

Concentrations of Substances in Freshwater Environments

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May 15, 2019

Science Research and Design-6th

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Abstract

This project served to ascertain whether or not the health of the marsh in Jones State Forest is sufficient to support aquatic lifeforms. Ten samples were taken from specific locations across the marsh; eight chemical tests were run on each of them to determine the quantity of certain chemicals in various areas around the marsh. The data from these tests were compared with official water safety guidelines to determine if the water was optimal for the support of aquatic life. Data on dissolved oxygen and the pH for the marsh was compared with Clear Lake and Murky Lake to assess the difference in health quality. It was found that the chemical composition of the marsh water was too poor to house most types of aquatic creatures, mainly due to only 27 percent of dissolved oxygen, a low hardness level, and a moderately acidic pH level of 6. It was also determined that the twin lakes had a high health quality, with a pH very close to 7 and a dissolved oxygen percentage greater than 50 percent, enabling it to be a habitat for many organisms.

Concentrations of Substances in Freshwater Environments

Water is the most important aspect of life on Earth. All organisms need some quantity of water to survive. Recently, pollution has reached into bodies of water, decreasing biodiversity by killing off organisms (Denchak, 2019). Oceans and lakes have become even more toxic as global warming causes water to become more acidic (Johnson & White, 2014). Other harmful sources of pollution include human waste treatments and agricultural runoff (Johnson & White, 2014).

Chloride is one of the most important chemicals in water. This is because it is one of the main components of salt, which is a necessity in aquatic environments (Hunt, Herron, & Green, 2014). Though chlorides in salts provide nutrients for organisms, a recent increase in chloride has caused many freshwater organisms, especially algae and planktonic crustaceans, to die. These increases are due to human waste, especially road salts, and agricultural runoff (Hunt, Herron, & Green, 2014).

In addition to chloride, phosphorus and nitrogen are crucial to plant life in freshwater environments (Perlman, 2017; Jin & Bierma, 2018). Also like chloride, an excessive supply of these chemicals can cause detrimental effects to the environment. When there is excessive nitrogen and phosphorus in the lakes, algae grows to the point that it deprives other organisms of oxygen and sunlight (Perlman, 2017; Jin & Bierma, 2018).

Carbon dioxide is another chemical that has caused marine life to decay. The overload of carbon dioxide that has been emitted into the air due to the burning of fossil fuels has heated the environment and acidified the oceans (Johnson & White, 2014; Rivera, 2019). This has caused many organisms to die due to the dangerous pH level, the decrease in the substance carbonate, and the increase in metals (Johnson & White, 2014; Rivera, 2019). An increase in metals

becomes toxic to organisms and a decrease in carbonate prevents organisms from building their skeletons.

Additionally, silica is one chemical that can prevent the growth of algae and decrease the amount of carbon dioxide in the water since it promotes the growth of diatoms (another type of algae) (NualgiAquarium, 2014). Though diatoms are algae, they produce oxygen, therefore stimulating the growth of other organisms. They also compete with other algae, decreasing the amount of algae that is depriving the fish of oxygen (NualgiAquarium, 2014). Furthermore, when diatoms die, they become food for fish.

However, the rise of synthetic chemicals in aquatic environments have caused death to many of aquatic organisms. Synthetic chemicals are highly toxic and can create acute or chronic effects on and in fish, algae, and invertebrates (Malaj et al., 2014). Excess amounts of organic chemicals come from sewers, fossil fuels, human waste products, and agricultural runoff.

All of the chemicals above were used to determine the overall health of aquatic ecosystems. Along with these chemicals, two properties were necessary to test in order to determine the pollution of the environment: pH and hardness. pH helps determine how well an ecosystem is doing, since all organisms can only live in a specific pH range. Water hardness is the sum of calcium and magnesium ions in water (Pourkhabbaz, Kasmani, Kiyani, & Hosynzadeh, 2011). It protects organisms from metal toxicity by forcing metals to compete with them over roaming freely in the water.

All these chemical and property tests were needed to be measured at least annually in aquatic environments to ensure the health of the organisms. Jones State Forest, located in The Woodlands, is one such place that has four aquatic environments that were needed to be

measured: the Northern lake, two twin lakes and a marsh. Every year the water quality in these areas is measured by the Academy of Science and Technology. This year, water quality was determined based on the pH of the water, the calcium and magnesium hardness, and the concentrations of phosphate, nitrate, chloride, silicate, zinc, and carbon dioxide. Samples were taken from the marsh, and data from the twin ponds and the northern lake were collected from the other Aquatic Chemistry groups. All this data was then used to compare the states of the different lakes and the marsh.

In the forest, ten samples were taken from different parts of the marsh and were tested to determine the pH and the concentrations of different substances. This data was then shared with 6th Period's Aquatic Biology group to determine the effects of the water quality on the organisms that live there. This was done by using the Aquatic Biology group's data on biodiversity along with the collected data on the water concentrations.

The samples were collected using a modified water gun named the Waterinator 2000. The Waterinator consists of multiple filters that decrease the mud content in samples. The carbon dioxide test was taken on the Floaty-Boaty PortaTable 3001 to get accurate measures from fresh water. The PortaTable consists of kickboards on top of an inner tube, making a perfect table to experiment on while in the wetland.

Methods

While in the forest, long pants were worn to minimize injuries from the trees. Any snakes or other dangerous organisms were not touched, which minimized the chance of being bitten or getting into contact with poison. Waders, with life jackets on, were worn when entering the water to decrease the chance of drowning and getting water on clothes. Due to the use of irritant

chemicals-such as sulfide reagent, chloride reagent, phosphorus reagent, silica reagent, etc-that could cause cancer, eye irritation, poison, burns, and lung problems (see safety data sheets for more information), goggles and latex gloves were worn, an eye sink was nearby, and a respirator and teacher was nearby when testing the water samples. All of the chemicals were also handled carefully to minimize it being spilled and accidentally getting on something or someone. All of the chemicals were diluted with water before pouring down the sink to prevent the chemicals from harming the environment. Glassware was used carefully and was not dropped.

Materials

- 10 mason jars that can hold 400 mL each
- 3 Medium-sized waders
- One pack of medium rubber bands
- One pack of 100 coffee filters
- One metal mesh kitchen drain
- Duct tape
- Standard sized inner tube
- Two kickboards
- One wine cork
- Stream Machine water pump toy, 42 cm. long
- Black marker
- GPS accurate to the millionth degree
- Low range phosphate testing kit
- Silica testing kit

- Zinc octa-slide 2, 0.0-1.4 ppm testing kit
- Carbon dioxide testing kit
- Hardness testing kit
- Kitchen timer
- Hand-held power drill
- Nitrate Nitrogen test kit
- Precision pH test kit
- Chloride test kit

Procedures

The Waterinator 2000 was built by taking the pull pump water gun and removing the plastic part that is over the nozzle to make a basic pump. A hole 1.905 cm. in diameter was drilled 4 cm. away from the opening of the pump. A coffee filter was wrapped over the nozzle of the water gun and duct taped to it so that the coffee filter's bottom was secured over the nozzle. The duct tape only touched the sides of the coffee filter, not the bottom (see Figure 1). Three more coffee filters were added to the gun in the same fashion.

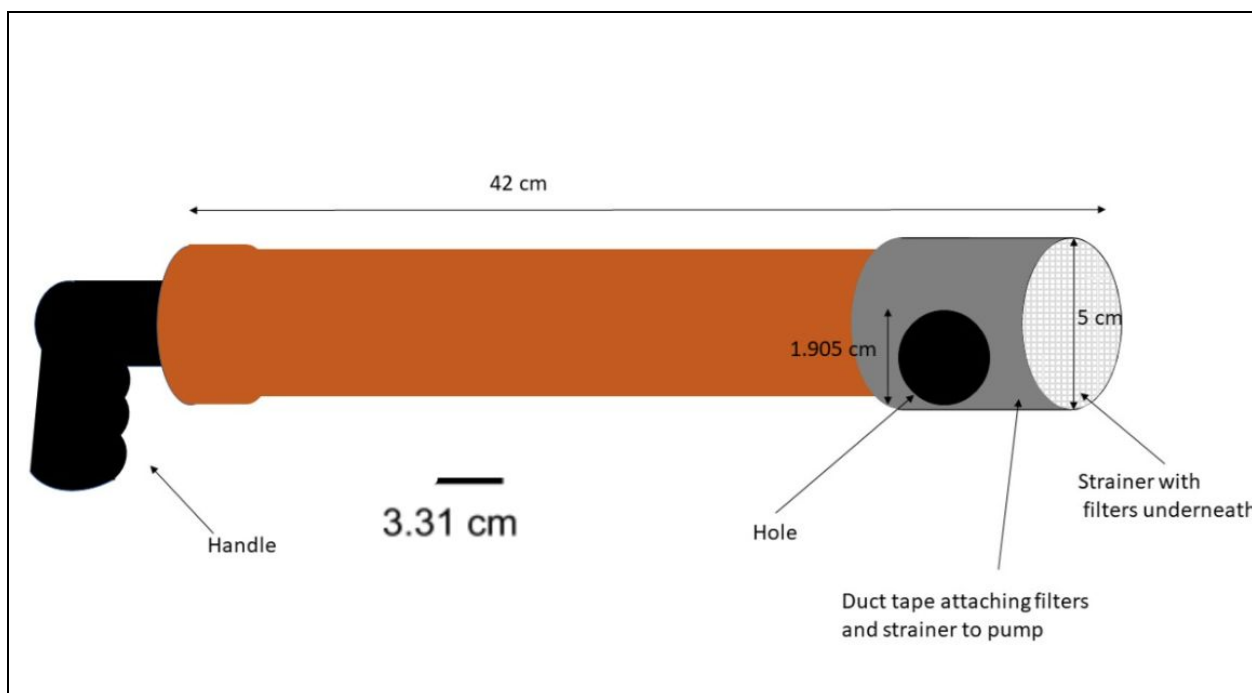


Figure 1: Waterinator 2000 - A pump with strainers to filter the mud from the water

The kitchen drain filter was placed over the opening the same way the coffee filters were placed over the opening and duct tape was wrapped around the drain filter, where the tape touched both the drain filter and the pump. This was repeated as many times as necessary to make the drain stick. The cork was inserted in the hole that was drilled earlier.

The Floaty-Boaty PortaTable 3001 was built by inflating the inner tube with air from the lungs until it was firm. The two kickboards were placed adjacent to each other so that the longer side of one kickboard touched one of the longer sides on the other kickboard. The kickboards were laid on a flat surface in this position. Duct tape was used to hold the kickboards together in this position so that they could be picked up roughly and still stay together. The duct-taped kickboards was placed over the inflated inner tube so that they covered the hole in the middle of the inner tube. The kickboards were laid as flatly as possible. Duct tape was used to tape the kickboards down to the inner tube. Five yards of duct tape were used to make sure that the

kickboards were secure enough to the inner tubes to withstand rough usage or withstand being dropped from five feet.

For the experiment, the Waterinator 2000's tip was put into the water within two meters of the northern edge of the forest's marsh (see Figure 2). The handle was pulled back to fill the Waterinator with water. The cork was pulled out over a mason jar to fill the jar with 300 mL of water. This water-filled jar was a sample. Once the sample was collected, the Waterinator was reset by pushing the handle all the way in.



Figure 2: Sample areas in the marsh - The abbreviations denote where the samples were taken:

north, west, east, south, middle

The lid was screwed onto the mason jar tightly to prevent water from leaking out. The jar was labeled with GPS coordinates and the sector of the marsh it was taken from (the north: N) for identification. The last paragraph and this paragraph of steps was repeated with the southern

(S), northeastern (N), southwestern (SS), northwestern (NW), western (W), eastern (NE), and southeastern (E) edges of the marsh (see Figure 2). This was done again with one sample in the center of the very middle of the marsh (MM) and another sample in the middle of the Eastern side of the marsh (M2).

For the low-range phosphate test, a test tube was filled to the 10 mL line with untreated sample water (Lamotte, n.d.c). It was then inserted in the rear hole on the top of the Low Range Comparator (see Figure 3). Another test tube was filled with 10 mL of sample water. A 1.0 mL pipet was used to add 1.0 mL of Phosphate Acid Reagent. This tube was then capped and swirled.

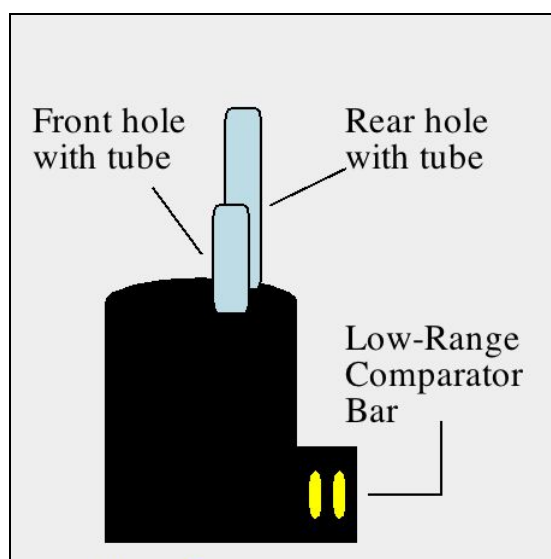


Figure 3: Low-Range Phosphate Comparator (not drawn to scale)

A 0.1 mL pipet was used to add one level measure of Phosphate Reducing Reagent to the tube. The tube was then capped and swirled. After five minutes-timed using the kitchen timer-the cap was removed from the test tube. The tube was inserted in the front hole on top of the Low Range Comparator. The Low Range Comparator Bar was inserted into the Low Range

Comparator. The comparator was positioned so that light shone down through the test tubes. The comparator was tilted until the color standards-a chart that the sample is compared to- and sample were illuminated. The color of the sample's reaction was matched to the color standards. The result from the Low Range Comparator Bar was read and recorded as "ppm Orthophosphate".

For the hardness test, a test tube was filled to the 12.9 mL line with sample water (Manuals Directory, n.d.). Five drops of Hardness Reagent #5 was added, then the tube was swirled. One Hardness Reagent #6 Tablet was added, with the tube capped and swirled afterward until the tablet disintegrated. If hardness was present, the solution turned red and the next procedures for the test were taken; otherwise, the solution turned blue and the rest of the instructions were skipped and the data was marked "zero ppm total hardness as CaCO_3 ".

The Direct Reading Titrator (see Figure 4) was filled with Hardness Reagent #7 then inserted into the center hole of the test tube cap. While the tube was gently swirled, the plunger was slowly pressed until the red solution changed to clear blue. The test was read directly from the scale on the large ring where the Titrator meets the Titrator barrel. The data was recorded as "ppm total hardness as CaCO_3 ".

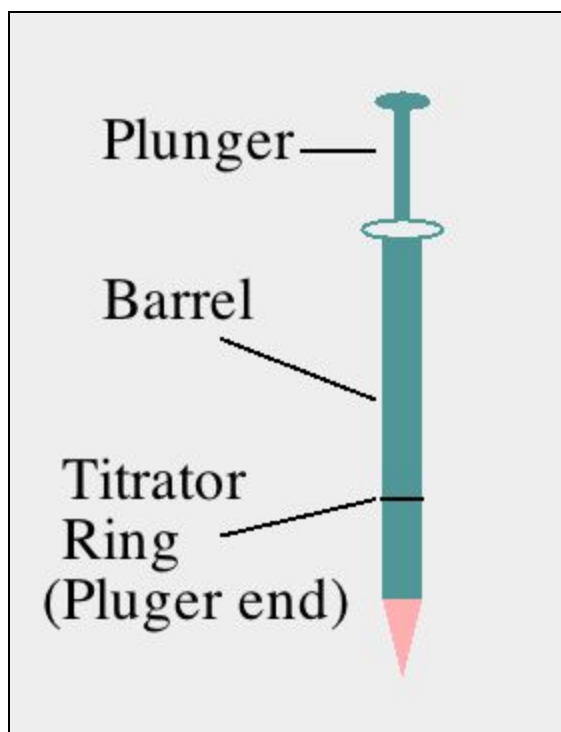


Figure 4: Direct Reading Titrator (not drawn to scale)

For the zinc test, the test tube was filled to the 10 mL line with sample water (LaMotte, n.d.g). Five drops of Zinc Conditioning Reagent were added, then the tube was capped and swirled. After waiting one minute, the 0.5 gram spoon was used to add one level measure of Zinc Reagent Powder. The tube was then capped and shaken for 15 seconds and no longer. After waiting one minute, the Zinc Octa-Slide 2 Bar was immediately placed into the Octa-Slide 2 Viewer (see Figure 5). The Octa-Slide 2 Viewer was held so that non-direct light entered through the back of the Viewer. The sample color was matched to a color standard within 30 seconds. This was recorded as “ppm zinc”.

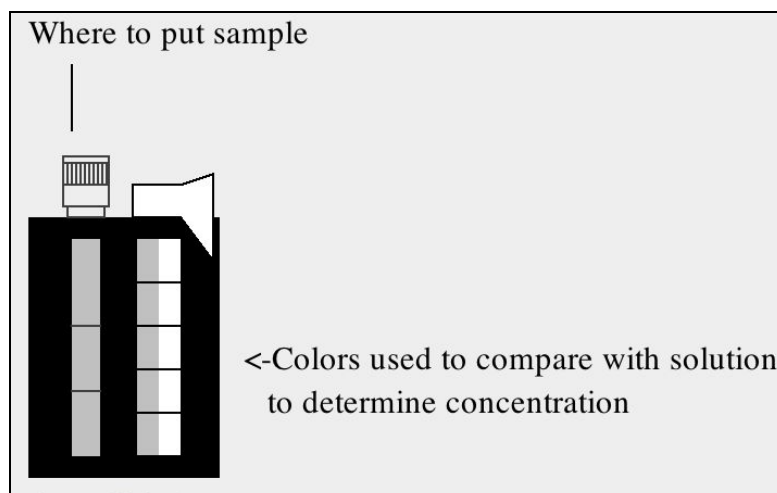


Figure 5: Octa-Slide 2 Viewer (not drawn to scale)

For the carbon dioxide test, the test tube was filled to the 20 mL line with sample water (LaMotte, n.d.a). Two drops of Phenolphthalein Indicator, 1% were added. If the solution remained colorless, the next step was taken; if the solution turned red, the rest of the procedures were skipped and the data was marked as “zero ppm Carbon Dioxide”. The Direct Reading Titrator (see Figure 4) was filled with Carbon Dioxide Reagent B. It was then inserted into the center hole of titration tube cap. While the tube was gently swirled, Carbon Dioxide Reagent B was added, one drop at a time, until a faint pink color was produced and the color persisted for thirty seconds. The test result was read directly from the scale where the large ring on the Titrator meets the Titrator barrel. This was recorded as “ppm Carbon Dioxide”.

For the silica test, the test tube was filled to 5 mL line with sample water (LaMotte, n.d.f). Seven drops of Silica Reagent #1 were added, and the tube was capped and mixed, by inverting, four times. Six drops of Silica Reagent #2 were added. The tube was capped and mixed. After waiting five minutes, six drops of Silica Reagent #3 were added and mixed into the solution. The solution sat for two minutes. The pipet was used to add two drops of Reducing

Reagent. The solution was then mixed. Within ten seconds, a blue color appeared if silica was present. If the blue color did not appear, the rest of the steps for the silica test were skipped and the result was recorded as “zero ppm Silica”. The Silica Octa-Slide 2 Bar was inserted into the Octa-Slide 2 Viewer (see Figure 5). The test tube was inserted into the Octa-Slide 2 Viewer. The color of the sample was compared to a color standard. This number was recorded as “ppm Silica”.

If the test color was darker than the 10.0 ppm standard, the test was repeated on the diluted sample since the color denoted that there was still more silica in the sample. The pipet was used to add 0.5 mL of water sample to the test tube. The sample was diluted to the 5 mL line with deionized water. The steps in the previous paragraph were repeated. The result was multiplied by ten and recorded as “ppm Silica”.

For the nitrate nitrogen tablet kit, the Nitrate-Nitrogen Octa-Slide 2 Bar was inserted into the Octa-Slide 2 Viewer (see Figure 5) (LaMotte, n.d.d). A test tube was filled to the 5 mL line with sample water. One Nitrogen #1 Tablet was added, and the solution was capped and mixed until the tablet disintegrated. One Nitrate #2 CTA Tablet was added. The test tube was immediately placed into the protective sleeve. It was then capped and mixed for two minutes in order to disintegrate the tablet. After letting the solution sit for five minutes, the test tube was removed from the protective sleeve. The test tube was then inserted into Octa-Slide 2 Viewer. The Octa-Slide 2 Viewer was held so non-direct light entered through the back of the Viewer. The Octa-Slide 2 Bar was inserted into the Viewer. The reacted sample was inserted into the top of the Viewer. The color of the reaction was matched to the color standards. The result was recorded as “ppm Nitrate Nitrogen”

For precision pH kit, the Wide Range pH Octa-Slide 2 Bar was inserted into the Octa-Slide 2 Viewer (see figure 5) (LaMotte, n.d.e). A test tube was filled to the 10 mL line with sample water. Ten drops of Wide Range pH Indicator were added. The tube was then capped and mixed. The tube was placed into the Octa-Slide 2 Viewer. The Octa-Slide 2 Viewer was held so non-direct light entered through the back of the Viewer. The sample color was matched to a color standard. This was recorded as pH.

For chloride test kit, a test tube was filled to the 15 mL line with sample water. One drop of Phenolphthalein Indicator, 1% was added (LaMotte, n.d.b). If the solution remained colorless, the next step was skipped. If the solution turned a pink color, Sulfuric Acid, 0.5N was added one drop at a time, with the solution mixed after each drop, until the pink color disappeared. Three drops of Chloride Reagent #1 were added, and the solution was capped and mixed. The solution turned yellow afterwards. The Direct Reading Titrator (see Figure 4) was filled with Chloride Reagent #2. The Titrator was inserted in the center hole of test tube cap. While the tube was gently swirled, the plunger was slowly pushed to add Chloride Reagent #2, one drop at a time, until the yellow color changed to orange-brown. The test result was read directly from the scale where the large ring on the Titrator met the Titrator barrel. If the plunger tip reached the bottom line on the Titrator scale (200 ppm) before the endpoint color change occurred, the Titrator was refilled and titration continued. When the test result was recorded, the original amount of reagent dispensed (200 ppm) was included. This was recorded as “ppm Chloride”.

Results

Ten samples were taken from the marsh at different coordinates, and each one was tested for their pH and hardness, and their concentrations of nitrate, phosphorus, carbon dioxide (CO₂),

silicate, zinc, and chloride. This data was shared with the other Aquatic Chemistry groups and vice versa (see Appendix). Data of organisms in the lakes was also shared by the Aquatic Biology group.

The Aquatic Biology group found small frogs and turtles in the marsh, though there weren't very many. In contrast to this, there were fish, frogs, snakes, and turtles in the twin lakes.

For the team data, the percent saturation of dissolved oxygen was seventy-two percent for Clear Lake, fifty-three percent for Murky Lake and twenty-seven percent for the marsh. The average pH for Clear Lake, Murky Lake, and the marsh was 6.916, 7.05, and 6, respectively. The standard deviation of each of the groups was calculated using the formula:

$$\sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$$

where x is an individual data point, \bar{x} is the mean of the data, and n is the number of plants in the group.

Standard Error formula:

$$\frac{\sigma}{\sqrt{n}}$$

where σ is the standard deviation and n is the number of plants in the group. Example for Group: $\frac{0.24}{\sqrt{2}} = 0.1697$ for Clear Lake

An ANOVA was calculated to compare the pH values between the bodies of water. With an F-value of 13.9 and a p-value of 0.00047, showing that there was a significant difference between the pH values of the groups. To determine which group caused this difference, the standard deviation and standard error was calculated for each body of water and displayed in Figure 6. The standard deviation was 0.24 for Clear Lake, 0.292 for Murky Lake, and 0.408 for

the marsh. The standard error was 0.1697 for Clear Lake, 0.13 for Murky Lake, and 0.129 for the marsh.

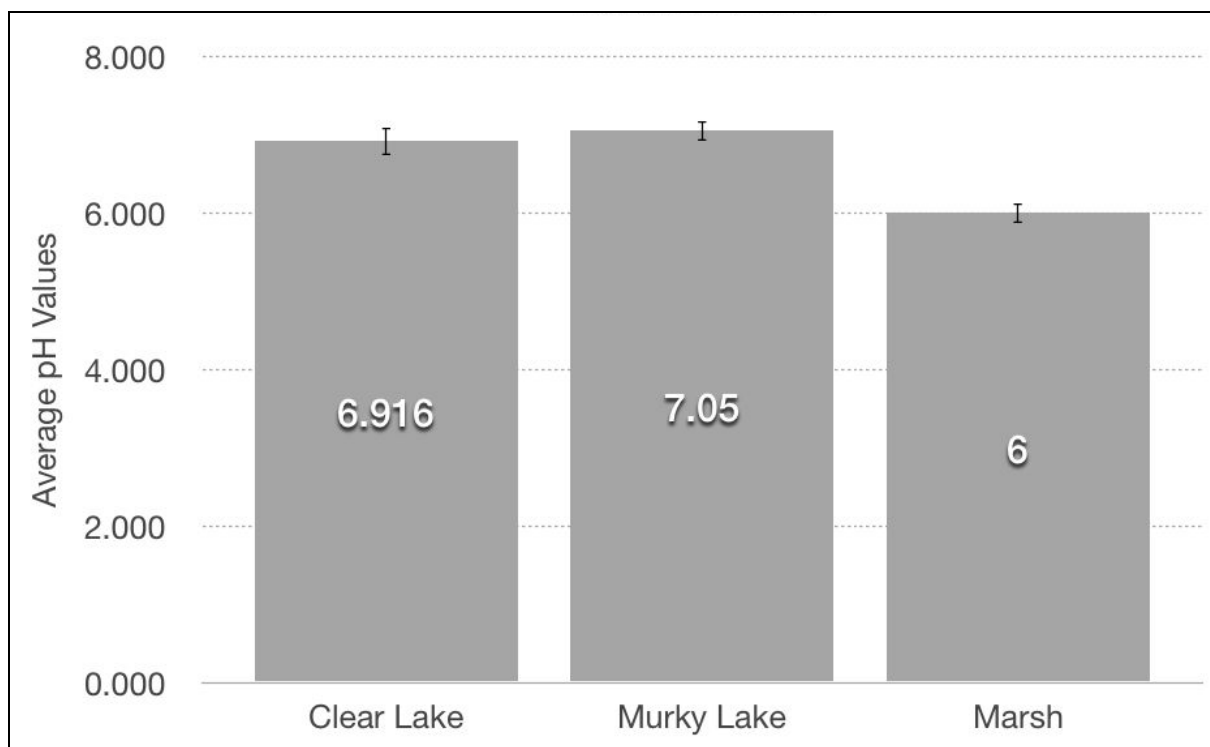


Figure 6: Average pH Values of Bodies of Water

For the individual marsh data, the measurements for phosphorus, nitrate, zinc, silicate, and pH all fell within the same range for each of the areas we tested, showing uniformity for all of these chemicals and the pH throughout the marsh. Chloride and CO₂ varied a little more, with a range of nineteen ppm for CO₂ and a range of thirteen ppm for chloride. Bar graphs of the chloride and CO₂ measurements of each of the individual data points, along with the average with and without the outliers, is shown in Figure 7 and 8. However, this variance is not that significant due to the large data points only being high for two of the areas: 42 (Special South) and 40 (Northeast) for CO₂ and 32 (North) and 32 (North Middle) for chloride. On the other hand, hardness varied widely with a range of seventy ppm (see Figure 9 for individual data with

the average). Two of the values were lower than the accepted range of 40-120 ppm, being at 32 ppm and 38 ppm. Most of the hardness data collected was quite low compared to the normal standard of 70 ppm.

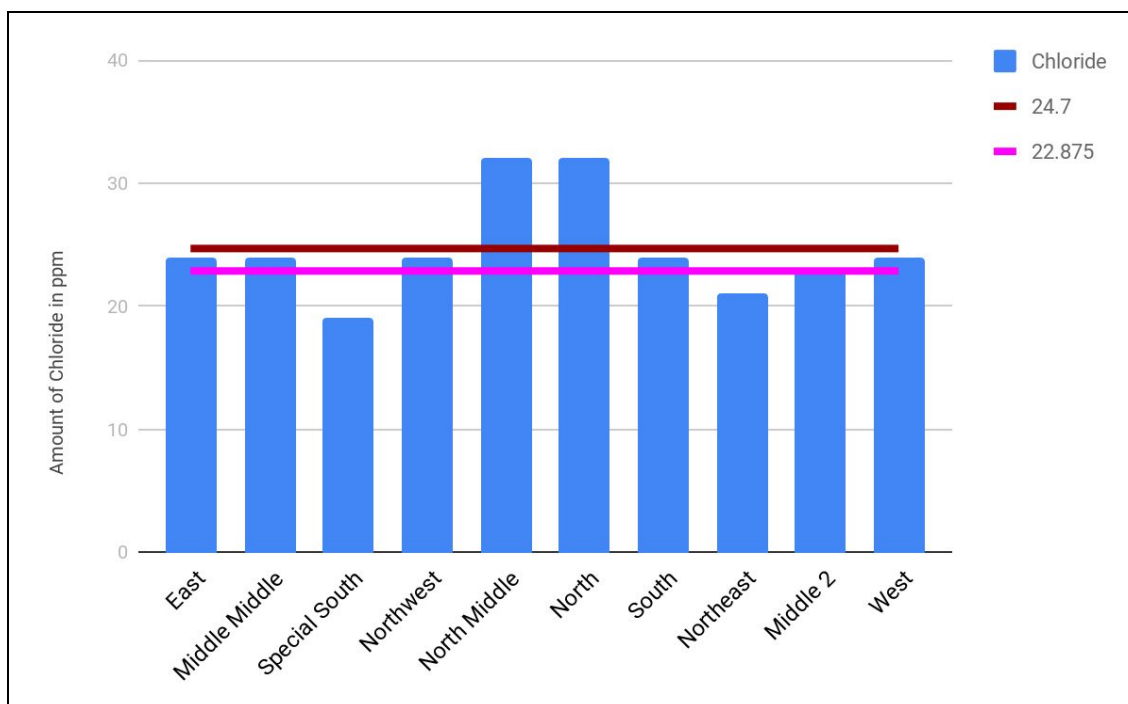


Figure 7: Chloride Measurements

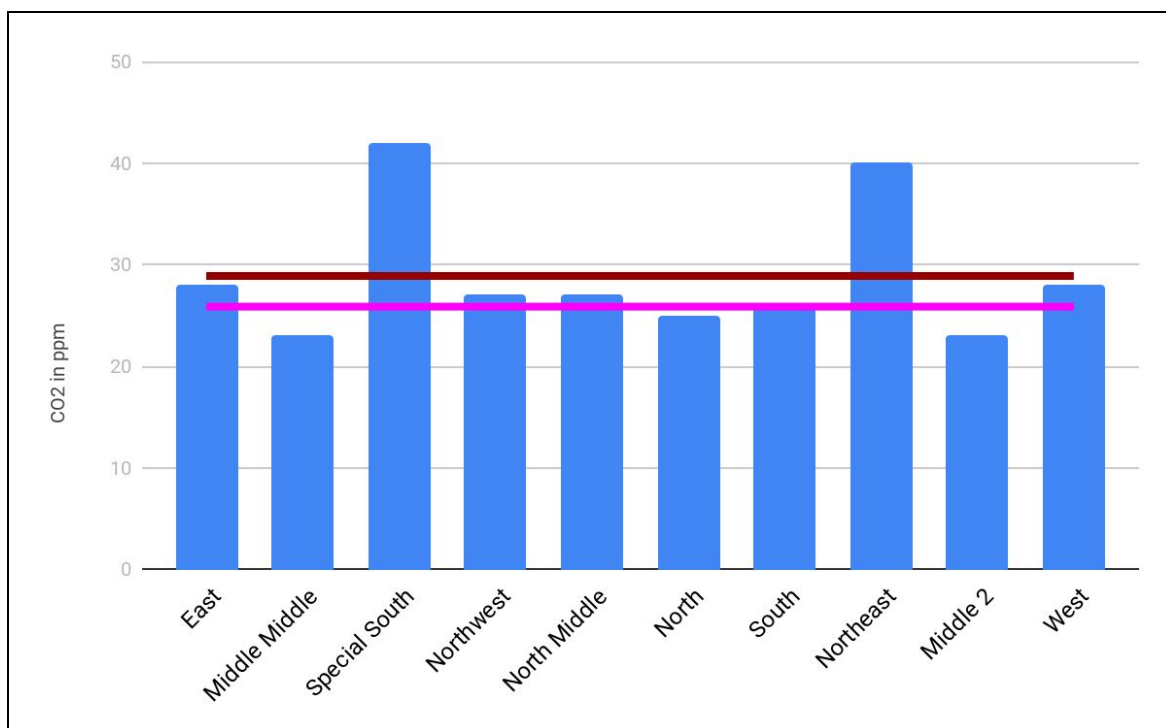


Figure 8: CO2 Measurements

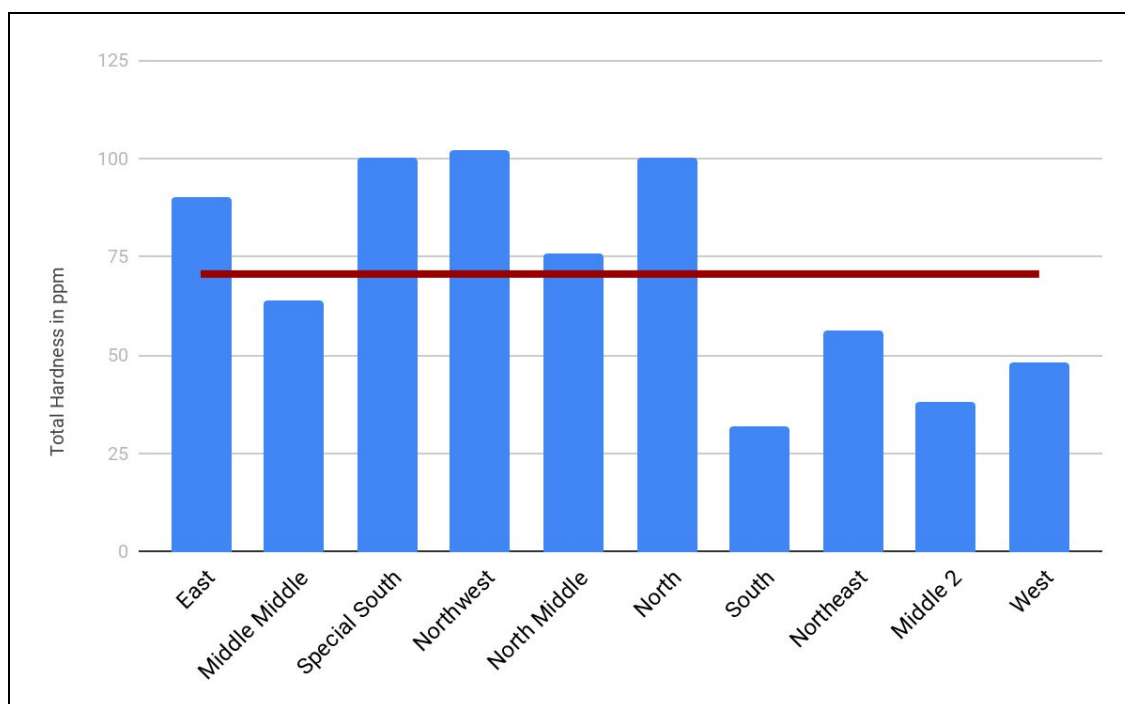


Figure 9: Total Hardness Measurements

Only pH and phosphate were outside the accepted range. Due to this, t-tests were run with the data to determine whether this would affect the quality of water. Phosphate had a p-value of 0.97, showing that the values collected were significantly different from the accepted value. The pH t-test had a p-value of 0.00377, showing that the pH was too far from the accepted range of seven.

Discussion

Chemical tests were done to the ten samples taken from different areas of the marsh to determine their pH and hardness, along with the concentrations of chloride, phosphate, nitrate, carbon dioxide, zinc, and silicate. These values were then compared with the accepted ranges to determine whether the contents of the lake were too high or too low.

Since the values of hardness, chloride, nitrate, carbon dioxide, silicate, and zinc all fell within the accepted range for a healthy freshwater environment, the marsh is seen to be a healthy aquatic environment. However, the pH and the phosphate values were not held within this acceptable range. The pH was too low, and the phosphate values were too high. Due to this, a t-test was calculated to determine whether this variation from the standard was normal or an anomaly.

A student's t-test gave a p-value of 0.97, which shows that the high phosphate value was not significantly different from the accepted value, meaning that the phosphate content of the lake was not abundant enough to create algae blooms or alter the health of the ecosystem. Despite that, the pH's p-value of 0.00377 from the t-test showed that the pH was significantly different from the accepted value of seven, meaning that the lake is too acidic for a normal freshwater environment. Although, this pH value of six is too low for most organisms, it just

means that the marsh is an acidic one, specifically a coastal plain marsh, explaining why the marsh was seen to only have some turtles and small frogs. However, the data was not compared to coastal plain marsh data, so there is still a question of whether the marsh meets the health criteria for a coastal plain marsh.

Furthermore, the data on the percent of dissolved oxygen solution in the water show that Clear Lake has the most capacity for housing animals, and the marsh has the least capacity for housing animals. Also, the data on the pH of the water show that Clear Lake and Murky Lake have the best pH value of 6.916 and 7.05, respectively, and the marsh has the worst pH value of 6. This is further supported by the standard error bars in Figure 6 showing that the marsh pH values don't overlap with the twin lakes' pH values, showing that the pH values for the marsh is significantly lower than the pH values for the twin lakes. This would explain the small number of organisms found in the marsh and the numerous amounts of aquatic creatures found in Clear Lake and Murky Lake since a low pH value is too acidic for most organisms to live in. This brings up a debate of whether Clear Lake or Murky Lake has better water quality. Due to this, a wide variety of chemical tests, not just pH and dissolved oxygen, should be done on the twin lakes to further understand the health quality of the lakes.

The data collected may have errors due to the way the data was calculated, especially for zinc. Most of the tests performed relied on color change. Due to slight variation of colors, the amount of substance predicted may have been wrong. In the zinc test, there was trouble determining what color the water matched, which may have caused the researchers to write down the wrong number. To make sure that the color matches, a program could be made to match the

water color to the correct shade. Probes could also be used to get an exact number instead of an estimate.

Another reason why the data may be slightly off is because the tests that were performed on the water had not been performed before. This could mean that the data of the first few tests may be off from the tests performed later, since the tools and the instructions of the tests became easier to use and understand. To prevent this from happening in future studies, these tests should be practiced on tap water multiple times before performing them on the actual test sample.

As stated before, to further understand the health of the marsh and all of the other lakes, tests on all of the chemicals should be performed: ammonia nitrate, potassium, absorbance at 340 nm, potassium, percent transmittance at 340 nm, and charge of current. The marsh values should be compared to accepted values for a coastal plain marsh to further determine whether the marsh is healthy enough for the organisms that live there. Also, the edges of the marsh, Clear Lake, and Murky Lake should be scouted out to determine how to get the best variety of samples, since this year the edges of the marsh had not been completely found. More samples from the twin lakes should also be taken in order to get a more accurate representation of the chemicals throughout the lake.

In addition, collaboration with the Air and Soil group could determine why the marsh is acidic and why there are different organisms in the area. Collaboration with the Botany group to determine the plant life in the area could also help determine the attraction of the environment to different organisms. This would determine whether the type of marsh or lake studied is healthy or not.

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Appendix

Marsh Raw Data

Water	CO2	Zinc	Silica	Hardness	Phosphate	pH	Nitrate	Chloride
E	28	0.6	<0.5	90	0.2	5.5	2	24
MM	23	0.4	<0.5	64	0.2	6	2	24
SS	42	0.6	<0.5	100	0.2	5.5	1	19
NW	27	0.6	<0.5	102	0.2	6.5	1	24
NM	27	0.6	<0.5	76	0.2	6	1	32
N	25	0.6	<0.5	100	0.2	5.5	2	32
S	26	0.6	<0.5	32	0.2	6.5	1	24
NE	40	0.2	<0.5	56	0.2	6	1	21
M2	23	0.4	<0.5	38	0	6	2	23
W	28	0.6	0.5	48	0	6.5	1	24

Murky and Clear Lake Data on pH

M: Shore	M: Near	M: Center	M: Runoff	M: Beside	C: Shore	C: Near
7.15	7.01	7.06	6.95	6.41	6.88	7.22