HIGH SCHOOL EDITION
Journal of Student Research

Effects of Salinity on Clay Flocculation in the Context of Mitigating Karenia Brevis Blooms in the Tampa Bay

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ABSTRACT

Objective: Tampa Bay Area is an estuary and thus has varying levels of salinity, so it has become imperative to determine whether or not the leading combat strategy (clay flocculation) to HABs is effective under these differing conditions. *Method:* an experiment was conducted that measured the settling rates of Kaolin clay at varying levels of salinity (0, 17.5, 30, and 45 Parts Per Thousand) within the range of *Karenia Brevis*. *Results:* There is no statistically significant difference between the settling rates of Kaolin clay within the range of tolerance of *K. Brevis,* and therefore can be used to combat *K. Brevis* blooms in Tampa Bay regardless of salinity.

Introduction

In 2019, Florida Governor Ron DeSanctis signed legislation that granted a total of 18 million dollars to The Red Tide Mitigation and Technology Development Facility at Mote Marine Lab. The researchers were left with a clear objective: reduce the harmful effects of Florida red tide.[8] The need for a solution to red tide is especially apparent in light of the mass red tide occurrence in 2021. In March of 2021, a leak was discovered in a reservoir pond at Piney Point, an abandoned phosphate mine near Tampa Bay. To prevent flooding and water contamination in local neighborhoods, the polluted phosphate-rich water from the damaged reservoir was redirected into Tampa Bay[4]. The addition of this nutrient-heavy water is significant in that it worsened pre-existing ride tide conditions. Because events like this happen, an immediate solution addressing such occurrences is necessary.[16]

However, before red tide can be combated, a solid understanding of what causes red tide is necessary. Florida red tide is a harmful algal bloom (HAB) that is composed of dinoflagellate *Karenia Brevis* (*K. Brevis)*, which is a microorganism that releases toxins that have several negative effects. Red tide has both natural and anthropogenic causes, both stemming from the addition of nutrients into Florida waters. In nature, water surface temperatures, Saharan dust storms, and rainfall add access to nutrients in the water. But nutrients are also added unnaturally by humans. Activities such as spraying chemical fertilizer can lead to nutrient-rich-runoff; thus, adding excess nutrients to Florida waters.[18] This eutrophication, or the addition of excess nutrients, can cause or exacerbate occurrences of red tide, since the conditions in nutrient-rich water better support expansive growth of microalgae such as *K. Brevis*, as opposed to water with stable levels of nutrients.

Red tide mitigation has been brought to the forefront of environmental conversation in recent years because of its negative effects. Florida red tide has caused significant damage to Florida's local lifestyles, especially economically. The Economic Impact Analysis Program at the University of Florida estimates that the Florida tourism industry lost more than 184 million dollars in 2017-2019 due to the Florida red tide because of a lack of incoming tourists.[6] This lack in tourism is linked to an environmental impact. According to a 2007 assessment of Florida red tide by Frank Alcock, director of Marine Policy Institute at Mote Marine Laboratory, during active red tides, an estimated 100 tons of fish die per day. These dead fish often wash ashore leading to rotting, and pungent smells.[1] Additionally, red tide poses a human health risk. Florida Health's Public Health Toxicology (Bureau of Epidemiology Division of Disease Control and Health Protection) lists symptoms of exposure to red tide ranging from teary eyes to respiratory irritation. [2]

While these are important concerns, perhaps the most significant issue is that a solution for mitigating red tide in the Tampa Bay has yet to be properly developed, and as more people experience red tide firsthand, the initiative for a solution has been gaining attention—thus the 18 million dollar grant. However, existing literature reveals that the locational challenges of Tampa Bay might make some solutions hard to generalize.

Literature Review

Multiple methods of mitigation have been proposed as a solution to Florida's red tide predicament in recent years. There were four main strategies of red tide mitigation outlined by *The Florida Harmful Algal Bloom State of the Science Symposium* in August of 2019: avoidance, chemical, biological, and physical.[9]

The avoidance strategy differs from the others because it aims to stop red tide long term, while the other strategies deal with the issue in the short term; this strategy's goal is to stop the anthropogenic red tide from occurring in Florida with major legislative reforms. However, because this strategy would involve imposing sweeping legislation or incentivizing companies dealing with nutrients such as phosphates and nitrogen to limit the number of stray nutrients that end up in run-off and Florida waters, it would be very difficult to implement and could end up taking decades to put into place[10]. Additionally, because this solution is legislation-based, it would only solve anthropogenic problems and not ones that occur naturally. The ideal solution for red tide would be able to address both natural and anthropogenic blooms immediately.

In the Chemical *Engineering Journal*, a review of recent advances in biological methods to reduce red tide, scientist Mohammad Shahadat and his co-researchers explore said immediate solutions. They analyze the use of bio-flocculants or the use of microorganisms to capture and kill red tide cells[12]. This idea is further elaborated on by others within the field such as researcher Rui Sun, among others, who published their findings on bio-flocculation in a review: *Microorganisms-based Methods for Harmful Algal Blooms Control.* They write that, although using biological tools such as bacteria can be highly effective at killing red tide, those tools still present concerns because of their high cost and unforeseen environmental effects that come with introducing unfamiliar organisms to an ecosystem[14].

Another, and one of the earliest red tide strategies to develop, is chemical flocculation. George Armytage Rounsefell (a fishery and research biologist) and John E. Evans (A writer-photographer for the Bureau of Commercial Fisheries) reported on the first attempt at the use of a compound called copper sulfate as a mitigation strategy in 1958. The addition of the toxic copper sulfate was successful at killing red tide but had the unintended consequence of being too toxic for the environment and killing many non-targeted organisms in its use[11]. For this reason, notable groups such as *The Florida Harmful Algal Bloom State of the Science Symposium* have advised against this strategy. However, far more recent studies pose an alternate solution with a more successful track record.

Woods Hole Oceanographic Institution has been looking in particular at a physical mitigation method called clay flocculation, which has been described as a process where, "Tiny but dense clay particles...combine with other particles in the water." As these particles aggregate, they form clusters that capture other particles in the water[7]. In the case of this research, that other particle is *K. Brevis* cells. In simplified terms, when the clay is added to water contaminated with *K. Brevis,* it bonds with itself and the red tide cells- essentially trapping the red tide, sinking it to the ground, and killing it. This is the leading method of mitigation today and has been successfully implemented around the world with little to no extraneous cost or environmental impact. In China, for example, clay flocculation has been included as a standard method in the "Technical Guidelines for Treatment with Red Tide Disaster" (which outlines the government's response to red tide) since 2014[17].

A 2021 review titled "An eco-environmental assessment of harmful algal bloom mitigation using modified clay" reviews cases of clay flocculation as a mitigation technique around the world for environmental consequences. They concluded that the times clay flocculation has been used, both in a controlled and real-life scenario, no adverse environmental impact can be observed. In other words, this strategy does not affect other organisms such as bottom dwelling animals and marine plants[13].

Clay flocculation has a promising history and seems relatively simple. However, the environmental makeup of Tampa Bay poses some additional challenges. For example, Tampa Bay is an estuary, meaning that it is a mixture of both fresh and saltwater. So, as a result of the mix (largely due to changing tides and freshwater from springs and rivers) Tampa Bay experiences varying levels of salinity[5]. Because of this, it now becomes necessary to examine the extent to which salinity differences within the range of tolerance of *K. Brevis* affect the flocculation of clay particles.

Clay flocculation is successful because the clay particles combine with one another and Red Tide cells, causing them to sink to the ground and die. Dr. Bruce Sutherland from the department of physics at the University of Alberta discusses the nuances of how salinity might affect such flocculation in a 2011 study. It states that because clay particles are generally "sheet-like" in figure and are negatively charged at their center and positively charged at their edges, they attach—or flocculate—to one another. Sutherland stipulates that adding salt and consequent sodium ions, would cause positive sodium ions to attach to the clay, thus increasing flocculation. Through a laboratory experiment that serves as inspiration for my own, Sutherland proves that adding salt will increase the rate of flocculation at high salinities[15].

While this addresses the question of how salinity affects flocculation, the answer was not given in the context of red tide or *K. Brevis*, but rather a sedimentation process in clay-laden estuaries. This reveals a glaring gap in the field as it applies to Tampa Bay. While clay flocculation is widely studied and experimentation has been done on salinity's effect on flocculation, there has been no laboratory-scale experimentation investigating the effect of salinity on clay flocculation specifically within the salinity range of tolerance of *K. Brevis*. Research that answers this question could be crucial in pinpointing when applications of clay to Tampa Bay would be most effective and when more clay or other methods might be more appropriate.

Method

To investigate the effect of salinity on clay flocculation specifically within the salinity range of tolerance of *K. Brevis*, an experiment was conducted. A controlled experiment was the ideal method because it allowed for a clear evaluation of how salinity impacted the clay. Another method such as field observations, or experimentation in an uncontrolled marine environment would not have been appropriate since it would allow for too many extraneous variables. By defining clear parameters for the experiment, confounding variables that emerge in marine environments (such as currents, tidal fluctuations, and pH) were eliminated, ensuring that the outcome of the experiment was a result of the changes in salinity. In practical application, such confounding variables would have to be considered in conjunction with salinity, however, the focus of this experiment was just on the effects of salinity as it applies to Tampa Bay, so addressing these other variables was not necessary for this specific experiment.

In this experiment, the independent variable was the salinity of the water. The amount of salt was determined based on the range of tolerance of *K. Brevis* or in other words how much or how little salt the red tide cells could survive in. The dependent variable of the experiment was the rate at which the clay settled in the water. And while clay settling rates don't explicitly equate to the clay's efficiencies at removing red tide cells, settling rates serve as a good indicator of whether or not the actual act of flocculation is impacted by salinity.

If the clay settled drastically faster in the saltier water, it is sufficient to say that the clay might flocculate faster, thus sinking red tide cells faster. However, if the clay did not settle any faster in saltwater within *K. Brevis*'s range of tolerance it can be assumed the mitigation method is effective across all salinities where *K. Brevis* blooms might occur.

Moreover, measuring settling rates as opposed to removal efficiencies made it possible to determine the effects of salinity without using red tide cells. By simply adding salt according to *K. Brevis*' range of tolerance, it is possible to observe results without the cells present, since their presence would have little effect on the differences in settling rates. This allowed for safe experimentation without the risk of exposure to *K.Brevis* toxins.

Experimental Design

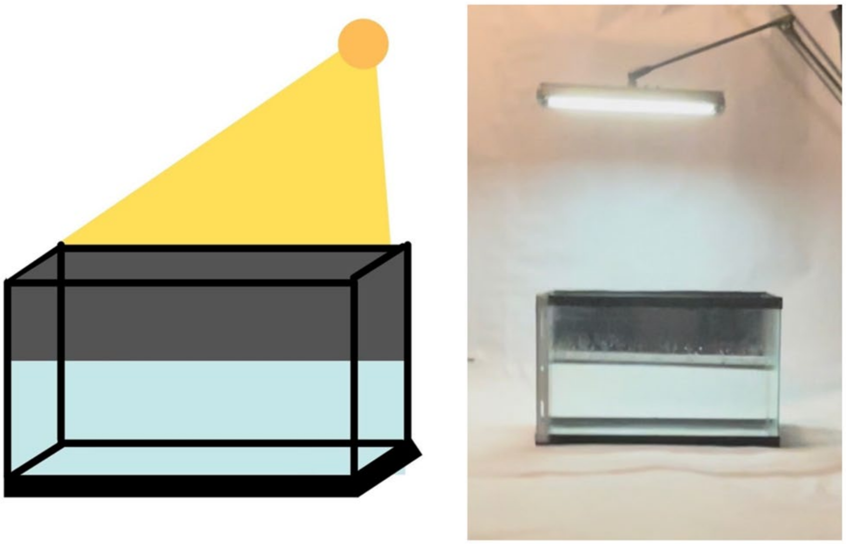


Figure 1. This figure consists of two images. One image is a screenshot of the preliminary design created for the experiment using the graphic design website Canva. The second is an image taken by the researcher shortly before experimentation. It shows how the experimental design was implemented practically.

Before experimentation could begin, a solid experimental design was necessary. A preliminary design was generated based on Sutherland's testing. The crux of Sutherland's experiment was adding clay to a tank filled with water and measuring the rate at which this clay settled with a multimedia recording device. While Sutherland's design effectively determined if salinity affected the settling of clay, it fails to address red tide entirely. This experiment builds off Sutherland's initial design while also making changes to address the question: does salinity impact the settling rate of clay within the range of tolerance of *K.Brevis*?

To gather clear qualitative results, Sutherland used specific lighting conditions when setting up his experiment. This experiment mimics those lighting conditions. For example, Sutherland uses black construction paper to isolate the main source of light and uses a bank of fluorescent bulbs to light the tank from the back. A mock-up was generated using this information with *Canva*, an online graphic design tool. This mock-up was then implemented in real life based on such stipulations *(Figure 1)* (See Appendix for full materials list).

Procedure

To measure any difference in settling rates across salinities, a 10-gallon glass tank was filled approximately halfway with tap water (Figure 2, A). Tap water was used as opposed to distilled water or purified water. Such a choice is justified within the broader context of this research since clay flocculation is used against red tide in marine environments where water is an initially turbid and heterogeneous mixture of particles. Moreover, Sutherland's experiment merely states that they use "freshwater" so it can be assumed using tap water instead of distilled water would have little to no broader impact on results. The water was added to a five-gallon fill line drawn with a marker to maintain accuracy.

Next, various levels of salt were added to the water according to K. Brevis' range of tolerance. The control was a tank with a salinity of 0 parts per thousand (ppt) or just water with no added salt. There were three more trials each with increasing levels of salinity. One had a salinity of 17.5 ppt, the minimum salinity within K. Brevis' range of tolerance. Another had a salinity of 30 ppt, the average salinity of Tampa Bay. A final tank had a salinity of 45 ppt, the maximum salinity within the range of tolerance of K. Brevis. Salinity was measured with a refractometer before each trial for consistency purposes (Figure 2, B). The saltwater was then thoroughly mixed using a wooden stirrer until the salt was completely dissolved. All experiments occurred at approximately 22 to 23 degrees Celsius.

At this time, 567 grams of Kaolin clay were measured using a generic kitchen scale and the clay to water ratio was based on the one used in Sutherland's experiment (Figure 2, C). The amount 567 g is a specific number generated from a ratio stipulated in Sutherland's experiment. Sutherland used 30 g of clay for a tank that had a volume of 1,000 cm3, so this experiment used 567 g of clay for a tank that had a volume of 18927.1 cm3 (5 gallons). In the experiment, White Kaolin clay from the brand Edgar Plastic Kaolin was used, chosen for its plasticity and use in pottery and ceramics, which is a feature of Kaolin clays used in mitigation noted by Woods Hole Oceanographic Institution7.

After the clay was measured, the 567 grams of Kaolin clay were added to the saltwater solution and stirred (Figure 2, D). When solution coloration was consistent, the stirring stick was removed indicating the trial has started (Figure 2, E). The experiment was recorded using a multimedia recording device propped on a tripod approximately two feet away from the tank. The experiment was backlit with a 15-watt fluorescent bulb and the top 15.24 cm of the tank was covered with black construction paper so that differences in light could be easily observed. Differences in the start and stop time (stop time being when the clay is completely settled within the bottom 1 cm of the tank) were recorded to determine if salinity affected settling rates (Figure 2, F)

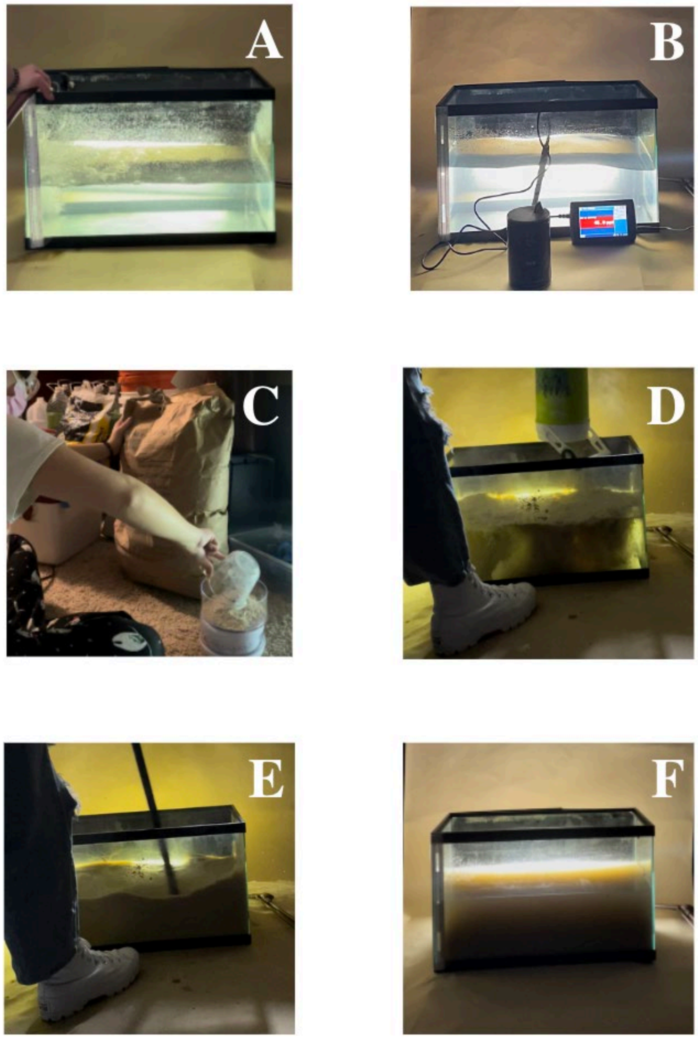


Figure 2. This figure consists of six images labeled A, B, C, D, E, and F. These figures were made by the researcher and show specific steps in the procedure being carried out.

The ultimate goal of this experiment is to determine how salinity impacts kaolin clay settling rates. My hypothesis for the experiment was as follows: if approximately 567 g of kaolin clay is added to 5 gallons of water at varying levels of salinity (0, 17.5, 35, and 45) then the settling rate of the clay will increase because the addition of sodium ions will neutralize the repulsive forces of the clay particles, thus speeding up flocculation.

The data both quantitative and qualitative, were analyzed by charts and pictures respectively. Quantitatively, the height of the clay was measured with a ruler every minute until the clay was settled. This was then recorded in a table comparing the height of the clay and time and analyzed for statistical significance. This data was then displayed qualitatively in a line graph showing the extent to which salinity changed how fast the clay settled. Additional video footage was analyzed to visually compare settling rates side-by-side in real-time. This additional footage allowed me to look back at images taken during the experiment and see how settling rates differed across trials.

While no hazardous chemicals or microorganisms were used in this experiment, precautions were still taken to ensure researcher and environmental safety. Kaolin clay consists of very fine particles, so a face covering and lab goggles were worn to protect against accidental inhalation of the clay as well as any irritation that may occur by exposing the eyes to the fine particles. The 5 gallons of water with settled clay were brought outside where it was carefully disposed of. When dispersed over a wide area, the clay had little to no effect on any grass or other organisms. After each trial, the glass tanks were thoroughly washed and dried before further experimentation. All safety actions involving clay were taken based on precautions recommended by Edgar Plastic Kaolin, or the clay Safety Data Sheet (See appendix for Safety Data Sheet).

Results

This experiment aimed to determine the effect of salinity on clay flocculation specifically within the salinity range of tolerance of *K. Brevis*. Ultimately, the experiment suggests that salinity does not impact the settling rates of Kaolin Clay within the range of tolerance of *K. Brevis*, which is apparent through the quantitative and qualitative data gathered from the experiment.

Qualitatively, images demonstrate a minute-by-minute difference between settling rates. *Figure 3* shows the height of the settling clay at exactly seven minutes. These particular images are footage from Trial 1. A digitally rendered ruler was added to the left of each image. The red line indicates approximately where the settling line ends. As seen in the images, this line remains very similar across the four salinities. To the eye or qualitatively, salinity seems to have had little effect on settling rates.

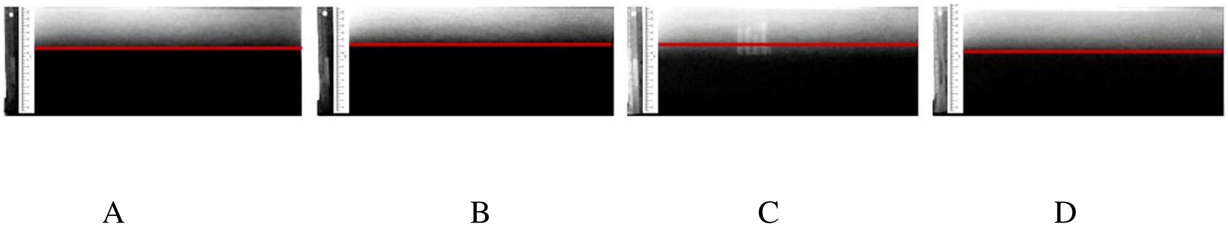


Figure 3. This figure is a combination of four images taken from video footage of experimentation (Trial 1). Footage has been edited to gray-scale for clear results. Red markers have been added as an additional visual cue.

This idea is only furthered by the quantitative data, which showed that clay settling was consistent across salinities, as is evident from the data presented in Table 1. The height of the clay across salinities was similar as time progressed. (See appendix for individual trial data). The similarity of these values lends itself to the conclusion: differences in salinity had little effect on the speed at which the clay settled. This similarity is made more clear when data is presented vertically, across time in a line graph (Figure 4).

Table 1. Mean Height of Clay Settling Over Time. This table represents the average height of the for each of the 20 tanks.

Height	A (0 Ppt)	B (17.5 Ppt)	C (30 Ppt)	D (45 Ppt)
0	15.24	15.24	15.24	15.24
1	15	14.7	14.4	14.4
2	14.3	13.82	13.68	13.38
3	13.28	12.92	12.46	12.28
4	12.14	11.86	11.5	11
5	11.12	10.96	10.22	9.98

6	9.92	9.58	9.32	8.82
7	9	8.7	8.4	8.16
8	7.94	7.84	7.68	7.26
9	6.9	7.08	6.74	6.26
10	6.02	5.84	5.36	5.24
11	5.08	5.04	4.36	4.08
12	3.94	4.06	3.3	3.16
13	3.38	3.5	2.28	2
14	2.08	2.12	1.88	1.54
15	1.62	1.2	1	1
16	1	1	1	1

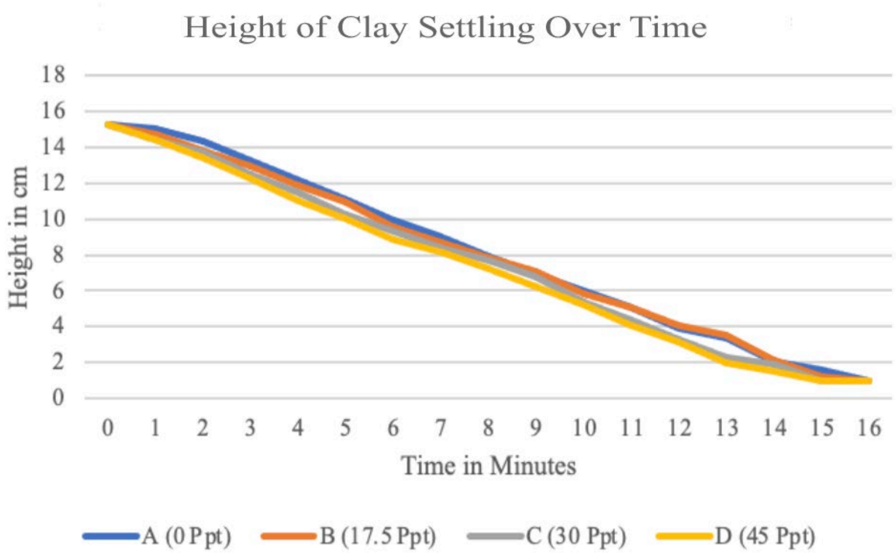


Figure 4. This graph shows the mean values for each trial every minute. The graph illustrates overlapping lines, proving similar settling patterns across salinities.

In all of the experiments, the clay was finished settling by 16 minutes. Additionally, experiments conducted at 45 ppt settled faster than those at the lowest salinity of 0 ppt. However, the difference between these averages is not drastic enough to say that salinity would impact the settling of clay in the context of red tide mitigation. This conclusion is made clear through statistical analysis that confirms results cannot be directly attributed to the independent variable of salinity.

Statistical Analysis

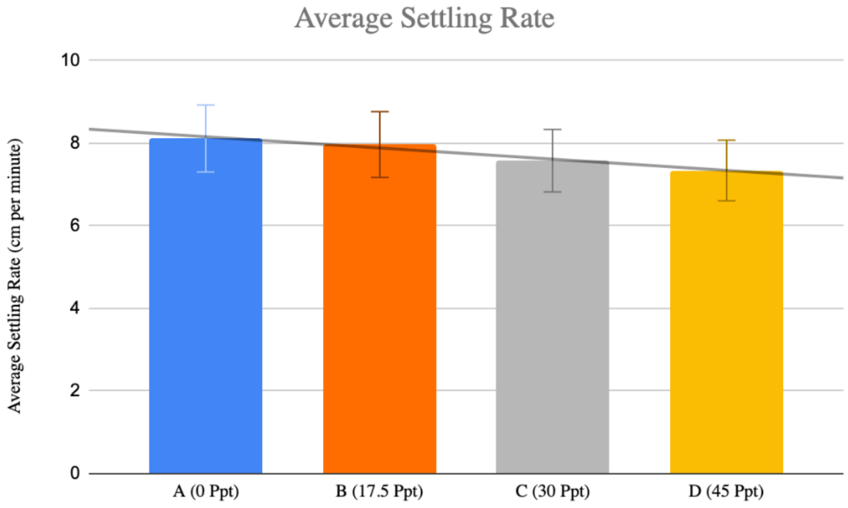


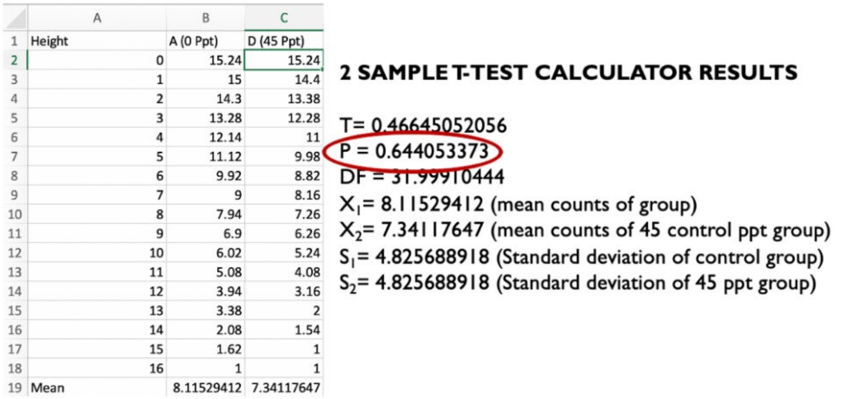
Figure 5. This bar graph displays the average settling rate of each salinity. Each bar represents an average of 5 trials. The graph included a trendline as well as error bars indicating a downward trend and significant experimental overlap.

Across all 20 trials, the results were very similar, an idea emphasized by the error bars in *Figure 5*. These error bars thoroughly overlap, suggesting a p-value higher than 0.05 and statistically non-significant results, ergo, results are unlikely to be attributable to a specific cause. To confirm this assumption, a standard statistical analysis *T-Test* and *ANOVA* test were performed.

In order to determine the statistical significance, data was reorganized into two columns, and the control and the highest salinity were compared via a standard student *T-Test*. These data sets were chosen because, according to the mean data presented in *Figure 5*, these were the two most different data sets. If these groups were non-significant, it is sufficient to say that the same will apply to the middling data groups.

T-Test

Null hypothesis- there is no statistical difference between mean settling rates of groups (45 ppt and control).



	A	B	C
1	Height	A (0 Ppt)	D (45 Ppt)
2	0	15.24	15.24
3	1	15	14.4
4	2	14.3	13.38
5	3	13.28	12.28
6	4	12.14	11
7	5	11.12	9.98
8	6	9.92	8.82
9	7	9	8.16
10	8	7.94	7.26
11	9	6.9	6.26
12	10	6.02	5.24
13	11	5.08	4.08
14	12	3.94	3.16
15	13	3.38	2
16	14	2.08	1.54
17	15	1.62	1
18	16	1	1
19	Mean	8.11529412	7.34117647

2 SAMPLE T-TEST CALCULATOR RESULTS

T= 0.46645052056
P = 0.644053373
DF = 31.99910444
X_1= 8.11529412 (mean counts of group)
X_2= 7.34117647 (mean counts of 45 control ppt group)
S_1= 4.825688918 (Standard deviation of control group)
S_2= 4.825688918 (Standard deviation of 45 ppt group)

Figure 6. This image is a screenshot of the means of experimental groups 0 ppt and 45 ppt from Microsoft Excel. To the right is a digital rendition of the calculator output when these values were compared in a standard two-sample T-Test. Red arrows were added to highlight critical information.

40.644 is not less than 0.05
The result is not significant when α=0.05

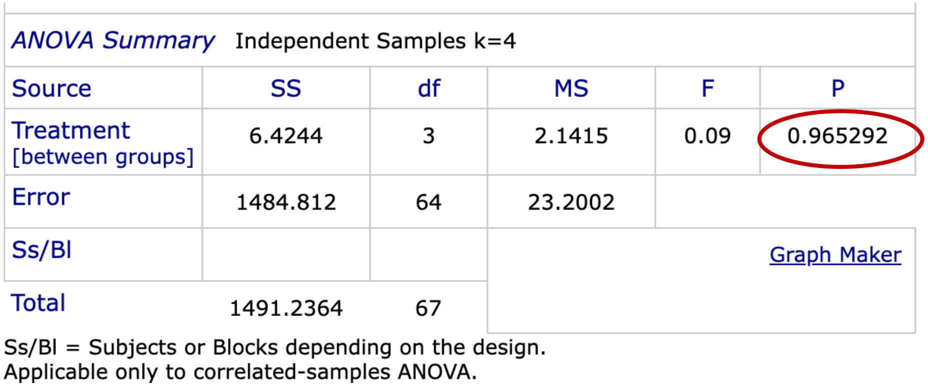
Fail to Reject Null Hypothesis

The results from the *T-Test* show that the findings of the experiment cannot be attributed to the salinity of the water *(Figure 6)*. Because the result is non-significant, there is no way to prove the small difference in settling rates that occurred because of the change in salinity or another external factor. Due to this test, it looks likely that salinity didn't impact the settling rate of the clay. However, it is still necessary to evaluate this difference among all groups in the experiment, hence the *ANOVA* test.

The *ANOVA* test is used to indicate a statistically significant difference between the mean values of a data set. In the case of this experiment, there were four groups being compared, 0 (control), 17.5, 30, and 45 ppt. The results of this comparison can be seen in *Figure 7.*

ANOVA Test

Null hypothesis- there is no statistical difference between mean settling rates of groups (control , 17.5, 30, and 45 ppt)



ANOVA Summary Independent Samples k=4

Source	SS	df	MS	F	P
Treatment [between groups]	6.4244	3	2.1415	0.09	0.965292
Error	1484.812	64	23.2002		
Ss/Bl					
Total	1491.2364	67			

Ss/Bl = Subjects or Blocks depending on the design.
Applicable only to correlated-samples ANOVA.

Figure 7. This image is a screenshot of the summary statistics for the ANOVA test from VassarStats (a website for statistical computation.) A red marker was added to highlight critical information.

The p-value of the results is 0.965… which is much greater than 0.05. So, these results corroborate the findings of the T-Test, furthering the idea that salinity had no significant impact on clay settling rates.

While these tests both indicate non-significant results, it is important to consider that this does not mean salinity had no impact on settling rates whatsoever. It simply suggests that statistically, it cannot be proven that salinity was the only reason for the differing rates. One possible explanation for such a difference could be uncontrollable inconsistencies during experimentation. For example, temperature fluctuations may have occurred. While attempts were made to conduct the experiment between 22 to 23 degrees Celsius, minor fluctuations could have impacted the results. Additionally, human error or experimenter bias may have influenced the measurements. Because measurements were taken by hand every minute, there is a possibility the measurements were taken invariably after the one-minute mark. For example, one trial might have been recorded at 1 minute and 0 seconds, while another might have been recorded at 1 minute 3 seconds, and so forth. So, there are possible explanations for the variations in settling rates beyond the independent variable of salinity.

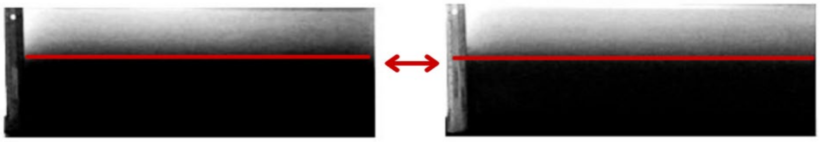
That being said, the statistical analysis proves the experiment is a good indicator that salinity will not have a drastic enough impact on settling rates to affect its role in red tide mitigation.

Discussion

My hypothesis stated that settling rates would increase with an increase in salt. Specifically, it was supposed that if approximately 567 g of kaolin clay is added to 5 gallons of water at varying levels of salinity (0, 17.5, 35, and 45) then the settling rate of the clay will increase because the addition of sodium ions will neutralize the repulsive forces of the clay particles, thus speeding up flocculation. However, my results and statistical analysis show that it does not increase within the range of tolerance of *K. Brevis*.

Synthesizing Research

Results at 7 minutes

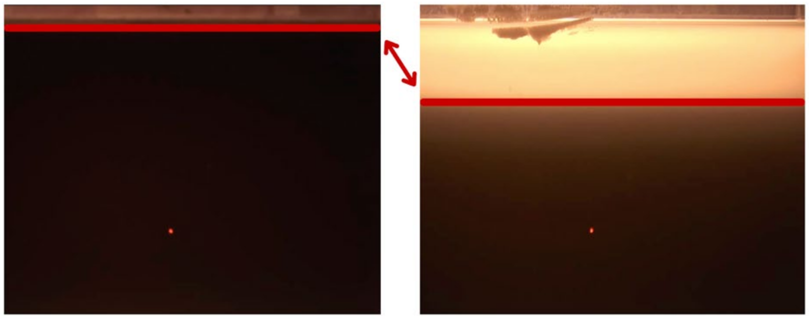


Freshwater (control)

Saltwater (about 45 ppt)

Figure 8. This figure is a combination of two images taken from video footage of experimentation (Trial 1). Footage has been edited to gray-scale for clear results. Red markers have been added as an additional visual cue.

Sutherland results at 7 minutes



Sutherland- Freshwater

Sutherland- Saltwater
(about 70 ppt)

Figure 9. This figure is footage from Sutherland's experimentation. Images were downloaded from the University of Alberta's webpage and edited to include red clarifying markers.

My hypothesis was largely based on the findings of Dr. Bruce Sutherland from the department of physics at the University of Alberta. *Figures 8 and 9* above show the results of this experiment compared to Sutherland's. Two main ideas can be extrapolated from this comparison.

First, the height of the clay in the freshwater in Sutherland's is much greater than the height of the clay in the freshwater in this experiment. One can determine that this difference is due to the scale at which the experiment was performed. Sutherland's experiment took place in a tank measuring 30cm in height, 5cm in width, and 20cm in length, nearly 13 times smaller than the tank used in this experiment.

Second, the difference between the height of the clay in freshwater and the height of the clay in saltwater is far more apparent in Sutherland's experiment. This is mostly because the water used in Sutherland's saltwater tank has a much higher salinity. Sutherland's saltwater footage is of water that has a salinity of about 70 ppt, a salinity much greater than the maximum salinity used here- 45 ppt. But unlike Sutherland's, this experiment was conducted under the pretense of red tide mitigation, therefore, accounting for such high salinities (outside the range of tolerance of *K. Brevis*) was unnecessary.

So, there are many justifiable differences between this experiment's results and Sutherland's. By comparing the two results, it becomes clear that in areas of exceedingly high salinities, flocculation is accelerated. This would be useful information if there was ever a need to use clay flocculation in areas of extremely high salinities. Before using clay flocculation in extremely salty water, further research would be necessary to then determine if this accelerated flocculation rate would hinder the objective of the clay. Yet, the results of this study indicate that such a case would not pertain to *K. Brevis* since salinity did not have a significant impact on the settling rates of Kaolin clay, supporting the idea that the mitigation technique can be used with consistent results across all salinities *K. Brevis* might be found.

Even so, further experimentation is required to determine how other factors not addressed in this research, such as temperature, pH, or currents would affect the technique. Only then will it be proven the strategy applies to all *K. Brevis* blooms. What's more, not all Harmful Algae blooms consist of K. Brevis[17]. While it is true that *K. Brevis* is the most predominant harmful algae. To address all potential instances of red tide, specific research into all HABs is required.

Conclusion

The search for the most effective red tide mitigation strategy is far from over. While this experiment does serve as a good indicator that salinity does not affect clay flocculation, further research is necessary to explore other nuances of the mitigation strategy. For example, research is necessary to determine how pH or tidal fluctuations might impact flocculation. Salinity isn't the only variable that might affect clay flocculation in Tampa Bay.

Moreover, this experiment does not address the use of additives such as Poly Aluminum Chloride to enhance flocculation. The addition of such chemicals has been proven to enhance flocculation and could possibly be impacted by changing salinities7. While experimentation using such additives is currently underway, it is necessary to see how this additive would then react in different salinities.

Additionally, experimentation that addresses how salinity affects removal efficiencies could be beneficial to a wider understanding of how clay interacts with salt. An experiment that uses K. Brevis cells and measures their presence in a water column before and after the clay is added at various salinities would be ideal to evaluate how salinity affects removal efficiencies. But for now, it can be said that clay flocculation can be used in Tampa Bay (regardless of salinity) as a way to effectively mitigate the long prevailing problem of red tide.

Limitations

There were anticipated challenges to implementing the chosen research method that gave way to some limitations within this experiment. For example, final settling times were an approximation since, due to the ambient nature of the settling clay, the exact time the clay is done settling can't be recorded. To address this "settled" was simply defined as when settling clay reached the bottom 1 cm of the tank.

Additionally, several repetitions of the experiment were conducted to obtain the most accurate results. 5 trials were conducted to examine the difference between 4 groups of data for a total of 20 runs.

Also, because of a high likelihood of visual inaccuracies in experiment results, a statistical analysis needed to be run to prove the hypothesis. The statistical analysis ultimately proved that since the results were non-significant, visual inaccuracies could be a contributor to the difference in data among groups.

Despite these limitations, this experiment lends itself to key conclusions in the field of red tide mitigation. While experimentation has been done on salinity's effect on clay flocculation and flocculation is widely studied, this research fills a critical gap in this research as it applies to red tide. The results of this experiment show that salinity has no significant effect on clay flocculation, specifically within the salinity range of tolerance of K. Brevis, information that is critical to the practical application of clay in the Tampa Bay Area.

Acknowledgments

Thank you to Mrs. Orrgeo for her endless patience and amazing advice, Mr. Koslin for his support, and J.W. Mitchell High School and the AP Capstone Program for this amazing opportunity.

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