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Nitrate and phosphate levels positively affect the growth of algae species found in Perry Pond.

STEFFII FRIED, BRENDAN MACKIE, and ERIN NOTHWEHR
Biology Department, Grinnell College, Grinnell, IA 50112, USA

Abstract
High rates of agricultural runoff can cause large quantities of nitrates and phosphates to enter the water system. When added to a water body, these nutrients can create a large proliferation of algae which is harmful to water quality. The blooms deplete oxygen levels in aquatic ecosystems and thus have a detrimental effect on the organisms within the system. We evaluated the effects of nine different combinations of nitrate and phosphate concentrations on algal growth by measuring relative chlorophyll levels. We concluded that both nitrates and phosphates have positive effects on algal growth. However, these variables affect algal growth independently of each other and there is no interaction between the two. This implies that both nitrates and phosphates are effective limiting nutrients that can be reduced to control algal proliferation.

Introduction

The contamination of the water systems with inorganic nitrogen and phosphorus from fertilizer runoff is a pressing concern given the prevalence of agriculture in the Midwest. To maintain a healthy hydrologic network, it is necessary to understand the effects of these increased chemical concentrations on water quality. Previous studies have shown that the addition of these nutrients to the water system would result in large proliferations of algae which have detrimental effects on the water quality. (Ryan *et al*. 1972, Frink and Machlis 1968, Sikka and Pramer 1968, and An and Park 2002). Algal blooms deplete the oxygen supply in the water system and are harmful to other aquatic species. Additionally, they cause taste and odor problems which result in reduced recreational use, and increased water treatment costs (An and Park 2002). In an effort to maintain a healthy water system and to minimize algal growth, the United States Environmental Protection Agency (USEPA) recommends that phosphate levels be kept below 0.1mg/l and nitrate levels be kept below 10mg/l (USGS 1996-1998). Our study will provide an evaluation of these standards and their effectiveness in preventing algal growth in Iowa ponds.

In systems with low algal growth, there is a shortage of either nitrogen or phosphorus which limits the algal growth. The cause of this shortage varies between different water systems. In Perry Pond, where we collected our samples, phosphate depletion most likely occurs because the phosphates are lost from the water column through sedimentation and because they do not have a gas phase (An and Park 2002). Nitrate and phosphate depletion in Perry Pond can also occur because of the accumulation of organic biomass in the pond. When this biomass dies, it sinks to the bottom of the pond, where, in a healthy system, decomposers will process it and release the nitrates and phosphates back into the system. However, in an unhealthy pond with a large proliferation of algae, there is not enough oxygen for the decomposers to survive, thus the dead biomass is not decomposed and nitrates and phosphates are not released into the water system. This results in nitrate and phosphate depletion which results in a decrease in algal growth (Freeman 2002).

Clearly, the scientific community generally agrees that an increase in nitrate and phosphate levels increases algal growth. Therefore, it is reasonable to expect that the growth of the algal species in Perry Pond will increase with the addition of nitrates and phosphates. However, we are also curious as to whether nitrate and phosphate levels affect algal growth independently of each other or if there is a significant interaction between the two nutrients.

Methods

We collected a random 19 liter sample of pond water from Perry Pond at the Conard Environmental Research Area near Kellog, IA on October 27, 2003. Perry Pond is surrounded by prairie and second growth woods, which act as a natural buffer strip on all sides. The nitrogen and phosphorus levels in the pond, as measured on October 27, 2003 were 0.259mg/l and 0.0442mg/l respectively. The sample contained the following genera of algae: *Spirogyra*, *Cladophora*, *Euglena, Protococcus*, and *Zygnema.*

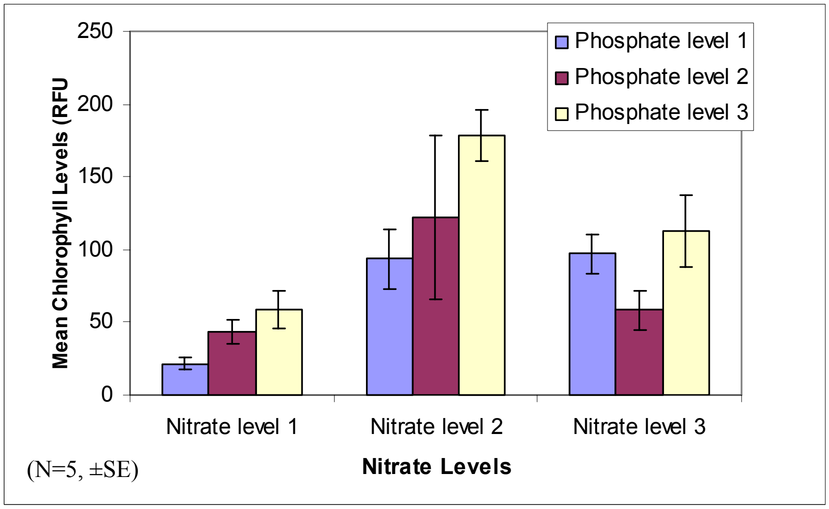

Figure 1: Mean chlorophyll levels measured in RFU's on a TD-700 Turner Designs flourometer day 7. N_1= 7.14x10^{-6}M nitrate, N_2= 9.61x10^{-4}M nitrate, N_3=.00928M nitrate. P_1=2.33x10^{-6}M, phosphate, P_2=3.23x10^{-6}M phosphate, P_3=7.47x10^{-6}M phosphate.

We used a centrifuge to separate the algae from the water to create an algal inoculate, .04ml of which we placed into 45 translucent plastic 500ml containers with 300mL of distilled water in each container. Each of these containers had one of nine different combinations of three levels of nitrogen and phosphorus with five replicates for each container. Based on a study by Tubea *et al* (1981) and the guidelines from the United States Environmental Protection Agency, the three levels of nitrogen we added were: N_1=7.14 *10^{-6}M, N_2=9.61 *10^{-4}M, N_3=.00928M. The three levels of phosphorus added were: P_1=2.33 *10^{-6}M, P_2=3.23 *10^{-6}M, P_3=7.47 *10^{-6}M. P_1 and N_1 are significantly lower then the USEPA standards and P_2 and P_3 and N_2 and N_3 are all higher then the USEPA's standards.

We placed translucent lids with three holes for gas exchange on the container to prevent excessive evaporation. We placed the containers in random order on a tray under uniform lighting at a level of 60.18 *u*M/m^2. They grew for five and seven days before we estimated the amount of algal growth by measuring the relative chlorophyll levels using a TD-700 Turner Designs fluorometer. The relative chlorophyll levels were based on a blank of distilled water at the level of 0 relative fluorescent units (RFU) and a solid state standard of 900 RFU. The data were analyzed with a two-way ANOVA to determine if there were differences among the nitrate and phosphate treatments and an interaction between treatments in algal growth.

Results

Two-way ANOVA revealed a significant difference in the relative chlorophyll levels, measured on the seventh day, for both the nitrate and the phosphate treatments (F_n=10.67, p_n=0.000 < 0.05, F_p=3.41, p_p=0.044 < 0.05). Figure 1 illustrates that in both N_1 and N_2, algal growth increased as the phosphate level increased. However, in N_3, algal growth did not uniformly increase as the phosphate level increased. This suggests the effects of the nutrients are dependent on each other. In N_3, the phosphate levels were not high enough for the N_3 level of nitrogen to significantly increase growth. However, the two-way ANOVA showed no significant interaction of the two treatments (F_{n*p}=0.92, p_{N*P}=0.462 > 0.05), suggesting that nitrogen and phosphorus affect algal growth independently. There were no significant effects on day 5 (the first day sampled).

Discussion

As expected, nitrate and phosphate levels both significantly affected algal growth. These results are supported by Ryan *et al*. (1972), Frink and Machlis (1968), and Sikka and Pramer (1968) who found that both nitrate and phosphate levels have a significant effect on algal growth. These results are contradicted by a study by B. Tubea *et al*. (1981), who found that phosphate levels had no significant effect on algal growth. However, their study did not isolate phosphate as a variable. Rather, they tested the effects several herbicides had on algal growth with high or low phosphate concentrations. They found that the phosphate levels had no significant impact on the herbicide's effects on algal growth. In contrast, our study directly analyzed the effects of the phosphate levels on algal growth.

The results of our study were also contradicted by Pietilainen and Niinioja (2001), who found that nitrate levels had no significant effect on algal growth. However, their study was conducted over the summer when the algae were in the logarithmic phase of growth. Our algae were collected in fall, when it was well past the logarithmic growth phase. Also, their samples were exposed to a diurnal light cycle with fifteen hours of daylight and nine hours or darkness whereas our samples experienced twenty four hours of light each day. Clearly, our study contradicts both of these study's results since it found that both nitrate and phosphate levels are factors limiting algal growth. Therefore, reducing the concentrations of either nutrient can be a useful tool for reducing algal growth.

It is interesting to note that the highest levels of relative chlorophyll (131.6 to 238.2 RFU) were found in the N_2P_3 treatment. This result is supported by studies by Havens *et al.*(1999) and Pietilainen and Niinioja (2001) which found that algae was most prolific in the high phosphorus and nitrogen treatments. The high growth levels in the N_2P_3 treatment as opposed to the N_3P_3 treatment imply that the effects of the nitrate and phosphate levels are dependent on each other. However, ANOVA revealed no significant interaction between the variables. This discrepancy between our intuitive logic and the statistical test most likely occurred because we preformed ANOVA over all three nitrate levels. Perhaps if we had used ANOVA to look for an interaction between nitrogen and phosphorus over N_2 and N_3 our results would have been significant.

Regardless of the significance of the interaction between the variables, we can conclude that if only one nutrient is substantially increased the other nutrient will limit the growth. For example, if nitrogen is present in very high concentrations, and phosphorus is not, the algae will proliferate until it has used up all of the phosphorus even if there is still an ample supply of nitrogen. It follows that, in an aquatic system with high nitrogen levels and low phosphorus levels, phosphorus is the limiting factor on algal growth. Therefore, if both nutrients are present in high concentrations neither nitrate nor phosphate will limit algal growth and an algal bloom will develop.

The data collected on day five, the first sampling, show that nitrate and phosphate levels have no significant effect on algal growth. Perhaps this is because all of the solutions initially contained enough nutrients to support algal growth. However, as the algae grew, it used up the nutrients and, in the solutions with low nitrogen and/or low phosphorus levels, nitrogen or phosphorus became a limiting factor and inhibited growth. These growth differences were not observed until the second sample on day seven, after the algae had a chance to grow.

However, a study by Pietilainen and Niinioja (2001) contradicts our results because it found that after the first three days the levels of chlorophyll in their samples almost doubled. This discrepancy most likely occurred because the algae they studied was in the logarithmic phase of growth at the beginning of the experiment and thus was reproducing at very rapid rates. In contrast, the algal inoculate in our samples was gathered from Perry Pond in the fall when it was well past the logarithmic stage of growth. Therefore, since it reproduced at a slower rate, we did not see significant results until the second sampling, seven days into the experiment.

The USEPA recommends that nitrogen concentrations be kept below 10mg/l and phosphorus concentrations be kept below 0.1mg/l to keep algae growth at a minimum. However, our P_1 level was 0.05mg/l and when substantial amounts of nitrogen were added, the algae still proliferated. This suggests that phosphate levels should be less then .05mg/l to substantially limit algal growth. In Perry Pond, the phosphate level was .0432mg/l and algal growth was sparse and confined to the littoral region at the time we took our sample. The low growth levels for N_1 support the USEPA's standards since our N_1 concentrations were below 10mg/l. Additionally, in Perry Pond where algae growth was sparse, the nitrate level was .259mg/l which is well below the standard of 10mg/l.

Our study shows that nitrogen and phosphorus levels both positively affect algal growth. Since these elements are two of the three main ingredients in fertilizers, they frequently enter the ground water, streams, and lakes through runoff from fields. Our study showed that the infiltration of these nutrients into Iowa ponds will increase the algal growth and thus decrease the water quality, and increase eutrophication. These detrimental effects of runoff justify the need for buffer strips and other method of conservation to reduce runoff. Our study site, Perry Pond, was surrounded by prairie and second growth forest, both of which act as natural buffer strips. Nonetheless, it does have inputs from streams that run through farm land. However, we collected our sample at the end of a drought so the runoff into the stream inputs was minimized. Thus, as expected, the nitrogen and phosphorus levels were low, 0.259mg/l and 0.0432mg/l respectively. As our study predicted, the low nutrient levels resulted in low algal growth.

Two of the three major ingredients in fertilizers are nitrates and phosphates. Our study showed that these nutrients increased algal growth which is harmful to water quality. It follows that fertilizer runoff has detrimental effects on water quality because it increases the concentrations of nitrates and phosphates in the water system and thus increases algal growth. However, most fertilizers contain nitrogen, phosphorus, and potassium. It would be interesting to test the effect potassium has on algal growth.

Our study focused on five algal species that lived in Perry Pond, which has relatively low nitrate and phosphate concentrations since it is surrounded by natural buffer strips. These strains of algae could be adapted to living in an environment with low nutrient concentrations, and thus our results are not representative of the entire spectrum of algae found in Iowa ponds. The experiment could be repeated on different strains of algae collected from different Iowa ponds with varying nutrient levels. Comparing the findings of these experiments to the findings of our study would yield a broader result that could be more universally applied.

The scientific community generally agrees that fertilizer runoff affects the entire aquatic community. However, it is unknown whether certain aspects of the community, such as the zooplankton, are affected by the increased nutrient concentrations, by the oxygen depletion that results from the algal blooms, or by another factor that is indirectly related to the fertilizer runoff. When we analyzed our samples we found several dead zooplankton. Further studies could analyze the effects of increased nitrogen and phosphorus concentrations on zooplankton and other aquatic species to determine if the other species are directly impacted by the increased nutrient concentrations.

Based on our results, it is safe to conclude that, at the time we took our sample, Perry Pond had a healthy level of nitrogen and phosphorus. Its levels are well below the USEPA's recommendations and, as our study predicted, algae growth is sparse and confined to the littoral region of the pond. Since the pond is surrounded by natural riparian and grass buffer strips, fertilizer runoff is low and, as a result, the nitrate and phosphate concentrations are low compared to farm ponds and other bodies of water that are prone to runoff. This result demonstrates the success of buffer strips in maintaining healthy nutrient levels and thus good water quality. However, further studies that test the nitrate and phosphate levels and their effects on algal growth in farm ponds are needed to more fully justify the need for buffer strips.

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