EVALUATION OF NUTRIENT LIMITATION TECHNIQUES IN THE GLOVER RIVER, MCCURTAIN, CO. OKLAHOMA

By

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CORRECTION

A systematic error was made in the interpretation of the output of a computer program for chlorophyll concentrations. Consequently,all values of chlorophyll and derivative data are in error by a factor of 1000.

To use the data in this thesis the reader needs to do the following:

1. Chlorophyll biomass : multiply values of mg chl.a/ square cm by 0.001.

2. Surplus P : multiply values of micrograms P/ mg chl.a by 1000.

3. Alkaline phosphatase activity (APA): multiply values of nM P/ micrograms chl.a/minute by 1000.

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CHAPTER I

INTRODUCTION

Lotic Nutrient Limitation

Nutrient limitation has been studied in lentic environments, but limited information is available in lotic systems. Nutrient limitation occurs when one or more elements are not available in excess of demand. Since periphyton is highly sensitive to changing nutrient concentrations, it may be used as indicators of past nutrient availability (Fairchild and Everett 1988). The most important nutrients affecting productivity in aquatic ecosystems are phosphorus (P) and nitrogen (N) (Wetzel 1983). Light, water velocity, discharge, and concentrations of trace elements are also important physical and chemical factors that affect productivity.

Techniques used to measure productivity in lentic environments may not provide sufficient information to determine nutrient demand in lotic systems. Productivity measurements may be confounded by physical and biotic factors such as current velocity and grazing (Elwood et al. 1981).

Nutrient availability and nutrient uptake are not completely understood in lotic environments. Nutrient

availability varies due to processes such as decomposition, nutrient pumping by aquatic macrophytes, runoff events, and input from point and non-point sources. Nutrient availability may be reduced when nutrients are not released by the stream heterotrophs.

Periphyton in river and streams may have special adaptations to reduce the potential of nutrient limitation. The epilithic community may tightly hold its nutrients at low ambient nutrient concentrations (Paul and Duthie 1988). Studies of river periphyton have shown that concentrations required to saturate growth rates of organisms mature mats are higher than concentrations needed to maximize growth of an immature mat (Bothwell 1985). The mat may sequester nutrients.

Water movement is an important factor controlling periphyton growth. High current velocities may scour periphyton or reduce the deposition of colonizers. Growth measured by chlorophyll a concentration decreased as velocity increased at low orthophosphate concentrations for algae grown on glass slides in artificial channels (Horner and Welsh 1981).

Several methods have been used to determine potential nutrient limitation of algal communities, including chemical water analysis, algal assemblages (Horner and Welsh 1981, Fairchild and Lowe 1988), algal nutrient concentrations (Healey and Hendzel 1979b, Rhee 1978, Smith 1983), substrate enrichment (Fairchild and Lowe 1984, Fairchild and

Everett 1988, Fairchild et al. 1984), stream fertilization (Peterson et al. 1983), surplus P (Fitzgerald and Nelson 1966, Wynne and Berman 1980, Wynne 1981), and alkaline phosphatase activity (APA) (Healey 1973, Healey and Hendzel 1979a and b, Petterson 1980, Wynne 1981, Wetzel 1981). Each technique measures potential limitation differently. It is often difficult to determine which nutrient is limiting because different techniques may be contradictory or nutrient limitation may vary seasonally. Thus, it is important to use several analyses (Petterson 1980).

Because enzymatic assays of APA are sensitive and rapid, measurement of APA alone might be a sensitive method for determining P limitation by periphyton. I evaluated the potential of an APA assay within streams by comparing results of APA assays to other methods. My objectives were to learn if,

1) an inverse relationship exists between APA and Molybdate Reactive Phosphorus of periphyton as exists in phytoplankton.

2) a relationship between the APA of periphyton and ambient soluble P or between APA of periphyton and the nitrogen:phosphate ratio of stream water exists.

3) a relationship between heterocysts frequency in bluegreen algae exists between treatments when N was limiting.

4) sestonic APA exceeded APA of periphyton if both were corrected for biomass.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The following techniques have been developed to study nutrient limitation in lakes, but have also been applied to stream studies. Each technique measures potential N and P nutrient limitation differently.

Point Source Enrichment

Point source enrichments are used to provide a localized supply of nutrients to periphyton. Periphyton biomass is compared to determine potential limitation. Nutrient diffusing substrates have been used to determine if a nutrient or a combination of nutrients is limiting algal growth. Substrates are made from clay pots or other vessels. Nutrients diffuse across the permeable surface of the substrates when they are submerged in water. Substrates provide a surface for algal colonization and a point source of nutrients. This technique provides an *in situ* bioassay of periphyton nutrient limitation (Fairchild et al. 1984). Controls releasing no nutrients are included in the experimental design.

Periphyton colonization is assumed to be equal and pas-

sive. The extent of periphyton growth is determined by estimating final biomass as areal chlorophyll a, biovolume, dry weight, or particulate carbon concentration. Experimenters compare biomass and community structure of the colonizing periphyton among replicated substrates releasing different nutrients (Fairchild and Lowe 1984). Significant differences in biomass and/or the presence of indicator species in a given treatment relative to the control have been used to test for nutrient limitation (Fairchild and Lowe 1984, Fairchild et al. 1985, Fairchild and Everett 1988, Short 1988).

Phosphorus Limitation

Although phosphorus (P) frequently is needed only in small amounts, it is the element most commonly limiting to algal growth (Wetzel 1983). Phosphorus cycles between aquatic and terrestrial environments. Unlike nitrogen, atmospheric sources of phosphorus do not exist. This limits phosphorus availability within the ecosystem to the dissolved and particulate forms weathered from rock. Organic phosphorus may be sorbed to particles and/or bound to sediments. Phosphate may be precipitated due to redox conditions, thus reducing its availability to primary producers in aquatic ecosystems (Goldman and Horne 1983). The form of phosphorus directly available for growth to phytoplankton and periphyton is orthophosphate (PO_4^{-3}). Algae have developed various strategies to compensate for the

limited availability of orthophosphate. These adaptations include the ability to store surplus P, the ability to take up low concentrations of phosphorus, and the ability to produce alkaline phosphatases. The detection of the latter and the former within the algal community provides important information in determining potential phosphorus limited growth.

Alkaline Phosphatase Activity

Enzymatic testing has been used to assess possible P limitation (Healey 1973, Healey and Hendzel 1979a and 1979b, Petterson 1980, Perrin, Bothwell and Slaney 1987, Pick 1987). The enzymatic analysis used to study phosphorus availability in lakes is alkaline phosphatase specific (attributed to algae) activity (APA). An increased concentration of algal cell surface enzyme alkaline phosphatase (AP) is an indicator that orthophosphate may be limiting (Healey and Hendzel 1979a and 1979b, Petterson 1980).

Phosphatase enzymes are a group of enzymes classified as either alkaline or acid depending on their pH optimum (Chrost et al. 1986, Reisen and Deirdre 1970). Alkaline phosphatase is a universal intracellular enzyme of mammalian and bacterial cells (Francko 1984b) and is also a cell surface enzyme of phytoplankton (Heath and Cook 1975).

Early studies demonstrated that AP may hydrolyze naturally occurring organic phosphate substances (Rigler 1961, Heath and Cook 1975). Thus, the production of AP allows the

algae to use organic phosphate compounds, such as polyphosphates and simple monoesters, when orthophosphate is not available (Healey and Hendzel 1979b, Heath and Cook 1975, Stewart and Wetzel 1983; Francko and Heath 1979). Phosphohydrolysis mediated by AP releases orthophosphate for direct use by algae and bacteria (Chrost and Albrecht 1986). The inverse relationship between phosphomonoesters and APA found in lakes has been used to further support the hypothesis that organic phosphorus containing compounds can provide an additional source of phosphorus to algae (Heath and Cook 1975). The specificity of the function of the enzyme has enabled experimenters to predict if orthophosphate is in demand to the algal assemblage (Heath and Cook 1975, Petterson 1980).

The actual contribution of phosphorus regeneration through AP hydrolysis is not completely understood. Phosphomonoesters are the compounds hydrolyzed by AP to provide an additional source of orthophosphate to algae. Theoretical rates of recycling have been calculated for different lakes. The range of rates of APA from 0.4 to 10.4 μg P/h emphasize the wide variation of rates of P regeneration and its significance to algae (Heath and Cook 1975, Pick 1987).

The increased activity of AP is the result of either an increased rate of production of the enzyme or the derepression of the enzyme at the cell surface (Stewart and Wetzel 1982). The repression of AP is caused by the presence of

orthophosphate (Petterson 1980). The lowering of ambient orthophosphate concentration potentially triggers derepression of the enzyme. The synthesis of AP occurred in culture when the concentration of orthophosphate supplied to Anabaena fell below 0.1 μg P/ 100 μg algal C (Healey and Hendzel 1979a). Phytoplanktonic alkaline phosphatase activity (APA) can increase to 10 times the normal activity under P limitation in nature (Petterson 1980). A laboratory study demonstrated that APA of cultured Peridinium increased as ambient orthophosphate concentrations decreased (Wynne 1981).

Many benefits of studying APA in relation to algal nutrient limitation exist. The simplicity of the reaction makes APA analysis relatively easy (Healey 1973). If information is available related to planktonic activity, the amount of phosphorus being released through APA can be predicted (Berman 1970). The procedures developed in past studies enabled analysis of APA in nature and culture (Perry 1972, Healey and Hendzel 1979a and 1979b). Cellular APA can be measured either colorimetrically or fluorometrically. Activities greater than 0.1 mg orthomethylfluorescein phosphate (o-MFP) hydrolyzed /mg dry wt/h are considered representative of P limitation in culture experiments (Healey and Hendzel 1979b).

Considerable variation exists in the APA of different species of algae under similar nutrient regimes. Some species do not produce AP (Healey and Hendzel 1979a). In

species that produce alkaline phosphatases, APA increases when the organism is phosphorus limited (Stevens and Parr 1977).

The hydrolysis of organic phosphorus compounds by AP appears to be restricted to phosphomonesters (PMEs). In Lough Neagh an increase in APA did not result in the decrease in soluble organic phosphate concentrations (Steven and Parr 1977). This suggests PMEs did not comprise a significant portion of the soluble organic phosphorus pool and that APA was not involved in algal phosphorus recycling. Knowledge of the composition of the pool of phosphorus compounds enables experimenters to predict the extent of phosphorus recycling and orthophosphate demand (Petterson 1980).

Total APA increases with the decrease of the orthophosphate concentration in eutrophic lakes (Suida and Chrost 1986). In one study maximum APA occurred at concentrations below 7.5 μg P/l and minimum APA occurred at concentrations above 50 μg P/l (Suida and Chrost 1986). This same study also compared the P uptake kinetics of phytoplankton and bacteria (Suida and Chrost 1986). Bacteria assimilated orthophosphate at lower concentrations than phytoplankton (Suida and Chrost 1986). The difference in assimilation rates of phytoplankton and bacteria suggests that phytoplankton may compensate for lowered P assimilation rates through AP production (Suida and Chrost 1986).

The presence of APA alone does not provide sufficient

evidence for P limitation. Many potential sources of APA exist in an aquatic environment. Total environmental APA is the result of enzyme production within the body of water by bacteria (Stewart and Wetzel 1982), phytoplankton (Petterson 1980), zooplankton (Janson 1976) and allochthonous sources (Stevens and Parr 1977). APA has been recognized as a component of sewage and may be added to a body of water through the discharge of wastes (Stevens and Parr 1977).

Total APA can be partitioned into a particulate and a dissolved fraction (Stewart and Wetzel 1982, Francko 1984a). Particulate APA is associated with organic matter, algae, bacteria, and detritral particles (Francko 1984b). Dissolved APA is that fraction not directly associated with a cell or cell membrane. Dissolved APA is frequently measured in an aliquot following filtration or partitioning by centrifugation (Healey and Hendzel 1979a). In four lakes in Michigan, the largest fraction of APA measured was the dissolved fraction and the smallest fraction of APA measured was the particulate fraction associated with algal particulate matter (Stewart and Wetzel 1982). In a study using water sampled from two Polish lakes, phytoplanktonic APA comprised 70% of total activity (Suida and Chrost 1987).

It is not known how APA differs between the seston and the periphyton in streams. Those cells within an algal mat might be depleted more rapidly than those cells at the surface (Paul and Duthie 1988). This could result in a difference in APA between cells recently broken free in

seston and attached algae deeper in the mat. Laboratory testing for nutrient limitation usually employs liquid cultures which may not be appropriate for streams, where the larger quantities of algae may be attached. Smith and Kalff (1981) suggested that differences in APA measured between algal species were the result of experimental, specifically culture conditions. Therefore, the results of culture studies may not be comparable to in situ experiments in streams.

Surplus P

Physiological adaptations enable algae to store P when orthophosphate (PO_4^{-3}) is in excess of demand (Darley 1982, Goldman and Horne 1983). Orthophosphate is the only form of P that algae can use directly. Algae take up and store orthophosphate as polyphosphates (Healey and Hendzel 1966). These polyphosphates are called surplus or luxury P (Healey and Hendzel 1966). Surplus P storage occurs when orthophosphate is available in concentrations greater than that required for basic cell metabolism. Surplus P is used by the organisms when external orthophosphate concentrations are depleted or absent. In natural systems and cultures surplus P enabled algal cells to grow and divide although ambient orthophosphate concentrations had fallen below 2 μg P/ l (Wynne and Berman 1980). Because of their ability to store P, ambient phosphorus concentrations may not reflect the actual nutrient status of algae (Healey 1973, Petterson

1980). Surplus P has been used to denote the status of P limitation of algae grown in culture and algal assemblages in lakes (Wynne 1981).

The method for determining surplus P involves extracting polyphosphates with heat from algal cells. In early studies, concentrations of surplus P below 0.08 μg P/ 100 μg dry wt were indicative of P limitation (Fitzgerald and Nelson 1973). Healey (1973) confirmed this value. Petterson (1980) found that 0.1 μg P/ 100 μg C was the threshold value of surplus P below which Lake Kinneret phytoplankton would produce AP. Therefore, 0.8 μg P/ 100 μg dry wt remains a good threshold for potential P limitation (assuming dry wt is 80% C).

Further studies revealed more information concerning the chemical nature of the surplus P. Hot water extracted surplus P is composed of a group of compounds that include both short chain (6-9 units with molecular weight 630-950) and long chain polyphosphates (greater than 9 units) with phosphate ester bonds (Wynne and Berman 1980). The majority of the short chain polyphosphates were non-molybdate reactive phosphorus (MRP) but hydrolyzable by alkaline phosphatase. Non-MRP denotes the inability of this fraction to evolve color when reacted with an ascorbic acid mixed reagent. The surplus P fraction is made up of long chain polyphosphates and is typically more abundant than non-MRP. This fraction is reactive with an ascorbic acid mixed reagent and known as MRP. This reagent is widely used to

develop color to measure orthophosphate and also some organic P in natural waters (Strickland and Parson 1968).

MRP becomes depleted when algal cells are grown under low orthophosphate concentrations (>0.05 mg P/l) (Wynne and Berman 1980). The shorter non-MRP appears to be a precursor to MRP (Wynne and Berman 1980). Wynne and Berman showed that surplus P concentrations were related to ambient orthophosphate concentrations under nonlimiting P conditions, reinforcing the importance of surplus P as a storage pool.

The significance of both of these fractions in relation to cell P status is not completely understood. In a Lake Kinneret study, hot water extractable P from cells grown at ambient concentrations of 0.55-6.6 mg P/l in culture and from Peridium grown in situ evolved almost equal amounts of MRP and non-MRP (Wynne 1981). Cells grown at low concentrations of orthophosphate (0.02-0.05 mg P/ l) depleted their intracellular reserves of MRP compounds before the non-MRP fraction was depleted (Wynne 1981). The presence of equal amounts of both the MRP and non-MRP fraction was found in cells grown in the lake during periods of algal blooms (Wynne 1981). The chemical properties of the surplus P fractions as well as preferential depletion and the presence of both fractions during periods of phosphorus abundance have lead Wynne to conclude the non-MRP fraction is a precursor MRP and that both fractions function as reserve P. The MRP fraction was measured in the following study.

The relationship between APA and surplus P has been

used to predict P limited growth. Surplus P and APA have been inversely correlated in many lake and culture studies (Petterson 1980, Healey and Hendzel 1979a). The increase in APA coupled with the decrease of MRP denotes potential P deficiency (Fitzgerald and Nelson 1966).

Nitrogen: Phosphate Ratios

Redfield (1958) studied the concentrations of nitrogen and phosphorus found in the phytoplankton in the ocean. Atoms of nitrogen and phosphorus were present in the phytoplankton in a ratio of 16:1. Atoms of nitrogen and phosphorus were present in a ratio of 15:1 in sea water (Redfield 1958). Redfield suggested these values denote the average nitrogen to phosphorus ratio in the various compartments of the biogeochemical cycle. Therefore, if the biogeochemcial cycle is self regulating, then the nutrient concentrations that deviate from these values denote an imbalance (i.e. potential limitation or overabundance). Theoretically cellular atomic N:P ratios that deviate on either side of the 16 to 1 atomic ratio are used to denote the potential nitrogen or phosphorus limited growth of the phytoplankton. If the inorganic nitrogen:phosphorus ratio in the water exceeded 16:1, phosphorus would limit phytoplanktonic growth. If the nitrogen:phosphorus ratio were less than 16:1, nitrogen would be limiting growth. Many scientists who have applied this idea have studied how the Redfield ratio relates to growth and productivity of aquatic produc-

ers in natural as well as in culture systems (Rhee 1978).

Theoretically, if the rate of supply of inorganic N or P (N:P ratio in water) differs from the 16:1 ratio, the potential for N or P limitation exists. Culture studies have shown, however, that there can be significant differences from the 16:1 Redfield ratio of nutrient supplies and final growth between species (Rhee 1973). Nevertheless, the measurement of the static soluble inorganic N:P ratio could provide information on the potential for nutrient limitation, if such ratios were widely different from the theoretical ratio.

Species Composition

In lakes, the presence of different dominant groups of algae follows species' demand for particular nutrients. For example, the succession of major planktonic species in Lake Michigan has been related to nutrient concentrations which were directly related to nutrient loading (Chang and Rossmen 1988). A change in species composition followed enrichment of holding ponds with three different concentrations of nutrients (O'Brien and Denoyelles 1976). A change in the composition of species of periphyton has also been demonstrated in streams (Fairchild and Everett 1985). This temporal succession is attributed to changes in nutrient concentration and current velocity. Diatom species succession has been demonstrated on glass slides (Reisen and Deirdre 1970). A study with nutrient diffusing substrates,

showed that significant differences existed among the genera colonizing different enrichment substrates (Fairchild and Lowe 1984). Diatoms and bluegreen algae showed a significant increase in density on P releasing substrates (Fairchild and Lowe 1984). Chlorophyta dominated the overall periphyton assemblage in another nutrient diffusing substrate study (Fairchild and Everett 1988). The supplement of nitrogen ($NaNO_3$): carbon (glucose) (N:C) and nitrogen : phosphorus (Na_2HPO_4):carbon (N:P:C) enrichments resulted in enhanced chlorophyceaen growth. In the same study, the addition of N and P in combination or P alone resulted in the enhanced growth of filamentous algae (Fairchild and Everett 1988).

Heterocysts

Nitrogen fixation permits certain algae and bacteria to grow when nitrate and ammonia have been depleted, even though N_2 fixation is more energetically costly (Goldman and Horne 1983). For example, the bluegreen Anabaena, has the ability to use ammonia, nitrate, and dinitrogen gas. Anabaena use nitrate and ammonia preferentially and switch to the more costly dinitrogen gas fixation, if supplies of nitrate or ammonia become depleted (Darley 1982). The presence of bluegreen algae containing a large number of heterocysts, the sites for N-fixation, usually denotes low levels of available nitrogen (nitrate and ammonia) (Wetzel 1983).

The relative numbers of heterocysts could be used to demonstrate N limitation (Wetzel 1983). But, this correlation may be confounded by phosphorus limited growth. When heterocystous Anabaena were grown under P deficient conditions, no heterocysts were detected in the culture after 160 h (Healey 1973). Nitrogen concentrations had not increased during this period. In bluegreen algae, a decline in the concentration of dissolved inorganic nitrogen and the decline of the intercellular ratio of C/N below 8:1, promotes heterocyst formation (Goldman and Horne 1983).

CHAPTER III

STUDY SITE

The study site is the Glover River which is in the Little River drainage basin in southeastern Oklahoma. The Little River has two tributaries in Oklahoma, the Mountain Fork and the Glover rivers. The Glover River originates in the Ouachita Mountains and flows southerly for approximately 90 km to its confluence with the Little River (Figure 1). The area of the drainage basin is 876 km^2. The stream gradient is from 19 m/km in the upper reaches and 1 m/km at the mouth (U.S. Army Corps of Engineers 1975). At the junction of the stream and the SH 3/7 bridge, the stream character changes markedly from a high gradient, rock substrate stream (mostly glides) to a low gradient gravel substrate stream (riffle/pool). The drainage basin is forested with mixed hardwood and pine and managed intensively by the Weyerhauser Corporation. The region surrounding the Glover River is currently being logged for hardwoods. Many poultry houses are in the vicinity. Clear-cut regions of the forests can be found within 5 to 10 km of Site I. Site I was not in direct contact with a clear-cut.

The Glover River is a soft water stream with conductivities of 38-46 μseimes/cm and a pH of 7.1-7.4 (U.S. Corps of

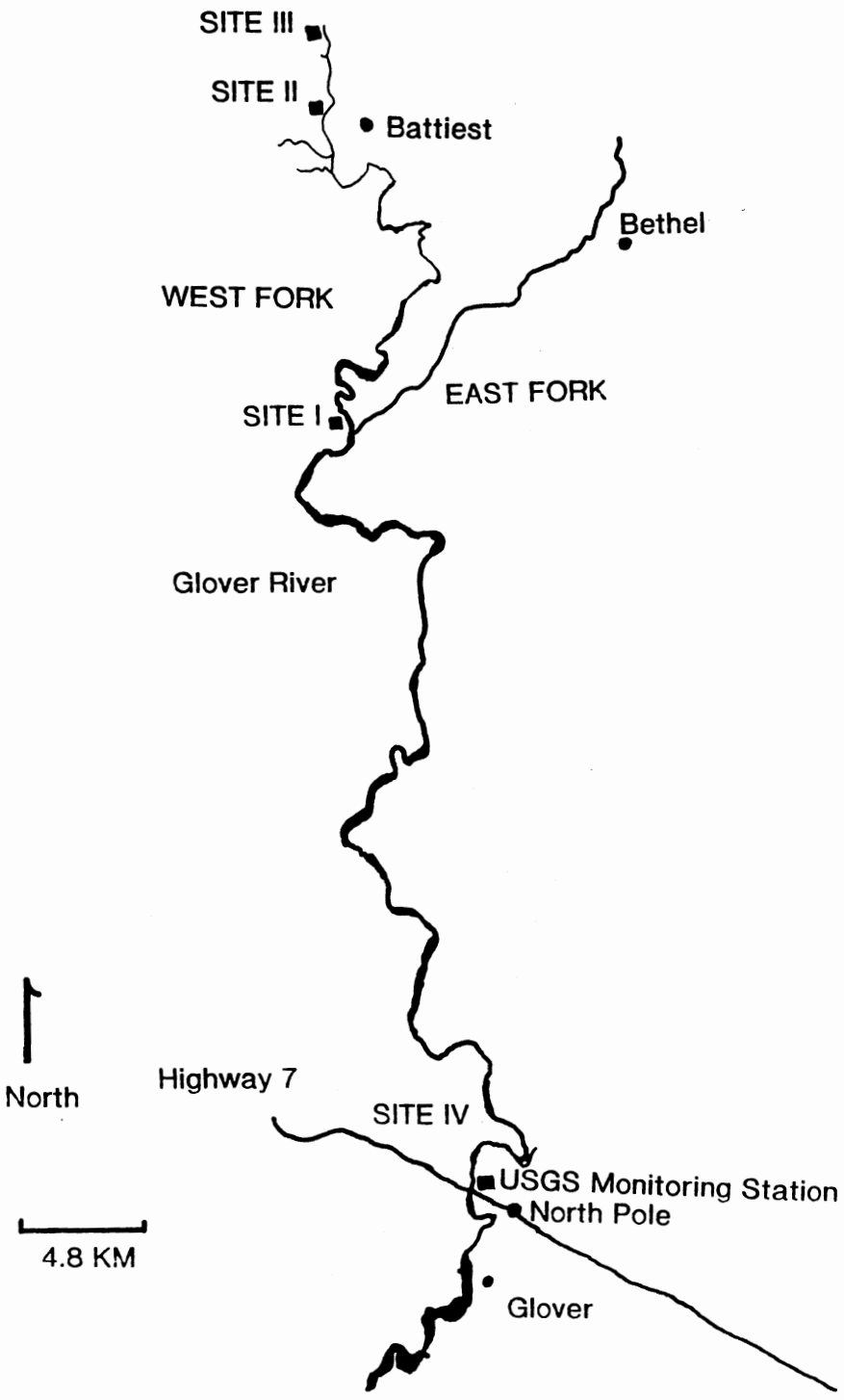


Figure 1. Study sites 1988, 1989, and 1990.

Army Engineers 1975). Discharge is extremely variable with some monthly ranges exceeding 9 m^3/s while others are as low as 0.4 m^3/s. Lowest discharge occurs in August and peak flow is in April. The river is not impounded and frequently floods in the spring and early summer (U.S. Army Corps of Engineers 1975).

The water depth of the Glover River was extremely variable between the fall of 1988 and the summer of 1990. The water depth varied along the river within each season and between seasons at particular locations. The depth of the river at Site II ranged from approximately 30 cm in the summer to 1.5 m in spring. The decrease in the flow and volume of the river in the fall of 1988 reduced stretches of the river to interconnecting pools. The estimated maximum depth at the sites studied during the spring and early summer of 1990 exceeded 6 m when the majority of southern Oklahoma experienced prolonged flooding.

The riverbed as observed from 1988 to 1990 is a rock substrate composed of rocks or a combination of large and small boulders. A heavy growth of periphyton was observed on the rocks of the riverbed and shoreline in the fall and summer of 1988 and 1989. Emergent and floating aquatic macrophytes grew along the shoreline. Grazers such as chironomids and immature odonates were a part of the benthic community and appear to be abundant in the fall and spring. Thick vegetation borders the shoreline with large elm trees and a dense growth of small understory trees and scrubs.

Four sites were sampled for nutrient concentrations, I,II III and VI, in the fall of 1988 and the spring and summer of 1989 (Figure 1), but only site I was sampled in 1990. Likewise site I and II were used for 1988-1989 substrate enrichment studies, but only site I was used in 1990.

Site I is located on a reach of the West Fork of the Glover River. The site is approximately 12-15 km south of the town of Battiest and is on open range used for grazing cattle. Cattle enter the river to drink water approximately 50 m downstream of site I, but did not come in direct contact with the enrichment substrates.

Site II is located approximately 2 km east of the town of Battiest on the west fork of the river. The area immediately bordering the river is heavily wooded and cattle were observed grazing near the water. A large rapids exists approximately 1000 m upstream of site II.

CHAPTER IV

METHODS

Introduction

Three analyses were used. Artificial substrates were used to detect periphyton response to point source nutrient enrichments. Additionally, the periphyton accumulated on the substrates was then used to perform enzymatic and surplus P observations in later observations (experiments 6-10). Grab water samples were also taken to monitor ambient nutrient concentrations.

Physical Parameters

Some physical parameters were also recorded at each study site. A turbidity reading was made at each site with a portable Hach turbidimeter model 16800 and a water temperature reading was determined with a hand held mercury thermometer. A 250 ml water sample was taken for measuring pH. The later was determined with a Corning Model 7 pH meter upon return to Stillwater.

Rainfall data were obtained from the Wes Watkins Agricultural Research Center, Lane OK. Rainfall data was measured in the city of Lane, which was located approximately 13 km to the southeast of Site I. Discharge

data was made available through the USGS. Discharge was measured at Site 07337900 Glover Creek, near Glover OK, which was immediately downstream of Site IV on the downstream side of the bridge on State Highway 3 and 7 and approximately 13 km south of Site I.

Substrate Enrichment Experiments

Substrate enrichment experiments were conducted at Site I and Site II in 1988 and in the spring 1989. Thirteen pots were set out each sample period. The experimental design for Experiments 1, 2, 3, 4, and 5 was,

Treatment	Number	Concentration
Control	5 replicates	0
Nitrogen	4 replicates	0.1 M $NaNO_3$
Phosphorus	4 replicates	0.1 M K_2PO_4

Substrate enrichment experiments were continued at Site I from July 1989 to October 1990. Thirty-two pots were set out each period, twice seasonally or as conditions permitted beginning August 1989. The experimental design for Experiments 6, 7, 8, 9, and 10 was,

Treatment	Number	Concentration
Control	8 replicates	0
Nitrogen	8 replicates	0.1 M $NaNO_3$
Phosphorus	8 replicates	0.1 M K_2PO_4
Nitrogen and Phosphorus	8 replicates	0.1 M $NaNO_3$ 0.1 M K_2PO_4

A list of all experiments by site, season and duration is summarized in Table I.

TABLE I

LIST OF EXPERIMENTS BY SITE, SEASON AND DURATION

EXPERIMENT	SITE	SEASON	DURATION	DAYS
1	I	WINTER	11/6-12/4/88	29
2	II	WINTER	11/6-12/5/88	30
3	I	SPRING	3/15-4/8/89	25
4	II	SPRING	3/15-4/7/89	24
5*	I	SUMMER	6/10-7/1/89	22
6	I	LATE SUMMER	7/29-8/19/89	21
7	I	FALL	9/29-10/21/89	23
8	I	SUMMER	6/6-6/26/90	11
9**	I	FALL	9/9-9/26/90	18
10	I	FALL	9/26-10/15/90	20

* No Nitrogen releasing substrates were recovered from Site I due to a flood.
** No Control Substrates were recovered due to a flood.

The enrichment experiments followed the technique outlined by Fairchild et al. (1985). Clay flower pots served as artificial substrates for periphyton colonization. Clay flower pots filled with 2% agar served as controls. Nitrate or phosphorus were added to the agar of the treatment pots. After exposure of 11 to 30 days, subsamples from four pots per treatment were used as sources for material for chl *a*, particulate carbon, and heterocyst density in experiments 1-10. In experiments 6 and 7 surplus P was determined using subsamples from four pots not sampled as above for biomass. Both surplus P and APA were determined using subsamples from four pots per treatment in experiments 8,9 and 10. Subsampling of the same substrate allowed for the direct comparison of APA to surplus P within each treatment.

The periphyton was scraped with a razor blade from the surface of the pots. The total area scraped was 180.6 cm^2 and did not include the lip or bottom of the pot. The samples used for chl *a*, particulate carbon analysis, and heterocyst density identification were placed in a polyethylene bottle, put on ice and transported back to the laboratory. The samples used for APA and surplus P analysis were placed in polyethylene bottles with distilled, deionized water and frozen with solid CO_2(dry ice) following Perrin et al. (1987) and transported to the laboratory. Freezing the algal cells prevented the dilution of APA as a result of cell division (Biggs, 1987).

TABLE II

LIST OF SUBSTRATE EXPERIMENTS AND PERIPHYTON ASSAYS

EXPERIMENT	ASSAYS	# OF SUBSTRATES PER ASSAY PER TREATMENT
1-5	PARTICULATE CARBON and CHL A	4
4	HETEROCYSTS	4
6-7	PARTICULATE CARBON and CHL A	4
6	HETEROCYSTS	4
	SURPLUS P	4
8-10	PARTICULATE CARBON and CHL A	4
8	HETEROCYSTS	4
	SURPLUS P and APA	4

Water Collection and Preservation

Surface water samples were collected both on the day substrates were set out and the day they were removed. Triplicate 500 ml subsurface water samples were taken upstream of the substrates. One of the samples was acidified using approximately 1-2 ml concentrated sulfuric acid and was later analyzed for total phosphorus (TP). Another sample was acidified and later analyzed for nitrate and ammonia after adjustment of pH. Another sample was preserved with 5 ml of chloroform proceeding analyzed for

soluble reactive phosphorus (SRP). After collection, all samples were placed in a 500 ml polyethylene bottle held in the dark and transported on ice. Transit time for all samples was 7-9 h.

Nutrient concentrations of water samples taken at the study site were measured within 12 h of their collection or they were frozen at -5° C until analysis could be completed.

Grab water samples were also collected for APA analysis. The water samples were frozen with solid CO_2, transported back to the laboratory, and kept frozen until the analysis.

Laboratory Analysis

Periphyton samples were diluted and replicates were analyzed for either particulate carbon or chlorophyll *a* concentration. The algal samples were diluted to a volume of 2 l. Replicate aliquots were then filtered through 1.2 μm Whatman 4.25 cm GF/C glass fiber filters pre-muffled at 232.2 °C for 3 h. Particulate carbon concentration of the sample on the filter was then measured by wet oxidation (Strickland and Parsons 1972).

Replicate aquilots from the 2 l dilution were used for the chl *a* analysis. Duplicate water samples were passed across 0.8 μm AA Millipore filters. The filters were frozen and stored in the dark at -5 °C until further analysis. Chl *a* concentrations were measured fluorometrically after extraction in 90% acetone (Strickland and Parsons 1972) with

a Turner model 111 fluorometer. The fluorometer was calibrated using known concentrations of chl a (Sigma Chemical Company). All samples from a treatment within an experiment were analyzed simultaneously.

Alkaline phosphatase activity was measured by the hydrolysis of 100 μM 3-O-methylfluorescein phosphate (o-MFP). Particulate APA was analyzed following Bothwell (1988). A 4.5 ml volume of periphyton was placed in a fluorometer tube. The addition of 0.5 ml of 100 μM o-MFP in 10 μM tris buffer was added to the sample to begin the reaction. The test tubes were then sealed with parafilm and inverted. During the next 1 to 2 h at intervals of approximately 30 min the test tubes were mixed by inversion and fluorescence was determined using a Turner model 111 fluorometer.

APA samples were compared to Tris controls. APA was measured as the average increase in fluorescence and converted to absolute units using a standard curve of fluorescence verses orthomethylflourescein (o-MF) concentration. Heated, distilled, deionized water was used as the blank. Activities were expressed in nM P/ unit of biomass per unit time. Parallel fluorometric analysis of chl a concentration of the same subsample were used to normalize particulate APA values to biomass (nM P/μg chl a x min^{-1}).

Sestonic and whole water APA were measured following the technique described by Perry (1972) and modified by Francko (1984). The analysis of whole water and a filtered

water fraction were used to determine dissolved and sestonic APA, respectively. The filtered water fraction was passed across pre-rinsed 1.2 μm Whatman 4.25 cm GF/C glass fiber filter at a vacuum pressure of 0.250 atm. Total APA was measured from a whole water sample. The filtered sample was analyzed for dissolved APA. The APA associated with organic matter, sestonic APA, was determined as the difference between dissolved APA and total APA. Whole and filtered water fractions were analyzed in duplicate.

Extraction of MRP was measured following a modified technique of Wynne and Berman (1980). Replicate volumes of algae were rinsed with 40 ml of distilled deionized water and extracted for 60 min at 100°C. The samples were cooled and centrifuged for 5 min. If samples remained turbid, they were filtered across 1.2 μm Whatman 4.25 cm GF/C glass fiber filters. A 2.7 ml volume of supernatant was placed in a 1 cm cuvette. After a 0.3 ml volume of mixed reagent (Strickland and Parsons 1968) was added to the supernatant, mixed and color was allowed to develop for 10 min. The absorbance of the samples was then measured at 885 nm against a distilled, deionized water blank.

In 1989, duplicate volumes of periphyton were filtered across a pre-muffled 1.2 μm Whatman 4.25 cm GF/C, glass fiber filter. The filter was then dried at 250°C for 2 h, weighed, muffled, and re-weighed. The difference in weight of the muffled and dried filter provided a measurement of the ash wt of the sample. Surplus P was initially normal-

ized to biomass as μg P/ 100 μg ash wt. In 1990, duplicate volumes of algae were filtered across 0.8 AA Millipore filters. The filters were ground with a hand held grinder and chl a concentration was measured fluorometrically following acetone extraction. Surplus phosphorus was normalized to biomass using chl a following Wynne and Berman (1980) (μg P/ mg chl a).

After return into the laboratory, water samples were prepared for nutrient analysis. Water samples were filtered through a Whatman 4.25 cm glass fiber filter with a pore diameter of 1.2 μm. All filters were pre-rinsed serially with two 100 ml washes of distilled and deionized water.

Dissolved nutrient analysis involved methods that involve color production in proportion to concentration. Absorbance was measured with a Shimadzu TB-85 spectrophotometer using standards that had been prepared daily. All samples were measured against distilled, deionized water as the absorbance blank. All samples were measured before reaction and that value was used as a turbidity correction.

SRP was measured with the ascorbic acid technique described by Strickland and Parson (1968). Triplicate 10.0 ml subsamples were combined and mixed with 1.0 ml of mixed reagent in a 5.0 cm cell. Absorbance was determined using distilled, deionized water as a blank at 885 nm.

Total phosphorus (TP) concentrations were measured following the ammonium persulfate digestion technique, EPA 365 2-4 (EPA Methods for Chemical Analysis of Water and

Wastes 1979). This water sample was not filtered. Absorbance was determined at 880 nm as above.

Ammonia concentration was measured following Solorzano (1968). Prior to reaction, the water sample was neutralized to a pH of 7.0 with 0.1 M NaOH. Glassware was steamed prior to use. Triplicate, 10 ml samples were prepared for each analysis. Sample color was developed in the dark for 1 hour. Absorbance was determined at 640 nm as above.

Nitrate concentration was measured as nitrite following reduction with cadmium (Strickland and Parsons 1968). Prior to reaction, the water sample was neutralized to a pH of 7. Triplicate or duplicate 10.0 ml samples were prepared for each analysis. Absorbance was determined at 543 nm as above.

Heterocyst density was measured from subsamples of periphyton scraped from the substrates. Samples were preserved in Lugol's solution. Samples were examined following Greeson et al. (1977). A ml fraction of a preserved sample was viewed under 400x magnification. Twenty random fields were examined and the organisms were identified as either Chrysophycota, Chlorophycota (filamentous or non-filamentous), or Cyanophycota using Prescott (1977). The cell number and heterocysts of the bluegreen algae were counted. Heterocyst frequency was expressed as total heterocysts/ total cell number.

Statistical Analysis

Mean biomass of periphyton that had accumulated on the different nutrient treatments were compared to each other and to controls for each experimental period. Areal particulate carbon and chl *a* concentrations respectively were used to estimate biomass. A rank transformation was performed on the biomass data before analysis (Conover and Iman 1981). Following transformation, a one-way ANOVA was performed to determine differences in accumulation among treatments. A Tukey analysis was then used to make pairwise comparisons of treatment at a 95% level of confidence unless otherwise noted.

CHAPTER V

RESULTS

Physical Parameters

The pH of the Glover River ranged from 5.2 to 7.5. Lower pH values may have been produced by the lag between sample collection and measurement. The pH values were neutral to slightly acidic (5.2). These values indicated that alkaline phosphatases could be monitored and were potentially functional. In highly acidic environments alkaline phosphatases do not function maximally, limiting their importance.

The turbidity of subsurface water samples of the Glover River as measured in Nephelometric Turbidity Units (NTU's) ranged from 2.3 to 25 NTU's throughout the study. Turbidity was highest during October 1989 and February, April, September, and October 1990 when values equaled or exceeded 20 NTU's. Light may have been limiting the growth of periphyton. Turbidity was attributed to suspended algae and sand particles.

Monthly rainfall and mean monthly discharge followed similar trends throughout the study. In November and December of 1988 total monthly rainfall was below 7.6 cm. Total monthly rainfall peaked in May and September of 1989

with rainfall exceeding 17.8 cm. In 1990, total monthly rainfall exceeded 12.7 cm in January and February and 20.3 cm from March to May. These conditions resulted in flooding during the first five months of 1990.

Throughout the study discharge was highly variable (Table III). Mean daily discharge rapidly increased following rainfalls. Discharge that exceeded 42 m^3/s displaced the nutrient releasing substrates. Discharge that exceeded 200 m^3/s probably disrupted attached algae by scouring artificial substrates. In Experiment 5 an hourly discharge exceeded 707 m^3/s, one of the highest hourly rates measured in the USGS sampling year from October 1988 to October 1989.

TABLE III

LIST OF MEAN AND MAXIMUM DAILY DISCHARGE BY EXPERIMENT

EXPERIMENT	DATE	MEAN m^3/s	MAXIMUM m^3/s
1,2		19.4	138.0
3,4		17.0	98.0
5		21.3	181.0
6		3.0	19.6
7		0.4	1.4
8		3.2	18.5
9		3.8	43.4
10		23.4	200.5

A rapid change in discharge that coincided with flooding in the Glover River potentially disrupted the periphyton community in all experiments except Experiments 6 and 7 when discharge did not exceed 11.3 m^3/s. In general it appears that high discharge which disrupted the attached community may have confounded biomass, surplus P, and APA measurements. Differences across treatments may have been erased by scouring, which likely occurred due to the extremely high discharge.

Nutrient Enrichment Data

Potential nutrient limitation is defined here as significant differences between the mean control and treatment biomass or between two treatment biomass estimates. In addition, the results as measured by particulate carbon and chl *a* estimates must agree. Using these criteria potential nutrient limitation was detected only in Experiments 4 and 6 and possibly 8 (Figures 3-6 and Table IV).

The results of Experiment 4 showed the mean areal particulate carbon concentration of the control, nitrogen (N) and phosphorus(P)-releasing substrates were 82.37, 134.37 and 120.17 μg C/ cm^2, respectively (Figure 3). The biomass of periphyton on the N-releasing substrate was significantly higher than the biomass accumulated on either of the other treatments. The chl *a* concentrations were 0.023, 0.090 and 0.045 mg chl *a* /cm^2 for the control, N, and P-releasing substrates, respectively. These differences

were statistically significant suggesting that nitrogen was limiting final periphyton biomass during this period.

The results of Experiment 6 also showed potential limitation (Figure 4). The mean particulate carbon concentration of periphyton on the control, N, and P-releasing substrates was 68.21, 154.40, and 92.69 μg C/ cm^2 respectively. The accumulation of periphyton on the N-releasing substrate was higher than the control and P-releasing treatment and statistically different than the control. The mean chl *a* concentration was 0.396, 1.18, and 0.588 mg chl *a* /cm^2 on the control, N, and P-releasing substrates. The chl *a* concentration of the periphyton on the N-releasing substrate was significantly higher than the other treatments, suggesting nitrogen limited algal growth during that period.

The third experiment where nutrient limitation appears to have been detected was Experiment 8 (Figure 5). These results, however, were not conclusive following the criteria for determining potential limitation. The mean areal chl *a* concentrations were 0.057, 0.249, 0.047, and 0.292 mg chl *a*/cm^2 for the control, N, P, and NP-releasing substrates, respectively. The N and NP-releasing substrates had significantly higher mean chl *a* concentrations than did the other treatments. The high chl *a* biomass of the NP-releasing substrate, but not the P-releasing substrate alone indicates that the nitrogen enrichment was responsible for the greater final biomass on the NP-releasing substrates. The mean particulate carbon concentrations were 2.39, 2.80, 1.81, and

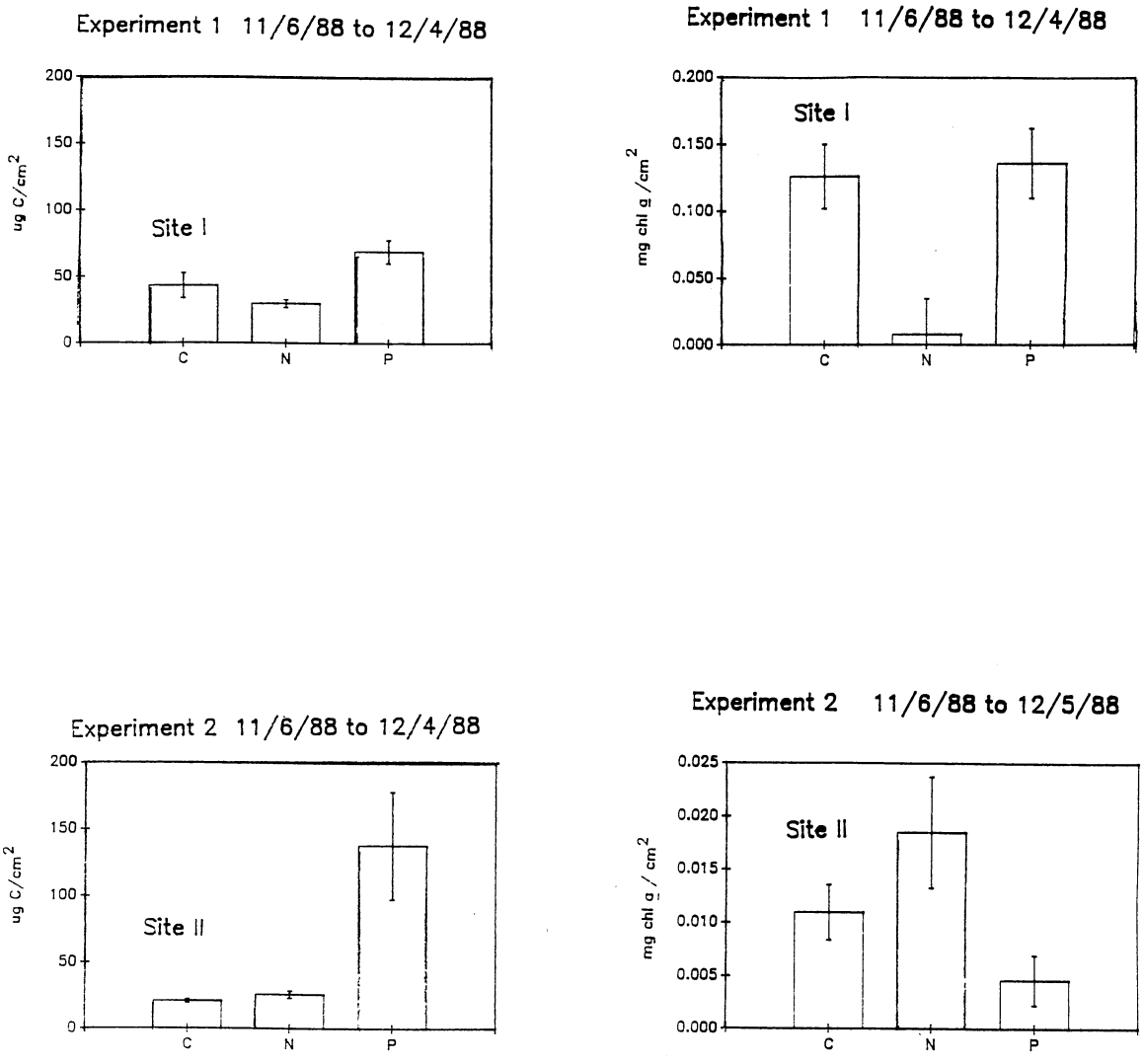


Figure 2. Biomass measured as particulate carbon and chlorophyll a 1988 in substrate experiments. C=control; N and P treatments. Error bars are Standard Error.

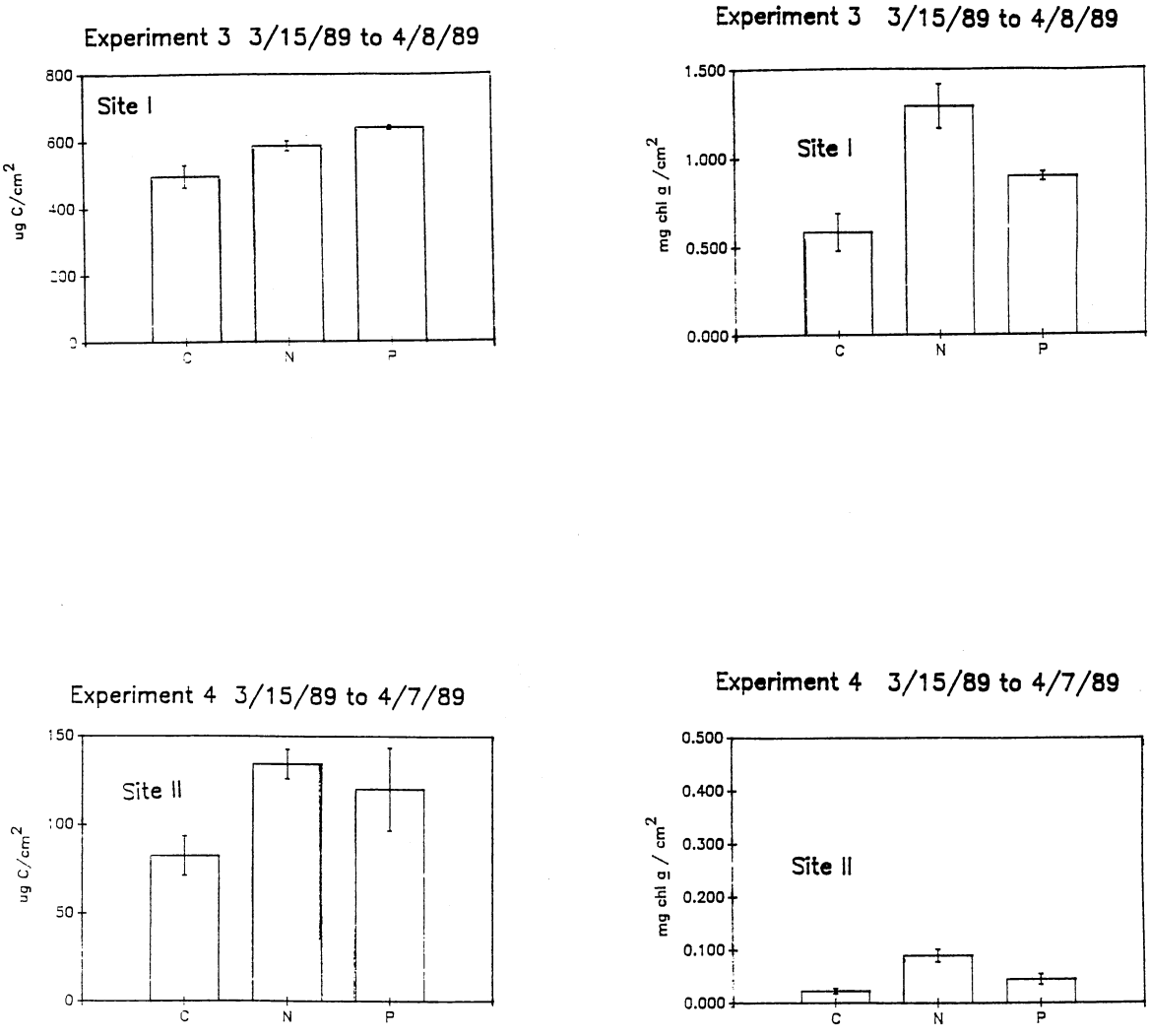


Figure 3. Biomass measured as particulate carbon and chlorophyll a 1989 in substrate experiments. C=control; N and P treatments. Error bars are Standard Error.

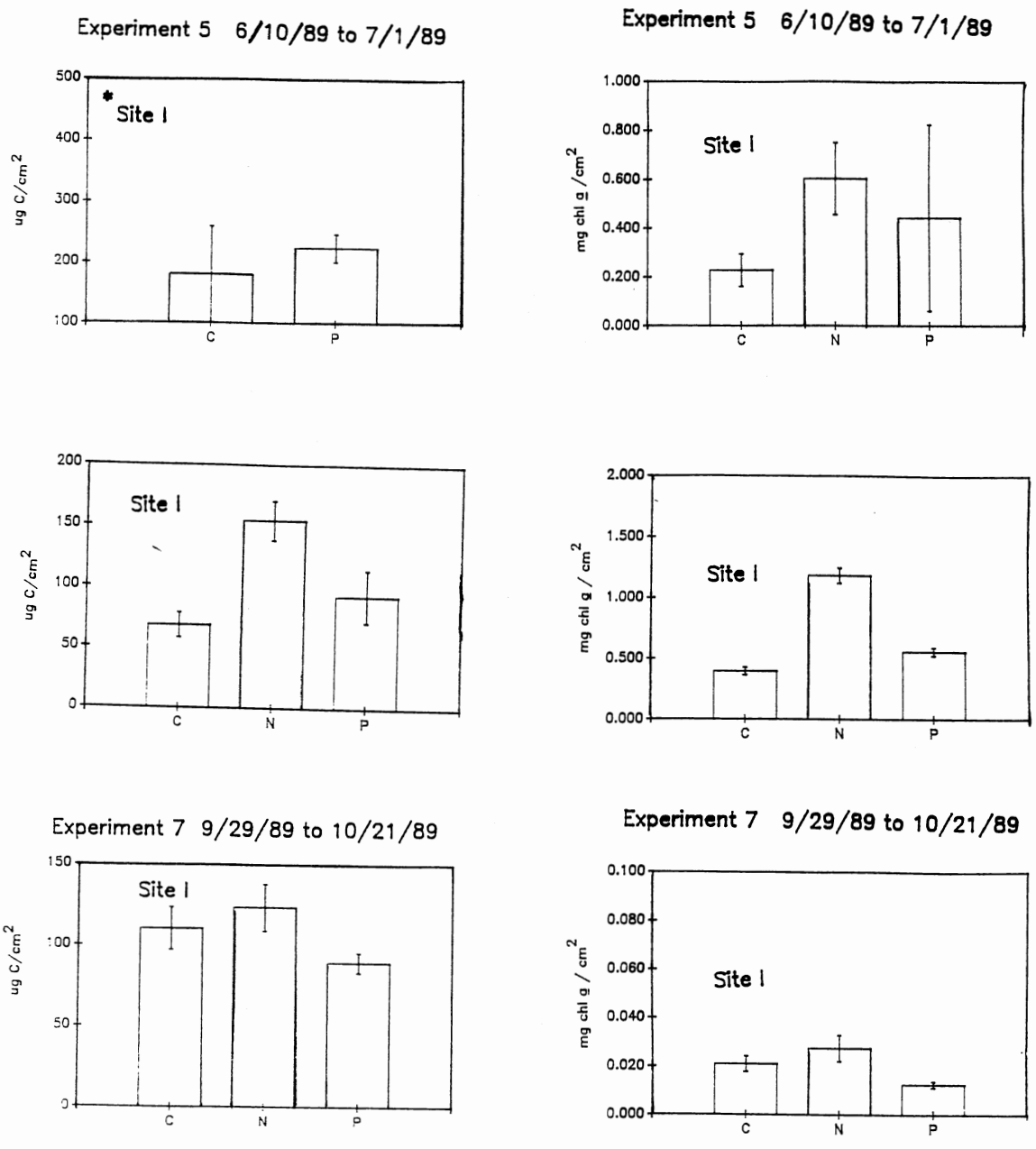


Figure 4. Biomass measured as particulate carbon and chlorophyll a 1989 in substrate experiments.
*Nitrogen treatments were lost in Experiment 5.
C=control; N and P treatments.
Error bars are Standard Error.

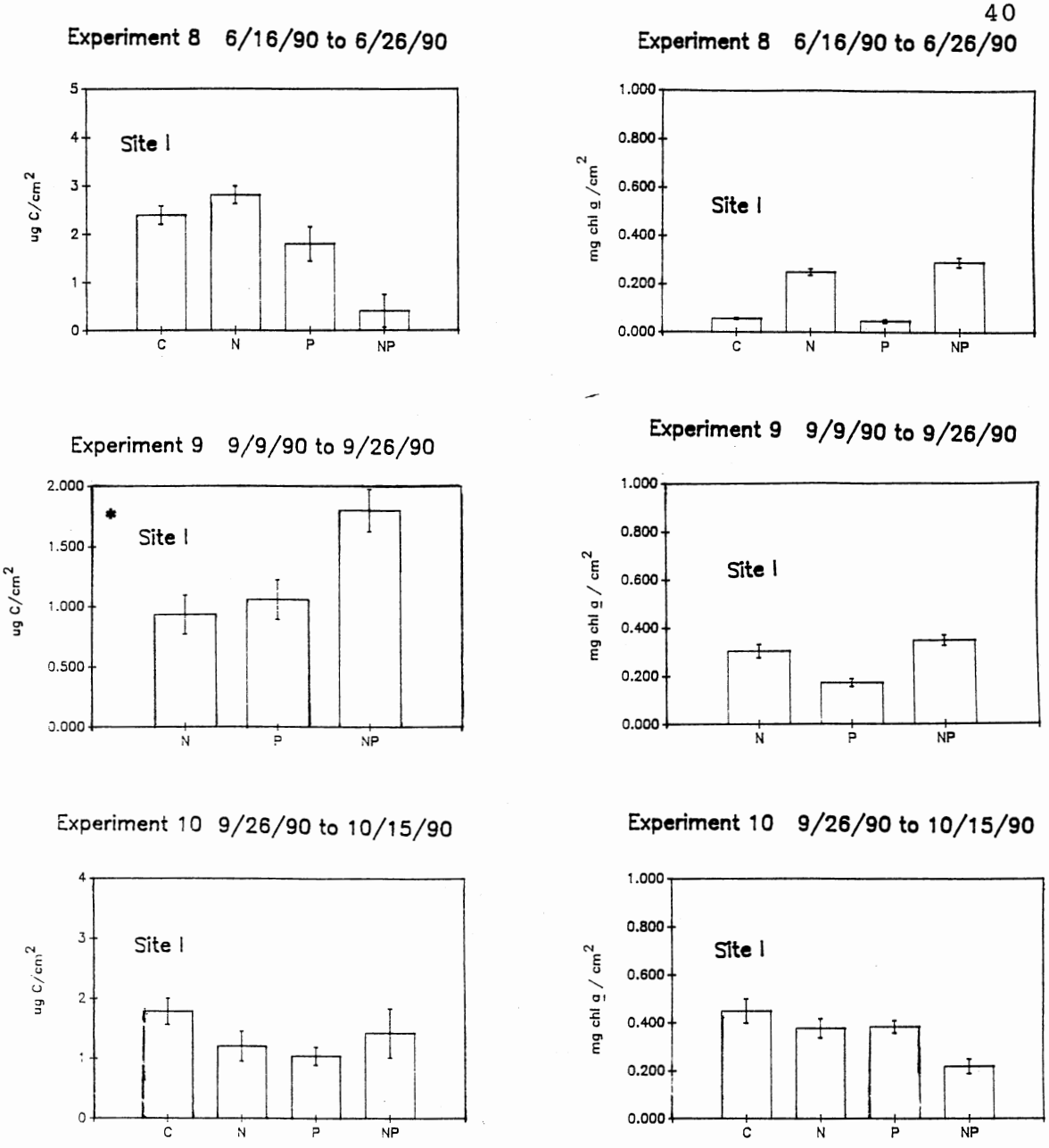


Figure 5. Biomass measured as particulate carbon and chlorophyll a 1990 in substrate experiments.
*Controls were lost in Experiment 9.
C= control; N and P treatments.
Error bars are Standard Error.

TABLE IV

HYPOTHESIS TESTING FOR NUTRIENT ENRICHMENT

+ = POTENTIAL LIMITATION - = NO POTENTIAL LIMITATION

EXPERIMENT	DATE	SITE	CHLOROPHYLL a		PARTICULATE CARBON	
			N	P	N	P
1	11/6-12/4/88	I	-	-	-	+
2	11/6-12/5/88	II	-	-	-	-
3	3/15-4/4/89	I	+	-	-	+
4	3/15-4/4/89	II	+	-	+	-
5	6/10-7/1/89	I	ND	-	ND	-
6	7/29-8/19/89	I	+	-	+	-
7	9/29-10/19/89	I	+	-	-	-
8	6/16-6/28/90	I	+	-	-	-
9	9/9-9/26/90	I	+	-	-	-
10	9/26-10/15/90	I	-	-	-	-

ND= NO DATA

0.41 for the control, N, P, and NP-releasing substrates, respectively.

Although not statistically significant, particulate carbon and chl a biomass trends mirrored each other in Experiments 1 and 7 (Figures 2 and 4). For rest of the experiments one or more of the treatments did not follow similar trends.

P limitation was not predicted for any experiment following the criteria of matching particulate carbon and chl a. P limitation was not predicted in any of the experiments where chl a was used as the biomass estimator. Potential P limitation was predicted in Experiment 1 and 3 when particulate carbon was used as the sole estimator of final areal biomass (Table IV).

APA

The APA activity of the periphyton (Table V) showed no significant differences across treatments within experimental period. The APA of periphyton accumulated in the control and nitrogen releasing substrates was ten times higher than that on the P or NP releasing substrates in Experiment 8. In Experiment 9, the APA of the P releasing substrate was the highest of any treatment and in Experiment 10 the APA of periphyton on the control, N-releasing and NP-releasing substrates was five times higher than that of the P-releasing substrates.

The results of total APA of whole water and dissolved

TABLE V

APA OF PERIPHYTON ACCUMULATED ON NUTRIENT RELEASING SUBSTRATES

		TREATMENT nM P/ μg chl a x min^{-1}			
EXPERIMENT	DATE	CONTROL	N	P	NP
8	6/16-6/28/90	0.235 (0.093)	0.155 (0.141)	0.017 (0.009)	0.019 (0.007)
9	9/9-9/26/90	ND	0.287 (0.054)	0.459 (0.079)	0.234 (0.019)
10	9/26-10/15/90	0.057 (0.011)	0.062 (0.004)	0.016 (0.004)	0.046 (0.015)

( )= STANDARD ERROR
ND= NO DATA

TABLE VI

APA OF WHOLE AND FILTERED WATER SAMPLES

X-I

DATA	WHOLE WATER (nM P/ μg chl a x min^{-1})	FILTERED WATER (nM P / μg chl a x min^{-1})
6/16/90	3.13	NO ACTIVITY DETECTED
6/28/90	4.31	NO ACTIVITY DETECTED
7/19/90	1.16	NO ACTIVITY DETECTED
9/9/90	6.43	NO ACTIVITY DETECTED
9/26/90	27.56	NO ACTIVITY DETECTED
10/15/90	9.8	2.08×10^{-3}

APA of filtered water samples are summarized in Table VI. The APA of the particulate fraction of a whole water sample exceeded that of the dissolved fraction. Most APA of the water samples appears to be attributed to particulate APA.

Surplus P

During 1989, surplus P concentration was measured as μg P/ 100 μg ash wt in experiments in which surplus P was measured in addition to biomass (Table VII). In Experiments 5, 6, and 7, the mean surplus P was largest in periphyton sampled from the P releasing substrates. Experiment 5 was the only experiment in which surplus P of algae of the P releasing substrate was significantly higher than any other treatment.

In Experiments 8-10, significant differences existed in surplus P between the P and N-releasing substrates (Table VIII). In all cases, surplus p was higher on the P or NP-releasing substrates. Surplus P was detected in the control as well as nutrient releasing substrates in Experiment 10. Surplus P was low in Experiments 8-10 and not significantly different from the control substrates suggesting that P demand was so high that P demand was not met with that supplied by the P or NP combination releasing substrates.

The surplus P data was ranked and then means compared following a Tukey procedure (Systat 4.1 1986) (Table IX). Differences in surplus P concentrations were observed

TABLE VII

SURPLUS P OF PERIPHYTON ACCUMULATED ON SUBSTRATES PLACED IN THE GLOVER RIVER

EXPERIMENT	TREATMENT (μg P/ 100 μg ASH WEIGHT)							
	CONTROL		N		P		NP	
5	0.0498	(0.0156)	0.0165	(0.0056)	0.1584	(0.0619)	ND	
6	0.0077	(0.0015)	0.0062	(0.0006)	0.0255	(0.0181)	ND	
7	0.0018	(0.0005)	0.0049	(0.0014)	0.0205	(0.0071)	ND	

ND= NO DATA
( )= STANDARD ERROR

TABLE VIII

SURPLUS P OF PERIPHYTON ACCUMULATED ON SUBSTRATES PLACED IN THE GLOVER RIVER

EXPERIMENT	TREATMENT (μg P/ mg chl a)			
	CONTROL	N	P	NP
8	$2.48 \times 10^{-3} (1.3 \times 10^{-3})$	1.0×10^{-3} (1×10^{-5})	2.9×10^{-3} (1.7×10^{-4})	2.67×10^{-3} (1.3×10^{-3})
9	ND	$4.95 \times 10^{-5} (6.82 \times 10^{-6})$	$7.38 \times 10^{-5} (6.1 \times 10^{-6})$	9.70×10^{-5} (1.6×10^{-5})
10	0 (0)	$6.45 \times 10^{-2} (4.27 \times 10^{-2})$	$8.35 \times 10^{-2} (3.7 \times 10^{-2})$	6.7×10^{-3} (4.45×10^{-3})

ND=NO DATA

TABLE IX

STATISTICAL COMPARISON OF SURPLUS P
ACROSS TREATMENTS WITHIN EXPERIMENT PERIODS USING THE TUKEY PROCEDURE.

+= SIGNIFICANT DIFFERENCE -= NO SIGNIFICANT DIFFERENCE.

DATE	EXPERIMENT	TREATMENT COMPARISONS				
		C:N	C:P	C:NP	N:P	N:NP
6/10-7/1/89	5	-	-	ND	+	ND
7/28-8/19/89	6	-	-	ND	-	ND
9/29-10/19/89	7	-	-	ND	-	ND
6/16-6/28/90	8	-	-	-	+	-
9/9-9/26/90	9	ND	ND	ND	-	+
9/26-10/15/90	10	-	-	ND	-	-

ND= NO DATA

between nutrient releasing (N:P or N:NP) treatments, but not between the control and various treatments.

APA Verses Surplus P

APA of the periphyton was plotted against surplus P by experiment and by treatment (Appendix A) across all experimental periods. When APA was plotted against surplus P in Experiment 8, the control and N-releasing substrates had slightly higher APA than the P and NP-releasing substrates. The plot resembled a vertical line. APA was detected on all substrate treatments in Experiment 8. The corresponding surplus P concentrations were low (>0.003 μg P/mg chl *a*). The plot of Experiment 9 also resembled a vertical line. In both plots APA's were high and surplus P concentrations were low. In Experiment 9 surplus P concentrations did not exceed 0.00004 μg P/mg chl *a*.

The plot of Experiment 10 demonstrated an inverse relationship with high APA values corresponding to low surplus P concentrations (Figure 6). The plot of Experiment 10 showed that the P and NP-releasing substrates had higher surplus P concentrations and lower APA than the N-releasing substrates and controls.

The pooled plot of controls and a pooled plot of APA versus surplus P for all experiments showed the strongest inverse relationship between APA and surplus P. (See Appendix A).

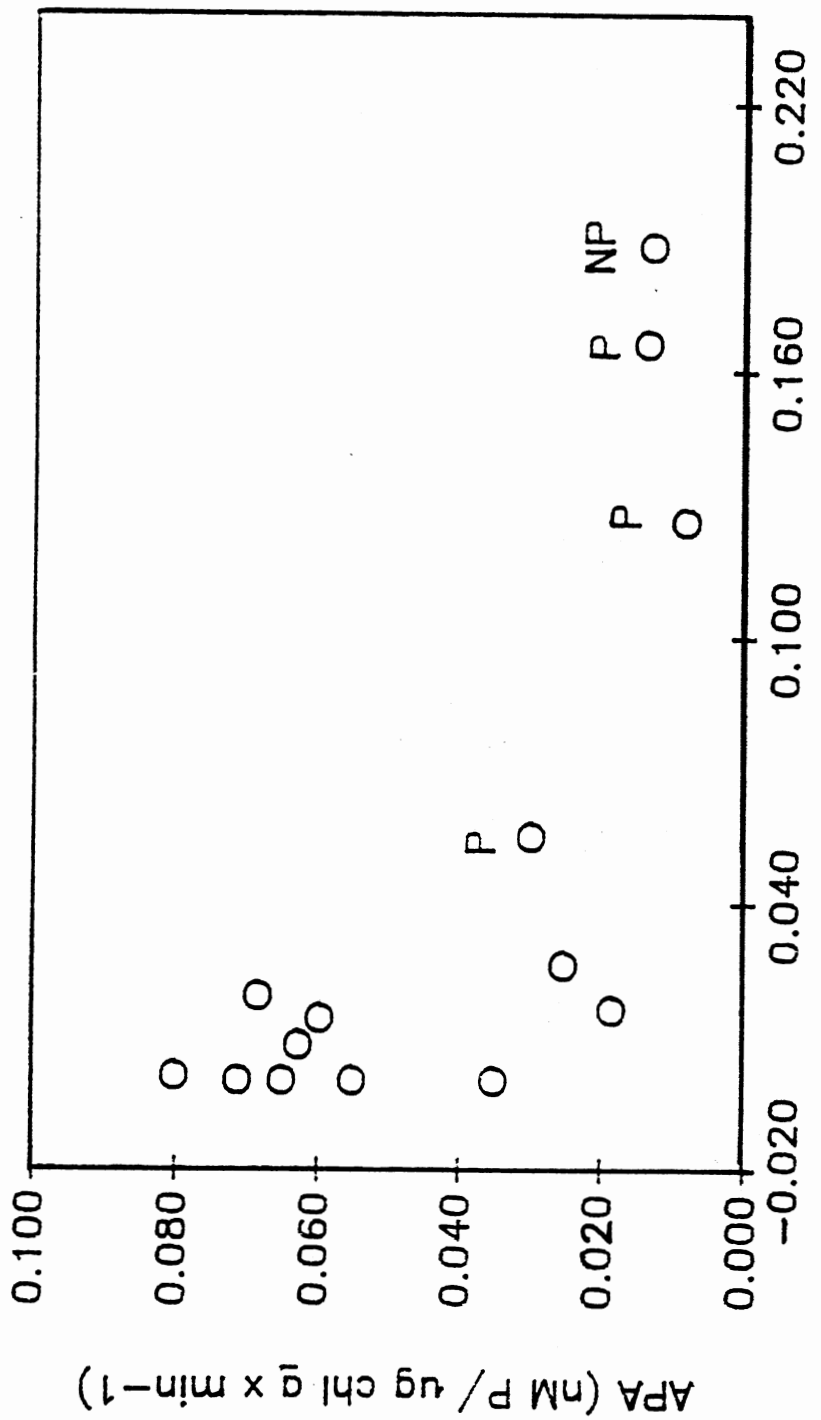


Figure 6. APA Versus Surplus P of all samples combined, Experiment 10.

Nutrient Data

When the study began in November of 1988, the SRP concentrations monitored in the Glover River were exceeded 10,000 μg P/l (Appendix B). The SRP concentrations declined in spring 1989 and then remained below 100 μg P/l from June to October. The mean SRP concentration was 1125 μg P/l in 1989. The ambient total phosphorus, SRP, ammonia and nitrate concentrations and sampling data are given in Appendix B.

Nitrate concentrations were above 380 μg NO_3-N/l in November and December of 1988. In 1989 nitrate concentrations changed markedly. The mean nitrate concentration was 85.83 μg/ l. Periods existed in 1989, when no nitrate was detected.

Ammonia concentrations were less variable than those of the other nutrients monitored in 1988 and 1989. Ammonia concentrations were between 196 and 5.2 μg NH_4-N/ l. Ammonia was not detected on only one of the sampling dates, 6-10-89. The mean ammonia concentration was 55.0 μg NH_4-N/l in 1989.

The SRP concentration was low throughout 1990 (Figure 8). SRP did not exceed 0.7 μg P/L throughout the 1990 sampling period. Nitrate concentration was highest at the beginning and end of the sampling season in 1990 ranging from 6.98 to 155 μg/l. Nitrate concentration exceeded 100 μg/l in January and then declined in the spring and summer.

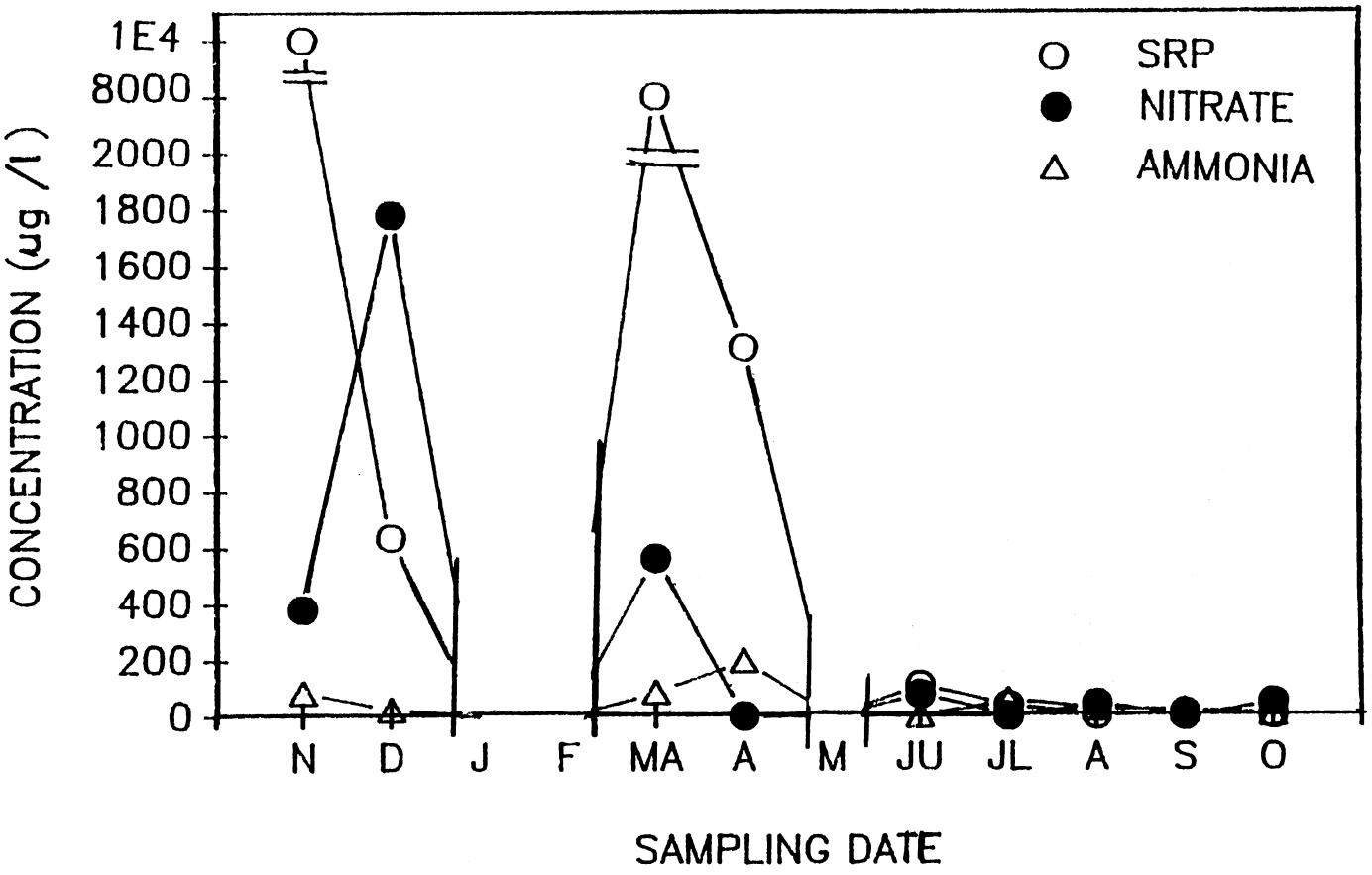


Figure 7. Nutrient Trends for Site 1, Glover River 1988 and 1989.

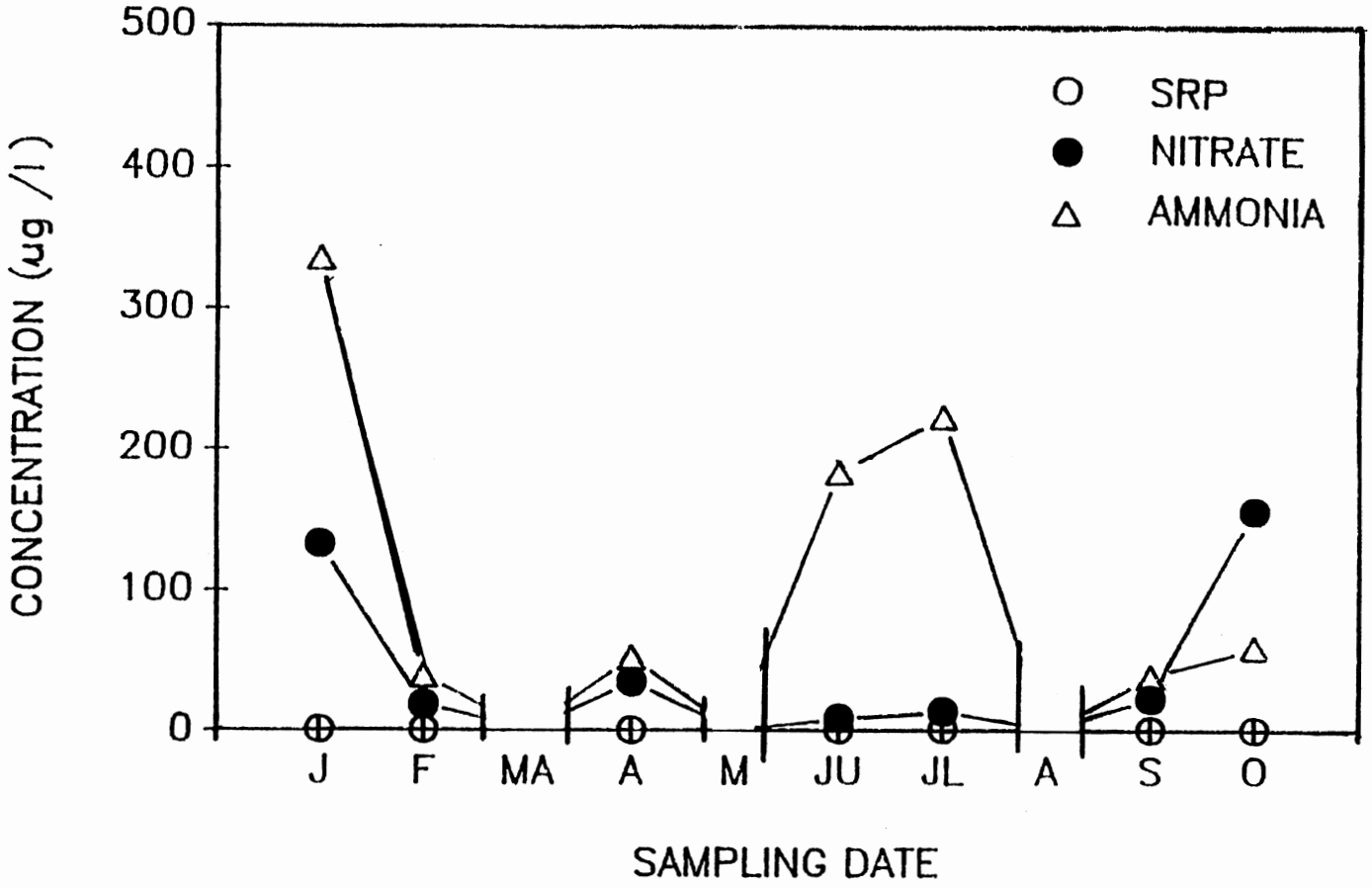


Figure 8. Nutrient Trends for Site 1, Glover River 1990.

Nitrate concentration rose again in late September and early October in 1990. The mean nitrate concentration was 46.25 µg/l. The pattern of ammonia concentration changed markedly
in 1990 (Appendix B). Ammonia concentrations were in excess of 300 µg/l in January and declined to concentrations below 87 µg/l until late June when concentrations rose above 200 µg/l. Ammonia concentrations then declined to the end of the 1990 sampling season. An increase in ammonia concentration from 1990 was probably the result of flooding that occurred in McCurtain County from January to June 1990. Rainfall washes ammonia from the watersheds forest floor before it can be converted to nitrate (Goldman and Horne 1983).

N:P Ratios

During 1989 N:P ratios were generally between 1:1-1:24, except for the values on July 1. During 1990 N:P ratios were greater than 80:1. These data indicate the stream changed from being potentially limited by N to being potentially limited by P during 1990.

Heterocysts

Periphyton were examined for heterocysts in Experiments 4, 6, and 8. In Experiments 4 and 6, potential N limitation was detected using the substrate enrichment technique. No heterocysts were detected in the control and P-releasing

TABLE X

ATOMIC NUTRIENT
NITROGEN(NO_3+NH_4):PHOSPHATE(PO_4^{-3}) RATIOS SITE I

+ = LIMITATION, - = NO LIMITATION, E= exponent, base 10

DATE	RATIO	LIMITATION N	LIMITATION P
11-5-88	1:4	+	-
12-4-88	1:1	+	-
3-15-89	1:24	+	-
4-7-89	1:7	+	-
6-10-89	1:34	+	-
7-1-89	1.68E18:0	-	+
7-29-89	1:3	+	-
8-19-89	2:1	+	-
9-29-89	2:1	+	-
10-21-89	2:1	+	-
1-27-90	640:1	-	+
2-23-90	80:1	-	+
6-16-90	184:1	-	+
6-29-90	448:1	-	+
7-19-90	323:1	-	+
9-9-90	9.5E17:0	-	+
9-26-90	92:1	-	+
10-15-90	2.31E18:0	-	+

substrate samples from Experiments 4 and 6 which conflicted with the substrate results. Heterocysts were found in the samples from Experiment 8. Heterocysts comprised 18% and 8% of the total number of bluegreen cells in the P-releasing and control substrates, respectively. Anabaena was the only genera identified as heterocystous. In Experiment 8 the heterocyst and particulate carbon substrate results agreed.

CHAPTER VI

DISCUSSION

Alkaline Phosphatase Activity

Since few studies of APA of periphyton exist, it is necessary to include comparisons of culture data and APA of phytoplankton in natural populations (Table XI). Bothwell (1985 and 1988) and Perrin et al. (1987) measured APA of periphyton accumulated on styrofoam substrates in the South Thompson and Koegh rivers of British Columbia. Values were 10 to 100 times greater than the activities measured on periphyton from the Glover River. The periphyton in the rivers of British Columbia were under extreme P limitation suggesting that the periphyton in the Glover River were not under as severe P limitation.

Following their extensive work with batch cultures and natural populations, Healey and Hendzel (1979a) suggested that APA in excess of 0.05 nM P/μg chl *a* x min^{-1} represented moderate P deficiency while activity exceeding 0.0833 nM P/μg chl *a* x min^{-1} represented severe P limitation of the algal community. The mean APA of control and N releasing substrates was in excess of 0.155 nM P/μg chl *a* x min^{-1} in Experiments 8 and 9 and exceeded Healey and Hendzel's (1979a) threshold of extreme limitation (controls were not

TABLE XI

MAXIMUM SPECIFIC ACTIVITY OF PHYTOPLANKTON AND PERIPHYTON IN NATURAL WATERS

ARTICLE	SITE	APA ($nM/\mu g$ chl $\underline{a}$ x min^{-1}) unless otherwise stated
	PERIPHYTON	
BOTHWELL(1988)	THOMPSON RIVER	3.3-6.6
BOTHWELL(1985)	THOMPSON RIVER	0.0833
PERRIN(1987)	KOEGH RIVER	3.3-6.6
THIS STUDY		
	Experiment 8 (control)	0.235
	Experiment 9 (control)	NO DATA
	Experiment 10 (control)	0.057
	PHYTOPLANKTON	
FRANCKO(1984)	LAKE LAWRENCE	9.8×10^{-4} nM P/ml x min^{-1}
HEATH AND COOK(1975)	EAST TWIN LAKE	.2833 nM P/ml x min^{-1}
PETTERSON(1980)	LAKE ERKEN	14
		0.22
		0.63
PETTERSON(1980)	LAKE WINDEREMERE	0.06 *
	LAKE GRASMERE	0.09 *
	LAKE HYMEJAURE	0.06
	LAKE STUGJON	0.7
	LAKE MAGNUSJAURE	0.7
	CHESAPEAKE BAY	0.7

*n-NP USED TO DETERMINE ACTIVITY
NOTE RATES HAVE BEEN CONVERTED FROM HOURLY TO PER MINUTE AND FROM μM TO nM FOR CONVENIENCE.

recovered in Experiment 9). The mean APA of control and N-releasing substrates exceeded 0.05 nM P/μg chl $\underline{a}$ x min^{-1} in Experiment 10 and fell into the category of slight deficiency.

Many published APA values have been analyzed in phyto-

plankton from natural systems. Maximum phytoplanktonic activity ranged from 0.06 to 0.7 nM P/μg chl *a* x min^{-1} in a variety of lakes (Table XI). Most of the periphytonic APA in this study fell within this range. This suggests that if periphyton APA is similar to phytoplanktonic APA, then periphyton growth was severely P limited during Experiments 8 and 9 and slightly P limited in Experiment 10.

Particulate APA appeared to comprise most of the APA in whole river water. Values ranged from 1.16 to 27.56 nM P/ μg chl *a* x min^{-1}. Low APA was detected in the filtered water (>2.1 x 10^{-4} nM P/μg chl *a* x min^{-1}). In filtered water samples, APA was attributed to dissolved and bacterial APA (filter size allowed bacteria to pass through). Therefore, most activity in the water was attributed to larger suspended particles.

In Lake Erken, Petterson found that particulate APA exceeded 0.44 nM P/μg chl *a* x min^{-1} when the phytoplankton demonstrated severe P limitation. Particulate APA values of Glover River seston were ten times greater than this value and in 1990 exceeded all the values listed in Table XII. This suggests that P was severely limiting to the seston in the Glover River throughout the Summer and Fall of 1990 when the APA of water samples was monitored.

Surplus P

The results of the surplus P experiments did not demonstrate significant differences in surplus P accumulation

across treatments. Differences between treatments were to serve as a criterion of P limitation. Theoretically, periphyton from the control and N-releasing substrates would have lower surplus P than periphyton from P-releasing substrates.

Fitzgerald and Nelson (1966) suggested 0.08 μg p/100 μg dry wt as a threshold below which P was limiting. Ash wt was used as a measure of biomass instead of dry wt in Experiments 5 to 7 due to the high sediment content of the periphyton samples. The surplus P concentration did not exceed the threshold value above in Experiments 5 to 7 except on the P-releasing substrates.

Surplus P of periphyton was corrected for chl *a* in Experiments 8-10. The surplus P concentrations did not exceed 0.003 μg P/ mg chl *a* for any treatment in Experiments 8-10. Surplus P was as low as 0.0005 μg P/ mg chl *a* in Experiment 10. The ratio of MR surplus P (ng x $cell^{-1}$) and chl *a* (ng x $cell^{-1}$) in Lake Kinneret was compared to surplus P concentrations measured in Experiments 8-10 (Wynne 1981). mg chl *a*. The Lake Kinneret data was collected during algal blooms of *Peridinium*, both from natural populations and batch culture studies. The surplus P of *Peridinium* were between 10 and 10,000 times greater than those measured in Glover River periphyton. The APA associated with the bloom was low and it was predicted that P was not limiting *Peridinium* growth. Therefore, the surplus P concentrations measured in the Lake Kinneret study represent true surplus

storage. Since these values were a magnitude or more greater than the values measured in the Glover River, it appears that a P surplus was not present.

During P limited periphyton growth, the P supplied by the P releasing substrate should release the periphyton colonizing the substrate to grow and provide luxury supplies. No significant differences existed in P storage within periphyton in the P, N, NP, or control substrates in Experiment 10. It appears that P was in such high demand during this period that the P supplied by the substrate did meet demand. No differences existed between control and P-releasing substrates suggesting that even surplus P data in combination with dissolved nutrient concentrations do not afford sufficient information to determine potential P limitation.

APA verses Surplus P

It appears that the periphytonic was under P limitation in Experiments 8, 9, and 10. The absence of surplus P and comparatively higher APA in Experiments 8 and 9 suggest that P limitation was severe. It appears P limitation was moderate in Experiment 10.

When APA was plotted against surplus P in Experiment 10 in a plot of combined data, an inverse APA/surplus P relationship was demonstrated. However, this relationship was not consistent and may be the result of extreme P limitation. When comparing APA across treatments within an

experiment, APA was not statistically higher in controls. Since the P-releasing substrates were providing a point source of P and were theoretically releasing the periphyton from P limitation, I anticipated lower activity on those P releasing substrates. It appears that the periphyton was under severe P limitation and P was not being stored.

Another explanation for this failure to demonstrate an inverse relationship between APA and surplus P may have been attributed to time. If periphyton had not appropriately colonized or had died and sloughed off substrates during high discharge, then biomass, surplus P and APA would be underestimated. Daily or weekly monitoring of APA of periphyton needs to be performed to compensate for scouring that may be removing biomass.

In some cases periphyton cells produced AP in addition to maintaining surplus P stores. This suggests that the periphyton needs to exceed some base storage concentration before AP production ceases. Surplus P measured in Experiment 10 in excess of 0.2 μg P/ mg chl *a* appears to provide some baseline storage at which APA was slightly less than the other treatments. Orthophosphate inhibits APA, production but due to low SRP it is unlikely that orthophosphate was responsible for APA inhibition in 1990.

Nutrients and Periphyton

In the nutrient-releasing experiments conducted in March and through August to September 1989 N limitation was

not detected. Potential P limitation was not detected with this technique. Flash flooding occurred in summer 1989 resulting in the loss of individual pots or whole treatments during summer. High discharge caused scouring which reduced the rate of accumulation on the substrates. High variation across substrates within treatments existed in the majority of the Experiments and reduced the success of this technique.

Nutrient Concentrations

Nutrient uptake kinetics follow Michaelis-Menten enzyme kinetics and can be compared to ambient nutrient concentrations. The half-saturation constants of nutrient uptake in algae in natural and culture experiments provides baseline information to predict potential nutrient demand. When half-saturation values for uptake are greater than ambient concentrations, the potential for nutrient limitation is high.

The half-saturation constant for uptake of nitrate as measured in Lake Windermere periphyton was >200 μg NO_3-N/l (Goldman and Horne 1983). Similar measurements were made in a series of eutrophic lakes for phytoplankton (Goldman and Horne 1983). Under eutrophic conditions, half-saturation constants for uptake exceeded 130 and 420 μg/l. The half-saturation constant of periphyton from Castle Lake ranged from 200-500 μg /l. The concentration of nitrate was below 50 μg NO_3-N/l for the most of 1989 and 1990 in the Glover

River. Nitrogen was potentially limiting periphyton growth under ambient nutrient conditions using this criterion.

The half-saturation constants for uptake of ammonia measured in natural populations ranged from 5 μg/l in oligotrophic lakes to 200-1000 μg /l in periphyton in Castle Lake (Goldman and Horne 1983). Ammonia concentrations did not exceed 350 μg NO_3-N/l in 1989 or 1990 in the present experiment, but these concentrations should be sufficient to saturate uptake and therefore should not have limited periphyton growth.

Phosphorus is needed in only small amounts to provide maximum growth in algae. Enzyme saturation constants vary among species. Concentrations in excess of 3 μg P/l provide enough phosphate-P to saturate the enzyme uptake systems in the majority of algal species tested in culture studies (Smith 1981). Ambient orthophosphate concentrations were in excess of 3 μg P/l in 1988 and the majority of 1989. If ambient concentration reflects availability in the Glover River, phosphorus was not limiting during 1989. Orthophosphate concentrations were consistently below this value or undetectable in 1990. During 1990 phosphorus was potentially limiting due to slow resupply rates as reflected by low ambient concentrations.

N:P Ratios

The atomic nitrogen to phosphate ratios of the water samples were well below the 16:1 ratio from December 1988 to

October 1989, except on 7-1-89 when no phosphorus was detected. Nitrogen was potentially limiting periphyton growth during this period. In 1990, the N:P ratio exceeded the 16:1 ratio from January to October 1990. P limitation was potentially limiting under these nutrient concentrations.

The results of the nitrogen: phosphorus ratios suggest that the conditions leading to potential nitrogen or phosphorus growth did not occur on a seasonal basis from 1989 to 1990. The amount of rainfall and flow in the river appeared to have a great effect on dissolved nutrient concentrations. The switch from an N:P ratio less than 16:1 to one greater than 16:1 occurred throughout 1990 when rainfall increased. The N:P ratios of water samples taken during flooding of 1990 and N:P ratios of samples taken immediately following rainfall suggest that precipitation or runoff of the surrounding watershed supplied inorganic nitrogen to the river. Nutrient retention in the watershed may be low due to the practice of clear-cutting. Therefore, precipitation may be important in adding dissolved and particulate nutrients into the Glover River. In similar studies, rainfall added organic nitrogen to a northern, temperate stream (Wetzel and Manney 1977) and added nitrate to a prairie stream when rainfall followed a period of low flow (Tate 1990).

Heterocysts

Heterocysts were detected in only one of the three experiments in which N was detected as potentially limiting

by the substrate enrichment technique. Therefore, heterocyst formation was not consistent under potentially limiting conditions.

CHAPTER VII

CONCLUSIONS

The substrate enrichment data, ambient nutrient concentrations, and N:P ratios indicate N was limiting the final biomass of periphyton in Winter 1988 and throughout 1989. The APA and surplus P data indicate P was limiting throughout 1990. The demand for P during September and October of 1990 was so high that the P supplied by the NP and P-releasing substrates failed to meet P demand.

The absence of dissolved APA in filtered water samples taken in 1990 when ambient SRP was low, suggests that bacterial activity does not comprise a significant portion of AP activity in the Glover River. APA of whole lakewater samples do not represent algal activity alone. Particulate APA consists of bacterial and algal components in lakes. APA was a practical monitoring device in the Glover River, where whole riverwater APA and periphytonic APA represent algal activity and potential algal P limitation.

Monitoring APA, surplus P of the periphyton, and ambient nutrient concentrations provided sufficient information to predict P limitation. The substrate enrichment technique was not successful for predicting potential N or P limitation or for monitoring differences in APA and surplus P

concentrations. Runoff diluted Glover River SRP concentrations and imported nitrate and ammonia. Runoff also scoured periphyton and increased turbidity. The effects of these physical and chemical factors were of greater magnitude than substrate enrichment on periphyton final biomass.

REFERENCES

Berman, J. 1970. Alkaline phosphatases and phosphorus availability in Lake Kinneret. Limnol. Oceanogr. 15: 663-674.

Biggs, B. J. F. 1987. Effects of sample storage and mechanical blending on the quantitative analysis of river periphyton. Freshwater Biol. 18: 197-203.

Bothwell, M. L. 1985. Phosphorus limitation of lotic periphyton growth rates: An intersite comparison using continuous-flow troughs (Thompson River system, British Columbia). Limnol. Oceanogr. 30: 527-542.

_____ , 1988. Growth rate responses of lotic periphyton diatoms to experimental phosphorus enrichment: the influence of temperature and light. Can. J. Fish. Aquat. Sci. 45: 261-270.

Carrick, H. J., R. L. Lowe, and J. T. Rotenberry. 1988. Guilds of benthic algae along nutrient gradients: relationships to algal community diversity. J. North Am. Benthol. Soc. 7: 117-128.

Chang, W. Y. B. and R. Rossman. 1988. Changes in the abundance of blue-green algae related to nutrient loading in the nearshore of Lake Michigan. Hydrobiologia 157: 271-278.

Chrost, R.J., W. Suida, D. Albrecht, and J. Overbeck. 1986. A method for determining enzymatically hydrolyzable phosphate (EHP) in natural waters. Limnol. Oceanogr. 31: 662-667.

Conover, W.J. and R.L. Iman. 1981. Rank Transformation as a Bridge Between Parametric and Nonparametric Statistics. Am. Stat. 3:124-129.

Cook, E. C. 1967. The Myxophyceae of North Carolina. Ed wards, Ann Arbor, Mich. 206 p.

Darley, W. M. 1982. Algal Biology: a physiological approach. Blackwell Sci. Boston, MA. 168 p.

Department of the Army, Tulsa District, Corps of Engineers, 1975. Draft of Environmental Statement of Glover Creek, Oklahoma. Tulsa, OK, 92 p.

Elwood, J. W., J. D. Newbold, A. F. Trimble, and R. W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62: 146-158.

Fairchild, G. W. and A. C. Everett. 1988. Effects of nutrient (N,P,C) enrichment upon periphyton standing crop, species composition and primary production in an oligotrophic softwater lake. Freshwater Biol. 19: 57-70.

_____ and R. L. Lowe. 1984. Artificial substrates which release nutrients: Effects on periphyton and invertebrate succession. Hydrobiologia 114: 29-37.

_____ ,R. L. Lowe, and W. B. Richardson. 1985. algal periphyton growth on nutrient-diffusing substrates: an in situ bioassay. Ecology 66: 465-472.

Fitzgerald, G. P. and T. C. Nelson. 1966. Extractive and enzymatic analyses for limiting or surplus phosphorus in algae. J. Phycol. 2: 32-37.

Fleet, R. J., D. W. Schindler, R. D. Hamilton, and N. E. R. Campbell. 1980 . Nitrogen fixation in Canadian Shield Lakes. Can. J. Fish. Aquat. Sci. 37: 494-505.

Francko, D. A. 1984a. Phytoplankton metabolism and cyclic nucleotides II. Nucleotide-induced perturbations of alkaline phosphatase activity. Arch. Hydrobiol. 100: 409-421.

_____ , 1984b. Relationships between phosphorus functional classes and alkaline phosphatase activity in reservoir lakes. J. of Freshwater Ecol. 2: 541-547.

Greeson, P.E., T.A.Ehlke, G.A. Irwin, B.W. Lium, and K.V. Slack (eds). 1977 Methods for collection and analysis of aquatic biological and microbiological samples. Book 5, Chapter A4. U.S. Government Printing Office, Washington, D.C.

Grimm, N.B. 1987. Nitrogen dynamics during succession in a desert stream. Ecology 68: 1157-1170.

Goldman, C. R. and A. J. Horne. 1983. Limnology. McGraw-Hill Inc., New York, NY. 464 p.

Healey, F. P. 1973. Characteristics of phosphorus deficiency in Anabaena. J. Phycol. 9: 383-394

_____ , and L. L. Hendzel. 1979a. Fluorometric measurement of alkaline phosphatase activity in algae. Freshwater Biol. 9: 429-439.

_____ , and L. L. Hendzel. 1979b. Indicators of phosphorus and nitrogen deficiency in five algae in culture. J. Fish. Res. Board Can. 36: 1364-1369.

Heath, R. T. and G. D. Cooke. 1975. The significance of alkaline phosphatase in a eutrophic lake. Verh. Internat. Verein. Limnol. 19: 959-965.

Hill, W. R., and A. W. Knight. 1988. Nutrient and light limitation of algae in two northern California streams. J. Phycol. 24: 125-132.

Horner, R. R. and E. B. Welsh. 1981. Stream periphyton development in relation to current velocity and nutrients. Can. J. Fish. Aquat. Sci. 38: 449-457.

Howarth, R. W. and R. Marino. 1988. Nitrogen fixation in freshwater, estuarine and marine ecosystems. 2. Biogeochemical controls. Limnol. Oceanogr. 33:688-701.

Janson, M. 1976. Phosphatases in lake Water: characterization of enzymes from phytoplankton and zooplankton by gel filtration. Science 194: 320-321.

Jorgensen, B. B., and N. P. Revbeck. 1985. Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnol. Oceanogr. 30: 111-122.

Lowe, R. L., and W. F. Gale. 1980. Monitoring river periphy ton with artificial benthic substrates. Hydrobiologia 69: 235-244.

Newbold, J. D., J. W. Elwood, R. V. O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiralling in streams. Can. J. Fish. Aquatic Sci. 38: 860-863.

O'Brien, W. J. and F. DeNoyelles, Jr. 1976. Response of three phytoplankton bioassay techniques in experimental ponds of known limiting nutrient. Hydrobiologia 49: 65-76.

Odum, E. P. 1983. Basic Ecology. CBS College Publishing, Philadelphia, PA. 613 p.

Ostrofsky, M. L. and F. H. Rigler. 1987. Chlorophyll-phosphorus relationships for subarctic lakes in western Canada. Can. J. Fish. Aquat. Sci. 44: 775-781.

Paul, B. J. and H. C. Duthie. 1988. Nutrient cycling in the epilithon of running waters. Canadian J. Bot. 67: 2302-2309.

Perrin, C. J., M. Bothwell, and P. A. Slaney. 1987. Experimental enrichment of a coastal stream in British Columbia: Effects of organic and inorganic additions on autotrophic periphyton production. Can. J. Fish. Aquat. Sci. 44: 1247-1256.

Perry, M. J. 1972. Alkaline phosphatase activity in subtropical Central North Pacific waters using a sensitive fluorometric method. Mar. Biol. 15:113.

Peterson, B. J., J. E. Hobbie, T. L. Corliss, and K. Kriet. 1983. A continuous flow periphyton bioassay: Tests of nutrient limitation in a tundra stream. Limnol. Oceanogr. 28: 583-591.

_____ , J.E. Hobbie, A.E. Hersey, M.A Lock, et al. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science 229:1383-1387.

Petterson, K. 1980. Alkaline phosphatase activity and algal surplus phosphorus as phosphorus-deficiency indicators in Lake Erken. Arch. Hydrobiol. 89: 54-87.

Pick, F. R. 1987. Interpretations of alkaline phosphatase activity in Lake Ontario. Can. J. Fish. Aquat. Sci. 44: 2078-2094.

Pick, F.R. and D.R.S. Lean. 1987. The role of macronutrients (C,N,P) in controlling cyanobacterial dominance in temperate lakes. New Zealand J. Marine Freshwater Research. 21: 425-434.

Power, M.E. and A.J. Stewart. 1987. Disturbance and recovery of an algal assemblage following flooding in an Oklahoma Stream. Am. Midl. Nat. 117:333-345.

Prescott, G.W. 1978. How to know the freshwater algae. Sparks Press, Raleigh, North Carolina. 342 p.

Pringle, C. M. 1987. Effects of water and substratum nutrient supplies on lotic periphyton growth: an integrated bioassay. Can. J. Fish. Aquat. Sci. 44: 619-629.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. American Scientist 205-221.

Reichardt, W. et al. 1967, Free dissolved enzymes in lake waters. Nature 216: 1345-1347.

Reisen, W. K. and J. S. Deirdre. 1970. Succession and Current demand relationships of diatoms on artificial substrates in Praters Creek, South Carolina. J. Phycol. 6: 117-121.

Rhee, G-Yull. 1978. Effects of N:P ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. Limnol. Oceanogr. 23: 10-25.

Rigler, F.H. 1956. A tracer study of the phosphorus cycle in lakewater. Ecology 37: 550-562.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. Science 195: 260-262.

Short, R.A. 1988. Final Report: Phosphorus Study on the Tributaries of the Colorado River. Interagency Cooperative Contract Number 14-70025. 75 p.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221: 669-671.

_____, 1986. Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. Can. J. Fish. Aquat.Sci. 43: 148-153.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799-801.

Stevens, R. J. and M. P. Parr. 1977. The significance of alkaline phosphatase activity in Lough Neagh. Freshwater Biology 7: 351-355.

Stewart, A. J. and R. G. Wetzel. 1982. Phytoplankton contribution to alkaline phosphatase activity. Arch. Hydrobiol. 93: 265-271.

Strickland, J. D. H. and T. R. Parsons. 1968. A Practical Handbook of Seawater Analysis. Bull. Fish. Res. Board Canada, 167. 311 p.

Suida, W. and R.J. Chrost. 1986. The relationship between alkaline phosphatase (APA) activity and phosphate availability for phytoplankton and bacteria in eutrophic lakes. Acta Microbiol. Polonica. 36: 247-257.

Tate, C.M. 1990. Patterns and controls of nitrogen in tallgrass prairie streams. Ecology 71: 2007-2018.

US Environmental Protection Agency. Methods for chemical analysis of water and waste. 1979. Environmental monitoring and support laboratory, Cincinnati, OH, 430 p.

Welsh, E. B., J. M. Jacoby, R. R. Horner, and M. R. Seeley. 1988. Nuisance biomass levels of periphytic algae in streams. Hydrobiologia 157: 161-168.

Wetzel, R. G. 1981. Long-term dissolved and particulate phosphatase activity in hardwater lake in relation to lake stability and phosphorus enrichment. Verh. Internat. Verein. Limnol. 21: 337-349.

_____ , 1983. Limnology. CBS College Publ.Co. Philadelphia, PA, 110 p.

_____ , R.G. and B.A. Manny. 1977. Seasonal changes in particulate and dissolved carbon and nitrogen in a hardwater stream. Archiv fur Hydrologie. 80: 20-39.

Wynne, D. 1981. Phosphorus, phosphatases and the Peridinium bloom in Lake Kinneret. Verh. Internat. Verein. Limnol. 21: 523-527.

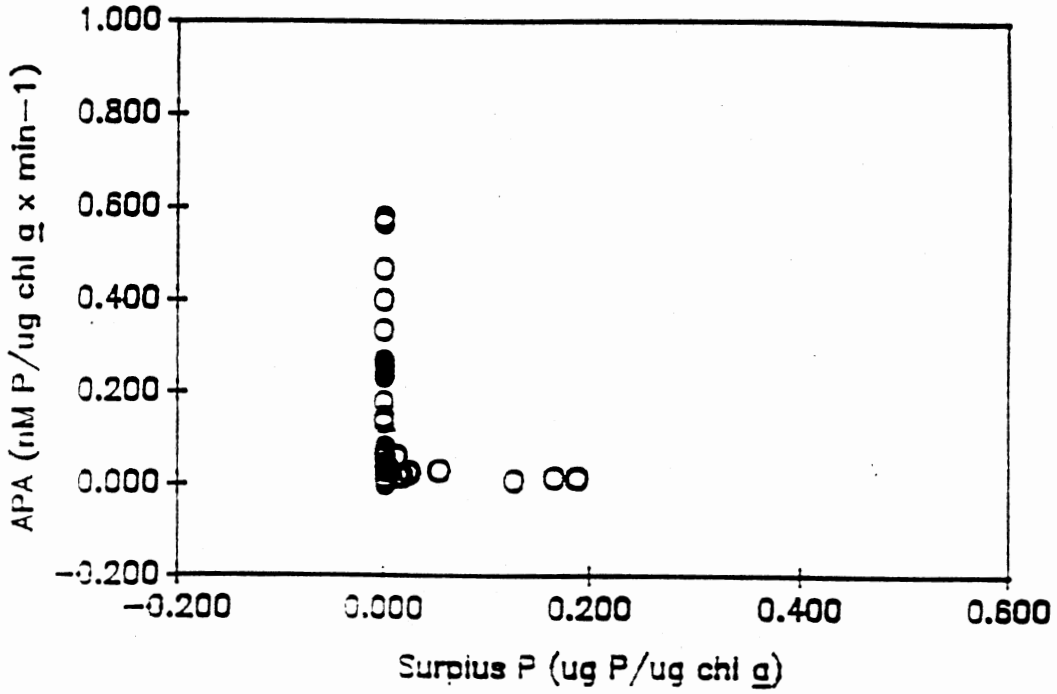
_____ , and Berman, T. 1980. Hot water extractable phosphorus-an indicator of nutritional status of Perdinium Cinctum (Dinophyceae) from Lake Kinneret (Isreal). J. Phycol. 16:40-46.

APPENDIXES

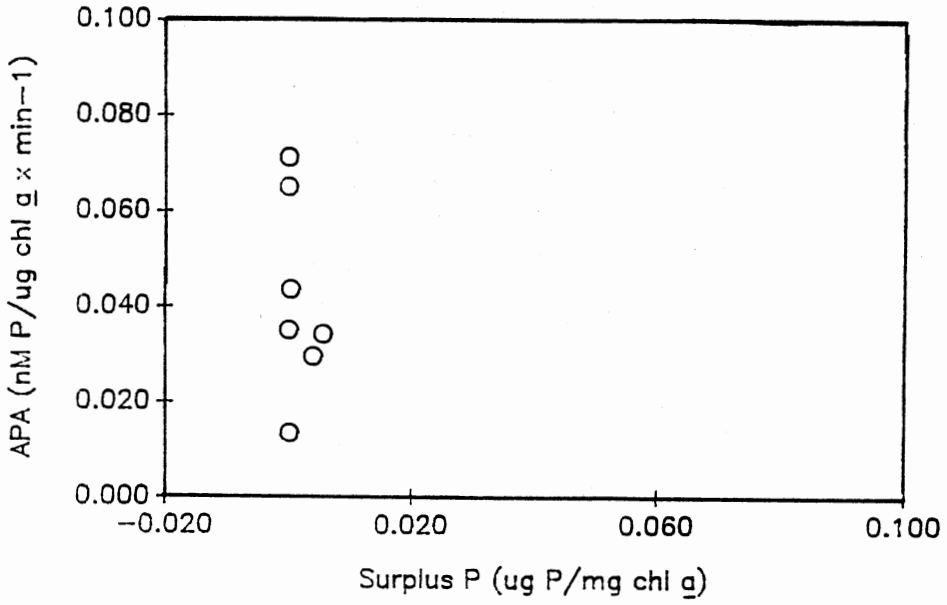
APPENDIX A

APA VERSUS SURPLUS P FOR 1990

APA Verses Surplus P of all Treatments



APA Versus Surplus P of Control Substrates



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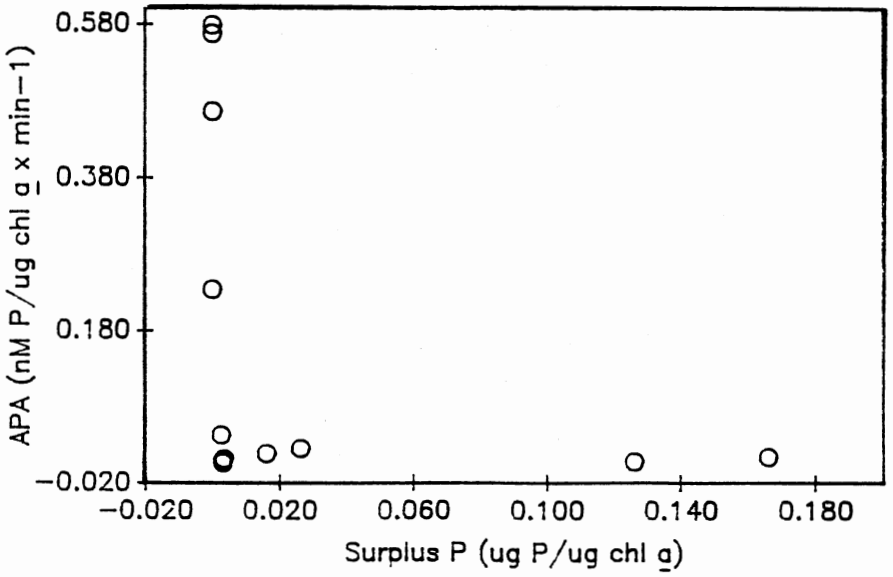

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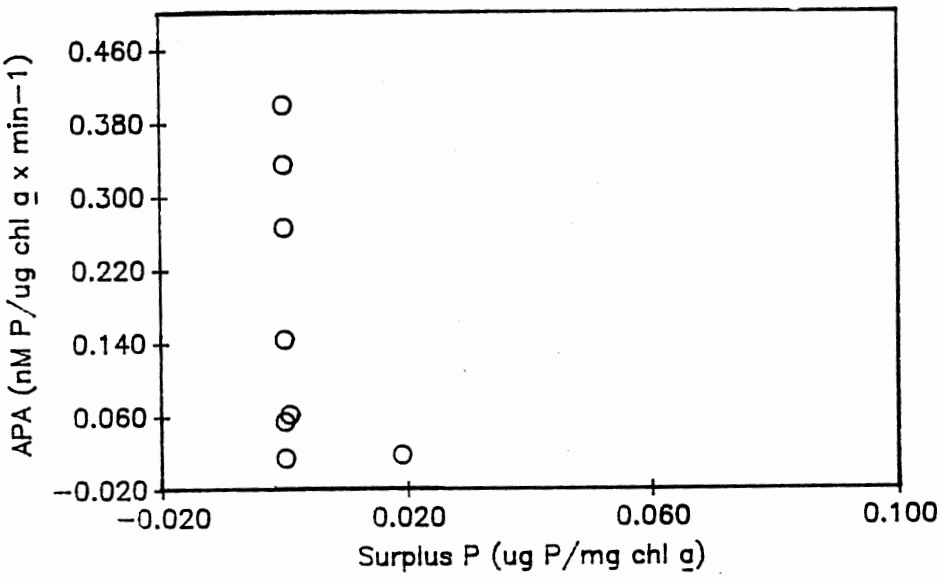
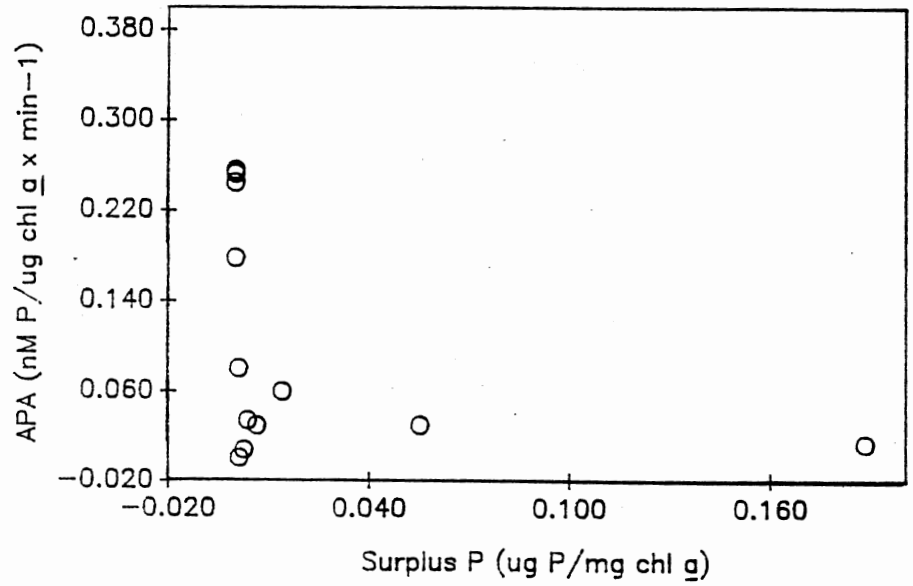


APA Versus Surplus P of NP releasing Substrates



APPENDIX B

NUTRIENT DATA FOR SITES I,II,III AND IV, GLOVER RIVER FOR 1988, 1989 AND 1990

NUTRIENT DATA FOR SITE I, GLOVER RIVER, FOR 1988, 1989 AND 1990.

DATE	TOTAL P (μg P/l)	SRP (μg P/l)	NITRATE (μg NO_3-N/l)	AMMONIA (μg NH_4-N/l)
11-5-88	2,383,000	10,099	380	85
12-4-88	46,000	635	1,780	23.5
3-15-89	ND	7,490	560	85
4-7-89	1,320	1,310	0	196
6-10-89	895	110	78	0
7-1-89	1,410	0	0	89.5
7-29-89	1,360	75.5	0.1	22
8-19-89	5,850	2.1	43.2	23.5
9-29-89	1,790	12.5	1.4	19.4
10-21-89	770	3.7	5.0	5.2
1-27-90	0.0	0.59	132.05	335.15
2-23-90	4.89	0.56	18.34	38.70
4-4-90	0.0	ND	34.29	51.53
6-16-90	ND	0.47	6.47	86.81
6-29-90	2.99	0.61	11.30	277.98
7-19-90	2.96	0.68	13.69	221.98
9-9-90	1.95	0.0	6.98	47.86
9-26-90	2.50	0.46	38.12	27.7
10-15-90	1.13	0.0	155.06	58.4

ND= NO DATA

NUTRIENT DATA FOR SITE II, GLOVER RIVER, FOR 1988 and 1989

DATE	TOTAL P (μg P/l)	SRP (μg P/l)	NITRATE (μg NO_3-N/l)	AMMONIA (μg NH_4-N/l)
11-5-88	ND	7,640	234	44.5
12-4-88	5,500	14,550	690	62.5
3-15-89	ND	7,500	560	85.5
4-7-89	4,840	1,380	600	848
6-10-89	0	8.5	14	160
7-1-89	245	0.4	152	169
7-29-89	ND	ND	0	13.5

ND=NO DATA

NUTRIENT DATA FOR SITE III, GLOVER RIVER, FOR 1988 AND 1989

DATE	TOTAL P (μg P/l)	SRP (μg P/l)	NITRATE (μg NO_3-N/l)	AMMONIA (μg NH_4-N/l)
11-5-88	2,190,000	7,990	280	28
12-3-88	65,000	14,350	1,240	12
4-7-89	26	1,355	0	245
6-10-89	0	0.8	0	160
7-1-89	0	193,000	0	498
7-29-89	0	ND	11.54	10.6

ND= NO DATA

NUTRIENT DATA FROM SITE IV, GLOVER RIVER, FOR 1988 AND 1989

DATE	TOTAL P (μg P/l)	SRP (μg P/l)	NITRATE (μg NO_3-N/l)	AMMONIA (μg NH_4-N/l)
11-5-88	2,304,000	9,630	224	31
12-3-88	1,146,000	14,750	940	671
4-7-89	195	1,390	0	294
6-10-89	0	8.5	72	0
7-29-89	1,340	0	40	14.45
8-19-89	1,620	ND	18.3	24.45
10-21-89	4,180	11.6	0	56.05

ND=NO DATA

APPENDIX C

PHYSICAL PARAMETERS

TURBIDITY OF THE GLOVER RIVER
AS MEASURED IN NTU'S FROM 1989 AND 1990.

	SITE			
DATE	I	II	III	IV
6/10/89	11	6.7	6.6	6.2
7/1/89	2.3	2.7	2.6	ND
7/29/89	13	14	15	16.8
8/19/89	21.5	ND	ND	3.0
10/2/89	11.5	ND	ND	ND
10/19/89	6.2	ND	ND	1.1
1/27/90	8.7	ND	ND	ND
2/24/90	22	ND	ND	ND
4/4/90	23	ND	ND	ND
6/16/90	0.6	ND	ND	ND
6/29/90	1.2	ND	ND	ND
7/19/90	13	ND	ND	ND
9/9/90	3.9	ND	ND	ND
9/26/90	20	ND	ND	ND
10/15/90	25	ND	ND	ND

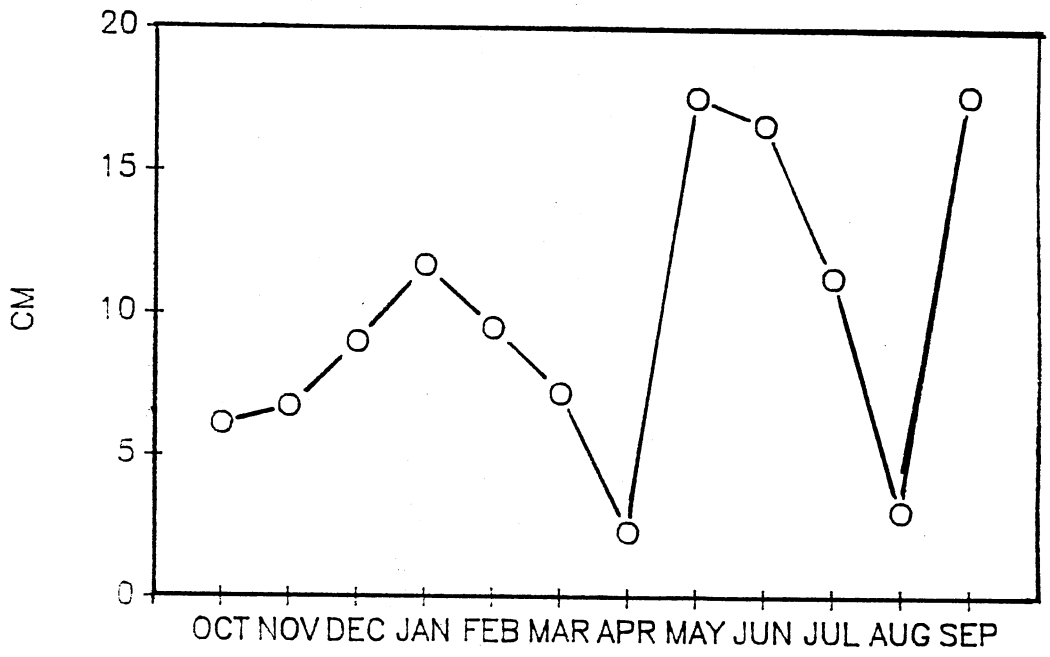
ND=NO DATA

GLOVER RIVER pH VALUES FROM 1989 AND 1990

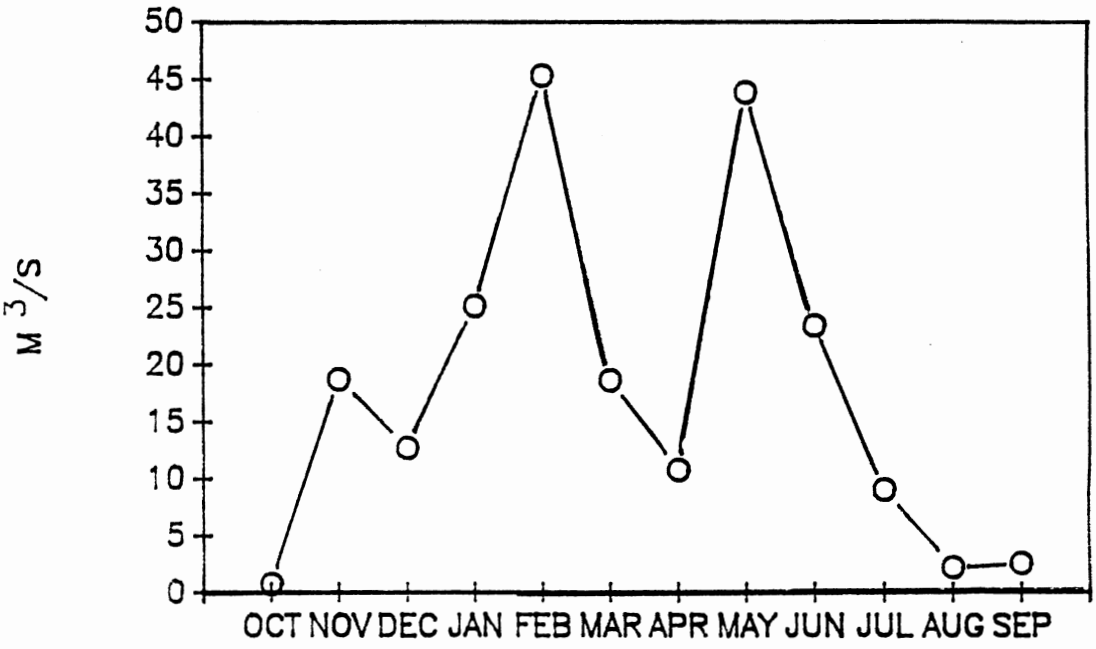
	SITE			
DATE	I	II	III	IV
6/10/89	6.0	5.7	5.6	6.0
7/1/89	6.8	5.4	5.8	ND
7/29/89	7.0	6.8	6.6	7.0
8/19/89	5.2	ND	ND	6.8
9/29/89	6.5	ND	ND	ND
10/19/89	6.5	ND	ND	ND
1/27/90	7.3	ND	ND	ND
2/24/90	ND	ND	ND	ND
4/4/90	6.5	ND	ND	ND
6/16/90	6.5	ND	ND	ND
6/29/90	7.0	ND	ND	ND
9/9/90	7.5	ND	ND	ND
9/26/90	6.5	ND	ND	ND
10/15/90	6.5	ND	ND	ND

ND=NO DATA

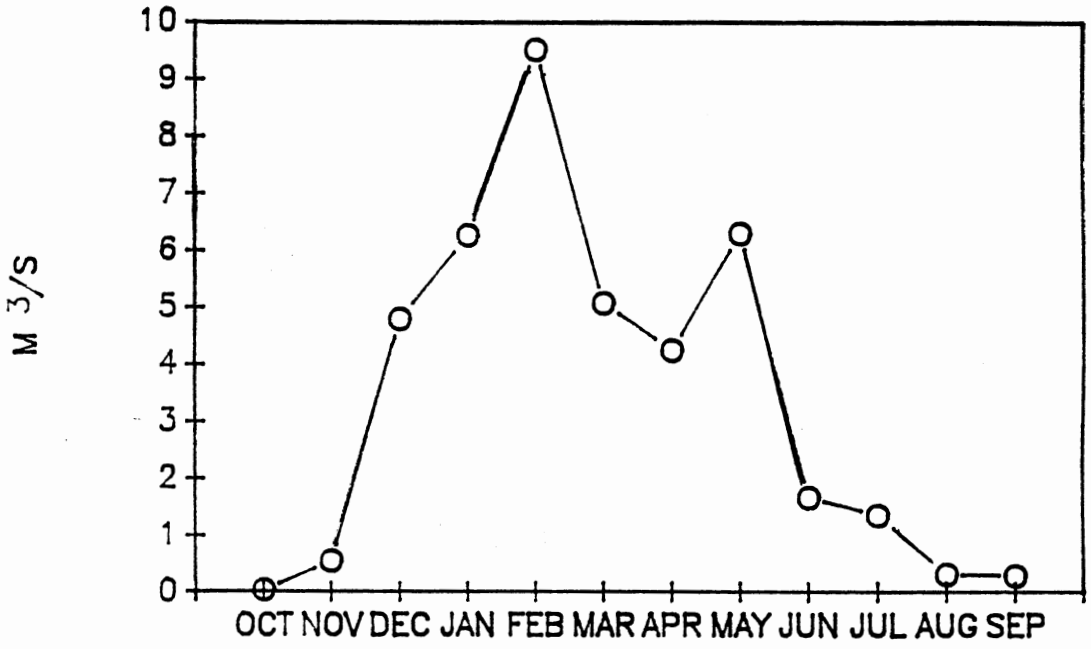
Total Monthly Rainfall Lane, OK October 1988 to September 1989



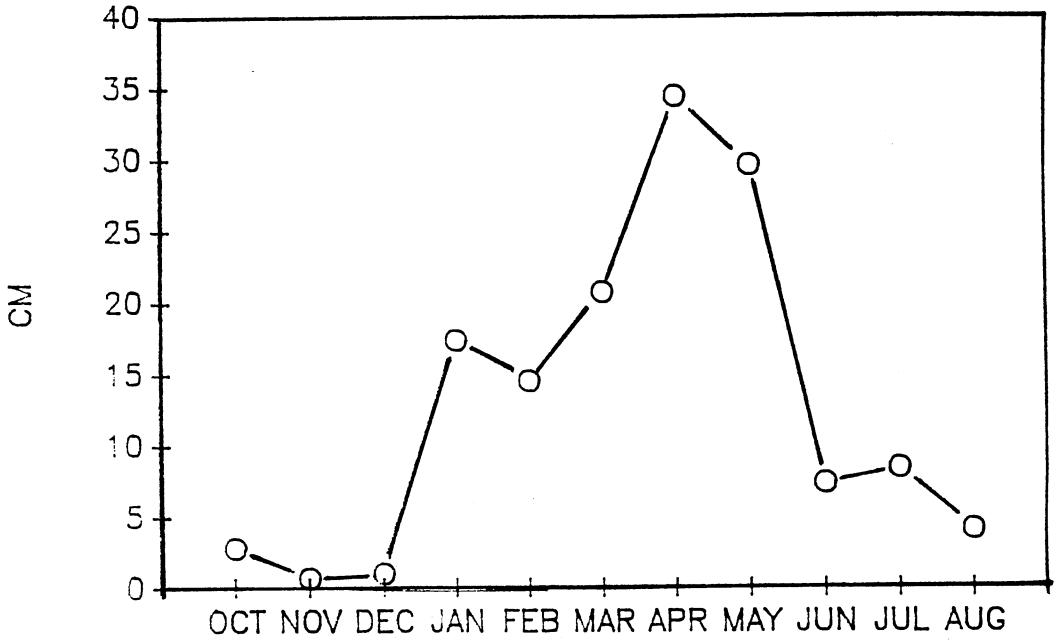
Mean Monthly Discharge Glover River, Glover OK
October 1988 to September 1989



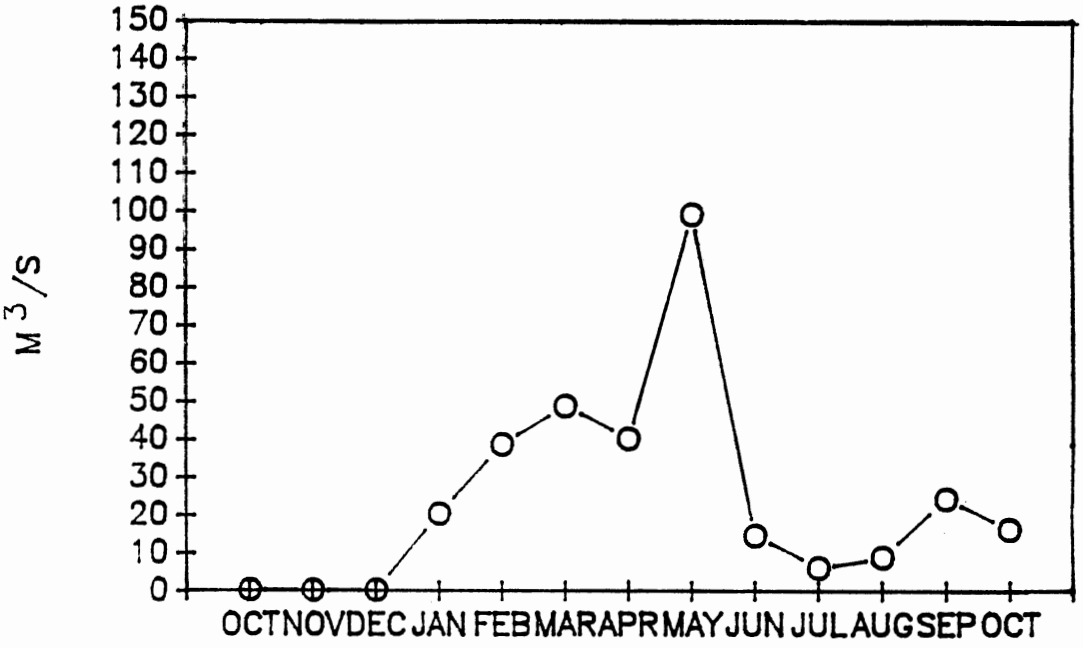
Minimum Monthly Discharge Glover River, Glover OK
October 1988 to September 1989



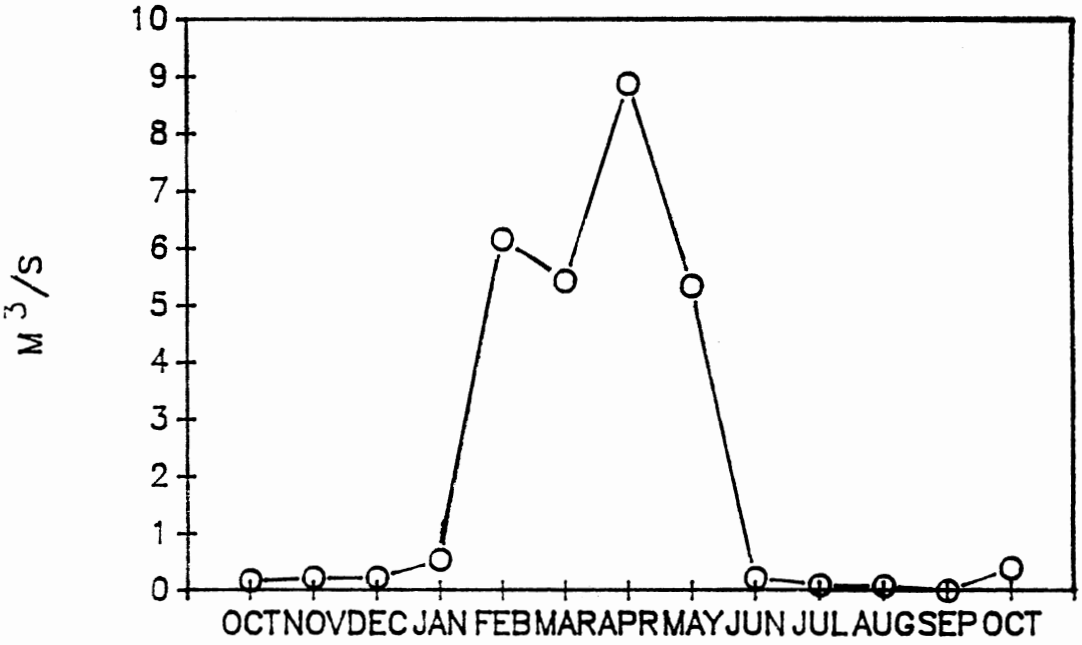
Total Monthly Rainfall Lane, OK October 1989 to August 1990



Mean Monthly Discharge Glover River, Glover OK
October 1989 to October 1990



Minimum Monthly Discharge Glover River, Glover OK
October 1989 to October 1990



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Master of Science

Thesis: EVALUATION OF NUTRIENT LIMITATION TECHNIQUES IN THE GLOVER RIVER MCCURTAIN, CO. OKLAHOMA

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