figure 7. Rapid-light curves were generated with a fast repetition rate fluorometer for dark acclimated samples from cultures of *Micromonas* CCMP 2099 grown under light intensities of 10, 20, 40, 80, 120, 160, and 200 μE m^{-2} s^{-1}. Plot shows average reoxidation of Q_A. Error bars are standard error.

Light Intensity (μE m^{-2} s^{-1})	Growth Rate	Fluorescence	Photosynthetic Efficiency	Sigma	Reoxidation
10	0.024±0.009	0.220±0.001	0.627±0.001	2740±20	16500±400
20	0.19±0.01	0.169±0.002	0.605±0.009	3050±50	17000±700
40	0.34±0.01	0.142±0.001	0.591±0.008	3120±10	11300±200
80	0.40±0.01	0.114±0.001	0.587±0.008	2980±30	10600±100
120	0.41±0.01	0.0923±0.0006	0.594±0.002	2900±20	11500±700
160	0.39±0.02	0.0851±0.0005	0.503±0.007	2300±60	9300±400
200	0.39±0.01	0.0754±0.0004	0.45±0.03	1700±200	7100±700

Table 1. Means and standard error of parameters measured for all cultures grown for the growth-light experiment. Averages were taken across the three biological replicates acclimated to each light intensity. Growth rates and fluorescence were averaged from data taken throughout the course of the experiment while F_v/F_m, sigma, and reoxidation were measured once for each culture.

PHOTOBIOREACTORS

Photosynthetic efficiency, calculated by dividing variation in fluorescence by maximum fluorescence, was measured throughout the experiment. Under the sine wave light regime, photosynthetic efficiency gradually decreased throughout the day, reaching a low of 0.2 at noon when the light intensity was highest. It then increased throughout the afternoon, as light intensity decreased. During the "night", photosynthetic efficiency gradually increased from about 0.57 to 0.59.

Under the square wave light regime, photosynthetic immediately decreased from about 0.57 to 0.20, rebounded to about 0.27, and gradually decreased to about 0.23 throughout the rest of the day. When the lights were turned off, photosynthetic efficiency rapidly returned to about 0.55 and slowly increased to about 0.57 throughout the night.

Under the 24-hours of light regime, photosynthetic efficiency maintained a slight increase and decrease pattern through the first day. By the second day, this petered out to an approximately flat line around 0.3.

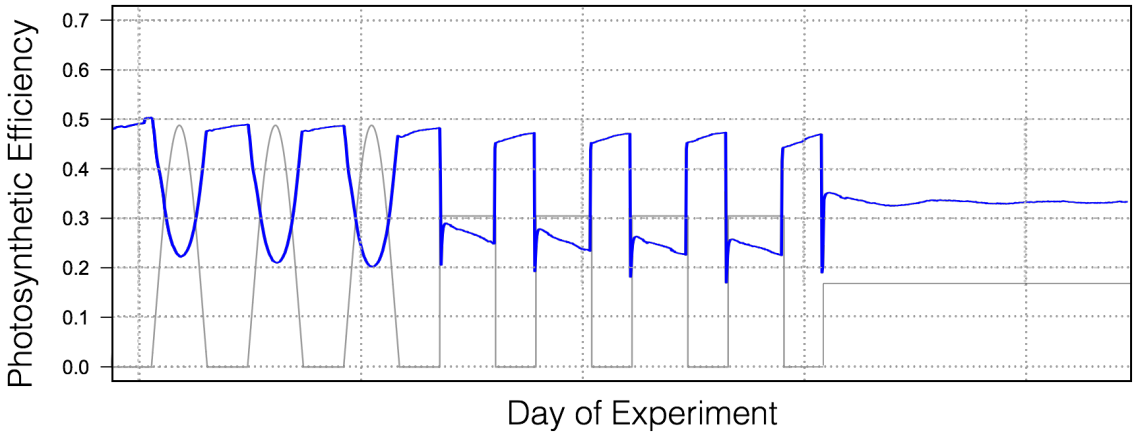

Figure 8. Photosynthetic efficiency was measured throughout the experiment by attaching a fast repetition rate fluorometer to the photobioreactors. Light intensity is shown in grey – the three portions of the experiment are the sine wave (mimicking a natural light regime), the square wave (demonstrating normal laboratory conditions), and continuous light. Daily photon dose was maintained throughout the experiment. Photosynthetic efficiency, calculated by dividing variation in fluorescence by maximum fluorescence, is shown in blue. It responded to the change in light regime.

Growth rates from the high resolution sampling during the experiment showed dramatic changes throughout the day, though they were similar under the sine wave and

square wave light regimes. The only pronounced difference between the pattern in growth rates was at 0300, when the algae grown under the sine wave light regime grew at a much higher rate than did those grown under the square wave light regime.

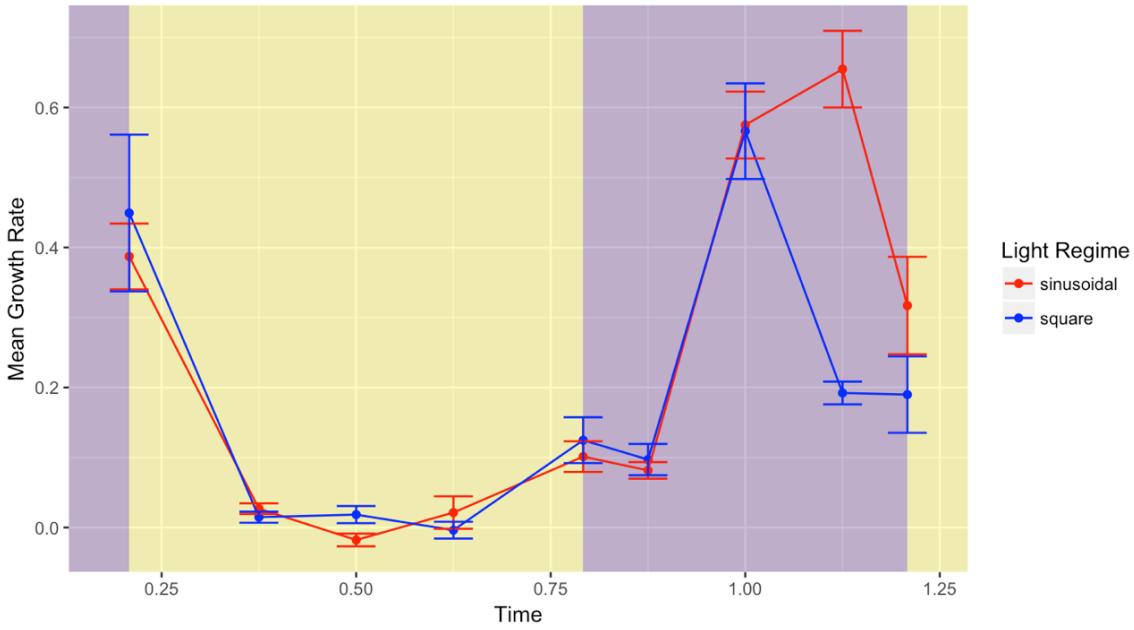

Figure 9. The mean growth rates across four biological replicates are plotted through time for the cultures grown under sinusoidal light wave and square light wave. The growth rate for the sine wave cultures is shown in red while the square wave is shown in blue. Error bars are standard deviation. Purple background denotes dark while yellow denotes lights on.

DISCUSSION

We find that *Micromonas* CCMP2099 is well adapted to low light intensity, and it can tolerate higher light levels fairly well. The low light adaptation is apparent in the low E_K value (Fig. 1; E_K=35 μE m^{-2} s^{-1}). At the higher light intensities, photosynthetic efficiency, functional absorption cross section of photosystem II, and reoxidation decreased substantially (Figs. 5, 6, & 7). We do not see these photochemical changes reflected in the growth rates, however, and *Micromonas* CCMP2099 maintained a stable growth rate through the highest light intensities tested (Fig. 1). This suggests that the algae are able to compensate for the increasing photostress and tolerate the higher light intensities.

The observed decrease in fluorescence with increasing light intensity was expected, as the measured red fluorescence is a proxy for chlorophyll concentration (Fig. 2). The signal we detect is likely due to algae at lower light levels bulking up on chlorophyll to better harvest light for photosynthesis. At high light intensities, they do not need to increase chlorophyll concentrations, and rather devote resources within the cell to other pigments.

We detected these other pigments from the ratios in absorption spectra normalized to the chlorophyll a peak (Fig. 4). The cultures grown at a higher light intensities had higher peaks in the carotenoid regions, indicating a higher ratio of carotenoids to chlorophyll a in their cells. Carotenoids can be photoprotective pigments in *Micromonas*, so at higher light intensities the algae are likely increasing the production of these pigments to protect their photosynthetic apparatus from light damage.

The sharp decrease in functional absorption cross section of photosystem II between 120 and 160 $\mu E\ m^{-2}\ s^{-1}$ (Fig. 6) is likely a sign of a state transition in the photosynthetic apparatus. The light-harvesting antenna complex migrates from photosystem II to photosystem I to better balance the absorbed light energy between the two reaction centers.

From the experiment in the photobioreactors, we find evidence that *Micromonas* CCMP2099 does not have endogenous rhythms in photosynthetic efficiency. Photosynthetic efficiency gradually decreased and increased corresponding to the gradual increase and decrease in light intensity under the sine wave light regime (Fig. 8). This can be understood through the increasing photostress the algae experience as the light intensity increases, reducing photosynthetic efficiency. They spend more energy on repairing from light damage, but cannot keep up with the damage as light intensity increases.

Under the square wave light regime, the dramatic decrease in photosynthetic efficiency is likely due the onslaught of high intensity light (Fig. 8). The dark reactions, collectively the Calvin Cycle, take some time to initiate and get up to speed. This is likely the cause of the rebound in photosynthetic efficiency we see shortly after the immediate drop. Photosynthetic efficiency then slowly decreases throughout the rest of the day as

repair rates cannot keep up with damage caused by photostress; light is a source of both energy and stress for the algae. The increase throughout the night is a sign of the repair mechanisms working to fix the photosynthetic apparatus without the onslaught of light stress.

Interestingly, two strains, *Micromonas* RCC299, a temperate strain of *Micromonas*, and *Ostreococcus tauri*, a close relative of *Micromonas*, the Worden Lab had previously tested under these light regimes showed a different pattern under the square wave regime (Fig. 10). Photosynthetic efficiency had a similar sharp decrease right when the lights were turned on. However, after a rapid rebound with the activation of the dark reactions, it slowly increased from there to a peak at midday and decreased throughout the rest of the day. This response was not due to any external input, as the light intensity did not change throughout the day, so it is likely a sign of an endogenous rhythm in the two temperate strains. *Micromonas* CCMP2099 does not seem to have this endogenous rhythm for photosynthetic efficiency.

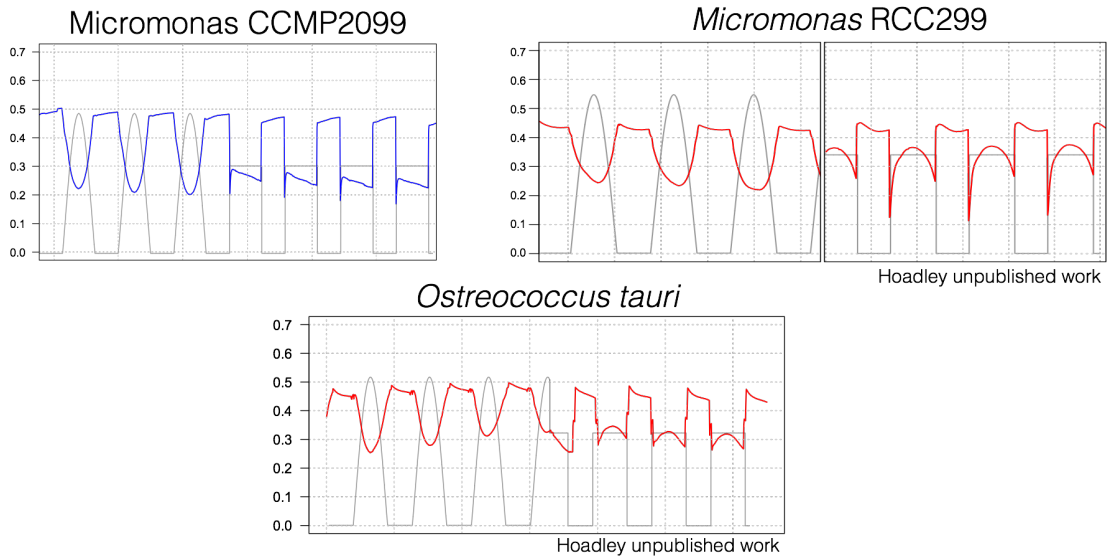

Figure 10. The photosynethic efficiency for three strains of algae plotted through time of the experiments. The colorful lines, blue for *Micromonas* CCMP2099 and red for *Micromonas* RCC299 and *O. tauri*, show photosynthetic efficiency while the grey lines show the light intensity throughout the experiment. *Micromonas* RCC299 and *O. tauri*, two temperate algal strains, display signs of an endogenous rhythm under the square wave light regime while *Micromonas* CCMP2099 does not.

A further sign of the lack of an endogenous rhythm in *Micromonas* CCMP2099 is seen in the response of photosynthetic efficiency to the continuous light regime. The slight trace of a cyclical rhythm quickly disappeared. This is a classic pattern for a trait without an endogenous rhythm.

O. tauri is a phylogenetic outgroup to the two *Micromonas* strains. This suggests that in their lineage, having the endogenous rhythm for photosynthetic efficiency is the basal trait that *Micromonas* CCMP2099 has lost, perhaps as an adaptation to the polar night and summer with extreme periods of dark and light.

CONCLUSION

Micromonas CCMP2099 is a low light adapted strain. It is able to maintain stable growth rates at higher light levels, although algae adapted to high light intensities show signs of photostress. They show a significant change in pigment ratios from those grown under low light intensities to those grown under high light intensities.

Growing *Micromonas* CCMP2099, *Micromonas* RCC299, and *Ostreococcus tauri* under different light regimes revealed a difference in circadian rhythm between the Arctic strain and the two temperate strains. CCMP2099 does not seem to have an endogenous rhythm in its photosynthetic efficiency while the other two exhibit such a rhythm. Going forward, we will use bioinformatics to examine the core molecular clock in *Micromonas* CCMP2099. We will blast the transcriptome and/or genome to see if any of the core genes are absent from the strain which could be causing the lack of an endogenous rhythm.

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