Effects of Nutrient Enrichment on the Growth of the Green Alga Spirogyra in Conesus Lake, N.Y.

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Abstract- Spirogyra is a filamentous green alga that inhabits the streams and lakes of North America, including Conesus Lake, NY. Two separate experiments were designed to determine if nutrient enhancement had an effect on Spirogyra growth. In one experiment, known quantities of Spirogyra were placed in 12 containers provided with mesh screens. The containers were separated into three groups and held in racks that were placed at increasing distances from a stream with a known effluent nutrient load. The average percent change in biomass calculated after two weeks for near, middle and far distances were 35.38, 16.62, and 4.12 (respectively). The growth rates of algae incubated near the stream were significantly higher than algae living farther away from the stream.
In a second experiment, Spirogyra was incubated in 12 closed one-liter containers. Each set of jars contained different concentrations of enhanced nitrogen and phosphorous; amounts of lake water and Spirogyra remained constant. Two sets of jars had enhanced concentrations of N and P, while a third contained ambient lake water. The average percent change in increased biomass determined after two weeks in the lake was 19.8 (high nutrient concentration), 10.8 (low nutrient concentration) and 7.5 (no nutrient addition).
There was a statistically significant difference in the growth rates of algae living in high concentration and control environments. The results obtained from these two experiments support our hypotheses that Spirogyra living in an environment with enhanced nutrient levels will have an accelerated growth rate compared to algae living in waters with ambient levels of N and P. Since the greatest Spirogyra growth was observed with increasing distance from stream outlets into the lake, artificial nutrient addition should be a concern to the Conesus Lake community.

Introduction

Spirogyra is a filamentous green alga that inhabits the streams and lakes of North America. Adapted to live in colder temperatures, Spirogyra can store large internal reserves of nutrients that can sustain maximum growth rates for several weeks. (O'Neal 1988). Nutrient enrichment in lake communities has been shown to change the original structure of the algae community (Havens 1999) and can lead to the increased growth of lake flora such as Spirogyra. Because of limitations in the availability of the nutrients nitrogen (N) and phosphorus (P) for growth, the addition of these nutrients to lake ecosystems may cause the Spirogyra population to increase dramatically (O'Neal 1988). This can bring changes to the species composition of algae and to the intrinsic dynamics of the lake (Havens 1999). Algal blooms, though considered by some to be a nuisance and a sign of environmental degradation, often play an important ecological role in supplying substrate and resources for epiphytic and browsing organisms (Lorenz 1991).

The distance of agricultural wastes and sewage into nearby lakes function as non-point sources of nutrient enrichment. A population explosion of Spirogyra in areas immediately downstream of an agricultural effluent or sewage is evidence of such enrichment. Resource use (cropland and livestock) and urbanization have been shown to be stronger determinates of algal communities than climatic factors (Hall 1999). Therefore, regulation of agricultural activity neighboring lakes is important to minimize the environmental impact resulting from nutrient additions (Holeck 1998). Studies have been conducted to address the long-term effects of nutrient additions on algae communities (Rice 1985). However, short-term enrichment experiments can indicate the potential for nutrient limitation of algal growth in the absence of other limiting factors (Kimmel 1986; Richardson 1986) and also demonstrate the effects of agriculture on nutrient enrichment.

Objectives

Algal population explosions in areas immediately surrounding run-off streams in Conesus Lake have increased over the past five years (Isidro Bosch, personal communication). Since many farms and roadways border Conesus Lake, it is likely that runoff nutrients are causing significant problems for Conesus Lake.

In 1992 Conesus Lake was colonized by the zebra mussel (Dreisenna polymorpha). Zebra mussels filter the water of phytoplankton, increasing light and reducing nutrient competition for filamentous algae by increasing the amount of nutrients present in the lake. Thus, the presence of these mussels can result in increased eutrophication (Bosch et al. 2001).

Historically, Conesus Lake has become increasingly eutrophic (Makarewicz 1986). Natural lake eutrophication can be accelerated by nutrient loading (Krohne 1998). Because eutrophications through non-point and biotic nutrient additions is a problem for the Conesus Lake community, the effects of increased levels of N and P associated with eutrophication should be examined to determine what effect, if any, the N and P entering the lake through streams is having on lake alga communities.

We hypothesize that:

> Spirogyra communities living near stream effluent where nutrient levels entering the lake are higher than normal lake levels will experience a greater increase in percent growth than those living in areas farther away from stream runoff.

> Spirogyra living in an environment where N and P levels are artificially enhanced will experience a greater percent growth over a two-week period compared to algal communities living in an environment with no nutrient additions.

Methods

Graywood Gully experiment

To measure the effects of non-point source nutrient enhancement of Conesus Lake on Spirogyra, an open experiment was designed to allow the algae to respond to the environmental changes. Experiments were conducted during October 2000 at Graywood Gully in Conesus Lake (Figure 1). The experimental apparatus used consisted of three plastic incubation racks containing four one-liter clear plastic jars (Figure 2a, Figure 2b). Canister lids affixed to a clear plastic sheet and supporting PVC pipes in each corner ensured that each set of canisters remained the same height from the lake bottom. This design simulates the ambient environmental conditions in which the Spirogyra normally grows in Conesus Lake. Sections

Figure 1- map of Conesus Lake with stream gullies. Algae were collected from Long Point Gully, and placed at increasing distances from Greywood gully (see Methods). Map adapted from Makarewicz et al. (2001)

of the canisters were removed and replaced with a thin wire screen to allow water flow between the lake and each canister while still enclosing the algae within the jar.

Algae were collected from Pebble-Beach Cove in Conesus Lake. Samples were filtered and rinsed to prevent sediment or organisms from adversely affecting the experiment. The algae were then blotted dry and divided into twelve separate samples of three grams each (blotted weight). Three additional samples of blotted dry Spirogyra were dried for 24 hours at 30o C to determine the conversion of blotted weight to dry weight.

The prevailing winds in Conesus Lake tends to force the stream runoff northward along the shore. Our incubation racks therefore, were placed 30 meters offshore from Graywood Gully and extended along the shoreline approximately 20-30 meters according to the wind current. Incubation racks were suspended below a buoy and held vertically within the water column by a cement anchor one meter below the surface. Each incubation rack held 3 jars containing Spirogyra.

The canisters were collected after two weeks. Canister numbers two and five were lost during the

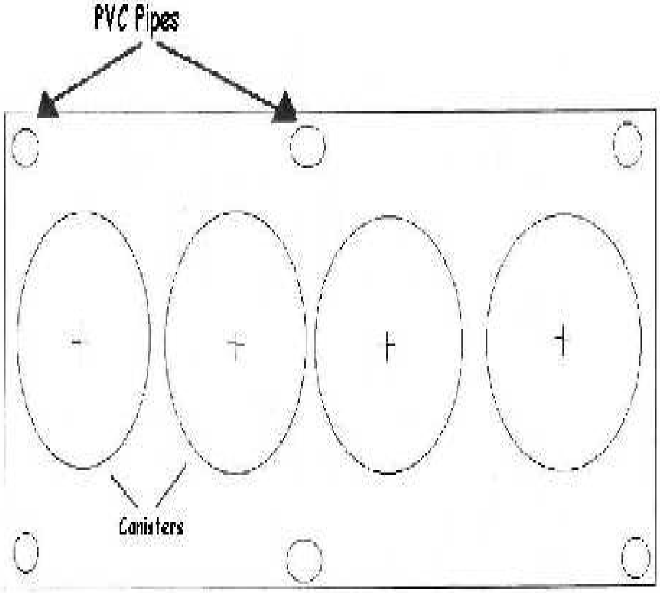

Figure 2a- top view of experimental apparatus. Four canisters were supported with PVC pipes.

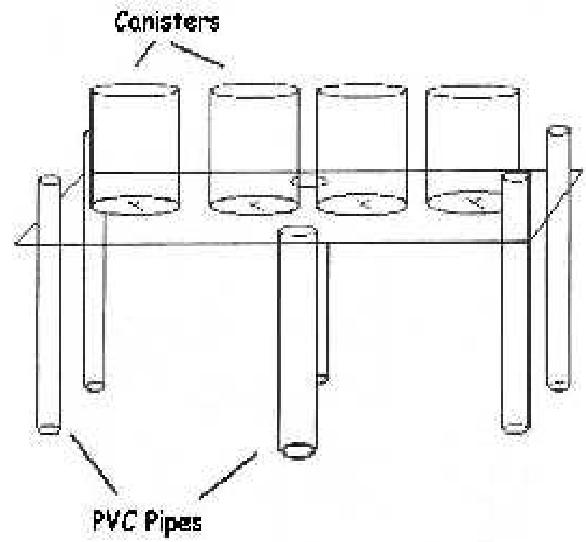

Figure 2b- Side view of experimental apparatus. Canisters were positioned to maximize exposure of algae to ambient light conditions.

course of the experiment, hence, only three replicates are used for the near and far racks. The algae from each canister were isolated and dried at 30oC for 24 hours to determine dry weights. The original dry weight of the sample was estimated by multiplying the blotted weight times the additional three samples of algae collected. Percent increases in dry weight were arcsine transformed. The percent changes in biomass from original to final dry weight were analyzed using an ANOVA to determine whether Spirogyra biomass exists between the three sets of canisters. A post hoc Tukey-test in SPSS was used to determine differences among the response variable (percent change in biomass) and the factor (distance from stream outlet).

Closed experiment

To measure the effects of phosphorus and nitrogen enrichment on Spirogyra, three separate samples of algae and lake water were collected from Long Point, Conesus Lake. The water collected was filtered as in the open experiment. Algae were treated in the same manner as the stream experiment.

Stock solutions of N and P were made using NaNO3 and K2HPO4 respectively. The maximum concentration of both N and P was based on the concentrations of stream effluent measured by Makarewicz (1999). The stock solution was measured at 3.76x10-3 g/mL N and 4.3x10-4 g/mL P for the maximum concentrations.

The incubation jars for the closed experiment had no wire mesh screen and were completely closed to the lake environment. The canisters were attached to the incubation rack and then filled with filtered lake water, algae, and one mL (maximum concentrations) of NO3- (final concentration: 3.76x10-3 g/mL) and one mL PO4-2 (final concentration: 4.3x10-4 g/mL) from the prepared stock solutions. Canisters five through eight, attached to a second incubation rack, were filled with filtered lake water, algae, at concentrations of 0.5 mL of NO3- (final concentration: 1.8x10-3 g/mL) and 0.5 mL PO4-2 (final concentration: 2.15x10-4 g/mL) from the prepared stock solutions (half of the maximum concentrations). Canisters nine through twelve contained filtered lake water and algae with no addition of N or P.

The three racks were attached to cinder blocks to prevent drifting and placed roughly 20 meters offshore of Pebble-Beach Cove. The racks were placed approximately one meter from each other. After one week the water from each canister were replaced with fresh filtered lake water and the original concentrations of N and P. The canisters were collected the following week. The algae from each canister was

Table 1- Results from the Graywood Gully experiment. There is a significant relationship between the canisters at near and far distances, (*) indicates significance at the 0.05 level (Tukey, p=0.029).

(I) Distance	(J) Distance	Mean Difference	Std. Error	Sig.
Near	Middle	0.4392	0.4932	0.663
	Far	1.7633*	0.5273	0.029
Middle	Near	-0.4392	0.4932	0.663
	Far	1.3242	0.4932	0.071
Far	Near	-1.7633*	0.5273	0.029
	Far	-1.3242	0.4932	0.071

isolated and dried at 30^{o} C for 24 hours to determine their dry weights. The original dry weight of the samples were calculated using the conversion factor obtained from the five additional samples collected. Percent increases in dry weight were arcsine transformed. The percent change in arcsine transformed biomass from original to final dry weight was analyzed using ANOVA to determine if a significant difference of Spirogyra biomass exists between the three sets of canisters. A post hoc Tukey-test was used to determine differences among the means for samples taken at the different distances from the stream outlets.

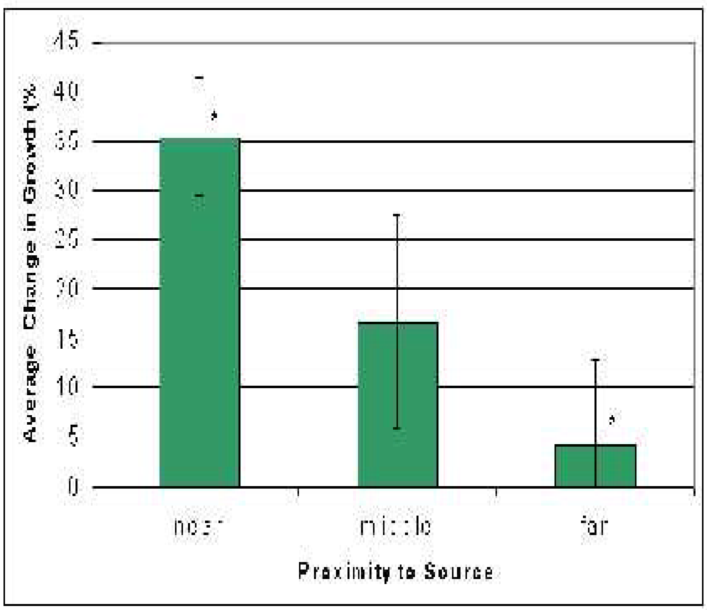

Figure 3- Average percent change in Spirogyra growth incubated at increasing distances from Graywood Gully. Error bars represent standard deviation. A Tukey indicated significantly different means in the near and far canisters (Tukey, df=9, p=0.029), a (*) denotes a significant relationship.

Appendix 1- Percent change in biomass for canisters at near, middle and far distances from Graywood Gully. Greatest percent changes were observed between near and far distances.

Location	Bottle	Initial Wet (g)	Initial Dry (g)	Final Dry (g)	Percent Change
Dry Weight Conversion Samples	A	1.5	0.20	-	-
	B	1.8	0.30	-	-
	C	2.1	0.30	-	-
Near	1	1.9	0.28	0.37	31.94
	3	2.0	0.30	0.42	42.28
	4	1.9	0.28	0.37	31.94
Middle	9	2.1	0.31	0.33	6.47
	10	2.0	0.30	0.34	15.18
	11	1.9	0.28	0.37	31.94
	12	2.1	0.31	0.35	12.92
Far	6	2.1	0.31	0.31	0.01
	7	1.9	0.28	0.32	14.11
	8	2.0	0.30	0.29	-1.76

Results

Graywood Gully Experiment

A single factor ANOVA (df=1, 9; p=0.029) indicated that the differences in growth were statistically significant (Table 1). The Tukey analysis indicated that there was a statistically significant difference of 31.26 percent change in biomass between the response variable and factor of the near and far incubators (df=1, 9; p= 0.029) (see Table 1). There was no statistical difference between the response

Table 2- Results from the closed experiment. There is a significant relationship between the canisters with high and no nutrient additions, (*) indicates significance at the 0.05 level (Tukey, p=0.027).

(I) Nutrient Addition	(J) Nutrient Addition	Mean Difference	Std. Error	Sig.
High	Low	0.2275	0.1313	0.246
	None	.4175*	0.1313	0.027
Low	High	-0.2275	0.1313	0.246
	None	0.19	0.1313	0.359
None	High	-0.4175*	0.1313	0.027
	Low	-0.19	0.1313	0.359

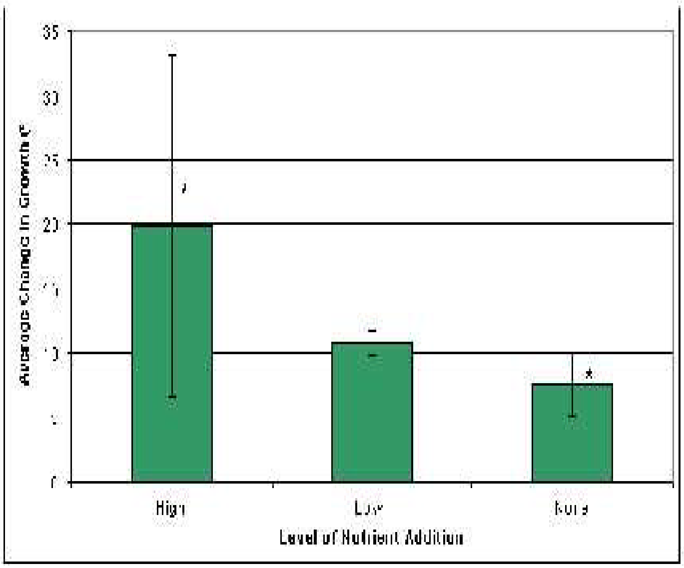

Figure 4- Average percent change in growth for three incubation racks versus nutrient addition for the closed experiment. Error bars indicate standard deviation. A Tukey indicated a significant difference between the high and low addition (Tukey, df=2, 11; p=0.027), a (*) denotes a significant relationship.

variable (biomass) and factor (distance) of the near and middle or the middle and far incubation racks (see Table 1).

Three days with high amounts of rainfall and runoff were observed during the incubation period. The average percent change in size for the bottles near the shore was 35.38 (+ 5.97) percent, mid-distance from the shore was 16.62 (+ 10.85) percent and farthest from the shore 4.12 (+8.69) percent (*Figure 3, see Appendix 1 for data from Graywood Gully experiment).*

Closed Experiment

A single factor ANOVA (df=11, p=0.034) indicated that the 11.64 percent increase in biomass between the high and low concentration containers were statistically significant. The Tukey results indicated that there was a statistically significant difference (df=2, 11; p=0.027) between the high nutrient enhancement and control rack. There was no statistical difference between the high and intermediate enhancement (p= 0.246) nor the intermediate and control (p=0.359) (Table 2). All canisters experienced a positive growth rate (Figure 4) that varied from 4.41 to 39.63 percent (Appendix 2).

Appendix 2:-Percent change in biomass for canisters with high, low, and no nutrient addition. Greatest percent changes were observed between high and no nutrient addition.

Location	Bottle	Initial Wet (g)	Initial Dry (g)	Final Dry (g)	Percent Change
Dry Weight	A	1.5	0.20	-	-
Conversion	B	1.8	0.30	-	-
Samples	C	2.1	0.30	-	-
	1	1.9	0.28	0.37	31.94
Near	3	2.0	0.30	0.42	42.28
	4	1.9	0.28	0.37	31.94
	9	2.1	0.31	0.33	6.47
Middle	10	2.0	0.30	0.34	15.18
	11	1.9	0.28	0.37	31.94
	12	2.1	0.31	0.35	12.92
	6	2.1	0.31	0.31	0.01
Far	7	1.9	0.28	0.32	14.11
	8	2.0	0.30	0.29	-1.76

Discussion

The results indicate that the combined additions of N and P have a positive effect on the growth rate of Spirogyra. In addition, we provide evidence for possible enhancement of algal growth by stream runoff. Our analysis shows that there was a statistical difference between the racks with the greatest nutrient addition and the racks with the lowest nutrient addition. In the open stream experiment, the Tukey test (df=11, p=0.029) indicated that the difference in percent growth between the near and far racks was not due to chance alone. This suggests a positive correlation between nutrient addition and high growth rates.

There was not a statistically significant relationship between the middle and far racks (Tukey: p=0.071). When comparing the average percent growth for the three racks, a gradient is apparent (Figure 3). The middle rack may be a true intermediate between the near and far racks. Other variables such as wind and water currents and pH levels may have effected the resulting percent growth in ways that we could not investigate. During the Graywood Gully experiment, three days with high amounts of rainfall and runoff were observed. This may have had an influence on the results as previous research has shown that the Graywood Gully commutes the highest levels of total phosphorus into the lake following rainfall events compared to other stream inputs in

Conesus Lake (Makarewicz et al., 2001).

The results from the open and closed experiments similar. The distinguishing result of both experiments were the identification of the limiting nutrients of N and P for Spirogyra growth. Since all of the algae were placed in similar environments, we are confident that there were no factors except the nutrient load that could have influenced the growth of the algae. As in the open experiment, there was no detectable statistical difference between the median (low nutrient level) rack and the high (maximum nutrient levels) and control racks (p= .246 and p= .359 respectively). But a comparison of the average percent growth for each of the three nutrient levels again shows that the median rack is an intermediate between the two extremes (Figure 4). This could be due to the difference in concentration between the high-low or the low-control is not enough to cause statistically differential growth in Spirogyra. That is not to say that the differences are not important biologically. Similar to the open experiment, the most important and significant difference is shown by the statistical difference between the high and control containers. Nutrient loading, for example from zebra mussel byproducts, have been shown to affect macrophyte populations within Conesus Lake (Bosch et al., 2001) by increasing light penetration, altering nutrient balance, and clearing the water of suspended material. The presence of zebra mussels along with nutrient loading from stream runoff can have an additive effect on nutrient levels causing an increase in macrophyte growth.

Since the greatest Spirogyra growth with increasing distance from the stream outlets into the lake, artificial nutrient addition should be a concern to the Conesus Lake community. Perhaps if the surrounding community focused on better agricultural practices the amount of nutrient enriched runoff could be reduced.

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