Bioassays To Determine Nutrient Limitations of Algal Biomass

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Abstract

Nutrient limitation in algal biomass is extremely important for the longevity of aquatic ecosystems. While algae is necessary for an ecosystem to strive, in excess, it can be extremely harmful to the organisms within the ecosystem, as well as, the ecosystem as a whole. In this experiment we determined which nutrients limit algae growth. The nutrients that we tested were nitrogen, phosphorous, and iron. To determine nutrient limitation, we performed a 6-day bioassay, measuring algal biomass each day, relative fluorescence unit (RFU). We collected water samples from two different water sources: Boomer Lake and a local neighborhood pond. We hypothesized that the pond water would be relatively nitrogen limited because of its close proximity to a neighborhood and point source runoff, and we hypothesized that Boomer Lake would not be nutrient limited, based on the lack of algae growth and high turbidity levels that were seen on in the water. We found that the local pond was N limited, while Boomer Lake samples seemed to not exhibit nutrient limitation at all.

Keywords: Toxic algal blooms, Eutrophication, Bioassay, Algal Blooms vs Nutrient addition, Chemical effects on aquatic ecosystems

Introduction

Algal biomass is affected by a number of factors, and several studies have been conducted to determine what exactly has the most influence on biomass, which will be further explained in the paper. Based on these studies there have been relatively common results that shows that nitrogen(N) and phosphorous(P) appear to be present in areas with large algal biomass. The presence of N and P can influence the amount of algae that grows in certain ecosystems, but they must be maintained at a certain ratio for there to be a healthy amount of growth (Harpole 2011; Rasdi *et al.* 2015). Algal biomass can be helpful or hindering to an aquatic ecosystem. It will affect each ecosystem differently depending on the size, and amount of organisms living within the ecosystem (Hayes *et al.* 2015). Algae, which represents the base of aquatic food webs, and its growth has a tremendous impact on ecosystems that have large amounts of organisms within it; such as fish, zooplankton, phytoplankton, and many other organisms in ecosystems (Dzialowski *et al.* 2005). Algal blooms can be toxic in nature; if the bloom is too large, it will bypass its benefit on the ecosystem and begin to destroy it from the inside out (Anne *et al.* 2015).

Algae is increasing in aquatic ecosystems, which is causing a large problem in certain water systems. The cause of this unnatural algae growth can be attributed to eutrophication. Eutrophication is the introduction of inorganic amounts of nutrients to an ecosystem, mainly N and P (Ryther *et al.* 1971). While originally eutrophication is not a bad thing, it is simply the aging of a water source; it has not become a major problem for many of our water systems (Falkowski *et al.* 2000). This process of eutrophication introduces inorganic N and P into an ecosystem at one time, which alters the N:P ratios and further affects the algal growth. This increased eutrophication can be attributed to an excess of man-made chemicals in run off, and the presence of several household cleaners and fertilizers that contain both N and P. In excess the processes of eutrophication process can increase algal growth, to an extent that cannot be carried by the ecosystem, which can negatively impact the ecosystem all together. If there is an excess in algal growth, it can decrease the amount of zooplankton, which will increase phytoplankton (because of no predation), that will increase the turbidity of the water, which will decrease the amount of sunlight that is able to penetrate the water's surface. If there is no sunlight passing through then it will negatively impact the plants. If the plants die then the primary consumers die, and if the primary consumers die than so do the secondary and tertiary consumers, which in the end will negatively impact the ecosystem as a whole (Porter 1977).

In this experiment, we hypothesized that different nutrients would limit algal growth in different water sources. The two water sources that

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we studied may have different nutrient concentration and ratios, which could cause the biomass to be greater in one than the other in response to different nutrient additions. We collected water from two sources. First, we collected from a local neighborhood pond, which we predicted would be limited by one nutrient or the other due to excess eutrophication. Second, we collected from Boomer Lake which we predicted would not be limited due to high turbidity levels.

Materials and Methods

To begin the experiment, we traveled to two different water sources, Boomer Lake and a local neighborhood pond. We collected samples in 40 L carboys and brought the samples back from each water source, to the laboratory at Oklahoma State and poured it through a 245 μm filter (to remove zooplankton). We then measured the baseline concentration of algae in each water source using a Turner Trilogy Fluorometer to attain Relative Fluorescence Units (RFU). The water was added to 1L mason jars, where each of the two water sources was added to twenty-four jars for a total of 48 sample jars. The water was poured into the 1 L jars adding approximately 250 mL of each to each jar at a time, to ensure that the water, to ensure that the water was mixed among the samples when it was poured from the carboys. The nutrients that we added were nitrogen (N), phosphorous (P), and iron (Fe). We added all possible combinations of the three nutrients to each sample, and each of the eight treatments were replicated in triplicate mesocosms. The first treatment was a control, which contained no nutrients. We then created treatments that were consistent of N, P, Fe, NP, NFe, PFe, and NPFe. The concentrations of added nutrients were 100 μg/L of P, 1600 μg/L of N, and 1 mg/L of Fe. We then placed the jars randomly on a shelf at room temperature under continuous light. We measured the RFU of each sample daily, after stirring each sample. After measurements were collected we put the jars back under the lights in a random patter until the next day, this was to ensure that they received similar amounts of light for a 24 hr. period.

Results

From Figure 1 (top) we determined that the pond was N limited, because RFU increased in all treatments that had N added to them. Those treatments that had P and Fe additions (except for those treatments that also included N, e.g., NP treatments) did not exhibit extensive algae growth relative to the control. Figure 2 (bottom) shows the algal biomass in Boomer Lake. In Boomer Lake the graphs shows a substantial growth in biomass for all of the treatments regardless of nutrient additions. Each treatment appears to show an increase in RFU from the second to sixth day; from the sixth to the ninth day there is a decrease in growth. In Figure 1, the treatments that showed substantial growth appeared to have a higher RFU than those in Figure 2.

Discussion

In this experiment our goal was to determine the nutrient that limited algal growth in two sources of water. We did this by performing bioassays with three different nutrients: N, P, and Fe. We hypothesized that our neighborhood pond would be N limited due to excess eutrophication based on its close proximity to a neighborhood. Our second hypothesis was based on our findings in Boomer Lake; we believed that nothing would be limited, because there appeared to be little algae growth and high turbidity in the lake. Both of our hypotheses were correct. We found based on the Figure 1 (top) that the pond was N limited because of the lack of growth that these treatments exhibited, the ratio of N:P in the absence of N additions was not favorable to growth. In Figure 2 (bottom) we found that nothing was limited, because there was substantial growth in all of the treatments. This could be due to the lack of eutrophication of unnatural N and P levels or due to high turbidity that was limiting algal growth in the lake.

There were also some other factors that could have contributed to these results. Our variables were extremely controlled in the 1 L bioassay jars, which mean that they were relatively unnatural. These samples were contained under light at all times (unnatural) meaning that the light dependent reactions were never stopped. They were also in room temperature at all times as well; there was not a fluctuation of temperature like there is in outside ecosystems. Based on these differences there could be another variable effecting algal biomass, and that is light. Based on these results the samples could have also not grown as well or could have grown better because of the excess light that they were exposed to.

Previous research has assessed algal growth compared to the amount of grazers present in the water (zooplankton). Researchers found somewhat

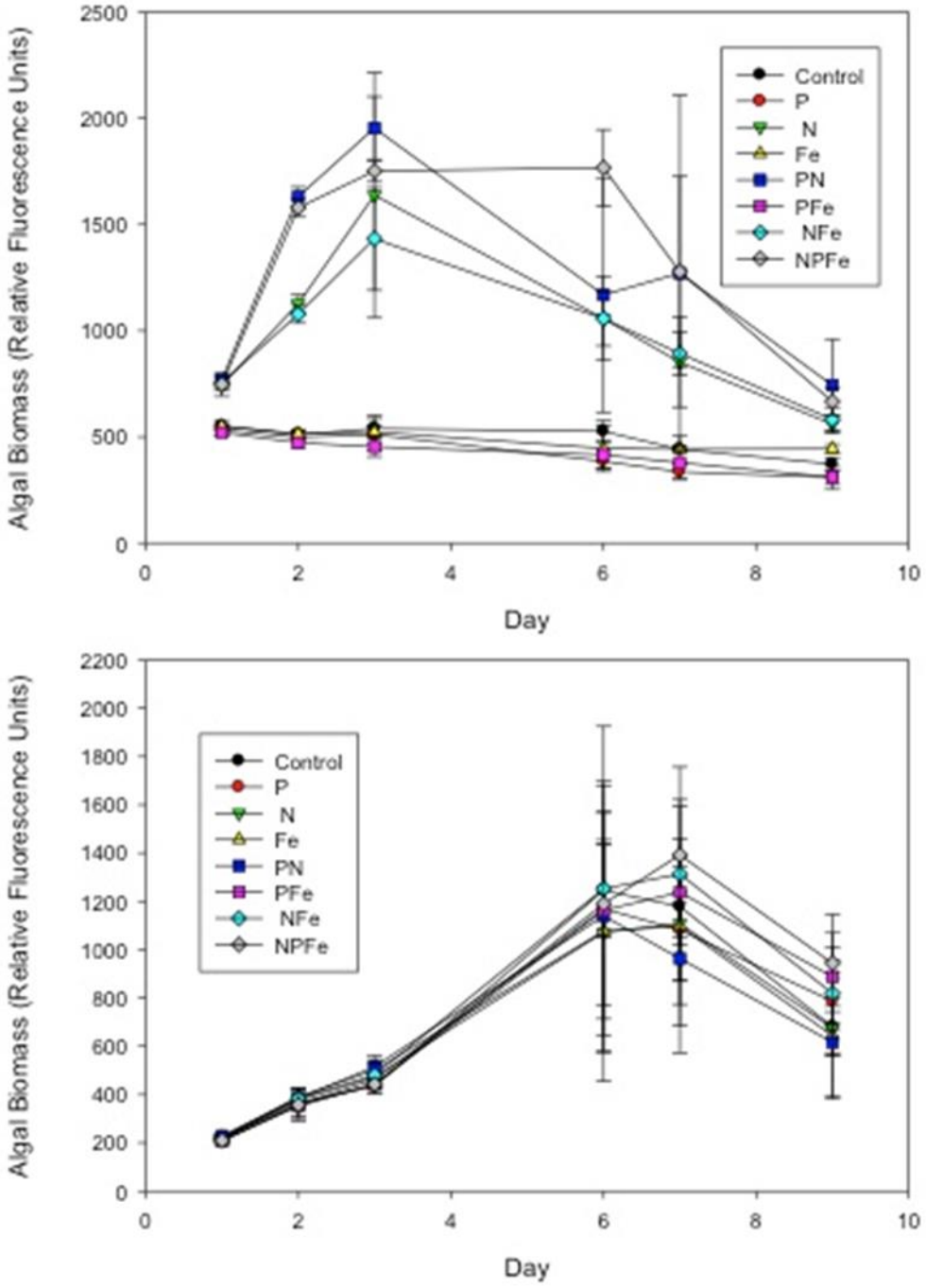

Figure 1 *- Algal growth (measured in RFU) in two water sources in response to phosphorous, nitrogen, and iron. The top graph shows results from a neighborhood pond while the bottom graph shows the results from Boomer Lake.*

of a negative correlation between zooplankton density and the algal growth (Harvey *et al.* 1935). This conclusion was reached because in the experiments conducted there was a noticeable difference between the amount of grazers present vs. the size of the alga blooms, because phytoplankton growth rates declined at the end of algal blooms, meaning that zooplankton (grazers) were most likely present. Those studies were mostly empirical, because of the lack of technology that was present at the time. More recent studies have been able to empirically measure the effects of grazing on algae growth. Porter (1977) was able to find that, based on the season, there were patterns in zooplankton grazing. Grazing by zooplankton was also dependent on the size and shape of the algal cell, if the cell was

too big the zooplankton would avoid feeding on it (Porter 1977). Other studies have been able to concur that algal blooms are regulated by zooplankton grazing (Mitra *et al.* 2006), but with increased eutrophication this is becoming a problem. While there are increased amounts of nutrients being introduced to the water there is not an equal amount of grazers being introduced to help regulate algal growth. With the increased eutrophication there have also been an increase in toxic algal blooms. The zooplankton are not able to filter through the algal blooms fast enough, nutrients are added to water at an alarming rate due to various reasons, and that increase algal blooms. Without the increase in predation of the blooms they are growing rapidly, and becoming increasingly toxic (Mitra *et al.* 2006). Algal blooms without normal predation begin to compete with another for survival. They adapt to their surroundings and become toxic in order to kill of any other competing algae, they are able to do this because nutrient demanded is never limited because of eutrophication (Mitra *et al.* 2006). Many of these blooms will disrupt the energy flow in trophic levels, and they have the ability to produce secondary metabolites (toxins), which make them less enticing to grazers (Teegarden 1999).

Further studies should be conducted to better understand the process of eutrophication and the development of toxic algal blooms. These two areas of study are dependent on each other most of the time. This continues to show that eutrophication is a growing problem in aquatic ecosystems, and many times the nutrients that are being added to these ecosystems are a direct result of man-made products.

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