The Impact of Stream Nutrient Loading on Metaphyton in Conesus Lake and the Use of Metaphyton Incubation Chambers for Measurement *In Situ* of Changes in Biomass

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This Work is Dedicated to Vincent Cacchione
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Abstract

Recently, the abundance of metaphyton (*Zygnema* and *Spirogyra*) in Conesus Lake has reached unprecedented levels. This dramatic increase has altered the ecological state of the littoral zone and may have cascading effects on the lake's ecosystem. Studies conducted at SUNY Brockport have demonstrated that stream effluent entering the lake contains high concentrations of soluble reactive phosphorus and nitrate. The hypothesis that stream effluent was having a positive effect on the biomass of metaphyton was tested using continuous flow-through incubation chambers. Metaphyton responded in a significant positive manner when exposed to stream effluent. Analysis of nutrient concentrations determined throughout the incubation chamber experiments and results of an enrichment experiment, suggest that metaphyton in Conesus Lake is limited by phosphorous and not nitrate.

Additionally, quantitative observations along transects were preformed to test the hypothesis that a close spatial relationship existed between the distribution of metaphyton and stream mouths. A close spatial relationship was not observed. Significantly higher percent cover of metaphyton was observed to the north or south of stream mouths (10 – 40 m away) when compared to percent cover directly in front of stream mouths. This pattern is attributed to disruptive forces of stream effluent during hydrometeorologic events.

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Introduction

Filamentous green algae (a type of metaphyton) have reached nuisance levels in the littoral areas of many freshwater and marine ecosystems (Howell *et al.* 1990; France and Welbourn 1992; Thybo-Chritesen *et al.* 1993a; Pillsbury *et al.* 1994; Planas *et al.* 1996; Pihl *et al.*1996, 1999). According to Hutchinson (1975) "metaphyton is a group of algae found aggregated in the littoral zone, which is neither strictly attached to substrata nor truly suspended." The metaphyton, which include single-celled (including diatoms), multi-celled, and filamentous algae, are a community adjacent, but not attached, to macrophytes. With shelter (often provided by macrophytes), abundant irradiance, a stable water column and ample nutrients, metaphyton can develop to nuisance levels (Stevenson *et al.* 1996). Development of metaphyton can be extensive enough to reduce swimming, fishing, and boating activity, adversely affect tourism and recreation, and raise public health concerns (The Conesus Lake Association 2002).

In general, growth of metaphyton communities depends on substrate type, irradiance, water movements, and nutrient availability (Pihl *et al.* 1999). A model proposed by Goldsborough and Robinson (1996), suggest that metaphyton will not dominate unless sheltered from physical disturbances and provided with a substrate, such as macrophytes, to anchor upon. Low light levels can significantly reduce metaphyton abundance and photosynthetic rates (Shortreed and Stockner 1983; Pihl *et al.* 1996; Scheffer *et al.* 1997), while full sunlight (especially on the surface of the water column) can lead to photoinhibition and a reduction in photosynthetic rates (Graham *et al.* 1995). Because water movement strongly affects the balance between metaphyton biomass accumulation and loss, as well as controlling nutrient supply, wave action and water currents greatly influence

metaphyton development (Howell *et al.* 1990; Dodds and Gudder 1992; Pihl *et al.* 1999; Grahm and Wilcox 2000). Some studies have shown a weak association between water-column nutrients and metaphyton (Grahm and Wilcox 2000), in contrast to the strong association seen with phytoplankton. However, metaphyton may be physiologically limited by several elements, primarily nitrogen and phosphorus (Murkin et al 1994; McDougal and Goldsborough 1995; Havens *et al.* 1999). In fresh water systems, phosphorous is frequently the limiting factor for algal growth. Relatively small increases in availability of inorganic phosphorus can affect algal production dramatically (Sridharan and Lee 1977; Dodds and Gudder 1992; Nicholls and Heintsch 1992; Planas *et al*. 1996; Havens *et al*. 1999 and McCormick *et al*. 2001).

The proliferation of filamentous green algae can also be influenced by exotic species such as zebra mussels (*Dreissena polymorpha*) and Eurasian watermilfoil (*Myriophyllum spicatum*). Zebra mussels can dramatically alter ecosystems by selectively filtering phytoplankton and concentrating the predominant energy flow in a lake to the benthos (Moss 1998; Lowe et al 1990). In Saginaw Bay in Lake Huron, the introduction of *Dreissena polymorpha* caused a shift from benthic diatoms to filamentous green algae. One year after the introduction, *Mougeotia* and *Spirogyra* became dominant. This was attributed to an increase in water clarity and light, less competition for nutrients, and deposits of fecal and pseudofecal pellets that increased available nutrients (Pillsbury and Lowe 1994; Fahnenstiel *et al*. 1995). Macrophytes can release nutrients into the water (Wetzel 1983; Burkholder and Wetzel 1989; Havens *et al* 2001) that may be utilized by metaphyton. In addition, Eurasian watermilfoil (*Myriophyllum spicatum*) offers an excellent structure for metaphyton to grow upon.

Eurasian watermilfoil and zebra mussels have invaded Conesus Lake (Bosch *et al.* 2000, Bosch *et al.* 2001, Bosch *et al.* 2002), the westernmost of the 11 Finger Lakes, located in Livingston County, New York (40° 54" N; 77° 43" W). During the summers of 2000, 2001, and 2002, the littoral areas of the lake supported massive blooms of filamentous green algae (*Zygnema* sp. and *Spirogyra* sp.) that were strongly associated with Eurasian watermilfoil and other macrophytes (Bosch *et al.* 2000, Bosch *et al.* 2001, personal observation). Studies have revealed that major macrophyte beds (predominately *Myriophyllum spicatum*) exist in shallow areas near mouths of streams (Bosch *et al.* 2000, Bosch *et al.* 2001, Bosch *et al.* 2002). As a result of extensive agricultural activity in the Conesus Lake watershed, streams entering the lake are known to carry large quantities of nitrate, total phosphorus and soluble reactive phosphorus, especially during precipitation events (Nitrate up to 1800 g/ha/day; total phosphorus up to 34 g/ha/day; soluble reactive phosphorus (SRP) up to 54 g/ha/day) (Makarewicz *et al.* 2001a; Makarewicz *et al.* 2002). A significant correlation existed (regression r^2= 0.65, p = 0.046) between annual hydrometeorologic event stream total phosphorus loading and the standing crops of macrophytes beds (Bosch *et al*. 2002). In Conesus Lake, metaphyton blooms appear to be associated with substrate provided by beds of Eurasian watermilfoil near mouths of streams that receive significant amounts of phosphorus and nitrate from the watershed.

Given the problems associated nutrient loading and massive metaphyton blooms, more information is needed to develop efficient metaphyton management strategies. To evaluate the relationship between stream nutrient loading and growth of metaphyton, I tested the following hypotheses in this study:

Hypothesis 1: Stream effluent high in soluble reactive phosphorus and nitrate had a stimulatory effect on Conesus Lake metaphyton biomass. To test this hypothesis, metaphyton were incubated in continuous flow-through incubation chambers, and percent growth was measured in order to determine stimulatory effects that stream effluent may have on the growth of the algae. Additionally, I analyzed water entering the incubation chambers for SRP and nitrate, and evaluated the relationship between these concentrations and the percent growth data.

Hypothesis 2: Phosphorus, rather than nitrate, was the cause of any stimulating effect of stream water on lake metaphyton. To test this hypothesis, I conducted a series of *in situ* nutrient enrichment experiments to investigate the effects of phosphorus and nitrate on metaphyton growth.

Hypothesis 3: A close spatial relationship existed between the distribution of metaphyton and stream mouths in Conesus Lake. To test this hypothesis, I determined percent metaphyton cover along transects near stream mouths and at varying distances from stream mouths.

Methods and Materials

Continuous Flow Metaphyton Incubation Chambers

***Overview and Experimental Units*:**

Weighed quantities (range: 0.23-1.5g) of metaphyton (*Zygnema* and *Spirogyra*) were placed in incubation chambers (Figure 2) that were continuously fed stream effluent or lake (offshore) water. After a period of three days, metaphyton were removed, weighed and the percent growth was calculated. The incubation chambers were placed in shallow water

(parallel to the shoreline), allowing the top 20 cm of each cylinder to extend above the water. Four incubation chambers were located in close proximity to the stream of interest so that the intake line or "bilge pump-filter apparatus" could be submerged directly in the stream. Another four incubation chambers were located to the south of each stream, with the bilge pump-filter apparatus submerged in the lake, 18 – 40 m from the stream intake line. Since Conesus Lake has a northerly flow, this ensured that stream effluent would not be pumped into the lake-fed incubation chambers. Each site was examined for shading potential and chambers were positioned in such a manner that little or no shading occurred throughout the day. The two experimental units were active at the same time (one being fed stream water and another being fed lake water). *Spirogyra* and *Zygnema* were selected for the incubation experiments because the two genera were ubiquitous throughout the lake during 2001 and 2002. The assemblage usually contained around 75 percent *Zygnema* and 25 percent *Spirogyra* (personal observation). Species identification of *Spirogyra* and *Zygnema* require visual observation of conjugation and zygotes. Since neither conjugation nor zygotes were observed, the species of the filamentous green algae incubated have not been determined.

Fourteen metaphyton incubation chamber experiments (~72 hour incubation period) were conducted during June, July and August of 2001 and 2002 at the following locations: Hanna's Creek, Graywood Gully, Sand Point Gully, Cottonwood Creek, North McMillan Creek, Densmore Gully and Wilkins Creek (Figure 1). All experiments were conducted during the summer of 2002 except for one Wilkins Creek experiment, which was started on 20 August 2001. The experiment conducted at North McMillan Creek was considered a control because the creek historically loads very little nitrate and phosphorus (less than 0.10

g/ha/d of total phosphorus during 2001) (Makarewicz *et al.* 2002), thus the creek's potential for metaphyton enhancement is limited.

Each experimental unit consisted of four components: four metaphyton incubation chambers (**A**), a water influx manifold (**B**), a bilge pump-filter apparatus (**C**), and a power source (**D**) (Figures 2, 3).

A) Metaphyton Incubation Chambers:

Incubation chambers were constructed from cylinders of transparent Plexiglas (height = 50 cm, interior diameter = 9.5 cm) (Figures 2, 4). Water, either from a stream or the lake, was pumped into the bottom of each chamber. As each chamber filled, water rose up the cylinder and eventually flowed out through four outlets located near the top of each cylinder. To prevent algal cells from escaping, a lattice of rubber bands served as an anchor for the filamentous algae and two rolled squares of 120 μm mesh were inserted into the outlets to serve as a filter (Figure 4).

B) Water Influx Manifold and Flow Control:

Water from the bilge pump flowed through 6.1 m of clear vinyl tubing (Inner Diameter = 1.6 cm, Outer Diameter = 1.9 cm) into a manifold (Figures 2, 3). The manifold had four outlets, each with a brass flow control knob, with 1 m of vinyl tubing (I.D. = 0.43 cm, O.D. = 0.63 cm) attached to each outlet. The small end of a 10 ml polystyrene pipet was connected to the tubing and the assembly was lowered into a chamber. This design allowed water to enter each cylinder from the bottom and drain from the top, resulting in continuous mixing throughout the chamber. The rate of water flow into each chamber was measured with a graduated cylinder and stopwatch. The flow control knobs were adjusted so that each chamber received

approximately 500 ml/min. This rate was maintained for 85% of the incubation period. Towards the end of the third day, the rate tapered off at to approximately 350 ml/min due to diminished battery voltage.

C) Bilge Pump-Filter Apparatus:

For each experimental unit, a bilge pump (Attwood #4204-1,893 L/h) was used to continually pump water through the four chambers. The introduction of sediment and other unwanted elements into the chambers was prevented by the use of a bilge pump-filter apparatus (Figure 5). Each bilge pump was wrapped in two sheets of 120 μm mesh secured by rubber bands, and placed in a plastic pail (13.5 L). The outlet of the pump exited through a hole near the base of the pail. A round piece (diameter = 40 cm) of 120 μm mesh was wrapped and securely tied over the mouth of the pail. Water was drawn down into the pail, through the bilge pump, into 6.1 m of clear vinyl tubing (I.D. = 1.6 cm, O.D. = 1.9 cm) and eventually to the bottom of the incubation chambers.

D) Power Source:

Each bilge pump was powered by a 12 volt deep cycle trolling battery.

Collection, Handling and Determination of Metaphyton Biomass:

I used a "spin dry weight method" to obtain wet weights of metaphyton without damaging or stressing cells. The method utilizes a salad spinner lined with unbleached paper towels to remove water from algal filaments before the filaments are weighed. To test the validity of this method, samples of algal filaments were spun for 2 min in a salad spinner lined with paper towels, weighed, dried in an oven at 60ºC for 48 hours and weighed again.

Linear correlation analysis showed that the relationship between "spun" weight and dry weight was strong ($r = 0.997$, $n = 26$) (Figure 6).

Metaphyton to be placed in incubation chambers were collected from littoral areas of Conesus Lake with a metal strainer and placed in a pail filled with lake water. The metaphyton were transported to S.U.N.Y. Geneseo where the algal filaments were spun for two minutes. After weighing on a Denver A200DS microbalance (0.10 mg resolution), approximately 2 mg of metaphyton were placed into 500 ml Nalgene bottles filled with lake water filtered through a 74 μm sieve, transported to the experimental sites and placed in incubation chambers with the use of a long-nosed funnel. After an incubation period of three days, the contents of each chamber were poured into a 74 μm sieve and spun dry before determining biomass. The change in biomass was calculated by subtracting the spun weight of the filaments after the incubation period from the spun weight of the filaments before incubation. The metaphyton were identified to genus following Prescott (1978), and Graham and Wilcox (2000).

Water Sampling and Nutrient Analysis:

Water samples were taken daily during experiments, 0.5 m from the bilge pump-filter apparatus in both the stream and lake. Samples were immediately filtered through 0.45 um MCI Magma Nylon 66 membrane filters and held at 4ºC until transported to the lab. If samples could not be analyzed immediately, they were stored at -10ºC for 1-5 days. Sampling bottles and filtering apparatus were pre-cleaned with phosphate-free RBS.

The samples were analyzed for nitrate and soluble reactive phosphorous at SUNY Brockport's NELAC certified "Water Chemistry" laboratory (NY # 11439). Nitrate levels were determined by the automated (Technicon Autoanalyser) cadmium reduction method

(APHA 1999) while soluble reactive phosphorus concentrations were determined by the automated (Technicon Autoanalyser) ascorbic acid method (APHA 1999). Lake and stream water temperatures and pH readings were obtained daily with a calibrated mercury thermometer and an Accumet portable pH meter.

Statistical Analysis:

To compare the percent growth of metaphyton that received stream effluent to the percent growth of metaphyton that received lake water at individual sites, one-tailed t-tests ($\alpha = .05$) were applied using Excel, version 2000. To test for differences between variances, f-tests were applied and appropriate t-tests were utilized (either t-tests assuming equal variance or t-tests assuming unequal variance). Individual incubation chambers were considered replicates ($n = 4$) despite the fact that all four chambers received water from the same bilge pump that was powered by the same battery. Pooling the variance of all experiments (all stream fed chambers vs. all lake fed chambers) was not appropriate because experiments were conducted at various times throughout the growing season and at various locations. Consequently, for each set of experiments, metaphyton were exposed to different physical, chemical, and ecological conditions. This design allowed experimentation under a wide range of environmental conditions (Hurlbert 1984, Zar 1999).

One-tailed t-tests were utilized to determine if concentrations of SRP and NO_3 were significantly higher in stream effluent than in adjacent lake water. Significant differences in pH and temperature between stream effluent and lake water were determined with the use of two-tailed t-tests ($\alpha = .05$).

***Spatial Differences in Metaphyton Growth Potential*:**

The growth potential of each stream (the ability of each stream to promote metaphyton growth) was determined by first calculating the average percent metaphyton growth of the four lake-fed chambers and then subtracting this value from the percent metaphyton growth of each of the four stream-fed chambers. The metaphyton growth potential at different locations was statistically compared using analysis of variance (ANOVA) and a Tukey test was utilized for multiple comparisons. Statistical analyses were conducted using SPSS, version 10.0.5 and Excel, version 2000.

Nutrient Limitation Experiments

Metaphyton were added to eight incubation chambers that were continually flushed with lake water and dosed with nutrients for a period of three days. This experiment was conducted three times with various concentrations of NO_3 and PO_4 continuously introduced into each chamber (Table 1). Experiment #1 had no replicates, but served as an aid in determining the nutrient concentrations used in experiment #2 and #3. The three enrichment experiments were conducted near Hanna's Creek at the northern end of the lake, from 19 August 2002 to 30 August 2002. Changes in metaphyton biomass were determined in exactly the same manner as in the incubation experiments above.

The enrichment experiments (Figure 7) consisted of the following components: eight metaphyton incubation chambers, two water influx manifolds, two bilge pump-filter apparatus, and two batteries. All components were constructed and used as previously described in the metaphyton incubation chamber experiments. In addition, a Harvard Apparatus Peristaltic pump (pump tubes = 0.484 ml/min) was used to pump nitrate and SRP

solutions into the incubation chambers at a rate of 1 ml/min. The nutrient solutions, contained in Nalgene carboys (4 L), were pumped through 90 cm of clear Tygon tubing (I.D. = 1.6 mm, O.D. = 3.2 mm) to the peristaltic pump, and then through another 300 cm of Tygon tubing and a 1 ml pipet into the bottom of the incubation chambers. The materials for enrichment (carboys and peristaltic pump) were positioned on dry land (Figure 8) while the incubation chambers were partly submerged in lake water.

Nutrient stock solutions were made by adding $NaNO_3$ and Na_2HPO_4 to de-ionized water. The concentrations of nitrogen (1 mg N/L) and phosphorus (16 µg P/L) used in the enrichment experiments were based on historical concentrations occurring in the lake (Makarewicz *et al.* 2001a; Makarewicz *et al.* 2001b) (Table 1). During the experiments, water in the incubation chambers was sampled daily. NO_3 and SRP analyses were conducted using methods mentioned above.

Statistical Analysis:

Statistical analyses were conducted using SPSS, version 10.0.5. A two-factor analysis of variance was used to analyze the effects that nitrate and soluble reactive phosphorus have on metaphyton biomass (Zar 1999). Data from two experiments (#2 and #3) were pooled, resulting in four replicates per treatment. Pooling the variability from two separate experiments was deemed acceptable because both experiments were conducted at the same location, three days apart, and under very similar physical and chemical conditions.

Metaphyton Spatial Distribution at Stream Mouths

Metaphyton percent cover was determined between 9 August 2001, and 11 August 2001 at Graywood Gully, Sand Point Gully, Long Point Gully, Cottonwood Creek, Sutton

Point Creek and North McMillan Creek (Figure 1). Grids consisting of five parallel transect lines, one meter apart, were constructed by fastening nylon rope marked at 1 m intervals to wooden stakes (1-2 m long) hammered into the substrate (Figure 9). During the construction of each grid, the two near-shore corners were disturbed and no data were obtained from those quadrats (Figure 9**,** areas *a* & *b*).

A grid was constructed in front of each stream mouth and at various undisturbed locations to the north and south of each stream (10 – 40 m from mouth of stream). Due to boat traffic and/or macrophyte removal, some areas to the north or south of stream mouths were not surveyed. The transects were parallel to the shoreline, with a maximum depth of 2 m and minimum depths of 0.4 to 1 m. Visual estimates of percent metaphyton cover were made from a canoe, using a 0.5 x 0.5 meter PVC-pipe frame with nylon twine criss-crossing the frame at approximately 8 cm intervals to form 36 squares. The PVC frame was placed at random locations inside each of the thirty quadrats of the grid, and the number of squares of the frame that were covered with metaphyton were recorded. Thus, 30 estimations of percent cover were made for each grid.

Statistical Analysis:

Statistical analyses were conducted using SPSS, version 10.0.5. and Excel, version 2000. An arcsine transformation was performed on all percent coverage data to ensure near normal distribution (Zar 1999). To compare percent metaphyton cover in front of each stream mouth to percent metaphyton cover to the north or south of each stream mouth, one-tailed t-tests were applied. The data from both grids in the proximity of each stream were combined in order to compare percent metaphyton cover between different locations throughout

Conesus Lake. Subsequently, analysis of variance (ANOVA) was applied and a Tukey test was utilized for multiple comparisons.

Results

Continuous Flow Metaphyton Incubation Chambers (Table 2).

Due to mechanical failures and disturbances resulting from public activity, only nine of the 14 incubation chamber experiments were considered valid. Most of the streams were dry by midsummer, except for Wilkins Creek and Hanna's Creek. Consequently, three experiments were conducted at Wilkins Creek.

Except for North McMillan Creek ($P = 0.469$, t-test), all sites showed significantly higher percent growth in the stream-fed metaphyton chambers ($P \leq 0.021$, t-test) (Figure 10) (Table 2). The overall average percent increase in stream-fed metaphyton was 268 % (range = 51- 515 %). The overall average percent increase for metaphyton that received lake water was 167 % (range = 29-369 %). During all experiments, stream SRP concentrations were significantly higher than lake concentrations ($P \leq 0.049$, t-tests) with the exception of Hanna's Creek ($P = 0.253$, t-test) and North McMillan Creek ($P = 0.237$, t-test) (Figure 11). Stream nitrate concentrations were dramatically higher than lake concentrations in five experiments (Figure 12). Despite having the same nitrate level in both streams and the lake, metaphyton biomass increased in the stream-fed chambers of Densmore Creek, Hanna's Creek and Wilkins Creek. North McMillan Creek had significantly higher concentrations of NO_3 than lake waters ($P = 0.005$, t-test), though no significant difference in metaphyton growth between the stream and lake experiments was evident.

Temperature differences between stream and lake-fed chambers were not statistically significant (P range = .19 - .88, two-tailed t-tests) (Figure 13) (Table 3). The average difference between stream and lake water temperatures at each site was 1.1°C (range of differences = 0.3-2.5° C). North McMillan Creek site had the largest temperature difference, with stream effluent averaging 21.8° C and lake water averaging 24.3° C. This difference was largely due to lower stream effluent temperature during the first 12 hours of the experiment (Appendix 1). Exposing the intake line of the stream fed chambers to direct sunlight after the first 12 hours increased the temperature of the stream fed incubation chambers to 24°C.

Metaphyton Growth and pH:

At all sites except Densmore Creek, stream effluent had lower daily pH values than corresponding lake water (Figure 14). Statistically, Greywood Gully had significantly (P = 0.039, two-tailed t-test) lower pH values than adjacent lake water but differences at all other sites were not significant (P range = .158 - .525, two-tailed t-tests). Percent metaphyton growth was plotted against corresponding ambient pH values (Table 3) and analysis by linear regression showed no significant correlation (P = 0.974, regression $r^2 < 001$).

***Spatial Differences in Metaphyton Growth Potential*:**

There was a highly significant difference (P < 0.000, ANOVA) in the growth potential between streams (Figure 16). Multiple comparison analysis (Tukey test) revealed that the areas surveyed fall into four overlapping statistical groups. Densmore Creek and Sand Point Gully had the highest growth potential followed by a group including Sand Point Gully, Cottonwood Creek, Wilkins Creek and Graywood Gully. Subsequently, Wilkins Creek, Graywood Gully, Hanna's Creek and North McMillan Creek were grouped together

having the lowest metaphyton growth potential. Overall, Densmore Creek had the highest (274 %) and North McMillan Creek had the lowest (2 %) growth potential.

Nutrient Limitation Experiments (Table 4).

Additions of SRP and SRP + NO_3 yielded metaphyton percent growth (mean = 129 percent) significantly higher (P = 0.002, Two-way ANOVA) than in chambers that received NO_3 only or no nutrients above ambient levels (mean = 60 percent growth) (Figure 17). Percent growth in chambers that were enriched with nitrate did not differ significantly (P= 0.540, Two-way ANOVA) from growth in chambers that received no additional nitrate above ambient levels. Also, interaction of nitrate and SRP did not affect percent growth of metaphyton during the experiment (P= 0.994, Two-way ANOVA) (Table 5). This experiment suggests that phosphorus enrichment stimulates metaphyton growth. Addition of NO_3 alone does not stimulate growth over the control, nor does addition of NO_3 to chambers also receiving phosphorus stimulate metaphyton growth beyond the levels observed in chambers just receiving phosphorus.

Metaphyton Spatial Distribution Near Stream Mouths (Table 6).

Metaphyton percent cover varied significantly between stream mouths (P = 0.000, ANOVA) (Figure 18). Multiple comparison analysis revealed that the areas surveyed fall into four statistical groups. Cottonwood Creek and Greywood Gully had the highest percent cover followed by Sand Point. Subsequently, Sutton Point and Long Point were grouped together having the second lowest percent cover, and finally North McMillan had the lowest percent cover. In other words, no significant difference was observed between Long Point and Sutton Point (P = 0.358, Tukey Test) or between Cottonwood Creek and Graywood Gully (P = 0.299, Tukey Test). The highest metaphyton percent cover occurred at

Cottonwood Creek (Average = 75% cover) and the lowest was observed at North McMillan Creek (Average = 3% cover).

Originally, we intended to survey metaphyton directly in front of and to the south of each stream mouth; however, the areas to the south of some streams were disturbed. Consequently areas to the north of these streams were surveyed. When comparing the percent metaphyton cover located in front of stream mouths to percent cover located to the north or south of stream mouths, all sites showed significantly higher percent cover in grids to the north or south ($P \leq 0.030$, t-test), except for Long Point Gully ($P = 0.301$, t-test) (Figure 19) (Table 7).

Discussion

Impact of Stream Effluent on Metaphyton in Conesus Lake

The metaphyton incubation chamber experiments demonstrate that stream effluent promotes metaphyton (*Spirogyra* and *Zygnema*) growth in Conesus Lake. In general, percent metaphyton growth in chambers that received stream effluent was dramatically higher than the percent metaphyton growth in chambers that received lake water (Figure 10). At Hanna's Creek, however, only modest but significant ($P = 0.021$, t-test) percent metaphyton growth was observed in chambers that received stream effluent, compared to the percent growth that occurred in the chambers that received lake water at that site. A possible explanation lies in the nature of the algae community at Hanna's Creek. Unlike all the other creeks in the incubation chamber experiments, Hanna's Creek was ecologically unique in possessing a massive bloom of *Hydrodictyon,* a type of green algae. Though *Hydrodictyon* is not known to exhibit allelopathy, the bloom had the potential to deplete the stream of

essential micronutrients required by the Zygnemataceae (*Zygnema* and *Spirogyra*). More importantly, the average soluble reactive phosphorus (SRP) concentration in Hanna's Creek was not significantly higher than lake concentrations (P = 0.253, t-test) (Figure 11). Where dramatic differences in percent growth of metaphyton occurred, experimental stream-fed incubation chambers had significantly higher concentrations of SRP. Percent growth in chambers that received effluent from North McMillan Creek were not significantly higher (P = 0.47, t-test) than percent growth in lake fed chambers; this result was expected since concentrations of SRP in that creek were not significantly different (P = 0.237, t-test) from concentrations of SRP in corresponding lake water.

Throughout all experiments, the temperatures of stream effluent did not significantly differ from lake water temperatures (P range = 0.19 – 0.88, t-tests) (Figure 13) (Table 3). However, temperatures that are not statistically different may be significant biologically to an organism. Experimental results suggest that the differences in temperature between the stream and lake-fed chambers were insignificant. This is emphasized by the fact that four out of eight of the experiments that showed higher percent metaphyton growth in stream-fed chambers, had slightly lower stream temperatures (range of differences = 0.7 – 1.7° C) than that of adjacent lake water. Alternatively, four out of eight of the experiments that showed higher percent metaphyton growth in stream-fed chambers had slightly higher stream temperatures (range of differences = 0.3-1.3° C). Additionally, based on the results of a study to determine optimal temperatures for net photosynthesis for an unknown species of *Spirogyra* (Graham *et al*. 1995), the differences in temperatures between stream and lake-fed incubation chambers were biologically insignificant.

The Effects of Phosphorus and Nitrate on Metaphyton Growth

I hypothesized that phosphorous was the cause of enhanced metaphyton growth and that nitrate was not a limiting factor to the growth of metaphyton in Conesus Lake. In the field experiments, metaphyton percent growth was significantly higher in the stream-fed chambers of Densmore Creek, Hanna's Creek and Wilkins Creek (20 August 2001) even though nitrate concentrations between the effluent of these steams and corresponding lake water were not significantly different (Densmore Creek P = 0.682, Hanna's Creek P = 1, and Wilkins Creek P = 1, t-tests). Also, no significant difference in percent metaphyton growth occurred between the North McMillan stream- and lake-fed chambers, even though significantly higher concentrations of NO_3 were observed in the lake as compared to the stream (P=0.005, t-test). Further corroboration of this hypothesis was provided by the enrichment experiments in which both nitrate and phosphorus levels were experimentally manipulated; filamentous green algae growth was significantly lower (Two-way ANOVA, P= 0.002) in chambers receiving only nitrate than in those receiving phosphorus. Furthermore, interaction effects were insignificant (P= 0.994, Two-way ANOVA), showing that the effect of SRP on metaphyton percent growth was not influenced by the presence of NO_3 (Table 5). Invariably, in the *in-situ* incubation experiments, all chambers that yielded higher percent metaphyton growth received stream effluent containing relatively higher SRP concentrations. It's evident that metaphyton biomass in Conesus Lake is strongly influenced by SRP levels.

Many other studies in fresh water bodies have demonstrated that filamentous green algae have flourished upon enrichment with phosphorus (Dodds and Gudder 1992; Planas *et al.* 1996; Browder *et al.* 1994) or both nitrate and phosphorus (Murkin *et al.* 1994;

McDougal and Goldsborough 1995; Sridharan and Lee 1977; Havens *et al.* 1999). Furthermore, in the northern Everglades, McCormick (2001) demonstrated that filamentous green algae accumulated phosphorus rapidly and in proportion to the loading rate.

PH and Growth

The Zygnemataceae are known to tolerate acidity and in many cases have dominated aquatic environments with a pH less than 6.0. (Lazarek 1981; Turner *et al.* 1986; Fairchild and Everett 1988; Howell *et al.* 1990; France and Welbourn 1992). Although the experiments were not designed to determine the effects of pH on Zygnematacean growth, six out of seven of the stream-fed chambers that had enhanced metaphyton growth also had lower pH values compared to the corresponding lake-fed chambers (mean difference = 0.35, range of difference = 0.13 - 0.57) (Table 3). Because dominance of Zygnematacean algae occurs in lakes with a pH below 6 (France and Welbourn 1992) differences in Ph between stream effluent and adjacent lake water were considered biologically insignificant throughout this study. The pH of manure ranges between 6.5 and 7.0. (Donham *et al.* 1985) and manure spreading in the Conesus watershed may be the cause of decreased pH values of stream effluent.

Spatial Differences in Metaphyton Growth Potential Throughout Conesus Lake

The experimental design was not entirely appropriate for comparison of the ability of effluent from different streams to stimulate metaphyton growth. In order to determine the affects of stream effluent on metaphyton biomass under a wide range of environmental conditions, experiments were run during different times of the growing season and under different physical conditions (irradiance and temperature). However, we considered spatial differences in the stream growth potentials as an important benchmark for future studies.

Not surprisingly, the ability of each stream to stimulate metaphyton growth (growth potential) varied greatly (Figure 16). For instance, the biomass of metaphyton in chambers that received effluent from Wilkins Creek (21 July 2002) increased 449 percent, while metaphyton in chambers that received effluent from Hanna's Creek increased only 69 percent. Several possible explanations exist. Water movements and irradiance are an important factor governing the proliferation of filamentous metaphyton (Graham and Wilcox 2000) and, as mentioned earlier, our experiments were run under different physical conditions. Also, inhibitory or stimulatory allelopathy induced by macrophytes or other types of algae (Havens *et al.* 2001) may be another contributing factor to such high variability. For example, a bed of *Chara* spp., which may release substances that inhibit algal growth (Van Donk, & Van De Bund 2002), was observed near metaphyton communities in the northern end of Conesus Lake near Hanna's Creek (personal observation). *Zygnema* and *Spirogyra* populations were relatively low at this site compared to other locations. Possible allelopathic interactions may exist, but were not investigated.

The Utility of Continuous Flow-Through Metaphyton Incubation Chambers

Numerous researchers have used *in-situ* methods for incubation and enrichment of algae (Owens *et al.* 1977; Sridharan and Lee 1977; Vanni 1987; Fairchild and Everett 1988; Nicholls and Heintsch 1992; Thybo-Christesen *et al.* 1993a; McDougal and Goldsborough 1994; Murkin *et al.* 1994; Twist et al 1997; Havens *et al.* 1999; Mososch *et al.* 1999; McCormick *et al.* 2001). Additionally, recirculating continuous flow methods have been developed, including "Benthic Algae Growth Chambers" (Milkie and Mulbry 2001) and artificial streams (Mulholland *et al.* 1991). However, to the best of the author's knowledge, continuous flow-through experiments involving filamentous green algae are unprecedented.

A continuous flow- through incubation system has certain advantages over closed incubation systems when working with filamentous algae. First, the system can simulate water movements present in littoral areas that are necessary for nutrient exchange throughout a metaphyton cloud. Also, the continuous flow of water may eliminate nutrient recycling that can occur in the interior of metaphytic clouds, allowing further isolation of the effects of the experimental variable (stream effluent, nutrient additions etc.).

Metaphyton Percent Cover

The geographic distribution of metaphyton in Conesus Lake has not been explored previous to this study. A significant difference existed in metaphyton cover amongst various locations. Because the streams adjacent to the surveyed areas historically load high amounts of nutrients (Makarewicz *et al. 2001a,* Makarewicz *et al. 2002*), I hypothesized that a close spatial relationship exists between the distribution of metaphyton and stream mouths in Conesus Lake. I expected a gradient to exist, in which percent metaphyton cover would diminish with distance from stream mouths, and a maximum amount of metaphyton would be observed directly in front of the mouths of creeks releasing high levels of phosphorus. This spatial distribution was not observed, and a significantly higher percent cover of metaphyton were observed to the north and south of stream mouths (10 – 40 m from mouth of stream). I attribute this pattern to the disruptive force of stream effluent entering the lake during hydrometeorologic events. Pihl *et al.* (1999) showed that spatial distributional patterns of *Cladophora* and *Enteromorpha* were unrelated to local nutrient inputs from streams, but distributional patterns of algal abundance were correlated with water movements. Similarly, a simulation by Goldsborough and Robinson (1996) suggests that

shelter from physical disturbances is extremely important for the proliferation of metaphyton.

While determining percent cover, I observed that filamentous green algae grew anchored to the featherlike structure of Eurasian watermilfoil fronds. The relationship between Eurasian watermilfoil and metaphyton in Conesus Lake is of interest. Since macrophytes can release nutrients obtained from sediments into the water (Cattaneo and Kalff 1979; Havens et al 2001), metaphyton associated with macrophytes may utilize these nutrients. Additionally, Eurasian watermilfoil beds increase water column stability by reducing water velocity, and offer an ideal anchor preventing filamentous green algae from sinking or being swept ashore. Macrophytes also enhance water clarity by reducing re-suspension of sediments (James and Barko 1990), which may allow metaphyton to receive higher irradiance levels and proliferate deeper in the water column. Major macrophyte beds (predominately *Myriophyllum spicatum*) exist in shallow areas near stream mouths, where significant amounts of soluble reactive phosphorous are released into the lake (Bosch *et al*. 2001; Makarewicz *et al.* 2001a; Bosch *et al*. 2002; Makarewicz *et al.* 2002). It is believed that the availability of nutrients and water column stability provided by macrophytes offer an ideal environment where filamentous green algae can proliferate. However, this is not true of all macrophytes; although the region directly in front of and to the south of North McMillan Creek supported a bed of eelgrass (*Vallisneria americana*), an extremely low percent metaphyton cover (mean = 3 %) was measured in this bed. Evidently, because of its blade-like structure, eelgrass is not a secure anchor for filamentous algae.

Bosch *et al*. (2001) found zebra mussels to be abundant on available substrate from the Conesus Lake shoreline to a depth of about 10 m. Hundreds of zebra mussels have been

found on macrophytes in the lake (Bosch, *et al.* 2001). Almost certainly, zebra mussels play a significant role in the bloom of metaphyton in Conesus Lake. Pillsbury and Lowe (1994) have demonstrated that the introduction of zebra mussels in Lake Huron has led to the dominance of filamentous green algae. This is attributed to filtering activities of *Dreissena*, leading to an increase in water clarity and irradiance; less competition with phytoplankton for nutrients; and deposits of fecal and pseudofecal pellets that increased available nutrients (Pillsbury and Lowe 1994).

The Dominance of Filamentous Green Algae

Why are filamentous green algae the dominant littoral algae of Conesus Lake? Attached *Cladophora* do grow extensively at various locations throughout the lake but appear to be limited by suitable substrate. Many *in situ* studies (Murkin *et al.* 1994; McDougal and Goldsborough 1995; Havens *et al.* 1999; Havens, unpublished data; McCormick 2001) indicate a shift towards filamentous metaphyton with nutrient enrichment. However, modeling by Valiela *et al.* (1997) suggests that at highest nutrient concentrations, phytoplankton will dominate over metaphyton because they have a lower compensation of irradiance than metaphyton (Havens et al 2001). Physiological adaptations may offer competitive advantages for filamentous Zygnematalians. Larger cells (i.e., *Spirogyra, Zygnema*) can take up and store more nutrients than smaller cells; this ability can offer a competitive advantage during periods of nutrient scarcity (Grahm and Wilcox 2000). Makarewicz *et al.* (2002) and Makarewicz *et al.* (2001a) have shown that nutrient loading during hydrometeorological events in Conesus Lake contributes massive amounts of phosphorus and nitrate to the lake in short pulses of time. The dynamic between periodic (pulse) loading events and the ability of filamentous green algae to store nutrients has not

been investigated, but periodic loading may offer a competitive advantage to filamentous green algae in Conesus Lake. Additionally, cells in filaments have a competitive advantage over single cells because nutrients can be transferred between cells along the filament, from regions of high ambient nutrient concentration to regions of low cellular concentrations (Stevenson *et al.* 1985, Riber and Wetzel 1987).

Clearly, the physical, chemical, and biological environment in littoral areas of Conesus Lake, coupled with the physical adaptations of filamentous green algae, creates an ideal scenario for the algae to flourish. Extensive agricultural applications of fertilizers and manure in the Conesus watershed provide an ample supply of phosphorus (Makarewicz *et al.* 2001a). Eurasian watermilfoil fronds provide anchorage, preventing the algal filaments from sinking or from being washed ashore. Additionally, macrophytes enhance water clarity and introduce nutrients from the sediment via their leaves and stems. Zebra mussels also assist in water clarification, eliminate competition from phytoplankton, and offer additional nutrients by depositing fecal pellets. Finally, physiological adaptations of *Zygnema* and *Spirogyra* permit nutrient storage and this ability allows the algae to capitalize on nutrient loading during hydrometeorological events.

Summary and Conclusion

Metaphyton were incubated in continuous flow-through chambers, and percent growth (percent change in biomass) was measured in order to determine stimulatory effects that stream effluent may have on the growth of filamentous green algae in Conesus Lake. Additionally, I analyzed water entering the incubation chambers for SRP and nitrate, and evaluated the relationship between these concentrations and the percent growth data. I conducted a series of enrichment experiments *in situ* to further investigate the effects of

these nutrients on growth. Finally, I performed quantitative observations along transects to test the hypothesis that a close spatial relationship existed between the distribution of metaphyton and stream mouths. I drew the following conclusions from the results of these experiments:

1. Stream effluent entering Conesus Lake has a positive effect on metaphyton biomass.
2. Metaphyton biomass in Conesus Lake significantly increases in the presence of SRP and, based on experimental evidence, metaphyton growth in Conesus Lake is limited by phosphorus.
3. In Conesus Lake, metaphyton exist in close association with macrophytes such as Eurasian watermilfoil, and the possibility of a symbiotic relationship between Eurasian watermilfoil and filamentous green algae warrants further investigation.
4. Relatively low percent cover of metaphyton directly in front of stream mouths emphasizes the importance of a stable water column for the proliferation of metaphyton.

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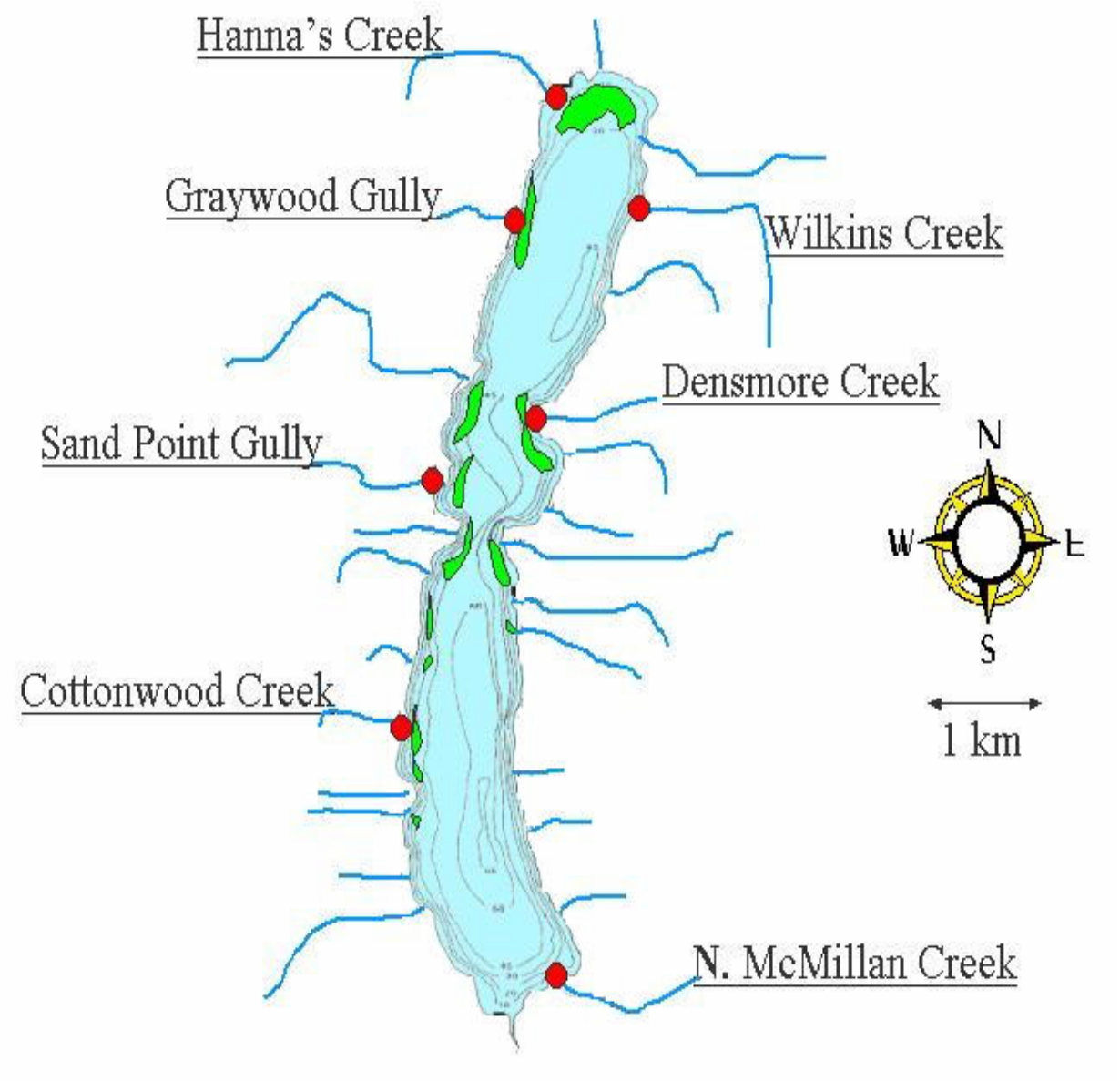

Figure 1. Conesus Lake. Red dots represent experimental sites and green areas represent macrophyte beds.

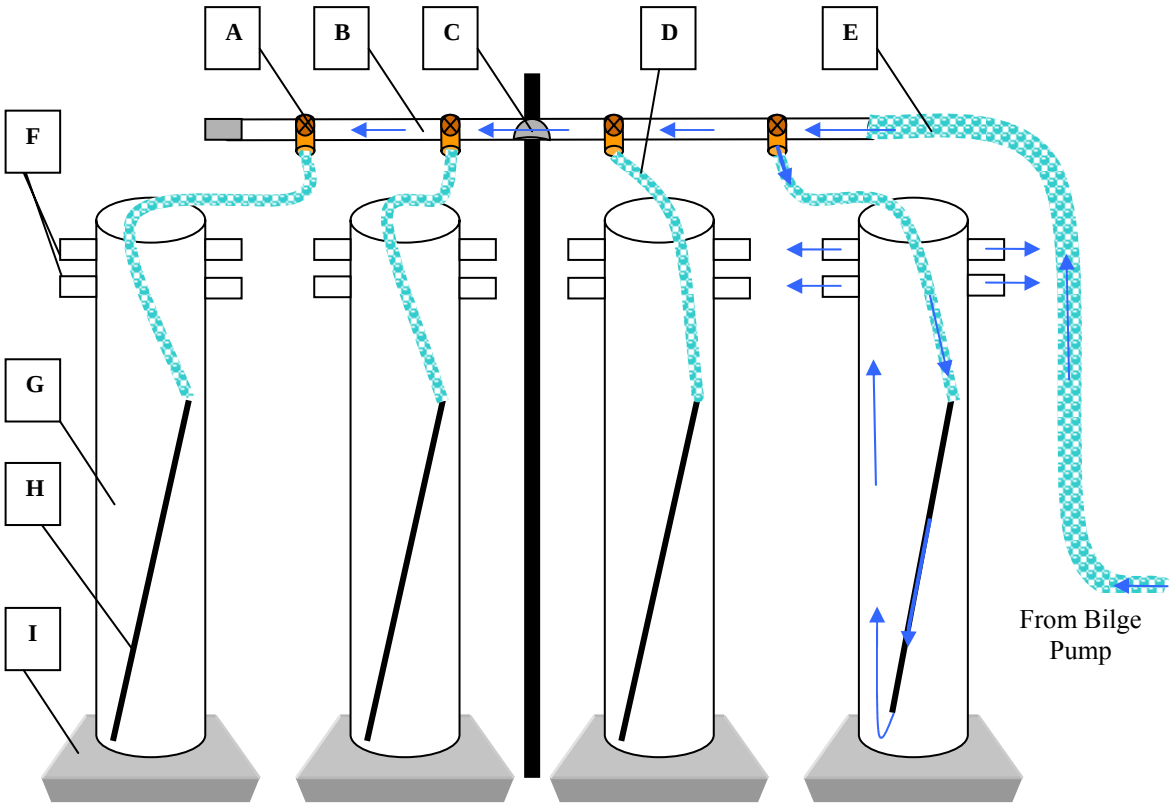

Figure 2. Metaphyton Incubation Chambers with manifold. Water from the bilge pump is fed into the bottom of the chambers. As the chambers fill, water exits through the outlets. A – Flow control valve, B – Manifold (PCV Pipe), C – Clamp for supporting manifold, D – Vinyl tubing (I.D. = 0.43 cm), E –Vinyl tubing (I.D. = 1.6 cm), F – Chamber outlets, G – Chamber, H – Pipet (10 ml), I – Base.

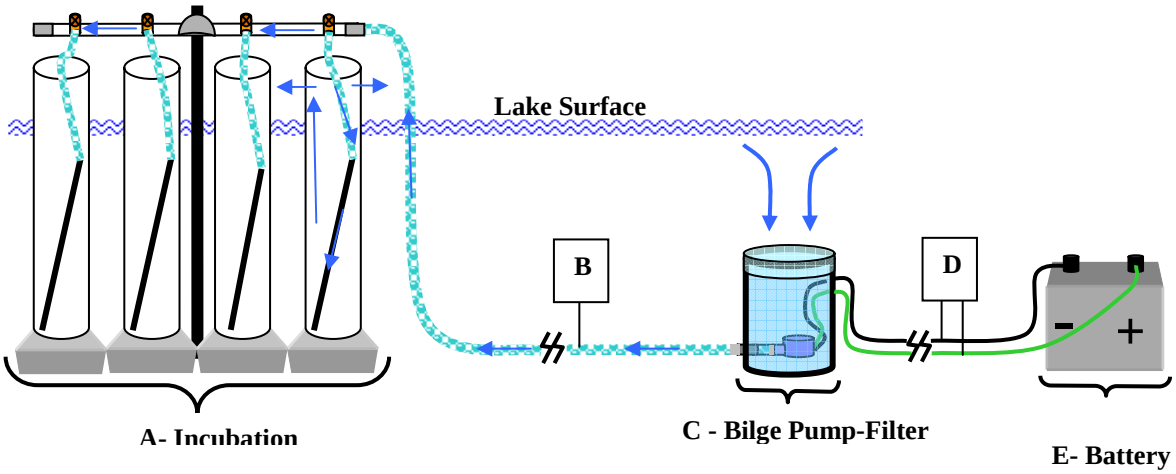

Figure 3. Experimental Unit. Blue arrows indicating flow of lake or stream water through the system. The incubation chambers are placed in shallow lake water allowing the top 20 cm of the chambers to extend above the water. A – Metaphyton incubation chambers, B – Vinyl tubing (L = 6.1m), C- Bilge pump-filter apparatus, D – Electrical Wire (L = 6.1 m), E – 12 V battery.

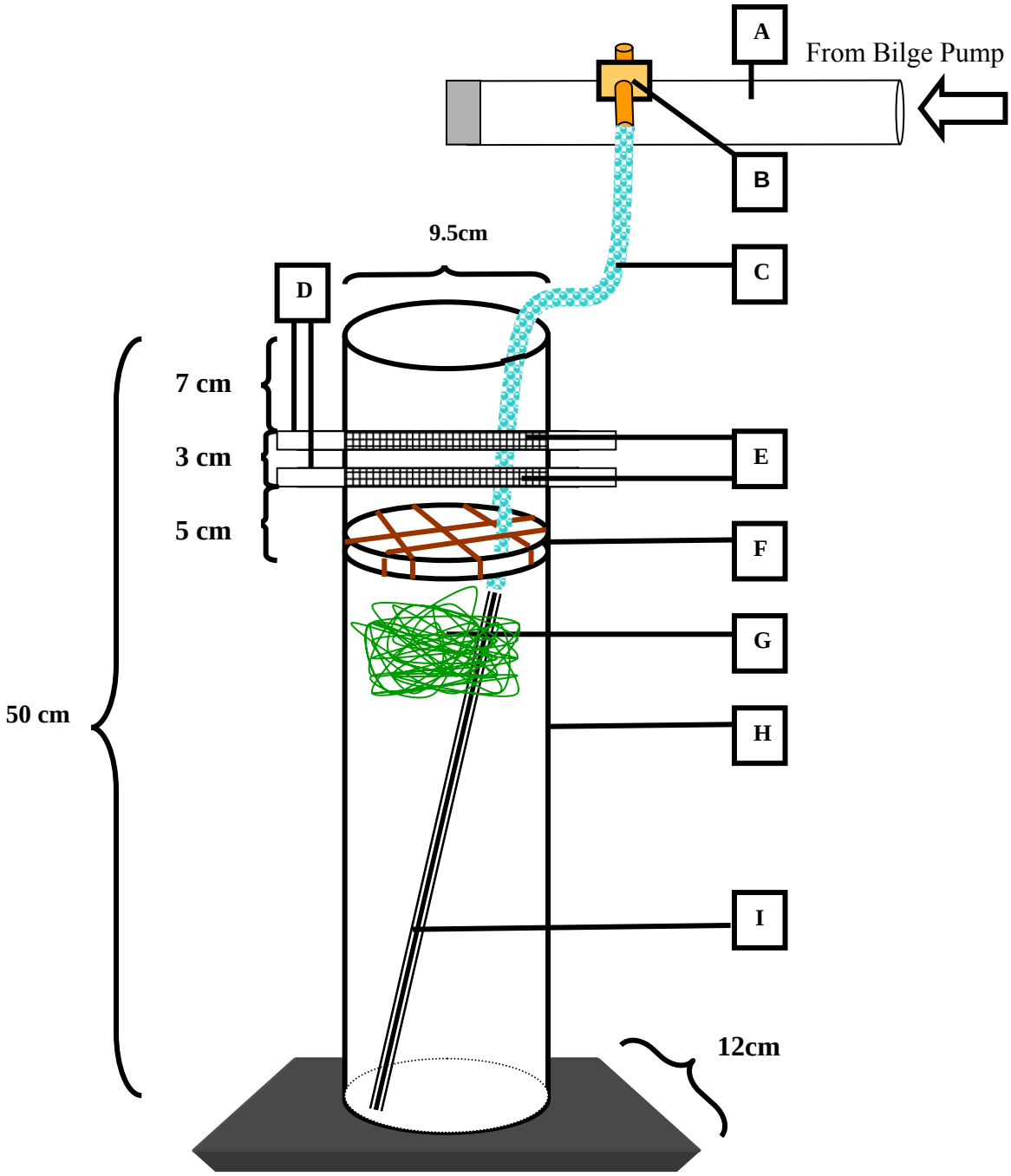

Figure 4. Metaphyton Incubation Chamber. A – Section of manifold, B – Flow control valve, C – Vinyl tubing (I.D. = .43 cm), D – Chamber outlet, E – 120µm mesh filter, F – Rubber band anchor, G – Metaphyton, H – Chamber, I – 10 ml pipet.

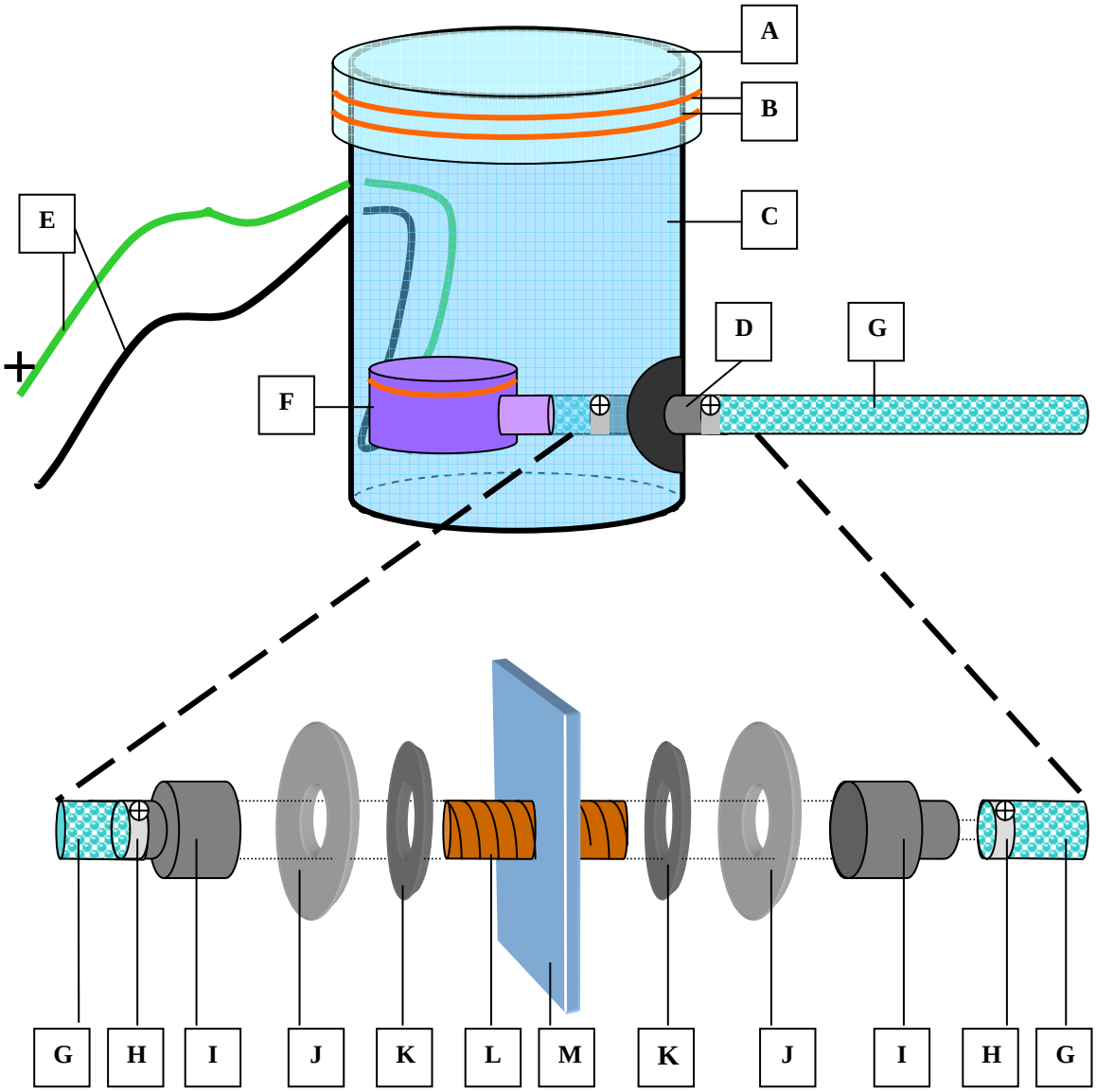

Figure 5. Bilge Pump-Filter Apparatus with exploded view of bilge pump-hose attachment assembly. A – 120**µ**m mesh, B – Nylon twine, C – 13.5L plastic pail, D – Female adaptor, E – Positive and negative electrical wires, F – Bilge pump with 120**µ**m mesh wrapped around it, G – Vinyl tubing (I.D. = 1.6 cm), H – Hose clamp, I – Female adaptor, J – Rubber washer, K – Steel washer, L – Brass pipe nipple, M – Wall of plastic pail.

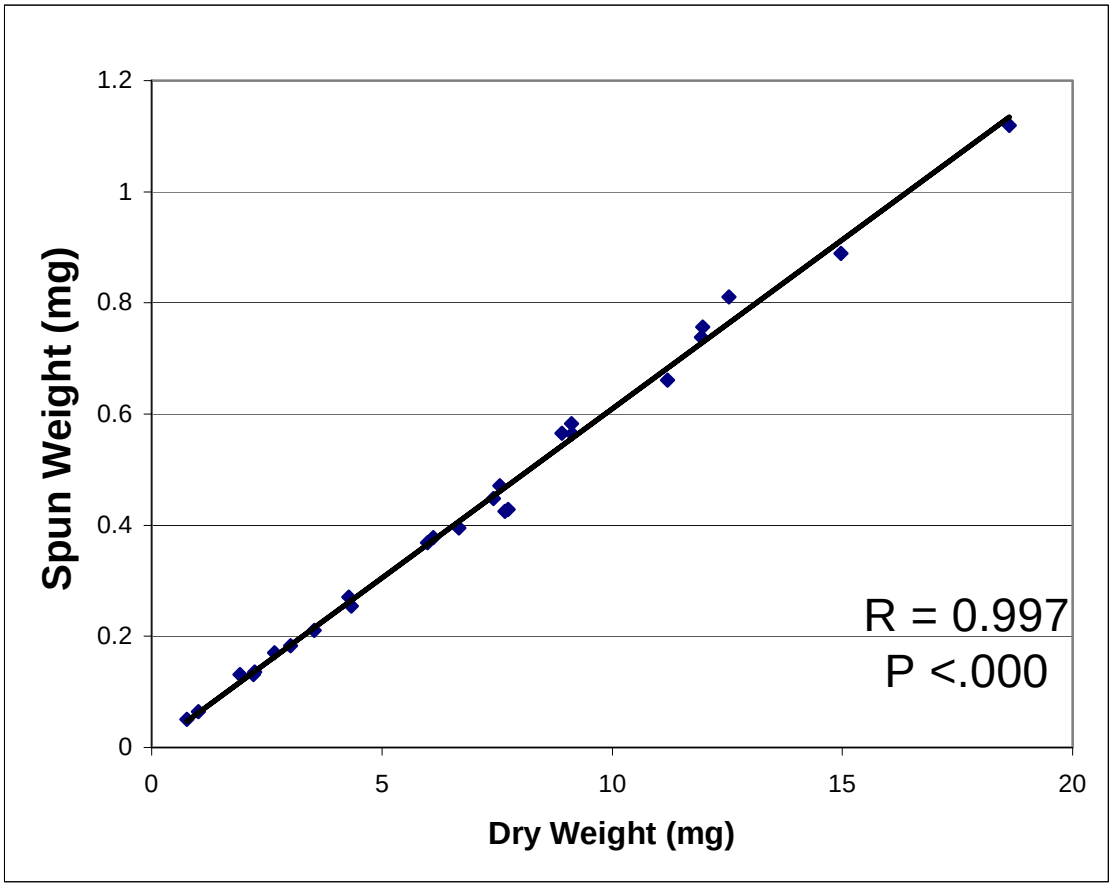

Figure 6. The relationship between the spun weight and dry weight of filamentous algae.

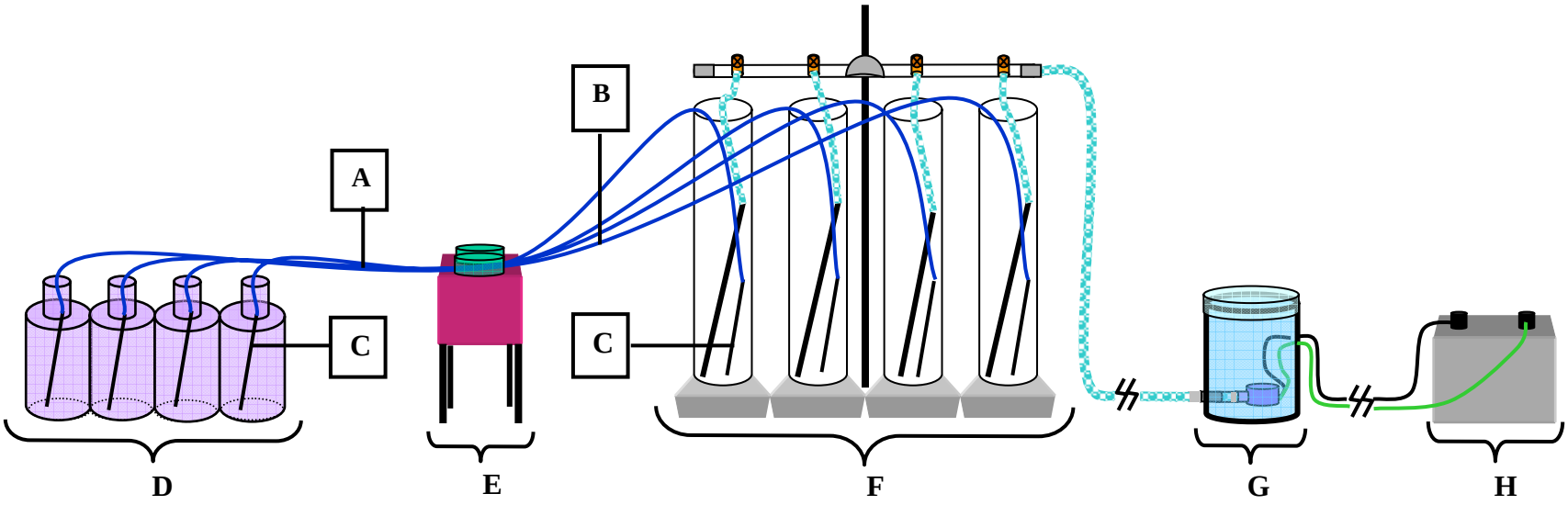

Figure 7. Enrichment Experiment. For clarity, only four incubation chambers and four carboys are shown. The actual experiments were run with eight incubation chambers, eight carboys, two filter / bilge pump assemblies, and two deep cycle batteries.
A – Tygon tubing (90 cm), B – Tygon tubing (300 cm), C – 1ml pipet, D – Carboys (4 L), E – Peristaltic pump,
F – Incubation chambers, G – Filter / bilge pump assembly, H – Deep cycle battery.

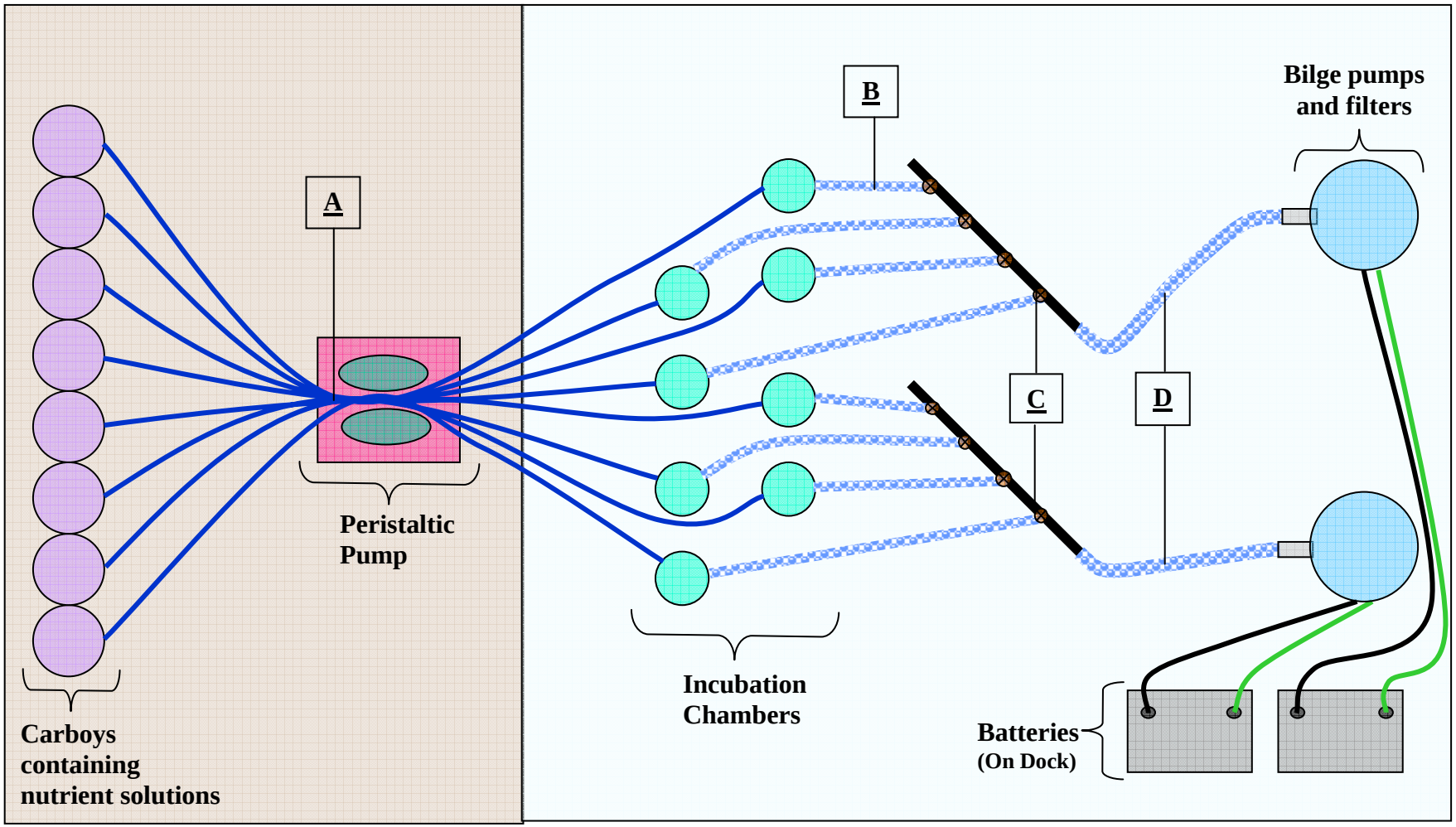

Figure 8. Design of enrichment experiment. The peristaltic pump brings a concentrated nutrient solution to the incubation chambers while the bilge pumps fill the chambers with lake water. The peristaltic pump and carboys were on dry land. The incubation chambers and bilge pumps were in the lake. A – Tygon tubing, B –Vinyl tubing (I.D. = 0.43cm), C- Manifolds, D –Vinyl tubing (I.D. = 1.6 cm).

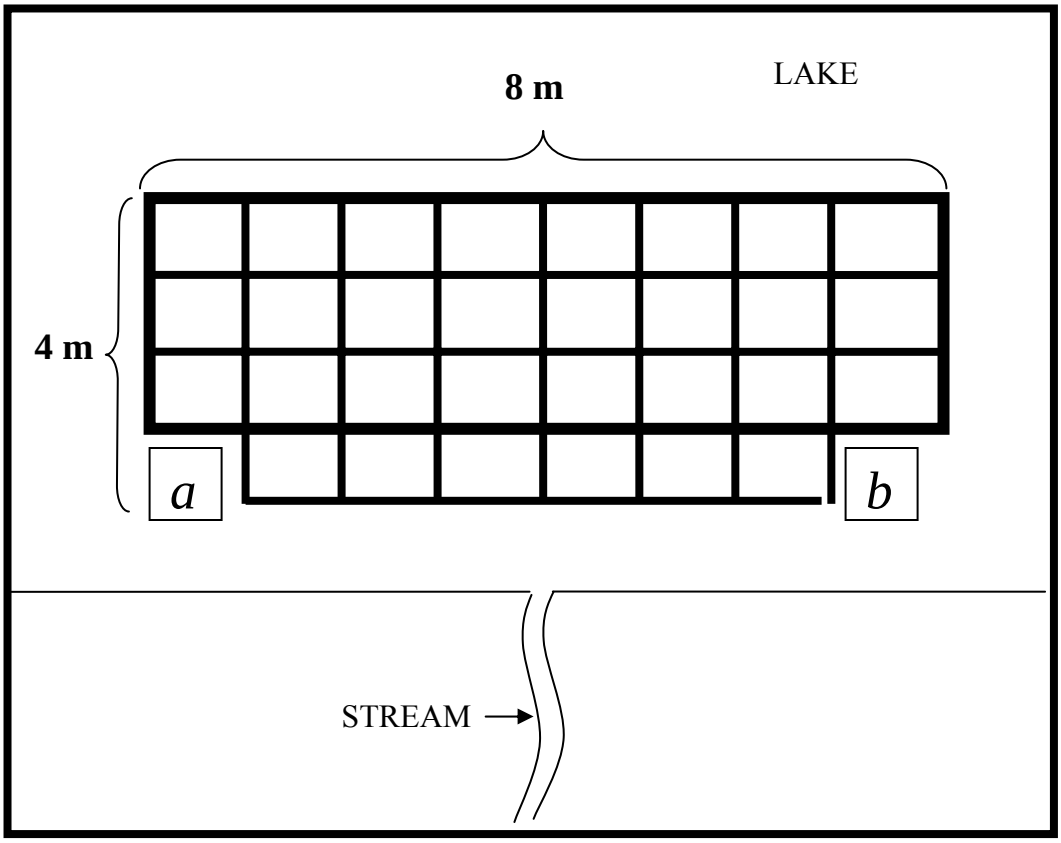

Figure 9. Design of grid used to determine percent cover of metaphyton. Percent cover was not determined in areas *a* and *b* because the metaphyton in these areas were disturbed during grid construction. Similar grids were constructed to the north and south of stream mouths.

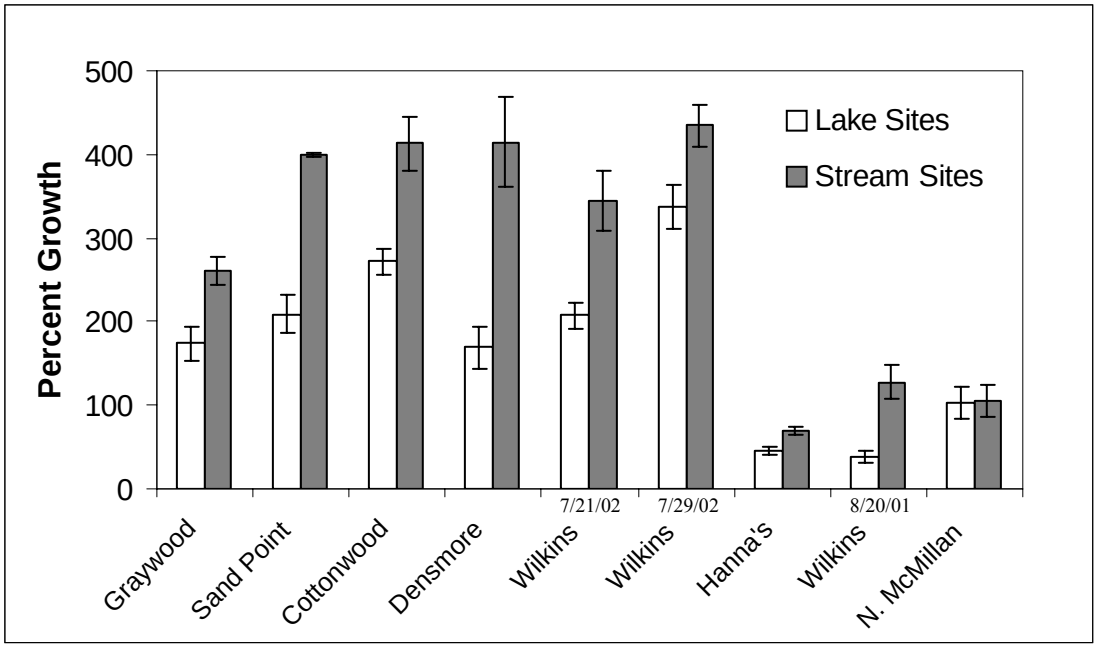

Figure 10. Comparison of percent growth between metaphyton that received stream effluent and metaphyton that received lake water. Error bars represent ± 1 SE.

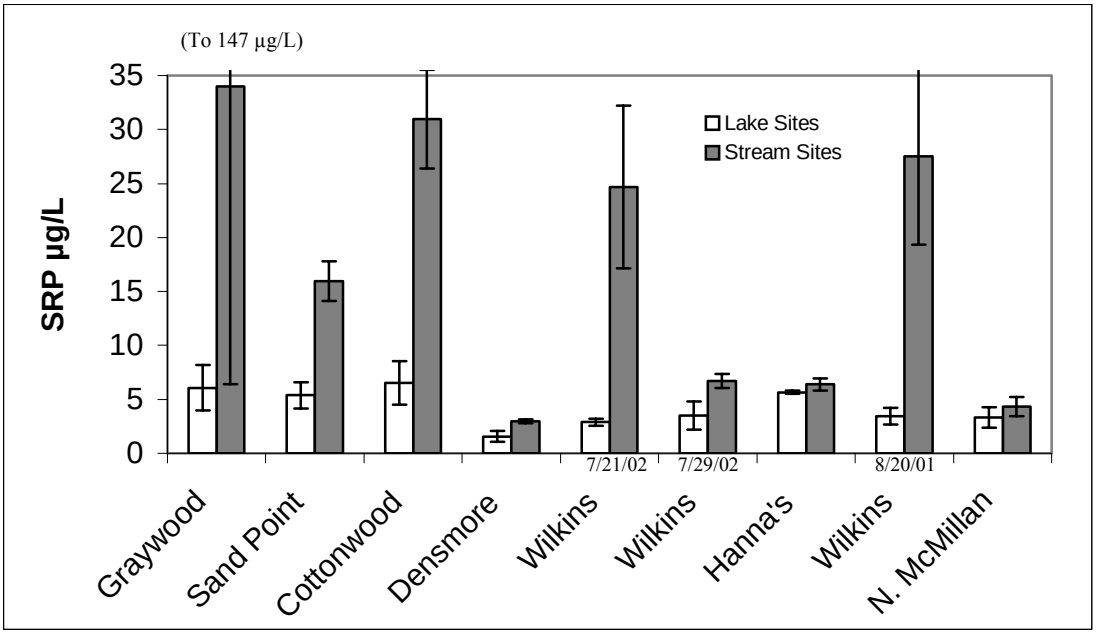

Figure 11. Comparison of SRP concentrations between lake and stream water. The SRP concentration in Graywood Gully was beyond the range of the graph (147 µg/L). Error bars represent ± 1 SE.

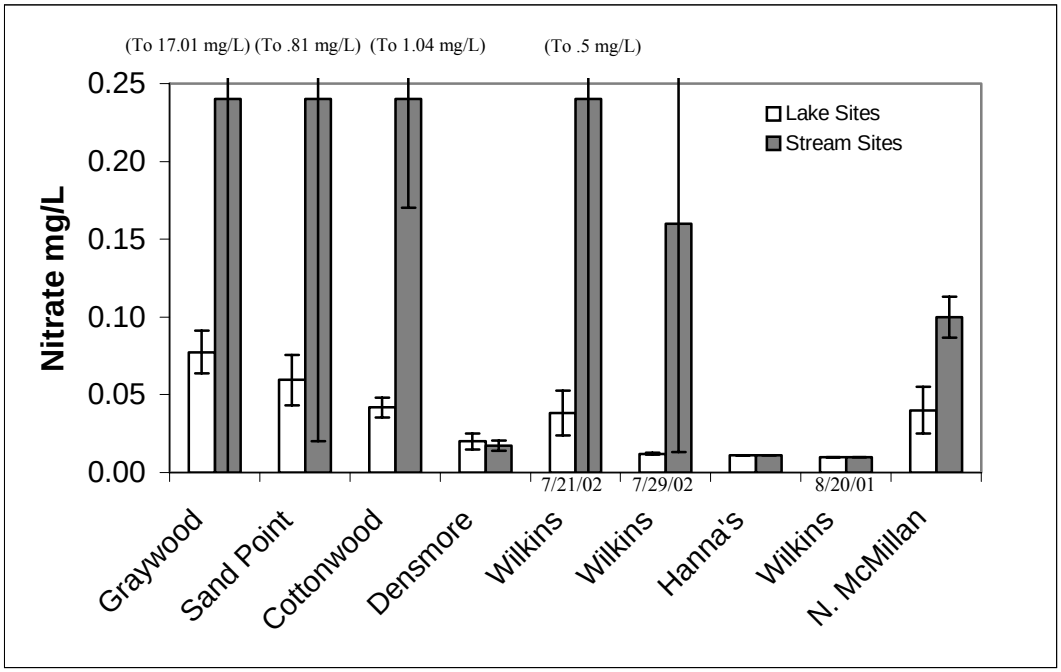

Figure 12. Comparison of NO_3 concentrations between lake and stream water. Nitrate concentrations in Graywood Gully, Sand Point Gully, Cottonwood Creek and Wilkins Creek were beyond the range of the graph and are depicted above each bar. Error bars represent ± 1 standard error.

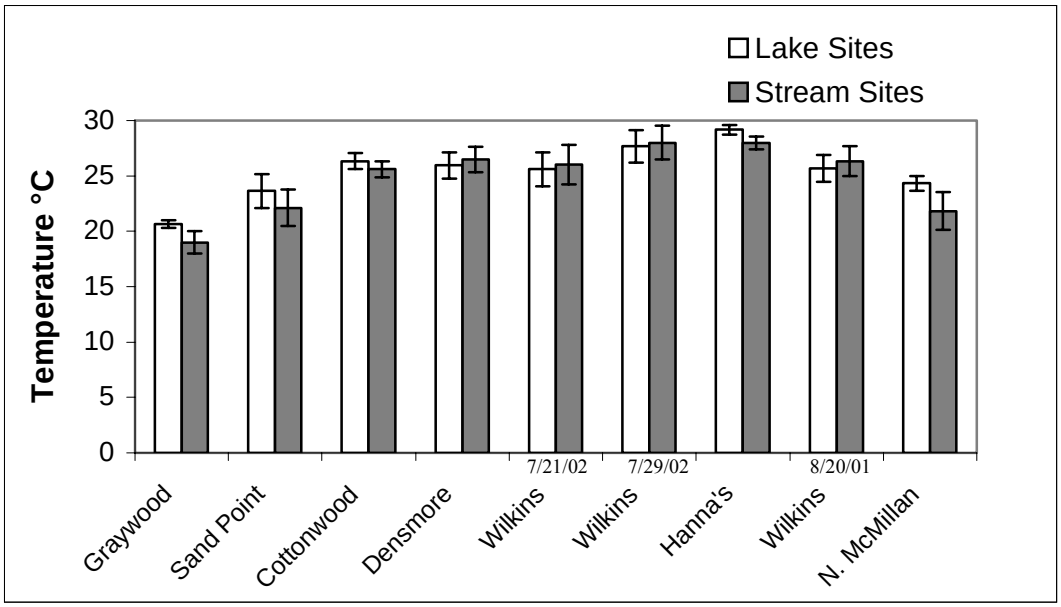

Figure 13. Comparison of average water temperatures in chambers that received stream effluent and chambers that received lake water. Error bars represent ± 1 SE.

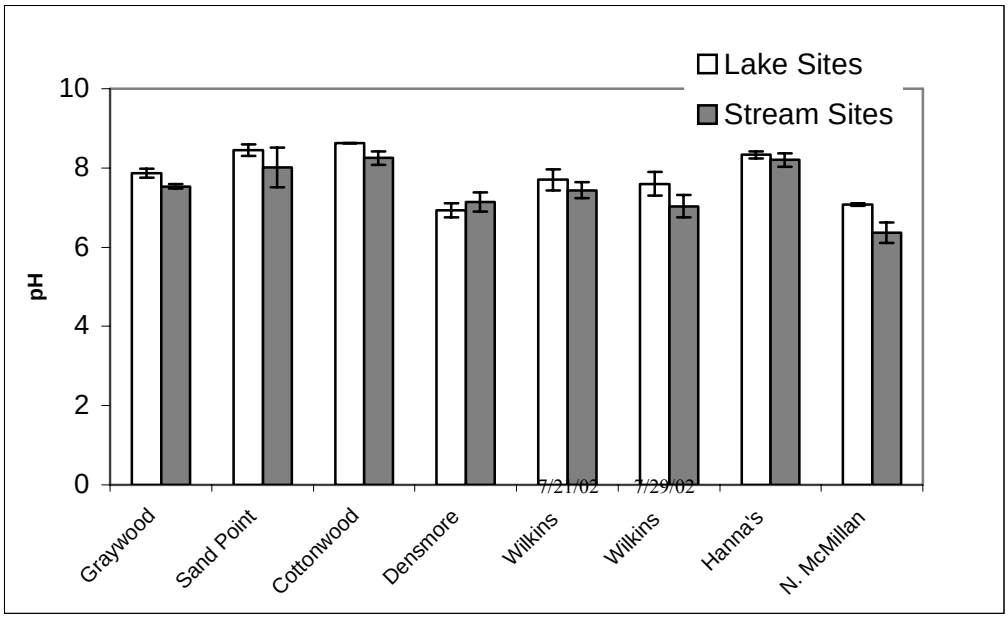

Figure 14. Comparison of average values of stream effluent pH and lake water pH at different sites. Error bars represent ± 1 SE. No pH data was obtained for the Wilkins Creek experiment on 08/20/01.

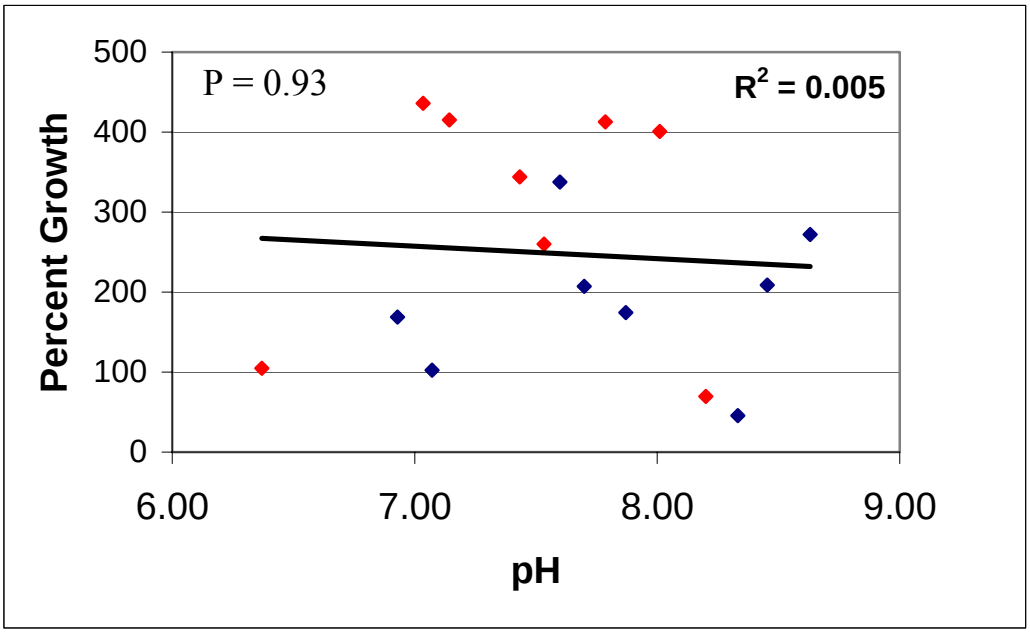

Figure 15. Percent Growth vs. pH. Red points represent values obtained from stream effluent and blue points represent values obtained from lake water.

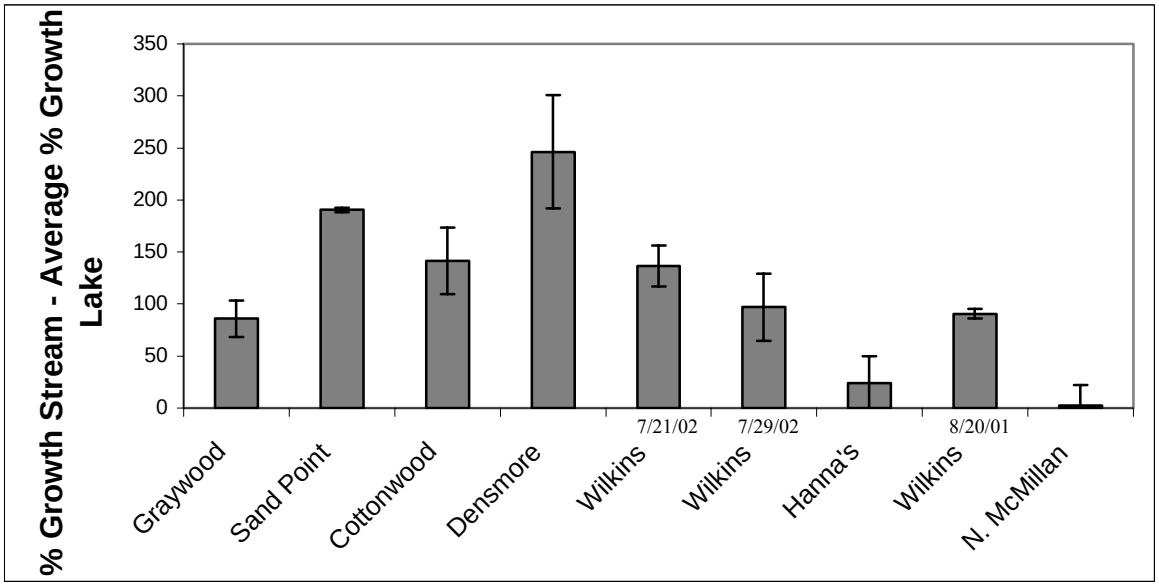

Figure 16. Spatial differences in metaphyton growth potential. Bars represent the difference between metaphyton percent growth in stream-fed chambers and metaphyton percent growth in lake-fed chambers at various locations. Error bars represent ± 1 SE.

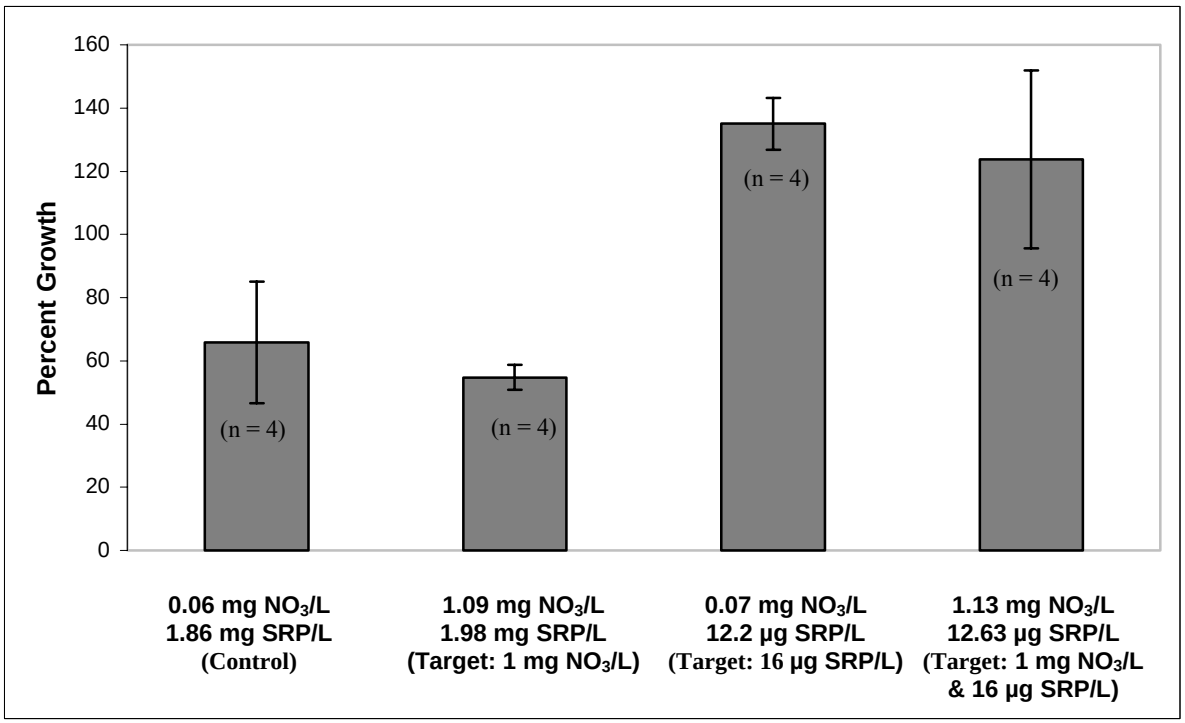

Figure 17. Results of the nitrate and phosphorus enrichment experiment determining nutrient limitation in Conesus Lake metaphyton. Nutrient concentrations are averages of actual levels inside incubation chambers. Error bars represent ± 1 SE.

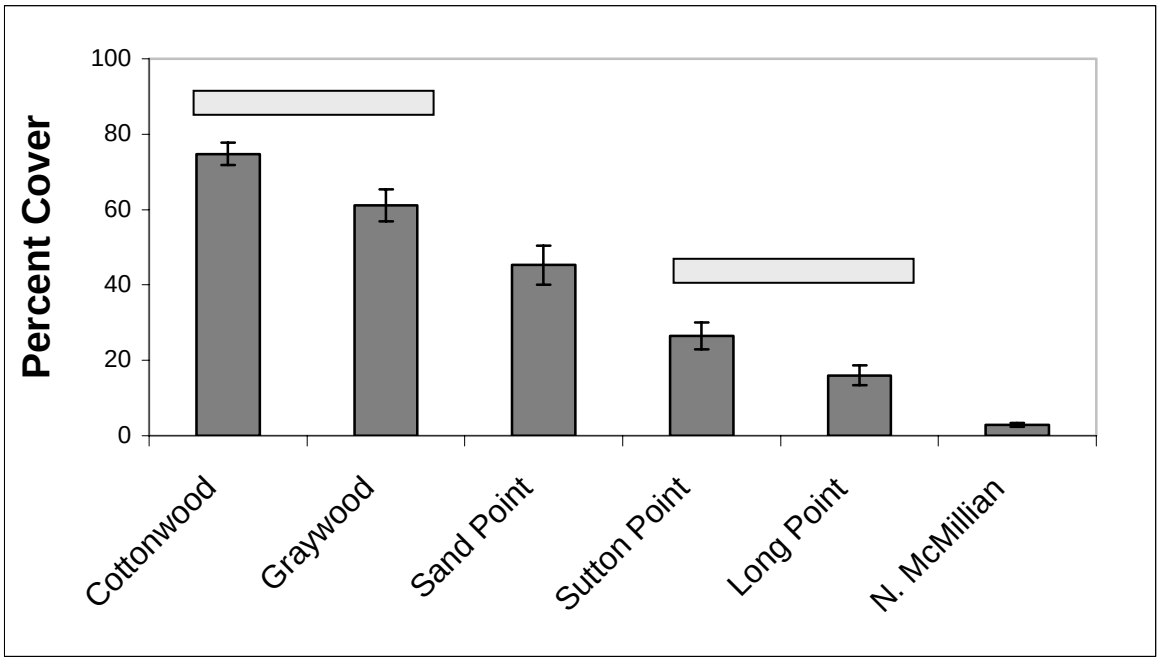

Figure 18. Percent metaphyton cover at different stream mouths. Bars above more than one column represent no significant difference between those sites as determined by Tukey HSD test. Error bars represent ± 1 SE.

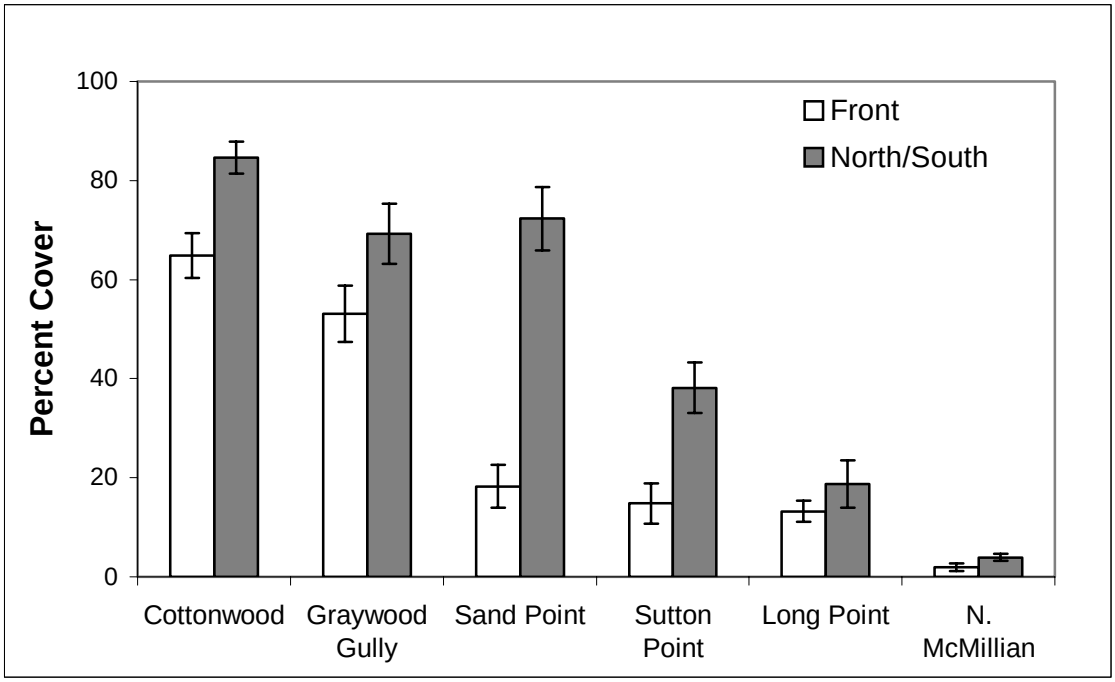

Figure 19. Percent metaphyton cover in front of stream mouths compared to percent metaphyton cover to the north or south of streams. Error bars represent ± 1 SE.

Table 1. Target concentrations for enrichment experiments. These levels do not include background concentrations.

Chamber	#1		#2		#3		#4		#5		#6		#7		#8	
	mg/L NO_3	µg/L PO_4	mg/L NO_3	µg/L PO_4	mg/L NO_3	µg/L PO_4	mg/L NO_3	µg/L PO_4	mg/L NO_3	µg/L PO_4	mg/L NO_3	µg/L PO_4	mg/L NO_3	µg/L PO_4	mg/L NO_3	µg/L PO_4
Experiment 1	0	0	0	16	2	4	2	8	4	4	4	8	8	0	8	16
Experiment 2	0	0	1	0	0	16	1	16	0	16	0	0	1	0	1	16
Experiment 3	0	0	1	16	0	16	1	0	0	16	1	16	1	0	0	0

Table 2. Statistical data for percent metaphyton growth including t-test results. Values in **bold** are averages ± 1 SE. The range is in parenthesis.

Site	Percent Growth	One tailed t-test p
Graywood Gully 6/13/02		P = 0.009
Stream	**260** ± 17 (208-282)	
Lake	**174** ± 20 (118-213)	
Sand Point Gully 6/27/02		P = 0.002
Stream	**399** ± 2 (394-404)	
Lake	**209** ± 23 (171-267)	
Cottonwood Creek 6/30/02		P = 0.003
Stream	**413** ± 32 (324-468)	
Lake	**271** ± 15 (245 - 314)	
Densmore Creek 7/7/02		P = 0.003
Stream	**415** ± 55 (260 -515)	
Lake	**168** ± 25 (99 - 215)	
North McMillan (Control) 7/14/02		P = 0.469
Stream	**105** ± 20 (80 - 163)	
Lake	**103** ± 19 (53 - 146)	
Wilkins Creek 7/21/02		P = 0.004
Stream	**344** ± 36 (266-423)	
Lake	**207** ± 16 (178-251)	
Wilkins Creek 7/29/02		P = 0.019
Stream	**434** ± 26 (383-496)	
Lake	**337** ± 26 (141-380)	
Hanna's Creek 8/1/02		P = 0.021
Stream	**69** ± 5 (52-71)	
Lake	**46** ± 4 (35-54)	
Wilkins Creek 8/20/01		P = 0.003
Stream	**128** ± 20 (85-173)	
Lake	**37** ± 7 (29-58)	

Table 3. Summary of data from metaphyton incubation chamber experiments. Values are averages ± 1 SE. The range is in parenthesis. ND = non-detectable.
* North McMillan was a control site. ** No pH measurements were taken.

Date	Site	Percent Growth	NO_3 (mg/L)	SRP (µg/L)	pH	T° C
6/13/02	**Graywood Gully**					
	Stream	**260** ± 17.38 (208-282)	**17.15** ± .61 (15.53-18.43)	**147** ± 27.57 (70.9-196.7)	**7.5 ± .05 (7.4-7.6)**	**19.0 ± 1.0 (18-21)**
	Lake	**174** ± 20 (118-213)	**0.08** ± .01 (.05-.11)	**6.1** ± 2.10 (3.4-12.3)	**7.9 ± .12 (7.6-8.1)**	**20.7 ± .33 (20-27)**
6/27/02	**Sand Point Gully**					
	Stream	**399** ± 2.03 (394-404)	**0.81** ± .23 (.32-1.34)	**16** ± 1.83 (11.9-20.3)	**8.0 ± .5 (7.2-8.9)**	**22.1 ± 1.64 (20-21)**
	Lake	**209** ± 23 (171-267)	**0.06** ± .02 (.04-.11)	**5.4** ± 1.21 (3.4-8.9)	**8.5 ± .15 (8.0-8.6)**	**23.6 ±1.5 (21-28)**
6/30/02	**Cottonwood Creek**					
	Stream	**413** ± 32 (324-468)	**1.04** ± .07 (.92-1.24)	**26.6** ± 4.58 (6.5-52.0)	**8.3 ± .17 (7.9-8.4)**	**25.6 ± .71 (23.5-26.5)**
	Lake	**271** ± 15 (245 - 314)	**0.04** ± .01 (.03-.05)	**6.5** ± 1.99 (4.0-12.4)	**8.6 ± .00 (8.6-8.6)**	**26.3 ± .71 (24-27)**
7/7/02	**Densmore Creek**					
	Stream	**415** ± 55 (260 -515)	**0.02** ± .00 (.01-.02)	**3** ± .18 (2.7-2.9)	**7.1 ± .24 (6.7-7.6)**	**26.5 ± 1.15 (25-29)**
	Lake	**168** ± 25 (99 - 215)	**0.02** ± .01 (.01-.03)	**1.6** ± .52 (.06-2.4)	**6.9 ± .18 (6.7-7.3)**	**25.9 ± 1.19 (24.5-2)**
7/14/02	**North McMillan***					
	Stream	**105** ± 20 (80 - 163)	**0.1** ± .01 **0.2** (.08-.13)	**4.3** ± .90 (1.9-6.0)	**6.4 ± .26 (6.1-7)**	**21.8 ± 1.69 (18.5-24)**
	Lake	**103** ± 19 (53 - 146)	**0.05** ± .02 (.03-.07)	**3.3** ± .95 (1.2-5.3)	**7.1 ± .03 (7-7.1)**	**24.3 ± .67 (23-25)**
7/21/02	**Wilkins Creek**					
	Stream	**344** ± 36 (266-423)	**0.5** ± .30 (.02-1.05)	**26.4** ± 7.5 (19.2-39.3)	**7.4 ± .20 (7.1-7.8)**	**26.3 ± 1.8 (24-29.8)**
	Lake	**207** ± 16 (178-251)	**0.04** ± .01 (.02-.07)	**2.9** ± .32 (2.4-3.5)	**7.7 ± .26 (7.2-8.1)**	**25.0 ± 1.5 (23-28)**
7/29/02	**Wilkins Creek**					
	Stream	**434** ± 26 (383-496)	**0.16** ± .15 (.01-.45)	**6.7** ± .66 (5.9-8.0)	**7.0 ± .29 (6.7-7.6)**	**28.0 ± 1.5 (25-30)**
	Lake	**337** ± 26 (141-380)	**0.01** ± .00 (.01-.01)	**3.5** ± 1.3 (.9-4.9)	**7.6 ± .31 (7-8)**	**27.7 ± 1.4 (25-30)**
8/1/02	**Hanna's Creek**					
	Stream	**69** ± 5 (52-71)	**0.01** ± .00 (.01-.01)	**6.4** ± .57 (5.5-7.3)	**8.2 ± .17 (7.9-8.5)**	**28.0 ± .58 (27-29)**
	Lake	**46** ± 4 (35-54)	**0.01** ± .00 (.01-.01)	**5.7** ± .15 (5.4-5.9)	**8.3 ± .09 (8.2-8.5)**	**29.2 ± .44 (28.5-30)**
8/20/01	**Wilkins Creek**					
	Stream	**128** ± 20 (85-173)	**ND**	**27.5** ± 8.2 (15.7-43.2)	**	**26.3 ± 1.3 (25-29)**
	Lake	**37** ± 7 (29-58)	**ND**	**3.4** ± .79 (4.9-2.2)	**	**25.7 ± 1.2 (24-28)**

Table 4. Data from nutrient enrichment experiments. Nutrient concentration, pH and temperature values are averages of data obtained over the three-day experimental period. Italicized values represent ambient concentrations. The concentration range is in parenthesis. ND = non-detectable.

Target Concentrations		Actual	Actual			
SRP (µg/L) / NO_3 (mg/L)	Rep	SRP (µg/L)	NO_3 (mg/L)	% Growth	pH	T(C)
16 / 1	**1**	15.17 (14.5 –16.0)	0.97 (.91-1.0)	174	7.9	24
16 / 1	**2**	13.37 (11.4-15.4)	1.00 (.92-1.09)	74	7.9	24
16 / 1	**3**	11.60 (10.5-12.7)	1.08 (1.01-1.15)	171	8.1	23
16 / 1	**4**	10.40 (9.5-11.3)	1.47 (1.02-1.92)	76	8.1	23
	Avg. =	**12.63**	**1.13**	**124**	**8.0**	**24**
16 / 0	**1**	15.07 (13.5-15.9)	*0.04 (.02-.09)*	133	7.9	24
16 / 0	**2**	12.83 (11.2-14.8)	*0.02 (.01-.02)*	113	7.9	24
16 / 0	**3**	11.50 (9.7-13.3)	*0.12 (.08-.16)*	143	8.1	23
16 / 0	**4**	9.40 (9.1-9.7)	*0.11 (.11-.11)*	151	8.1	23
	Avg. =	**12.20**	***0.07***	**135**	**8.0**	**24**
0 / 1	**1**	*3.00 (2.1-4.5)*	0.87 (.77-.92)	43	7.9	24
0 / 1	**2**	*2.20 (1.7-2.6)*	1.01 (.87-1.16)	57	7.9	24
0 / 1	**3**	*1.47 (1.2-1.7)*	1.43 (1.05-1.80)	58	8.1	23
0 / 1	**4**	*1.24 (ND-1.2)*	1.08 (1.01-1.14)	61	8.1	23
	Avg. =	***1.98***	**1.09**	**55**	**8.0**	**24**
0 / 0	**1**	*2.27 (2.0-2.7)*	*0.02 (<.02 -.03)*	28	7.9	24
0 / 0	**2**	*2.60 (2.0-3.8)*	*0.06 (.10-.04)*	38	7.9	24
0 / 0	**3**	*1.35 (1.3-1.4)*	*0.08 (.06-.09)*	106	8.1	23
0 / 0	**4**	*1.24 (ND-1.2)*	*0.08 (.07-.08)*	91	8.1	23
	Avg. =	***1.86***	***0.06***	**66**	**8.0**	**24**

Table 5. Results of two-way ANOVA applied to data from enrichment experiment.

	SUM OF SQUARES	DF	F	P
SRP	19113.063	1	15.325	.002
NITRATE	495.063	1	.397	.540
SRP * NITRATE	6.250E-02	1	.000	.994

Table 6. Metaphyton Spatial Distribution at Stream Mouths. Grids were located in front of stream mouths, to the north of stream mouths, and to the south of stream mouths. Each grid consists of 30 square meters. Values in each quadrat are percent cover. The average percent cover is given above each grid along with the distance from the center of the stream mouth. Depth values in the far left column indicate the range of depths for grids surveyed.

	North	Stream Mouth (Center)	South
Graywood Gully 8/9/01 Depth: .4m-2m		AVG: 53 % 81 56 89 22 100 14 17 33 100 44 25 44 50 25 50 31 67 100 100 33 39 83 33 56 100 11 22 100 56 11	(20 m) AVG: 69 % 100 100 100 22 64 11 19 58 50 14 14 86 83 50 67 11 44 100 100 100 100 89 94 100 100 100 100 94 50 56
Sand Point 8/9/01 Depth: .1m-2m	Macrophyte Removal Area 100 100 100 100 100 83	0 0 0 0 0 0 0 0 0 17 19 8 0 0 0 0 33 36 0 25 42 42 28 28 83 41 83 41 6 14	
Long Point 8/10/01 Depth: 1m-2m	(20 m) AVG: 72 % Swimming Area	AVG: 18 % 14 11 17 6 8 8 3 8 25 22 14 17 14 11 6 11 8 11 50 25 44 11 17 0 11 0 0 25 0 0	8 11 8 6 11 6 6 1 6 0 .05 42 1 0 67 86 50 75 83 0
Cottonwood 8/10/01 Depth: 1.5-2m	Swimming Area	AVG: 12 % 22 28 33 42 33 42 64 58 34 67 50 44 86 58 67 56 72 64 28 100 89 70 100 100 86 75 50 100 100 100	(40 m) AVG: 19 % 56 56 67 50 86 100 100 89 67 100 100 75 83 86 83 94 92 97 61 100 100 100 100 100 42 83 83 94 94 100
Sutton Point 8/11/01 Depth: 1-2m	14 83 86 33 70 6 14 42 14 25 00 20 33 1 42 Boat Activity 33 42 39 39 39 39	AVG: 65 % 25 3 17 0 3 11 0 6 11 0 0 11 50 3 83 6 8 0 78 0 0 0 0 25 14 39 3 3 8 39	(40 m) AVG: 85 %
N. McMillan 8/11/01 Depth: 1.5-2m	(10 m) AVG: 38 %	AVG: 15 % 0 0 0 0 6 0 0 0 14 8 11 0 0 0 0 0 17 0 0 0 0 0 0 0 0 0 0 0 3 0	14 0 0 6 8 11 8 11 0 8 8 0 Boat Activity 0 0 3 3 3 3 3 3
		AVG: 2 %	(30 m) AVG: 4 %

No metaphyton

Table 7. Statistical data for percent metaphyton cover. Values in **bold** are averages of percent cover ± 1 SE. The range is in parenthesis. Average percent cover data was arcsine transformed to normalize before t-tests were applied. Percent metaphyton cover was determined directly in front of stream mouths, and to the south or north of stream mouths. The northern sides were surveyed only if southern sides were disturbed.

Site	Average Percent Cover	One tailed t-test p values
Cottonwood Creek		0.001
In Front	**65** ± 5 (22-100)	
South	**85** ± 3 (42-100)	
Graywood Gully		0.030
In Front	**53** ± 6 (11-100)	
South	**69** ± 6 (11-100)	
Sand Point Gully		0.000
In Front	**18** ± 4 (0-83)	
North	**72** ± 6 (6-100)	
Sutton Point Creek		0.000
In Front	**15** ± 4 (0-83)	
North	**38** ± 5 (6-100)	
Long Point Gully		0.301
In Front	**12** ± 2 (0-50)	
South	**19** ± 5 (0-86)	
North McMillan Creek		0.001
In Front	**2** ± 1 (0-17)	
South	**4** ± 1 (0-14)	

Appendix 1. Data obtained from the metaphyton incubation experiments: Chambers 1-4 were fed stream water and chambers 5-6 were fed lake water. The weight of the algae before incubation was subtracted from the weight of the algae after the incubation period and the percent growth was calculated. Daily values for nitrate, soluble reactive phosphorous, pH and temperature are recorded on the right side of each chart. Percent growth data that are in bold face and italicized were outliers and discarded. Replacement values are averages of remaining data utilized during statistical analysis.

Graywood Gully			6/13/2002			Water sample analysis				
Chamber	**Wt. Before (g)**	**Wt. After (g)**	**Difference (g)**	**% Growth**	**% Growth Replacement Value**	**Date**	**NO_3 mg/L**	**SRP µg/L**	**pH**	**T° C**
1	0.62	2.32	1.71	277.80		6/18	18.43	176.2	7.7	18.0
2	1.23	4.69	3.46	282.33		6/19	17.60	196.7	7.5	18.0
3	0.94	2.90	1.96	208.46		6/20	17.05	144.0	7.5	21.0
4	1.37	5.10	3.73	272.30		6/21	15.53	70.9	7.5	
5	0.92	2.02	1.09	118.03		6/18	0.05	12.3	8.2	21.0
6	0.96	2.81	1.85	191.94		6/19	0.09	4.9	7.8	20.0
7	0.85	2.67	1.82	213.30		6/20	0.06	3.7	7.9	21.0
8	0.64	2.63	1.98	***308.36***	**174.42**	6/21	0.11	3.4	7.6	

Sand Point Gully			6/27/2002			Water sample analysis				
Chamber	**Wt. Before (g)**	**Wt. After (g)**	**Difference (g)**	**% Growth**	**% Growth Replacement**	**Date**	**NO_3 mg/L**	**SRP µg/L**	**pH**	**T° C**
1	0.29	1.44	1.15	394.20		6/25	1.34	14.3		21.0
2	0.29	1.44	1.15	404.14		6/26	1.01	11.9	7.2	20.5
3	0.64	1.90	1.26	***198.68***	**399.17**	6/27	0.58	20.3	7.8	20.0
4	0.92	2.76	1.84	***199.13***	**399.17**	6/28	0.32	17.4	9.0	27.0
5	0.69	1.88	1.19	173.12		6/25	0.04	5.1	8.5	21.0
6	0.72	1.95	1.23	170.95		6/26	0.11	4.1	8.7	22.5
7	0.89	2.89	2.00	224.14		6/27	0.05	8.9	8.2	23.0
8	0.64	2.36	1.72	266.89		6/28	0.04	3.4		28.0

Cottonwood Creek			6/30/02			Water sample analysis				
Chamber	**Wt. Before (g)**	**Wt. After (g)**	**Difference (g)**	**% Growth**	**% Growth Replacement**	**Date**	**NO_3 mg/L**	**SRP µg/L**	**pH**	**T° C**
1	0.57	1.48	0.91	***159.18***	**413.49**	6/30	1.24	14.8		23.5
2	0.23	1.26	1.03	448.93		7/1	0.92	23.9	8.5	26.0
3	0.31	1.76	1.45	467.69		7/2	1.02	33.1	7.9	26.4
4	0.42	1.79	1.37	323.85		7/3	0.98	52.0	8.4	26.5
5	0.61	2.09	1.49	244.75		6/30	0.05	4.0	8.6	24.2
6	0.46	1.91	1.45	313.93		7/1	0.05	4.1	8.6	27.0
7	0.49	1.78	1.29	263.86		7/2	0.03	5.6	8.6	27.1
8	0.50	1.83	1.32	264.22		7/3	0.04	12.4		27.0

Densmore Creek			7/7/02			Water sample analysis				
Chamber	Wt. Before (g)	Wt. After (g)	Difference (g)	% Growth	% Growth Replacement	Date	mg/L NO_3	µg/L SRP	pH	T° C
1	0.92	3.31	2.39	259.53						
2	0.71	3.87	3.16	443.58		7/8	0.02	2.9	6.8	28.7
3	0.64	3.91	3.27	515.18		7/9	0.01	3.3	7.0	24.8
4	0.58	3.15	2.57	441.93		7/10	0.02	2.7	7.6	26.0
5	1.12	3.52	2.40	214.85						
6	1.11	3.28	2.17	195.42		7/8	0.03	0.6	6.8	28.3
7	1.30	2.59	1.29	99.38		7/9	0.01	1.7	6.7	25.0
8	1.00	2.65	1.65	165.54		7/10	0.02	2.4	7.3	24.5

North McMillan			7/14/02			Water sample analysis				
Chamber	Wt. Before (g)	Wt. After (g)	Difference (g)	% Growth	% Growth Replacement	Date	NO_3 mg/L	SRP µg/L	pH	T° C
1	1.13	2.18	1.06	93.64		7/14	0.08	4.1		
2	0.80	1.44	0.64	80.25		7/15	0.09	5.3	6.1	18.5
3	0.75	1.97	1.22	163.10		7/16	0.13	6.0	6.1	24.0
4	1.19	2.16	0.98	82.38		7/17	0.12	1.9	7.0	23.0
5	0.67	1.66	0.98	145.70		7/14	0.03	4.5		
6	1.15	2.32	1.17	101.22		7/15	0.07	5.3	7.0	23.0
7	1.77	2.71	0.94	52.93		7/16	ND	2.3	7.1	25.0
8	1.16	2.45	1.29	110.77		7/17	ND	1.2	7.1	25.0

Wilkins Creek			7/21/02			Water sample analysis				
Chamber	Wt. Before (g)	Wt. After (g)	Difference (g)	% Growth	% Growth Replacement	Date	NO_3 mg/L	SRP µg/L	pH	T° C
1	1.69	2.73	1.04	***61.20***	**344.44**					
2	1.72	2.55	0.83	***48.39***	**344.44**	7/22	0.02	39.3	7.8	29.8
3	1.09	5.73	4.63	423.28		7/23	1.05	20.6	7.4	24.0
4	1.16	4.23	3.07	265.60		7/24	0.44	19.2	7.1	25.0
5	1.07	2.96	1.90	177.84						
6	1.22	3.78	2.56	208.86		7/22	0.02	3.5	8.1	28.0
7	1.19	3.48	2.29	191.99		7/23	0.07	2.8	7.2	24.0
8	0.81	2.85	2.04	251.09		7/24	0.03	2.4	7.8	23.0

Wilkins Creek			7/29/02			Water sample analysis				
Chamber	**Wt. Before (g)**	**Wt. After (g)**	**Difference (g)**	**% Growth**	**% Growth Replacement**	**Date**	**NO_3 mg/L**	**SRP µg/L**	**pH**	**T° C**
1	1.01	5.64	4.63	456.51						
2	0.70	3.36	2.66	383.23		7/30	0.45	6.2	7.6	25.0
3	1.15	5.76	4.62	401.73		7/31	<.01	8.0	6.8	29.0
4	0.68	4.06	3.38	496.28		8/1	0.01	5.9	6.7	30.0
5	0.90	4.22	3.32	369.51						
6	1.70	6.17	4.47	263.50		7/30	<.01	0.9	7.0	25.0
7	1.03	4.96	3.92	379.84		7/31	<.01	4.7	8.0	28.0
8	0.88	2.11	1.24	***141.34***	**337.62**	8/1	0.01	4.9	7.8	30.0

Hanna's Creek			8/1/02			Water sample analysis				
Chamber	**Wt. Before (g)**	**Wt. After (g)**	**Difference (g)**	**% Growth**	**% Growth Replacement**	**Date**	**NO_3 mg/L**	**SRP µg/L**	**pH**	**T° C**
1	1.21	1.88	0.67	55.44						
2	1.02	1.55	0.53	51.80		8/2	0.01	5.5	8.2	28.0
3	0.94	1.59	0.64	68.04		8/3	<.01	7.3	7.9	27.0
4	1.19	2.03	0.84	70.72		8/4	.01	7.10	8.5	29.0
5	1.39	1.88	0.48	34.69						
6	1.41	2.17	0.76	54.13		8/2	0.01	5.4	8.3	29.0
7	1.09	1.61	0.52	47.64		8/3	<.01	5.9	8.5	28.5
8	1.33	1.94	0.61	45.75		8/4	.01	5.60	8.2	30.0

Wilkins Creek			8/20/2001			Water sample analysis				
Chamber	**Wt. Before (g)**	**Wt. After (g)**	**Difference (g)**	**% Growth**	**% Growth Replacement**	**Date**	**NO_3 mg/L**	**SRP µg/L**	**pH**	**T° C**
1	0.98	2.01	1.03	104.50		8/21	ND	43.2		25.0
2	1.07	1.98	0.91	84.89		8/22	ND	23.7		29.0
3	0.63	1.57	0.94	149.91		8/23	ND	15.7		25.0
4	0.51	1.38	0.88	172.68						
5	0.77	1.21	0.45	58.45		8/21	ND	3.2		24.0
6	1.11	1.44	0.34	30.41		8/22	ND	4.9		28.0
7	0.57	0.75	0.18	31.14		8/23	ND	2.2		25.0
8	0.58	0.75	0.17	29.38						