Euglena gracilis Growth Rate Under Different Light Exposure Length and the Underlying Relationship

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Abstract

In this project, the relationship between the growth rate of *Euglena gracilis* and the exposure time to light was investigated by developing three experimental groups, collecting the group populations over 12 days and using the values to create a growth curve for each. As light encourages the growth of chlorophyll, we predicted that with longer periods of light exposure, *Euglena gracilis* culture would grow at a much higher rate compared to the groups that did not receive as much light. The mean growth rate was 0.2004 ± 0.1274 day $^{-1}$ for 0 hours, 0.3846 ± 0.3426 day $^{-1}$ for 12 hours, 0.2971 ± 0.3492 day $^{-1}$ for 24 hours. It was determined that the exposure time to light had no significant effect on the growth rate of *Euglena gracilis* ($p = 0.7570$) and suggested that no extensive light source is needed when growing an *E. gracilis* culture.

Introduction

This study will be focusing on how light affects the growth of *Euglena gracilis* which are mixotrophic algae that rely on photosynthesis and phagocytosis for food (Ahn et al., 2019). Previous research has studied how temperature, iron, and pH affect the growth of *Euglena*. When iron was used to study the growth rate of *Euglena* (Chen et al., 2020), there was no significant difference found between different conditions despite affecting the formation of chlorophyll. However, when the temperature was a factor, it was found that *Euglena* had higher

growth rates in phototrophic conditions compared to mixotrophic and heterotrophic conditions (Wang et al., 2018). In terms of pH, a neutral environment was found to be most favorable for the growth of *E. gracilis*; pH values above 8 and below 4 are detrimental to the organism (Danilov, Ekelund, 2001). We are therefore building on previous research to help identify if light alone affects the growth of *E. gracilis.* The optimal temperature for growth was found to be around 25-30 degrees Celsius (Ko, Spekmaier, Wang., 2020). Since *E. gracilis* cultures are mixotrophic they can survive in both light and dark conditions. When the *E. gracilis* were studied under dark conditions, it was found that their chloroplast length decreased because they were unable to produce chlorophyll (Ahn et al., 2019). This, therefore, showed a positive correlation between growth rate and light exposure, proposing that as long as both photosynthesis and organic carbon assimilation can proceed simultaneously, the growth rate is expected to be higher than that achieved under photoautotrophic or heterotrophic conditions (Ogbonna, J., et al.). Hence, we predict that the *E. gracilis* in the incubator with 24h light will have the highest growth compared to 0h of light and 12h of light.

Methods

To test the effect of light exposure time on the *E. gracilis'* growth rate, three treatment levels were created. The three treatment levels were 24 hours light exposure every day, 12 hours light exposure every day, and 0 hours light exposure every day. Within each treatment level, there were four 25 mL test tubes to provide more reliable experimental data.

To prepare the experimental groups, a 150 mL master mix was first generated by mixing the *E. gracilis* stock solution and the growth medium, ensuring that we did not reach nutritional conditions that may restrict cell multiplication (Nigon, V., Heizmann, P.). Then, 10 mL of the master mix was distributed into each test tube to ensure the same initial cell concentration. The *E. gracilis* concentration in the stock solution was determined using a hemocytometer, and after

averaging three counts, the value was calculated to be 3.33 x 10^5 cells/mL. Based on previous studies, it was suggested that a starting concentration of around 5 x 10^4 cells/mL is optimal to develop a growth curve within a population (Zhu, Wakisaka, 2018). Therefore, 22.52 mL of stock solution and 127.48 mL of growth medium were mixed to generate the master mix. After the master mix was developed, the cell density was recalculated to confirm that the desired value

was achieved. Following the same procedure, the cell concentration in the master mix was 6.1 x 10^4 cells/mL, which conformed with the desired initial concentration.

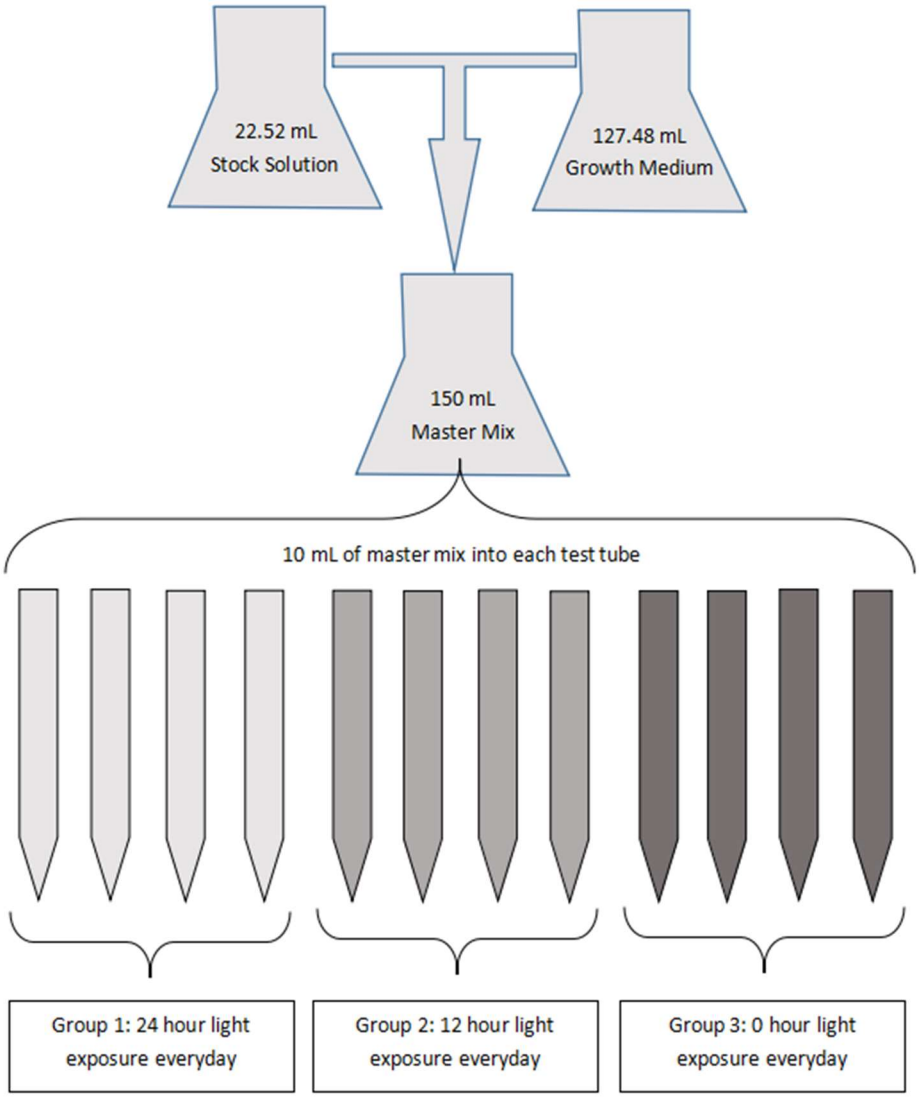

Figure 1: Flowchart of experimental groups preparation.

After 10 mL of the master mix was transferred into each test tube following the sterilization procedure, test tubes were separated into three groups and stored in the designated incubators for cell cultures to grow. Among all 12 test tubes, four of them were incubated under light throughout the experiment, four were incubated in the dark all the time, and the last four were incubated under a 12h-12h light-dark cycle. All the experimental groups were kept under 25℃ to reduce the effect of temperature on the growth rate. The day of preparing the master

mix was designated as Day 1, and samples were collected from each test tube on Days 3, 5, 8, 10, and 12 to obtain data and to construct the growth curve. During the collection of samples, the same sterilization procedure was followed to reduce the influences of other organisms that might affect the growth of the culture.

When collecting samples, 100 μL of cell culture was extracted from the test tube and combined with 10 μL of fixative. All the samples were kept in the fridge until Day 12 when the cell densities of all the samples were counted using hemocytometers. After obtaining the raw data, the concentrations of each treatment group were plotted to compare any difference in the growth rate, and the growth rates in each culture were calculated for further analysis. Furthermore, the data collected was analyzed using a one-way ANOVA test to determine whether the difference in the growth rate was significant enough to conclude that light exposure time affects the growth rate of *Euglena*.

Results

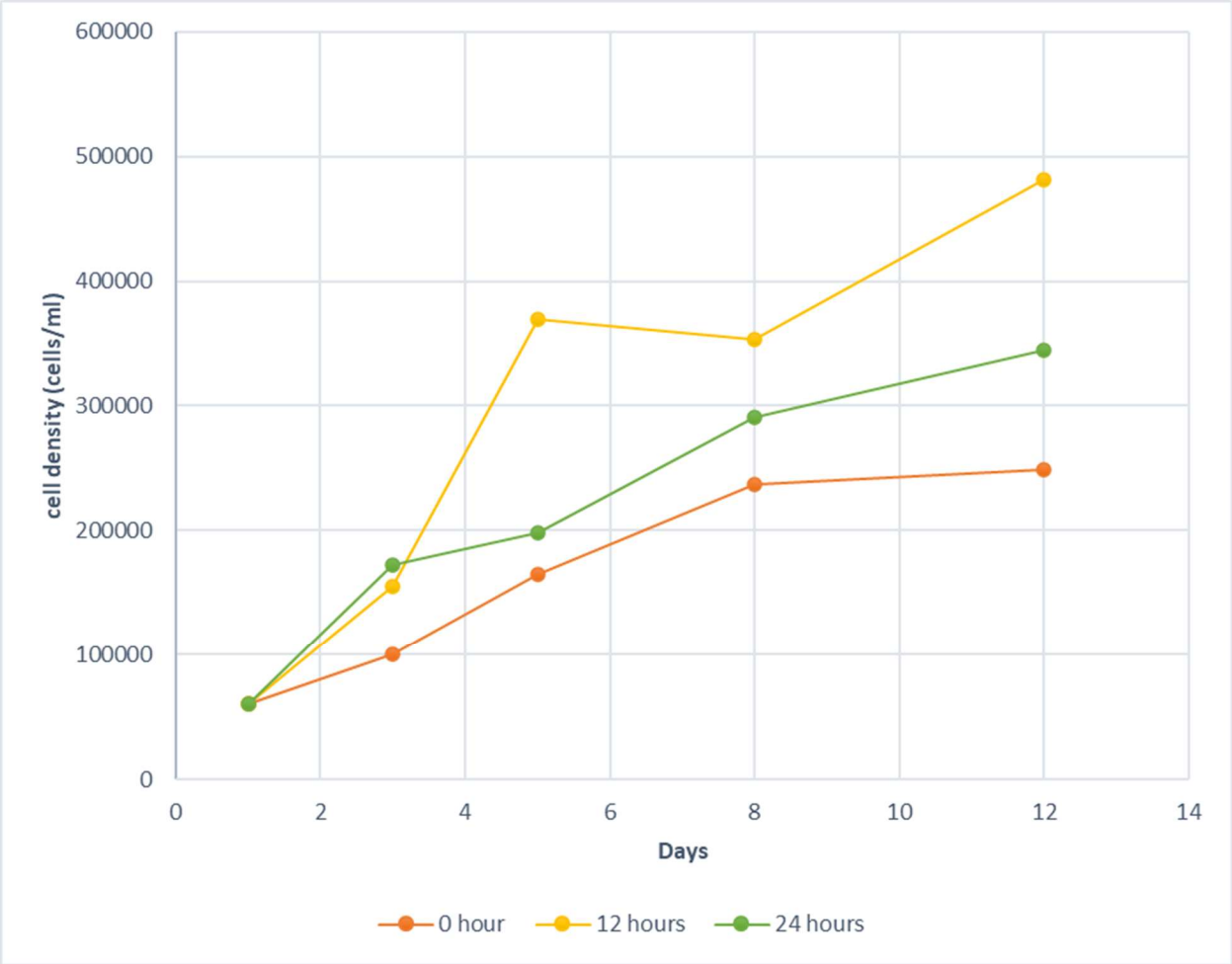

Figure 2: Mean cell densities (cells/mL) for each treatment for *Euglena gracilis* grown in different exposure times to light- 0 hour (n = 4), 12 hours (n = 4), and 24 hours (n = 4) over the 12-day time period of the experiment.

Figure 2 shows the cell density for each individual treatment. Initially, the cell density was maximum for the population which was exposed to light for 24 hours. After Day 3, the group which was exposed to light for 12 hours had the highest cell densities among the different treatments and this trend was maintained till the end of the experiment on Day 12. Based on the graph projection in Figure 2, by the end of the experiment, only the treatment with 0 hours of

sunlight exposure reached a plateau compared to the other two treatments which did not plateau.

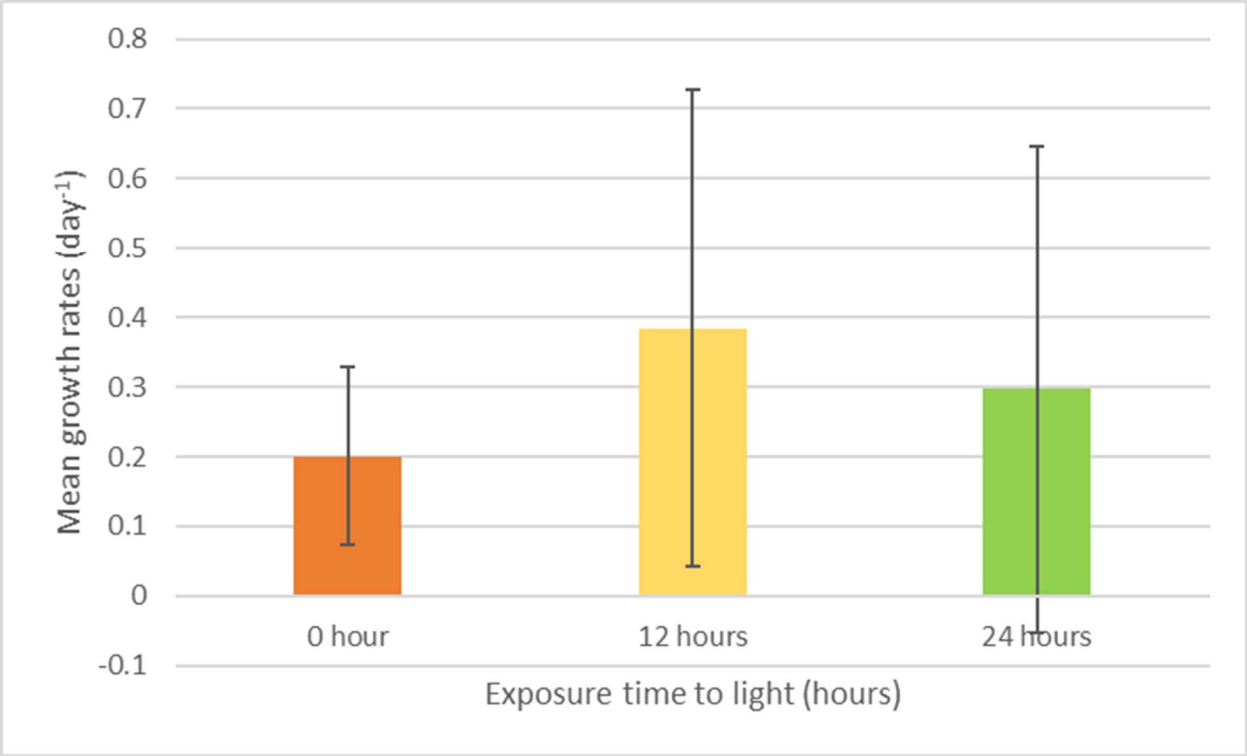

Figure 3: Mean cell growth rates (day $^{-1}$) for each treatment for *Euglena gracilis* grown in different exposure times to light- 0 hour (n = 4), 12 hours (n = 4), and 24 hours (n = 4) over the 12-day time period of the experiment. The error bars show a 95% confidence interval with the upper and lower whiskers extending to the minimum and maximum values ($p>0.05$).

The mean growth rates (Figure 3) with the 95% confidence intervals were 0.2004 ± 0.1274 day $^{-1}$, 0.3846 ± 0.3426 day $^{-1}$, 0.2971 ± 0.3491 day $^{-1}$ for the 0 hour, 12 hours, and 24 hours treatments respectively. A one-way ANOVA test calculated a p-value of 0.7570 meaning the results were not significant.

Discussion

Despite the clear increase in growth rate observed towards the end of the experiment, we found that with a P-value of greater than 0.05, we cannot reject the null hypothesis that is: the duration of light exposure has no effect on the growth rate of *Euglena gracilis* cultures. This conclusion may be due to the data collection being limited by time and not including the all-important exponential growth rate of the different cultures. Due to the lack of significant difference between the calculated growth rates, we cannot support our initial prediction that increased duration of exposure to light will increase the growth rate of *E. gracilis* cultures.

Our results do not correlate with what past research has found. According to our experiment, increased exposure to light does not affect the cell division and the overall growth curve of *E. gracilis* which is most likely due to the high error calculated and the inaccuracies during data collection. The observation that exposure to darkness increases the degradation of chloroplast in *E. gracilis* suggests that there should be a positive correlation between light exposure and growth rate (Ahn, 2019). Our results also contradict the increased light intensity resulting in an increased growth rate observed in other previous research, further suggesting that our margin of error was too high (Constantopoulos, 1967 and Wang, 2018). However, our results do share similarities with increased iron levels resulting in reduced chloroplast length but overall, statistically similar growth rates (Chen, 2020).

Over the course of the experiment, there were multiple sources of error that may have influenced the experiment. Since the beginning, there is a low chance that our *E. gracilis* samples were contaminated when we were mixing our master mix. While this is unlikely due to our sterilization procedure, if it did occur, we would have no way of knowing. Another possible

source of error was in the incubators we used. Previous studies have indicated that increased light intensity has a positive effect on *E. gracilis* growth rates, therefore, if the light intensity in our incubators was not identical, then it may have interfered with our results (Beneragama, 2021). One major source of error for our experiment and the one responsible for the anomalously low cell counts from the samples collected on 2022-03-16, was inconsistent resuspension and collection of sample test tubes when fixing the counting tubes (Figure 4) which we decided not to use in our data analysis due to the inaccurate collection methods employed. We attempted to ensure that all samples were thoroughly resuspended before samples were collected, however, without a way to measure the distribution of *E. gracilis* throughout the tube, we have no way of knowing when maximum resuspension has taken place. Another possible source of human error towards the end of the experiment was the cell counts themselves using the hemocytometer. While we did make use of a standard procedure and clickers to ensure we did not lose track, it is possible that over the 120 counts mistakes may have occurred. Another insurance we used against possible mistakes was that we counted all samples twice, reducing the chances of a counting error influencing our final conclusions.

Through our experiment, we came across multiple unexpected results. The first was the anomalously high cell count that occurred in sample 3 of the 12-hour light exposure group collected on 2022-03-11 (Figure 4). This cell count occurred for both counts of the sample, suggesting an error in resuspension or collection occurred during the fixation of the counting tube, and as such, it was removed from our data analysis as it only served to confuse our results and we had enough samples to continue our experiment without it. Similarly, all the samples collected on 2022-03-16 had cell counts that were much too low to be accurate (Figure 4). This suggested that the data collection was inconsistent with data collected throughout the rest of the experiment and would only serve to muddle our conclusions. Therefore, we decided not to use this data as well (Figure 5). The final unexpected result that was noted was the jump

in the growth rate of both the 24-hour and 12-hour light exposure groups right at the end of the experiment (Figure 6). This result suggested that they were entering an exponential phase of growth, which we had hoped to use to calculate the growth rates. This suggests that our starting concentration of *E. gracilis* was too low for the duration of our experiment and as such, we should have continued data collection until the end of this exponential phase.

For further research, identifying if a low correlation between light exposure and growth rate is consistent within other phototrophic microorganisms could prove useful in determining the levels of light required to achieve desired growth rates and whether or not investing in 24-hour light exposure is worthwhile during an experiment. Additionally, sample collection occurring more often, perhaps once a day, would ensure that inaccurate sampling would have less of an impact on the final results. In ideal conditions, increasing the number of samples collected and the number of counts made per sample would allow for more accurate conclusions and may facilitate the development of formulas that allow for the calculation of growth rate based on available growth formulas and light exposure, reducing the number of variables present in future experiments.

Conclusion

Using the results of this study, we fail to reject the null hypothesis and thus conclude that the duration of light exposure does not affect the growth rate of *E. gracilis* cultures. As the duration of light exposure increases, there is no statistically significant increase in the growth rate of *E. gracilis* cultures. This does not align with many previous studies so may be due to a high margin of error, alternatively, this may suggest that the effect of light exposure is considerably less than the effect of growth mediums and temperature and is thus harder to prove the effects of.

Acknowledgments

We would first like to acknowledge the University of British Columbia Vancouver which is located on the traditional, ancestral, and unceded territory of the Musqueam people. We would like to thank Dr. Celeste Leander and Tessa Blanchard for their constant support and guidance during the entire term and for making this project possible. We would also like to thank our lab technician, Mindy Chow for all the assistance in acquiring our equipment and preparing our culture. We would also like to thank our peers for reviewing our project and providing us with constructive feedback.

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Appendix

Graphs of data

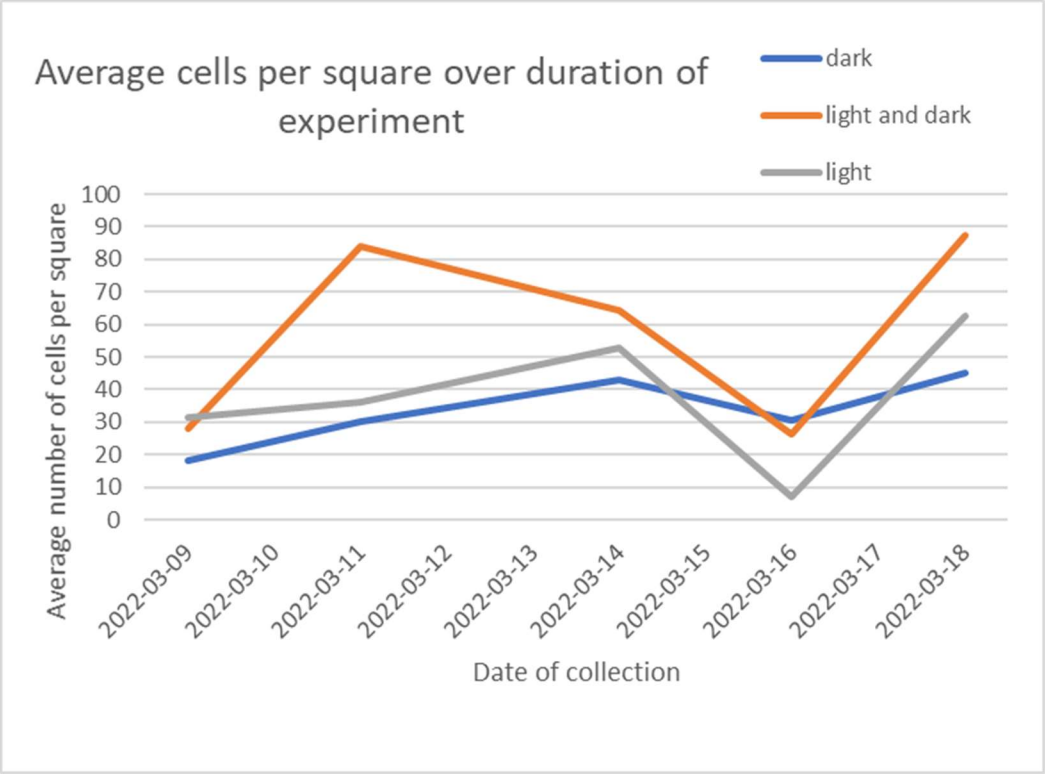

Figure 4: displays the average counted cell density per Hemocytometer square over the duration of the experiment. Note the high value of the light and dark sample at 2022-03-11 and the dip in concentration for all samples at 2022-03-16 that suggest anomalies in data collection and/or analysis.

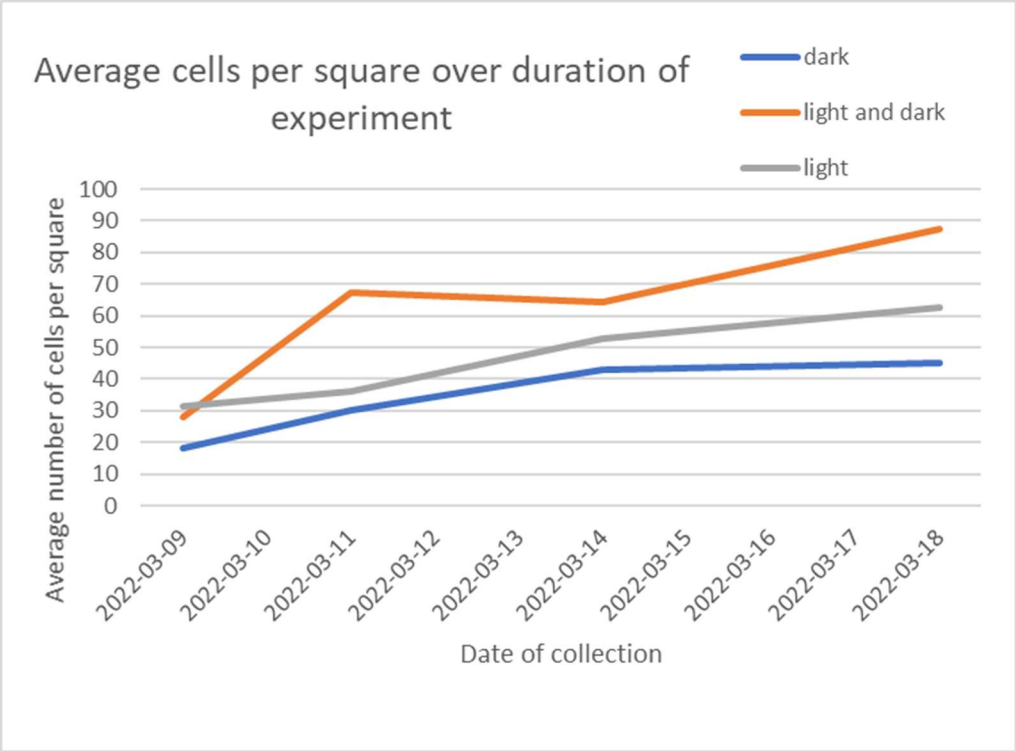

Figure 5: displays the average counted cell density per Hemocytometer square over the duration of the experiment with the aforementioned anomalies removed allowing for a cleaner display of cell density increased over time.

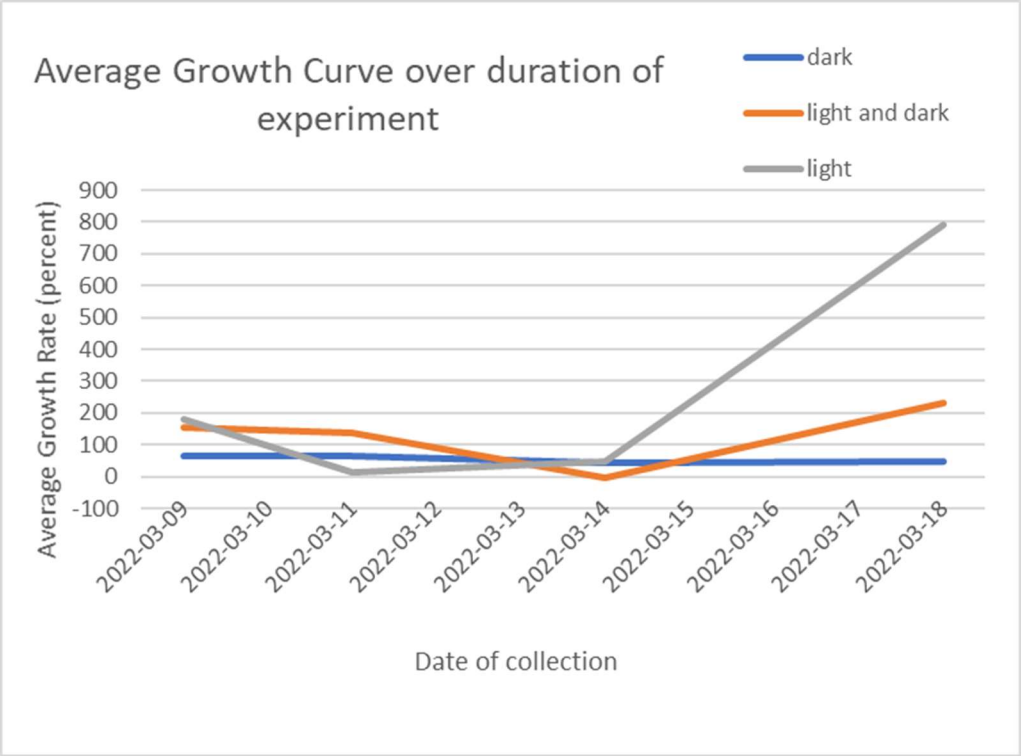

Figure 6: displays the calculated cell growth rates over the course of the experiment. Note the drastic increase in growth rate observed as a part of the light, and light and dark samples suggesting they were entering an exponential phase of growth.

			cells counted		boxes counted			
date of sample collection	time exposed to light (h)	sample number	count 1	count 2	count 1	count 2	average number of cells per box	cells/mL for time
3/7/2022	ALL	ALL	101	121	10	10	11.1	61050
3/9/2022	0	4	112	108	6	8	15.7142857	
3/9/2022	0	3	109	110	12	11	9.52173913	
3/9/2022	0	2	116	103	4	5	24.3333333	
3/9/2022	0	1	127	109	5	5	23.6	100607.87
3/9/2022	12	4	115	112	4	7	20.6363636	
3/9/2022	12	3	116	101	5	5	21.7	
3/9/2022	12	2	125	173	4	2	49.6666667	
3/9/2022	12	1	102	124	7	4	20.5454546	154754.17
3/9/2022	24	4	104	113	1	2	72.33333333	

3/9/2022	24	3	106	101	5	8	15.92307692	
3/9/2022	24	2	105	101	8	8	12.875	
3/9/2022	24	1	115	102	6	3	24.11111111	172208.47
3/11/2022	0	4	224	118	1	8	38	
3/11/2022	0	3	139	109	2	10	20.66666667	
3/11/2022	0	2	105	111	3	3	36	
3/11/2022	0	1	125	101	4	5	25.11111111	164694.44
3/11/2022	12	4	121	198	2	2	79.75	
3/11/2022	12	3	115	153	1	1	134	
3/11/2022	12	2	133	122	3	2	51	
3/11/2022	12	1	104	109	2	1	71	369875
3/11/2022	24	4	123	122	3	4	35	
3/11/2022	24	3	110	127	3	3	39.5	
3/11/2022	24	2	117	144	3	3	43.5	
3/11/2022	24	1	136	101	4	5	26.33333333	198458.33
3/14/2022	0	4	116	124	4	6	24	
3/14/2022	0	3	137	114	4	3	35.85714286	
3/14/2022	0	2	147	106	2	2	63.25	
3/14/2022	0	1	115	133	3	2	49.6	237472.32
3/14/2022	12	4	118	117	4	2	39.16666667	
3/14/2022	12	3	116	146	2	3	52.4	
3/14/2022	12	2	152	148	2	2	75	
3/14/2022	12	1	187	174	2	2	90.25	353122.92
3/14/2022	24	4	143	135	2	2	69.5	
3/14/2022	24	3	118	124	4	3	34.57142857	
3/14/2022	24	2	101	125	2	3	45.2	
3/14/2022	24	1	142	170	3	2	62.4	291048.21
3/16/2022	0	4	115	105	8	6	15.71428571	
3/16/2022	0	3	107	109	12	12	9	
3/16/2022	0	2	114	102	3	3	36	
3/16/2022	0	1	112	134	2	2	61.5	168044.64
3/16/2022	12	4	114	107	7	9	13.8125	
3/16/2022	12	3	116	102	7	8	14.53333333	
3/16/2022	12	2	104	129	5	6	21.18181818	
3/16/2022	12	1	144	138	3	2	56.4	145650.52
3/16/2022	24	4	103	125	5	9	16.28571429	
3/16/2022	24	3	29	27	16	32	1.166666667	
3/16/2022	24	2	22	19	16	16	1.28125	
3/16/2022	24	1	104	112	13	10	9.391304348	38671.786

3/18/2022	0	4	131	145	3	3	46	
3/18/2022	0	3	114	100	3	2	42.8	
3/18/2022	0	2	129	111	3	3	40	
3/18/2022	0	1	100	108	2	2	52	248600
3/18/2022	12	4	143	115	2	1	86	
3/18/2022	12	3	145	171	2	2	79	
3/18/2022	12	2	159	106	2	2	66.25	
3/18/2022	12	1	121	117	1	1	119	481593.75
3/18/2022	24	4	114	105	2	2	54.75	
3/18/2022	24	3	151	113	2	2	66	
3/18/2022	24	2	105	128	2	2	58.25	
3/18/2022	24	1	136	152	2	2	72	345125

Table 1: The collection of samples' raw data.

Day (treatment)	growth rate (day-1)
3 (0H)	0.323979259
3 (12H)	0.767437892
3 (24H)	0.910388754
5 (0H)	0.318496846
5 (12H)	0.695040521
5 (24H)	0.076215377
8 (0H)	0.14729879
8 (12H)	-0.015097067
8 (24H)	0.155515231
12 (0H)	0.011714711

12 (12H)	0.090953339
12 (24H)	0.046450024

Table 2: Data for the growth rate along with the days and the treatment.

Exposure to sunlight (hours)	Mean growth rate ($day^{(-1)}$)	SD	95 CI
0 hour	0.200372401	0.130039006	0.127435884
12 hours	0.384583671	0.349615601	0.342616994
24 hours	0.297142347	0.356295089	0.349162771

Table 3: The mean growth rate for the treatments along with their 95% CI

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.067926	2	0.033963	0.287185	0.756999	4.256495
Within Groups	1.06435	9	0.118261			
Total	1.132275	11				

Table 4: One-way ANOVA analysis of the mean growth rates of the treatments