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A Seasonal Quantitative Analysis of Phytoplankton Populations in Freshwater Quarries of North-Eastern Illinois

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A SEASONAL QUANTITATIVE ANALYSIS OF PHYTOPLANKTON POPULATIONS IN
FRESHWATER QUARRIES OF NORTH-EASTERN ILLINOIS

By

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ABSTRACT

The populations of two phytoplankton genera, *Stigeoclonium*, a green algae, and *Tabellaria*, a diatom were surveyed and monitored over a period of 19 months in two different freshwater quarries in North Eastern Illinois. Populations were compared by the amount of cells per milliliter. Water samples were collected at different depths and throughout the year in both quarries. The autumn season showed the largest amount of phytoplankton populations, and *Tabellaria* was most abundant genus. Of the two quarries sampled, the one located in an urban center had a larger abundance of algal cells. A statistically significant relationship was determined between algal cell abundance and depth, with the surface water have the largest amount of cells.

Keywords: Phytoplankton, Green Algae, Freshwater, Quarry, Illinois

INTRODUCTION AND REVIEW OF LITERATURE

The importance of phytoplankton has been recognized and accepted in the scientific community for many years. Planktonic production is the base of almost all aquatic food chains as well as provides about 70% of the world's oxygen (Reynolds, 1984). An established definition of plankton is "the living fraction of material which floats in the sea or freshwater and is moved passively by wind or current" (Boney, 1975). Phytoplankton are the mostly autotrophic and microscopic organisms that fit the planktonic definition. Almost all species of phytoplankton are unicellular, eukaryotic and exist either singularly or in colonies. Early research showed relationships between excessive or depleted phytoplankton populations and problems in the water and fishing industries. Because of these findings, a thorough scientific understanding of phytoplankton ecology was needed, and over the past fifty years numerous scientists have been working toward this goal (Reynolds, 1984).

There are many classifications of phytoplankton. This research focused on micro plankton taken from freshwater lentic, or stagnant, environments. The most prominent of freshwater phytoplankton is the algae. While there are other groups of freshwater phytoplankton, for the purpose of this research, the closest attention was paid to algal specimens. Algae have an extremely wide range of characteristics. Its range of sizes is in proportion to the range from an egg to a small house (Reynolds, 1984). A.D. Boney outlines some specific characteristics to look for when identifying phytoplankton organisms: Cell

shape, cell dimensions, cell wall, mucilage layers, chloroplasts, flagella, and reserve substances (1975).

Two specific genera of phytoplankton were analyzed: *Tabellaria* and *Stigeoclonium*. These two genera were chosen because preliminary population analysis showed them to be the two most abundant genera, respectively, in the water systems. *Tabellaria* is one of the widest distributed freshwater diatom described as rectangular in shape and existing in colonies (Kopen, 1973). Diatoms are characterized by the presence of a silica based shell and a yellow-brown color. Members of the genus *Tabellaria* are autotrophic, using sunlight and chlorophyll (Diatom, 2013). The specific specie of *Tabellaria* is often dependent on the alkalinity of the water in which it is found (Knudson, 1954). Photo A1 (Schimmelpfennig, 2012) is a *Tabellaria* colony found in the initial population analysis. *Stigeoclonium* is a common genus of freshwater green algae. It is described as having branched filaments, which are limited by nutrient abundance, particularly phosphate. Members of this genus are able to flourish in many water conditions, including heavily polluted water (John, 2002). Photo A2 (Schimmelpfennig, 2012) is a *Stigeoclonium* specimen taken from the initial population analysis.

Preserving the samples is important for later use of those samples. The samples from the initial analysis were preserved by a solution made from Lugol's, distilled water, and glacial acetic acid. The publishers of "Phytoplankton in a transitional ecosystem of the Northern Adriatic Sea and its putative role as an

indicator for water quality assessment," used a formaldehyde solution to preserve their samples, which is another method tested method of preservation (2009).

The research manual created by UNESCO describes the use of different types of microscopes used for phytoplankton observation: standard and inverted. The standard microscope was used in this research. An inverted microscope is one in which the light source and condenser are positioned above and the specimens are viewed from below the cell (Sournia, 1978). This is particularly useful in the counting of nanoplankton populations using a Palmer cell. However, as the two genera of phytoplankton explored in this research were microplankton, the use of an inverted microscope was unnecessary.

Relationships between phytoplankton and different aspects of the water they reside in have been researched and discovered by many. Relationships between pH and water temperature were researched by Biologists in Estonia in 2008 (Haidna), and many more teams have researched the relationship between nutrient levels and phytoplankton populations. One method of discovering the nutrient-phytoplankton correlation is by taking dry-ash measurements of nutrients within the phytoplankton itself. This is the most accurate method for nutrient-phytoplankton comparison, as the measurements are the actual amount absorbed by the phytoplankton. The amount of different nutrients needed in functioning phytoplankton is known and includes eleven macronutrients, the

main three being carbon, nitrogen, and phosphorous, and nine micronutrients (Reynolds, 1984).

Both of the collection sites were man-made and maintained as recreational freshwater quarries. By understanding the seasonal fluctuations of the major phytoplankton taxa, the maintainers will have better insight into how to treat their respective properties. The collection sites were Haigh Quarry and Bird Park Quarry (41.116982°N 87.863866°W and 41.346667° N, 88.186389° W respectively). Haigh Quarry is located in Bradley, Illinois and is approximately four miles from the campus of Olivet Nazarene University. Bird Park quarry is located in Kankakee, Illinois and is approximately three miles from Olivet's campus.

Observations show that there are flocks of ducks and geese using Bird Park Quarry throughout the year, which would provide a constant biotic input. This is compared to Haigh Quarry, where only one duck individual has been seen wading during preliminary observations. These locations were selected as research sites because of the lack of published research pertaining to phytoplankton in lentic systems of North East Illinois.

These quarries have also been selected due to information about their history, primary input, and local influence. Haigh Quarry is currently operated by the Haigh family who has done so for many years. Bird Park Quarry is facilitated by the Kankakee Valley Park District. Haigh Quarry, has agricultural input from surrounding farms and fields while Bird Park Quarry has a majority of input from

storm runoff, as it is located in the center of the city of Kankakee (lake-link, 2013). This information helps to provide insight in to how the location affects the microbiological life of two similar water systems.

Differences were expected between quarries, depths, and seasons. Seasonally, the spring season is expected to show the largest amount of algal cells due to spring mixing of the water column in lentic systems. This takes place partly because the sun heats the surface water where nutrients are rich and have been trapped during the winter season, allowing the nutrients to be utilized by the organisms. The autumn season should follow spring as the second largest amount, with summer and winter closely matched (Levinton, n.d.). As depth increases, the amount of algal cells is expected to decrease, as the amount of light is more available toward the surface of a water column. Finally, there is expected to be a larger amount of total algal cells in Bird Park Quarry than in Haigh Quarry due to its storm runoff and biotic input of the countless ducks and geese that use the quarry.

MATERIALS AND METHODS

The methods of collection are extremely important in studies analyzing water samples. The quality of data derived from analysis depends directly on the quality of the samples. The United States Geological Survey (USGS) has standard methods for water sampling. A nearly two hundred-page field manual outlines all necessary information for collecting samples for research in both free-flowing and still-water sites. The published methods for still-water sites were followed

for this research. The manual lays out six steps to sampling at still-water sites: preparing, locating site, selecting depths, collecting samples, processing samples, and cleaning equipment. The key to preparation is to obtain thoroughly cleaned and decontaminated glass or plastic bottles and to keep them away from possible contaminants as much as possible in route to the collecting site. Once at the sample site, rinsing the bottles with the water from the site can help to eliminate any remaining soap residue and condition the device to the environment (USGS, 2006).

The USGS states that it is not correct in assuming, and very unlikely, that because a water system is not free-flowing, that the properties are homogeneous throughout. Therefore they suggest "a single sampling point generally is not adequate to describe the physical and chemical properties of the water body, or the distribution and abundance of the inhabiting biological community" (USGS, 2006). In order to obtain the best variation, samples were collected at three increments starting at the surface, and moving toward the bottom (0 meters, two meters, and five meters). Five meters was not always a feasible depth at Bird Park Quarry because it does not reach a depth of Five meters in all locations. Samples were also taken at 10 and 15 meters at Haigh Quarry when location allowed.

According to the USGS standards, the next step is collecting samples. The initial population analysis samples were collected by hand held grab bottles using SCUBA equipment, a depth gauge, four one-liter water bottles, and a mesh bag

to hold the bottles. All official samples included in the data sets were collected using a “thief-type sampler” called a Kemmerer device (Model SKU 1510-C22 from Wildlife Supply Company). The Kemmerer is a water-sampling device that allows a person to collect water samples from a boat above the water. The Kemmerer is dropped in to the water and when it reaches the desired depth, set by the operator, it snaps closed, trapping water inside.

As quickly as possible, the sample was transferred to the sampling bottle. This technique was chosen for sampling because it allows the depth of the sample to be controlled. This allows samples to be taken during the autumn and winter seasons when the temperature is too low to collect samples by SCUBA. Neither quarry involved in the research allowed the use of a motorized boat. Therefore both a canoe and a kayak were used to collect samples. The Kemmerer device was dropped from the boat to collect samples at different depths.

A preliminary sample was taken from Haigh Quarry on October 17, 2012 before permission to sample at Bird Park Quarry was acquired. Official samples were taken from both quarries on January 6, March 23, August 28, and November 12 of 2013, as well as March 8 and May 12 of 2014. Sampling took place over time to deduce seasonal variations.

After each collection, following USGS protocol, the samples were transferred to the laboratory at Olivet Nazarene University. The samples were analyzed using a compound microscope and a Sedgwick Rafter Cell (Model SKU

1801-G20, Wildlife Supply Company). The Sedgwick Rafter Cell holds 1 milliliter of water in a gridded chamber containing 100 squares, each representing 1 microliter of fluid. Samples were microcentrifuged before extracting 1 milliliter of fluid using a micropipette. All samples were treated in this manner to create consistency between the many samples. Twenty grids were selected at random for each sample and the number of *Tabellaria* and *Stigeoclonium* cells in each square were counted. After the 20 random cells were counted, the cell numbers were summed and then divided by 20 for an average per square. The average was multiplied by 100, as there are 100 squares per Sedgwick-Rafter cell. The resulting value represents the amount of cells per milliliter in any given sample. These values were analyzed by quarry, season, and depth. These different comparisons are shown graphically in the results.

RESULTS

The total algal cell count by season, in Bird Park Quarry and Haigh Quarry, followed a similar pattern. The two genera followed the same pattern as the total cell count, with *Stigeoclonium* being the less prevalent throughout the seasons. Figure 1 shows the change over time of both *Stigeoclonium* and *Tabellaria* in Haigh Quarry. Figure 2 shows the change over time of the total cells per milliliter of both algal genera in Bird Park Quarry.

Figure 1: Seasonal variation in algal cells/mL in Haigh Quarry

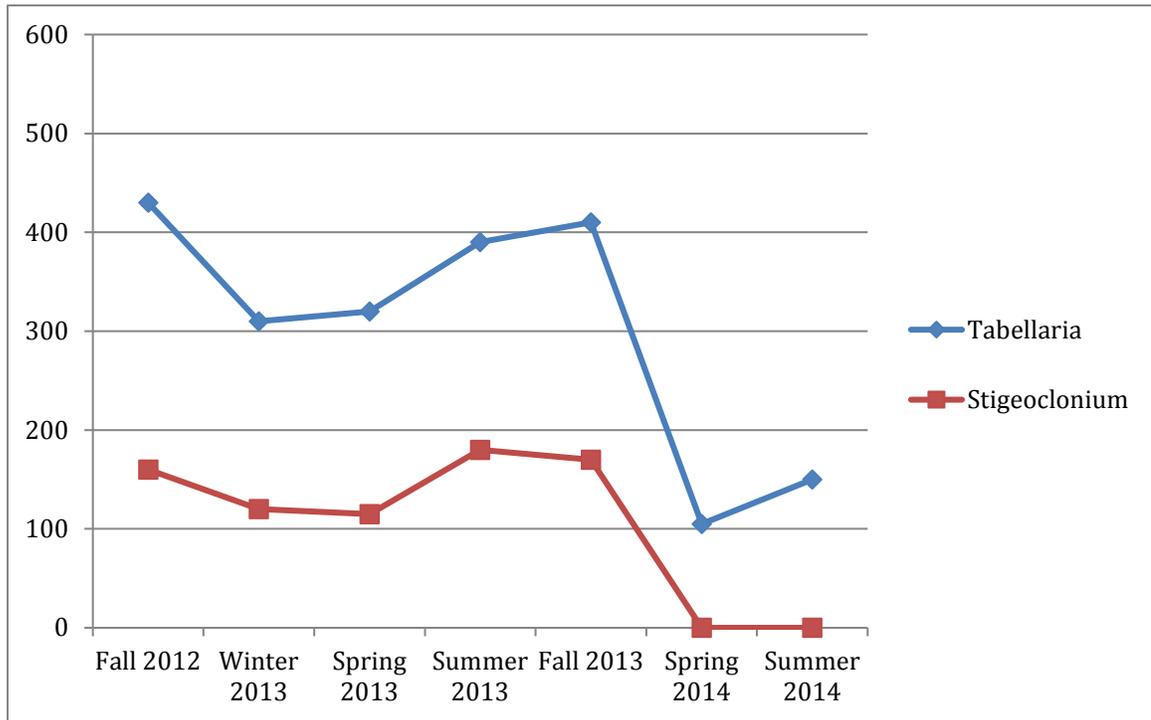
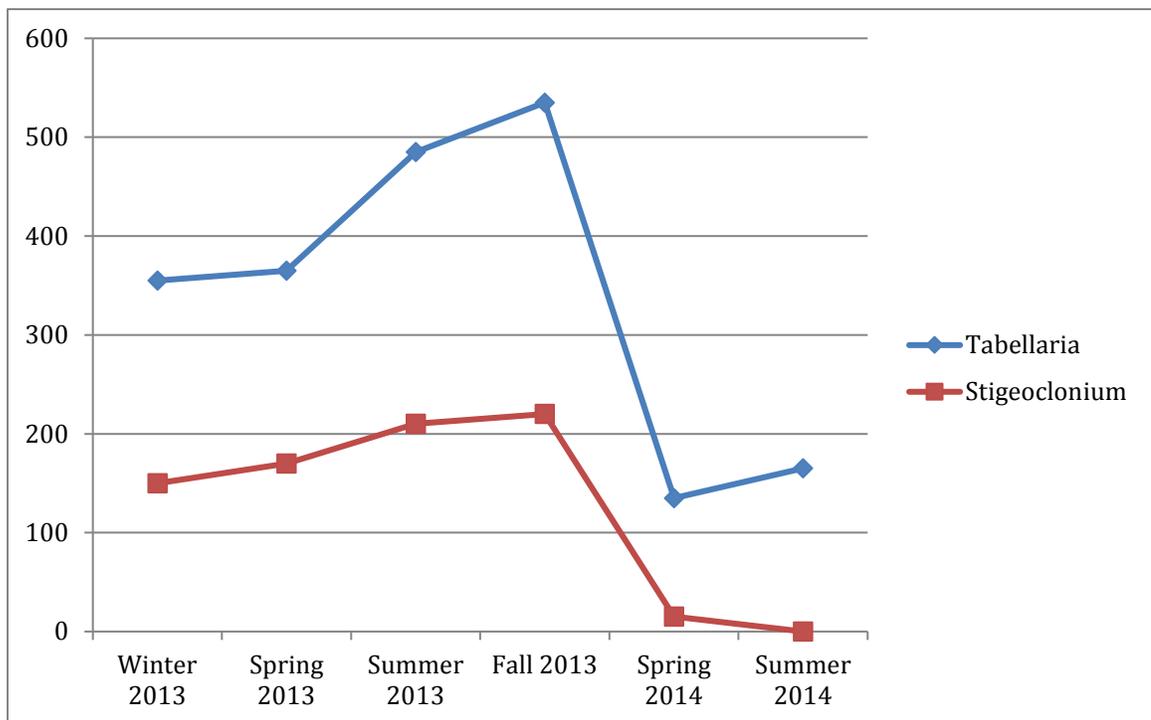
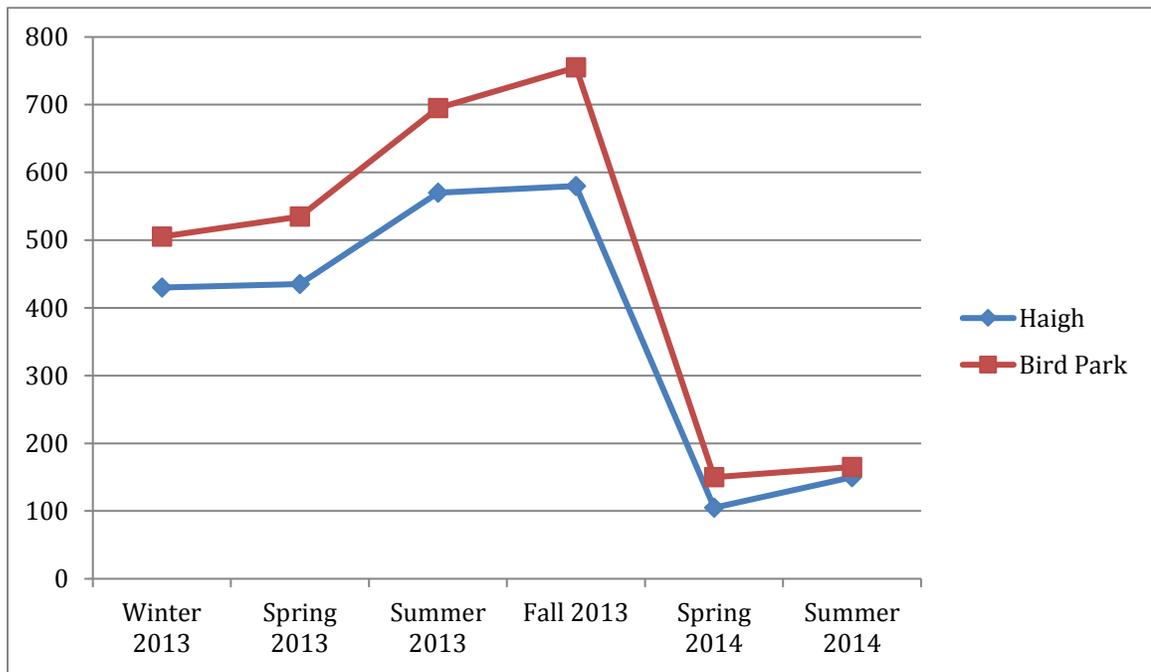


Figure 2: Seasonal variation in algal cells/mL in Bird Park Quarry



The two quarries were compared by examining the seasonal variation of algal cell count per milliliter. These values were determined by summing the cells per milliliter of each genera for Bird Park Quarry and Haigh Quarry. This relationship is shown in Figure 3, where the red points represent summed values for Bird Park and the blue represents the summed values for Haigh Quarry.

Figure 3: Change in total algal cells in both quarries



As seen in figures 1 through 3, the values increase gradually from the winter of 2013 to the autumn of 2013 before dropping drastically for the sample collected in the spring of 2014. The winter of 2014 is missing from all data sets, and resultantly from all graphs, due to a longer than normal period of time of ice covering the quarries. It was not until the beginning of March that the ice

thawed enough for samples to be taken. A small increase can be seen from the spring to the summer of 2014 in all figures except in regards to *Stigeoclonium*. This genus remained constant at zero cells per milliliter in Haigh Quarry, and slightly decreased in Bird Park Quarry. As can be seen from the graphical representations, an overall pattern is consistent. The *Tabellaria* values are consistently larger and tend to have greater fluctuation than those of *Stigeoclonium*.

Data collected from Bird Park Quarry was not consistent enough to draw conclusions relating to depth; however, Figure 4 shows the relationship of depth to cells/mL of *Tabellaria* in Haigh Quarry. Figure 5 shows the same relationship in regards to *Stigeoclonium* in Haigh Quarry.

Figure 4: *Tabellaria* cells/mL in Haigh Quarry by depth

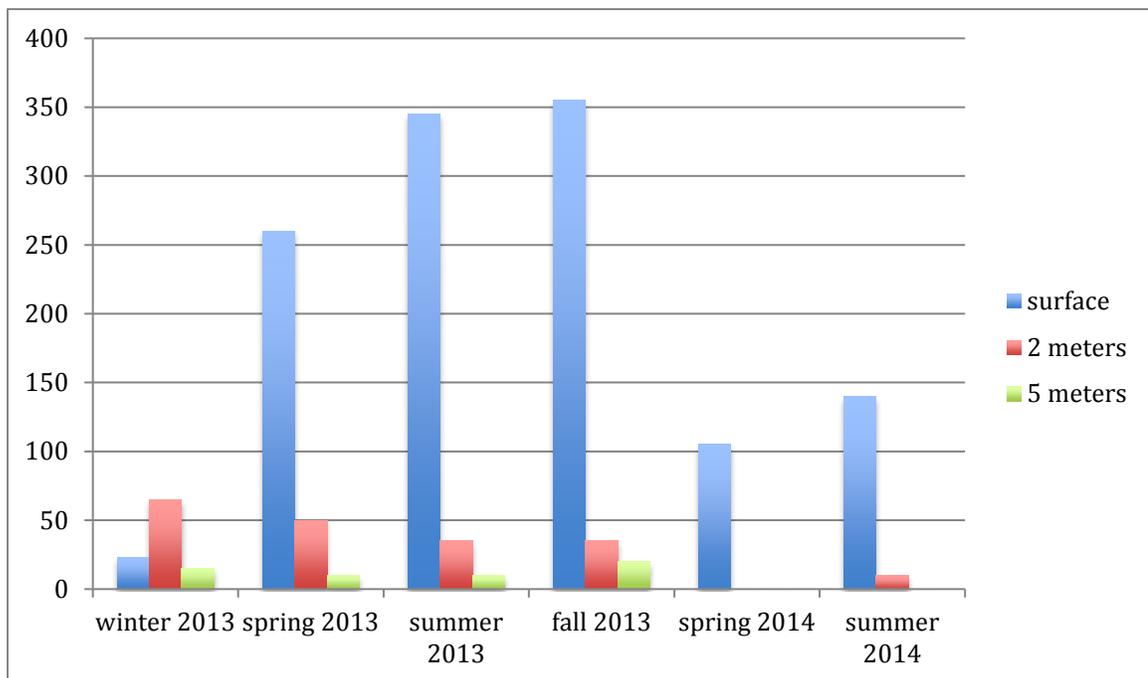
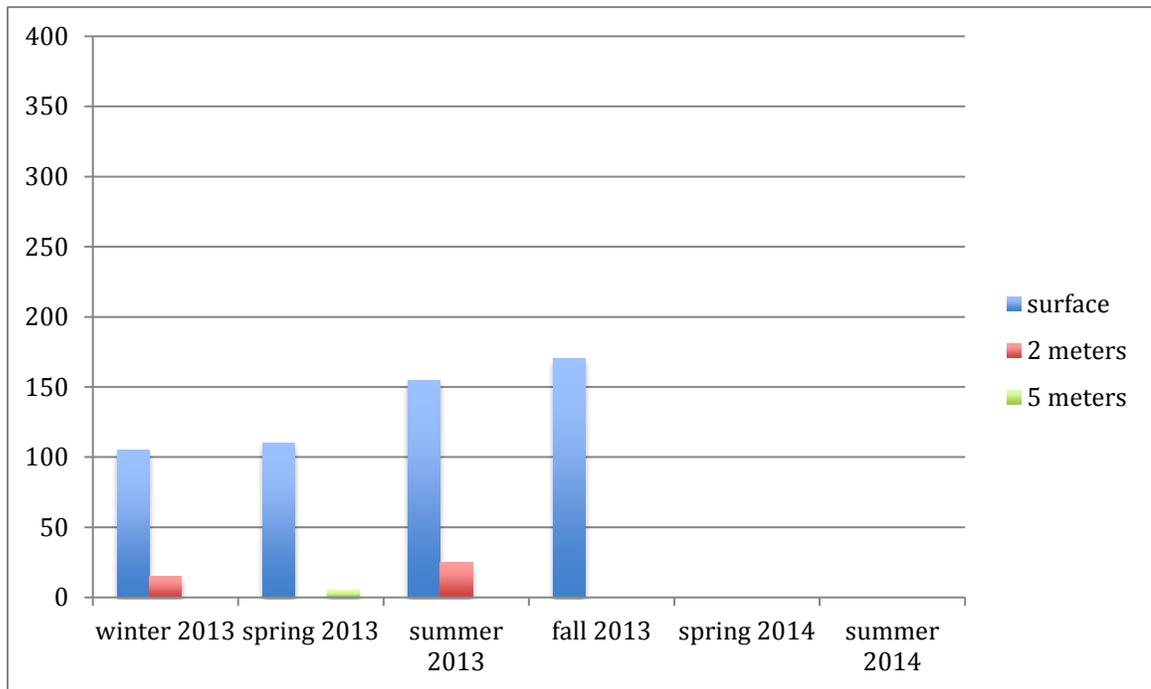


Figure 5: *Stigeoclonium* cells/mL in Haigh Quarry by depth



Both figures 4 and 5 show a dominant amount of the population of both genera being present at the surface as compared to the other depths. Again, *Tabellaria* is more abundant overall than *Stigeoclonium*.

Student t tests comparing the two quarries to each other reveal a p value of 0.2612, which is above the accepted standard value of statistical significant: 0.05. This means that the difference in cell amounts between the two quarries is not a statistically significant difference. An ANOVA test analyzing the seasonal variation of algal cells/mL in Haigh Quarry produced a p value of 0.2221, which is also not statistically significant. A similar insignificant p value is determined from the total algal count over time in Bird Park Quarry. Statistically significant data

was found when comparing depths. In Haigh Quarry, the depth comparison of *Tabellaria* gives a p value of <0.0001 , which meets the significance criteria. Similarly the depth comparison of *Stigeoclonium* in Haigh Quarry produced a statistically significant value of 0.006791. When comparing the zero to 15 meter depth samples that were taken at Haigh Quarry a p value of 0.003816 is produced, showing the comparison to be significant. However when comparing the data from all depth intervals (2, 5, 10, and 15 meters) excluding the surface the p value increases drastically to 0.449023, which is not significant. (Vassarstats, 2014). Table A3 summarizes the statistical analyses and their corresponding p values.

DISCUSSION

The hypothesis that the largest volume of algal cells would occur during the spring seasons was not supported by the data. Instead, the autumn season(s) claimed the most abundant populations of both genera in both quarries. The summer and winter seasons were relatively similar within each genus. The spring season of 2014 showed the lowest values of all samples. While this is opposite of the hypothesis there are some potential explanations. Both of the quarries were covered with ice from December through early March. This provided for an anomaly of a spring season and it is plausible that the algae did not have significant time to bloom. However, the sample that was taken in May of 2014 did not show much of an increase in growth either, showing barely half of the total cell volume of the preceding autumn season. It is possible that

the extreme conditions of the winter will have an effect on the water columns that is farther-reaching than one or two seasons. Continued monitoring over the next few years could shed some light on this possibility. While the value of cells per milliliter did fluctuate over time, there was not a statistically significant difference between the seasons. A possible explanation for this could be that both quarries are small systems and a comparison of the quarry data to data from larger lentic systems could possibly support this hypothesis.

The total amount of algal cells per milliliter was larger in Bird Park Quarry than in Haigh Quarry. This supports the hypothesis, which was based on the assumption of there being more available nutrients due to storm runoff and avian biotic input in Bird Park Quarry. However, a second explanation could be the shallow environment of Bird Park Quarry. In shallow systems, nutrient exchange occurs continuously and there is a lesser-defined water column (Levinton, n.d.). This could allow more nutrients to be available throughout the year as compared to Haigh Quarry.

Within both quarries the genus *Tabellaria* was more abundant than the genus *Stigeoclonium*, as can be seen in both Figures 1 and 2. It has been shown that *Stigeoclonium* is limited primarily by phosphate abundance (John, 2002). A chemical analysis of these quarries compared to others with a larger abundance of *Stigeoclonium* could help determine if phosphate was indeed the limiting factor in this case.

The statistically significant relationship determined from this data is between cell volume and depth. There was a significant difference between the different depths in Haigh Quarry for the genus *Stigeoclonium*, and even more so for the genus *Tabellaria*. This supports the hypothesis, which was based on the assumption that more photosynthetically active radiation would be available at the surface than at lower depths. Measuring the radiation, nutrient levels, and pH at these different depths could support this assumption.

One example, as mentioned earlier, is that *Stigeoclonium* grows better in acidic freshwater (Baker, 2012). Perhaps the pH at the surface of these systems is lower than at two meters and below. However, when the surface data was taken out of the ANOVA analysis, the p value grew out of the significance threshold. This suggests that while the difference between depths was shown to be significant, it is the surface level data that created the significant p value. This means that the remaining depth intervals were not significantly different from each other.

As with most field-based research, there is a risk of contamination in the transfer of samples from the field to the laboratory. As outlined in the materials and methods section, precautions were taken to decrease the chance of contamination; however, there is a chance that organisms could have not transferred properly. With repeated sampling the procedure became more efficient, limiting the contamination risk even more. The more efficient the sampling process, the less chance of contamination. Further research in this area

could utilize two or more researchers to make the sampling more efficient. Further research should include a more complete chemical analysis as well as a continuum of the genera surveys. This will strengthen the basis for assumptions and hypotheses.

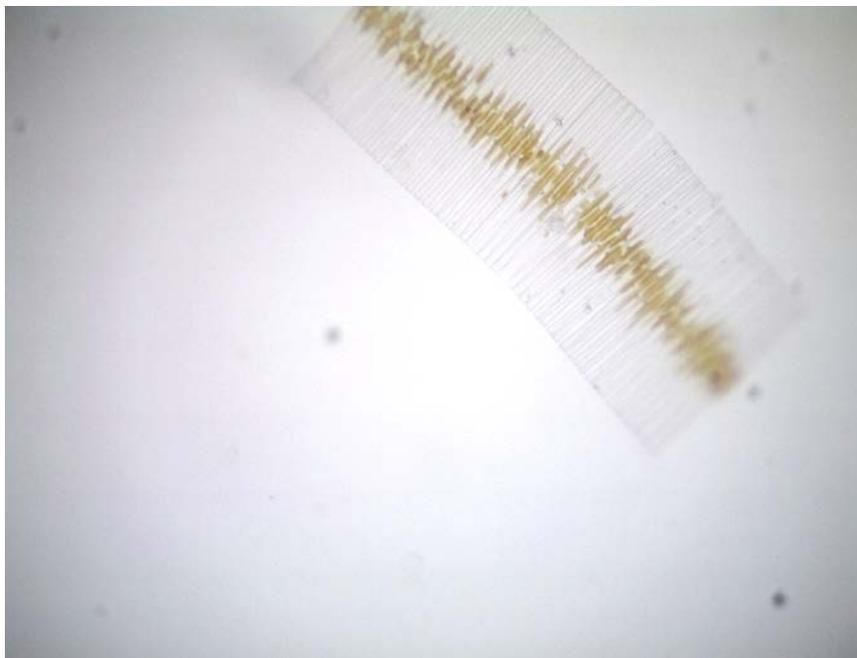
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APPENDIX

A1: *Tabelarria* cells at 1000x magnification



A2: *Stigeoclonium* cells at 1000x magnification

