The effect of fertilizer on the growth rate of the diatom *Licmophora abbreviata*

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Abstract

The main objective of this study was to examine the effect that increasing concentrations of liquid fertilizer at 0%, 20%, 40% and 60% has on the organism *Licmophora abbreviata*, a type of benthic diatom. This allows us to gain a better understanding of the effects of agricultural fertilizer runoffs on rivers, its surrounding environment, and the conditions of the community these microalgae live in. The methods consisted of growing diatoms in the four different concentrations for a period of two weeks, with three replicates per treatment. The diatom growth rate was measured on six different days with a hemocytometer where the change in concentration of cells was determined by counting the number of cells per volume. The significance of the data was analyzed with a one-way ANOVA test. Results show significant evidence to suggest that there is a difference in the growth rate of diatoms between the 0%, 20%, 40% and 60% environments. The number of cells in the 0% treatment increased with time, analogous to their natural growth in rivers. The 20% , 40% and 60% treatments of liquid fertilizer, had a negative effect on diatom growth. This is explained by the disturbance the added nutrients from the fertilizer caused on the optimal ratio of nutrients that diatoms need (Danielsson, 2008). Thus, we conclude that agricultural fertilizer runoffs have a negative effect on the growth rate of the benthic diatom community in rivers.

Introduction

Microalgae play an important role in marine communities as primary producers. They represent the base of the food chain for marine ecosystems since zooplankton use them as their food source which indirectly impacts the availability of food for larger organisms such as salmon. Indicators such as abundance and distribution patterns can help detect the ecosystem conditions (Facca et al. 2004).

The optimal growth of diatoms occur in a balanced nutrient ratio of nitrogen(N):phosphorus(P):silica(Si). When this ratio is disturbed, diatom growth is significantly hindered as suggested in a study conducted at Huanghai Sea (Fu et al. 2012). In silica-limiting

environments, diatoms that depend on silica are outcompeted by non-silica dependent phytoplankton, which lead to a turnover of phytoplankton composition and algae blooms (Howarth et al., 2011). This can lead to a drastic change in the marine environment leading to hypoxia, a low oxygen condition, which can be detrimental to other organisms, such as salmon, living in the water column (Howarth et al., 2011).

The aim of our study is to gain a better understanding of the effects that agricultural runoffs have on ecosystems, and the conditions of the community these microalgae live in by looking at their growth rate as an indicator of these conditions. In our study, the growth rate of the diatom species, *Licmophora abbreviata,* was monitored under different concentrations of liquid fertilizer. Our null hypothesis is that there is no difference in diatom growth rate between the control and fertilizer treated groups. By our understanding of how nutrients affect diatom growth rate, our alternate hypothesis is that there will be a difference in the growth rate of the diatom, *Licmophora abbreviata*, with the increase of fertilizer concentrations.

Methods

We prepared 4 treatments using different fertilizer concentrations of 0%, 20%, 40% and 60% under which the diatoms grew for a period of 2 weeks. The environment of the diatoms' growth was controlled using an incubator of 20 degrees Celsius with regulated light intervals. Each treatment had three replicates that allowed for an average value of cell growth to be calculated per treatment.

We first prepared the 100% fertilizer solution. We did this by following bottle instructions for a volume of 250mL of fertilizer designed for a garden (using medium optimal for diatom growth as solvent instead of water).

Then we diluted the corresponding mL of the prepared 100% fertilizer with 2mL of stock solution containing diatoms ($7.5x10^5$ cells/mL) and corresponding mL of medium for a total volume of 10mL for each treatment. As seen in Fig.1 below:

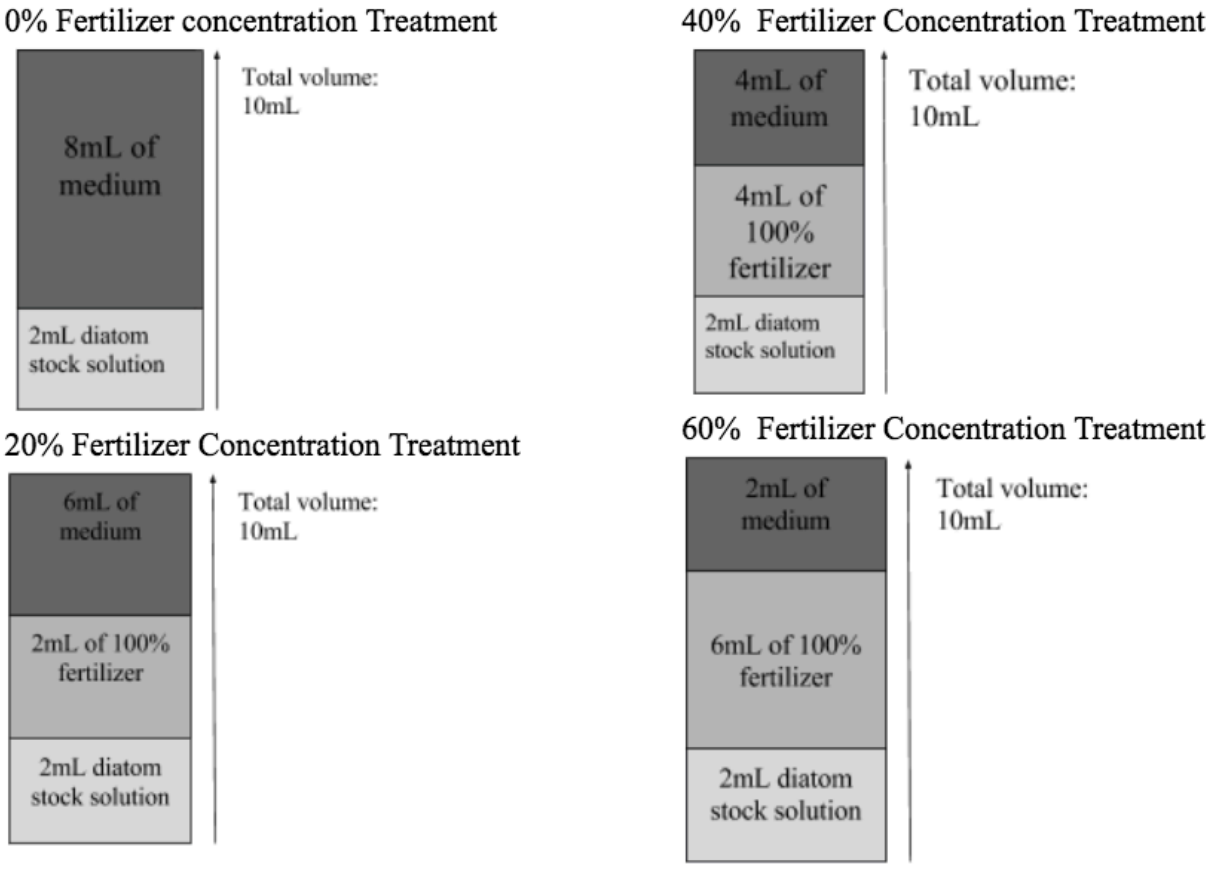


Fig.1 Preparation of each treatment with corresponding ratio of fertilizer to medium

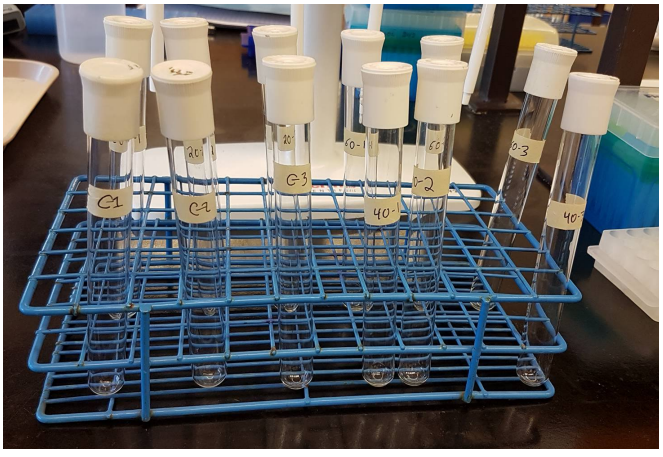


Fig. 2 Test tube rack holding the replicates of each treatment labelled as treatment-replicate (ie.- Replicate 1 for 0% fertilizer concentration is seen as C-1).

Once all the treatments and their corresponding replicated were prepared, we set them in a test tube rack as seen in Fig.2. A sample of 100µl from each replicate was taken following a sterile technique, and fixed with 10µm of fixative three times a week, (Monday, Wednesday, Friday). The following steps were taken:

1. Use ethyl alcohol to sterilize the bench
2. Pipette 10µl into each of the 12 Eppendorf tubes. For this step the same pipette tip was used
3. Vortex the replicate test tube for 10 seconds at low intensity, to not disrupt the cells too much.
4. Use a flame to sterilize the mouth of the replicate test tube
5. Use a new pipette tip to sample 100µl from the test tube, without sinking the tip more than halfway into the test tube. Assumption that the vortex mixed it well enough so that the top layer has the same concentration of cells/mL as the bottom layer.
6. Use flame to sterilize the mouth of the replicate test tube before capping it and placing it on test tube rack
7. Mix the 100µl sample with the fixative in the Eppendorf tube for at least 5 repetitions.
8. Place Eppendorf tubes with samples in the fridge when done.

The picture below of Fig.3 shows the labelling of all the Eppendorf tubes, ready to be placed into the fridge.

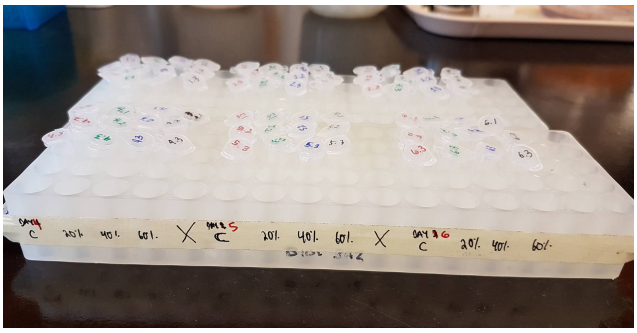


Fig. 3 Arrangement of Eppendorf tubes according to the day they were fixed, the treatment they belong to and the replicate they represent.

The second part of our involved the counting of the cells in each Eppendorf tube, using a hemocytometer and microscope.

The steps followed were:

1. Mix the contents of Eppendorf tube with pipette tip several times, using a volume of 80µm
2. Once contents are well mixed, take 20µl of sample and place on a hemocytometer slide
3. Place slide on microscope and count the number of cells

In order to reduce error, a standard counting method was agreed upon. This encompasses including cells which are touching the middle borderline in the count for a box, as well as making sure organelles are visible within the diatom shell. Then, using the dilution factors of the hemocytometer as seen in Fig.3 below, the total cell count per corresponding square color was then multiplied by the dilution factor to get the cells/mL.

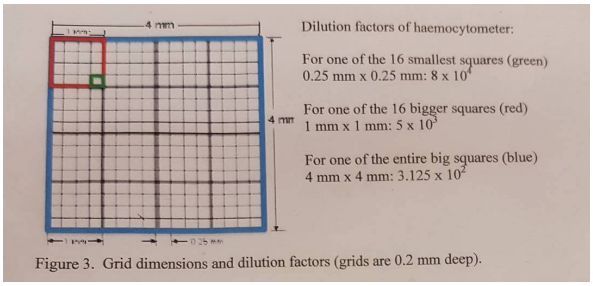
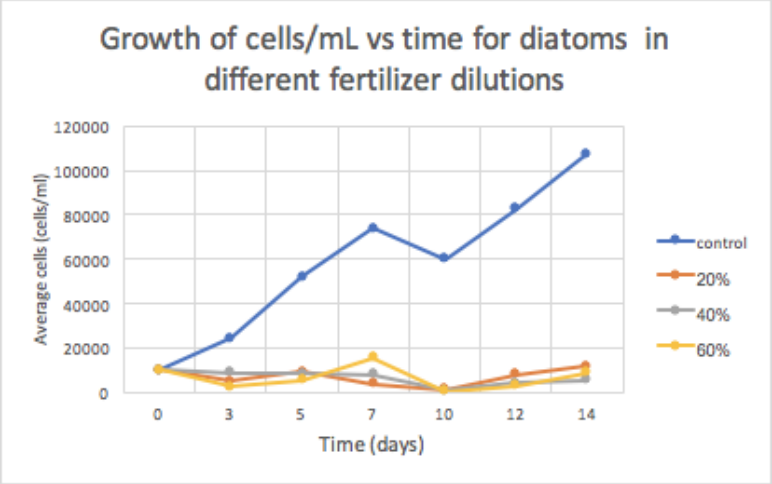


Fig.3 Grid dimensions and dilution factors of hemocytometer

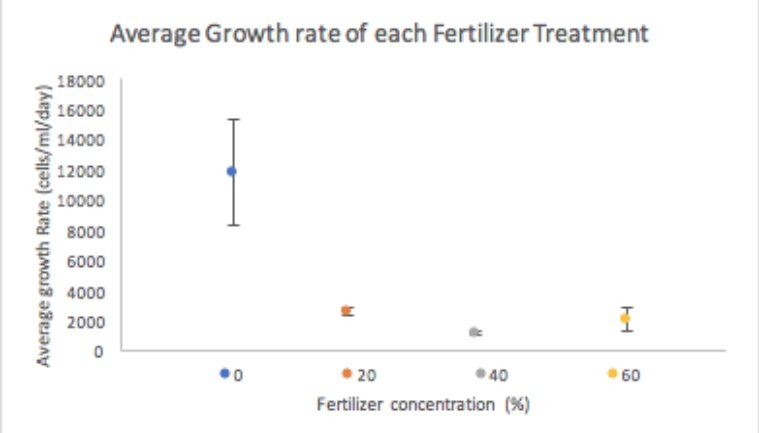
Once each replicate had been counted per treatment, the average of the 3 replicates were graphed against time. This showed the trend of the diatom populations in each group over the two week period, as seen in Graph 1.

Results

Graph 1 below includes the cell count averages obtained from the 3 replicates of each treatment against time. We observed a trend of increased cell counts in the control over the two week period. However, the three treatments appear to have very limited growth, much lower than the control. There is a decrease in average cell count on day 10 for all of the treatments.



Graph 1. Comparison of all 4 treatments average cell number taken from its 3 replicates, during the two week period.



Graph 2. Comparison of the average growth rate (cells/mL/day) for the control, 20%, 40% and 60% fertilizer concentration treatments. Values obtained from the one-way ANOVA test for days 10-14.

Graph 2 above includes the average growth rates over day 10 to day 14 for the control (0%), and 20%, 40%, and 60% fertilizer treatments. The 0% group growth rate is higher than the 20%, 40%, and 60% growth rates, and the 20% treatment's growth rate is greater than the 40% treatment.

The data was further analyzed with a one-way ANOVA statistical test in order to compare whether a significant difference was found in the number of cells (cells/mL) between the control, 20%, 40% and 60% fertilizer treatments from days 10 to 14. This was done by plotting the cell counts of each group over time from days 10-14 to obtain a slope. These slopes in units of cell/ml/day were used in the one-way ANOVA test. Graph 2 above shows the results from this analysis. The ANOVA test gave the following conclusions: at the 5% significance level we find that the p-value= 0.000271, and the f-ratio value= 23.076. Therefore, there is a significant difference between the treatments at the 5% confidence level.

A second one-way ANOVA test compared the replicates of treatments 20%, 40%, and 60% from day 10-14. This would allow us to determine whether the concentration of fertilizer causes a significant difference in the growth of cells from day 10 on. The results show that at the 5% significance level, the f-ratio value=6.64 , and the p-value=0.03. Therefore, the difference is significant at the 5% confidence level, and the specific concentration of fertilizer will impact the growth rate of the diatoms over time. The trend in Graph 1 suggests that this difference may be due to less harmful effect of 20% than of 40% or 60% fertilizer.

Discussion:

Our data showed that the growth rate of *L.abbreviata* was significantly less when grown with fertilizer treatment, at 20%, 40%, or 60%, than in the controls. The trend in Graph 1 clearly shows that diatom growth increased with time in the control, while there was limited growth in the fertilizer treatments. Graph 2 shows that this difference is highly significant ($p=0.0003<0.005$) over days 10-14. Therefore, the null hypothesis that there is no difference in diatom growth rate between the control and fertilizer treated groups can be rejected**.** This result supports the research performed by Hillebrand and Sommer (2000), that diatom growth decreases with the addition of fertilizer.

Further analysis of Graph 1 demonstrates differences between the 20%, 40% and 60% treatments. Random growth with a general pattern of decrease between day 0 to day 10 and an increase of growth rate between day 10 to day 14 is seen. According to the results of ANOVA test between day 10 and 14; the p-value is 0.03, which shows a significant difference between the treatments after day 10.

The fertilizer that was used as the stock solution consisted of 20% total nitrogen, 20% available phosphoric acid, and 20% soluble potash, as well as trace quantities of other minerals but no silica. The diatoms of the control treatment grew in an optimal environment used by lab technicians, containing silica, and other nutrients, therefore the addition of fertilizer to the optimal environment changes the relative ratio of nitrogen and phosphorus to silica in the external environment of the diatoms.

This growth behavior in Graph 1 can be explained by the importance of the Redfield ratio. The Redfield ratio describes the internal ratio of nutrients in a cell body that allows for the most efficient growth (Cleveland, 2007). Organisms have adapted such that this ratio is similar to their external environments. In diatoms, this ratio of nitrogen, phosphorus, and silica is 16:1:15 (Danielsson, 2008). By keeping the concentration of Si constant while changing the concentration of N and P produces an environment an unbalanced nutrient ratio. These ratios will affect the growth of the diatoms in a negative way which explains the decrease in growth from day 0 to day 10 in Graph 1 for the 20%, 40% and 60% fertilizer treatments.

Analysis of graph 1 shows a small increase in growth from day 10 to day 14 for the fertilizer treatments. As stated by Rogato et al. (2015), the dependence of diatom growth rate on nutrient uptake rate is a function of the external and internal concentrations of nutrient with the maximum assimilation rate. The active process of nutrient uptake by enzymes can be altered by changes to the affinity of nutrients to transporters (Rogato, 2015). Intracellular changes help diatoms adapt to their environment which may explain the increase in growth rate from day 10 to day 14. A question that arises is whether the diatoms with small intracellular adaptations might

grow at the same rate as the control. Further research would be needed to determine long term trends.

A possible interpretation of graph 1 is that the diatom population was severely impacted by the fertilizer, with major die-off by day 10, and then possible recovery of the population from cells selected for their ability to cope with the altered nutrient ratio. However, the recovery was still slight compared with the rate of growth of the control population.

The two main sources of error in our study were in data collection and determining the concentration of the initial stock solution. In order to reduce the errors of counting cells with the hemocytometers, a counting standard was established and 3 replicates were counted at each treatment level. To reduce the error is in determining the concentration of the initial stock solution, the average number of cells of 3 counts was used to determine the stock solution.

Conclusion

The null hypothesis stated that there is no difference in diatom growth rate between the control (0%) and 20%, 40% and 60% fertilizer concentration treatments. We reject the null hypothesis at the 5% confidence level. We conclude that fertilizer treatment (20%, 40%, 60%) has a very strong deleterious effect of diatom growth rate.

The trend of our data demonstrates the dependence of diatom growth rate to the optimal nutrient Redfield ratio of N:P:Si (Danielsson, 2008). The decrease in cell numbers calculated up to day 10, is likely a consequence of the disturbance that the fertilizer caused on the nutrient ratio. We conclude that runoff fertilizer will have a negative impact on the short term growth rate of the diatoms in their natural environment. The rise after day 10 suggests the possibility of

population recovery. Yet because of the organism adapting its intracellular composition to match with the external conditions (Rogato et al. 2015), diatoms, such as *L.abbreviata,* might adapt to their surroundings and grow into their environment. A longer period of growth would give us insight into how steadily they would grow, and the long term impact of fertilizer on diatoms' growth rate in the ecosystem.

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