The Ability of *Cladophora* to Adapt to Nutrient Changes in the Watab Watershed

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The Ability of *Cladophora* to Adapt to Nutrient Changes in the Watab Watershed.

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and the Degree Bachelor of Arts
In the Department of Biology

by
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Abstract

Over the past several years, excessive algal growth has occurred in the lakes associated with Saint John's University campus. To assess the causes of this growth, the physical, chemical and biological characteristics of Lower Stumpf Lake were monitored for approximately four months. Environmental parameters of temperature, pH, dissolved oxygen, and nutrients were assessed to determine which parameter influenced growth of the green alga *Cladophora*. Based on these results, controlled laboratory culture experiments were conducted to determine the nitrate and phosphate requirement and uptake for *Cladophora*. This alga grew well in nutrient rich Lower Stumpf Lake. However, in culture, *Cladophora* was resilient to low concentrations of nitrate and phosphate. This suggested *Cladophora* may have a mechanism for nutrient uptake and storage. Understanding the uptake and storage capabilities of this alga may help determine affective management strategies to improve the quality of Lower Stumpf Lake.
Introduction

Aquatic systems have an important impact on all forms of life. These systems provide habitat for aquatic organisms and water for drinking, and industrial and domestic uses. They also help to keep the earth cool. These systems offer beauty and fulfill recreational purposes. Aquatic systems are a major source of oxygen, which is released through the photosynthesis of algae and aquatic plants (Horne and Goldman 1994). Understanding how the chemical, physical and biological factors within an aquatic system interact is key to understanding the changes occurring within these systems. Through this understanding, we can help keep these systems pristine, not only for the organisms which find a home within them, but for the future.

Saint John’s University (SJU) is fortunate to be surrounded by several aquatic systems. Lower Stumpf Lake, the focus of this study, is one of these unique systems. Created by the Benedictine monks in 1868 (Bapt 1934), this artificial lake was formed by damming the Watab River. The dam, located at the north end of the lake where the SJU access road currently exists, caused the Watab River to rise and eventually form Lower Stumpf Lake. During the lake’s first freeze, the emergent trees, which had originally lined the river banks, were cut (Bapt 1934). The stumps disfigured the lake for years, giving Lower Stumpf its name.

Over the past several years, Lower Stumpf Lake appears to have undergone some water quality changes. Visible differences in algal and plant distribution and abundance become more evident as summer progresses. The most notable of the changes in vegetation has occurred in the green filamentous alga, Cladophora.

Representatives of the genus Cladophora are distributed worldwide and often dominate the benthos in fresh and marine waters (Dodds and Gudder 1992). Recently, Cladophora has
been extensively studied because of its wide distribution and abundance in the Great Lakes and irrigation systems throughout western United States (Lembi et al. 1988). This alga can also be observed in many other lakes and streams throughout North America.

Typically temperate species, *Cladophora* appears soon after ice-out and before the establishment of vascular plants in the spring (Engel 1985). *Cladophora* is generally a benthic alga, growing attached to rocky bottom areas and other similar stable substrates. However, as the summer progresses, oxygen bubbles collect within the branches and this alga detaches from its substrate. Once *Cladophora* detaches, it forms free-floating mats as are occurring in Lower Stumpf Lake. Detachment or sloughing (Canale et al. 1982), becomes troublesome once the mats are free. These large free-floating mats can clog water intake pipes, foul fish nets, cause various odor and taste problems in drinking water, and create changes in the type of fish and other aquatic organisms present. *Cladophora* mats can also become a nuisance when the wiry filaments wash up on shore. The decaying mat left on shore is aesthetically unpleasant and creates a barrier to recreation. In 1975, the problem caused by these mats was so great in Lake Erie and Lake Ontario, front-end loaders and dump trucks were used to remove *Cladophora* from their shores (Auer et al. 1982).

The proliferation of *Cladophora* is a great concern to researchers and the general population, not only because of the aesthetic displeasures created, but because of the environmental factors, including light, temperature, and nutrient availability, often necessary to create such growth. High nutrient concentrations, most notably nitrate and phosphate, are generally associated with nuisance growth of algae. Eutrophication is the enrichment process of a water body due to an increase in nutrient loading (Horne and Goldman 1994). It is a gradual
process occurring for all but a few smaller lakes (Horne and Goldman 1994). Eutrophic lakes are characterized by high nutrient levels, and the abundance of planktonic or attached algae (primary production). Low water clarity may be the simplest indicator of a eutrophic lake.

Cultural eutrophication is another process occurring through the human-related input of domestic wastewater rich in nutrients. The use of detergents once high in phosphate stimulated cultural eutrophication. Intensive agriculture, excessive fertilization and storm runoff are other sources of nutrients for aquatic systems. Since eutrophication is so frequently linked with pollution or excessive fertilization, the point may be missed that eutrophic environments are not always undesirable (Horne and Goldman 1994). For instance, eutrophic lakes may produce large quantities of fish for food or sport. Eutrophication can be reversed, although the process is expensive and extremely slow.

Growths of *Cladophora* reach nuisance proportions often as a result of eutrophication (Dodds and Gudder 1992). Specifically, increased phosphate loading is strongly suspected to support increases in *Cladophora* growth (Auer and Canale 1982b). Several studies seem to confirm this notion in the Great Lakes as well as in most smaller freshwater systems.

The nutrients, nitrogen and phosphorus, responsible for algal growth are found in several different forms within an aquatic system (Horne and Goldman 1994). Nitrogen can be present as nitrogen gas ($\text{N}_2$), nitrite ($\text{NO}_2$), nitrate ($\text{NO}_3$), ammonia ($\text{NH}_3$), ammonium ($\text{NH}_4$) and urea ($\text{CO(NH}_2\text{)}_2$). Nitrogen gas can be mixed in the lake, but is broken down quickly. Nitrite is generally present only in trace quantities in water exposed to oxygen, where it is transformed to nitrate (Horne and Goldman 1994). High nitrite levels are toxic. Algae utilize nitrogen in the form of nitrate or ammonia. Nitrate is the most abundant of the two and is metabolized by algae
only after transformation by nitrate reductase, an enzyme that reduces nitrate to nitrite (Horne and Goldman 1994). Nitrite is then further reduced to an amino group (-NH$_2$) and can be assimilated into the cells. Therefore, ammonia is preferred by algae because it is already in this reduced state, but is not as readily available in the aquatic system.

Nitrate concentrations tend to follow seasonal patterns (Horne and Goldman 1994), which may be evident through algal blooms. During the spring and summer, nitrate levels typically decrease due to algal uptake and in the fall and winter, these levels are replenished from the soil, water inflows and precipitation. An adequate amount of nitrate is not always present and may therefore limit algal growth. Competition for this nutrient occurs and often proceeds by enzyme kinetics (Horne and Goldman 1994).

Enzyme kinetics is exhibited by many different types of algae under both nitrate and phosphate limitations. The rate of nitrate uptake and the affinity of the enzyme for its substrate varies with species and cell size (Horne and Goldman 1994). Algal kinetics can be measured experimentally and is extremely useful in determining whether or not nitrate-limiting conditions exist. Nevertheless, in aquatic systems, nitrate is often found in excess amounts. This excess can result from natural changes in the vegetation, application of agricultural fertilizers to surrounding land and sewage treatment (Horne and Goldman 1994). The concentration and supply rate of nitrate is intimately connected with land practices because nitrate ions move easily through the soil and are quickly lost from the land through runoff and soil erosion.

Phosphorus is an essential nutrient for algal growth. It plays an irreplaceable role in cell construction of nucleic acids, ATP (energy source), proteins and lipid membranes. Most phosphorus is held in a biologically unavailable form by particles in lake water (Horne and
Goldman 1994). These forms are unavailable for algal use because they are adsorbed onto soil and other organic particles, making access very difficult. Out of the phosphorus compounds in an aquatic system, algae can only use soluble phosphate (PO₄) for growth.

Phosphate is a common growth-limiting factor for phytoplankton in lakes worldwide. A common nitrogen to phosphorus ratio (N:P) needed to support plant and algal growth is 10:1 (Horne and Goldman 1994). A ratio greater than 10 indicates phosphate-limitation and a ratio less than 10 indicates nitrate-limitation. Algae have, however, evolved special mechanisms that reduce the severity of phosphate limitation (Horne and Goldman 1994). Alkaline phosphatase is an enzyme which can cleave the chemical bond between phosphate and the bound organic molecule. Thus, it allows normally unavailable phosphate to be utilized. In times of high phosphate, algae can store excess in polyphosphate granules. This luxury consumption allows for the cell to divide several times when external phosphate supplies are depleted (Horne and Goldman 1994). The enzyme kinetics observed in phosphate-limited algae is similar to the kinetics of nitrate-limited algae.

When an essential nutrient becomes limiting, its rate of supply will determine the algal growth rate (Riegman and Mur 1986). Uptake and assimilation are regulated by the cell to enable optimal growth under the environmental conditions. Through enzyme kinetics several species of algae can uptake and store excess nitrate and phosphate. Most of these species are blue-green algae such as Oscillatoria agardhii. In these algae, two important processes are considered during growth under nutrient-limited conditions (Riegman and Mur 1984). First, the nutrient is taken up as reflected by uptake capacity, which most often follows Michaelis-Menton enzyme kinetics. Second, this substrate is assimilated into cell material as reflected in the
internal nutrient content of the cells. In phosphate-limited cells, alkaline phosphatase activity can also be used as an indicator of limitation (Riegman and Mur 1986). Studies of nitrate-limitation are less frequent, yet have been conducted with the blue-green species *Oscillatoria agardhii*. Similar to phosphate uptake, these studies suggest that short term rates of nitrate or ammonium uptake by this alga are a function of external concentrations and Michaelis-Menton kinetics (Zevenboom and Mur 1981). Zevenboom and Mur (1981) concluded that growth rate also has an influence on the nitrate and ammonium uptake kinetics of this species. Cells depleted of nitrate have reduced capacity for photosynthesis and assimilate nitrate rapidly (Eppley and Renger 1974). Essentially all nitrate taken up by growing cells is assimilated into cell material (Eppley and Renger 1974).

In summary, nutrients especially nitrate and phosphate, are important to algal growth. Nutrient rich or eutrophic waters typically support algal blooms such as those seen in Lower Stumpf Lake. Several studies have been conducted concerning the uptake of nitrate and phosphate in blue-green algae. Uptake mechanisms have been demonstrated in several blue-greens, they have not been firmly established in a green filamentous alga such as *Cladophora*. The widespread abundance of this alga in Lower Stumpf Lake provided the perfect opportunity to study *Cladophora* growth, the problems associated with this growth and this alga's nutrient requirements.

This study had two main hypotheses. First, of the environmental parameters tested in Lower Stumpf Lake, nutrients would have the greatest influence on *Cladophora* growth. Additionally, algal growth would be directly correlated with nutrient abundance in Lower Stumpf Lake and in culture. Ultimately the question, "would reductions in nutrient input into Lower
Stumpf Lake have a significant influence on the biomass of the weedy filamentous alga, *Cladophora*" will be addressed.

In order to test these hypotheses, this study was conducted with three main goals. First, the seasonal distribution and abundance of *Cladophora* were observed. Next, the most important environmental parameters influencing *Cladophora* growth, within Lower Stumpf Lake, were determined. Lastly, if nutrients influenced algal growth, the determination of nutrient requirements in culture was necessary. The first two goals were met by conducting a field survey of the lake. Through this field study, *Cladophora* biomass was collected and the environmental parameters of temperature, pH, dissolved oxygen, nitrate and phosphate were monitored for approximately four months. In the laboratory, experimental nutrient cultures were established. These cultures subjected *Cladophora* to different external nitrate and phosphate concentrations to determine the role of nutrients in this alga's growth.
Materials and Methods

Field procedures

An initial survey of Lower Stumpf Lake was conducted prior to monitoring the physical, chemical and biological changes within the system. This survey consisted of determining sampling sites, and making observations of the general topography, vegetation and general morphometry of Lower Stumpf Lake (map). Physical, chemical, and biological changes in Lower Stumpf Lake were monitored weekly between June 25 and October 11, 1996. Each week, Cladophora biomass was collected from three of six different sites using an open ended metal barrel quadrant (diameter= 40.6 cm). The barrel was placed randomly in the lake, approximately 2 to 3 meters from shore, and all plant and algal material within the barrel collected. Plant material and Cladophora were separated and rinsed. These plant and algal samples were dried overnight (105°F), weighed and relative biomass estimated. Relative biomass was determined by taking dry weight of algal or plant material and dividing by the volume of the barrel quadrant (in liters). Microscopic observations of Cladophora health were also recorded.

In addition to these biological observations, physical and chemical characteristics of the lake were examined. Air temperature and water temperature (just under the surface) were monitored using an alcohol thermometer. Chemical analysis included monitoring pH, dissolved oxygen, and nutrient levels (nitrate and phosphate). The pH was monitored using a Hach chemical kit. Dissolved oxygen (DO) content of the water was measured using a Hach chemical kit (modified Winkler method). This test gave an indication of how photosynthetically active the plants and algae were in the lake. Initial nutrient levels were monitored using LaMotte colorometric chemical kits specific for nitrate and phosphate. However, nutrient levels found
within the lake were below the detection range of these kits, and alternative methods for nutrient analysis were eventually employed. Methods are discussed in the laboratory experimental procedures section. Meanwhile, water samples were collected, filtered, and stored frozen until further nutrient analysis could be conducted. Small algal samples were also collected at the water sampling sites to determine internal nutrient levels in comparison to external nutrient levels. *Cladophora* was rinsed, dried and stored for analysis at a later date.

**Laboratory experimental procedures**

Previously frozen water samples were thawed overnight. A persulfate oxidation procedure (Ravek and Avnimelech 1979) was adopted as the initial digestion procedure for nutrient analysis in the water samples (appendix I). Approximately two grams of potassium persulfate were added to 60 ml water samples in labeled 250 ml Erlenmeyer flasks. The flasks were covered with aluminum foil and autoclaved for approximately one hour at 21 atm pressure and 121°C. Autoclaved samples were allowed to cool for nutrient analysis.

Nitrate content of Lower Stumpf Lake water samples was determined according to Greenburg *et al.* (1992) (appendix II). This method used a cadmium reduction column to reduce all nitrates present to nitrite\(^1\). After the persulfate digestion procedure, water samples were prepared for reduction of the nitrates. First, 25.0 ml of the autoclaved water sample was added to 75.0 ml \(\text{NH}_4\text{Cl-EDTA}\) solution and mixed. The mixed sample was poured into the column and collected at a rate of 10 ml/minute. The first 25 ml of each sample was collected and discarded.

\(^1\) Since nitrite levels found in natural waters should be negligible, the nitrite concentrations determined in this method were assumed to be due completely to the reduction of the nitrates.
The following 50 ml was collected, and as soon as possible, 2.0 ml of the color reagent was added. A pink solution was formed. Between 10 minutes and two hours after the addition of a color reagent, absorbance was measured at 543 nm using a Hewlett Packard 8452A Diode Array Spectrophotometer. Sample absorbance was measured against a deionized water-reagent blank and compared to a standard nitrate calibration curve.

The standard nitrate calibration curve was prepared using a stock nitrate solution of 100 μg NO₃/ml. Standards were prepared at the following concentrations: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0 and 15.0 μg NO₃/ml by diluting appropriate volumes of stock solution to 100 ml. All nitrate standards were digested and analyzed with the above nitrate reduction procedure. The calibrated curve was created on MathCad using the concentration of the standard solutions and their absorbance at 543 nm (appendix III).

Total phosphate content of water samples was analyzed according to Greenburg et al. (1992) (appendix IV). This method originally used sulfuric and nitric acid to digest all the phosphate present. However, this initial acid digestion procedure was modified according to Ravek and Avnimelech (1979). The persulfate oxidation digestion procedure was adopted because it is considered to be a fast and reliable method for the determination of total phosphorus (APHA 1971). After cooling, the digested water sample was neutralized (pH 7) with dilute sodium hydroxide. This sample solution was transferred using a 25.0 ml volumetric pipet to a clean dry 125 ml Erlenmeyer flask. Then 1.0 ml of the molybdate reagent was added and mixed thoroughly. The tin(II)chloride solution (0.25 ml) was added and mixed. A blue solution was formed and after 10 minutes, the absorbance at 650 nm was measured using a Hewlett Packard 8452A Diode Array Spectrophotometer. Sample absorbance was measured against a deionized
water-reagent blank. The observed absorbance of the samples was recorded and compared to a standard phosphate calibration curve.

The standard phosphate calibration curve was prepared using standard phosphate solutions at concentrations of 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, and 10 μg PO₄/ml. These standards were made using 0.5, 1.0, 5.0, 10, 25, 50, and 100 ml of a stock phosphate solution (50.0 μg PO₄/ml) and diluting to 500 ml, respectively. All standard phosphate solutions were digested and analyzed with the above total phosphate procedure. The calibrated curve was created on MathCad using the concentration of the standard solutions and their corresponding absorbance at 650 nm (appendix V).

Internal levels of nitrate and phosphate in Cladophora from Lower Stumpf Lake and nutrient experiments were determined following a similar protocol as used for the nitrate and phosphate determination in water samples. The initial persulfate procedure had two modifications due to the presence of organic material (Ravek and Avnimelech 1979). Samples of 10 to 15 mg dried, ground Cladophora was added to the two grams potassium persulfate and 60 ml deionized water in 250 ml Erlenmeyer flasks. Flasks were labeled, covered with aluminum foil and autoclaved for approximately two hours to ensure complete digestion of the internal nitrate and phosphate released from the algal cells. Cooled samples were filtered to make sure that organic material was not present to clog the nitrate reduction column. Both nitrate and phosphate analysis was conducted in the same manner as lake water samples.

Cladophora nutrient experiments

A Cladophora filament was isolated from Lower Stumpf Lake. This filament was grown
in a CLII nutrient medium based on Gerloff and Fitzgerald (1976) (appendix VI). In an attempt
to create unialgal cultures, filaments were pulled across agar plates to help remove epiphytes
from the algal cells and transferred aseptically into autoclaved 250 ml Erlenmeyer flasks with
125 ml sterile CLII every two weeks (more often in early establishment of cultures). Once
cultures were established, nutrient experiments were conducted to determine the critical nutrient
requirement range for *Cladophora* growth. Nitrate or phosphate concentration was varied by
modification of the original CLII medium (appendix VII). To determine critical nitrate
requirements, approximately equal amounts of healthy *Cladophora* were transferred by spot plate
technique to flasks containing various concentrations of nitrate. The spot plate technique was
conducted using a porcelain mixing plate. This plate had 12 small engraved wells. *Cladophora*
filaments were placed into the wells to determine if approximately equal algal masses (by visual
comparison) were used as starting inocula for nutrient cultures. The various nitrate
concentrations were 0, 50, 100, 500 and 1200 μM NO₃. Unmodified or standard CLII had a
nitrate concentration of 1200 μM NO₃. Nitrate cultures, conducted in triplicate, were allowed to
incubate for 28-37 days. These cultures were analyzed for general health using a compound light
microscope. Dry weights were measured to monitor growth. The growth rate of each
experimental culture was determined using the Guillard (1973) growth equation:

\[ k = \ln \left( \frac{n_i}{n_0} \right) * 1.443 / t \]

where \( n_i = \) final *Cladophora* dry weight (mg)

\( n_0 = \) initial or starting *Cladophora* dry weight (mg)

\( t = \) number of days incubated

\( k = \) growth rate (doublings/day).
Guillard’s growth equation was used as a way to compare or standardize the experimental trials. Additionally, analysis of internal nitrate content of the *Cladophora* filament was conducted.

To determine critical phosphate requirements, approximately equal amounts of healthy *Cladophora* were transferred again by spot plate technique to flasks containing various concentrations of phosphate. These phosphate concentrations were 0, 5, 10, 50, and 90 μM PO₄. Unmodified or standard CLII had a phosphate concentration of 90 μM PO₄. Phosphate cultures, conducted in triplicate, were allowed to incubate for between 28-37 days. These cultures were analyzed for general health using a compound light microscope. Dry weights were obtained to monitor growth. Growth rate was determined and analysis of internal phosphate content of *Cladophora* filaments was conducted.

**Statistical analysis**

Statistical tests were conducted on data samples to show if by comparison, data sets had any correlation. Two data sets, for example algal biomass over time versus external nitrate concentration over time, were entered as x and y variables, respectively. These data comparisons were plotted as XY- scatter plots to determine if any trends were visible. A least ordinary squares test was then conducted to determine the best fit line. The p value from the least ordinary squares test was then examined. Typically, data sets having significant correlation will have p values less than or equal to 0.05. Significance between data sets would indicate that as the x-variable changed the y-variable tested showed a corresponding change. For instance, in the above example, data on algal biomass and external nitrate concentration showed significance, meaning that as an increase in biomass occurred, an increase in nitrate concentration also
occurred at the same time. This was true for decreases as well.
Field Results

Biomass

*Cladophora* biomass in Lower Stumpf Lake significantly changed between June 26 and October 11, 1996 (figure 1). In late June, *Cladophora* was abundant, approximately 56 g dry weight/L. Biomass never decreased below this amount, until the end of the growing season. *Cladophora* biomass reached its first peak, 142 g dry weight/L, in mid-July (7/12/96). Algal biomass slightly declined at the end of July. This mid-summer decline in biomass continued for approximately three weeks. *Cladophora* then reached greatest abundance, 164.9 g dry weight/L, during the first part of August. Following this peak abundance, *Cladophora* biomass declined from 164.9 g dry weight/L to 0 g dry weight/L at the end of September.

Vascular plant biomass followed a similar trend to *Cladophora* biomass (figure 2). Vascular plants present included, *Ceratophyllum* (coontail), *Potamogeton* (narrow leaf pondweed), *Myriophyllum* (milfoil), *Megaodon* (water marigold), *Elodea* (water weed), *Chara*, and *Nymphaea* (white water lily). *Lemna* (duckweed) was highly associated with areas of *Cladophora* growth. Biomass indicated that plant growth flourished early in the season, reaching greatest abundance in mid-July (474 g dry weight/L). Plant abundance greatly decreased during the last part of July, but increased again during the first two weeks of August. The second peak (8/8/96) for plant biomass occurred one week after *Cladophora*'s greatest abundance. This second peak, 320.8 g dry weight/L, in plant biomass was considerably less than the first peak of greatest abundance and was followed by a decrease in biomass. The most significant drop in plant biomass occurred at this time, the end of September, when it reached the lowest amount of biomass for the entire growing season, 56.5 g dry weight/L. During the observed time period
plant biomass did not reach zero abundance, as did *Cladophora* biomass.

**Air and water temperature**

Air temperature varied throughout the sampling period. Temperatures in June and early July are fairly steady around 20°C. In mid-July, temperature exhibited more extreme changes (table 1). In late July, temperature remained stable and in August the temperature increased. This rise was followed by a sharp and sudden decrease in air temperature as fall began.

Water temperature remained fairly stable throughout the entire growing season. Small variations, between 1-2°C, usually occurred, and over a period of almost two months, the greatest temperature variation was only 3.6°C. This two-month period did show a gradual warming of water temperature. Water temperature dropped rapidly from its high, 26.4°C on 8/16/96, to the low, 16.2°C on 9/29/96.

**Dissolved oxygen and pH**

Dissolved oxygen (DO) levels measured in late June and early July were quite high, 15 to 16 ppm (table 1). These levels decreased significantly from 15.39 to 9.7 ppm in mid-July and fluctuated slightly, 8.5 to 10.5 ppm, throughout the end of July. DO levels never returned to the initial high. In contrast, DO levels reached the lowest level, 4.0 ppm, at the end of July, and increased to 11 ppm in early August (8/1/96). In August, DO levels again fluctuated, yet remained between 8 and 10 ppm until late September when levels dropped to 6.67 ppm.

The pH level of Lower Stumpf Lake remained fairly consistent over the entire summer, ranging from approximately 8.2 to 9.1. Slight increases occurred throughout mid to late July
(7/12/96 to 7/24/96) and in mid August (8/8/96 to 8/16/96). The lower pH levels occurred during late June into early July, and again in late July and September.

**Nitrate and phosphate**

External nitrate concentrations within Lower Stumpf Lake water samples showed great variation throughout the sampling period (figure 3). The maximum external nitrate concentration (28.7 µg NO₃/ml) occurred on 7/12/96. The minimum external nitrate concentration (3.41 µg NO₃/ml) was detected on 8/1/96. Winter nitrate level was measured as 15.9 µg NO₃/ml. Statistical analysis was conducted to determine if changes in external nitrate levels corresponded to changes in *Cladophora* biomass (table 2). A p value of 0.008 was calculated, suggesting strong correlation between the two data sets.

Internal nitrate concentration within *Cladophora* filaments, collected in conjunction with water samples, also greatly varied over the sampling period (figure 4). The internal nitrate high (1.40 µg NO₃/ml) occurred on 7/17/96, approximately one week following the external nitrate peak. The lowest concentration of internal nitrate (0.293 µg NO₃/ml) occurred early in the sampling period (7/9/96). To determine if internal nitrate levels corresponded to *Cladophora* biomass (table 2) or external nitrate levels (table 3), statistical analysis was conducted. A p value of 0.174 was calculated for algal biomass and internal nitrate and a p value of 0.331 was calculated for internal nitrate versus external nitrate. Both values indicated little or no significance between data sets.

External phosphate concentrations detected in water samples from Lower Stumpf Lake also varied over the sampling period (figure 5). Minimum external phosphate concentration
occurred on 7/24/96 and was detected as 0.050 µg PO₄/ml. The maximum external phosphate concentration (1.89 µg PO₄/ml) occurred on 8/1/96. This external phosphate maximum occurred in conjunction with maximum *Cladophora* biomass. Winter phosphate level was measured as 0.137 µg PO₄/ml. Statistical analysis was conducted to determine if there was a correlation between external phosphorus and algal biomass (table 2). A p value of 0.066 was calculated, suggesting the changes within data sets corresponded.

Internal phosphate content within *Cladophora* filaments collected from Lower Stumpf Lake, also varied over the sampling period (figure 6). The minimum internal phosphate concentration (0.039 µg PO₄/ml) was detected on 7/12/96 with the maximum (0.155 µg PO₄/ml) being detected on 7/8/96. Internal phosphate content increased slightly toward the end of the sampling period. Statistical indicated that internal phosphate levels did not greatly correspond to either *Cladophora* biomass, p= 0.114 (table 2), or external phosphate levels, p= 0.682 (table 3).
Field Discussion

Biomass

The presence of *Cladophora* in June is quite typical for this alga. *Cladophora* is well established by late spring and often first appears following ice-off and before vascular plant abundance in early spring (Engel 1985). The changes in algal biomass observed throughout Lower Stumpf Lake were similar to the traditional seasonal growth pattern reported in the literature. *Cladophora* follows a bimodal seasonal distribution pattern, having maximum abundance in spring and autumn with a period of summer decline (Lorenz and Herdendorf 1982). A similar distribution pattern was observed in Lower Stumpf, as indicated by the presence of the two peaks. However, the time period during which these peaks were observed was quite different from what literature suggested. The first peak did not occur in early spring but rather in early-July and the second peak in late-July and early-August, not autumn. The summer decline was observed in mid-July. This difference in timing could be partially due to the unusually late spring experienced in 1996. Comparison of plant and algal biomass did not have strong correlation, p=0.116. This suggests that plant and algal growth did not influence the other significantly. Therefore, environmental parameters promoting growth were present in abundance to support both plant and algal growth. The changes within *Cladophora*’s seasonal distribution and abundance could be attributed to several other factors.

In a natural aquatic system many environmental factors can influence algal growth. However, in this study only temperature, pH, dissolved oxygen, nitrate and phosphate were analyzed. Changes in air temperature have little influence on submerged algal growth (p=0.186). Air temperature does, however, impact water temperature. Water temperature changes, in turn,
may play a role in supporting, or limiting *Cladophora* growth. *Cladophora*’s optimal temperature range in the field is reported to be 19-24°C (Lembi *et al.* 1988). Lower Stumpf Lake water temperature remained near the upper limit of this optimal temperature range for most of the season. However, in late August water temperature rose to 26°C then fell sharply. This increase and sudden decrease in temperature was followed by a decline in biomass which eventually reached zero suggesting that water temperature may have a slight impact on *Cladophora* growth. However, statistical analysis indicated that water temperature (p=0.118) did not have as direct of an influence on algal growth as other environmental parameters tested in Lower Stumpf Lake. Water temperature may have also been important in the detachment of *Cladophora* (Millner *et al.* 1982), although no specific temperature range was given indicating when this phenomenon may occur.

Initial DO levels were high, which is indicative of better algal and plant growth. The increase in DO suggest increased levels of photosynthesis and thus presumably growth had already occurred. As biomass decreased in mid summer, DO levels also decreased and in August DO levels again peaked along with *Cladophora* and plant abundance. DO levels dropped at the end of the growing season as did all biomass. These fluctuations in DO are likely the result of changes in biomass and the photosynthetic activity of the alga and plants present. Therefore DO cannot be used to predict how much growth will occur. Rather, levels are used as an indicator of the oxygen production (photosynthetic activity) and health of algal and plant material over a given period of time. Dissolved oxygen was not a statistically significant (p=0.608) environmental parameter influencing growth.

The slight increase in pH seemed to correspond more closely to decreases in *Cladophora*
biomass. The first increase in pH occurred on 7/12/96 and was followed by the mid-summer decline in algal biomass (7/17/96 to 7/30/96). During this decline, pH remained higher and on 7/30/96 the pH dropped slightly (9.0 to 8.2) which was followed by the greatest abundance of C. Cladophora 8/1/96. After this peak abundance, pH again increased to approximately nine as biomass decreased. At the end of the season pH decreased, and algal biomass reached zero. Data seemed to indicate that C. Cladophora grew best when pH levels were less than nine. In fact (Lembi et al. 1988) found that high pH, temperature and NH₄⁺ concentration may decrease C. Cladophora growth. However, no evidence was found to indicate what levels constitute high pH. Even though pH may have a slight influence on algal growth in general, statistical analysis indicated that a strong correlation (p=0.189) did not exist between pH and C. Cladophora growth in Lower Stumpf Lake.

Statistical analysis conducted indicated that nutrients, nitrate and phosphate, had the greatest correspondence to changes in C. Cladophora biomass. These two environmental parameters exhibited the lowest p values, 0.008 and 0.066, respectively. This correlation meant that algal abundance corresponded to changes in nutrient levels found in Lower Stumpf Lake. Therefore, of the environmental parameters tested, nitrate and phosphate are the best candidates for influencing C. Cladophora growth most within the lake.

Two goals of this study have been answered. First, field data indicated that C. Cladophora had a summer distribution pattern within Lower Stumpf Lake. However, this distribution varied slightly from that which is generally reported in the literature. Secondly, of the environmental parameters tested and compared to algal abundance, nitrate and phosphate levels in the lake had the greatest influence on C. Cladophora growth.
*Cladophora* is common in eutrophic, or nutrient rich waters (Horne and Goldman 1994). Nitrate and phosphate levels of Lower Stumpf Lake, ranging from 3.41 to 28.7 μg NO₃⁻/ml and 0.05 to 1.89 μg PO₄³⁻/ml classified Lower Stumpf Lake as eutrophic to hypereutrophic as reported by Wetzel (1983). Lower Stumpf Lake nitrate levels measured were higher than most levels reported in the Great Lakes. The low phosphate levels measured in Lower Stumpf Lake were well within the ranges reported in the Great Lakes. However, the high levels were much higher. This suggests that Lower Stumpf Lake has an abundance of nutrients needed for algal growth. An understanding of how these nutrients directly influence productivity would help to determine what type of modification, if any, could be made to limit this algal productivity. In order to determine what influence nutrients had and what levels were needed for growth, controlled experiments were conducted in the laboratory.
Experimental Results

Nitrate experiment

Indoor nitrate (NO₃) experiments were conducted in triplicate and allowed to incubate for 28-37 days. Experimental cultures were grown in modified CLII media at five different nitrate concentrations, 0 µM, 50 µM, 100 µM, 500 µM, and 1200 µM NO₃. Average starting dry weights of inocula Cladophora were 9.05, 11.7, and 14.4 mg for each trial, respectively. Final Cladophora dry weights ranged from 21.0 mg to 83.0 mg. The mean growth rate of the three experimental trials at various nitrate concentrations was determined (figure 7). Mean growth rates ranged from 0.062 doublings/day to 0.067 doublings/day. The indoor nitrate experiments indicated an increase in Cladophora growth rates at the higher nitrate concentrations, 0.065 doublings/day and 0.067 doublings/day at 100 µM and 500 µM NO₃, respectively. The growth rate steadily increased from 0.062 doublings/day at 0 µM NO₃ to 0.067 doublings/day at 500 µM NO₃. A slight decrease in growth rate, 0.067 to 0.065 doublings/day, occurred when nitrate concentrations changed from 500 µM NO₃ to 1200 µM NO₃. Overall, growth rate appeared to follow the trend, the greater concentration of nitrate present in the growth media, the faster Cladophora grew. Statistical analysis was conducted to determine if growth rate was in fact impacted by the available nitrate concentration (figure 8). A p value of 0.405 indicates a weak correlation between growth and external nitrate levels.

Internal nitrate concentrations were also determined for Cladophora grown at the differing external nitrate concentrations. Internal concentrations for each trial, were determined using the same standard nitrate calibration curve prepared for Lower Stumpf Lake external nitrate levels. The mean internal nitrate concentration was determined and compared to the external
nitrate concentration (figure 9). Internal concentrations ranged from 1.08 to 1.62 μg NO₃⁻/ml per mg Cladophora. Statistical analysis was conducted to determine if external nitrate increases corresponded with increases in internal nitrate concentration. A p value of 0.055 was calculated thereby indicating significant correlation between internal and external nitrate levels. The greatest changes in internal nitrate levels were detected between 0 μM to 50 μM and 500 μM to 1200 μM NO₃⁻. Very little change in internal nitrate concentrations occurred between external concentrations of 50 μM to 500 μM NO₃⁻. Internal nitrate concentrations were 1.08, 1.32, 1.34, 1.35, and 1.62 μg NO₃⁻/ml per mg Cladophora for cultures grown at 0 μM, 50 μM, 100 μM, 500 μM and 1200 μM NO₃⁻, respectively. Internal nitrate levels were also compared statistically to Cladophora growth (figure 10). A p value of 0.145 was calculated suggesting that Cladophora growth corresponded more closely to internal nitrate levels than it did to external levels.

**Phosphorus experiment**

Indoor phosphate (PO₄) experiments were conducted in triplicate and allowed to incubate for 28-37 days. Average starting dry weights of inocula Cladophora were 8.1 mg, 10.9 mg, and 13.8 mg for trials one, two, and three, respectively. Final Cladophora dry weights ranged from 27.6 mg to 92.5 mg dry weight. These experimental cultures were grown in modified CLII medium, at five different phosphate concentrations, 0 μM, 5 μM, 10 μM, 50 μM, and 90 μM PO₄. Growth rate of cultures was calculated according to the Guillard (1973) growth equation.

The mean growth rate of Cladophora in the three experimental trials at the various phosphate concentrations was calculated (figure 11), ranging from 0.058 doublings/day to 0.069 doublings/day. Indoor phosphate experiments demonstrated an increase in Cladophora growth
rate at the highest phosphate concentrations, 0.068 and 0.069 doublings/day at 50 μM and 90 μM PO₄. The growth at 10 μM PO₄ was slightly lower than the rate at 5 μM PO₄, 0.060 doublings/day compared to 0.063 doublings/day. A growth rate of 0.058 doublings/day at 0 μM PO₄ occurred and gradually increased to 0.069 doublings/day at 90 μM PO₄ concentration. Statistical analysis was conducted to determine possible correlation between growth rate and external phosphate (figure 12). A p value of 0.044 was calculated, indicating that Cladophora growth responded to increases in phosphate concentration.

Internal phosphate concentrations were determined for Cladophora grown at the various external phosphate concentrations. The mean internal phosphate concentration for the three trials was calculated. Mean internal phosphate concentrations ranged from 0.166 μg PO₄/ml to 0.362 μg PO₄/ml per mg dry weight Cladophora. The general trend seems to be, as external phosphate concentration increased, internal phosphate concentrations followed, p= 0.024 (figure 13). The greatest change in internal phosphate concentration (0.246 to 0.347 μg PO₄/ml per mg Cladophora) occurred between 10 μM and 50 μM PO₄, which corresponds to the greatest change in external phosphate concentration. Cultures grown at 0 μM, 5 μM, and 90 μM PO₄ also varied, 0.166, 0.205, and 0.362 μg PO₄/ml per mg Cladophora, respectively. Internal phosphate levels were compared statistically to Cladophora growth (figure 14). A p value of 0.021 was calculated suggesting algal growth corresponded closely to internal phosphate levels.

**Microscopic observations**

Cladophora filaments grown in the various external CLII nitrogen concentrations were observed using a compound microscope (table 4). Control cultures (1200 μM NO₃) appeared
green in color with an abundance of oxygen bubbles throughout the algal mat. Filaments were thin, and cells were densely packed with chloroplasts and many (greater than 10) branches. The 500 μM NO$_3$ cultures appeared light green to green in color, with oxygen bubbles present in the algal mats. Under the microscope, filaments appeared green with no brown tint. Branching was moderate (5 to 10) and chloroplasts numbers were comparable to the control. However, new filaments had a slightly decreased number of chloroplasts. Cultures at 100 μM NO$_3$ were also light green and oxygen bubbles were present throughout the mat. Filaments had fewer chloroplasts appearing pale green-brown. A moderate amount of branching (5 to 10) was visible. The 50 μM NO$_3$ cultures were light green in appearance and had oxygen bubbles present. These filaments were brown-green, with moderate branching (5 to 10). Some filaments had cells with densely packed chloroplasts, but several cells had chloroplasts that had shriveled and lost color. At 0 μM NO$_3$, cultures became light green and in contrast to cultures grown at higher nitrate concentrations, no oxygen bubbles were apparent. These filaments appeared more brown-green, were still thin, but few branches (5 or less) were present. The number of chloroplasts was not as dense as in the control culture.

Observations were made for phosphate experimental cultures. Control cultures (90 μM PO$_4$) appeared green with an abundance of oxygen bubbles in the algal mats. Moderate branching (5 to 10) of filaments was observed. Individual filaments were light green with many chloroplasts present. However, chloroplasts in the control phosphate culture were not as numerous as chloroplasts found in cultures grown at various nitrate concentrations. The 50 μM PO$_4$ cultures were dark green, with many oxygen bubbles. Filaments were green showing many new branches (greater than 10). Chloroplasts were numerous. At 10 μM PO$_4$ cultures were dark
green and the mats contained many oxygen bubbles. Individual filaments appeared green and showed a moderate number of new branches (5 to 10). Chloroplast numbers did not compare with the control phosphate culture, but were still present. The 5 μM PO₄ cultures appeared green with many oxygen bubbles present in the algal mats. Filaments observed were green, had few (less than 5) branches, and chloroplasts had begun to break down. Filaments were more difficult to pull apart than with the 0 μM PO₄ cultures, suggesting that slightly more branching may have occurred. The 0 μM PO₄ cultures appeared green with oxygen present in the mats. Each filament was green but chloroplasts appeared somewhat broken down. Filaments had few (less than 5) branches and these new branches contained almost no chloroplasts. Overall, phosphate cultures seemed to show more branching than found in nitrate cultures. The apparent trend showed branching and filament health to increase as the level of phosphate in the culture media increased.
Experimental Discussion

Nitrate experiments

*Cladophora* is typically found in nutrient rich waters. Therefore, high levels of external nitrate and phosphate are expected in lakes where this alga grows. These levels were present within Lower Stumpf Lake, but in culture *Cladophora* grew at much lower levels than expected. Indoor nitrate experiments indicated that as external nitrate concentration increased from 0 μM to 500 μM NO$_3$ a slight increase in algal growth occurred. Correlation between external levels and growth was weak (p=0.405) suggesting that *Cladophora* growth was only slightly influenced by external nitrate availability. The slight decrease in growth rate from 500 μM to 1200 μM NO$_3$ may greatly affect the correlation between external nitrate levels and growth, and can be explained by the fact that too much nitrate can also have a limiting effect on algal growth (Zevenboom and Mur 1978).

Many types of algae, especially blue-greens, exhibit the ability to uptake nitrate. Their uptake depends greatly on the external substrate level (Droop 1973). Therefore the more nitrate present, the more the cell will be able to uptake and use for cell growth. This was apparent, by the slight increase in *Cladophora* growth rate at the higher nitrate levels. However, according to Auer and Canale (1982a) growth of algae is governed by internal not external nutrient levels. Therefore, a greater correlation should be seen between growth and internal nitrate levels.

External nitrate concentration was significantly correlated to the internal content of the cell (p=0.055). This suggests that internal levels were greatly influenced by the amount of nitrate present around the cells. If more nitrate was available, cells were able to uptake and utilize more of this nutrient. For instance, high external nitrate levels created higher internal nitrate levels.
This was true within the experimental cultures and is supported by Droop (1973). Therefore, external nitrate levels did regulate internal nitrate levels.

If external concentrations do not directly govern growth, but instead regulate internal levels, do these internal levels influence growth? Droop (1973) states that growth depends on the internal substrate concentration. In this experiment, \textit{Cladophora} growth rate was compared statistically to internal nitrate levels and a p value of 0.145 was calculated. This suggests that \textit{Cladophora} growth was influenced more by internal nitrate levels than by external levels (p=0.405). Yet, the external nitrate levels should control the internal nitrate concentration. At high internal nitrate concentration growth increased slightly and at low nitrate levels growth was somewhat slowed. This difference in growth rate is supported by primary literature. Previous studies have shown that growth rate of phytoplankton is a direct function of cellular levels of nutrients (Rhee 1978).

Significant growth, 0.062 doublings/day and 0.063 doublings/day, was noticed at low 0 \(\mu\text{M}\) and 50 \(\mu\text{M}\) NO\(_3\) concentrations, respectively. This growth was unexpected. How can it be explained? Growth at these low nitrate levels suggests that \textit{Cladophora} may be able to use internal sources of nitrate for growth. Thus, \textit{Cladophora} must have the ability to store nitrate for later use. Data on internal levels further supports this conclusion because internal levels of nitrate at low external concentration are somewhat depleted in comparison with internal levels at high nitrate concentrations. Primary literature also supports this conclusion. Droop (1973) states that, internal levels depend on the uptake of an external substrate. Thus, the external levels determine the internal nutrient content of the cell, which in turn then determine algal growth. A decrease in internal nutrients corresponded to decreased nutrients in the nutrient medium.
Microscopic observations also indicate that *Cladophora* has the ability to store nutrients. At high nitrate levels, *Cladophora* filaments appeared healthy. Filaments were green, chloroplasts were intact, many branches were found and cultures exhibited high oxygen evolution. In contrast, *Cladophora* filaments at low nitrate concentrations appeared unhealthy. Green coloration was lost, few branches were noticed, chloroplasts were deteriorated, and a low oxygen evolution was seen. When algae uptake nitrate, it is assimilated into cellular components such as nucleic acids, proteins, and chlorophyll. In this way, nitrate is stored within the cell. If external levels of nitrate reach a low level, uptake is decreased leading to decreased assimilation and storage. When external levels are limited for a long period of time, the internal nutrient reserves are utilized. Cellular components will begin to break down to support growth of the algae. This was observed through the filament health of *Cladophora* exposed to the various nitrate levels. During the approximate 30-day incubation period *Cladophora* appeared to use external nutrient sources first, if present, and then utilized internal reserves. This was also observed by the slight decrease in internal nitrate content found at low external concentrations. When external nutrients were not present, these reserves were used immediately. The amount of chlorophyll, along with the rate of photosynthesis, may further decrease if nitrate deficiency continues (Lewin 1962). Nitrate reductase, the enzyme, or carrier responsible for nitrate uptake, is an adaptive system and responds quickly to the changes in the cell’s nutritional status (Bonner and Varner 1965). Algal detachment may also be associated with reduced internal nitrate concentration (Millner et al. 1982).
Phosphate experiments

*Cladophora* is highly sensitive to changes in phosphate (Auer and Canale 1982b). This was evident through statistical analysis comparing nitrate culture experiments to phosphate culture experiments. In general, algal growth was influenced more (smaller p values) by both external and internal phosphate than nitrate levels. In culture an increase in external phosphate levels increased growth. This seemed to suggest that *Cladophora* was able to uptake and utilize the available phosphate. If given a sufficient supply of phosphate, algae will uptake in quantities far in excess of their actual needs and can use this excess to grow when no phosphate is present (Lewin 1962). Experimental data supported this conclusion. Growth rates at the low phosphate concentrations were still significant, indicating that *Cladophora* must have the capability to uptake nutrients and store for this growth. Auer and Canale (1980) state that *Cladophora* does exhibit the ability to store phosphate in excess of growth requirements. Internal phosphate content determined from the cultures suggested that this alga was exhibiting this storage capability. As external phosphate levels increased, more uptake occurred and cell phosphate content was increased. Statistical analysis indicated that this relationship between the experimental external and internal levels was, in fact, significant (p=0.024). Growth also increased as internal phosphate increased (p=0.021). The overall vitality of *Cladophora* varies directly with tissue phosphorus content (Auer and Canale 1980). Microscopic analysis of algal filaments also suggested that internal phosphate content is important to *Cladophora* growth. Filaments at high phosphate concentrations exhibited green coloration, completely intact chloroplasts, many branches and good oxygen evolution. These characteristics were also exhibited in filaments grown at high nitrate concentrations. In contrast, filaments at low
phosphate concentrations exhibited decreased filament health. *Cladophora* lost green coloration, few branches were observed, chloroplasts were deteriorated and oxygen evolution decreased. This suggests that at the low external phosphate concentrations, internal storage was utilized to support growth. The internal phosphate levels determined by nutrient analysis also provided support for this storage ability. Internal levels were slightly decreased at low external levels because internal nutrients were being utilized for growth.
Conclusions

Overall, the results of this study were surprising. *Cladophora* was expected to grow at high nutrient levels. These levels were present in the lake, but in culture *Cladophora* exhibited extreme resilience to low nitrate and phosphate levels. Reasons for this growth may be due to an exceptional nutrient uptake system, in which this alga can absorb nitrate and phosphate even when low concentrations are present. Competition may also greatly influence algal growth. In culture competition is minimized allowing for *Cladophora* to utilize all nutrients present. Thus, low concentrations can sustain growth for a limited time.

Growth rate did correlate with external nutrient concentrations as indicated by the increased growth rate at higher nutrient levels. This was also evidence for a good uptake system. Therefore, *Cladophora* can uptake and use the high nutrient levels for growth. Changes in biomass seemed to correspond more closely with phosphate levels than nitrate levels. According to Auer and Canale (1982a) these changes are directly and immediately proportional to phosphate uptake. However, under nutrient limitation growth is directly related to size of internal pool (Auer and Canale 1982b).

The dependence on the internal nutrient pool was exhibited by *Cladophora*'s ability to store excess nutrients. This ability was observed through differences in filament health and the slight variation in internal nutrient content occurring between filaments grown at low versus high external concentrations. During the 30 days incubation *Cladophora* used external nutrient sources first, if present, and then used internal reserves. When external nutrients were not present, these reserves were used immediately.

Contrary to what is found with many other types of green filamentous algae, such as
Spirogyra, Cladophora exhibited extreme resilience to low nutrient levels due to its uptake and storage ability. This alga is able to survive under a wide variation of nutrient conditions because it is extremely efficient at scavenging essential nutrients and will colonize suitable substrates given the slightest nutritional advantage (Jackson and Hamdy 1982). This information is intriguing, yet discouraging for aquatic systems similar to Lower Stumpf Lake. Growth of this alga will not be controlled by simply limiting nutrients. Alternative management strategies are required to control the “super alga” Cladophora.
Further Research

Upon conclusion of this study, many other avenues can be explored concerning the aquatic system of Lower Stumpf Lake. Future research should involve the continuation of experimental nutrient cultures. Some aspects to consider when continuing these cultures are; extending the time period for culture growth, replenishment of starting nutrient concentration and creation of cultures at more nutrient concentrations, specifically at low levels nitrate and phosphate. Determination of nutrient point sources within the lake would also be critical for a couple of reasons. First, algal growth is often associated with point sources of nutrients and second, by finding sources we may be able to divert these nutrient inputs. It would also be important to determine nutrient requirements needed for growth of other algae and macrophytes that may compete with Cladophora for growth.
Table 1: Environmental parameters tested in Lower Stumpf Lake, June 26 to September 29, 1996.

<table>
<thead>
<tr>
<th>Date</th>
<th>Air Temp (°C)</th>
<th>Water Temp (°C)</th>
<th>Dissolved Oxygen (ppm)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/26/96</td>
<td>-</td>
<td>22.8</td>
<td>16.7</td>
<td>8.7</td>
</tr>
<tr>
<td>7/9/96</td>
<td>19.3</td>
<td>23.8</td>
<td>15.4</td>
<td>8.9</td>
</tr>
<tr>
<td>7/12/96</td>
<td>21.9</td>
<td>24.1</td>
<td>9.70</td>
<td>9.0</td>
</tr>
<tr>
<td>7/17/96</td>
<td>26.6</td>
<td>-</td>
<td>10.5</td>
<td>9.1</td>
</tr>
<tr>
<td>7/20/96</td>
<td>18.2</td>
<td>24.2</td>
<td>8.46</td>
<td>9.0</td>
</tr>
<tr>
<td>7/24/96</td>
<td>21.5</td>
<td>24.5</td>
<td>10.1</td>
<td>9.0</td>
</tr>
<tr>
<td>7/30/96</td>
<td>19.5</td>
<td>23.5</td>
<td>4.00</td>
<td>8.2</td>
</tr>
<tr>
<td>8/1/96</td>
<td>25.5</td>
<td>24.3</td>
<td>11.0</td>
<td>9.0</td>
</tr>
<tr>
<td>8/8/96</td>
<td>21.5</td>
<td>26.0</td>
<td>9.10</td>
<td>9.1</td>
</tr>
<tr>
<td>8/13/96</td>
<td>25.0</td>
<td>26.0</td>
<td>8.33</td>
<td>9.0</td>
</tr>
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<td>8/16/96</td>
<td>23.0</td>
<td>26.4</td>
<td>8.50</td>
<td>9.0</td>
</tr>
<tr>
<td>9/19/96</td>
<td>26.0</td>
<td>19.8</td>
<td>9.83</td>
<td>8.3</td>
</tr>
<tr>
<td>9/29/96</td>
<td>6.67</td>
<td>16.2</td>
<td>6.67</td>
<td>8.2</td>
</tr>
</tbody>
</table>
Table 2: Statistical analysis. A least squares test was conducted comparing Cladophora abundance to various environmental parameters over time within Lower Stumpf Lake. A \( p \) value of less than or equal to 0.05 was considered significant, meaning algal abundance and the parameter showed corresponding or similar changes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>External nitrate</td>
<td>3.41 to 28.7 ( \mu )g/ml</td>
<td>0.008</td>
</tr>
<tr>
<td>Internal nitrate</td>
<td>0.293 to 1.40 ( \mu )g/ml</td>
<td>0.174</td>
</tr>
<tr>
<td>External phosphate</td>
<td>0.050 to 1.89 ( \mu )g/ml</td>
<td>0.066</td>
</tr>
<tr>
<td>Internal phosphate</td>
<td>0.039 to 0.155 ( \mu )g/ml</td>
<td>0.114</td>
</tr>
<tr>
<td>Water temperature</td>
<td>16.2 to 26.4 °C</td>
<td>0.118</td>
</tr>
<tr>
<td>pH</td>
<td>8.17 to 9.13</td>
<td>0.189</td>
</tr>
<tr>
<td>Plant biomass</td>
<td>56.5 to 474 g/L</td>
<td>0.116</td>
</tr>
</tbody>
</table>
Table 3: Statistical analysis. A least squares test was conducted, comparing external and internal nutrient changes over time within Lower Stumpf Lake. A p value of less than or equal to 0.05 was considered significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>0.331</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.682</td>
</tr>
</tbody>
</table>
Table 4: Microscopic Observations of Cladophora filaments grown at experimental nitrate or phosphate concentrations. Color, oxygen evolution and branching was observed.

<table>
<thead>
<tr>
<th>Nutrient concentration</th>
<th>Color</th>
<th>Oxygen Evolution</th>
<th>Branching</th>
</tr>
</thead>
<tbody>
<tr>
<td>1200 µM NO₃</td>
<td>green</td>
<td>yes</td>
<td>greater than 10</td>
</tr>
<tr>
<td>500 µM NO₃</td>
<td>light green</td>
<td>yes</td>
<td>5 to 10</td>
</tr>
<tr>
<td>100 µM NO₃</td>
<td>light green</td>
<td>yes</td>
<td>5 to 10</td>
</tr>
<tr>
<td>50 µM NO₃</td>
<td>light green</td>
<td>yes</td>
<td>5 to 10</td>
</tr>
<tr>
<td>0 µM NO₃</td>
<td>light green</td>
<td>no</td>
<td>less than 5</td>
</tr>
<tr>
<td>90 µM PO₄</td>
<td>green</td>
<td>yes</td>
<td>5 to 10</td>
</tr>
<tr>
<td>50 µM PO₄</td>
<td>dark green</td>
<td>yes</td>
<td>greater than 10</td>
</tr>
<tr>
<td>10 µM PO₄</td>
<td>dark green</td>
<td>yes</td>
<td>5 to 10</td>
</tr>
<tr>
<td>5 µM PO₄</td>
<td>green</td>
<td>yes</td>
<td>less than 5</td>
</tr>
<tr>
<td>0 µM PO₄</td>
<td>green</td>
<td>yes</td>
<td>less than 5</td>
</tr>
</tbody>
</table>
Figure 1: Biomass changes for *Cladophora* in Lower Stumpf Lake, June 26 to October 11, 1996.
Figure 2: Biomass changes for algae and vascular plants in Lower Stumpf Lake, June 26 to October 11, 1996.
Figure 3: Average external nitrogen concentration within Lower Stumpf Lake water samples, June 26 to October 11, 1996.
Figure 4: Internal nitrate concentration of *Cladophora* samples taken from Lower Stumpf Lake, June 26 to October 11, 1996
Figure 5: Average external phosphorus concentration within Lower Stumpf Lake water samples, June 26 to October 11, 1996.
Figure 6: Internal phosphate concentration of *Cladophora* samples taken from Lower Stumpf Lake, June 26 to October 11, 1996
Figure 7: *Cladophora* growth in experimental cultures at various external nitrate concentrations. A p value of 0.405 was calculated through statistical analysis, indicating external nitrate had a small influence on growth.
Figure 8: Statistical analysis (best fit line) of algal growth in experimental cultures at various external nitrate concentrations. Least ordinary squares test gave a p value of 0.405, indicating external nitrate had a small influence on growth.
Figure 9: Internal nitrate content of *Cladophora* grown in experimental cultures of various external nitrate levels. Upon statistical analysis a p value of 0.055 was calculated thereby indicating significant correlation between internal and external nitrate levels.
Figure 10: *Cladophora* growth and internal nitrate content at the experimental nitrate concentrations (represented by circles). Statistical analysis indicated that algal growth corresponded more closely with internal nitrate levels ($p=0.145$) than external nitrate levels ($p=0.405$).
Figure 11: *Cladophora* growth in experimental cultures at various external phosphate concentrations. A p value of 0.044 was calculated through statistical analysis indicating growth is influenced by increases in phosphate concentration.
Figure 12: Statistical analysis of algal growth in experimental cultures at various external phosphate concentrations. Least squares test gave a p value of 0.044, indicating growth is influenced by increases in phosphate concentration.
Figure 13: Internal phosphate content of *Cladophora* grown in experimental cultures of various external phosphate levels. Upon statistical analysis a p value of 0.024 was calculated indicating significant correlation between internal and external phosphate levels.
Figure 14: *Cladophora* growth and internal phosphate content at the experimental phosphate concentrations (represented by circles). Statistical analysis indicated that algal growth corresponded more closely with internal phosphate levels \((p=0.021)\) than external phosphate levels \((p=0.044)\).
Map: Lower Stumpf Lake

Size = 71 acres
87% area is < 4.6 m depth
Maximum depth = 11 meters

- Red = Cladophora
- Black = sampling site
Appendix

I. Persulfate Oxidation Digestion Procedure.

(Based on Raveh and Avnimelech, 1979, Total Nitrogen Analysis in Water, Soil and Plant Material with Persulphate Oxidation.)

Two grams of potassium persulfate ($K_2S_2O_4$) were added to a 50 ml water sample in a 100 ml Erlenmeyer flask. Similarly, 1.5 to 5 g $K_2S_2O_4$ and 50 ml water were added to 100 mg soil or plant samples. (The quantity of potassium persulfate depends on the organic matter contents of the sample and should be at least 50 times higher than the quantity of organic matter in the sample.) The flasks were covered with aluminum foil and autoclaved for 1 hour (water samples) or 2 hours (soil or plants) at $1.5 \times 10^5$ Pa (1.5 atm).

The nitrate analysis was changed to fit the needs of this project.
II. Cadmium reduction column


Reagents

a. Copper-cadmium granules: Washed 25 g new or used 40- to 60- mesh cadmium granules with 6 M HCl and rinsed with deionized water. Swirled cadmium with 100 ml 2 % CuSO₄ solution for 5 minutes or until blue color partially faded. Decanted and repeated with fresh CuSO₄ until a brown colloidal precipitate developed. Gently flushed with water to remove all precipitated copper.

b. Color reagent: To 800 ml deionized water added 100 ml 85 % phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, added 1 g N-(1-naphtyl)-ethylenediaminedihydrochloride. Mixed to dissolve, then diluted to 1 L with water. Solution was stable for 1 month in dark bottle. Refrigerated.

c. Ammonium chloride-EDTA solution: Dissolved 13 g NH₄Cl and 1.7 g disodium ethylenediaminetetraacetate in 900 ml water. Adjusted pH to 8.5 with concentrated ammonium hydroxide and diluted to 1 L.

d. Dilute ammonium chloride-EDTA solution: Diluted 300 ml NH₄Cl-EDTA solution to 500 ml with water.

e. Copper sulfate solution, 2 %: Dissolved 20 g CuSO₄·5H₂O in 500 ml water and diluted to 1 L.

f. Stock nitrate solution: Dried potassium nitrate in an oven at 105°C for 24 hours. Dissolved 0.7218 g in water and diluted to 1000 ml. (1.00 ml = 100 μg NO₃-N) Preserved with 2 ml chloroform. Solution stable for 6 months.

g. Intermediate nitrate solution: Diluted 100 ml stock nitrate solution to 1000 ml with water. (1.00 ml = 10.0 μg NO₃-N) Preserved with 2 ml chloroform. Solution stable for 6 months.
Preparation of reduction column.

Inserted a small plastic tube into bottom of reduction column and filled with water. Added sufficient Cu-Cd granules to produce a column 18.5 cm long. Maintained water level above Cu-Cd granules to prevent entrapment of air. Washed column with 200 ml dilute NH₄Cl-EDTA solution. Activated column by passing through it, at 7 to 10 ml/minute, at least 100 ml of a solution composed of 25 % 1.0 mg μg NO₃-N/ L standard and 75 % NH₄Cl-EDTA solution.

Sample reduction.

To 25.0 ml sample, added 75 ml NH₄Cl-EDTA solution and mixed. Poured sample into column and collected at a rate of 7 to 10 ml/minute. Discarded the first 25 ml. Collected the rest in original sample flask. There was no need to wash columns between samples, but if columns were not to be reused for several hours or longer, poured 50 ml dilute NH₄Cl-EDTA solution onto the top and let it pass through the system. Stored Cu-Cd column in this solution and never let it dry.

Color development and measurement.

As soon as possible, and not more than 15 minutes after reduction, added 2.0 ml color reagent to 50 ml sample and mixed. Between 10 minutes and 2 hours afterward, measured absorbance at 543 nm against a distilled water-reagent blank.
III. Nitrate Standard Calibration Curve

\[ y_i = mx_i + b \]

\( m := \text{slope}(x, y) \quad m = 0.05324 \)
\( b := \text{intercept}(x, y) \quad b = 0.00508 \)
\( c := \text{corr}(x, y) \quad c = 0.96035 \)
IV. Total phosphate


Reagents.

a. Sulfuric-Nitric Acid Solution: Added slowly 300 ml concentrated sulfuric acid to approximately 600 ml distilled water. Cooled. Added 4.0 ml concentrated nitric acid and diluted to 1 L.

b. Ammonium Molybdate Reagent: Dissolved 25 g ammonium molybdate, \( \text{(NH}_4\text{)}_6\text{Mo}_7\text{O}_{24} \ \text{V}_2\text{H}_2\text{O} \), in about 200 ml distilled water. Added slowly with care 280 ml concentrated sulfuric acid to 400 ml distilled water and cooled to room temperature. Added the molybdate solution to the sulfuric acid solution, mixed and diluted to 1 L.

c. Tin(II) Chloride Solution: Dissolved 1.25 g tin(II) chloride, \( \text{Sn}_2\ \text{V}_2\text{H}_2\text{O} \), in 100 ml glycerol. Heated in a water bath and stirred until dissolution was complete.

d. Standard Phosphate Solution: Dissolved 0.7165 g potassium dihydrogen phosphate, \( \text{KH}_2\text{PO}_4 \), in distilled water and diluted to 1 L. Transferred exactly 100.0 ml of this standard stock solution to a 1-liter volumetric flask and diluted to mark with distilled water. Each ml of this solution contained 50.0 \( \mu \)g PO\(_4\).

Procedure.

Transferred a 100 ml aliquot of water sample to a 250 ml conical flask and added one drop of a 0.5 % phenolphthalein indicator. If indicator gave color, added the sulfuric-nitric acid mixture until the color was discharged. Added 1 ml of this acidic solution. Boiled for approximately 2 hours, adding distilled water to maintain a volume of about 50 ml. After cooling the solution, neutralized with dilute sodium hydroxide. Transferred to a 100 ml volumetric flask and diluted to mark with distilled water.

Transferred 50.0 ml of this sample solution to a dry 125 ml conical flask. Added 2.0 ml of the molybdate reagent and mixed thoroughly. Added 0.5 ml of the tin(II) chloride solution and mixed thoroughly. After exactly 10 minutes, measured the
absorbance at 650 nm using 1.000 cm cells. Compared with calibration curve prepared in an analogous manner.
IV. Phosphate Standard Calibration Curve

![Graph showing the relationship between Phosphate concentration (g/ml) and Absorbance (650 nm).](image)

\[ y = mx + b \]

- \( m := \text{slope}(x, y) \) \quad m = 0.152
- \( b := \text{intercept}(x, y) \) \quad b = 0.058
- \( c := \text{corr}(x, y) \) \quad c = 0.994
VI. PREPARATION OF CLII MEDIUM

Modifications by
C.A. Lembi & H. Adrian

(Based on Gerloff & Fitzgerald, 1976, The Nutrition of Great Lakes Cladophora, EPA-600/3-76-044. Modification up-to-date, 6/88)

I. Prepared 500 ml stock solutions as follows. Used deionized water. Stored stocks in refrigerator.

<table>
<thead>
<tr>
<th>Salt</th>
<th>g/ 500 ml stock</th>
<th>ml/ 1.5 L medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Disodium EDTA</td>
<td>0.0500</td>
<td>15</td>
</tr>
<tr>
<td>2. Na₂SiO₃ - 9H₂O</td>
<td>2.8805</td>
<td>15</td>
</tr>
<tr>
<td>3. Na₂CO₃*</td>
<td>1.0000</td>
<td>15</td>
</tr>
<tr>
<td>4. Fe citrate + Citric acid</td>
<td>0.3000 each</td>
<td>15</td>
</tr>
<tr>
<td>5. Ca(NO₃)₂ - 4H₂O</td>
<td>6.9100</td>
<td>15</td>
</tr>
<tr>
<td>6. KCl</td>
<td>1.7200</td>
<td>15</td>
</tr>
<tr>
<td>7. Na₂HPO₄</td>
<td>0.6400</td>
<td>15</td>
</tr>
<tr>
<td>8. MgSO₄ - 7H₂O</td>
<td>3.7500</td>
<td>15</td>
</tr>
<tr>
<td>9. Trace elements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>1.8640</td>
<td>0.3</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.7730</td>
<td>0.3</td>
</tr>
<tr>
<td>MnSO₄ - H₂O</td>
<td>0.4225</td>
<td>0.3</td>
</tr>
<tr>
<td>ZnSO₄ - 7H₂O</td>
<td>0.2875</td>
<td>0.3</td>
</tr>
<tr>
<td>CuSO₄ - 5H₂O</td>
<td>0.0625</td>
<td>0.3</td>
</tr>
<tr>
<td>(NH₄)₂Mo₇O₂₄·4H₂O</td>
<td>0.0092</td>
<td>0.3</td>
</tr>
</tbody>
</table>

II. Prepared vitamins. The modifications of Gerloff & Fitzgerald’s methods are based on Stein, Phycological Methods, pp 39-40. The concentrations used were the same.

B₁
- made 1X solution when needed:
  0.0400 g Thiamine-HCl
  100 ml deionized water
- acidified to pH 4.5-5.0 (if necessary)
- dispensed in screw-cap tubes in dosages needed for addition to autoclaved medium; 10 ml per 1L CLII, 15 ml per 1.5L CLII
- stored unused tubes frozen
- thawed and added aseptically to autoclaved medium as was necessary
B₁₂  
- made 10X stock solution when needed:
  0.0010 g Cyanocobalamin
  1 L deionized water
- diluted stock to 1X solution using 3 g/ 100 ml water (10 ml stock to 100 ml)
- acidified to pH 4.5-5.0 with 1 M HCl
- dispensed in screw-cap tubes (dosages 5 ml per 1L CLII, 7.5 ml per 1.5L CLII)
- autoclaved 15-20 min at 121°C
- stored unused tubes frozen
- thawed and added aseptically to autoclaved medium as was necessary

III. Once all stocks, inorganic and vitamin, had been prepared:
- added the correct amounts of inorganic stock solutions, which had been set out to warm to room temperature, to flask partially filled with deionized water. It was helpful to have a set of pipets labeled for solutions 1-9, exclusively. Brought to volume (1.5 L), mixed well and autoclaved 15-29 min at 121°C
- when medium had cooled, thawed and added vitamins B₁, B₁₂ and modified NaHCO₃ if used

* Modification Bicarbonate source and pH.

Omitted stock solution #3 (Na₂CO₃).
After autoclaving medium, added (in addition to the vitamins) a sterile NaHCO₃ solution:

NaHCO₃ was added at the rate of 0.3 g/L CLII.
We made up a solution of 3 g/ 100 ml deionized water, filter-sterilized it (0.45 µM millipore filter), and added 15 ml/ 1.5 L CLII.
We found that the final pH of the freshly prepare CLII is 8.1 to 8.4.
According to Lembi, using this modification improves the buffering capacity of the medium and reduces precipitation during autoclaving.
VII. Modified CLII for Nutrient Experiments.

*Nitrate Experiment*

Replaced original stock solution #5 with two different stock solutions. The first replaced the calcium concentration in standard CLII, the second allowed the nitrate concentration to be varied.

<table>
<thead>
<tr>
<th>New stock</th>
<th>g/ 250 ml stock</th>
<th>ml/ 1.5 L medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.088 M CaCl₂</td>
<td>2.4424</td>
<td>10</td>
</tr>
<tr>
<td>0.030 M NaNO₃</td>
<td>0.6466</td>
<td>-</td>
</tr>
</tbody>
</table>

The nitrate concentration was modified by adding the following quantities of 0.030 M NaNO₃ to 350 ml prepared CLII media, which contained the CaCl₂ replacement, and normal amounts of the other standard solutions, vitamins and sodium bicarbonate (appendix VI).

<table>
<thead>
<tr>
<th>Modified nitrate concentration (approx.)</th>
<th>0.030 M NaNO₃ (ml added)</th>
<th>Water (ml added)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>5.8</td>
</tr>
<tr>
<td>50</td>
<td>0.6</td>
<td>5.2</td>
</tr>
<tr>
<td>100</td>
<td>1.2</td>
<td>4.5</td>
</tr>
<tr>
<td>500</td>
<td>5.8</td>
<td>0</td>
</tr>
</tbody>
</table>

*Phosphate Experiment*

Replaced original stock solution #7 with a different concentration Na₂HPO₄ stock solution.
New stock | g/250 ml stock | ml/1.5 L medium
--- | --- | ---
0.0031 M Na$_2$HPO$_4$ | 0.1110 | -

The phosphate concentration was modified by adding the following quantities of 0.0031 M Na$_2$HPO$_4$ to 350 ml prepared CLII media, which contained the normal amounts of the other standard solutions, vitamins and sodium bicarbonate (appendix VI). An extra 10 ml water was added to this media to have the same volume (approx. 1.5 L) as standard CLII.

<table>
<thead>
<tr>
<th>Modified phosphate concentration (approx.)</th>
<th>0.0031 M Na$_2$HPO$_4$ (ml added)</th>
<th>Water (ml added)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>5.8</td>
</tr>
<tr>
<td>5</td>
<td>0.6</td>
<td>5.2</td>
</tr>
<tr>
<td>10</td>
<td>1.2</td>
<td>4.5</td>
</tr>
<tr>
<td>50</td>
<td>5.8</td>
<td>0</td>
</tr>
</tbody>
</table>
References


Bapt, Abb. S. Joannis. 1934. Natural History of Collegeville MN. Archivum, St. John’s Abbey Archives. Collegeville MN.


