Examining the Effects of Light Wavelengths on the Growth Rate of *Chlamydomonas reinhardtii*

BIOL 342
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Abstract

Chlamydomonas reinhardtii is a unique alga that acts as a major food source for salmon species. It has the unique ability to grow autotrophically in the light, and heterotrophically in the dark. In this study, we aimed to explore the behaviour of *C. reinhardtii* in different light conditions. Specifically, we examined the growth rate of *C. reinhardtii* under different light treatments. These light treatments varied in light wavelength, and consisted of white light (positive control), red light (lower visible light frequency), blue light (higher visible light frequency), and green light (intermediate visible light frequency). We used a hemocytometer to do cell counts over a period of 14 days and displayed the growth rate *C. reinhardtii* on growth curves. We then calculated the overall growth rate, and performed perform a one-way ANOVA test to determine if there is a significant difference in the growth rate between the different light treatment groups. The ANOVA test revealed an F-value of 24.37 and a p-value of less than 0.0001. Since ANOVA found a significant difference between the four treatment groups ($p\text{-value} < 0.05$), we performed a Tukey-Kramer test to determine which treatment groups are significantly different. The multiple comparison test revealed that the growth rates of the control group was significantly different to the growth rate of the red, blue, and green treatment groups ($p\text{-values} < 0.05$), and the growth rates of the red, blue, and green treatment groups were not significantly different from each other ($p\text{-values} > 0.05$).

Introduction

Chlamydomonas reinhardtii is a haploid, unicellular green alga that forms the planktonic portion of the salmon food web. They are about 10 μm in diameter and their cell wall is made of hydroxyproline-rich glycoproteins (Harris, 2001; Gallagher et al, 2015). *C. reinhardtii* is one of the major food sources for fish and other species integral to our environment, including salmon (Norembuena et al., 2015). As such, it is important to understand their reproduction and growth habits, and the possible factors that can affect their growth rate. Given their array of unique characteristics, *C. reinhardtii* is considered a model organism for understanding algal metabolism (Scranton, 2015). They have the ability to grow both photoautotrophically and heterotrophically in the dark, depending on the conditions of their surrounding environment (Stavis et al., 1973). Moreover, they can be easily cultured and examined under a variety of environmental conditions (Erickson et al., 2015).

All photosynthetic organisms require light for growth, however, the frequency and conditions of the available light will vary in their natural habitat, ranging from sunlight with very high light intensity (around 2000 μmol photons m^{-2} s^{-1}) to shaded areas with little light available (Erickson et al., 2015). Thus, it is important to be aware of the optimal light conditions for maximum growth. According to Erickson et al. (2015), there is a correlation between the amount of light absorption and the rate of photosynthesis. If a photosynthetic organism absorbs too much light, a phenomenon called light stress occurs, leading to photo-oxidative by-products that damage the photosynthetic centers and decrease the efficiency and rate of photosynthesis (Erickson et al., 2015).

When *C. reinhardtii* is exposed to blue light, the growth rate was faster and higher compared to when exposed to red light (Oldenhof et al., 2006). However, Mooij et al. (2016), argue that blue and red lights are less preferred for cell growth compared to warmer lights such as white and green-yellow. Based on their findings, exposure to blue and red lights causes photosystem over-saturation, effectively decreasing the photosynthetic efficiency. They further argued that green or yellow lights that are weakly absorbed will increase the photosynthetic efficiency (Mooij et al., 2016).

The purpose of this study is to measure cell growth under four different conditions of exposure to white, green, blue, and red light, in order to find the optimal light conditions for growth. Based on an initial literature review, we predicted that the highest growth rate will be observed when exposed to green light, as it encompasses the middle, intermediate range of wavelengths. Our null hypothesis stated that there is no significant difference in mean growth rate between the four treatment groups, and our alternative hypothesis indicates that there will be a significant difference in mean growth rate between different treatments.

Methods

In order to determine the effects of light wavelength on the growth rate of *C. reinhardtii*, we first found the concentration of our initial sample of cells. We did this by using a hemocytometer and a compound microscope to determine the number of cells in a given area. Since *C. reinhardtii* are mobile organisms, we mixed in IKI fixative to prevent them from moving. 100 µL of the cell sample was mixed with 10 µL of fixative. 10 µL of this fixative-cell mixture was loaded onto the hemocytometer and viewed under

a compound microscope. The number of cells counted in certain sized squares allowed us to determine the initial concentration of our cell solution. Using Equation 1, we determined the concentration of our cell sample based on the number of cells we count in certain sized squares on the hemocytometer.

(Equation 1) (Number of cells counted ÷ number of squares) × (dilution factor of square) x 1.1

For our initial cell sample, we counted 58 cells in one 1 mm x 1 mm square, which corresponded to an initial concentration of 6.38 x 10^5 cells/mL. Since we want to count around 200 cells/10 µL (2.0 x 10^4 cells/mL), we needed to dilute our initial cell sample to 2.0 x 10^4 cells/mL. In order to produce enough cell sample solution for all our replicates, we needed to dilute our initial sample of 6.38 x 10^5 cells/mL to a final concentration of 2.0 x 10^4 cells/mL in a volume of 100 mL (Figure 1). Using Equation 2, we determined that we needed to mix 319 µL of our initial cell sample with 99.681 mL of cell media to get our desired final concentration.

(Equation 2) $C_1V_1 = C_2 V_2$

After this dilution, we viewed our sample again under a hemocytometer and counted 2 cells in one 1 mm x 1 mm square. This corresponded to a concentration of 2.2 x 10^4 cells/mL. Since the concentration of our sample after dilution was close to 2.0 x 10^4 cells/mL, we confirmed that our calculations and dilutions were successful.

After diluting our cell sample, we set up our light treatment groups. We planned to place 4 replicates under four different light treatment groups: a positive control, a red light group, a blue light group, and a green light group (16 replicates total). To create the different light wavelength treatment groups, we wrapped our replicate culture tubes with

red acetate filters, blue acetate filters, green acetate filters, and white cloths (for the control). The white cloth was used to ensure equal light intensity among all our groups. We then pipetted 10 mL of our diluted *C. reinhardtii* sample each into our 4 replicate culture tubes for our 4 treatment groups (Figure 1). We performed an initial cell count for our replicates using the hemocytometer and compound microscope, and counted each replicate 3 times to account for sampling bias. Once the initial counts were complete, we placed our replicates in a 17 ^{o}C incubator, running under light and dark cycles, to grow overnight. The replicates were later moved on Day 7 to a 25 ^{o}C incubator after observing a slow initial growth rate.

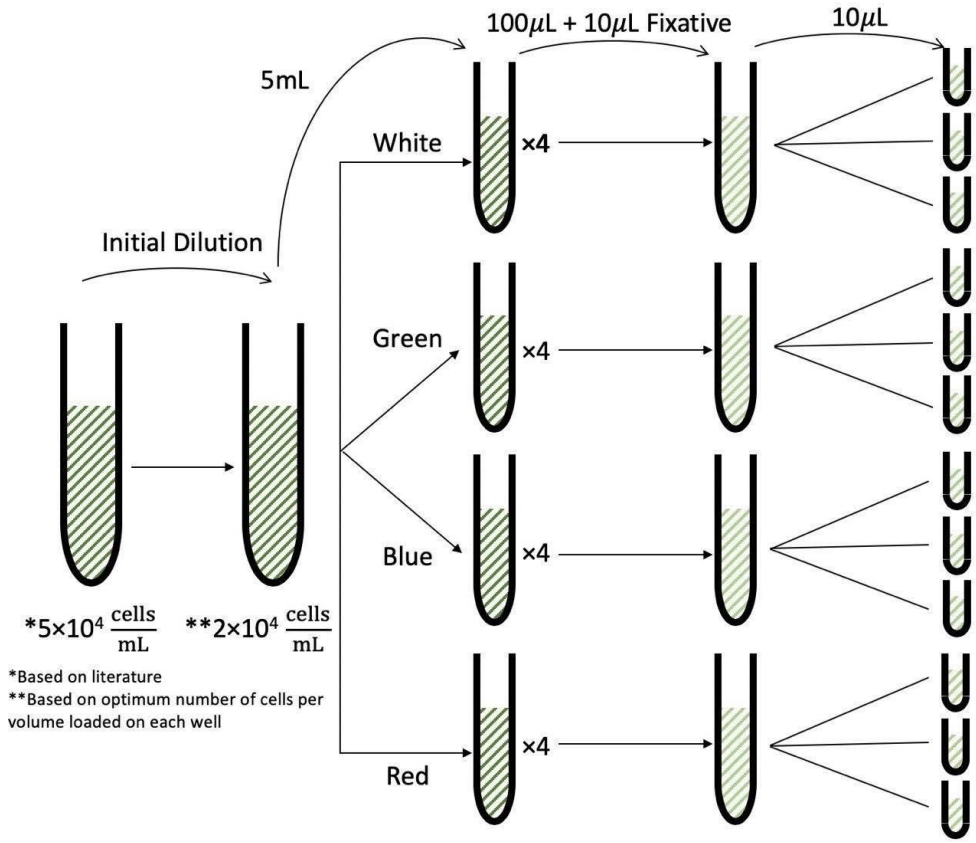

Figure 1. Initial Dilutions and Counting Procedure of *C. reinhardtii* replicates.

Since we are exploring the growth rate of *C. reinhardtii*, we conducted cell counts of our replicates for several days, once the treatment groups were all set up. The procedure was similar to the initial cell count: mix 100 µL of cells with 10 µL of fixative, load 10 µL of this mixture onto a hemocytometer, count the number of cells using a compound microscope, and repeat this process for all the replicates and count each replicate 3 times. In total, we observed the growth of *C. reinhardtii* over a period of 14 days and did cell counts for 7 of those days. The number of cells for each replicate was converted to average concentration, and plotted on growth curves to quantify and observe trends in the growth rate of *C. reinhardtii*. We then wanted to determine if there was a significant difference in growth rate between the four treatment groups. To do this, we calculated the overall growth rate (from t = 0 days to t = 14 days) for each replicate, and performed a one-way ANOVA test. Since our ANOVA test found a significant difference in our data (i.e. p-value < 0.05), we needed to perform a Tukey-Kramer test to determine which treatment groups were significantly different from each other. All growth curves, graphs, and statistical tests were performed on GraphPad.

Results

The average concentration of each treatment group was plotted on a growth curve as a function of time (in hours). The objective of creating a growth curve was to observe the growth trends of *C. reinhardtii* among the four treatment groups.

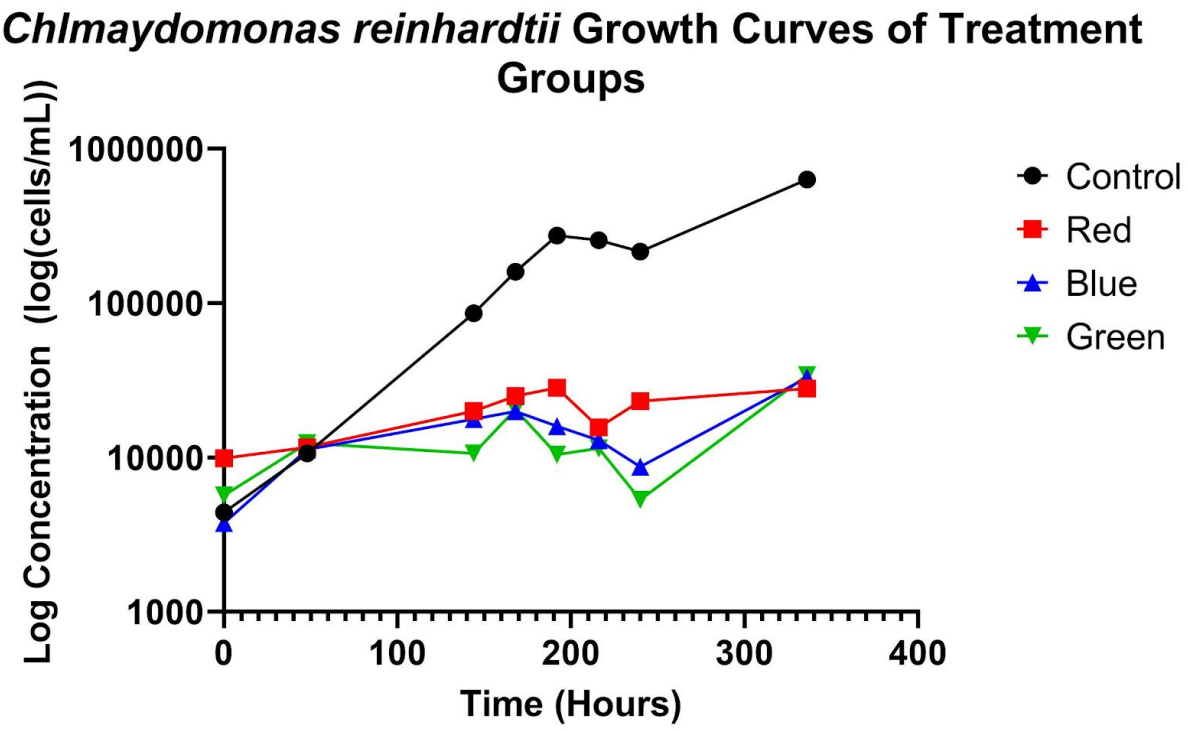

Figure 2. *C. reinhardtii* Growth Curves of the Four Treatment Groups Over 14 Days (336 hours).

Based on these growth curves, there are a few interesting observations of the growth rate of *C. reinhardtii* between the different treatment groups. Most noticeably, the control treatment group seemed to grow at a much faster rate compared to the red, blue, and green treatment groups. On the other hand, the red, blue, and green treatment groups seem to have a similar growth rate over the 14 day period. Lastly, an interesting, yet unexpected observation we found from the growth curves was the amount of fluctuation in the cell concentration of *C. reinhardtii* over time. We speculate that these abnormalities are due to subjective cell counting errors, and argue that these fluctuations would become negligible if more data points were taken.

While growth curves help observe trends in the growth rate of *C. reinhardtii*, it cannot determine if there are significant differences in the growth rate between the treatment groups. As a result, we need to display variation in growth on a different graph and perform statistical tests to determine if such a difference exists. We calculated the overall growth rate (from t = 0 hours to t = 336 hours) for each replicate, displayed the growth rate data on a boxplot, and performed an ANOVA test to test for differences in growth rate. A box-plot is the most appropriate graph because we are displaying the effects of light wavelength (categorical variable) on the growth rate of *C. reinhardtii* (numerical variable).

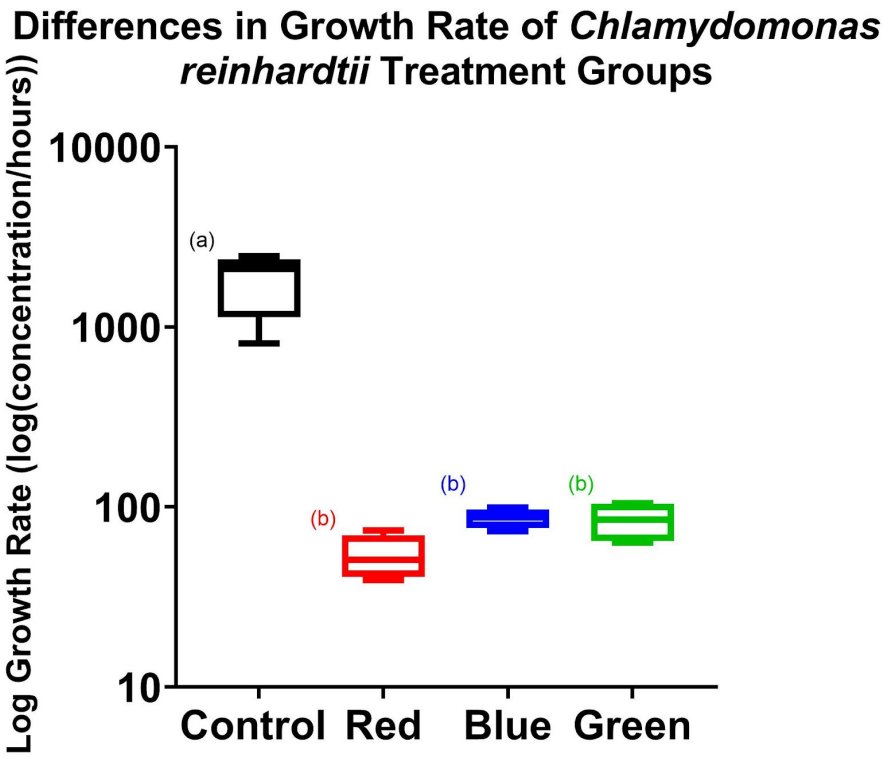

Figure 3. Differences in Log Mean *C. reinhardtii* Growth Rate Between the Four Treatment Groups (n = 4, F-value = 24.37, p-value < 0.0001). Treatment groups with the same letter are not significantly different, while treatment groups with different letters are significantly different.

The graph reveals a dramatic increase in growth rate for the control group compared to the other light treatment groups, while the red, blue, and green light treatment groups seem to have a similar growth rate. Further calculation of the mean growth rates for each treatment group found an average growth rate of 1866.42 $\text{cells} \cdot \text{mL}^{-1} \cdot \text{hours}^{-1}$ for the control group, 53.74 $\text{cells} \cdot \text{mL}^{-1} \cdot \text{hours}^{-1}$ for the red light group, 86.75 $\text{cells} \cdot \text{mL}^{-1} \cdot \text{hours}^{-1}$ for the blue light group, and 84.57 $\text{cells} \cdot \text{mL}^{-1} \cdot \text{hours}^{-1}$ for the green light group. A one-way ANOVA test between the four treatment groups finds an F-value of 24.37 and a p-value of less than 0.0001. Since the ANOVA test revealed a significant difference in the growth rate between the four treatment groups, a Tukey-Kramer test was performed to determine which groups are significantly different from each other. The multiple comparisons test revealed that the growth rates of the control group was significantly different from the growth rates of the red, blue, and green treatment groups (p-values < 0.05), and the growth rates of the red, blue, and green treatment groups were not significantly different from each other (p-values > 0.05).

Discussion

After performing the one-way ANOVA test, we found a p-value < 0.0001. Since our p-value was less than 0.05, we reject the null hypothesis that there is no significant difference in mean growth rate between the four treatment groups, and lend support to our alternative hypothesis. A further Tukey-Kramer multiple comparison test revealed that the growth rate for the control group was significantly greater than that of the red,

blue, and green treatment groups, whereas there was no statistically significant difference in growth rates among the red, blue, and green treatment groups.

Although the difference is not statistically significant, we found that, of the three colour treatments, blue light produced the highest mean growth rate, followed by green and then finally red light. These results were not completely expected as at the start of the experiment, we predicted the treatment exposed to green light to yield the highest growth rate. Prior literature on *C. reinhardtii* suggests that exposure to blue and red light can lead to a decrease in photosynthetic efficiency due to the oversaturation of the photosystem (Mooij et al., 2016). Whereas on the other hand, softer colours, which have weaker absorption properties such as green and yellow have been shown to increase photosynthetic efficiency.

Nonetheless, the results reported in this research should be considered in light of some limitations. The major limitation in interpreting our data stems from our small sample size. Our experiment used a very small sample size (n = 4), and as a result, we violated one of our assumptions of the one-way ANOVA and Tukey-Kramer tests (i.e. large sample size). This condition could have impacted our ANOVA and Tukey-Kramer results, potentially leading to false inferences between treatment groups. Another limitation we had was having all group members perform cell counts under a compound microscope, rather than having just one designated member. As this method is inherently subject to individual bias and confounding, it is possible that our results may have been influenced. The second potential source of uncertainty is associated with a miscalculation at the start of the experiment, where we incorrectly calculated the initial

concentration for our sample. This led to our initial sample being much too dilute, thus extending the lag phase of each of the treatment groups. Another possible source of error includes moving our samples on day three from a 17 ^{o}C incubator to a 25 ^{o}C incubator. A recent study conducted by Vitova et al., (2011) looked at the effect of temperature on cultures of *C. reinhardtii.* They concluded that temperature can play a major role in influencing growth rates and cell division cycles. Therefore, it is possible that our samples, when placed in the 17 ^{o}C, may not have been exposed to optimal growing conditions. To further improve the accuracy of results in future studies, we suggest increasing the number of replicates for each treatment group in an attempt to mitigate sampling bias. Similarly, an increase in incubation time can also work to possibly create a greater variation in cell density between the treatments.

Conclusion

In this study, we examined the effects of different wavelengths of light on the growth rate of *Chlamydomonas reinhardtii,* specifically under blue, red, green and normal white light. We observed and counted the cell growth rate of the organism under the various light treatments over the course of 14 days. Data analysis led to the rejection of our null hypothesis that the mean growth rates of the four treatment groups are not significantly different (p-value < 0.0001). A further multiple comparison test found that the growth rate of *C. reinhardtii was* significantly higher when exposed to the white light treatment group (control) as compared to the red, green and blue treatment groups (p-values < 0.05). On the other hand, the growth rates of the red, blue, and

green treatment groups were not significantly different from each other (p-values > 0.05). Further research should examine the growth rate of *C. reinhardtii* over a longer period of time. With more accurate growth curves, better inferences on the growth rate of *C. reinhardtii* can be made between the treatment group.

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Appendix

Initial Cell Count (t = 0): October 29, 2019

Replicate	Control	Red	Blue	Green
1	2/9, 4/9, 4/9	9/9, 8/9, 4/9	2/9, 2/9, 4/9	3/9, 5/9, 4/9
2	5/9, 7/9, 9/9	8/9, 10/9, 8/9	2/9, 2/9,4/9	5/9, 4/9, 4/9
3	5/9, 1/9, 3/9	12/9, 15/9, 10/9	6/9, 3/9, 5/9	7/9, 5/9, 7/9
4	5/9, 1/9,2/9	7/9, 5/9, 12/9	3/9, 4/9, 4/9	7/9, 5/9, 6/9

Incubator Abiotic Factors:

Temperature: 17.0 ^{o}C

Control Light Intensity: 1547 lux
Red Light Intensity: 1479 lux
Blue Light Intensity: 1565 lux
Green Light Intensity: 1559 lux

1st Counting Day (t = 2): October 31, 2019

Replicate	Control	Red	Blue	Green
1	7/9, 5/9, 1/9	11/9, 2/9, 7/9	9/9, 13/9, 12/9	6/9, 3/9, 10/9
2	9/9, 14/9, 32/9	7/9, 8/9, 16/9	13/9, 16/9, 19/9	9/9, 9/9, 10/9
3	5/9, 6/9, 2/9	11/9, 14/9, 13/9	6/9, 6/9, 5/9	7/9, 14/9, 12/9
4	13/9, 8/9, 14/9	16/9, 6/9, 16/9	4/9, 17/9, 3/9	16/9, 15/9, 24/9

2nd Counting Day (t = 6): November 4, 2019

Replicate	Control	Red	Blue	Green
1	102/7, 54/9, 34/9	15/9, 17/9, 18/9	12/9, 8/9, 14/9	10/9, 9/9, 9/9
2	76/9, 72/9, 82/9	23/9, 19/9, 17/9	14/9, 30/9, 17/9	17/9, 11/9, 8/9
3	107/9, 79/9, 85/9	16/9, 16/9, 10/9	23/9, 27/9, 29/9	6/9, 5/9, 6/9
4	87/9, 56/9, 60/9	23/9, 18/9, 26/9	6/9, 4/9, 9/9	8/9, 14/9, 13/9

3rd Counting Day (t = 7): November 5, 2019

Replicate	Control	Red	Blue	Green
1	156/4, 151/5, 156/5	24/9, 26/9, 24/9	15/9, 14/9, 14/9	15/9, 23/9, 35/9
2	47/9, 54/9, 123/6	16/9, 14/9, 33/9	12/9, 21/9, 17/9	16/9, 27/9, 29/9
3	42/9, 94/9, 79/9	13/9, 12/9, 17/9	17/9, 16/9, 21/9	13/9, 6/9, 13/9
4	60/9, 61/9, 96/9	20/9, 40/9, 34/9	21/9, 28/9, 21/9	25/9, 10/9, 11/9

New Incubator Abiotic Factors:

Temperature: 25.0 ^{o}C
Light Intensity: 1830 lux

4th Counting Day (t = 8): November 6, 2019

Replicate	Control	Red	Blue	Green
1	178/5, 157/6, 157/5	20/9, 24/9, 20/9	19/9, 21/9, 26/9	9/9, 10/9, 6/9
2	190/4,180/4, 163/6	35/9, 25/9, 15/9	12/9, 8/9, 10/9	5/9, 12/9, 7/9
3	139/9, 85/9, 154/9	33/9, 56/9, 15/9	15/9, 19/9, 15/9	44/9, 28/9, 37/9
4	130/9, 160/6, 90/9	30/9, 21/9,14/9	10/9, 9/9, 10/9	18/9, 14/9, 9/9

5th Counting Day (t = 9): November 7, 2019

Replicate	Control	Red	Blue	Green
1	88/9, 95/9, 56/9	24/9, 22/9, 22/9	16/9, 8/9, 13/9	56/9, 25/9, 38/9
2	141/3, 149/3, 159/3	22/9, 16/9, 11/9	10/9, 9/9, 10/9	25/9, 35/9, 40/9
3	155/7, 151/7, 167/8	11/9, 5/9, 8/9	17/9, 12/9, 13/9	23/9, 27/9, 23/9
4	138/9, 103/9, 164/9	11/9, 7/9, 12/9	9/9, 13/9, 11/9	17/9, 19/9, 10/9

6th Counting Day (t = 10): November 8, 2019

Replicate	Control	Red	Blue	Green
1	172/6, 151/9, 120/9	23/9, 19/9, 21/9	9/9, 5/9, 8/9	6/9, 4/9, 5/9
2	161/6, 171/7, 177/7	30/9, 25/9, 28/9	11/9, 6/9, 6/9	1/9, 2/9, 2/9
3	162/9, 175/7, 169/7	22/9, 21/9, 12/9	8/9, 3/9, 3/9	8/9, 5/9, 6/9
4	130/9, 115/9, 130/9	11/9, 25/9, 16/9	15/9, 14/9, 7/9	6/9, 7/9, 6/9

7th Counting Day (t = 14): November 12, 2019

Replicate	Control	Red	Blue	Green
1	151/6, 155/6, 171/7	26/9, 22/9, 24/9	34/9, 25/9, 31/9	29/9, 31/9, 45/9
2	197/3, 204/3, 183/3	16/9, 9/9, 12/9	27/9, 32/9, 16/9	40/9, 15/9, 21/9
3	199/3, 150/2, 172/2	31/9, 12/9, 30/9	36/9, 28/9, 28/9	29/9. 32/9, 16/9
4	160/4, 187/2, 174/3	20/9, 29/9, 43/9	22/9, 41/9, 39/9	40/9, 30/9, 44/9