

The effect of pH levels on the growth rate of diatoms

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SUMMARY

The ocean's acidification levels and harmful microalgae blooms have increased over the past several decades and are attributed to climate change. Diatoms are microalgae that produce large amounts of oxygen in the atmosphere and serve as important food sources for other marine organisms. Because of our limited understanding of diatoms, we do not know for sure how climate change will impact different species of diatoms. We hypothesized diatom growth rates would be reduced as water pH level decreases. In this research experiment, we conducted two different trials comparing diatom growth rates at the ocean's current pH level (8.1) with pH levels 7.5 and 6.8, respectively. The results suggest that diatoms could continue to thrive when the ocean pH level decreases slightly to pH 7.5. However, this study also suggests there is a limitation of diatom adaptability if the ocean pH level decreases further to pH 6.8.

INTRODUCTION

The level of carbon dioxide in the atmosphere from the burning of fossil fuels has increased ocean acidity and impacted marine ecosystems by decreasing the amount of calcium carbonate ions available to hard shell organisms, causing the shells to thin (1). The ocean absorbs around 30% of the carbon dioxide in the atmosphere, and as a result, the ocean's pH level has decreased from approximately 8.2 to 8.1 in about 200 years (1). In addition, as ocean water continues to increase in acidity, it is hypothesized marine ecosystems and ocean food chains will decline due to the decrease of hard shell organisms and some fish populations (1).

Microalgae play an important role as a food source for other marine organisms (2). Climate change has been linked to an increase in microalgae blooms that are harmful to other marine organisms (2). As algal blooms die and decompose, the bacteria takes away oxygen from the waters and causes harm to other marine organisms (2). More research is needed to determine if microalgae such as diatoms will be impacted by further ocean acidification (3).

One of the most important microalgae in the ocean is diatoms. Due to their abundance, diatoms produce 20–30% of the oxygen in the atmosphere through photosynthesis (3). Diatoms are also important sources of food for other organisms such as zooplankton, aquatic insects, fish, and whales. They can be found in almost every aquatic ecosystem, including saltwater and freshwater. Because of their importance in oxygen production as well as the ecosystem, we investigated how lower pH ocean water affects the growth of diatoms.

Despite there being several studies on how ocean pH affects diatoms, the understanding of how it affects the growth of diatoms is still limited. A climate change study that used global modeling predicted microalgae, including diatoms, would have lower growth rates due to ocean acidification (4). A review of several studies on pH effects on diatoms suggests diatoms have a lower growth rate when pH is higher than 8.4 (5-6). However, these studies have limited testing at lower pH levels between 6.8 and 7.5, and have found conflicting results where lower pH may either negatively impact diatoms or have no impact (5-7).

We aimed to fill in the knowledge gap by testing diatoms at different pH levels not tested in the studies reviewed. We hypothesized that diatoms would likely have a decrease in growth rate in more acidic water. This research experiment tested how diatoms grow on tiles placed in tubs of aquarium salt water at varying pH levels. The control tub was at a pH of 8.1, and the two experimental tubs were at a pH of 7.5 and 6.8. Specifically, pH of 7.5 was chosen because climate change projections indicate that a drop to this pH level could happen around year 2100, and pH of 6.8 was chosen to test the effect when pH falls slightly below neutral (8). We measured and recorded the observations weekly on how diatoms grew and spread onto surrounding tiles. We then calculated the percentage of diatom coverage, which is defined by the area of diatom growth as compared to the overall area of the tiles. This study suggests that diatom growth rate was nearly constant from pH 8.1 to 7.5, but may decline if pH drops further to 6.8. These findings contribute additional information of diatoms at different pH levels that should further enhance our understanding on the impacts of ocean acidification.

RESULTS

Our original experimental design was a single trial comparing the growth of diatoms at two pH levels, 8.1 (control) and 7.5 (experimental). The water in our tubs was taken from the saltwater system used in the Cabrillo Aquarium aquatic nursery where the experiment was conducted. Both tubs began with only the center tile completely covered with diatoms (one tile out of 11 total tiles = 9% coverage). After the second week of experimentation, we observed slightly more diatom growth in pH 7.5 than in the control. But by the end of the four weeks of the first trial, the diatom coverage was about the same in both tubs. We observed 41% coverage at pH 7.5 versus 37% coverage at pH 8.1 (**Figure 1**). Therefore, the results suggest diatoms were not negatively impacted at pH 7.5 ($p=0.09$). We also observed that the diatoms in pH 7.5

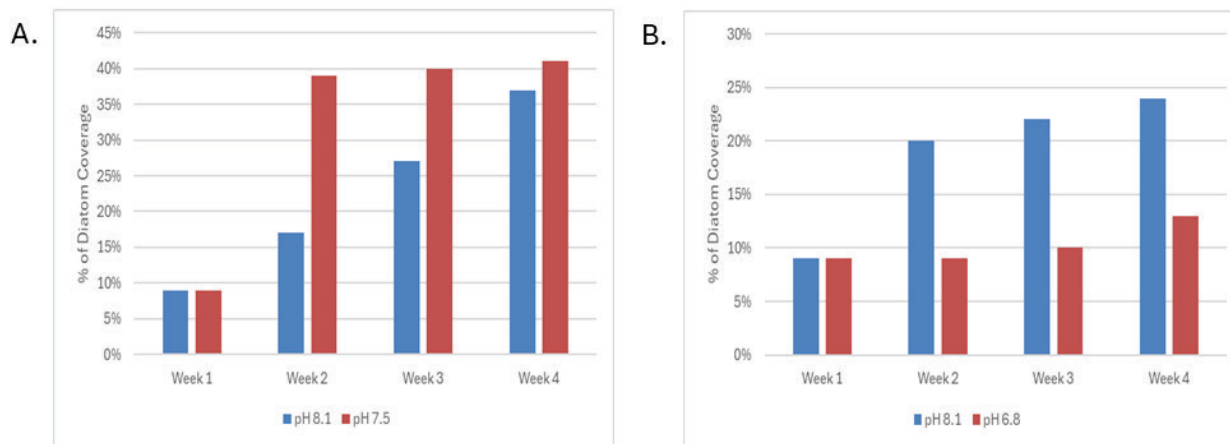


Figure 1: Percent of diatom coverage. A) Bar graph showing diatom coverage of control tub (pH 8.1) versus experimental tub (pH 7.5) ($n=1$ tub per pH level). Unpaired t -test from week 2 to week 4, $p=0.09$. B) Bar graph showing diatom coverage of control tub (pH 8.1) versus experimental tub (pH 6.8) ($n=1$ tub per pH level). Unpaired t -test from week 2 to week 4, $p=0.002$.

were slightly darker in color after four weeks.

With the growth of diatoms in pH 7.5 not much different than in pH 8.1, we decided to conduct a second trial at pH 6.8, which is slightly below neutral pH. Like the first trial, we started with two tubs with 9% diatom coverage. During the first two weeks, the diatoms in pH 6.8 did not have any measurable growth. By the end of four weeks, we observed 13% coverage in pH 6.8 versus 24% coverage in pH 8.1 (**Figure 1**). The results suggest diatom growth rate was significantly reduced at pH 6.8 ($p=0.002$). We also observed that the diatoms in pH 8.1 were darker in color compared to the diatoms in pH 6.8.

DISCUSSION

After two different trials at three different pH levels and over eight weeks of experimentation, our results suggested that the growth of diatoms remained similar to the control at pH 7.5 and was reduced at pH 6.8. Therefore, if ocean acidification continues to occur, diatoms might continue to thrive as the pH lowers from 8.1 to 7.5. However, this experiment also suggested that the diatom growth rate might decrease if the ocean pH level continues to drop to 6.8.

This experiment relied on the measurement of diatom spread on the tiles to represent growth. Diatoms reproduce asexually by binary fission (3). They can grow on tiles using self-secreted polymers, which allow them to build colonies (3). As the colonies grow, the diatoms spread onto other tiles. During the experiment, we also observed that color intensity variation was affected by diatom population density. We speculate this color difference may be due to density variations, since the density of diatoms affects their color (9). The darker color intensity of diatoms corresponded with better tile area coverage, which indicated increased growth. Conversely, the lighter color of diatoms corresponded with sparse growth. Although we took note of these observations, we did not quantify the density of diatoms. Therefore, a future improvement to our method would be weighing the tiles before and after the experiment to also determine growth using weight differences. Another method is to scrape off the diatoms from the tiles and weigh the diatoms after drying to exclude water

weight variations, although this alternative could only be done at the end of the experiment.

Future testing improvements could involve conducting trials with three to five replication processes per pH condition, which would enable further statistical analysis and account for variability. Another improvement can also involve varying the water temperature to research the heating of ocean waters from climate change.

The National Oceanic and Atmospheric Administration (NOAA) ran scenarios predicting future ocean pH levels. In a high carbon dioxide emissions scenario, NOAA projected that the ocean pH would fall to 7.67 by the year 2100 and could continue to fall further (8). Even though NOAA has no projected ocean pH level beyond year 2100 at this time, the results of this experiment might complement future projection models due to the importance of diatoms in oxygen production and carbon dioxide absorption.

MATERIALS AND METHODS

Growth Conditions and pH

In this research experiment, we tested diatoms from Cabrillo Marine Aquarium in different plastic tubs (12 inches x 16 inches x 4 inches) containing aquarium salt water at pH 8.1, 7.5, or 6.8. Because there were only two tubs, the experiment was done in two different trials with one control tub and one experimental tub. The first trial compared pH 8.1 to 7.5, while the second trial compared pH 8.1 to 6.8. For the control tub of each trial, the aquarium salt water at pH 8.1 was used. The pH of these tubs was monitored each week before and after the weekly water changes. For the two experimental tubs at lower pH, the saltwater's pH was adjusted using distilled white vinegar with 5% acidity, which was used in a previous study (10). For the tub with pH 7.5, two mL of vinegar was added in 1-mL increments with mixing of the water in between. The same process was used in the second trial until the tub reached pH 6.8, which took a total of 3 mL of vinegar.

Diatom Growth Measurements

A total of eleven 4-inch square tiles were placed on the bottom of each tub, with only the center tile being covered

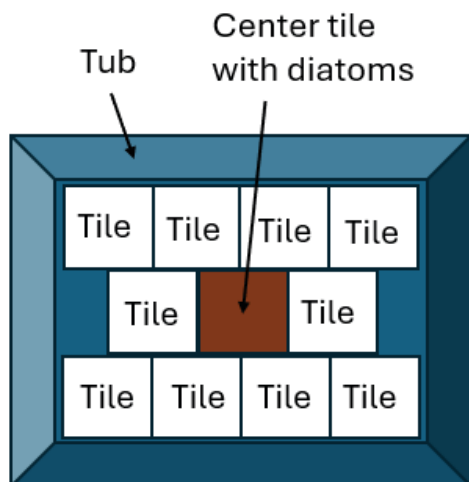


Figure 2: Experimental setup. Each trial began with the tub's center tile covered with diatoms and surrounded by 10 empty tiles with no diatoms. Diatom coverage on all the tiles was measured weekly using a quadrat.

with diatoms, provided by the aquatic nursery (Figure 2). The center tile was measured using a quadrant to ensure the starting amount was the same for each tub. The 10 surrounding tiles were empty with no diatoms. Each tub was then placed under a grow light of the same brightness. These tubs were kept on a counter in the aquatic nursery, where it was room temperature at approximately 70 degrees Fahrenheit. In each trial, the growth of diatoms was measured each week for a total of 4 weeks using a quadrat to measure the percentage of diatom coverage on all the tiles. A quadrat is a four-sided frame that was used to isolate each 4-inch square tile. Each tile was measured for diatom coverage, and the total amount of diatom coverage was then summed. Water changes were conducted weekly using a siphon tube to dispose of the water. Then the steps to adjust the pH using distilled white vinegar were repeated to maintain the pH of the water in the experimental tubs.

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