The Effects of Ocean Acidification on *Emiliania huxleyi* and *Thalassiosira pseudonana*

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Abstract:

Ocean acidification is the process where carbon dioxide is absorbed into the ocean, creating different compounds that change the chemistry of the ocean. The changes could be harmful to microorganisms, like phytoplankton. One aspect that could be negatively affected by ocean acidification is the growth rates of phytoplankton. Many studies have looked into how phytoplankton react to ocean acidification, but many of the results are contradicting. This study looked into how ocean acidification affects the growth rate of two phytoplankton species: *Thalassiosira pseudonana* and *Emiliania huxleyi*. Another objective of this study was to find a method to measure cell density other than counting cells under a microscope. Understanding the effects of ocean acidification on microorganisms could help us understand how big of a problem ocean acidification really is. Supporting evidence was found that *Thalassiosira pseudonana* reacts negatively to pH changes due to ocean acidification due to pH, and *Emiliania huxleyi* was almost unaffected as it adjusted quickly to the different conditions. It was found that cell density had a direct relationship with light absorbance, suggesting that cell measurements could be found using the absorbance of the cells.

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Introduction:

Climate change and increasing concentrations of greenhouse gases has been a well-known problem since the early 2000s. However, there is a lesser known problem caused by the major increase in carbon dioxide (CO_2) concentrations that poses an equal or greater threat: ocean acidification. The overall consequences to the environment due to ocean acidification are not well known. What is known about the effects of ocean acidification is that the metabolic rates and immune systems of some organisms can be negatively affected. Ocean acidification and also decreases the oxygen in the ocean, which could negatively affect many microorganisms like algae and phytoplankton. Ocean acidification and its effects happen at a rapid rate that could prevent many organisms from adapting to the changing environment of the ocean. One species of microorganism that could experience harsh effects is phytoplankton. Phytoplankton, one of the basic organisms of the marine ecosystem, are unicellular autotrophic plankton that usually reside near the upper layer of the ocean. Phytoplankton are vital components of marine food webs. There has recently been more research conducted on different species of phytoplankton and on ocean water chemistry manipulation to see the potential effects of ocean acidification (Armbrust et al. 2004; Borchard et al., 2011; Franklin et al. 2012; Iglesias-Rodríguez et al. 2008; Kranz et al. 2010; Langer et al. 2009; Rost et al. 2008; Schulz et al. 2009; Shi et al. 2012; Yang et al. 2012).

Since the Industrial Revolution, partial pressure of carbon dioxide (pCO_2) has increased from < 300 ppmv to 390 ppmv, and is estimated to reach over 750 ppmv by the end of the century (Houghton et al. 2001, as quoted by McCarthy et al. 2012). A pCO_2 increase from 390 to 750 ppm would lead to an average pH decrease of 0.5 (Caldeira and Wickett, 2003, as quoted by Borchard et al., 2011) through the process of ocean acidification. Since the ocean is generally a carbon sink, the ocean absorbs about 33% of atmospheric carbon dioxide (IPCC, 2001), which can stay dissolved in the ocean in the form of dissolved inorganic carbon, or react with the contents of the ocean. Ocean acidification results when the CO_2 absorbed in the ocean reacts with the water to form carbonic acid (H_2CO_3). Most of the H_2CO_3 formed from the reaction then splits into a hydrogen ion (H^+) and bicarbonate (HCO_3^-) ions since H_2CO_3 is a weak acid. The H^+ ions then react with carbonate ions to create more HCO_3^- ions (Doney et al. as quoted by Das and

Mangwani, 2015). The significant increase in atmospheric CO_2 in the last 100 years has led to a drastic increase in CO_2 diffusion into the ocean, which has caused the pH to decrease and ocean chemistry to change drastically. A change in ocean chemistry would have a bottom-up effect on marine systems by first affecting the growth of primary producers such as phytoplankton.

Phytoplankton play an important role in nutrient dynamics through primary production, which is the process where organic compounds are synthesized from atmospheric or aqueous CO_2, usually through the process of photosynthesis. However, primary processes and photosynthesis rely on enzyme activity, and the lowering pH of the ocean can cause the enzymes to denature preventing the processes from occurring at an optimal pace, and limiting cell growth as a result. The chemistry change in the ocean can also have a negative impact on phytoplankton by limiting nutrient availability (Shi et al., 2012). Since phytoplankton are responsible for supporting marine food webs through primary production, a negative impact on phytoplankton could result in a significant population decrease of higher-order organisms in the ocean. The rates of photosynthesis, however, increases with the availability of CO_2 (Riebesell et al., 1993; Rost et al. 2008), which could increase growth rate.

Many studies have been conducted to understand the overall effects of ocean acidification on phytoplankton and the marine ecosystem. Some studies looked into specific species of phytoplankton and approached different methods of manipulating the water chemistry. One study conducted by Rost et al. (2008) analyzed the effects of ocean acidification on coccolithophores, a type of phytoplankton that go through the process of calcification. Calcification is the process where calcium salts are accumulated in the body tissue of the organisms. Calcification in coccolithophores creates a protective layer of plates around the cells, so a negative impact on calcification rates of coccolithophores could be detrimental to their survival. The study found that different species of coccolithophores have different reactions to ocean acidification. It was hypothesized that this happened due to the different species having different requirements for homeostasis, and increased concentrations of CO_2 from ocean acidification stimulate photosynthesis. However, it was initially thought that ocean acidification would have an overall negative effect on phytoplankton like coccolithophores due to the limitation of calcium carbonate ($CaCO_3^-$) from ocean acidification. One species of coccolithophores in particular is *Emiliania*

huxleyi (E. huxleyi). *E. huxleyi* is a large contributor to primary production in the ocean through the multiple processes, including photosynthesis. *E. huxleyi* is one of the most abundant species of coccolithophores in the ocean, and it was observed that *E. huxleyi* would have lower calcification rates negatively, affecting its growth rate. However, Iglesia-Rodriguez et al. (2008) observed conflicting results that showed *E. huxleyi* had increased calcification and photosynthesis rates therefore increasing its growth rate and resistance. These conflicting results make it difficult to conclude how certain phytoplankton will react to the increasing threat of ocean acidification, and is why it is important to obtain more data. Knowing how ocean acidification affects the growth of organisms such as coccolithophores that rely on shells can also predict what happens to larger organisms that grow shells in a similar process.

The change in pH caused by ocean acidification can also have a large effect on phytoplankton. Enzymes can denature if they are not in their optimal pH, so many marine organisms could see a negative affect in their biogeochemical processes due to ocean acidification. Another species of phytoplankton that are large contributors of primary production is the diatom, contributing about 45% of total oceanic primary production. Diatoms do not have a shell-like layer like coccolithophores, so the effects of ocean acidification on the species could differ significantly from that of *E. huxleyi*. One species of diatom that is studied in particular is *Thalassiosira pseudonana (T. pseudonana)*. Franklin et al. (2012) suggested that the rates of biogeochemical cycling are reliant on the physiological state (condition) of the phytoplankton. Franklin's study shows that the changes of the ocean can also have an effect on the physiological state of phytoplankton without coccoliths or shells, therefore affecting their primary production processes and growth rate.

The changes in the ocean may not be completely negative to phytoplankton and other marine organisms. For example, not all CO_2 is completely converted to acid. Instead, a significant amount stays in the form of dissolved inorganic carbon (DIC). The increasing amount of CO_2 being absorbed into the ocean increases concentrations of DIC in the ocean, making it easier for phytoplankton and other organisms to go through photosynthesis. Higher concentration of DIC could also have a stimulatory effect on phytoplankton growth, making the effects of acidification on phytoplankton growth complex. This highlights the need to test on multiple

species of phytoplankton since combined factors will have variable effects on different species. It is also important to continue tests on different species of phytoplankton multiple times to validate data on how ocean acidification affects marine organisms.

Many studies have been conducted on potential effects of ocean acidification on certain species of phytoplankton, but many contradict each other, so it is difficult to predict what will actually happen in the next 50-100 years. The effects of ocean acidification on ocean chemistry are also not completely understood. Apart from the changes to the carbonate cycle and pH, there could also be small changes in other compounds that could have a drastic effect sometime in the future. Performing more experiments on ocean manipulation and how marine organisms react to the change in ocean chemistry can give us an idea of how drastic the effects of ocean acidification will be in the next century.

Statement of Purpose:

The main goal of this study was to measure specific growth rates and doubling times in the diatom, *T. pseudonana*, and the coccolithophore, *E. huxleyi*. This was done by comparing growth of species in a control medium (F/2; Guillard) and two experimental treatments (F/2 plus acid, fF2 with varied carbonate chemistry) where the pH and carbonate concentrations were lower than the normal pH (8.2) and carbonate concentrations of the ocean. It was hypothesized that the growth rates of both species would decrease as pH decreased, with *E. huxleyi* having a much greater difference in growth rate due to the added burden of coccolith maintenance in lower carbonate.

A secondary goal of this study was to identify the relationship between light absorbance and cell density for both *E. huxleyi* and *T. pseudonana* to provide a more rapid method of measuring cell density. This would allow more accurate experiments in a shorter amount of time. It was also hypothesized that there would be a positive relationship between cell density and absorbance due to the increasing amount of cells contributing to photosynthesis, and that finding cell density from absorbance would be possible.

Methodology:

Culture Conditions-

Strains of the coccolithophore *Emiliania huxleyi* and the diatom *Thalassiosira pseudonana* were received from the National Center for Marine Algae and Microbiota at Bigelow Laboratory. About 12 liters of seawater from Playland Park Beach, in Rye, New York, were collected and about five liters were filtered. The filtered seawater was made into a F/2 medium (Guillard and Ryther, 1962; Guillard, 1975) with salinity measured out to be 15 ppt. The F/2 medium was used since it is a widely used enriched seawater medium that helps growing algae and phytoplankton, especially diatoms.

Twenty-two 125 mL pyrex flasks were acid washed with 0.1 molar (M) hydrochloric acid (HCl) and autoclaved. Once the flasks were sterile, each of the two species of phytoplankton were transferred into five flasks, and were grown in controlled conditions for about three weeks to get them acclimated to the light and temperature cycles of the lab: estimated light intensity of 21.7 footcandles from ambient light grown in an 11:13 hour light:dark cycle, and an average air temperature of 24°C.

Spectrophotometry-

After becoming acclimated to the conditions of the lab, one flask each of the initial cultures of *E. huxleyi* and *T. pseudonana* were transferred into the F/2 medium and put into a spectrophotometer to measure the absorbances according to wavelength. This was to find the peak of absorbance for *E. huxleyi* and *T. pseudonana*; a wavelength range of 400-800 nanometers (nm) was used to find this. The data was plotted (Figure 1) and the wavelength range of 660-680 nm was found to have the most absorbance for both species. This range was then used to measure the absorbances of the two phytoplankton at different growth points. The relationship between growth rate and absorbance was conducted only on group 1 of the control group for both *E. huxleyi* and *T. pseudonana*. Absorbance was measured using a spectrophotometer on the same days that cell density was measured.

Manipulating Media-

Using methods similar to those used by Langer and Bode (2011), manipulated F/2 media of pH and carbonate were created. Langer and Bode used a constant ratio of carbonate to bicarbonate at different concentrations of carbonate. Using their data for carbonate concentrations, a carbonate to bicarbonate concentration ratio was found to be 5.43. A volume of 400 mL of the F/2 medium was isolated to use for the carbonate concentration manipulation, and the supervising scientist calculated the amount, in grams, of carbonate and bicarbonate needed for a pCO^2 concentration of 750 ppm for the volume isolated. A similar process was conducted for the lowered pH medium, but without using a ratio. A volume of 600 mL of the F/2 medium was isolated, and the supervising scientist calculated the amount of 0.1 M HCl needed to change the initial pH of the medium to around 7.6. Each medium (control, pH, and carbonate) was transferred into four flasks each, and filled to 75 mL. Seven mL of *E. huxleyi* and *T.pseudonana* were transferred into two flasks of each medium using a sterile pipette.

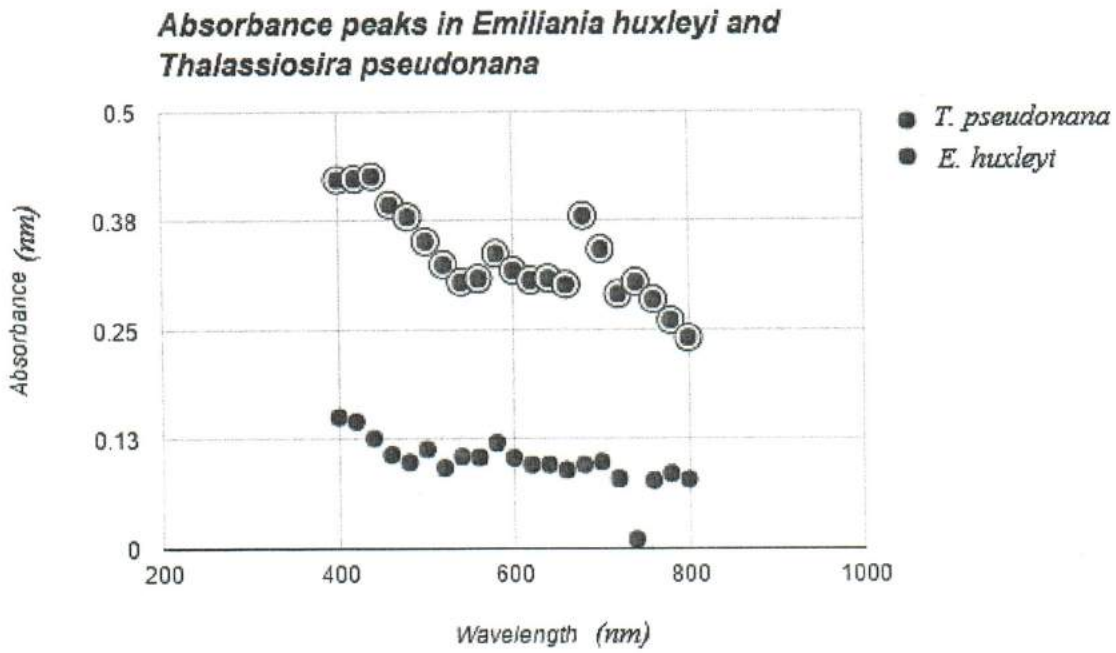


Figure 1: Absorbance at different wavelengths were found for both species. An interval was noted to incorporate the range where absorbance would be highest before a sharp decrease.

Microscopy-

The growth rates of *E. huxleyi* and *T. pseudonana* were measured based on cell density using a one mL gridded microscope slide (Sedgewick Rafter Slide from WildCo.) and a compound light microscope. Cell density was measured by focussing on a μL box on the gridded slide and counting the number of cells in that one box. This was repeated three times and the cell counts of the three trials were averaged and multiplied by 1,000 to give an average cell density for one mL. Cell count data was measured and calculated for six days, with a few break days in between. The averages of each flask were plotted on a graph for each species to show the growth rates of the two species of phytoplankton in control, manipulated pH, and carbonate settings.

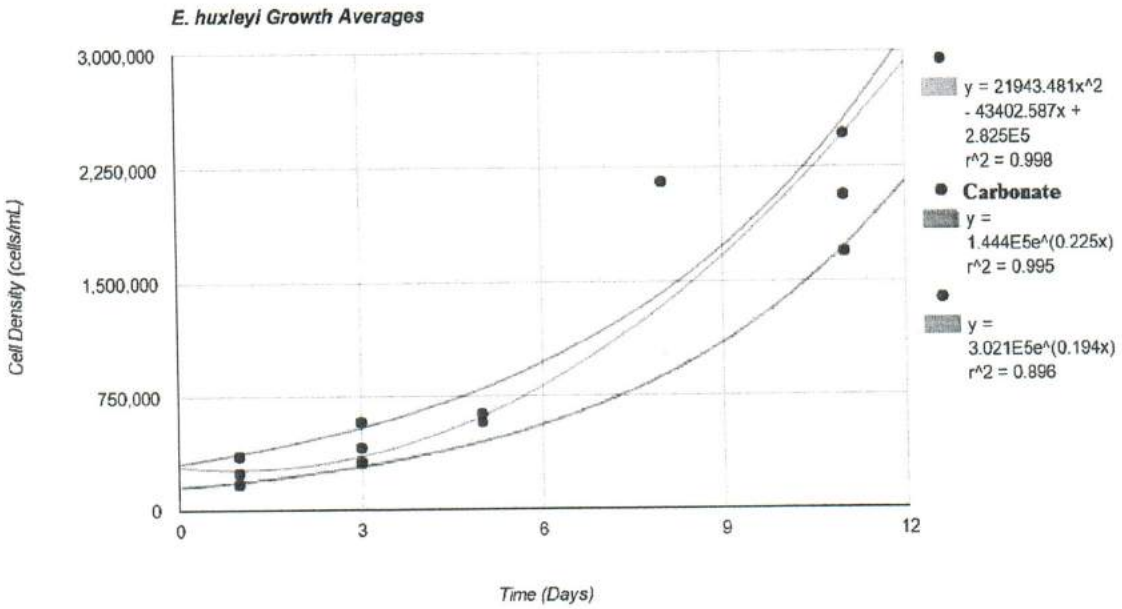
Statistical Analysis-

Using the cell count data, graphs plotting average cell density over time for both species were made and regressions were run. Using the exponential equations from the regression analyses, doubling times were calculated for all trials of each species. A one-way ANOVA was conducted on the results, for each species.

Another set of graphs were made for both species in group 1 control, comparing their absorbance at a specific wavelength to their cell density. A regression analysis was applied.

Results:

Figure 2.1: Average cell density was measured over eleven days for the three groups of *E. huxleyi*. Regression analyses were run on the three groups of data.



Based on the graph (Figure 2.1) for the average growth rates of *E. huxleyi*, the pH and control groups had similar growth rates, while the carbonate had a similar function shape, but a slightly lower growth rate. However, all three groups grew at similar rates up until day three. The control also showed a decrease in cell density on day 11, which could mean the group reached carrying capacity between day eight and 11.

The graph for *T. pseudonana* (Figure 2.2), on the other hand, has three very different growth rates. A lower pH had a detrimental effect on the growth rate of *T. pseudonana*, while the carbonate only decreased in growth rate by a small amount. Similarly to *E. huxleyi*, *T. pseudonana* has a decrease in cell density on the last day.

Our p-values for the *E. huxleyi* and *T. pseudonana* doubling times were both significantly higher than 0.05 (0.23 for *E. huxleyi* and 0.64 for *T. pseudonana*). This means that our data for

the doubling time is statistically insignificant. The data is also seen to be insignificant for *E. huxleyi* by looking at the group-mean-squared values. The group-mean-squared-between is lower than the group-mean-squared-within, which made a lower F-value. On the other hand, *T. pseudonana* had a higher group-mean-squared-between than the group-mean-squared-within, which made a higher F-value, making it slightly more significant than that of *E. huxleyi*, but still insignificant.

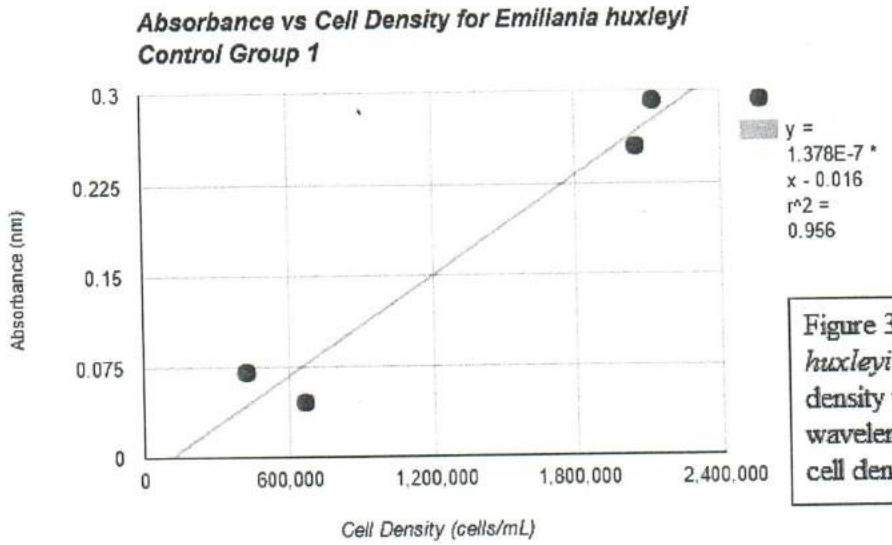
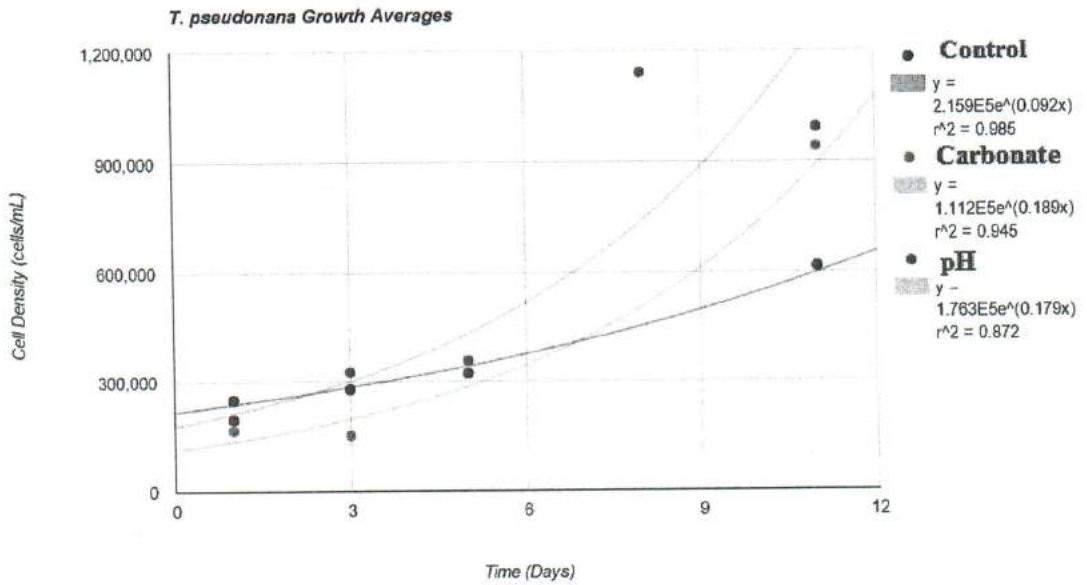


Figure 3.1: The absorbance of *E. huxleyi* was measured on the days cell density was. The absorbance at a wavelength of 660 nm was plotted with cell density to see the relationship.

Figure 2.2: Average growth rate was measured over 11 days for three different groups of *T. pseudonana*. Regressions were run on the three group's data based on the equation with the highest r^2 value 1 <.



T. pseudonana- Doubling Time (in days)	pH Group	Carbonate Group	Control Group
Group 1	16.0	20.4	3.18
Group 2	6.30	0.334	2.34

Table 1.1: Approximate doubling time of *T. pseudonana* using the equations from graphs.

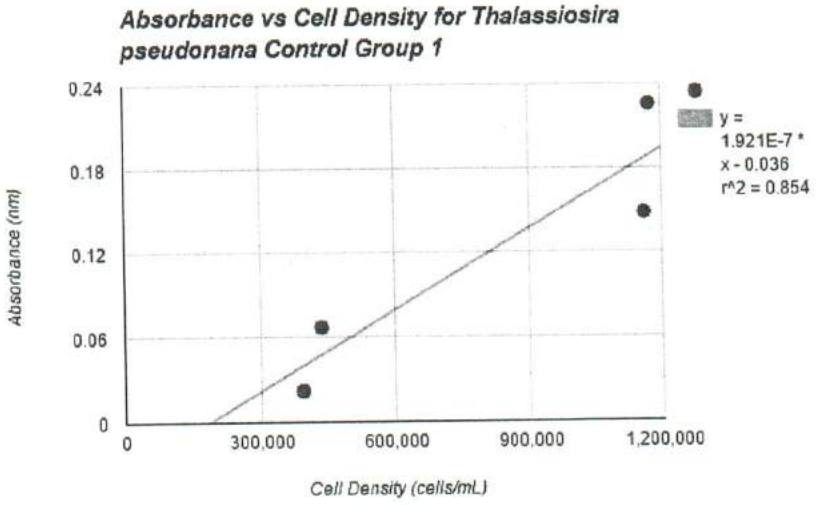


Figure 3.2: The absorbance of T. pseudonana was measured on the days cell density was. The absorbance at wavelength 640 nm was plotted with cell density to see the relationship

Figure 3.1, shows that there is a positive relationship between absorbance and cell density in *E. huxleyi* is shown to be linear. This is the same with *T. pseudonana* based on Figure 3.2, however, in both graphs there are points where similar cell densities have differing absorbances.

Discussion-

Based on the regression analysis of the *E. huxleyi* trials in Figure 2.1, the growth rates of the control and pH groups were very similar. This suggests that pH will not be a big factor in *E. huxleyi* growth in the next century. However, the carbonate group for *E. huxleyi* had a slower growth rate than the other two. This supports the findings of Langer and Bode (2011), since they concluded that the main factor of ocean acidification that affects *E. huxleyi* will be the change in carbonate chemistry. The carbonate concentrations in the ocean heavily influence the process of calcification in coccolithophores, which limits the production of their protective coccolith layer.

Table 2.1: One-way ANOVA of *E. huxleyi* doubling times.

ANOVA	*E. huxleyi*					
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.80	2	0.898	2.45	0.234	9.55
Within Groups	1.10	3	0.367			
Total	2.90	5				

Table 2.2: One-way ANOVA of *T. pseudonana* doubling times.

ANOVA	*T. pseudonana*					
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	86.2	2	43.1	0.518	0.641	9.55
Within Groups	249	3	83.2			
Total	336	5				

The three groups of *T. pseudonana,* on the other hand, reacted very differently from each other. The control for *T. pseudonana* had a similar growth curve to that of *E. huxleyi*, and the carbonate group for *T. pseudonana* reacted similarly to that of *E. huxleyi* as well in that both had

a similar curve shape as the control, but *T. pseudonana* had a slightly lower growth rate. However, the pH group for *T. pseudonana* had a significantly reduced growth rate compared to the control. This supports the idea in the introduction that diatoms are sensitive to pH change. There could be many reasons why diatoms are sensitive to pH. One possibility is that the pH-level affects enzymes and enzyme activity. Since changing pH denatures enzymes, the rates of important biological processes, like photosynthesis and primary productions, could significantly decrease, which would result in a significant decrease in growth rate. However, it is not certain that the decrease growth rate due to pH is from decreased enzyme activity based on the methods of this research. In order to understand pH's effects on *T. pseudonana* and diatom growth rates, more research would have to be done focussing on pH sensitive characteristics of the species.

It is important to note that in the average growth rates of both species were found to, both *E. huxleyi* and *T. pseudonana* decrease in cell density on the last day measured. This could be due to both species reaching carrying capacity (the stationary growth phase) before day eleven. A decrease in cell density around day eleven due to lack of resources would result. If this is the case, then, from what we see in the graphs, lowered pH and manipulated carbonate concentrations increase the time it takes for the two species to reach their carrying capacity.

Both species of phytoplankton reacted similarly in the first three days of the experiment. More research is necessary to understand how other species acclimates to the conditions. As we see from the graph, both *E. huxleyi* and *T. pseudonana* in manipulated carbonate concentrations began to acclimate and behaved similarly to that of their control groups. This was a rapid adaptation in both species. This could give an optimistic view toward phytoplankton survivability to ocean acidification in the future. However, more research would have to be conducted on this specific idea to have a better understanding of phytoplankton's abilities to adapt to ocean acidification.

The graphs of the average growth rates in both species (Figures 2.1 and 2.2) show that there are major differences in growth rate and doubling time in different conditions. However, in the ANOVAs conducted on both species, there was very little significance in difference in doubling time. This contradiction in statistical analysis suggests that more trials are necessary to

verify trends. To solve this problem, multiple trials and groups could be conducted to gather more data on this subject.

This study also looked at the relationship between light absorbance and cell density of both species. The graphs shown (Figures 3.1 and 3.2) are based on one group from the control with four absorbances. Although the graphs shown a positive correlation, which suggests a relationship between the two, a larger study would have to be conducted comparing more data points on a graph to support a linear relationship between absorbance and cell density. However, if absorbance is found to be related to cell density, a new method could be used to measure cell density. Instead of counting the cells, a graph could be used to find the cell density based on a specific absorbance. However, this could only be used for a control group since different conditions of the ocean could affect light absorbance within cells.

Conclusion-

The two objectives of this study were to see how ocean acidification affects the growth rates of *E. huxleyi* and *T. pseudonana*, and to see if there is a relationship between absorbance and cell density. For *E. huxleyi*, all three groups reacted similarly, with the pH group having a very similar growth rate to the control, while the carbonate group started off with a lower growth rate, but quickly acclimated to the conditions. *T. pseudonana*, on the other hand, had an overall negative response to the effects of ocean acidification. *T. pseudonana* was able to acclimate in manipulated carbonate conditions, but struggled in lowered pH.

The relationship between light absorbance and cell density for both species was shown to be positive, however the sample size was too small to make an actual conclusion on the relationship.

The next step would be to conduct similar studies on different species of phytoplankton and to focus on individual growth rates, and on the time it takes for the species to acclimate to the conditions, if they acclimate at all, and to assess how rapid they are able to adapt. This study would also have to be repeated with a larger sample size to validate the conclusions made.

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