

Effects of ocean acidification on the photosynthetic ability of *Chaetoceros gracilis* in the Monterey Bay

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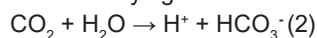
SUMMARY

Ocean pH levels have been dropping since the Industrial Revolution due to increased carbon dioxide gas (CO₂) in the atmosphere. When CO₂ is absorbed by the ocean, hydrogen ions are released, resulting in a decrease in pH. Dangerous levels of carbonic acid in the ocean ecosystem as a result of CO₂ absorption may have an effect on the health of marine algae, such as diatoms. Marine diatoms account for an estimated 20% of oxygen production in the atmosphere and are essential to the aquatic food chain. I hypothesized that increased ocean acidity would decrease the photosynthetic ability of *Chaetoceros gracilis*, a diatom prolific in Monterey Bay, because of the usually corrosive effects of carbonic acid on both seashells and cells' internal structures. I altered pH of algae environments and measured the photosynthetic ability of diatoms over four days by spectrophotometer. Surprisingly, a decrease in pH improved the photosynthetic ability of *C. gracilis*, but only within a specific pH range. The diatoms grown in pH 7.5 medium had the highest average absorbance value; therefore, this pH value is a "sweet spot" that optimizes the growth of this species of diatom. These findings indicate that *C. gracilis* may become more abundant in Monterey Bay as the pH of the ocean continues to drop, potentially contributing to harmful algal blooms.

INTRODUCTION

Climate change is a multifaceted issue that is the result of the burning of fossil fuels (1). Greenhouse gases are released when fossil fuels are used as an energy source; these gases, specifically carbon dioxide (CO₂), absorb heat from solar radiation and reflect it back to Earth's surface. The Industrial Revolution led to the creation of many large-scale factories that emit excess carbon dioxide gas, along with other fossil fuels, to provide energy to their businesses. The potentially detrimental effects carbon dioxide may have on the pH levels of the world's oceans has become a highly contested topic in recent years in conjunction with raising concerns about climate change in general (1). An estimated 30% of CO₂ in the atmosphere is absorbed by the ocean and reacts with water (H₂O) to form carbonic acid (H₂CO₃) (1, 2), which then breaks down into hydrogen and bicarbonate ions. The chemical

formula underlying this ocean acidification phenomenon is:



One way to quantify this phenomenon is by measuring a solution's pH, a value that represents its acidity or basicity. Therefore, as more carbon dioxide gas is absorbed into the ocean, levels of hydrogen ions will increase, leading to decreased oceanic pH levels. The current pH level of the ocean surface has been measured at pH 8.1, which is slightly basic (1). According to the National Oceanic and Atmospheric Administration, "the pH of surface ocean waters has fallen by 0.1 pH units [since the Industrial Revolution]" (3). This may not seem significant but, because the pH scale is logarithmic, a 0.1 pH unit drop is equivalent to a 30% increase in acidity. My experiment explored the behavior of a specific species of diatom when grown in medium of the ocean's original surface pH (pH 8.2), in a more acidic environment with pH 7.5, and at a much more acidic medium at pH 7.0. These pH levels were chosen to represent the steady decline of ocean pH levels following the Industrial Revolution, as our control condition was set to the pH level of the sea before that point in history.

Diatoms are unicellular microalgae that are abundant in freshwater and seawater environments. Increased acidity may contribute to the breakdown of diatoms' internal cellular structures (4). However, other studies have found opposite results with different species of marine diatoms (5). Lower pH levels could be beneficial to the growth of marine diatoms, indicating that these microorganisms could be acidophilic, or able to grow in environments of high acidity (5). Rapid increases in algae populations may lead to algal blooms, which have the capability to decimate entire aquatic ecosystems. When toxin-producing algae multiply rapidly and then die, they starve the water column of oxygen; their remains are decomposed by bacteria, which depletes the dissolved oxygen present in marine environments (6). Fish and other organisms in the water die from anoxia and lack of sunlight. Poisoned seafood can send people to the hospital or even be fatal (7). The effects that increased acidity may have on these microorganisms in the Monterey Bay National Marine Sanctuary (MBNMS) could be catastrophic. The MBNMS, stretching from San Francisco to Cambria on the California coast, is the largest federally protected underwater sanctuary in the United States. It is home to hundreds of diverse and endangered species that are indigenous to this portion of the Pacific Ocean (8). A decrease in the health of the marine diatoms present in the MBNMS could reflect the poor health of diatoms globally which, given that diatoms are responsible

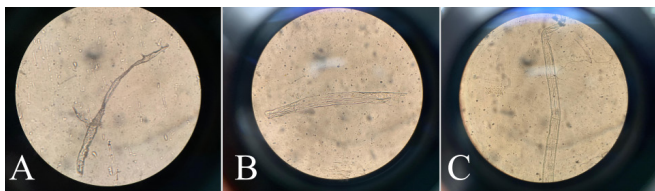


Figure 1. *Chaetoceros gracilis* from sample group cuvettes after 4 days in the pH buffer/seawater mixture. A) *C. gracilis* diatoms in medium of pH 8.2. B) *C. gracilis* diatoms in medium of pH 7.5. C) *C. gracilis* diatoms in medium of pH 7.0. Images taken at 400X.

for generating 20% of the oxygen in Earth’s atmosphere, would catastrophically affect oxygen levels around the world (9).

Limited research has been done specifically on the effects of decreased pH levels on the photosynthetic ability of marine diatoms. The ramifications of ocean acidification on these microorganisms could also vary from species to species because of the differences in individual characteristics across the thousands of species of diatoms in the oceans. The diatom species *Chaetoceros gracilis* was the focus of this experiment because its genus, *Chaetoceros*, is prolific in the Monterey Bay, as observed by an ongoing University of California, Santa Cruz study funded by the California Harmful Algal Bloom Monitoring and Alert Program, or CalHABMAP (10). The sheer diversity of diatom species makes it incredibly difficult for scientists to observe each one’s behavior and characteristics; *C. gracilis*, for example, is only distinguishable by its long, narrow shape and spindles (11). One cannot make an educated assumption about *C. gracilis*’s reaction to a change in the pH of its medium because of the lack of research done on this species.

I hypothesized that a decrease in the pH of media containing *C. gracilis* would cause a decrease in the photosynthetic ability of the diatom. Diatoms exposed to a higher pH (less acidic) will be able to photosynthesize more efficiently than diatoms exposed to a lower pH (more acidic). Using a spectrophotometer, I measured the amount of light the diatoms grown at different pH values absorbed in relation to their photosynthetic ability. The more light the MBNMS samples retained, the more efficiently the assayed diatoms were able to photosynthesize. This data can be used to plot the photosynthetic ability of the samples over time. It was found that, over the experimental timeframe, *C. gracilis* diatoms performed photosynthesis most efficiently at a range from pH 8.2 to pH 7.5. As pH falls past pH 7.5, the photosynthetic ability of the diatoms decreases noticeably.

RESULTS

We used a spectrophotometer to measure the light absorbance of the MBNMS diatom samples, which were kept in three different pH environments and assayed twice daily for four days (Table 1, Figure 1). The spectrophotometer shot light through the specimens and measured absorbance values to quantify the photosynthetic ability of the samples

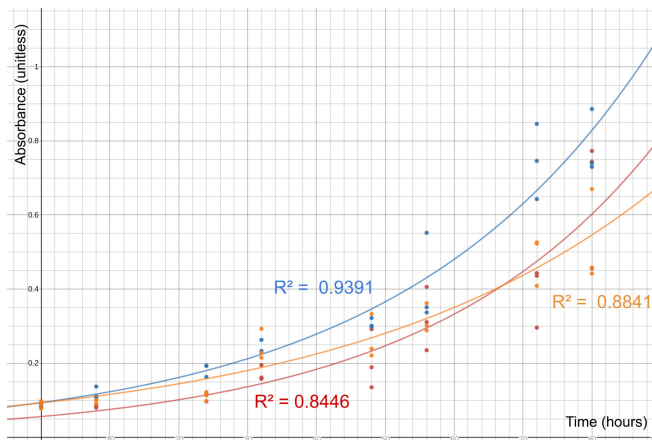


Figure 2. Absorbance Values Over Time. The experiment was performed over the course of 80 hours (four days), and the growth rate of each sample group was quantified by measuring the absorbance rate of each cuvette twice a day. For all sample groups, the absorbance values increased as time increased. Samples grown in medium of pH 8.2 are represented by the red exponential function with the regression equation $y = 0.0560 \cdot 10.3^x$. Samples grown in medium of pH 7.5 are represented by the blue exponential function with the regression equation $y = 0.0937 \cdot 1.03^x$. Samples grown in medium of pH 7.0 are represented by the orange exponential function with the regression equation $y = 0.0928 \cdot 1.02^x$. The coefficient of determination values (R^2) for diatoms in medium of pH 8.2, pH 7.5, and pH 7.0 are 0.8446, 0.9391, and 0.8841, respectively.

(Table 2). The graph of the absorbance values with respect to time clearly displays an upward trend for each sample group (Figure 2). The photosynthetic ability of the diatoms in all samples increases over time, indicating exponential growth of the diatoms’ photosynthetic ability. The regression equations for each pH value are in exponential form: $y = a \cdot b^x$, where “a” is the initial absorbance value, or y-intercept, and “b” is the change factor, or, in this case, the growth rate. This is representative of the growth dynamics of micro-algae. According to Baert’s FAO Fisheries Aquaculture Manual, cultures typically go through different stages of growth: “the lag or induction phase, the exponential phase, phase of declining growth rate, stationary phase, and death or ‘crash’ phase” (12). When our samples were delivered before experimentation, they had already been growing long enough to complete the lag phase. During the timeframe of my experiment, the algae were in the exponential phase, and therefore their growth can be tracked by an exponential regression equation. The diatoms were not cultured long enough to reach the other

pH Level	Experimental Groups			Blanks (B)		
	pH 8.2	pH 7.5	pH 7.0	pH 8.2	pH 7.5	pH 7.0
Distilled Water (mL)	2	2	2	4	4	4
Seawater Medium (mL)	2	2	2	4	4	4
Algae (mL)	4	4	4	0	0	0
Total Volume (mL)	8	8	8	8	8	8
pH Buffer Powder (g)	0.001746	0.001746	0.001746	0.001746	0.001746	0.001746

Table 1. Components in Sample Groups and Blanks. Amounts of each component—distilled water, seawater, pH buffer powder, and *Chaetoceros gracilis* algae—added to the sample group and reference blank test tubes.

	Time (hours)	Cuvette Sample Number	Absorbance of Experimental Groups		
			pH 8.2	pH 7.5	pH 7.0
Day 1; Time 1	0	1	0.097	0.089	0.098
		2	0.081	0.086	0.078
		3	0.083	0.089	0.089
Day 1; Time 2	8	1	0.084	0.108	0.100
		2	0.089	0.110	0.101
		3	0.080	0.137	0.090
Day 2; Time 1	24	1	0.114	0.193	0.098
		2	0.097	0.163	0.122
		3	0.119	0.193	0.113
Day 2; Time 2	32	1	0.158	0.233	0.293
		2	0.161	0.263	0.215
		3	0.195	0.230	0.228
Day 3; Time 1	48	1	0.292	0.299	0.221
		2	0.189	0.301	0.333
		3	0.135	0.322	0.239
Day 3; Time 2	56	1	0.406	0.552	0.362
		2	0.311	0.337	0.302
		3	0.235	0.351	0.289
Day 4; Time 1	72	1	0.436	0.846	0.523
		2	0.443	0.643	0.526
		3	0.296	0.746	0.409
Day 4; Time 2	80	1	0.773	0.886	0.670
		2	0.744	0.730	0.458
		3	0.456	0.740	0.442
Average for all Days			0.253	0.360	0.267

Table 2. Absorbance Values for each Sample Group. Twice a day for four days, the absorbance values for each sample group were measured by spectrophotometer. Reference blanks were used to calibrate the spectrophotometer. Three cuvettes for each sample group were compared with their respective blanks. The table shows the exact absorbance values measured for each sample group on each day, as well as the total average absorbance value for each sample group across all days.

three phases characteristic of algae growth.

At the end of the four-day experiment, absorbance values taken from the two daily time points at each pH value were averaged and used to compare photosynthetic ability across samples (Figure 3). This value represented how the algae grew and increased their surface area to absorb light energy. This simplification was used as a supplement to the exponential functions for data analysis. Diatoms grown at a pH of 7.5 had a 35.13% greater average absorbance value than those at pH 7.0 and a 42.36% greater average than

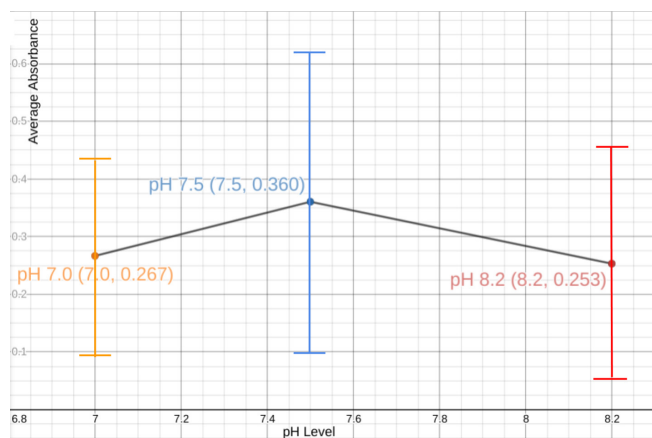


Figure 3. Average Absorbance Values. For each sample group, the absorbance values across all days were averaged to determine the average absorbance value for each of the three different pH levels. The error bars for each pH value represent the standard deviation of the data set.

those at pH 8.2. Samples grown at pH 7.0 had a 5.35% higher absorbance average than those grown at pH 8.2 (Figure 3). However, the average absorbance value for each pH level is not definitive due to the fact that it does not account for the growth rate of the diatoms over time. To further the accuracy of the data, the exponential regression graphs and equations were also taken into account.

The regression equations for each pH level were derived by plotting the absorbance values for each individual cuvette sample at the various pH levels on an absorbance-time graph. The equations represented the relationship between the absorbance values and time; the graph showed an exponential growth trend. A regression equation was fitted to the plotted points using Desmos graphing software for each pH value. The regression equations were very accurate according to their values, or coefficients of determination (Figure 2). A value greater than 0.8 indicates a strong correlation between the independent variable (time) and dependent variable (absorbance values). The coefficients of determination for samples in mediums of pH 8.2, pH 7.5, and pH 7.0 were 0.8446, 0.9391, and 0.8841, respectively. Therefore, the regression equations of the different pH levels fit the data well.

While the average absorbance value for diatoms in medium of pH 7.0 was greater than the overall average for algae grown in medium of pH 8.2, the growth rate (*b*) for those in pH 8.2 was greater than that of those in pH 7.0. This indicates that diatoms in pH 8.2 performed photosynthesis at a faster rate than diatoms at pH 7.0. This is represented by the crossing of the diatom growth rates in pH 8.2 and pH 7.0 cross at approximately hour 67 (Figure 2). At that point, the absorbance values of the samples in pH 8.2 become greater than those of the pH 7.0 samples (Table 2).

The algae samples grown in medium of pH 7.5 were determined to be the best-performing group due to their

high growth rate and high average absorbance; they had a relatively modest initial value (*a*) and a high growth rate (*b*) (Figure 2). Diatoms in pH 8.2 had the same growth rate as those in pH 7.5, indicating that both sample groups performed photosynthesis at roughly the same rate. Nonetheless, samples grown in pH 7.5 had the highest average absorbance of all sample groups.

DISCUSSION

The data observed indicate that a decrease in the pH of media containing *Chaetoceros gracilis* will cause a decrease in the photosynthetic ability of the diatoms. According to my experiment, a decrease in the pH of medium (equivalent to an increase in acidity) can result in *C. gracilis* performing photosynthesis more efficiently. However, there is a specific range within which this species of diatom photosynthesizes most effectively. Diatoms in a medium of pH 7.5 photosynthesized more effectively than those at pH 7.0 (Figure 2). While samples at pH 8.2 and pH 7.5 had the same growth rate, those at pH 7.5 had a greater overall average absorbance (Figure 3).

C. gracilis appeared to perform photosynthesis well at a range from pH 8.2 to pH 7.5. As the pH drops below 7.5, the photosynthetic ability of the diatoms greatly decreases. Current surface ocean water is measured to be at pH 8.1, indicating that *C. gracilis* may not be currently performing photosynthesis at its peak potential (1). As the hydrogen ion concentration in the world's oceans continues to rise, the photosynthetic efficiency of the marine diatom *C. gracilis* may increase, boosting the population of marine algae present in the Monterey Bay National Marine Sanctuary.

The results of this study reveal that ocean acidification is potentially causing rapid algae growth, or "algal blooms." Algal blooms have severe negative impacts on the marine ecosystem, and unanticipated algal blooms could wreak havoc on the Monterey Bay National Marine Sanctuary if measures are not taken to decrease the effects of ocean acidification.

The effects of ocean acidification on the MBNMS are being tracked by the Monterey Bay Aquarium Research Institute (MBARI). They found that oceanic pH, an indicator of acidity, has decreased over the last 20 years in Monterey Bay, which correlates with trends in global ocean pH (13). The results seen in this experiment are an indication that these diatoms may not only prosper in seawater of lower pH in the MBNMS, but also in oceans across the Earth, given the global similarities in acidity change.

Throughout the experiment, some unavoidable potential errors were encountered due to the scope of the study and the materials used. There is the possibility that the ingredients present in the pH buffer powder may have inadvertently acted as a nutrient agent for the diatoms. It is unlikely, however, that the pH buffer had a significant impact, as it was created specifically for marine environments. The algae colonies were ordered from a science supply company, Carolina

Biological Supply, so their initial health was dependent on the company's quality.

Each of the samples' initial absorbance value at hour 0 was slightly different; ideally, they would all be exactly the same. When the samples were received, they likely were not measured out by cell count, but rather by volume. This insignificant difference in cell quantity is likely what determined the initial absorbance value of the diatoms. The average absorbance values of diatoms in mediums of pH 8.2 and pH 7.5 may have been closer if this flaw did not exist in the experiment.

This project can be expanded upon by exploring the effects of pH changes on different species of diatoms in the Monterey Bay National Marine Sanctuary or in different oceanic regions altogether. Additionally, measuring the diatoms' response to increased acidity over a longer period of time could be performed to expand our understanding of the long-term effects of ocean acidification. Studying media containing diatoms with even lower pH levels could provide insight into what may occur if the current trend of increasing carbon dioxide emissions continues. Future studies should focus on long-term effects of pH level changes on different species of diatoms to better understand the phenomenon of ocean acidification and how it may be linked to algal blooms.

MATERIALS AND METHODS

Setup Process

Prior to experimentation, all test tubes were sterilized in an autoclave at 121°C for 15 minutes at 1.157 atm. In order to distinguish between the different pH levels of the samples, three test tube lids were labeled "pH 8.2," "pH 7.5," and "pH 7.0." The other three test tube lids were labeled "pH 8.2 B," "pH 7.5 B," and "pH 7.0 B" with the letter "B" representing the reference blanks. An incubation chamber used for culturing the diatoms was prepared by attaching a 12W LED white light strip to the top of a mylar-walled 46cm • 46cm • 56cm grow box using duct tape. The light was set on a timer for a 12 hours light/dark cycle. The chamber was protected from external light sources to eliminate any effect they could have had on the growth of the diatoms.

Alteration of pH Levels

Before adjusting the acidity of the distilled water for the experiment, an electronic pH meter was calibrated with liquids of known pH (pH 4.01, pH 6.86, and pH 9.18). Buffers are solutions designed to maintain a specific pH value by selectively releasing or absorbing hydrogen ions and are useful when a constant pH is desired in scientific experiments (14). The amount of marine pH buffer in grams needed for 1000 mL of distilled water was calculated for each sample group and reference blank (pH 8.2, pH 7.5, and pH 7.0) to generate solutions that (for the sample groups only), would result in the desired pH. The pH buffer's instructions called for two teaspoons of pH powder to be added to 10 gallons of distilled water; this was converted to milliliters of distilled

water and grams of pH powder (2 teaspoons of pH buffer were measured on a scale to be 8.265 grams).

$$(10 \text{ gallons of distilled water}) \div (2 \text{ teaspoons of pH buffer}) = (37,854.1 \text{ mL of distilled water}) \div (8.265 \text{ g of pH buffer})$$

$$(37,854.1 \text{ mL}) \div (8.265 \text{ g}) = (8.000 \text{ mL of total solution in each test tube}) \div x$$

$$x = 0.001746 \text{ g (of pH buffer for 8 mL of total solution)}$$

Using this information, the amount of pH buffer in grams needed for the sample groups (test tubes with pH 8.2, pH 7.5, and pH 7.0 labels) was calculated.

$$(2.000 \text{ mL of distilled water}) \div (0.001746 \text{ g of pH buffer}) = (1000 \text{ mL of distilled water}) \div (y)$$

$$y = 0.8734 \text{ g (of pH buffer for 1000 mL of distilled water)}$$

Next, the amount of pH buffer in grams needed for the reference blanks (test tubes with pH 8.2 B, pH 7.5 B, and pH 7.0 B labels) was quantified.

$$(4.000 \text{ mL of distilled water}) \div (0.001746 \text{ g of pH buffer}) = (1000 \text{ mL of distilled water}) \div (z)$$

$$z = 0.4367 \text{ g (of pH buffer for 1000 mL of distilled water)}$$

Using the calculations above, the appropriate amount of pH 8.2 buffer was added to a volumetric flask and labeled "pH 8.2." This step was repeated for all test tubes, and the appropriate amount of pH buffer was added to one of the volumetric flasks for each test tube (with respect to the pH level in **Table 1**). The flasks were labeled according to their pH value, just as this information was labeled on the test tubes. A magnetic stir bar was placed inside each volumetric flask, and the flasks were set on stir plates to mix for one hour.

Preparing the Samples and Blanks for Experimentation

Samples were prepared with two mL of saltwater medium and four mL of *Chaetoceros gracilis* marine diatoms in test tubes with pH 8.2, pH 7.5, and pH 7.0 labels. Next, four mL of saltwater medium was transferred into test tubes with pH 8.2 B, pH 7.5 B, and pH 7.0 B labels. For test tube pH 8.2 B, four mL of distilled water with pH buffer was added to the test tube. This process was repeated for all reference blanks (test tubes pH 7.5 B and pH 7.0 B) using the distilled water and pH buffer mixture from volumetric flasks labeled with their respective pH value (pH 7.5 B and pH 7.0 B); this altered the pH of the solution in each test tube. For the sample groups (pH 8.2, pH 7.5, and pH 7.0), two mL of distilled water with pH buffer added was transferred from their respective volumetric flasks to the sample group test tubes. An electronic pH meter was used to measure the pH value of each test tube to ensure that

the solutions were at the desired pH values as indicated in **Table 1**. Finally, rubber stoppers were used to seal each test tube, and the samples were mixed.

Preparing the Cuvettes for Measurement

Exactly 80 μ L of the pH 8.2 mixture was pipetted into three 100 μ L cuvettes, and cuvette lids were labeled "pH 8.2." This process was repeated for sample groups pH 7.5 and pH 7.0, and the lids of their cuvettes were labeled accordingly. For the reference blank pH 8.2 B, 80 μ L of the test tube pH 8.2 B mixture was transferred into one 100 μ L cuvette and the cuvette lid labeled "pH 8.2 B." The process was repeated for reference blanks pH 7.5 B and pH 7.0 B, with their cuvette lids being labeled accordingly.

Measuring Absorbance Values

A spectrophotometer is an instrument used to measure the amount of light absorbed or transmitted through a given sample. Absorbance is quantified by a spectrophotometer when a blank or reference solution is compared against a sample. The output value is a unitless measurement that is the logarithm of the intensity of the light passing through the reference cell divided by the intensity of the light passing through the sample cell, $A = \log_{10}(I_0/I)$ (15). When algae photosynthesize, they absorb light from the sun to begin a chemical reaction that results in the production of glucose ($C_6H_{12}O_6$) and oxygen (O_2), shown in the chemical formula for photosynthesis:



Since absorbance is a measure of how much light is taken in by a given sample, this metric was used to evaluate the photosynthetic ability of *C. gracilis*, similar to methods employed by other studies in the field (17). The solution in each cuvette was mixed by pipetting the solution in and out once. The parameters (the desired 450 nm wavelength and absorbance setting) were inputted into a Genesys 10S UV-Vis Spectrophotometer. The three cuvettes labeled "pH 8.2" for sample group pH 8.2 were inserted into slots 1, 2, and 3 of the 6-position cell holder. The reference blank pH 8.2 B cuvette, labeled "pH 8.2 B," was inserted into slot B (blank) of the 6-position cell holder. The absorbance test was run by the spectrophotometer for sample group pH 8.2 with reference blank pH 8.2 B and the absorbance value for each cuvette was recorded into a scientific notebook (**Table 2**). This process was repeated for sample group pH 7.5 with reference blank pH 7.5 B and then for sample group pH 7.0 with reference blank pH 7.0 B. After the tests were completed, the cuvettes were transferred back into the grow box with their caps slightly ajar to allow airflow. The *C. gracilis* diatoms were left to grow over the course of the next four days (**Figure 1**). These absorbance tests were repeated two times daily for the duration of the growth period at 7:00 AM (time 1) and 3:00 PM (time 2) PST.

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