BIOMONITORING OF PERIPHYTON AND NUTRIENTS AS INDICATORS OF ENVIRONMENTAL STRESS IN OZARK STREAMS

By

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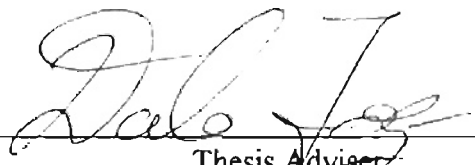

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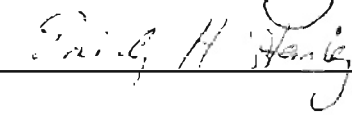

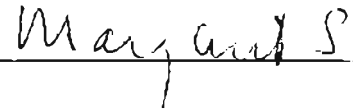

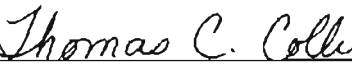

Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Lakes and streams are vital sources of drinking water. The need to preserve these resources led to the Clean Water Act of 1972. One goal of this Act was to maintain the "physical, chemical, and biological integrity of the waters of the United States." As a result, the nation's water quality has improved (USEPA 1987). However, pollution still is occurring twenty years after this law was enacted.

According to the United States Environmental Protection Agency's (EPA) 1986 National Water Quality Inventory Report to Congress, nonpoint source (NPS) pollution is the leading cause of pollution of freshwater. Nonpoint sources, such as agricultural runoff, are responsible for 75% of polluted lakes and 65% of polluted streams in the United States (USEPA 1987).

Nonpoint source loading of nitrogen and phosphorus in watersheds accelerates eutrophication of lakes, reservoirs, streams and rivers. Wetzel (1983) defines eutrophication as nutrient enrichment. Effects of this enrichment include increased primary productivity and hypolimnetic anoxia during summer stratification of lakes. Cultural eutrophication of streams has been linked to increased nutrients from NPS pollution originating in the watershed and can lead to excess algal growth and water

quality degradation.

Limnologists generally agree that the primary causal agents of eutrophication are increased loads of phosphorus and nitrogen (Vallentyne 1970; Schindler 1974). Phosphorus has been shown to be the main agent of eutrophication; the greatest potential for increased biomass exists as a result of P loading (Vollenweider 1968; Vallentyne 1970; Hutchinson 1973). Controlling concentrations of nitrogen or carbon in a lake or reservoir are not feasible because of their atmospheric "sinks" (Schindler 1977). By controlling phosphorus loads that reach lakes, phytoplankton could be forced into phosphorus limitation and their productivity limited.

Attenuation of NPS pollution to lotic systems would provide managers with a method to control phosphorus loading of lentic ecosystems. Such attenuation requires knowledge of ephemeral and spatial attributes of NPS pollution and the effects on the biota. Knowledge of possible sources of the NPS pollution also is necessary in development of a pollution management plan. Development of a biomonitoring approach to assess the rate at which nutrients are being loaded into a particular water body is also necessary if effective management of such loadings is to be achieved. Biomonitoring of nutrient concentrations is the topic of this proposal.

Problem Statement

Nutrient loadings from NPS is a major contributor to pollution of lotic

ecosystems (USEPA 1987). Quantification of NPS nutrient loads has been difficult because NPS pollution usually occurs during runoff events (Omernick 1976). Problems arise in sampling procedures because grab samples fail to give true levels of nutrients unless taken in series after a major runoff event. Thus, traditional measurement of water quality alone often underestimates nutrient loads and thus underestimates potential cultural eutrophication of the receiving water.

The water quality of a lotic ecosystem is manifested inherently within the structure and productivity of its biota (Hughes et al. 1990; Round 1991). Thus, measurement of the community of attached biota should provide information about possible ecosystem stress when coupled with water quality data. Several technical problems arise in the implementation of these approaches. Measurement of biota in the stream requires collection of a mature, thick biofilm. Such integral data collection is hindered by heavy grazing and the unknown maximum age the biofilm reaches prior to sloughing.

A holistic biomonitoring approach is needed to fully assess degradation or improvement of impacted watersheds. Monitoring efforts are being sought to determine effectiveness of implemented best management practices (BMP) on Peacheater Creek in northeastern Oklahoma. Best management practices are in place along Peacheater Creek in an effort to reduce the amount of NPS phosphorus that probably result from intensive agricultural practices (primarily poultry rearing) in the drainage basin. The goal of the BMP is to control NPS phosphorus loading into the Illinois River and Tenkiller Ferry Reservoir. This river has been designated a wild and scenic river by the State of

Oklahoma (SCS 1992). In theory, best management practices will curtail the process of cultural eutrophication of both waters. This study was part of a paired watershed project whose goal was to determine if implemented BMP in Peacheater Creek watershed were effective in lowering nutrient loading as determined by monitoring biological and water chemistry parameters. No BMPs were implemented along Tyner Creek, located in close proximity to Peacheater Creek. Peacheater Creek is the manipulated watershed in the study and Tyner Creek is the reference watershed.

Research Objectives

The goals of this research were to use biomonitoring techniques to compare biofilm nutrient concentrations and to determine rates of biomass accrual in two impacted watersheds. Surplus P concentrations in the biofilm were correlated with nutrient concentrations in two watersheds to define biofilm stress. Specifically, my objectives were to:

1. construct an apparatus to hinder non-swimming grazers from using artificial substrates,

2. determine the optimal age of a mature, thick biofilm prior to sloughing for the purpose of monitoring,

3. evaluate differences in nutrient concentrations in Tyner Creek and Peacheater Creek,

4. determine biofilm phosphorus stress measured by surplus phosphorus in Tyner Creek and Peacheater Creek,

5. determine seasonal difference in the optimal age of the biofilm and nutrient concentrations in Peacheater Creek and Tyner Creek,

6. evaluate alkaline phosphatase activity in Tyner and Peacheater Creeks during the winter.

The statistical null hypotheses are stated as follows:

$\mathbf{H_o}$: time for biofilm development is the same in Peacheater and Tyner Creeks,

$\mathbf{H_o}$: concentrations of biofilm surplus phosphorus in Peacheater Creek do not deviate significantly from concentrations in Tyner Creek,

$\mathbf{H_o}$: biofilm development time does not significantly deviate seasonally between Peacheater and Tyner Creeks,

$\mathbf{H_o}$: concentrations of biofilm surplus phosphorus are the same in the summer and winter in Peacheater and Tyner Creeks,

$\mathbf{H_o}$: alkaline phosphatase concentrations are the same in Peacheater and Tyner Creek during the winter,

$\mathbf{H_o}$: nutrient concentrations are the same in Peacheater and Tyner Creek.

CHAPTER II

STUDY SITES

The study sites were Peacheater Creek and Tyner Creek in the Illinois River watershed in northeastern Oklahoma (Figure 1). Both streams are tributaries to the Baron Fork River which is a tributary of the Illinois River. The Illinois River is impounded and creates Tenkiller Ferry Reservoir.

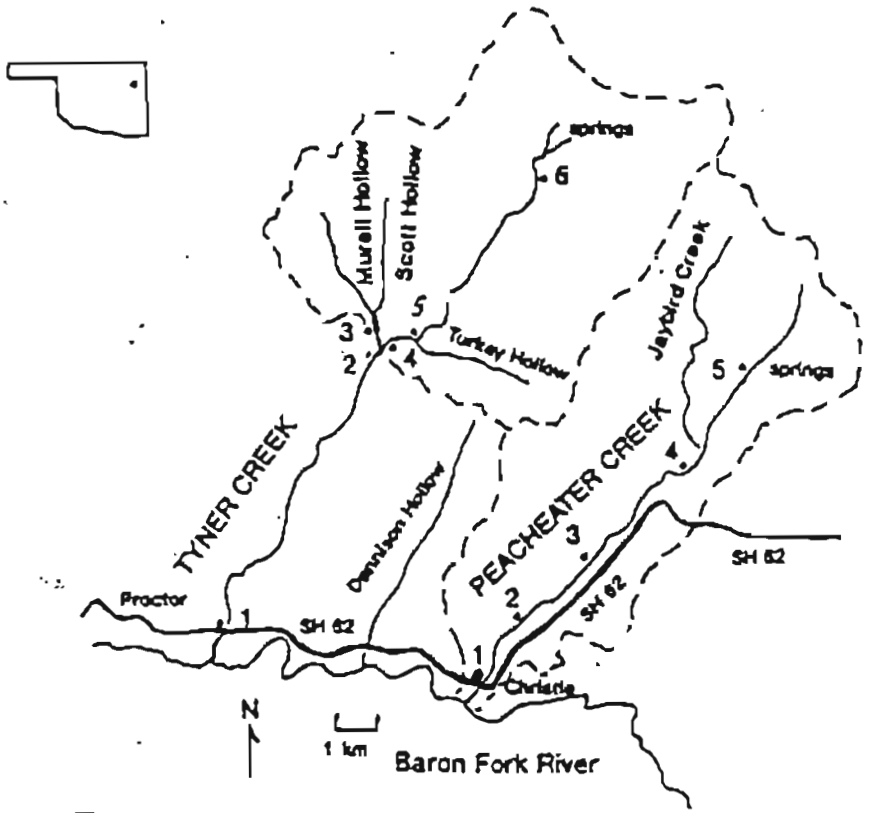

Figure 1. Map of Peacheater and Tyner Creek watersheds. The watershed boundaries are denoted by dotted lines. Solid lines denote waterways. State highway 62 is marked in bold. United States Geological Survey gauging station on Peacheater Creek is located at station 1.

Water quality in the Illinois River has experienced accelerated degradation for over 20 years (Gakstatter and Katko 1986). Approximately 95% of nutrient loading in the Illinois River basin has been attributed to NPS nutrient enrichment (Gakstatter and Katko 1986). Increased nutrient loading has occurred concomitantly with increased agricultural activity in the watershed. Litter produced by more than 200 million chickens reared in the basin is used as fertilizer and often results in excess P concentrations in the soil and hence in water (SCS 1992).

This study was done as part of a paired watershed project developed by the Oklahoma Conservation Commission. The focus of the study was two adjacent watersheds in Adair County, Oklahoma: Peacheater Creek and Tyner Creek. Peacheater Creek was the experimental watershed in this study and Tyner Creek served as the reference watershed. Best management practices (BMP) implemented in the Peacheater watershed include minimizing fertilizer application and proper disposal of dead chickens. The goal of BMP is to reduce NPS nutrient loading originating from agricultural activity into the streams.

The Peacheater watershed is a second order stream located east of Tyner Creek and has an area of 6,560 ha (SCS 1992). The study site was located approximately 1.5 km upstream from the Highway 62 bridge transversing Peacheater Creek. Peacheater Creek site in this study was located about 610 m upstream from site 2 (Figure 1). Tyner Creek is a third-order stream; its watershed area is 6,475 ha. This stream was sampled at site 2 in the upper Tyner Creek watershed approximately 50 m downstream from the confluence of Tyner Creek and Murell Hollow and Scott Hollow tributaries (Figure 1).

Both watersheds are located in the Ozark Highlands. Topography is primarily rough, steep hills, thus a high potential for surface runoff exists in these watersheds (SCS 1992). The major land use in the both watersheds is improved pastureland, brush pastureland-rangeland, and oak-hickory forestland.

Large numbers of poultry and dairy farms occur in both watersheds. It has been estimated that 88,596 metric tons of poultry and dairy manure are produced in Peacheater watershed annually (SCS 1992). This waste contains approximately 748,437 kg of N and 474,839 kg of P (SCS 1992); therefore, runoff events allow considerable NPS loading of these nutrients to the stream.

The stream bottom of Tyner Creek and Peacheater Creek is composed of rocky substrata between 10 and 15 cm in diameter. The submersed macrophyte, *Ceratophyllum* sp., waspresent in the summer of 1996 and winter of 1997 in both streams.

Cattle had direct access to the site sampled at Peacheater Creek, but not to the Tyner Creek site. Periphyton mats were not observed growing on rocky substrata in either stream in summer months of 1996 despite probable nutrient loading. High grazer density, primarily snails (*Elimnia* sp.), was observed when periphyton growth was scarce on natural substrata. However, periphyton growth on the bottom of the streams was noticeable in winter months of 1997 when snail densities were low. Ephemeropteran grazers were found on artificial substrata in both streams in the winter of 1997. Abundance of Ephemeroptera ranged from 3 to 6 organisms on each respective substratum sampled during experiment 4.

Chapter III

MATERIALS AND METHODS

Introduction

Four experiments were performed. Periphyton growth on suspended pine boards was monitored over time to assess growth of the biofilm prior to sloughing by using chlorophyll *a* (chl. *a*) and ash free dry weight (AFDW) as indicators of biomass, and surplus P and alkaline phosphatase activity (APA) as measures of nutrient limitation. Ambient nutrient concentrations were also determined.

Floating boards containing artificial substrata were used to prevent grazer activity. The apparatus used consisted of untreated 0.61 x 1.22 m boards, attached by aerial steel cable to steel posts set in the stream bottom. Boards were placed in an upstream-downstream direction. An additional steel bar was attached underneath the board to ensure submersion of substrata during incubation.

Initially, two different artificial substrata were used and biomass was determined by measuring chl. *a* content of accumulated periphyton. Substrata on which periphyton accumulated were fixed to both sides of the boards. Styrofoam substrata, approximately 5.08-cm thick and 30.48-cm long, were bolted to the sides of the board and sampled with a 1.1-cm diameter cork borer. A grid was marked on the styrofoam and a number

assigned to each. Sampling grids were chosen at random. Silicated disc substrata (5.067 cm^2) were glued in recesses on the sides of boards such that their faces were flush with the side surface of the board. The discs were harvested randomly as single units.

Experiments

Periphyton growth on artificial substrata was sampled at one site in both Peacheater and Tyner Creeks, respectively (Table 1). One disc or one core from the styrofoam was considered a sample. In pilot experiments 1 and 2, one board for each one substratum type was deployed in each stream. The purpose of the pilot experiments was to determine which type of substrata to use.

Grazers were never observed on silicated disc substrata. Disc substrata diameter was larger and thus proved easier to handle in the field and to transport back to the laboratory. Silicated discs were also easier to attach to boards and samples were never lost. Portions of the styrofoam substratum attached to the sides of the boards dislodged and were lost in Tyner Creek during pilot experiment 1. Harvesting of substrata ceased after a colonization time of 42 to 56 days.

The purposes of experiments 3 and 4 were to determine colonization time necessary for a biofilm to reach peak biomass, and to determine colonization time necessary for no differences in surplus P and APA to exist. Three boards were deployed in each stream in experiment 3 and 4; one for each parameter measured, and substrata were silicated discs only. Three random samples were collected weekly from each board. Three samples taken from board 1 were used to analyze chl. *a*. Samples from

TABLE I

EXPERIMENTAL DESIGN OF STUDIES CONDUCTED IN PEACHEATER CREEK AND TYNER CREEK

Experiment †	Experiment Date	Number of Samples‡							
		Silicated Discs				Styrofoam			
		Chl. *a*	Surplus P	AFDW	APA	Chl. *a*	Surplus P	AFDW	
Pilot 1	13 May - 24 Jun 1996	3				3			
Pilot 2	24 Jun - 17 Aug 1996	3				3			
Experiment 3	5 Aug - 27 Sep 1996	3	3	3					
Experiment 4	5 Jan - 17 Feb 1997	3	3	3	3				

† All experiments conducted in the shade.
‡ 1 board deployed per substratum in pilot experiments in each stream. 3 boards deployed in experiment 3 and 4 in each stream. Designated numbers indicate weekly sample number taken from each board. The same board was sampled every week for determination of a set parameter. In experiment 4, chl. *a* and APA were analyzed from the same sample.

board 2 were used in determination of surplus P. Samples from board 3 were used in determination of AFDW (Table 1). However, in experiment 4, APA and chl. *a* measurements were made on the same sample, hence boards were not separate treatments.

Colonization time necessary for development of biofilm prior to sloughing and surplus P concentrations and APA were assessed in experiments 3 and 4. Three samples were taken from each respective board in experiment 3 to measure AFDW, surplus P, and chl. *a* (Table 1). In experiment 4, the boards in Peacheater Creek was moved upstream approximately 50 m from the site of previous experiments due to fencing which had been put in place across the stream. The boards in Tyner Creek were in the same

location in the stream as in previous experiments. In experiment 4, APA was measured in addition to parameters measured in experiment 3. AFDW was not measured in experiment 4.

In the field, silicated discs and styrofoam substrata sampled for chl. *a* and surplus P analyses were wrapped in aluminum foil and placed on ice for transport back to the laboratory. Samples used for determination of APA and chl. *a* in experiment 4 were placed in polyethylene bottles with 150 ml of stream water and transported on solid CO_2 so as not to dilute APA. Transport time from the field to the laboratory was approximately 3 hours.

Physical Parameters

Physical parameters were determined at both sites each time the streams were sampled. Water temperature was taken *in situ* using a hand-held thermometer. Water samples used in determining physical parameters were collected in polyethylene bottles and transported on ice back to the laboratory. A laboratory Corning Model 7 pH meter was used to take pH readings. A YSI model 33 S-C-T conductivity meter and Hach turbidimeter were used to determine conductivity and turbidity of stream water. Rainfall data were obtained from the National Weather Service for Stilwell, Oklahoma. Discharge data were collected downstream from the sampling site on Peacheater Creek at United States Geological Survey (USGS) gauging station 07196973 which is located on Peacheater Creek at Highway 62 bridge (Figure 1).

Collection and Preservation of Water

Six grab samples of water were collected at each site on all sampling dates. Water samples were taken in 250-ml acid-washed polyethylene bottles and transported on ice back to laboratory. Aliquots to be tested for soluble reactive P (SRP) and nitrate (NO_3-N) were filtered with a prerinsed 0.8-µm AA Millipore membrane filter upon returning to the laboratory. Samples were stored in the dark at 0°C until analyses could be performed, usually within 7 days.

Laboratory Analyses

Water chemistry parameters analyzed included soluble reactive P (SRP), total P (TP), nitrate-N, chloride and sulfate-S. Standards were freshly prepared for each analysis. A spike, laboratory blank of deionized water, and a field blank were used to ensure accuracy and precision. Certified EPA samples also were analyzed simultaneously during selected analyses and the concentrations were compared to the true value.

The ascorbic acid technique was used to measure SRP (Wetzel and Likens 1991). Absorbance was determined at 885 nm using deionized water as a blank standard. Optical density was measured using a Shimadzu UV 120-02 spectrophotometer. This method is applicable in a range of 1 to 500 μg PO_4-P /L (Wetzel and Likens 1991). Concentrations of TP in unfiltered stream water were measured using the potassium persulfate digestion technique (APHA 1980). Absorbance was determined at 885 nm. A

Dionex DX-100 ion chromatograph was used to measure concentrations of chloride, nitrate-N, and sulfate-S (USEPA 1989).

Chl. *a* concentration was used to estimate periphyton biomass on artificial substrata. Discs were placed in 50-ml beakers and 10 ml of 90% alkaline acetone was added to each sample. Styrofoam plug substrata were placed in graduated centrifuge tubes and 10 ml of 90% alkaline acetone was added to each sample. Samples were then extracted in a refrigerator for 24 hours in the dark.

The extract from both substrata was then sonicated with a Fisher model 300 Sonic Dismembranator for 90 s at 50% and 35% power for the discs and plugs, respectively. Ultrasonication has been shown to effectively remove algal cells from artificial substrata (Gale 1975). The substrata appeared clean after sonication. The extract was then transferred to graduated centrifuge tube and centrifuged for 15 min.

In experiment 4, substrata collected for chl. *a* and APA determination and river water used in transport were placed into a 250-ml beaker. The samples were sonicated at 50% power for 90 s to dislodged algal cells from the silicated disc. Two 50-ml aliquots were taken from the slurry and filtered across Whatman 934AH glass fiber filters for chl. *a* analyses. Filters were folded in half, wrapped in aluminum foil, and frozen for no longer than 24 hr. The remaining slurry was placed in glass vials and frozen at -80°C until APA analysis could be completed. Samples were not stored for more than 14 days.

Filters to be used for chl. *a* determination in experiment 4 were thawed, then ground in 3 ml of 90% alkaline acetone with a hand held tissue grinder. Final extract volume was 10 ml in all experiments. Samples were extracted for 24 hr in a refrigerator.

Samples were then centrifuged for 15 min and read spectrophometrically with a Seacomam model S1000 spectrophotometer using a 1-cm path length (Wetzel and Likens 1991). Absorbance of the extract was measured at 665 and 750 nm before and after the addition of 0.3 ml of 1 N HCl to correct for phaeophytins. In each analysis, 2 unexposed substrata were also processed and functioned as blanks. Chl. *a* measurements in weeks 1 and 2 were not corrected for phaeophytins due to analytical error. Therefore, in statistical tests, uncorrected chl. *a* data were used to ensure internal consistency of data.

Surplus P was extracted by boiling periphyton samples in 50 ml deionized water for 1 hr in a 100°C water bath (Wynne and Berman 1980). New standards were prepared for each analysis. Uncolonized substrata served as the blank. The extract was filtered through a prerinsed 0.8-µm AA Millipore membrane filter and concentrations measured according to the ascorbic acid technique previously described. Surplus P measured in this manner yielded a measurement of available intracellular stored P extractable by hot water, hereafter called available stored P (surplus P_i), as an indicator of the P status of algal cells (Fitzgerald and Nelson 1966).

In experiment 4, the P content of the biofilm was determined by measuring both the available stored P and the total cellular P component extractable by hot water, hereafter called total surplus P (surplus P_t). The P_t fraction has been shown to represent a potentially important P pool in algal cells (Wynne and Berman 1980). P_t was determined by placing silicated discs in 100-ml beakers with 100 ml deionized, distilled water. The beakers were then placed in a hot water bath for 60 minutes as previously described. A

50-ml aliquot was taken from each sample to measure the surplus P_t fraction, using the potassium persulfate digestion technique for TP. To estimate surplus P_p the remaining 50 ml of extract was filtered and measured as previously described for SRP. Surplus P concentrations were normalized to biomass (chl. *a* and AFDW), respectively.

Determination of AFDW was done by placing silicated discs in preweighed crucibles with deionized water. The disc was then sonicated at 70% power for 2 minutes to dislodge algal cells. Silicated discs were then removed from the slurry. The slurry was then dried at 105°C for 24 hrs, cooled in a desiccator, then reweighed. Samples were ignited for 1 h at 500°C. To correct for clay hydration, the samples were rehydrated with deionized water and dried at 105°C. Ash-free dry weight was calculated according to APHA (1989).

Alkaline phosphatase activity was measured by the hydrolysis of 3-O-methylfluorescein phosphate (MFP) following Perry (1972) modified by Franco (1984). A stock solution of 20 μg MFP/ml was prepared with Tris buffer and frozen at -80°C in small aliquots. Working substrate was diluted to 1 μg MFP/ml with Tris buffer. A 5.0-ml aliquot of periphyton sample was placed in a fluorometer tube. The addition of 0.6 ml of 1 μg MFP/ml substrate in Tris buffer began the reaction. Fluorometer tubes were immediately sealed with parafilm and inverted once. Fluorometric measurements were taken immediately and after 1 hr. Fluorescence was determined on a Turner model 111 fluorometer against a distilled, deionized water blank.

APA was measured as an increase in fluorescence as the substrate (MFP) was enzymatically hydrolyzed to 3-O-methylfluorocein which is fluorescent. Fluorometric

readings were converted to absolute units via a standard curve of fluorescence versus MF concentration. APA was expressed as nM MFP/ unit of biomass per unit time. Activity was normalized to chl. *a* measured from subsamples taken from the same initial slurry as APA.

Statistical Analysis

Periphyton data were tested for normality using Kolmogorov-Smirnov normality test (Sigma Stat, Jandel Scientific, San Rafael, CA 1992). Parameters which were not normal were rank transformed before appropriate tests were performed (Table 2).

Periphyton parameters (chl. *a*, surplus P, AFDW, and APA) were plotted as a function of time. Peak concentrations were determined by statistical comparison of measurements sampling dates to identify the colonization time necessary to achieve maximum concentrations. Differences in weekly concentrations over time in each stream were determined by a one-way ANOVA test (Sigma Stat, Jandel Scientific, San Rafael, CA 1992). A Tukey's pairwise comparison test was then employed to discern differences between specific weeks (Systat, Evanston, IL 1992). Peak biomass was defined to occur the second week in which no statistical difference existed between subsequent biomass concentrations. This definition assured the estimate of peak biomass would be made in the upper asymptote of logarithmic growth.

In pilot experiment 1 and 2, styrofoam and silicated disc substratum time courses were compared within each stream. This comparison was done using Kruskall-Wallace 1-way ANOVA on ranks (Sigma Stat, Jandel Scientific, San Rafael, CA 1992). Pairwise

comparisons of chl. *a* time courses were done using Dunn's Method (Sigma Stat, Jandel Scientific, San Rafael, CA 1992).

In all experiments, periphyton and water chemistry data were compared between streams. Data collected in experiments 3 and 4 were compared within each stream to determine seasonal differences. In experiment 4, surplus P fractions were compared within each stream. All comparisons were performed by t-test or Mann-Whitney comparison test depending on normality (Sigma Stat, Jandel Scientific, San Rafael, CA 1992). Weekly data were combined prior to analyses.

Relationships between surplus P, APA, and SRP were determined using Pearson Product Moment correlation test (Sigma Stat, Jandel Scientific, San Rafael, CA 1992). Significance was defined at $p < 0.05$ for all tests.

TABLE II

STATISTICAL TRANSFORMATIONS PERFORMED ON TIME COURSE DATA

SITE	EXPERIMENT	PARAMETER	NORMALITY TEST	TRANSFORMATION
Peacheater	2	Chl. a Styrofoam	Passed	
Tyner	2	Chl. a Styrofoam	Failed	Rank
Peacheater	2	Chl. a Disc	Passed	
Tyner	2	Chl. a Disc	Passed	
Peacheater	3	Chl. a	Passed	
Tyner	3	Chl. a	Passed	
Peacheater	4	Chl. a	Passed	
Tyner	4	Chl. a	Passed	
Peacheater	3	AFDW	Passed	
Tyner	3	AFDW	Passed	
Peacheater	3	Areal Surplus P	Passed	
Tyner	3	Areal Surplus P	Passed	
Peacheater	3	Surplus P/chl. a	Failed	Rank
Tyner	3	Surplus P/chl. a	Failed	Rank
Peacheater	3	Surplus P/AFDW	Failed	Rank
Tyner	3	Surplus P/AFDW	Failed	Rank
Peacheater	4	Areal Surplus P_t	Passed	
Tyner	4	Areal Surplus P_t	Passed	
Peacheater	4	Surplus P_t/chl. a	Failed	Rank
Tyner	4	Surplus P_t/chl. a	Failed	Rank
Peacheater	4	Areal Surplus P_i	Passed	
Tyner	4	Areal Surplus P_i	Passed	
Peacheater	4	Surplus P_i/chl. a	Failed	Rank
Tyner	4	Surplus P_i/chl. a	Failed	Rank
Peacheater	4	APA	Passed	
Tyner	4	APA	Failed	Rank

If no transformation is indicated, raw data were used in statistical tests. If indicated, rank transformed data was used in statistical tests.

CHAPTER IV

LITERATURE REVIEW

The Periphytic Community

Definition

Periphyton is a complex, diverse community which occupies an important role in stream ecosystems (Odum 1983). Originally, the term periphyton was used to describe algal communities attached to artificially submerged objects (Cole 1988). This definition was expanded to mean a complex community, sometimes termed aufwuchs, of microbiota (algae, bacteria, fungi, animals, inorganic and organic detritus) that is attached to and associated with substrata. However, this definition has been largely abandoned due to its ambiguity (Wetzel 1983).

Round (1981) categorized periphyton according to the growth form of the associated algal community. Algae growing on macrophytic surfaces are termed epiphytic. Algae growing on rock surfaces are epilithic. Epizoic algae grow on the surfaces of animals and epipelic algae grow on sediment (Round 1981). Periphyton is commonly used to describe the combined communities (Round 1981). In this study, the term periphyton will be defined as the microfloral community living upon the surfaces of

submersed objects in water (Wetzel 1983).

Autotrophy and the Periphytic Community

Lotic ecosystems can be described by the dominant trophic state (i.e., autotrophy or heterotrophy) (Cummins 1974). Autotrophy is the ability of an ecosystem to photosynthetically convert light energy from the sun to chemical energy which is required for growth and maintenance of organisms in that system (Wetzel 1983). The fraction of the periphytic community which is photosynthetic is responsible for the majority of autochthonous primary productivity in most lotic ecosystems (Hill et al. 1992).

Early evaluations of the importance of primary productivity in lotic ecosystems focused on comparisons with allochthonous inputs of organic material (Cummins 1974). Fisher and Likens (1973) demonstrated that only 1% of the energy input in a particular first-order stream originated from autochthonous sources, that is, from in-stream photosynthetic activity of the periphyton as well as other macrophytes. The remainder was derived from allochthonous inputs of organic material. This suggested that primary production was inconsequential in stream production. These and other studies (e.g., Hynes 1970) focused on small first-order streams which were heavily shaded by forest canopies, which reduced primary production of algae during much of the growing season. As a result, streams were referred to as heterotrophic because greater amounts of organic matter were decomposed than autochthonously produced within the stream (Wetzel and Ward 1992).

However, other studies (Odum 1957, Naiman 1976) showed autotrophs to be the primary contributor of organic matter to streams. Minshall (1978) compared known energy contributions from different streams having different sources (autochthonous and allochthonous) based on the geography of the watershed region and stream order. Patterns of increased autotrophy with increased stream order were found in similar comparison studies by Minshall et al. (1983) and Naiman (1983). Streams tend toward autotrophy as the canopies open in higher-order streams due to increased irradiance and photosynthetic activity (Wetzel 1983).

One definitive type of autotrophic lotic ecosystem is characterized by a small periphytic standing crop acting as the primary producers. Though not abundant, the periphyton may demonstrate high productivity (Minshall 1978). McIntire (1973) pointed out that periphyton is not as abundant as are allochthonous detrital inputs, a reason why the importance of autotrophic production to often overlooked (McIntire 1973). Using computer modeling of periphyton dynamics, McIntire (1973) demonstrated that an increased turnover rate of algae could enable a small periphyton community to maintain a large standing stock of consumers. Lamberti and Resh (1983) found similar results in a study involving grazing activity of the caddisfly, *Helicopsyche borealis*, which resulted in a low standing crop of periphyton and a concomitant accelerated algal turnover rate (O_2 evolved per unit chl. *a*).

Controls of Periphyton Growth

Top Down Control of Periphyton

In lotic ecosystems, periphyton is consumed by a variety of herbivorous scrapers including macroinvertebrates and fish (Cole 1988). Grazing activity, the consumption of living plants by animals, is important in supporting trophic structure and food web dynamics (Lamberti and Resh 1983). Periphyton communities are susceptible to grazer consumption. This consumption represents an initial step in transfer of energy resources to higher trophic levels. Most periphytic productivity has commonly been thought to be transferred to higher trophic levels (Wetzel and Ward 1992). However, periphyton production which directly enters detrital pathways has not been quantified directly under natural conditions (Wetzel and Ward 1992).

Modeling studies predict that in an even-numbered trophic level system (e.g., periphyton herbivore interaction), the autotrophic fraction will be controlled by herbivory (Fretwell 1987). This control has been termed "top down." Strong (1992) also concluded that where simple direct linkages of periphyton and grazers exist, autotrophs would be subject to top down control. Elwood and Nelson (1972) found grazing rates in a stream to be equivalent to rates of net primary production and concluded that herbivores control algal biomass. Rosemond et al. (1993) demonstrated that top down effects were more significant when simultaneously coupled with bottom up effects of growth-limiting resources. Interaction of both nutrients and herbivores were considered in determination of algal biomass and productivity (Rosemond et al. 1993).

Effects of Grazing on Periphyton

Reduction in Biomass. Grazers can have marked effects on the structure and function of the periphytic community (Hart 1985). Consumption by grazers, dislodgement and export of cells due to current velocity, and cell mortality resulting from senescence or pathogens all result in reduction of periphytic biomass (Hill et al. 1992). Reduction in periphyton biomass as a result of grazing activity and subsequent prevention of biomass accumulation has been demonstrated in numerous studies. These studies compared grazer- excluded periphytic communities and communities where grazing was not prevented (i.e., grazer-included communities) (Lamberti and Resh 1983; Stewart 1987; Lamberti et al. 1987; Mulholland et al. 1991).

Grazer Influenced Succession. Grazers have been shown to simplify the taxonomic structure and physiognomy of periphyton (Steinman et al. 1987; Mulholland et al. 1991). Rosemond et al. (1993) found grazed communities were dominated by cyanobacteria and chlorophytes, which were overgrown by diatoms when grazing pressure was released. Power et al. (1988) showed that grazers tended to support an increase in algae of particular growth forms (i.e., prostrate algae with tightly attached filaments having basal cell division). Mulholland et al. (1991) also found grazing activity resulted in periphyton composed of a thin mat of algae with a prostrate growth form. Ungrazed periphyton was characteristically a thick, complex mat representing algae with filamentous, gelatinous, and prostrate growth forms (Mulholland et al. 1991). Lamberti et al. (1989) found that grazing by snails delayed algal succession by increasing the abundance of adanate diatoms and reducing the abundance of erect and non-attached algae.

Morphological differences in algae lead to preferential grazing. Herbivores tend to remove algal taxa with upright, erect, or filamentous structures relative to taxa with prostrate morphologies (Steinman et al. 1992). Rosemond et al. (1993) found that herbivores consumed faster growing species more readily than slower growing species, which suggested competitive ability was eventually replaced by mechanisms of resistance to herbivory.

Areal Productivity. Areal productivity (the fixation of carbon per unit area) of the periphyton has been demonstrated to be limited by grazing because the reduction of biomass due to cropping and hindrance of chl. specific productivity (Lamberti et al. 1987; Hill et al. 1992). Reduction in areal productivity of periphyton has been found to occur even with addition of nutrients, suggesting that grazing can control periphyton growth (biomass accrual) (Stewart 1987).

Beneficial Effects of Grazing. Although grazers reduce biomass and areal productivity of periphyton, positive effects on grazer removal have been noted (Lamberti and Resh 1983; Stewart 1987; Power et al. 1988). Loss of biomass due to grazing activity has been demonstrated to increase periphyton productivity per unit standing crop of chl. *a* (Lamberti and Resh 1983). Grazing by *Campostoma* sp. increased biomass-specific primary production of periphyton, which was attributed to removal of the upper story of algae thus reducing self-shading to understory algae (Stewart 1987). In comparing ungrazed and grazed periphyton communities, Power et al. (1988) found that thin cyanobacteria mats were sustained when periphyton was subjected to grazing. The nitrogen-fixing capabilities of the cyanobacteria increased periphyton productivity by

maintenance of high levels of nitrogen fixers in producer assemblages. Hill et al. (1992) attributed the increase in biomass specific productivity of periphyton to cropping of senescent cells, selection for the establishment of rapidly dividing algal taxa, and an increase in recycled limiting nutrients.

Interaction With Nutrient Availability. Grazers also affect nutrient accrual of remaining periphyton. In nutrient-limited systems, regeneration of nutrients due to grazing releases nutrients back to the water to be taken up by periphyton (Mulholland et al. 1983). Grazers deter vertical biomass accrual. Because nutrients are not sequestered in a thick mat community, nutrient diffusion capabilities are increased (Hill et al. 1992). However, with increased nutrient availability, increased rates of downstream transport of particles occur, thus available nutrients move farther downstream, increasing spiraling length and making the nutrients essentially unavailable to the remaining periphyton (Newbold et al. 1982). Removal of biomass by grazers also creates exposed abiotic surfaces which can act as phosphorus "sinks" rendering the nutrient unavailable (Mulholland et al. 1983).

Bottom-Up Regulation of Periphyton

Periphyton is not only controlled by herbivorous activity, but may also be affected from the "bottom up", that is by limited resources needed for sustained growth (Rosemond et al. 1995). Leibig (1849), in a terrestrial study, stated that the resource available in the smallest quantity relative to the requirements of the plant, would limit its growth, Leibig's law of minimum. But nutrient limitation in aquatic ecosystems is more

complicated and often two nutrients are co-limiting.

Nutrient Limitation. Wetzel (1983) stressed the importance of phosphorus and nitrogen as limiting resources in aquatic systems. This was illustrated by comparing the essential elements required for algal growth. Nitrogen and phosphorus have the highest ratios of plant nutrient content to nutrient supply available in freshwater (Vallentyne in Wetzel 1983). Phosphorus, nitrogen, or both have commonly been shown to limit primary productivity in lentic ecosystems (Vollenweider 1976). Bothwell (1989) has shown the same phenomenon in experimental streams.

Phosphorus limitation of algal growth in streams has been reported in numerous studies (Bothwell 1988; Peterson et al. 1985; Pringle 1987; Hart and Robinson 1990). In these studies, concentrations of available phosphorus as soluble reactive phosphorus (SRP) were less than or equal to 15 μg/l. Most authors reported values less than 5 μg/l as limiting. Nitrogen has been found to be limiting in streams with ambient inorganic nitrogen concentrations less than 60 μg/l (Grimm and Fisher 1986; Hill and Knight 1988). Chessman et al. (1992) found that nutrient limitation is widespread in streams whose watersheds vary in vegetation and land use.

Determination of Limitation

Methods of analyzing the trophic status of streams, phosphorus limitation in particular, include biomass measurements, fertilization techniques, and alkaline phosphatase activity (APA) bioassays (Toetz 1995). Measurements of biomass include measurement of luxury phosphorus (surplus P) and determination of cellular atomic N:P

ratios. A ratio below 17:1 by atoms was reported by Rhee and Gotham (1980) as the critical point when phytoplankton are phosphorus limited. Fertilization techniques involved nutrient additions by two different mechanisms: artificial substrates which diffuse nutrients (Fairchild et al. 1985) and direct additions to artificial stream channels (Bothwell 1985).

Internal Nutrient Dynamics of the Periphyton Mat. The periphyton mat is essentially a microcosm of tightly packed autotrophic and heterotrophic assemblages of algae, bacteria, etc. with a self-generated boundary layer (Sand-Jensen 1983). This mat community is efficient at recirculating and conserving essential, limited inorganic nutrients such as phosphorus and nitrogen (Sand-Jensen 1983). Internal recycling of nutrients is an important survival mechanism for the mat community. Even under high nutrient conditions, nutrients can be limiting to the periphyton mat because diffusion restricts transport of ions into the mat (Bothwell 1989). Autogenic nutrient cycling has also been observed to increase as concentration gradients increased between the water and the periphyton mat, suggesting cycling may be controlled by diffusion (Riber and Wetzel 1987; Mulholland et al. 1994).

Peterson and Grimm (1992) noted temporal changes in biomass accrual in nutrient enriched treatments. This was attributed to increased recycling and nitrogen fixation within the mat suggesting that allochthonous nutrient sources are eventually replaced by autogenic recycling as the main supplier of nutrient to the mat community. Steinman et al. (1995) demonstrated that increasing thickness of the periphytic matrix reduced P turnover rate and concluded that biomass accrual influenced autogenic cycling

because resistance to diffusional nutrient exchange is associated with increased biomass. Paul and Duthie (1988) found that the overstory layer of the periphyton mat was responsible for most of the uptake of nutrients because of its higher density of actively metabolizing cells. After sufficient thickness is obtained , nutrient cycling can increase in response to reductions in ambient nutrient concentrations. Nutrient demands of the periphyton may be met by nutrient cycling within the mat (Mulholland et al. 1991).

Concomitant Limitation of Periphyton Growth

Periphyton biomass accrual is often limited by both nutrients and grazers, that is from the bottom up and the top down (Rosemond et al. 1993). Rosemond et al. (1993) concluded that when considered separately, effects of grazing and nutrient limitation on periphyton accrual were less significant than when their combined effects were considered. Similar studies also found periphyton to be limited by nutrients and grazing activity (Hart and Robinson 1990; Hill et al. 1992). However, Stewart (1987) concluded that grazing primarily limited biomass-specific productivity of periphyton even with nutrient enrichment. Rosemond (1994) found that productivity and biomass remained constant, although seasonal variations in nutrient levels and irradiance occur which could potentially limit periphyton biomass and concluded that when heavy grazing pressure was reduced, multiple factors could have been significant in periphyton biomass growth and productivity.

The number of trophic levels a system supports also is involved in the ability of periphyton to accumulate biomass. When exposed to nutrient additions, ecosystems

exhibiting even numbered trophic levels (i.e., periphyton, grazer system) showed an increase in herbivore biomass and a subsequent decrease in algal biomass (Power 1992). Rosemond et al. (1993) showed increased enrichment response in ungrazed substrata (odd number of trophic levels) but not in substrata which were grazed (even number of trophic levels). Increases in plant biomass in response to nutrient enrichment should be greater in systems with odd numbered trophic levels (Rosemond et al. 1993).

Artificial Substrata

Periphyton communities are diverse, hence they may colonize extreme habitats (Wetzel 1983). Artificial substrata provide a known colonizable area of homogeneous composition in which to study attached growth. Traditionally, glass slides were used as artificial substrates (Aloi 1990). This type of substrate has fallen out of favor due to the different periphyton assemblages colonizing glass slides compared to natural substrates (Loeb 1981), but not entirely abandoned (Pringle 1990). Tuchman and Stevenson (1980) popularized clay tiles, which are used commonly today in studies of periphyton biomass. Styrofoam also has been used as an artificial substrate for periphyton (Bothwell 1985). Bothwell (1985) used anchored styrofoam sheets as substrates for algal colonization. The sheets were sampled using a cork borer. Biomass was found to be lower on such substrata than on naturally occurring epilithon in the sublittoral zone of Lake Tahoe. This was attributed to either insufficient colonization time or differential herbivory between the two substrata (Aloi 1990). Gibeau and Miller (1989) developed a method which used porcelain or fused silica discs were attached to small, agar filled vials as

substrates in nutrient enrichment experiments. These substrata proved to be easy to harvest and were easily replicated (Gibeau and Miller 1989).

Mechanisms to hinder grazers from artificial substrates are necessary in field studies which compare productivity and biomass of grazed and ungrazed periphyton and those which use the productivity of the biomass as stress indicators. Insecticides (Peterson and Grimm 1992) and exclusion pens (Stewart 1987) have been used to reduce grazer density. Suspension of substrates and petroleum jelly barriers also have been used to eliminate non-swimming grazer pressure (Lamberti and Resh 1983; Lamberti et al. 1987; Hill et al. 1992).

Periphyton Growth Processes

Extrinsic Influences on Growth

Light Limitation. The rate of algal growth and photosynthesis are both affected by light intensity (Wetzel 1983). In clear headwater streams, turbidity does not cause modifications in intensity, spectral composition, or duration of light reaching the periphyton (Reynolds 1992). Turbidity in the form of fine, non-living suspended particles interferes with underwater light penetration, which can affect algal growth and distribution (Reynolds 1992). Steinman and McIntire (1987) found that irradiance can directly affect algal biomass, taxonomic structure, and physiognomy. In laboratory streams exposed to 150 and 400 $\mu E \cdot m^{-2} \cdot s^{-1}$, the relative abundance of chlorophytes was greater and substrata were covered with thick algal mats as compare to channels exposed

to 15 and 50 $\mu E \cdot m^{-2} \cdot s^{-1}$. Succession was distinct on high and low irradiance substrata (Steinman and McIntire 1987). Losee and Wetzel (1992) described the periphyton matrix as a complex optical system whose heterogeneity of components results in an abundance of scattering and absorbing interactions. Peterson and Grimm (1992) suggested that seasonal changes in temperature may also affect the competitive abilities of certain taxa. There exists a great diversity of preferred temperature ranges among algae (Wetzel 1983).

Phosphorus. As discussed previously, nutrient availability, primarily phosphorus and nitrogen, directly affects periphyton growth. Phosphorus is an essential nutrient involved in energy transformations (Newbold 1992). Although only needed in small amounts, it is the element which most frequently limits algal growth (Wetzel 1983) and is associated with the greatest potential increases in biomass when in excess (Vollenweider 1968 and Hutchinson 1973). Nitrogen is readily available in the atmosphere, but atmospheric sources of phosphorus do not exist. Phosphorus is thus only available from dissolved and particulate forms of weathered rock or anthropocentric origins. It is cycled or transported from this terrestrial source to the aquatic environment. Organic phosphorus is tightly bound either to sediments or sorbed to particles (Wetzel 1983).

Phosphorus is categorized in aquatic systems as either dissolved or particulate on the basis of whether it passes through a 0.45-µm filter. As a result, the dissolved constituent may include a component which is colloidal. Inorganic dissolved phosphorus occurs as orthophosphate (PO_4^{-2}) which is biologically available for growth of

periphyton. This fraction is less than 5% of total phosphorus in most natural waters. More than 90% of the P in fresh water occurs as organic phosphates and cellular components in the biota adsorbed to inorganic and dead particulate organic substances (Wetzel 1983). Soluble reactive phosphorus (SRP) is the amount of phosphorus which is hydrolyzed by the molybdate blue method (orthophosphate, organic and colloidal bound forms) (Strickland and Parsons 1972).

Phosphorus is regenerated in aquatic ecosystems from the biota. This process occurs by excretion of dissolved inorganic P (DIP) or dissolved organic P (DOP) from living algae and bacteria, the release of P upon death and lysis of cells, and ingestion followed by egestion, excretion, and death of animal consumers (Newbold 1992). Production of alkaline phosphatase and storage of luxury consumption of P (surplus P) are mechanisms algae use to compensate for depleted levels of biologically available P.

Nitrogen. Nitrogen, a primary constituent of protein, can also be found at levels which limit algal growth in lotic systems (Grimm and Fisher 1986). Unlike P, cycling of N between the terrestrial and aquatic environments involves exchanges with the atmosphere and dissolved inorganic N is available in two forms: NH_4^+ and NO_3^- rather than one, PO_4^{3-}. Nitrogen does not bind to sediments as tightly as does P, therefore N remains dissolved in water as it percolates in ground water. Precipitation as a source of N is variable depending on local weather conditions, watershed usage, and wind patterns (Wetzel 1983). Many blue-green algae have the ability to fix nitrogen, that is to reduce atmospheric nitrogen (N_2) to form ammonium nitrogen (NH_4^+-N) in the process of N-fixation thus making N available to the biota (Wetzel 1983). This phenomena has been

studied in N-limited streams as a mechanism blue-green algae use to compensate for low N levels (Grimm and Fisher 1984; Peterson and Grimm 1992).

Both N and P have been found to limit algal growth in stream ecosystems (Grimm and Fisher 1986; Elwood et al. 1981). Streams can also exhibit simultaneous limitation of algal growth by N and P. Tate (1990) reported enhanced algal growth on clay saucers enriched with both N and P over controls and individual nutrient enrichments in Konza Prairie streams in Kansas. Neither P nor N limited algal growth individually, thus the algae were N and P limited (Tate 1990).

Nonpoint Sources of Nutrients. Allochthonous inputs of phosphorus and nitrogen in lotic ecosystems have been shown to contribute to eutrophication in downstream impoundments. Because P is often limiting, it has been described as the primary causal agent of eutrophication in lentic systems because of the direct influence it has on algal standing crop (Vollenweider 1968; Hutchinson 1973). Novotny and Chesters (1981) state that 50% of P and a greater amount of N originate from nonpoint sources (NPS), that is, the source of input cannot be traced to a single source. Data collected from the National Eutrophication Survey suggest correlations between land use in the watershed and NPS contributions of nutrients (Omernick 1976; Omernick 1977). Streams flowing through agricultural land demonstrated higher P and N levels than those draining forested watersheds (Omernick 1976). Novotny and Chesters (1981) attributed this to fertilizer application on agricultural land followed by runoff events which allow P and N (usually inorganic) to reach the stream. Watersheds with agricultural usage exported 3.7 times more P and 2.2 times more N than non-agricultural watersheds (Omernick 1977). This

NPS pollutant loading has a significant impact on the rate of eutrophication in downstream reservoirs.

Succession. Changes in algal biomass are often difficult to quantify due to stream heterogeneity (Pringle 1988) and limiting factors previously described. Succession is the process of colonization and subsequent change following a disturbance (Fisher 1983). Algal biomass during low winter growth follows characteristic successive phases (Sand-Jensen 1983). This is one type of succession in which colonizers are never completely eliminated. Succession has also been demonstrated on artificial substrates (Lamberti et al. 1989; Pringle 1990; Peterson and Grimm 1992; Rosemond 1994) and after disturbances such as floods (Fisher et al. 1982).

The primary successional phase is characteristic of a thin, relatively simple biological mat community dominated by fast growing sessile algal species and bacteria (Sand-Jensen 1983). Losses due to sloughing and grazing are presumably small. Changes in the overlying water chemistry are tightly coupled with changes in periphyton biomass in early successional stages (Sand-Jensen 1983). Bothwell (1993), found that reduction of photosynthetically active radiation (PAR) and ultraviolet light (UV) to 90% initially inhibited algal accumulation, but eventually the effect was reversed allowing a succession to stalked diatom genera. Peterson and Grimm (1992) showed initial diatom dominance on artificial substrates eventually changed to a dominance by cyanobacteria. Fisher et al. (1982) found post-flood colonizers to be diatoms, which were subsequently replaced by blue-green or *Cladophora*-dominated assemblages. Reynolds (1992) also reported pioneering communities dominated by diatoms, particularly *Ceratoneis* sp.,

following a spate in which stones were turned over exposing new surfaces. This community gave way to larger diatom species of *Synedra* sp. and *Aulacoseira* sp. or to blue-green algae in other streams (Reynolds 1992).

As succession continues, other factors influence biomass accrual (e.g., grazing) and internal processes within the mat assume a greater role in periphyton growth (Sand-Jensen 1983). The relationship of these factors can be quantified as the change in algal biomass (ΔB) over a particular time interval equal to growth of the algae (G) plus colonization (C) minus grazing losses (Gr) and mechanical detachment or sloughing (M) (Sand-Jensen 1983):

$$\Delta B=(G+C)-(Gr+M) \qquad (1)$$

In the present experiment, the pressure of grazing on the mat community was released by suspending the artificial substrates from the surface (i.e., floating substrata). Hence, mechanical detachment or sloughing is the variable which negatively affected biomass accrual of the periphyton community.

Mechanical Detachment

Velocity of the surrounding water functions in controlling periphyton growth. A direct result of high stream velocity is scouring of the periphyton or reduction of colonizer deposition. Horner and Welsh (1981) showed that algal growth on glass slides, as measured by chl. *a*, was inversely proportional to velocity at low orthophosphate conditions in artificial channels. Similarly, Ghosh and Gaur (1994) demonstrated that

stimulatory effects of P enrichment on algal growth was particularly enhanced at low flow. Periphyton is thought to achieve an optimum age prior to detachment or sloughing. Biomass accrual is positive when no factors are limiting that is:

$$\frac{\Delta B}{\Delta t} < 0 \qquad (2)$$

where B represents biomass and t represents time. As the cells comprising the periphyton mat mature, sloughing occurs or:

$$\frac{\Delta B}{\Delta t} > 0 \qquad (3)$$

Rosemond et al. (1993) observed periods of algal increase followed by sloughing in treatments excluded from grazing activity with high nutrients. Grazed treatments were more stable, changing little over time regardless of nutrient level. This suggests that physical and chemical factors affect rates of sloughing. Rates of sloughing have been shown to be variable from stream to stream (Mulholland et al. 1991).

Senescence of the periphyton mat prior to sloughing is a function of biomass accrual and optimal allowable colonization and succession time. This stage represents the period when change in biomass over change in time approaches zero. Exposure periods of artificial substrates vary but are commonly 2 weeks to 1 month depending on water quality, water temperature (seasonality), and purpose of the study (Aloi 1990). In this study, periphyton is being used to determine nutrient status of a lotic system. Hence,

knowledge of maximum maturity of the periphyton mat, as determined by thickness and chl. *a* content, was essential.

Biomarkers for Biofilm Assessment

Alkaline Phosphatase Activity

Phosphatases are enzymes which promote the degradation of complex P compounds into orthophosphate (PO_4^{-3}) and an organic moeity (Jansson et al. 1988). They are classified as acid or alkaline by the pH which hydrolyzing potential is optimum (Jansson et al. 1988). Most natural waters have a pH above 7; thus alkaline phosphatase (AP) is important in many aquatic environments.

Alkaline phosphatases are found intracellularly in mammalian and bacterial cells (Francko 1984) and extracellularly in phytoplankton (Heath and Cook 1975). Alkaline phosphatase activity (APA) has been used as an enzymatic test of nutrient limitation in lentic ecosystems. Increases in extracellular APA are indicative of low orthophosphate (P_i) levels (P limitation) (Perry 1972; Petterson 1980; Wetzel 1981; Wynn 1981).

Phosphatase also has been demonstrated to be a sensitive indicator of P limitation in lotic periphyton communities (Bothwell 1985, 1989). Mulholland and Rosemond (1992) used inverse trends in APA and ambient SRP concentrations to determine periphyton responses to nutrient gradients. Mulholland et al. (1995) used chl. specific APA and C:nutrient ratios in periphyton to determine nutrient deficiency along a continuum of SRP in a stream.

Heath and Cook (1975) demonstrated that AP may hydrolyze organic phosphate resources. The most substantial pool of organic dissolved phosphate in lakes is polyphosphate phosphomonoesters (PME), a low molecular weight substrate of AP (Heath and Cook 1979). When inorganic P (P_i) is low in ambient water, AP is produced which allows the algae to use organic phosphate compounds, such as PME, as sources of biologically available P (Francko and Heath 1979; Stewart and Wetzel 1983). Hydrolysis of P serves to mitigate the effects of P limitation by releasing P_i to algae and bacteria (Jansson et al. 1988).

Phosphomonoesters are the organic phosphorus compounds hydrolyzed by extracellular APA. Heath and Cook (1975) suggested that the significance of APA is dependent on the concomitant occurrence of PME substrates. An inverse relationship between APA and PME concentration was detected in their study (Heath and Cook 1975). This supports the concept of the ability of AP to provide alternative sources of P_i to the biota. However, in Lough Neagh this inverse relationship did not exist (Stevens and Parr 1977). Increases in APA did not result in decreased soluble organic phosphate concentrations suggesting that PME did not comprise a significant part of the organic phosphate pool (Steven and Parr 1977). Alkaline phosphatase activity could be suppressed if ample PME concentrations are not available to convert to P_i

Berman (1970) estimated that only 30-66% of total P was hydrolyzed by APA in epilimnetic waters. Petterson (1980) showed that the composition of the P pool is needed to predict the extent P recycling and P_i demand. How much AP hydrolysis contributes to the soluble P pool in an aquatic system is not fully understood. Heath and

Cook (1975) calculated maximum potential hydrolysis rates of PME by APA (V_{max}) to be 10.4 µmoles phosphate released per hour per liter of lakewater. Berman (1970) found V_{max} to range from 0.2 - 1.26 $\mu M \cdot h^{-1}$ in Lake Kinneret, Israel. Pick (1987) calculated a V_{max} value of 0.26 $\mu M \cdot h^{-1}$ in Lake Ontario. Differences in hydrolysis rates demonstrate the diversity in the soluble P pool.

The increased activity of AP has been attributed to increased rates of production of the enzyme or derepression of the enzyme at the surface of the cell (Stewart and Wetzel 1982). Orthophosphate acts as a repressor of AP (Jansson et al. 1988). When ambient P_i concentrations are lowered, the enzyme is potentially derepressed. Fitzgerald and Nelson (1966) demonstrated APA increased 25 times its normal activity in P-limited algae. Stevens and Parr (1977) reported an increase in APA in response to spring algal blooms which had reduced the ambient P levels to below 10 $\mu gP \cdot l^{-1}$. Petterson (1980) showed APA of phytoplankton to increase to 10 times the normal activity under P limiting conditions. Wetzel (1981) found a decrease in APA under P enrichment conditions.

Although studies have implicated phytoplankton as the primary source of APA in lentic ecosystems (Petterson 1980; Heath and Cook 1975), other potential sources exist Total APA in an aquatic environment encompasses all the enzyme production by bacteria, zooplankton, and phytoplankton within that body of water (Jansson et al. 1988). Stevens and Parr (1977) found additional APA contributions to Lough Neagh from sewage outfalls and the watershed.

Dissolved APA is defined as "free" enzymes which will pass through a 0.45µm

membrane filter (Jansson et al. 1988). This fraction of total APA, though short-lived (Petterson 1980), has been found to be significant, contributing as much as 70% of the total activity (Stewart and Wetzel 1982; Suida and Chrost 1987). Stewart and Wetzel (1982) found that dissolved APA values often were overestimated due to the small size of many free-living aquatic bacteria which could pass through the filter separating the fractions. Particulate APA is that which is associated with organic matter, algae, bacteria, and detrital particles (Francko 1984). Stewart and Wetzel (1982) found the maximum algal contribution to the total activity was less than 34% suggesting that non-algal particulate APA can be a significant component of the particulate fraction of APA. Total APA also has been shown to increase with the subsequent decreases in P_i concentrations in eutrophic lakes (Suida and Chrost 1986).

As previously described, the thickness of the mat community can affect nutrient uptake. Cells at the surface of the mat may not become devoid of nutrients as quickly as cells within the mat community (Paul and Duthie 1988). This could result in differing activity rates associated with the algal particulate fraction depending on where the particular cell is located within the periphyton mat.

Surplus Phosphorus

Algae have mechanisms which allow assimilation of excess levels of phosphorus when ambient inorganic P levels are at growth-limiting levels (Wetzel 1983). This storage of surplus or luxury P is essentially as polyphosphates. Storage of surplus P occurs when ambient inorganic P concentrations are available at higher levels than

required for basic cell maintenance and used when inorganic P concentration are depleted. Gage and Gorham (1984) demonstrated that algae store P in excess of need at or above 10 µg P mg•dry weight^{-1}. Surplus P is used by algae when ambient P_i concentrations are growth limiting. Petterson (1980) demonstrated that during P-limiting conditions in Lake Erken, surplus P levels decreased 4 to 5 times.

Ambient P_i levels may not be an accurate indicator of the nutrient status of the algae because of the ability to store surplus P (Petterson 1980). Wynne (1981) showed that *Peridinium* blooms persisted although ambient concentrations of inorganic P in Lake Kinneret were consistently below levels thought to be indicative of P limitation.

Wynne and Berman (1980) used surplus P as an indicator of the status of nutrient limitation of the algae in Lake Kinneret. Concentrations of < 0.08 µg P· 100 µg C^{-1} have been found to be indicative of P limitation (Fitzgerald and Nelson 1966). Petterson (1980) drew similar conclusions demonstrating production of AP would be triggered at surplus P concentrations of 0.1 µg P· 100 µg C^{-1}. Therefore, concentrations below 0.1 µg P· 100 µg C^{-1} are indicative of P limitation.

Surplus P is the amount of total cellular P that can be extracted using hot water (i.e., hot water extractable P or surplus P_i). Surplus P_i is composed of long and short chain polyphosphates with phosphate ester bonds (Wynn and Berman 1980). Most short chain polyphosphates are hydrolyzable by APA and comprised the bulk of the non-molybdate-reactive P (non-MRP) pool (i.e. phosphorus not reactive with an ascorbic acid mixed reagent). These short chains also appeared to be a precursor for a more permanent P storage (Wynn and Berman 1980). Long chain polyphosphates are typically the more

abundant fraction of surplus P. When algal cells are grown in low inorganic P conditions, SRP becomes depleted (Wynn and Berman 1980).

The interactions of these fractions in relation to P status have demonstrated a preference of MRP by algal cells (Wynn 1981). Hot water extractable P from lake *Peridinium* and from cells grown in culture at high ambient P_i contained equal amounts of non-MRP and MRP suggesting that both fractions served as P reserves. At low inorganic P concentrations, MRP was first depleted by *Peridinium*. From this result it was suggested that non-MRP remains available to the cell at low ambient inorganic P concentrations and may function as an intermediary P-storage compound (Wynn 1981).

Relationship of APA and Surplus P

Surplus P concentrations and APA have been used concomitantly to predict P limitation of algal growth. An increase in APA correlated with a decrease in surplus P (as MRP) indicates possible P limitation (Fitzgerald and Nelson 1966). Petterson (1980) demonstrated this inverse relationship between APA and surplus P as MRP in lake and culture studies. This relationship implies that when inorganic P is limiting, APA is increased to hydrolyze unusable forms of organic P to inorganic P and surplus P levels are concomitantly depleted as the algal cell responds to limitation by utilizing stored inorganic P.

Longitudinal Gradients in Lotic Systems

River Continuum Concept

The river continuum concept (Vannote et al. 1980) associates changes in lotic communities to the downstream gradient of abiotic factors from the headwaters to the mouth of the river. Successive, interrelating geomorphical, chemical, and physical factors in lotic ecosystems are accompanied by communities evolving to each state of morphological and hydraulic conditions. The river continuum concept (RCC) states that consideration of the gradient of physical factors formed by the watershed which the lotic system drain, is vital in understanding biological processes and stream dynamics (Vannote et al. 1980).

According to the RCC, streams in undisturbed deciduous forest streams demonstrate a longitudinal resource gradient in which communities are predictably structured. Headwater streams are primarily heterotrophic, relying heavily on allochthonous detritus in the form of course particulate organic matter (CPOM) as the primary energy source. Gross primary productivity to community respiration ratios (P/R) are generally less than 1 in lower order streams. Algal growth is often limited by light in the headwaters of a stream dominated by groundwater inputs. As the canopy opens in the middle reaches, autochthonous primary productivity and autotrophy dominates (P/R>1). The lower reach river is again heterotrophic (P/R<1), being light-limited once again due to turbidity and water depth. Large rivers receive significant amounts of fine particulate organic matter (FPOM) from upstream. This FPOM is processed CPOM which originated in the headwaters. This demonstrates the interdependence of organic resources and invertebrate functional feeding groups which process the resources and

how they change along the continuum (Vannote et al. 1980).

Nutrient Spiraling

Nutrient spiraling initially described the joint processes of nutrient cycling and downstream transport in lotic systems (Webster 1975; Webster and Patten 1979). This was then expanded to an ecological framework. This concept holds that upstream nutrient cycling will affect downstream communities and processes because forms and concentrations of nutrients and organic matter in transport will be changed (Newbold et al. 1982, 1983; Elwood et al. 1983).

Spiraling explains downstream progress of the aqueous, particulate, and consumer fractions of a nutrient cycle. The cycle of a nutrient includes biological assimilation (uptake) and subsequent biological processing and movement through the food web resulting in eventual regeneration into the inorganic form. Spiraling length consists of the average downstream distance traveled by a dissolved nutrient atom until uptake (uptake length) plus the downstream distance traveled within the biota until regeneration (turnover length) is attained (Newbold et al. 1981).

Newbold et al. (1983) released $^{32}PO_4$ into the stream to measure spiraling in Walker Branch, a small stream in Tennessee. Downstream transport of P occurred at a velocity of 10.4 $m \cdot h^{-1}$ completing one cycle every 18.4 days. The average downstream distance of one spiral (transport of one P atom from the water compartment and back again) was 190 m. Transport within the water column contributed the most to the spiraling length (165 m) and consumer turnover length contributed the least with 0.05 m

(Newbold et al. 1983). Phosphorus remained in FPOM for the greatest amount of time (99 days). Only 2.8% of P uptake from particulate matter was transferred to consumers suggesting most of the P was released from this fraction to the water (Newbold et al. 1983).

Mulholland et al. (1985) noted seasonal variations in spiraling. The uptake of $^{32}PO_4$ from the water by CPOM was greatest in the fall after leaf fall and lowest in August prior to leaf fall. During fall and early winter periods when CPOM is abundant due to leaf fall and uptake length is short, P spiraling exerts strong controls over biotic processes downstream (Mulholland et al. 1985). Guasch et al. (1995) found despite high algal biomass, summer photosynthetic capacity decreased following enrichment. This was attributed to mat thickness and subsequent self-shading which could have prevented an increase in photosynthetic activity via enrichment (Guasch et al. 1995). Grazing has been demonstrated to increase spiraling length of P by reducing periphyton biomass and subsequently reducing uptake of P from the water (Mulholland et al. 1983). Steinman et al. (1995) concluded that P turnover in the periphyton mat was highest under grazed conditions with low biomass. This study also determined that irradiance had no significant effect on P turnover.

Shorter spiraling lengths (tight spirals) are indicative of more efficient utilization of nutrients relative to nutrient supply. This increased efficiency is intuitive in that a nutrient atom cycles through the biota a greater number of times as it travels the length of the stream in a tight spiral (Newbold et al. 1983).

Patch Dynamics

The concept of patch dynamics can be used to consider effects of biological communities in one patch on communities in patches directly downstream (Pringle et al. 1988). A patch is defined as a distinctive spatial unit that is determined by processes which are attributed to its uniqueness. In lotic systems, patches may be determined by interactions of topography, substrata conditions, current patterns, organisms, and disturbance (Pringle et al. 1988).

Patch dynamics are more pronounced in streams with low ambient nutrient levels where periphyton would be located in adjacent patches in response to localized nutrient sources (Pringle et al. 1988). Periphyton is more evenly distributed in nutrient rich environments negating the effects of patch dynamics (Pringle et al. 1988). A stream can thus be defined as a mosaic of nutrient micro-patches which differ in chemical nature. These micro-patches may reveal variable but conceivably predictable biotic responses among patches (Pringle et al. 1988; Pringle 1990).

Patch characteristics determine how nutrients are transported along the stream continuum. This temporal and spatial heterogeneity of nutrient supply allows algae to maintain diversity in nutrient-poor systems (Pringle et al. 1990). Thus, nutrient spiraling must be evaluated in the context of patch dynamics. Processes of nutrient spiraling are affected by substrata patch arrangements (riffle-pool) and larger stream-order variability. Substrata types are variable and often influenced by stream order (Vannote et al. 1980). Abiotic and biotic retention of nutrients is determined by patch characteristics such as

this. Hart (1985) showed that grazing caddisflies can create spatial variability of periphyton (patches) which influence nutrient cycling. Surplus P and APA can be thought of as mechanisms periphyton possess which allow maximization of the ability to capitalize on nutrient variations attributed to patch dynamics. This increases overall ability of the system to retain nutrients (i.e., shorter spiraling lengths) (Pringle et al. 1988).

Upstream Downstream Linkage in Periphyton

Streams are longitudinally connected ecosystems which demonstrate upstream downstream linkages (Mulholland et al. 1995). Fisher et al. (1982) showed linkages in N uptake in Sycamore Creek, Arizona. Uptake of N upstream resulted in reduced nitrate concentrations downstream and subsequent dominance by blue-green algae. This demonstrates longitudinal linkages in community structure and stream metabolism which are a direct result of nutrient transport in the overlying water (Fisher et al. 1982).

Mulholland and Rosemond (1992) demonstrated upstream-downstream biotic linkages in Walker Branch, Tennessee. This study demonstrated that nutrient uptake can reduce nutrient concentrations in the overlying water and influence the structure and function of periphyton communities downstream. Responses to longitudinal depletion of nutrients by the periphyton community were found to be limited to P-cycling indices of APA and P content of the periphyton. Concentration of SRP declined along a downstream gradient (Mulholland and Rosemond 1992). Periphyton assemblages continued to respond at SRP concentrations of 1-5$\mu g \cdot l^{-1}$ suggesting algal APA

compensated for low SRP levels. Increased APA levels were reported as SRP decreased longitudinally. The P levels of the periphyton remained low under P-limiting conditions. Mulholland and Rosemond (1992) suggested that this may indicate reduced surplus P when supplies of P are limiting or may indicate a more rapid rate of nutrient recycling within the mat.

Upstream-downstream longitudinal patterns also have been reported in laboratory stream studies (Mulholland et al. 1995). Significant declines in SRP, NO_2-NO_3, and N:P ratios were reported with distance downstream. The composition of the periphyton community also was reported to change with distance downstream. Alkaline phosphatase activity increased significantly with distance. Decline of the ratio of net:total P uptake rate with distance suggested downstream communities recycled P more efficiently. The ratio of total P uptake rate : GPP also declined longitudinally indicating greater P cycling withing the periphyton downstream. Mulholland et al. (1995) concluded that longitudinal depletion of nutrient concentrations can be mitigated by increased nutrient cycling which would prevent large longitudinal changes in algal biomass and productivity.

CHAPTER V

RESULTS

Chl. *a* Time Courses

Time necessary for peak biomass was determined as occurring when further significant accumulation of biomass stopped. Specifically, peak biomass was defined as the second week in which no statistical difference existed between subsequent biomass parameters (chl. *a* and AFDW), and assured that logarithmic growth phase had ceased. Results of ANOVA tests and Tukey's pairwise comparisons are reported in Appendix D.

Pilot Experiment 1

Due to analytical problems regarding spectrophotometry, data on biological parameters measured during the experiment were not valid. Water chemistry data are described below.

Pilot Experiment 2

Styrofoam Substrata. There was a significant effect of time on chl. *a* concentrations in Peacheater Creek (1-way ANOVA, $F=5.039$, $p=0.004$) and Tyner Creek ($F=5.894$, $p=0.002$) (Table 3) (Figure 2). In Peacheater Creek, accumulation measured

after 2 weeks colonization time was not significantly different from measurement in all following weeks ($p<0.05$). Week 3 chl. *a* accumulation was also not significantly different from all following weeks($p<0.05$). Based on the criterion that peak biomass occurs after the second week when no statistical difference between subsequent weeks was detected, peak biomass occurred after 3 weeks colonization time (Table 3, Figure 2). In Tyner Creek, time necessary for peak biomass occurred at 4 weeks, using the same criterion, i.e. it was the second week after which no statistical difference in chl. *a* concentrations existed between weeks ($p<0.05$) (Table 3, Figure 2).

Fused Silicated Disc Substrata. There was a significant effect of time on chl. *a* concentrations measured on silicated discs in Peacheater Creek (1-way ANOVA, $F=57.639$, $p<0.001$) and Tyner Creek ($F=3.605$, $p=0.025$). In Peacheater Creek, there were many significant differences in biomass between weeks in pairwise comparisons ($p<0.05$) and it was not possible to define the time of peak biomass. However, it was estimated that peak biomass was reached after 7 weeks colonization time because by 8 weeks biomass had declined statistically ($p<0.05$) (Table 3, Figure 3). Peak biomassoccurred after 3 weeks colonization in Tyner Creek (Table 3, Figure 3).

Styrofoam Versus Silicated Disc Substrata. Mean or median values are given in parentheses. Units have been given previously. In Peacheater Creek, mean chl. *a* concentrations on styrofoam substrata (9.90) were not significantly different than for those on silicated discs (10.26) in a paired t-test ($t=-0.191$, $df=42$, $p=0.850$). In Tyner Creek, median chl. *a* concentrations on styrofoam substrata (8.94) were significantly different than on silicated discs (11.38) (Mann-Whitney rank sum test, $T=549.0$,

TABLE III

SIGNIFICANT DIFFERENCES IN CHL. *a* CONCENTRATIONS BETWEEN WEEKS OF COLONIZATION TIME

Colonization	Peacheater Creek							Tyner Creek						
Pilot 2-Styro														
Week 1	2	3	4	5	6	7	8	2	3	4	5	6	7	8
Week 2		3	4	5	6	7	8		3	4	5	6	7	8
Week 3 †			4	5	6	7	8			4	5	6	7	8
Week 4 §				5	6	7	8				5	6	7	8
Week 5					6	7	8					6	7	8
Week 6						7	8						7	8
Week 7							8							8
Pilot 2-Disc														
Week 1	2	3	4	5	6	7	8	2	3	4	5	6	7	8
Week 2		3	4	5	6	7	8		3	4	5	6	7	8
Week 3 §			4	5	6	7	8			4	5	6	7	8
Week 4				5	6	7	8				5	6	7	8
Week 5					6	7	8					6	7	8
Week 6						7	8						7	8
Week 7							8							8
Experiment 3														
Week 1	2	3	4	5	6	7		2	3	4	5	6	7	
Week 2		3	4	5	6	7			3	4	5	6	7	
Week 3			4	5	6	7				4	5	6	7	
Week 4				5	6	7					5	6	7	
Week 5 †§					6	7						6	7	
Week 6						7							7	
Experiment 4														
Week 1	2	3	4	5	6			2	3	4	5	6		
Week 2		3	4	5	6				3	4	5	6		
Week 3 †§			4	5	6					4	5	6		
Week 4				5	6						5	6		
Week 5					6							6		

The level of significance is 0.05. Weeks which share a common underline are not significantly different in chl. *a* concentration from the week in the corresponding column at the left. †Indicates peak biomass occurred in Peacheater and § in Tyner Creek.

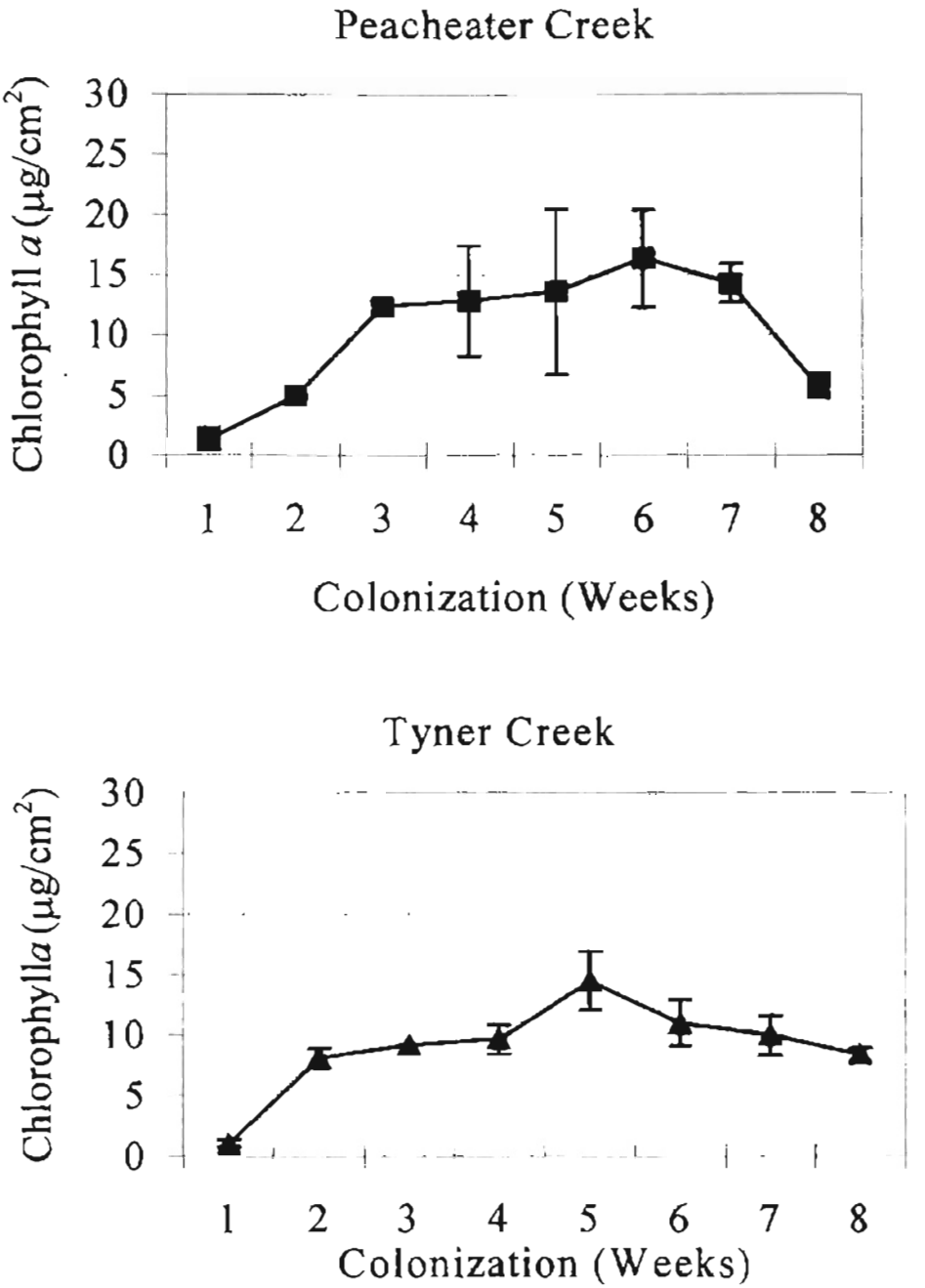

Figure 2. Biomass accumulation as measured by mean chl. *a* concentration on styrofoam substrata summer 1996 in pilot experiment 2. Error bars are standard deviation of mean (n=3). Peak biomass occurred during week 3 in Peacheater Creek and week 4 in Tyner Creek.

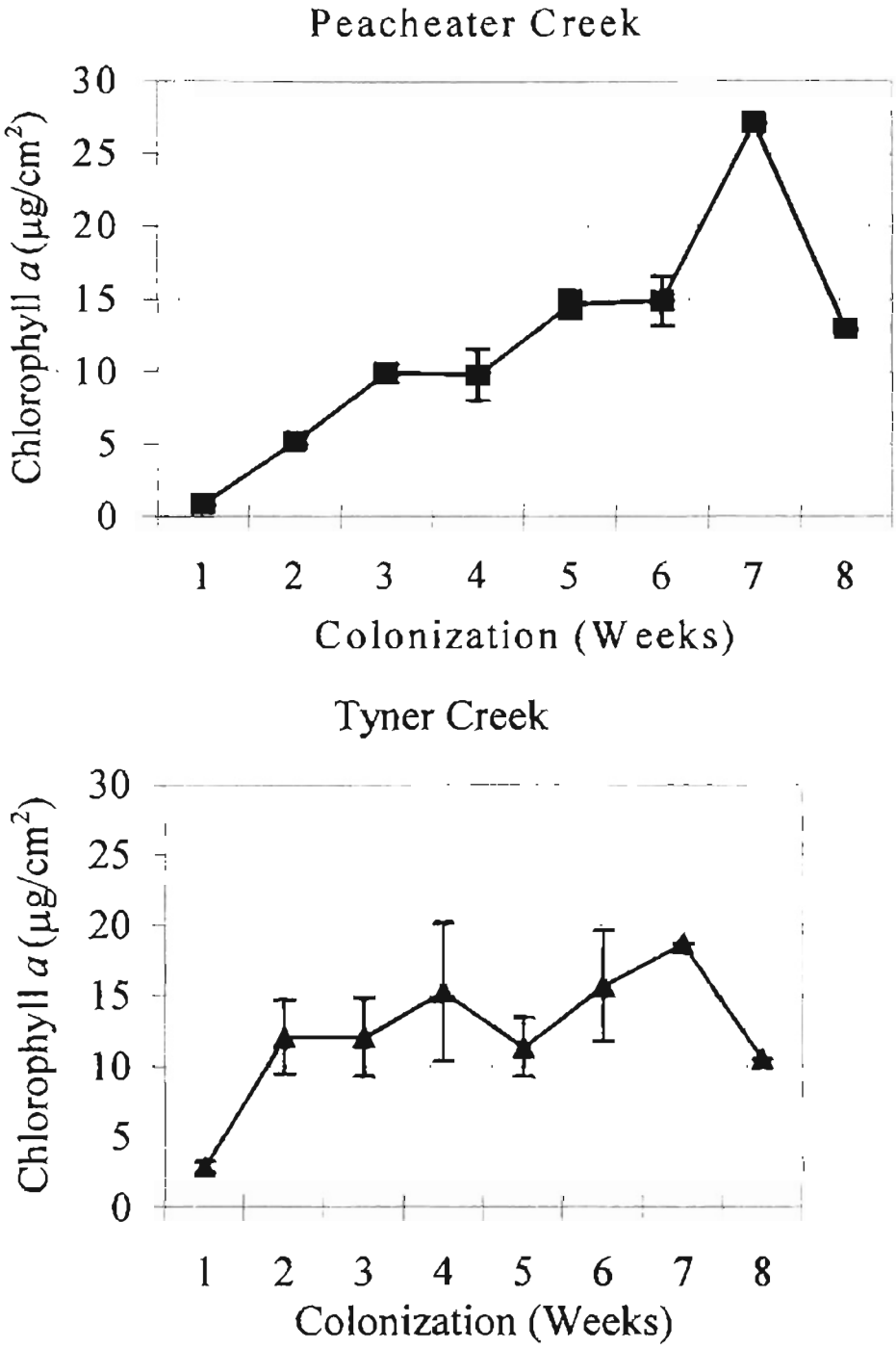

Figure 3. Biomass accumulation as measured by mean chl. *a* concentration on fused silicated disc substrata summer 1996 in pilot experiment 2. Error bars are standard deviation of mean (n=3). Peak biomass occurred during week 7 in Peacheater Creek and week 3 in Tyner Creek.

p=0.020). Two grazing snails were noticed on styrofoam substrata in Peacheater Creek during sampling in weeks 5 and 6. No grazers were present on the silicated discs during the sampling period. Silicated discs were used in remaining experiments as described in materials and methods.

Experiment 3

No data were collected after 8 weeks of colonization due to a flood that washed boards downstream. There was a significant effect of time on chl. *a* concentrations in Peacheater Creek (1-way ANOVA, F=11.455, p<0.001) and Tyner Creek (F=19.408, p<0.001). Peak biomass was measured after 5 weeks in both Peacheater Creek and Tyner Creek (Table 3, Figure 4).

Experiment 4

Weekly chl. *a* concentrations in experiment 4 showed a significant effect of time in Peacheater Creek and Tyner Creek (1-way ANOVA, F=8.814, p=0.000; F=18.347, p=0.000). In Peacheater Creek, peak biomass was reached after 3 weeks colonization (Table 3, Figure 5). Data collected in Tyner Creek did not meet the criterion used to detect time necessary for peak biomass accrual (Table 3, Figure 5). Biomass accumulation in weeks 3 through 5 was not significantly different from week 2. However, accumulations in weeks 3 through 5 were different from week 6 accumulation (Table 3). For this reason, peak biomass was determined to have occurred after 3 weeks colonization time.

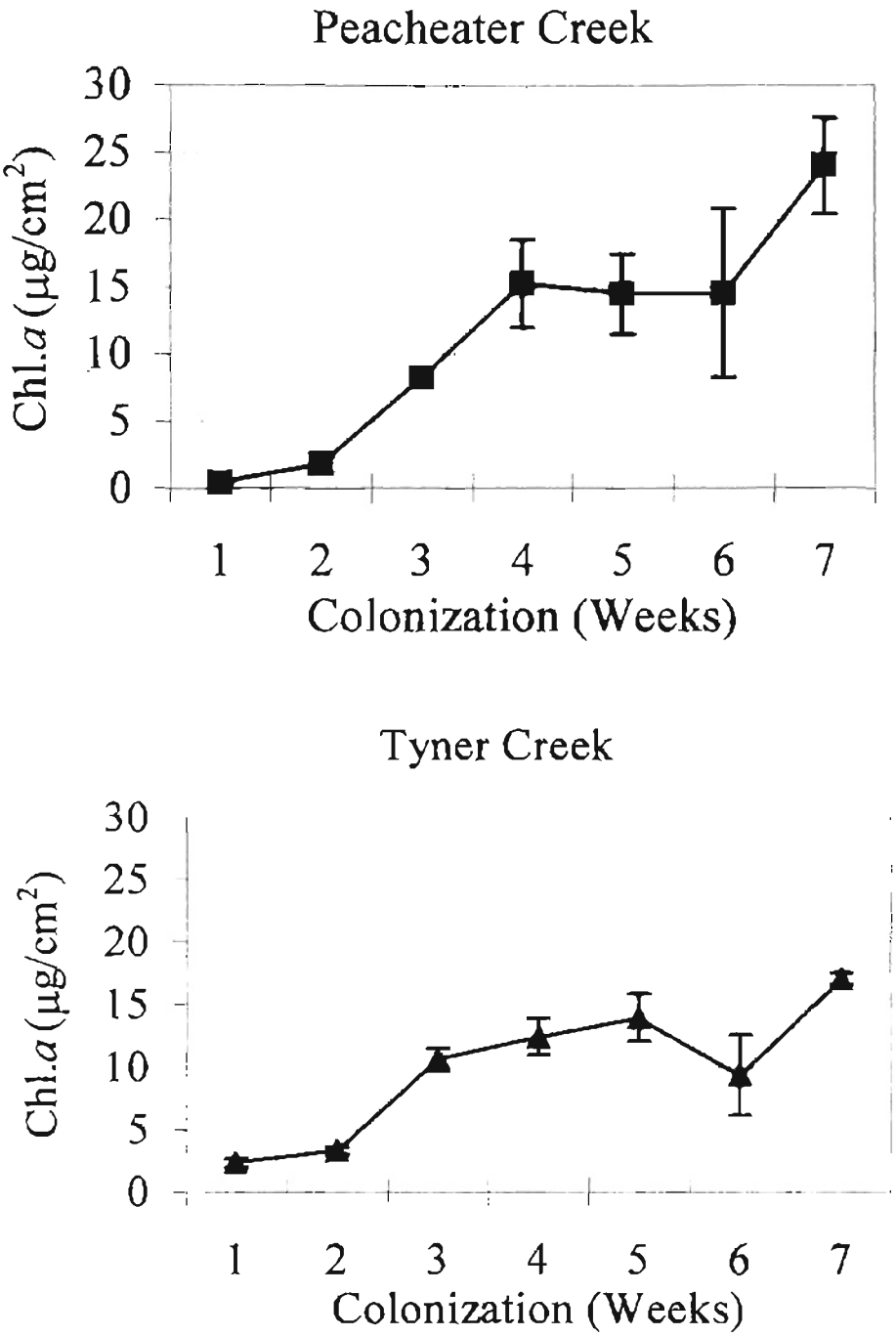

Figure 4. Biomass accumulation as measured by chl. *a* concentration in late summer 1996 in experiment 3. Error bars are standard deviation of mean (n=3) .Peak biomass occurred during week 5 in both Peacheater Creek and Tyner Creek.

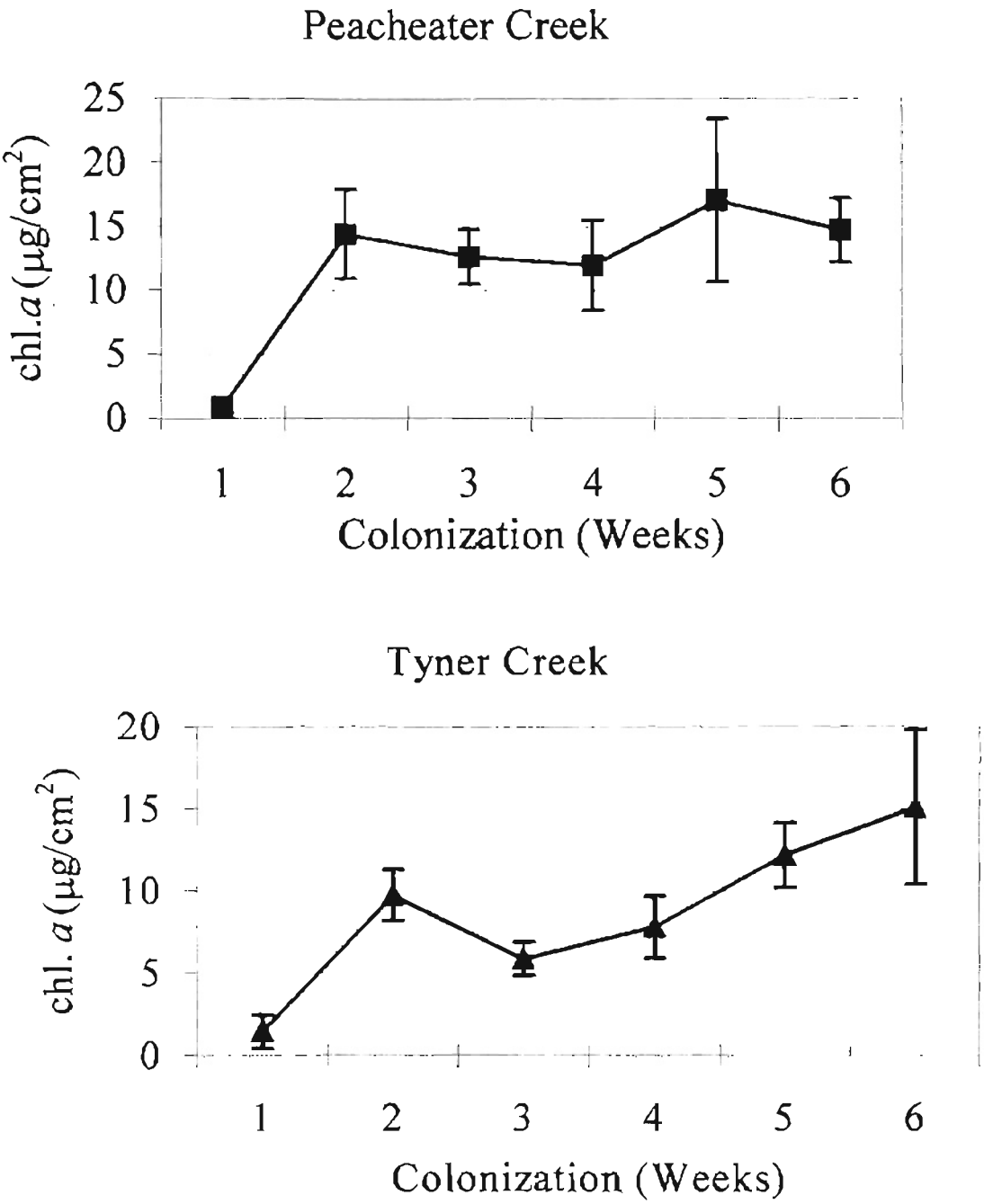

Figure 5. Chl. *a* concentrations in Peacheater Creek and Tyner Creek during experiment 4. Error bars are standard deviations of mean (n=3). Peak biomass occurred during week 3 in both Peacheater and Tyner Creek.

Time Course Comparison

Differences existed in weekly chl. *a* concentrations between time courses (Kruskill-Wallace 1-way ANOVA on ranks, H=28.8, df=3, $p<0.001$). Pairwise comparisons were made using Dunn's test. Differences in ranks are given in parentheses.

In Tyner Creek, chl. *a* concentrations during the last time course (experiment 4) were significantly different than the first time courses (pilot experiment 2) on styrofoam (33.4) and silicated disc (28.0) in early summer and in experiment 3 (31.5) in late summer in Peacheater Creek ($p<0.05$). In Peacheater Creek, chl. *a* concentrations during experiment 4 were significantly different than in experiment 2 on styrofoam substrata (26.23) and silicated discs (23.71) ($p<0.05$). Chl. *a* concentrations during experiment 4 were also significantly different than during experiment 3 (25.54) ($p<0.05$). Statistical tables are presented in Appendix D.

AFDW Time Courses

Experiment 3

In experiment 3, AFDW was analyzed as another measure of biomass. Data were not collected for week 1 due to analytical error. Statistical tables and AFDW means are presented in Appendix D. F-ratios were significant in Peacheater Creek suggesting significance of time (1-way ANOVA, F=11.069, p=0.001). In Peacheater Creek, no significant difference existed between AFDW accumulated after 2 weeks and following

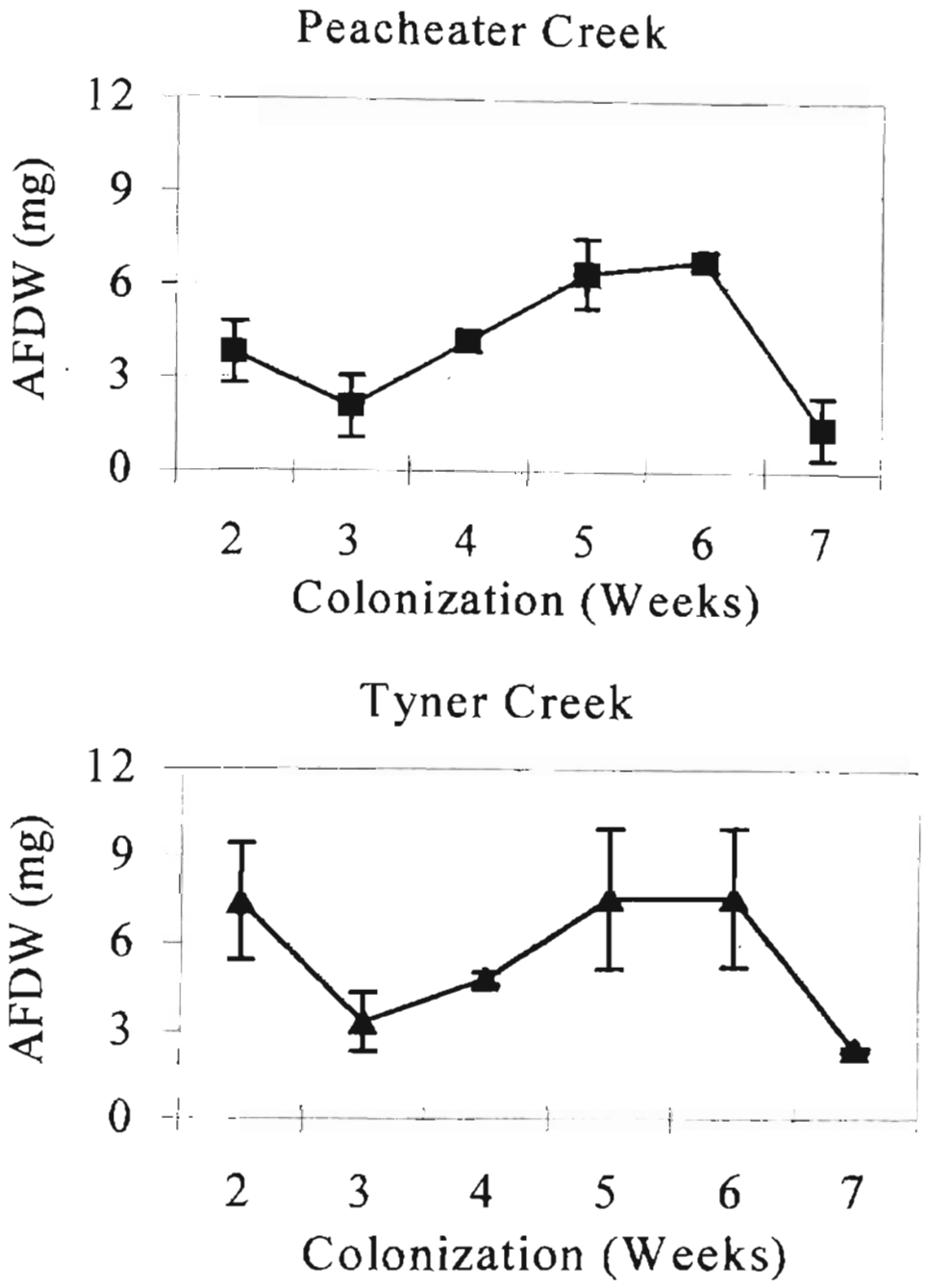

Figure 6. Biomass accumulation as measured by AFDW in late summer 1996 in experiment 3. Error bars are standard deviations of mean (n = 3). Peak biomass occurred during week 4 in Peacheater Creek and week 3 in Tyner Creek.

TABLE IV

SIGNIFICANT DIFFERENCES IN AFDW BETWEEN WEEKS OF COLONIZATION TIME DURING EXPERIMENT 3

Colonization Time	Peacheater Creek					Tyner Creek				
Week 2	3	4	5	6	7	3	4	5	6	7
Week 3§		4	5	6	7		4	5	6	7
Week 4†			5	6	7			5	6	7
Week 5				6	7				6	7
Week 6					7					7
Week 7										

The level of significance is 0.05. Weeks that share a common underline are not significantly different in AFDW concentrations from the week in the corresponding column at the left. † Indicates peak AFDW occurred in Peacheater Creek and § in Tyner Creek

weeks ($p<0.05$) (Table 4). Differences did exist between week 3 accumulation and weeks 5 through 7 ($p<0.05$). The second week in which all subsequent measurements were statistically the same was week 4. Thus, peak AFDW was reached after 4 weeks colonization time in Peacheater Creek (Table 4, Figure 6).

A similar trend in AFDW was followed in Tyner Creek (Figure 6). However, F-ratios were not significant (1-way ANOVA, $F=3.290$, $p=0.046$). This suggests no difference existed between weekly AFDW measurements in Tyner Creek after 3 weeks. Therefore, peak biomass occurred after 3 weeks colonization time using the criterion above (Table 4, Figure 6). Tyner Creek developed a mature periphytic mat slightly sooner than Peacheater Creek as measured by AFDW.

Chlorophyll *a* Versus AFDW Time Courses

Both biomass parameters were measured in experiment 3. No significant correlation was detected in either stream ($p>0.1$). Correlation coefficients were $r = 0.296$ and $r = 0.300$ in Peacheater and Tyner Creeks, respectively.

Time necessary for peak biomass to occur in all time courses is summarized in Table 5. In Peacheater Creek, peak biomass occurred after similar colonization periods in all time courses (3 to 5 weeks). The exception was the first time course which occurred 24 June through 28 August 1996 on silicated discs. Peak biomass occurred in this experiment after 7 weeks colonization; the latest of all time courses. Peak biomass occurred later in experiment 3 (5 weeks) than in experiment 2 on styrofoam substrata (3 weeks) and experiment 4 (3 weeks).

Time necessary for peak biomass accumulation, as measured by chl. *a*, also varied in Tyner Creek (Table 5). Peak biomass on silicated disc substrata occurred sooner during experiments 2 and 4 (3 weeks) than during experiment 3 (5 weeks). Longer colonization time was necessary for styrofoam substrata. Unlike Peacheater Creek, the longest time period needed for periphyton to achieve maximum biomass in Tyner Creek was 5 weeks during experiment 3. The greatest discrepancy between the two streams occurred during the second pilot experiment. Peak concentrations of chl. *a* occurred sooner on silicated discs in Tyner Creek (2 weeks) as compared to Peacheater Creek (7 weeks).

In Peacheater Creek peak AFDW occurred one week later (week 4) than peak chl. *a* (Table 5). Peak biomass occurred after the same colonization period for both chl. *a* and AFDW in Tyner Creek (Table 5).

TABLE V

COMPARISON OF TIME NECESSARY FOR PEAK BIOMASS ACCUMULATION IN PEACHEATER CREEK AND TYNER CREEK

EXPERIMENT	PARAMETER	PEACHEATER CREEK	TYNER CREEK
Pilot 2 - Styrofoam	Chl. *a*	3 weeks	4 weeks
Pilot 2 - Silicated Disc	Chl. *a*	7 weeks	3 weeks
Experiment 3	Chl. *a*	5 weeks	5 weeks
Experiment 3	AFDW	4 weeks	3 weeks
Experiment 4	Chl. *a*	3 weeks	3 weeks

Necessary colonization time for peak biomass accumulation, as measured by chl. *a*, occurred sooner in the winter than the summer in most time courses in both streams (Table 5).

Surplus P Time Courses

Significance in weekly concentrations was determined with a 1-way ANOVA test. Differences between weekly measurements were determined by Tukey's pairwise comparisons test. Statistical tables are reported in Appendix D.

Experiment 3

Areal Surplus Phosphorus. Surplus P not normalized to a biomass parameter is referred to as areal (μg surplus P · disc^{-1}). Surplus P of periphyton accumulation was measured beginning at 2 weeks colonization time. There was a significant effect of time in concentrations in Peacheater Creek ($F=8.814$, $p<0.001$) and Tyner Creek ($F=5.691$,

p=0.008).

In Peacheater Creek, areal surplus P increased with increasing colonization time (Figure 7). In pairwise comparisons, week 7 areal surplus P was statistically greater than concentrations in weeks 2 through 4 ($p<0.05$) (Table 6). No statistical differences existed in areal surplus P concentrations after 5 weeks colonization time ($p>0.05$).

In Tyner Creek, concentrations of areal surplus P also increased with colonization time (Figure 7). Pairwise comparisons of week 2 surplus P with all other weeks revealed concentrations measured during week 2 were significantly less than weeks 5 through 7 ($p<0.05$) (Table 6). No significant differences between areal surplus P were detected after 3 weeks colonization time ($p>0.05$) (Table 6).

Surplus P Normalized to Chl. *a*. Weekly concentrations of surplus P normalized to chl. *a* were significantly different in Peacheater Creek ($F=10.919$, $p=0.001$) and Tyner Creek ($F=11.638$, $p<0.0001$). When normalized to chl. *a*, surplus P did not increase with increasing colonization time as when expressed on an areal basis (Figure 8).

In Peacheater Creek, pairwise comparisons of week 2 surplus P concentrations with all other weeks revealed significant differences with between week 2 and weeks 4, 6, and 7 surplus P ($p<0.05$) (Table 6). Pairwise comparisons with week 3 and following weeks also revealed week 4 surplus P was significantly less than week 3 ($p<0.05$) (Table 6). No significant differences existed between surplus P concentrations after week 4 ($p>0.05$) (Table 6, Figure 8).

In Tyner Creek, pairwise comparisons of week 2 surplus P revealed statistical differences with all other weeks ($p<0.05$) except week 6 ($p>0.05$) (Table 6). No

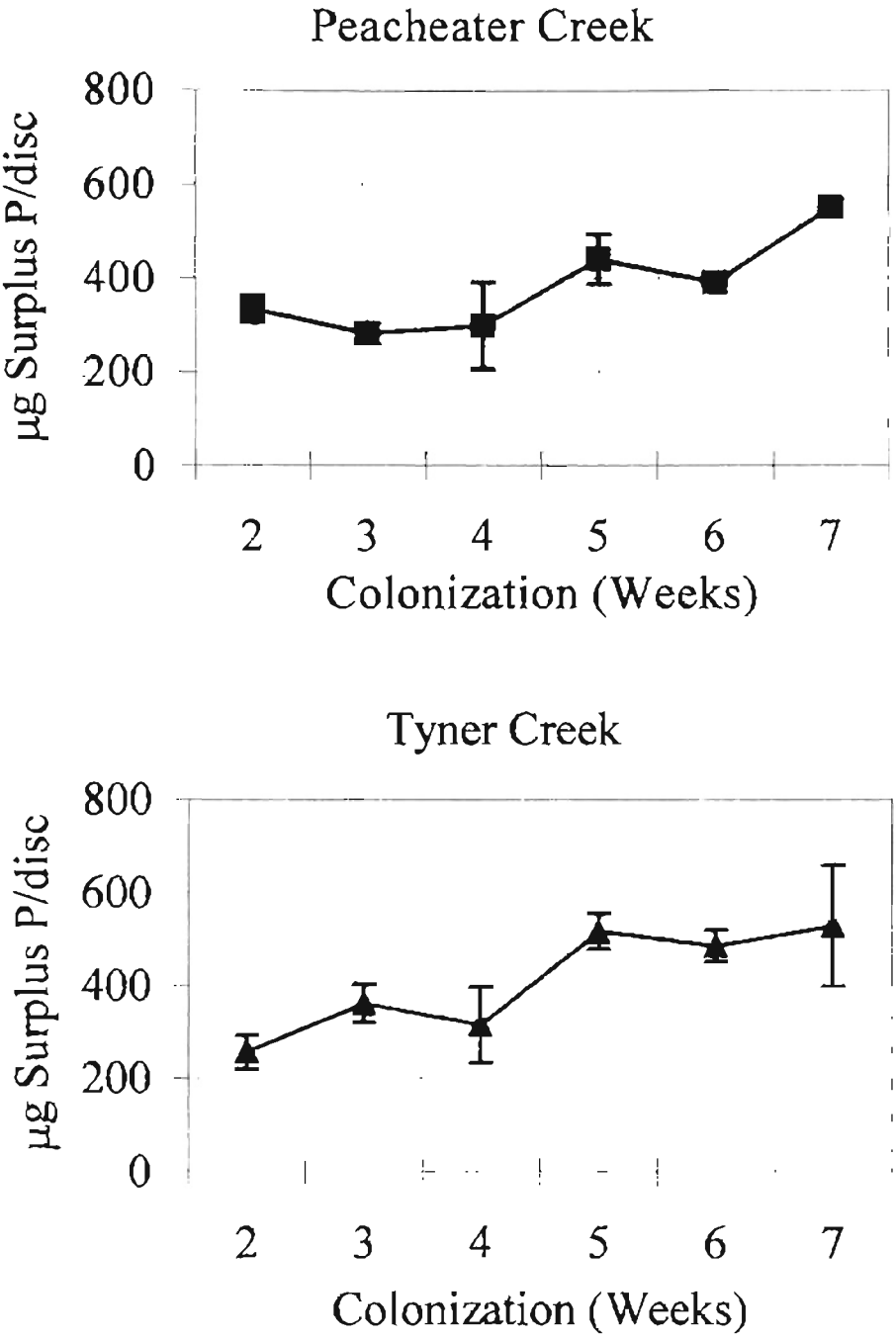

Figure 7. Areal surplus P content of periphyton accumulated on silicated disc substrata over time during experiment 3. Error bars are standard deviation of mean (n=3).

TABLE VI

SIGNIFICANT DIFFERENCES IN SURPLUS P CONCENTRATIONS BETWEEN WEEKS OF COLONIZATION TIME

Colonization Time	Peacheater Creek	Tyner Creek
Areal		
Experiment 3		
Week 2	3 4 5 6 7	3 4 5 6 7
Week 3	4 5 6 7	4 5 6 7
Week 4	5 6 7	5 6 7
Week 5	6 7	6 7
Week 6	7	7
Week 7		
Normalized to Chl. *a*		
Experiment 3		
Week 2	3 4 5 6 7	3 4 5 6 7
Week 3	4 5 6 7	4 5 6 7
Week 4	5 6 7	5 6 7
Week 5	6 7	6 7
Week 6	7	7
Week 7		
Normalized to AFDW		
Experiment 3		
Week 2	3 4 5 6 7	3 4 5 6 7
Week 3	4 5 6 7	4 5 6 7
Week 4	5 6 7	5 6 7
Week 5	6 7	6 7
Week 6	7	7
Week 7		

The level of significance is 0.05. Weeks which share a common underline are not significantly different in surplus P concentration from the week in the corresponding column at the left.

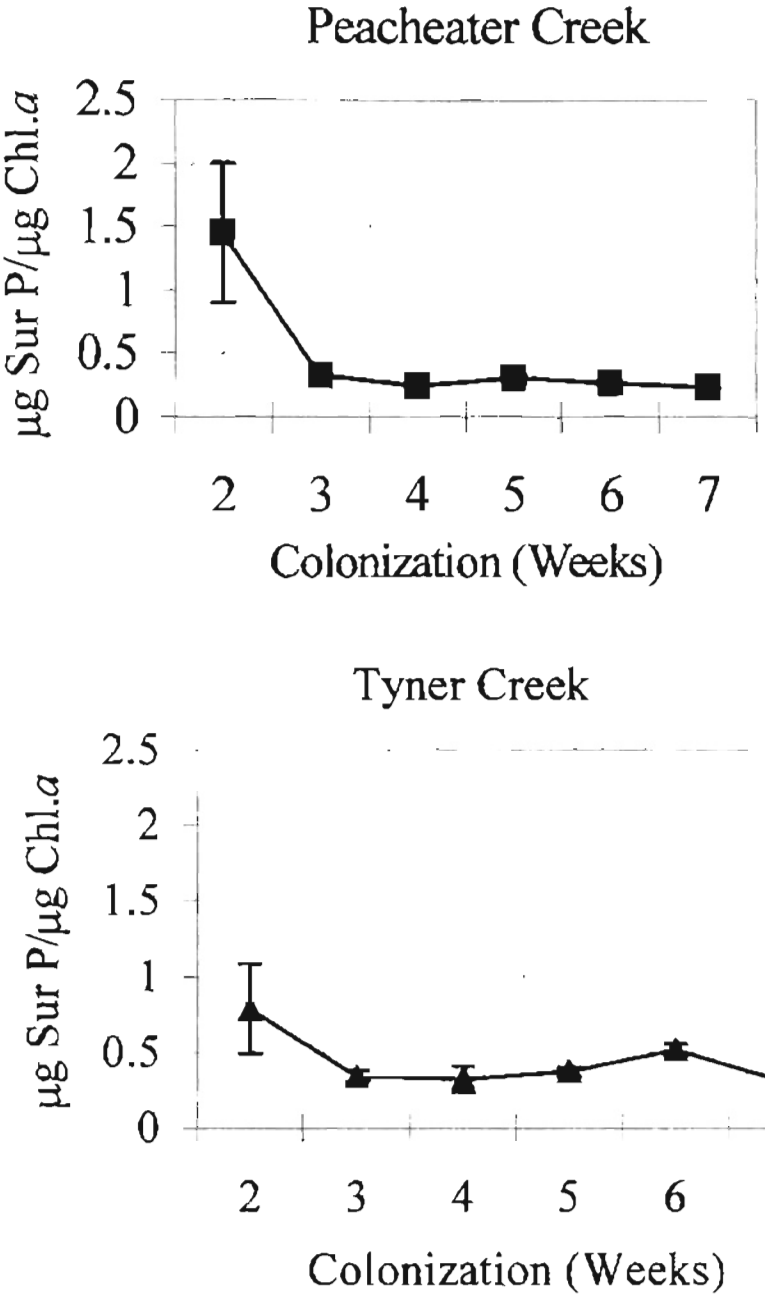

Figure 8. Surplus P content of periphyton normalized to chl. *a* accumulated on silicated disc substrata over time during experiment 3. Error bars are standard deviation of mean (n=3).

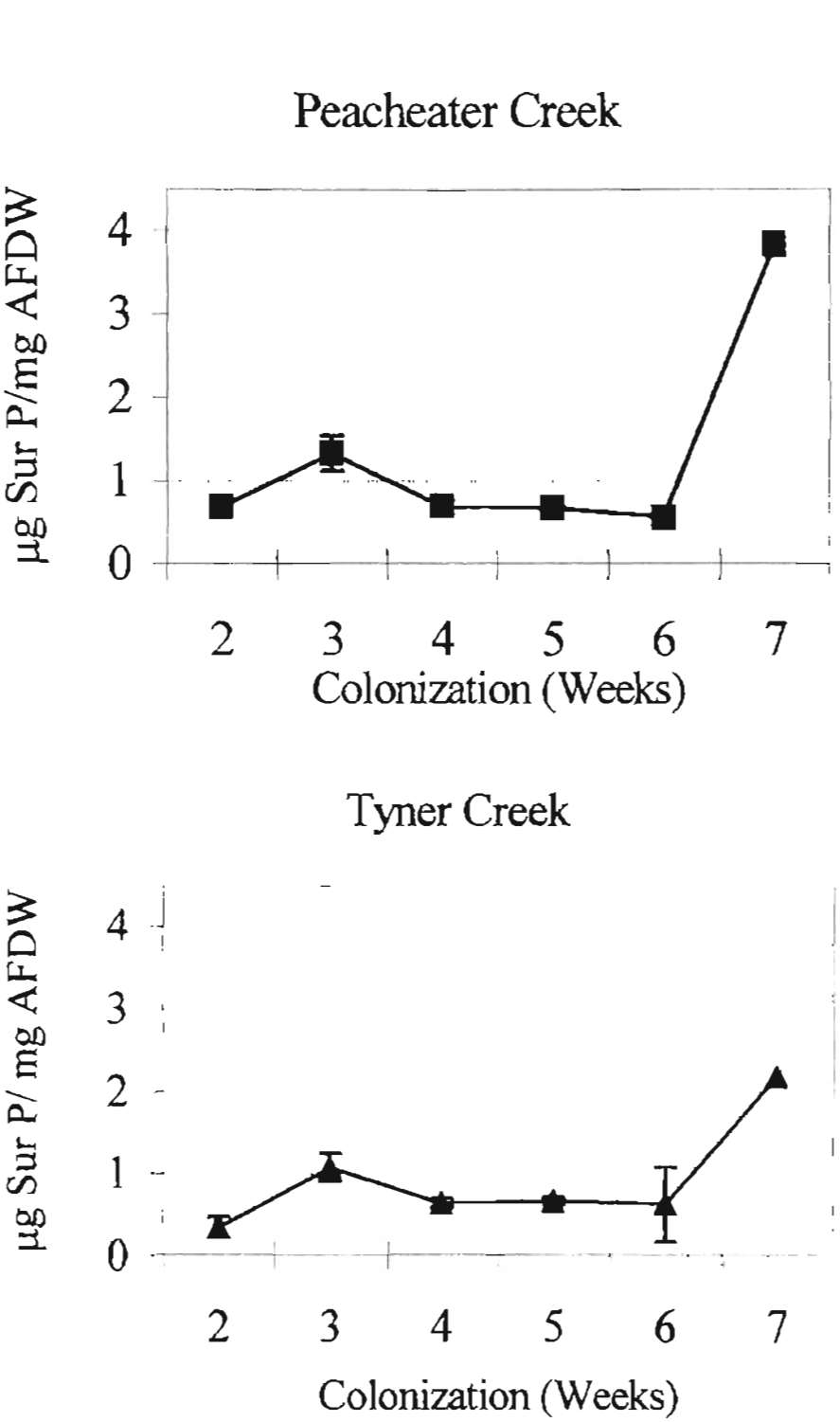

Figure 9. Surplus P content of periphyton normalized to AFDW accumulated on silicated disc substrata over time during watershed comparison experiment 3. Error bars are standard deviation of mean (n=3).

significant difference existed between week 3 through 7 in pairwise comparisons of week 3 surplus P with following weeks ($p>0.05$) (Table 6). However, other pairwise comparisons revealed week 6 surplus P was significantly greater than for week 4 and 7 ($p<0.05$) (Table 6). Other than week 6 surplus P, no significant differences existed in surplus P normalized to chl. *a* in Tyner Creek after 3 weeks colonization time.

Surplus P Normalized to AFDW. Surplus P concentration in periphyton normalized to AFDW (μg surplus P $\cdot$ (mg AFDW)$^{-1}$) also showed a significant effect of time in Peacheater Creek and Tyner Creek, respectively (1-way ANOVA, $F=13.144$, $p<0.001$; $F=5.249$, $p=0.010$). In both streams, surplus P concentrations normalized to AFDW increased initially, then declined and increased again (Figure 9). In Peacheater Creek and Tyner Creek, week 6 surplus P was significantly less than weeks 3 through 5 and week 7 concentrations ($p<0.05$) (Table 6). This made it difficult to use these data to predict an accurate time to sample surplus P normalized to AFDW.

Experiment 4

Total Surplus P. There was a significant effect of time on areal surplus P_t concentrations in Peacheater Creek ($F=8.661$, $p=0.001$) and Tyner Creek ($F=10.336$, $p=0.001$). In Peacheater Creek, the same trend of increasing surplus P_t concentrations with increasing colonization time was apparent (Figure 10). No significant difference existed in areal surplus P_t after 2 weeks colonization time ($p<0.05$) (Table 7). Tyner Creek data indicated some deviation from this trend (Figure 10). In pairwise comparisons of weeks 1, 2, and 3 with following weeks, areal surplus P_t after 4 weeks

colonization was significantly greater than all other weeks ($p<0.05$) (Table 7, Figure 10). No significant differences between areal surplus P_t concentrations occurred after 4 weeks colonization time in Tyner Creek ($p>0.05$) (Table 7).

A significant effect of time also existed among concentrations of weekly surplus P_t normalized to chl. *a* in Peacheater Creek ($F=6.300$, $p=0.004$) and Tyner Creek ($F=15.380$, $p=0.000$). In Peacheater Creek, the same inverse curvilinear relationship between surplus P_t concentrations, normalized to chl. *a*, and time was observed as in experiment 3 (Figure 11). Pairwise comparisons of weeks 1 concentrations with following weeks revealed week 1 surplus P_t was significantly greater that weeks 3 through 6 (Table 7). Further, week 5 surplus P_t was significantly less than week 2 surplus P_t ($p<0.05$) (Table 7). No statistical differences existed in surplus P_t concentrations after 3 weeks colonization ($p>0.05$) (Table 7).

Temporal trends in Tyner Creek were distinct from those in Peacheater Creek (Figure 11). Pairwise comparisons of week 1 with following weeks indicated weeks 3, 5, and 6 were significantly less than concentrations during week 1 ($p<0.05$) (Table 7). Week 2 surplus P_t was also significantly greater than weeks 3, 5, and 6 ($p<0.05$) (Table 7). Further, week 4 was significantly greater that week 6 ($p<0.05$) (Table 7). No difference existed in surplus P_t concentrations after 3 weeks colonization time ($p>0.05$) except week 6 was significantly less than week 4 surplus P_t ($p<0.05$) (Table 7).

Available Surplus P. There was a significant effect of time on estimates of areal surplus P_i in Peacheater Creek ($F=6.008$, $p=0.003$) and Tyner Creek ($F=8.499$, $p=0.001$). In Peacheater Creek, compared to week 1, concentrations of surplus P_i increased to 3

TABLE VII

SIGNIFICANT DIFFERENCES IN SURPLUS P CONCENTRATIONS BETWEEN WEEKS OF COLONIZATION TIME DURING EXPERIMENT 4

Colonization Time	Peacheater Creek					Tyner Creek				
Areal Surplus P_t										
Week 1	2	3	4	5	6	2	3	4	5	6
Week 2		3	4	5	6		3	4	5	6
Week 3			4	5	6			4	5	6
Week 4				5	6				5	6
Week 5					6					6
Week 6										
Surplus P_t Normalized to Chl. *a*										
Week 1	2	3	4	5	6	2	3	4	5	6
Week 2		3	4	5	6		3	4	5	6
Week 3			4	5	6			4	5	6
Week 4				5	6				5	6
Week 5					6					6
Week 6										
Areal Surplus P_i										
Week 1	2	3	4	5	6	2	3	4	5	6
Week 2		3	4	5	6		3	4	5	6
Week 3			4	5	6			4	5	6
Week 4				5	6				5	6
Week 5					6					6
Week 6										
Surplus P_i Normalized to Chl. *a*										
Week 1	2	3	4	5	6	2	3	4	5	6
Week 2		3	4	5	6		3	4	5	6
Week 3			4	5	6			4	5	6
Week 4				5	6				5	6
Week 5					6					6
Week 6										

The level of significance is 0.05. Weeks sharing common underlines are not significantly different from the week in the corresponding column at the left.

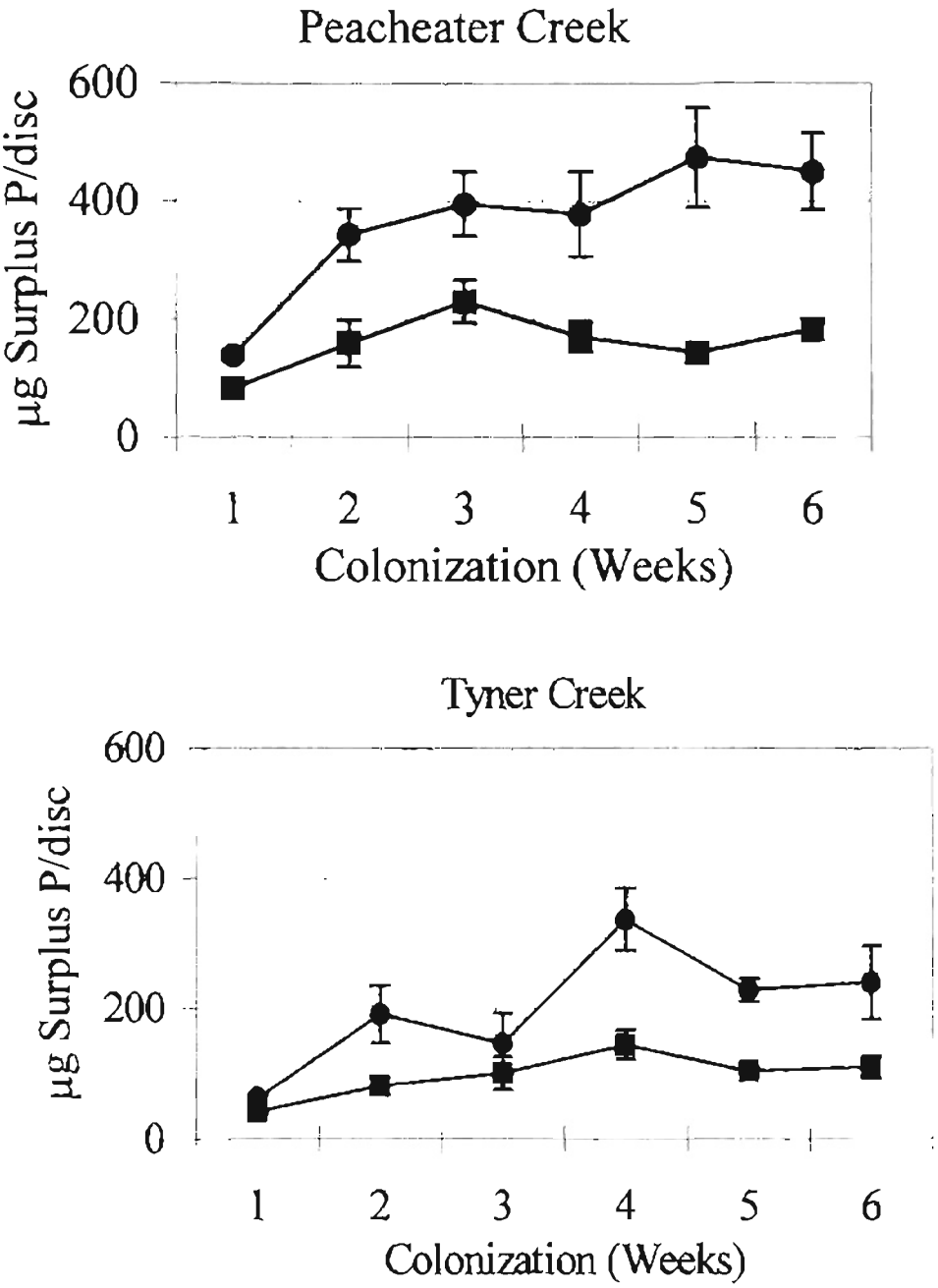

Figure 10. Total and available areal surplus P concentrations measured from 13 January through 17 February 1997 during experiment 4 in Peacheater Creek and Tyner Creek. Circles denote total surplus P and squares denote available surplus P. Error bars are standard deviation of mean (n=3).

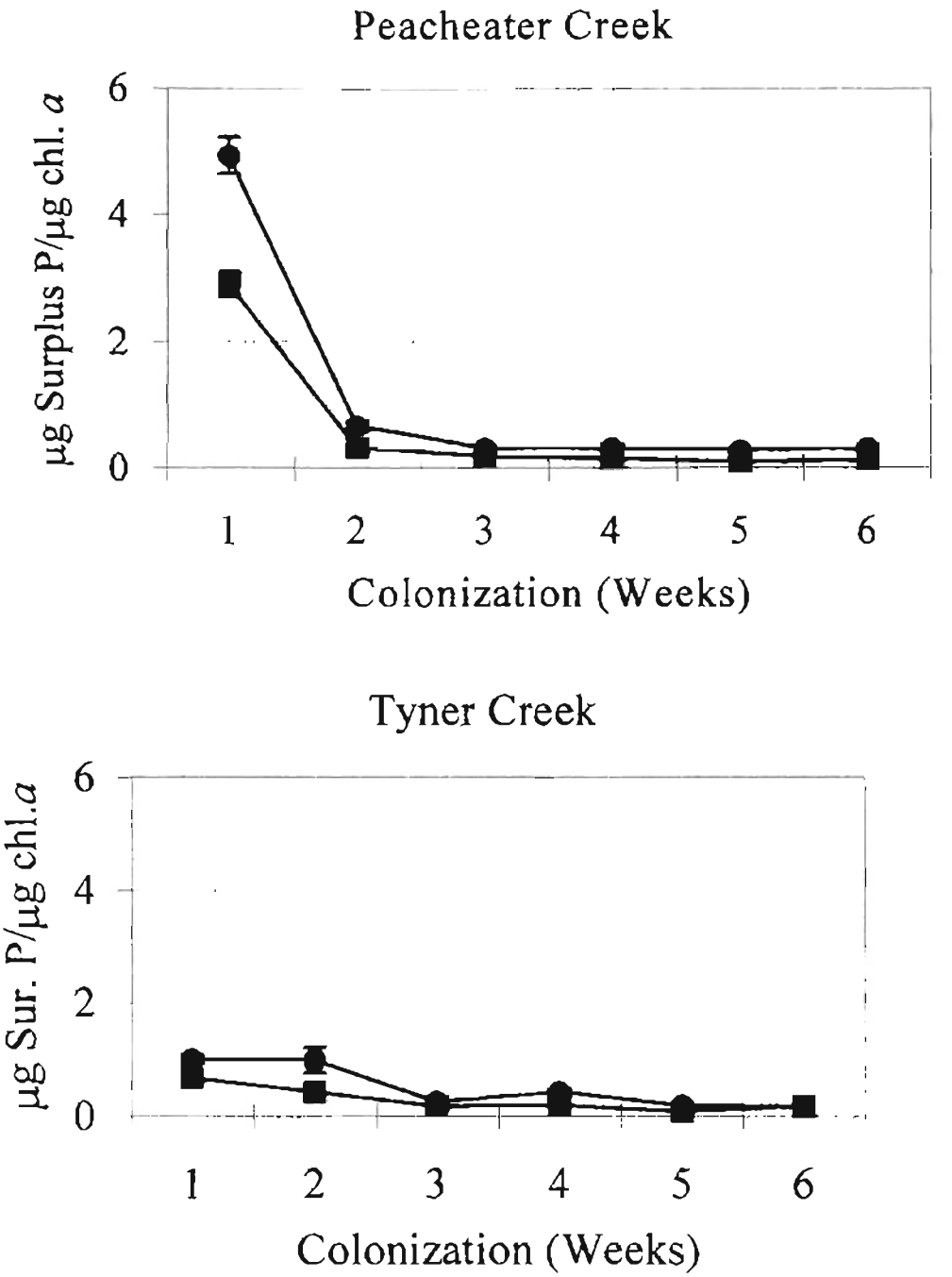

Figure 11. Total and available surplus P concentrations normalized to chl. *a* measured from 13 January through 17 February 1997 during experiment 4 in Peacheater Creek and Tyner Creek. Circles denote total surplus P and squares denote available surplus P. Error bars are standard deviation of mean (n=3).

weeks colonization time, decreased, and increased again at 6 weeks colonization (Figure 10). Weeks 3 and 6 were significantly greater than week 1 surplus P_i ($p<0.05$) (Table 7). No significant differences existed after 2 weeks colonization ($p>0.05$) (Table 7).

In Tyner Creek, concentrations of areal surplus P_i increased up to 3 weeks colonization time and then level off (Figure 10). Week 1 surplus P_i was significantly less than weeks 3 through 6 ($p<0.05$) (Table 7). Further, week 2 was significantly less than week 4 ($p<0.05$) (Table 7). No statistical differences existed in pairwise comparisons of surplus P_i after 3 weeks colonization time ($p>0.05$) (Table 7).

Significant differences in time also existed in concentrations of P_i when normalized to chl. *a* in Peacheater Creek ($F=41.438$, $p=0.000$) and Tyner Creek ($F=35.642$, $p=0.000$). In Peacheater Creek, surplus P_i demonstrated an inverse curvilinear relationship over time (Figure 11). Considerable differences were evident in pairwise comparisons of surplus P_i concentrations (Table 7). Surplus P_i in weeks 1 and 2 were significantly greater than all other measurements ($p<0.05$). The trend for surplus P in one week to be significantly greater than in following weeks held true throughout the time course with the exception of week 5 (Table 7). Week 5 surplus P_i was consistently less than other weeks in all pairwise comparisons ($p<0.05$) (Table 7). No pairwise comparisons revealed a point where statistical differences ceased to exist (Table 7).

In Tyner Creek, surplus P_i concentrations decreased from week 1 to week 6 (Figure 11). Considerable differences also existed in pairwise comparisons of surplus P_i concentrations in Tyner Creek (Table 7). Pairwise comparisons of surplus P_i measured in weeks 1 and 2 showed these weeks to be significantly greater than all other weeks

($p<0.05$) (Table 7). Pairwise comparison of weeks 3 and 4 with following weeks showed these weeks were significantly greater than weeks 5 and 6 ($p<0.05$) (Table 7). As in Peacheater Creek, no pairwise comparisons revealed a point where statistical differences ceased to exist (Table 7).

Time Course Comparisons

Comparisons of summer surplus P data collected during experiment 3 and winter surplus P data collected during experiment 4 were made for available surplus P only. In reporting the results below, the mean or median is given in parentheses. Units are omitted, but have been given previously.

Median concentrations of areal surplus P_i in Peacheater Creek during experiment 3 (364.5) were significantly different than during experiment 4 (158.2) in a Mann-Whitney rank sum test ($T=439.0$, $p<0.001$, $n=18$). In Tyner Creek, areal surplus P_i concentrations during experiment 3 (405.4) were significantly different than during experiment 4 (100.2) in a Mann-Whitney rank sum test ($T=459.0$, $p<0.001$, $n=18$). Normalized to chl. *a*, median concentrations of surplus P_i in Peacheater Creek during experiment 3 (0.280) were also significantly different than during experiment 4 (0.160) in a Mann-Whitney rank sum test ($T=376.0$, $p=0.022$, $n=18$). In Tyner Creek, surplus P_i concentrations normalized to chl. *a* during experiment 3 (0.380) were significantly different than experiment 4 (0.195) in a Mann-Whitney rank sum test ($T=381.0$, $p=0.014$, $n=18$).

Colonization times necessary for no statistical differences between subsequent

surplus P_i concentrations were different between experiments in Peacheater Creek. Areal surplus P_i demonstrated no significant differences after 5 weeks of colonization in experiment 3. This plateau was reached considerably sooner in experiment 4 (2 weeks). The point where statistical differences ceased to exist were consistent across experiments in Tyner Creek (3 weeks).

Comparisons of surplus P_i normalized to chl. *a* between experiments were not possible since statistical differences always existed in experiment 4 in either stream. Table 8 summarizes colonization times in which statistical differences between surplus P_i concentrations ceased to exist.

TABLE VIII

COMPARISON OF COLONIZATION TIME
NECESSARY FOR NO STATISTICAL DIFFERENCES IN
SURPLUS P CONCENTRATIONS TO EXIST BETWEEN EXPERIMENTS

EXPERIMENT	SURPLUS P_i	PEACHEATER CREEK	TYNER CREEK
Experiment 3	Areal	5 Weeks†	3 Weeks†
Experiment 3	Normalized to Chl. *a*	4 Weeks†	3 Weeks†
Experiment 4	Areal	2 Weeks	3 Weeks
Experiment 4	Normalized to Chl. *a*	‡	‡

† Surplus P was not measured in periphyton colonized 1 week
‡ There did not exist a time when no statistical differences occurred

Winter Total Surplus P Versus Available Surplus P. In Peacheater Creek, mean concentration of areal surplus P_t (355.7) was significantly different than that of P_i (160.3) in a paired t-test ($t=-5.43$, $p<0.0001$, $df=34$). Available and total areal surplus P increased together during weeks 1 through 4 (Figure 10). Total P stored increased in

unison with available P in week 5. With increasing colonization time, periphyton tended to store more P_t in Peacheater Creek.

In Tyner Creek, mean concentration of areal surplus P_t (201.1) was significantly different than that of P_t (97.3) in a paired t-test (t=-4.27, p=0.0002, df=34). Total and available surplus P tended to follow the same trend during weeks 1 through 4. The exception was week 3, when mean concentration of total (100.8) and available (146.8) surplus P concentrations were not significantly different in a paired t-test (t=-1.21, df=4, p=0.291) (Figure 10). Periphyton allowed to colonize for longer periods stored less total P in Tyner Creek.

When normalized to chl. *a*, median concentration of surplus P_t (0.335) in Peacheater Creek was significantly different than that of P_t (0.160) (Mann-Whitney rank sum test: T=234.5, p=0.019, n=18). In Tyner Creek, median concentration of surplus P_t (0.335) was also significantly different than that of P_t (0.195) (Mann-Whitney rank sum test: T=265.0, p=0.033, n=18). In both streams, the relative difference between total surplus P and available surplus P tended to decline with increasing periphyton maturity (Figure 11).

APA Time Course

A significant effect of time was detected in weekly measurements of APA in Peacheater Creek (F=4.543, p=0.005) and Tyner Creek (F=6.605, p=0.001). In Peacheater Creek, APA decreased from week 1 to 2 colonization then increased to 4 weeks colonization (Figure 12). APA was consistently below 1.01 nM MF•μg chl. *a*$^{-1}$

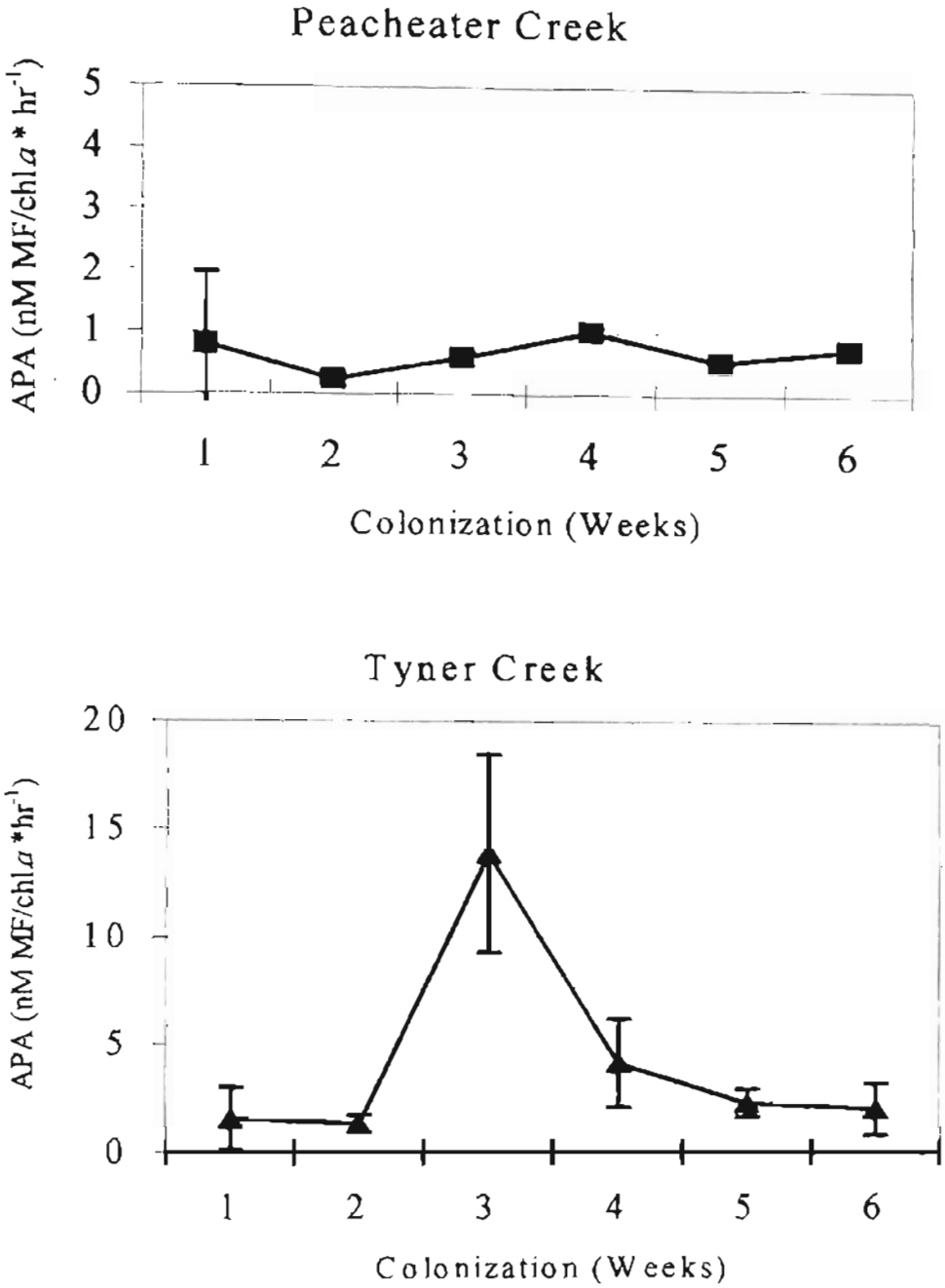

Figure 12. APA in Peacheater Creek and Tyner Creek measured 13 January through 17 February 1997 during experiment 4. Error bars are standard deviation of mean (n=3).

TABLE IX

SIGNIFICANT DIFFERENCES IN APA CONCENTRATIONS
BETWEEN WEEKS OF COLONIZATION TIME DURING EXPERIMENT 4

Colonization Time	Peacheater Creek					Tyner Creek				
Week 1	2	3	4	5	6	2	3	4	5	6
Week 2		3	4	5	6		3	4	5	6
Week 3			4	5	6			4	5	6
Week 4				5	6				5	6
Week 5					6					6
Week 6										

The level of significance is 0.05. Weeks which share a common underline are not significantly different in APA from the week in the corresponding column at the left.

^{1}•hr^{-1}. Pairwise comparisons of week 1 APA with all other weeks revealed week 1 APA was significantly greater than week 2 ($p<0.05$) (Table 9). Further, week 4 APA was significantly greater than week 2 APA ($p<0.05$) (Table 9). No statistical differences existed in APA after 3 weeks colonization time (Table 9).

APA in Tyner Creek increased from week 2 to 3 then decreased through week 6 (Figure 12). Week 1 APA was significantly less than week 3 APA ($p<0.05$) (Table 9). Week 2 was significantly less than weeks 3 and 4 APA ($p<0.05$). Week 3 and 4 APA were statistically similar ($p>0.05$) and significantly greater than weeks 5 and 6 ($p<0.05$) (Table 9). No differences existed in APA in Tyner Creek after 4 weeks colonization time ($p>0.05$) (Table 9).

Watershed Comparisons

Biological Parameters

In experiment 2, mean concentration of chl *a* on styrofoam substrata in Peacheater Creek (9.90) was not significantly different than Tyner Creek (8.83) in a paired t-test (t=0.724, p=0.473, df=46). Mean concentration of chl. *a* on silicated discs during experiment 2 in Peacheater Creek (10.3) was not significantly different than Tyner Creek (11.8) (paired t-test, t=-0.842, p=0.405, df=38). In experiment 3, mean chl. *a* in Peacheater Creek (10.6) was not significantly different than Tyner Creek (9.50) (paired t-test, t=0.506, p=0.616, df=38). In experiment 4, mean chl. *a* in Peacheater Creek (12.77) was significantly different than Tyner Creek (9.12) (paired t-test, t=2.75, p=0.008, df=66). In experiment 3, mean AFDW in Peacheater Creek (4.26) was not significantly different than Tyner Creek (5.70) (paired t-test, t=-1.68, p=0.102, df=32).

In experiment 3, mean areal surplus P_i in Peacheater Creek (359.9) was not significantly different than Tyner Creek (403.2) in a paired t-test (t=-1.05, p=0.304, df=32). When normalized to chl. *a*, median surplus P_i in Peacheater Creek (0.280) was not significantly different than Tyner Creek (0.380) (Mann-Whitney rank sum test, T=245.5, p=0.076, n=17). When normalized to AFDW, median surplus P_i in Peacheater Creek (0.787) also was not significantly different than Tyner Creek (0.693) (Mann-Whitney rank sum test, T=314.0, p=0.582, n=17).

In experiment 4, mean areal surplus P_i in Peacheater Creek (160.3) was significantly different than Tyner Creek (97.3) in a paired t-test (t=4.14, p=0.002, df=34). When normalized to chl. *a*, median surplus P_i in Peacheater Creek (0.160) was not significantly different than Tyner Creek (0.195) (Mann-Whitney rank sum test, T=345.5, p=0.704, n=18). Mean areal surplus P_i in Peacheater Creek (355.7) was significantly

different than Tyner Creek (201.1) (paired t-test, t=3.80, p=0.0006, df=34). When normalized to chl. *a*, median surplus P_i in Peacheater Creek (0.335) was not significantly different than Tyner Creek (0.335) (Mann-Whitney rank sum test, T=360.5, p=0.393, n=18). Median APA in Peacheater Creek (0.670) was significantly lower than median APA in Tyner Creek (2.840) (Mann-Whitney rank sum test, T=1088.5, p<0.0001, n=28).

Water Chemistry Parameters

SRP and TP During Experiments 1 - 3. Mean SRP in Peacheater Creek (41.3) was significantly different than Tyner Creek (19.8) in a paired t-test (t=7.55, p<0.0001, df=129). Mean TP in Peacheater Creek (50.8) was also significantly different than Tyner Creek (30.7) in a paired t-test (t=4.63, p<0.0001, df=84). Peacheater Creek SRP and Tyner Creek SRP were significantly correlated (p<0.01, r = 0.96) (Figure 13). Both TP and SRP increased in concentration in response to a flood which occurred 26, 27 September 1996 (Figure 13). Mean TP, SRP, nitrate-N, chloride, and sulfate-S concentrations and sampling data are reported in Appendix A.

At the beginning of the study in May, 1996, SRP concentrations in Peacheater Creek exceeded 40 μg/L, then declined in early June 1996 to below 30 μg/L (Figure 13). Concentrations exceeded 30 μg/L from early June to late August 1996. The lowest mean concentration occurred in early September 1996 (21 μg/L). Following a major flood, mean SRP concentration in Peacheater Creek was four times higher than the previous week (109 μg/L). SRP concentration after the flood (109) was significantly different than the previous week (27) (paired t-test, t=45.3, p<0.0001, df=4). Mean SRP

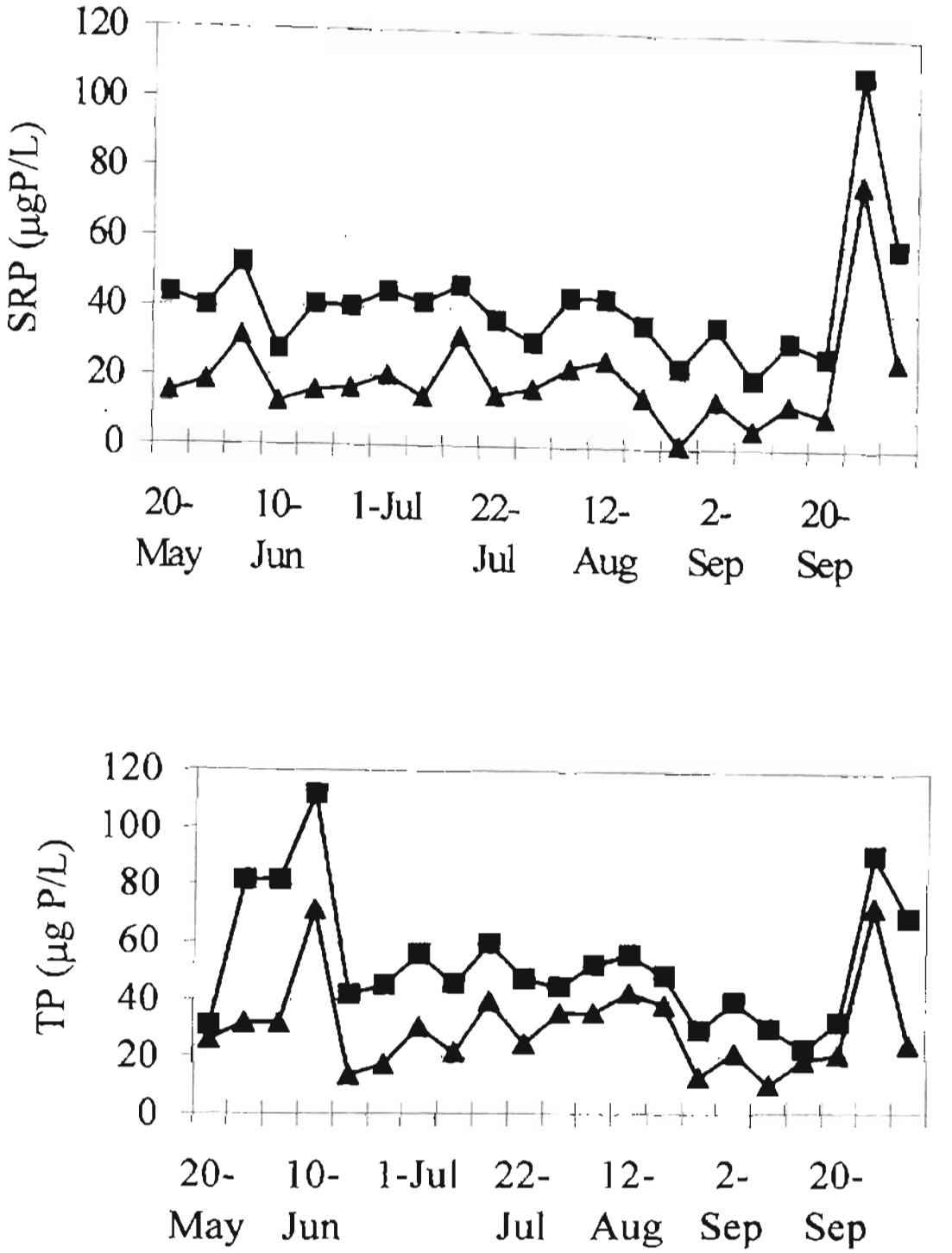

Figure 13. Mean SRP and TP concentrations in Peacheater Creek and Tyner Creek during May through October 1996 during experiments 1, 2, and 3. Squares denote Peacheater Creek and triangles denote Tyner Creek.

concentration was 41.3 μg/L during 20 May through 3 October 1996.

Concentrations of SRP in Tyner Creek were less than 20 μg/L in May, 1996, then rose to above 30 μg/L in early June and mid July (Figure 13). These were the highest concentrations reported and were not in response to a flood. Mean concentrations of SRP remained near 20 μg/L throughout the summer. In late August, SRP concentrations declined to below 14 μg/L. The minimum mean SRP concentration was 1 μg/L on 24 August 1996. In response to the flood event in late September, SRP concentrations rose 8 times to 78 μg/L. Mean SRP concentration measured after the flood event (78) was significantly different than the previous week (10) (paired t-test, $t=-34.5$, $p<0.0001$, $df=4$). Mean SRP concentration during the sampling period was 20 μg/L.

Total P also followed similar patterns in both streams (Figure 13). There was a significant correlation of TP concentrations between Peacheater Creek and Tyner Creek from May to October 1996 ($p<0.05$, $r = 0.81$). Maximum concentrations of TP occurred on 10 June 1996 in both streams. On this date, concentrations of TP in Peacheater Creek and Tyner Creek were 111 μg/L and 71 μg/L, respectively. Concentrations of SRP were relatively low on this date. Mean TP concentration in Peacheater Creek during 20 May through 3 October 1996 was 51 μg/L. In Tyner Creek, mean concentration of TP during this period was 31 μg/L. Concentrations of TP did rise in response to the flood event in late September, 1996, however, these concentrations were lower than those reported in June, 1996 (Figure 13).

SRP and TP Experiment 4. During 13 January to 17 February 1997, mean SRP concentration in Peacheater Creek (21.8) was significantly higher than in Tyner Creek

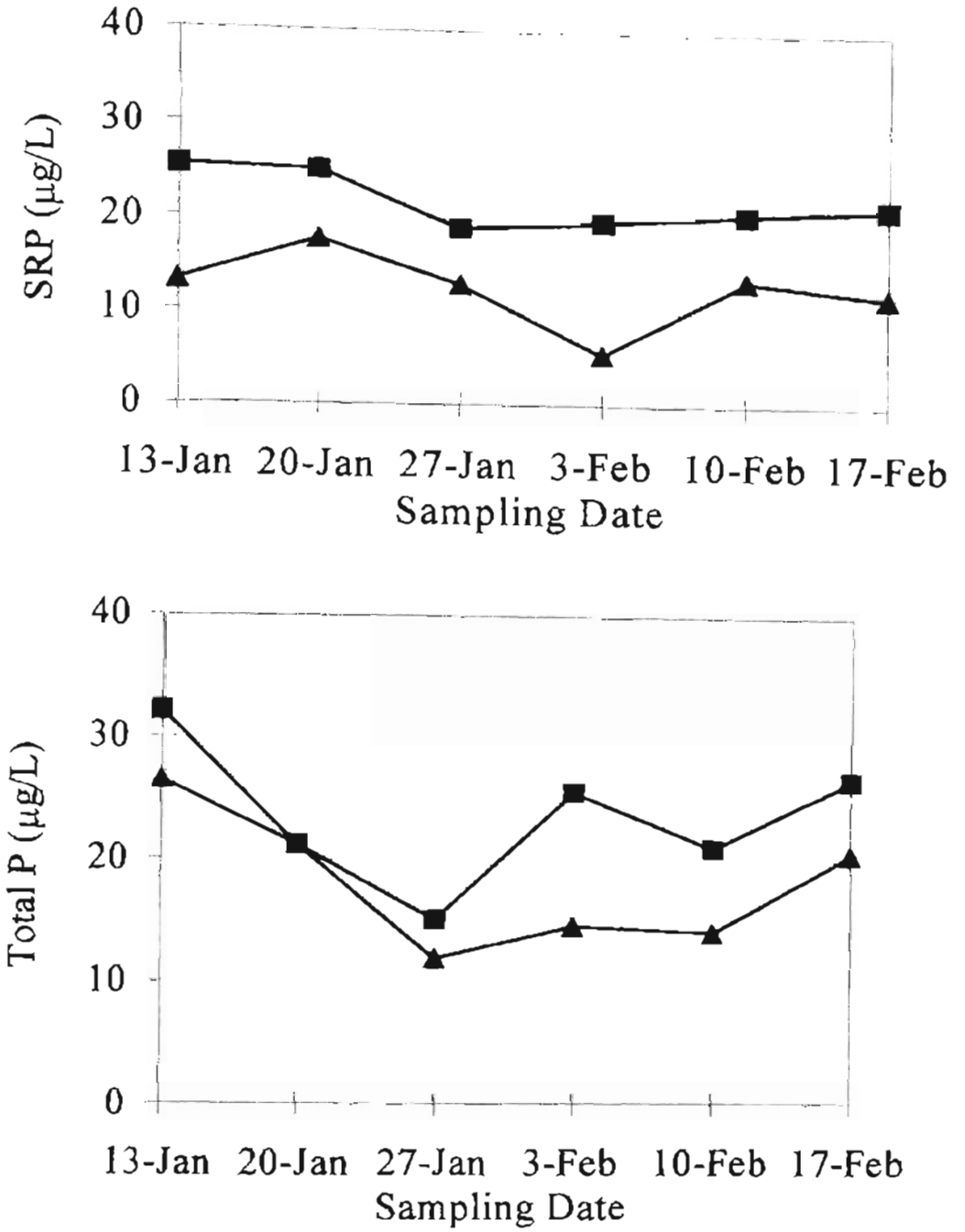

Figure 14. Concentrations of SRP and TP in Peacheater Creek and Tyner Creek measured from 13 January through 17 February 1997 during experiment 4 (i.e. winter). Squares denote Peacheater Creek and triangles denote Tyner Creek.

(12.3) (paired t-test, $t=8.48$, $p<0.0001$, $df=34$). Mean TP concentration in Peacheater Creek (23.6) was also significantly higher than TP in Tyner Creek (18.1) (paired t-test, $t=2.40$, $p=0.255$, $df=22$) (Figure 14). Water chemistry data are reported in Appendix A.

SRP concentrations in Peacheater Creek and Tyner Creek were significantly correlated ($p<0.05$, $r=0.50$). At the beginning of this experiment in January, 1997, SRP concentrations in Peacheater Creek were 26 μg/L (Figure 14). Concentrations of SRP declined in late January 1997 to below 20 μg/L, and then increased to 21 μg/L by mid February, 1997. In Tyner Creek, SRP concentrations never exceeded 20 μg/L. Maximum SRP occurred on 20 January 1997 (18 μg/L). SRP concentrations declined to 5 μg/L in early February 1997. Other measurements ranged from 11 μg/L to 13 μg/L in Tyner Creek.

Total P concentrations followed similar trends in both streams (Figure 14). TP concentrations in Peacheater Creek and Tyner Creek were significantly correlated ($p<0.01$, $r = 0.72$). Mean concentrations were the same (21μg/L) in both streams on 20 January 1997. In Peacheater Creek, TP concentrations at the beginning of experiment exceeded 30 μg/L. By the end of January 1997, TP concentrations had declined to 15.1 μg/L. Total P then increased in February 1997 to more than 26 μg/L. The same decline in TP concentrations was also seen in Tyner Creek. On 13 January 1997, TP concentration in Tyner Creek was 26.5 μg/L. The minimum TP concentration was recorded in late January (11.9 μg/L). Total P concentration then increased to above 20 μg/L in mid February.

Summer Versus Winter P Data. Mean SRP concentration measured in Peacheater

Creek during the summer (20 May to 3 October 1996) (41.3) was significantly higher than during the winter (13 January to 17 February 1997) (21.8) (paired t-test, t=-4.65, p<0.0001, df=85). In Tyner Creek, mean SRP concentration in the summer (19.8) was also significantly higher than during the winter (12.3) in a paired t-test (t=-2.09, p=0.040, df=81).

Anion Concentrations During Experiment 1-3

Units for means or medians given in parentheses for nitrate-N, chloride, and sulfate are mg/L. From 20 May to 3 October 1996, the mean nitrate-N concentration in Peacheater Creek (4.70) was not significantly different than Tyner Creek (3.92) (paired t-test, t=0.995, p=0.321, df=142). Early in the study, nitrate-N concentrations were above 3 mg NO_3-N/L in both streams. Nitrate concentrations steadily declined as the summer progressed (Figure 15). Minimum values occurred on 24 August 1996 in both streams. Minimum concentration in Peacheater Creek was 0.90 mg NO_3-N/L and 0.92 mg NO_3-N/L in Tyner Creek. Nitrate concentrations then rose to concentrations similar to those recorded in early summer (Figure 15).

Mean chloride concentration in Peacheater Creek (12.81) was also not significantly different than Tyner Creek (11.82) in a paired t-test (t=0.625, p=0.533, df=142). Chloride concentrations were consistently below 10 mg/L in May through August 1996 in both streams (Figure 15). Concentrations in Tyner Creek were somewhat more variable. Chloride concentrations increased six fold in Tyner Creek and eight fold in Peacheater Creek from 24 August to 3 September 1996. Maximum chloride

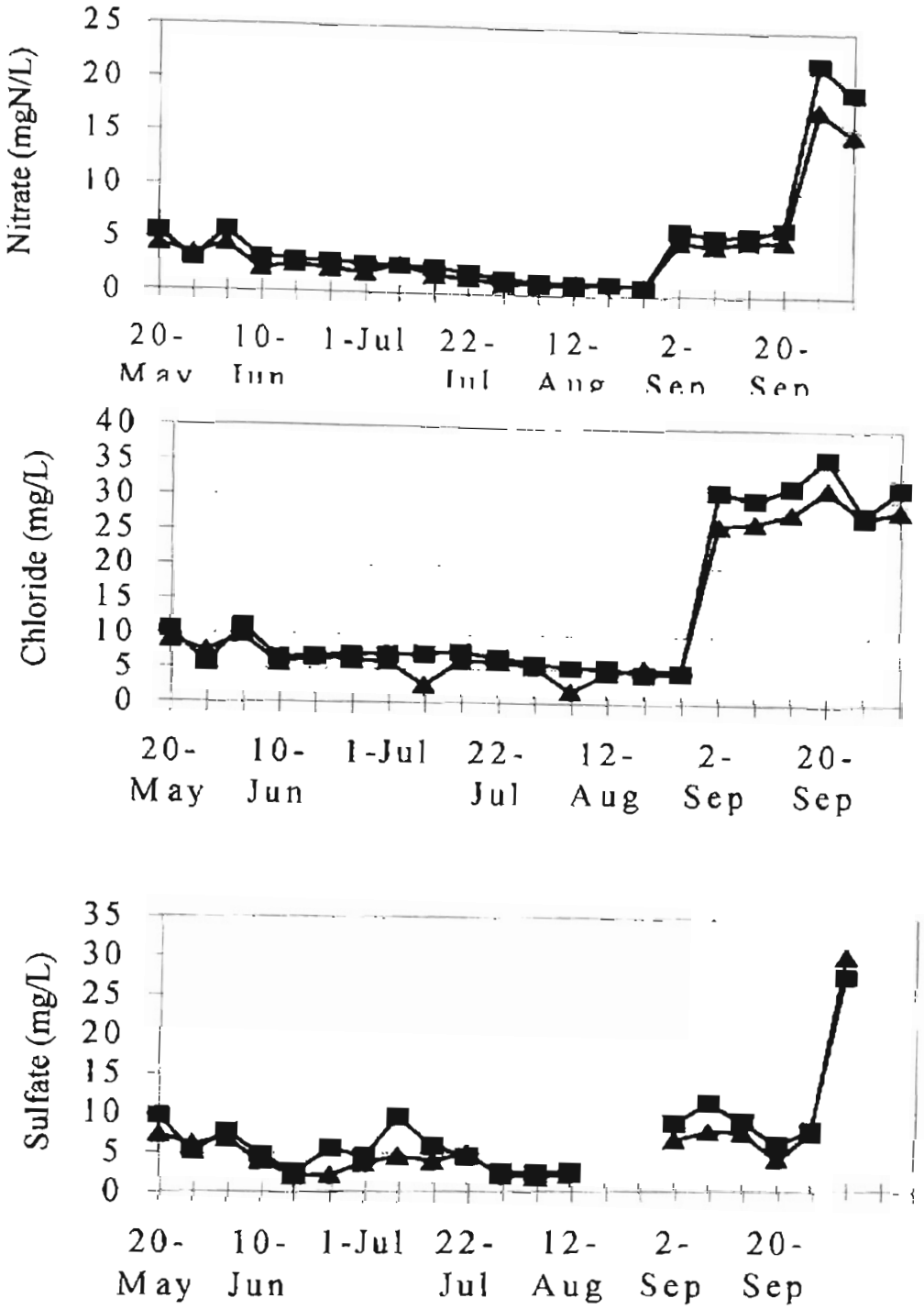

Figure 15. Mean nitrate, as NO_3-N, chloride, and sulfate concentrations in Peacheater Creek and Tyner Creek in experiments 1 through 3 May through October 1996. Squares denote Peacheater Creek and triangles denote Tyner Creek.

concentration in Peacheater Creek was 31.5 mg/L and 36.1 mg/L in Tyner Creek measured 20 September 1996. Concentrations of chloride remained at this elevated level for the remainder of the sampling period in both streams.

Mean sulfate concentration in Peacheater Creek (7.01) was not significantly different than Tyner Creek (6.47) in a paired t-test ($t=0.419$, $p=0.676$, $df=130$). Sulfate concentrations were variable for both streams during the sampling period (Figure 15). No data were available for 17 - 24 August 1996. In Peacheater Creek, concentrations ranged from 2.4 mg/L to 11.5 mg/L prior to a flood event in late September. Sulfate concentrations ranged from 1.9 mg/L to 7.7 mg/L in Tyner Creek during the same period. Proceeding the flood, sulfate concentrations rose to 27.7 mg/L and 30.3 mg/L in Peacheater Creek and Tyner Creek.

Anion Data During Experiment 4

From 13 January to 17 February 1997, the mean nitrate-N concentration in Peacheater Creek (3.32) was significantly different than Tyner Creek (2.57) (paired t-test, $t=5.57$, $p<0.0001$, $df=34$). Nitrate-N concentrations in Peacheater Creek ranged from 3.6 mg NO_3-N/L in early January to 2.9 mg NO_3-N/L in mid February. In Tyner Creek, concentrations ranging from 3.2 mg NO_3-N/L to 1.8 mg NO_3-N/L were observed. In both streams, maximum concentration of NO_3-N occurred on 13 January and minimum concentration occurred on 17 February 1997 (Figure 16).

Mean chloride concentration in Peacheater Creek (7.53) was significantly different than Tyner Creek (5.97) (paired t-test, $t=11.3$, $p<0.0001$, $df=34$).

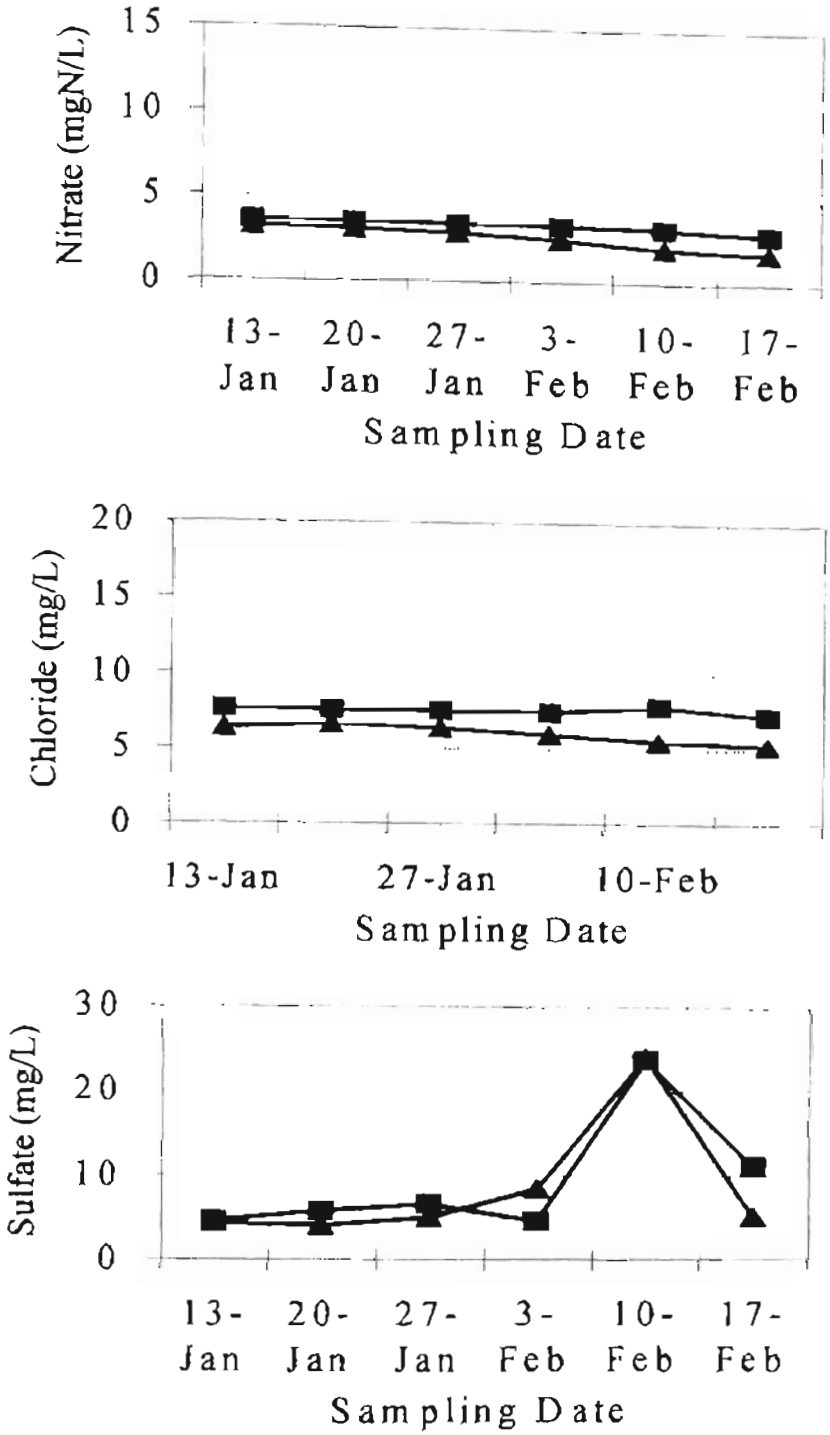

Figure 16. Concentrations of nitrate, chloride, and sulfate in Peacheater Creek and Tyner Creek during experiment 4 13 January 1997 through 17 February 1997. Squares denote Peacheater Creek and triangles denote Tyner Creek.

Concentrations of chloride in Peacheater Creek ranged from 7.2 mg/L to 7.6 mg/L. In Tyner Creek, chloride concentrations ranged from 5.2 mg/L to 6.6 mg/L. Minimum mean chloride concentrations were recorded on 17 February 1997 in both streams (Figure 16).

Sulfate concentrations followed a different trend (Figure 16). Mean sulfate concentration in Peacheater Creek (9.43) was not significantly different than Tyner Creek (8.55) (paired t-test, t=0.297, p=0.768, df=34). In Peacheater Creek, mean sulfate concentration increased through January 1997 from 4.7 mg/L to 6.6 mg/L. A similar increase was documented in Tyner Creek; however, the increase continued through February. Maximum mean sulfate in Peacheater Creek and Tyner Creek was 23.75 mg/L and 24.14 mg/L, respectively reported on 10 February 1997. This represented a 5 fold and 3 fold increase in Peacheater Creek and Tyner Creek, respectively. Sulfate concentration declined in both streams the following week.

Winter Versus Summer Anion Data

Mean nitrate-N concentration measured in Peacheater Creek during the summer (4.70) was not significantly different than in the winter (3.32) (paired t-test, t=-1.13, p=0.263, df=88). In Tyner Creek, mean nitrate-N concentration in the summer (3.92) was also not significantly different than in the winter (2.57) (paired t-test, t =-2.49, p=0.178, df=88).

Differences between seasons were established with the chloride data. Mean chloride concentration measured in Peacheater Creek during the summer (12.84) was

significantly different than in the winter (7.53) (paired t-test, t=-2.09, p=0.039, df=88). In Tyner Creek, mean chloride concentration in the summer (11.77) was also significantly different than in the winter (5.97) (paired t-test, t=-2.49, p=0.015, df=88).

Mean sulfate-S concentration measured in Peacheater Creek during the summer (7.01) was not significantly different than in the winter (9.43) (paired t-test, t=-1.38, p=0.170, df=82). In Tyner Creek, mean sulfate-S concentration in the summer (6.47) was also not significantly different than in the winter (8.55) (paired t-test, t=0.900, p=0.371, df=82).

Ecological Relationships

SRP Versus Surplus P

Experiment 3. On an areal basis, there was no detectable relationship between SRP and surplus P in Peacheater Creek or Tyner Creek during experiment 3 ($p>0.05$) (Table 10). SRP was the dependent variable in all correlations. Respectively, surplus P , normalized to chl. *a*, and SRP were positively correlated in Peacheater Creek ($p<0.05$, $r = 0.51$) (Table 10). No significant correlation was detected in Tyner Creek ($p>0.05$) (Table 10). In both streams, the highest mean SRP concentration was associated with the highest mean surplus P concentration normalized to chl. *a* (Figure 17). A significant relationship of surplus P and SRP only existed when surplus P concentrations were normalized to chl. *a*. Concentrations of surplus P normalized to AFDW showed no relationship to SRP in Peacheater Creek or Tyner Creek ($p>0.1$) (Table 10).

TABLE X

STATISTICAL CORRELATIONS OF ECOLOGICAL PARAMETERS

Experiment	Relationship	Peacheater Creek		Tyner Creek	
		r	p-Value	r	p-Value
3	Areal SP_i - SRP	-0.277	0.286	-0.280	0.277
3	SP_i/chl. a - SRP	0.507	**0.037**	0.441	0.076
3	SP_i/AFDW - SRP	-0.121	0.645	-0.360	0.156
4	Areal SP_i - SRP	-0.000	0.999	0.345	0.363
4	SP_i/chl. a - SRP	0.515	0.156	-0.293	0.444
4	Areal SP_t - SRP	-0.566	0.112	-0.049	0.901
4	SP_t/chl. a - SRP	0.513	0.158	-0.353	0.351
4	APA - SRP	-0.055	0.829	-0.165	0.526
4	Areal SP_i - APA	-0.568	0.087	0.317	0.373
4	SP_i/chl. a - APA	0.531	0.115	-0.587	0.075
4	Areal SP_t - APA	-0.587	0.074	0.567	0.087
4	SP_t/chl. a - APA	0.473	0.167	-0.554	0.097

SP = Surplus P
SP/chl. a = Surplus P normalized to chl. *a*
SP/ AFDW = Surplus P normalized to AFDW
r is the correlation coefficient.
Bold p-value indicates significant relationship. The level of significance is 0.05.
The first parameter given is the independent variable and the second is the dependent variable in all correlations.

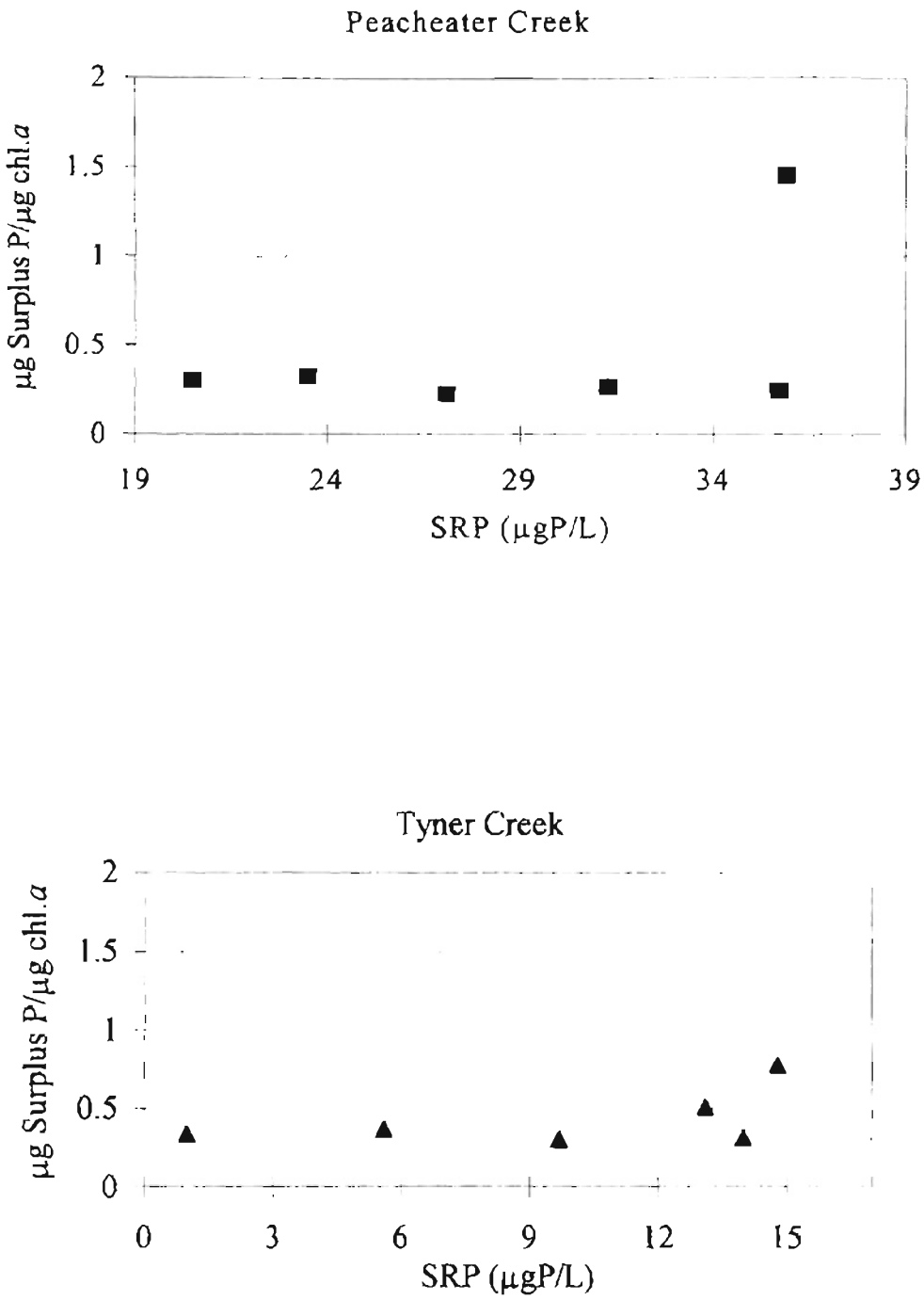

Figure 17. Relationship between surplus P normalized to chl. *a* and SRP in Peacheater Creek and Tyner Creek measured during experiment 3 8 Aug through 27 Sep 1996.

Experiment 4. Surplus P_t and SRP and surplus P_i and SRP were not significantly correlated in either stream ($p>0.05$) (Table 10). No relationship between these parameters existed in any expression of surplus P (Tables 10). Plots of this relationship are in Appendix C.

APA versus SRP

No relationship existed between APA and SRP in either stream ($p>0.1$) (Table 10). Correlation coefficients were $r = 0.83$ and $r = 0.53$ in Peacheater Creek and Tyner Creek, respectively.

APA Versus Surplus P

APA of periphyton was plotted against surplus P_i and surplus P_t concentrations (areal and normalized to chl. *a*) (Appendix C). No significant correlation existed between APA and surplus P_i (areal and normalized) in either stream ($p>0.05$) (Table 10). There was also no statistical relationship between APA and surplus P_t (areal and normalized) in Peacheater Creek or Tyner Creek ($p>0.05$) (Table 10).

Physical Parameters

Temperature of stream water in Peacheater Creek ranged from 11 °C to 24 °C during the summer (20 May to 3 October). In Tyner Creek, temperatures ranged from 12 °C to 22 °C during the same period. Maximum temperatures occurred 9 September 1996 in both streams. Minimum temperatures occurred 3 October 1996 in both streams.

Mean temperatures in the summer were 21.2°C and 19.4°C in Peacheater Creek and Tyner Creek, respectively. Water temperature was consistently higher in Peacheater Creek. Chl. *a* concentrations were not statistically different in this period. This suggests that temperature did not limit periphyton biomass accrual between streams for experiments 1 through 3.

During experiment 4 (winter), water temperature ranged from 7°C to 13°C in Peacheater Creek. In Tyner Creek, water temperature ranged from 6°C to 12°C during the same period. Minimum temperature in Peacheater Creek was measured 20 January 1997. In Tyner Creek, minimum temperature was measured 27 January 1997. Maximum temperature was measured 17 February 1997 in both streams. Tyner Creek had higher water temperatures in the first two weeks measured (13 January and 20 January). Peacheater Creek had warmer water temperatures in all other weeks. Chl. *a* concentrations were significantly less during the winter. This suggests that temperature could have potentially limited biomass accrual during experiment 4. Water temperatures are reported in Appendix B.

The pH of both streams ranged from slightly acidic to neutral; Tyner Creek from pH 6.7 to 7.8 and Peacheater Creek from 6.6 to 7.7. Limestone dominated bedrock in both watersheds undoubtedly caused buffering potential of streams, thus pH values were often greater than 7.0. Thus, alkaline phosphatases were functional and could be monitored. Slightly acidic values may have been a result of the pH being taken in the laboratory after samples had been transported rather than in the field. Values for each experiment are recorded in Appendix B.

Turbidity of Tyner and Peacheater Creek was measured as Nephelometric Turbidity Units (NTU). The turbidity of Tyner Creek ranged from 0.21 to 15.0 NTU. During May through early August, 1996, turbidity values did not exceed 1.0 NTU. Turbidity was highest in late August through October, 1996 and January, 1997 when values consistently exceeded 1.5 NTU. The turbidity of Peacheater Creek ranged from 0.11 to 11.5 NTU throughout the duration of the study. Turbidity was highest in January 1997 as NTU averaged 4.38. The overall mean for Peacheater Creek was 1.79 NTU. Rocky substrata on the stream bottom were visible at both sites. Relatively low turbidity values in both streams suggests that light was able to penetrate to stream bottom in both Peacheater Creek and Tyner Creek. Turbidity measured during the study are reported in Appendix B.

Conductivity in Tyner Creek ranged from 143 to 196 μmhos. The mean value was 167.5 μmhos. Highest values occurred in August, 1996. Conductivity in Peacheater Creek was consistently lower; values ranged from 103 to 174 μmhos. The mean value was 150.03 μmhos and was rarely lower than 140 μmhos. These data are recorded in Appendix B.

Total monthly rainfall was highest in September 1996 (25.8 cm) (Table 11). A 10 year flood occurred in late September (USGS 1996). On September 27 and 28, 1996, total daily precipitation was 16.4 cm and 4.8 cm, respectively. This resulted in the termination of pilot experiment 2 before data for week 8 were collected as all boards were washed away. No rainfall data were available for October 1996.

Discharge data were obtained from USGS gauging station on Peacheater Creek.

located where the stream is transversed by the Highway 62 bridge near station 1 (Figure 1). There is no gauging station located on Tyner Creek. It is assumed that due to the close proximity of the two watersheds, trends in discharge recorded for Peacheater Creek

TABLE XI

MONTHLY DISCHARGES FROM PEACHEATER CREEK AND PRECIPITATION RECORDED FROM STILWELL, OK DURING 1996

DATE	MEAN DISCHARGE $m^3 \cdot s^{-1}$	MAXIMUM DISCHARGE $m^3 \cdot s^{-1}$	TOTAL DISCHARGE $m^3 \cdot s^{-1}$	TOTAL PRECIPITATION cm
MAY	0.590	1.730	18.450	11.201
JUNE	0.180	0.420	5.280	11.354
JULY	0.040	0.070	1.160	16.383
AUGUST	0.009	0.050	0.280	11.481
SEPTEMBER	1.280	20.970	66.610	25.756
OCTOBER	0.490	1.950	15.170	N/A

could be applied to Tyner Creek. Most discharge observations were made at base flow (Table 11). Discharges recorded in July, August, and October are indicative of base flow conditions. Maximum mean monthly discharge is most often reached in May (USGS 1996). However, as previously mentioned, an unusually high amount of precipitation fell during late September and caused a rapid increase in discharge. Total daily discharge for 27 September 1996 was 20.998 $m^3 \cdot s^{-1}$. Total daily discharge remained above 2.35 $m^3 \cdot s^{-1}$ through 30 September 1997. These values were the highest total daily discharge values measured during the USGS sampling year from October, 1995 to October, 1996 (USGS 1996).

CHAPTER VI

DISCUSSION

Biofilm Time Courses

The time necessary for peak biomass development varied both between watersheds and experiments. In Peacheater Creek, a period ranging from 3 to 7 weeks was necessary for peak biomass development. Colonization time ranging from 3 to 5 weeks was required in Tyner Creek to achieve peak biomass. Peak biomass occurred sooner in Peacheater Creek in experiment 2 on styrofoam substrata. Colonization periods were shorter in Peacheater Creek during experiment 2 on silicated disc substrata and during experiment 3 for AFDW. Other time courses, biomass measured as chl. *a*, yielded the same colonization time periods in both streams.

Various artificial substrata exposure periods have been used or suggested to achieve a community of a known age which resembles that on natural substrata. Exposure periods of 4 weeks were used in rivers with high nutrient concentrations by Gale et al. (1979). However, the investigators reported that the data did not resemble growth fluctuations on natural substrata. Experiments on upstream-donwstream linkages of surplus P and APA used 8 week colonization periods (Mulholland et al. 1995). Phosphorus limitation of periphyton growth rate was evaluated on periphyton allowed to

colonize 17 to 24 days in continuous-flow troughs in the Thompson River (Bothwell 1985). Neilson et al. (1984) used colonization times of 12 weeks in the winter and only 6 weeks in the summer. Biomass on artificial substrata during these colonization periods were still found to be less than that measured on natural substrata. In a recent study, glass slides were allowed to colonize 8 weeks to develop a periphytic community whose taxonomic abundance could be compared along a nutrient gradient in the Everglades (McCormick and Odell 1996).

Comparison of biomass accrual periods necessary for the periphytic community to resemble natural substrata were made in New Zealand rivers of differing nutrient regimes (Biggs 1988). Periphytic growth on etched glass slides in moderately enriched rivers (SRP=3 - 4 μg/L) required 8 weeks exposure period to resemble growth on natural substrata (i.e. potentially peak biomass). In enriched rivers (SRP=20-72 μg/L), time for biomass to approximate natural concentrations was 4 weeks (Biggs 1988). Peacheater Creek mean SRP concentration for the summer and autumn was 41.3 μg/L, making it comparable to results in the enriched river in Biggs (1988) study. Peak biomass was reached after 4 weeks only as measured by AFDW. Chl. *a* accrual periods were shorter (3 weeks) and longer (7 weeks) in other experiments.

Tyner Creek mean SRP concentration during the same period was 19.7 μg/L. This concentration , though significantly less than Peacheater Creek, was also indicative of enriched streams in the New Zealand study. Peak biomass was reached after 4 weeks colonization period in experiment 2 in Tyner Creek. Peak biomass occurred after 3 to 5 weeks colonization period in all other experiments in Tyner Creek.

Biomass accumulated in any of the accrual periods was not the same given the same station, water quality, and velocity similarities. These discrepancies could be a function of propagule availability and the ability of planktonic organisms to attach and colonize artificial substrata (Biggs 1988; Jones 1978).

Experiment 4 data suggested peak biomass was reached after 3 weeks in both streams. These results were not expected. Irradiance is usually lower in the winter and water temperature was markedly lower (Appendix A). The winter time course was not performed at the same site, but rather upstream from the summer time course site in Peacheater Creek. Recent studies have shown that SRP concentrations vary upstream to downstream (Mulholland et al. 1995). The taxonomic composition of the periphytic community also has been shown to vary with along a nutrient gradient in the Everglades (McCormick and Odell 1996). Accrual time necessary to achieve a community on artificial substrata which resembles that on natural substrata, but of a known age, also could vary along a longitudinal gradient in response to nutrient availability and canopy cover. Substrata in Tyner Creek were placed at the identical site as previous experiments therefore may give a more true picture of seasonal variation in biomass accrual time.

The increase in biomass over time did not always adhere to the expected sigmoidal accrual curve in each time course (see Figures 3, 4, 5 and 12). Logarithmic growth could have occurred more rapidly than sampling every 7 days could have detected. If logarithmic growth did occur more rapidly than detected by the sampling regime, rapid sloughing and recolonization could have occurred and not been detected. The absence of sigmoidal curve in biomass time courses was also noted by Biggs (1988).

This study warned against expectations of comparable results from sampling one site at different times based on fixed colonization times due to the dynamic nature of periphyton colonization and rapid community turnover time (Biggs 1988). Therefore, it may be more advantageous to define a window of time in which to sample a biofilm grown on an artificial substrata in which one could conclude with reasonable accuracy that a mature community had developed.

This study suggests that a time of 3 to 5 weeks would be an appropriate incubation period in both streams for peak biomass to be attained on artificial substrata. This window of accumulation time is suggested to be the most accurate time to monitor ecosystem stress as defined by periphytic nutrient dynamics. However, not all time courses measured fell within this suggested time. In experiment 2, peak biomass on silicated discs occurred later in Peacheater Creek than the suggested time to incubate artificial substrata.

Grazing could have affected both absence of the expected sigmoidal curve of biomass accrual and the time necessary for periphyton to reach peak biomass. Grazing pressure by nonswimming invertebrates appeared not to be a factor in experiments 2 or 3. However, algal grazing by *Campostoma* sp. has been noted as being heavy in other studies on Baron Fork River, the receiving river of Peacheater Creek and Tyner Creek (Power et al. 1988). *Campostoma* sp. was not observed in Tyner Creek on any visit to the sampling site. This grazer was observed in Peacheater Creek, however, no observations of grazing artificial substrata were made on any visit to the stream. This does not discount the possibility that grazing did occur in Peacheater Creek. Comparisons of

periphyton communities exposed to grazing by *Campostoma* sp. to communities where grazers were excluded demonstrated algal species domination was controlled by grazing (Power et al. 1988). However, *Campostoma* sp. in Baron Fork River typically graze at depths greater than 15-20 cm (Matthews et al. 1986). In the Baron Fork of the Illinois River, grazing activity in channel margins and shallower areas has been shown to be dominated by snails rather than by *Campostoma* sp. (Matthews et al. 1986; Power et al. 1988). These spatial characteristics matched the stream conditions in which the boards were placed in this study.

During experiment 4, Ephemeroptera were abundant at every sampling date in both streams. In this experiment, invertebrate grazing by insect larvae could have caused algal declines. Grazing losses could occur fast enough in periods of high productivity, so that the turnover time of the periphytic community was less than the colonization time necessary for peak biomass accrual (Biggs 1988). Lamberti and Resh (1983) showed patterns of periphyton accrual to follow a pattern of biomass increase and decrease related to competitive spacing of grazers. Therefore, grazing could result in fluctuating biomass measurements over time as found in experiment 4 in both streams (Figure 12).

Surplus P

A concentration of 0.8 μg surplus P/mg dry weight has been suggested as a threshold below which P is limiting (i.e. warning level for P accumulation) (Fitzgerald and Nelson 1966). Recalculated, based on chl. *a* equaling 0.5 - 2% dry weight (Reynolds 1984), this threshold is 0.04 - 0.16 μg surplus P/μg chl. *a*. In experiment 3

concentrations of surplus P were consistently above this threshold in both streams. This suggests that P was potentially not limiting to algal growth during experiment 3. However, in experiment 4 surplus P concentrations were below the maximum value of the threshold (0.16 μg surplus P/μg chl. *a*) in three of the six weeks measured in both streams. Accumulation of P in periphyton allowed to colonize 4, 5, and 6 weeks in Peacheater Creek and 3, 5, and 6 weeks in Tyner Creek was below the threshold. Periphyton could potentially have been P-limited in the latter portion of experiment 4. This would suggest that more mature periphyton communities demonstrated possible P-limitation as defined by surplus P better during experiment 4 than during experiment 3.

Surplus P concentrations, both P_i and P_t, were measured in Lake Kinneret, Israel during a bloom of *Peridinium* both in the lake and in batch cultures (Wynne 1981). The study suggested that *Peridinium* growth was not P-limited based on high surplus P concentrations and low APA associated with the bloom. Therefore, comparisons of the ratio of surplus P_i from Lake Kinneret, expressed as ng•cell^{-1}, and chl. *a*, also expressed as ng•cell^{-1}, and surplus P_i concentrations normalized to chl. *a* from experiments 3 and 4 could be another indicator of possible P-limitation in Peacheater Creek and Tyner Creek.

The surplus P accumulated in both streams were within the range of surplus P accumulated in the *Peridinium* bloom, both in Lake Kinneret and in batch culture. Peak surplus P in both streams was much as 3 times greater compared to ratios of surplus P in Lake Kinneret. This was true for experiment 3 and experiment 4. However, surplus P in weeks 5 and 6 in Tyner Creek during experiment 4 were close to the minimum ratio detected in Lake Kinneret. Because surplus P concentrations measured in Lake Kinneret

were indicative of true surplus P accumulation, this would suggest that Peacheater Creek was never P-limited during experiment 3 or 4. This comparison also suggests Tyner Creek might tend toward P-limitation as measured in mature biofilms in experiment 3, but not in experiment 4.

Surplus P accumulation in periphyton has not been studied extensively. Concentrations of surplus P detected on P diffusing substrata in the Glover River, Oklahoma were considerably less (<30 μg P•mg chl. a^{-1}) than accumulations measured in Peacheater Creek and Tyner Creek (Nord 1991). It was suggested that the Glover River periphyton was under such P stress that periphyton did not accumulate surplus P in spite of the P released by substrata (Nord 1991). Because periphyton growth in Peacheater Creek and Tyner Creek accumulated considerably more P than in the Glover River, it is possible that P was not limited in this study.

The validity of these comparisons is unknown. The surplus P accumulations measured in this study were measured in periphyton of varying maturity. Few studies of surplus P in periphyton exist, especially periphyton of a known age. Surplus P measured early in biofilm development would lead to a different conclusion regarding nutrient limitation than would surplus P measured later in biofilm development. During experiment 3, surplus P concentrations were constant after 4 weeks colonization time in Peacheater Creek. No differences were detected after 3 weeks colonization time in Tyner Creek, with the exception that week 6 was significantly lower than other weeks. In experiment 4 no similarities between weeks were consistently detected. However, an inverse curvilinear relationship was again established, suggesting sampling later in

biofilm development would be better.

These results suggest that surplus P is best sampled in the suggested time of 3 to 5 weeks. Surplus P monitored in periphyton in early logarithmic stage may be artificially elevated due to normalization by low levels of chl. *a*. Sampling later in biofilm development may provide a more accurate indicator of ecosystem stress as defined by periphytic surplus P concentrations.

APA

APA in Tyner Creek was significantly higher than APA in Peacheater Creek. No statistical differences were detected in APA after 3 weeks colonization time in Peacheater Creek and after 4 week in Tyner Creek. As noted in surplus P discussion, few studies exist regarding APA levels in periphyton, especially APA of periphyton of a known age.

Periphytic APA measured in the summer on styrofoam substrata in the Thompson River system, British Columbia was 100 times greater than APA measured in Peacheater Creek and more than 10 times the levels measured in Tyner Creek (70 - 145 nM MFP•μg chl. a^{-1} hr.$^{-1}$) (Bothwell 1988). In Koegh River, British Columbia, APA ranged from 70 - 106 nM MU•μg chl. a^{-1} hr.$^{-1}$ measured in January, February, and March (Perrin et al. 1987). The maximum APA measured in Peacheater Creek during these months was 1.103 nM MFP•μg chl. a^{-1} hr.$^{-1}$ and 13.875 nM MFP•μg chl. a^{-1} hr.$^{-1}$ in Tyner Creek; considerably less than that measured in Koegh River. Periphyton in Thompson River and Koegh River was under severe P limitation. This suggests that periphyton in

Peacheater Creek and Tyner Creek were not under severe P limitation.

In extensive planktonic studies, Healey and Hendzel (1979) suggested that APA less than 3.0 nM MF•μg chl. a^{-1} hr.$^{-1}$ was indicative of no P deficiency and activity exceeding 5.0 nM MF•μg chl. a^{-1} hr.$^{-1}$ was indicative of severe P deficiency of the algal community. Measurements falling between these two threshold values were suggested to be indicative of slight P deficiency (Healey and Hendzel 1979). Peacheater Creek activity did not exceed 1.013 nM MF•μg chl. a^{-1} hr.$^{-1}$. According to Healey and Hendzel's (1979) threshold for P deficiency, Peacheater Creek was not P-limited. However, in Tyner Creek, activity measured in 3 weeks accumulation of periphyton exceeded the threshold for severe P deficiency and week 4 APA suggested slight P deficiency according to Healey and Hendzel (1979).

In phytoplankton studies, APA values of 12 to 42 nM MF•μg chl. a^{-1} hr.$^{-1}$ have been reported in various P-limited lakes (Petterson 1980). In Tyner Creek, APA exceeded 12 nM MF•μg chl. a^{-1} hr.$^{-1}$ after 3 weeks colonization suggested that if periphytic APA is similar to phytoplanktonic APA, Tyner Creek demonstrated P-limitation briefly in experiment 4.

In Tyner Creek, APA after 3 to 5 weeks colonization was 4 times greater than activity in other weeks. This supports the hypothesis that biofilms sampled during a window of 3 to 5 weeks of accumulation time are representative. In Peacheater Creek, mean APA measured in weeks 3 through 5 was 1.3 times greater than measured in other weeks. These data also support the conclusion that knowledge of the age of the biofilm is imperative when using periphytic parameters to assess stream ecosystem stress.

Ecological Relationships

Surplus P and SRP have been shown to have a direct proportional relationship as algal cells harbor P when available in excess of need. In Lake Erken, surplus P concentrations in phytoplankton decreased 4 to 5 times during P-limitation (Petterson 1980). Phosphorus content of periphytic communities has been shown to decrease along a longitudinal gradient of decreasing SRP concentrations in Walker Branch, Tennessee (Mulholland and Rosemond 1992). A weak positive relationship between surplus P normalized to chl. *a* and SRP was detected in Peacheater Creek during experiment 3.

The weak or absence nature of a relationship between surplus P and SRP in these streams could be the result of nutrient loading via NPS pollution. Pulses of P enter Peacheater Creek and Tyner Creek after rainfall events in the form of runoff (see Figure 13 and Appendix B). Depending when on the hydrograph the particular stream was sampled, SRP concentrations could increase and this not immediately be reflected by surplus P concentrations of periphyton. Biological uptake of P might not be reflected until after the initial pulse of P as measured by SRP has ceased. The analysis of SRP has also been shown to overestimate true orthophosphate in natural waters (Bothwell 1985).

Observed grazing by Ephemeroptera in experiment 4 and the possibility of grazing by *Campostoma* sp. in experiment 3 could also have influenced P dynamics of the periphyton community. When algal cells are grazed, stored P is released and, if limiting conditions exist, it is recycled back to the algal cells in the periphytic community (Mulholland et al. 1983). However, if P is not limited, as in most of this

study, nutrient spiraling length increases thus causing nutrients to be unavailable for uptake by remaining periphyton (Newbold et al. 1982). Therefore, grazing could deter the observation of a relationship between ambient P concentrations and stored P by releasing P sequestered in the periphyton thus making it available for downstream transport.

The relationships between APA and SRP, and APA and surplus P have been shown to be inversely proportional in phytoplankton (Perry 1972; Petterson 1980) and periphyton (Bothwell 1985, 1988). The hydrolysis of P from an organic compound by AP allows algae to mitigate the detrimental effects of P limitation by cleaving P from the organic moeity rendering the P available to the algal cell (Jansson et al. 1988). Algae only store P in times when P is available in abundance. Therefore it would follow that high APA and low surplus P concentrations of periphyton would be indicative of P-limitation. No detectable relationship existed between APA and SRP or APA and surplus P (available or total) in either stream during experiment 4.

In Lake Kinneret, APA of *Peridinium* was measured both in batch culture and in natural conditions (Wynne 1981). In batch culture, *Peridinium* exposed to decreasing ambient orthophosphate concentrations (6600 μg/L - 20 μg/L) demonstrated corresponding increased APA levels (Wynne 1981). However, no such relationship was found in *Peridinium* bloom of the lake. APA measured at the end of the bloom was 10 times greater than previously measured activity. Corresponding orthophosphate concentrations were 2 μg/L; among the lowest recorded in Lake Kinneret during the experiment. The substantial increase in APA at the end of the bloom was not related to

orthophosphate concentrations or surplus P concentrations, both of which remained constant over the bloom period (Wynne 1981). Wynne (1981) suggested that APA in *Peridinium* probably reflected the state of various P storage pools other than that extractable by hot water (i.e. surplus P). Periphyton from both Peacheater Creek and Tyner Creek stored surplus P and produced AP. This also suggests that there could be other internal P pools reacting with AP other than that extractable by hot water.

A model based on a study done in Peacheater Creek by Toetz (1995) suggested that SRP concentrations in February must be reduced from 23 μg/L to concentrations indicative of pristine conditions (i.e. 3 μg/L) for a substantial increase in APA to occur. A basin wide study of the same area documented a SRP threshold of 5 μg/L beyond which APA demonstrated an inverse curvilinear relationship with SRP (Tang, *unpublished data*). McCormick and Odell (1996) also found that the inverse relationship of oligotrophic indicator diatom species and total P was detectable after a threshold of 5 - 10 μg TP/L was exceeded. Data from the studies cited suggest that no inverse relationship was detected between APA and SRP in the present study because SRP concentrations did not decrease to a threshold concentration where APA would dramatically increase, thus demonstrating an inverse curvilinear relationship.

The bacterial component of the periphyton community could also have skewed interpretations of data. Perrin et al. (1987) discovered an increase in periphytic APA in response to organic matter additions in the Koegh River, British Columbia. This was attributed to a possible increase in heterotrophic bacteria which out competed algae for P resources. Increased bacterial biomass on the substrata was also suggested to have

resulted in higher APA levels when normalized to chl. *a* as a biomass estimation (Perrin et al. 1987). The periphytic community is a diverse community of microbiota including not only algae, but bacteria, fungi, animals, and inorganic and organic detritus. Stewart and Wetzel (1982) suggested that non-algal sestonic phosphatases could be a major component of the particulate associated (periphytic) phosphatase pool. Therefore, bacterial associated activity could cause misinterpretations of APA thought to be associated only with the autotrophic portion of periphyton and surplus P.

CHAPTER VII

CONCLUSIONS

The floating board apparatus appeared to relieve grazing pressure by non-swimming grazers. This apparatus was a useful tool in studying periphyton of known age on artificial substratum in streams heavily impacted by non-swimming grazers. However, in Peacheater Creek and Tyner Creek, grazer dominance seemed to shift to insect larvae in the winter. Observations were consistently made of grazing Ephemeroptera on the apparatus and thus the substrata in both streams. This suggests that the board apparatus is only useful in streams dominated by non-swimming grazers during summer months when insect larvae densities were seemingly low.

Peak biomass did not occur after the same incubation time in either stream regardless of season. However, a consistent window of peak biomass development was established. Peak biomass tended to occur after 3 to 5 weeks colonization time in both streams. Possible grazing activity and rapid sloughing with subsequent slow community overturn could have affected biomass accrual. This period of colonization time is suggested for incubating artificial substratum prior to sampling in order to accurately monitor ecosystem stress.

Indicators of ecosystems stress in this study were defined by periphytic surplus P

and APA. After 3 to 4 weeks colonization time, surplus P showed little variation in either stream during experiment 3. Considerable differences in surplus P concentrations between weeks were detected in experiment 4. Observed grazing in experiment 4 could have affected surplus P concentrations by increasing spiraling length of P released by algal cells via grazing. These data suggest that surplus P is best sampled after 3 weeks.

No statistical differences existed in APA after 3 to 4 weeks colonization time in either stream. More APA occurred in both streams' periphyton during 3 to 5 weeks colonization time. A representative sample of APA would be available in this suggested colonization period.

Surplus P data indicate that Peacheater Creek was never P-limited in experiments 3 or 4. Evidence of slight P-limitation existed in Tyner Creek as defined by mature periphytic surplus P concentrations in experiments 3 and 4. APA data indicate that Peacheater Creek periphyton was never P-limited. In Tyner Creek, P-deficiency was indicated during the suggested sampling period of 3 to 5 weeks.

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APPENDIX A

WATER CHEMISTRY DATA

WATER CHEMISTRY DATA FOR PEACHEATER CREEK FOR 1996 AND 1997

DATE	TOTAL P (µg P/L)	SRP (µg P/L)	NITRATE (mg N/L)	CHLORIDE (mg/L)	SULFATE (mg/L)
5-20-96	30.9	43.4	5.571	10.590	9.718
5-27-96	81.7	40.0	3.103	5.665	5.212
6-3-96	81.7	52.4	5.756	11.049	7.826
6-10-96	111.6	27.6	3.228	6.564	4.867
6-17-96	41.9	40.7	3.014	6.782	2.552
6-24-96	45.2	40.1	2.968	7.114	5.611
7-1-96	55.7	44.6	2.747	7.073	4.591
7-8-96	45.5	41.4	2.556	7.136	9.644
7-15-96	59.7	46.5	2.427	7.452	5.930
7-22-96	47.5	36.6	2.025	6.705	4.570
7-29-96	44.7	30.3	1.476	5.938	2.643
8-5-96	52.4	43.5	1.299	5.306	2.416
8-12-96	55.7	43.3	1.205	5.272	2.745
8-17-96	48.6	35.9	1.096	4.412	ND
8-24-96	29.6	23.5	0.903	4.646	ND
9-2-96	39.4	35.7	6.295	31.099	8.911
9-9-96	30.4	20.5	5.73	29.952	11.525
9-13-96	23.0	31.3	5.929	31.83	9.145
9-20-96	32.6	27.1	6.483	36.047	6.124
9-28-96	91.1	109.2	22.041	27.85	8.02
10-3-96	69.1	59.0	19.412	31.838	27.708
1-13-97	32.2	25.4	7.64	3.56	4.70
1-20-97	21.2	25	7.56	3.49	5.81
1-27-97	15.1	18.8	7.52	3.42	6.60
2-3-97	25.6	19.6	7.41	3.33	4.61
2-10-97	21.0	20.5	7.82	3.20	23.75
2-17-97	26.6	21.3	7.22	2.91	11.11

ND = NO DATA

WATER CHEMISTRY DATA FOR TYNER CREEK FOR 1996 AND 1997

DATE	TOTAL P (µg P/L)	SRP (µg P/L)	NITRATE (mg N/L)	CHLORIDE (mg/L)	SULFATE (mg/L)
5-20-96	26.3	15.1	4.335	9.013	7.320
5-27-96	31.8	18.4	3.564	7.453	6.243
6-3-96	31.8	31.3	4.443	9.789	6.861
6-10-96	71.3	12.5	2.120	5.675	4.027
6-17-96	13.7	15.8	2.629	6.576	2.165
6-24-96	17.5	16.6	2.198	6.263	2.221
7-1-96	30.5	20.4	1.918	6.001	3.710
7-8-96	21.8	14.3	2.706	2.694	4.621
7-15-96	39.8	32.0	1.638	63228	3.939
7-22-96	24.9	14.9	1.460	6.061	4.993
7-29-96	35.4	16.9	0.986	5.430	2.253
8-5-96	35.4	23.0	0.991	1.912	1.912
8-12-96	42.6	25.3	0.905	4.474	2.308
8-17-96	38.2	14.8	1.068	5.266	ND
8-24-96	13.1	1.0	0.923	4.718	ND
9-2-96	21.4	14.0	5.18	26.142	6.713
9-9-96	10.3	5.6	4.88	26.539	7.803
9-13-96	18.9	13.1	5.339	27.931	7.629
9-20-96	21.0	9.7	5.297	31.459	4.290
9-28-96	72.7	77.6	17.703	27.399	7.693
10-3-96	24.6	26.2	15.415	28.496	30.262
1-13-97	13.1	26.5	3.15	6.32	4.44
1-20-97	17.6	21.2	3.03	6.58	4.08
1-27-97	12.8	11.9	2.89	6.32	5.00
2-3-97	5.4	14.6	2.52	5.94	8.49
2-10-97	13.4	14.1	2.06	5.47	24.14
2-17-97	11.8	20.5	1.79	5.22	5.17

ND = NO DATA

APPENDIX B

PHYSICAL PARAMETERS

PEACHEATER CREEK AND TYNER CREEK
pH VALUES FROM 1996 AND 1997

DATE	PEACHEATER CREEK	TYNER CREEK
5-20-96	7.1	7.2
5-27-96	7.1	7.1
6-3-96	7.4	7.2
6-10-96	7.0	6.8
6-17-96	7.3	7.1
6-24-96	7.4	7.5
7-1-96	7.3	7.1
7-8-96	7.3	7.3
7-15-96	7.2	6.7
7-22-96	7.5	7.3
7-29-96	7.2	6.9
8-5-96	6.9	7.5
8-12-96	7.1	7.2
8-17-96	7.3	7.2
8-24-96	7.0	7.4
9-2-96	7.5	7.3
9-9-96	7.6	7.5
9-13-96	6.6	6.9
9-20-96	6.8	6.9
9-28-96	7.7	7.8
10-3-96	7.3	7.6
1-13-97	7.4	7.2
1-20-97	7.3	7.1
1-27-97	7.3	7.3
2-3-97	7.3	7.1
2-10-97	7.3	7.1
2-17-97	7.3	7.1

Mean pH values (n=3)

TURBIDITY OF PEACHEATER CREEK AND TYNER CREEK
AS MEASURED IN NTU FROM 1996 AND 1997

DATE	PEACHEATER CREEK	TYNER CREEK
5-20-96	0.8	0.9
5-27-96	1.4	1.5
6-3-96	0.9	0.7
6-10-96	0.9	1.0
6-17-96	0.9	0.9
6-24-96	3.4	3.2
7-1-96	0.5	0.8
7-8-96	0.3	0.3
7-15-96	0.7	0.8
7-22-96	0.4	0.4
7-29-96	0.3	0.2
8-5-96	0.7	0.5
8-12-96	0.5	0.5
8-17-96	1.3	10.3
8-24-96	1.5	1.8
9-2-96	1.2	1.3
9-9-96	0.7	3.0
9-13-96	0.1	4.2
9-20-96	1.2	1.1
9-28-96	5.6	3.8
10-3-96	1.5	1.5
1-13-97	11.5	15.0
1-20-97	ND	ND
1-27-97	1.2	1.1
2-3-97	2.2	4.7
2-10-97	3.5	3.0
2-17-97	3.5	3.0

ND = No Data
Turbidity values are mean (n=3)

CONDUCTIVITY OF PEACHEATER CREEK AND TYNER CREEK
AS MEASURED IN μmhos FROM 1996 AND 1997

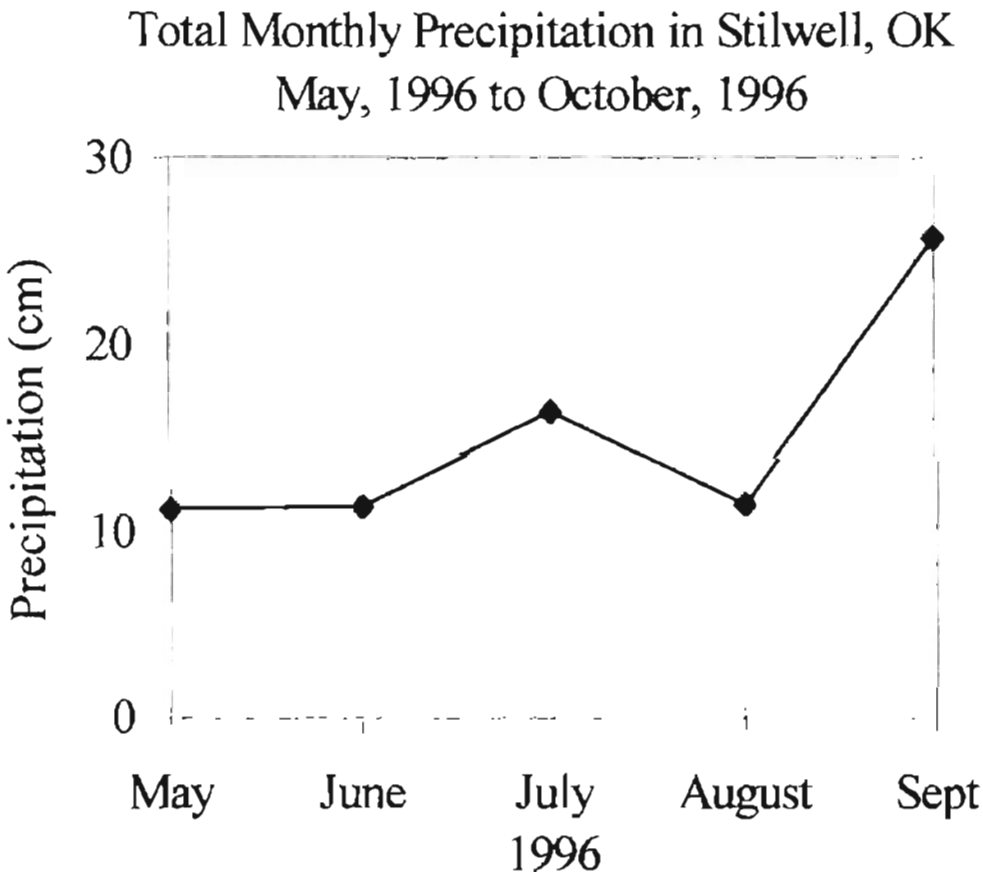
DATE	PEACHEATER CREEK	TYNER CREEK
5-20-96	155	165
5-27-96	103	146
6-3-96	162	165
6-10-96	161	173
6-17-96	141	149
6-24-96	140	143
7-1-96	151	180
7-8-96	174	162
7-15-96	160	169
7-22-96	169	179
7-29-96	161	181
8-5-96	159	170
8-12-96	167	196
8-17-96	125	143
8-24-96	113	168
9-2-96	160	191
9-9-96	ND	ND
9-13-96	ND	ND
9-20-96	ND	ND
9-28-96	ND	ND
10-3-96	ND	ND
1-13-97	ND	ND
1-20-97	ND	ND
1-27-97	ND	ND
2-3-97	ND	ND
2-10-97	ND	ND
2-17-97	ND	ND

ND = No Data
Conductivity values are means (n=3)

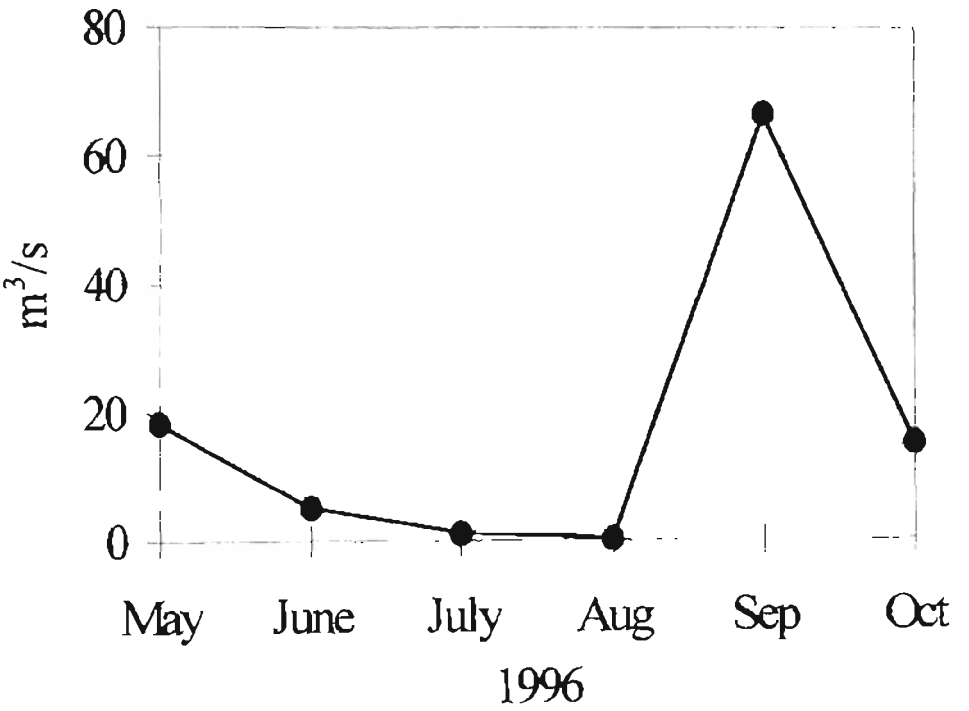
SURFACE WATER TEMPERATURES IN °C FOR PEACHEATER CREEK AND TYNER CREEK FROM 1996 AND 1997

DATE	PEACHEATER CREEK	TYNER CREEK
5-20-96	19	17
5-27-96	ND	ND
6-3-96	21	20
6-10-96	20	19
6-17-96	21	20
6-24-96	21	20
7-1-96	23	20
7-8-96	23	20
7-15-96	23	20
7-22-96	23	21
7-29-96	23	22
8-5-96	23	20
8-12-96	23	20
8-17-96	23	20
8-24-96	23	20
9-2-96	22	20
9-9-96	24	22
9-13-96	20	20
9-20-96	18	16
9-28-96	18	19
10-3-96	11	12
1-13-97	8	10
1-20-97	7	9
1-27-97	8	6
2-3-97	11	9
2-10-97	11	9
2-17-97	13	12

ND = NO DATA

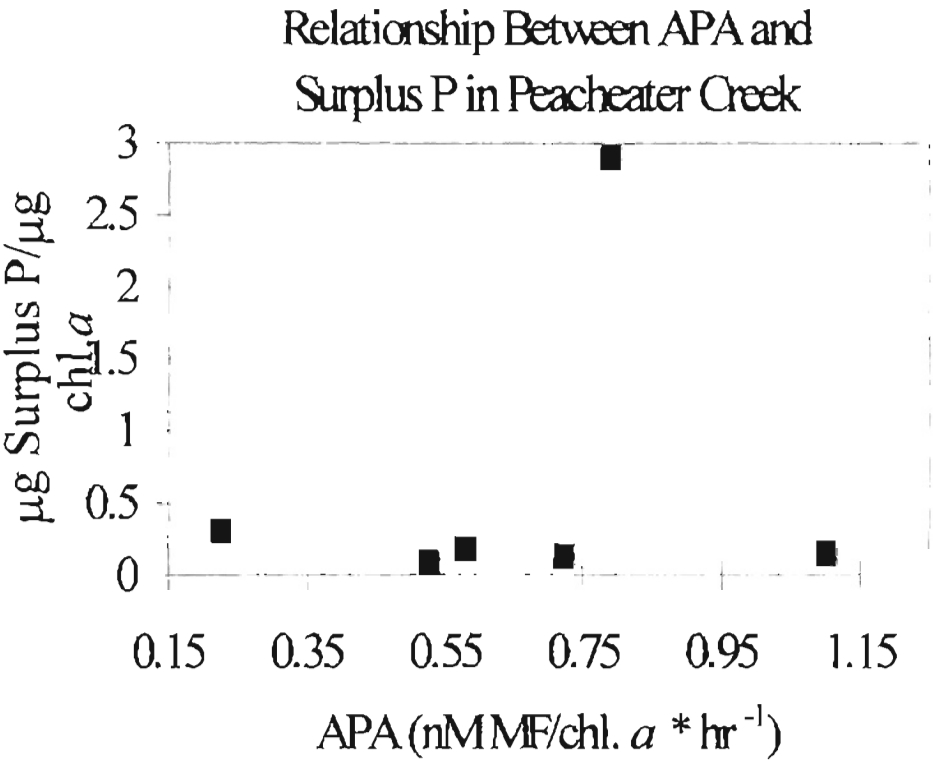

Total Monthly Precipitation in Stilwell, OK
May, 1996 to October, 1996
Precipitation (cm)
30
20
10
0
May
June
July
August
Sept
1996

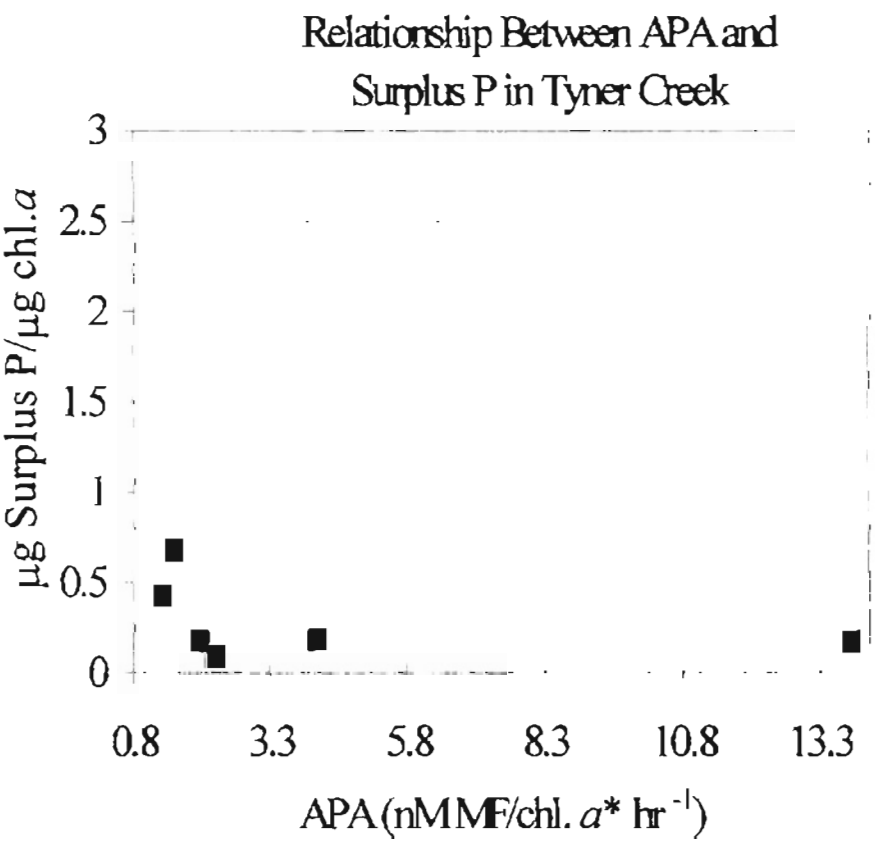
Total Monthly Discharge Peacheater Creek, Christie, OK, May, 1996 to October, 1996

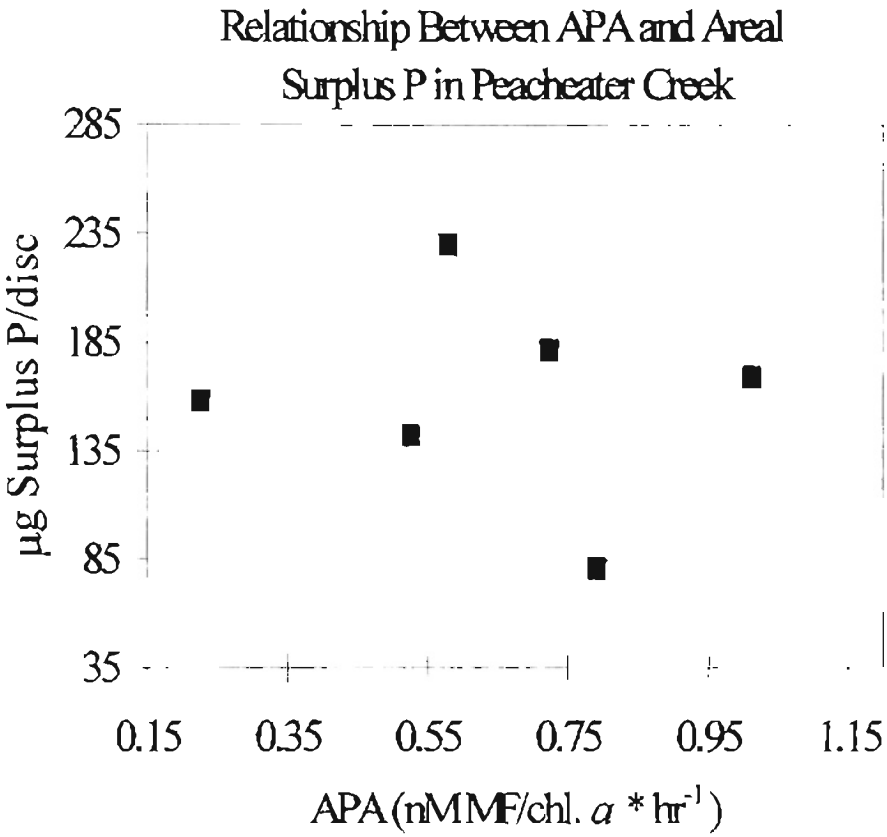


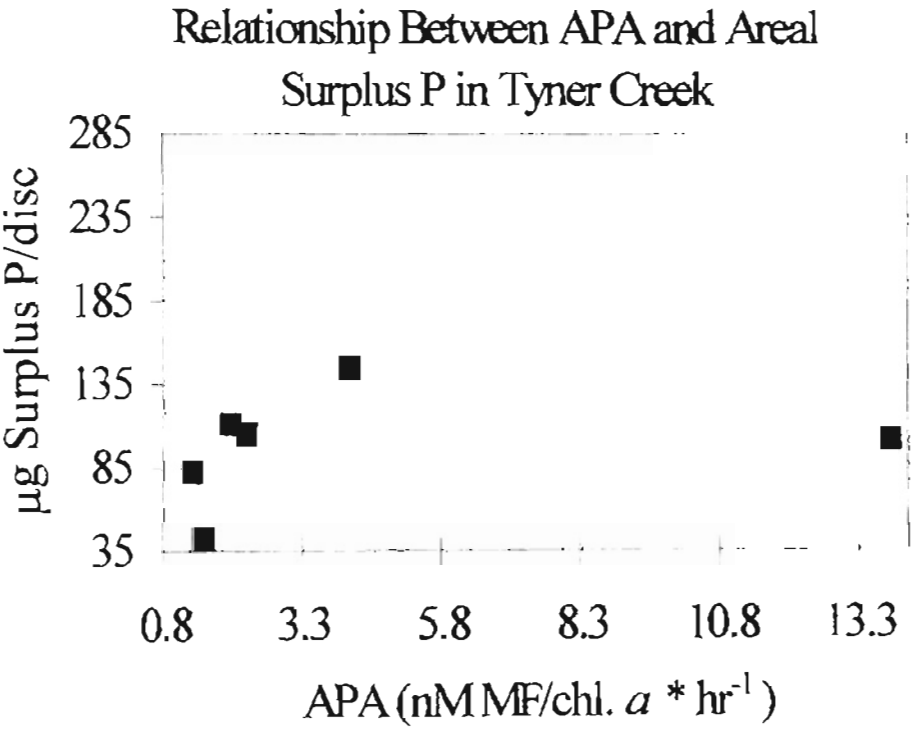
APPENDIX C

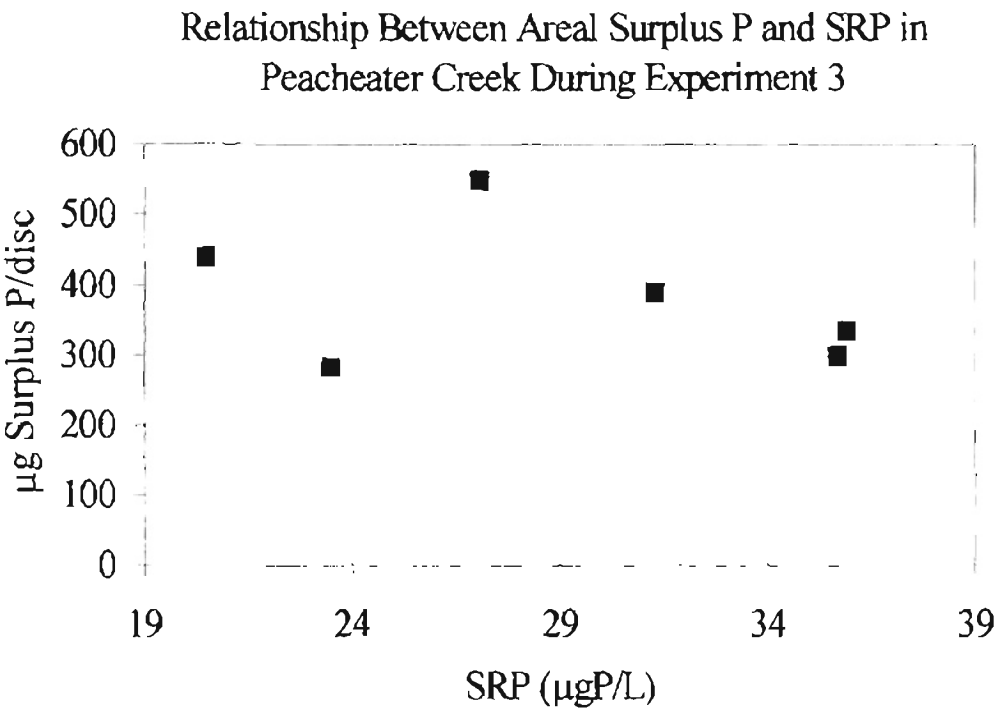
ECOLOGICAL RELATIONSHIPS


Relationship Between APA and
Surplus P in Peacheater Creek
μg Surplus P/μg chl.a
3
2.5
2
1.5
1
0.5
0
0.15
0.35
0.55
0.75
0.95
1.15
APA (nM MF/chl. a * hr -1)

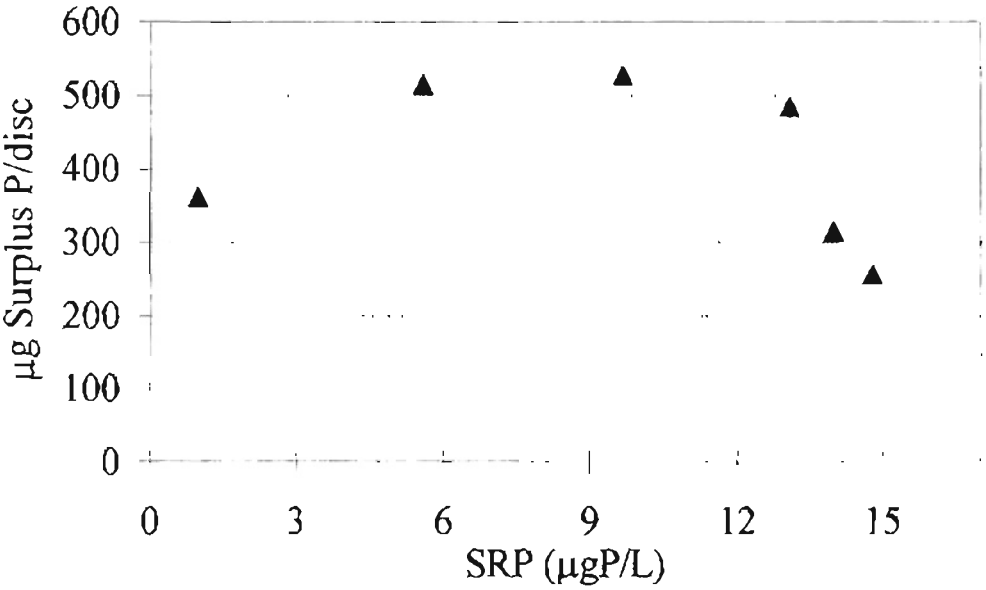

Relationship Between APA and
Surplus P in Tyner Creek
µg Surplus P/µg chl.a
3
2.5
2
1.5
1
0.5
0
0.8
3.3
5.8
8.3
10.8
13.3
APA (nM MF/chl. a* hr -1)

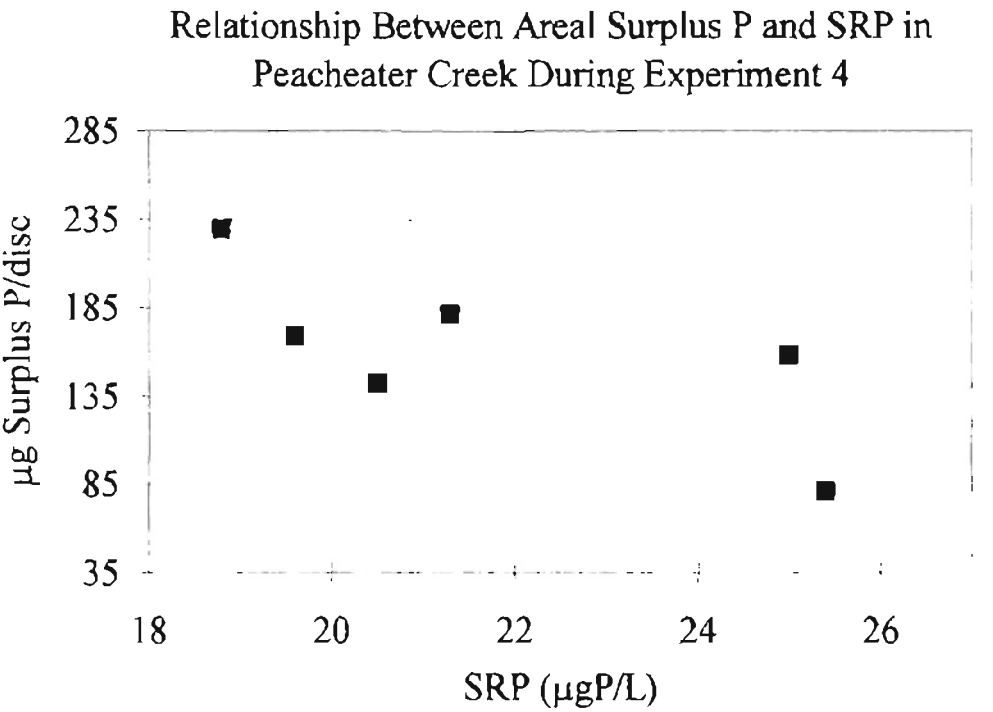

Relationship Between APA and Areal Surplus P in Peacheater Creek
µg Surplus P/disc
285
235
185
135
85
35
0.15
0.35
0.55
0.75
0.95
1.15
APA (nMMF/chl. a * hr-1)

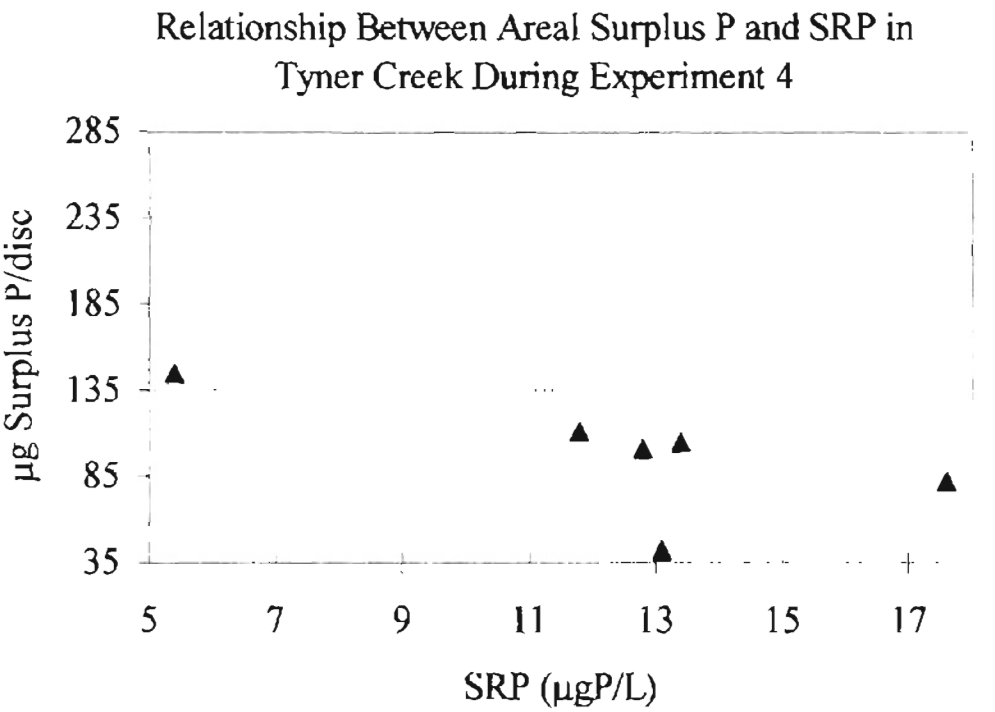

Relationship Between APA and Areal Surplus P in Tyner Creek
µg Surplus P/disc
285
235
185
135
85
35
0.8
3.3
5.8
8.3
10.8
13.3
APA (nM MF/chl. a * hr-1)


Relationship Between Areal Surplus P and SRP in Peacheater Creek During Experiment 3
µg Surplus P/disc
600
500
400
300
200
100
0
19
24
29
34
39
SRP (µgP/L)

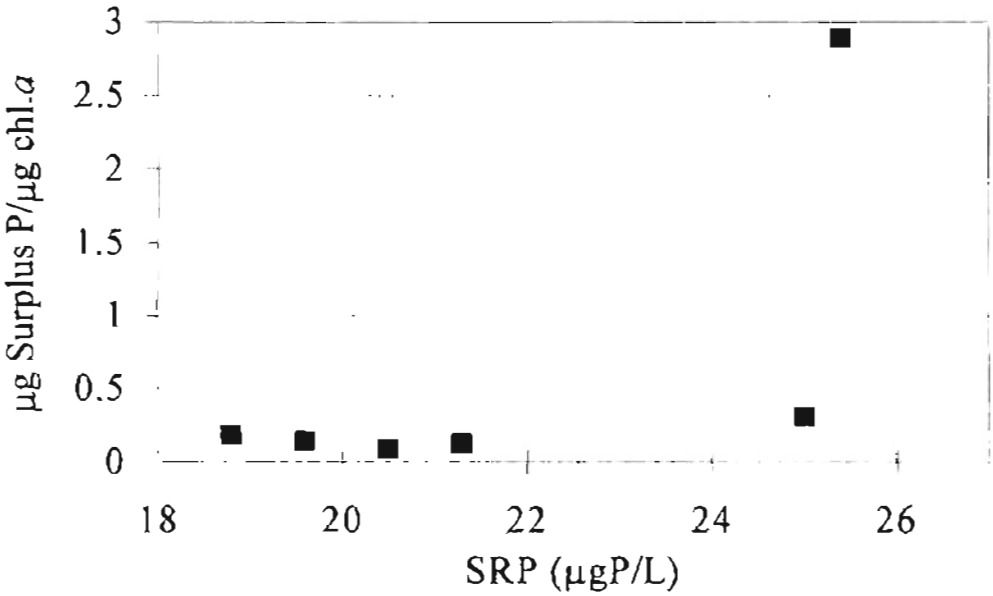
Relationship Between Areal Surplus P and SRP in Tyner Creek During Experiment 3

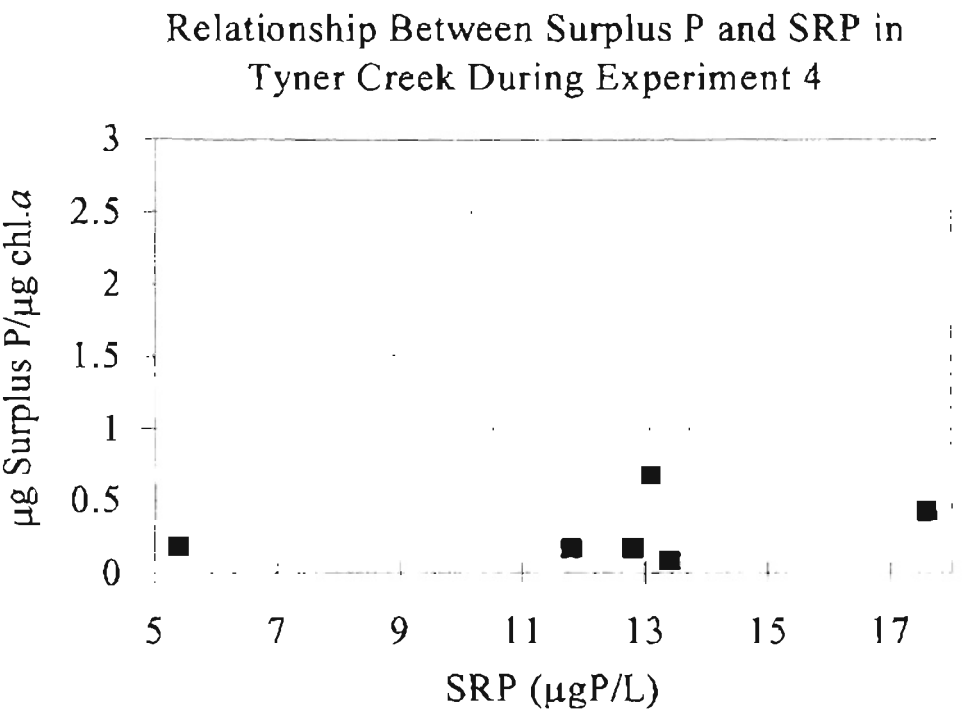



Relationship Between Areal Surplus P and SRP in Peacheater Creek During Experiment 4
285
235
185
135
85
35
µg Surplus P/disc
18
20
22
24
26
SRP (µgP/L)


Relationship Between Areal Surplus P and SRP in Tyner Creek During Experiment 4
285
235
185
135
85
35
µg Surplus P/disc
5
7
9
11
13
15
17
SRP (µgP/L)

Relationship Between Surplus P and SRP in Peacheater Creek During Experiment 4




Relationship Between Surplus P and SRP in Tyner Creek During Experiment 4
µg Surplus P/µg chl.a
3
2.5
2
1.5
1
0.5
0
5
7
9
11
13
15
17
SRP (µgP/L)

APPENDIX D

STATISTICAL TABLES

TABLES 1-25: One-way ANOVA of **time courses** in Tyner and Peacheater Creeks. Tukeys multiple range test shows significant differences between weeks ($p<0.05$).

TABLE 1: Styrofoam substrata in Peacheater Creek for Experiment 2
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **Chl. *a***

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	1109.798	7	85.032	5.039	0.004
ERROR	270.001	16	16.875		

TUKEYS TEST FOR VARIABLE: **Chl. *a***. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE $\mu g/cm^2$.

				WEEK n=3			
1.331	4.893	10.323	12.780	13.569	16.345	14.252	5.716
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8

TABLE 2: Styrofoam substrata in Tyner Creek for Experiment 2
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE:**Chl. *a***

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	828.667	7	118.381	5.894	0.002
ERROR	321.333	16	20.083		

TUKEYS TEST FOR VARIABLE: **Chl. *a***. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE $\mu g/cm^2$.

				WEEK n=3			
1.037	8.050	9.16	9.598	14.43	10.949	9.934	8.332
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8

TABLE 3: Silicated Disc Substrata in Peacheater Creek for Experiment 2
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE:**Chl. *a***

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	758.150	7	108.307	57.639	0.000
ERROR	22.549	12	1.879		

TUKEYS TEST FOR VARIABLE: **Chl. *a***. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE $\mu g/cm^2$.

				WEEK n=3			
0.822	5.144	9.890	9.765	14.627	14.835	27.123	12.878
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8

TABLE 4: Silicated Disc Substrata in Tyner Creek for Experiment 2
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE:**Chl. *a***

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	828.667	7	53.114	3.605	0.025
ERROR	176.785	12	14.732		

TUKEYS TEST FOR VARIABLE: **Chl. *a***. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE $\mu g/cm^2$.

				WEEK n=3			
2.848	12.045	12.056	15.295	11.356	15.670	9.934	8.332
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8

TABLE 5: Silicated Disc Substrata in Peacheater Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE:**Chl. *a***

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	1075.424	6	179.237	11.455	0.000
ERROR	203.421	13	15.648		

TUKEYS TEST FOR VARIABLE: **Chl. *a***. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE $\mu g/cm^2$.

			WEEK n=3			
0.397	1.801	8.262	15.261	14.469	14.514	23.986
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 6: Silicated Disc Substrata in Tyner Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE:**Chl. *a***

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	471.437	6	78.573	19.408	0.000
ERROR	52.094	13	4.007		

TUKEYS TEST FOR VARIABLE: **Chl. *a***. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE $\mu g/cm^2$.

			WEEK n=3			
2.327	3.342	10.58	12.428	13.945	9.345	17.076
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 6: Silicated Disc Substrata in Peacheater Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **AFDW**

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	66.894	5	13.379	11.069	0.001
ERROR	13.295	11	1.209		

TUKEYS TEST FOR VARIABLE: **AFDW**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE mg

WEEK
n=3

3.79	2.08	4.21	6.40	6.84	1.41
Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 7: Silicated Disc Substrata in Tyner Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **AFDW**

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	71.333	5	14.267	3.290	0.046
ERROR	47.695	11	4.336		

TUKEYS TEST FOR VARIABLE: **AFDW**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE mg

WEEK
n= 3

7.43	3.32	4.81	7.56	7.58	2.39
Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 8: Silicated Disc Substrata in Peacheater Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE:**Chl. *a***

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	734.899	5	146.980	8.814	0.000
ERROR	466.894	28	16.675		

TUKEYS TEST FOR VARIABLE: **Chl. *a***. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE $\mu g/cm^2$.

			WEEK n=3		
0.923	14.375	12.608	11.964	17.013	14.686
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 9: Silicated Disc Substrata in Tyner Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE:**Chl. *a***

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	585.429	5	117.086	18.347	0.000
ERROR	178.688	28	6.382		

TUKEYS TEST FOR VARIABLE: **Chl. *a***. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE $\mu g/cm^2$.

			WEEK n=3		
1.451	9.759	5.882	7.834	12.186	15.096
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 10: Silicated Disc Substrata in Peacheater Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **AREAL SURPLUS P**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	734.899	5	146.980	8.814	0.000
ERROR	466.894	28	16.675		

TUKEYS TEST FOR VARIABLE: **AREAL SURPLUS P**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/disc

			WEEK n=3		
333.547	281.644	298.557	440.284	389.528	548.397
Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 11: Silicated Disc Substrata in Tyner Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **AREAL SURPLUS P**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	179554.05	5	35910.81	5.691	0.008
ERROR	69410.55	11	6310.05		

TUKEYS TEST FOR VARIABLE: **AREAL SURPLUS P**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/disc

			WEEK n=3		
252.668	360.897	314.292	515.247	483.953	527.207
Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 12: Silicated Disc Substrata in Peacheater Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **SURPLUS P**

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	338.333	5	67.667	10.919	0.001
ERROR	68.167	11	6.197		

TUKEYS TEST FOR VARIABLE: **SURPLUS P**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/μg chl. *a*

			WEEK n=3		
1.828	0.322	0.193	0.300	0.265	0.226
Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 13: Silicated Disc Substrata in Tyner Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **SURPLUS P**

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	341.875	5	68.375	11.638	0.000
ERROR	64.625	11	5.875		

TUKEYS TEST FOR VARIABLE: **SURPLUS P**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/μg chl. *a*

			WEEK n=3		
0.746	0.337	0.250	0.365	0.511	0.305
Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 14: Silicated Disc Substrata in Tyner Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **SURPLUS P**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	349.500	5	69.900	13.144	0.000
ERROR	58.500	11	5.318		

TUKEYS TEST FOR VARIABLE: **SURPLUS P**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/mg AFDW

			WEEK n=3		
0.3438	1.0727	0.6448	0.6725	0.6300	2.1767
Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 15: Silicated Disc Substrata in Peacheater Creek for Experiment 3
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **SURPLUS P**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	287.500	5	57.500	5.249	0.010
ERROR	120.500	11	10.955		

TUKEYS TEST FOR VARIABLE: **SURPLUS P**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/mg AFDW

			WEEK n=3		
0.6880	1.3362	0.6998	0.6788	0.5620	3.8379
Week 2	Week 3	Week 4	Week 5	Week 6	Week 7

TABLE 16: Silicated Disc Substrata in Peacheater Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **AREAL SURPLUS P_t**

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	272545.16	5	54509.031	8.661	0.001
ERROR	75523.03	12	3293.586		

TUKEYS TEST FOR VARIABLE: **AREAL SURPLUS P_t**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/disc

			WEEK n=3		
138.025	343.296	395.097	377.454	473.184	449.526
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 17: Silicated Disc Substrata in Tyner Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **AREAL SURPLUS P_t**

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	128825.612	5	25765.122	10.336	0.001
ERROR	29912.300	12	2492.692		

TUKEYS TEST FOR VARIABLE: **AREAL SURPLUS P_t**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/disc

			WEEK n=3		
62.585	191.557	146.830	336.826	228.676	240.310
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 18: Silicated Disc Substrata in Peacheater Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **SURPLUS P_t**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	348.667	5	69.733	6.300	0.004
ERROR	132.833	12	11.069		

TUKEYS TEST FOR VARIABLE: **SURPLUS P_t**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/μg chl. *a*

WEEK
n=3

4.935	0.655	0.309	0.311	0.274	0.302
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 19: Silicated Disc Substrata in Tyner Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **SURPLUS P_t**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	418.667	5	83.733	15.380	0.000
ERROR	35.333	12	5.444		

TUKEYS TEST FOR VARIABLE: **SURPLUS P_t**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/μg chl. *a*

WEEK
n=3

1.004	0.995	0.246	0.424	0.185	0.157
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 20: Silicated Disc Substrata in Peacheater Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **AREAL SURPLUS P_i**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	35918.970	5	7183.794	6.008	0.003
ERROR	12661.546	12	1055.129		

TUKEYS TEST FOR VARIABLE: **AREAL SURPLUS P_i**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/disc

			WEEK n=3		
80.850	158.360	229.633	169.175	142.444	181.575
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 21: Silicated Disc Substrata in Tyner Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **AREAL SURPLUS P_i**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	17445.480	5	3489.096	8.499	0.001
ERROR	4926.203	12	410.517		

TUKEYS TEST FOR VARIABLE: **AREAL SURPLUS P_i**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/disc

			WEEK n=3		
41.915	81.436	100.810	144.747	104.319	110.835
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 22: Silicated Disc Substrata in Peacheater Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **SURPLUS P_i**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	454.667	5	90.933	41.438	0.000
ERROR	26.333	12	2.194		

TUKEYS TEST FOR VARIABLE: **SURPLUS P_i**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/μg chl. *a*

WEEK
n=3

2.891	0.302	0.180	0.140	0.083	0.122
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 23: Silicated Disc Substrata in Tyner Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **SURPLUS P_i**

SOURCE	ANOVA SS	DF	ANOVA MS	F-RATIO	P
WEEK	556.405	5	111.281	35.642	0.000
ERROR	46.833	15	3.122		

TUKEYS TEST FOR VARIABLE: **SURPLUS P_i**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE μg Surplus P/μg chl. *a*

WEEK
n= 3

0.673	0.423	0.169	0.182	0.084	0.072
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 24: Silicated Disc Substrata in Peacheater Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **APA**

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	2.177	5	0.435	4.543	0.005
ERROR	2.108	22	0.096		

TUKEYS TEST FOR VARIABLE: **APA**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE nM MF•μg chl. a^{-1}•hr^{-1}

			WEEK n=3		
0.792	0.227	0.581	1.013	0.528	0.724
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLE 25: Silicated Disc Substrata in Tyner Creek for Experiment 4
ONE-WAY ANALYSIS OF VARIANCE-DEPENDENT VARIABLE: **APA**

SOURCE	**ANOVA SS**	**DF**	**ANOVA MS**	**F-RATIO**	**P**
WEEK	1001.008	5	200.202	6.605	0.001
ERROR	636.492	21	30.309		

TUKEYS TEST FOR VARIABLE: **APA**. MEANS WITH IDENTICAL UNDERLINE ARE NOT SIGNIFICANTLY DIFFERENT. UNITS ARE nM MF•μg chl. a^{-1}•hr^{-1}

			WEEK n=3		
1.530	1.323	13.875	4.141	2.296	1.991
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

TABLES 1-2: One-way ANOVA of **time course comparisons** in Peacheater Creek. Significance level is $p<0.05$.

TABLE 1: Chl. *a* time courses in Peacheater Creek for Experiments 2-4.
KRUSKAL-WALLIS ONE-WAY ANALYSIS OF VARIANCE ON RANKS
DEPENDENT VARIABLE: **Chl. *a* TIME COURSE**

SOURCE	MEDIAN	25%	75%	H	DF	P
STYROFOAM	22.3	14.59	32.2	16.7	3	0.0008
SILICATE DISC	22.7	13.19	28.9			
EXP. 3	27.5	5.58	42.0			
EXP. 4	12.6	9.97	16.9			

ALL PAIRWISE MULTIPLE COMPARISON PROCEDURE USING DUNN'S METHOD FOR VARIABLE: **Chl. *a* TIME COURSE**.

COMPARISON	DIFF. OF RANKS	p	Q	p<0.05
Styrofoam vs. Exp. 4	26.23	4	3.36	Yes
Styrofoam vs. Disc	2.52	3	0.29	No
Styrofoam vs. Exp. 3	0.69	2	0.08	No
Exp.3 vs. Exp. 4	25.54	3	3.09	Yes
Exp.3 vs. Disc	1.83	2	0.20	No
Disc vs. Exp. 4	23.71	2	3.00	Yes

TABLE 2: Chl. *a* time courses in Tyner Creek for Experiments 2-4.
KRUSKAL-WALLIS ONE-WAY ANALYSIS OF VARIANCE ON RANKS
DEPENDENT VARIABLE: **Chl. *a* TIME COURSE**

SOURCE	MEDIAN	25%	75%	H	DF	P
STYROFOAM	21.1	16.1	26.9	28.8	3	<0.0001
SILICATE DISC	23.1	15.5	30.7			
EXP. 3	25.4	8.9	32.1			
EXP. 4	8.8	5.9	11.7			

ALL PAIRWISE MULTIPLE COMPARISON PROCEDURE USING DUNN'S METHOD FOR VARIABLE: **Chl. *a* TIME COURSE**.

COMPARISON	DIFF. OF RANKS	p	Q	p<0.05
Styrofoam vs. Exp. 4	33.4	4	4.4	Yes
Styrofoam vs. Disc	5.4	3	0.6	No
Styrofoam vs. Exp. 3	1.9	2	0.2	No
Exp.3 vs. Exp. 4	31.5	3	4.1	Yes
Exp.3 vs. Disc	3.5	2	0.4	No
Disc vs. Exp. 4	28.0	2	3.6	Yes

VITA

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