The Effect of Acidification on the Ability of *Euglena gracilis* to Perform Positive Phototaxis

Ran Bi, Gabrielle Chan, Jasmine Hyun, Josie Duncan

Abstract

The effect of acidification on the ability of *Euglena gracilis* to perform positive phototaxis, movement towards a light source, was observed. The importance of this study was to simulate and explore the effects ocean acidification due to climate change on the photosynthetic ability of this marine algae. The trials included pH 5, 6, and 7, with 7 being optimal and any pH outside this range being lethal to the organism. A stock solution of cultured *E. gracilis* was separated into three beakers and hydrochloric acid was used to decrease the pH for each respective treatment. In three separate petri dishes, there was a positive control, negative control, and test treatment. The positive control was completely exposed to light, the negative control was completely covered by dark, opaque plastic, and the test treatment was covered with a small cut-out window for light to enter. From a two-way ANOVA, the p-value for light was found to be 0.220, 0.119 for pH, and 0.162 for the interaction between light and pH. Since the p-values for all three are greater than the level of significance (0.05), we fail to reject the three null hypotheses, concluding that light has no effect on positive phototaxis, pH has no effect on positive phototaxis, and there is no interaction between light and pH. These findings do not support our prediction that a decrease in pH will reduce the ability of *E. gracilis* to perform positive phototaxis.

Introduction

Euglena gracilis are unicellular photosynthetic freshwater flagellates that form the basis of trophic webs and thus, play a key role in supporting the upper trophic aquatic populations such as salmon (Richer et al., 2014). The ability of *E. gracilis* to perform photosynthesis through phototaxis, which is the bodily ability to move toward or away in response to light, allow these primary producers to actively search for and stay in an area of optimal light conditions for growth and survival (Häider & Häider, 1988). Through phototaxis, *E. gracilis* are able to swim toward a light source if the intensity is less than a critical value (positive phototaxis) and swim away if the intensity is above the critical value (negative phototaxis) to perform photosynthesis and become a key player in oxygen production in marine ecosystems (Ogawa et al., 2016).

During the last decade, the increase of carbon dioxide inputs from industrialization has resulted in ocean acidification, becoming a serious problem for marine ecosystems where the effects of a decrease in pH can be detrimental to all trophic levels, including important primary producers like *E. gracilis* (Danilov & Ekelund, 2001). Due to the increase in abundance and solubility of carbon dioxide in the world's waters, it has become one of the leading problems in freshwater ecosystems, where the absence of a strong buffering system compared to oceans make the freshwater ecosystem more vulnerable to acidification. With the highest growth rate of *E. gracilis* detected at pH 7, and death of cells at pH less than 4, ocean acidification and pH play a crucial role in the survival and growth of photosynthetic aquatic algal communities like *E. gracilis* (Danilov & Ekelund, 2001).

The purpose of this study is to examine the effects of increased acidity on the ability of *E. gracilis* to perform positive phototaxis. This study will help us determine the magnitude of change and sensitivity in *E. gracilis* motility by examining the abundance in light and dark regions when we decrease the pH. The following are the hypotheses for the experiment:

H_o (1): Light has no effect on positive phototaxis

H_o (2): pH has no effect on positive phototaxis

H_o (3): There is no interaction between light and pH

H_A (1): Light has an effect on positive phototaxis

H_A (2): pH has an effect on positive phototaxis

H_A (3): There is an interaction between light and pH

We predict that decreasing the pH will negatively affect the ability of *E. gracilis* to perform positive phototaxis, which will be shown by having lower abundance in light regions when we increase the acidity of the *E. gracilis* solution.

Materials and Methodology

Materials

Stock solution of *E. gracilis* and 1M hydrochloric acid (HCl) was obtained from Celeste Leander's laboratory at the University of British Columbia.

Preparation of the 3 pH treatments

The sterile stock solution was first swirled to ensure that relatively equal amounts of *E. gracilis* will be used for the different pH treatments. From Figure 1, the culture was then separated into three sterile 250 mL beakers, such that each contained 200 mL. For each pH treatment, the solution was mixed using a magnetic stir bar as 1M HCl was added until the pH decreased from 8 to 7, 6, and 5. A pH probe connected to a Texas Instrument TI-84 calculator was used to monitor the pH of the solution. The beakers were covered immediately after pH adjustment to ensure sterility.

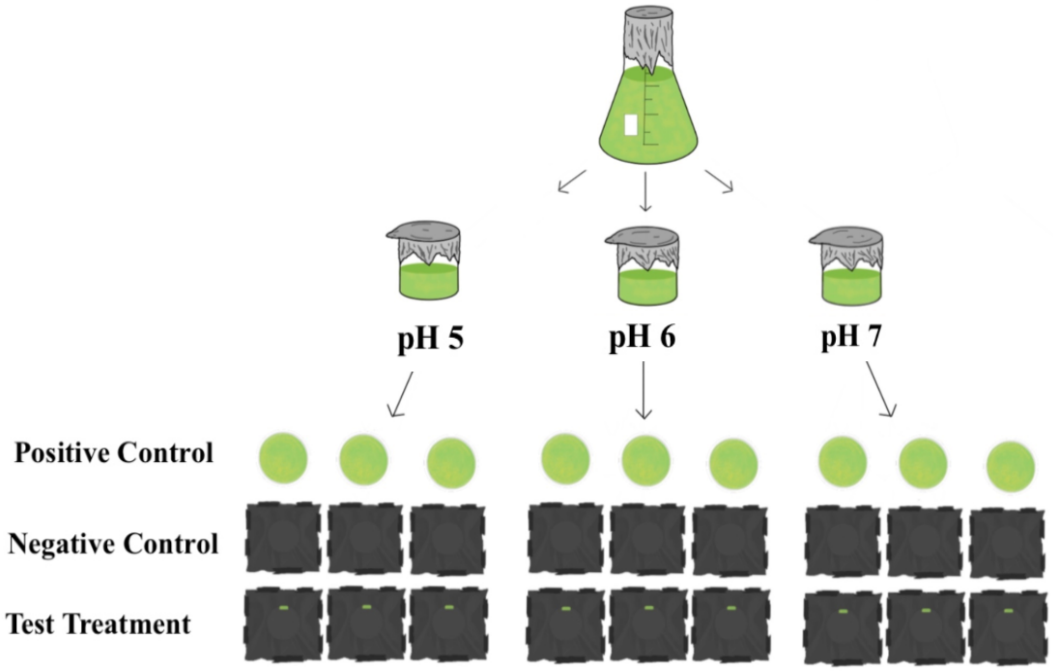

Figure 1. 27 trials were conducted (3 positive, 3 negative, and 3 test treatments for each pH condition).

Experimental Protocol

The experimental protocol was inspired from a procedure proposed by Michael Espey (2012). In total, 27 trials were performed (3 positive controls, 3 negative controls, and 3 test treatments for each pH level), which are discussed below. In each trial, a 100 mm petri dish was filled with 20 mL of the appropriate pH treatment, which was just enough to cover the bottom. The petri dishes within each pH treatment were randomly assigned to be a positive control, negative control, or test, in order to reduce bias. After every trial, a 100 µL sample was taken from the top edge of the plate (or near the light source in the test treatment) and added to a microcentrifuge tube filled with 2 µL of 3% glutaraldehyde fixative. Immediately after, a 100 µL sample was taken from the opposite bottom edge (or in the dark for the test treatment) and added to another microcentrifuge tube filled with 2 µL of fixative.

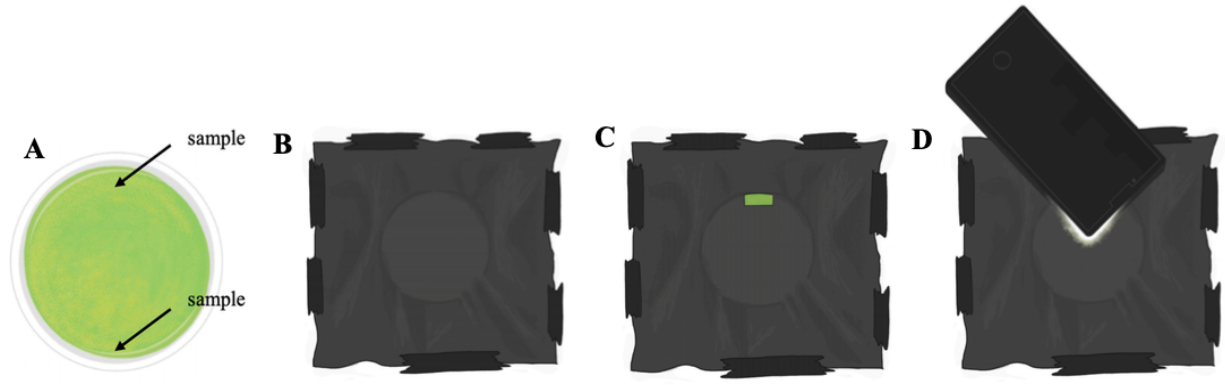

Figure 2. Visual reference for the experimental set up of the positive control (A), negative control (B), and test treatment (C and D). A. For the positive control, the petri dish was left uncovered and two samples were taken—one on the top edge and another on the opposite bottom edge. For consistency, the samples were taken in the same place for all trials. **B**. In the negative control, a black opaque plastic waste bag was used to cover the entire petri dish, so light could not penetrate. **C**. For the test treatment, a 2 cm by 1 cm window was cut out of the waste bag to allow light localization at the top edge of the petri dish. **D**. For the light source, the flashlight of an iPhone X was placed directly above the window.

For the positive control, Figure 2A, the petri dishes were left uncovered and undisturbed on the bench for 1 minute. The petri dishes for the negative control, Figure 2B, were covered with a thick black waste bag, and black electrical tape was used to tape the sides down. In the test treatment, Figure 2C, a 2 cm by 1 cm window was made in the waste bag to allow light penetration in a localized manner. Shown in Figure 2D, the flashlight of an iPhone X set to maximum (~2650 lux) was placed above the window and the petri dish was left undisturbed for 1 minute.

In total, 54 samples were taken from the 27 trials—two samples for each of the positive control, negative control, and test treatment. Due to time constraints, the samples were mixed using a micropipette then stored in a fridge to be counted a week later.

Counting the number of E. gracilis

A Zeiss compound light microscope and a Fuchs Rosenthal Haemocytometer, which has 16 1 mm x 1 mm squares, was used to count the *E. gracilis*. To evenly distribute the *E. gracilis*, the solution was mixed with a micropipette before loading onto the haemocytometer. The number of squares containing 100-300 *E. gracilis* was used to determine the average number of *E. gracilis*, which was subsequently multiplied by the dilution factor and correction factor to obtain the density (number of *E. gracilis*/mL). It is important to mention that for two of the pH 7 samples (pH 7 positive control at bottom edge, and pH 7 negative control at light source), an extra 2 μL of fixative was added, as some of the *E. gracilis* were observed to be moving.

Statistics

A two-way ANOVA was performed to see if the positive phototactic responses from the different pH treatments and light conditions were statistically different. In all cases, the criterion for significance level was set to $\alpha = 0.05$. Although data was collected from the dark regions, they were not included in the statistical analysis. If the data from the dark regions were taken into account, a three-way ANOVA would be required for the analysis of an interaction between light and pH on phototaxis across the different regions (upper and lower). Regrettably, we neither had the statistical tool nor knowledge to perform such a test. As the data collected from the lower region were only subjected to pH differences due to being in the dark, it was decided to only use the data collected from the upper region. Since both pH and light were both present in the upper region, the chosen data was sufficient to answer our experimental question—whether pH has an effect on the positive phototactic ability of *E. gracilis* in the presence of light.

Results

Two-way ANOVA

Since we would like to investigate the mean difference between groups that are split on two explanatory variables, a two-way ANOVA was the appropriate statistical test to use, with pH and light being the explanatory variable, and phototactic ability being the response variable. Data was collected from both the upper and lower part of each of the 27 petri-dishes, where the upper regions were subjected to different light conditions, and the lower regions were always dark and covered in plastic.

Table 1. Two-way ANOVA result summary table.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Light Condition	2.05E+10	2	1.03E+10	1.648	0.220	3.555
pH	2.99E+10	2	1.5E+10	2.402	0.119	3.555
Interaction	4.62E+10	4	1.16E+10	1.856	0.162	2.928
Within	1.12E+11	18	6.23E+09			
Total	2.09E+11	26				

Based on information in Table 1, we conclude that the test statistic for light condition has a calculated F-value of 1.648. Using alpha value of 0.05, we find a critical $F_{0.05;2}$ value of 3.555. The calculated F (1.648) is smaller than critical F (3,555), so we fail to reject null hypothesis 1, which states that light has no effect on positive phototaxis. Since the p-value for the calculated F (1.648) is 0.220, we conclude that effect of light is insignificant at that level.

The calculated F-values for both pH (2.402), and interaction between pH and light (1.856), do not exceed their F-critical value (3.555 and 2.928 respectively). Thus, we fail to

reject null hypothesis 2 and 3. Moreover, the effect of pH and the interaction between pH and light condition have p-values of 0.119 and 0.162, which are both greater than 0.05. As a result, their effects on positive phototaxis are insignificant.

Table 2. Summary of amount of variance for each of the 9 combinations of pH and light condition, and the total variance for each of the three pH and light conditions

Summary	pH5	pH6	pH7	Total (light condition)
No light				
Variance	8.20e+7	2.21e+9	8.37e+9	6.4e+9
Light				
Variance	8.48e+7	7.68e+8	1.51e+8	4.95e+8
Window				
Variance	7.53e+7	1.46e+8	4.42e+10	1.66e+10
Total (pH)	1.71e+8	5.34e+9	1.68e+10	

From Table 2, the largest variance in the data exists in the pH 7 and window experimental setting ($4.42e^{10}$). The large variance value tells us that the data we collected for this experimental setting was very spread out from their average. The variances for all three light conditions under pH 5 were all relatively small when compared to other variance values, as they have a magnitude of 10^7, while other variance values are at least in the magnitude of 10^8. The small variance values suggest that the data for all three light conditions at pH 5 are relatively closer to their averages when compared with other groups.

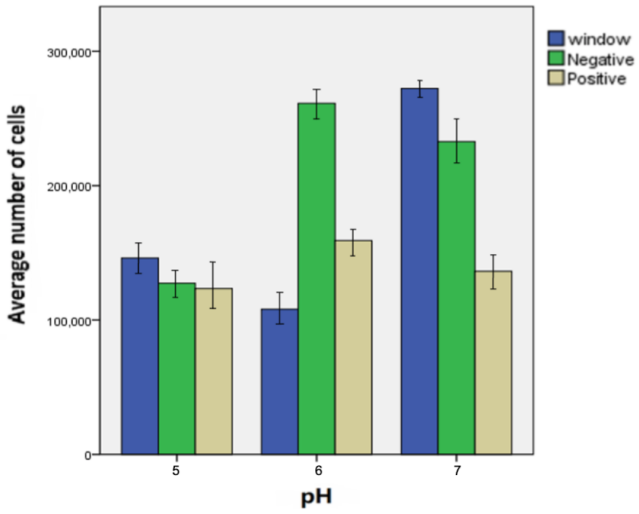

Figure 3. Mean cell density of *E. gracilis* under different experimental combinations of pH and light conditions.

Based on Figure 3, we see that the cell density under the window, and negative and positive light conditions, do not appear to follow a consistent pattern. Cell density was highest in the window light condition for pH 5 and 7, while it was lowest in window light condition under pH 6. Potential factors and errors leading to these observations are discussed in the following section.

Discussion

From the statistical analyses using a two-way ANOVA, we fail to reject the null hypotheses [H_o (1), H_o (2), and H_o (3)]. We cannot support that decreasing the pH of the medium containing cultured *E. gracilis* affects the organism's ability to perform positive phototaxis. There is no significant movement toward a light source under the conditions of this experiment. The results confirm this by showing no significant difference in abundance of cells in the

window, light, and dark regions of the petri dishes across pH 5, 6, and 7. With a time period of one minute exposure to light in our test treatment, either the organism did not respond to the presence of light, or they were not given enough time to adjust and provide a reaction of phototaxis. Further experimentation, particularly with different time periods and more trials, is required to determine why there is an insignificant instance of positive phototaxis.

Although there was no significant difference in abundance of cells within a pH, there was a difference in abundance across the pH trials. We found an overall smaller abundance of cells in the pH 5 trial compared to pH 6 and 7, possibly due to an exceedingly large discrepancy between the treatment pH and optimal pH of the organism. Danilov (2001) found that the highest growth rate of *E. gracilis* occurred at a pH of 7 and did not survive below pH 4 or above pH 8. Additionally, Danilov (2001) stated that the most efficient photosynthetic activity of *E. gracilis* occurred at pH 6, however, pH had a stronger effect on growth rate than photosynthesis. Both processes are equally important when considering the sustainability of the ecosystem they share with higher trophic level populations, such as salmon. If growth rate is more sensitive to a varying pH, depleting numbers of *E. gracilis* will have a significant consequence for the following trophic level, creating a positively correlating pattern for the ecosystem such that all levels will decrease in tandem. Furthermore, Alexander (1931) found that the photosynthetic activity of *E. gracilis* is pH dependent and suggests that it plays an important role in the carbon cycle in nature.

Our methodology was justified by having both positive and negative controls to compare with cell counts in light and dark conditions. With a small window in the treatment, we were able to localize light in a specific area of the petri dish to determine if there was a difference in

motility of cells directly exposed to light and those in darkness. Although failing to reject the null hypothesis was largely dependent on the methodology of the study, it is possible that under real conditions, *E. gracilis* and other photosynthetic organisms are able to adapt to the changing pH of their environment. If this hypothesis was proven true, it would be extremely beneficial, considering the current state of increasing ocean acidification, to acquire resiliency to a changing climate and shifts in biota of the Earth (Harley et al., 2006). It is also important to consider other byproducts of climate change that may affect organismal functions, such as phototaxis. In our experiment, we only tested the effects of pH, which decreases when excess carbon dioxide from greenhouse gases dissolves in water (Harley et al., 2006). It is possible that changing the temperature of the environment of *E. gracilis*, another effect of climate change, will impact phototaxis. Since temperature remained constant across our trials, we are unable to determine if it was a contributing factor.

Past studies have shown that *E. gracilis* does have a response to light, such that cell division still occurs in darkness, photosynthetic activity increases linearly over 8 to 10 hours of a light period, and the cells maintain circadian rhythms of phototaxis, even in continuous light trials (Walther & Edmunds, 1973). These findings contradict our results since we did not observe positive phototaxis, regardless of the pH of the treatment. Walther and Edmunds (1973) used a brighter light source of 12,000 lux and had longer time periods for their treatments, which may have contributed to the differences in our results.

If our methodology was to be repeated, sources of error we found should be closely considered. The largest source of error would be the insufficient volume of fixative added to each microcentrifuge tube after a sample was extracted from the petri dish. The majority of our

samples contained completely dead cells while a significant number contained a few or many motile cells. Since this error was only discovered a week later when cell counting commenced, it is possible that some cells replicated during that time period and therefore, increased the number of cells in that sample. Another source of error could be insufficient mixing of the medium before transferring volume since the denser algae cells would sink to the bottom of the container. Lux was not considered as a variable, but it is possible that the flashlight used on the iPhone X would not produce comparable effects to real sunlight. Tally counters were used to minimize human counting error.

Time constraints played a large role in the parameters of our measurements. In future experiments, longer time periods of the test treatment could be manipulated, although we relied on the assumption that the *E. gracilis* cells are able to move relatively quickly. The potential effects of climate change on phototaxis may also be studied by changing the temperature to a more representative state of real environmental conditions.

Conclusion

We fail to reject all three null hypotheses, as there was no significant movement toward the light source in all three pH conditions. Thus, contrary to our predictions, decreasing the pH of the medium containing *E. gracilis* culture does not decrease the ability of the organism to perform positive phototaxis. The results of this study do not support the significance of the impact of climate change on the motile function of *E. gracilis*.

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Appendix

Work Distribution

Ran Bi - statistical analysis, results

Gabrielle Chan - abstract, data analysis, materials and methodology, acknowledgements, literature cited

Josie Duncan - abstract, discussion, conclusion, literature cited

Jasmine Hyun - introduction, literature cited

Raw Data and extra tables

Table 1. Raw data showing the average number of *E. gracilis*.

		Light		
pH 5		Trial 1	Trial 2	Trial 3
	Window	29	27	30
	Negative	23	27	25
	Positive	23	24	26
pH 6				
	Window	24	21	19
	Negative	61	50	43
	Positive	29	27	37
pH 7				
	Window	30	29	101
	Negative	34	37	65
	Positive	29	27	24
		Dark		
pH 5		Trial 1	Trial 2	Trial 3
	Window	17	25	26
	Negative	29	36	36
	Positive	20	26	39
pH 6				
	Window	27	31	41
	Negative	41	39	29
	Positive	41	19	26
pH 7				
	Window	27	35	39
	Negative	20	60	41
	Positive	30	29	32

Table 2. Cell density for each of the three trials under 9 different experimental conditions, with the average of the three trials at the rightmost column.

dilution factor 1	5100		dilution factor 2	5200				
					Light			
pH 5		Trial 1	Density(cells/mL)	Trial 2	Density(cells/mL)	Trial 3	Density(cells/mL)	Average
	Window	29	149175	27	136425	30	153000	146200
	Negative	23	118320	27	136425	25	127500	127415
	Positive	23	116280	24	120360	26	133875	123505
pH 6								
	Window	24	121380	21	105060	19	97750	108063
	Negative	61	311100	50	255000	43	217600	261233
	Positive	29	149175	27	137700	37	190400	159092
pH 7								
	Window	30	153000	29	149175	101	515100	272425
	Negative	34	171700	37	188700	65	338000	232800
	Positive	29	147900	27	137700	24	123420	136340
					Dark			
pH 5		Trial 1	Density(cells/mL)	Trial 2	Density(cells/mL)	Trial 3	Density(cells/mL)	Average
	Window	17	85000	25	124950	26	132600	114183
	Negative	29	147900	36	181900	36	183600	171133
	Positive	20	102000	26	130050	39	200600	144217
pH 6								
	Window	27	137700	31	158100	41	210800	168867
	Negative	41	209100	39	200600	29	146625	185442
	Positive	41	209100	19	96900	26	132600	146200
pH 7								
	Window	27	136425	35	176800	39	197200	170142
	Negative	20	99450	60	303450	41	207400	203433
	Positive	30	150450	29	148200	32	163200	153950

RESULTS

Window	Light	Dark		**Negative**	Light	Dark		**Positive Control**	Light	Dark
pH 5	146200	114183		pH 5	127415	171133		pH 5	123505	144217
pH 6	108063	168867		pH 6	261233	185442		pH 6	159092	146200
pH 7	272425	170142		pH 7	232800	203433		pH 7	136340	153950

Table 3. Mean, standard error and 95% confidence interval.

1.pH

Dependent Variable: number

pH	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
pH=5	132373.333	35839.665	32866.470	231880.197
pH=6	176129.333	35839.665	76622.470	275636.197
pH=7	213855.000	35839.665	114348.136	313361.864

2.Light Condition

Dependent Variable: number

Light Condition	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
window	175562.667	35839.665	76055.803	275069.530
Negative	207149.333	35839.665	107642.470	306656.197
Positive	139645.667	35839.665	40138.803	239152.530