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Fate and Effects of Nitrogen and Phosphorus in Shallow Vegetated Aquatic Ecosystems

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Fate and Effects of Nitrogen and Phosphorus in Shallow Vegetated Aquatic Ecosystems

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Notice

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Foreword

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This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

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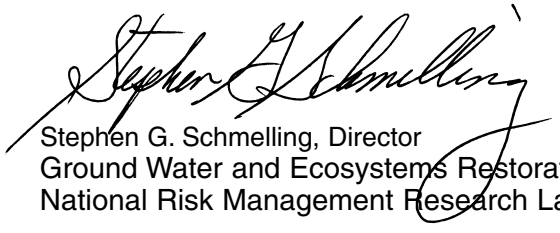

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Executive Summary

Nitrate concentrations have greatly increased in streams and rivers draining agricultural regions of the Midwestern United States. Increasing nitrate transport to the Gulf of Mexico has been implicated in the hypoxic conditions that threaten the productivity of marine fisheries. Increases in nitrate concentrations have been attributed to a combination of factors including agricultural expansion, increased nitrogen application rates, increased tile drainage, and loss of riparian wetlands. These landscape-level changes have resulted in a decreased natural capacity for nitrogen uptake, removal, and cycling back to the atmosphere. Land managers are increasingly interested in using wetland construction and rehabilitation as a management practice to reduce loss of nitrate from the terrestrial systems. Yet, relatively little is known about the limnological factors involved in nitrate removal by wetland systems.

We conducted a series of studies from 1999-2000 to investigate the functional capacity of shallow, macrophyte-dominated pond wetland systems for uptake, assimilation, and retention of nitrogen (N) and phosphorus (P). We evaluated four factors that were hypothesized to influence nutrient uptake and assimilation: 1) nitrate loading rates; 2) nitrogen to phosphorus (N:P) ratios; 3) frequency of dosing/application; and 4) timing of dose initiation.

Nutrient assimilation was rapid; more than 90% of added nutrients were removed from the water column in all treatments. Neither variation in N:P ratios (evaluated range: <13:1 to >114:1), frequency of application (weekly or bi-weekly), nor timing of dose initiation relative to macrophyte development (0%, 15–25%, or 75–90% maximum biomass) had significant effects on nutrient assimilation or wetland community dynamics. Maximum loading of nitrate (60 g N/m^2; 2.4 g P/m^2) applied as six weekly doses stimulated algal communities, but inhibited macrophyte communities.

Predicted shifts from a stable state of macrophyte- to phytoplankton-dominance did not occur due to nutrient additions. Macrophytes, phytoplankton, and the sediment surface were all significant factors in the removal of nitrate from the water column. Overall, these shallow, macrophyte-dominated systems provided an efficient means of removing nutrients from the water column. Construction or rehabilitation of shallow, vegetated wetlands may offer promise as land management practices for nutrient removal in agricultural watersheds.

Introduction

Anthropogenic eutrophication of water bodies has been a major aquatic research and management focus since the 1950's (Jansson et al. 1994; Smith, 2003). Point sources of nutrients, such as effluents from municipal and industrial facilities, have largely been identified and controlled via mechanical and engineering approaches to water pollution prevention. However, non-point sources of nutrients to water bodies have continued to rise due to expanded agricultural activities, increased application of fertilizers, fossil-fuel combustion, over-application of manure to crops, and runoff from urban areas (Vitousek et al. 1997; Carpenter et al. 1998). These non-point sources of nutrients are continuing to rise due to difficulties in source identification, lack of effective nutrient management strategies, and the lack of regulatory focus (Carpenter et al. 1998).

Shallow aquatic systems such as wetlands and ponds can act as sinks for nutrients, thereby significantly decreasing watershed export (Vitousek et al. 1997). Jansson et al. (1994) suggest that shallow ponds can provide the best means of nitrogen retention through sedimentation, uptake by vegetation, and denitrification. Assimilative processes may be facilitated in shallow environments because of the high surface areas of sediments and aquatic plants compared to pelagic systems (Gasith and Hoyer 1998). Phosphorus may likewise be assimilated into vegetation or retained in sediments during periods of high nutrient loading. However, under senescent or anoxic conditions, sediments may act as a nutrient source and result in release of nutrients to the water column (Scheffer 1998).

The establishment of macrophyte stands in shallow systems can increase nutrient retention and recycling (de Haan et al. 1993). During the growing season, macrophytes act as a sink by accumulating nutrients in developing tissues (Engel 1990). Weisner et al. (1994) demonstrated that removal of nitrate from the water column was significantly higher in vegetated than non-vegetated mesocosms due to uptake and denitrification. Macrophytes stimulate denitrification by lowering the redox potential in microzones at the sediment surface and releasing dissolved organic carbon. Therefore, shallow ponds utilized to reduce nutrients in surface waters may be most effective if macrophyte communities develop and persist (Jansson et al. 1994).

One factor that may diminish the establishment and persistence of macrophyte stands is a dense community of phytoplankton that may develop with nutrient enrichment. Lake and reservoir investigations have generally found an inverse relationship between macrophyte and phytoplankton communities where two alternative conditions may exist: 1) a macrophyte-dominated system containing clear water and low phytoplankton biomass, or 2) high phytoplankton biomass, with turbid water and poor macrophyte development. These "alternative stable states" of macrophyte or phytoplankton dominance are relatively persistent and do not readily alternate unless conditions are disrupted by external or internal forces (Scheffer 1990, 1998).

Nutrient ratio is a primary determinant of primary production in aquatic systems (Sakamoto 1966, Wetzel 1983). Optimum ratios of nitrogen:phosphorus (N:P ratio) are approximately 13 (mass:mass basis) in aquatic systems; ratios that are under 10 are generally considered nitrogen-limited, and ratios above 17 are generally considered phosphorus-limited (Redfield et al. 1963; Sakamoto 1966). Thus, aquatic systems that receive nutrient inputs near the optimum level will achieve a maximum level of primary productivity with efficient utilization of nutrients and minimal dissolved nutrient accumulation in the water column. Nutrient ratios that are limiting in one nutrient frequently exhibit elevated dissolved forms of the nutrient in excess. Thus, nutrient uptake and retention is maximal when the N:P ratio is near the 13:1 optimum. Nutrients other than nitrogen or phosphorus can be limiting (e.g., silica, carbon dioxide) in some systems, but most are typically limited by phosphorus or nitrogen. Other factors can cause departures from expectations based on ratios of dissolved nutrients. For example, internal sources of nutrients from sediments are an especially critical component in shallow aquatic systems such as wetlands (Sand-Jensen and Borum 1991; Scheffer 1998). In addition, intense grazing of phytoplankton by zooplankton (such as in the absence of fish predators) can increase turnover rates of phosphorus and sustain productivity under conditions of low P supply and low algal biomass (Wetzel 1983).

Another primary factor influencing whether a system is macrophyte or phytoplankton- dominated is nutrient loading (Scheffer 1998). When total phosphorus is below 20 µg P/L, algal turbidity and shading are minimal, thereby allowing for the proliferation of the macrophyte community (Mjelde and Faafeng 1997). Conversely, at high N and/or P loadings, algal biomass can rapidly increase beyond zooplankton grazing demands and thus dominate aquatic systems due to shading of macrophytes. Dominance, however, is not absolute because other factors such as non-algal turbidity, water depth, and season can alter predictions and outcomes (Scheffer 1998).

Timing of nutrient loading, rather than the absolute amount of nutrient input, may also be influential in the determination of macrophyte or phytoplankton dominance. Algal communities stimulated by nutrient enrichment early in the growing season can result in significant shading effects and thus hinder development of macrophytes (Phillips et al. 1978). However, some macrophytes may out-compete phytoplankton by early season accumulation and storage of available nutrients (Ozimek et al. 1990). It has been demonstrated that established macrophyte stands can maintain dominance despite increases in loading (Balls et al. 1989). Such communities may respond with a change in composition to tall-growing species that are better able to compete with epiphytes and phytoplankton shading (Moss 1990). Established macrophyte stands can also reduce the amount of nitrogen in the water column, thereby inhibiting algal taxa that are not able to fix atmospheric nitrogen. These nitrogen decreases may be the result of macrophyte uptake or the facilitation of denitrification. Much less is known regarding the uptake and assimilation of phosphorus in shallow vegetated systems (Scheffer 1998).

There are other biological factors that may influence the relative contribution of algal and macrophyte communities to aquatic productivity and nutrient cycling. For example, shallow ponds and wetlands may not support fish communities because of extreme temperature fluctuations and low dissolved oxygen (Bronmark and Hansson 1998). In the absence of fish predation, large-bodied zooplankton frequently dominate and exert extreme grazing pressure on the phytoplankton (Brooks and Dodson 1965) which can promote water clarity and increase growth and stability of macrophyte communities (Moss 1995, Scheffer 1998). Zooplankton can exert variable grazing pressure on phytoplankton, though, and therefore may not always be inversely related to phytoplankton biomass (Mitchell et al. 1988). Grazing may be ineffectual in controlling a filamentous algal community, which is less palatable to grazers than smaller-celled micro-algal species (Mayer et al. 1997). It has also been observed that nitrogen-limited systems may have decreased grazing pressure by zooplankton due to proliferation of large and generally unpalatable cyanobacteria (Jensen et al. 1994).

Much of the research regarding eutrophication of aquatic systems has been conducted using fertilization experiments. Studies have demonstrated that macrophytes show a variable response to nutrient loading, and that the relative capacity of a system for nutrient retention may depend on the resulting dominant community. Mulligan et al. (1976) used experiments at two fertilization levels in shallow ponds without fish to evaluate the fate of added nutrients and effects on the macrophyte community. With the highest load, they found that dense communities of phytoplankton inhibited or eliminated macrophyte development. Balls et al. (1989) conducted enrichment experiments in constructed ponds to explore the mechanisms of macrophyte loss in local water bodies that had lost submerged plant communities. In their experiments, macrophytes strongly buffered against all levels of nutrient enrichment and maintained dominance whether or not fish were present; however, experimental treatments included several phosphorus levels but only one nitrogen level. Stachowicz et al. (1994) fertilized a field pond over several years across various states of macrophyte and phytoplankton dominance to evaluate nutrient retention. They found that phosphorus retention was high under both phytoplankton and macrophyte dominance; however, macrophytes were far more effective at reducing the export of nitrogen. Therefore, research has demonstrated the complexities between nutrient enrichment and community interactions; yet few studies have comprehensively evaluated the full range of factors that may influence the uptake, assimilation, and retention of nitrogen and phosphorus in experimental wetland systems. Such extensive studies are logistically complex, but are necessary to isolate the relative effects of nutrient loading, ratios, frequency, and timing on retention and community dynamics (Moss 1995; Havens et al. 1999).

The objective of this study was to systematically evaluate the assimilative capacity of shallow, vegetated experimental wetlands for the uptake, removal, and retention of nitrogen and phosphorus. Three factors were evaluated: 1) the effect of N:P ratios; 2) the effect of loading or dosing rates of N and P; and 3) the effect of timing of dosing of N and P. The studies were conducted to explore the response of these experimental systems to nutrient manipulation under controlled, experimental conditions. The results are provided to explore the functional utility of using constructed wetlands as mitigation tools for removal of nutrients in runoff from agricultural watersheds.

Methods

Study Site

Studies were conducted at the experimental mesocosm facility located at the U.S. Geological Survey's Columbia Environmental Research Center (CERC), Columbia, MO. This facility was constructed in 1968 to provide a controlled experimental complex to evaluate the effects of environmental stressors on shallow aquatic systems. Individual impoundments are approximately 0.1 ha in area and range in depth from 0.1 to 1.5 m. Macrophyte communities are dominated by *Najas guadalupensis* and *Chara* sp. Physical, chemical, and biological characteristics of the mesocosms have been previously described (Fairchild et al.1992,1994; Fairchild and Sappington, 2002).

Corral Construction and Design

Circular corrals were used as the experimental treatment unit because they are highly replicable and reduce statistical variation typical of whole mesocosms. Multiple corrals were placed within each of 4 mesocosms; the mesocosm served as the experimental block.

Corrals were constructed of impermeable Scrimweave™ (StoCote Products, Chicago, IL) to create a circular enclosure of approximately 4-m diameter. Corral walls were secured to a circular ring of 2.5 cm diameter black polyethylene water pipe supported on steel fence posts (driven outside the corral) to maintain the upper edge of the corral approximately 20 cm above the water surface. The bottom edges of the corral wall were wrapped outward beneath a piece of circular metal garden edging, which was then driven approximately 8 cm into the sediments before the ponds were filled with water. The sides of the corrals were then weighted down with bricks while the ponds were filled with well water over a 2-d period. Once flooded, the mesocosms were allowed to mix and allow mobile biota to freely move within the system. Prior to dosing, the corral edges were raised and secured to effectively isolate the contents (water, sediment, and biota) within each individual corral.

Water exchange between the corral and the outside water was minimal as indicated by visual inspection (i.e., turbidity during wading on the outside of the corral) and analytical chemical data. Ponds were occasionally refilled with water during the season to maintain an average depth near 1 meter; water additions were conducted during non-critical periods of the dose/monitoring schedule to minimize artifacts of corral management. Depths ranged among the corrals from 0.74–1.16 m and averaged 0.91 m. Levels of water in each individual corral remained similar to that outside of the corral due to slow water diffusion through the sediments.

Dosing

The ranges of dose concentrations were selected based on the range of published spring and summer concentrations of N and P from Midwestern streams subject to agricultural runoff (Hauck et al. 1997). Magnitude of dosing was chosen based on literature reviews of studies (Johnston 1991; Mitsch et al. 1999) that indicate that natural wetlands can assimilate a range of 0.03 - 28 g N /m^2/yr and 0.07-3.48 g P/m^2/yr depending on a range of factors including wetland type, depth, vegetative structure, and hydrologic residence time.

Granular agricultural fertilizers (soda of nitrate and triple super phosphate) were used to dose the corrals. Amendments were calculated according to corral volumes and the percent of available N and P in the fertilizer. Fertilizer was pre-weighed, placed into a cotton bag, and agitated under the surface of the water inside the perimeter of the corral for approximately 5 minutes. The water was then gently mixed with a paddle to ensure nutrient distribution. Once granules were mostly dissolved, the bag was suspended in the water column to allow for release of residual nutrients in the material. Laboratory experiments prior to the start of the study indicated that nutrients were rapidly released into the water column. Rapid nutrient dissolution was also verified by measured nutrient concentrations in the corrals (see results). Nutrient bags were specific for each corral and were used throughout the experiment. Control corrals were similarly mixed to prevent experimental bias due to the physical disturbance of mixing.

Water Chemistry

Water samples were collected with a tube sampler (cylindrical sampler 7.62 cm diameter by 1 m length; vol.= 4560 cm^3) deployed in a rapid, vertical motion to collect a depth-integrated water sample. Three vertical samples were composited in a clean 20-L polyethylene bucket. The composite was thoroughly mixed and then sub-sampled with a 1-L polyethylene bottle. The 1-L samples were immediately chilled on ice and transported to the laboratory for analysis. Unfiltered samples were kept on ice or refrigerated until processed or analyzed. All samples were analyzed within recommended time limits according to EPA standards (U.S. EPA 1979).

Approximately 250 mL of each water sample was filtered for dissolved nutrients using a 0.45 µm membrane (47 mm nitrocellulose filter; Whatman Inc., Clifton, NJ). All filtering equipment was thoroughly rinsed with deionized water between samples to minimize contamination. The filtrate (for dissolved constituents) was stored refrigerated ($\leq$ 4°C) in 60-mL Nalgene HDPE bottles or 10-mL capped, borosilicate disposable tubes until analysis.

Soluble reactive phosphorus (SRP) was determined using a color reagent and an MR 1201 Spectrophotometer as described by U.S. EPA Method 365.3 (U.S. EPA 1979). A Lachat 8000 Flow Injection Analyzer (Lachat Instruments, Milwaukee, WI) was used for analysis of nitrogen. The combined total of nitrate (NO_3-N) and nitrite (NO_2-N) was measured by a colorimeter after cadmium reduction to nitrite (Lachat Instruments 1997). Ammonia (NH_3-N) was measured colorimetrically after reactions with alkaline phenol, sodium hypochlorite, and sodium nitroprusside (Lachat Instruments 1997).

Unfiltered water was analyzed for total phosphorus and total nitrogen. Samples to be analyzed for total nitrogen and total phosphorus were stored frozen (<0°C) in 60-mL HDPE bottles. Before analysis, the samples were thawed, mixed, and pipetted into 10-mL glass tubes. Samples for total phosphorus were oxidized using potassium persulfate (Fisher Scientific, Fairlawn, NJ) and then analyzed for orthophosphorus on an MR 1201 Spectrophotometer (U.S. EPA 1979). The spectrophotometer was also used to determine total nitrogen as N after persulfate oxidation (Crumpton et al 1992).

The pH of each sample was determined using an Orion Model SA 290A pH meter in accordance with the manufacturer's recommendations. Alkalinity was determined by burette titration with 0.02 N sulfuric acid and expressed as mg

$CaCO_3$/L. Hardness (mg $CaCO_3$/L) was measured using a color indicator and burette titration with EDTA (APHA 1992). Conductivity was determined using an YSI Model 33 S-C-T meter (YSI Corp., Yellow Springs, OH) and expressed as µS/cm at room temperature (20–30°C). The HACH Model 2100A Turbidimeter (Hach Co., Loveland, CO) was used to estimate the turbidity (NTU's).

Phytoplankton

Phytoplankton biomass was based on the chlorophyll *a* content of algae. A measured amount of sample (25–250 ml) was filtered through a 47 mm glass fiber filter (Gelman type A/E; Fisher Scientific, Fairlawn, NJ). The filter was then placed in a 15-ml vial of 90% buffered acetone and refrigerated overnight for extraction. This extract was subsequently analyzed using a fluorometer (Turner Designs 10-AU-Fluorometer; La Jolla, CA) using EPA Method 445.0 (APHA 1992). Particulate organic carbon samples were filtered onto a 47-mm Gelman type A/E filter and then combusted and analyzed using a Coulometrics Model 2010 Total Carbon Analyzer (UIC Corporation, Wheaton, IL). Phytoplankton were sampled and preserved for taxonomic analysis on a monthly basis by preservation of 40 mL of the unfiltered water sample using 1 mL Lugol's solution. Phytoplankton were counted and identified by John Beaver of BSA Environmental Services, Beachwood, OH.

Periphyton

Periphyton biomass and accrual rates in the corrals were evaluated as chlorophyll concentrations extracted from growth on artificial substrates (1 cm by 10 cm strips of Scrimweave™ suspended vertically just below the surface of the water). In June of 1999, four strips were exposed for four weeks and then analyzed individually for chlorophyll. Future exposures were shorter to reflect the rapid growth of periphyton that was observed. In July of 1999, four strips were deployed. Two replicate strips were collected after 1-week and 2-week exposure intervals. In August and September, six strips were deployed, and three strips were collected and analyzed as a composite after each of 1-week and 2-week exposures. Strips were carefully collected with forceps in the field and immediately put on ice in vials of 15 mL of 90% buffered acetone. Periphyton chlorophyll was estimated using the same methods as for the phytoplankton; however, values were expressed as accrual rates (µg Chl/cm^2/wk).

Zooplankton

Monthly zooplankton samples were collected. On May 12, 1999, (Study 1) zooplankton samples were collected using a 63 µm Wisconsin net and vertical tows to effectively sample a 10-L volume. Thereafter, zooplankton were sampled using vertical migration samplers modified from the design of Whiteside et al. (1978). Samplers consisted of a funnel and 2-L bottle assembly inverted and positioned in the water column just above the macrophyte layer. The funnel and bottle used were clear so as to minimize avoidance due to darkened conditions. Samplers were deployed at dusk and retrieved at dawn. These samplers passively trapped zooplankton during diurnal feeding movements. On retrieval, the samplers were poured through a 63 µm Wisconsin net to isolate the zooplankton. Samples were stored in 90% ethanol. Samples were analyzed by Bill Mabee, Missouri Dept. of Conservation, Columbia, MO. Sample numbers were then calculated on an area basis by dividing zooplankton number by the surface area of the funnel surface.

Macrophytes

Macrophytes were qualitatively assessed each month based on visual assessment and ranking of the benthic plant and filamentous algae communities; separate estimates were made within each of four quadrants of each corral. Assessments included estimates of percent cover, height, species composition, and color. There were only two species of macrophytes in the corrals (*Chara* sp. and *Najas guadalupensis*) which were easily distinguishable based on color and morphometrics. *Chara* sp. is a macroalgae and has an upright and branched thallus, and is attached to the substrate by rhizoids (Smith 1950; Kufel and Kufel 2002). *Najas* sp. is a submerged, branched macrophyte.

Macrophytes were quantitatively sampled each month from pre-set, buried standardized rings to minimize disturbance and sampling bias. Each ring (5 cm height; 10 cm diameter) was cut from a cross-section of white PVC water pipe. Replicate sampling rings were deployed in each of four corral quadrants to account for spatial variation within each corral. Prior to the study initiation, the rings were pushed into the sediments until flush with the top of the sediment layer. This technique made the rings easy to locate, but minimized shading or enclosure effects.

Monthly composite macrophyte samples were collected (one ring from each of the four quadrants) from each corral by divers wearing Neoprene wet suits. Wet suits allowed the divers to maintain neutral buoyancy and caused minimal disturbance to surrounding sediments and macrophytes (Madsen 1993). Collection involved diving to locate a ring and digging underneath it with a Plexiglas board. The board created a bottom for the encircled sediment and macrophyte sample, and enabled it to be brought to the surface for careful processing. Macrophyte material originating from the area enclosed by the ring was collected, including all above and belowground biomass. Composites of four rings per corral were stored in plastic bags on ice during transport to the lab. In the lab, the macrophytes were washed on a small mesh screen (<1 mm mesh) and any debris or attached sediment was carefully removed. Samples were then

placed in pre-weighed aluminum foil packets, dried at 105°C, and weighed to get an estimate of dry weight biomass (Madsen 1993). Biomass was expressed as dry weight (g/m^2). A Wiley Mill was used to grind the dried samples, which were then stored in airtight vials.

Dried and ground macrophyte samples were subsequently analyzed for total nitrogen and total phosphorus content. Samples analyzed for N content were weighed (0.2 g) and then combusted in a LECO FP-528 Analyzer. This apparatus transformed sample nitrogen to N_2, which was then measured by thermal conductivity detection and expressed as percent of dry weight.

Total phosphorus in macrophyte tissues was determined in pre-weighed samples (0.2 g) using perchloric acid digestion (6% perchloric acid) based on the procedure of Sommers and Nelson (1972). This digestion process converts all phosphorus to orthophosphate in a clear supernatant. Orthophosphate was then determined using the Lachat 8000 FIA (Lachat Instruments, Inc. 2000). The results, expressed as µg P/L, were converted to a percent basis (mass:mass) normalized to the amount of plant material used in the digestion.

Sediment

Sediment samples were collected concurrently with macrophytes. One sediment plug of the top 2–5 cm of sediment was taken from each macrophyte ring and deposited in a plastic bag. Care was taken not to include any plant material with the sediment plug. The composite of sediment plugs for the four rings sampled in each corral was homogenized by hand and dried in foil pans at 105°C. Dried samples were ground using a Wiley mill and stored in airtight vials. Methods for analysis of nitrogen and phosphorus content of the sediments were the same as for macrophytes. At the beginning of the season, four separate rings were placed in the ponds. The sediment enclosed by them was collected, dried, and weighed to determine an average mass of sediment within a ring. Estimates of sediment nutrient pools (g N or P/(m^2*5 cm deep)) were calculated by multiplying the percent content of nutrient by the average mass of sediment enclosed by the sampling ring, and then converting to a square meter of surface area.

System Metabolism

System metabolism was measured each week as a variation of the diurnal oxygen method outlined by Lind (1985). This method was chosen over traditional light-dark bottle techniques because evaluations were desired for the total system including macrophytes, phytoplankton, and sediments. Dissolved oxygen and temperature readings were taken with a YSI Model 54 Oxygen Meter in every corral on a consecutive morning, evening, and morning sequence. The first readings of each sequence coincided with water collection. Before field use, probes were calibrated in saturated air according to manufacturers' specifications. Dissolved oxygen and temperature were measured by submerging the probe to mid-depth of the water column to ensure homogeneity. All corrals were sampled in less than an hour to decrease temporal variability. Oxygen readings (mg O_2/L) were designated M1 (morning 1), E1 (evening 1), and M2 (morning 2). Gross production (GP) was calculated by: GP = (E1-M1)+(E1-M2). Gross respiration (GR) was calculated by: GR=2*(E1-M2) as a modification of Lind (1985).

Statistical Analysis

Data were tested for normality of distribution by treatment using Proc Univariate in the Statistical Analysis System, Release 6.12 (1996). The strong seasonal nature of the data (see Appendix 1) resulted in data that were not normally distributed with homogeneous variance. Therefore, all datasets were subsequently transformed using the rank procedure prior to analysis (Conover and Iman 1981). Although some statistical power was lost by rank transformation, this method provided the best means of analyzing all of the datasets uniformly (Snedecor and Cochran 1967, Green 1979). Transformed data were analyzed as a randomized complete block design using Analysis of Variance (ANOVA) to determine influences due to N-dose, P-dose, time, and their interactions (using pond as the block and corrals as experimental units). When ANOVA indicated significant main effects, we statistically compared individual treatments using the Student-T test. Significant differences between rank-transformed values were determined at the $p \leq 0.05$ level.

Quality Assurance Summary for Nutrient Analyses

A summary of quality assurance results for nutrient analyses is presented in Table 1. Results indicated that recovery of spiked standards ranged from 79–108% across the two years of study. Recoveries were within the range of acceptable results for these analyses.

Table 1. Summary of Recovery Data for Nutrient Analyses Recoveries. Numbers Represent Mean + 1 Standard Deviation. Number in Parenthesis is Number of Independent Standards Analyzed Each Year

	Year	
Nutrient	**1999**	**2000**
NH_3	90.6 + 15.2 (63)	85.9 + 14.7 (38)
NO_2NO_3	87.6 + 19.0 (64)	95.4 + 15.7 (38)
SRP	94.2 + 4.6 (64)	108 + 18.6 (38)
TN	85.9 + 21.1 (30)	79.1 + 19.4 (18)
TP	94.3 + 2.0 (30)	101.6 + 3.7 (18)

STUDY 1: Effects of N:P Ratio and Biweekly Nutrient Loading

The nitrogen to phosphorus ratio (N:P ratio) is known to be a primary determinant of the response of phytoplankton to nutrient enrichment in deep, lentic aquatic ecosystems (Sakamoto 1966; Wetzel 1983). However, far less is known regarding the response of phytoplankton and macrophyte communities in shallow, vegetated wetlands (Scheffer 1998). Thus, the first experiment performed in 1999 evaluated the effects of N:P ratios on nutrient cycling, system metabolism, and structural dynamics of the experimental mesocosms.

There were three objectives in Study 1: 1) determine how nutrient loading and the N:P ratio influenced the concentration and relative distribution of nutrients in the water column, macrophytes, and sediments; 2) evaluate how nutrient load and the N:P ratio influenced species composition, biomass, and/or abundance of macrophytes, phytoplankton, and periphyton; and 3) characterize the assimilation and retention capabilities of shallow ponds for nutrient loads under varying N:P ratios.

Experimental Design

Ponds were drained the April 15, 1999, and corrals were constructed over a 2-week interval. A total of 36 corrals were constructed with nine corrals within each of 4 replicate ponds (experimental blocks). Ponds were reflooded with well water on May 1 and allowed to biologically re-colonize from sediments. On May 18, the sides of the corrals were raised above the water surface and secured to isolate each individual corral and its contents. Water sampling began on May 20.Three levels of nitrogen (0, low, and high) and three levels of phosphorus (0, low, and high) were studied in a balanced factorial design as described in Figure 1. Two dosing phases were evaluated: Early (May 20–July 27, 1999) (Table 2) and Late (July 29–September 21, 1999) (Table 3). In the Early phase (Table 2), targeted nominal concentrations of nitrogen ranged from 0 to 5 mg N/L; whereas, targeted nominal phosphorus concentrations ranged from 0 to 88 µg/L. Thus, N:P ratios ranged from approximately 16:1 (approximate Early ambient conditions) to 114:1. In the Late phase (Table 3), the amount of phosphorus was increased to standardize the N:P ratio at 13:1 (optimum N:P ratio for productivity), 25:1 (lower end of phosphorus limitation), and 50:1 (high phosphorus limitation). Over the 12 weeks, the total loads of added nutrients ranged from 0 g NO_3-N/m^2 and 0 g P/m^2 in the Control, to 30 g NO_3-N/m^2 and 0.864 P/m^2 in the treatment receiving the highest concentrations of both nutrients. The frequency (6 doses) and timing (every two weeks) of dosing were held constant and were initiated on June 2, 1999, when macrophytes had attained approximately 25% surface cover as viewed from above.

Corrals were dosed six times during the study (Early 6/2/99, 6/16/99, 6/30/99; and Late 7/28/99, 8/11/99, 8/25/99). In all additions, target concentrations for nitrate were 0, 2.5, and 5 mg NO_3-N/L for ambient, low, and high treatments, respectively. Phosphorus was applied in two phases constituting the Early (first three doses) and Late season (last three doses). Target levels were 0, 44, and 88 µg P/L in the Early season, and 0, 100, and 200 µg P/L in the Late season.

The nitrogen or phosphorus portion of the dose was referred to as N or P, and for ambient target levels, the designation 0N or 0P was used. In some parameter analyses, P-dose did not significantly influence the results. For those analyses, the three phosphorus treatments were combined within the nitrogen treatment for ease of presentation, i.e., (0N:0P), (0N:loP), and (0N:hiP) were averaged to create 0N. Likewise, in analyses where P-dose was determined to be the stronger influence, all nitrogen treatments (0P, loP, and hiP) were combined. Relative P-designations were used for simplicity of presentation because dosing amounts were changed during the season. Analyses were conducted across the entire season, and on subsets of the Early (May 20–July 27) and Late (July 29– September 21) seasons to evaluate treatment effects.

Results

Macrophytes

Macrophyte Biomass

Macrophytes grew rapidly from May to July until a maximum biomass of approximately 800 g/m^2 was reached; thereafter, macrophyte biomass decreased during the remainder of the study in all treatments (Figure 2). There was a statistically significant effect of Day on macrophyte biomass during the Early-, Late-, and Full-season analysis. However, there were

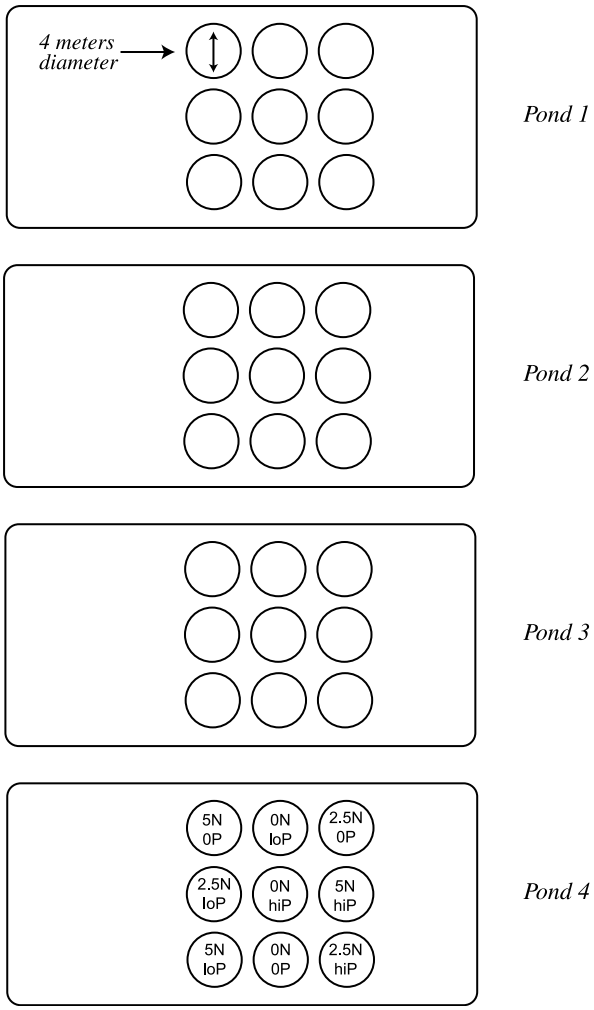


Figure 1. *Pond and corral diagram for Study 1 experiments indicating corral orientation and diameter. Pond 4 shows an example of the random assignment of the nine treatments.*

Table 2. Target Dose Ratios (N:P) for Treatments Receiving Both Nutrients in the Early Dosing Period of Study 1. Treatments Consisting of at Least One Ambient Level are not Represented Because Initial Ambient Levels for Both nutrients were at or below the Method Limit of Detection

		N (mg/L)		
		Ambient	**2.5**	**5**
P (µg/L)	**Ambient**			
	44		**57:1**	**114:1**
	88		**28:1**	**57:1**

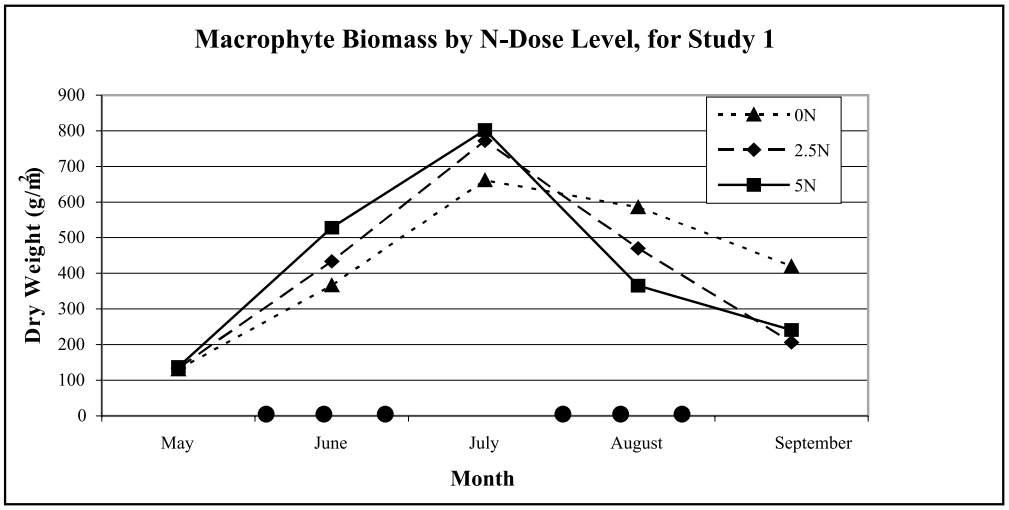
Table 3. Target Dose Ratios (N:P) for Treatments Receiving Both Nutrients in the Late Dosing Period of Study 1. Treatments Consisting of at Least One Ambient Level are not Represented Because Initial Ambient Levels for Both Nutrients were at or below the Method Limit of Detection

		N (mg/L)		
		Ambient	**2.5**	**5**
P (µg/L)	**Ambient**			
	100		**25:1**	**50:1**
	200		**13:1**	**25:1**

no significant main effects of N or P dosing. In May, all treatments had initial dry weight biomass averaging 135 g/m^2. The midseason point (July 27) coincided with maximum macrophyte biomass, which reached 661 g/m^2 (5 times initial levels) in the 0N treatment. However, there were no significant differences among N treatments at peak biomass levels reached in July. Senescence during the Late season (August and September) resulted in a loss of one-third of the biomass in 0N, and final stands were significantly smaller than the maximums ($p<0.05$). Treatments 2.5N and 5N lost over two-thirds of their maximum stand, and by September, had macrophyte biomass levels, 206 and 241 g/m^2, respectively, that were significantly lower ($p<0.05$) than 0N (419 g/m^2). Differences between 2.5N and 5N were not significant. Nutrients appeared to slightly increase both macrophyte growth rates and rates of senescence; however, overall main effects of nutrients were not significant. On the last sampling date, there were significant differences among treatments when evaluated using a single LS Means test in which the 2.5N and 5N treatments contained significantly lower macrophyte biomass than 0N, implying that nutrient addition enhanced decomposition processes late in the study.

Variability in macrophyte biomass was caused to some degree by variation in depth across the experimental pond blocks. Topography of the pond bottom varied from 0.91 to 1.16 m depth across locations in the 36 individual corrals. Macrophyte biomass levels "crashed" to zero in two of the deepest corrals in pond 4 in August. These corrals were treated with intermediate levels of nutrients (2.5N:loP and 5N:loP) which would indicate that the crashes were not related to necessarily high levels of enrichment (5N or hiP) or a particular TN:TP ratio. Rather, these two corrals were proximal to each other in the deepest section of the pond. Therefore, we conclude that the macrophyte crashes were probably due to a factor of location which was light-limited due to depth as opposed to algal-generated turbidity.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Macro. Biomass	Full	0.5045	0.1360	0.5157	0.0001	0.0447	0.9171	0.9382
	Early	0.0717	0.1389	0.1696	0.0001	0.8030	0.5546	0.9122
	Late	0.0942	0.1988	0.4978	0.0001	0.0496	0.8974	0.8360



		Month					Season
		M	J	J	A	S	Avg.
N-dose	0	130	366	661	585	419[a]	422
	2.5	134	433	772	470	206[b]	395
	5	137	528	802	365	241[b]	412

Figure 2. *Changes in macrophyte biomass over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced macrophyte biomass in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of macrophyte biomass over the entire experimental season. Because N-dose*Day was a significant influence in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (g dry weight/m^2) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

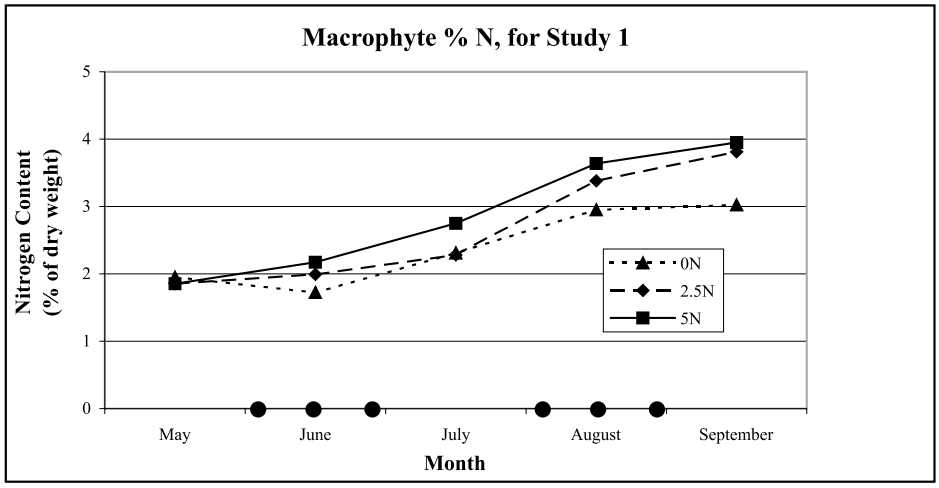
Macrophyte Taxa

Qualitative, visual observations of the macrophyte and algal communities during Study 1 revealed seasonal succession, although variability within and across treatments was high. *Chara*, an attached macroalgae, dominated most corrals in early June. These populations declined by late June, and were not detectable by late July. The rooted macrophyte community consisted of only one genus, *Najas*, throughout the season. These plants succeeded *Chara* in all corrals except two that experienced biomass crashes (treatments 2.5N:loP and 5N:loP). In those cases, *Chara* populations were replaced by filamentous algae, and *Najas* re-growth never occurred. Filamentous algal growth was observed in all of the corrals on at least one date; however, filamentous biomass was qualitatively greater in treated corrals compared to Control ponds. Filamentous algae, however, was not a major contributor of total macrophyte biomass in any treatment.

Macrophyte Nutrients

Day was a significant main effect for both N (Figure 3) and P (Figure 4) content of macrophytes. Both N and P content of macrophyte tissue significantly increased over the season in all treatments in 1999. Prior to dosing, macrophytes averaged 1.89% N (Figure 3). Initial samples were composed of both *Chara* sp. and *Najas guadalupensis*. The measured N concentrations were intermediate between published literature values for similar species including *Chara vulgaris* (2.43–3.19% N; Dykyjova and Kvet 1982) and *Najas maritima* (1.05–1.87% N; Royle and King 1991). There was a significant main effect of N-dose on nitrogen content of macrophytes ($p < 0.05$) during the Late- and Full-season analysis. The nitrogen content of 0N macrophytes increased from 1.95% N to 3.02% N during the season. Following dose initiation, macrophytes in N-dosed treatments had higher N content than 0N, and differences among treatments

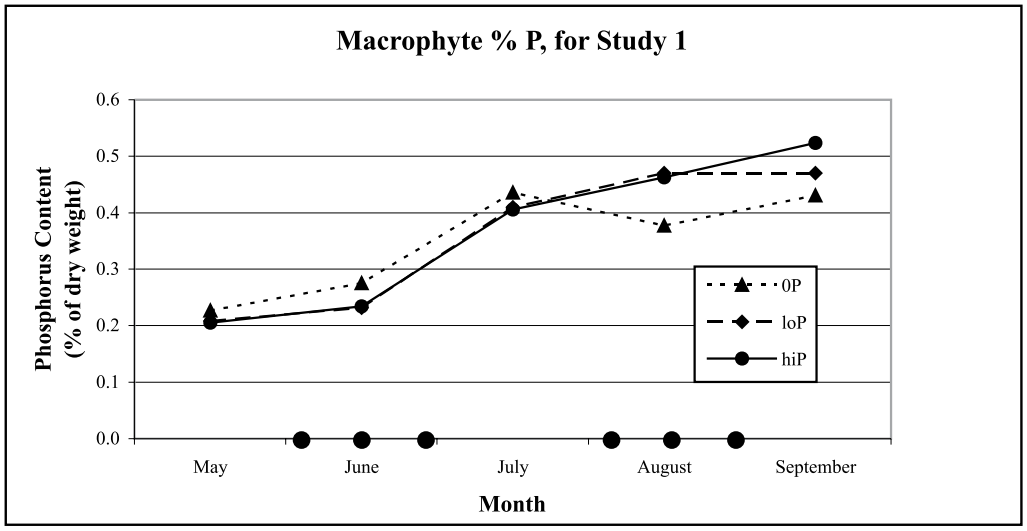
		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Macro. % N	Full	0.0011	0.6122	0.8228	0.0001	0.0432	0.1891	0.8810
	Early	0.2393	0.6444	0.9359	0.0001	0.0583	0.1489	0.5412
	Late	0.0001	0.8591	0.4992	0.0001	0.0493	0.2010	0.8406



		Month					Season
		M	J	J	A	S	Avg.
N-dose	0	1.95	1.73[a]	2.31[a]	2.95[a]	3.02[a]	2.39[a]
	2.5	1.85	1.99[ab]	2.28[a]	3.38[ab]	3.81[b]	2.66[b]
	5	1.86	2.18[b]	2.75[b]	3.64[b]	3.95[b]	2.87[c]

Figure 3. *Changes in macrophyte nitrogen content over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced N content in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of N content over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (% N of dry weight) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Macro. % P	Full	0.2639	0.5910	0.7526	0.0001	0.8113	0.0378	0.9077
	Early	0.9591	0.1689	0.8386	0.0001	0.7392	0.7026	0.9231
	Late	0.0895	0.0324	0.7838	0.0558	0.8552	0.1081	0.4913



		Month					Season
		M	J	J	A	S	Avg.
P-dose	0	0.23	0.28	0.44	0.38[a]	0.43[a]	0.35
	lo	0.21	0.23	0.41	0.47[b]	0.47[ab]	0.36
	hi	0.21	0.23	0.41	0.46[b]	0.52[b]	0.37

Figure 4. *Changes in macrophyte phosphorus content over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced P content in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of P content over the entire experimental season. Because P-dose*Day was a significant influence in ANOVA, values are pooled by P-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (% P of dry weight) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

increased during the season. The final nitrogen concentrations in 2.5N (3.81% N) and 5N (3.95% N) were nearly 30% higher than final levels in the 0N treatment (3.02% N; significant difference $p<0.05$). There was no significant effect of P-dose on nitrogen content of macrophytes.

Macrophytes contained an average phosphorus content of 0.22% P at the beginning of the study (Figure 4). As with the percent N, that value was an intermediate between published literature values for *Chara vulgaris* (0.36–0.46% P; Dykyjova and Kvet 1982) and *Najas guadalupensis* (0.16% P; Boyd 1970). Day had a significant effect on P content of macrophytes during the Early- and Full-season comparisons. P-dose had a significant effect on P content of macrophytes during the Late season ($p<0.05$). The phosphorus content of 0P macrophytes increased by 0.2% P from May to July, but did not change substantially during senescence. Macrophytes in loP and hiP were similar in P content to 0P during the growing season, but continued to accumulate phosphorus in senescence. Phosphorus content of hiP macrophytes was nearly 20% higher than 0P in August and September (significant difference $p<0.05$). The P content in loP was only 10% greater than that in the 0P treatment, and was only significantly higher in August ($p<0.05$). N-dose had no significant effects on P content of macrophytes.

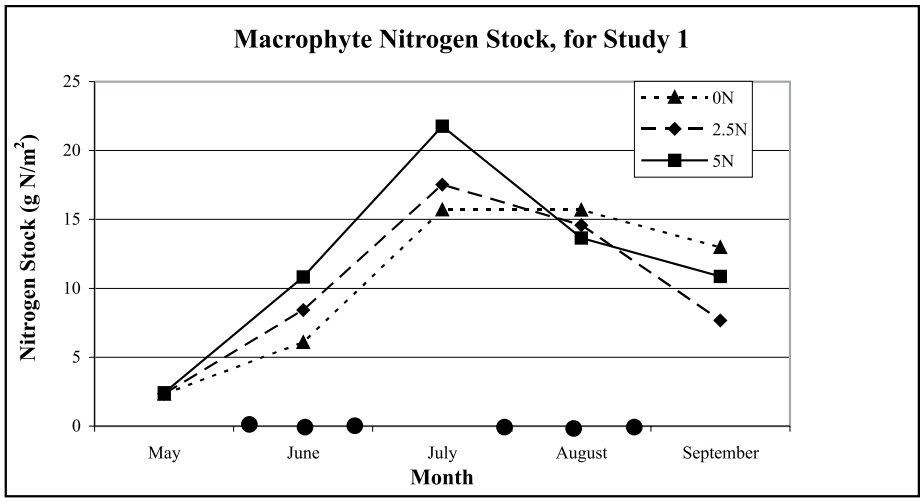
Macrophyte Nutrient Stock

The stock of N and P in macrophytes, expressed as g N/m^2 or g P/m^2, was calculated by multiplying the dry weight biomass (g/m^2) by the tissue content (g N/g dry wt or g P/g dry wt). Results are provided in Figures 5 and 6.

Nitrogen stocks in macrophytes followed seasonal trends of increase and decrease similar to biomass patterns. Day was a significant main effect ($p<0.0001$) controlling N stock of macrophytes (Figure 5) during the Early dosing, Late dosing, and Full-season analysis; N-dose had significant effects on N stocks of macrophytes during the Early season but had no effect during the Late dosing season. Control N stocks increased 7-fold from May (initially 2.3 g N/m^2) to July (average 15.7 g N/m^2 in July) (Figure 5). The 2.5N and 5N treatments developed maximum stocks of 17.5 and 21.8 g N/m^2, respectively, in July, which were significantly higher than Control levels on the average. During the senescent, Late treatment period nitrogen stocks decreased significantly ($p<0.05$) and declined to 13.0, 7.7, and 10.9 in the Control, 2.5N, and 5N treatments, respectively. P-dose had no significant effects on nitrogen stocks of macrophytes.

Phosphorus stock in macrophytes increased 20-fold (from 0.15 to 3.28 g P/m$^{2)}$ in Control corrals during the May–July Early period and was reflected in a significant main effect of Day. However, there were no significant main effects of N-dose or P-dose in phosphorus stocks of macrophytes. Phosphorus pools were similar among treatments during all phases of macrophyte growth. Phosphorus loss during biomass decline was weakly related to N-dose ($p=0.11$). During senescence, the store of P in 0N decreased by 25% to 2.21 g P/m^2, but this loss was not significant (Figure 6). In N-dosed treatments, however, phosphorus stocks in September were <40% of July maximums (significant difference $p<0.05$). Final values in 2.5N and 5N were 0.96 and 1.24 g P/m^2, respectively, significantly lower than concurrent pools in 0N ($p<0.05$).

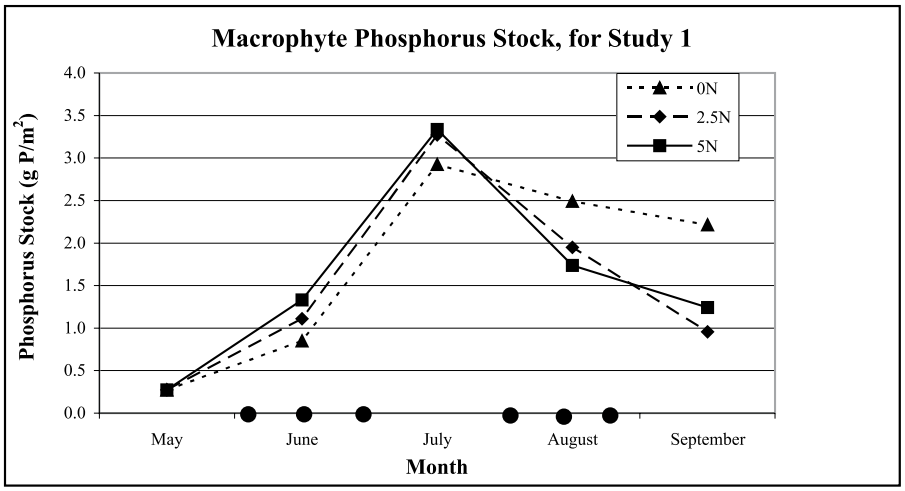
		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
N Stock	Full	0.6425	0.0950	0.6361	0.0001	0.1680	0.9058	0.9421
	Early	0.0265	0.0843	0.0999	0.0001	0.5542	0.5710	0.662
	Late	0.7499	0.2515	0.6855	0.0001	0.1688	0.9804	0.8861



		Month					Season
		M	J	J	A	S	Avg.
N-dose	0	2.3	6.1	15.7	15.7	13.0	10.6
	2.5	2.3	8.4	17.5	14.6	7.7	10.1
	5	2.4	10.8	21.8	13.7	10.9	11.9

Figure 5. *Changes in macrophyte nitrogen stock over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced the N stock in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of N stock of macrophytes over the entire experimental season. There were no significant influences based on the full season. Because N-dose was a significant influence in ANOVA of the early season, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (g N/m^2) represented in the graph.*

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
P Stock	Full	0.3382	0.3881	0.6646	0.0001	0.0684	0.9121	0.9391
	Early	0.1972	0.0519	0.0681	0.0001	0.8858	0.7631	0.8715
	Late	0.1154	0.6334	0.9263	0.0001	0.1109	0.5589	0.9337



		Month					Season
		M	J	J	A	S	Avg.
N-dose	0	0.27	0.85	2.92	2.49	2.21	1.75
	2.5	0.27	1.11	3.27	1.95	0.96	1.51
	5	0.27	1.33	3.33	1.74	1.24	1.58

Figure 6. *Changes in macrophyte phosphorus stock over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced the P stock in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of P stock of macrophytes over the entire experimental season. Because N-dose*Day was weakly significant (p<0.1) in ANOVA based on the entire season, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (g P/m^2) represented in the graph.*

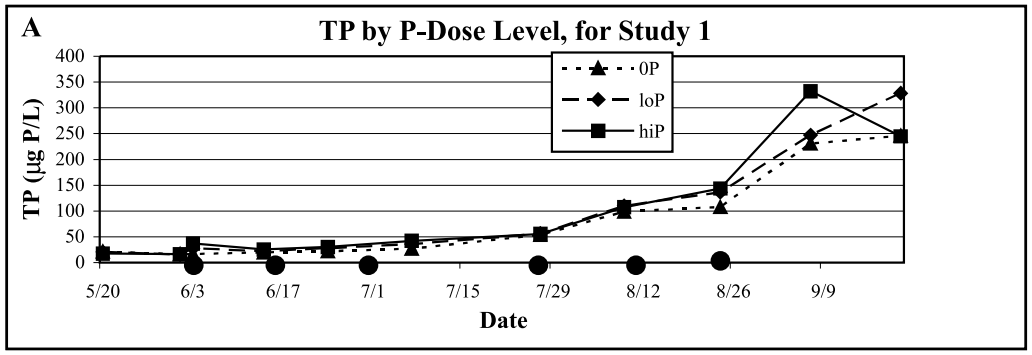
Water Chemistry

Phosphorus

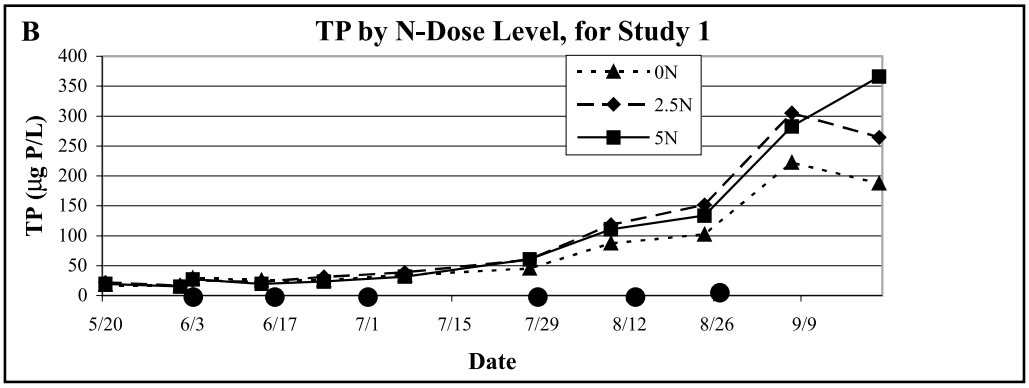
Total phosphorus (TP) in water increased 8-fold in the Control and >14-fold in P or N-dosed treatments over the course of the study. Day had a highly significant effect on TP ($p<0.001$), and both N- and P-dose had significant main effects during the Early- and Full-season analysis; however, there was also significant P-dose*Day and N-dose*Day interactions which complicate the interpretation of the effects of N-dose and P-dose alone. During the Early season, TP in 0P rose from 21 to 54 µg P/L (Figure 7A). In the amended treatments, TP was up to 20 µg P/L higher than 0P from dose initiation to July 7, but midseason values were similar. During macrophyte senescence, TP levels in 0P quadrupled to a final maximum of nearly 250 µg P/L, indicating internal loading from the sediments and/or macrophytes. LoP and hiP maximums in September were >325 µg P/L, but were not significantly different from each other or 0P.

When evaluated by N-dose, total phosphorus in 0N increased 11-fold during the season from 20 to 220 µg P/L (Figure 7B). During the Early season, TP in 0N increased from 18 to 45 µg P/L, and N-dosed treatments were within 10% of 0N values. At midseason, TP in N-dosed treatments was 60 µg P/L, a third greater than in the 0N Control (45 µg P/L; significant difference $p<0.05$). During the Late dosing period, which corresponded with macrophyte senescence, TP increased in all treatments, and differences between N-dosed and 0N treatments increased significantly when compared on single dates. TP in 0N and 2.5N peaked at 222 and 305 µg P/L, respectively, on September 7, and then dropped 15% in both treatments by September 21. TP in 5N on September 7 and 21, was 283 and 366 µg P/L, respectively, but due to variability among replicates, these levels were not significantly different from the other treatments.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
TP	Full	0.0422	0.0003	0.1006	0.0001	0.0014	0.0001	0.8995
	Early	0.0341	0.0001	0.4947	0.0001	0.0056	0.0001	0.9075
	Late	0.0763	0.3278	0.1972	0.0001	0.7635	0.2091	0.9026



		Date											Season
		5/20	6/1	6/3	6/14	6/24	7/7	7/27	8/9	8/24	9/7	9/21	Avg.
P-dose	0	21[a]	16	17[a]	20[a]	22[a]	27[a]	54	99	108[a]	231	245	78[a]
	lo	21[a]	16	28[b]	22[ab]	28[b]	36[b]	56	110	136[b]	247	328	93[b]
	hi	17[b]	16	37[c]	25[b]	30[b]	42[c]	55	107	143[b]	332	244	96[b]

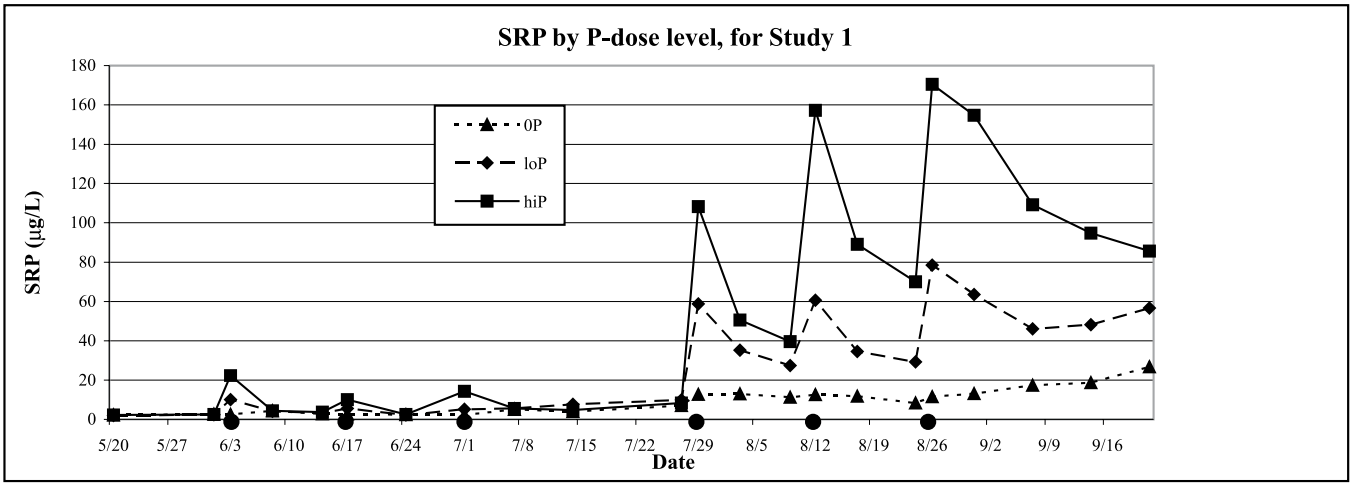


		Date											Season
		5/20	6/1	6/3	6/14	6/24	7/7	7/27	8/9	8/24	9/7	9/21	Avg.
N-dose	0	18[a]	17	29	25[a]	26[a]	35[ab]	45[a]	87[a]	102[a]	222[a]	188	72[a]
	2.5	22[b]	17	25	23[ab]	31[b]	39[a]	60[b]	118[b]	151[b]	305[b]	264	96[b]
	5	19[ab]	15	27	19[b]	23[a]	31[b]	60[b]	111[ab]	134[b]	283[ab]	366	99[a]

Figure 7. *Changes in total phosphorus by phosphorus and nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced TP in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The upper graph (A) is a plot of TP over the entire experimental season. Because P-dose and P-dose*Day were significant influences in ANOVA, values are pooled by P-dose in the graph. Dark circles on X axis mark dose dates. The table below Graph (A) lists LS Means (mg P/L) represented in Graph (A), along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in Graph (B). The table below Graph (B) lists LS Means represented in Graph (B) and follows the format described above.*

Soluble reactive phosphorus concentrations demonstrated cyclical response patterns due to significant main effects of both Day and P-dose in Early-, Late-, and Full-season analysis ($p<0.0001$) (Figure 8). However, there were also significant P-dose*Day interactions. Initial SRP concentrations (2 µg/L) were at or below the limit of detection. SRP in the 0P treatments fluctuated around the detection level during the first half of the experiment; SRP concentrations gradually increased during the Late dosing period to an average of 27 µg/L by late September. Peaks in SRP in the P-dosed treatments reflected the six amendments. During the Early season, SRP concentrations measured 24 hours after dosing reflected <20% of the calculated additions (i.e., 80% loss/day) which is indicative of the rapid assimilative capacity of the wetlands for dissolved phosphorus. SRP measurements taken one week after dosing were similar to the Control values. Calculated dissipation rates, based on weekly declines, were <4 µg P/L/day during the Early study (first 3 doses) but were probably underestimates because additions were rapidly and completely dissipated within that time period (Figure 9). After midseason, dissipation rates increased (Figure 9). During the Late dosing period there was a net accumulation of SRP in the P-dosed corrals due to dose modifications and/or internal loading from the sediments and/or macrophytes (Figure 8). In the week following doses three and four, dissipation rates in hiP were over twice those in loP ($p<0.05$), but in neither treatment did SRP return to Control levels. On the final sample date (September 21, 1999), SRP levels in hiP averaged 86 µg/L and were significantly higher than Control (27 µg/L) and loP (57µg/L) treatments (Figure 8). Final SRP concentrations did not differ between loP and hiP treatments.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
SRP	Full	0.7891	0.0001	0.2883	0.0001	0.5486	0.0001	0.9999
	Early	0.2794	0.0001	0.3087	0.0001	0.8351	0.0001	0.9939
	Late	0.8821	0.0001	0.5087	0.0001	0.0001	0.0001	0.9726



		Date																						Season
		5/20	6/1	6/3	6/8	6/14	6/17	6/24	7/1	7/7	7/14	7/27	7/29	8/3	8/9	8/12	8/17	8/24	8/26	8/31	9/7	9/14	9/21	Avg.
P-dose	**0**	2	3	3^{a}	4	3	3^{a}	2	2^{a}	5	4	7	13^{a}	13^{a}	11^{a}	13^{a}	12^{a}	8^{a}	12^{a}	13^{a}	17^{a}	19^{a}	27^{a}	89^{a}
	lo	2	2	11^{b}	4	3	6^{b}	2	5^{b}	6	8	10	59^{b}	35^{ab}	28^{b}	61^{b}	35^{b}	29^{b}	79^{b}	64^{b}	46^{b}	48^{a}	57^{a}	27^{b}
	hi	2	3	22^{c}	4	4	10^{c}	3	14^{c}	6	5	8	108^{c}	51^{b}	40^{c}	157^{c}	89^{c}	70^{c}	171^{c}	155^{c}	109^{c}	95^{b}	86^{b}	55^{c}

Figure 8. *Changes in soluble reactive phosphorus by phosphorus dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced SRP in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of SRP over the entire experimental season. Because P-dose and P-dose*Day were significant influences in ANOVA, values are pooled by P-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (mg P/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

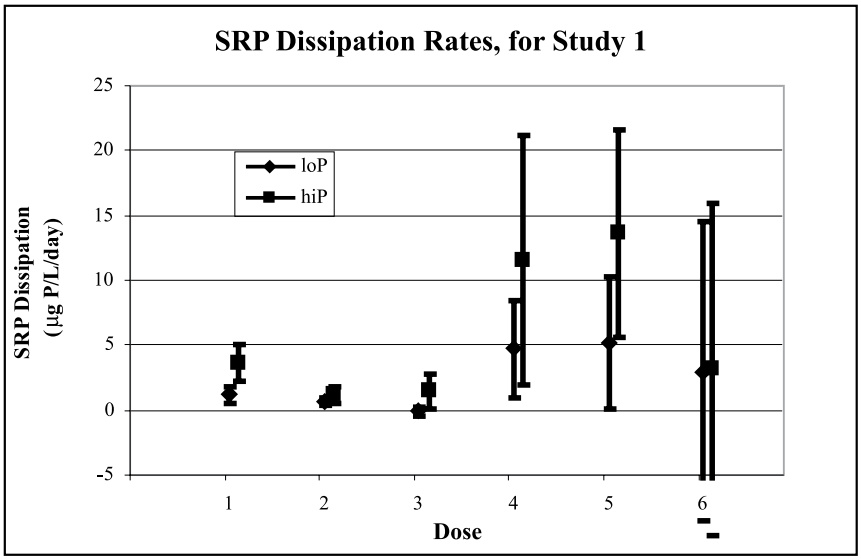
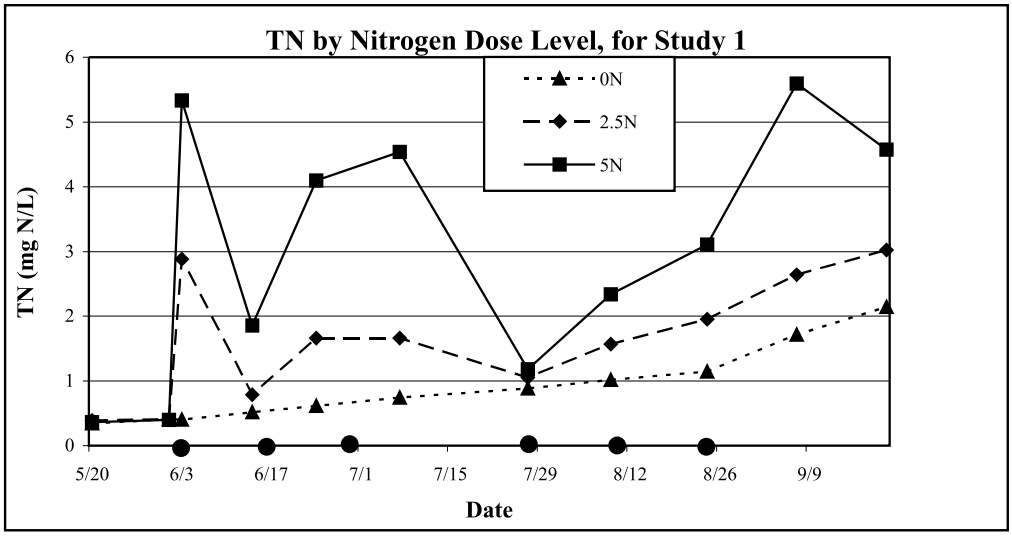


Figure 9. *Changes in soluble reactive phosphorus dissipation rates over dose periods. Phosphorus dissipation (mg P/L/day) was calculated from concentrations one day and one week after each dose.*

Nitrogen

There were significant main effects of Day, N-dose, and N-dose*Day interactions on total nitrogen (TN) levels in the study; however, P-dose had no effect on TN levels. TN significantly increased in all treatments during the study and averaged a 6-fold increase over the 4-month study interval. In 0N treatments, TN increased from 0.35 to 2.14 mg N/L by the end of the study (Figure 10). During macrophyte growth, nitrate constituted 95% and 88% of the total nitrogen in 5N and 2.5N, respectively, due to inputs from the dosing regime (Figure 11). Nitrate values decreased to near Control values at the end of the Early dosing period (approximately 1 mg/L; July 27) which illustrates the assimilative capacity for nitrate when macrophytes were rapidly growing. During the Late dosing intervals, which corresponded to the observed period of normal macrophyte senescence, TN in 0N, 2.5N, and 5N increased 2, 3, and 4-fold, respectively. In the Late dosing period, the relative proportion of TN as nitrate was <1% in 2.5N, and dropped from 42% to 9% in 5N, indicating that a portion of the added nitrate was transformed and maintained in the water column in the organic form. A net increase of nearly 2 mg N/L in 0N treatment during the season indicated that these systems had large, internal sources of nitrogen derived from sediment stores. In the final samples, TN concentrations in 5N (4.57 mg N/L) were significantly larger than 2.5N (3.02 mg N/L) and the 0N Control ($p<0.05$).

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
TN	Full	0.0001	0.1046	0.1833	0.0001	0.0001	0.1399	0.8312
	Early	0.0001	0.6390	0.0720	0.0001	0.0001	0.4482	0.1774
	Late	0.0001	0.1000	0.4224	0.0001	0.1947	0.1809	0.8512



		Date											Season
		5/20	6/1	6/3	6/14	6/24	7/7	7/27	8/9	8/24	9/7	9/21	Avg.
N-dose	0	0.35	0.41	0.40[a]	0.52[a]	0.61[a]	0.74[a]	0.88[a]	1.01[a]	1.14[a]	1.71[a]	2.14[a]	0.90[a]
	2.5	0.39	0.41	2.89[b]	0.79[b]	1.66[b]	1.66[b]	1.05[ab]	1.57[b]	1.95[b]	2.64[b]	3.02[b]	1.64[b]
	5	0.36	0.40	5.33[c]	1.86[c]	4.10[c]	4.54[c]	1.18[b]	2.34[c]	3.11[c]	5.59[c]	4.57[c]	3.03[c]

Figure 10. *Changes in total nitrogen by nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced TN in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of TN over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (mg N/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Nitrate in the 0N treatments never exceeded 0.01 mg NO_3-N/L, and was at or below the limit of detection during most of the study (Figure 12). Nitrate values the day following the first dose showed that target levels of 2.5 and 5 mg NO_3-N/L were achieved by the additions (Figure 12). Statistical analyses confirmed that N-dose was a significant main effect on nitrate (<0.0001), but that P-dose was not influential. Following dose initiation, nitrate in 5N remained significantly higher than 0N through the end of the season. Nitrate levels in 2.5N were similar to 0N at the midseason point (July 27), and in the last three post-treatment samples.

Nitrate dissipation rates were calculated each week after dosing (Figure 13). During the Early dosing period, uptake in 5N averaged over 0.4 mg NO_3-N/L/day (8% applied N loss/day) and was significantly greater than rates in 2.5N which averaged less than 0.3 mg NO_3-N/L/day (12% applied P/day). Following the fourth amendment, when phosphorus additions were increased (Table 3), the nitrate dissipation only slightly increased (0.42 mg NO_3-N/L/day; 8% applied P loss/day); however, nitrate uptake decreased thereafter. Nitrate dissipation of the last dose in 5N was less than 0.06 mg NO_3-N/L/day (1% applied N loss/day). This rate was substantially lower than previous 5N rates and only 15% of concurrent 2.5N rates.

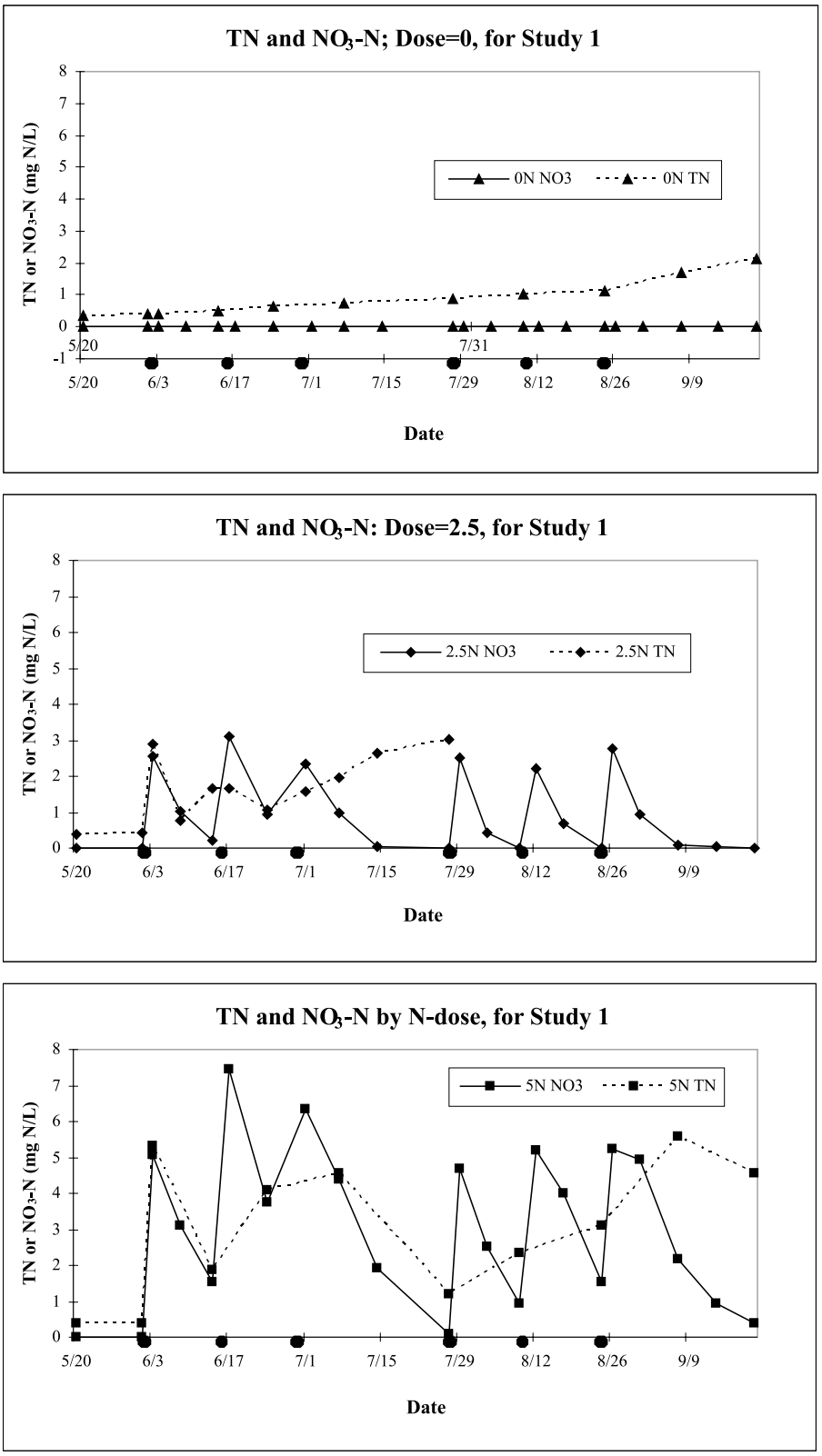
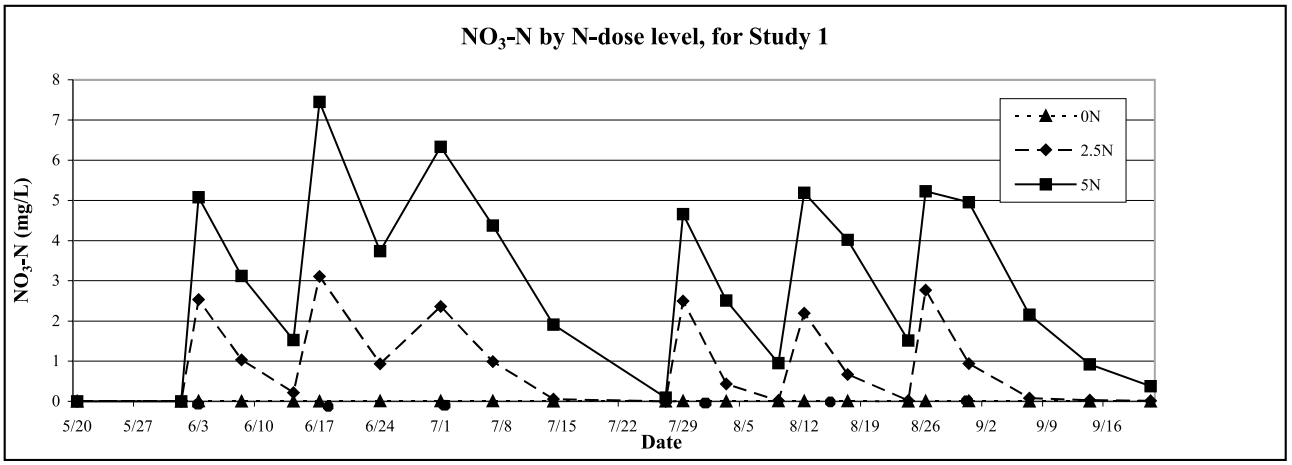


Figure 11. *Total and nitrate nitrogen over the season pooled by N-dose level. Nitrate values for 0N are not visible due to scale. Dark circles on X axis mark dose dates.*

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
NO_3-N	Full	0.0001	0.8444	0.7058	0.0001	0.0001	0.8022	0.8125
	Early	0.0001	0.5900	0.8230	0.0001	0.0001	0.4141	0.9868
	Late	0.0001	0.8762	0.6241	0.0001	0.0001	0.7471	0.4127



		Date																					Season
		5/20	6/1	6/3	6/8	6/14	6/17	6/24	7/1	7/7	7/14	7/27	7/29	8/3	8/9	8/12	8/17	8/24	8/26	8/31	9/7	9/14	9/21	Avg.
N-dose	0	0.005	0.005	0.007[a]	0.005[a]	0.005[a]	0.005[a]	0.006[a]	0.007[a]	0.007[a]	0.006[a]	0.005[a]	0.006[a]	0.006[a]	0.007[a]	0.007[a]	0.008[a]	0.006[a]	0.009[a]	0.007[a]	0.006[a]	0.005[a]	0.005[a]	0.026[a]
	2.5	0.006	0.005	2.535[b]	1.031[b]	0.215[b]	3.105[b]	0.934[b]	2.360[b]	0.984[b]	0.058[b]	0.005[a]	2.493[b]	0.434[b]	0.021[a]	2.196[b]	0.665[b]	0.020[b]	2.770[b]	0.937[b]	0.083[a]	0.034[a]	0.017[a]	0.951[b]
	5	0.006	0.005	5.080[c]	3.120[c]	1.528[c]	7.453[c]	3.735[c]	6.333[c]	4.374[c]	1.910[c]	0.093[b]	4.660[c]	2.509[c]	0.949[b]	5.187[c]	4.018[c]	1.517[c]	5.229[c]	4.957[c]	2.160[b]	0.921[b]	0.379[b]	3.006[c]

Figure 12. *Changes in nitrate by nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced NO_3-N in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of NO_3-N over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (mg NO_3-N/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

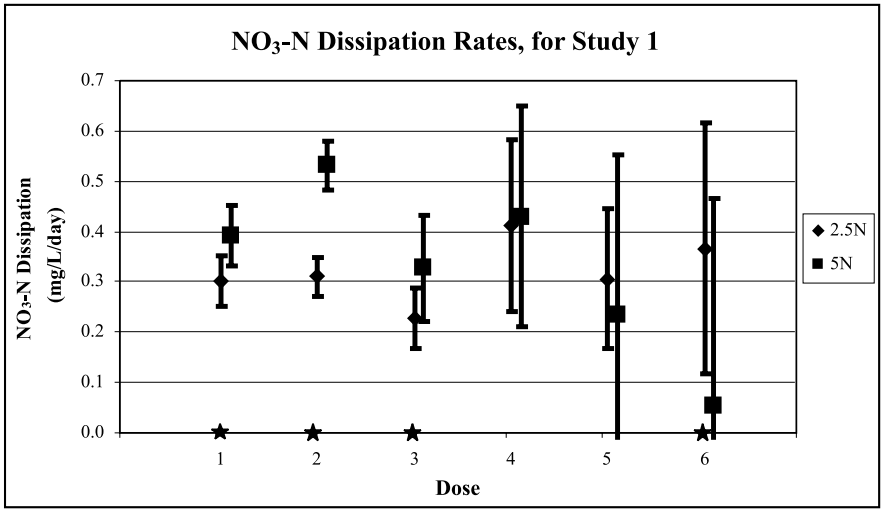


Figure 13. *Changes in nitrate dissipation rates over dose periods. Nitrate dissipation (mg NO_3-N/L/day) was calculated from concentrations one day and one week after each dose. Analyses showed that dissipation rates were 30- 70% greater in 5N than 2.5N in the first half of the season. Mean dissipation rates of the 6th dose in 5N were only 15% of concurrent 2.5N rates. Values plotted are LS Means pooled for N-dose, and bars represent one standard deviation above and below the mean. Stars indicate significant difference (p<0.05) between 2.5N and 5N treatments.*

Nitrate dissipation during the midseason inter-treatment period (July 1–27) and at the end of the season (Aug. 26–Sept. 1) resulted in complete nitrate uptake in 2.5N, and nearly complete uptake in 5N (Figure 14). During both periods, nitrate concentrations in 2.5N fell below 0.05 mg NO_3-N/L within three weeks following the amendment, and were similar to ambient levels in 0N ($p>0.05$). Nitrate in 5N decreased below 0.4 mg NO_3-N/L within four weeks, but remained significantly higher ($p<0.05$) than 0N and 2.5N. The sustained uptake of nitrate indicated that the assimilative capacity of these shallow, macrophyte-dominated systems was still high, but that the time required for nitrate assimilation >5 mg NO_3-N/L was increasing.

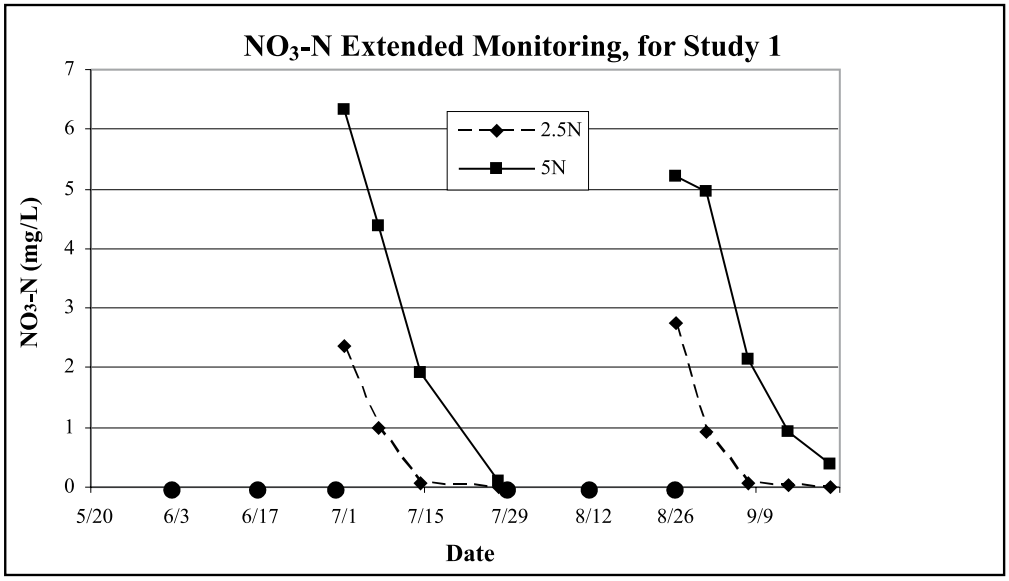
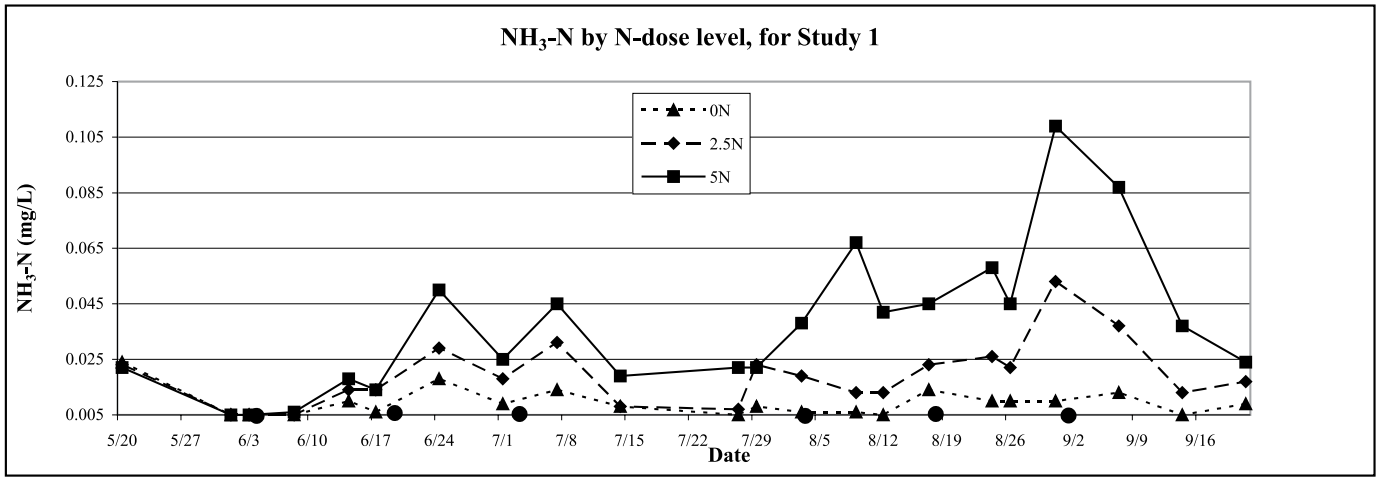


Figure 14. *Nitrate concentrations by N-dose over the 4-week extended monitoring periods at the midseason and the end. Dark circles on X axis mark dose dates. Nitrate in 2.5N and 5N was significantly different ($p<0.05$) on all but the last day of each period.*

Ammonia levels in 0N treatment fluctuated between the limit of detection (0.005 mg NH_3-N/L) and 0.025 mg NH_3-N/L during the study (Figure 15). Both N-dose ($p<0.0001$) and Day ($p<0.0001$) had significant main effect on ammonia concentrations. P-dose had no significant main effect on ammonia. Ammonia generally peaked the week following additions, resulting in concentrations in 5N that were 2-11 times those in concurrently measured 0N corrals. Ammonia in the 2.5N corrals was intermediate between the 0N and 5N levels, and likewise showed periodic increases the week after dosing. Simultaneous but smaller peaks (<0.02 mg NH_3-N/L) also occurred in the 0N corrals, indicating a possible effect of the stirring procedure on sediment release of ammonia. Ammonia in 0N averaged 0.009 mg NH_3-N/L during the study. Seasonal averages in the 5N (0.037 mg NH_3-N/L) and 2.5N (0.024 mg NH_3-N/L) treatments were significantly greater (four and three times, respectively) than those in the 0N treatment.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
NH_3-N	Full	0.0001	0.8565	0.8264	0.0001	0.0001	0.0533	0.9998
	Early	0.0001	0.2373	0.1956	0.0001	0.0021	0.0309	1.0000
	Late	0.0001	0.8563	0.7506	0.0001	0.0014	0.1746	0.8346



		Date																					Season
		5/20	6/1	6/3	6/8	6/14	6/17	6/24	7/1	7/7	7/14	7/27	7/29	8/3	8/9	8/12	8/17	8/24	8/26	8/31	9/7	9/14	9/21	Avg.
N-dose	0	0.024	0.005	0.005	0.005	0.010^{a}	0.006^{a}	0.018	0.009^{a}	0.014^{a}	0.008^{a}	0.005^{a}	0.008^{a}	0.006^{a}	0.006^{a}	0.005^{a}	0.014^{a}	0.010^{a}	0.010^{a}	0.010^{a}	0.013^{a}	0.005^{a}	0.009^{a}	0.009^{a}
	2.5	0.023	0.005	0.005	0.005	0.014^{ab}	0.014^{b}	0.029	0.018^{b}	0.031^{b}	0.008^{a}	0.007^{a}	0.023^{b}	0.019^{b}	0.013^{b}	0.013^{b}	0.023^{b}	0.026^{b}	0.022^{b}	0.053^{b}	0.037^{b}	0.013^{b}	0.017^{ab}	0.024^{b}
	5	0.022	0.005	0.005	0.006	0.018^{b}	0.014^{b}	0.050	0.025^{b}	0.045^{b}	0.019^{b}	0.022^{b}	0.022^{b}	0.038^{b}	0.067^{c}	0.042^{c}	0.045^{c}	0.058^{c}	0.045^{c}	0.109^{b}	0.087^{c}	0.037^{c}	0.024^{b}	0.037^{c}

Figure 15. *Changes in ammonia by nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced NH_3-N in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of NH_3-N over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (mg NH_3-N/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Nitrogen:Phosphorus Ratio

Sakamoto (1966) observed an optimum range of TN:TP ratio of approximately 13 (range 10:1–17:1; mass:mass basis) for algal productivity. Sakamoto (1966) proposed that above a TN:TP ratio of 17:1 algal populations were limited by phosphorus; a TN:TP ratio less than 10:1 was likely nitrogen-limited. All treatments had TN:TP ratios >17 during most of the Early dosing period, indicating they were P-limited. N:P ratios decreased below 10:1 in some treatments (0N:loP, 0N:hiP, 2.5N:loP, 2.5N:hiP) during the Late dosing period which indicates that some nitrogen limitation may have occurred (Figure 16). The Control (0N:0P) was P-limited through August (TN:TP>17) but approached 15:1 (near optimum) in the final two samples. Analyses indicated that TN:TP was significantly influenced by N-dose ($p<0.0001$), P-dose ($p<0.02$), and N-dose*P-dose interactions ($p<0.02$). In samples taken the day following the first dose, TN:TP ratios in the P-dosed treatments were 2–3 times larger than predicted based on the loading ratios, whereas ratios in all 0P treatments were within 12% of calculations. Therefore, added nitrogen remained in the water column, while phosphorus additions were rapidly lost through sedimentation or uptake by periphyton and macrophytes. At midseason, TN:TP ratios in all treatments ranged from 17:1 and 25:1. Following the P-dose increase during the Late dosing period (beginning July 29), N:P ratios decreased (range 4–30). Internal P-loading (evidenced in SRP and TP data) and P-dose modifications lowered ratios to between 10 and 17 in all treatments at some point in the Late season.

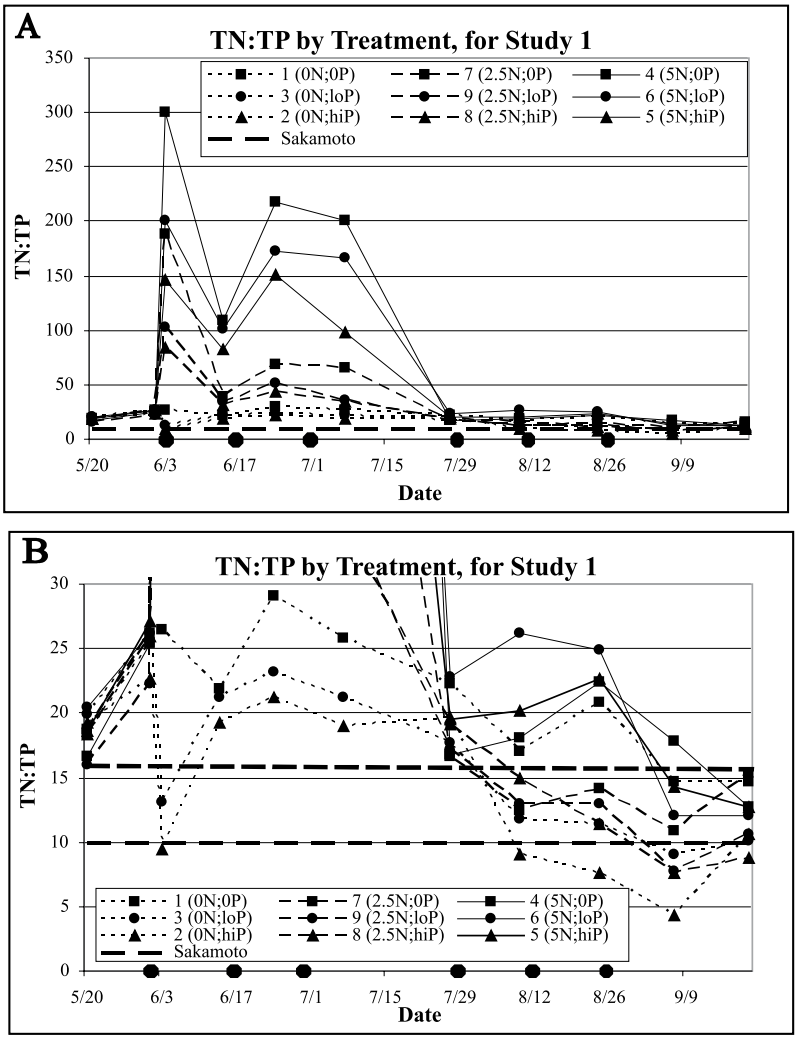
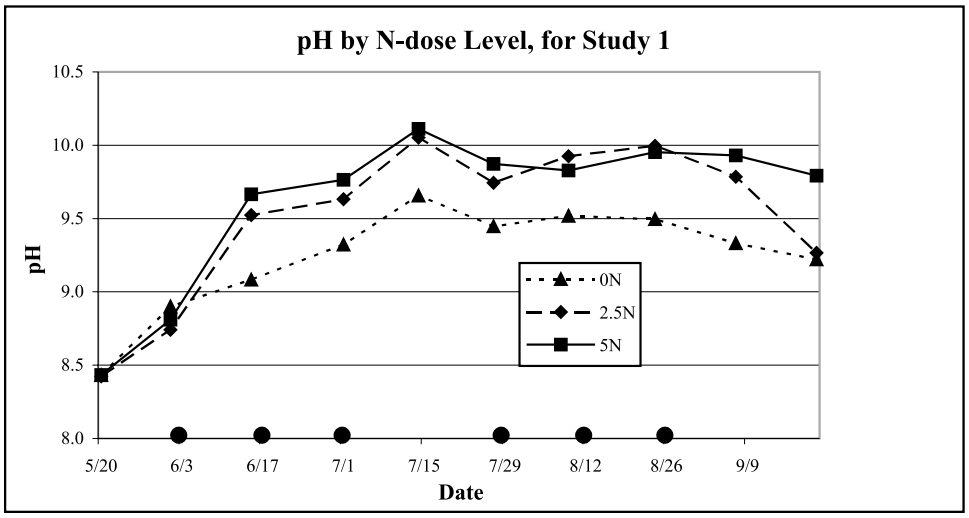


Figure 16. *Changes in the ratio of total nitrogen to total by treatment over time. A) TN:TP ratios in all treatments presented with a heavy dashed line representing the range for optimal algal growth proposed by Sakamoto (1966). B) The lower graph is the same as (A), but with the scale enlarged to delineate ratios in the late season. Dark circles on X axis mark dose dates.*

pH

N-dose, Day, and the N-dose*Day interaction had significant effects on pH; however, P-dose had no significant effect. Levels of pH in 0N increased from 8.4 to 9.7 during the Early season, and then decreased to 9.2 by September (Figure 17). The pH levels significantly increased ($p<0.001$) in the N-dosed treatments compared to the 0N treatment within two weeks of the first dose and remained above 9.5 the remainder of the season. This increase in pH occurred concurrently with the observed increase in primary productivity which is expected as available dissolved carbon dioxide decreases due to increase photosynthetic uptake of carbon (Wetzel 1983). Thus pH was a good surrogate indicator of the positive effects of N-dose and Day on primary productivity. However, there were significant interactions among N-dose and Day during the Early, Late, and Full-study components which indicate that neither main effect, alone, was solely responsible for observed increases in pH.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
pH	Full	0.0001	0.0844	0.9726	0.0001	0.0001	0.6194	0.9955
	Early	0.0001	0.1901	0.8664	0.0001	0.0033	0.8201	0.9996
	Late	0.0001	0.0506	0.9818	0.0001	0.0001	0.2298	0.2191



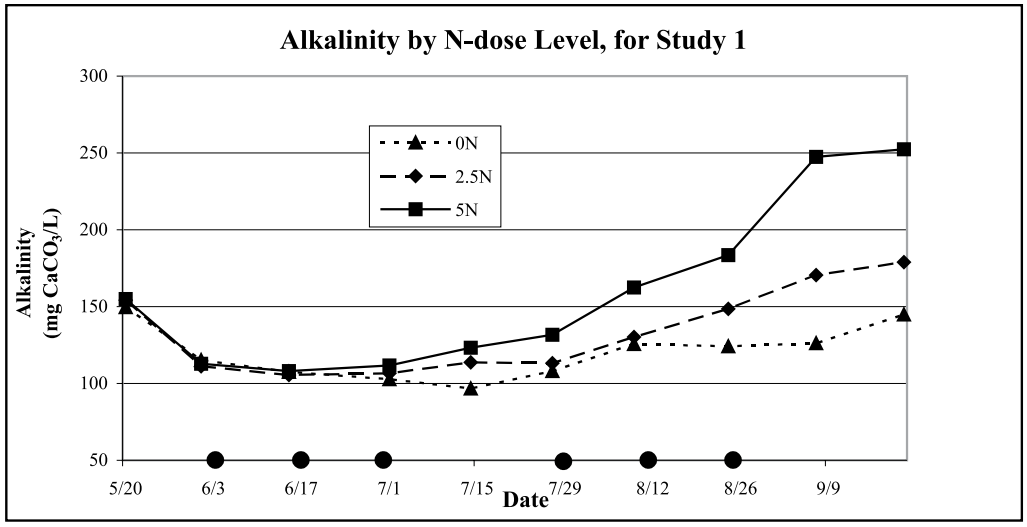
		Date										Season
		5/20	6/1	6/14	7/1	7/14	7/27	8/9	8/24	9/7	9/21	Avg.
N-dose	0	8.4	8.9	9.1[a]	9.3[a]	9.7[a]	9.5[a]	9.5[a]	9.5[a]	9.3[a]	9.2[a]	9.1[a]
	2.5	8.4	8.7	9.5[b]	9.6[b]	10.1[b]	9.7[ab]	9.9[b]	10.0[b]	9.8[b]	9.3[b]	9.1[b]
	5	8.4	8.8	9.7[b]	9.8[b]	10.1[b]	9.9[b]	9.8[b]	10.0[b]	9.9[c]	9.8[c]	9.2[c]

Figure 17. *Changes in pH by nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced pH in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of pH over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose (data were averaged by H-ion concentration, then converted to pH: calculated pH= - log (H-ion). Dark circles on X axis mark dose dates. The lower table lists pH represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Alkalinity and Hardness

Alkalinities in 0N ranged from 97–150 mg $CaCO_3$/L during the study; the lowest values of alkalinity occurred in mid-July (Figure 18). N-dose, Day, and the N-dose*Day interaction had significant effects on alkalinity, which rose continuously after dose initiation to over 175 and 250 mg $CaCO_3$/L in 2.5N and 5N, respectively. Alkalinities in the 5N treatment were significantly higher ($p<0.05$) than 0N and 2.5N from July through the end of the season. Values in the 2.5N were only significantly higher than 0N on July 14 and the last three dates of the season.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Alk.	Full	0.0001	0.7345	0.7961	0.0001	0.0001	0.3286	0.8250
	Early	0.0033	0.8571	0.6605	0.0001	0.0674	0.2470	0.9088
	Late	0.0001	0.4856	0.6464	0.0001	0.0001	0.9018	0.2777

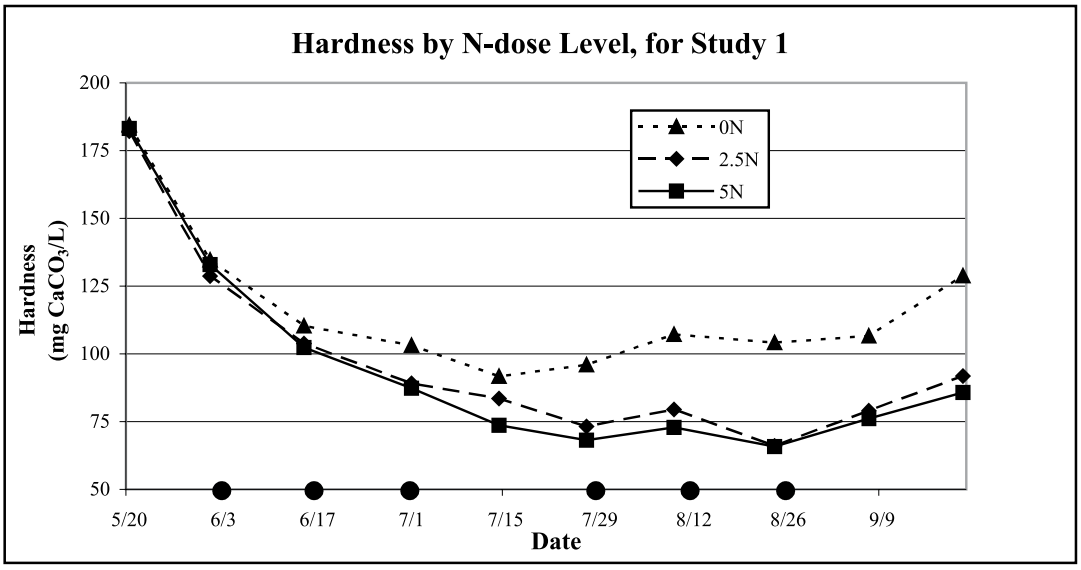


		Date										Season
		5/20	6/1	6/14	7/1	7/14	7/27	8/9	8/24	9/7	9/21	Avg.
N-dose	0	150	115	108	103[a]	97[a]	108[a]	126[a]	124[a]	126[a]	145[a]	120[a]
	2.5	154	112	106	107[ab]	114[b]	113[a]	130[a]	149[b]	171[b]	179[b]	133[b]
	5	155	113	108	112[b]	123[c]	132[b]	163[b]	184[c]	248[c]	252[c]	159[c]

Figure 18. *Changes in alkalinity by nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced alkalinity in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of alkalinity over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (mg $CaCO_3$/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Hardness values in 0N decreased from May (184 mg $CaCO_3$/L) to a seasonal minimum in mid-July (92 mg $CaCO_3$/L), and then increased to 129 mg $CaCO_3$/L by the end of September (Figure 19). Hardness values were negatively influenced by N-dosing and Day ($p<0.0001$). The apparent effect of Day corresponds to a decrease in cations in the water column over time following flooding of the experimental wetlands with CERC well water. Loss of hardness levels over time is frequently observed in these systems due to precipitation losses as dissolved carbon dioxide decreases and pH increases due to primary productivity. In treatments receiving either N-dose, hardness values were significantly lower ($p<0.05$) than in the 0N treatment which corresponds to overall positive effects of N-dosing on primary productivity and carbon dioxide removal. Hardness did not differ significantly in the 2.5N and 5N treatments, however.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Hard.	Full	0.0001	0.6185	0.2112	0.0001	0.0001	0.9282	0.7965
	Early	0.0001	0.1554	0.6675	0.0001	0.0001	0.5679	0.8249
	Late	0.0001	0.5589	0.2905	0.0001	0.2906	0.9411	0.5647



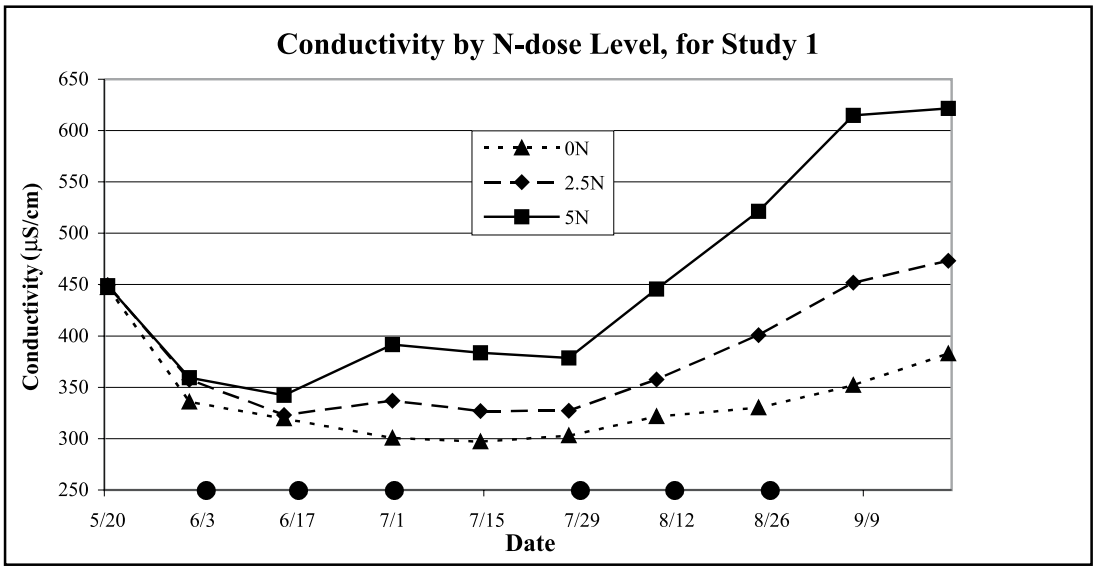
		Date										Season
		5/20	6/1	6/14	7/1	7/14	7/27	8/9	8/24	9/7	9/21	Avg.
N-dose	0	184	135	110[a]	103[a]	92[a]	96[a]	107[a]	104[a]	107[a]	129[a]	117[a]
	2.5	182	128	104[ab]	89[b]	84[b]	73[b]	80[b]	66[b]	79[b]	92[b]	98[b]
	5	183	133	102[b]	87[b]	74[c]	68[b]	73[b]	66[b]	76[b]	86[b]	95[b]

Figure 19. *Changes in hardness by nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced hardness in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of hardness over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (mg $CaCO_3$/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Conductivity

Both N-dose, Day, and N-dose*Day interaction had significant effects on conductivity of the corrals. Conductivity in 0N corrals declined from initial levels of 450 µS/cm to seasonal minimums (297 µS/cm) at mid-season (Figure 20). During the Late season, 0N conductivity steadily rose to 383 µS/cm. Conductivity was contributed by two major factors: 1) well water used to fill the corrals prior to study initiation, and 2) N-dosing using sodium nitrate. Conductivity decreased early in the study due to the gradual loss of ions due to precipitation reactions in the water column. N-dosing, initiated in early June, significantly increased conductivity ($p<0.0001$) in the 2.5N and 5N treatments due to the influence of sodium (Na) in the fertilizer. Conductivity in 5N was significantly higher ($p<0.05$) than both 0N and 2.5N from June 14 through the end of the season, attaining over 600 µS/cm by September. The 2.5N treatments had significantly higher conductivity than the 0N treatments from July 1 through September. Thus, conductivity was an artifact of the experimental treatment as opposed to a response variable related to eutrophication.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Cond.	Full	0.0001	0.7040	0.8247	0.0001	0.0001	0.9623	0.9696
	Early	0.0001	0.9665	0.4561	0.0001	0.0001	0.9785	0.9157
	Late	0.0001	0.7032	0.7437	0.0001	0.0001	0.1003	0.3626



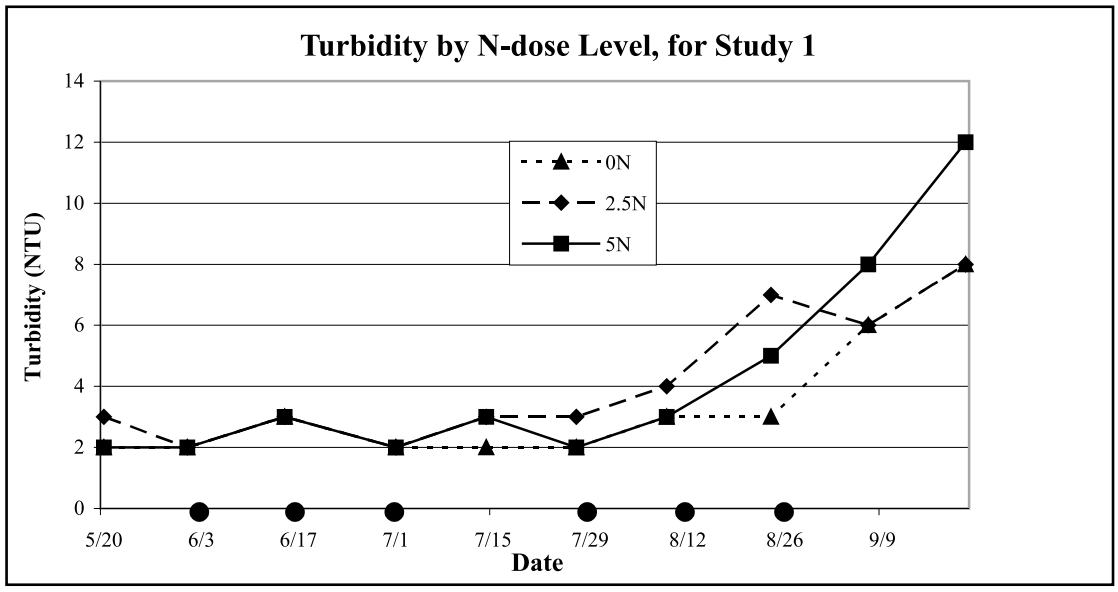
		Date										Season
		5/20	6/1	6/14	7/1	7/14	7/27	8/9	8/24	9/7	9/21	Avg.
N-dose	0	447	336	319[a]	301[a]	297[a]	303[a]	322[a]	330[a]	352[a]	383[a]	339[a]
	2.5	450	357	323[a]	337[b]	327[b]	327[b]	358[b]	401[b]	452[b]	473[b]	380[b]
	5	449	359	342[b]	392[c]	384[c]	379[c]	446[c]	521[c]	615[c]	622[c]	451[c]

Figure 20. *Changes in conductivity by nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced conductivity in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of conductivity over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (µS/cm) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Turbidity

The experimental enclosures were minimally affected by wave action, in-flow, and disturbance. Therefore, turbidity values primarily reflected living and detrital material as opposed to suspended sediment. In the Early dosing period, turbidity values were similar among treatments and only ranged from 2–3 NTU's (Figure 21). Turbidity increased during the Late dosing period due to the main treatment effects of N-dose, Day, and the N-dose*Day interaction. P-dose had no significant effect on turbidity; however, the P-dose*Day and the N-dose*P-dose*Day interactions were significant due to the Late dosing regime. Overall, turbidity increased in all treatments (4 to 6-fold). Although this increase was significantly related to N-dose, the increases in the 0N treatment indicated that turbidity increases, in part, were due to release of nutrients and organic matter as macrophytes senesced as discussed below in relation to phytoplankton dynamics.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Turb.	Full	0.0292	0.8732	0.5126	0.0001	0.0001	0.2042	0.2958
	Early	0.6693	0.9171	0.1474	0.0001	0.5685	0.5483	0.9378
	Late	0.0033	0.3784	0.6334	0.0001	0.0005	0.0295	0.0200



		Date										Season
		5/20	6/1	6/14	7/1	7/14	7/27	8/9	8/24	9/7	9/21	Avg.
N-dose	0	2	2	3	2	2	2	3^{a}	3^{a}	6^{a}	8^{a}	3^{a}
	2.5	3	2	3	2	3	3	4^{b}	7^{b}	6^{a}	8^{ab}	4^{b}
	5	2	2	3	2	3	2	3^{b}	5^{b}	8^{b}	12^{b}	4^{b}

Figure 21. *Changes in turbidity by nitrogen dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced turbidity in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of turbidity over the entire experimental season. Because N-dose and N-dose*Day were significant influences in ANOVA, values are pooled by N-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (NTU) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not signifi-*

Phytoplankton

Chlorophyll *a* was used as an indicator of phytoplankton biomass. Day ($p<0.0001$), and to a lesser extent phosphorus ($p=0.0399$), had significant effects on chlorophyll during the Early dosing period. Chlorophyll remained <25 µg/L in all treatments from May through July (Figure 22) during the period of initial macrophyte growth. Neither N-dose or P-dose had significant effects on chlorophyll during the Late dosing period, in part due to the high inherent variability among replicates; standard deviations frequently exceeded 100 µg/L (n=4 replicates). In the Control (0N:0P), chlorophyll was <12 µg/L until the last two days of the season when values increased to above 50 µg/L. In the Control, chlorophyll was significantly correlated with turbidity ($r^2= 0.82$; $p<0.0001$), TN ($r^2= 0.70$; $p<0.0001$), and TP ($r^2= 0.66$; $p<0.0001$). Relations between chlorophyll and turbidity, TN, and TP were generally weaker in the dosed treatments than in the Control. These results indicate that there was tight coupling between macrophyte growth and nutrient uptake which limited phytoplankton growth during the Early dosing period. As macrophytes began to senesce, macrophyte:nutrient relationships were less tightly coupled as reflected in increased chlorophyll, turbidity, and dissolved nutrients.

Monthly planktonic algal identifications yielded 99 species during Study 1, representing the divisions Chlorophyta (greens), Cyanophyta (cyanobacteria or blue-greens), Cryptophyta (cryptomonads), Bacillarophyta (diatoms), Euglenophyta (euglenoids), Xanthophyta (yellow-greens), Pyrrophyta (dinoflagellates), and Chrysophyta (golden-browns) (Table 4). Total species richness was similar among treatments and averaged approximately eight species during summer, with slightly fewer species in May and September.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Chl	Full	0.0986	0.4311	0.8651	0.0001	0.5062	0.0513	0.8492
	Early	0.3438	0.0399	0.8861	0.0001	0.9225	0.4139	0.5958
	Late	0.1668	0.1284	0.8800	0.0001	0.0236	0.2754	0.8347

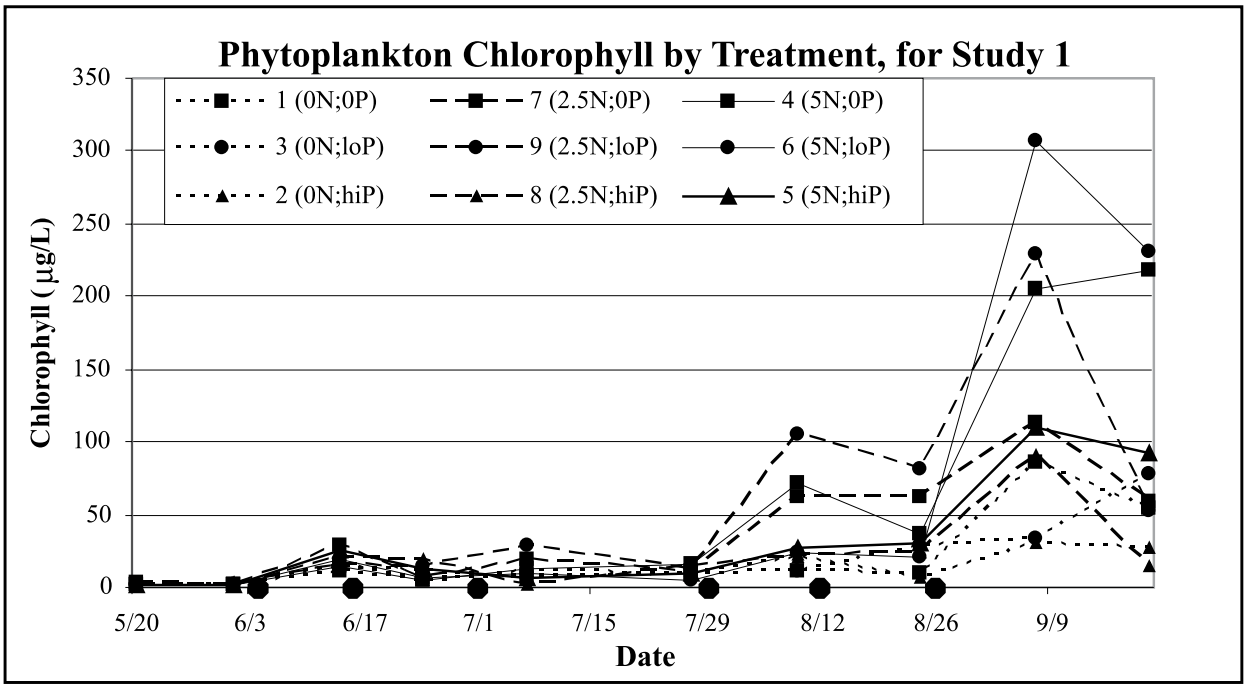


Figure 22. *Changes in phytoplankton chlorophyll by treatment over time. The table presents probabilities that dose, day, and interactions of dose and day influenced chlorophyll in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of chlorophyll over the entire experimental season. Dark circles on X axis mark dose dates.*

Table 4. List of Phytoplankton Species Collected During Study 1

Kingdom	Phylum/ Division	Class	Order	Family	Genus	Species
Monera	Cyanophycota	Cyanophyceae	Nostocales	Nostocaceae	Anabaena	sp.
					Anabaena	flosaquae
					Anabaena	inequalis
					Anabaena	planctonica
					Anabaena	variabilis
					Aphanizomenon	sp.
					Pseudanabaena	sp.
				Oscillatoriaceae	Oscillatoria	sp.
					Oscillatoria	tenuis
				Rivulariaceae	Calothrix	sp.
					Gloeotrichia	sp.
			Chroococcales	Chroococcaceae	Aphanocapsa	sp.
					Aphanothece	sp.
					Chroococcus	sp.
					Chroococcus	limneticus
					Chroococcus	varius
					Merismopedia	punctata
					Microcystis	aeruginosa
Plantae	Bacillariophyta	Bacillariophyceae	Achnanthales	Achnanthaceae	Achnanthes	sp.
				Cocconeidaceae	Cocconeis	sp.
					Cocconeis	placentula
			Bacillariales	Bacillariaceae	Nitzschia	sp.
			Cymbellales	Cymbellaceae	Cymbella	sp.
					Cymbella	minuta
				Gomphonemataceae	Gomphonema	sp.
					Gomphonema	augur
					Gomphonema	olivaceum
				Rhoicospheniaceae	Rhoicosphenia	curvata
			Eunotiales	Eunotiaceae	Eunotia	sp.
					Eunotia	pectinalis
			Naviculales	Naviculaceae	Navicula	sp.
Plantae	Bacillariophyta	Bacillariophyceae	Naviculales	Pinnulariaceae	Pinnularia	sp.
			Rhopalodiales	Rhopalodiaceae	Epithemia	sp.
					Rhopalodia	gibba
			Surirellales	Surirellaceae	Cymatopleura	sp.
					Surirella	sp.

Table 4. List of Phytoplankton species Collected During Study 1 (Continued)

Kingdom	Phylum/ Division	Class	Order	Family	Genus	Species
					Surirella	brebissonii
			Biddulphiales	Biddulphiaceae	Hydrosera	sp.
			Melosirales	Melosiraceae	Melosira	islandica
					Melosira	varians
			Thalassiosirales	Stephanodiscaceae	Cyclotella	meneghiniana
		Fragilariophyceae	Fragilariales	Fragilariaceae	Diatoma	sp.
					Fragilaria	sp.
					Fragilaria	capucina
					Fragilaria	crotonensis
					Synedra	sp.
					Synedra	delicatissima
					Synedra	ulna
			Tabellariales	Tabellariaceae	Tabellaria	sp.
					Tabellaria	fenestrata
	Chlorphyta	Chlorophyceae	Chlorococcales	Oocystaceae	Ankistrodesmus	falcatus
					Chlorella	sp.
					Oocystis	sp.
					Oocystis	pusilla
					Quadrigula	sp.
				Scenedesmaceae	Coelastrum	sp.
					Coelastrum	microporum
					Crucigenia	sp.
					Crucigenia	apiculata
					Crucigenia	irregularis
					Crucigenia	quadrata
					Crucigenia	retangularis
					Scenedesmus	sp.
					Scenedesmus	acuminatus
Plantae	Chlorphyta	Chlorophyceae	Chlorococcales	Scenedesmaceae	Scenedesmus	arcuatus
					Scenedesmus	bijuga
					Scenedesmus	brasiliensis
					Scenedesmus	quadricauda
			Oedogoniales	Oedogoniaceae	Bulbochaete	sp.
					Oedogonium	sp.
			Tetrasporales	Palmellopsidaceae	Pseudosphaerocystis	sp.

Table 4. List of Phytoplankton Species Collected During Study 1 (Continued)

Kingdom	Phylum/ Division	Class	Order	Family	Genus	Species
					Sphaerocystis	sp.
			Ulotrichales	Ulotrichaceae	Ulothrix	sp.
					Ulothrix	tenerrima
			Volvocales	Chlamydomonadaceae	Chlamydomonas	sp.
					Chlamydomonas	globosa
					Chlamydomonas	ovata
				Volvocaceae	Eudorina	sp.
					Eudorina	elegans
					Pleodorina	californica
			Zygnematales	Desmidiaceae	Closterium	sp.
					Closterium	littorale
					Cosmarium	sp.
					Cosmarium	botrytis
					Micrasterias	sp.
					Staurastrum	sp.
				Zygnemataceae	Mougeotia	sp.
					Spirogyra	sp.
		Cryptophyceae	Cryptomonadales	Cryptomonadaceae	Rhodomonas	minuta
					Rhodomonas	minuta var. nannoplanctica
	Chrysophyta	Chrysophyceae	Ochromonadales	Synuraceae	Synura	sp.
	Euglenophycota	Euglenophyceae	Euglenales	Euglenaceae	Euglena	sp.
					Phacus	longicauda
					Trachelomonas	sp.
					Trachelomonas	scabra
	Pyrrophycophyta	Dinophyceae	Peridiniales	Glenodiniopsidaceae	Hemidinium	nasutum
	Xanthophyta	Xanthophyceae	Vaucheriales	Vaucheriaceae	Vaucheria	sp.

Algal enumeration at the division level revealed seasonal patterns in total and relative abundances that were similar among treatments (Figure 23). In general, total algal densities were highest in mid-August (mean = $7.1*10^6$ cells/L). Densities declined by September to approximately half of August levels. In the Early season, cryptomonads averaged $8.8*10^5$/L and were the numerically dominant taxa in May (92% of populations) and June (38% of populations) during the early dosing interval. However, during the Late summer season, cryptomonad populations contributed <5% to the total phytoplankton community. Blue-green populations peaked in summer and averaged $5*10^5$ cells/L during the June–August period, but were never the numerically dominant taxa. Green algae were poorly represented in May samples ($<1*10^4$ cells/L) but represented 30% of the algae ($5*10^5$ cells/L) in June. Chlorophytes were the dominant taxa in July, August, and September, comprising 48, 91, and 90% of the community, respectively. Chlorophyte populations peaked in August (average $>6*10^6$ cells/L). Diatoms represented <5% of the total phytoplankton community throughout the 4-month study. Euglenophyta, Pyrrophyta, Xanthophyta, and Chrysophyta were infrequently encountered; their occurrence was not consistent among corrals within a given treatment and did not show a seasonal relationship.

Cyanobacteria observed during the study included *Anabaena, Aphanizomenon, Aphanothece, Calothrix, Gloeotrichia, Oscillatoria*, and *Pseudanabaena* sp. Statistical analysis indicated that there was not a substantial difference in the total abundance of these genera among treatments. Two corrals from the Control (0N:0P) and one corral from 5N:0P experienced blooms of *Anabaena* and *Aphanizomenon* in July and August. TN:TP ratios in those corrals prior to the blooms (>16:1) indicated that nitrogen was not strongly limiting. Likewise, TN in bloom corrals was similar to replicate corrals prior to and subsequent to the blooms. Although cyanobacteria are capable of "fixing" atmospheric nitrogen under nitrogen-limited conditions, there were no trends to indicate that nitrogen fixation was a nominal factor in nitrogen dynamics or primary productivity in the loP or hiP treatments during the study.

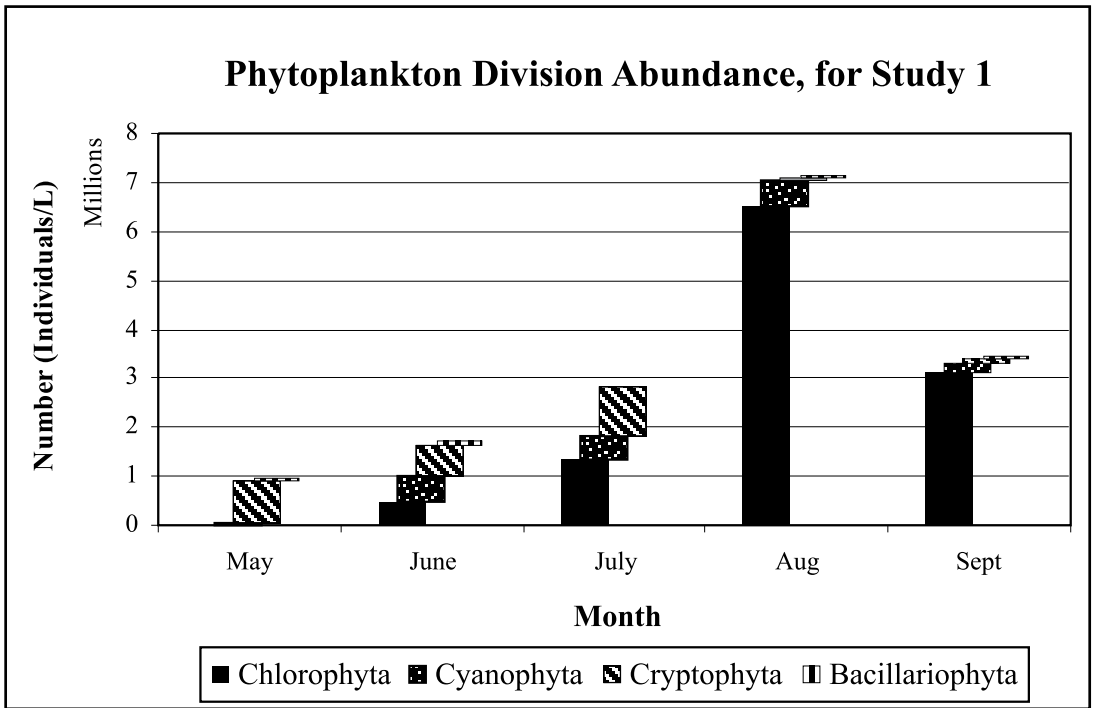


Figure 23. *Changes in phytoplankton abundance of the four dominant divisions over time. Columns represent averages of all corrals in each month to show general successional trends over the season.*

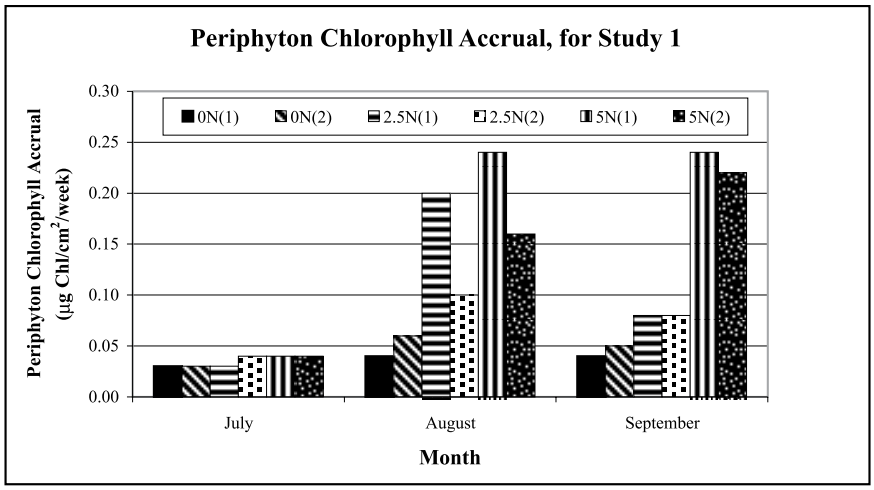
Periphyton

Periphyton biomass was measured as the accrual rate of chlorophyll *a* on Scrimweave™ strips. Accrual rates of periphyton biomass (expressed as µg Chl/cm^2/wk) were measured in July, August, and September which corresponded to the Early, Late, and post-dosing periods. During each interval, strips were incubated and retrieved for both 1-week and 2-week intervals to determine periphyton accrual rates related to nutrient dosing. In this analysis, the data were statistically analyzed for main effects using the entire combined dataset.

Day ($p<0.0001$), N-dose ($p<0.0001$), and P-dose ($p=0.0336$) had significant effects on periphyton accrual rates in the 1-week periphyton growth interval; the N-dose*Day interaction ($p=0.0043$) was also significant (Figure 24). Day ($p<0.0001$) and N-dose ($p<0.0001$) had significant effects on the 2-week periphyton response; however, P-dose had no effect (Figure 24).

Control periphyton accrual rates averaged 0.05 µg Chl/cm^2/wk for both 1-week and 2-week growth intervals during the July, August, and September sampling intervals. Neither N-dose nor P-dose was a significant factor in the July data, however, which corresponded to the Early dosing interval. In contrast, nutrient dosing significantly increased the 1-week periphyton accrual rates during August which occurred during the Late dosing period; however, there were no significant nutrient dosing effects on the 2-week data. The weaker associations between periphyton accrual and dosing in the 2-week exposures, in which accrual rates began to decrease, could have been due to nutrient limitation, biomass

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Peri. 1-wk	Full	0.0001	0.0336	0.9892	0.0001	0.0043	0.7177	0.9591
Peri. 2-wk	Full	0.0001	0.0913	0.9296	0.0001	0.1312	0.4456	0.2106



		Month						Season Avg.	
		July		Aug		Sept			
		1	2	1	2	1	2	1	2
N-dose	0	0.03	0.03	0.04^a	0.06	0.04^a	0.05	0.04^a	0.04^a
	2.5	0.03	0.04	0.20^b	0.10	0.08^b	0.08	0.10^b	0.07^b
	5	0.04	0.04	0.24^b	0.16	0.24^c	0.22	0.17^b	0.14^c

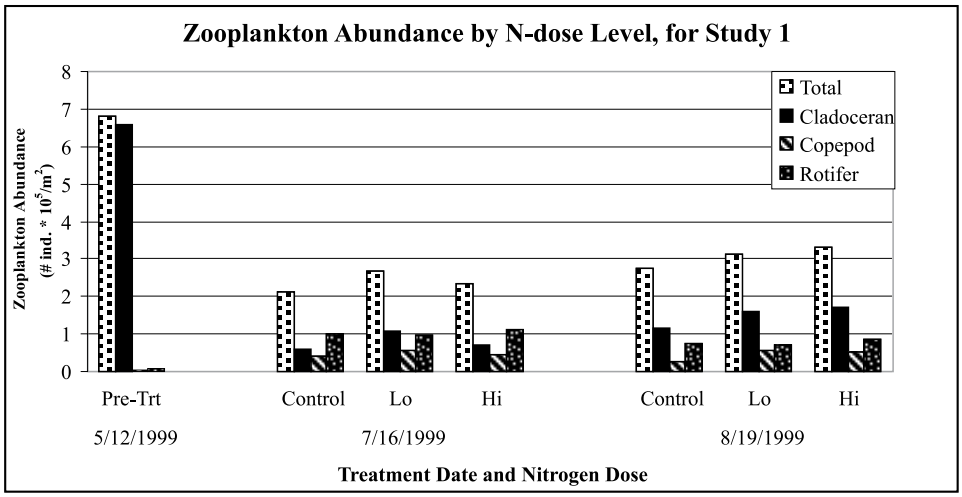
Figure 24. *Changes in periphyton chlorophyll accrual rates over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced periphyton accrual in ANOVA of rank-transformed data. Data from one and two-week exposures were analyzed separately. Darkened values are not significant ($p>0.05$). The graph is a plot of accrual rates in both datasets. Solid and striped bars represent (1) and (2) week exposures, respectively. Because N-dose was a significant influence in ANOVA of both datasets, values are pooled by N-dose in the graph. The lower table lists LS Means (mg Chl/cm^2/wk) for both datasets represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different. Significant differences between monthly values within the 2-week data set are not shown due to the lack of significant influence on the model.*

loss, or shifts in the species composition of the periphyton community. N-dose at the 5N dosing level had significant effects on both the 1-week and 2-week accrual rates in September during the post-dosing interval. Both N-dose and P-dose had significant effects on 1-week and 2-week periphyton accrual rates when tested across the seasonal average, resulting in an average of 0.4, 0.1, and 0.17 µg Chl/cm²/wk for the Control, 2.5N, and 5N treatments, respectively (1-week data); and 0.4, 0.07, and 0.14 µg Chl/cm²/wk for the Control, 2.5N, and 5N treatments, respectively (2-week data). Collectively, the data indicated that both N-dose and P-dose influenced periphyton accrual during the Late and post-dosing periods but had minimal effects early in the study. Thus, periphyton productivity most likely played a much greater role in overall system productivity late in the study compared to the early component of the study when macrophytes probably were more dominant factors.

Zooplankton

N-dose had significant effects (p=0.0295) on total numbers of copepods; however, neither N-dose or P-dose had significant effects (p>0.05) on total numbers of zooplankton, total numbers of cladocerans, or total numbers of rotifers in Study 1 (Figure 25). However, Day was a significant factor for total numbers of zooplankton (p= 0.0212) and total numbers of cladocerans (p=0.0013). Highest actual numbers of zooplankton occurred during the early, pre-treatment period when total numbers reached approximately 6.8 * 10^5 zooplankton/m²; over 95% of total zooplankton was represented by cladocerans during this pre-treatment period. Total numbers of zooplankton appeared to decline across treatments

	N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Total # zooplankton	0.3928	0.2251	0.7789	0.0212	0.7276	0.9380	0.5520
Total # cladocerans	0.1517	0.1311	0.6708	0.0013	0.2643	0.8668	0.5696
Total # copepods	0.0295	0.2877	0.9829	0.3235	0.0689	0.6800	0.8138
Total # rotifers	0.9359	0.2544	0.5545	0.1962	0.7919	0.2445	0.6261



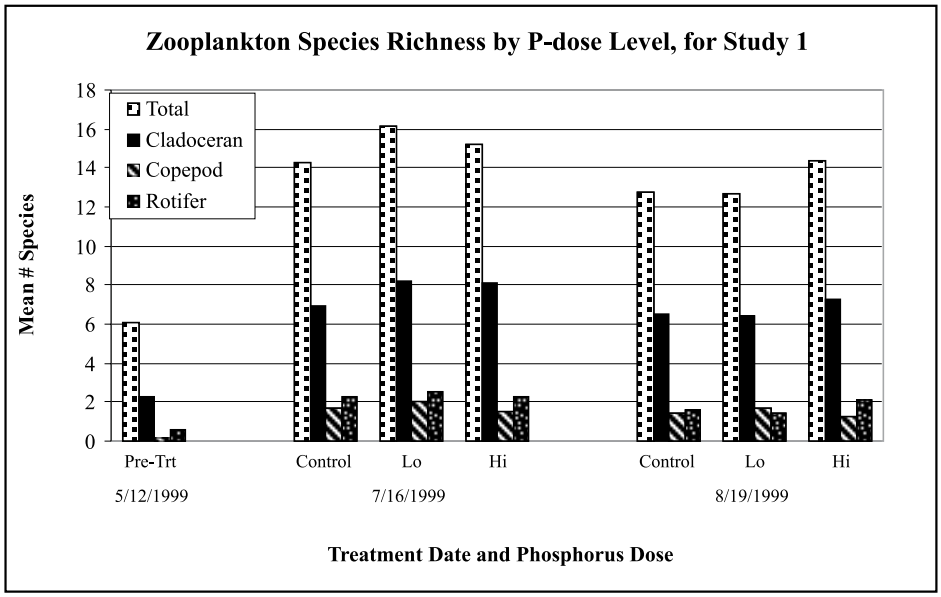
		Average number of copepods (# x10^5/m²)		
		5/12/1999	7/16/1999	8/19/1999
N-dose	Control	0.3	0.42[a]	0.26[a]
	2.5	0.3	0.57[a]	0.56[b]
	5	0.3	0.44[a]	0.52[a]

Figure 25. *Changes in total numbers of copepods over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced total numbers of zooplankton by major group in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). Because N-dose was a significant influence in ANOVA for total number of copepods, values are pooled by N-dose in the graph. The lower table lists LS Means (total number of copepods) along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

in the July and August sampling periods to less than 3.2 * 10^5 zooplankton/m^2; however, these samples were taken with the passive activity traps as opposed to the pre-treatment period which was net samples corrected to the same sq. meter basis of comparison. In addition, proportions of copepods and rotifers increased compared to cladocerans. The observed increase in total numbers of copepods was statistically related to N-dose (p=0.0295) but not P-dose or Day. Total number zooplankton species was significantly related to Day (p=0.0005) (Figure 26). Total number cladoceran species was significantly related to P-dose (p=0.0041), Day (p=0.0003), and the P-dose*day interaction (p=0.0281).

There were a total of 31 species of zooplankton species identified in Study 1; the species list is presented in Table 5. Thirteen species were cladocerans; 5 species were copepods; 13 species were rotifers; and one species was an ostracod. Prior to corral construction (May 12, 1999), the zooplankton community was dominated by two species of cladocera: *Ceriodaphnia reticulata* and *Daphnia pulex* in a 4:1 ratio. By July, however, the zooplankton community had shifted to a dominance of seven species: *Hexartha mira* (Rotifera), *Cypridopsis* sp. (Ostracoda), *Microcylcops rubellus* (Copepoda), *Platyias patulus* (Rotifera), *Chydorus brevilabrus* (Cladocera), *Alona monocantha* (Cladocera),

	N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Total # sp.	0.3192	0.0878	0.0874	0.0005	0.0069	0.1360	0.4634
# Cladoceran sp.	0.8617	0.0041	0.0600	0.0003	0.0602	0.0281	0.3907
# Copepod sp.	0.5020	0.1857	0.9454	0.1233	0.3202	0.9434	0.9771
# Rotifer sp.	0.3515	0.2822	0.6119	0.0871	0.0038	0.1967	0.7883



		Average number of cladoceran species		
		5/12/1999	7/16/1999	8/19/1999
P-dose	Control	2.3	6.9[a]	6.5[a]
	Lo	2.3	8.2[b]	6.4[a]
	Hi	2.3	8.1[b]	7.3[b]

Figure 26. *Changes in zooplankton species richness over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced zooplankton species richness by major group in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). Because P-dose was a significant influence in ANOVA for number of cladoceran species, values are pooled by P-dose in the graph. The lower table lists LS Means (number cladoceran species) along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Table 5. List of Zooplankton Species Collected During Study 1

Phylum	Class	Subclass	Order	Suborder	Family	Genus	Species
Arthropoda	Branchiopoda	Phyllopoda	Diplostraca	Cladocera	Chydoridae	Alona	guttata
						Alona	monocantha
						Alona	setulosa
						Chydorus	brevilabris
						Dunhevedia	crassa
						Kurzia	latissima
						Leydigia	acanthocercoides
						Pleuroxus	denticulatus
					Daphniidae	Ceriodaphnia	reticulata
						Daphnia	pulex
						Scapholeberis	armata
						Simocephalus	vetulus
					Sididae	Diaphanosoma	brachyurum
	Maxillopoda	Copepoda	Calanoida		Diaptomidae		pallidus
			Cyclopoida		Cyclopidae	Eucyclops	agilis
						Macrocyclops	albidus
						Microcyclops	rubellus
	Ostracoda	Podocopa			Cypridopsidae	Cypridopsis	sp.
Rotifera	Monogononta		Flosculariaceae		Filiniidae	Filinia	longiseta
					Hexarthridae	Hexarthra	mira
			Ploima		Asplanchnidae	Asplanchna	sieboldi
					Brachionidae	Euchlanis	sp.
						Keratella	cochlearis
						Keratella	testudo
						Mytilina	ventralis
						Platyias	patulus
						Platyias	quadricornis
					Lecanidae	Lecane	leontina
						Lecane	luna
						Monostyla	quadridentata
					Synchaetidae	Polyarthra	remata

and *C. reticulata* (Cladocera). Slight shifts in dominance of the top 7 species were observed in August: *Cypridopsis sp.* (Ostracoda), *P. patulus* (Rotifera), *Dunhevedia crassa* (Cladodera), *Hexartha mira* (Rotifera), *Chydorus brevilabrus* (Cladocera), *M. rubellus* (Copepoda), and *C. reticulata* (Cladocera). Total numbers of cladocerans were significantly correlated with chlorophyll *a* (r=0.258; p=0.0285); however, no single species of cladoceran was correlated with chl *a*. *Ceriodaphnia reticulata* was negatively correlated with macrophyte biomass; however, no other cladoceran species demonstrated statistical associations with macrophytes.

Sediment

Nitrogen content of sediment in the Control nearly doubled between May (0.21% N) and June (0.37% N), and then remained near that level for the remainder of the season. ANOVA did not indicate treatment influences, but concentrations in all treatments in May were significantly lower ($p<0.05$) than the other months (Figure 27). Sediments contained around 84 g N/(m^2*5 cm deep) in May, and between 150 and 160 g N/(m^2*5 cm deep) during the remainder of the season. The apparent sediment pool of nitrogen increased seasonally in all treatments due to a combination of macrophyte mobilization/deposition and possibly physical remixing of sediments due to macrophyte and sediment sampling activity. Thus, it is apparent that even in the highest dosing treatment where a total of 30 g of nitrogen was applied (5N treatment), it was difficult to measure temporal increases in loading and transfer of dosed nitrogen to sediments due to the inherent error involved in the procedures used. In fact, early attempts using deposition trays demonstrated significant, yet highly variable accumulations of sediment and detritus in trays due to various factors including biomass sloughing, disturbance by physical activity, and disturbance by gaseous evolution by sediment decomposition processes. Denitrification, not directly measured in this study, may also have been a factor in our inability to detect nitrogen accumulation in sediments over time.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Sed N	Full	0.5556	0.2262	0.8142	0.0001	0.1300	0.7739	0.7751
	Early	0.4488	0.1085	0.4518	0.0001	0.1261	0.5689	0.5757
	Late	0.6950	0.3225	0.7425	0.2731	0.2529	0.6824	0.5818

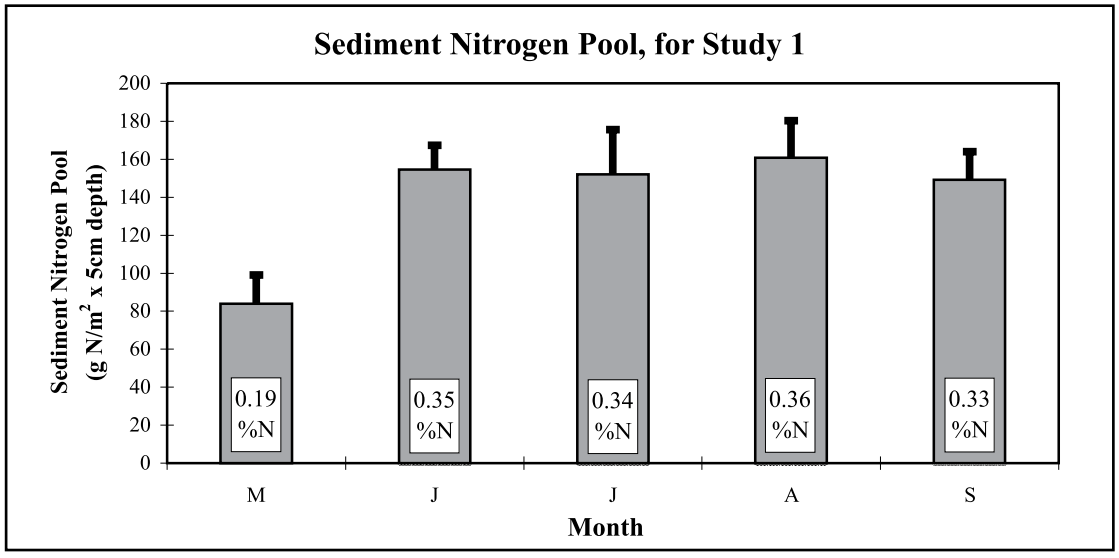


Figure 27. *Changes in sediment nitrogen pool over time. The table presents probabilities that dose, day, and interactions of dose and day influenced N content in sediments in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of the estimated pool of N in the sediments for an area of 1 m^2 and a depth of 5 cm. Because treatment influences were not significant, monthly values are presented as averages of all treatments. The average %N in sediments is shown within the column for each month.*

Phosphorus content of the Control sediments fluctuated between 0.05 and 0.06% P during the experiment (Figure 28). ANOVA indicated that treatment responses were not influenced by N- or P-dosing, and that sediment phosphorus was significantly lower ($p<0.05$) in September than earlier months although differences were slight. Sediments contained between 23 and 25 g P/(m^2*5 cm deep) during the season. Dosing in the highest treatments (hiP) delivered 0.86 g/m^2; therefore, the background levels of phosphorus in sediments inhibited our ability to measure additional phosphorus accumulation. However, due to the fact that phosphorus is conserved (i.e., not cycled to the atmosphere), it is assumed that phosphorus not accounted for in macrophytes or the water column was transferred to sediment.

		N-dose	P-dose	N-dose * P-dose	Day	N-dose * Day	P-dose * Day	N-dose * P-dose * Day
Sed P	Full	0.6170	0.3018	0.5109	0.0001	0.6327	0.9244	0.6701
	Early	0.1828	0.1247	0.6567	0.0011	0.8508	0.9531	0.5493
	Late	0.7065	0.7123	0.3755	0.0001	0.3865	0.6731	0.7113

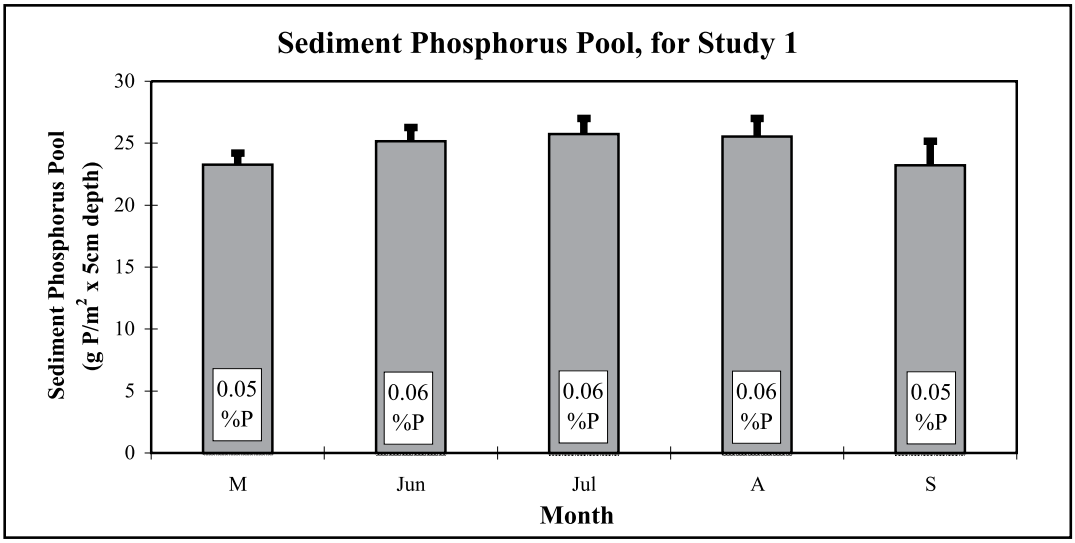
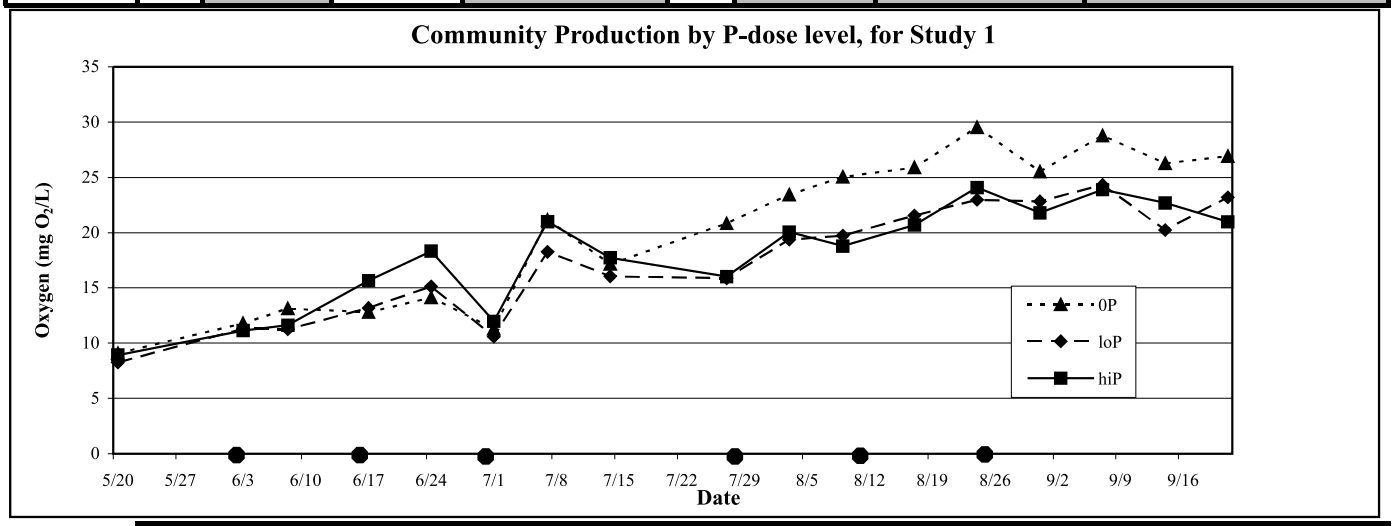


Figure 28. *Changes in sediment phosphorus pool over time. The table presents probabilities that dose, day, and interactions of dose and day influenced P content in sediments in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of the estimated pool of P in the sediments for an area of 1 m^2 and a depth of 5 cm. Because treatment influences were not significant, monthly values are presented as averages of all treatments. The average %P in sediments is shown within the column for each month.*

System Metabolism

Dissolved oxygen was measured on a consecutive morning, evening, and morning sequence and was used to evaluate community metabolism of the corrals through estimations of gross community primary production and respiration. Day ($p<0.0001$), P-dose ($p=0.0430$), and the P-dose*Day (0.0249) interaction were significant factors related to community gross primary production during the 4-month study (i.e., Full dataset) (Figure 29). Prior to dosing, all treatments had similar levels of gross primary productivity and averaged 9 mg/L. As dosing began, gross primary productivity increased in all treatments concurrently with macrophyte development. N-dose was a significant, positive main effect which increased productivity compared to Controls during the Early dosing period; P-dose had no significant effect during the Early dosing period. P-dose had a significant, negative effect on gross primary production. Production estimates in loP and hiP were 20% lower than 0P when averaged over the course of the study; however, the loP and hiP treatments did not differ between each other.

		N-dose	P-dose	N-dose*P-dose*Day	Day	N-dose*Day	P-dose*Day	N-dose*P-dose*Day
Gross Production	Full	0.7528	0.0430	0.7831	0.0001	0.5315	0.0249	0.9966
	Early	0.0470	0.1078	0.8292	0.0001	0.9509	0.1153	1.0000
	Late	0.9622	0.0126	0.4070	0.0001	0.1123	0.8879	0.7420

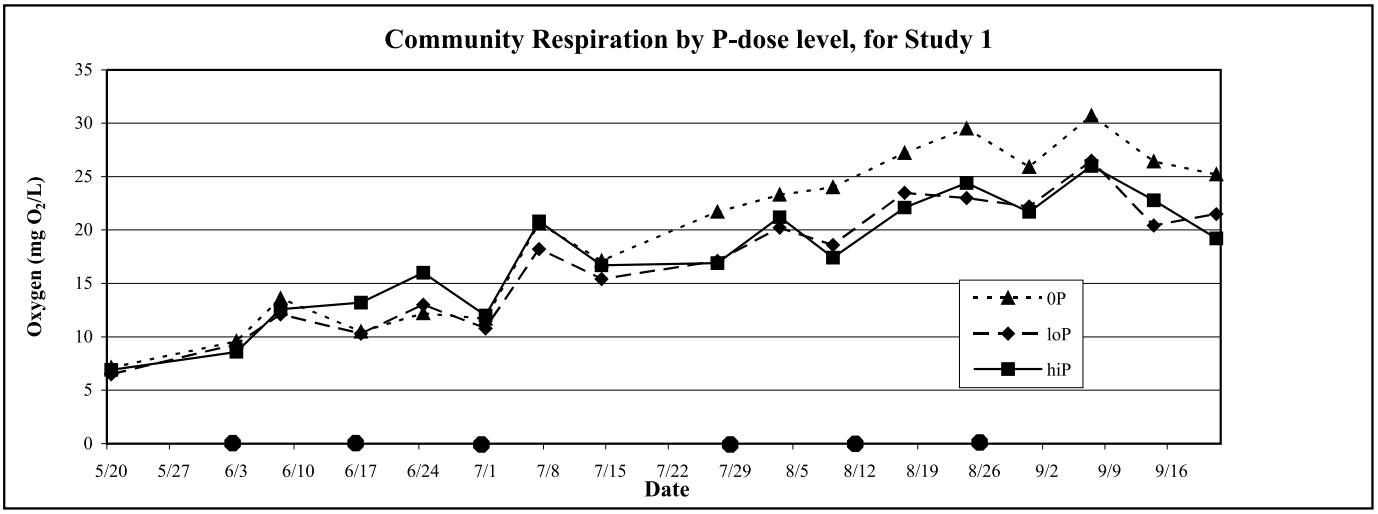


		Date																	Season
		5/20	6/3	6/8	6/17	6/24	7/1	7/7	7/14	7/27	8/3	8/9	8/17	8/24	8/31	9/7	9/14	9/21	Avg.
P-dose	0	9.1	11.8	13.1	12.8	14.1^{a}	11.3	21.1	17.2	20.8^{a}	23.4^{a}	25.0^{a}	25.9^{a}	29.5^{a}	25.5	28.8	26.3^{a}	26.9^{a}	20.2^{a}
	lo	8.2	11.3	11.3	13.2	15.1^{a}	10.6	18.3	16.0	15.9^{b}	19.4^{b}	19.7^{b}	21.6^{ab}	23.0^{b}	22.8	24.3	20.2^{b}	23.2^{ab}	17.3^{b}
	hi	8.9	11.1	11.6	15.7	18.3^{b}	12.0	21.0	17.7	16.0^{b}	20.1^{ab}	18.8^{b}	20.7^{b}	24.1^{b}	21.8	23.9	22.7^{ab}	21.0^{b}	18.0^{ab}

Figure 29. *Changes in community gross oxygen production by phosphorus dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced production in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of production over the entire experimental season. Because P-dose and P-dose*Day were significant influences in ANOVA, values are pooled by P-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (mg O_2/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Trends in community gross respiration were similar to gross primary production in direction and magnitude (Figure 30). Day was a significant factor influencing community respiration; however, N-dose had no effect, and P-dose was significant for only the Late dosing interval and had negative effects compared to the Control 0P treatment. The negative effect of P on both gross community primary productivity and community respiration reflects, and perhaps exacerbates to some degree, the observed macrophyte senescence. Macrophyte stands declined during the Late dosing period in all treatments including the Controls. HiP treatments accumulated more dissolved phosphorus than other treatments (Figure 8), which indicates to some degree that the nutrient assimilatory capacity of the corrals declined along with macrophyte biomass. Phytoplankton productivity increased late in the study in response to increased nutrient availability (Figure 22); however, it was not sufficient to maintain levels of productivity observed in Control treatments. Macrophytes dominate the productivity of these systems, and therefore as macrophyte productivity declined so did the overall estimates of community metabolism.

		N-dose	P-dose	N-dose*P-dose*Day	Day	N-dose*Day	P-dose*Day	N-dose*P-dose*Day
Gross Respiration	Full	0.6750	0.0599	0.8156	0.0001	0.6619	0.0705	0.9955
	Early	0.0615	0.1136	0.8077	0.0001	0.9641	0.2840	1.0000
	Late	0.8474	0.0337	0.5473	0.0001	0.1069	0.8474	0.6095



		Date																Season
		5/20	6/3	6/8	6/17	6/24	7/1	7/7	7/14	7/27	8/3	8/9	8/17	8/24	8/31	9/7	9/14	9/21	Avg.
P-dose	0	7.1	9.6	13.6	10.5	12.2	11.7	20.6	17.1	21.7	23.3	24.0	27.2	29.5	25.9	30.7	26.4	25.2	19.8
	lo	6.5	9.3	12.1	10.3	13.0	10.8	18.2	15.4	17.1	20.2	18.6	23.5	23.0	22.2	26.5	20.4	21.5	17.0
	hi	6.9	8.6	12.6	13.2	16.0	12.0	20.8	16.7	16.9	21.2	17.4	22.1	24.4	21.7	26.0	22.8	19.2	17.6

Figure 30. *Changes in community gross respiration of oxygen by phosphorus dose levels over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced respiration in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of respiration over the entire experimental season. Because P-dose and P-dose*Day were weakly significant (p<0.1) in ANOVA, values are pooled by P-dose in the graph. Dark circles on X axis mark dose dates. The lower table lists LS Means (mg O_2/L) represented in the graph. Statistical comparisons are not presented.*

Net Nutrient Balance

During this study, a maximum of 30 g N/m^2 and 0.86 g P/m^2 was applied. A final mass balance of nutrients was calculated among various nutrient pools to determine the net efficiency of uptake and assimilation of nutrients in these experimental systems (Table 6). At the end of the study, macrophytes contained a total of 13.0, 7.7, and 10.9 g N/m^2 in the Control, 2.5N, and 5N treatments, respectively; water contained an additional 2.14, 3.02, and 4.57 g N/m^2 in the Control, 2.5N, and 5N treatments, respectively. Combined (macrophytes + water), these two nutrient pools contained 15.14, 10.72, and 15.47 g N/m^2 stocks at the end of the study. Thus, a total of 71% (2.5 N treatment) and 52% (5N treatment) of total nitrogen added during the study were found in these two major biological pools at the end of the study. This implies, under simple mass balance conditions (i.e., no loss to the atmosphere), that up to 29% (2.5N treatment) and 48% (5N treatment) of total nitrogen added were absorbed or lost to the sediments as detritus. Actual attempts to measure the amount of nitrogen in sediments were unsuccessful due to a combination of the large mass of pre-existing nitrogen in sediments and the error contributed by our sampling procedures. However, we know that these numbers are conservative, since the Control treatment alone exhibited a total sequestration of 15.14 g N/m^2 in the absence of external nitrogen addition. Thus, it is evident that macrophytes, algae, and sediments combined as an efficient biological, chemical, and physical sink for nitrogen under the study as designed.

Phosphorus, likewise, was efficiently assimilated and retained in the study. At the end of the study, macrophytes contained a total stock of 2.21, 0.96, and 1.24 g P/m^2 in the Control, loP, and hiP treatments, respectively; water contained an additional stock of 0.24, 0.33, and 0.24 P/m^2 in the Control, loP, and hiP treatments, respectively. Thus, macrophytes alone contained more phosphorus, including the Control treatment, than the total externally added to even the hiP treatment. Thus, the 2.5N and 5N treatments at the end of the study had negative sediment transfer coefficients, which mean that even under these conservative assumptions, the macrophytes and water contained more phosphorus than could be explained by external addition and that phosphorus assimilation from water (i.e., added dose) was extremely efficient.

Table 6. Summary Table of the Final Store of Phosphorus and Nitrogen in Water and Macrophytes in Study 1

Nitrogen	**Load** (g N/m^2)	**Macrophytes** (g N/m^2)	**Water** (g N/m^2)	**Macrophytes + Water Total** (g N/m^2)	**Presumed Sediment Transfer** (g P/m^2)
Control	0	13.0	2.14	15.14	NA
2.5	15	7.7 (51%)	3.02 (20%)	10.72 (71%)	4.28 (29%)
5.0	30	10.9 (36%)	4.57 (15%)	15.47 (52%)	14.53 (48%)
Phosphorus	**Load** (g P/m^2)	**Macrophytes** (g P/m^2)	**Water** (g P/m^2)	**Macrophytes + Water Total** (g P/m^2)	**Presumed Sediment-Transfer** (g P/m^2)
Control	0	2.21	0.24	2.45	NA
Lo	0.43	0.96 (223%)	0.33 (77%)	1.29 (300%)	-0.85 (-198%)
Hi	0.86	1.24 (144%)	0.24 (28%)	1.48 (172%)	-0.62 (-72%)

STUDY 2: Effects of Dosing Prior to Macrophyte Development

Study 2 was conducted in year 2000 to evaluate the effect of nutrient loading on nutrient assimilation, cycling, and community responses to enrichment in shallow, vegetated aquatic systems. There were four objectives in Study 2: 1) determine how nutrient loads influenced the concentration and relative distribution of nutrients in the water column, macrophytes, and sediments; 2) evaluate how nutrient loads influenced species composition, biomass, and/or abundance of macrophytes, phytoplankton, periphyton, and zooplankton; 3) determine if nutrient enrichment prior to macrophyte growth induced a phytoplankton-dominated state that persisted throughout the season; and 4) characterize the assimilation and retention capabilities of shallow ponds receiving set weekly nutrient additions, starting prior to macrophyte development.

The hypothesis for Study 2 was that weekly additions of N and P starting prior to macrophyte development would have a negative impact on the macrophytes due to shading by stimulated periphyton and phytoplankton communities in both Lo and Hi treatments. This hypothesis was based on findings in Study 1 that indicated that 25% macrophyte coverage had provided a stable state that could not be shifted by nutrient addition. We predicted that phytoplankton would establish dominance early in the season; zooplankton grazing would not maintain algal biomass at a low level, because nutrient stimulation would allow for an algal growth rate that was higher than the grazing rate. Ultimately, phytoplankton would persist and reduce macrophyte development by shading (Scheffer 1990,1998). Alternatively, in the absence of fish predators, large-bodied zooplankton communities would graze expanding algal populations and maintain water clarity and macrophyte dominance (Brooks and Dodson 1965).

Experimental Design

In Study 2, the frequency of dosing was increased to six weekly additions rather than six bi-weekly additions studied in Study 1 of 1999. We standardized N:P ratios at 25:1 based on the evidence that N:P ratio was not a significant factor in nutrient dynamics or production in Study 1. Finally, nutrient enrichment in Study 2 was initiated prior to observable macrophyte growth. Mesocosms were drained April 11, 2000, for corral construction. A total of 12 corrals were constructed in each of 4 mesocosms (blocks) (Figure 31). After a 26-d draw-down period for corral construction, the ponds were refilled over a 2-day period (May 7-8) and allowed to mix for 2 days prior to raising of sides of corrals on May 9. Water sampling was begun on May 10 and terminated September 12. Dosing began on May 11, 2000, and continued weekly for 6 weeks. A different set of ponds were used in Study 2 to prevent bias due to the previous year's study. There were four replicate corrals for each of the three experimental treatments (n=12 total corrals). There were three treatments in Study 2: 1) a Control, in which no nutrients were added; 2) "Lo", in which the load was 30 g NO_3-N/m^2 and 1.2 g P/m^2 (dosed as 5 mg/L N and 200 µg/L P each of 6 weeks; and 3) "Hi", in which the load was 60 g NO_3-N/m^2 and 2.4 g P/m^2 (dosed as 10 mg/L N and 400 µg/L P each of 6 weeks). The loads in these dosed treatments were two-fold greater than the maximum in 1999 (total load 30 g NO_3-N/m^3 and 0.864 g P/m^3).

Results

Macrophytes

Macrophyte Taxa

The aquatic macrophyte community consisted of >99% *Najas* during the experiment. The macroalgae *Chara* sp., was sparsely present in May but was not noted in subsequent months due to the dominance of *Najas guadalupensis*. The relative absence of *Chara* sp. in the ponds in Study 2 was in contrast to the results observed in Study 1. The lack of *Chara sp.* may have resulted due to the fact that ponds were drawn down longer (26-d draw-down) in year 2000 compared to 1999 (14 days) which may have altered normal seasonal succession of the macrophyte community.

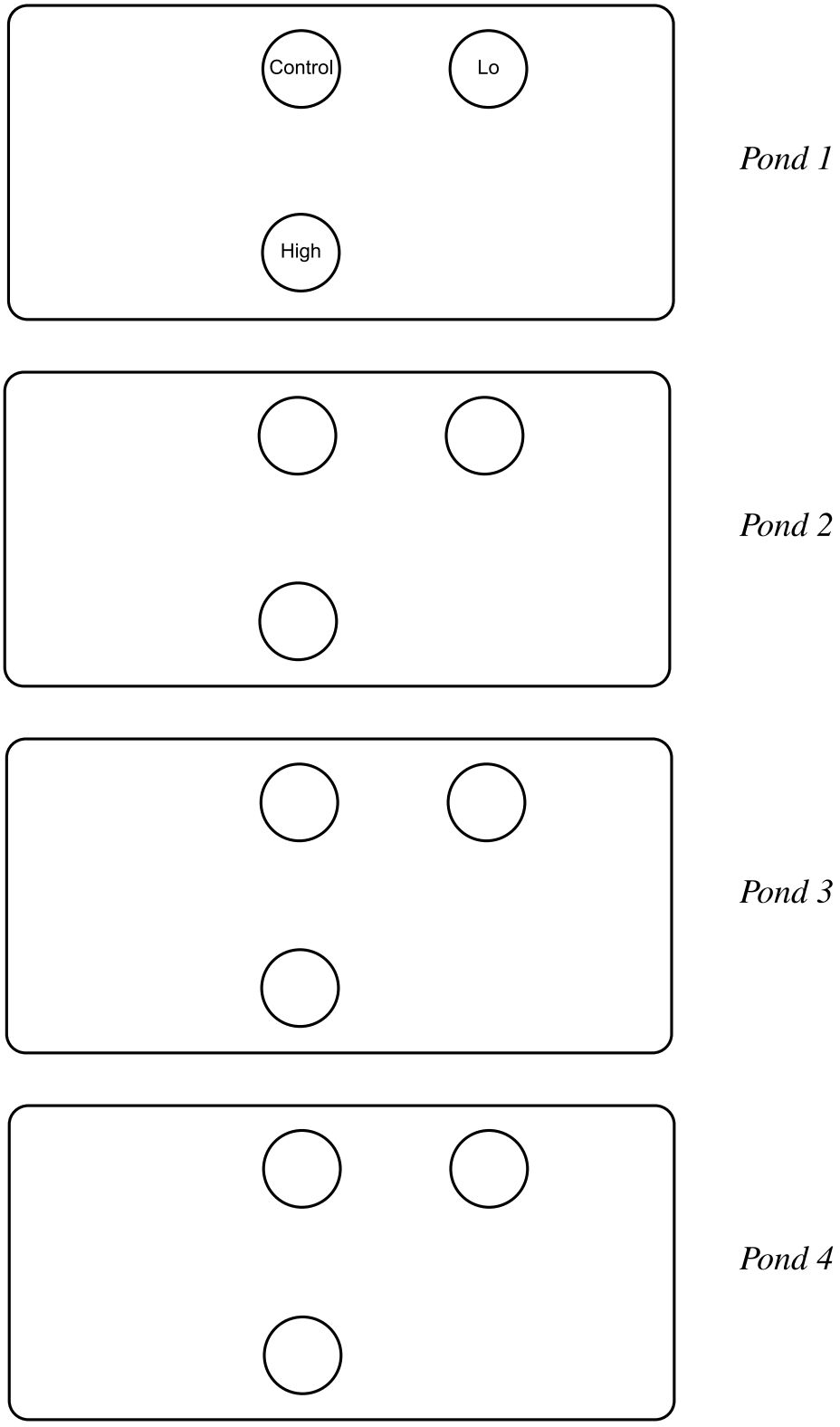
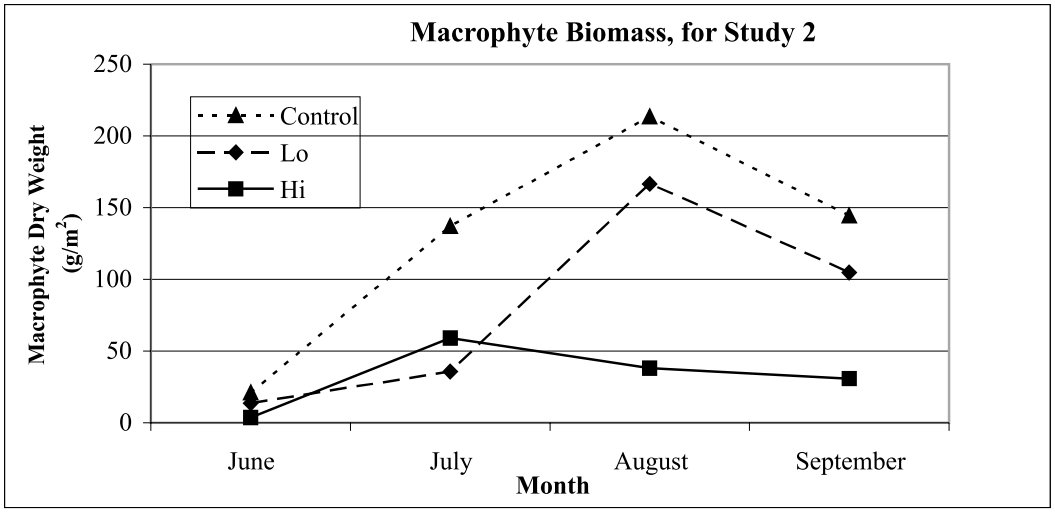


Figure 31. *Corral and pond diagram for Study 2 experiments indicating orientation of corrals. Pond 1 shows an example of the random assignment of the treatments.*

Macrophyte Biomass

There was no measurable growth of macrophytes above the sediment surface at the initiation of the study; macrophyte surface coverage was <1%. Macrophytes grew rapidly in the Control from May to early August and reached a maximum biomass of 213 g/m^2 (Figure 32). Thereafter, macrophytes lost biomass (32%) between August and September. Both Day and Dose had significant effects on macrophyte biomass. Nutrient enrichment negatively influenced macrophyte growth, resulting in stands in the dosed treatments that were significantly smaller than the Control when averaged across the season ($p<0.0013$). However, ANOVA indicated that enrichment did not significantly influence biomass in any given month. Biomass in Lo peaked in August (167 g/m^2) at 75% of Control stands, and subsequently decreased to 105 g/m^2 in September. In Lo, timing of growth and senescence periods was similar to the Control. In Hi, however, the growth period ended in July with a maximum biomass of 59 g/m^2. During senescence, biomass in Hi declined by 53%, ending the season at only 31 g/m^2. These results were contrary to those in Study 1 in which neither macrophyte dosing or nutrient ratio had significant effects on macrophyte biomass. Macrophyte biomass in Study 2 was only 25% of that observed in Study 1. However, different ponds were used in each study.

	Dose	Day	Dose*Day
Macrophyte Biomass	0.0013	0.0001	0.1364



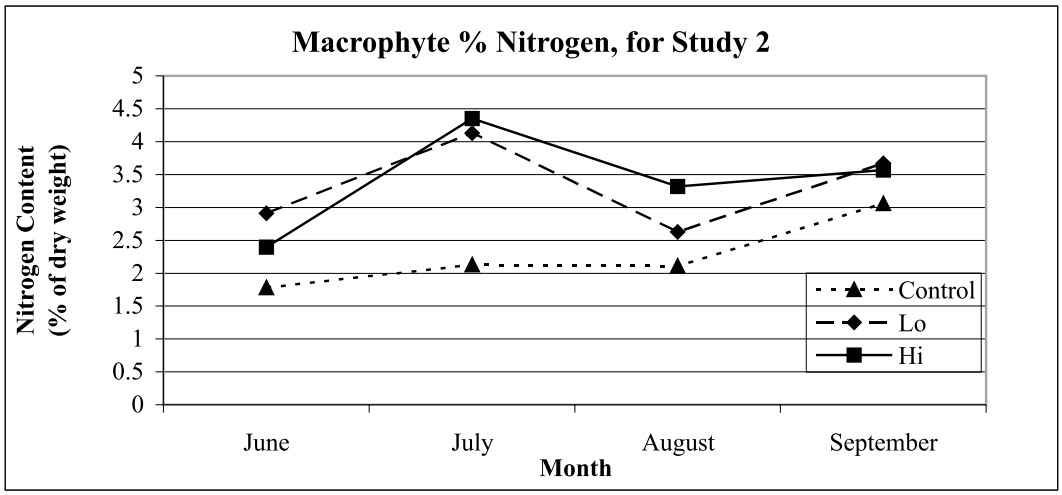
		Month				Season
		June	July	August	September	Avg.
Dose	Control	21	137	213	144	130[a]
	Lo	14	36	167	105	80[b]
	Hi	4	59	38	31	33[c]

Figure 32. *Changes in macrophyte biomass over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced macrophyte biomass in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of macrophyte biomass over the experimental season pooled by treatment. The final dose occurred one week subsequent to June samples. The lower table lists LS Means (g dry weight/m^2) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Macrophyte Nutrients

Day (p= 0.0001), Dose (p= 0.0061), and the Dose*Day interaction (P= 0.0129) had significant effects on nitrogen content of macrophytes in Study 2 (Figure 33). Nitrogen content of macrophytes in the Control significantly increased ($p \leq 0.05$) from 1.78% N in June to 3.06% N in September. Nitrogen uptake in the amended treatments was enhanced during the dose period. Nitrogen content in Lo and Hi peaked at >4% N in early July at levels 2-fold higher than Controls. Thereafter, N levels in macrophytes decreased in both the Lo and Hi treatments. Macrophytes in the Lo treatment contained significantly higher nitrogen ($p \leq 0.05$) compared to Controls in June, July, and September. Macrophytes in the Hi treatment were significantly higher ($p \leq 0.05$) than Controls in July and August; percentage nitrogen in macrophytes in Hi was significantly higher ($p \leq 0.05$) than those in Lo in August, only. Nitrogen concentrations were similar in macrophytes in Study 2 (Figure 33) compared to Study 1 (Figure 3)

	Dose	Day	Dose*Day
Macrophyte % N	0.0061	0.0001	0.0129

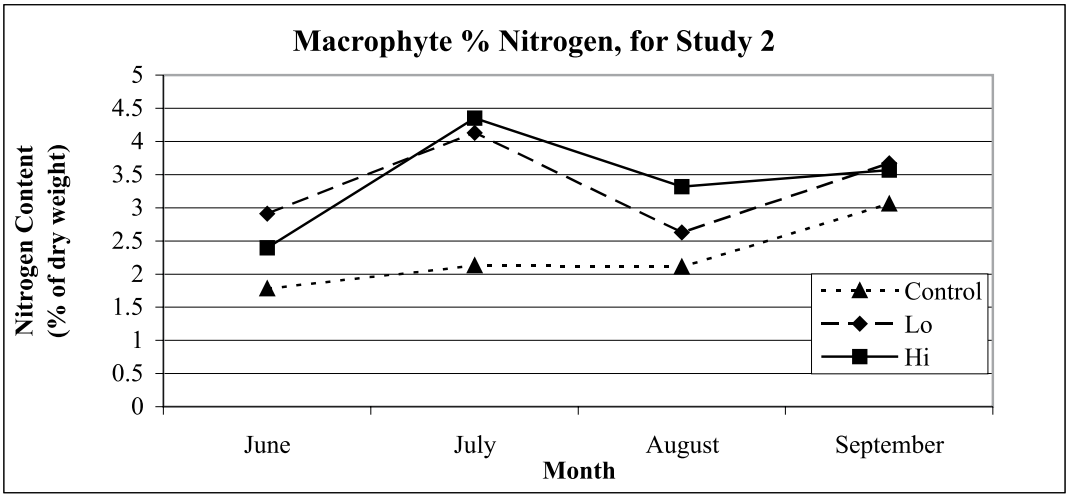


		Month				Season Avg.
		June	July	August	September	
Dose	Control	1.78[a]	2.13[a]	2.11[a]	3.06[a]	2.27[a]
	Lo	2.91[b]	4.13[b]	2.63[a]	3.67[b]	3.34[b]
	Hi	2.40[ab]	4.35[b]	3.32[b]	3.57[ab]	3.41[b]

Figure 33. *Changes in macrophyte nitrogen content over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced N content in ANOVA of rank-transformed data. The graph is a plot of N content over the experimental season pooled by treatment. The final dose occurred one week subsequent to June samples. The lower table lists LS Means (% N of dry weight) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Dose (p=0.0061) and Day (p= 0.0001) had significant effects on phosphorus content of macrophytes (Figure 34). Phosphorus content of macrophytes in the Control averaged 0.26% P in June, and increased to a maximum of 0.54% P in September. Seasonal averages of phosphorus in macrophytes were significantly higher in the Lo and Hi treatments, with peak concentrations in early July at 0.68% P and 0.95% P, respectively. Phosphorus concentrations of macrophytes in Study 2 (Figure 34) exceeded those in Study 1 (Figure 4) in all treatments including the Control.

	Dose	Day	Dose*Day
Macrophyte % N	0.0061	0.0001	0.0129



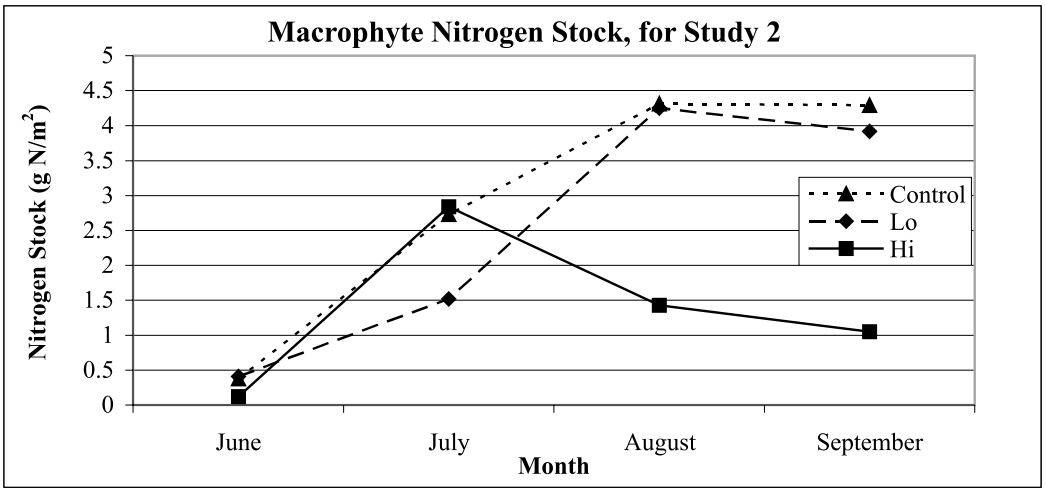
		Month				Season Avg.
		June	July	August	September	
Dose	Control	1.78[a]	2.13[a]	2.11[a]	3.06[a]	2.27[a]
	Lo	2.91[b]	4.13[b]	2.63[a]	3.67[b]	3.34[b]
	Hi	2.40[ab]	4.35[b]	3.32[b]	3.57[ab]	3.41[b]

Figure 34. *Changes in macrophyte phosphorus content over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced P content in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of P content over the experimental season pooled by treatment. The final dose occurred one week subsequent to June samples. The lower table lists LS Means (% P of dry weight) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Macrophyte Nutrient Stock

Both Day (p= 0.0001) and Dose (p= 0.0157) had significant effects on stocks of nitrogen in macrophytes. The N stock in macrophytes in the Control treatment increased 10-fold, from 0.4 g N/m^2 to a maximum of 4.3 g N/m^2 over the course of the study (Figure 35). The N stock in Controls did not decrease during macrophyte senescence because the actual percentage of nitrogen continued to increase late in the study (Figure 35). Nitrogen stocks of macrophytes were similar in the Lo and Control treatments. The N stocks of macrophytes in the Hi treatment were similar to the Control in June and July. However, due to premature senescence, N stocks in Hi in August (1.4 g N/m^2) and September (1.1 g N/m^2) were 30% lower than in the Control and Lo treatments.

	Dose	Day	Dose*Day
N Stock	0.0157	0.0001	0.2508

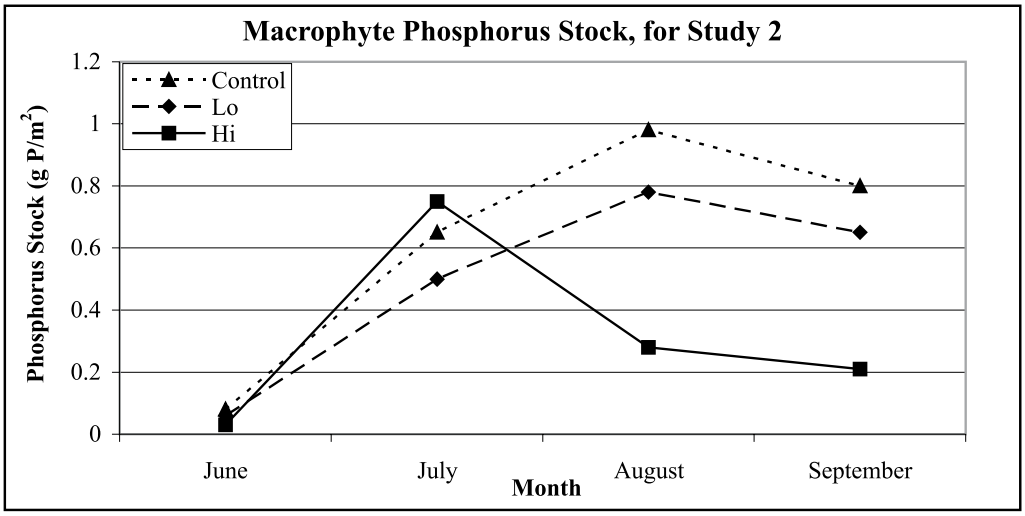


		Month				Season Avg.
		June	July	August	September	
Dose	Control	0.4	2.7	4.3	4.3	2.9[a]
	Lo	0.4	1.5	4.3	3.9	2.5[a]
	Hi	0.1	2.8	1.4	1.1	1.4[b]

Figure 35. *Changes in macrophyte nitrogen stock over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced N stock in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of N stock over the experimental season pooled by treatment. The final dose occurred one week subsequent to June samples. The lower table lists LS Means (g N/m^2) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Both Day (p= 0.0001) and Dose (p= 0.0274) had significant effects on P stocks of macrophytes (Figure 36). The P stock in Control macrophytes increased 12-fold, from 0.08 g P/m^2 to a maximum of 0.98 g P/m^2 in August. During senescence, P stock in the Control decreased to 0.80 g P/m^2, but was not significantly lower than the August maximum. The P stock in Lo was 25% lower than the Control from July through September, but the treatments were not significantly different overall (p>0.05). The P stock in Hi peaked in July at levels comparable to the Control (0.75 g P/m^2 g P/m^2) but declined thereafter to 0.28 and 0.21 g N/m^2 in August and September, respectively, due to macrophyte senescence. Overall, the P stock in Hi was significantly smaller (p<0.05) than the Control and Lo treatments.

	Dose	Day	Dose*Day
P Stock	0.0274	0.0001	0.4455



		Month				Season Avg.
		June	July	August	September	
Dose	Control	0.08	0.65	0.98	0.80	0.58[a]
	Lo	0.06	0.50	0.78	0.65	0.45[a]
	Hi	0.03	0.75	0.28	0.21	0.30[b]

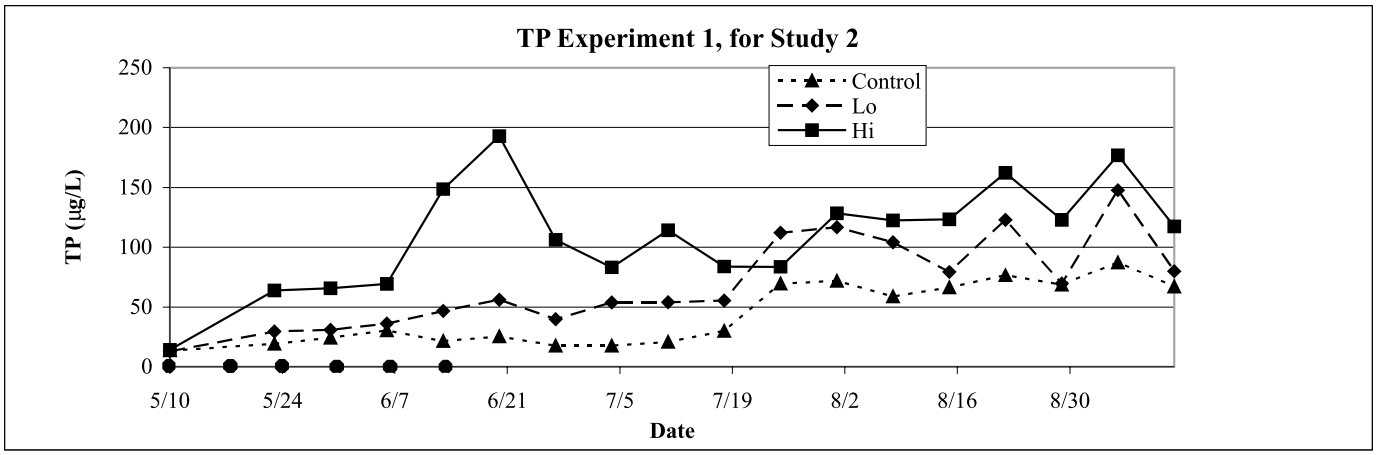
Figure 36. *Changes in macrophyte phosphorus stock over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced P stock in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of P stock over the experimental season pooled by treatment. The final dose occurred one week subsequent to June samples. The lower table lists LS Means (g P/m^2) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Water Chemistry

Phosphorus

Day (p=0.0001), Dose (p=0.0048), and the Dose*Day interaction were significant factors controlling total phosphorus concentrations (Figure 37). Total phosphorus concentrations increased in the Controls from 14 µg P/L in early May to a maximum of 87 µg P/L in early September (Figure 37). Total phosphorus concentrations averaged 110 µg/L in the Hi treatment and was significantly greater ($p<0.05$) than the Control on all but two dates. Concentrations of TP in the Lo treatment did not differ from the Control treatment on a pooled, study basis but was frequently greater than Control levels on individual dates.

	Dose	Day	Dose*Day
TP	0.0048	0.0001	0.0001

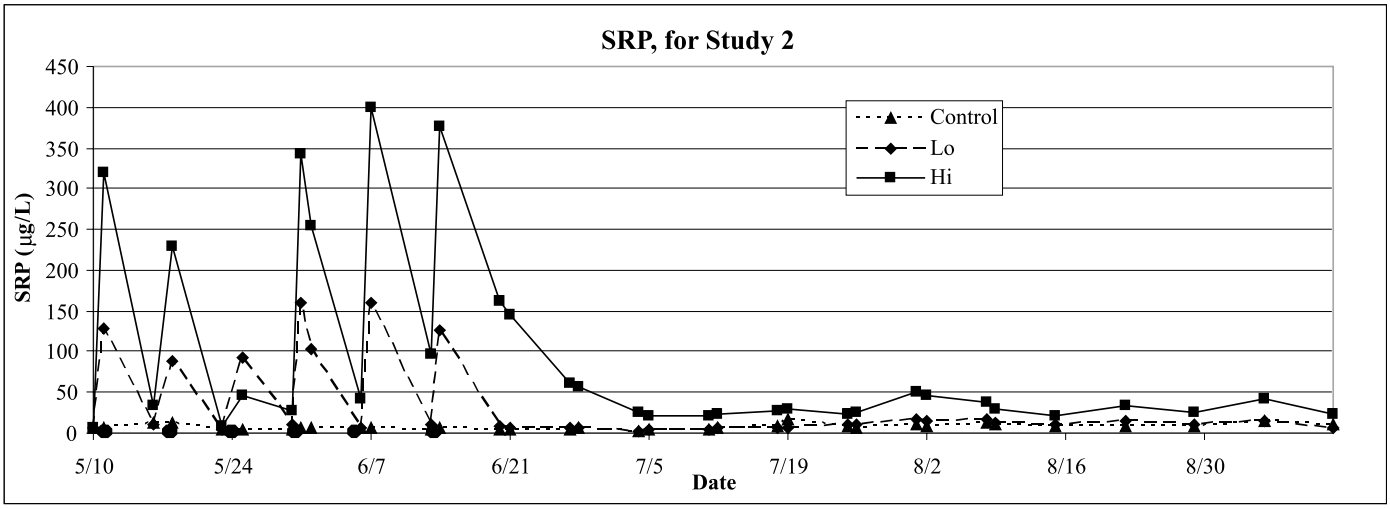


		Day																	Season
		5/10	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	14	19^{a}	24^{a}	31^{a}	22^{a}	25^{a}	18^{a}	18^{a}	21^{a}	30^{a}	70	72^{a}	59^{a}	66^{a}	77^{a}	69^{a}	87^{a}	67^{a}	44^{a}
	Lo	13	30^{a}	31^{a}	36^{a}	47^{b}	56^{b}	40^{b}	54^{b}	54^{b}	55^{b}	112	117^{ab}	104^{ab}	79^{a}	123^{a}	70^{a}	148^{ab}	80^{a}	69^{a}
	Hi	14	64^{b}	66^{b}	69^{b}	148^{c}	193^{c}	106^{c}	83^{c}	114^{c}	84^{b}	84	128^{b}	122^{b}	123^{b}	162^{b}	123^{b}	177^{b}	117^{b}	110^{b}

Figure 37. *Changes in total phosphorus concentrations over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced TP in ANOVA of rank-transformed data. The graph is a plot of TP over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Soluble reactive phosphorus in the Controls averaged 7 µg P/L (range 2 µg to 16 µg P/L) during the season (Figure 38). Dose (p=0.0001), Day (p=0.0001), and the Dose*Day interaction (p= 0.0001) were all significant factors in SRP dynamics. Due to rapid loss of SRP observed in Study 1, we sampled SRP in Studies 2 and 3 within approximately 1 hr of application. Peaks in SRP in Lo and Hi indicated an average of 60% and 70% dose recovery, respectively. On May 25, the recovery of only 11% of the third amendment in Hi indicated some unexplained problem in dosing. Dissipation in the dosed treatments was rapid. The Lo treatment assimilated 100% of each dose within a week (approximately 29 µg P/L/day; 14% P loss/day); whereas, the SRP in the Hi treatment had not yet dissipated to Control levels prior to the subsequent dose. Calculated dissipation rates in the Hi treatment averaged 40 µg P/L/day (25% applied P loss/day; excluding anomalous dose three) during the May to early June dosing period.

	Dose	Day	Dose*Day
SRP	0.0001	0.0001	0.0001



Day

Dose	5/10	5/11	5/16	5/18	5/23	5/25	5/30	5/31	6/1	6/6	6/7	6/13	6/14	6/20	6/21	6/27	6/28
Control	5.55	5.93	12.58	13.25	4.55	4.15	4.35	5.35	6.45	6.08	5.68	4.80	7.15	3.60	3.55	4.08	5.70
Lo	5.58	128.40	11.33	89.30	3.73	93.00	9.73	160.15	102.48	6.90	159.88	10.78	127.10	9.00	5.78	5.60	5.70
Hi	5.75	320.30	33.80	229.08	8.93	46.25	28.10	342.08	255.13	41.18	398.53	96.18	375.65	161.28	144.10	60.85	57.60

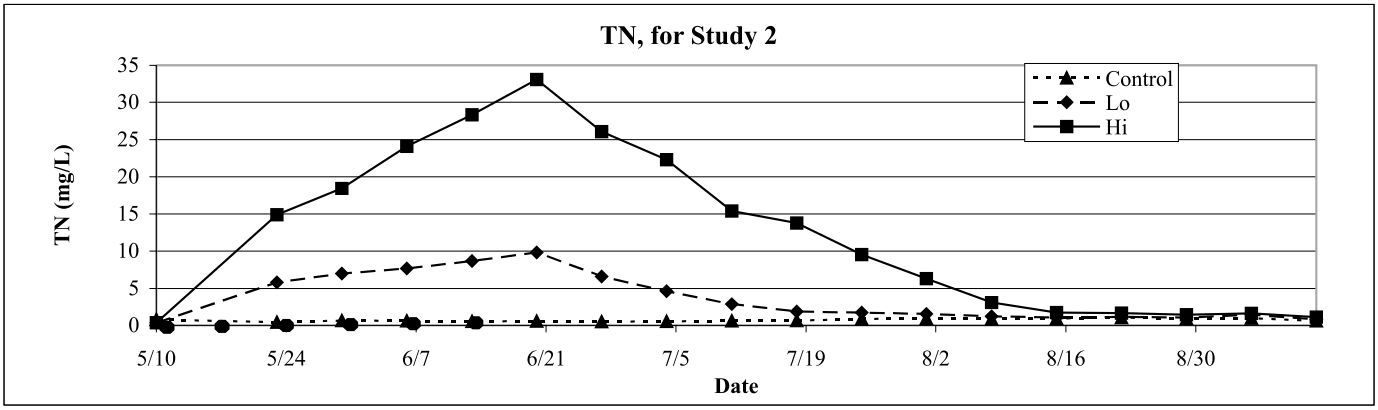
Dose	7/4	7/5	7/11	7/12	7/18	7/19	7/25	7/26	8/1	8/2	8/8	8/9	8/15	8/22	8/29	9/5	9/12	**Season Avg.**
Control	2.05	3.80	3.43	6.78	9.40	16.33	8.85	7.23	9.95	8.00	11.98	9.53	7.75	8.83	8.18	14.35	9.95	7.33[a]
Lo	3.00	3.23	3.58	5.28	5.65	6.23	9.78	9.60	16.90	13.90	16.63	12.48	10.35	14.65	10.25	14.48	6.00	32.25[b]
Hi	25.43	20.50	20.68	23.35	26.58	30.13	22.95	25.05	50.13	47.03	37.13	29.95	21.13	33.80	24.53	42.70	22.23	91.41[c]

Figure 38. *Changes in soluble reactive phosphorus concentrations over time. The table presents probabilities that dose, day, and interactions of dose and day influenced SRP in ANOVA of rank-transformed data. The graph is a plot of SRP over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (µg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Nitrogen

Dose ($p<0.001$), Day ($p<0.0001$), and the Dose*Day interaction ($p<0.0001$) were all significant factors related to total nitrogen dynamics (Figure 39). Total nitrogen in the Control ranged from lows of 0.43 mg N/L in mid-May to highs of approximately 1 mg N/L in August. From the start of the season to July 18, TN in the Control fluctuated between 0.4 and 0.8 mg N/L. Between July 18 and 25, TN increased by 33%, and subsequent concentrations (range: 0.85–1.03 mg N/L) were significantly larger ($p<0.05$) than values before July 18. The Lo and Hi treatments exhibited TN levels that were significantly larger ($p<0.05$) than the Control on most dates ($p<0.05$). During the dose period, most of the measured TN consisted of nitrate (90–100% nitrate) in the Lo and Hi treatments; whereas, nitrate was at background levels in the Control (Figure 40). Following dose six, TN in the Lo (9.83) and Hi (33.08 mg N/L) treatments reached seasonal maximums. TN declined throughout the remainder of the season and averaged approximately 1 mg N/L at the end of the study. Final TN levels in the Lo (0.83 mg N/L) and Hi (1.10 mg N/L) treatments were significantly greater ($p<0.05$) than the Control (0.63 mg N/L).

	Dose	Day	Dose*Day
TN	0.0001	0.0001	0.0001

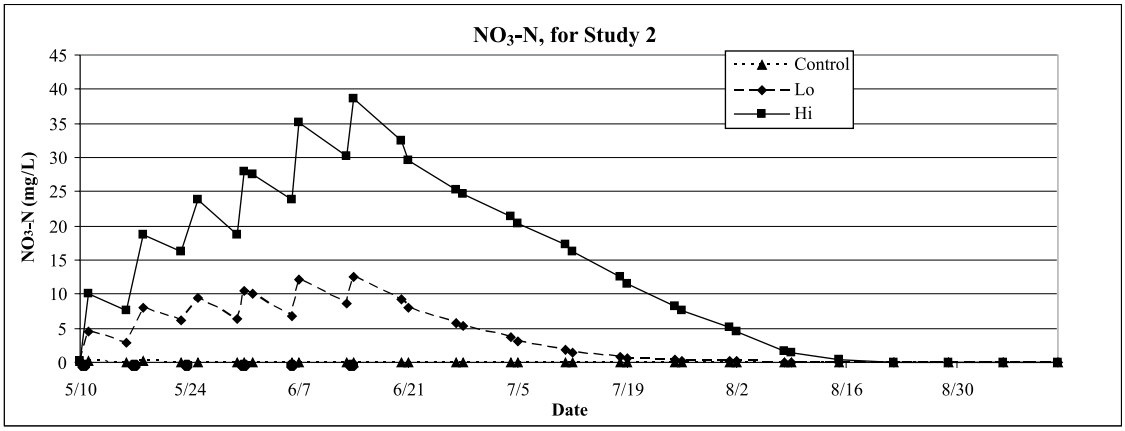


		Day																		Season
		5/10	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	0.78	0.43^{a}	0.65^{a}	0.63^{a}	0.53^{a}	0.58^{a}	0.50^{a}	0.53^{a}	0.60^{a}	0.65^{a}	0.88^{a}	0.93^{a}	0.85^{a}	0.95^{a}	1.03^{a}	0.95^{a}	0.98^{a}	0.63^{a}	0.70^{a}
	Lo	0.33	5.80^{b}	6.98^{b}	7.68^{b}	8.68^{b}	9.83^{b}	6.60^{b}	4.63^{b}	2.88^{b}	1.88^{b}	1.73^{b}	1.55^{b}	1.23^{b}	1.08^{a}	1.15^{a}	1.03^{a}	1.43^{b}	0.83^{b}	3.63^{b}
	Hi	0.38	14.88^{c}	18.43^{c}	24.10^{c}	28.33^{c}	33.08^{c}	26.08^{c}	22.30^{c}	15.38^{c}	13.78^{c}	9.53^{c}	6.30^{c}	3.08^{c}	1.73^{b}	1.65^{b}	1.45^{b}	1.63^{b}	1.10^{c}	12.40^{c}

Figure 39. *Changes in total nitrogen concentrations over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced TN in ANOVA of rank-transformed data. The graph is a plot of TN over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Dose ($p<0.0001$), Day ($p<0.0001$), and the Dose*Day interaction ($p<0.0001$) were all significant factors in nitrate dynamics. Nitrate in Control fluctuated near the limit of detection (0.005 mg NO_3-N/L) during most of the season. During the dose period, nitrate peaks in Lo and Hi indicated that dosing achieved 87% and 96% of target concentrations, respectively (Figure 40). Rates of nitrate dissipation in the week following each amendment did not differ between Lo and Hi, but did significantly increase ($p<0.05$) from an average of 0.43 mg NO_3-N/L/day (4% N loss/day) after the first two doses, to 0.75 mg NO_3-N/L/day (7.5% N loss/day) after the final four doses as macrophyte biomass increased (Figure 41). Nitrate amendments were not completely dissipated within a week, and therefore, dissipation rates were accurate estimates of uptake in these systems in May and early June. Following the final amendment, nitrate in the Lo and Hi treatments peaked at 12.63 and 38.57 mg NO_3-N/L, respectively, and subsequently decreased throughout the remainder of the season. Due to slower dissipation rates with each successive week following the final dose, nitrate in Lo did not fall below the limit of detection until August 15 (Figure 40). Nitrate in the Hi treatment steadily decreased at a rate of 0.59 mg NO_3-N/L/day (6% N loss/day) from June 21 (29.67 mg NO_3-N/L) to August 8 (1.71 mg NO_3-N/L) (Figure 40). After August 9, nitrate dissipation in the Hi treatment continued at <0.2 mg NO_3-N/L/day until concentrations fell below the limit of detection on August 29.

	Dose	Day	Dose*Day
NO_3-N	0.0001	0.0001	0.0001



		Day																
		5/10	5/11	5/16	5/18	5/23	5/25	5/30	5/31	6/1	6/6	6/7	6/13	6/14	6/20	6/21	6/27	6/28
Dose	Control	0.207	0.156	0.073	0.168	0.030	0.018	0.009	0.011	0.009	0.009	0.009	0.008	0.014	0.010	0.010	0.011	0.014
	Lo	0.180	4.586	2.878	8.081	6.134	9.451	6.454	10.489	10.149	6.849	12.072	8.685	12.634	9.155	8.078	5.657	5.294
	Hi	0.226	10.141	7.681	18.676	16.156	23.740	18.771	27.882	27.510	23.793	35.209	30.191	38.566	32.561	29.666	25.343	24.572

		7/4	7/5	7/11	7/12	7/18	7/19	7/25	7/26	8/1	8/2	8/8	8/9	8/15	8/22	8/29	9/5	9/12	Season Avg.
Dose	Control	0.015	0.010	0.017	0.009	0.015	0.013	0.011	0.010	0.007	0.008	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.027[a]
	Lo	3.630	3.147	1.785	1.539	0.811	0.706	0.323	0.250	0.150	0.122	0.013	0.017	0.006	0.005	0.006	0.005	0.005	4.098[b]
	Hi	21.345	20.251	17.197	16.299	12.475	11.509	8.273	7.588	5.079	4.580	1.708	1.364	0.329	0.013	0.005	0.005	0.005	15.256[c]

Figure 40. *Changes in nitrate concentrations over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced nitrate in ANOVA of rank-transformed data. The graph is a plot of nitrate over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

	Dose	Day	Dose*Day
N Dissipation	0.1179	0.0001	0.4453

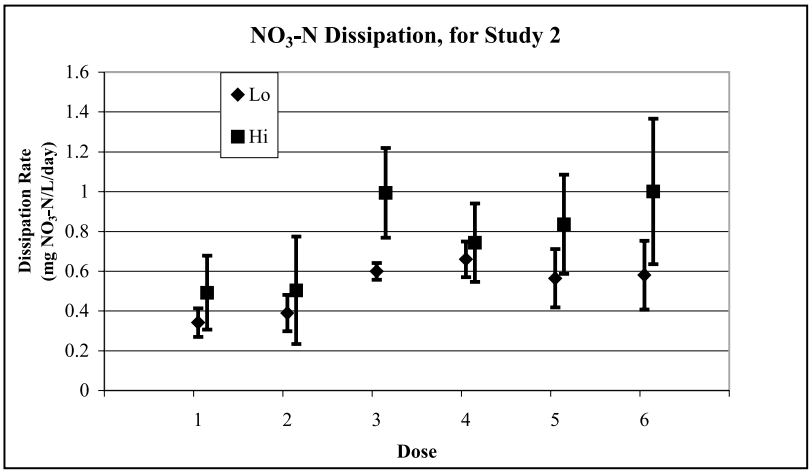
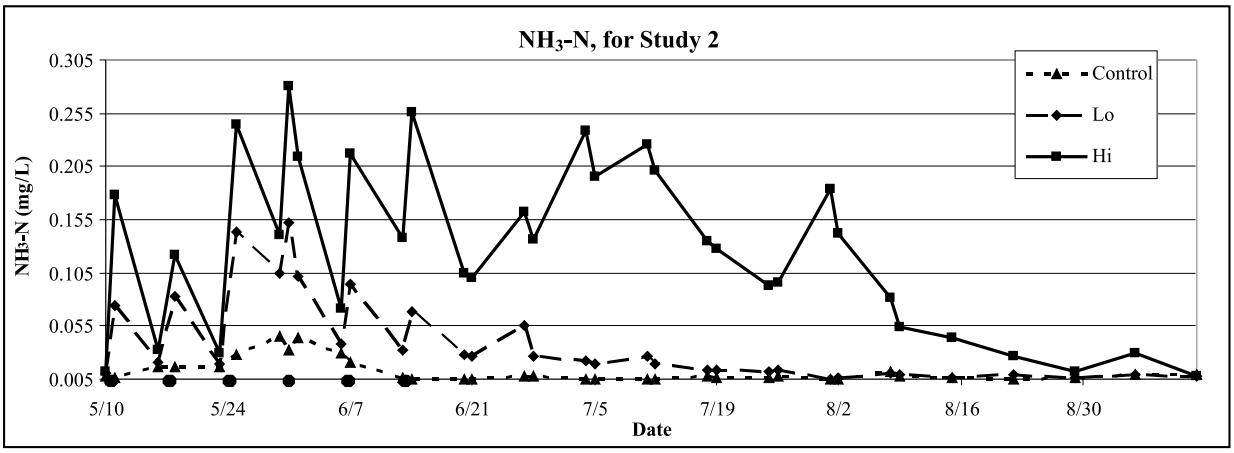


Figure 41. *Changes in nitrate dissipation rates over dose periods. Nitrate dissipation rates (mg NO_3-N/L/day) in the week following each dose shown as LS Means for Lo and Hi treatments. Bars indicate the one standard deviation above and below the mean. Dissipation rates were not significantly different between Lo and Hi (p>0.05).*

Ammonia in the Control was near the limit of detection (0.005 mg NH_3-N/L) throughout most of the season (Figure 42). During the dose period, however, ammonia in the Control rose to 0.045 mg NH_3-N/L, possibly due to the disturbance of stirring. Dose (p=0.0003), Day (p<0.0001), and the Dose*Day interaction (p<0.0001) were significant factors in dynamics of ammonia. Ammonia in the amended treatments peaked on dates of nutrient application at 4 times Control levels. Seasonal maximums in the Lo treatment (0.152 mg NH_3-N/L) and Hi treatment (0.281 mg NH_3-N/L) occurred following the fourth dosing (May 31). For four weeks following the dose period, ammonia in the Lo treatment was significantly greater (p<0.05) than Control levels. After July 18, ammonia in Lo was not significantly different (p>0.05) from the Control. After the dose period, ammonia in the Hi treatment varied between 0.1 and 0.25 mg NH_3-N/L until August 8. Thereafter, ammonia in the Hi treatment decreased >85%, and in September, concentrations were comparable to the Control. Overall, study averages of ammonia in the Lo treatment (0.038 mg NH_3-N/L) and Hi treatment (0.127 mg NH_3- N/L) were significantly different from each other and exceeded the Control average (0.012 mg NH_3-N/L).

	Dose	Day	Dose*Day
NH_3-N	0.0003	0.0001	0.0001



		Day																
		5/10	5/11	5/16	5/18	5/23	5/25	5/30	5/31	6/1	6/6	6/7	6/13	6/14	6/20	6/21	6/27	6/28
Dose	Control	0.008	0.006a	0.016	0.017a	0.017a	0.028a	0.045	0.033a	0.044a	0.030	0.021a	0.006a	0.005a	0.005a	0.005a	0.008a	0.008a
	Lo	0.011	0.074b	0.021	0.083b	0.020ab	0.144b	0.105	0.152b	0.101ab	0.038	0.094b	0.033b	0.069b	0.028b	0.027b	0.055b	0.027ab
	Hi	0.012	0.178b	0.032	0.122b	0.030b	0.244b	0.141	0.281b	0.214b	0.071	0.217b	0.137b	0.256b	0.105c	0.100c	0.162b	0.136b

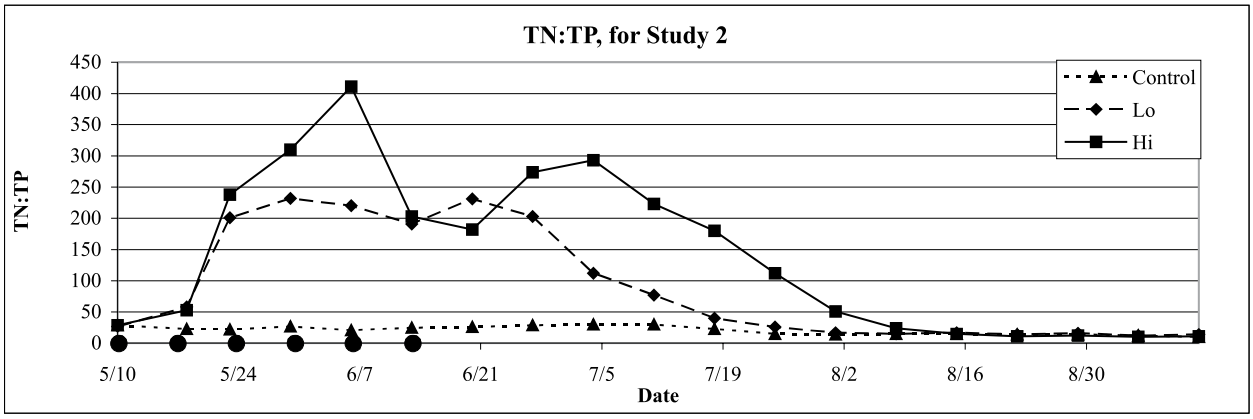
		7/4	7/5	7/11	7/12	7/18	7/19	7/25	7/26	8/1	8/2	8/8	8/9	8/15	8/22	8/29	9/5	9/12	**Season Avg.**
Dose	Control	0.005a	0.005a	0.005a	0.005a	0.008a	0.007a	0.006a	0.008a	0.005a	0.005a	0.012a	0.008a	0.006a	0.005a	0.006	0.010	0.009	0.012[a]
	Lo	0.022b	0.020b	0.026b	0.020b	0.014a	0.014a	0.012a	0.013a	0.005a	0.006a	0.011a	0.009a	0.007a	0.009b	0.007	0.009	0.008	0.038[b]
	Hi	0.238c	0.195c	0.226c	0.201c	0.135b	0.128b	0.093b	0.096b	0.184b	0.142b	0.082b	0.054b	0.044b	0.027b	0.012	0.029	0.008	0.127[c]

Figure 42. *Changes in ammonia over time. The table presents probabilities that dose, day, and interactions of dose and day influenced ammonia in ANOVA of rank-transformed data. The graph is a plot of ammonia over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Nitrogen:Phosphorus Ratio

Dose ($p<0.0001$), Day ($p<0.0001$), and the Dose*Day interaction ($p<0.0001$) were significant factors influencing the TN:TP ratio. The TN:TP ratio in the Control fluctuated between 20 and 30 from the beginning of the experiment through July 18, indicating P-limitation (Figure 43). The TP increase on July 25 dropped TN:TP to 15:1, and ratios were between 10 and 20 during the remainder of the season. The calculated TN:TP of the amendments was 25:1, but after the first two doses, ratios in Lo and Hi had doubled to more than 50:1, indicating that added phosphorus was rapidly lost from the water column whereas nitrate accumulated (Figures 38 and 40). Following the third amendment, TN:TP ratios in the dosed treatments exceeded 200, nearly ten times the Control. During July, TN:TP ratios in Lo and Hi decreased because of the TN decline, and in August reached levels between 10 and 20, comparable to the Control.

	Dose	Day	Dose*Day
TN:TP	0.0001	0.0001	0.0001



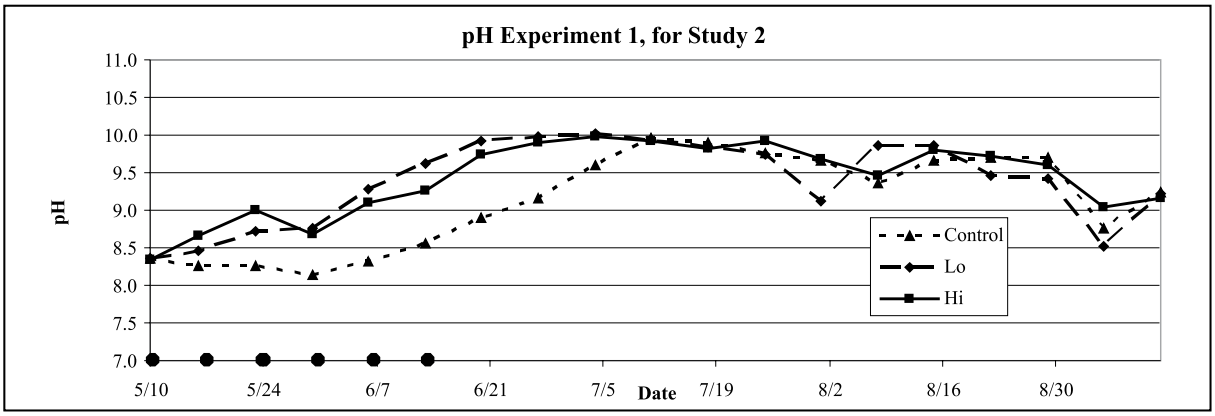
		Day																			Season
		5/10	5/18	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	29	23^{a}	22^{a}	27^{a}	21^{a}	25^{a}	26^{a}	29^{a}	30^{a}	30^{a}	23^{a}	15^{a}	14^{a}	15^{a}	15	14	14	12	10^{ab}	21^{a}
	Lo	27	58^{b}	201^{b}	232^{b}	220^{b}	191^{b}	231^{b}	203^{b}	112^{b}	77^{a}	40^{a}	26^{a}	17^{a}	15^{a}	17	14	16	12	14^{a}	91^{b}
	Hi	29	53^{b}	238^{b}	310^{b}	411^{b}	203^{b}	182^{b}	274^{b}	293^{c}	223^{b}	180^{b}	112^{b}	51^{b}	24^{b}	15	11	12	10	11^{b}	139^{c}

Figure 43. *Changes in the ratio of total nitrogen to total phosphorus over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced TN:TP in ANOVA of rank-transformed data. The graph is a plot of TN:TP over the experimental season pooled by treatment. Dark circles on X axis circles indicate dose dates. The lower table lists LS Means represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

pH

Day ($p<0.0001$) was a significant main effect on pH response; however, dose had no effect. Levels of pH in the Control averaged 8.3 in May and early June, but then rose steadily to nearly 10 by mid-July (Figure 44). With the exception of September 5, when pH was 8.8, pH in the Control fluctuated between 9 and 10 during July to September. Nutrient enrichment did not significantly influence pH ($p>0.05$) in analyses based on the entire season. However, values in the Lo and Hi treatments averaged >0.5 pH units higher than the Control during the May-June dosing period.

	Dose	Day	Dose*Day
pH	0.1495	0.0001	0.2657



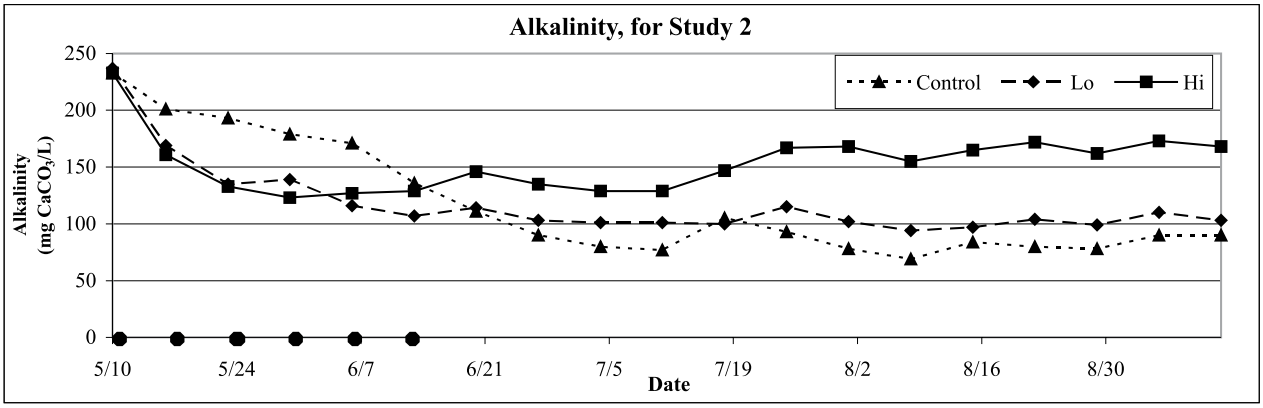
		Day																		Season Avg.
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	
Dose	Control	8.4	8.3	8.3	8.1	8.3	8.6	8.9	9.2	9.6	10.0	9.9	9.8	9.7	9.4	9.7	9.7	9.7	8.8	9.2	8.7
	Lo	8.4	8.5	8.7	8.8	9.3	9.6	9.9	10.0	10.0	9.9	9.8	9.7	9.1	9.9	9.9	9.5	9.4	8.5	9.2	9.0
	Hi	8.3	8.7	9.0	8.7	9.1	9.3	9.7	9.9	10.0	9.9	9.8	9.9	9.7	9.5	9.8	9.7	9.6	9.0	9.2	9.1

Figure 44. *Changes in pH over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced pH in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of pH over the experimental season pooled by treatment (data were averaged by H-ion concentration, then converted to pH: calculated pH= - log (H-ion)). Dark circles indicate dose dates. The lower table lists LS Means represented in the graph.*

Alkalinity and Hardness

Alkalinity significantly responded to Day ($p<0.0001$), Dose ($p=0.0019$), and the Dose*Day interaction ($p<0.0001$) (Figure 45). Alkalinity in the Control decreased from a maximum of 233 mg $CaCO_3$/L on May 10 to 80 mg $CaCO_3$/L in early July, and then fluctuated between 60 and 110 mg $CaCO_3$/L through September. Initial alkalinity in 2000 (233 mg $CaCO_3$/L) was higher than initial values observed in 1999 (150 mg $CaCO_3$/L) because water samples in 2000 were collected within two days of filling the ponds, whereas over two weeks elapsed between filling and sampling in 1999. Alkalinities ranged from 100–170 mg $CaCO_3$/L in the Lo and Hi treatments during the dosing period and were 25% lower than Control levels. Following the dosing period, alkalinities in the Lo treatment ranged from 94 to 105 mg $CaCO_3$/L and were similar to Control values. Alkalinities in the Hi treatment remained between 129 and 173 mg $CaCO_3$/L through September and were significantly higher than the other treatments ($p<0.05$).

	Dose	Day	Dose*Day
Alkalinity	0.0019	0.0001	0.0001

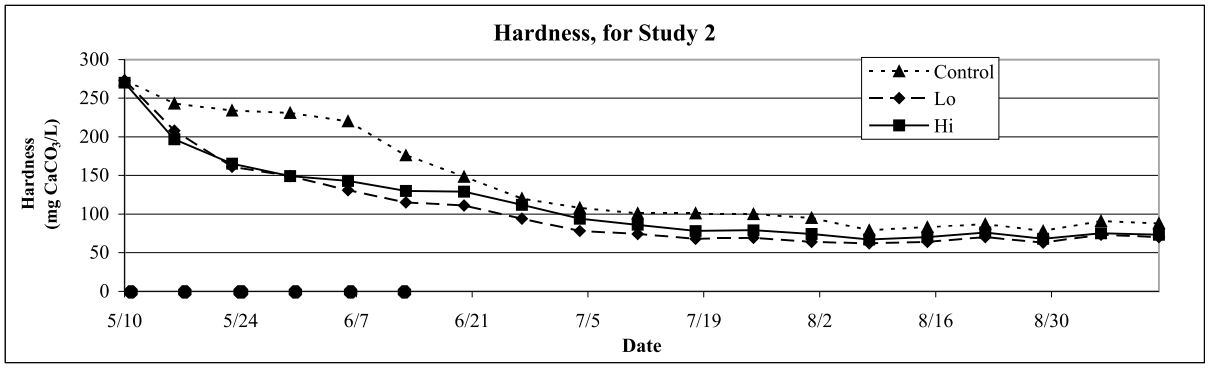


		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	233	201	193^{a}	179^{a}	171^{a}	136^{a}	111^{a}	90^{a}	80^{a}	77^{a}	105^{a}	93^{a}	78^{a}	69^{a}	84^{a}	80^{a}	78^{a}	90^{a}	90^{a}	117^{a}
	Lo	237	169	135^{b}	139^{ab}	116^{b}	107^{b}	114^{a}	103^{a}	101^{a}	101^{b}	100^{a}	115^{b}	102^{b}	94^{b}	97^{a}	104^{b}	99^{b}	110^{b}	103^{a}	118^{a}
	Hi	233	161	133^{b}	123^{b}	127^{b}	129^{a}	146^{b}	135^{b}	129^{b}	129^{c}	147^{b}	167^{c}	168^{c}	155^{c}	165^{b}	172^{c}	162^{c}	173^{c}	168^{b}	154^{b}

Figure 45. *Changes in alkalinity over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced alkalinity in ANOVA of rank-transformed data. The graph is a plot of alkalinity over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (mg $CaCO_3$/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Hardness was similarly affected by Day (p=0.0001) but not by Dose (p=0.1353). Trends in hardness paralleled those of alkalinity early in the study when water quality was highly influenced by the groundwater source at corral filling. Maximum hardness (273 mg $CaCO_3$/L) was observed at the beginning of the season (Figure 46). Hardness values decreased approximately 50% by June 21 in the Control and averaged 148 mg $CaCO_3$/L. Hardness values in the Lo and Hi treatments were approximately 25% lower compared to the Control during the dosing period, but were similar to Control values late in the study. Hardness averaged 140, 105, and 112 mg/L mg $CaCO_3$/L in the Control, Lo, and Hi treatments, respectively, over the course of the entire study.

	Dose	Day	Dose*Day
Hardness	0.1353	0.0001	0.3848



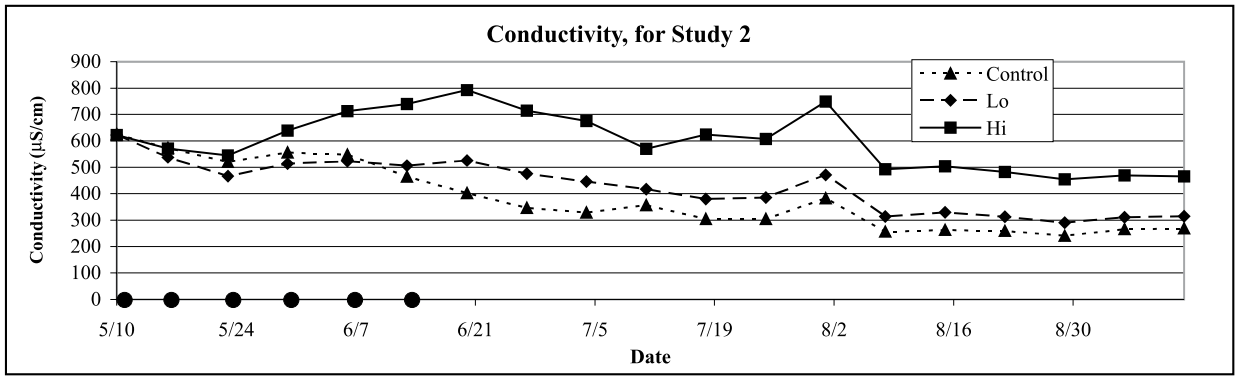
		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	273	243	234	231	220	176	148	120	108	101	101	100	95	79	83	87	78	91	88	140
	Lo	273	208	161	149	131	115	111	94	78	74	68	69	64	62	64	70	63	73	70	105
	Hi	270	197	165	149	143	130	129	112	94	86	78	79	74	67	70	76	68	75	73	112

Figure 46. *Changes in hardness over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced hardness in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of hardness over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (mg $CaCO_3$/L) represented in the graph.*

Conductivity

Conductivity averaged 382, 429, and 601 µS/cm in the Control, Lo, and Hi treatments, respectively; Day (p=0.0001), Dose (p=0.0007), and the Dose*Day (p=0.0001) had significant effects (Figure 47). Conductivity in the Control decreased 60% during the season, from an initial maximum of 625 µS/cm, to 268 µS/cm in September. Conductivity in the Lo treatment was similar to the Control during the dose period, and around 25% higher than the Control during the remainder of the season. Conductivity in the Hi treatment increased substantially after dose two, and fluctuated between 600 and 800 µS/cm during most of June and July. In early August, conductivity in Hi decreased to 500 µS/cm, but remained significantly larger than the other treatments through the end of the season (p<0.05).

	Dose	Day	Dose*Day
Conductivity	0.0007	0.0001	0.0001



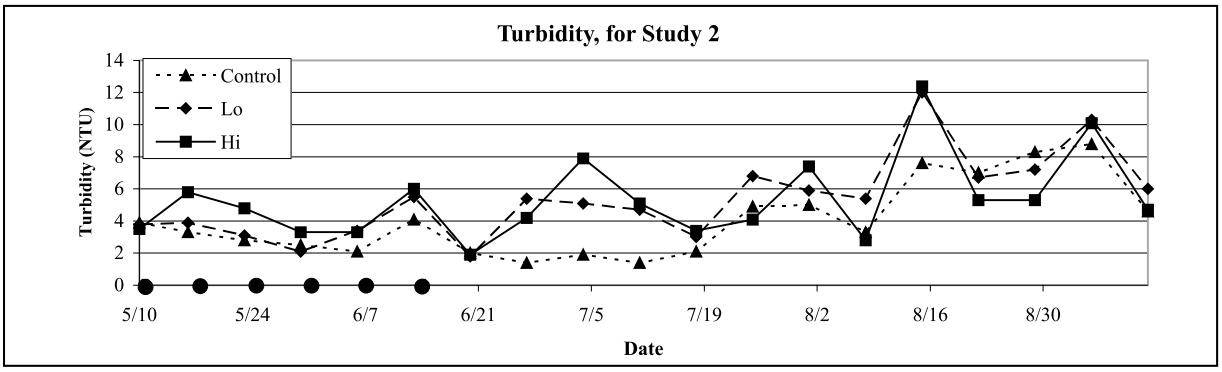
		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	625	573	520^{ab}	556^{a}	548^{a}	464^{a}	402^{a}	346^{a}	329^{a}	357^{a}	305^{a}	305^{a}	383^{a}	256^{a}	263^{a}	259^{a}	241^{a}	266^{a}	268^{a}	382^{a}
	Lo	622	538	466^{a}	514^{a}	523^{a}	506^{a}	526^{b}	476^{b}	446^{b}	417^{a}	380^{b}	385^{b}	471^{b}	314^{b}	330^{b}	313^{b}	291^{a}	311^{b}	315^{b}	429^{a}
	Hi	622	571	545^{b}	639^{b}	712^{b}	739^{b}	792^{c}	714^{c}	675^{c}	570^{b}	624^{c}	607^{c}	749^{c}	492^{c}	503^{c}	482^{c}	454^{b}	469^{c}	465^{c}	601^{b}

Figure 47. *Changes in conductivity over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced conductivity in ANOVA of rank-transformed data. The graph is a plot of conductivity over the experimental season pooled by treatment. Dark circles on X axis indicate dose dates. The lower table lists LS Means (mS/cm) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Turbidity

Turbidity ranged from 1.4 to 12.0 NTU's during the study. Day and the Dose*Day interaction had significant effects; Dose had no effect. Turbidity in the Control ranged from 1 to 4 NTU's through mid-July, and then increased to a maximum of 8.8 NTU's on September 5 (Figure 48). Overall, turbidity in Lo and Hi was similar to the Control, but significant differences ($p<0.05$) were observed on four dates during the season based on Day-specific T-tests. After two doses, turbidity in the Hi treatment (4.8 NTU's) was significantly greater than the Control (2.8 NTU's) ($p<0.05$). Also, for three weeks in late June and early July, amended treatments exhibited turbidities of 4–8 NTU's, 2–3 times Control levels.

	Dose	Day	Dose*Day
Turbidity	0.4085	0.0001	0.0199



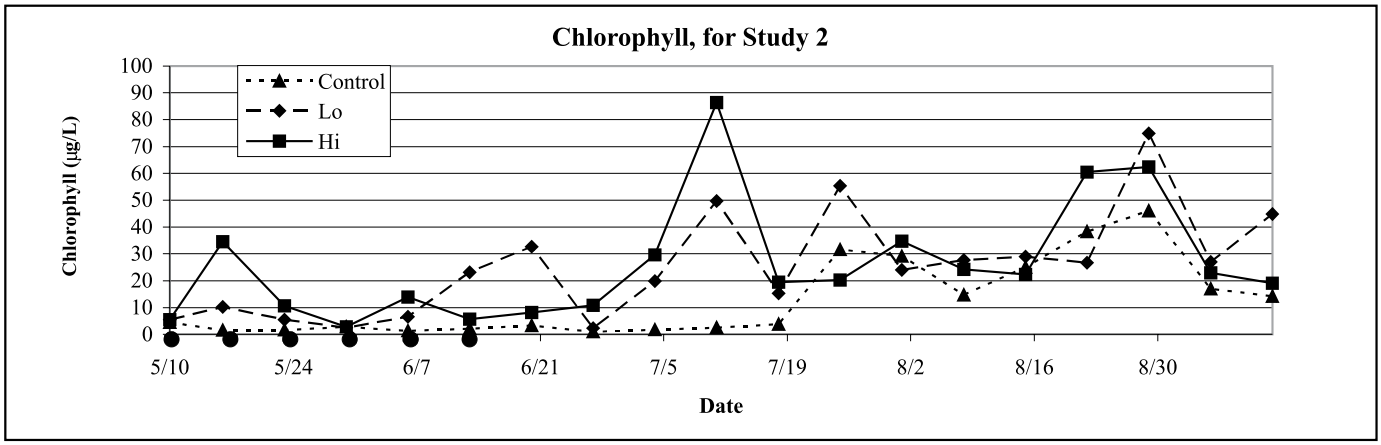
		Day																		Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	3.9	3.3	2.8[a]	2.5	2.1	4.1	2.0	1.4[a]	1.9[a]	1.4[a]	2.1	4.9	5.0	3.3	7.6	7.0	8.3	8.8	4.6	4.0
	Lo	3.8	3.9	3.1[ab]	2.1	3.4	5.5	1.8	5.4[b]	5.1[b]	4.7[b]	3.0	6.8	5.9	5.4	12.0	6.7	7.2	10.3	6.0	5.4
	Hi	3.5	5.8	4.8[b]	3.3	3.3	6.0	1.9	4.2[b]	7.9[b]	5.1[b]	3.4	4.1	7.4	2.8	12.4	5.3	5.3	10.1	4.7	5.3

Figure 48. *Changes in turbidity over time. This combination of tables and a graph presents the data and statistical information pertaining to turbidity (NTU). The upper table presents probabilities that dose, day, and interactions of dose and day influenced turbidity in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of turbidity over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (NTU) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Phytoplankton

Phytoplankton biomass, measured as chlorophyll *a*, responded significantly to the effects of Day and the Dose*Day interaction (Figure 49). Chlorophyll *a* averaged 13, 25, and 26 µg/L Chl in the Control, Lo, and Hi treatments, respectively; however, levels varied seasonally within treatments, and therefore, there was no significant main effect of Dose. In the Control, phytoplankton biomass as Chl was <4 µg/L from May through mid-July. In late July, as macrophytes matured, Chl increased to 32 µg/L by late July, and then varied between 14 and 46 µg/L through September. Significant deviations from the Control were noted in the Lo and Hi treatments between dose initiation and mid-July ($p<0.05$), but not during the remainder of the study. In the Hi treatment, chlorophyll peaked on May 15 (35 µg/L) and again on July 11 (86 µg/L). Chlorophyll in the Lo treatment was not significantly different from Hi, but exhibited peaks of 33 µg/L on June 20, and 50 µg/L on July 11.

	Dose	Day	Dose*Day
Phytoplankton Chlorophyll	0.1474	0.0001	0.0086



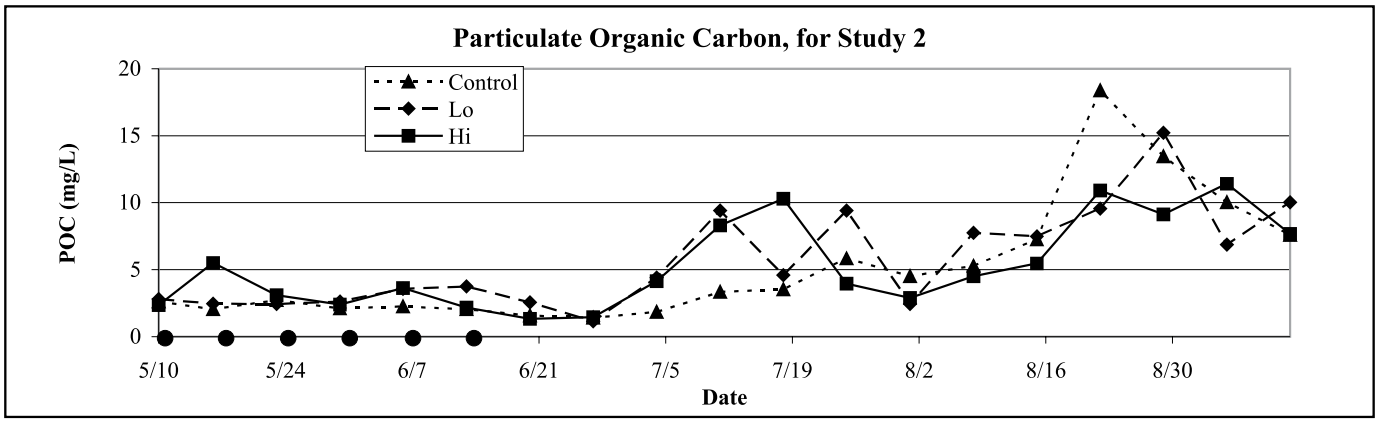
		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	5	2^{a}	2^{a}	3	1^{a}	2^{a}	3^{a}	1^{a}	2^{a}	3^{a}	4	32	29	15	25	38	46	17	14	13
	Lo	6	10^{b}	6^{ab}	3	7^{b}	23^{b}	33^{b}	2^{ab}	20^{b}	50^{b}	15	55	24	28	29	27	75	27	45	25
	Hi	6	35^{b}	11^{b}	3	14^{b}	6^{ab}	8^{ab}	11^{b}	30^{b}	86^{b}	19	20	35	24	22	60	63	23	19	26

Figure 49. *Changes in phytoplankton chlorophyll concentrations over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced Chl in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of Chl over the experimental season pooled by treatment. Dark circles on X axis indicate dose dates. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Particulate organic carbon (POC) significantly increased over time ($p<0.0001$) but did not respond to nutrient dosing (Figure 50). The Control, Lo, and Hi treatments averaged 5.14, 5.71, and 5.29 mg C/L, respectively, for the entire study. Control POC levels fluctuated around 2 mg C/L from May through early July; POC's gradually increased in the Control treatment to a maximum of 18.4 mg C/L on August 22, and then decreased to 7.6 mg C/L by the end of the season.

Day ($p<0.0001$), Dose ($p=0.0099$), and the Dose*Day interaction ($p=0.0028$) had significant effects on the POC:Chl ratio of water (Figure 51). The POC:Chl ratio averaged 1044, 530, and 485 in the Control, Lo, and Hi treatments, respectively, when averaged over the entire study. Control POC:Chl ratios were significantly greater than those in Lo and Hi treatments during the early part of the study due to the observed increase in chlorophyll from phytoplankton stimulation (Figure 49). POC:Chl ratios in the Control ranged from 1000:1 to 2500:1 from late May to mid-July, but decreased to less than 500:1 during the remainder of the season as chlorophyll concentrations increased in water (Figure 51). Though carbon and chlorophyll increased in August and September, the smaller ratio was due to the greater proportional contribution of

	Dose	Day	Dose*Day
POC	0.8625	0.0001	0.6071



		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	2.61	2.05	2.71	2.10	2.25	2.04	1.58	1.40	1.84	3.32	3.52	5.84	4.50	5.28	7.26	18.38	13.45	10.02	7.59	5.14
	Lo	2.80	2.46	2.44	2.61	3.56	3.75	2.56	1.14	4.41	9.41	4.59	9.40	2.43	7.75	7.49	9.54	15.21	6.87	10.01	5.71
	Hi	2.36	5.49	3.09	2.40	3.63	2.16	1.33	1.46	4.12	8.29	10.29	3.95	2.89	4.51	5.47	10.90	9.12	11.42	7.67	5.29

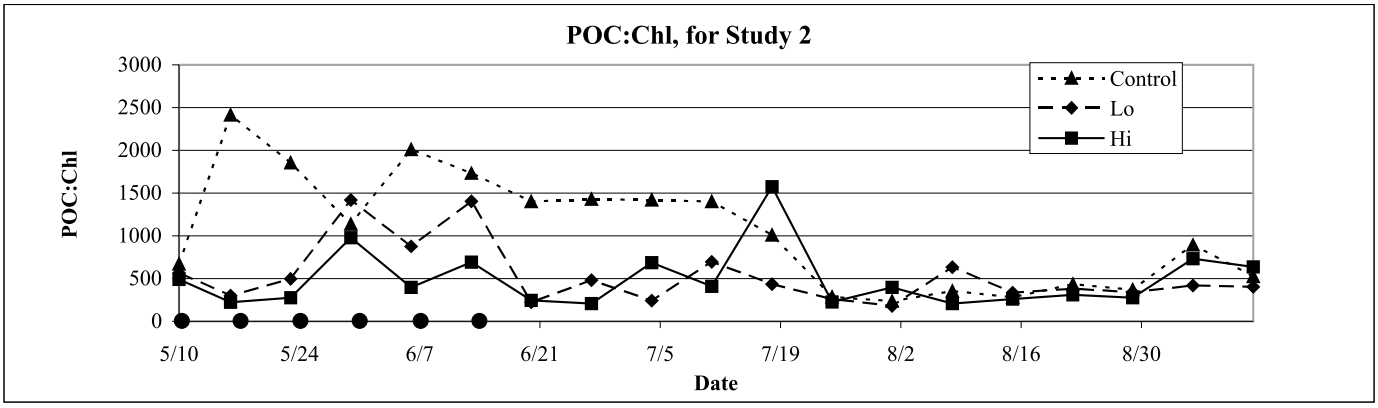
Figure 50. *Changes in particulate organic carbon concentrations over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced POC in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of POC over the experimental season pooled by treatment. Dark circles on X axis indicate dose dates. The lower table lists LS Means (mg POC/L) represented in the graph.*

chlorophyll. POC:Chl ratios in the dosed treatments averaged <1000:1 and were significantly lower than the Control on most dates between May and mid-July. Because POC values were similar at that time, lower POC:Chl ratios in the dosed treatments reflect a greater proportion of living algal biomass in the suspended carbon pool as compared to the Control. POC:Chl ratios in the dosed treatments averaged <500:1 in August and September, similar to the Control.

Dose had no significant effects (p>0.05) on the major taxa of phytoplankton; however, Day was a significant factor (p=0.0001) as phytoplankton increased seasonally (Figure 52). Initial numbers of phytoplankton were less than $0.4*10^6$ cells/L in May samples, and numbers were evenly distributed across major divisions. Phytoplankton numbers increased in June, however, to a community dominated by chlorophytes. Phytoplankton numbers increased further in July, after dosing had ended, to an average of $11.8*10^6$ cells/L; approximately 80% of the community was chlorophytes, whereas cyanophytes comprised 20% of the community. Total numbers of algae declined between July and August to approximately $6.0*10^6$ cells/L but shifted in proportions to equal numbers of Chlorophytes and Cyanophytes. By September, however, algal cell numbers had increased to a seasonal high of $25.7*10^6$ cells/L and a community dominated by Cyanophytes. On all dates Euglenophytes, Bacillariophytes, and Cryptophytes were rare. Thus, the phytoplankton community was more sensitive to seasonal changes in light and temperature than to nutrient dosing.

A total of 137 species of algae/cyanobacteria were observed in Study 2; the list of observed phytoplankton species is presented in Table 7. We observed 62 species of Chlorophytes; 37 species of Bacillarophytes; 15 species of Cyanophytes; 13 species of Euglenophytes; 5 species of Chysophytes; 4 species of Cryptophytes; and 1 species of Pyrrophycota. Early in the season, the Chlorophytes were dominated by *Gleocystis*, *Scenedesmus*, and *Oedegonium sp.*; whereas, the Cyanophytes were dominated by the filamentous *Oscillatoria sp.* By the end of Study 2, the algal community was dominated by the filamentous cyanophyte *Oscillatoria sp.* and the chlorophytes *Pleodorina*, *Oocystis*, *Characium*, and *Oedeogonium* sp.; lesser amounts of the Euglenophytes (*Trachlemonas* and *Euglena* sp.) and the Bacillariophytes (*Navicula*, *Nitzschia*, *Fragilaria*, and *Gomphonema* sp.) were observed.

	Dose	Day	Dose*Day
POC:Chl	0.0099	0.0001	0.0028



		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Dose	Control	670	2413[a]	1854[a]	1135	2008[a]	1730[a]	1400[a]	1427[a]	1419[a]	1400[a]	1010[a]	285	235	353[ab]	281	434	369	892	523	1044[a]
	Lo	562	301[b]	496[b]	1418	875[ab]	1404[b]	222[b]	479[b]	242[b]	695[b]	434[b]	259	181	632[a]	334	380	342	419	404	530[b]
	Hi	488	223[b]	276[b]	979	397[b]	689[ab]	245[b]	206[c]	684[b]	409[b]	1572[a]	226	397	208[b]	260	310	276	732	634	485[b]

Figure 51. *Changes in the ratio of particulate organic carbon to phytoplankton chlorophyll over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced POC:Chl in ANOVA of rank-transformed data. The graph is a plot of POC:Chl over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

	5/11/00	6/7/00	7/5/00	8/2/00	9/5/00
Total	0.3479	1.0865	11.8264	6.0000	25.7251
Chlorophyta	0.2280	0.9270	8.3968	2.6262	7.9720
Euglenophyta	0.0010	0.0003	0.0143	0.0003	0.8118
Bacillariophyta	0.0725	0.1225	0.4033	0.0711	0.4051
Cryptophyta	0.0357	0.0000	0.0120	0.0079	0.0738
Cyanophyta	0.0107	0.0367	3.0000	2.1320	16.4624

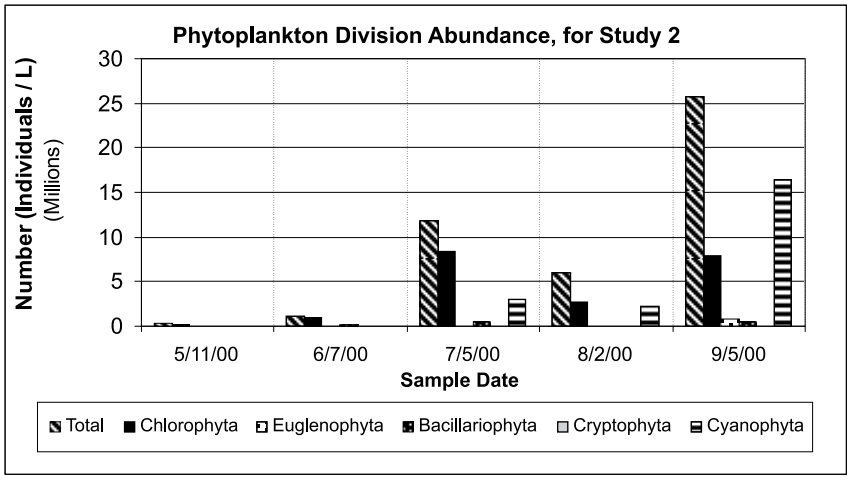


Figure 52. *Changes in phytoplankton abundance of the dominant divisions over time. Columns represent averages of all corrals in each month to show general successional trends over the season.*

Table 7. List of Phytoplankton Species Collected During Studies 2 and 3

Kingdom	Phylum/Division	Class	Order	Family	Genus	Species
Monera	**Cyanophycota**	**Cyanophyceae**	**Chroococcales**	**Chroococcaceae**	*Aphanocapsa*	**sp.**
					Chroococcus	**sp.**
					Coelosphaerium	**sp.**
					Gloeocapsa	**sp.**
					Gloeothece	**sp.**
					Merismopedia	**sp.**
					Microcystis	**sp.**
			Nostocales	**Nostocaceae**	*Anabeana*	**sp.**
					Nostoc	**sp.**
				Oscillatoriaceae	*unknown*	**sp.**
					Arthrospira	**sp.**
					Oscillatoria	**sp.**
				Rivulariaceae	*Gloeotrichia*	**sp.**
					Gloeotrichia	*echinulata*
					Rivularia	**sp.**
Plantae	**Bacillariophyta**	**Bacillariophyceae**	**Achnanthidiaceae**	**Achnanthidiaceae**	*Achnanthidium*	**sp.**
			Bacillariales	**Bacillariaceae**	*Hantzschia*	**sp.**
					Nitzschia	**sp.**
					Nitzschia	*intermedia*
					Nitzschia	*linearis*
					Nitzschia	*pura*
					Nitzschia	*sigma*
					Tryblionella	*acuta*
			Cymbellales	**Anomoeoneidaceae**	*Anomoeoneis*	*sphaerophora*
				Cymbellaceae	*Cymbella*	**sp.**
				Gomphonemataceae	*Gomphonema*	*acuminatum*
					Gomphonema	*angustatum*
					Gomphonema	*augur*
					Gomphonema	*ventricosum*
			Fragilariales	**Fragilariaceae**	*Fragillaria*	*crotonensis*
					Synedra	**sp.**
					Synedra	*acus*
					Synedra	*berolinensis*
Plantae	**Bacillariophyta**	**Bacillariophyceae**	**Fragilariales**	**Fragilariaceae**	*Synedra*	*rumpens*
					Synedra	*ulna*
			Naviculales	**Naviculaceae**	*Navicula*	**sp.**
					Navicula	*trivialis*
				Pinnulariaceae	*Caloneis*	*hyalina*

Table 7. List of Phytoplankton Species Collected During Studies 2 and 3 (Continued)

Kingdom	Phylum/Division	Class	Order	Family	Genus	Species
					Pinnularia	**sp.**
					Pinnularia	*lundii*
				Stauroneidaceae	*Stauroneis*	*phoenicenteron*
			Rhopalodiales	**Rhopalodiaceae**	*Epithemia*	**sp.**
					Epithemia	*argus*
					Epithemia	*turgida*
					Epithemia	*adnata*
					Rhopalodia	*gibba*
			Surirellales	**Surirellaceae**	*Surirella*	*capronii*
			Thalassiophysales	**Catenulaceae**	*Amphora*	**sp.**
					Amphora	*exiqua*
					Amphora	*normanii*
					Amphora	*pediculus*
					Amphora	*veneta*
	Chlorophyta	**Chlorophyceae**	**Chaetophorales**	**Chaetophoraceae**	*Gongrosira*	*lacustris*
			Chlorococcales	**Characiaceae**	*Characium*	**sp.**
					Characium	*limnetica*
				Chlorococcaceae	*Schroederia*	**sp.**
					Schroederia	*judayi*
					Schroederia	*setigera*
					Tetraedron	*arthrodesmiforme*
					Tetraedron	*constrictum*
				Coccomyxaceae	*Dispora*	*crucigenoides*
					Gloeocystis	**sp.**
					Gloeocystis	*ampla*
					Gloeocystis	*gigas*
					Gloeocystis	*major*
				Dictyosphaeriaceae	*Botryococcus*	**sp.**
					Pediastrum	*duplex*
				Oocystaceae	*Ankistrodesmus*	**sp.**
					Ankistrodesmus	*falcatus*
					Kirchneriella	**sp.**
					Langerheimiella	*subsalsa*
					Oocystis	**sp.**
			Cladophorales	**Cladophoraceae**	*Pithophora*	**sp.**
	Chlorophyta	**Chlorophyceae**	**Chlorococcales**	**Scenedesmaceae**	*Coelastrum*	*microporum*
					Scenedesmus	*abundans*
					Scenedesmus	*arcuatus*

Table 7. List of Phytoplankton Species Collected During Studies 2 and 3 (Continued)

Kingdom	Phylum/Division	Class	Order	Family	Genus	Species
					Scenedesmus	*armatus*
					Scenedesmus	*bernardii*
					Scenedesmus	*bijuga*
					Scenedesmus	*dimorphus*
					Scenedesmus	*longus var. naegelii*
					Scenedesmus	*quadricauta*
			Microsporales	**Microsporaceae**	*Microspora*	**sp.**
			Oedogoniales	**Oedogoniaceae**	*Bulbochaete*	**sp.**
					Oedogonium	**sp.**
			Tetrasporales	**Palmellopsidaceae**	*Sphaerocystis*	*schroeteri*
			Ulotrichales	**Ulotrichaceae**	*Ulothrix*	**sp.**
					Ulothrix	*tenerrima*
			Volvocales	**Chlamydomonadaceae**	*Carteria*	**sp.**
					Chlamydomonas	**sp.**
					Chlorogonium	*spirale*
				Haematococcaceae	*Haematococcus*	*lacustris*
				Volvocaceae	*Eudorina*	*elegans*
					Gonium	*pectorale*
					Pandorina	**sp.**
					Pleodorina	*californica*
					Volvox	**sp.**
Plantae	**Chlorophyta**	**Chlorophyceae**	**Zygnematales**	**Desmidiaceae**	*Closterium*	**sp.**
					Closterium	*incurvum*
					Closterium	*leibleinii*
					Closterium	*turgidum*
					Closterium	*venus*
					Cosmarium	**sp.**
					Cosmarium	*comminsurale var. crassum*
					Cosmarium	*cosmetum*
					Staurastrum	**sp.**
					Staurastrum	*cingulum*
					Staurastrum	*ophiura*
				Zygnemataceae	*Mougeotia*	**sp.**
					Mougeotia	*elegantula*
					Mougeotiopsis	*calospora*
					Spirogyra	*fallax*

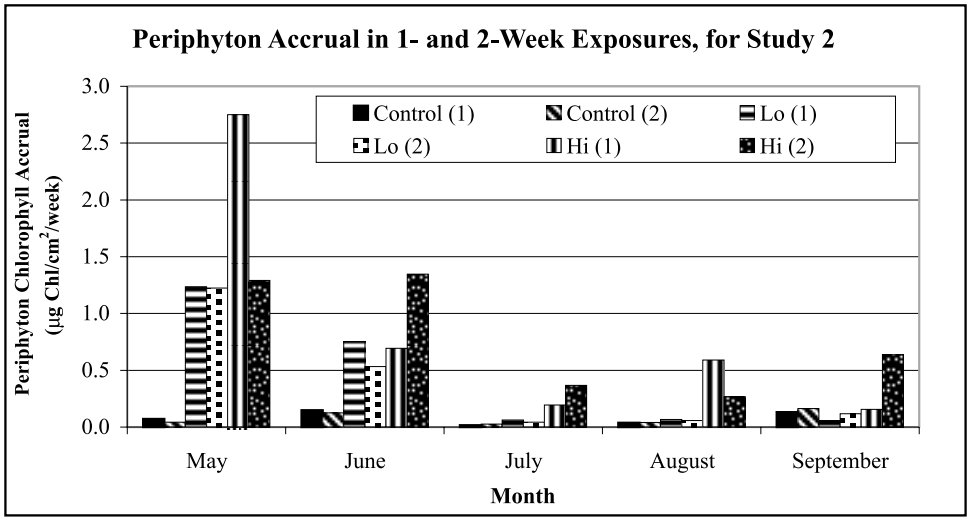
Table 7. List of Phytoplankton Species Collected During Studies 2 and 3 (Continued)

Kingdom	Phylum/Division	Class	Order	Family	Genus	Species
					Zygnema	**sp.**
	Chrysophyta	**Chrysophyceae**	**Chromalinales**	**Chromulinaceae**	*Chromulina*	**sp.**
				Chrysococcaceae	*Chrysococcus*	**sp.**
			Ochromonadales	**Synuraceae**	*Mallomonas*	**sp.**
					Synura	**sp.**
			Rhizochrysidales	**Rhizochrysidaceae**	*Chrysamoeba*	**sp.**
		Cryptophyceae	**Cryptomonadales**	**Cryptomonadaceae**	*unknown*	**sp.**
					Chroomonas	**sp.**
					Rhodomonas	**sp.**
					Rhodomonas	*minuta*
	Euglenophycota	**Euglenophyceae**	**Euglenales**	**Euglenaceae**	*Euglena*	**sp.**
					Euglena	*acus*
					Euglena	*caudata*
					Euglena	*convoluta*
					Phacus	*anocoelus*
					Phacus	*chloroplastes*
					Phacus	*hilikoides*
Plantae	**Euglenophycota**	**Euglenophyceae**	**Euglenales**	**Euglenaceae**	*Phacus*	*longicauda*
					Phacus	*triqueter*
					Trachelomonas	**sp.**
					Trachlemonas	*volvocina*
					Trachlemonas	*hispida*
					Trachlemonas	*robusta*
	Pyrrophycophyta	**Dinophyceae**	**Peridiniales**	**Peridiniaceae**	*Peridinium*	*willei*

Periphyton

Periphyton accrual rates were significantly affected by Day ($p<0.0001$) and Dose ($p<0.003$) for both the one- and two-week accrual intervals (Figure 53). In addition, the Dose*Day interaction was significant ($p=0.0174$) for the 1-week accrual data. One-week periphyton accrual rates averaged 0.08, 0.44, and 0.88 µg Chl/cm^2/wk in the Control, Lo, and Hi treatments, respectively. Two-week periphyton accrual rates averaged 0.08, 0.39, and 0.78 µg Chl/cm^2/wk, respectively, in the Control, Lo, and Hi treatments. Control values peaked in June and September, averaging 0.14 µg Chl/cm^2/wk, and were 3-fold rates in May, July, and August (average 0.04 µg Chl/cm^2/wk). Highest levels of periphyton accrual in the Lo (1.23 µg Chl/cm^2/wk) and Hi (2.75 µg Chl/cm^2/wk) treatments occurred in May following the initiation of dosing when nutrients and light were un-limited. Accrual levels in this study were 10-fold higher than in Study 1 due to the earlier timing of dosing, higher levels of dosing, and decreased competition with macrophytes due to the study design. Biomass remained significantly higher in the Lo and Hi treatments in June and July but at lower levels than in May as nutrients and light began to limit periphyton growth. In general, the 1-week and 2-week accrual rates were similar within both the Control and Lo treatments. However, the 1-week and 2-week accrual rates varied in the Hi treatment most likely due to variation in levels of self-shading of periphyton and macrophytes.

	Dose	Day	Dose*Day
Periphyton 1-week	0.0011	0.0001	0.0174
Periphyton 2-week	0.0026	0.0001	0.0912



		Month										Season	
		May		June		July		August		September		Avg.	
		1-wk	2-wk	1-wk	2-wk	1-wk	2-wk	1-wk	2-wk	1-wk	2-wk	1-wk	2-wk
Dose	Control	0.07^a	0.04	0.15^a	0.13	0.02^a	0.03	0.04^a	0.04	0.13	0.16	0.08^a	0.08^a
	Lo	1.23^b	1.22	0.75^b	0.53	0.06^b	0.04	0.07^a	0.06	0.06	0.12	0.44^b	0.39^b
	Hi	2.75^b	1.29	0.69^{ab}	1.35	0.20^b	0.37	0.59^b	0.27	0.16	0.64	0.88^c	0.78^b

Figure 53. *Changes in accrual rates of periphyton chlorophyll in 1- and 2-week exposures over time. The 1- and 2-week datasets were analyzed separately. The upper table presents probabilities that dose, day, and interactions of dose and day influenced accrual rates in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of accrual rates over the experimental season pooled by treatment. Solid and striped bars represent 1-and 2-week exposures, respectively. The lower table lists LS Means (mg Chl/cm^2/wk) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

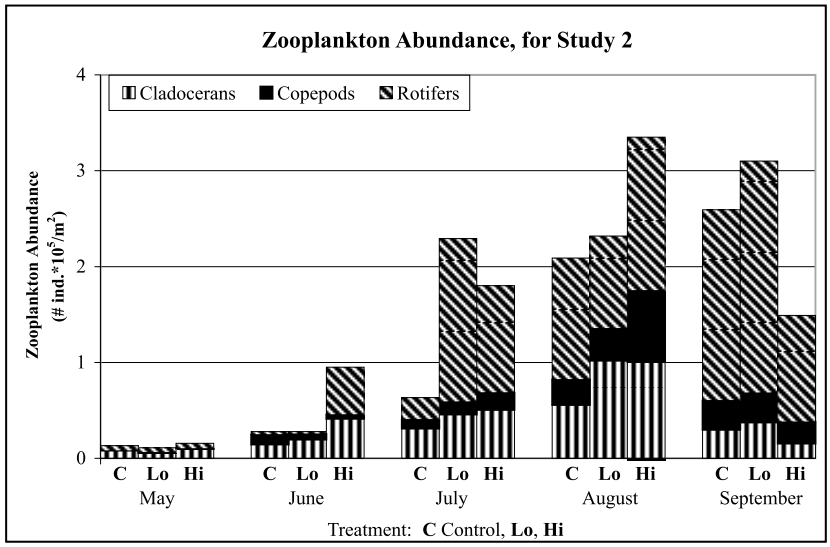
Zooplankton

Day (p=0.0001) was a significant main effect influencing total numbers of zooplankton as well as numbers of cladocerans, copepods, and rotifers (Figure 54). Dose had no effect on zooplankton numbers. The Dose*Day interaction was significant only for total zooplankton numbers.

Identification of monthly zooplankton samples yielded 35 genera, including 16 rotifers, 12 cladocerans, and 7 copepods. A list of observed species is presented in Table 8. Numerically, macrozooplankton (cladocerans and copepods) and microzooplankton (rotifers and nauplii) represented 40% and 60%, respectively, of the total organisms collected each month. This relative abundance did not significantly change (p>0.05) during the season or with treatment.

In the Control, abundance of zooplankton increased 20-fold from May to September (Figure 54). Total zooplankton abundance was at a seasonal minimum in May ($0.1*10^5$ zooplankton/m^2) and was similar among treatments. In June, total zooplankton increased 6-fold in the Hi treatment ($1.0*10^5$ zooplankton /m^2) and was significantly larger ($p<0.05$) than in the Control ($0.3*10^5$ zooplankton /m^2) and Lo ($0.3*10^5$ zooplankton /m^2) treatments. In July total zooplankton abundance increased in both the Lo ($2.3*10^5/m^{2)}$ and Hi ($1.8*10^5/m^{2)}$ treatments compared to the Control. Total zooplankton numbers were similar across treatment in August and September. Regressions of Chl to total zooplankton abundance indicated that relationships were significant for the Control ($r^2=0.41$; $p<0.005$) but not for the Lo ($r^2= 0.16$; $p<0.1$) or Hi ($r^2= 0.08$; $p<0.5$) treatments.

	Dose	Day	Dose*Day
Total Zooplankton	0.0550	0.0001	0.0167
Cladocerans	0.6252	0.0013	0.7403
Copepods	0.6669	0.0001	0.9821
Rotifers	0.3717	0.0001	0.4090



		Month					Season
		May	June	July	August	September	Avg.
Dose	Control	13337	30719[a]	65669[a]	223446	271863[ab]	121007
	Lo	11420	29409[a]	232999[b]	238095	335594[a]	169503
	Hi	16108	98059[b]	181778[b]	339782	154545[b]	158055

Figure 54. *Changes in zooplankton abundance over time. The upper table presents probabilities that dose, day, and interactions of dose and day influenced abundance of the total zooplankton community, cladocerans, copepods, and rotifers in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of abundance over the experimental season (Control (C), Lo, Hi). Abundances in the respective categories (Cladocerans- stripes; Copepods- solids; Rotifers- checks) are stacked to indicate totals. The lower table lists LS Means (# of indiv./m^2) for abundance of the total zooplankton community, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Table 8. List of Zooplankton Species Collected During Studies 2 and 3

Phylum	Class	Subclass	Order	Suborder	Family	Genus	Species
Arthropoda	**Branchiopoda**	**Phyllopoda**	**Diplostraca**	**Cladocera**	**Chydoridae**	*Alona*	**sp**.
						Alona	*affinis*
						Alona	*karua*
						Alona	*guttata*
						Alonella	**sp**.
						Alonella	*globulosa*
						Chydorus	*sphaericus*
						Kurzia	*latissima*
						Leydigia	*acanthocercoides*
						Pleuroxus	*bidentata*
						Pleuroxus	*denticulus*
					Daphniidae	*Ceriodaphnia*	**sp.**
						Ceriodaphnia	*quadrangula*
						Ceriodaphnia	*reticulata*
						Daphnia	*laevis*
						Daphnia	*magna*
						Daphnia	*pulex*
						Moina	**sp.**
						Scapholeberis	*mucronata*
						Simocephalus	**sp.**
						Simocephalus	*expinosus*
						Simocephalus	*serrulatus*
					Macrothricidae	*Macrothrix*	*rosea*
					Sididae	*Sididae*	**sp.**
						Diaphanosoma	*brachyurum*
	Maxillopoda	**Copepoda**	**Calanoida**		**Calanidae**	*Calanoid*	*copepodid*
					Diaptomidae	*Diaptomus*	*sanguineus*
						Diaptomus	*virginiensis*
						Leptodiaptomus	*ashlandii*
						Skistodiaptomus	*mississippiensis*
						Skistodiaptomus	*pallidus*
			Cyclopoida		**Cyclopidae**	*Acanthocyclops*	*vernalis*
						Cyclopoid	*copepodid*
Arthropoda	**Maxillopoda**	**Copepoda**	**Cyclopoida**		**Cyclopidae**	*Diacyclops*	*thomasi*
						Eucyclops	*agilis*
						Tropocyclops	*prasinus*

Table 8. List of Zooplankton Species Collected During Studies 2 and 3. (Continued)

Phylum	Class	Subclass	Order	Suborder	Family	Genus	Species
Arthropoda	**Ostracoda**						
Rotifera	**Bdelloidea**		**Bdelloida**				
	Monogononta				**Conochilidae**	*Conochilus*	*unicornis*
					Filiniidae	*Filinia*	*longiseta*
					Hexarthridae	*Hexarthra*	*mira*
			Ploima		**Asplanchnidae**	*Asplanchna*	**sp.**
					Brachionidae	*Brachionus*	*calyciflorus*
						Brachionus	*caudatus*
						Brachionus	*patulus*
						Brachionus	*quadradentatus*
						Euchlanis	*dialata*
						Keratella	*cochlearis*
						Keratella	*quadrata*
						Lepadella	*ovalis*
						Platyias	*quadricornis*
					Lecanidae	*Lecane*	*sp.*
						Lecane	*crenata*
						Monostyla	*sp.*
						Monostyla	*bulla*
					Notommatidae	*Cephalodella*	*sp.*
					Synchaetidae	*Ploesoma*	*sp.*
						Polyarthra	*vulgaris*
						Synchaeta	*pectinata*
					Trichocercidae	*Trichocerca*	*longiseta*
						Trichocerca	*multicrinis*
						Trichocerca	*pusilla*
						Trichocerca	*similis*

Rotifers accounted for approximately 75% of the zooplankton numbers during the season. Rotifer populations significantly increased ($p<0.05$) during the season, but the effect of nutrient enrichment on rotifer numbers was not significant ($p>0.05$). In May and June, rotifers averaged $<0.5*10^5/m^2$ and were dominated by *Bdelloid* spp., *Euchlanis* spp., *Hexartha mira*, *Lecane* spp., and *Monostyla* spp. Rotifers increased from an average of $1.0*10^5/m^2$ in July, to $1.8*10^5/m^2$ in September, when *Brachionus* spp., *Euchlanis* spp., *H. mira,* and *Monostyla bulla* dominated.

Cladocerans and copepods were at seasonal minimums in May ($0.1*10^5/m^2$), peaked over $0.8*10^5/m^2$ in August, and averaged $0.6*10^5/m^2$ in September. Cladocerans represented 97% of the macrozooplankton in May and were dominated by *Sididae* spp. In June and July, cladocerans accounted for 75% of the macrozooplankton and were dominated by *Simocephalus serrulatus* and *Ceriodaphnia* spp. Copepod populations in June and July were approximately 43% calanoids and 57% cyclopoids. In August, at the peak of macrozooplankton abundance, cladocerans accounted for 67% of macrozooplankton numbers and were predominantly *Alona* spp., *Ceriodaphnia* spp., *Chydorus sphaericus*, and *Simocephalus serrulatus*. Copepods were primarily cyclopoids (>95%) in August. Cladoceran populations declined at the end of the season, and final macrozooplankton communities were close to 1:1 cladocerans to copepods. In September, dominant genera in both cladocerans and copepods were the same as in August.

Sediment

Nitrogen and phosphorus in sediments were significantly influenced by Day ($p=0.0043$), but were not affected by nutrient amendments. Nitrogen content in sediments was greatest in June and September, averaging 0.35% N (Figure 55) which was similar to nitrogen concentrations observed in Study 1 (Figure 27). In July and August, N content averaged 0.29% N. Sediments contained approximately 157 g N/(m^2*5 cm deep) in June and September, and around 137 g N/(m^2*5 cm deep) during July and August. Total nitrogen pools in sediment were similar to those observed in Study 1 (Figure 27).

	Dose	Day	Dose*Day
Sediment Nitrogen	0.9842	0.0043	0.4733

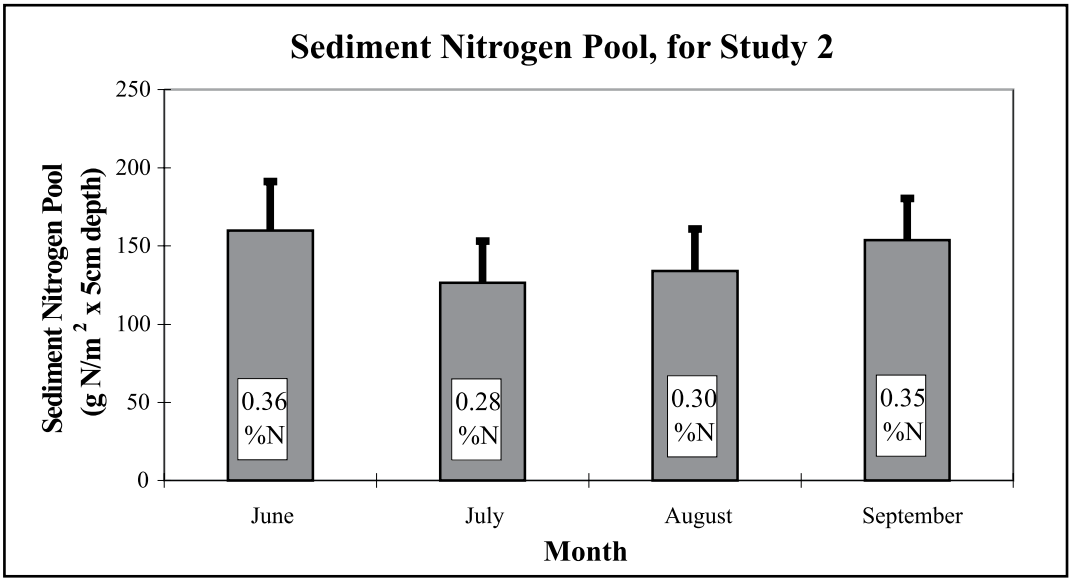


Figure 55. *Changes in the sediment nitrogen pool over time. The table presents probabilities that dose, day, and interactions of dose and day influenced N content in sediments in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of the estimated pool of N in the sediments for an area of 1 m^2 and a depth of 5 cm. Because treatment influences were not significant, monthly values are presented as averages of all treatments. The average %N in sediments is shown within the column for each month.*

Sediment phosphorus pools were not statistically related to Dose. Day (p=0.0004) was a significant main effect as sediment concentrations declined slightly over time (Figure 56). Sediment phosphorus concentrations were at a maximum in June at 0.07% P and decreased slightly in subsequent months to approximately 0.06% P. Sediment phosphorus pools ranged from 26 to 32 g P/(m^2*5 cm deep) during the season. Sediment phosphorus data was similar to that observed in Study 1 (Figure 28).

System Metabolism

Day had a significant main effect on observed values of gross primary production and community respiration (Figure 57). Dose had no significant effect on either parameter when combined over the entire study; however, lack of effect is biased by the large number of observations in the dataset, and differences among treatments were significant on several dates during the dosing period (p<0.05).

	Dose	Day	Dose*Day
Sediment Phosphorus	0.6012	0.0004	0.4261

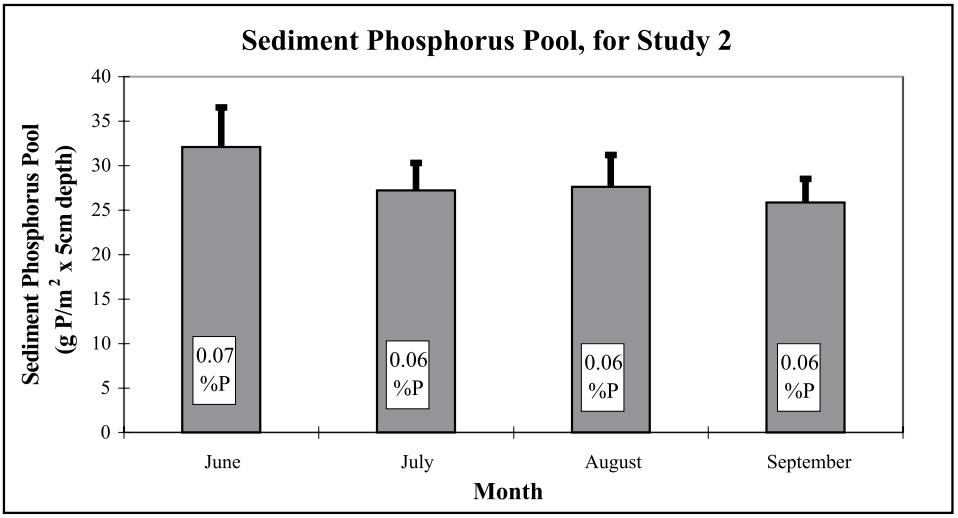
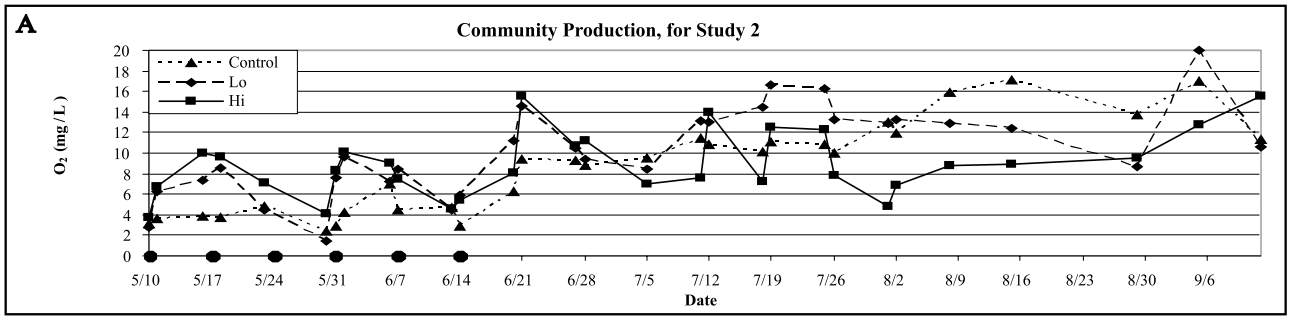
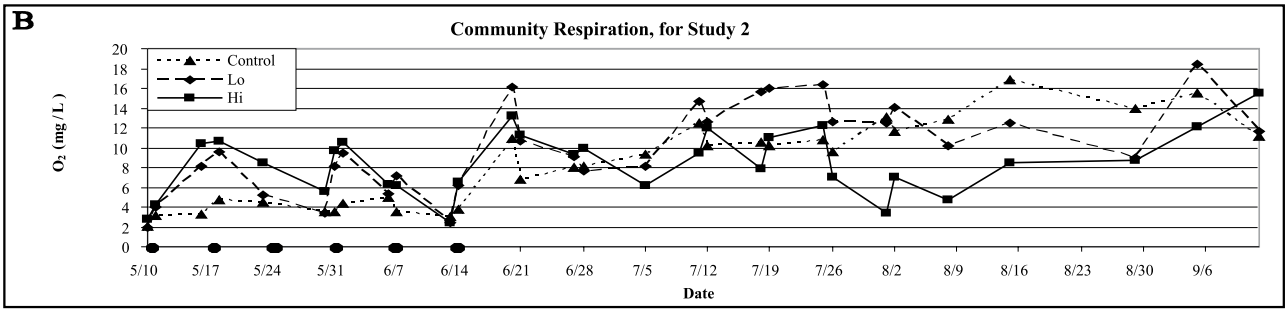


Figure 56. *Changes in the sediment phosphorus pool over time. The table presents probabilities that dose, day, and interactions of dose and day influenced P content in sediments in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of the estimated pool of P in the sediments for an area of 1 m^2 and a depth of 5 cm. Because treatment influences were not significant, monthly values are presented as averages of all treatments. The average %P in sediments is shown within the column for each month.*

Estimates of both diel gross primary production and community respiration increased 3-fold over the season, from around 4 mg O_2/L in May and early June, to around 15 mg O_2/L in August and September (Figure 57). Oxygen production in the Lo treatment was significantly larger than the Control on May 18, May 31, June 1, and June 7. Oxygen production in the Hi treatment was significantly larger ($p<0.05$) than the Control on May 16, May 18, May 31, June 1, and June 21. Near the end of the season, oxygen production in the Hi treatment was significantly smaller than the Control on August 1, August 2, August 8, and August 15. Respiration values were similar to production in magnitude, seasonality, and responses to enrichment. Oxygen respiration in Lo was significantly larger than the Control on May 16, May 18, May 31, and June 1. Community respiration in the Hi treatment was significantly greater than the Control on May 16, May 18, May 31, June 1, and June 21. Respiration in the Hi treatment was significantly less than the Control ($p<0.05$) throughout much of August. Collectively, the data reflect the significant increases in primary productivity of phytoplankton (Figure 49) and periphyton (Figure 53) due to dosing of nutrients early in the season prior to macrophyte development.

	Dose	Day	Dose*Day
Production	0.6341	0.0001	0.0001
Respiration	0.6352	0.0001	0.0001





		Day															
		1	2	7	9	14	21	22	23	28	29	35	36	42	43	49	
Dose	Control	3.08	3.65	3.90	3.75	4.78	2.45	2.85	4.25	6.95	4.43	4.65	2.88	6.28	9.45	9.33	
	Lo	2.80	6.25	7.40	8.55	4.50	1.50	7.63	9.63	7.28	8.45	4.43	5.90	11.23	14.58	10.50	
	Hi	3.68	6.78	10.00	9.60	7.10	4.10	8.33	10.10	9.08	7.43	4.55	5.48	8.08	15.50	10.78	
		50	57	63	64	70	71	77	78	84	85	91	98	112	119	126	**Season Avg.**
Dose	Control	8.80	9.55	11.50	10.85	10.08	11.03	10.83	9.98	12.98	11.88	15.88	17.10	13.75	17.00	11.33	8.51
	Lo	9.40	8.43	13.15	13.03	14.45	16.65	16.23	13.25	12.95	13.20	12.88	12.38	8.73	20.03	10.65	10.20
	Hi	11.25	7.00	7.65	13.93	7.25	12.55	12.30	7.88	4.83	6.90	8.75	8.88	9.53	12.78	15.55	8.92

Figure 57. *Changes in community oxygen and respiration production over time. The table presents probabilities that dose, day, and interactions of dose and day influenced production and respiration in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graphs are plots of production (A) and respiration (B) over the experimental season pooled by treatment. Dark circles indicate dose dates. The lower table lists LS Means (mg/L) represented in the graph.*

Net Nutrient Balance

During Study 2, a maximum of 60 g N/m^2 and 2.4 g P/m^2 was applied. A final mass balance of nutrients was calculated among various nutrient pools to determine the net efficiency of uptake and assimilation of nutrients in these experimental systems (Table 9). At the end of the study, macrophytes contained a total of 4.29, 3.92, and 1.05 g N/m^2 in the Control, Lo, and Hi treatments, respectively; water contained an additional 0.63, 0.83, and 1.10 g N/m^2 in the Control, Lo, and Hi treatments, respectively. Combined (macrophytes + water), these two nutrient pools contained 4.92, 4.75, and 2.15 g N/m^2 stocks at the end of the study. Thus, a total of 16% (Lo) and 4% (Hi treatment) of total nitrogen added during the study were found in these two major nitrogen pools at the end of the study. This implies, under simple mass balance conditions (i.e., no loss to the atmosphere) that up to 84% (Lo treatment) and 96% (Hi treatment) of total nitrogen added were absorbed or lost to the sediments as detritus. Attempts to measure sediment nutrient dynamics did not reveal the amount of nitrogen transferred due to a combination of the large mass of pre-existing nitrogen in sediments and the error contributed by our sampling procedures. However, we know that these numbers are conservative, since the Control treatment alone exhibited a total sequestration of 4.92 g N/m^2 (combined macrophytes and water) at the end of the study in the absence of external nitrogen addition. The large percentage of nitrogen that was not accounted for at the end of the study (84%, Lo treatment; 96% Hi treatment) indicates that these shallow, vegetated aquatic systems served as efficient biological, chemical, and physical sink for nitrogen.

Phosphorus, likewise, was efficiently assimilated and retained in the study. At the end of the study, macrophytes contained a total stock of 0.80, 0.65, and 0.21 g P/m^2 in the Control, Lo, and Hi treatments, respectively; water contained an additional stock of 0.07, 0.08, and 0.12 g/P/m^2 in the Control, Lo, and Hi treatments, respectively. Using conservative mass balance estimates, subtracting the amount of phosphorus in macrophytes and water from that applied in dosing indicates that sediments had a net accrual of 0.47 (Lo treatment) and 2.07 (Hi treatment) g P/m^2. Macrophytes in the Control treatment contained 4-fold more phosphorus than the Hi treatment at the end of the study even though no phosphorus was applied. This difference in macrophyte storage is largely due to the significant higher biomass observed in the Control compared to the Hi treatment (Figure 32). Thus, even though the Hi treatment significantly reduced macrophyte biomass compared to Controls, the system efficiently retained phosphorus.

Table 9. Summary Table of the Final Store of Phosphorus and Nitrogen in Water and Macrophytes in Study 2. Load Represents the Total Amount of Fertilizer P or N Added in Each Treatment. Stores in Macrophytes are Considered as Grams Per Cubic Meter Because Water Depth was 1 m

Nitrogen	**Load** (g N/m^2)	**Macrophytes** (g N/m^2)	**Water** (g N/m^2)	**Macrophytes + Water Total** (g N/m^2)	**Presumed Sediment Transfer** (g N/m^2)
Control	0	4.29	0.63	4.92	NA
Lo	30	3.92 (13%)	0.83 (3%)	4.75 (16%)	25.25 (84%)
Hi	60	1.05 (2%)	1.10 (2%)	2.15 (4%)	57.85 (96%)
Phosphorus	**Load** (g P/m^2)	**Macrophytes** (g P/m^2)	**Water** (g P/m^2)	**Macrophytes + Water Total** (g P/m^2)	**Presumed Sediment Transfer** (g P/m^2)
Control	0	0.80	0.07	0.87	NA
Lo	1.2	0.65 (54%)	0.08(7%)	0.73 (61%)	0.47 (39%)
Hi	2.4	0.21 (9%).	0.12 (5%)	0.33 (14%)	2.07 (86%)

STUDY 3: Effects of Dosing in Relation to Macrophyte Stage

Study 3 was conducted concurrently with Study 2 in 2000 to evaluate the effect of the timing of nutrient additions in relation to stage of macrophyte development. The results of Study 2 demonstrated that early, intense dosing of nutrients significantly reduced macrophyte biomass compared to the Control treatment, but that shallow, vegetated aquatic systems were still efficient in removing both nitrogen and phosphorus. There were three objectives in Study 3: 1) to determine how the stage of macrophyte development influenced the concentration and relative distribution of nutrients in the water column, macrophytes, and sediments; 2) to determine if the timing of nutrient addition in relationship to macrophyte stage influenced species composition, biomass, and/or abundance of macrophytes, phytoplankton, periphyton, and zooplankton; and 3) to characterize the assimilation and retention capabilities of shallow ponds receiving doses at different stages of macrophyte development.

The hypothesis for Study 3 was that timing of additions would be influential in determination of community dominance; formation of alternative stable states (i.e., phytoplankton or macrophyte dominance) would be created based on the stage of macrophyte development at the initiation of nutrient dosing. At one extreme, phytoplankton and periphyton would be stimulated by enrichment in the Early treatment. With little initial competition from the macrophytes, algal communities would establish and maintain dominance throughout the season due to a growth rate that was higher than the grazing rate of zooplankton consumers (Scheffer 1998), and by imposing light limitation on the macrophytes (Phillips et al. 1978). In addition, overall nutrient uptake and assimilation would be reduced in the Early treatment. Mid and Late treatments, however, would be macrophyte dominated and resist a shift to phytoplankton dominance due to removal of nutrients by epiphytes/macrophytes (Scheffer 1990, 1998) and shifting to tall growth forms or species (Moss 1990). Large-bodied zooplankton would promote water clarity through grazing, which would further stabilize macrophyte dominance (Brooks and Dodson 1965, Scheffer 1998). Systems dominated by macrophytes at the timing of nutrient addition would be more efficient in nutrient uptake compared to a phytoplankton dominated system.

Experimental Design

Mesocosms were drained April 11, 2000 for corral construction. A total of 16 corrals were constructed in each of 4 mesocosms (blocks). Ponds were refilled over a 2-day period (May 7–8) and allowed to mix for 2 days prior to raising of sides of corrals on May 9. Water sampling began on May 10 and terminated September 12. Dosing began on May 11, 2000 and continued weekly for 6 weeks. A different set of ponds was used in Study 3 than in Study 1 to prevent bias due to the previous study. Study 3 was conducted over the period of May 10 to September 12 of 2000. In Study 3, nutrient additions were added during one of three stages of macrophyte growth: Early (0% cover; initiated May 11), Mid (15–25% cover; June 12), or Late (75–90% cover; July 5) (Figure 58). The three dosed treatments received the same nutrient load (30 g NO_3-N/m^2; 1.2 g P/m^2) applied as six successive weekly additions of 5 mg NO_3-N/L and 200 µg P/L (25:1 N:P ratio).

Results

Macrophytes

Macrophyte Taxa

The aquatic macrophyte community consisted of >99% *Najas* during the experiment. *Chara*, an attached macroalgae, was sparsely present in May, and was not noted in subsequent months in any treatment. The lack of *Chara* sp. may have resulted due to the fact that ponds were drawn down longer (26-day draw-down) in year 2000 compared to 1999 (16 days) which may have altered normal seasonal succession of the macrophyte community.

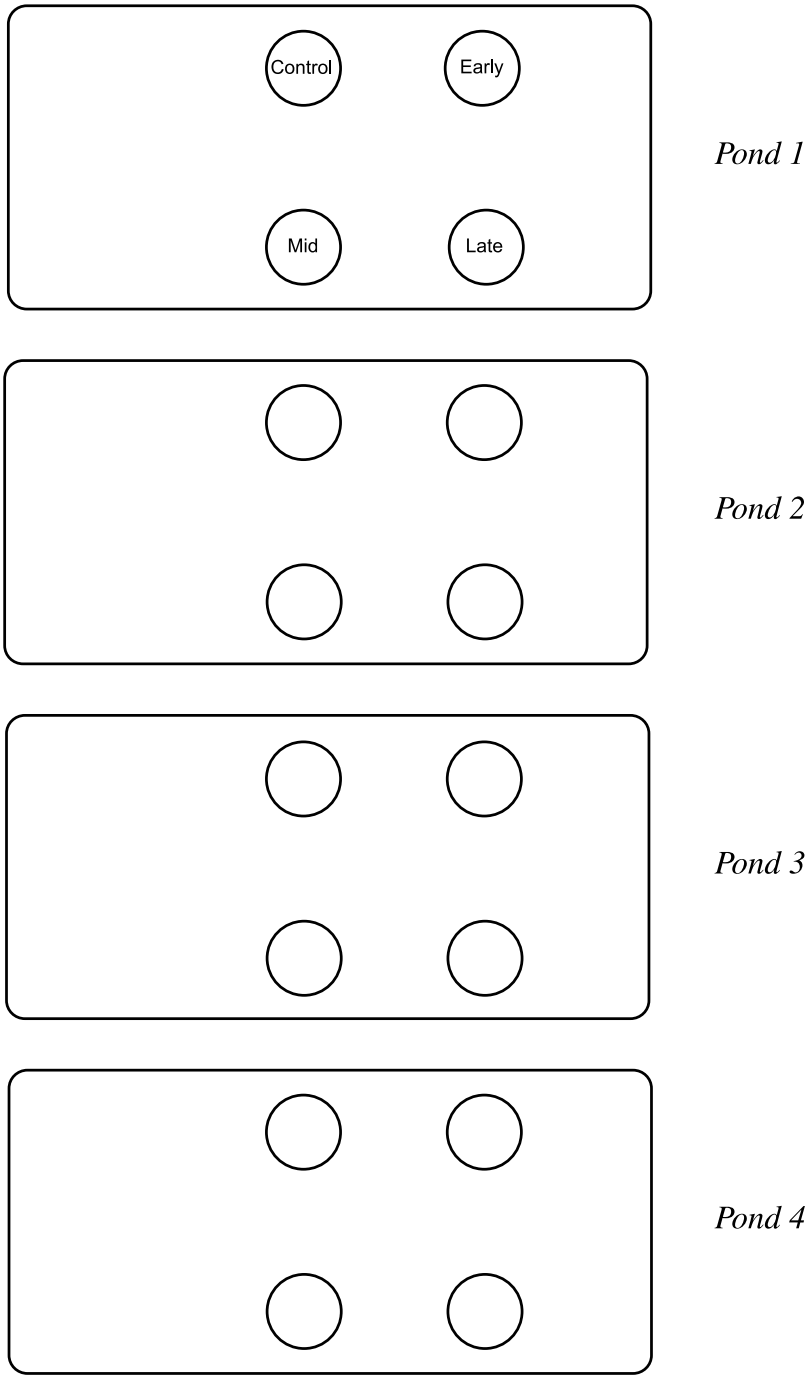
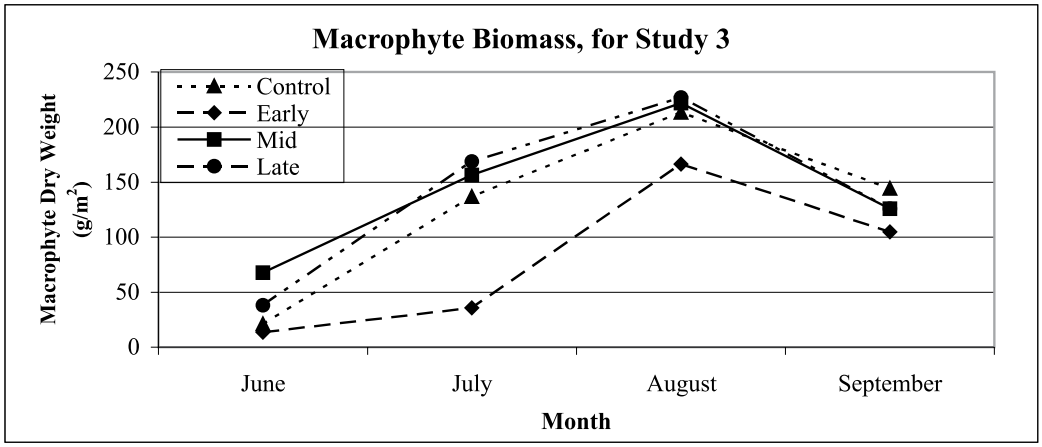


Figure 58. *Corral and pond diagram for Study 3 experiments indicating orientation of corrals. Pond 1 shows an example of the random assignment of the treatments.*

Macrophyte Biomass

Study 3 was initiated on May 10, 2000. There was no measurable growth of macrophytes above the sediment surface at the beginning of the study (i.e., Early dosing). Macrophyte stage (Stage) had no significant effect on observed macrophyte biomass (Figure 59). However, Day ($p<0.0001$) had a significant effect. Macrophyte biomass averaged 130, 80, 143, and 139 g/m^2 in the Control, Early, Mid, and Late treatments, respectively, when averaged over the four monthly sample dates (Figure 59). Maximum macrophyte biomass occurred in all treatments in early August; thereafter, macrophytes began to senesce. Macrophyte biomass was highly variable within treatments; at the peak of the growing season biomass in replicate corrals differed by as much as one order of magnitude. In the Control, the maximum biomass was 213 g/m^2 in August. Control biomass decreased by 32% to a final biomass of 144 g/m^2 in September. Nutrient enrichment had a weak influence on macrophyte development in the Early treatment ($p<0.1$), but had no influence in either the Mid or Late treatments. Biomass in the Early treatment was only 36 g/m^2 in early June, or approximately 25% of macrophyte biomass in the other treatments. During July, biomass in the Early treatment increased 4-fold and peaked at 167 g/m^2. Peak macrophyte biomass (August sampling) in the Early treatment was 75% of the maximum biomass in the Control (213 g/m^2), Mid (222 g/m^2), and Late (227 g/m^2) treatments, but differences were not significant. Percent loss of macrophytes due to senescence was similar in the Control and dosed treatments.

	Stage	Day	Stage*Day
Biomass	0.0981	0.0001	0.2285



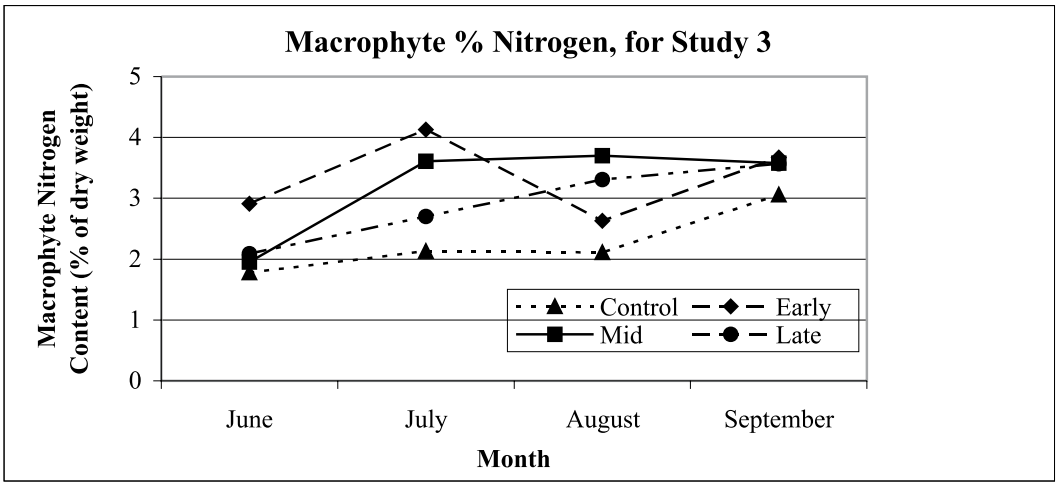
		Month				Season Avg.
		June	July	August	September	
Stage	Control	21	137	213	144	130
	Early	14	36	167	105	80
	Mid	68	157	222	126	143
	Late	38	169	227	126	139

Figure 59. *Changes in macrophyte biomass over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced macrophyte biomass in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of macrophyte biomass over the experimental season pooled by treatment. Corresponding arrows mark dose periods. The lower table lists LS Means (g dry weight/m^2) represented in the graph.*

Macrophyte Nutrients

Stage (p=0.0033), Day (p=0.0001), and the Stage*Day interaction (p=0.0421) were significant factors affecting concentrations of nitrogen in aquatic macrophytes. In the Control, N in macrophyte tissues increased during the season (Figure 60). Nitrogen content in the Control averaged around 2% N from June to August, then increased to 3.06% N in September. In amended treatments, nitrogen uptake was enhanced during and immediately following the dose period. In the Early treatment, N content in June (2.91% N) and July (4.13% N) was significantly higher than the Control ($p<0.05$), but subsequent values were similar. Nitrogen content of macrophytes in the Mid treatment significantly increased in July (3.61% N) after dose initiation, but was similar to the Control in August and September. Nitrogen content in the Late treatment significantly increased in August (3.31% N) but was similar to the Control in September. Total season averages indicated that the Late (2.92% N), Mid (3.21% N), and Early (3.34% N) treatments were significantly greater than the Control average (2.27% N) which indicates the positive response to nitrogen dosing in all treatments.

	Stage	Day	Stage*Day
Macrophyte % N	0.0033	0.0001	0.0421

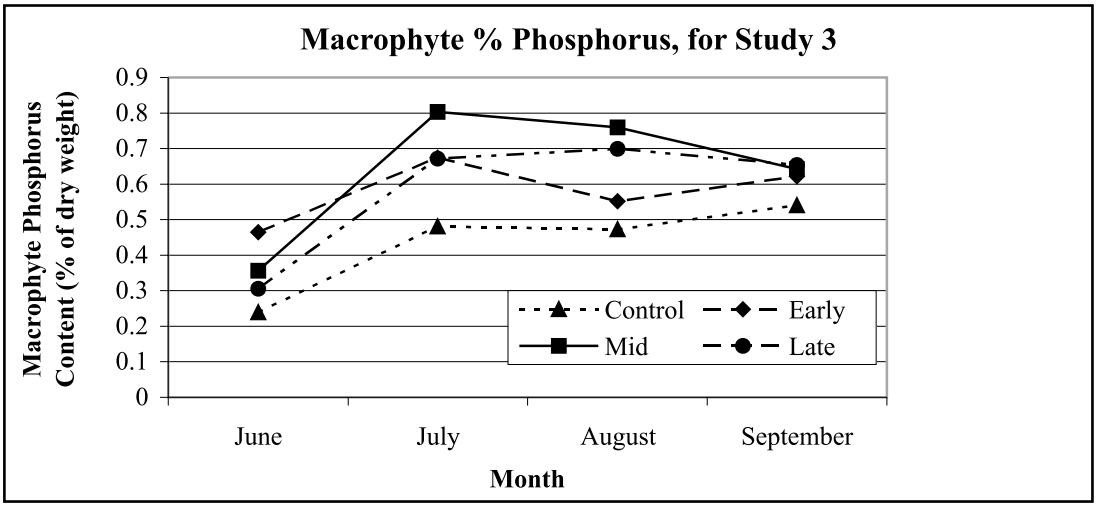


		Month				Season Avg.
		June	July	August	September	
Stage	Control	1.78[a]	2.13[a]	2.11[a]	3.06	2.27[a]
	Early	2.91[b]	4.13[b]	2.63[ac]	3.67	3.34[b]
	Mid	1.95[a]	3.61[bc]	3.70[b]	3.58	3.21[b]
	Late	2.09[ab]	2.70[ac]	3.31[bc]	3.57	2.92[b]

Figure 60. *Changes in macrophyte nitrogen content over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced N content in ANOVA of rank-transformed data. The graph is a plot of N content over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (% N of dry weight) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Stage (p=0.0128) and Day (p<0.0001) were significant main effects on phosphorus concentration of macrophytes; however, there was no Stage*Day interaction (Figure 61). Season total averages were significantly higher in the Early (0.58% P), Mid (0.64% P), and Late (0.58% P) treatments compared to the Control (0.43% P). Phosphorus content of macrophytes in individual treatments within the season did not reflect the effect of dosing due to high variability with treatments.

	Stage	Day	Stage*Day
Macrophyte % P	0.0128	0.0001	0.5899



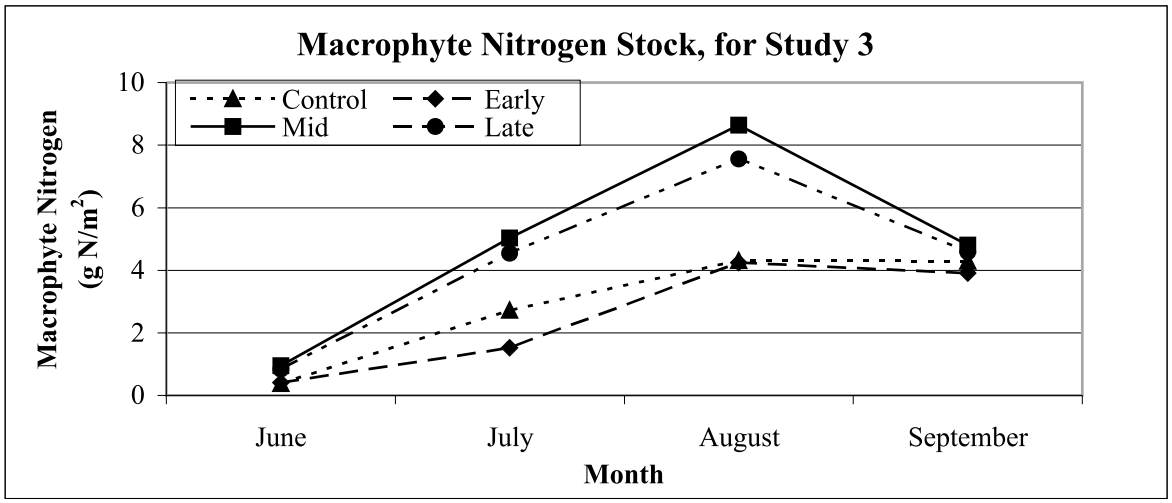
		Month				Season Avg.
		June	July	August	September	
Stage	Control	0.24	0.48	0.47	0.54	0.43[a]
	Early	0.47	0.67	0.55	0.62	0.58[b]
	Mid	0.36	0.80	0.76	0.64	0.64[b]
	Late	0.31	0.67	0.70	0.65	0.58[b]

Figure 61. *Changes in macrophyte phosphorus content over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced P content in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of P content over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (% P of dry weight) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Macrophyte Nutrient Stock

Stage had no significant effect on nitrogen stock of macrophytes; however, Day was a significant main effect (p=0.0001) as nitrogen stocks increased seasonally in all treatments (Figure 62). The N stock in the Control increased 10-fold during the growing season, from 0.4 to 4.3 g N/m^2, but did not substantially decrease during senescence. The maximum stocks in the Mid (8.6 g N/m^2) and Late (7.6 g N/m^2) treatments in August were nearly 2-fold those in the Early (4.3 g N/m^2) and Control (4.3 g N/m^2) treatments; however, by the end of the study, total macrophyte stocks were similar among all treatments (range 2.5–4.9 g N/m^2).

	Stage	Day	Stage*Day
N Stock	0.0902	0.0001	0.6595

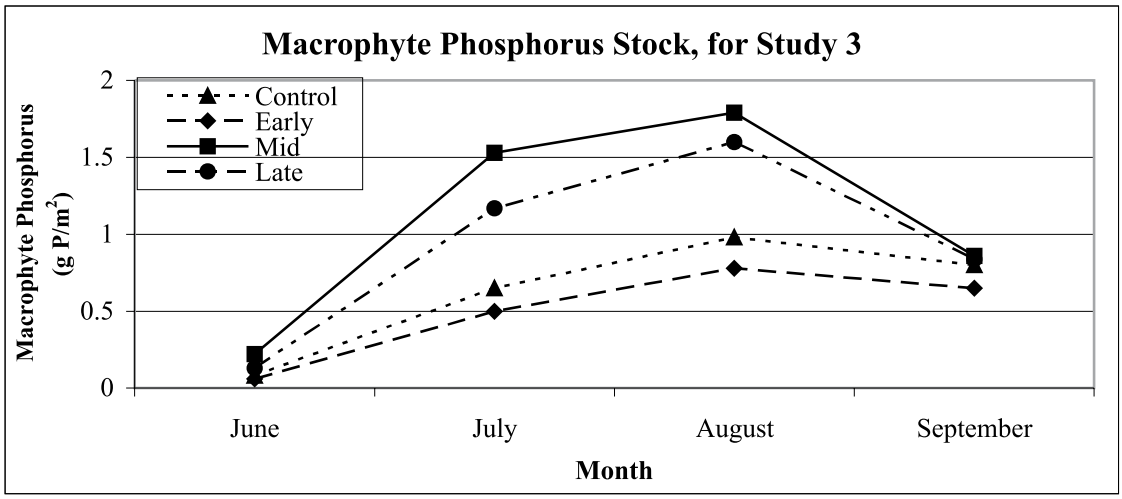


		Month				Season Avg.
		June	July	August	September	
Stage	Control	0.4	2.7	4.3	4.3	2.9
	Early	0.4	1.5	4.3	3.9	2.5
	Mid	1.0	5.0	8.6	4.8	4.9
	Late	0.8	4.6	7.6	4.6	4.4

Figure 62. *Changes in macrophyte nitrogen stock over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced N stock in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of N stock over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (g N/m^2) represented in the graph.*

Both Stage (p=0.0308) and Day (p=0.0001) significantly affected phosphorus macrophyte stocks (Figure 63). The phosphorus stock in the Control increased 12-fold during macrophyte growth, from 0.08 to 0.98 g P/m^2 (August peak) and then decreased to 0.80 g P/m^2 during senescence. The Mid (0.95 g P/m^2) and Late (0.94 g P/m^2) treatments were significantly greater than Controls (0.58 g P/m^2) at the end of the study period.

	Stage	Day	Stage*Day
P Stock	0.0308	0.0001	0.5054



		Month				Season
		June	July	August	September	Avg.
Stage	Control	0.08	0.65	0.98	0.80	0.58^{ab}
	Early	0.06	0.50	0.78	0.65	0.45^{a}
	Mid	0.22	1.53	1.79	0.86	0.95^{bc}
	Late	0.13	1.17	1.60	0.84	0.94^{c}

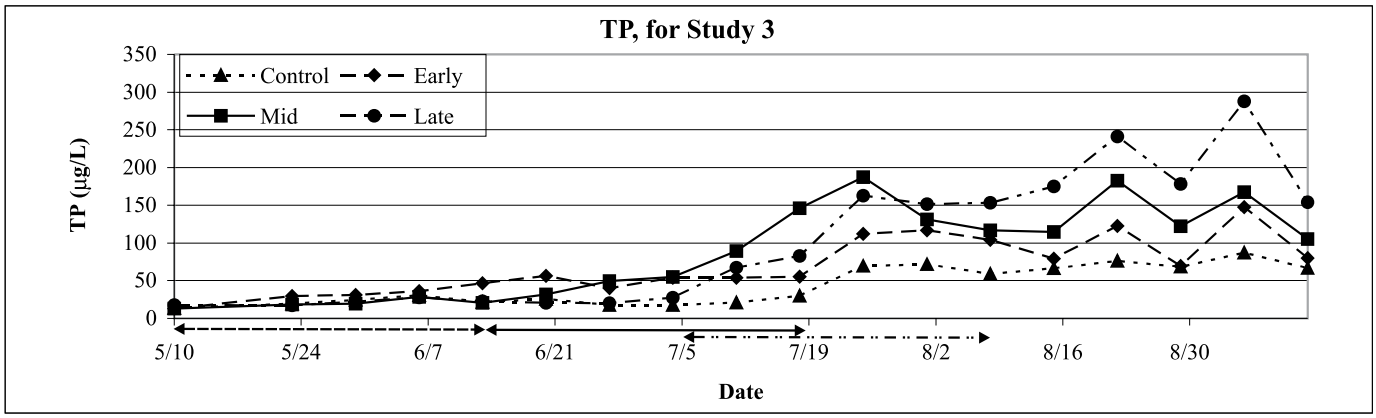
Figure 63. *Changes in macrophyte phosphorus stock over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced P stock in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of P stock over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (g P/m^2) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Water Chemistry

Phosphorus

Stage (p=0.0287), Day (p<0.0001), and the Stage*Day interaction (p=0.0001) each were significant factors related to TP concentrations in water (Figure 64). Total phosphorus concentrations averaged over the entire study were 102, 89, 69, and 44 µg/L TP in the Late, Mid, Early, and Control treatments, respectively; all treatments were significantly greater than the Control (p<0.05). Total phosphorus increased from early May (14 µg P/L) to a maximum in September (87 µg P/L). From May through mid-July, TP in the Control ranged from 14 to 30 µg P/L on a weekly basis. Total phosphorus in the Control more than doubled in late July from 30 to 70 µg P/L. After July 25, values in the Control continued to increase. Nutrient additions increased TP during and following dose periods; dosing effects were more pronounced in the Mid and Late treatments compared to the Control and Early treatments.

	Stage	Day	Stage*Day
TP	0.0287	0.0001	0.0001

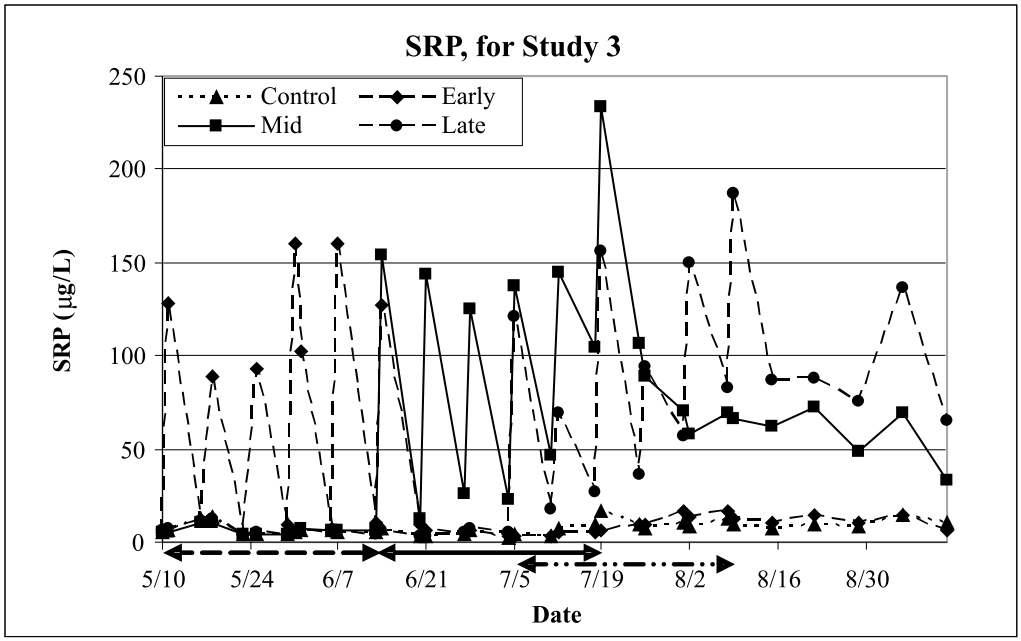


		Day																		Season
		5/10	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Stage	Control	14	19^{a}	24^{ab}	31	22^{a}	25^{a}	18^{a}	18^{a}	21^{a}	30^{a}	70^{a}	72^{a}	59^{a}	66^{a}	77^{a}	69^{ab}	87^{a}	67^{ab}	44^{a}
	Early	13	30^{b}	31^{a}	36	47^{b}	56^{b}	40^{b}	54^{b}	54^{b}	55^{b}	112^{a}	117^{ab}	104^{ab}	79^{a}	123^{a}	70^{a}	148^{ab}	80^{a}	69^{b}
	Mid	13	18^{a}	20^{b}	29	21^{a}	32^{a}	49^{b}	55^{b}	90^{c}	146^{c}	187^{b}	131^{b}	117^{b}	115^{ab}	183^{b}	122^{bc}	167^{b}	105^{ab}	89^{b}
	Late	18	17^{a}	22^{ab}	28	23^{a}	21^{a}	20^{a}	27^{a}	68^{bc}	83^{b}	163^{b}	152^{b}	153^{b}	175^{b}	241^{b}	178^{c}	288^{b}	154^{b}	102^{b}

Figure 64. *Changes in total phosphorus concentrations over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced TP in ANOVA of rank-transformed data. The graph is a plot of TP over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Stage (p=0.0001), Day (p=0.0001) and the Stage*Day interaction (p=0.0001) were significant factors in SRP concentrations (Figure 65). Soluble reactive phosphorus (SRP) in the Control and Stage treatments ranged between 2 and 16 µg/L until the initiation of dosing within Stages. Soluble reactive phosphorus dissipated rapidly after addition, indicating that SRP was rapidly assimilated or lost from the water column. Dissipation rates averaged 20 µg/L/day in the Early treatment, but were probably underestimates because additions were completely dissipated in 5 days; calculations based on loss of nominal concentrations applied indicated an approximately 25% P loss/day (Figure 66). Following the final amendment, SRP in the Early treatment was <20 µg/L and similar to the Control. In the Mid and Late treatments, dissipation rates were only 17 and 13 µg/L/day (8 and 6% P loss/day), respectively, and SRP accumulated in the water column during the dosing period within each Stage. At the end of the season, SRP in the Mid and Late treatments decreased to 33 and 65 µg/L, respectively, but remained significantly higher than in the Control and Early treatments (p<0.05).

	Stage	Day	Stage*Day
SRP	0.0001	0.0001	0.0001

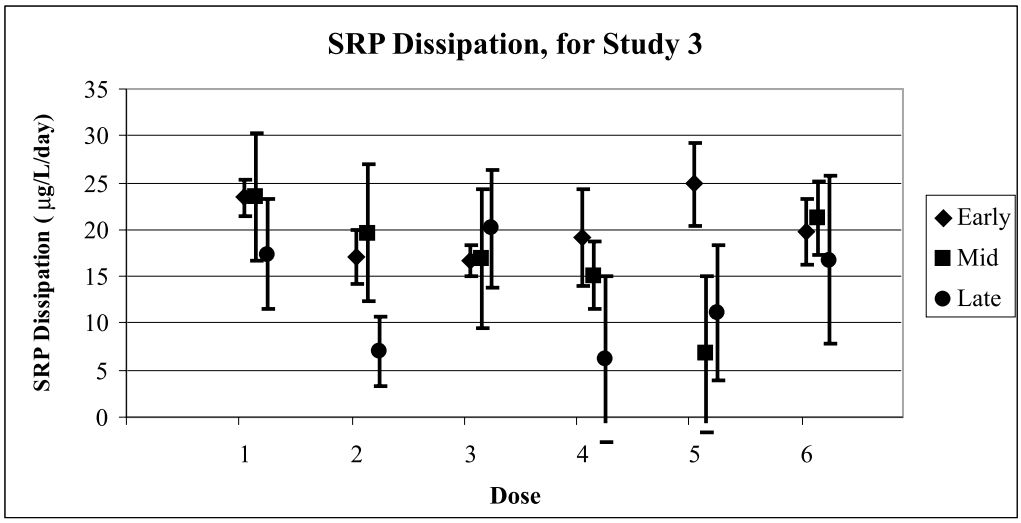


		Day																
		5/10	5/11	5/16	5/18	5/23	5/25	5/30	5/31	6/1	6/6	6/7	6/13	6/14	6/20	6/21	6/27	6/28
Cover	Control	6	6	13	13	5	4	4	5	6	6	6	5	7	4	4	4	6
	0%	6	128	11	89	4	93	10	160	102	7	160	11	127	9	6	6	6
	25%	5	6	11	11	4	4	4	6	7	6	6	6	154	13	143	26	125
	75%	6	7	11	13	4	5	4	5	7	5	5	4	6	3	3	5	8

		7/4	7/5	7/11	7/12	7/18	7/19	7/25	7/26	8/1	8/2	8/8	8/9	8/15	8/22	8/29	9/5	9/12	Season Avg.
Cover	Control	2	4	3	7	9	16	9	7	10	8	12	10	8	9	8	14	10	7[a]
	0%	3	3	4	5	6	6	10	10	17	14	17	12	10	15	10	14	6	32[b]
	25%	23	137	47	144	104	233	106	89	70	58	70	66	62	72	48	70	33	58[c]
	75%	6	121	17	69	27	156	36	94	57	149	82	187	86	88	76	137	65	46[b]

Figure 65. *Changes in soluble reactive phosphorus concentrations over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The table presents probabilities that stage, day, and interactions of stage and day influenced SRP in ANOVA of rank-transformed data. The graph is a plot of SRP over the experimental season pooled by treatment. Corresponding arrows mark dose periods. The lower table lists LS Means (µg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

	Stage	Dose	Stage*Dose
SRP Dissipation	0.0236	0.0015	0.0030



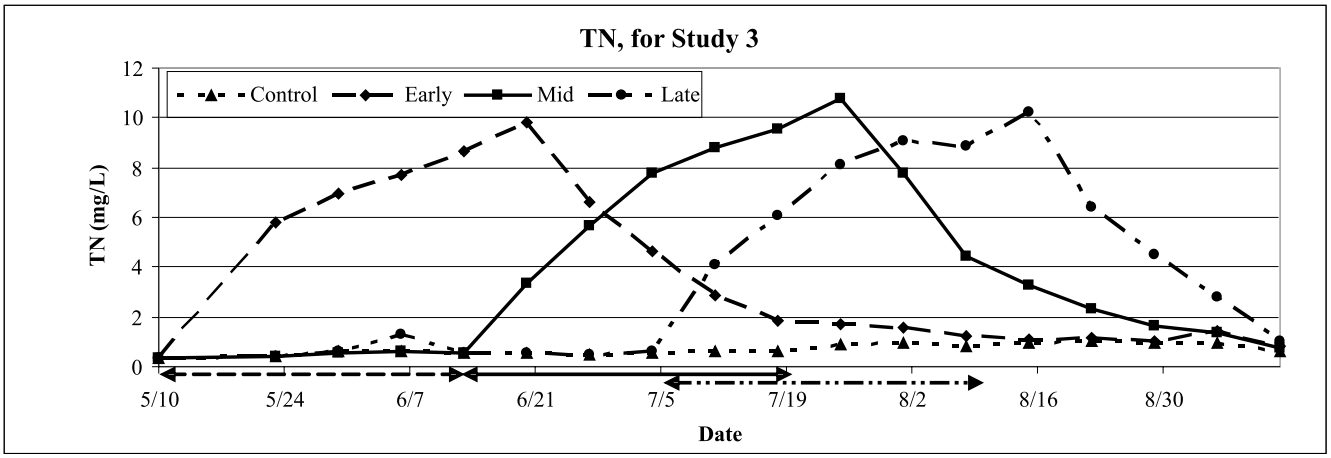
		Dose						Season Avg.
		1	2	3	4	5	6	
Stage	Early	23^{a}	17^{a}	17	19^{a}	25^{a}	20	20^{a}
	Mid	24^{ab}	20^{a}	17	15^{ab}	7^{b}	21	17^{ab}
	Late	17^{b}	7^{b}	20	6^{b}	11^{b}	17	13^{b}

Figure 66. *Changes in soluble reactive phosphorus dissipation over dose periods. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, dose, and interactions of stage and dose influenced SRP dissipation in ANOVA of rank-transformed data. The graph is a plot of SRP dissipation rates for each dose pooled by treatment. Bars indicate one standard deviation above and below the mean. The lower table lists LS Means represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Nitrogen

Stage ($p<0.0001$), Day ($p<0.0001$), and the Stage*Day interaction ($p<0.0001$) were significant factors related to TN in the water column (Figure 67). Total nitrogen averaged 0.70, 3.63, 3.89, and 3.67 mg N/L in the Control, Early, Mid, and Late treatments, respectively. Total nitrogen in the Control ranged from 0.38 to 0.98 mg N/L over the course of study. Total nitrogen exhibited a pattern of increase in dosed treatments that corresponded to the dosing period and peaked near 10 mg N/L the week following the final dose (Figure 67). During a dose period, TN in an amended treatment was composed of 90% added nitrate, indicating that persistence of the dose was driving the TN pattern. Total nitrogen values in the Early, Mid, and Late treatments dropped to around 1 mg N/L within four weeks of the sixth weekly nutrient addition. Total nitrogen in the Early and Mid treatments was comparable to the Control near the end of the season.

	Stage	Day	Stage*Day
TN	0.0001	0.0001	0.0001

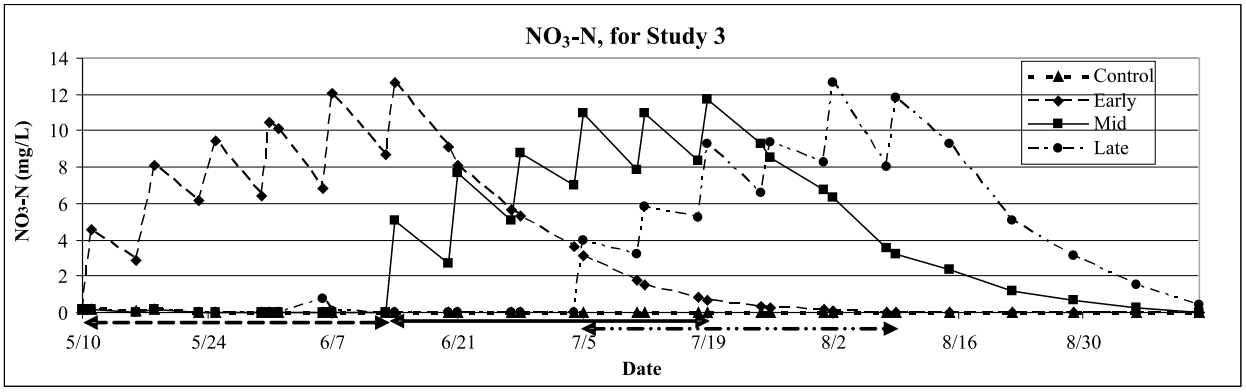


		Day																		Season
		5/10	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Stage	Control	0.38	0.43^{a}	0.65^{a}	0.63^{a}	0.53a	0.58^{a}	0.50^{a}	0.53^{a}	0.60^{a}	0.65^{a}	0.88^{a}	0.93^{a}	0.85^{a}	0.95^{a}	1.03^{a}	0.95^{a}	0.98^{a}	0.63^{a}	0.70^{a}
	Early	0.33	5.80^{b}	6.98^{b}	7.68^{b}	8.68b	9.83^{b}	6.60^{b}	4.63^{b}	2.88^{b}	1.88^{b}	1.73^{b}	1.55^{b}	1.23^{a}	1.08^{a}	1.15^{a}	1.03^{a}	1.43^{ab}	0.83^{b}	3.63^{b}
	Mid	0.35	0.40^{a}	0.55^{a}	0.60^{a}	0.58a	3.35^{c}	5.65^{b}	7.75^{c}	8.80^{c}	9.53^{c}	10.78^{c}	7.75^{c}	4.45^{b}	3.30^{b}	2.33^{a}	1.65^{a}	1.38^{ab}	0.78^{ab}	3.89^{bc}
	Late	0.38	0.40^{a}	0.60^{a}	1.28^{a}	0.55a	0.55^{a}	0.50^{a}	0.60^{a}	4.13^{b}	6.08^{d}	8.13^{c}	9.05^{c}	8.88^{c}	10.25^{c}	6.40^{b}	4.48^{b}	2.80^{b}	1.03^{b}	3.67^{c}

Figure 67. *Changes in total nitrogen concentrations over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced TN in ANOVA of rank-transformed data. The graph is a plot of TN over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Stage ($p<0.0001$), Day ($p<0.0001$), and the Stage*Day interaction ($p<0.0001$) were also significant factors related to nitrate in the water column (Figure 68). Nitrate in the Control fluctuated near the limit of detection (0.005 mg NO_3-N/L) during the study. In contrast, nitrate concentrations in the Stage treatments closely reflected temporal patterns of dosing. Calculations of nitrate dissipation rates indicated that post-dose sampling accounted for 87, 80, and 71% of nitrate added in the Early, Mid, and Late treatments, respectively (Figure 69). Nitrate dissipation rate was inversely related to Stage: Early (0.52 mg NO_3-N/L/day; 10% N loss/day); Mid (0.42 mg NO_3-N/L/day; 8% N loss/day); and Late (0.34 mg NO_3-N/L/day; 7% N loss/day). Day was a significant main effect ($p<0.0001$) in addition to the Stage*Day interaction ($p=0.0041$) (Figure 69). Nitrate dissipation continued at similar rates in amended treatments after the termination of dosing until concentrations fell below the limit of detection (August 22, Early treatment; September 12, Mid and Late treatments).

	Stage	Day	Stage*Day
NO_3-N	0.0001	0.0001	0.0001

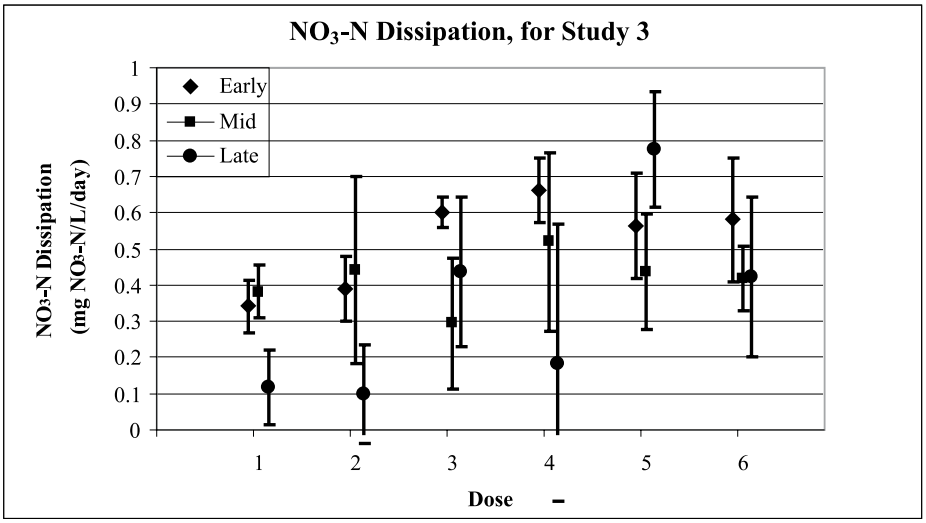


		Day																
		5/10	5/11	5/16	5/18	5/23	5/25	5/30	5/31	6/1	6/6	6/7	6/13	6/14	6/20	6/21	6/27	6/28
Cover	Control	0.207	0.156	0.073	0.168	0.030	0.018	0.009	0.011	0.009	0.009	0.009	0.008	0.014	0.010	0.010	0.011	0.014
	0%	0.180	4.586	2.878	8.082	6.134	9.451	6.454	10.489	10.149	6.849	12.072	8.685	12.634	9.155	8.078	5.657	5.294
	25%	0.212	0.159	0.034	0.152	0.012	0.014	0.011	0.023	0.029	0.023	0.024	0.025	5.030	2.739	7.694	5.038	8.807
	75%	0.202	0.151	0.037	0.187	0.015	0.017	0.007	0.014	0.022	0.737	0.096	0.017	0.036	0.013	0.041	0.012	0.032

		7/4	7/5	7/11	7/12	7/18	7/19	7/25	7/26	8/1	8/2	8/8	8/9	8/15	8/22	8/29	9/5	9/12	**Season Avg.**
Cover	Control	0.015	0.010	0.017	0.009	0.015	0.013	0.011	0.010	0.007	0.008	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.027[a]
	0%	3.630	3.147	1.785	1.539	0.811	0.706	0.323	0.250	0.150	0.122	0.013	0.017	0.006	0.005	0.006	0.005	0.005	4.098[b]
	25%	7.043	11.000	7.887	10.959	8.343	11.745	9.252	8.543	6.730	6.364	3.560	3.200	2.359	1.226	0.716	0.295	0.005	3.801[b]
	75%	0.037	3.955	3.245	5.861	5.275	9.242	6.617	9.346	8.238	12.654	8.004	11.779	9.254	5.077	3.120	1.498	0.462	3.097[b]

Figure 68. *Changes in nitrate concentrations over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The table presents probabilities that stage, day, and interactions of stage and day influenced nitrate in ANOVA of rank-transformed data. The graph is a plot of nitrate over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

	Stage	Dose	Stage*Dose
NO_3-N Dissipation	0.0543	0.0001	0.0041

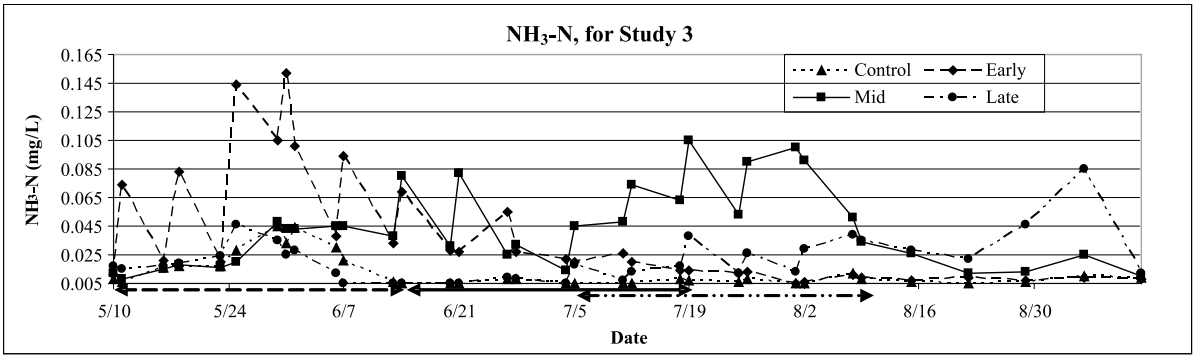


		Dose						Season Avg.
		1	2	3	4	5	6	
Stage	Early	0.34^{ab}	0.39^{a}	0.60^{a}	0.66^{a}	0.56^{ab}	0.58	0.52
	Mid	0.38^{a}	0.44^{a}	0.29^{b}	0.52^{a}	0.44^{a}	0.42	0.42
	Late	0.12^{b}	0.10^{b}	0.44^{ab}	0.18^{b}	0.78^{b}	0.42	0.34

Figure 69. *Changes in nitrate dissipation over dose periods. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, dose, and interactions of stage and dose influenced nitrate dissipation in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of nitrate dissipation rates for each dose pooled by treatment. Bars indicate one standard deviation above and below the mean. The lower table lists LS Means represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Stage (p=0.0046), Day (p<0.001) and the Stage*Day interaction (p=0.0001) had significant effects on ammonia (Figure 70). Ammonia in the Control was near the limit of detection (0.005 mg NH_3-N/L) during most of the season. During the Early dose period, however, ammonia in the Control, Mid, and Late treatments rose to 0.045 mg NH_3-N/L, possibly due to disturbance of the bare sediment surface during stirring. A similar response was not seen in the Control during other periods because disturbance of the stirring effect may have been dampened by macrophytes. In amended treatments, ammonia peaked on the day of additions. Ammonia peaks during the dose period decreased in the order of dosing initiation (Early, 0.07 to 0.15 mg NH_3-N/L; Mid, 0.03 to 0.11 mg NH_3-N/L; and Late, 0.01 to 0.04 mg NH_3-N/L); indicating that ammonia responses were less as the growing season progressed. Seasonal maximums in the Early (0.152 mg NH_3-N/L) and Mid (0.105 mg NH_3-N/L) treatments occurred during their respective dose periods; however, maximum ammonia concentration in the Late (0.085 mg NH_3-N/L) treatment occurred latently in September. Following the dose period, ammonia concentrations in the Early and Mid treatments gradually decreased over time and were comparable to the Control after July 12 and August 29, respectively. Ammonia in the Late treatment was similar to the Control with the exception of a sudden observed increase in early September. The proportion of ammonia compared to nitrate was minimal (<1%) in all treatment/day combinations.

	Stage	Day	Stage*Day
NH_3-N	0.0046	0.0001	0.0001



		Day																
		5/10	5/11	5/16	5/18	5/23	5/25	5/30	5/31	6/1	6/6	6/7	6/13	6/14	6/20	6/21	6/27	6/28
Cover	Control	0.008	0.006a	0.016	0.017a	0.017	0.028a	0.045	0.033a	0.044ab	0.030ab	0.021a	0.006a	0.005a	0.005a	0.005a	0.008a	0.008a
	0%	0.011	0.074b	0.021	0.083b	0.020	0.144b	0.105	0.152b	0.101b	0.038b	0.094b	0.033b	0.069b	0.028b	0.027b	0.055b	0.027ab
	25%	0.012	0.008a	0.015	0.018a	0.016	0.020a	0.048	0.043a	0.043ab	0.045a	0.045ac	0.038ab	0.080b	0.031ab	0.082b	0.025c	0.032b
	75%	0.017	0.015a	0.017	0.019a	0.024	0.046ab	0.035	0.025a	0.028a	0.012a	0.005c	0.005a	0.005a	0.005a	0.005a	0.009ac	0.008a

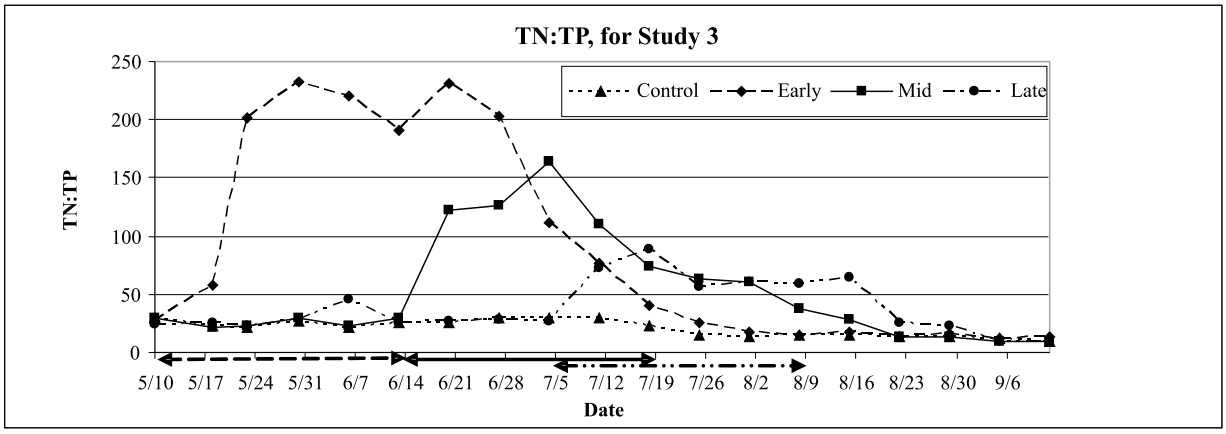
		7/4	7/5	7/11	7/12	7/18	7/19	7/25	7/26	8/1	8/2	8/8	8/9	8/15	8/22	8/29	9/5	9/12	Season Avg.
Cover	Control	0.005a	0.005a	0.005a	0.005a	0.008a	0.007a	0.006a	0.008a	0.005a	0.005a	0.012a	0.008a	0.006a	0.005a	0.006ab	0.010ab	0.009ab	0.012[a]
	0%	0.022b	0.020b	0.026bc	0.020bc	0.014ab	0.014ac	0.012a	0.013ac	0.005a	0.006a	0.011ac	0.009a	0.007a	0.009ac	0.007a	0.009a	0.008a	0.038[b]
	25%	0.014b	0.045c	0.048c	0.074c	0.063b	0.105b	0.053b	0.090b	0.100b	0.091b	0.051bc	0.034b	0.026b	0.012b	0.013bc	0.025bc	0.010b	0.043[b]
	75%	0.005a	0.018bc	0.007ab	0.013ab	0.017ab	0.038bc	0.012ab	0.026bc	0.013b	0.029b	0.039b	0.035b	0.028b	0.022bc	0.046c	0.085c	0.012ab	0.021[b]

Figure 70. *Changes in ammonia concentrations over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The table presents probabilities that stage, day, and interactions of stage and day influenced ammonia in ANOVA of rank-transformed data. The graph is a plot of ammonia over the experimental season pooled by treatment. Corresponding lines and arrows on X axis mark dose periods. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Nitrogen:Phosphorus Ratio

The TN:TP ratio was significantly related to Stage (p=0.0001), Day (p<0.0001), and the Stage*Day interaction (p<0.0001) (Figure 71). Nutrients were added during each Stage at a targeted N:P ratio of 25:1; in the absence of internal loading, this ratio was expected to be phosphorus limited. Similarly, the N:P ratio in the Control fluctuated between 20 and 30 from May through July 18, indicating the potential for P limitation; thereafter, the TN:TP decreased to between 10 and 20 during the remainder of the season. Although the calculated TN:TP of the amendments was 25:1, the ratio in the Early treatment doubled to more than 50:1 after the first dosing because the added P was rapidly lost from the water column in proportion to nitrate. Following the third amendment, TN:TP ratios in the Early treatment exceeded 200 (10-fold greater than the Control) and remained around that level through June. During July, TN:TP ratio in the Early treatment dropped due to the decline in TN, and thereafter, levels were similar to the Control. In the Mid treatment, the TN:TP ratio increased from 29 to 122 following the first amendment, and peaked at 164 following the third dose. The ratio in the Mid treatment decreased through July and was comparable to the Control (<20) by mid-August. The TN:TP ratio peaked at 89:1 following the second dose and gradually decreased to around 9:1 by September. Seasonal averages of TN:TP ratios were 21, 91, 52, and 38 in the Control, Early, Mid, and Late treatments, respectively, which revealed the overall effect of Stage on the TN:TP ratio of water.

	Stage	Day	Stage*Day
TN:TP	0.0002	0.0001	0.0001



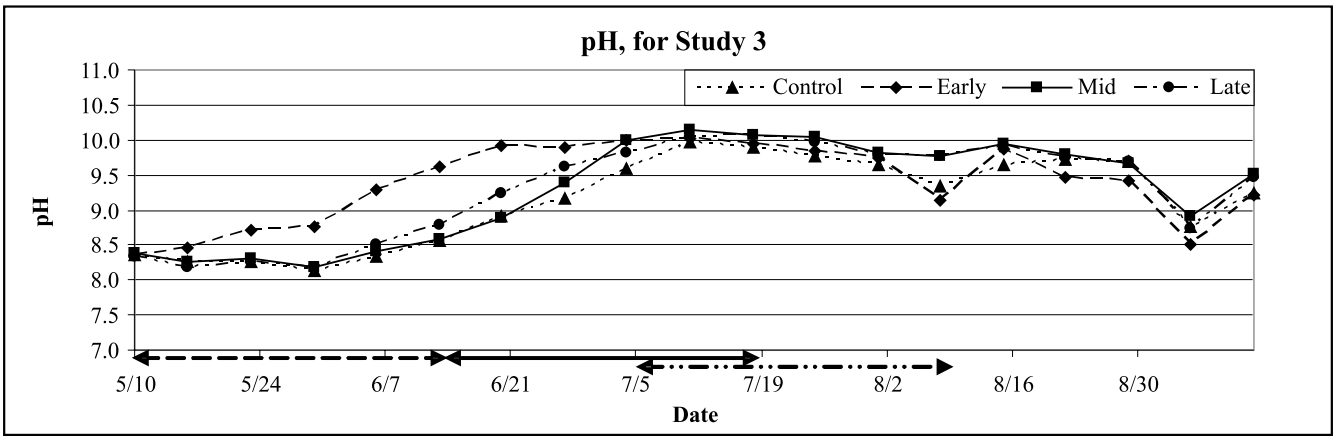
		Day																			Season Avg.
		5/10	5/18	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	
Stage	Control	29	23^{a}	22^{a}	27^{a}	21^{a}	25^{a}	26^{a}	29^{a}	30^{a}	30^{a}	23^{a}	15^{a}	14^{a}	15^{a}	15^{a}	14^{a}	14^{a}	12	10	21^{a}
	Early	27	58^{b}	201^{b}	232^{b}	220^{b}	191^{b}	231^{b}	203^{b}	112^{b}	77^{ab}	40^{a}	26^{a}	17^{a}	15^{a}	17^{a}	14^{a}	16^{ab}	12	14	91^{b}
	Mid	29	22^{a}	23^{a}	29^{a}	23^{a}	29^{a}	122^{b}	127^{b}	164^{b}	110^{b}	74^{b}	63^{b}	60^{b}	37^{b}	28^{b}	14^{a}	13^{a}	9	10	52^{b}
	Late	24	25^{a}	23^{a}	28^{a}	46^{b}	25^{a}	27^{a}	28^{a}	27^{a}	72^{b}	89^{b}	56^{b}	60^{b}	59^{b}	64^{c}	25^{b}	23^{b}	10	9	38^{b}

Figure 71. *Changes in the ratio of total nitrogen to total phosphorus over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced TN:TP in ANOVA of rank-transformed data. The graph is a plot of TN:TP over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

pH

Both Stage (p=0.0160) and Day (p<0.0001) had significant effects on pH of the water column (Figure72). Seasonal averages of pH in the Early treatment (pH=9.0) were significantly greater (p<0.05) than in the Control (pH=8.7) treatment; however, the seasonal average pH in the Mid and Late treatments (pH=8.8) were similar to the Control. Initial pH values in all treatments ranged from 8.3–8.4 and continually increased over time. Dosing in the Early treatment led to increases in pH in the Early treatment of approximately 0.5 units. In early July, the pH levels in all treatments converged to approximately 10 and remained similar among treatments for the remainder of the study. Thus, nutrient amendments influenced pH only when added prior to macrophyte growth (Early treatment). The observed increase in pH reflects the stimulation of periphyton communities (Figure 81) as discussed below.

	Stage	Day	Stage*Day
pH	0.0160	0.0001	0.2352



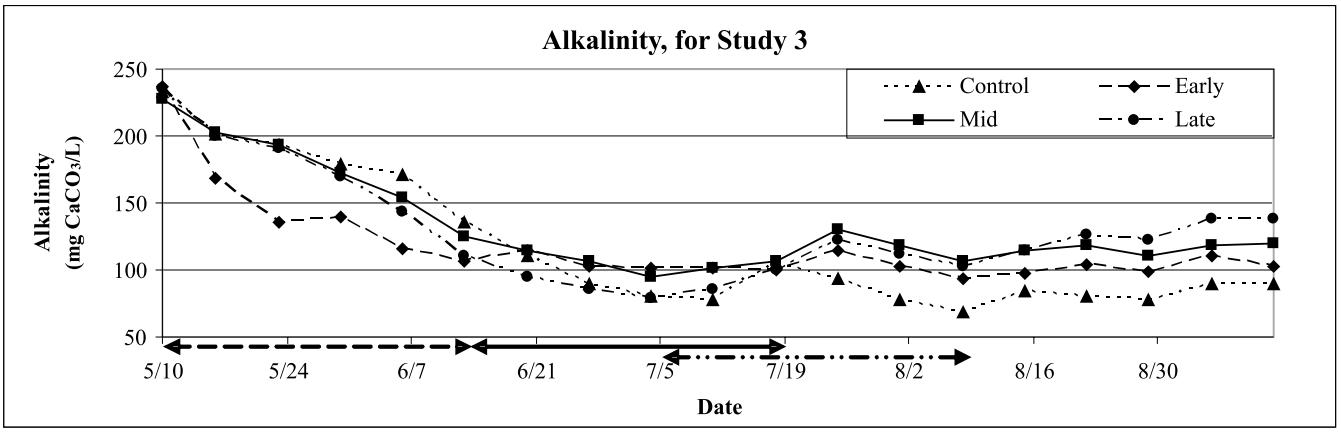
		Day																			Season Avg.
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	
Stage	Control	8.4	8.3	8.3	8.1	8.3	8.6	8.9	9.2	9.6	10.0	9.9	9.8	9.7	9.4	9.7	9.7	9.7	8.8	9.2	8.7[a]
	Early	8.4	8.5	8.7	8.8	9.3	9.6	9.9	9.9	10.0	10.0	9.9	9.8	9.7	9.1	9.9	9.5	9.4	8.5	9.2	9.0[b]
	Mid	8.4	8.2	8.3	8.2	8.4	8.6	8.9	9.4	10.0	10.2	10.1	10.0	9.8	9.8	10.0	9.8	9.7	8.9	9.5	8.8[a]
	Late	8.3	8.2	8.3	8.2	8.5	8.8	9.2	9.6	9.8	10.1	10.1	10.0	9.8	9.8	9.9	9.7	9.7	8.7	9.5	8.8[a]

Figure 72. *Changes in pH over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced pH in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of pH over the experimental season pooled by treatment (data were averaged by H-ion concentration, then converted to pH: calculated pH= - log (H-ion)). Corresponding lines and arrows mark dose periods. The lower table lists LS Means represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Alkalinity and Hardness

Stage (p=0.0111), Day (p<0.0001), and the Stage*Day interaction (p<0.0001) had significant effects on alkalinity (Figure 73). Seasonal averages of alkalinity indicated that the Mid (133 mg $CaCO_3$/L) and Late (130 mg $CaCO_3$/L) treatments were significantly greater (p=0.05) than the Control and Early treatments (117–118 mg $CaCO_3$/L). Alkalinity in the Control decreased from a maximum of 233 mg $CaCO_3$/L soon after corral filling on May 10 to 80 mg $CaCO_3$/L in early July prior to stabilization in a range between 60 and 110 mg $CaCO_3$/L through the remainder of the season. Early dosing substantially decreased alkalinities by 25% compared to Control levels in May and early June due to loss of carbonate to primary productivity. However, alkalinity in the Early treatment was similar to the Control by late June. Alkalinities in the Mid and Later treatments increased above Control levels as dosing was initiated at each respective Stage. Thus, Early treatment decreased alkalinities soon after dosing; whereas, dosing had the opposite effect in the Mid and Late treatments which indicates a differential system response in periphyton-dominated communities (Early treatment) compared to macrophyte-dominated systems (Mid and Late treatments).

	Stage	Day	Stage*Day
Alkalinity	0.0111	0.0001	0.0001

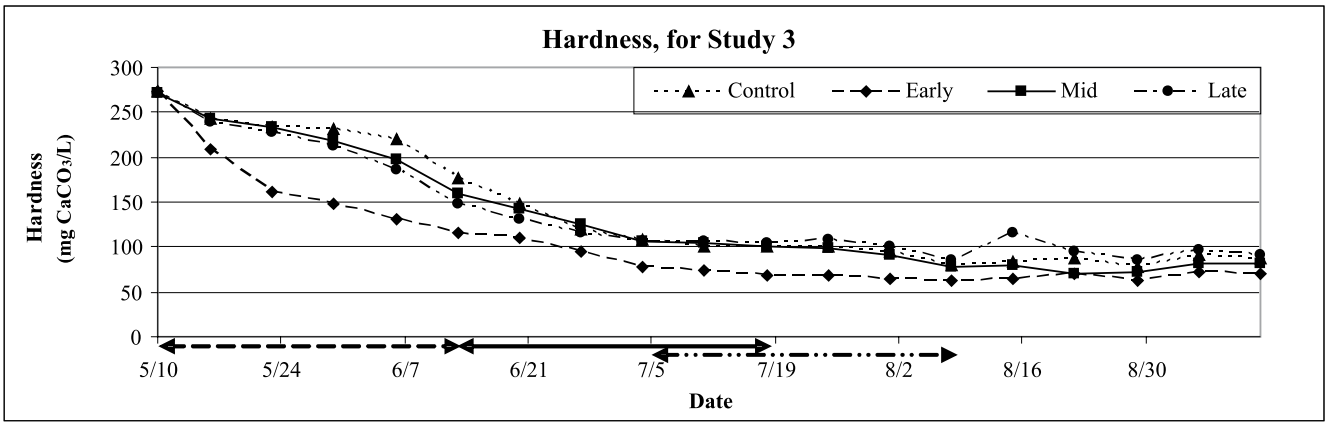


		Day																		Season Avg.
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	
Stage	Control	233	201	193	179	171^{a}	136^{a}	111^{ab}	90^{ab}	80^{a}	77^{a}	105	93^{a}	78^{a}	69^{a}	84^{a}	80^{a}	78^{a}	90^{a}	90^{a}	117^{a}
	Early	237	169	135	139	116^{b}	107^{b}	114^{a}	103^{a}	101^{b}	101^{b}	100	115^{b}	102^{b}	94^{b}	97^{ab}	104^{b}	99^{b}	110^{b}	103^{ab}	118^{ab}
	Mid	228	203	194	173	154^{a}	125^{ab}	114^{ab}	107^{a}	95^{ab}	101^{b}	106	130^{b}	118^{b}	107^{b}	114^{bc}	119^{b}	111^{b}	118^{b}	120^{bc}	133^{b}
	Late	236	200	191	170	144^{ab}	110^{ab}	95^{b}	85^{b}	79^{a}	86^{ab}	100	123^{b}	112^{b}	102^{b}	114^{c}	126^{b}	123^{b}	138^{b}	138^{c}	130^{b}

Figure 73. *Changes in alkalinity over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced alkalinity in ANOVA of rank-transformed data. The graph is a plot of alkalinity over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (mg $CaCO_3$/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different*

Stage (p=0.0048), Day (p=0.0001), and the Stage*Day interaction (p=0.0001) were all significant factors affecting hardness (Figure 74). Hardnesses, averaged over the entire study, were 140, 105, 135, and 139 mg $CaCO_3$/L in the Control, Early, Mid, and Late treatments, respectively; the Early treatment was significantly higher ($p<0.05$) than the Control on a total-study basis. Hardness averaged 273 mg $CaCO_3$/L at the beginning of the study and reflected conditions within the well water source. Hardness values declined from May to September in a linear trend over time. Hardness decreased relative to Control values due to the Early treatment dosing which resulted in an approximate 30% decrease in hardness values. However, neither the Mid nor Late treatments influenced hardness values. The decrease in hardness in the Early treatment reflected the precipitation losses of major divalent cations as carbonate was consumed due to increased productivity of periphyton in the absence of macrophytes. Hardness continued to decline in all treatments as macrophytes developed. Macrophyte biomass, and hence overall system primary productivity, was relatively similar in the Mid, Late, and Control treatments; hence, hardness levels exhibited similar trends over time as carbonate was consumed and cations were precipitated.

	Stage	Day	Stage*Day
Hardness	0.0048	0.0001	0.0001



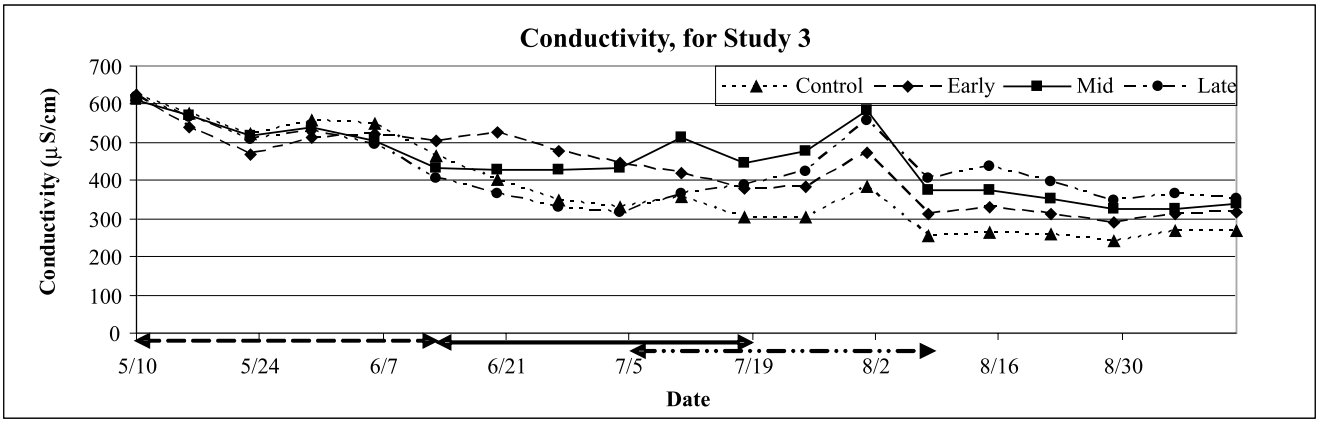
		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Stage	Control	273	243	234^{a}	231^{a}	220^{a}	176^{a}	148^{a}	120^{a}	108^{a}	101^{a}	101^{a}	100^{a}	95^{ac}	79^{ab}	83^{a}	87^{ab}	78	91^{ab}	88	140^{a}
	Early	273	208	161^{b}	149^{b}	131^{b}	115^{b}	111^{b}	94^{b}	78^{b}	74^{b}	68^{b}	69^{b}	64^{b}	62^{a}	64^{a}	70^{a}	63	73^{a}	70	105^{b}
	Mid	272	243	233^{a}	218^{a}	198^{a}	160^{ab}	142^{ab}	125^{a}	106^{a}	104^{a}	101^{a}	98^{a}	92^{a}	78^{ab}	79^{a}	71^{a}	72	82^{a}	81	135^{a}
	Late	271	239	228^{ab}	213^{ab}	187^{ab}	149^{ab}	131^{ab}	116^{a}	106^{a}	107^{a}	105^{a}	109^{c}	101^{c}	86^{b}	115^{b}	95^{b}	86	97^{b}	92	139^{a}

Figure 74. *Changes in hardness over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced hardness in ANOVA of rank-transformed data. The graph is a plot of hardness over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (mg $CaCO_3$/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Conductivity

Stage (p=0.0046), Day (p=0.0001), and the Stage*Day interaction (p=0.0001) were highly significant factors controlling changes in conductivity (Figure 75). All three dosing stages (Early, 429 µS/cm; Mid, 451 µS/cm; and Late, 430 µS/cm) contained significantly higher ($p<0.05$) conductivity values compared to the Control (382 µS/cm). Conductivity in the Control decreased 60% during the season, from an initial maximum of 625 µS/cm, to 268 µS/cm in September. The primary decrease in conductivity (50%) occurred by early July, and values fluctuated around 260 µS/cm the final six weeks of the experiment. Although conductivity was significantly influenced by amendments ($p<0.05$), the dosed treatments were not significantly different from each other.

	Stage	Day	Stage*Day
Conductivity	0.0046	0.0001	0.0001



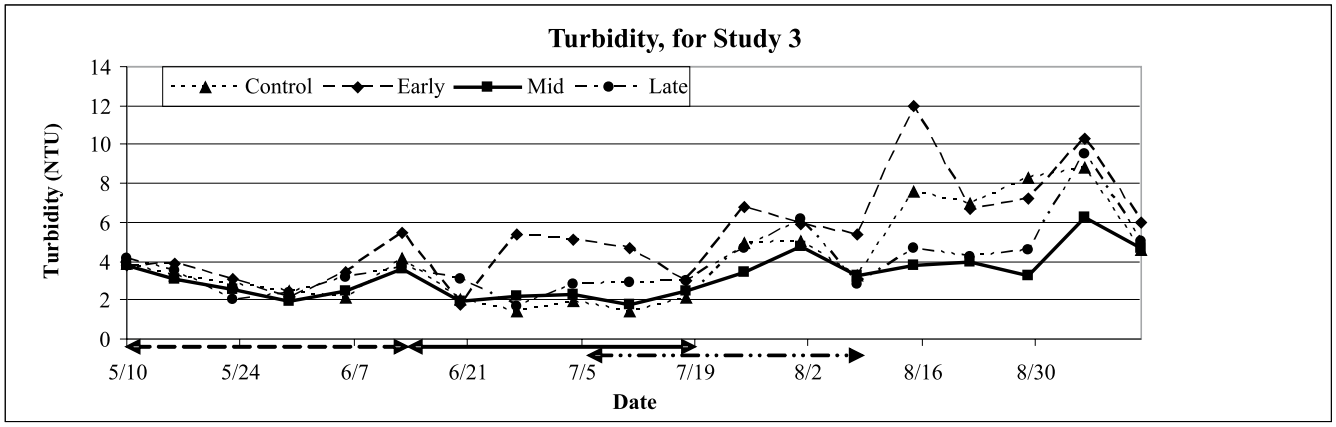
		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Stage	Control	625	573	520	556	548	464^{ab}	402^{ac}	346^{a}	329^{a}	357^{a}	305^{a}	305^{a}	383^{a}	256^{a}	263^{a}	259^{a}	241^{a}	266^{a}	268^{a}	382^{a}
	Early	622	538	466	514	523	506^{a}	526^{b}	476^{b}	446^{b}	417^{b}	380^{b}	385^{b}	471^{a}	314^{b}	330^{b}	313^{b}	291^{ab}	311^{ab}	315^{ab}	429^{b}
	Mid	609	572	516	538	505	432^{bc}	429^{a}	426^{c}	432^{b}	511^{c}	444^{c}	478^{c}	582^{b}	375^{c}	376^{b}	353^{bc}	325^{bc}	327^{b}	337^{b}	451^{b}
	Late	620	568	510	530	493	404^{c}	364^{c}	330^{a}	318^{a}	367^{ab}	389^{b}	422^{b}	557^{b}	404^{c}	436^{c}	395^{c}	348^{c}	364^{b}	354^{b}	430^{b}

Figure 75. *Changes in conductivity over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced conductivity in ANOVA of rank-transformed data. The graph is a plot of conductivity over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (mS/cm) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Turbidity

Turbidity changes were associated with Day ($p<0.0001$) but not with Stage (Figure 76). Overall, study averages of the Control (4.0 NTU's), Early (5.4 NTU's), Mid (3.2 NTU's), and Late (3.9 NTU's) treatments were quite similar with a range of less than 2.2 NTU's. Turbidity in the Control ranged from 1 to 4 NTU's through mid-July, and then increased to a maximum of 8.8 NTU's on September 5 (Figure 76). Although the Stage of dosing had no significant main effect on turbidity, average values were generally highest in the Early Stage due to the significant effect of Early dosing on phytoplankton biomass discussed below. Such effects were not observed in the Mid and Late treatments due to the dominance of macrophytes on system productivity and nutrient dynamics.

	Stage	Day	Stage*Day
Turbidity	0.1898	0.0001	0.1681



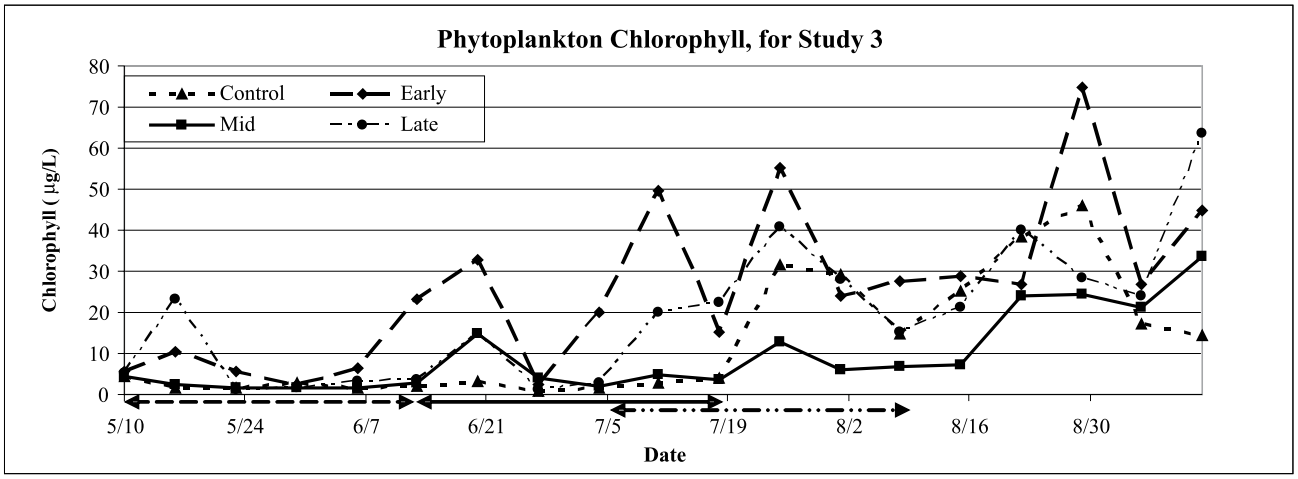
		Day																			Season Avg.
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	
Stage	Control	3.9	3.3	2.8	2.5	2.1	4.1	2.0	1.4	1.9	1.4	2.1	4.9	5.0	3.3	7.6	7.0	8.3	8.8	4.6	4.0
	Early	3.8	3.9	3.1	2.1	3.4	5.5	1.8	5.4	5.1	4.7	3.0	6.8	5.9	5.4	12.0	6.7	7.2	10.3	6.0	5.4
	Mid	3.8	3.1	2.6	1.9	2.5	3.6	1.9	2.2	2.3	1.8	2.5	3.4	4.8	3.3	3.8	4.0	3.3	6.3	4.7	3.2
	Late	4.1	3.5	2.0	2.4	3.2	3.7	3.1	1.7	2.8	2.9	3.0	4.7	6.2	2.8	4.7	4.2	4.6	9.5	5.0	3.9

Figure 76. *Changes in turbidity over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced turbidity in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of turbidity over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (NTU) represented in the graph.*

Phytoplankton

Stage (p=0.0279), Day (p=0.0001), and the Stage*Day interaction (p=0.0461) were all significant effects controlling changes in phytoplankton biomass as measured as Chl *a* (Figure 77). Total study averages indicated that Chl *a* concentrations in the Early treatment (25 µg/L) were significantly greater (p<0.05) than the Control (13 µg/L), Mid (9 µg/L), and Late (19 µg/L) treatments. Chl *a* averaged 5 µg/L across all treatments at study initiation in early May. Chl *a* ranged from 1–5 µg/L from May through mid-July; increased to 31 µg/L by late July; and then varied between 14 and 46 µg/L through September. Early nutrient enrichment resulted in significant increases (p<0.05) in Chl *a* from May through mid-July; during this period macrophyte biomass was low, and both phytoplankton and periphyton increased due to the nutrient subsidy. Phytoplankton in the Mid and Late treatments frequently departed from Control values during the study but varied and were not significantly different from the Controls.

	Stage	Day	Stage*Day
Phytoplankton Chl	0.0279	0.0001	0.0461

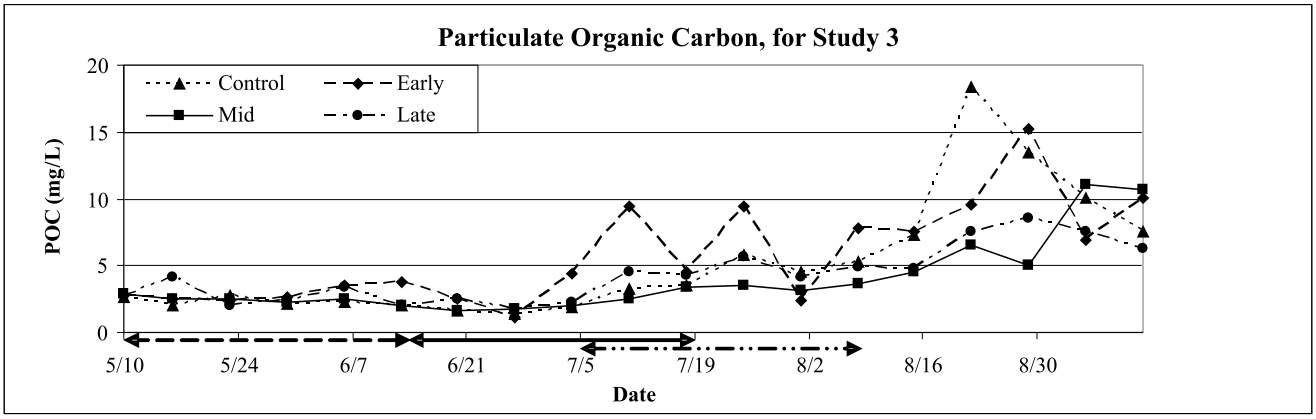


		Day																			Season
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	Avg.
Stage	Control	5	2^{a}	2^{a}	3	1^{a}	2^{a}	3^{a}	1	2^{a}	3^{a}	4^{ab}	32	29^{a}	15	25^{a}	38	46	17	14	13^{a}
	Early	6	10^{b}	6^{b}	3	7^{b}	23^{b}	33^{b}	2	20^{b}	50^{b}	15^{a}	55	24^{a}	28	29^{ab}	27	75	27	45	25^{b}
	Mid	5	2^{a}	2^{a}	2	1^{a}	3^{a}	15^{bc}	4	2^{a}	5^{a}	4^{b}	13	6^{b}	7	7^{b}	24	24	21	34	9^{a}
	Late	5	23^{a}	1^{a}	2	3^{ab}	4^{a}	15^{ac}	1	3^{a}	20^{b}	22^{a}	41	28^{a}	15	21^{ab}	40	29	24	64	19^{ab}

Figure 77. *Changes in phytoplankton chlorophyll concentrations over time. This combination of tables and a graph present the data and statistical information pertaining to phytoplankton chlorophyll (mg/L). Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced Chl in ANOVA of rank-transformed data. The graph is a plot of Chl over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (mg/L) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Particulate organic carbon (POC) was not influenced by Stage, but did significantly increase over time due to the main effect of Day ($p<0.0001$) (Figure 78). Levels of POC averaged 5.14, 5.71, 3.91, and 4.29 mg/L in the Control, Early, Mid, and Later treatments, respectively. Initial levels of POC ranged from 2.61 to 2.84 mg/L among treatments and remained less than 5 mg/L through July 4. Thereafter, POC levels increased in all treatments, typical of the late season senescent period dominated by internal nutrient release.

	Stage	Day	Stage*Day
POC	0.5435	0.0001	0.8359

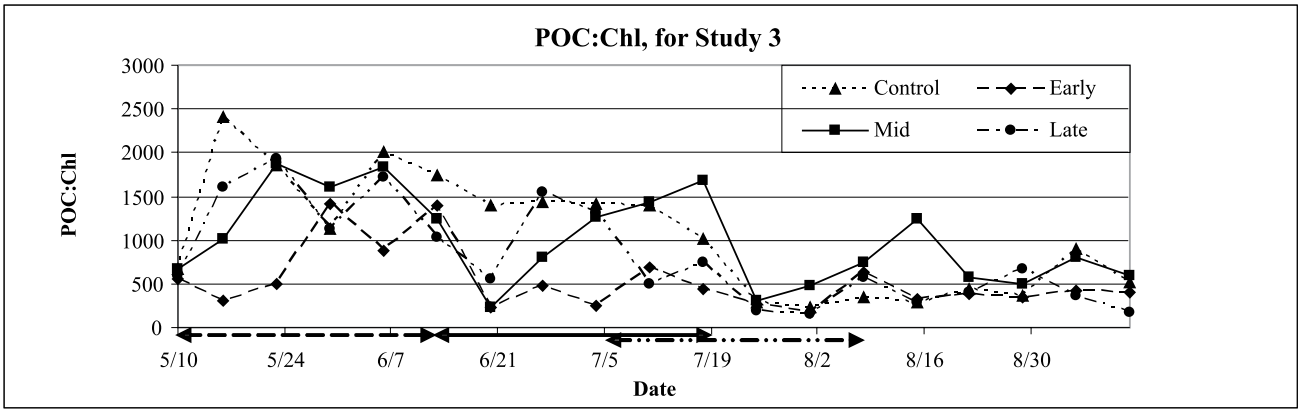


		Day																				Season Avg.
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	
Stage	Control	2.61	2.05	2.71	2.10	2.25	2.04	1.58	1.40	1.84	3.32	3.52	5.84	4.50	5.28	7.26	18.38	13.45	10.02	7.59	5.14
	Early	2.80	2.46	2.44	2.61	3.56	3.75	2.56	1.14	4.41	9.41	4.59	9.40	2.43	7.75	7.49	9.54	15.21	6.87	10.01	5.71
	Mid	2.84	2.55	2.49	2.26	2.52	2.06	1.68	1.75	2.05	2.57	3.42	3.50	3.09	3.65	4.55	6.54	5.07	11.10	10.70	3.91
	Late	2.72	4.17	1.97	2.43	3.40	2.01	2.46	1.73	2.31	4.53	4.27	5.69	4.16	4.91	4.81	7.51	8.59	7.57	6.26	4.29

Figure 78. *Changes in particulate organic carbon concentrations over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced POC in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of POC over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means (mg C/L) represented in the graph.*

POC:Chl ratios varied significantly in relation to Stage (p=0.0028), Day (p=0.0001), and the Stage*Day interaction (p=0.0323) (Figure 79). Total season averages of the POC:Chl ratio were significantly lower in the Early (530) and Late (813) treatments compared to the Control (1044); POC:Chl ratio was lowest in the Mid treatment (992) which was significantly greater (p< 0.05) than in the Early treatment. On May 10, the POC:Chl ratios ranged from 562–670 among treatments and increased in all treatments (upper limit 2500) as productivity began to increase. The POC levels significantly decreased (p<0.05) in the Early treatment as dosing was initiated due to the stimulatory effect on phytoplankton. Similar decreases in POC:Chl ratios were observed in the Mid and Late treatments as dosing was initiated in relation to macrophyte stage; however, the dominance of macrophytes dampened the level of response to dosing.

	Stage	Day	Stage*Day
POC:Chl	0.0028	0.0001	0.0323



		Day																			Season Avg.
		5/10	5/16	5/23	5/30	6/6	6/13	6/20	6/27	7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12	
Stage	Control	670	2413[a]	1854[a]	1135	2008	1730[a]	1400[a]	1427[a]	1419[a]	1400[a]	1010[a]	285	235	353	281	434	369	892	523[a]	1044[a]
	Early	562	301[b]	496[b]	1418	875	1404[b]	222[b]	479[b]	242[b]	695[b]	434[b]	259	181	632	334	380	342	419	404[ab]	530[b]
	Mid	668	1009[a]	1879[a]	1610	1833	1240[a]	224[b]	796[ab]	1264[a]	1431[ab]	1683[a]	310	482	742	1238	569	488	798	593[a]	992[ac]
	Late	589	1599[a]	1924[a]	1128	1724	1026[ab]	562[ab]	1549[a]	1324[a]	492[b]	748[b]	187	144	580	285	398	662	355	175[b]	813[c]

Figure 79. *Changes in the ratio of particulate organic carbon to phytoplankton chlorophyll over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced POC:Chl in ANOVA of rank-transformed data. The graph is a plot of POC:Chl over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods. The lower table lists LS Means represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Stage of treatment had no significant effects ($p>0.05$) on the distribution of the five major classes of phytoplankton; however, Day was a significant ($p=0.0001$) factor. Low numbers of phytoplankton were observed in May sampling which occurred soon after corral-filling (Figure 80). By June, the phytoplankton community was dominated by the chlorophytes with the cyanophytes being low in number. Total number of algae significantly increased in July to a total of $9.9*10^6$ cells/L; approximately 71% of the phytoplankton were Chlorophytes, and 25% were Cyanophytes. Phytoplankton numbers decreased in August but remained similar in distributions among major groups. Peak seasonal numbers of phytoplankton were observed in September, when total numbers of phytoplankton reached $25.4*10^6$ cells/L, and the community shifted to a community slightly dominated by Cyanophytes (50% of the community) compared to Chlorophytes (44% of the community); numbers of Euglenophytes and Baccillariophytes also increased but comprised less than 6% of the total community.

A total of 137 species of algae/cyanobacteria were observed in Study 2; the list of observed phytoplankton species is presented in Table 7. We observed 62 species of Chlorophytes; 37 species of Bacillarophytes; 15 species of Cyanophytes; 13 species of Euglenophytes; 5 species of Chysophytes; 4 species of Cryptophytes; and 1 species of Pyrrophycota. Early in the season, the Chlorophytes were dominated by *Gleocystis*, *Scenedesmus*, and *Oedegonium sp.*; whereas the Cyanophytes were dominated by the filamentous *Oscillatoria sp*. By the end of Study 2, the algal community was dominated by the filamentous cyanophyte *Oscillatoria sp*. and the chlorophytes *Pleodorina*, *Oocystis*, *Characium*, and *Oedeogonium* sp.; lesser amounts of the Euglenophytes (*Trachlemonas* and *Euglena* sp.) and the Bacillariophytes (*Navicula*, *Nitzschia*, *Fragilaria*, and *Gomphonema* sp.) were observed.

	5/11/00	6/7/00	7/5/00	8/2/00	9/5/00
Total	0.3870	0.8640	9.9290	6.0210	25.4040
Chlorophyta	0.2541	0.7711	7.0589	3.9591	11.3575
Euglenophyta	0.0009	0.0008	0.0049	0.0005	0.7509
Bacillariophyta	0.0733	0.0874	0.2259	0.0854	0.5586
Cryptophyta	0.0468	0.0000	0.0938	0.0050	0.0553
Cyanophyta	0.0121	0.0043	2.5453	1.9706	12.6819

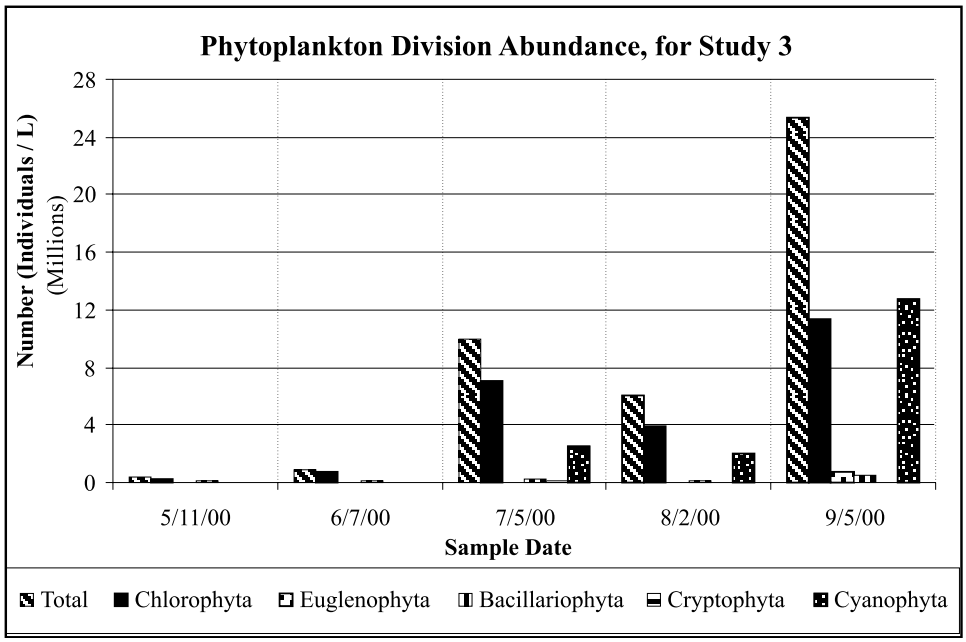
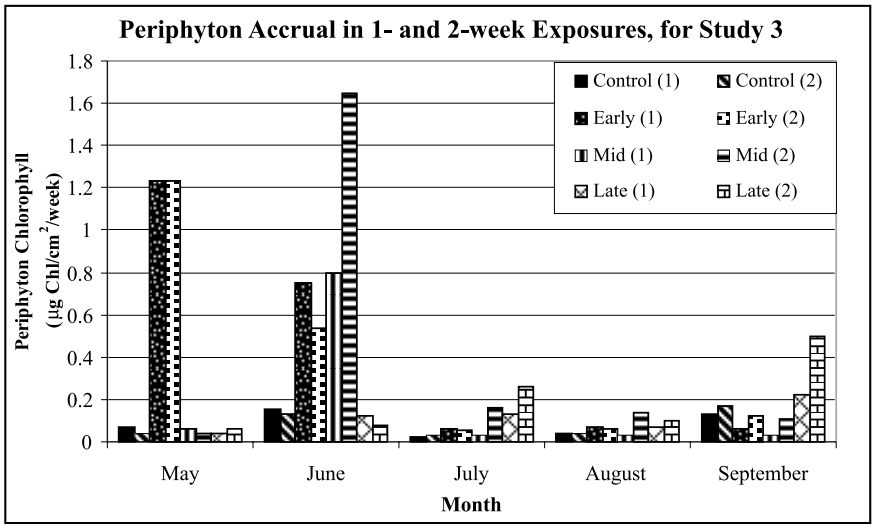


Figure 80. *Changes in phytoplankton abundance of the dominant divisions over time. Columns represent averages of all corrals in each month to show general successional trends over the season.*

Periphyton

Both 1-week and 2-week periphyton accrual rates responded to Stage (p=0.0173), Day (p=0.0020), and Stage*Day interaction (p=0.0042) (Figure 81). Periphyton accrual averaged 0.08 µg Chl/cm²/wk for both 1-week and 2-week accrual intervals in the Control when averaged across all dates. Individual weekly accrual rates in the Control ranged from a minimum of 0.02 µg Chl/cm²/wk in July to a maximum of 0.17 µg Chl/cm²/wk in September. One- and two-week accrual rates in the Control were similar. Periphyton accrual was substantially enhanced by nutrient enrichment, but the response varied with the timing of the dose period. Dosing in the Early macrophyte stage resulted in a 19-fold increase (1.23 µg Chl/cm²/wk) in periphyton accrual rates. However, during the post-dosing period from July through September, rates in Early treatments averaged <0.1 µg Chl/cm²/wk and were comparable to the Control. Mid treatment rates peaked at 1.65 µg Chl/cm²/wk in 2-week exposures in June; 2-week rates were twice those based on 1-week exposures. These differences may be because 2-week exposures received two amendments, whereas 1-week exposures had received only one amendment. Rates in Mid treatments averaged <0.2 µg Chl/cm²/wk from July through September. Accrual rates in Late treatments were minimally enhanced during the dose period in July, averaging 0.13 and 0.26 µg Chl/cm²/wk in 1- and 2-week exposures, respectively, due to the substantial competition by increased macrophyte stands late in the study.

	Stage	Day	Stage*Day
Periphyton 1-week	0.0173	0.0020	0.0042
Periphyton 2-week	0.0119	0.0001	0.0002



		Month										Season	
		May		June		July		August		September			
		1-wk	2-wk	1-wk	2-wk	1-wk	2-wk	1-wk	2-wk	1-wk	2-wk	1-wk	2-wk
Stage	Control	0.07^{a}	0.05^{a}	0.15^{ab}	0.13^{ab}	0.02^{a}	0.03^{a}	0.04	0.04	0.13^{ab}	0.17	0.08^{a}	0.08^{a}
	Early	1.23^{b}	1.23^{b}	0.75^{a}	0.54^{a}	0.06^{b}	0.05^{ab}	0.07	0.06	0.06^{a}	0.12	0.43^{b}	0.40^{b}
	Mid	0.06^{a}	0.04^{a}	0.80^{ab}	1.65^{a}	0.03^{ab}	0.16^{bc}	0.03	0.14	0.03^{ab}	0.11	0.18^{a}	0.42^{ac}
	Late	0.04^{a}	0.06^{a}	0.12^{b}	0.08^{b}	0.13^{b}	0.26^{c}	0.07	0.10	0.22^{b}	0.50	0.12^{ab}	0.18^{bc}

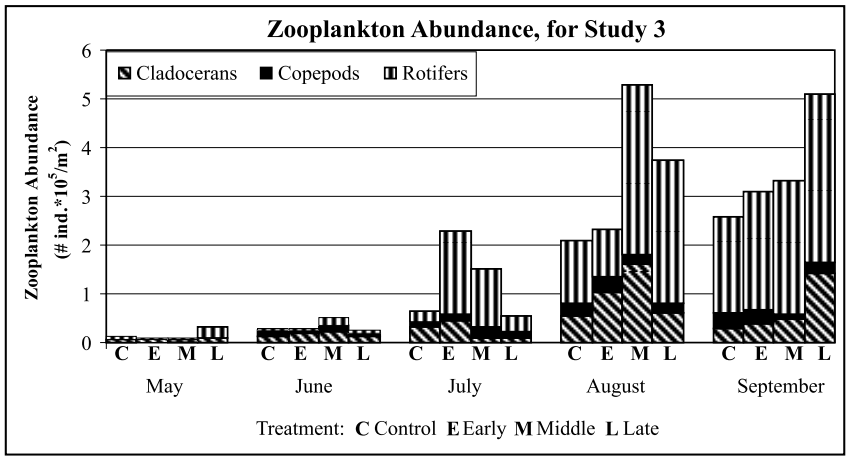
Figure 81. *Changes in accrual rates of periphyton chlorophyll in 1- and 2-week exposures over time. The 1- and 2-week datasets were analyzed separately. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced accrual rates in ANOVA of rank-transformed data. The graph is a plot of accrual rates over the experimental season pooled by treatment. The lower table lists LS Means (mg Chl/cm²/wk) represented in the graph, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different (p>0.05). In columns without letters, values are not significantly different.*

Zooplankton

Dosing in relation to macrophyte development (i.e., Stage) had no statistically significant effect on the zooplankton community. However, Day ($p<0.0001$) and the Stage*Day interaction ($p=0.0108$) had significant effects (Figure 82). Total numbers of zooplankton, in addition to major group classifications (cladocerans, copepods, and rotifers) increased in numbers over time in all treatments. A total of 36 genera, including 16 rotifers, 13 cladocerans, and 7 copepods, were identified in this study. A list of zooplankton taxa observed in Study 3 are presented in Table 8.

Lowest numbers of zooplankton were observed in all treatments in May soon after corral filling (Figure 82). Observed significant differences among treatments at study initiation, and prior to treatments, indicate the relative variability of the zooplankton data. For example, the Late treatment contained significantly higher numbers of zooplankton compared to the Early and Mid treatments even though dosing had not begun. Similarly, there were no significant differences in total zooplankton in June in any treatment. Dosing effects became apparent, however, in July as total numbers of zooplankton significantly tripled in the Mid ($2.3*10^5$ zooplankton/m^2) and Late ($1.6*10^5$ zooplankton/m^2) treatments compared to the Control ($0.6*10^5$ zooplankton/m^2). Total zooplankton numbers declined in the Early treatment in August but remained significantly higher than the Control in the Mid and Late treatments. In September, total numbers of zooplankton were elevated in all treatments compared to the Control; however, only the Late treatment was significantly greater than the Control.

	Stage	Day	Stage*Day
Total Zooplankton	0.0508	0.0001	0.0108
Cladocerans	0.2337	0.0001	0.5621
Copepods	0.6790	0.0001	0.8000
Rotifers	0.0548	0.0001	0.1494



		Month					Season
		May	June	July	August	September	Avg.
Stage	Control	13337[ab]	30719	65669[a]	223446[a]	271863[a]	121007
	Early	11420[a]	29409	232999[b]	238095[a]	335594[ab]	169503
	Mid	9821[a]	56406	165363[b]	534564[b]	343311[ab]	221893
	Late	31544[b]	25099	64462[a]	393359[ab]	523995[b]	207692

Figure 82. *Changes in zooplankton abundance over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The upper table presents probabilities that stage, day, and interactions of stage and day influenced abundance of the total zooplankton community, cladocerans, copepods, and rotifers in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of abundance over the experimental season by treatment (Control (C); Early (E); Mid (M); Late (L)). Abundances in the respective categories (Cladocerans-diagonals; Copepods-solids; Rotifers-stripes) are stacked to indicate totals. The lower table lists LS Means (# of indiv./m^2) for abundance of the total zooplankton community, along with statistical information based on the rank transformed data. Within a column, values sharing a letter are not significantly different ($p>0.05$). In columns without letters, values are not significantly different.*

Rotifers accounted for approximately 95% of the microzooplankton numbers during the season. In the Control, rotifer abundance increased significantly from May to September ($p<0.05$). Overall, nutrient enrichment substantially increased rotifer communities in Mid and Late treatments ($p<0.1$), but not in the Early treatments. In May and June, rotifer abundance was $<0.2*10^5/m^2$ in all treatments, and communities were dominated by *Bdelloid* spp., *Euchlanis* spp., *Hexartha mira*, *Lecane* spp., and *Monostyla* spp. In the Control, rotifers increased from $0.2*10^5/m^2$ in July to $2.0*10^5/m^2$ in September, and were dominated by *Brachionus* spp., *Euchlanis* spp., *H. mira,* and *Monostyla bulla*. Rotifer abundance in the dosed treatments was up to 3-times that in the Control, but dominant genera were similar among treatments.

Macrozooplankton populations in the Control were at a minimum in May ($0.1*10^5/m^2$) and peaked in August at $0.8*10^5/m^2$. Nutrient enrichment did not significantly influence cladoceran or copepod numbers, or the cumulative macrozooplankton community ($p>0.05$). In May, cladocerans represented 96% of the macrozooplankton in all treatments, and were dominated by *Sididae* spp. In June and July, cladocerans accounted for 67% and 59% of the macrozooplankton, respectively, and were dominated by *Simocephalus serrulatus* and *Ceriodaphnia* spp. Copepod populations in June and July in all treatments averaged 50% calanoids and 50% cyclopoids. In August, at the peak of macrozooplankton abundance, cladocerans accounted for 77% of the macrozooplankton numbers in all treatments. In August, cladocerans were predominantly *Alona* spp., *Ceriodaphnia* spp., *Chydorus sphaericus*, and *Simocephalus serrulatus*, and copepods were primarily cyclopoids (>94%). Cladoceran abundance declined at the end of the season, but still accounted for 68% of final macrozooplankton numbers. Dominant genera of cladocerans and copepods in September were the same as in August.

Sediment

N and P stocks in sediments changed significantly during the season ($p<0.01$), but were not significantly affected by nutrient amendments at various macrophyte stages. The N content of sediments was highest in June and September, averaging 0.35% N (Figure 83). Sediment content in July and August averaged 0.28% N. Sediments contained around 149 g N/(m^2*5 cm deep) in June and September, and around 127 g N/(m^2*5 cm deep) during July and August. Sediment P was at a maximum in June at 0.07% P (Figure 84). In subsequent months, P content fluctuated around 0.06% P. Sediment phosphorus pools ranged from 25 to 29 g P/(m^2*5 cm deep) during the season.

	Stage	Day	Stage*Day
Sediment Nitrogen	0.1886	0.0033	0.9331

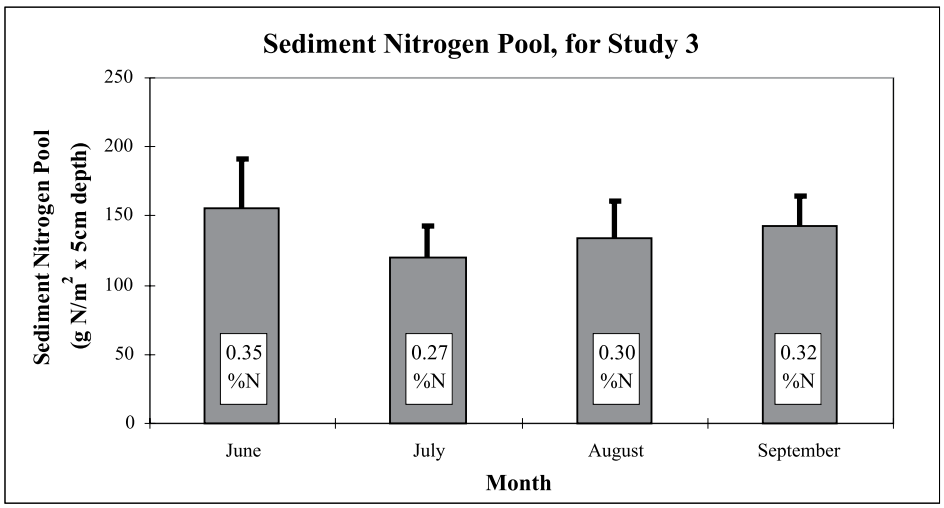


Figure 83. *Changes in sediment nitrogen pool over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The table presents probabilities that stage, day, and interactions of stage and day influenced N content in sediments in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graph is a plot of the estimated pool of N in the sediments for an area of 1 m^2 and a depth of 5 cm. Because treatment influences were not significant, monthly values are presented as averages of all treatments. The average %N in sediments is shown within the column for each month.*

	Stage	Day	Stage*Day
Sediment Phosphorus	0.5330	0.0051	0.5811

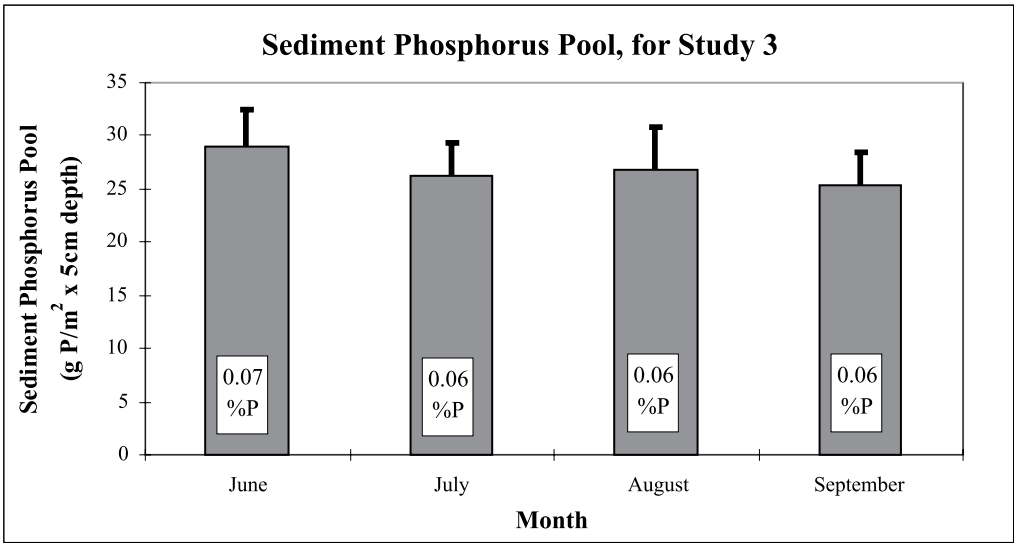


Figure 84. *Changes in sediment phosphorus pool over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The table presents probabilities that stage, day, and interactions of stage and day influenced P content in sediments in ANOVA of rank-transformed data. Darkened values are not significant (p>0.05). The graph is a plot of the estimated pool of P in the sediments for an area of 1 m^2 and a depth of 5 cm. Because treatment influences were not significant, monthly values are presented as averages of all treatments. The average %P in sediments is shown within the column for each month.*

System Metabolism

Estimates of gross community primary production (GCPP) and community respiration (CR) increased 3-fold over the season among all treatments; the effect of Day was significant ($p<0.0001$) (Figure 85). In addition, there was significant effect of the Stage*Day interaction ($p=0.0374$) on GCPP. Stage had no significant effect on either parameter.

Gross community primary production in the Control increased from around 4 mg O_2/L in May and early June, to around 15 mg O_2/L in August and September. Levels of GCPP significantly increased ($p<0.05$) in the Early treatment in early May and ranged from 5.9–9.6 mg O_2/L, or 2-fold Control levels. Thereafter, GCPP was similar in the Early treatment and Controls through the end of the season with the exception of August 29 when the Early (8.7 mg O_2/L) treatment was lower than the Control (13.8 mg O_2/L). Production in the Mid treatment was similar to the Control on all but two dates during the season. On June 20, the Mid treatment (10.2 mg O_2/L) was significantly higher ($p<0.05$) than the Control (6.3 mg O_2/L), and on August 15, Mid (11.6 mg O_2/L) was lower than the Control (17.1 mg O_2/L). Oxygen production in the Late treatment was similar to the Control throughout most of the season.

Community respiration was similar in magnitude and seasonality to GCPP. There was no stimulation of CR due to the Early treatment. Therefore, CR was less sensitive to dosing than GCPP.

	Stage	Day	Stage*Day
Production	0.4523	0.0001	0.0374
Respiration	0.3811	0.0001	0.0524

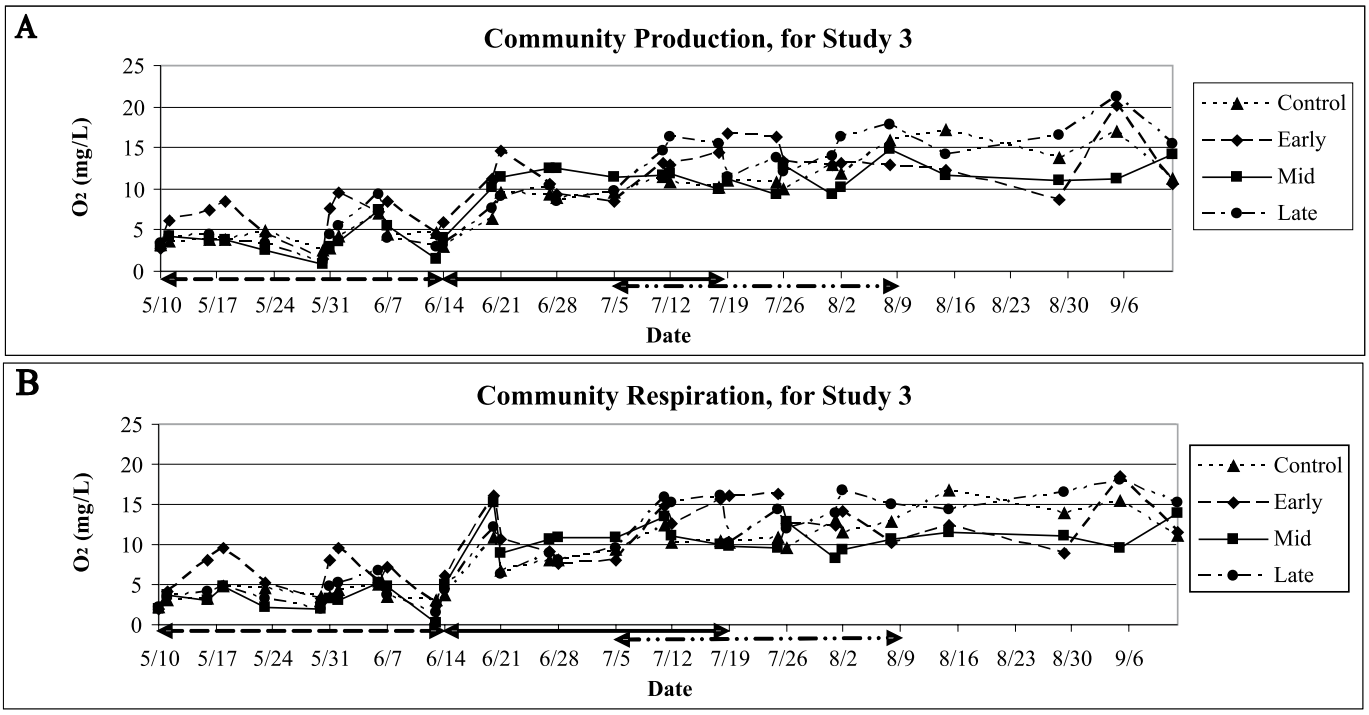


Figure 85. *Changes in community oxygen and respiration production over time. Treatments are differentiated by stage, which refers to the stage of macrophyte development at the start of the dose period. The table presents probabilities that stage, day, and interactions of stage and day influenced production and respiration in ANOVA of rank-transformed data. Darkened values are not significant ($p>0.05$). The graphs are plots of production (A) and respiration (B) over the experimental season pooled by treatment. Corresponding lines and arrows mark dose periods.*

Net Nutrient Balance

In Study 3, we applied equivalent loads of nutrients at different stages of macrophyte growth: Early (0% cover; initiated May 11), Mid (15–25% cover; June 12), or Late (75–90% cover; July 5) (Figure 59). The three dosed treatments received the same nutrient load (30 g NO_3-N/m^2; 1.2 g P/m^2) applied as six successive weekly additions of 5 mg NO_3-N/L and 200 µg P/L (25:1 N:P ratio). We calculated a final mass balance of nutrients among various nutrient pools to determine the net efficiency of uptake and assimilation of nutrients in these experimental systems (Table 10).

At the end of the study, macrophytes contained a total of 4.29, 3.92, 4.81, and 4.60 g N/ m^2 in the Control, Early, Mid, and Late treatments, respectively (Table 10). Water contained an additional 0.63, 0.83, 0.78, and 1.03 g N/m^2 in the Control, Early, Mid, and Late treatments, respectively. Combined (macrophytes + water), these two nutrient pools contained 4.92, 4.75, 5.59, and 5.63 g N/m^2 stocks at the end of the study. Thus, a total of 16% (Early treatment), 19% (Mid treatment), and 19% (Hi treatment) of total nitrogen added during the study were found in these two major nitrogen pools at the end of the study. This implies, under simple mass balance conditions (i.e., no loss to the atmosphere) that up to 84% (Lo treatment) and 81% (Mid and Late treatments) of total nitrogen added was sorbed to sediments or lost to the sediments as detritus. Attempts to measure actual sediment transfers were unsuccessful due to a combination of the large mass of pre-existing nitrogen in sediments and the error contributed by our sampling procedures. However, we know that these numbers are conservative, since the Control treatment alone revealed a total sequestration of 4.92 g N/m^2 (combined macrophytes and water) at the end of the study in the absence of external nitrogen addition. The large percentage of nitrogen that was not accounted for at the end of the study (84%, Early treatment; 81% Mid and Late treatments) indicates that these shallow, vegetated aquatic systems served as efficient biological, chemical, and physical sinks for nitrogen.

Phosphorus, likewise, was efficiently assimilated and retained in the study (Table 10). At the end of the study macrophytes contained a total stock of 0.80, 0.65, 0.86, and 0.84 g P/m^2 in the Control, Early, Mid, and Late treatments, respectively. Water contained an additional stock of 0.07, 0.08, 0.11, and 0.15 g P/m^2 in the Control, Early, Mid, and Late treatments, respectively. Using conservative mass balance estimates, subtracting the amount of phosphorus in macrophytes and water from that applied in dosing indicates that sediments had a net accrual of 0.47 (Early treatment), 0.23 (Mid treatment), and 0.21 (Hi treatment) g P/m^2. Macrophytes in the Control, Mid, and Late treatments contained equivalent amounts of phosphorus at the end of the study even though no phosphorus was applied to Controls. In contrast, the Early treatment contained 25% less phosphorus than the other treatments. This difference in macrophyte storage is largely due to the significantly lower biomass (Figure 59) and P stock (Figure 63) in macrophytes observed in the Early treatment compared to the Control, Mid, and Late treatments. Thus, Early treatment, prior to macrophyte development, somewhat inhibited the growth of macrophytes, though the overall phosphorus uptake and storage were similar and efficient across treatments.

Table 10. Summary Table of the Final Store of Phosphorus and Nitrogen in Water and Macrophytes in Study 3. Load Represents the Total Amount of Fertilizer P or N Added in Each Treatment. Stores in Macrophytes are Considered as Grams Per Cubic Meter Because Water Depth was 1 m

Nitrogen	**Load** (g N/m^2)	**Macrophytes** (g N/m^2)	**Water** (g N/m^2)	**Macrophytes + Water Total** (g N/m^2)	**Presumed Sediment Transfer** (g P/m^2)
Control	0	4.29	0.63	4.92	NA
Early	30	3.92 (13%)	0.83 (3%)	4.75 (16%)	25.25 (84%)
Mid	30	4.81 (16%)	0.78 (3%)	5.59 (19%)	24.41 (81%)
Late	30	4.60 (15%)	1.03 (3%)	5.63 (19%)	24.37 (81%)
Phosphorus	**Load** (g P/m^2)	**Macrophytes** (g P/m^2)	**Water** (g P/m^2)	**Macrophytes + Water Total** (g P/m^2)	**Presumed Sediment Transfer** (g P/m^2)
Control	0	0.80	0.07	0.87	NA
Early	1.2	0.65 (54%)	0.08 (7%)	0.73 (61%)	0.47 (39%)
Mid	1.2	0.86 (72%)	0.11 (9%)	0.97 (81%)	0.23 (19%)
Late	1.2	0.84 (70%)	0.15 (13%)	0.99 (83%)	0.21 (18%)

[1]Mean, (n); [STD,COV]

Discussion

Three studies were conducted over a 2-year period to determine the nutrient assimilation capacity of shallow vegetated aquatic systems. We evaluated the effects of magnitude of dosing; N:P ratio; frequency of dosing, and timing of dosing. We hypothesized that these various nutrient loading regimes would stimulate periphyton and phytoplankton growth, and subsequently, macrophyte development would be inhibited due to light limitation. An alternative stable state, dominated by phytoplankton, would occur that was less efficient than the original macrophyte community in assimilating and retaining nutrients from the water column. Ultimately, these results were not obtained. Under all scenarios, the macrophyte-dominated system persisted, and nutrient uptake and assimilation were maintained at over 90% efficiency. In some cases, macrophyte biomass was reduced below control levels; however, these experimental aquatic systems retained their ability to sequester excess nutrients, as described below.

Nutrient Dissipation Rates

Both nitrogen and phosphorus rapidly dissipated in these studies regardless of nutrient loading rate, N:P ratio, or timing of nutrient application in relation to macrophyte stage. Target concentrations of nitrogen were usually quite close to nominal dose applied. However, phosphorus losses were rapid, and concentrations were frequently less than 25% of nominal (i.e., Study 1). Rapid loss rates of phosphorus cannot be explained by biological uptake alone; rather, much of the phosphorus was probably lost to precipitation with iron and calcium carbonate similar to that observed in high alkalinity marl ponds (Otsuki and Wetzel 1972, Wetzel 1983). Phosphorus precipitation was greatest early in dosing intervals when hardness and alkalinity were still high due to the influence of the well water source. Therefore, nutrient dissipation rates on a mass basis varied and underestimated actual losses in the case of phosphorus due to rapid uptake and precipitation. Percentage losses, calculated as the percentage nominal concentration applied to the amount remaining immediately prior to the next dose, indicate that phosphorus dissipation rates (14-80% P/day) exceeded nitrogen dissipation rates (4-12% N/day) but that both were rapid. Nutrient uptake was greatest during the period ranging from May–July. Thereafter, however, nutrient uptake decreased due in part to macrophyte senescence, decomposition, and possible release of nutrients from sediments. Highest nutrient additions, added in the Hi treatment in Study 2 (60 g/m^2 N; 2.4 g/m^2 P) resulted in an overall loss of 95% of dosed N and 86% of dosed P by the end of the study. Similar results were observed by Balls et al. (1989) when various rates of nitrogen (up to 29 g N/m^2) and phosphorus (up to 2.3 g P/m^2) were added to vegetated ponds over the period May–October: half of added nutrients were gone in 2 days, and nutrients were near background levels within a 2-week period.

Nutrient Stocks

Nutrient stocks were evaluated in major ecosystem compartments (macrophytes, water, and sediments) to determine relative pools of nutrients over time. Sediments represented the single highest nutrient stock (and remained stable over time in spite of dosing) and contained an average of 150 g/m^2 N and 23 g/m^2 P. Macrophytes contained the second highest stock of nutrients. Maximum macrophyte nutrient stocks ranged from 2.8–22 g/m^2 N and from 0.7–3.3 g/m^2 P at peak macrophyte biomass; macrophyte nutrient stocks were usually lower at the end of each study (1.05-13.00 g/m^2 N and from 0.65-2.21 g/m^2 N) due to loss at senescence. Thus, macrophytes at peak biomass contained less than 15% nitrogen and phosphorus even at maximum stock levels. Water contained significantly lower nutrient stocks compared to macrophytes or sediments, and only became significant pools of nutrients at the end of the study as macrophytes senesced.

Phytoplankton Dynamics

Phytoplankton Biomass and Growth Rates

Phytoplankton contributes the greatest amount of primary productivity to many deep aquatic ecosystems including oceans, large lakes, and reservoirs (Wetzel 1983). However, the contribution of phytoplankton productivity to shallow aquatic systems is variable and depends on a combination of physical (e.g., depth, turnover time, inorganic turbidity), chemical (e.g., nutrient status), and biological (e.g., presence or absence of macrophytes, zooplankton grazing pressure). Phytoplankton, under unlimited conditions of light and nutrients, have greater potential for production per unit carbon compared to epiphytes or macrophytes for several reasons: 1) phytoplankton have a greater surface area: volume ratio

compared to epiphytes or macrophytes; 2) cell walls are thinner in phytoplankton; 3) diffusional gradients are less for phytoplankton due to the combination of thin cell walls and frequent mixing in the water column; and 4) phytoplankton are less light limited due to water mixing, and in some cases motility, which maintains cells in the photic zone (Sand-Jensen and Borum 1991).

We hypothesized that nutrient additions, under some cases, would stimulate phytoplankton to the point where shading would result in light limitation of macrophytes and epiphytes; system dominance would then shift from macrophyte dominance to an alternative stable state (Scheffer 1990) dominated by phytoplankton (Scheffer 1998). This shift is similar to that documented in a eutrophic, shallow lake in Austria by Mayer et al. (1997). These predictions, however, did not occur in our studies. In Study 1, we varied N:P ratio and loading rates throughout the summer. Early in the season, phytoplankton biomass responded positively to phosphorus dosing, and Chl *a* significantly increased up to 35 µg/L through late July; however, water clarity was retained. Maximum phytoplankton biomass rapidly increased in all treatments in September in Study 1 (up to 200-320 µg/L Chl *a*) approximately 2 weeks after the last dose. However, only Day and the N-dose*Day were significant factors late in the study; main effects of N dose and P dose were not significant. Chl *a* increased in all treatments (unrelated to treatment effects) as nutrients were released from senescing macrophytes in combination with possible sediment release of nutrients. In a field study, Landers (1982) reported that phytoplankton chlorophyll increased from 10 to around 90 µg/L during senescence in macrophyte enclosures, but chlorophyll remained around 10 µg/L in denuded enclosures. Based on those findings, he concluded nutrient release by senescing macrophytes caused the proliferation of phytoplankton.

In Study 2, both Lo and Hi dosing early in the study resulted in phytoplankton increases up to 33 µg/L Chl *a*; however, Chl *a* did not linearly increase with dosing due to internal damping mechanisms. Maximum phytoplankton biomass in Study 2, which intensified loading of nutrients, was observed in the Hi dose in early July (90 µg/L Chl *a*) just prior to the end of dosing; however, chlorophyll declined soon thereafter and was not maintained. In Study 3, we varied nutrient dosing by applying nutrients (Early, Mid, and Late) under varying stages of macrophyte development. Chlorophyll *a* was significantly related to timing of the dose, but varied in 2-week cycles. In contrast, nitrates gradually increased with dosing, whereas SRP cycled in 7-day spikes due to rapid dissipation. Therefore, a combination of differential nutrient controls and increasing grazing pressure by zooplankton acted to maintain Chl *a* levels below what would be expected in the absence of internal control mechanisms. Maximum phytoplankton biomass in Study 3, which varied nutrient loading in relation to macrophyte stage, peaked in July and late August (55 and 75 µg/L Chl *a,* respectively) in the treatment receiving the Early dosing. However, phytoplankton biomass in this treatment was not consistently different from the Control, which indicates that internal mechanisms again masked any lasting effect of nutrient timing or dose.

In summary, phytoplankton responses in all three studies were similar in that we saw positive phytoplankton responses due to nutrient dosing early in the study. However, the degree of phytoplankton response was less than expected. Balls (1989) observed similar responses in mesocosm experiments when phytoplankton response to nutrient enrichment was much lower than predicted in bioassays. They concluded that macrophytes and epiphytes may have assimilated nutrients thereby reducing nutrient availability to phytoplankton; they also suggested that zooplankton grazing became important in the regulation of algal biomass at some point in late spring. Therefore, phytoplankton was regulated due to a combination of top-down (zooplankton grazing) and bottom-up (nutrient competition by epiphytes) internal control forces (Bronmark and Hansson 1998; Sondergaard and Moss 1998). In our studies, peak phytoplankton biomass ranged from 75-310 µg/L Chl *a* across all studies, which is extremely high for aquatic systems such as lakes and reservoirs and would lead to a trophic classification of hypereutrophy and loss of macrophytes (Carlson 1977). However, numerous studies have indicated that traditional trophic state indices based on phytoplankton do not apply to shallow, vegetated aquatic systems because of interacting internal mechanisms that allow macrophytes and clear-water conditions to persist even though nutrient loading is high (Gasith and Hoyer 1998; Bachman et al. 2002).

Phytoplankton Species Composition

None of the experimental treatments in our three studies resulted in significant shifts in dominant in phytoplankton community composition. Some of our treatments were designed to result in nitrogen limitation, which should favor development of cyanophytes due to their ability to fix atmospheric oxygen (Wetzel 1983). In spite of nitrogen-limited conditions in some treatments, we observed a dominance of chlorophytes rather than blue-greens. Research has demonstrated that some macrophytes such as *Ceratophyllum demersum* (Kogan and Chinnova 1972; Koerner and Nicklisch 2002; Gross et al. 2003), *Myriophyllum spicatum* (Koerner and Nicklisch 2002), *Najas marina* (Gross et al. 2003), and *Chara sp*. (van Donk and van de Bund 2002) can produce allelopathic substances that inhibit growth of both green algae and cyanobacteria. However, allelopathic effects are rather species specific as opposed to acting on general taxonomic groups of algae and cyanobacteria (Koerner and Nicklisch 2002; Gross et al. 2003). It is unclear in our study if macrophytes produced allelopathic substance that may have inhibited some species of the phytoplankton.

Others have suggested that in shallow lakes the sediments may release nutrients that promote chlorophytes over blue-greens. Based on their survey of 178 lakes, Jensen et al. (1994) concluded that chlorophytes, with a higher growth rate

than blue-greens, may have a competitive advantage in shallow systems where sediments release pulses of nutrients. In Lake Sobygard, a system in which sediment release has been the major source of P for phytoplankton, chlorophytes dominate in the summer and autumn (Sondergaard et al. 1990). In our study, sediment release of P was consistent among treatments, and may explain the continued dominance of chlorophytes into the late season.

In studies on small, shallow, macrophyte-dominated ponds, Mulligan et al. (1976) observed that phytoplankton in control treatments were dominated by chlorophytes and cyanophytes. Following heavy fertilization (total load: 75 mg N/L; 7.5 mg P/L), they observed Chl increases up to 300 µg/L and dominance by different phytoplankton species that were not observed in the Control; however, the community was still dominated by chlorophytes and cyanophytes. We observed similar results in our studies. Therefore, relative shifts of phytoplankton under nutrient-enriched conditions may differ than that frequently observed in deeper, limnetic systems.

Periphyton Dynamics

Periphyton Biomass and Growth Rates

Periphyton is defined as the sessile assemblage of diatoms and algae that forms on underwater surfaces including macrophytes, sediments, and other surfaces such as corral sides. The role and dynamics of periphyton have received far less focus in studies compared to macrophytes and phytoplankton because of the difficulty in sampling and subsequent bias associated with incubations for productivity estimates. Therefore, researchers frequently use colonization rates of artificial substrates for estimates of periphyton productivity. In our studies, we incubated Scrimweave™ strips below the water surface to measure accumulation of Chl *a* at 1 and 2-week intervals. This technique does not account directly for periphyton on plants, sediments, or corral sides which are subject to differential influence of light and invertebrate grazing. It does, however, standardize the effects of surface area and light in order to partition the relative influence of experimental dosing and nutrient availability.

Periphyton productivity, or accrual rate, was low in July of Study 1 and ranged from 0.03–0.04 µg Chl/cm^2/wk; accrual rates were similar across treatments. Maximum biomass accrual (0.24 µg Chl/cm^2/wk) was observed in the August and September samples as nutrient dosing rates were increased. Biomass levels were comparable to those observed on artificial substrates in other studies (Cattaneo and Kalff 1980; Brock et al. 1995; James et al. 2000). Both N and P dosing had significant effects on periphyton growth rates; however, the N dose had a greater relative effect than P dose. In his review of nutrient cycling in shallow lakes, Lijklema (1994) concluded that spring algal biomass is determined by initial nutrient concentrations and external loading, whereas later in the season, internal loading may play a greater role as a nutrient source. This theory is supported by the periphyton dynamics in our study. In June, periphyton accrual rates were higher in P-dosed than 0P treatments, indicating P-limitation. Later in the season, August and September, periphyton responded to N-dosing; P availability was not dependent on phosphorus amendments, as indicated by equal or higher levels of periphyton growth in 0P than P-dosed treatments. Water column SRP and TP increased during the late season in all treatments indicating that internal P loading, whether from macrophytes or sediments, was occurring.

Periphyton dynamics differed in Studies 2, however, when nutrient doses were applied prior to macrophyte development. Highest periphyton biomass accrual (2.75 µg Chl/cm^2/wk) was observed in May in the Hi dose treatment. Thereafter, biomass accrual decreased as nutrient competition became more intense in June. A residual dosing effect was observed in July, August, and September, but biomass was much lower (<0.6 µg Chl/cm^2/wk) after the dosing period (end June 10) when nutrients became limiting. Periphyton biomass accrual in Study 3 was also greatest in May and June (maximum 1.65 µg Chl/cm^2/wk) during the Early and Mid dosing intervals; however, dosing had much less effect during the Late dosing interval. These observations imply that nutrient availability was high early in the study when both macrophyte and phytoplankton biomass was low, but that late in the study, as phytoplankton biomass increased, periphyton was limited by both nutrients and light.

We did not measure actual nutrient pools in periphyton. However, approximate nutrient stocks in periphyton can be estimated using conversion rates of chlorophyll and nutrient relationships from the literature. Stelzer and Lamberti (2002) found that chlorophyll, nitrogen, and phosphorus comprised approximately 0.03%, 0.7%, and 0.06%, respectively, of periphyton on a dry-wt basis. Therefore, chlorophyll conversion factors of 23 and 2 could be used to estimate nutrient stocks of periphyton in our study. Using these conversion factors, peak levels of periphyton observed (2.75 µgChl/cm^2; Study 2), respectively, and the total area of Scrimweave™ material in each corral (12.54 m^2), we estimate that the entire nutrient stock associated with periphyton on the Scrimweave™ corral sides would be approximately 9.0 g N and 0.69 g P per corral. Values adjusted to an area basis (12.56 m^2 surface area of each corral) would be 0.71 g N/m^2 and 0.06 g P/m^2. Thus, on a standing crop basis, periphyton associated with the corral sides in Study 2 contained approximately equivalent amounts of nutrients as water; 18% compared to macrophytes; but less than 1% as compared to sediments. Percentages in Studies 1 and 3 are much less than this calculation due to proportionately less periphyton biomass in these studies.

It is difficult to estimate the total surface area of macrophyte stands due to the high level of surface area associated with individual leaves or whorls. However, Bachman et al. (2002) estimated that periphyton biomass associated with macrophytes accounted for approximately 1.8% dry weight of submerged macrophytes in a survey of 319 shallow, vegetated lakes in Florida. In addition, the percentage of nitrogen (2-4% dry wt) and phosphorus (0.2-0.5% dry wt) of macrophyte tissue in our study exceeds that estimated for periphyton based on the findings of Stelzer and Lamberti (2002). Thus, it is apparent that periphyton represented a relatively minor component of nutrient pools associated with macrophytes or the corral sides in this study.

However, biomass is not a good estimator of productivity for periphyton. Periphyton can exhibit high rates of productivity under conditions of high nutrient availability even though standing crop is held low due to grazing (Cattaneo and Kalff 1980, Sand-Jensen and Borum 1991). Nutrient uptake by periphyton can be rapid from the water column. Cell walls of periphyton are thicker than phytoplankton, but thinner than macrophytes, which allow intermediate rates of nutrient uptake; furthermore, the diffusional boundary of periphyton can vary depending on nutrient concentrations and the degree of mixing of the water column (Sand-Jensen and Borum 1991). In this study, however, mixing was undoubtedly low due to the confinement of the water column by the corral sides which minimized wind-mixing; this may have decreased productivity of periphyton due to decreased nutrient exchange. Highest levels of periphyton growth rates were observed early in Study 2 when dosing occurred and invertebrate grazing was low due to the lack of colonization time; in addition, macrophyte shading was minimal due to low biomass. In contrast, in Study 1 and portions of Study 3, it is likely that macrophyte shading and nutrient limitation led to decreased periphyton productivity. Thus, we believe that periphyton productivity was probably not a major sink for nitrogen or phosphorus compared to other compartments. In some systems, however, constant nutrient renewal and invertebrate grazing can mask high rates of periphyton productivity as long as light is not limiting by turbidity or macrophyte shading. The relative role of periphyton in nutrient uptake and assimilation therefore is a priority for future studies of nutrient dynamics in shallow water bodies.

Macrophyte Dynamics

Macrophyte Biomass and Growth Rates

Macrophyte growth rates were robust in these studies; however, there were differences across years in maximum biomass. In Study 1 (1999) macrophyte biomass in Controls peaked at maximum dry weight biomass of 800 g/m^2 in July. Macrophyte biomass in Studies 2 and 3 (2000) peaked in August at 213 g/m^2, which is 75% less than the previous year. Macrophyte biomass can vary substantially within these experimental systems. For example, maximum biomass averaged 122 g/m^2 (Fairchild and Sappington 2002), 170 g/m^2 (Fairchild et al. 1994), and 330 g/m^2 (Fairchild et al. 1992) among mesocosms in three different studies conducted at CERC. The peak macrophyte biomass observed in Study 1 (800 g/m^2) is higher than previously observed; however, in these corral studies, sampling was restricted to waters < 1-m deep. Lower biomass observed in year 2000 likely occurred due to the extended drawdown period for corral construction in 2000 (26 days) compared to 1999 (16 days)**.**

Various nutrient regimes applied during these studies had variable effects on macrophyte growth. Maximum macrophyte biomass was not affected by nitrogen load, phosphorus load, or the N:P ratio when loads of up to 30 g N/m^2 and 0.86 g P/m^2 were applied on a bi-weekly basis to corrals containing approximately 25% surface coverage of macrophytes (Study 1). Similarly, there was no effect of nutrient dosing (30 g N/m^2 and 1.2 g P/m^2) when dose frequency was increased to weekly dosing (Study 3) when nutrients were applied either mid-season (i.e., Mid; 15-25% macrophyte coverage, June 12) or late-season (i.e., Late, 75-90% cover; July 5). Balls et al. (1989) also found macrophytes to persist in 0.8-m deep ponds dosed over 5 months (thirteen doses totaling loads of 29 g N/m^2 and 2.3 g P/m^2). In contrast, we found that nutrient dosing prior to macrophyte development (early in growing season) caused significant decreases in macrophyte growth and biomass when applied at weekly low (30 g N/m^2 and 1.2 g P/m^2) and high dose (60 g N/m^2 and 2.4 g P/m^2) levels. However, early dosing did not totally eliminate macrophyte stands or shift the systems to a phytoplankton-dominated alternative stable state (Scheffer 1990, 1998). These results indicate that shallow, macrophyte-dominated systems can persist at dosing of up to 60 g N/m^2 and 2.4 g P/m^2; however, this may be the upper limit of early loading that will maintain a macrophyte-dominated system structure. Results also indicated that *Najas* relied primarily on the sediments as its primary nutrient source since added nutrients did not stimulate macrophyte growth. Laboratory studies have also shown that sediments are the primary source of nutrients for *Najas* sp. (Moeller et al. 1988).

There are several mechanisms that can limit the existence and persistence of macrophytes in shallow aquatic systems. Inorganic turbidity, for example, can decrease light penetration and therefore eliminate submerged macrophytes (Wetzel 1983). Inorganic turbidity can result from erosional runoff, wind activity/wave action, and bioturbation due to fish and invertebrates (Engel 1990; Gasith and Hoyer 1998; Horppila and Nurminen 2003). None of these factors were present in our study due to the experimental design and tight control of experimental conditions. Depth is also a significant factor limiting growth of submerged macrophytes (Wetzel 1983; Haekanson and Boulion 2002). Macrophytes can persist in clear, oligotrophic systems at depths of up to 10 meters due to a combination of light attenuation and increasing hydrostatic pressure (Wetzel 1983). However, actual, realized maximum depth of macrophyte distributions in most mesotrophic-eutrophic littoral aquatic systems is much less.

We observed the loss of macrophytes in Study 1 in two dosed corrals in the deepest part of the mesocosm (approximately 1.1 m depth); in these corrals, phytoplankton biomass and resulting turbidity were noteably higher, suggesting that loss of macrophytes occurred due to light attenuation by phytoplankton. Phytoplankton turbidity due to nutrient enrichment is a primary limiting factor for macrophytes in eutrohpic systems (Moss 1976). Mulligan et al. (1976) demonstrated that high loading of nutrients (75 g N/m^3; 7.5 g P/m^3) prolonged phytoplankton blooms (>3 months with Chl >100 µg/L), which in turn eliminated some plants (*Chara* spp., *Myriophyllum spicatum*), and temporarily inhibited others (*Elodea canadensis*) by shading. The highest loading rates applied in our studies (60 g N/m^2 and 2.4 g P/m^2) prior to macrophyte development did not result in phytoplankton blooms; chlorophyll levels remained less than 40 µg/L during the dose period, and turbidity was less than 10 NTU's. Zooplankton numbers were low in the first month of the study, and therefore zooplankton grazing did not appear intense. It is probable that epiphytes on macrophytes, corral sides, and perhaps sediments were primary factors in nutrient dissipation that minimized phytoplankton dominance. Subsequently, light penetration remained sufficient for macrophyte survival, growth, and persistence. Seasonal timing of nutrient additions can also be a significant factor in the ability of phytoplankton to out-compete macrophytes. As macrophytes grow, the canopy moves higher in the water column and light is less of a limiting factor (Scheffer 1998). Therefore, factors that reduce light, such as phytoplankton or inorganic turbidity, will have the greatest impacts in the early stages of macrophyte development.

Periphyton can also limit the growth rates and ultimate standing crop of macrophytes by excessive growth on leaves and stems resulting in shading and light limitation (Phillips et al. 1978; Cattaneo and Kalff 1980; Sand-Jensen and Borum 1991). In laboratory enrichment experiments, Phillips et al. (1978) demonstrated that periphyton restricted the growth of *Najas marina* under enriched conditions; in separate field experiments, they linked poor development and premature decline of macrophytes (including *Najas marina*) in the field to light limitation imposed by dense epiphytic growth. Sand-Jensen and Borum (1991) also suggest that periphyton may also inhibit macrophyte growth by slowing diffusion of carbon dioxide and oxygen. In our experiments, periphyton accrual rates in the early, high weekly dosing regimes were 40-times and 5-times rates measured in the Controls in May and June, respectively. Therefore, persistent development of periphyton may have reduced light reaching macrophyte surfaces, thereby inhibiting macrophyte growth.

Self-shading of macrophytes is also a significant factor in many aquatic ecosystems. As macrophytes grow in height, the amount of light reaching the lower leaves becomes limiting, and may result in "shedding" of old leaves that cannot receive enough light to be photosynthetically active. This is a form of self pruning, and can present a significant mechanism of nutrient deposits to sediments in the form of detrital material (van Donk et al. 1993). Such "leaf shedding" was not measured directly in this study. However, the significance of the effect was often noted late in the studies during deployment of zooplankton traps. It became difficult to place the traps into the corrals without disturbing accumulated organic debris resulting from senescent macrophytes. In addition, overnight release of gases from sediment respiratory processes entrained significant amounts of organic material from sediments and plants which were carried up into the funnel traps.

Macrophyte Species Composition

Macrophyte stands in the CERC experimental mesocosms are dominated by two species of macrophytes: *Chara* sp., a macroalgae; and *Najas guadalupensis*, a submerged vascular angiosperm (Fairchild et al. 1992, 1994; Fairchild and Sappington 2002). *Najas* normally comprises approximately 70-90% of the biomass in these systems, whereas *Chara* ranges from 10-30% biomass (Fairchild et al. 1994; Fairchild and Sappington 2002). *Chara* populations were observed early in Study 1 which was conducted in year 1999. In Study 1, *Chara* was observed early in the study but declined in abundance during June until a monospecific stand of *N. guadalupensis* developed. *Chara* sp. was not observed in Studies 2 and 3 that occurred in year 2000, macrophyte stands consisted of a monospecific stand of *N. guadalupensis*. The lack of *Chara* sp. early in 2000 was most likely due to the extended draw-down period of 2000 (26 days) compared to 1999 (16 days).

Seasonal succession from *Chara* to stands of *Najas*, *Potamogeton*, and other angiosperms has frequently been observed in aquatic systems (e.g., Crawford 1977; Wood 1950). Seasonal declines in *Chara* abundance can occur due to excessive nutrients, lack of nutrients, and light limitation. Forsberg (1964) observed poor charophyte development in the laboratory at phosphorus levels around 20 µg P/L; similar field observations were made. Forsberg (1964) proposed that phosphorus concentrations at or above 20 µg P/L had an inhibitory effect on *Chara,* although the mechanisms of inhibition were not known. Such an inhibitory effect was probably not a factor in this study, because Control populations of *Chara* declined in Study 1 when TP was <20 µg P/L.

Nutrient limitation can also be a factor in *Chara* declines. *Najas* and *Chara* are capable of absorbing nutrients from the surrounding water, but their ability to utilize nutrients from the sediments depends on the efficiency of roots in *Najas* and rhizoids (root-like filaments) in *Chara*. *Chara* has demonstrated the ability to assimilate nutrients from water through its rhizoid and other structures (Box 1986, 1987; Kufel and Kufel 2002). *Najas* has been shown to take up N and P in fertilized sediments, and in other lab experiments *Najas* relied on the sediment for nearly all of its phosphorus requirement

(Moeller et al. 1988). A limited supply of nutrients in the water column, especially phosphorus, necessitates utilization of sediment stores for growth. *Najas* may have had an advantage over *Chara* under those circumstances, because roots provided a more efficient means of nutrient uptake than rhizoids (Moeller et al. 1988; Kufel and Kufel 2002).

Other authors have indicated that light limitation was the main factor causing *Chara* replacement due to turbidity or shading by macrophytes (Blindow 1992; Crawford 1977). Turbidity observed in May and June of 1999 (<4 NTU's) was not at a level that would likely cause *Chara* to decline. However, physical shading may have been a factor. Fairchild et al. (1994) previously provided evidence that shading by *Najas* was a factor in competition with *Chara*, when application of 50 µg/L atrazine reduced *Najas* populations, released *Chara* sp. from light limitation, and allowed *Chara* sp. to dominate the aquatic macrophyte community. *Chara* was only observed in Study 1 and nutrient addition itself did not negatively alter *Chara* incidence or macrophyte growth. Collectively, these results indicate that shading by *Najas* and epiphytes was the primary factor causing decline of *Chara*.

Macrophytes as Nutrient Sinks

Although nutrient dosing did not increase the growth rates of macrophytes, it did significantly increase the apparent nutrient concentrations of macrophytes. Macrophytes in the Control treatment averaged 1.90%N and 0.22%P at the beginning of Study 1 and increased to 3.02%N and 0.43%P by the end of the study. Nutrient dosing significantly increased concentrations of both N and P in macrophytes in Study 1 at up to 3.95%N and 0.52%P in the high dose treatments. In Studies 2 and 3, we observed similar concentrations and trends of N and P in the Control treatment. We also observed significant increases in nutrient content of macrophytes due to the effect of Dose (Study 2) and Stage (Study 3) of macrophytes related to timing of dose; nutrient concentrations in macrophytes increased in proportion to dosing of both N and P and in some cases increased over 200% greater (4.35%N and 0.95%P; July, Study 2) than nutrient concentrations compared to the Control (2.13%N and 0.48%P; July, Study 2). Although nutrient concentrations of macrophytes increased under all treatments, macrophyte nutrient stocks varied and strongly reflected trends in macrophyte biomass. For example, in Study 2, when Early dosing significantly decreased macrophyte biomass, the nutrient stock declined accordingly.

Our measurements of N and P concentrations in *Najas* from Control macrophytes are similar to those in the literature. Royle and King (1991) examined nutrient concentrations in macrophytes in Lake Liddell, New South Wales; nutrient concentrations in *Najas marina*, *Vallisneria spiralis*, *Potomogeton perfoliatus*, and *Potomogeton pectinatus* were similar when based on means by species (1.51-1.91% N; 0.15-0.19% P). Boyd (1970) also found similar concentrations of P (0.15% P) in *Najas guadalupensis*; concentrations ranged from 0.12% to 0.27% P among five other submerged species. Therefore, *Najas* appears to be intermediate in its ability to store nutrients.

Macrophytes as Nutrient Sources

Macrophytes do not provide significant dissolved nutrient sources to the water column during periods of active growth. However, macrophytes serve as significant sources to sediments via nutrient translocation from roots and through shedding and sloughing of leaves and associated periphyton (Barko et al 1991). In addition, senescing macrophytes release significant amounts of dissolved phosphorus and nitrogen to the water column that are utilized by periphyton and phytoplankton (Landers 1982; Engel 1990).

Release of nutrients during macrophyte senescence varied in our studies. In Study 1, we applied varying levels of nitrogen and phosphorus over a 16-week period. Macrophyte growth was robust, reaching a maximum of biomass levels in July of 661-802 g/m^2 dry weight among treatments. Subsequent macrophyte senescence resulted in a loss of up to 50% of N and P stocks. Much of this loss was not accounted for in the water column; therefore, it is likely that the majority of these nutrients were transferred to sediments as observed by van Donk et al (1993) and Stachowicz et al. (1994). However, these transfers were not observed in actual measurements of sediment stocks due to the degree of error of the chosen sampling method as compounded by the large pool of N in sediments (160 g/m2). Significant amounts of nitrogen and phosphorus were also transferred to the water column as indicated by dramatically increasing amounts of phytoplankton chlorophyll *a* and concurrent 3-fold increases in TN and TP across all treatments.

Similar, but less dramatic results were obtained in Study 3 in the Mid and Late dosing. In contrast, macrophyte senescence and nutrient releases were less profound in Studies 2 and 3 even though total nutrient loading was increased. In Study 2, when nutrients were applied before macrophytes emerged, total macrophyte biomass reached a maximum of 213 g/m^2 dry weight in the Control in August. Macrophytes began to senesce over the next 30 days, but N and P stocks in macrophytes were maintained, and phytoplankton biomass, as indicated by chl *a*, TN, and TP did not substantially increase. The decreased effects of nutrient release in Studies 2 and 3 may be due to the proportionately smaller macrophyte biomass (75% less; maximum 213-227 g/m^2 dry weight) compared to Study 1 (maximum 661-802 g/m^2 dry weight). Therefore, fewer nutrients were available for release, and degree of senescence at the end of the study was less due to decreased macrophyte self-shading and perhaps a truncated sampling duration. It is evident, however, that macrophytes can serve as both sources and sinks for nutrients in shallow vegetated aquatic systems, but likely

depend on factors such as biomass, species, and other physical and chemical factors (Barko et al. 1991; van Donk et al. 1993; Stachowicz et al. 1994).

Zooplankton Dynamics

Zooplankton are known to play a major role in water quality and clarity of deep-water limnetic systems (Brooks and Dodson, 1965; Irvine et al. 1989). Zooplankton, especially the cladocerans, are efficient filter feeders that serve to graze down zooplankton populations in systems where fish are absent. When fish are present, they frequently selectively feed on larger bodied zooplankton such as the cladocerans; under high levels of fish predation, the zooplankton community often shifts to small-bodied rotifers and copepod nauplii which are less efficient in grazing phytoplankton. Subsequently, in systems containing fish predators, cladoceran zooplankton numbers decrease; algae tends to increase; and water clarity deceases.

In Study 1, the zooplankton community was dominated by the cladoceran *Ceriodaphnia reticulata* early in the study prior to corral construction and treatment. In the absence of fish, *C. reticulata* was able to effectively graze down phytoplankton and maintain a high level of water clarity. Subsequently, macrophyte growth and biomass development were high. Cladocerans continued to dominate the zooplankton community throughout the study in spite of increased nutrient loading. Late in the study, algae began to increase due to nutrient release from macrophytes, and total cladocerans increased concordantly; therefore, water clarity was maintained (turbidity < 7 NTU's) until the final two weeks of the study. These findings support those of Hansson (1992), which suggested that in systems with two main trophic levels (algae and zooplankton), phytoplankton biomass would show a minor increase with nutrient enrichment, but algal populations would be largely regulated by grazers. Dominance of cladocerans in Study 1 contrasted results in a previous study at CERC when fish were present and rotifers dominated the zooplankton community (Boyle et al. 1996).

In contrast, in Studies 2 and 3, zooplankton numbers were low early in the study in May and June in spite of early nutrient dosing. Low numbers were likely due to the early flooding of the corrals prior to macrophyte emergence. Although cladocerans, primarily *C. reticulata,* were dominant early in Studies 2 and 3, they were replaced in July and August by high numbers of rotifers including *Brachionis spp.*, *Eudhlanis spp., H. mira*, and *Monostyola bulla.* Larger cladocerans and copepods generally exert greater grazing pressure on phytoplankton than smaller zooplankton species due to high intake rates and a large range of particle sizes that they are able to ingest (Thorpe and Covich 1991). However, Jeppesen et al. (1990) found that phytoplankton declines in shallow Lake Sobygard were associated with proliferation of rotifers and/or cladocerans. In our Studies 2 and 3, rotifer abundance increased midsummer and persisted as the numerically dominant division (60% of the cumulative zooplankton abundance), regardless of date or treatment. Rotifers may have provided a steady grazing pressure on the algae and may have influenced the size, structure, and abundance of the phytoplankton by regulating small algal species (Thorpe and Covich 1991). Nutrient dosing regime apparently had little effect on zooplankton communities in our studies. Rather, differences in zooplankton community dynamics among years (1999, Study 1 versus 2000, Studies 2 and 3) were more likely due to differences in the operational aspects of corral construction and flooding chronology.

Although abundance in this study can be expressed volumetrically (number/m^3) based on the 1 meter depth, it must be noted that we sampled the zooplankton using behavioral traps set overnight as opposed to instantaneous tow samples frequently used in limnological studies. Therefore, our zooplankton counts were based on numbers vertically migrating overnight on an area basis (number/m^2). Therefore, highly active zooplankton taxa are more likely to appear in samples compared to those less prone to vertical night-time migration.

The average abundance of rotifers in our study (range 0.7-1.0*10^5/m^2 across 3 studies) was comparable to the rotifer abundance reported by Irvine et al. (1989) in other macrophyte-dominated mesocosms devoid of fish (average: 1.0*10^5/m^2; range: 0.3 *10^5/m^2–1.7*10^5/m^3). The seasonal average of cladoceran abundance (range 0.3-1.0*10^5/m^2) was similar to the average of 0.3*10^5/m^2 reported by Irvine et al (1989). Maximum cladoceran abundance in the Control (1.1*10^5/m^2; Study 1) was also comparable to abundance (range: 0.4*10^5/m^2–0.8*10^5/m^2) measured using vertical migration samplers in Lake Itasca in August by Williams (1983). Irvine et al. (1989) reported copepod abundance around 2.0*10^5/m^2, which was an order of magnitude greater than averages in our study (range 0.2-0.3*10^5/m^2). Vertical migration in copepods is well noted in Hutchinson (1967). Therefore, it is not known why preferential samples of vertical migrants accounted for fewer copepods, but comparable numbers of rotifers and cladocerans, than tube samples used by Irvine et al. (1989). Site and species variability may have been more influential in estimates of zooplankton abundance than sampling techniques.

Sediments as Nutrient Sources and Sinks

Sediments are known to be the primary pool of nutrients in shallow aquatic systems (Johnston 1991; Barko et al. 1991). The stock of nutrients in the sediments varies with consideration of depth, but in general is at least one or two orders of magnitude greater than the macrophytes or water (Johnston 1991). Sediments in our study contained approximately 150 g N/m^2 and 23 g P/m^2 as measured in the nutrient pool in the upper 5 cm of sediment. Comparatively, macrophytes

had less than 22 g N/m^2 and 3.3 g P/m^2 under maximum conditions dosage and biomass conditions. Water contained less than 1 g N/m^2 and 0.1 g P/m^2 under Control conditions and up to 10 g N/m^2 and 0.4 g P/m^2 under highest dosage conditions; higher levels generally were due to high levels of dissolved nutrients due to dosing as opposed to true steady-state conditions. Thus, sediments dominated as the primary source and sink of nutrients in these studies. We did not observe an increase in total nutrient pools in sediments, however, due to the size of the nutrient pool and the inherent error in our measurement technique.

Phosphorus amendments were effectively conserved in our experimental corrals because these systems had no outflow. Sediments served as both a source and a sink for phosphorus in this study. For example, Control macrophytes accrued up to 3.3 g P/m^2 from sediments even though no external phosphorus was added. In contrast, phosphorus additions to the corrals rapidly dissipated in as little as 7 days; the major portion of these additions were transferred to sediments either directly (sorption or precipitation) or indirectly due to detrital transfer. Johnston (1991) reviewed the literature regarding uptake and retention of P by natural wetlands and indicated that values averaged 0.34 g P/m^2/yr (range 0.07-3.48 g P/m^2/yr). Richardson and Qian (1999) evaluated a North American wetland database and determined that the assimilative capacity of most wetlands for phosphorus is around 1 g P/m^2/yr. Our studies support this estimate because at dosing levels of 0.86 g P/m^2 (Study 1) and 1.2 g P/m^2 (Study 2) phosphorus was effectively assimilated without significant effects on community or nutrient dynamics. In our highest dose (2.4 g P/m^2; Study 2), macrophyte biomass decreased, which indicates that there is an upper limit to phosphorus assimilation by submerged macrophytes.

Nitrogen removal and transfer to sediment was also efficient. Effective doses of up to 60 g N/m^2 were assimilated in our studies. Mitsch et al. (1999) reviewed the literature regarding nitrogen assimilation in natural wetlands and indicated that assimilation of up to 28 g N/m^2/yr can occur; even higher assimilation can occur in engineered wetlands. In contrast with phosphorus, nitrate is not necessarily conserved because it may be lost to the atmosphere as a gas via denitrification processes (Seitzinger 1988). Johnston (1991) indicated that denitrification can be a major factor in loss of nitrogen in wetland soils; denitrication losses can range from 0.002 - 0.34 g N/m^2/yr (mean 0.19 g N/m^2/yr) at unammended sites and from 16-134 g N/m^2/yr (mean 60 g N/m^2/yr) in sites amended with inorganic nitrogen. Denitrification involves the microbial transformation of nitrate to N_2 under saturated anaerobic conditions (Scheffer 1998). Macrophytes can stimulate denitrification by providing a source of organic carbon to sediments; as organic matter decomposes, near-surface layers of sediments become anoxic and stimulate denitrification processes (Weisner et al. 1994). If denitrification is related to loading and water TN, as suggested by Jensen et al. (1992), nitrate additions may enhance loss rates. We did not measure actual denitrification rates in our studies. Greater denitrification in relation to nitrate amendments in our study may explain why N-stores in N-dosed treatments were not as high, compared to the Control, as their loading would have predicted.

Conclusions, Management Implications, and Research Needs

We determined that corral experiments, used to simulate shallow vegetated aquatic systems, were highly efficient in removal of nitrogen (applied as nitrate) and phosphorus (applied as phosphate) at dose levels of up to 60 g N/m^2 and 2.4 g P/m^2; nutrient uptake, assimilation, and retention were efficient regardless of magnitude of dose, timing of dose in relation to macrophyte development, or frequency of dose. In one treatment (high early dose), we observed significant reductions in macrophyte biomass; however, stands persisted throughout the study, and nutrient removal was efficient. Total nutrient removal was over 90% as indicated by dissolved nutrients remaining at the end of the study.

Sediments served as the largest storage pool of nutrients, followed by macrophytes, phytoplankton, epiphytes, and water (dissolved forms). Sediment was such a dominant factor in nutrient dynamics because it provided the primary source of nutrient for macrophytes; this was dramatically illustrated by macrophyte production in Control treatments in the absence of macrophyte dosing. Much of the applied nutrients were returned to the sediments by macrophyte senescence, shedding of leaves, grazing of epiphytes, and zooplankton grazing. Precipitation of phosphorus was also a likely factor based on observed decreases in hardness over time which was greater in dosed treatments. In this study, we attempted to determine the rates of sediment deposition of nutrients using precipitation trays. However, these attempts were unsuccessful due to the disturbance created in macrophyte sampling. Other ancillary observations support the contention that grazing of epiphytes and shedding of macrophyte leaves were significant factors, including high accumulations of fine particulate organic matter in zooplankton traps and observed particles settled on macrophyte surfaces. Future studies should focus more closely on direct quantification of detrital nutrient transfer to sediments for epiphytes and macrophytes. In addition, direct measurements of denitrification rates are needed.

Although the results of these studies demonstrated that shallow, vegetated aquatic systems are highly efficient in nutrient uptake, assimilation, and removal, there may be some limitations in the direct application of these data. Our wetlands were operated as “closed systems” which eliminated any losses of nutrients due to hydrologic discharge. In addition, our systems were isolated from wind and wave action, common in natural systems, which can increase turbidity and decrease availability to macrophytes. Wetlands in the actual environment experience variable inputs and outputs of water and nutrients based on the season, frequency, and magnitude of rainfall events, size of the watershed, and wetland dimensions. Therefore, dissolved nutrients and suspended particles can be lost from the system as “leakage” in overflow or runoff. In addition, we focused primarily on the active growing season of the wetland cycle; the over-winter period was not studied. Under aerobic conditions, phosphate forms insoluble complexes with iron which facilitate phosphorus retention in sediments; however, under anaerobic conditions, phosphorus is released to interstitial waters and the water column. During the over-winter period, biological activity is low and so dissolved phosphorus and nitrogen can be leached from wetland systems during colder temperatures. In addition, newly constructed wetlands may lack the fine organic sediments that promote macrophyte development and critical sediment processes. Each of these factors could alter the efficiency of wetlands for nutrient removal. Therefore, additional studies on the nutrient removal efficiencies of both constructed and natural wetlands are needed to determine the ultimate role of wetlands as management tools for nutrient reduction streams and other receiving bodies.

Literature Cited

American Public Health Association. 1992. Standard methods for the examination of water and wastewater. Amer. Pub. Health Assoc. Inc., New York.

Bachman, R.W., C.A. Horsburgh, M.V. Hoyer, L.K. Mataraza, and D.E. Canfield Jr. 2002. Relations between trophic state indicators and plant biomass in Florida lakes. Hydrobiologia 470: 219-234.

Balls, H., B. Moss, and K. Irvine. 1989. The loss of submerged plants with eutrophication: experimental design, water chemistry, aquatic plant and phytoplankton biomass in experiments carried out in ponds in the Norfolk Broadland. Fresh. Biol. 22:71-87.

Barko, J.W., D. Gunnison, and S.R. Carpenter. 1991. Sediment interactions with submersed macrophyte growth and community dynamics. Aquat. Bot. 41:41-65.

Blindow, I. 1992. Decline of charophytes during eutrophication: comparison with angiosperms. Freshwat. Biol. 28:9-14.

Box, R.J. 1986. Quantitative short-term uptake of inorganic phosphate by the *Chara hispida* rhizoid. Plant, Cell and Environ. 9:501-506.

Box, R.J. 1987. The uptake of nitrate and ammonium nitrogen in *Chara hispida* L.: the contribution of the rhizoid. Plant, Cell, and Environ. 10:169-176.

Boyle, T.P., J.F. Fairchild, E. F. Robinson-Wilson, P.S. Haverland, and J.A. Lebo. 1996. Ecological restructuring in experimental aquatic mesocosms due to the application of diflubenzuron. Environ. Tox. Chem. 15:1806-1814.

Boyd, C.E. 1970. Chemical analyses of some vascular aquatic plants. Arch. Hydrobiol. 67(1):78-85.

Brock, T.C.M., R.M.M. Roijackers, R. Rollon, F. Bransen, and L. Van der Heyden. 1995. Effects of nutrient loading and insecticide application on the ecology of Elodea-dominated freshwater microcosms II: responses of macrophytes, periphyton, and macroinvertebrate grazers. Arch. Hydrobiol. 134:53-74.

Bronmark, C. and L. Hansson. 1998. The biology of lakes and ponds. Oxford University Press, Oxford.

Brooks, J.L. and S.I. Dodson. 1965. Predation, body size and composition of the plankton. Science 150:28-35.

Carlson, R.E. 1977. A trophic state index for lakes. Limnol. Ocean. 22:361-369.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8(3):559-568.

Cattaneo, A., and J. Kalff. 1980. The relative contribution of aquatic macrophytes and their epiphytes to the production of macrophyte beds. Limnol. Oceanogr. 25(2):280-289.

Conover, W.I., and R.L. Iman. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. Amer. Statistician 35:124-128.

Crawford, S.A. 1977. Chemical, physical, and biological changes associated with *Chara* succession in farm ponds. Hydrobiol. 55(3):209-217.

Crumpton, W.G., T.M. Isenhart, and P.D. Mitchell. 1992. Nitrate and organic N analyses with second derivative spectroscopy. Limnol. Oceanogr. 37:907-913.

de Haan, H., L. Van Liere, S.P. Klapwijk, and E. Van Donk. 1993. The structure and function of fen lakes in relation to water table management in The Netherlands. Hydrobiol. 265:155-177.

Dykyjova, D. and J. Kvet, 1982. Mineral nutrient economy in wetlands of the Trebon Basin Biosphere Reserve, Czechoslovakia, *in* B. Gopal, E.R. Turner, R.G Wetzel, D.F. Whigham, eds. Proceedings of the Interational Conference of Wetlands: Ecology and Management. International Scientific Publications, Jaipur, India.

Engel, S. 1990. Ecosystem responses to growth and control of submerged macrophytes: a literature review. Technical Bulletin No. 170. Dept. Natural Resources., Madison, WI.

Fairchild, J.F., T.W. La Point, J. Zajicek, M.K. Nelson, F.J. Dwyer, and P.A. Lovely. 1992. Population, community, and ecosystem-level responses of mesocosms to pulsed doses of a pyrethroid insecticide. Environ. Toxicol. Chem. 11:115-130.

Fairchild, J.F., T.W. La Point, and T. Schwartz. 1994. Effects of an herbicide and insecticide mixture in experimental aquatic mesocosms. Arch. Env. Tox. Chem. 27:527-533.

Fairchild, J.F. and L.S. Sappington. 2002. Fate and effects of the triazinone herbicide metribuzin in outdoor aquatic mesocosms. Archives. Environ. Toxicol. Chem. 43(21):198-2002.

Forsberg, C. 1964. Phosphorus, a maximum factor in the growth of Characeae. Nature 201:517-518.

Gasith, A. and M.V. Hoyer. 1998. Structuring role of macrophytes in lakes: changing influence along lake size and depth gradients, *in* Jeppesen, E., M. Sondergaard, M. Sondergaard, and K. Christoffersen, eds. The structuring role of submerged macrophytes in lakes. Springer, New York.

Green, R.H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. Wiley and Sons, New York, NY.

Gross, E.M., D. Erhard, and E. Ivanyi. 2003. Allelopathic activity of Ceratophyllum demersum L. and Najas marina ssp intermedia (Wolfgang) Casper. Hydrobiologia 509: 583-589.

Haekanson, L., and V.V. Boulion. 2002. Empirical and dynamical models to predict the cover, biomass, and production of macrophytes in lakes. Ecol. Model. 151:213-243.

Hansson, L. 1992. The role of food chain decomposition and nutrient availability in shaping algal biomass development. Ecology 73: 241-247.

Hauck, H.S., L.G. Huber, and C.D. Nagal. 1997. Water Resources Data Missouri Water Year 1996. U.S. Geological Survey Water Data Report MO-96-1. Rolla, MO. 292 p.

Havens, K.E., T.L. East, A.J. Rodusky, and B. Sharfstein. 1999. Littoral periphyton responses to nitrogen and phosphorus: an experimental study in a subtropical lake. Aquat. Bot. 63:267-290.

Horppila, J., and L. Nurminen. 2003. Effects of submerged macrophytes on sediment resuspension and internal phosphorus loading in Lake Hiidenvesi (southern Finland). Water Res. 37:4468-4474.

Hutchinson, G.E. 1967. A treatise on limnology. Volume II: Introduction to lake biology and the limnoplankton. Wiley, New York.

Irvine, K., B. Moss, and H. Balls. 1989. The loss of submerged plants with eutrophication, II. Relationships between fish and zooplankton in a set of experimental ponds, and conclusions. Freshwat. Biol. 22:89-107.

James, M.R., I. Hawes, and M. Weatherhead. 2000. Removal of settled sediments and periphyton from macrophytes by grazing invertebrates in the littoral zone of a large, oligotrophic lake. Fresh. Biol. 44:311-326.

Jansson, M., R. Andersson, H. Berggren, and L. Leonardson. 1994. Wetlands and lakes as nitrogen traps. Ambio 23(6):320-325.

Jensen, J.P., E. Jeppesen, P. Kristensen, P.B. Christensen, and M. Sondergaard. 1992. Nitrogen loss and denitrification as studied in relation to reductions in nitrogen loading in a shallow, hypertrophic lake (Lake Sobygard, Denmark). Int. Revue ges. Hydrobiol. 77(1):29-42.

Jensen, J.P., E. Jeppesen, K. Olrik, and P. Kristensen. 1994. Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. Can. J. Fish. Aq. Sci. 51:1692-1699.

Jeppesen, E., M. Sondergaard, O. Sortkjaer, E. Mortensen, and P. Kristensen. 1990. Interactions between phytoplankton, zooplankton and fish in a shallow, hypertrophic lake: a study of phytoplankton collapses in Lake Sobygard, Denmark. Hydrobiol. 191:149-164.

Johnston, C.A. 1991. Sediment and nutrient retention by freshwater wetlands: effects on surface water quality. Crit. Rev. in Env. Control. 21(5,6):491-565.

Koerner, S., and A. Nicklisch. 2002. Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. J. Phycol. 38:862-871.

Kogan, S.J., and G.A. Chinnova. 1972. Relations between Ceratophylum demersum and some blue-green algae. Hydrobiol. J. [Gidrobiol. Zh.] 8:14-19.

Kufel, L., and I. Kufel. 2002. Chara beds acting as nutrient sinks in shallow lakes--a review. Aquat. Bot. 72:249-260.

Lachat Instruments, Inc. 1997. Determination of Ammonia by Flow Injection Analysis Colorimetry. QuikChem Method 10-107-06-1-A. Milwaukee, WI.

Lachat Instruments, Inc. 1997. Determination of Nitrate/Nitrite in Surface and Wastewaters by Flow Injection Analysis. QuikChem Method 10-107-04-1-B. Milwaukee, WI.

Lachat Instruments, Inc. 2000. Orthophosphate in Waters. QuikChem Method 10-115-01-1-A. Milwaukee, WI.

Landers, D.H. 1982. Effects of naturally senescing aquatic macrophytes on nutrient chemistry and chlorophyll a of surrounding waters. Limnol. Oceanogr. 27(3):428-439.

Lijklema, L. 1994. Nutrient dynamics in shallow lakes: effects of changes in loading and role of sediment-water interactions. Hydrobiol. 275/276:335-348.

Lind, O.T. 1985. Handbook of common methods in limnology. Kendall/Hunt, Dubuque, IA.

Madsen, J. D. 1993. Biomass techniques for monitoring and assessing control of aquatic vegetation. Lake and Reserv. Manage. 7(2):141-154.

Mayer, J., M. T. Dokulil, M. Salbrechter, M. Berger, T. Posch, G. Pfister, A.K.T. Kirschner, B. Velimirov, A. Steitz, and T. Ulbricht. 1997. Seasonal successions and trophic relations between phytoplankton, zooplankton, ciliate and bacteria in a hypertrophic shallow lake in Vienna, Austria. Hydrobiol. 342/343:165-174.

Mitchell, S.F., D.P. Hamilton, W.S. MacGibbon, P.K.B. Nayar, and R.N. Reynolds. 1988. Interrelations between phytoplankton, submerged macrophytes, black swans (*Cygnus atratus*) and zooplankton in a shallow New Zealand lake. Int. Revue ges. Hydrobiol. 73(2):145-170.

Mitsch, W.J., J.W. Day, Jr., J.W. Gilliam, P.M. Groffman, D.L. Hey, G.W. Randall, and N. Wang. 1999. Reducing nutrient loads, especially nitrate-nitrogen, to surface water, ground water, and the Gulf of Mexico. Topic 5 Report for the Integrated Assessment on Hypoxia in the Gulf of Mexico, NOAA Coastal Ocean Program Decision Analysis Series No. 19. 111 p.

Mjelde, M. and B.A. Faafeng. 1997. Ceratophyllum demersum hampers phytoplankton development in some small Norwegian lakes over a wide range of phosphorus concentrations and geographical latitude. Freshw. Biol. 37:355-365.

Moeller, R.E., J.M. Burkholder, and R.G. Wetzel. 1988. Significance of sedimentary phosphorus to a rooted submersed macrophyte (*Najas flexilis* (Willd.) Rostk. and Schmidt) and its algal epiphytes. Aquat. Bot. 32:261-281.

Moss, B. 1976. The effects of fertilization and fish on community structure and biomass of aquatic macrophytes and epiphytic algal populations: an ecosystem experiment. J. Ecol. 64:313-342.

Moss, B. 1990. Engineering and biological approaches to the restoration from eutrophication of shallow lakes in which aquatic plant communities are important components. Hydrobiol. 200/201:367-377.

Moss, B. 1995. The microwaterscape: a 4-dimensional view of interactions among water chemistry, phytoplankton, periphyton, macrophytes, animals, and ourselves. Water Sci. Technol. 32:105-116.

Mulligan, H.F., A. Baranowski, and R. Johnson. 1976. Nitrogen and phosphorus fertilization of aquatic vascular plants and algae in replicated ponds. I. Initial response to fertilization. Hydrobiol. 48(2):109-116.

Otsuki, A., and R.G. Wetzel. 1972. Coprecipitation of phosphate with carbonates in a marl lake. Limnol. Oceanogr. 17:763-767.

Ozimek, T., R.D. Gulati, and E. van Donk. 1990. Can macrophytes be useful in biomanipulation of lakes? The Lake Zwemlust example. Hydrobiol. 200/201:399-407.

Phillips, G.L., D. Eminson, and B. Moss. 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4:103-126.

Redfield, A.C., B.H. Ketchum, and F.A. Richards. 1963. The influence of organisms on the composition of seawater, *in* Hill, M.N. (ed.), The Sea, Vol. 2, Wiley Interscience, New York, NY, p. 26-77.

Richardson, C.J. and S.S. Qian. 1999. Long-term phosphorus assimilative capacity in freshwater wetlands: A new paradigm for sustaining ecosystem structure and function. Environ. Sci. and Technol. 33(10):1545-1551.

Royle, R.N. and R.J. King. 1991. Aquatic macrophytes in Lake Lindell, New South Wales: biomass, nitrogen and phosphorus status, and changing distribution from 1981 to 1987. Aquat. Bot. 41(4):281-298.

Sakamoto, M. 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. Arch. Hydrobiol. 62:1-28.

Sand-Jensen, K. and J. Borum. 1984. Epiphyte shading and its effect on photosynthesis and diel metabolism of *Lobelia dortmanna* during the spring bloom in a Danish lake. Aquat. Bot. 20:109-120.

Sand-Jensen, K. and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat. Bot. 41:137-175.

SAS Institute, Inc. 1996. Statistical Analysis System, Release 6.12., SAS Institute, Inc., Cary, N.C.

Scheffer, M. 1990. Multiplicity of stable states in freshwater systems. Hydrobiol. 200/201:475-486.

Scheffer, M. 1998. Ecology of shallow lakes. Chapman & Hall, London. 357 p.

Seitzinger, S.P. 1988. Denitrification in freshwater and marine coastal ecosystems: ecological and geochemical significance. Limnol. Oceanogr. 33:702-724.

Smith, G.M. 1950. The freshwater algae of the United States. McGraw-Hill, New York.

Smith, V. 2003. Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environ. Sci. Res. Int. 10:126-139.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. University of Iowa Press, Ames, IA.

Sommers, L.E., and D.W. Nelson. 1972. Determination of total phosphorus in soils: a rapid perchloric acid digestion procedure. Soil Sci. Soc. Amer. Proc. 36:902-904.

Sondergaard, M., E. Jeppesen, P. Kristensen, and O. Sortkjaer. 1990. Interactions between sediment and water in a shallow and hypertrophic lake: a study on phytoplankton collapses in Lake Sobygard, Denmark. Hydrobiol. 191:139-148.

Sondergaard, M. and B. Moss. 1998. Impact of submerged macrophytes on phytoplankton in shallow freshwater lakes, *in* Jeppesen, E., M. Sondergaard, M. Sondergaard, and K.Christoffersen, eds. The structuring role of submerged macrophytes in lakes. Springer, New York.

Stachowicz, K., M. Czernoch, and E. Dubiel. 1994. Field pond as a sink for nutrients migrating from agrocenoses to freshwaters. Aquat. Sci. 56(4):363-375.

Stelzer, R.S., and G.A. Lamberti. 2002. Ecological stoichiometry in running waters: periphyton chemical composition and snail growth. Ecology 83:1039-1051.

Thorpe, J.H. and A.P. Covich. 1991. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego.

U.S. Environmental Protection Agency (USEPA). 1979. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency, Washington, D.C. 430 p.

van Donk, E., R.D. Gulati, A. Iedema, and J.T. Meulemans. 1993. Macrophyte-related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shallow lake. Hydrobiol. 251:19-26.

van Donk, E., and W.J. van de Bund. 2002. Impact of submerged macrophytes including charophytes on phyto-and zooplankton communities: allelopathy verus other mechanisms. Aquat. Bot. 72:261-274.

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7(3):737-750.

Weisner, S.E.B., P.G. Eriksson, W. Graneli, and L. Leonardson. 1994. Influence of macrophytes on nitrate removal in wetlands. Ambio 23(6):363-366.

Wetzel, R.G. 1983. Limnology (2nd ed.). Saunders College Publishing, Philadelphia, PA.

Whiteside, M.C., J.B. Williams, and C.P. White. 1978. Seasonal abundance and pattern of Chydorid Cladocera in mud and vegetative habitats. Ecol. 59(6):1177-1188.

Williams, J.B. 1983. A study of summer mortality factors for natural populations of Chydoridae (Cladocera). Hydrobiol. 107:131-139.

Wood, R.D. 1950. Stability and zonation of Characeae. Ecol. 31(4):642-647.

Appendix 1: Experimental Error Associated with Physical, Chemical, and Biological Variables in Shallow, Vegetated, Outdoor Experimental Corrals

Abstract

In years 1999 and 2000 the U.S. Geological Survey was funded by the U.S. Environmental Protection Agency to conduct a series of experiments to evaluate the fate and effects of nutrients in simulated shallow, vegetated wetlands. These studies were conducted in outdoor experimental corral systems located at the Columbia Environmental Research Center in Columbia, MO. Results of these studies were published in a final report by Fairchild and Vradenburg (2004). Data from these studies were not normally distributed and exhibited heterogeneity of variance. Therefore, the data were statistically analyzed using non-parametric statistics. Although data means by treatment were presented, the actual experimental error rates, defined as the coefficient of variation (COV), were not reported. This study re-evaluates the experimental error rates of the control treatments from the 1999 and 2000 studies to allow a relative comparison of the variability of numerous physical, chemical, and biological variables across treatments. Results indicated that physical parameters, including pH and temperature exhibited low experimental error rates (COV range <10%). Chemical variables ranged widely in experimental error rates. For example, alkalinity, hardness, and conductivity ranged from 2 - 25% COV. Total nutrients in water (total nitrogen and total phosphorus) ranged from 0 - 55% COV. Dissolved nutrients, including ammonia, nitrate-nitrite, and soluble-reactive phosphorus, ranged from 5 - 200% COV because they frequently were low and near detection limits. Nutrient content (total nitrogen and total phosphorus) of macrophytes and sediments exhibited relatively low experimental error rates and ranged from 5 - 55% due to the inherently large, static pools of nutrients. Highest rates of experimental error were associated with biological variables such as macrophyte biomass (28 - 96% COV), periphyton biomass (20 - 110% COV), chlorophyll *a* (i.e., algal biomass; 20 - 125% COV), phytoplankton community structure (50 - 450% COV), and zooplankton community structure (30 - 200% COV). Relative rates of experimental error of physical, chemical, and biological variables varied with season. For example, error rates of physical parameters were lowest early in the season since these were driven by characteristics of the well water source. In contrast, experimental error rates of biological variables were highest early in the season when numbers were low, and species were rapidly colonizing. Highest average rates of experimental error were also associated with variables influenced by biological processes (e.g., nutrient supply, zooplankton grazing, etc.) and seasonal species succession controlled by nutrient supply and temperature constraints. An understanding of the ranges and sources of experimental error can be valuable in planning experiments where statistical power to differentiate among treatments is desired. However, increased statistical power often requires large increases in statistical replication which may have tradeoffs in terms of the number of treatment effects that may be studied. Ultimately, design of such studies must be driven by the goals and objectives of a study as constrained by the experimental error rates that occur in complex ecological test systems.

Introduction

The U.S. Environmental Protection Agency (USEPA) is the primary federal agency whose mission is to conduct research to support environmental regulation in the United States. Research sponsored by the USEPA is conducted across a vast array of experimental test systems ranging from standardized in-vivo assays, conducted at the cellular or enzyme level, to landscape level analysis reflecting broad spatial and temporal scales. Each of these test systems has its own intent and merit.

In vitro cellular assays are intended to be highly replicable (e.g., across laboratories) and repeatable (i.e., over time within a laboratory). Such assays allow rapid through-put of large numbers of samples. For example, an in-vivo cellular assay may allow one to determine the response to a given chemical stressor. However, in many cases, these tests may only reflect a relative response of a cell or tissue to one factor, with no potential homeostasis or inherent capacity to recover from an insult.

In contrast, large scale biological experiments, conducted at the landscape level, allow studies of broad temporal and spatial scales to examine environmental changes. For example, long-term ecological experiments can be conducted to determine the effects of climate change on forest productivity. Such studies, while having perhaps the ultimate environmental relevance, may take years to decades to determine a trajectory or response. Such experiments are difficult to repeat or replicate due to inherent differences across landscapes and stochastic error.

Other experimental approaches fall in the middle of the continuum of environmental realism and statistical confidence. For example, mesocosm experiments allow a researcher to study the responses of a simulated ecosystem or community to an environmental stressor or manipulation. Examples of mesocosms include experimental ponds, streams, field plots, or other physical models of natural ecosystems. Mesocosms can be cost-effective approaches for ecological studies that allow a researcher to replicate treatments and derive statistical inference regarding system responses.

The USEPA, in 1999 and 2000, funded the U.S. Geological Survey under IAG DW14938559-01 to conduct mesocosm experiments to determine the assimilative capacity of simulated wetlands for nutrients. The results of these studies were reported to the USEPA in a final report entitled "Fate and Effects of Nitrogen and Phosphorus in Shallow, Vegetated Aquatic Ecosystems" (Fairchild and Vradenburg, 2004). These studies examined the response of aquatic mesocosms to several factors: 1) nitrate loading rates; 2) nitrogen to phosphorus (N:P) ratios; 3) frequency of dosing/application; and 4) timing of dose initiation.

Analysis of the data in these studies indicated that in most cases the data were not normally distributed and exhibited non-homogeneous variance. In spite of several statistical attempts to normalize the data, the data did not fit the assumptions required for Analysis of Variance (ANOVA). Therefore, the data were analyzed using ANOVA of ranked data as suggested by numerous statistical references (Snedecor and Cochran, 1967; Green, 1979). ANOVA of ranked data allows a researcher to evaluate the differences among experimental treatments using a robust, unbiased approach. However, there are tradeoffs in some cases in terms of estimates of statistical power or the true degree of differences among treatments because the actual data are analyzed in terms of relative rank differences only.

Even though the use of ranked data in ANOVA is widely accepted and commonly done, there is still some utility in evaluating the experimental error of variables in a dataset. Calculations of experimental error can be useful in designing future experiments. For example, a researcher may wish to determine the number of replicates needed, for example, to detect a 50% difference among variables (Snedecor and Cochran, 1967; Ellersieck and LaPoint, 1995). One might also be interested in evaluating the relative experimental error among different response variables in order to choose those that respond to experimental manipulations yet vary little in time and space due to inherent and external forces.

Objective

Herein, we analyze the results of Fairchild and Vradenburg (2004) to determine the experimental error of various physical, chemical, and biological variables. This analysis is done to allow comparison of this mesocosm data to other datasets derived from other experimental test systems used in research sponsored by the USEPA. Actual comparisons to other data are not conducted in this analysis. Rather, these data are provided to researchers and quality assurance personnel of the USEPA for use in experimental design of future experiments, and as an objective dataset for use in evaluating the cost effectiveness, utility, and value of various test systems currently used in ecological research. As previously described, the choice of the test system is driven by multiple factors which range from ecological relevance to other objectives such as high statistical precision and accuracy. There are tradeoffs associated with each choice.

Methods

Data for this analysis were derived from Fairchild and Vradenburg (2004). Experimental error was defined as the coefficient of variation (COV), which is calculated as follows:

COV = STD/Mean x 100, where

STD = standard deviation, and

Mean = arithmetic average of the data

The COV is the proportion of variation of a variable reflected as the average value of the observations. Ideally, the lower the COV the fewer replicates are needed to determine a desired difference in an experiment. For this analysis, we calculated the COV using the grand mean, in addition to the mean within a given date for each variable. Note that a different set of ponds were used in year 2000 compared to year 1999 to minimize effects due to the previous year's experimental manipulations. Differences across years can be due to inherent pond differences in addition to annual differences in the timing of flooding, temperature, etc. Therefore, data were calculated separately for each year of the study. Only the control data are used for this analysis, since they represent the true variability of a metric in the absence of the experimental manipulation. All statistics were calculated using the Statistical Analysis System (SAS, 2000).

Results and Discussion

Raw data summaries (grand mean, mean, standard deviation, and COV by week) for 1999 are presented in Tables A1-A6. Raw data summaries (grand mean, mean, standard deviation, and COV by week) for 2000 are presented in Tables A7-A11. The data (years 1999 and 2000) are combined and plotted for visualization of trends in the COV over time for each variable (Figures A-1–A-43).

Water quality

The COV for algal biomass, measured as chlorophyll *a* content of water, ranged from 24 - 128% across the two years of study. Chlorophyll varied much more in year 2000 than in 1999, and appeared to cycle in intensity over 30-day intervals. Chlorophyll data fluctuated seasonally in 1999, but to a lesser extent than in year 2000 (Figure A-1). The COVs for periphyton biomass, represented as chlorophyll *a* content associated with the surface of Scrimweave^{tm} strips, ranged from 21 - 110% over the two years. Relative differences in the COV for periphyton varied within a year, but the ranges were similar across years 1999 and 2000.

The COVs for particulate nutrients (TN and TP) are presented in Figures A-3 and A-4. The COV for TN ranged from 0 -41% (Figure A-3). Higher temporal variation occurred in year 2000 (COV range 0 - 41%) than in year 1999 (COV range 13 - 27%). The COV in TP values ranged from 14 - 52 % in Year 1999 and from 11 - 54% in year 2000 (Figure A-4).

The COVs for dissolved nutrients, including ammonia (NH_3), nitrate-nitrite (NO_2NO_3), and soluble reactive phosphorus (SRP) are presented in Figures A-5–A-7. The COVs for NH_3 ranged from 5 - 200% within a year but were similar in ranges across years (Figure A-5). The COVs for NO_2NO_3 also ranged from 5 - 200% in a manner similar to NH_3 (Figure A-6). The COVs for SRP ranged from 3 - 63% and were considerably lower than those for forms of dissolved nitrogen (Figure A-7). The high COVs for dissolved nutrients were in part due to the extremely low concentrations in the control corrals which were near the limits of detection in most cases (Tables A-1 and A-7). In contrast to the control corrals, dosed corrals typically (data not shown) exhibited COV values of dissolved nutrients in the range of 10 - 20% on dosing days due to the high levels of nutrients added to those corrals. The COVs in treated corrals increased with time after nutrient addition, however, as dissolved nutrients were being rapidly being assimilated by plants.

The COVs of physical/chemical parameters of pH, alkalinity, hardness, turbidity, conductivity, and temperature ranged from 0 - 30% and were much more uniform across time and years compared to the chlorophyll/nutrient data. This decreased variation is largely due to the highly buffered water quality conditions derived from the well water source used to fill the experimental corrals. Routinely, the COVs for these variables were lowest early in the study soon after corral flooding and prior to divergence due to biological, chemical, and physical influences. The COVs for pH were similar across years and varied little across the control corrals (COV 0 - 5%) (Figure A-8). Highest COVs occurred in late June in both years when primary productivity was generally highest; during this period, carbon dioxide is highly assimilated leading to an increase in both magnitude and variation of pH. The COV values for alkalinity, hardness, and conductivity were uniform (within and across years) and generally ranged from 2 - 20% (Figures A-9, A-10, and A-11). Although absolute values of these parameters changed over time (Fairchild and Vradenburg, 2004) due to internal processes, the actual level of variation among control replicates did not change much over time. These variables are much less variable since they are driven by physical/chemical limnological conditions (e.g., precipitation, dissolution) as opposed to biological interactions (e.g., photosynthesis, grazing, etc.). The COVs for turbidity, in contrast, fluctuated both within and across years and ranged from 0 - 89%. Although turbidity values themselves were less than 10 NTU's, the COVs can vary due to many factors including algal turbidity, physical disturbance due to sampling, and in some cases bubbling of gaseous releases from sediments. Therefore, variation in turbidity is caused by numerous biological and chemical factors.

The COVs for temperature, measured at dusk (Figure A-13) and dawn (Figure A-14) varied less than any other variable in these studies and ranged from 1 - 7% across experimental replicates. Although absolute measures of temperature range considerably in the diurnal and annual cycle, the COV does not because of the large latent thermal mass of the experimental systems. Since the systems are constructed to uniform standards (depth, circumference, and volume), temperature variations are damped across replicates.

Macrophytes

The COV of macrophyte weights (macrophyte biomass) was two-fold higher in year 2000 (62 - 97%) compared to year 1999 (28-58%) (Figure A-15). Differences across years may have varied due to differences in the draw-down/flooding regime as well as inherent pond differences since different ponds were used each year. For both years, the COV of macrophyte biomass decreased over the season as macrophyte biomass increased and stand patchiness decreased.

The COVs for nutrient content of aquatic macrophytes ranged from 6 - 30 % for TN (Figure A-16) and from 6 - 53% for TP (Figure A-17). The COVs for nutrients in macrophytes decreased seasonally for both constituents. It is not clear why such a dramatic trend in nutrient content occurred over time; however, it is likely due to changes in growth status with higher variation occurring early in the season prior to stand maturity.

Sediments

The COV of TN in sediments ranged from 12 - 32% across years (Figure A-18) and did not vary appreciably across years. There was also no appreciable seasonal trend in the pattern of COV for TN in sediments. The COV for TP in sediments ranged from 8 - 18% (Figure A-19); there was likewise no seasonal trend in the COV for TP in sediments. The relative lack of variation in nutrient content of sediments was due to two factors: 1) the large, relatively static pool of nutrient stocks and 2) the use of composite sampling which reduced error.

Zooplankton

The COV for total zooplankton densities ranged from 21 - 94% (Figure A-20). Highest variation occurred in year 2000 at the beginning of the study. Such high variation is likely due to the rapid rate of flooding that occurred prior to macrophyte development. Variation in zooplankton numbers decreased over time as the systems matured in year 2000. In contrast, in year 1999 the highest variation in total zooplankton density was observed at the end of the year, most probably due to macrophyte senescence and altered physical habitat conditions. The COV for total zooplankton species

richness ranged from 4 - 23% across and within years (Figure A-21); this variation was much lower than that observed for total zooplankton numbers. The COV for Simpson's dominance ranged from 9 - 77% over the two years of study (Figure A-22).

The COV for density of cladocerans ranged from 12 - 28%, and was similar between the two years (Figure A-23). Average COV for cladoceran species richness ranged from 12 - 30% between the two years, and fluctuated little during the year (Figure A-24). Similarly, Simpson's dominance of the cladoceran community ranged from 18 -50% across and within years (Figure A-25). Average variation among the cladoceran community parameters varied less than those calculated based on the combined, total zooplankton community which is ecologically significant since the cladocerans generally contribute the greatest to overall zooplankton grazing pressure in aquatic systems (Brooks and Dodson, 1965; Wetzel, 1983).

The COV for copepod numbers ranged from 6 - 278% across years (Figure A-26). The COV for copepod species richness ranged from 23 - 141% over two years (Figure A-27). The COV for Simpson's dominance of the copepod community ranged from 9 - 130% (Figure A-28). Highest COV for copepod parameters, as previously noted, occurred on the first sampling date in year 2000 due to the early flooding effects on zooplankton variability.

The COVs for rotifer numbers were intermediate between those of cladocerans and copepods and ranged from 50 -175% within a given year but were relatively similar across years (Figure A-29). The COVs for rotifer species richness varied considerably across years and ranged from 0 - 100% in year 1999 but only ranged from 16 - 40% in year 2000 (Figure A-30). The COVs for Simpson's dominance of the rotifer community were similar to that for species richness both among and across years, with higher variation noted in year 1999 compared to year 2000 (Figure A-31).

Phytoplankton

The COVs for parameters associated with the phytoplankton community were some of the highest observed for any biological, physical, or chemical variable observed (Figures 32 - 43). For example, the COV for total phytoplankton numbers ranged from 195 - 460% in year 1999 but ranged from 80 - 100% in year 2000 (Figure A-32). The COVs for phytoplankton species richness, however, were lower than for total numbers and ranged from 10 - 88% (Figure A-33). Similar levels of variation were noted for total numbers and species richness of major groups of phytoplankton (e.g., Bacillariophyta, Chlorophyta, Cryptophyta, Cyanophyta, and Euglenophyta) (Figures 34-43). The high levels of variation in the phytoplankton community parameters across years are commonly observed due to many factors including changes in temperature, nutrient supply, and intensity of zooplankton grazing pressure (Wetzel, 1983) or simply differences due to inherent pond differences across years.

Conclusions

Coefficients of variation (COVs) ranged from 1 - 450% in outdoor aquatic experimental corrals used by studies in Fairchild and Vradenburg (2004). Lowest COVs were associated with physical parameters such as temperature and pH (range 1 - 8%). Some chemical parameters, such as alkalinity, hardness, and conductivity exhibited low COVs (range 1 - 26%) because they were not strongly influenced by biological interactions. Other parameters, such as nutrient concentrations, varied widely in COVs. Total nutrients (e.g., TN and TP) in water, macrophytes, and sediment COVs ranged from 8 - 55%. Dissolved nutrients varied much more across replicates (range 3 - 200% COV) because they were frequently low, near detection limits and were intimately tied to biological processes such as plant uptake and decomposition. Highest levels of variation occurred in some biological endpoints such as zooplankton, phytoplankton, and macrophyte community structure. The COV for chlorophyll *a*, used to estimate phytoplankton biomass, ranged from 22 - 130% due to differences in nutrient supply and zooplankton grazing pressure. The COVs for macrophyte biomass (28 - 96%) and periphyton biomass (20 - 110%) reflected spatial and temporal variation in response to shading and other factors. Although common groups of zooplankton (e.g., cladoceran species richness; COV range 12 - 30%) and phytoplankton (e.g., chlorophyte species richness; COV range 10 - 120%) exhibited moderate levels of variation, rare community groups were much more variable, with COVs commonly exceeding 200%. An understanding of these levels of variance can be used to design experiments based on anticipated statistical (e.g., precision) and ecological (e.g., relevance) objectives.

References

Brooks, J.L. and S.I. Dodson. 1965. Predation, body size and composition of the plankton. Science 150:28-35.

Ellersieck, M.R. and T.W. LaPoint. 1995. Statistical analysis. *In* Rand, G., ed., Fundamentals of aquatic toxicology: effects, environmental fate, and risk assessment. Taylor and Francis Publishers, Washington, D.C. p. 307-344.

Fairchild, J.F. and L. Vradenburg. 2004. Fate and effects of nitrogen and phosphorus in shallow, vegetated aquatic ecosystems. Final Report to the U.S. Environmental Protection Agency, IAG DW14938559-01 Sept. 30, 2004. 187 p.

Green, R.H. 1979. Sampling design and statistical methods for environmental biologists. Wiley and Sons, New York, N.Y.

Snedecor, G.W. and W.G Cochran. 1967. Statistical methods. University of Iowa Press, Ames, IA.

Statistical Analysis System Institute (SAS). 2000. SAS/STAT Guide for personal computers, Version 6, 4th ed., Volumes 1 & 2. SAS Institute Inc., Cary, N.C. 1685 p.

Wetzel, R.G. 1983. Limnology (2nd ed.). Saunders College Publishing, Philadelphia, PA.

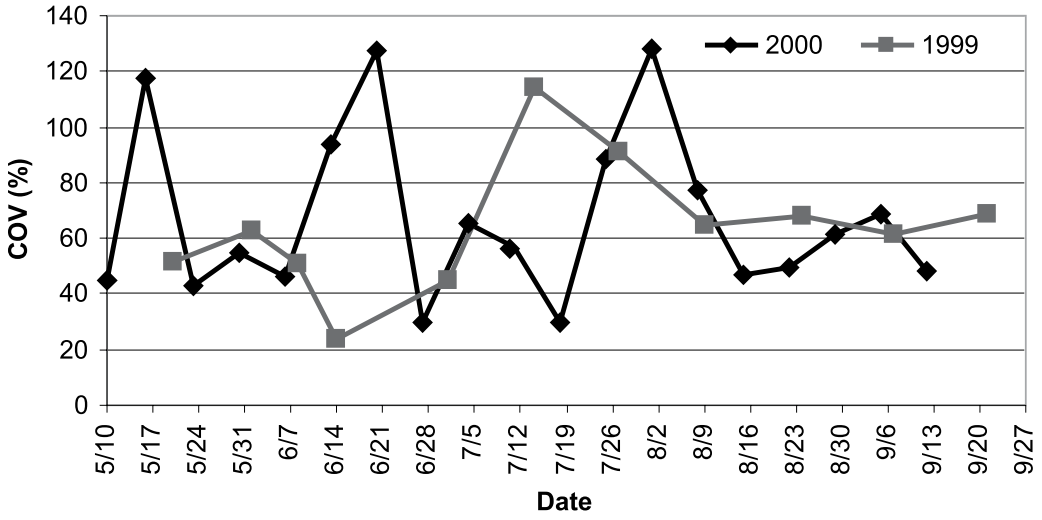


Figure A-1. *Coefficient of variation (%) of chlorophyll a in water (µg/L) over time for two-yr study.*

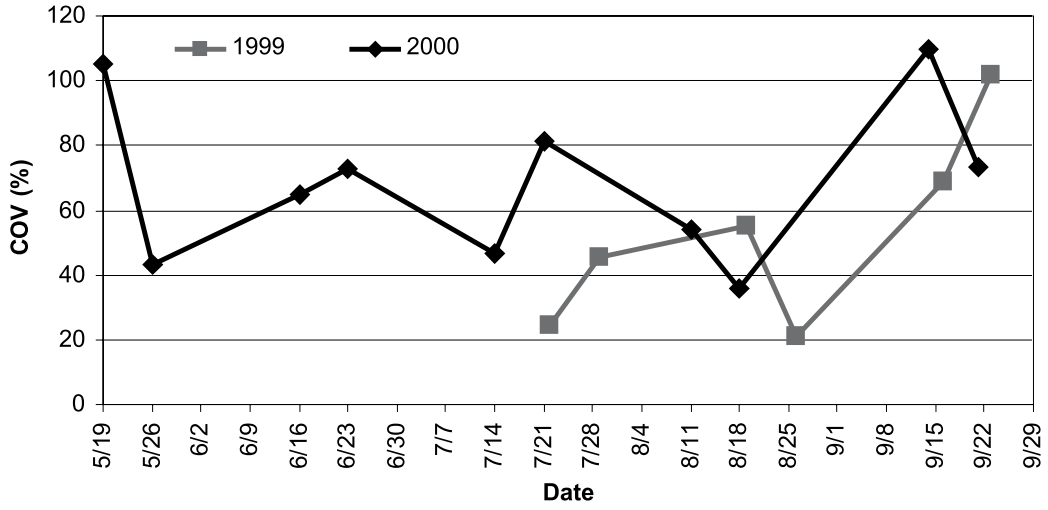


Figure A-2. *Coefficient of chlorophyll a in periphyton (µg/cm^2) over time for two-yr study.*

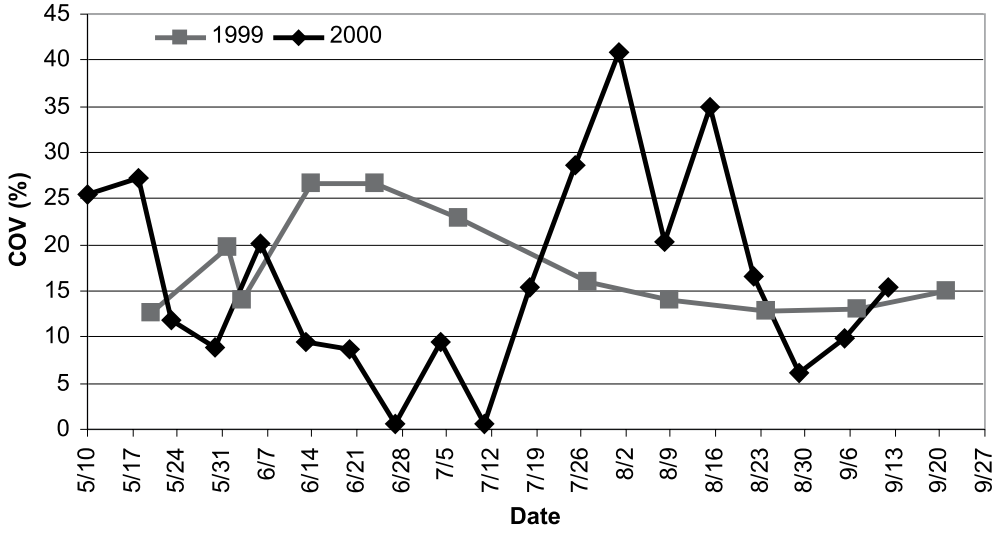


Figure A-3. *Coefficient of variation (%) of total nitrogen (mg/L) over time for two-yr study.*

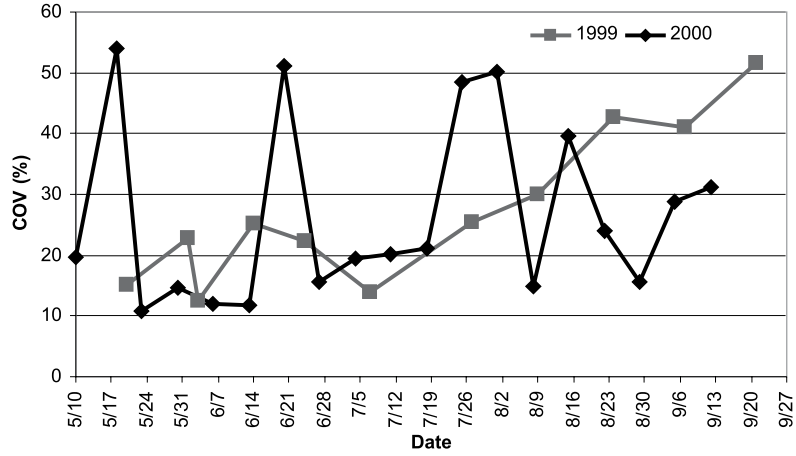


Figure A-4. *Coefficient of variation (%) of total phosphorus (µg/L) over time for two-yr study.*

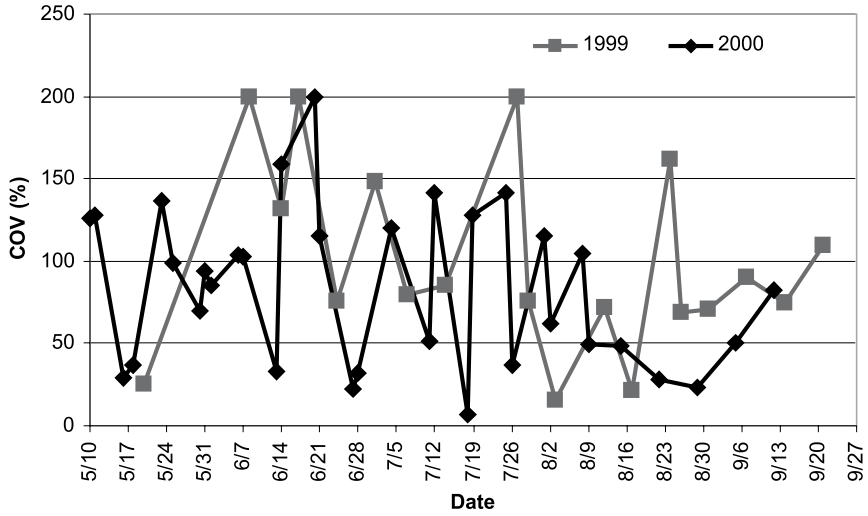


Figure A-5. *Coefficient of variation (%) of ammonia (µg/L) over time for two-yr study.*

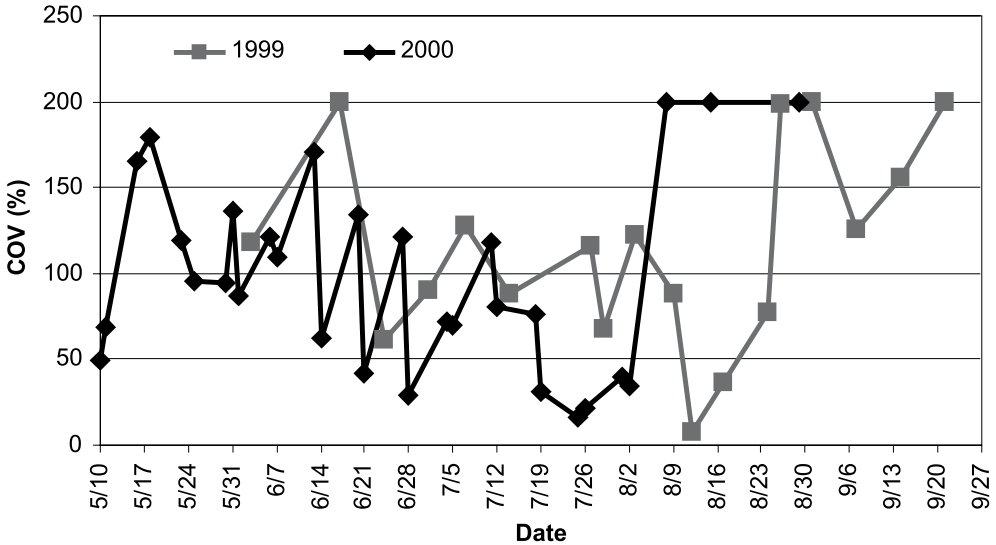


Figure A-6. *Coefficient of variation (%) of nitrate / nitrite (µg/L) over time for two-yr study.*

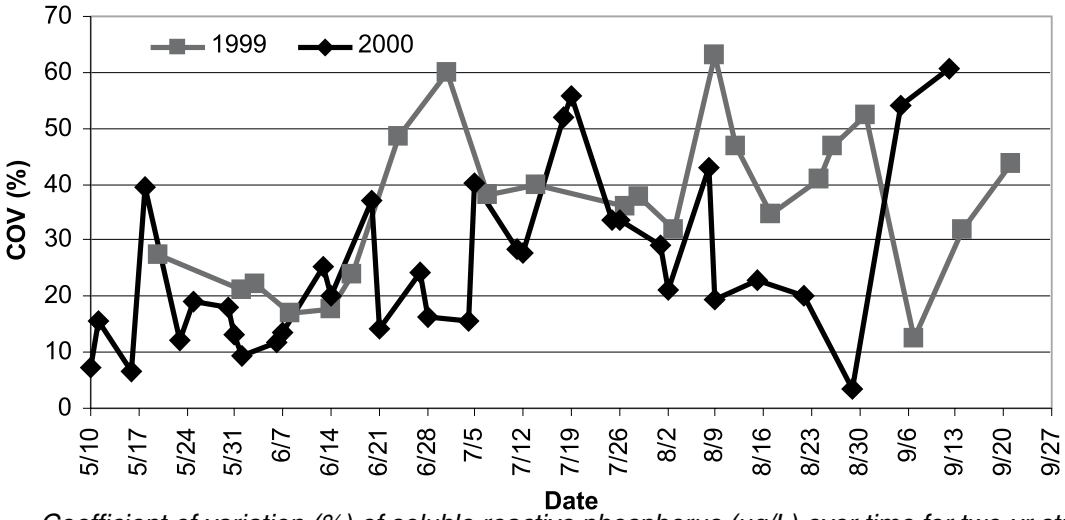


Figure A-7. *Coefficient of variation (%) of soluble reactive phosphorus (µg/L) over time for two-yr study.*

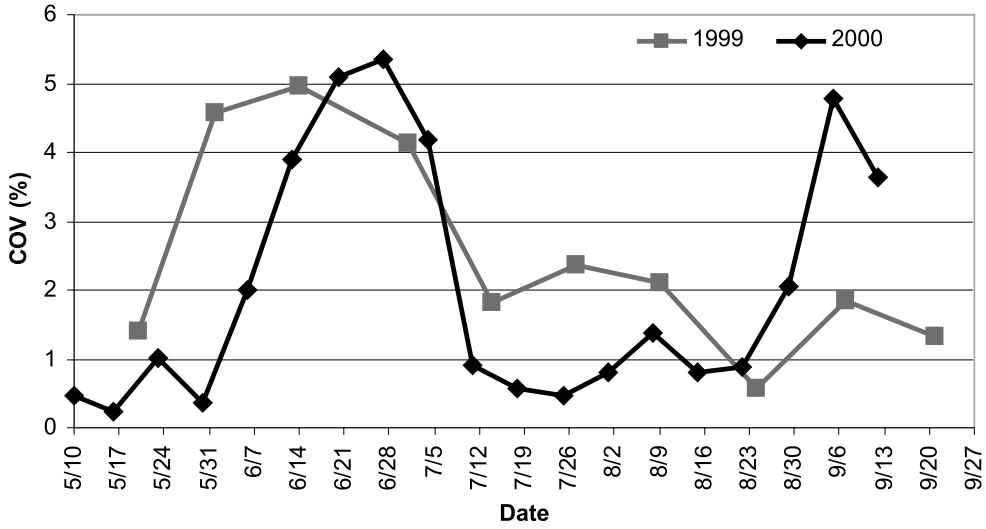


Figure A-8. *Coefficient of variation (%) of pH over time for two-yr study.*

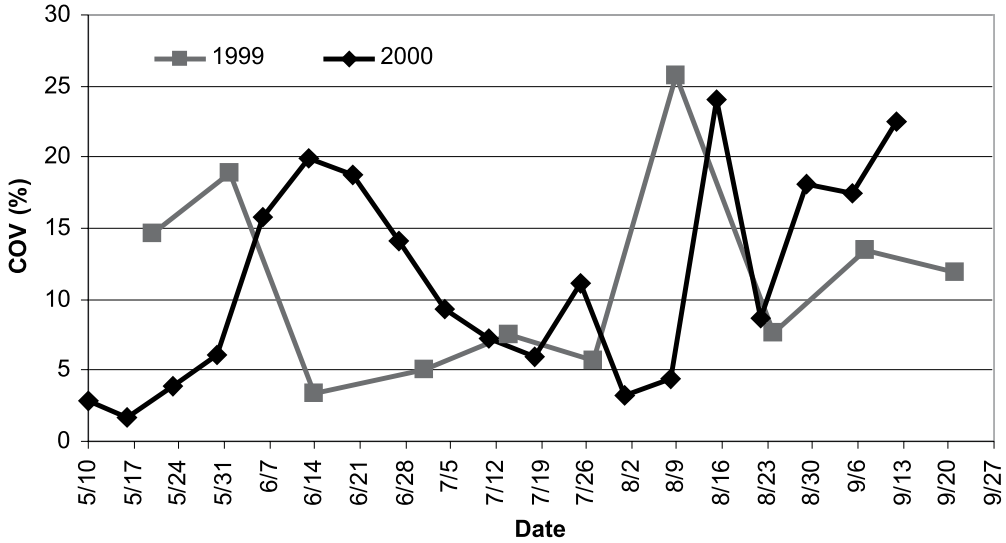


Figure A-9. *Coefficient of variation (%) of alkalinity (mg/L) over time for two-yr study.*

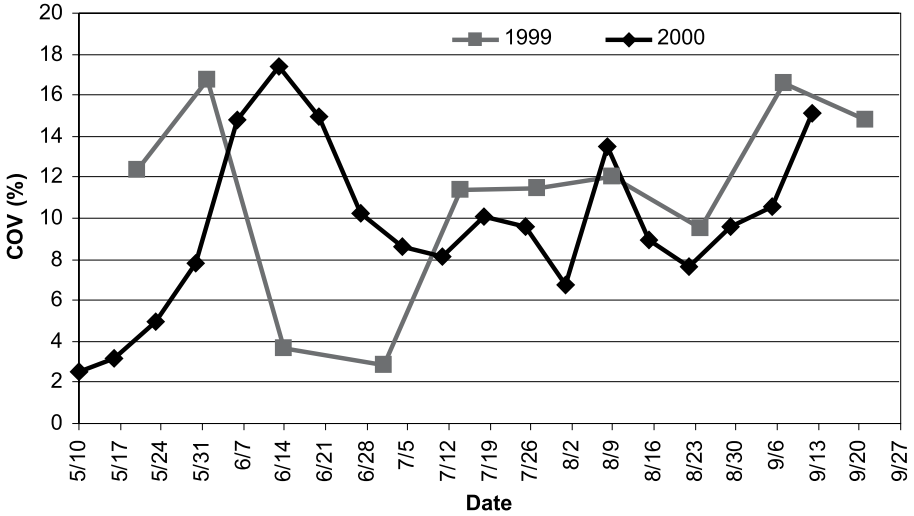


Figure A-10. *Coefficient of variation (%) of hardness (mg/L) over time for two-yr study.*

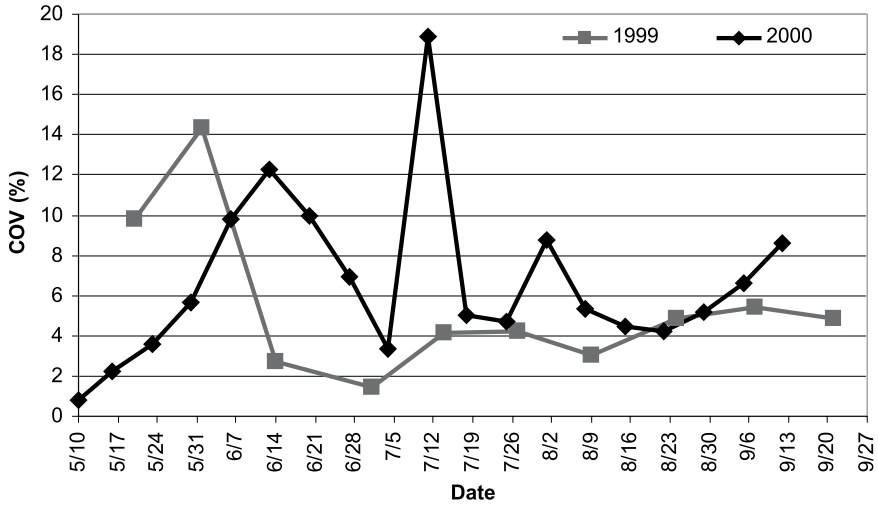


Figure A-11. *Coefficient of variation (%) of conductivity (mS/cm) over time for two-yr study.*

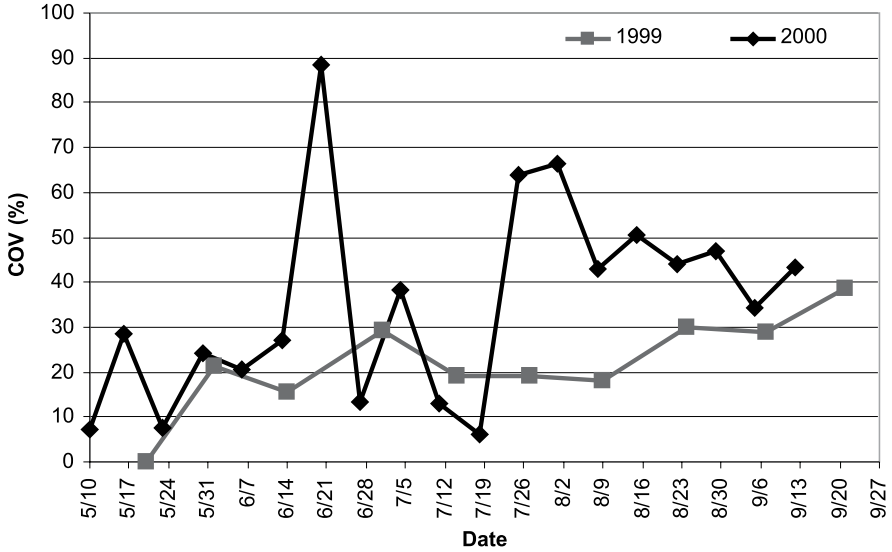


Figure A-12. *Coefficient of variation (%) of turbidity (NTU) over time for two-yr study.*

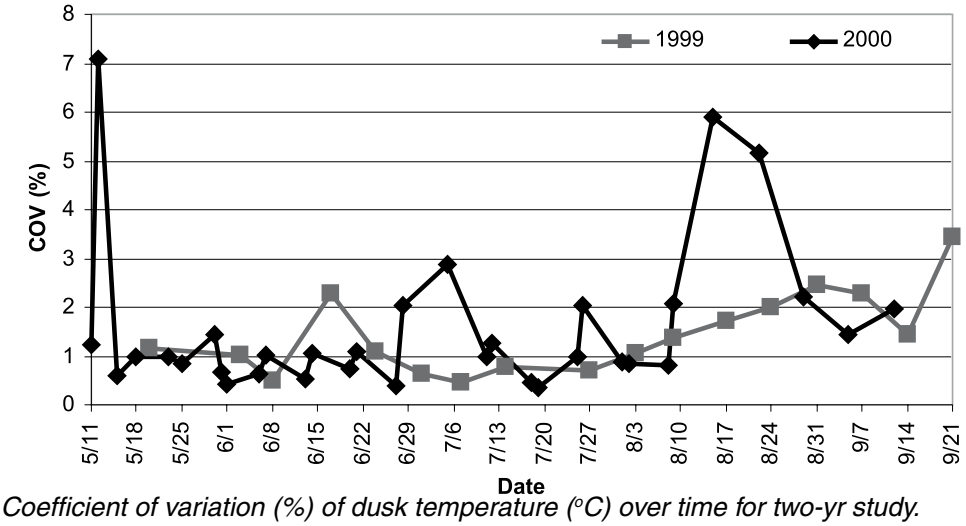


Figure A-13. *Coefficient of variation (%) of dusk temperature (°C) over time for two-yr study.*

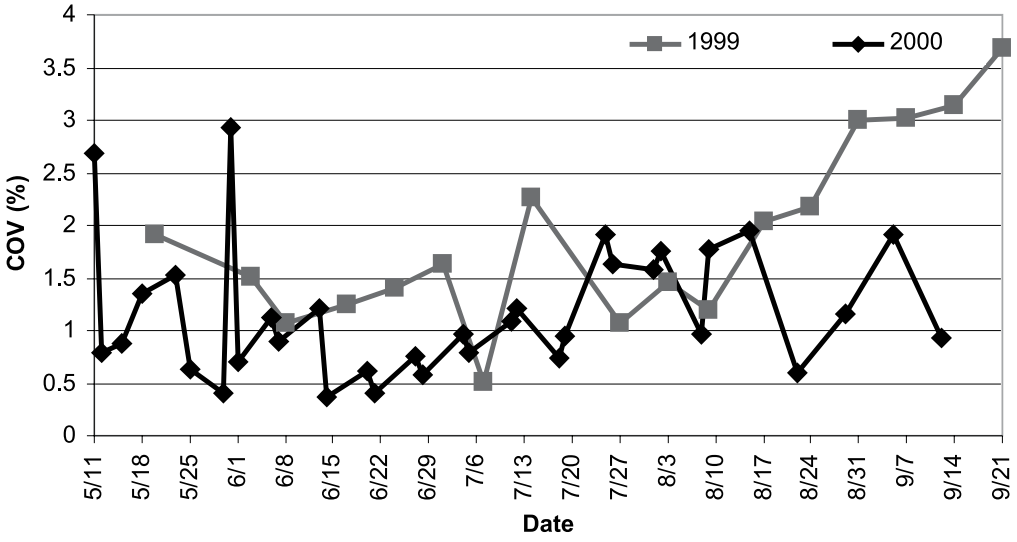


Figure A-14. *Coefficient of variation (%) of dawn temperature (°C) over time for two-yr study.*

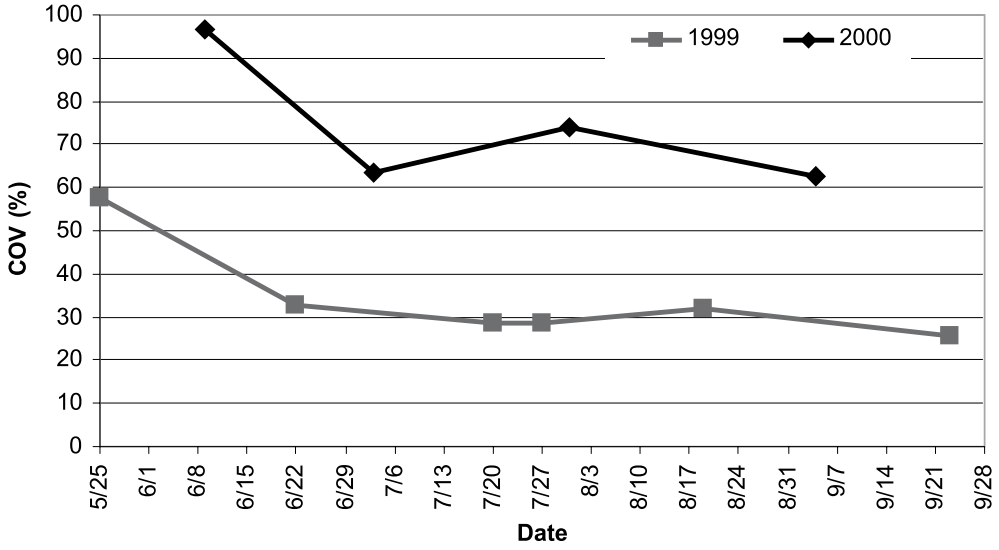


Figure A-15. *Coefficient of variation (%) of macrophytes (g dry wt.) over time for 1999 study.*

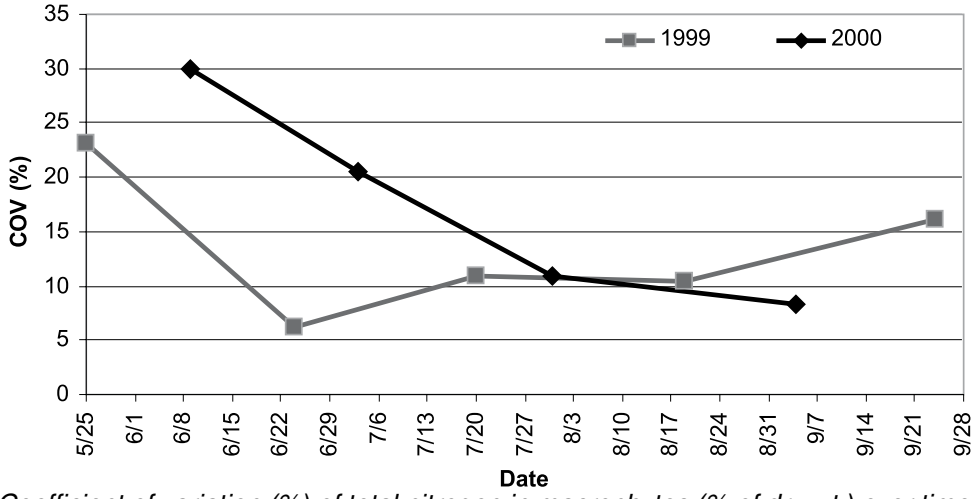


Figure A-16. *Coefficient of variation (%) of total nitrogen in macrophytes (% of dry wt.) over time for two-yr study.*

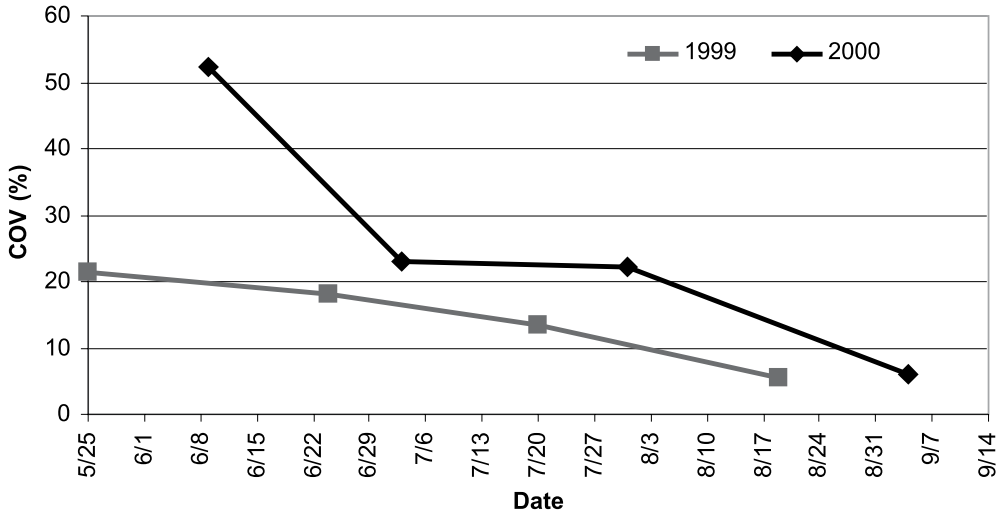


Figure A-17. *Coefficient of variation (%) of total phosphorus in macrophytes (% of dry wt.) over time for two-yr study.*

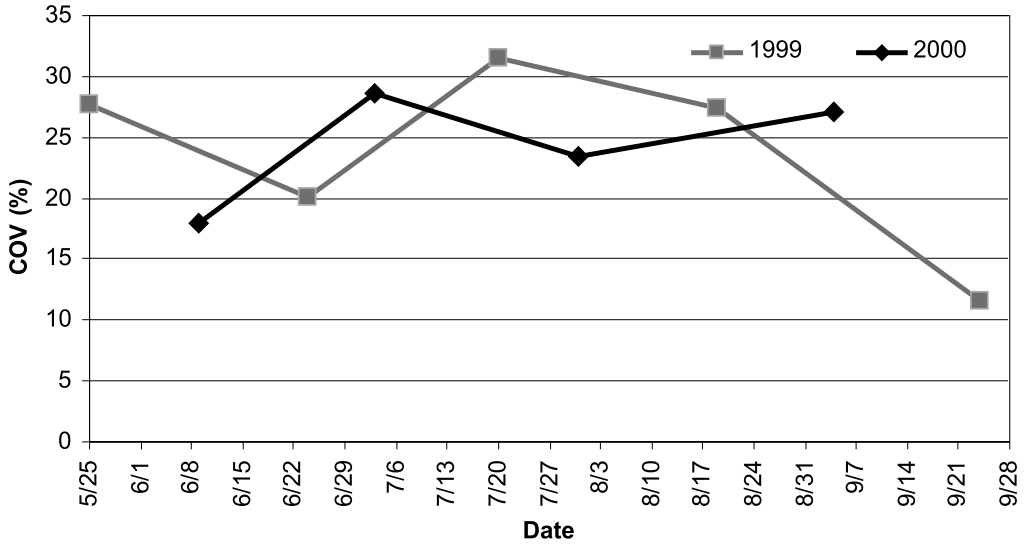


Figure A-18. *Coefficient of variation (%) of total nitrogen in sediment (g N/m^2 * 5 cm depth) over time for two-yr study.*

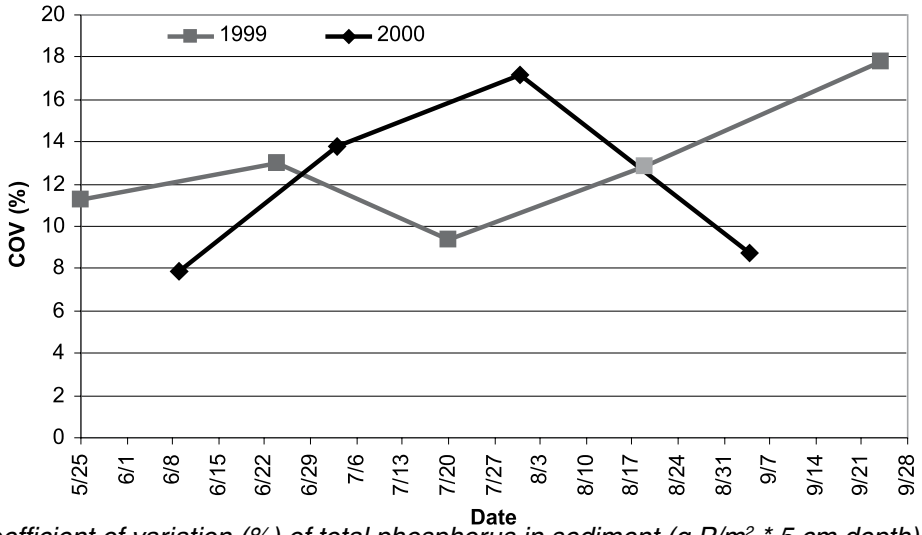


Figure A-19. *Coefficient of variation (%) of total phosphorus in sediment (g P/m² * 5 cm depth) over time for two-yr study.*

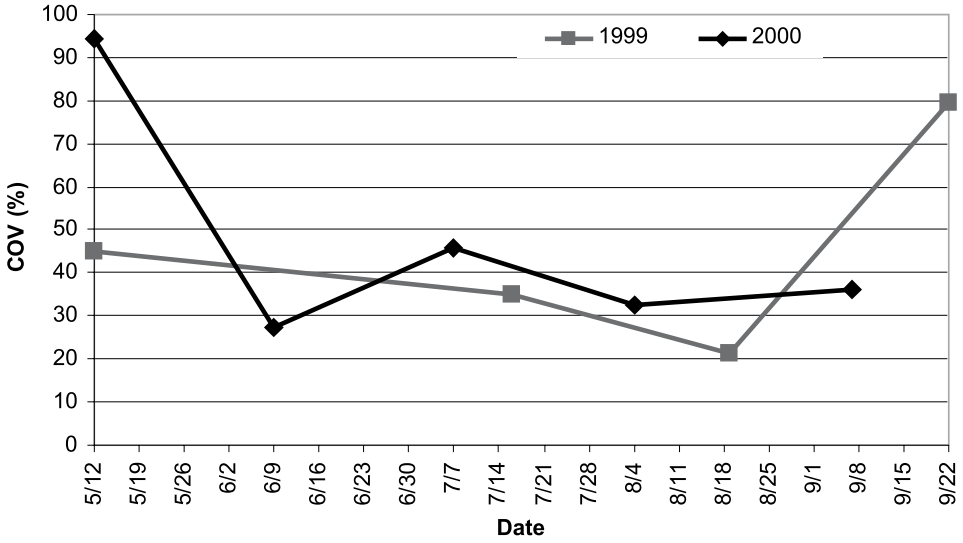


Figure A-20. *Coefficient of variation (%) of total number zooplankton (# individuals/m²) over time for two-yr study.*

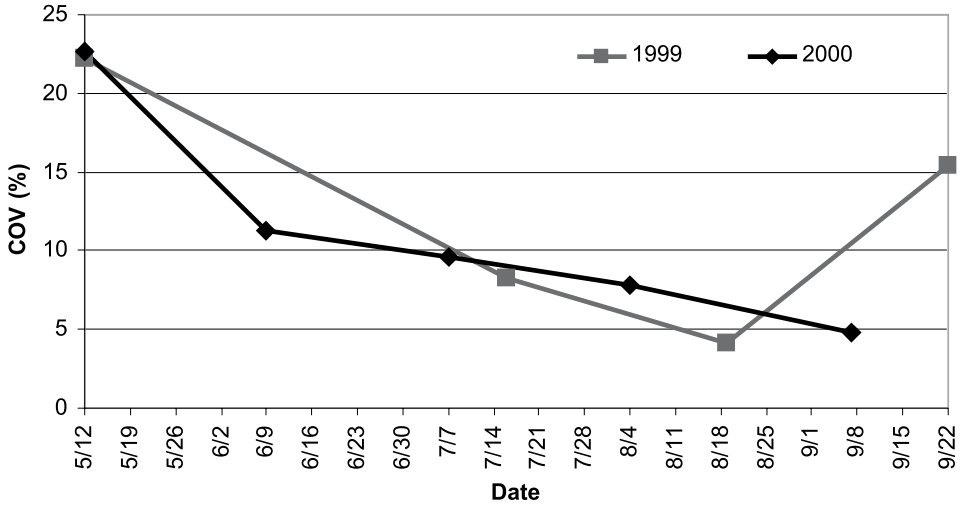


Figure A-21. *Coefficient of variation (%) of species richness total zooplankton over time for two-yr study.*

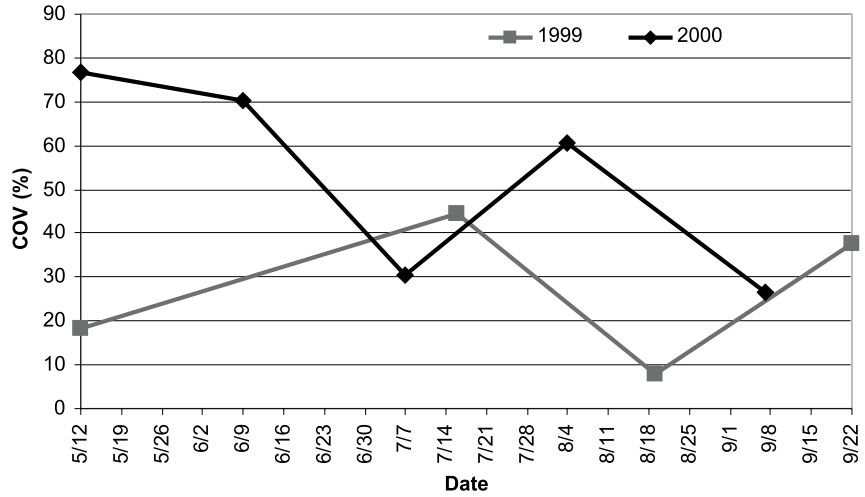


Figure A-22. *Coefficient of variation (%) of Simpson's dominance of total zooplankton over time for two-yr study.*

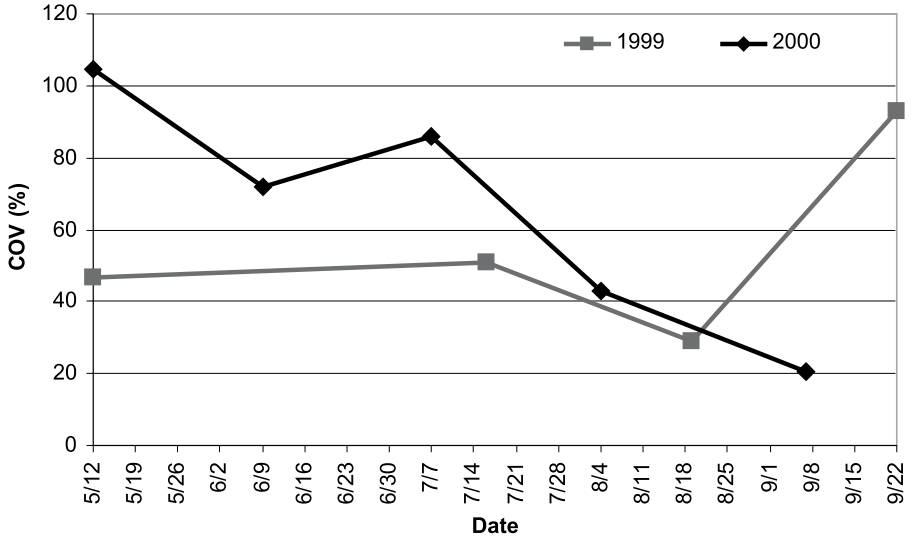


Figure A-23. *Coefficient of variation (%) of total numbers of cladoceran zooplankton (#/m^2) over time for two-yr study.*

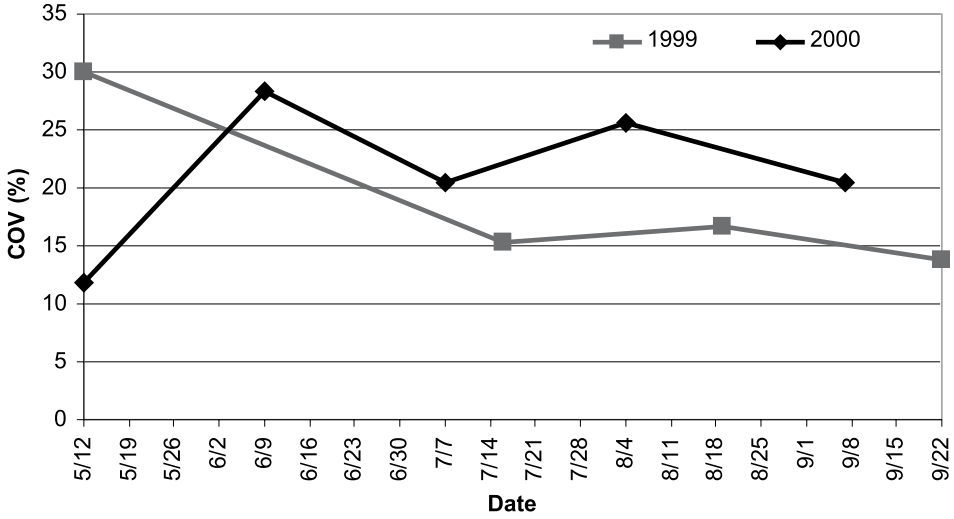


Figure A-24. *Coefficient of variation (%) of species richness of cladoceran zooplankton over time for two-yr study.*

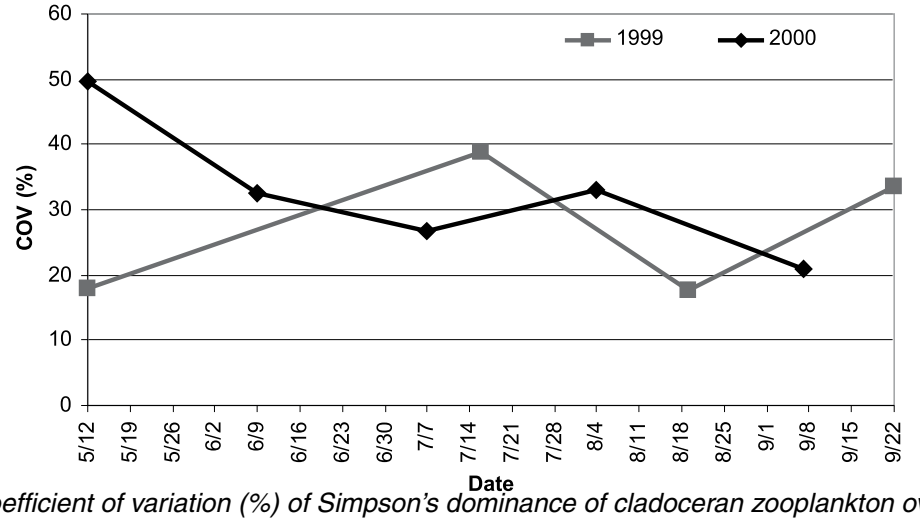


Figure A-25. *Coefficient of variation (%) of Simpson's dominance of cladoceran zooplankton over time for two-yr study.*

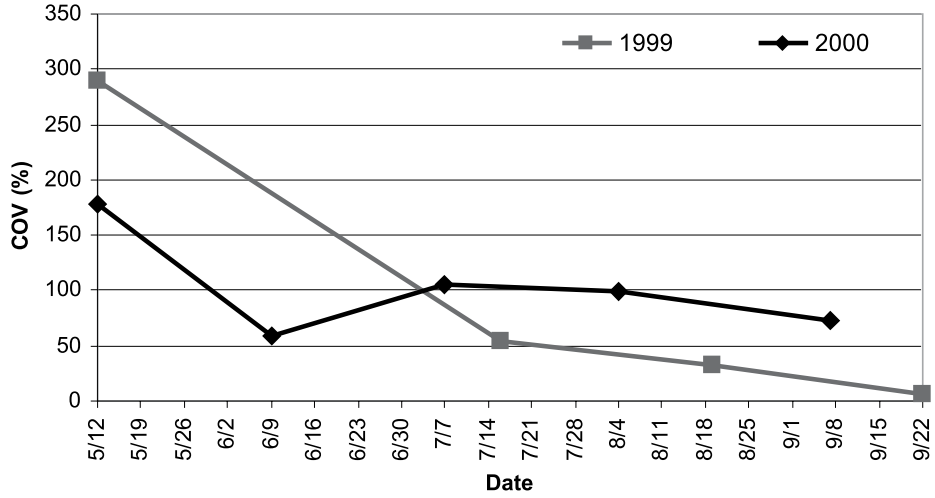


Figure A-26. *Coefficient of variation (%) of total numbers copepod zooplankton (#/m^2) over time for two-yr study.*

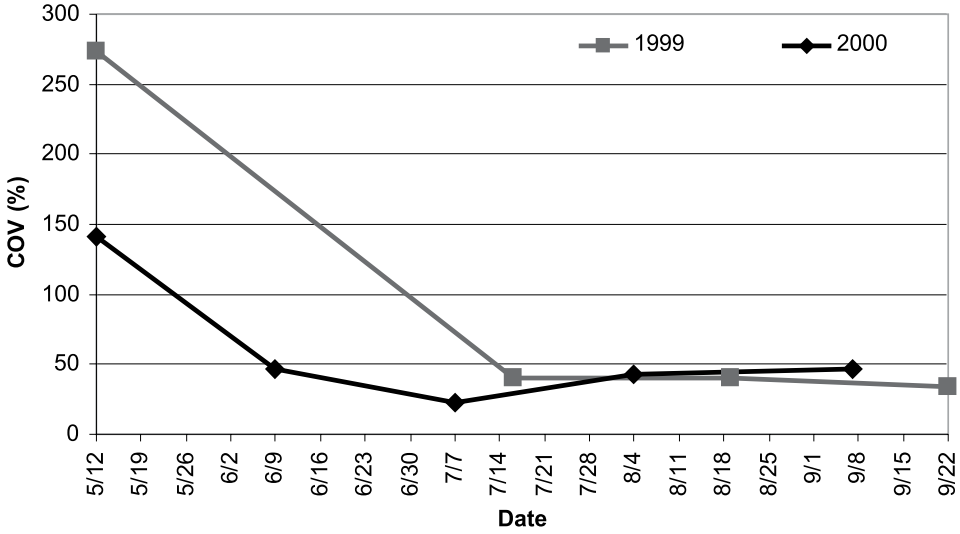


Figure A-27. *Coefficient of variation (%) of species richness of copepod zooplankton over time for two-yr study.*

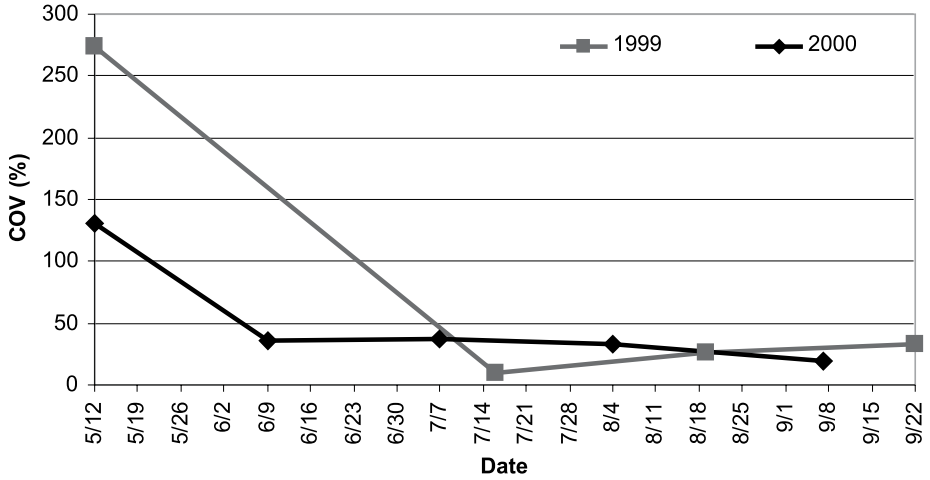


Figure A-28. *Coefficient of variation (%) of Simpson's dominance of copepod zooplankton over time for two-yr study.*

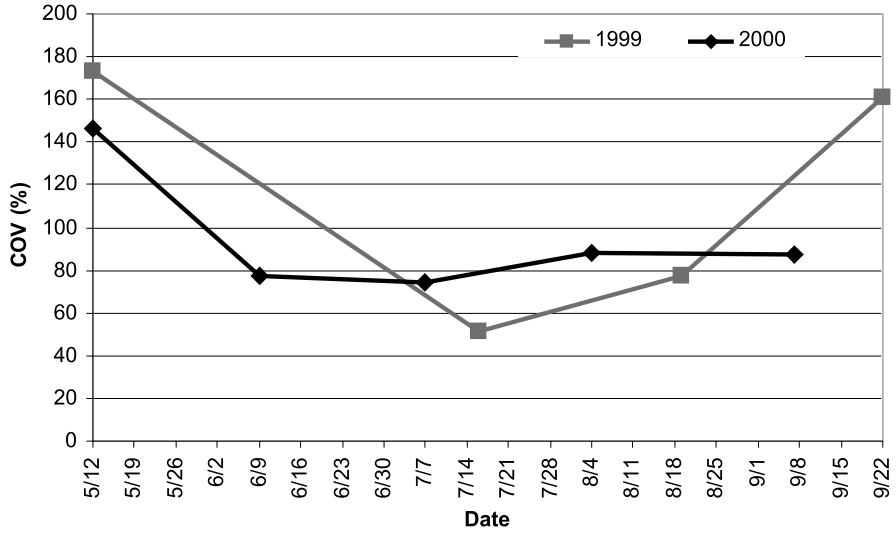


Figure A-29. *Coefficient of variation (%) of # individuals of rotifer zooplankton (#/m^2) over time for two-yr study.*

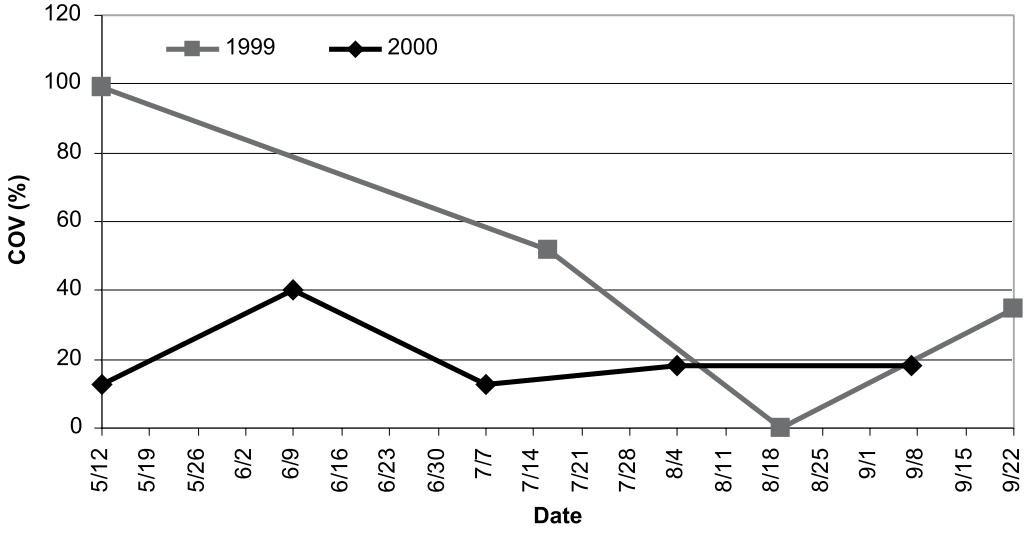


Figure A-30. *Coefficient of variation (%) of species richness of rotifer zooplankton over time for two-yr study.*

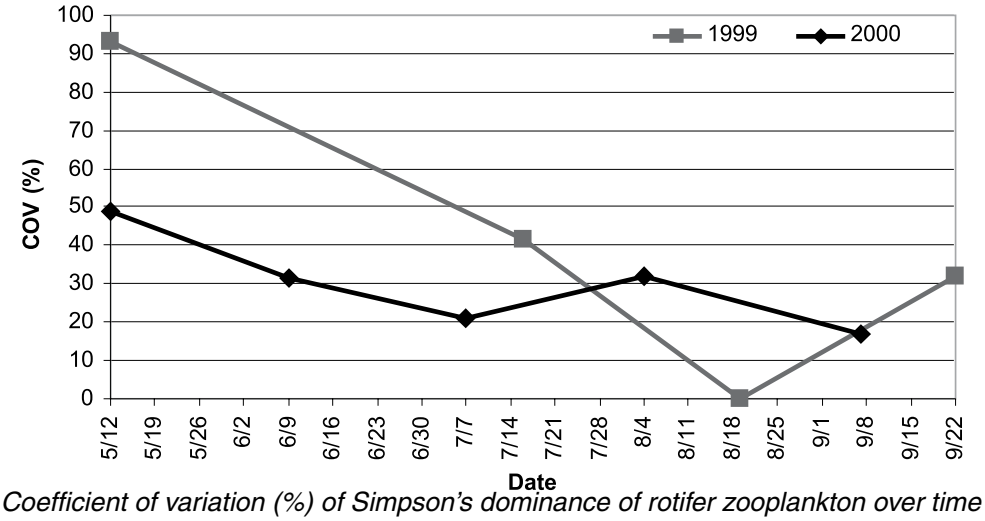


Figure A-31. *Coefficient of variation (%) of Simpson's dominance of rotifer zooplankton over time for two-yr study.*

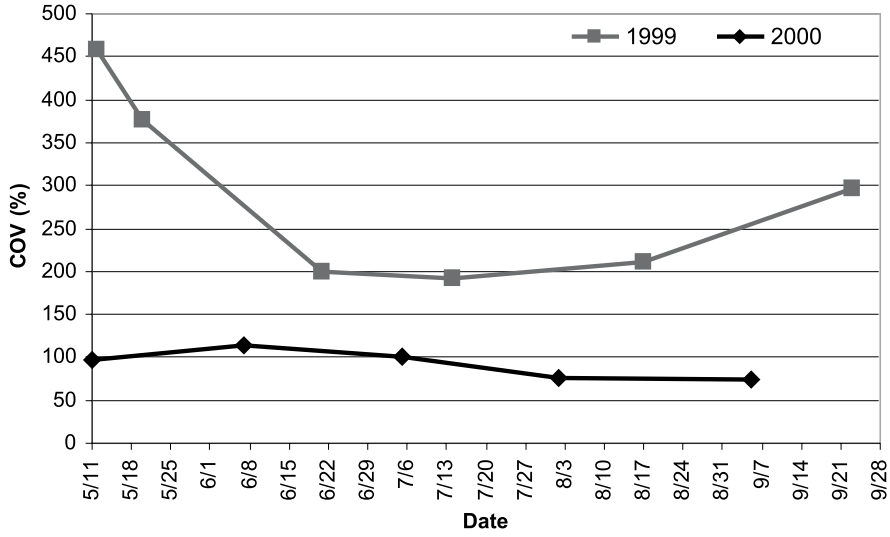


Figure A-32. *Coefficient of variation (%) of total numbers phytoplankton (# cells/L) over time for two-yr study.*

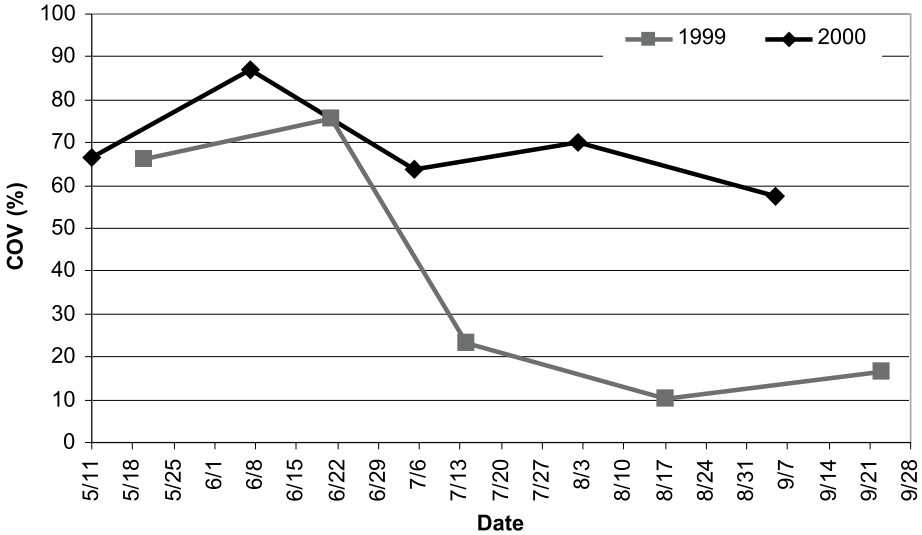


Figure A-33. *Coefficient of variation (%) of species richness of phytoplankton (# species/ L) over time for two-yr study.*

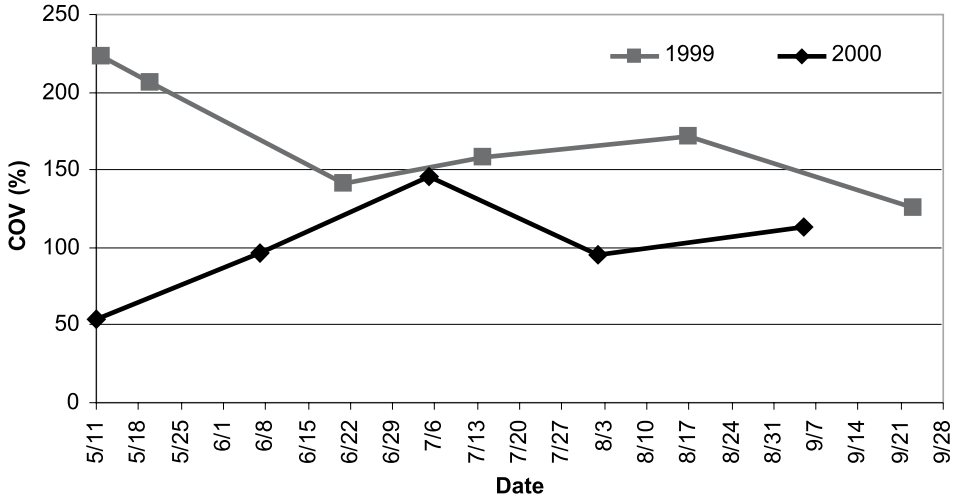


Figure A-34. *Coefficient of variation (%) of total numbers bacillariophyta (# cells /L) over time for two-yr study.*

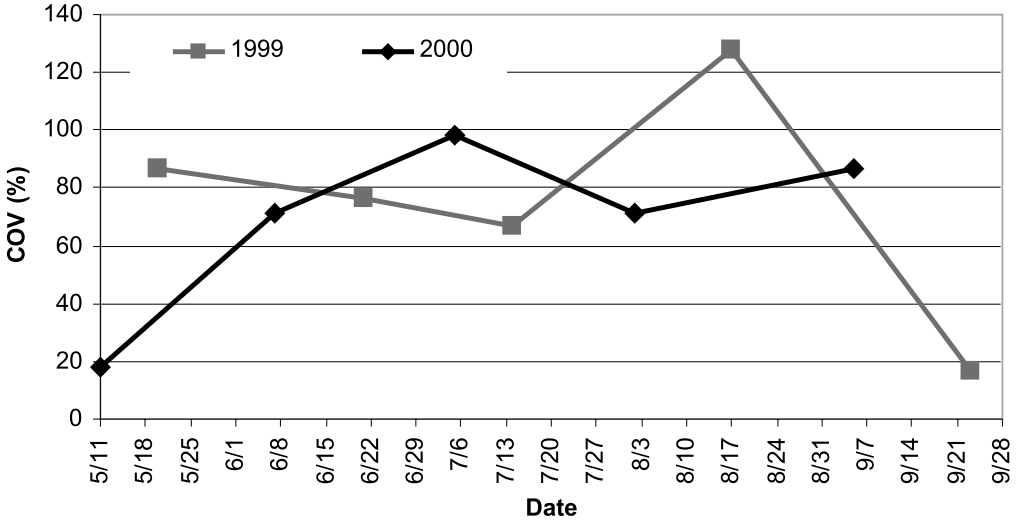


Figure A-35. *Coefficient of variation (%) of species richness bacillariophyta (# species/L) over time for two-yr study.*

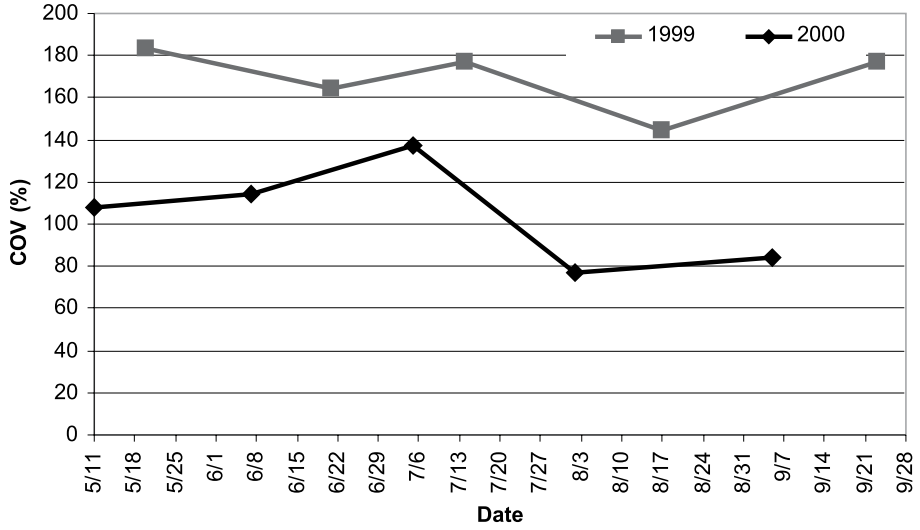


Figure A-36. *Coefficient of variation (%) of total numbers chlorophyta (# cells/L) over time for two-yr study.*

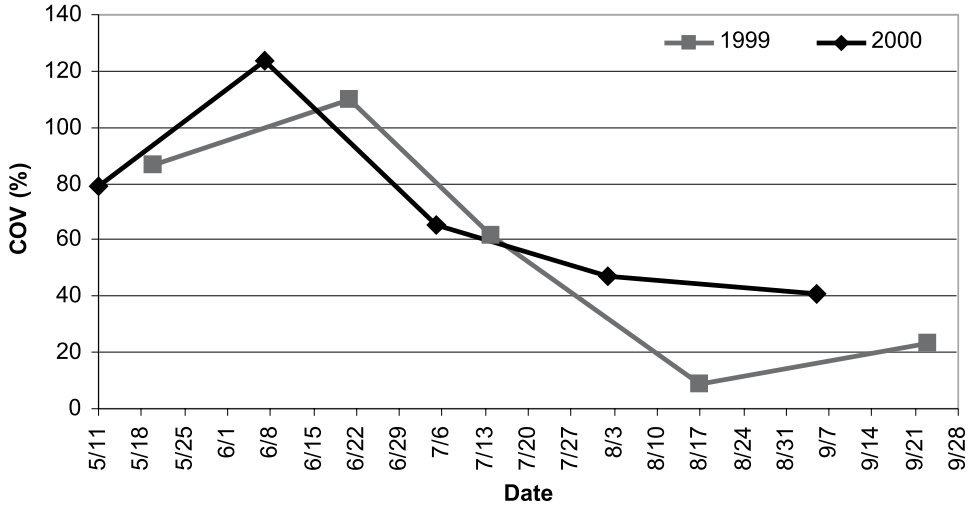


Figure A-37. *Coefficient of variation (%) of species richness of chlorophyta (# species/L) over time for two-yr study.*

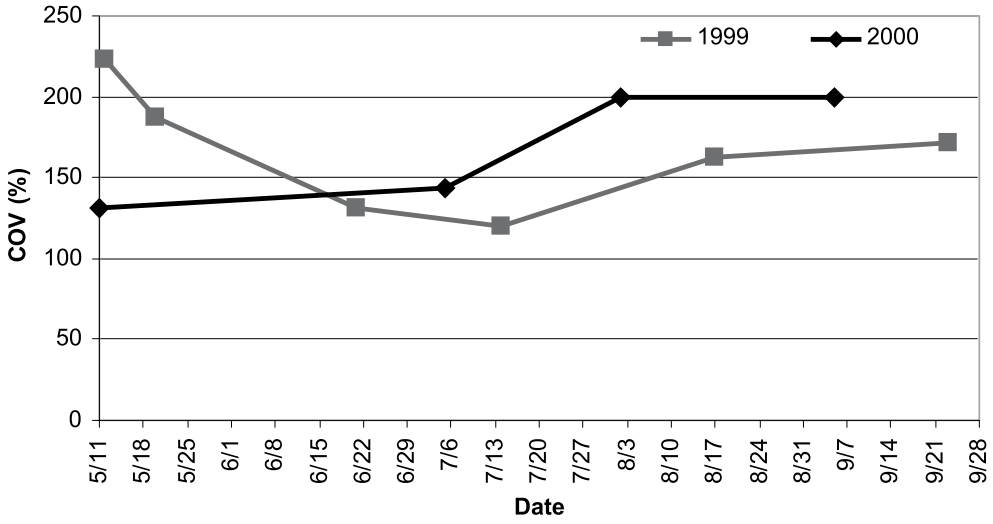


Figure A-38. *Coefficient of variation (%) of total numbers of cryptophyta (# cells/L) over time for two-yr study.*

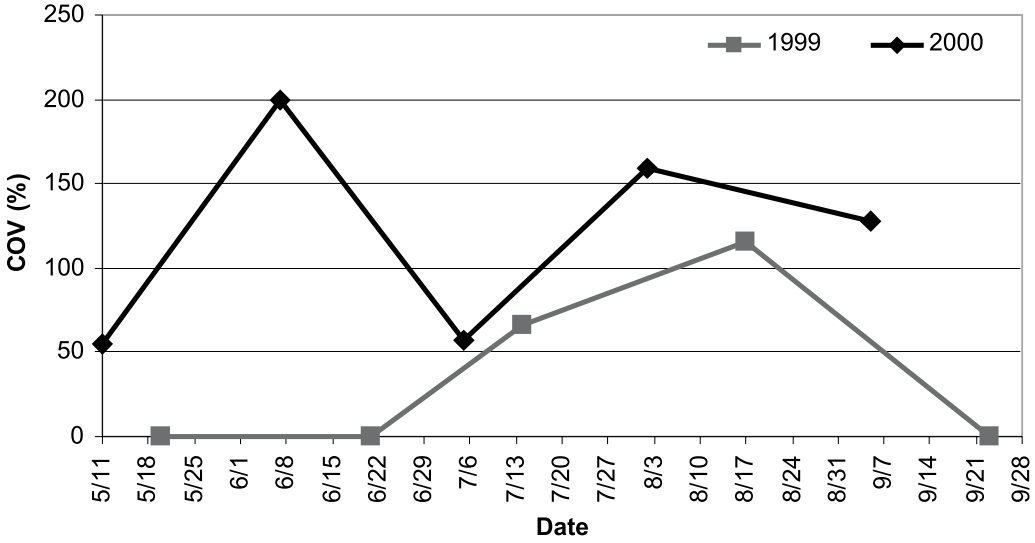


Figure A-39. *Coefficient of variation (%) of species richness cryptophyta (# species/L) over time for two-yr study.*

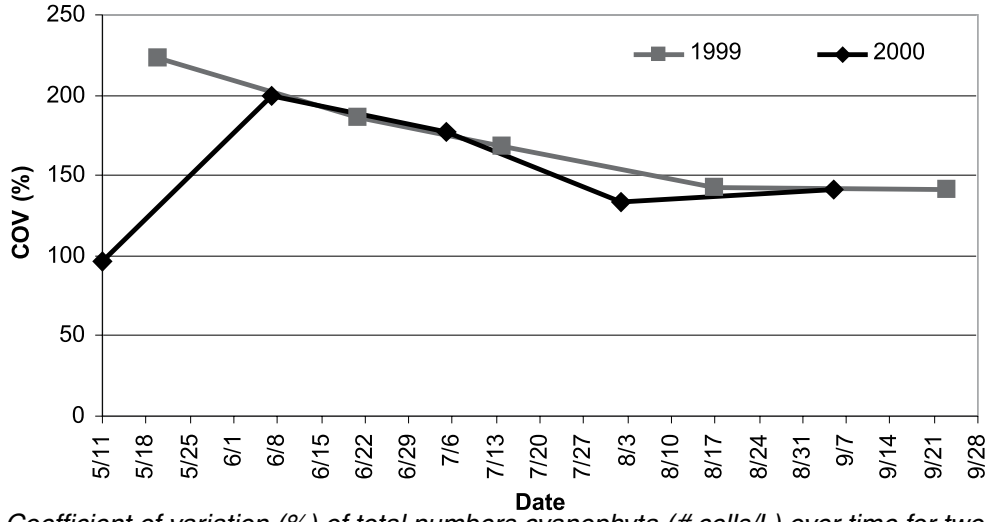


Figure A-40. *Coefficient of variation (%) of total numbers cyanophyta (# cells/L) over time for two-yr study.*

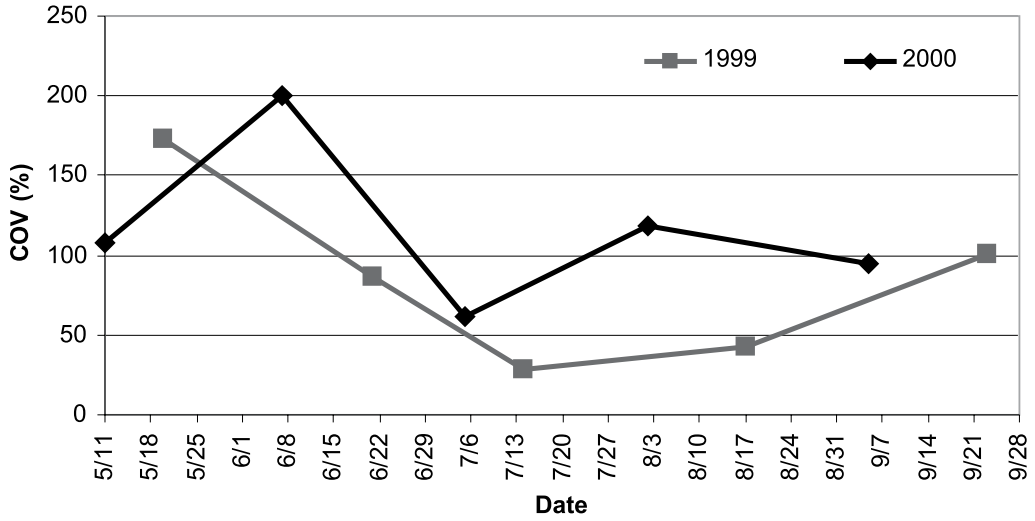


Figure A-41. *Coefficient of variation (%) of species richness cyanophyta (# species/L) over time for 1999 study.*

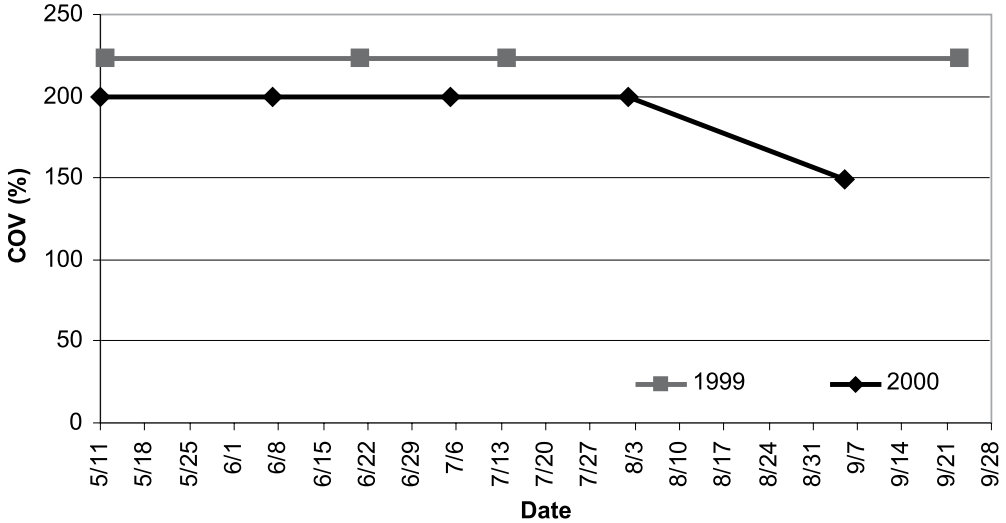


Figure A-42. *Coefficient of variation (%) of total numbers euglenophyta (# cells/L) over time for two-yr study.*

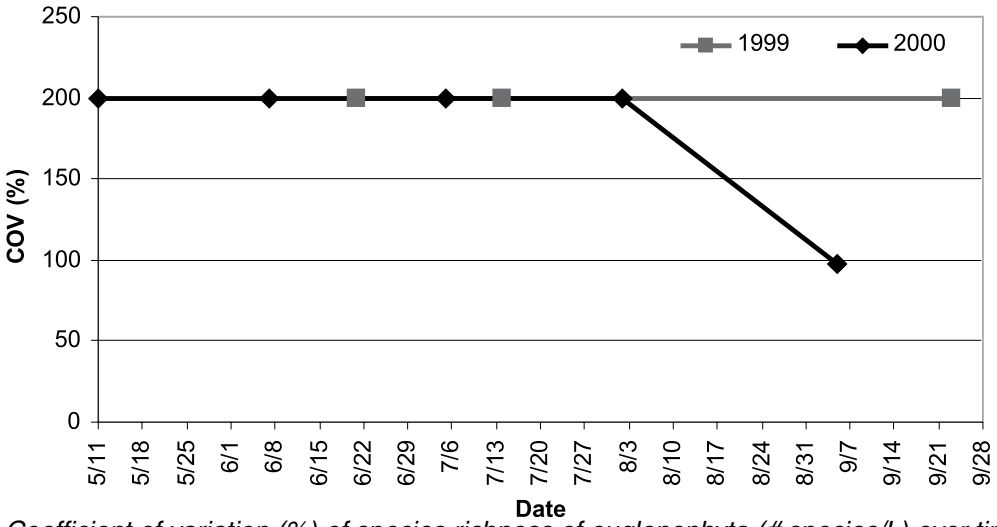


Figure A-43. *Coefficient of variation (%) of species richness of euglenophyta (# species/L) over time for two-yr study.*

Table A-1. Experimental Error Statistics for Nutrient Data from 1999 Study

Variable	Grand Mean	Mean By Date[1]				
		5/20/1999	6/1/1999	6/3/1999	6/8/1999	6/14/1999
Chlorophyll in water (µg/L)	8.57 (44) [14.91,174]	0.75 (4) [0.39,51]	0.66 (4) [0.41,63]	-[2]	0.74 (4) [0.38,51]	5.30 (4) [1.28,24]
Chlorophyll in periphyton(µg/cm^2)	0.04 (22) [0.03,83]	-[2]	-[2]	-[2]	-[2]	-[2]
TN (mg/L)	0.87 (44) [0.63,72]	0.33 (4) [0.04,13]	0.44 (4) [0.09,20]	0.43 (4) [0.06,14]	-[2]	0.46 (4) [0.12,27]
TP (µg/L)	48.7 (44) [52.93,109]	17.68 (4) [2.68,15]	17.13 (4) [3.90,23]	16.23 (4) [2.01,12]	-[2]	20.98 (4) [5.28,25]
NH_3 (µg/L)	0.01 (88) [0.01,125]	0.03 (4) [0.01,25]	0 (4) [0,0]	0 (4) [0,0]	0 (4) [0,200]	0.01 (4) [0.01,1327]
SRP (µg/L)	5.98 (88) [4.66,78]	1.38 (4) [0.38,27]	2.13 (4) [0.45,21]	2.03 (4) [0.45,22]	4.65 (4) [0.79,17]	3.1 (4) [0.55,18]
NO_2/NO_3 (µg/L)	0.06 (88) [0.42,672]	0 (4) [0,0]	0 (4) [0,0]	0 (4) [0,118]	0 (4) [0,0]	0 (4) [0,0]

Variable	Mean By Date[1]					
	6/17/1999	6/24/1999	7/1/1999	7/7/1999	7/14/1999	7/27/1999
Chlorophyll in water (µg/L)	-[2]	-[2]	2.07 (4) [0.93,45]	-[2]	4.09 (4) [4.68,114]	4.46 (4) [4.07,91]
Chlorophyll in periphyton (µg/cm^2)	-[2]	-[2]	-[2]	-[2]	-[2]	0.01 (3) [0,24]
TN (mg/L)	-[2]	-[2]	0.46 (4) [4.20,27]	-[2]	0.67 (4) [0.15,23]	0.78 (4) [0.12,16]
TP (µg/L)	-[2]	-[2]	18.72 (4) [4.20,22]	-[2]	26.03 (4) [3.60,14]	34.85 (4) [8.84,25]
NH_3 (µg/L)	0 (4) [0.01,200]	0.01 (4) [0.01,76]	0.01 (4) [0.01,148]	0.01 (4) [0.01,80]	0.01 (4) [0.01,85]	0 (4) [0,200]
SRP (µg/L)	2.3 (4) [0.55,24]	1.35 (4) [0.66,49]	0.98 (4) [0.59,60]	4.03 (4) [1.54,38]	3.85 (4) [1.53,40]	4.33 (4) [1.56,36]
NO_2/NO_3 (µg/L)	0 (4) [0,200]	0 (4) [0,61]	0.01 (4) [0,90]	0 (4) [0,128]	0 (4) [0,88]	0 (4) [0,115]

[1] Mean, (n); [STD,COV]
[2] Denotes no sample taken.

Table A-1. Experimental Error Statistics for Nutrient Data from 1999 Study (Continued)

Variable	Mean By **Date**[1]					
	7/29/1999	**8/3/1999**	**8/9/1999**	**8/12/1999**	**8/17/1999**	**8/24/1999**
Chlorophyll in water (µg/L)	-[2]	-[2]	5.47 (4) [3.53,65]	-[2]	-[2]	4.17 (4) [2.84,68]
Chlorophyll in periphyton ($\mu g/cm^2$)	0.03 (3) [0.01,45]	-[2]	-[2]	-[2]	0.03 (4) [0.01,55]	0.07 (4) [0.02,21]
TN (mg/L)	-[2]	-[2]	0.84 (4) [0.12,14]	-[2]	-[2]	1.05 (4) [0.14,13]
TP (µg/L)	-[2]	-[2]	49.33 (4) [14.82,30]	-[2]	-[2]	50.65 (4) [21.60,43]
NH_3 (µg/L)	0.01 (4) [0,76]	0 (4) [0,15]	0 (4) [0,0]	0 (4) [0,72]	0.02 (4) [0,21]	0.01 (4) [0.01,162]
SRP (µg/L)	9.08 (4) [3.42,38]	11.7 (4) [3.72,32]	8.05 (4) [5.08,63]	9.7 (4) [4.55,47]	10.3 (4) [3.57,35]	7.08 (4) [2.89,41]
NO_2/NO_3 (µg/L)	0 (4) [0,68]	0 (4) [0,122]	0.01 (4) [0,88]	0.01 (4) [0,8]	0 (4) [0,36]	0 (4) [0,77]

Variable	**Mean By Date1**				
	8/26/1999	**8/31/1999**	**9/7/1999**	**9/14/1999**	**9/21/1999**
Chlorophyll in water (µg/L)	-[2]	-[2]	40.88 (4) [25.04,61]	-[2]	25.64 (4) [17.64,69]
Chlorophyll in periphyton ($\mu g/cm^2$)	-[2]	-[2]	-[2]	0.03 (4) [0.02,69]	0.05 (4) [0.06,102]
TN (mg/L)	-[2]	-[2]	2.05 (4) [0.26,13]	-[2]	2.12 (4) [0.32,15]
TP (µg/L)	-[2]	-[2]	140 (4) [57.39,41]	-[2]	144 (4) [74.55,52]
NH_3 (µg/L)	0.01 (4) [0.01,69]	0.01 (4) [0.01,70]	0.02 (4) [0.02,90]	0 (4) [0,75]	0 (4) [0,110]
SRP (µg/L)	7.55 (4) [3.54,49]	6.53 (4) [3.40,52]	5.6 (4) [0.71,13]	10 (4) [3.19,32]	15.98 (4) [6.99,44]
NO_2/NO_3 (µg/L)	0.90 (4) [1.78,198]	0.41 (4) [0.81,200]	0.01 (4) [0.01,125]	0 (4) [0,155]	0 (4) [0,200]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-2. Experimental Error Statistics for Water Quality Data from 1999 Study

Variable	Grand Mean	Mean By **Date**[1]				
		5/20/1999	**6/1/1999**	**6/14/1999**	**7/1/1999**	**7/14/1999**
pH	9.35 (40) [0.48,5]	8.43 (4) [0.12,1]	8.87 (4) [0.41,4]	9.15 (4) [0.45,5]	9.64 (4) [0.40,4]	9.77 (4) [0.18,2]
Alkalinity (mg/L)	119.78 (40) [23.08,19]	155 (4) [22.72,15]	131 (4) [24.79,19]	108 (4) [3.65,3]	101 (4) [5.03,5]	101.5 (4) [7.55,7]
Hardness (mg/L)	114.03 (39) [33.05,29]	186.25 (4) [22.95,12]	154.5 (4) [25.89,17]	112.5 (4) [4.12,4]	100 (4) [2.83,3]	97.5 (4) [11.12,11]
Turbidity (NTU)	2.97 (40) [2.31,78]	2.00 (4) [0,0]	1.68 (4) [0.36,21]	3.25 (4) [0.50,15]	1.65 (4) [0.48,29]	1.73 (4) [0.33,19]
Conductivity (mS/cm)	337.9 (40) [52.87,16]	449.25 (4) [43.92,10]	379.75 (4) [54.41,14]	322.75 (4) [8.77,3]	297 (4) [4.32,1]	294 (4) [12.25,4]

Variable	**Mean By Date**[1]				
	7/27/1999	**8/9/1999**	**8/24/1999**	**9/7/1999**	**9/21/1999**
pH	9.76 (4) [0.23,2]	9.70 (4) [0.20,2]	9.57 (4) [0.05,1]	9.38 (4) [0.17,2]	9.21 (4) [0.12,1]
Alkalinity (mg/L)	104 (4) [5.89,6]	128.25 (4) [32.99,26]	112.5 (4) [8.54,8]	116.5 (4) [15.61,13]	140 (4) [16.73,12]
Hardness (mg/L)	93 (4) [10.65,11]	103.5 (4) [12.48,12]	93 (4) [8.87,10]	94 (4) [15.58,17]	103.3 (3) [15.28,15]
Turbidity (NTU)	1.73 (4) [0.33,19]	1.83 (4) [0.33,18]	1.93 (4) [0.57,30]	7.88 (4) [2.29,29]	6.00 (4) [2.31,38]
Conductivity (mS/cm)	296 (4) [12.57,4]	310 (4) [9.42,3]	314.25 (4) [15.33,5]	340 (4) [18.51,5]	376 (4) [18.35,5]

[1]Mean, (n); [STD,COV]

Table A-3. Experimental Error Statistics for Diurnal Temperature Data from 1999 Study

Variable	Grand Mean	Mean By **Date**[1]					
		5/20/1999	**6/3/1999**	**6/8/1999**	**6/17/1999**	**6/24/1999**	**7/1/1999**
Dusk	27.49 (68) [3.84,14]	21.4 (4) [0.41,2]	21.95 (4) [0.33,2]	27.95 (4) [0.30,1]	20.13 (4) [0.25,1]	25.3 (4) [0.36,1]	24.85 (4) [0.40,2]
Dawn	23.78 (68) [3.68,15]	25.8 (4) [0.29,1]	24.2 (4) [0.24,1]	31.08 (4) [0.15,0]	23.68 (4) [0.54,2]	29.68 (4) [0.32,1]	26 (4) [0.16,1]

Variable	**Mean By Date**[1]						
	7/7/1999	**7/14/1999**	**7/27/1999**	**8/3/1999**	**8/9/1999**	**8/17/1999**	**8/24/1999**
Dusk	27.9 (4) [0.14,1]	25.03 (4) [0.57,2]	30.75 (4) [0.33,1]	25.94 (4) [0.38,1]	25.25 (4) [0.30,1]	25.73 (4) [0.53,2]	23.35 (4) [0.51,2]
Dawn	31.93 (4) [0.15,0]	30.55 (4) [0.24,1]	34.25 (4) [0.24,1]	26.93 (4) [0.29,1]	31.05 (4) [0.42,1]	29.45 (4) [0.51,2]	26.1 (4) [0.52,2]

Variable	**Mean By Date**[1]			
	8/31/1999	**9/7/1999**	**9/14/1999**	**9/21/1999**
Dusk	21.88 (4) [0.66,3]	23.1 (4) [0.70,3]	18.28 (4) [0.57,3]	15.5 (4) [0.57,4]
Dawn	26.9 (4) [0.66,2]	28.73 (4) [0.66,2.]	22.08 (4) [0.32,1]	18.98 (4) [0.65,3]

[1]Mean, (n); [STD,COV]

Table A-4. Experimental Error Statistics for Macrophyte (dry wt., TN, and TP) and Sediment (TN,TP) Data from 1999 Study

		Mean By **Date**[1]			
Variable	**Grand Mean**	**5/25/1999**	**6/24/1999**	**7/20/1999**	**7/27/1999**
Macrophyte (g dry wt.)	14.6 (24) [8.53,58]	2.07 (4) [1.19,58]	9.48 (4) [3.11,33]	22.67 (4) [6.48,29	22.67 (4) [6.48,29]
Macrophyte TN (% of dry wt.)	2.48 (20) [0.60,24]	2.18 (4) [0.50,23]	1.84 (4) [0.11,6]	2.32 (4) [0.25,11]	-[2]
Macrophyte TP (% of dry wt.)	13.9 (16) [4.64,33]	9.86 (4) [2.12,21]	9.66 (4) [1.75,18]	18.68 (4) [2.53,14]	-[2]
Sediment TN (g N/m^2*5 cm depth)	0.33 (20) [0.10, 31]	0.21 (4) [0.06,28]	0.37 (4) [0.07,20]	0.35 (4) [0.11,32]	-[2]
Sediment TP (g N/m^2*5 cm depth)	0.06 (20) [0.01,13]	0.05 (4) [0.01,11]	0.06 (4) [0.01,13]	0.06 (4) [0.01,9]	-[2]

	Mean By Date	
Variable	**8/19/1999**	**9/24/1999**
Macrophyte (g dry wt.)	16.35 (4) [5.24,32]	14.38 (4) [3.70,26]
Macrophyte TN (% of dry wt.)	2.85 (4) [0.30,10]	3.22 (4) [0.52,16]
Macrophyte TP (% of dry wt.)	17.41 (4) [0.93,5]	-[2]
Sediment TN (g N/m^2*5 cm depth)	0.42 (4) [0.11,27]	0.33 (4) [0.04,12]
Sediment TP (g N/m^2*5 cm depth)	0.06 (4) [0.01,13]	0.05 (4) [0.01,18]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-5. Experimental Error Statistics for Zooplankton Data from 1999 Study

Category	Variable	Grand Mean	Mean By **Date**[1]			
			5/12/1999	**7/16/1999**	**8/19/1999**	**9/22/1999**
Zooplankton	totnum	172575 (11) [84650,49]	-[2]	128137 (4) [44710,35]	200703 (4) [42998,21]	194322 (3) [154322,79]
Zooplankton	nsptot	14.45 (11) [2.25,16]	-[2]	15.25 (4) [1.26,8]	12.25 (4) [0.50,4]	16.33 (3) [2.52,15]
Zooplankton	simpdom	0.21 (11) [0.07,33]	-[2]	0.23 (4) [0.10,45]	0.20 (4) [0.02,8]	0.19 (3) [0.07,38]
Cladoceran	totnum	86189 (11) [83034,96]	-[2]	46733 (4) [23687,51]	71462 (4) [20683,29]	158437 (3) [147012,93]
Cladoceran	nsptot	6.64 (11) [1.43,22]	-[2]	6.25 (4) [0.96,15]	5.75 (4) [0.96,17]	8.33 (3) [1.15,14]
Cladoceran	simpdom	0.34 (11) [0.12,36]	-[2]	0.32 (4) [0.12,39]	0.44 (4) [0.08,18]	0.24 (3) [0.08,34]
Copepods	totnum	24040 (11) [12950,54]	-[2]	22096 (4) [11991,54]	34607 (4) [11348,33]	12544 (3) [729.07,6]
Copepods	nsptot	1.64 (11) [0.67,41]	-[2]	2 (4) [0.82,41]	1.25 (4) [0.50,40]	1.67 (3) [0.58,35]
Copepods	simpdom	0.86 (11) [0.18,21]	-[2]	0.90 (4) [0.08,9]	0.88 (4) [0.24,27]	0.80 (3) [0.26,33]
Rotifers	totnum	37192 (11) [33315,90]	-[2]	50369 (4) [25876,51]	49234 (4) [38116,77]	3569 (3) [5738,161]
Rotifers	nsptot	1.73 (11) [1.01,58]	-[2]	2.5 (4) [1.29,52]	1 (4) [0,0]	1.67 (3) [0.58,35]
Rotifers	simpdom	0.85 (11) [0.24,28]	-[2]	0.70 (4) [0.29,42]	1 (4) [0,0]	0.84 (3) [0.27,32]

[1]Mean, (n); [STD,COV]
[2]Different sampling method taken.

Table A-6. Experimental Error Statistics for Total Numbers of Phytoplankton (#/m^2) Data from 1999 Study

Category	Variable	Grand Mean	Mean By **Date**			
			5/12/1999	**5/20/1999**	**6/21/1999**	**7/14/1999**
Phytoplankton	totnum	219650 (191) [576313,262]	8073 (23) [36995,458]	77495 (29) [292374,377]	193927 (34)	330473 (36) [635370,192]
Phytoplankton	nsptot	8.1 (20) [3.61,45]	6 (1)	4 (3) [2.65,66]	7.75 (4) [5.85,76]	7.5 (4) [1.73,23]
Bacillariophyta	totnum	20936 (24) [43964,210]	0 (4) [0,0]	12885 (4) [13392,104]	914 (4) [1348,148]	1164 (4) [1629,140]
Bacillariophyta	nsptot	1.75 (20) [1.45,83]	4 (1)	2 (3) [1.73,87]	1.25 (4) [0.96,77]	0.75 (4) [0.50,67]
Chlorophyta	totnum	1166782 (24) [3110909,267]	0 (4) [0,0]	66.67 (3) [115,173]	348010 (4)	2617842 (4)
Chlorophyta	nsptot	3.90 (20) [2.77,71]	0 (1)	0.67 (3) [0.58,87]	3.75 (4) [4.11,110]	4 (4) [2.45,61]
Cryptophyta	totnum	390337 (24) [827294,212]	0 (4) [0,0]	1460085 (4) [1603986,110]	375039 (4)	492572 (4) [599606,122]
Cryptophyta	nsptot	0.85 (20) [0.37,43]	1 (1)	1 (3) [0,0]	1 (4) [0,0]	0.75 (4) [0.50,67]
Cyanophyta	totnum	244625 (24) [483733,198]	0 (4) [0,0]	92124 (4) [113131,123]	661615 (4)	350367 (4) [216299,62]
Cyanophyta	nsptot	1.4 (20) [1.10,78]	0 (1)	0.33 (3) [0.58,173]	1.5 (4) [1.29,86]	1.75 (4) [0.50,29]
Euglenophyta	totnum	119 (24) [583,490]	0 (4) [0,0]	714 (4) [1428,200]	0 (4) [0,0]	0 (4) [0,0]
Euglenophyta	nsptot	0.20 (20) [0.41,205]	1 (1)	0 (3) [0,0]	0.25 (4) [0.50,200]	0.25 (4) [0.50,200]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-6. Experimental Error statistics for Total Numbers of Phytoplankton (#/m^2) Data from 1999 Study (Continued)

		Mean By **Date**	
Category	**Variable**	**8/17/1999**	**9/23/1999**
Phytoplankton	totnum	394908 (32) [836675,212]	233126 (36) [692818,297]
Phytoplankton	nsptot	9.25 (4) [0.96,10]	11.5 (4) [1.91,17]
Bacillariophyta	totnum	17531 (4) [12631,72]	93118 (4) [76066,82]
Bacillariophyta	nsptot	0.75 (4) [0.96,128]	3.5 (4) [0.58,16]
Chlorophyta	totnum	3774233 (4) [6261811,166]	260560 (4) [238524,92]
Chlorophyta	nsptot	5.75 (4) 0.50,9]	5.5 (4) [1.29,23]
Cryptophyta	totnum	14325 (4) [28651,200]	0 (4) [0,0]
Cryptophyta	nsptot	0.5 (4) [0.58,115]	1 (4) [0,0]
Cyanophyta	totnum	286507 (4) [385525,135]	77137 (4) [154273,200]
Cyanophyta	nsptot	2.25 (4) [0.96,43]	1.25 (4) [1.26,101]
Euglenophyta	totnum	0 (4) [0,0]	0 (4) [0,0]
Euglenophyta	nsptot	0 (4) [0,0]	0.25 (4) [0.50,200]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-7. Experimental Error Statistics for Nutrient Data from 2000 Study

Variable	Grand Mean	Mean By **Date**[1]				
		5/10/2000	**5/11/2000**	**5/16/2000**	**5/18/2000**	**5/23/2000**
Chlorophyll in water (µg/L)	6.09 (76) [8.94,147]	2.16 (4) [0.97,45]	-[2]	0.77 (4) [0.91,118]	-[2]	0.79 (4) [0.34,43]
Chlorophyll in periphyton (µg/cm^2)	1.01 (40) [0.01,114]	-[2]	-[2]	-[2]	0.01 (4) [0.01,105]	-[2]
TN (mg/L)	0.68 (76) [0.26,38]	0.38 (4) [0.10,26]	-[2]	-[2]	0.3 (4) [0.08,27]	0.43 (4) [0.05,12]
TP (µg/L)	42.22 (76) [29.11,69]	13.68 (4) [2.68,20]	-[2]	-[2]	14.18 (4) [7.67,54]	19.43 (4) [2.08,11]
NH_3 (µg/L)	0.01 (136) [0.02,155]	0.01 (4) [0.01,126]	0 (4) [0,128]	0.02 (4) [0,29]	0.02 (4) [0.01,37]	0.01 (4) [0.02,137]
SRP (µg/L)	7.32 (136) [4.30,59]	5.55 (4) [0.40,7]	5.93 (4) [0.91,15]	12.58 (4) [0.84,7]	13.25 (4) [5.23,39]	4.55 (4) [0.56,12]
NO_2/NO_3 (µg/L)	0.03 (136) [0.07,288]	0.21 (4) [0.10,50]	0.16 (4) [0.11,69]	0.07 (4) [0.12,165]	0.17 (4) [0.30,179]	0.03 (4) [0.03,119]

Variable	**Mean By Date**[1]					
	5/25/2000	**5/30/2000**	**5/31/2000**	**6/1/2000**	**6/6/2000**	**6/7/2000**
Chlorophyll in water (µg/L)	-[2]	1.35 (4) [0.74,55]	-[2]	-[2]	0.68 (4) [0.31,46]	-[2]
Chlorophyll in periphyton (µg/cm^2)	1.01 (4) 1.02 [0,43]	-[2]	-[2]	-[2]	-[2]	-[2]
TN (mg/L)	-[2]	0.65 (4) [0.06,9]	-[2]	-[2]	0.63 (4) [0.13,20]	-[2]
TP (µg/L)	-[2]	24.42 (4) [3.56,15]	-[2]	-[2]	30.5 (4) [3.68,12]	-[2]
NH_3 (µg/L)	0.03 (4) [0.03,99]	0.05 (4) [0.03,70]	0.03 (4) [0.03,94]	0.04 (4) [0.04,85]	0.03 (4) [0.03,103]	0.02 (4) [0.02,103]
SRP (µg/L)	4.15 (4) [0.79,19]	4.35 (4) [0.79,18]	5.35 (4) [0.70,13]	6.45 (4) [0.60,9]	6.08 (4) [0.72,12]	5.68 (4) [0.76,13]
NO_2/NO_3 (µg/L)	0.02 (4) [0.02,96]	0.01 (4) [0.01,94]	0.01 (4) [0.01,136]	0.01 (4) [0.01,87]	0.01 (4) [0.01,121]	0.01 (4) [0.01,109]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-7. Experimental Error Statistics for Nutrient Data from 2000 Study (Continued)

	Mean By **Date**[1]					
Variable	**6/13/2000**	**6/14/2000**	**6/20/2000**	**6/21/2000**	**6/27/2000**	**6/28/2000**
Chlorophyll in water (µg/L)	1.04 (4) [0.98,94]	-[2]	1.59 (4) [2.02,127]	-[2]	0.47 (4) [0.14,30]	-[2]
Chlorophyll in periphyton ($\mu g/cm^2$)	-[2]	0.01 (4) [0.01,65]	-[2]	0.02 (4) [0.02,73]	-[2]	-[2]
TN (mg/L)	0.53 (4) [0.05,10]	-[2]	0.58 (4) [0.05,9]	-[2]	0.5 (4) [0,0]	-[2]
TP (µg/L)	21.58 (4) [2.53,12]	-[2]	25.48 (4) [1302,51]	-[2]	17.75 (4) [2.76,16]	-[2]
NH_3 (µg/L)	0.01 (4) [0,33]	0 (4) [0,159]	0 (4) [0,200]	0 (4) [0,115]	0.01 (4) [0,22]	0.01 (4) [0,32]
SRP (µg/L)	4.8 (4) [1.21,25]	7.15 (4) [1.43,20]	3.6 (4) [1.33,37]	3.55 (4) [0.50,14]	4.08 (4) [0.99,24]	5.7 (4) [0.92,16]
NO_2/NO_3 (µg/L)	0 (4) [0.01,170]	0.01 (4) [0.01,62]	0.01 (4) [0.01,134]	0.01 (4) [0,42]	0.01 (4) [0.01,122]	0.01 (4) [0,29]

	Mean By Date[1]					
Variable	**7/4/2000**	**7/5/2000**	**7/11/2000**	**7/12/2000**	**7/18/2000**	**7/19/2000**
Chlorophyll in water (µg/L)	0.85 (4) [0.56,66]	-[2]	1.24 (4) [0.70,56]	-[2]	1.82 (4) [0.55,30]	-[2]
Chlorophyll in periphyton ($\mu g/cm^2$)	-[2]	-[2]	-[2]	0 (4) [0,47]	-[2]	0 (4) [0,81]
TN (mg/L)	0.53 (4) [0.05,10]	-[2]	0.6 (4) [0,0]	-[2]	0.65 (4) [0.10,15]	-[2]
TP (µg/L)	17.85 (4) [3.49,20]	-[2]	21.05 (4) [4.27,20]	-[2]	30.13 (4) [6.35,21]	-[2]
NH_3 (µg/L)	0 (4) [0,120]	0 (4) [0,0]	0 (4) [0,52]	0 (4) [0,141]	0.01 (4) [0,6]	0 (4) [0.01,128]
SRP (µg/L)	1.83 (4) [0.29,16]	3.8 (4) [1.52,40]	3.43 (4) [0.97,28]	6.78 (4) [1.88,28]	9.4 (4) [4.88,52]	16.33 (4) [9.08,56]
NO_2/NO_3 (µg/L)	0.02 (4) [0.01,71]	0.01 (4) [0.01,70]	0.02 (4) [0.02,118]	0.01 (4) [0.01,80]	0.01 (4) [0.01,76]	0.01 (4) [0,31]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-7. Experimental Error Statistics for Nutrient Data from 2000 Study (Continued)

	Mean By **Date**[1]					
Variable	**7/25/2000**	**7/26/2000**	**8/1/2000**	**8/2/2000**	**8/8/2000**	**8/9/2000**
Chlorophyll in water (µg/L)	15.04 (4) [13.32,89]	-[2]	13.89 (4) [17.80,128]	-[2]	7.01 (4) [5.41,77]	-[2]
Chlorophyll in periphyton ($\mu g/cm^2$)	-[2]	-[2]	-[2]	-[2]	-[2]	0 (4) [0,54]
TN (mg/L)	0.88 (4) [0.25,29]	-[2]	0.93 (4) [0.38,41]	-[2]	0.85 (4) [0.17,20]	-[2]
TP (µg/L)	69.58 (4) [33.74,49]	-[2]	71.78 (4) [36.06,50]	-[2]	58.88 (4) [8.81,15]	-[2]
NH_3 (µg/L)	0 (4) [0,141]	0.01 (4) [0,37]	0 (4) [0,115]	0 (4) [0,62]	0.01 (4) [0.01,104]	0.01 (4) [0,49]
SRP (µg/L)	8.85 (4) [2.98,34]	7.23 (4) [2.42,34]	9.95 (4) [2.91,29]	8 (4) [1.69,21]	11.98 (4) [5.15,43]	9.53 (4) [1.84,19]
NO_2/NO_3 (µg/L)	0.01 (40 [0,17]	0.01 (4) [0,22]	0.01 (4) [0,40]	0.01 (4) [0,35]	0 (4) [0,200]	0 (4) [0,0]

	Mean By Date[1]					
Variable	**8/15/2000**	**8/22/2000**	**8/29/2000**	**9/5/2000**	**9/12/2000**	**9/21/2000**
Chlorophyll in water (µg/L)	11.90 (4) [5.56,47]	18.26 (4) [9.07,50]	21.89 (4) [13.41,61]	8.11 (4) [5.58,69]	6.77 (4) [3.26,48]	-[2]
Chlorophyll in periphyton ($\mu g/cm^2$)	1.01 (4) [0,36]	-[2]	-[2]	-[2]	0.01 (4) [0.01,110]	0.03 (4) [0.02,74]
TN (mg/L)	0.95 (4) [0.33,35]	1.03 (4) [0.17,17]	0.95 (4) [0.06,6]	0.98 (4) [0.10,10]	0.63 (4) [0.10,15]	-[2]
TP (µg/L)	66.25 (4) [26.22,40]	76.68 (4) [18.32,24]	68.65 (4) [10.74,16]	87.38 (4) [25.09,29]	66.93 (4) [20.88,31]	-[2]
NH_3 (µg/L)	0.01 (4) [0,48]	0 (4) [0,29]	0.01 (4) [0,24]	0.01 (4) [0.01,51]	0.01 (4) [0.01,83]	-[2]
SRP (µg/L)	7.75 (4) [1.77,23]	8.83 (4) [1.78,20]	8.18 (4) [0.29,4]	14.35 (4) [7.75,54]	9.95 (4) [6.05,61]	-[2]
NO_2/NO_3 (µg/L)	0 (4) [0,200]	0 (4) [0,0]	0 (4) [0,200]	0 (4) [0,0]	0 (4) [0,0]	-[2]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-8. Experimental Error Statistics for Water Quality Data from 2000 Study

Variable	Grand Mean	Mean By **Date**[1]				
		5/10/2000	**5/16/2000**	**5/23/2000**	**5/30/2000**	**6/6/2000**
pH	9.17 (76) [0.67,7]	8.37 (4) [0.04,0]	8.26 (4) [0.02,0]	8.28 (4) [0.08,1]	8.14 (4) [0.03,0]	8.35 (4) [0.17,2]
Alkalinity (mg/L)	116.65 (75) [52.43,45]	233 (4) [6.48,3]	200.75 (4) [3.40,2]	192.5 (4) [7.55,4]	178.75 (4) [10.75,6]	170.5 (4) [26.80,16]
Hardness (mg/L)	139.66 (76) [66.63,48]	273 (4) [6.83,3]	243 (4) [7.75,3]	234 (4) [11.66,5]	231 (4) [18.07,8]	220.25 (4) [32.54,15]
Turbidity (NTU)	4.04 (76) [2.93,73]	3.88 (4) [0.28,7]	3.28 (4) [0.94,29]	2.78 (4) [0.21,7]	2.48 (4) [0.60,24]	2.1 (4) [0.43,21]
Conductivity (µs/L)	382.39 (76) [126.66,33]	625.25 (4) [4.79,1]	572.75 (4) [12.92,2]	519.5 (4) [18.70,4]	556.25 (4) [31.67,6]	548.25 (4) [53.92,10]

Variable	Mean By Date[1]					
	6/13/2000	**6/20/2000**	**6/27/2000**	**7/4/2000**	**7/11/2000**	**7/18/2000**
pH	8.64 (4) [0.34,4]	9.05 (4) [0.46,5]	9.41 (4) [0.50,5]	9.77 (4) [0.41,4]	9.97 (4) [0.09,1]	9.90 (4) [0.06,1]
Alkalinity (mg/L)	135.5 (4) [26.95,20]	110.5 (4) [20.74,19]	89.5 (4) [12.58,14]	79.75 (4) [7.41,9]	76.5 (4) [5.51,7]	79.67 (3) [4.73,6]
Hardness (mg/L)	175.5 (4) [30.51,17]	147.5 (4) [22.05,15]	119.75 (4) [12.23,10]	108.25 (4) [9.32,9]	101 (4) [8.25,8]	100.75 (4) [10.18,10]
Turbidity (NTU)	4.1 (4) [1.12,27]	2.03 (4) [1.79,88]	1.43 (4) [0.19,13]	1.85 (4) [0.70,38]	1.35 (4) [0.17,13]	2.05 (4) [0.13,6]
Conductivity (mS/cm)	464 (4) [56.91,12]	402 (4) [39.88,10]	345.75 (4) [24.10,7]	328.75 (4) [10.94,3]	357 (4) [67.46,19]	305 (4) [15.36,5]

[1]Mean, (n); [STD,COV]

Table A-8. Experimental Error Statistics for Water Quality Data from 2000 Study (Continued)

	Mean By **Date**[1]					
Variable	**7/25/2000**	**8/1/2000**	**8/8/2000**	**8/15/2000**	**8/22/2000**	**8/29/2000**
pH	9.76 (4) [0.05,0]	9.66 (4) [0.08,1]	9.37 (4) [0.13,1]	9.66 (4) [0.08,1]	9.71 (4) [0.08,1]	9.74 (4) [0.20,2]
Alkalinity (mg/L)	92.5 (4) [10.25,11]	77.5 (4) [2.52,3]	68.5 (4) [3.00,4]	84 (4) [20.20,24]	80 (4) [6.93,9]	78 (4) [14.14,18]
Hardness (mg/L)	99.5 (4) [9.57,10]	94.5 (4) [6.40,7]	79 (4) [10.65,13]	82.5 (4) [7.37,9]	87 (4) [6.63,8]	78.25 (4) [7.50,10]
Turbidity (NTU)	4.93 (4) [3.14,64]	5.03 (4) [3.34,66]	3.28 (4) [1.41,43]	7.58 (4) [3.84,51]	6.98 (4) [3.06,44]	8.33 (4) [3.92,47]
Conductivity (mS/cm)	305 (4) [14.45,5]	382.75 (4) [33.48,9]	256.25 (4) [13.72,5]	263.25 (4) [11.70,4]	259 (4) [10.95,4]	241 (4) [12.49,5]

[1]Mean, (n); [STD,COV]

Table A-9. Experimental Error Statistics for Diurnal Temperature Data from 2000 Study

		Mean By **Date**[1]					
Variable	**Grand Mean**	**5/11/2000**	**5/12/2000**	**5/15/2000**	**5/18/2000**	**5/23/2000**	**5/25/2000**
Dusk	27.82 (132) [3.17,11]	16.55 (4) [0.44,3]	19.08 (4) [0.15,1]	19.75 (4) [0.17,1]	21.89 (4) [0.30,1]	22.7 (4) [0.35,2]	23.88 (4) [0.15,1]
Dawn	24.71 (136) [2.98,12]	20.63 (4) [0.25,1]	23.78 (4) [1.69,7]	21.63 (4) [0.13,1]	24.7 (4) [0.24,1]	25.13 (4) [0.25,1]	26.73 (4) [0.22,1]

	Mean By Date[1]						
Variable	**5/30/2000**	**5/31/2000**	**6/1/2000**	**6/6/2000**	**6/7/2000**	**6/13/2000**	**6/14/2000**
Dusk	24.08 (4) [0.10,0]	24.63 (4) [0.72,3]	26.74 (4) [0.19,1]	19.78 940 [0.22,1]	21.18 (4) [0.19,1]	25.75 (4) [0.31,1]	26.03 (4) [0.10,0]
Dawn	27.33 (4) [0.39,1]	29.9 (4) [0.20,1]	30.05 (4) [0.13,0]	23.88 (4) [0.15,1]	25.19 (4) [0.25,1]	29.13 (4) [0.15,1]	26.93 (4) [0.29,1]

	Mean By Date[1]						
Variable	**6/20/2000**	**6/21/2000**	**6/27/2000**	**6/28/2000**	**7/4/2000**	**7/5/2000**	**7/11/2000**
Dusk	24.43 (4) [0.15,1]	23.33 (4) [0.10,0]	24.51 (4) [0.18,1]	24.1 (4) [0.14,1]	27.9 (4) [0.27,1]	28.13 (4) [0.22,1]	30.38 (4) [0.33,1]
Dawn	25.48 (4) [0.19,1]	25.7 (4) [0.28,1]	25.98 (4) [0.10,0]	28 (4) [0.57,2]	-[2]	32.73 (4) [0.95,3]	33.55 (4) [0.33,1]

	Mean By Date[1]						
Variable	**7/12/2000**	**7/18/2000**	**7/19/2000**	**7/25/2000**	**7/26/2000**	**8/1/2000**	**8/2/2000**
Dusk	29.6 (4) [0.36,1]	27.1 (4) [0.20,1]	26.58 (4) [0.25,1]	24.68 (4) [0.47,2]	25.13 (4) [0.41,2]	25.3 (4) [0.40,2]	26.55 (4) [0.47,2]
Dawn	32.7 (4) [0.42,1]	28.75 (4) [0.13,0]	27.53 (4) [0.10,0]	29.1 (4) [0.28,1]	29.83 (4) [0.61,2]	29.88 (4) [0.26,1]	28.6 (4) [0.24,1]

	Mean By Date[1]						
Variable	**8/8/2000**	**8/9/2000**	**8/15/2000**	**8/22/2000**	**8/29/2000**	**9/5/2000**	**9/12/2000**
Dusk	24.85 (4) [0.24,1]	26.65 (4) [0.47,2]	27.08 (4) [0.53,2]	25.13 (4) [0.15,1]	28.48 (4) [0.33,1]	23.63 (4) [0.45,2]	24.7 (4) [0.23,1]
Dawn	29.38 (4) [0.24,1]	32.28 (4) [0.67,2]	30.68 (4) [1.81,6]	28.68 (4) [1.48,5]	32.05 (4) [0.70,2]	25.93 (4) [0.38,1]	26.35 (4) [0.52,2]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-10. Experimental Error Statistics for Macrophyte and Sediment Data from 2000 Study

Variable	Grand Mean	Mean By **Date**[1]			
		6/9/2000	**7/3/2000**	**7/31/2000**	**9/4/2000**
Macrophyte (g dry wt.)	1.05 (16) [0.94,89]	0.17 (4) [0.16,97]	1.11 (4) [0.71,64]	1.73 (4) [1.28,74]	1.17 (4) [0.73,62]
Macrophyte TN (% of dry wt.)	2.27 (16) [0.60,26]	1.78 (4) [0.53,30]	2.13 (4) [0.44,21]	2.11 (4) [0.23,11]	3.06 (4) [0.26,8]
Macrophyte TP (% of dry wt.)	0.45 (15) [0.13,30]	0.26 (3) [0.14,52]	0.48 (4) [0.11,23]	0.47 (4) [0.11,22]	0.54 (4) [0.03,6]
Sediment TN (g N/m^2*5 cm depth)	0.33 (16) [0.08,25]	0.39 (4) [0.07,18]	0.27 (4) [0.08,29]	0.32 (4) [0.07,23]	0.34 (4) [0.09,27]
Sediment TP (g N/m^2*5 cm depth)	0.06 (16) [0.01,14]	0.07 (4) [0.01,8]	0.06 (4) [0.01,14]	0.06 (4) [0.01,17]	0.06 (4) [0.01,9]

[1]Mean, (n); [STD,COV]

Table A-11. Experimental Error Statistics for Zooplankton Data from 2000 Study

			Mean By **Date**[1]				
Category	**Variable**	**Grand Mean**	**5/12/2000**	**6/9/2000**	**7/7/2000**	**8/4/2000**	**9/7/2000**
Zooplankton	totnum	61996 (20) [47761,77]	9699 (4) [9140,94]	32395 (4) [8879,27]	51647 (4) [23553,46]	119845 (4) [39122,33]	96395 (4) [34670,36]
Zooplankton	nsptot	13 (20) [2.60,20]	10.5 (4) [2.38,23]	13.25 (4) [1.50,11]	12 (4) [1.15,10]	12.25 (4) [0.96,8]	17 (4) [0.82,5]
Zooplankton	simpdom	0.36 (20) [0.22,60]	0.48 (4) [0.37,77]	0.33 (4) [0.23,70]	0.40 (4) [0.12,31]	0.35 (4) [0.21,60]	0.24 (4) [0.06,26]
Cladoceran	totnum	32522 (20) [26900,83]	9080 (4) [9485,104]	16838 (4) [12094,72]	36472 (4) [31391,86]	65376 (4) [27971,43]	34845 (4) [7145,21]
Cladoceran	nsptot	4.2 (20) [0.95,23]	4.25 (4) [0.5,12]	5 (4) [1.41,28]	4 (4) [0.82,20]	3.75 (4) [0.96,26]	4 (4) [0.82,20]
Cladoceran	simpdom	0.57 (20) [0.19,33]	0.58 (4) [0.29,50]	0.47 (4) [0.15,33]	0.68 (4) [0.18,27]	0.60 (4) [0.20,33]	0.53 (4) [0.11,21]
Copepods	totnum	17997 (20) [21714,121]	188.29 (4) [334.9,178]	11941 (4) [6997,59]	11075 (4)	30844 (4) [30551,99]	35934 (4) [26162,73]
Copepods	nsptot	2.35 (20) [1.31,56]	1 (4) [1.41,141]	3 (4) [1.41,47]	2.5 (4) [0.58,23]	2.25 (4) [0.96,43]	3 (4) [1.41,47]
Copepods	simpdom	0.65 (20) [0.30,47]	0.37 (4) [0.48,130]	0.69 (4) [0.24,35]	0.68 (4) [0.26,37]	0.67 (4) [0.22,33]	0.82 (4) [0.15,19]
Rotifers	totnum	3894 (20) [2.12,38]	290.98 (4) [426.0,146]	174.25 (4) [134.6,77]	1230 (4) [918.2,75]	6909 (4) [6091,88]	10864 (4) [9501,87]
Rotifers	nsptot	5.5 (20) [2.12,38]	4.5 (4) [0.58,13]	4.25 (4) [1.71,40]	4.5 (4) [0.58,13]	5.25 (4) [0.96,18]	9 (4) [1.63,18]
Rotifers	simpdom	0.44 (20) [0.14,33]	0.43 (4) [0.21,49]	0.38 (4) [0.12,31]	0.46 (4) [0.10,21]	0.55 (4) [0.18,32]	0.36 (4) [0.06,17]

[1]Mean, (n); [STD,COV]
[2]Denotes no sample taken.

Table A-12. Experimental Error Statistics for Total Numbers of Phytoplankton (#/m^2) Data from 2000 Study

			Mean By **Date**[1]		
Category	**Variable**	**Grand Mean**	**5/11/2000**	**6/7/2000**	**7/5/2000**
Phytoplankton	totnum	5700600 (20) [6698397,118]	335000 (4) [323417,97]	1754250 (4) [1987828,114]	5227500 (4) [5262102,101]
Phytoplankton	nsptot	15.9 (20) [10.74,68]	12.25 (4) [8,66]	10 (4) [9,87]	15.5 (4) [10,64]
Bacillariophyta	totnum	221150 (20) [422420,191]	45000 (4) [24083,54]	47750 (4) [45828,96]	195500 (4) [284288,145]
Bacillariophyta	nsptot	4.8 (20) [3.78,79]	2.75 (4) [1,18]	5 (4) [4,71]	4.50 (4) [4,99]
Chlorophyta	totnum	2766000 (20) [3392408,123]	214000 (4) [230853,108]	1693000 (4) [1933972,114]	3594000 (4) [4946223,138]
Chlorophyta	nsptot	7.2 (20) [4.77, 66]	5.5 (4) [4,79]	4.25 (4) [5,124]	6 (4) [4,65]
Cryptophyta	totnum	23400 (20) [44882,192]	62000 (4) [81191,131]	0 940 [0,0]	22500 (4) [32388,144]
Cryptophyta	nsptot	1.25 (20) [1.37,110]	1.75 (4) [1,55]	0.25 (4) [1,200]	2 (4) [1,58]
Cyanophyta	totnum	2635650 (20) [5394883,205]	13000 (4) [12490,96]	9500 940 [19000,200]	1413000 (4) [2509204,178]
Cyanophyta	nsptot	2.1 (20) [2.27,108]	2 (4) [2,108]	0.25 (4) [1,200]	2.75 (4) [2,62]
Euglenophyta	totnum	57263 (19) [220880,386]	1000 (4) [2000,200]	1000 (4) [2000,200]	2500 (4) [5000,200]
Euglenophyta	nsptot	0.58 (19) [1.02,176]	0.25 (4) [1,200]	0.25 (4) [1,200]	0.25 (4) [1,200]

[1]Mean, (n); [STD,COV]

Table A-12. Experimental Error Statistics for Total Numbers of Phytoplankton (#/m^2) Data from 2000 Study (Continued)

		Mean By **Date**[1]	
Category	**Variable**	**8/2/2000**	**9/5/2000**
Phytoplankton	totnum	9569500 (4) [7340385,77]	11619750 (4) [8508006,73]
Phytoplankton	nsptot	18.75 (4) [13,70]	23 (4) [13,58]
Bacillariophyta	totnum	118250 (4) [112760,95]	699250 (4) [795404,114]
Bacillariophyta	nsptot	5 (4) [4,71]	6.75 (4) [6,87]
Chlorophyta	totnum	3095750 (4) [2391557,77]	5233250 (4) [4417499,84]
Chlorophyta	nsptot	9 (4) [4,47]	11.25 (4) [5,41]
Cryptophyta	totnum	20000 (4) [40000,200]	12500 (4) [25000,200]
Cryptophyta	nsptot	1.5 (4) [2,159]	0.75 (4) [1,128]
Cyanophyta	totnum	6334500 (4) [8421168,133]	5408250 (4) [7633214,141]
Cyanophyta	nsptot	3 (4) [4,119]	2.5 (4) [2,95]
Euglenophyta	totnum	1000 (4) [2000,200]	355333 (3) [529881,149]
Euglenophyta	nsptot	0.25 (4) [1,200]	2.33 (3) [1.53,65]

[1]Mean, (n); [STD,COV]

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