Effect of photoperiod on exponential growth rates of *Chlamydomonas reinhardtii* and the downstream impacts on juvenile salmon populations

Nick Hsieh, Shayan Molaei, Pavneet Virk, Shaylen Young, Irena Zivkovic

Abstract

Chlamydomonas reinhardtii are photoautotrophic green algae. Given this characteristic, the purpose of our experiment was to determine if photoperiod has an effect on the population growth rate of *C. reinhardtii.* Our experiment consisted of our Control, Treatment 1, and Treatment 2, for which the algae were kept in climate controlled incubators and exposed to 8-hour, 3-hour, and 21-hour photoperiods, respectively. We collected samples of cultures for all treatment replicates over a nine day period. The growth curves obtained show a trend of increasing cell concentration with increased photoperiod. Statistical analysis using one-way ANOVA on maximum growth rates and Tukey HSD test showed a significant difference between Treatment 1 and 2 maximum growth rates ($p= 0.0395$); as such, we rejected our null hypothesis, providing support for the alternate hypothesis that *C. reinhardtii* maximum growth rate changes as photoperiod changes. These results demonstrate that the exponential growth rate of *C. reinhardtii* is significantly higher at a 21-hour photoperiod, compared to a 3-hour photoperiod. This suggests that during spring months, when juvenile salmon emerge from the nest and photoperiod is high, increased growth rates of *C. reinhardtii* will be observed. This affects upper trophic levels, as zooplankton and invertebrates feed on green algae, such as *C. reinhardtii*; newly emerged juvenile salmon will in turn feed on the zooplankton and invertebrates. We conclude that that *C. reinhardtii* exponential growth rates are significantly higher at a 21-hour photoperiod compared to a 3-hour photoperiod; this poses a profound implication on the surrounding ecosystem.

Introduction

Chlamydomonas reinhardtii is a photoautotrophic species of green alga (Janssen *et al.,* 2012). Algae such as *C. reinhardtii* are crucial to ecosystems as they are a food source for small invertebrate species, planktonic crustaceans, zooplankton, and fish species (Norambuena *et al.,* 2015). Previous studies have noted a positive effect in growth of fish species that contain algae in their diets (Norambuena *et al.,* 2015). The depletion of algae would therefore affect upper trophic levels, including salmon and other species that feed on small invertebrates and zooplankton. For

this reason, it is important to understand the significant role that algae such as *C. reinhardtii* play in marine ecosystems. Furthermore, it is crucial to analyze the effects of environmental factors that could alter the growth and abundance of *C. reinhardtii*.

The purpose of our investigation was to determine if photoperiod has an effect on the growth of *C. reinhardtii.* Photoperiod is the length, in hours, of daylight to which a species is exposed. Our null hypothesis was that the exponential growth rate of *C. reinhardtii* does not change as photoperiod changes. The alternate hypothesis was that the exponential growth rate of *C. reinhardtii* changes as photoperiod changes. We predicted that the highest exponential growth rate of *C. reinhardtii* would result from our maximum experimental photoperiod of 21 hours, which is close to the 15-hour photoperiod experienced by salmon during fry emergence; we also expected to see a trend of increasing exponential growth rate with increased photoperiod (Gerson *et al.,* 2016; Time and Date, 2017).

Marcel Janssen and colleagues conducted a study similar to this investigation to understand the energy requirements for photoautotrophic growth of *C. reinhardtii* (Janssen *et al.,* 2012). Specifically, Janssen *et al.* (2012) focused on the amount of light given to the green algae; their results demonstrated that the amount of light *C. reinhardtii* is exposed to is directly proportional to the population growth rate. Janssen *et al.* (2012) concluded that only a moderate amount of light is required to sustain *C. reinhardtii*. Furthermore, Janssen *et al.* (2012) learned that the population growth rate of *C. reinhardtii* is higher at a lower irradiance; at high irradiance levels, growth falters as algae can only use limited quantities of light (Janssen *et al.,* 2012).

Although Janssen et al. did not directly study photoperiod effects on *C. reinhardtii,* this factor still played a role in their study as the algae were kept under a 16:8 hour day and night cycle.

Methods

Algae culture

We cultured 50 mL of *Chlamydomonas reinhardtii* algae stock, grown in standard *C. reinhardtii* medium, during a one-week period prior to the experiment start date. We obtained the algae stock and medium from the University of British Columbia BIOL 342 Laboratory.

Procedure

We determined the initial concentration of the stock using a hemocytometer, counting and extrapolating to get cells per mL of solution. To perform the cell counts, we used a micropipette to transfer 100 µL of stock solution and 10 µL of IKI fixative to a 500 µL microcentrifuge (MCF) tube (Figure 1a). We then utilized the micropipette to mix the stock and fixative, before transferring 10 µL of solution to a hemocytometer slide (Figure 1a). We then used an Axiostar compound microscope to view the slide, and counted cells with a click counter to determine cell concentration (cells/mL). We performed this same procedure for all subsequent measurements of cell concentration in this experiment.

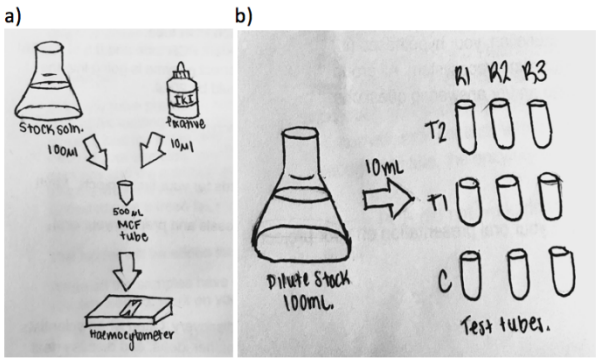

Figure 1. a) General procedure used to fix *C. reinhardtii* samples and load the hemocytometer. b) Division of dilute stock solution to 9 test tubes.

We then diluted our *C. reinhardtii* stock using the standard medium to create a 100 mL solution at 2.0 x 10^5 cells/mL (Figure 1b). Using a 10 mL pipette, we transferred 10 mL of the diluted stock to each of nine test tubes (Figure 1b). We labelled three test tubes as our Control replicates, three as our Treatment 1 replicates, and three as our Treatment 2 replicates (Figure 1b). A different pipette was used when transferring the dilute algae stock to the three treatments, to minimize risk of contamination. We then placed the tubes from each individual treatment in separate test tube holders, and transferred the Treatment 1 and 2 holders to a single incubator, and the Control to another (Figure 2). The Control consisted of *C. reinhardtii* under conditions of an 8-hour photoperiod, Treatment 1 consisted of a 3-hour photoperiod, and Treatment 2 had a 21-hour photoperiod. The incubators simulated photoperiod for Treatment 2 and Control, while we manually manipulated photoperiod for Treatment 1 using a cardboard box to simulate darkness. Both incubators operated at an identical light intensity and at a temperature of 20 degrees Celsius, controlling for the effects of light and temperature on algae growth.

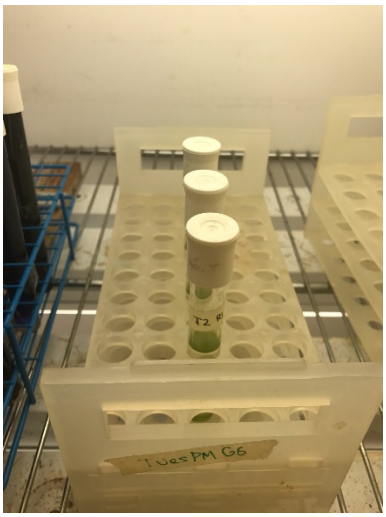

Figure 2. Image of Treatment 2 set up in an incubator. Identical set-up was used for all treatments.

We manually manipulated the photoperiod of Treatment 1 for a nine-day period by removing and replacing the cardboard box. Each Monday, Wednesday, and Friday, we fixed samples of all replicates using the fixing procedure outlined previously; we stored these samples in the BIOL 342 fridge and labelled them with the date, treatment, and replicate number. During this period, we recorded qualitative observations of the level of algae growth, based upon the relative amount of green algae visible in the test tubes. During the weekend, we moved all treatments to the Control incubator, as we were unable to access the lab during these days to manually manipulate the photoperiod of Treatment 1. At the end of the data collection period, we calculated and recorded the *C. reinhardtii* cell concentration for all the fixed samples, using the counting procedure outlined previously.

Data Analysis

We plotted our measurements of *C. reinhardtii* cell concentration on a line graph, displaying a growth curve of mean cell concentration (cells/mL) versus time (days) for the

treatments. We then fit a line to the exponential growth phase of each curve to determine the mean exponential growth rate, in units of cells/mL/day. Subsequently, we used one-way ANOVA to compare the mean exponential growth rates of our treatments; this determined whether there was a significant difference among our treatments. We then used the Tukey Kramer HSD test to determine the exact treatments that were significantly different from one another.

Results

As time progressed, Treatment 2 developed a thick green algae layer at the top of the liquid. The green algae layer could also be seen on the top of the test tubes corresponding to Treatment 1 and Control as well, but the layer was progressively thinner (T2>>T1>Co). Towards the end of the experiment, the algae layers could be observed both at the top and at the bottom of all treatment test tubes.

After counting *C. reinhardtii* cells in each fixed sample and obtaining an average cell concentration for each replicate over time, we constructed the growth curves for each treatment, as shown in Figure 3. This figure shows the cell concentration in each treatment on different days, with the slope of the curve representing the growth rate. Initially looking at Figure 3, we can see that the growth curves corresponding to Treatment 1 and Control are quite similar, while the growth curve for Treatment 2 is much steeper. Treatment 2 shows a much higher cell concentration at all points along the x-axis compared to the Control and Treatment 1.While Treatment 2 experiences a lag phase between days 2 and 4, *C. reinhardtii* concentration in Treatment 1 and the Control group starts to increase. The cell concentration in the Control group reaches a maximum at day 4 before starting to decrease, while the Treatment 1 cell concentration

continues to increase. Meanwhile, cell concentrations in Treatment 2 start to increase between days 4 and 7.

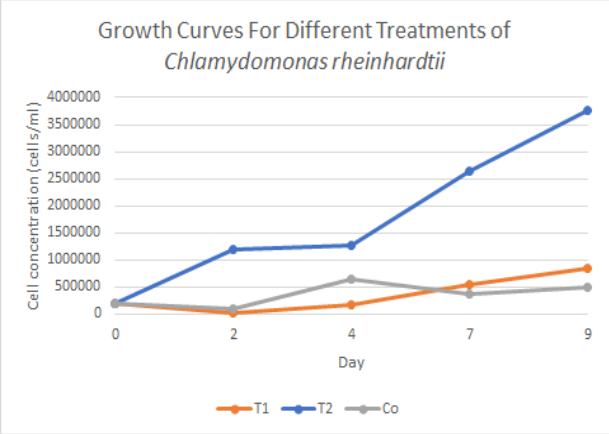

Figure 3: Growth Curves of *C. reinhardtii*

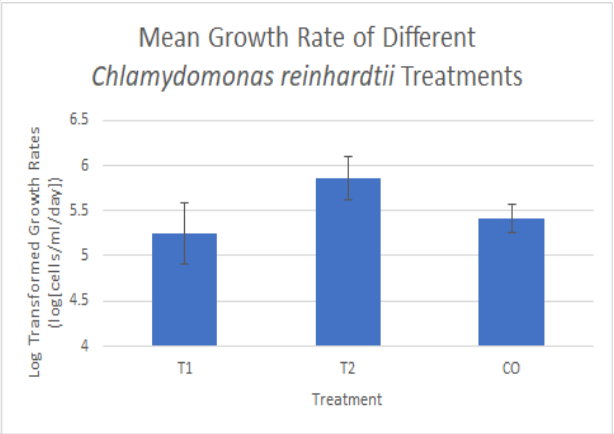

Figure 4: Mean exponential growth rates during treatments

Mean exponential growth rates for each treatment were calculated based on the average of the maximum growth rate of each replicate. This data is represented in Figure 4 with 95% confidence intervals. Figure 4 shows that Treatment 2 has a higher growth rate compared to the other two treatments. Additionally, the Control group has a higher growth rate compared to Treatment 1.

We analyzed the exponential growth rates for each treatment using 1-way ANOVA and Tukey HSD statistical analysis to see if the differences are statistically significant. A p-value of 0.0395 resulted from our ANOVA test, demonstrating a significant difference between two or more of the growth rates. The Tukey HSD test shows that the mean exponential growth rates of T1 and T2 are significantly different from each other, with the rate of T2 being significantly higher than that of T1 (Figure 4). The exponential growth rate of the Control group is not

significantly different from either T1 or T2 (Figure 4). During data analysis we found that the exponential growth rate data for T2 had a high variation compared to the rest of the treatments.

Equation 1: Sample calculation for max growth rate of C. reinhardtii T2:

T2 growth rate=slope between days 0 and 2= (1200833-200000)/(2-0)=500417 (cells/ml/day)

Table 1: ANOVA sample calculations for T2 and p-value using log transformed data

Treatments	A
Observations (N)	3
Sum (xi)	17.5672
Mean (x)	5.8557
Sum of squares (xi2)	102.9564
Sample variance (s2)	0.0440
Sample std. Dev. (s)	0.2098
Std. dev. of mean (SE_x)	0.1211

source	Sum of squares SS	Degree of freedom v	Mean square	F static	p-value
Treatment	0.5949	2	0.2974	5.8120	0.0395
Error	0.3071	6	0.0512		
Total	0.9020	8			

Discussion

Based upon the results of our statistical analysis, we rejected the null hypothesis and therefore supported our alternate hypothesis that *C.reinhardtii* exponential growth rate changes as photoperiod changes. We determined that at their maximum growth, *C. reinhardtii* under conditions of a 21-hour photoperiod, Treatment 2, grew significantly faster than those under an 8-hour photoperiod, Treatment 1. However, the exponential growth rate of *C. reinhardtii* under conditions of an 8-hour photoperiod was not significantly different from the other two treatments, demonstrating that differences in exponential growth rate are only found between extreme photoperiod treatments. Our results therefore support our prediction that the maximum exponential growth rate of *C. reinhardtii* would result from the maximum experimental photoperiod of 21 hours. Furthermore, our data showed a trend of increasing exponential growth rate with increasing photoperiod, as expected due to the phototrophic nature of *C. reinhardtii.* These findings are supported by those of Fortes and Lüning (1980), Bouterfas *et al.* (2006), and Wahidin *et al.* (2013) all of which found that growth rate of phototrophic algae increases with photoperiod length.

Although we have successfully rejected the null hypothesis, there are errors that may have influenced our results. One of the main sources of error is the variation in the lab environment. Specifically, we noted inconsistencies in incubator temperatures, which fluctuated between 18-22 degrees Celsius; this could have impacted our results as growth rates of photoautotrophic algae are found to increase with increased temperature (Eppley, 1972). Additionally, the transfer of all treatments to the 8-hour photoperiod incubator during the weekend temporarily disrupted the experimental growth conditions. This may have decreased

algal growth rates, and may account for the lack of significant difference between the Control and the two treatments. Finally, human procedural error, such as mistakes during counting may have also impacted our results, as this could correspond to incorrect calculations of growth rate.

The *C. reinhardtii* populations had the greatest growth rate in treatment two where they were exposed to the greatest photoperiod, twenty one hours of light. This is considered high light exposure. Many phototrophic algae, such as algal symbionts of coral, are damaged by high light exposure (Lesser & Farrell, 2004). However, *C. reinhardtii* are capable of maintaining high growth rates during high light exposure, corresponding to our results. Metabolic changes during a photoacclimation period make this possible (Davis *et al.*, 2013). One way to do this is by increasing thermal energy dissipation within photosystems one and two, which is triggered by lumen acidification of the chloroplast during high light exposure. This plays a photoprotective role during acclimation (Allorent *et al.*, 2013).The lag phase recorded from the cells in treatment two during days two to four suggests a possible photoacclimation process.

C. reinhardtii make up the base of the salmon food chain. Salmon emerge from gravel nests in the spring, a time during which photoperiod ranges from 14-16 hours of light (Time and Date, 2017). Our results show that maximum *C. reinhardtii* exponential growth rates occur at a 21-hour photoperiod, indicating that algal growth rates during salmon emergence are high. After emergence and yolk sac depletion, salmon feed on zooplankton and invertebrates, which in turn feed on *C. reinhardtii*; low algae growth would therefore reduce juvenile salmon food sources, reducing survival to smoltification (Groot & Margolis, 1991; Norambuena *et al.,* 2015). Furthermore, the timing of emergence influences the likelihood of juvenile salmon survival, and is correlated to *C.reinhardtii* population levels (Ackerman, 2017). Early emergence poses a

tradeoff between early access to food and increased predation risk; however, low *C. reinhardtii* growth rates are expected to promote early emergence, as this would ensure access to limited food resources (Jones *et al.,* 2003; Ackerman, 2017).

This experiment provides the foundation needed to investigate the effects of climate change on the growth rate of photoautotrophic algae, as climate change is found to alter photoperiod lengths (Blanchard, 2012). In addition to photoperiod investigations, further studies are needed to examine the effects of light lux and temperature on *C.reinhardtii* maximum growth rate. This would allow for a more in-depth examination of the impacts of climate change on algae growth.

Conclusion

In this experiment, we rejected our null hypothesis and therefore provided support for the hypothesis that the exponential growth rate of *C. reinhardtii* changes as photoperiod changes. Specifically, the exponential growth rate of *C. reinhardtii* is significantly higher at a 21-hour photoperiod compared to a 3-hour photoperiod. The trends demonstrated in our data follow those outlined by our prediction; the maximum algal exponential growth rate corresponds to the highest photoperiod level of 21 hours, and that exponential growth rate generally increases as photoperiod increases. Our results demonstrate that maximal exponential growth rates of *C. reinhardtii* occur at photoperiod levels similar to those present during salmon fry emergence, indicating the profound implications on the ecosystem if algae growth was inhibited or altered (Gerson *et al.* 2016; Time and Date, 2017).

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Appendix

Table 1. *Chlamydomonas reinhardtii* Cell Concentration: Treatment 1

Day	Replicate 1 Concentration (cells/mL)	Replicate 2 Concentration (cells/mL)	Replicate 3 Concentration (cells/mL)	Mean Cell Conce tration (cells/mL)
Nov. 1st	33000	11000	33000	25667
Nov. 3rd	225500	126500	181500	177833
Nov. 6th	550000	522500	588500	553667
Nov. 8th	396000	748000	1364000	836000

Table 2. *Chlamydomonas reinhardtii* Cell Concentration: Treatment 2

Day	Replicate 1 Concentration (cells/mL)	Replicate 2 Concentration (cells/mL)	Replicate 3 Concentration (cells/mL)	Mean Cell Concentration (cells/mL)
Nov. 1st	1276000	1204500	1122000	1200833
Nov. 3rd	550000	918500	2304500	1257667
Nov. 6th	2068000	2420000	3410000	2632667
Nov. 8th	4554000	3371500	3344000	3756500

Table 3. *Chlamydomonas reinhardtii* Cell Concentration: Control

Day	Replicate 1 Concentration (cells/mL)	Replicate 2 Concentration (cells/mL)	Replicate 3 Concentration (cells/mL)	Mean Cell Concentration (cells/mL)
Nov. 1st	16500	132000	159500	102667
Nov. 3rd	374000	814000	715000	634333
Nov. 6th	264000	467500	374000	368500
Nov. 8th	533500	390500	550000	491333

Table 5. Mean Exponential Growth Rate for Control, Treatment 1, and Treatment 2.

Treatment	Exponential Growth Rate (cells/mL/ day)
Control	265833
1	141167
2	500417