

The Effects of pH on the Abundance of Phytoplankton for Mariculture

Team: Saber-Toothed Salmon

**Micah Briola, Matthew Guevara, Duncan Menzies,
Hana Oshima, Keenan Sanderson**

**Ketchikan High School
2610 Fourth Avenue
Ketchikan, Alaska 99901**

Primary Contact: Micah Briola

micahcbriola@yahoo.com

Coach: Julie Landwehr

julie.landwehr@kgsd.org

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Abstract

Very little research has been done on the effects changing ocean pH has on the food source of shellfish. However, since one of the issues shellfish have with changing ocean pH is that they have less energy to take care of other vital needs like obtaining food, researching how changing ocean pH affects the phytoplankton could be significant. We focused on whether or not lower pH levels would cause the phytoplankton populations to decrease. We conducted an experiment that placed four different species of phytoplankton in three different pH levels and see how their populations and growth would be affected. While there was no statistically significant change in the results of our experiment, our results could suggest a possible trend. This combined with anecdotal evidence from a mariculture expert was adequate for us to be able to suggest that other mariculture facilities perform similar experiments with bigger sample sizes. We would also suggest doing more counts during their experiment, using broader pH ranges to determine optimum pH for algae growth and so better maximize the growth of their phytoplankton cultures. Our team did not have enough replicates within each sample group, which led to us having no statistically significant change, and so those extra counts with broader pH ranges would perhaps help provide a more statistically significant change.

Introduction

The decrease of the ocean's pH due to the increase of carbonic acid, called ocean acidification, can have disastrous effects on the ocean and the organisms it contains. This is especially true when it concerns calcifiers, particularly: shellfish, gastropods, and calcareous plankton (Henriques, 2010). The reason is that those organisms rely on calcium carbonates, such as aragonite and calcite, to build their shells and skeletons. While it is relatively easy to obtain calcium carbonates in pH-balanced water, a decrease in the ocean's pH makes it harder for calcifiers to pull the calcium carbonates from the water. This reduction in the availability of calcium carbonates, and causes larval and juvenile-stage calcifiers to have slower growth rates and higher mortality rates (Henriques, 2010). When these organisms must use more energy to obtain the calcium carbonates, they have less energy for other essential needs, such as obtaining food or defending themselves (Adelsman, 2012). It is because of this that most researchers have put a lot of effort into looking at how ocean acidification affects the shellfish.

We found that there is very little information available about how other phytoplankton might be affected, specifically how the food for shellfish is affected. It was only after a local scientist pointed it out to our team that we realized that most researchers hadn't really looked into these phytoplankton issues with regard to effects of ocean acidification. Because of this, our team decided to conduct an experiment that would help us determine how to best maximize the growth of phytoplankton and to see what effect lower pH would have on the phytoplankton cultures. This seemed like the best course of action to us, for two main reasons. The first, is because within Alaska, the shellfish mariculture industry an important part of economy, particularly in Southeast Alaska. The second reason is because our team had convenient access

to the OceansAlaska facility, which meant we had access to both resources and information.
(Alaska Department of Fish and Game, n.d)

Biological and Physical Aspects of the Issue

Ecology of Phytoplankton

Ocean acidification has taken its toll on phytoplankton, shellfish, and mariculture overall. Progressively over the years, carbon dioxide has been entering the waters, decreasing pH levels, as well as calcification rates of many marine animals such as calcareous phytoplankton and shellfish (Fabry, et al, J, 2008). Along with lower calcification rates, shortages of carbonate ions in the ocean have affected organisms that have calcifying shells. Phytoplankton go through growth, sex, quiescence, and cell death. Growth is where they reproduce asexually, and then follows sex where the fusion of haploid cells and the process of meiosis occur. Quiescence is the stage when metabolic rates are reduced and division ceases and soon leads to its death. With more acidic water, species such as coccolithophores, foraminifera, and euthecossomatous pteropods are prone to having decalcified cells. This affects the health and viability of the phytoplankton and if it is damaged enough that can also lead to affecting those organisms depending on it. Phytoplanktonic life is essential to the ocean's food chain and produces most of the oxygen in the atmosphere. Biodiversity of the ocean will change as the health of phytoplankton change (Rogelj, et al. 2009) (Nicholls, et al. 1999).

Ecology of Shellfish

Shellfish are also in danger from the effects of ocean acidification. For example, oysters utilize phytoplankton as a food source. After three years of age, oysters are at their height of

reproductive maturity and by this time oysters need to have consumed large amounts of phytoplankton to start spawning. Oyster ripening can take up to two months before they can spawn. The fertilized eggs then undergo cell division to form larvae. These larvae start out swimming with the current to follow phytoplankton and after two weeks they reach the pediveliger stage. At this stage they move to the bottom of a river or estuary to search for a substrate to attach to. As spat, the oyster starts to secrete calcium carbonate for developing their shells. Every year on average in the wild, oysters grow an inch. After one year it becomes juvenile, then after three years it becomes an adult. Depending on the temperature and salinity of the water it is in it may grow faster or slower (Meritt, et al. n.d.).

Mariculture

In Alaska, there are a number of different types of shellfish that are being harvested through mariculture. These include littleneck clams, blue mussels, geoducks, purple-hinged rock scallops, cockles, razor clams, and spiny scallops. In addition, there is one species is not native to Alaskan waters: Pacific oysters. (Alaska Department of Fish and Game, n.d). All of these species are in at least one facility or location where they can be harvested. Of all the different species of shellfish, Pacific oysters far surpass all of the other species in terms of number harvested by all of the shellfish farms around Alaska and the amount of total revenue generated by the shellfish. Of the total number of shellfish harvested including all of the species that are involved in mariculture, Pacific oysters account for approximately 93% of the total profit for the industry. The Pacific oysters are very popular because they cannot reach sexual maturity in the cold waters of Alaska. This is a win for the environment because it prohibits the Pacific oysters from

becoming an invasive species, which has the potential to have a massive impact on the environment (Alaska Department of Fish and Game, n.d).

Mariculture Facilities: OceansAlaska

There are numerous mariculture facilities around Alaska, but only a few shellfish hatcheries and setting facilities. One that we would like to point out is OceansAlaska. OceansAlaska is located in Southern Southeast in the waters around Ketchikan, Alaska. It is a non-profit organization whose funds come from local, state, and federal grants. This facility is built on a mobile, floating platform, meaning that it can move from place to place. At this facility, they conduct research in order better understand the best practices for setting oysters and geoducks. The shellfish that they raise at their hatchery are the Pacific oyster and the geoduck. Like any other living organism, they require energy. At their facility, they feed the shellfish with different types of phytoplankton. It is crucial that they keep the water at a certain pH (usually 8.2-8.4 is recommended) so that the phytoplankton won't die off before they feed it to the shellfish (OceansAlaska Marine Science Center, n.d).

Problems in Washington

In Puget Sound, Washington, there is a major mariculture industry. One of the primary species that is harvested in Puget Sound harvest is the pacific oyster. Puget Sound is the site of three major oyster hatcheries, that have until recently supplied all the mariculture industry in Alaska with what the oyster seed needs. There are some recent and worrisome events that are affecting the ability of the hatcheries in Puget Sound from producing enough seed for Alaska. Deep-sea ocean upwellings are bringing the dissolved CO₂ from the bottom of the ocean up to

the Puget Sound area making the water more acidic. When the water is more acidic, it makes it more difficult for the shellfish to extract aragonite from its surroundings in the early stages of its life cycle. This example of ocean acidification is affecting the economy of the mariculture industry in Washington because pacific oysters aren't surviving past the larval stage. It is also affecting the mariculture industry in Alaska, as there is not enough seed to supply the growers in the rest of the Pacific Northwest and have seed to send to Alaska (Puget Sound Restoration Fund, n.d).

Experiment to Determine pH tolerance of Phytoplankton

Purpose of the Experiment

The purpose of our experiment was to find the effect pH change had on the growth of phytoplankton in general as well as on specific species. It would then be possible to apply the gathered knowledge to the shellfish mariculture industry in hopes of helping them maximize the growth of their shellfish food source. The reason we decided on using phytoplankton is that phytoplankton are key species for both our ecology and the ocean's ecology, acting as the producers at the bottom of the food chain for most ocean organisms, as well as creating almost 50% of the world's available oxygen (Sage, et al, 2013). Our hypothesis is that the lower the pH, the less the algae would thrive even if there was difference in the tolerance limits between different kinds of algae.

Understanding the pH tolerances of individual algal species would help maximize the growth, which in turn could help the shellfish mariculture industry. To compare the different kinds of phytoplankton we chose to compare four different species of phytoplankton, two diatoms and two flagellates and we placed the four species within three different pH levels.

The diatoms, unicellular organisms without flagella, we used were *Thalassiosira pseudonana*(3H) and *Chaetoceros calcitrans*(CCal), and flagellates, organisms with one or more flagella, we used were *Isochrysis sp.*(Clso) and *Tetraselmis suecica* (TetC). *Thalassiosira pseudonana* and *Chaetoceros calcitrans* were ordered from Whiskey Creek Shellfish Hatchery (Morgan, 2013). *Isochrysis sp.* and *Tetraselmis suecica* came from Bigelow Labs (Morgan, 2013). Since the average worldwide pH levels in the ocean range from 7.9 to 8.4, we decided to use the pH levels 8.0, 7.8, and 7.6. This allowed us to determine how more acidic environments might affect the phytoplankton worldwide. The lowest pH, 7.6, was thought to be too acidic for good algae culture, based on the experience of the algae culturist at OceansAlaska, who has seen that cultures of algae that drop that low in pH do not survive.

In this experiment, we had thirty-six 50 mL test flasks altogether, separated into four different groups of nine to represent the four species. These nine were then further separated according to the different pH levels: 7.6, 7.8, and 8.0, accordingly. We then grouped together the pHs and species, creating three sets of twelve, one for each pH containing three of each species.

Protocols and Materials of the Experiment

A five-micron filter was used for filtering the seawater that was collected. We used thirty-six 50 mL water flasks because using smaller flasks would be more efficient in comparing the color (indicating the concentration level of each algal culture), and visual differences would be easier to observe in a smaller flask than a larger one. To set constant factors we used a Biotronette Mark III Environmental Chamber to control the amount of light and temperature the algal cultures get. Barbara Morgan from OceansAlaska provided us with four species of algae, and the growth solution. As mentioned previously, the four algal species we used were

Thalassiosira pseudonana and *Chaetoceros calcitrans* for diatoms and *Isochrysis sp.* and *Tetraselmis suecica* for flagellates. Hydrochloric acid was used to adjust the pH and 10 mL of F2 modified growth medium and concentrated silica for the growth solution with pH 8 Tris bases acting as a buffer.

Experimental Methods

For this experiment to be successful we had to set clear limits on what our controlled factors would be and what our variable would be. The only variable we changed, and thus the only variable in the environment of the phytoplankton we are comparing per species in this experiment is the pH levels. The pH levels we have tested were 8.0, 7.8, and 7.6. To keep the other factors constant, the same filtered seawater of pH 8.0 was used to create the growth solution was used, which has the same amount of F2 modified growth medium, concentrated silicate, and tris base in pH8 for all of the flasks. Hydrochloric acid was used to control the pH environment, so the results we obtained would show the effect of only the pH on the algal cultures, and not any other potential effects such as a response of the algae to a carbon source. The amount of algae put into each flask using graduated pipettes was 1 mL. To control conditions for the growth difference in the algal cultures we used a Biotronette mark III Environmental Chamber to set the temperature at 70F° and the light for 24 hours for 10 days of growth all together. At the end, pictures of the cultures were taken so we would be able to compare their growth depending on the pH and species. In those pictures, you can see that there are few visible difference between pH levels, except for Figure 6, which is clearly different (see Figures 3-6 in Appendix 1). The likely reason for this difference is that the clear flasks were not inoculated.

Seawater was collected from 8ft below surface (below the freshwater layer) and filtered through a 5 micron filter. The growth solution was created by adding 10 mL of nutrients (an F2 modified growth medium), concentrated silicate (30 g/L in dH₂O which is listed as being 1.06 X 10⁻⁴ M), and tris base in pH8. This growth solution acts as a buffer to the 10 L of filtered seawater. This is the same growth solution used to grow algae culture at OceansAlaska. The original pH of our water was 8.0, and by adding hydrochloric acid to two different samples of that water, the technician reduced the pH of the two samples down to 7.8 and 7.6. It was those three samples we used for our experiment.

Each flask was clearly marked. Thirty (30) mL of the growth solution was put in each 50 mL flask. The technician kept the level of the solution low enough for the surface area to maximize the efficiency of exchange between the culture and the air. The top of the flasks were covered by wadded up cotton cheesecloth, then wrapped with tinfoil. All of the flasks were sterilized by autoclaving. Each flask was inoculated with 1 mL of one type of phytoplankton. The process was repeated with the three other species. Each sample was placed in the Biotronette mark III Environmental Chamber. Flasks were swirled at least once every 24 hours to increase growth. Some photos were taken to track the growth visually. As a final step, record and analyze the results of the pH.

Growth was measured by turbidity of the solution and the number of algal bodies in selected sample. Turbidity was measured by the visible change in color from sample to sample. All samples were photographed by setting them on a white piece of paper under and behind the flasks and care was taken to have uniform light on the samples. A darker color of the samples indicated more growth within the algal culture. The number of algal cells in a selected sample was measured by using a microscope. One drop of swirled flask solution was placed on a gridded

slide, using a Leica CME microscope, focused on 40x on square E4, and photographed with a Vivitar ViviCam X022 camera. The count was done within the 1000 x1000 pixels box on Apple Preview. Since the pipettes we used only held 26 drops, we only looked at approximately .04 of a millimeter. The pictures at times didn't clearly identify an organism, so we had to make judgement calls on those. As a final step, the results were tested by the Ketchikan High School Staff and US Forest Service scientist for statistical validity at both 90 and 95% confidence levels.

Results of the Experiment

No significant change in color was seen in the various samples of the photos. Even considering that, the data was interesting to examine. The average change in numbers of organisms from low pH to high pH was similar within the types of algae (see Table 1). Even though the statistical analysis did not show a significant difference in the growth of the algae for the different pH treatments, it appeared there were some slight trends that could be seen.

Species	Total pH 7.6	Total pH 7.8	Total pH 8.0
<i>Thalassiosira</i>	22	13	8
<i>Chaetoceros</i>	17	10	5
<i>Isochrysis</i>	51	68	16
<i>Tetraselmis</i>	4	15	9

Table 1: Average numbers from 3 slides of individual phytoplankton by species and pH after 10 days of incubation. Cell E4 was read per slide.

Thalassiosira and *Chaetoceros* are diatoms increased in number as the pH decreased. The number of diatoms decreased at the relatively constant rate of 60% for every 0.2 increase of pH. *Isochrysis* and *Tetraselmis* are flagellates had their numbers peaked at pH 7.8. Flagellates had non-uniform, volatile results that differed from sample to sample.

Conclusion of Experiment

Though we had valid numbers in our data, there wasn't enough difference in abundance to be statistically significant, likely due to our small experimental data size and the lack of other counts. Using the ANOVA and other statistical tools, both the confidence levels of 90% and 95% showed no significant differences in the abundance of phytoplankton. However, trends could be seen from the data we collected which appeared to show they did not clearly support our original hypothesis. The trend seen in the flagellates showed us that there was a peak in which the algal species could thrive the best, which appears to be pH 7.8. Since the diatoms did not reach a peak, this may indicate that their peak could be still lower ($> \text{pH}7.6$). If this experiment was done more extensively at more pH levels, it would be possible to find the best suited pH for the specific type of phytoplankton desired. Also, growing the cultures for a longer period of time, and sampling them more frequently might more clearly show the effect of pH on the cultures. Individual species need to be tested so that the hatchery operators know the optimum pH of the food-algae used in their hatchery. As we were analyzing all the data, we had to take care to remember that, due to the small size of our samples (3 samples per group), that our results were not statistically valid. So while these trends are interesting, they need to be replicated more to validate the results. It would be important for future researchers to be aware of the need to test different phytoplankton species because this experiment demonstrated that different types of algae reacts differently to different levels of pH.

Algae is necessary for the culturing of shellfish so the knowledge of the effect of pH on algae is important for the hatcheries. This experiment could yield recommendations for making shellfish hatcheries more productive. Matching the pH to the desired species of algae is

important because each type of algae reacts differently. The flagellates were most abundant at pH 7.8 and diatoms at pH 7.6. This experiment could be used with any species cultured in the hatchery in order to determine the optimum pH. It would be best to monitor every species you would want to use for shellfish food with the local seawater.

Research, Monitoring and Suggestions

Successful Management

After speaking with a mariculture expert and hearing about their experience with crashing algal cultures in relation to pH levels, our team conducted research and discovered that lower pH levels actually benefitted the growth of our algal cultures, while higher pH levels affected their growth negatively. However, our research was not statistically significant, and our results didn't match the results of the mariculture expert. Because of this, we recommend that mariculture facilities that would be affected (positively/negatively) should conduct this type of experiments because, depending on what kind of marine organisms are grown, the organism population could decline, or it could increase from the change in pH.

Our suggestion for mariculture facilities is to conduct an experiment like ours before they start growing their own phytoplankton. They should do this because this could prohibit crashes due to local seawater pH changes. To do this experiment, the facility would need to take water and algae from their immediate area and use a wide range of pH levels to achieve more accurate results. They should also do counts regularly. In our experiment, HCl was used to adjust the pH of the water instead of CO₂, because CO₂ would have affected photosynthesis, thus affecting the final results of our experiment. This operation would be very low cost because there are already

monitoring protocols in place to monitor ocean conditions at these facilities. The only cost would be extra labor hours.

Conclusion

Shellfish mariculture is an important and growing part Southeast Alaska's economy, and as the Puget Sound incident shows, it is an industry that is highly susceptible to ocean acidification. Ocean acidification makes it more difficult for the shellfish to develop their shells in their larval and juvenile stages by making it more difficult for them to pull calcium carbonates from the water. For this reason, the shellfish expend more energy to obtain the carbonates, which leaves them less energy to obtain food. Due to this, most researchers put their efforts into finding out exactly how the ocean acidification affects the shellfish, not what other aspects that ocean acidification affects. As a result of this, our team did an experiment to try and confirm our hypothesis that phytoplankton would thrive less in lower pH levels, regardless of differences in their tolerance limits. So, though the results didn't perhaps show conclusive evidence to support our hypothesis, we did see a trend that may have indicated that somewhat lower pH levels do not necessarily mean less prosperous growth. Our results showed that it is important for the mariculture industry to monitor the pH in the waters that they use to grow their shellfish.

If these facilities follow our recommendations and conduct this kind of experiment in order to determine how their algae respond to the combination of their local seawater and the input of CO₂ for culturing, it will make their facilities more cost efficient. We hope that they would have more success at growing shellfish against the detrimental effects of ocean acidification and the lowering of the oceans pH .

APPENDIX 1: TABLES AND FIGURES

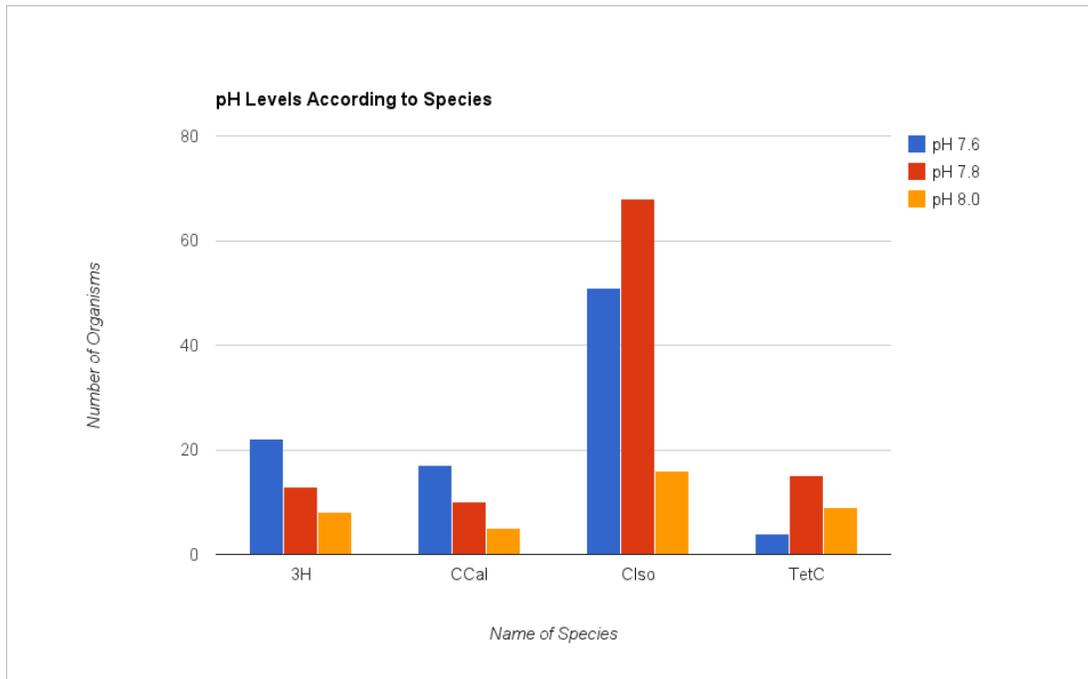


Figure 1: Abundance of the Species According to pH

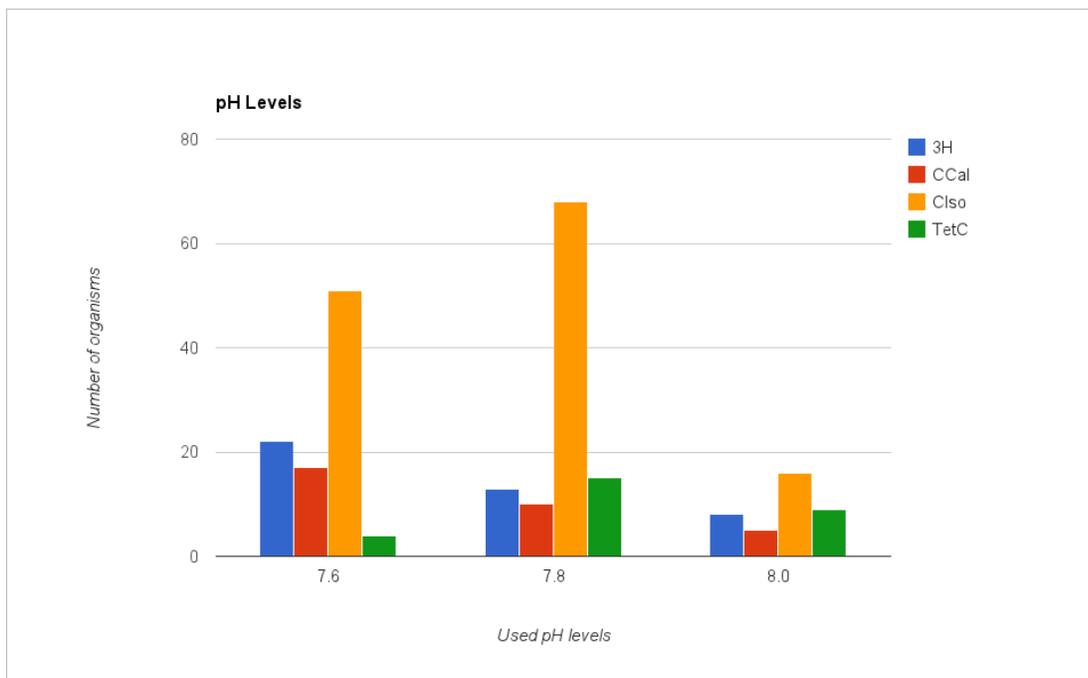


Figure 2: Abundance Levels in the pH According to Species

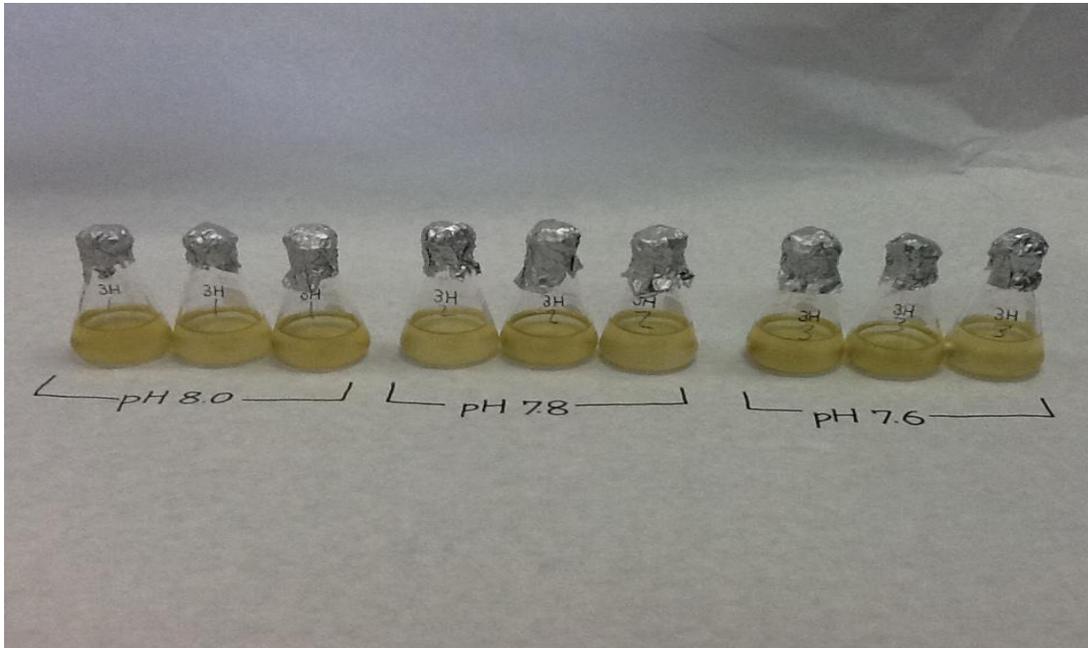


Figure 3: Comparison of *Thalassiosira* samples by pH



Figure 4: Comparison of *Chaetoceros* Samples by pH

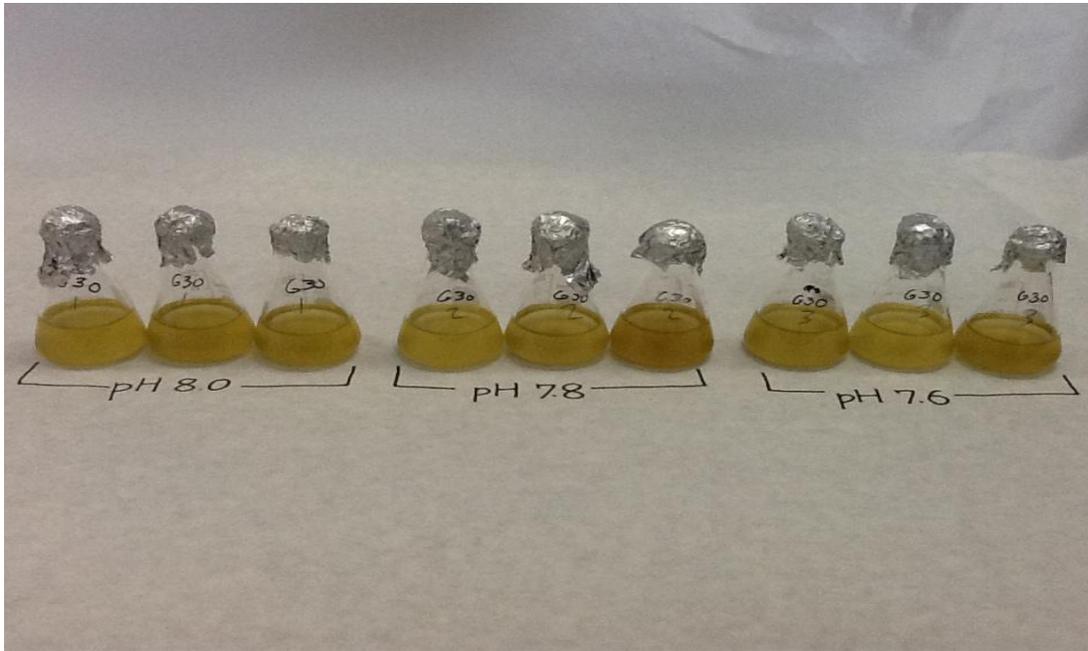


Figure 5: Comparison of *Isochrysis* samples by pH

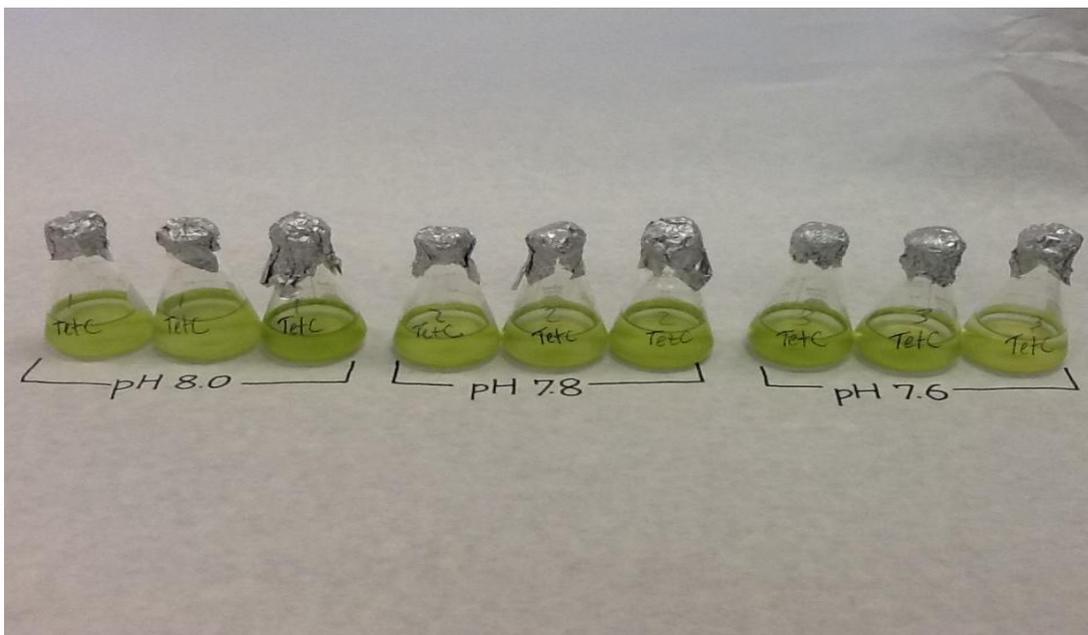


Figure 6: Comparison of *Tetraselmis* sample by pH



Figure 7: Team standing around CCal comparison according to pH

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