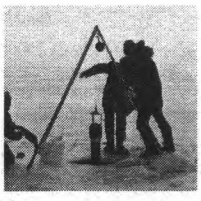
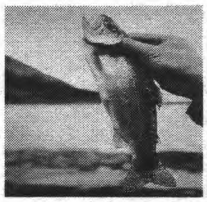
Canada Alberta Northwest Territories

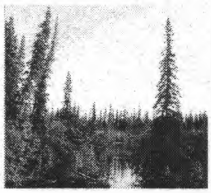
Northern River Basins Study





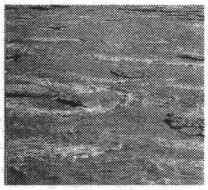


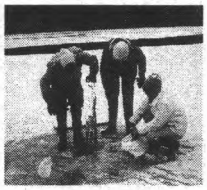





NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 46

GROWTH RATE AND BIOMASS RESPONSES OF PERIPHYTIC ALGAE TO NUTRIENT ENRICHMENT OF STABLE AND UNSTABLE SUBSTRATA ATHABASCA RIVER






Prepared for the
Northern River Basins Study
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by

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NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 46

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PREFACE:

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

It is explicit in the objectives of the Study to report the results of technical work regularly to the public. This objective is served by distributing project reports to an extensive network of libraries, agencies, organizations and interested individuals and by granting universal permission to reproduce the material.

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NORTHERN RIVER BASINS STUDY
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Whereas the above publication is the result of a project conducted under the Northern River Basins Study and the terms of reference for that project are deemed to be fulfilled,
IT IS THEREFORE REQUESTED BY THE STUDY OFFICE THAT;
this publication be subjected to proper and responsible review and be considered for release to the public.

(Dr. F. J. Wrona, Ph.D., Science Director) — (Date) 10 April 95

Whereas it is an explicit term of reference of the Science Advisory Committee "to review, for scientific content, material for publication by the Board",
IT IS HERE ADVISED BY THE SCIENCE ADVISORY COMMITTEE THAT;
this publication has been reviewed for scientific content and that the scientific practices represented in the report are acceptable given the specific purposes of the project and subject to the field conditions encountered.
SUPPLEMENTAL COMMENTARY HAS BEEN ADDED TO THIS PUBLICATION: [ ] Yes [ ] No

(Dr. P. A. Larkin, Ph.D., Chair) — (Date) 12 April 95

Whereas the Study Board is satisfied that this publication has been reviewed for scientific content and for immediate health implications,
IT IS HERE APPROVED BY THE BOARD OF DIRECTORS THAT;
this publication be released to the public, and that this publication be designated for: **[ ] STANDARD AVAILABILITY [ ] EXPANDED AVAILABILITY**

(Lucille Partington, Co-chair) — (Date) 12/04/95

(Robert McLeod, Co-chair) — (Date) 12/04/95

GROWTH RATE AND BIOMASS RESPONSES OF PERIPHYTIC ALGAE TO NUTRIENT ENRICHMENT OF STABLE AND UNSTABLE SUBSTRATA, ATHABASCA RIVER

STUDY PERSPECTIVE

A particular area of concern related to municipal and industrial effluent discharges in the northern river basins is the effect of nutrients (nitrogen and phosphorus) on the aquatic environment. Nutrients enter a river from municipal and industrial effluents, agricultural and timber-harvesting runoff, natural runoff, ground water sources and tributary inflow. Added nutrients can cause changes in abundance and production of benthic biota and production, reproduction and survivorship of fish. Nutrients may also affect dissolved oxygen concentrations as a result of enhanced plant growth, which is, in turn, decomposed by bacteria that consume oxygen. The changes to the biological communities resulting from the addition of nutrients and their subsequent effect on the chemical and physical components of the ecosystem is referred to scientifically as eutrophication. Understanding the impacts of nutrients on the aquatic environment is therefore critical for managing industrial and municipal effluent discharges to the Peace, Athabasca and Slave rivers in order to minimize eutrophication and safeguard ecosystem health.

Related Study Questions

4a)	*What are the contents and nature of the contaminants entering the system and what is their distribution and toxicity in the aquatic ecosystem with particular reference to water, sediments and biota?*
5	*Are the substances added to the rivers by natural and man-made discharges likely to cause deterioration of the water quality?*
13b)	*What are the cumulative effects of man-made discharges on the water and aquatic environment?*

This report presents the results of an experiment conducted in artificial streams in which the growth of benthic algae was studied in relation to phosphorus and nitrogen concentrations in the water. The goal of this study is to determine whether benthic algal biomass in the upper Athabasca River is limited by the availability of phosphorus or nitrogen. This was accomplished by growing benthic algae in artificial streams (periphyton flumes) enriched with phosphorus or nitrogen to establish the type and degree of nutrient limitation. The flumes were located on-site, adjacent to the Athabasca River at Hinton.

Phosphorus addition was found to increase benthic algal biomass by as much as 100 times that of the river water control and nitrogen enriched streams. This strong response to phosphorus addition and little effect of nitrogen addition indicates that the benthic algal community was phosphorus limited. Examination of the benthic algal species composition showed that diatoms were dominant at phosphorus concentrations < 1 µg/L. At higher concentrations of phosphorus a green algae (*Chlorella sp.*) dominated on the tile substrata, whereas diatoms remained dominant on the silt substrata. *Chlorella* was later discovered to be a contaminant introduced on the tile. However, because *Chlorella* is routinely used in bioassays, results from the contaminated substrata can be compared with published bioassay tests on *Chlorella*. Examination of algal growth on the silt substrata showed that peak biomass increased with increasing phosphorus concentration and no obvious saturation up to 50 µg/L, although the greatest change was at phosphorus concentrations ≤10 µg/L.

Results from this study showed that growth of benthic algae in the upper Athabasca River is limited by phosphorus. Addition of phosphorus to the upper Athabasca River would increase benthic algal growth in the absence of other growth-limiting factors. Experiments are continuing with artificial streams to assess seasonal differences in the response of benthic algae to phosphorus addition and these studies, along with similar experiments involving benthic invertebrates, will be used to evaluate food web responses to nutrient additions.

REPORT SUMMARY

A phosphorus enrichment experiment was run in flow-through troughs at Hinton, Alberta to examine taxonomic, growth and biomass responses by periphytic algae to PO_4-P additions in the Athabasca River. A standard test for nutrient deficiency showed that growth of algal periphyton was limited by phosphorus. More detailed growth and biomass responses to P additions were examined at PO_4-P additions of 0 (control), 0.1, 0.2, 1, 5, 10, 25 and 50 $\mu g \cdot L^{-1}$ applied to randomly selected troughs from which tile and silt substrata were sampled to measure the time-course accrual of chlorophyll *a*. In unamended river water, the algal community was dominated by diatoms on both substrata but at P concentrations ≥ 1 $\mu g \cdot L^{-1}$, the green alga, *Chlorella sp.* became the most abundant single genus on tile, comprising almost 70% of total cell numbers. The remaining 30% included up to nine diatom species. This taxonomic shift indicated a change in competitive dominance with P addition. *Chlorella sp.* did not become established on the silt substrata regardless of P additions. This finding, coupled with evidence to show that *Chlorella sp.* does not naturally occur in the Athabasca River, indicated that *Chlorella sp.* was a contaminant that was not removed when the tile was cleaned prior to the experiment. Relative specific growth rates ($\mu:\mu_{max}$) demonstrated Monod kinetics with saturation occurring between 0.2 and 1 $\mu g \cdot L^{-1}$ of P added. Relative peak biomass ($PB:PB_{max}$) was proportional to community growth rate. A model describing change in $PB:PB_{max}$ with P addition was determined using data from the Athabasca troughs and experimental troughs used on the South Thompson River. The effect of *Chlorella sp* on growth adaptation to P addition in the Athabasca was removed by eliminating data from the tile substrata. Despite the theoretical possibility that a model from one river could be extrapolated to another, our comparison showed significant differences between models that was related to substrata differences between experiments. This finding indicated that functional models of periphyton response to nutrient augmentation cannot be extrapolated from one river to another unless factors that can affect biomass accrual have similar effects between the sites being compared. Contamination of tile substrata by *Chlorella sp.* in the Athabasca experiment served as a warning that attention to quality control in outside mesocosm experiments cannot be overemphasized.

ACKNOWLEDGEMENTS

Funding for this project was made available from the Federal/Provincial/Territorial Northern River Basins Study. We thank Weldwood of Canada Ltd. and particularly staff at the Hinton site including Andy Hartery and Laudy Lickacz for access through mill property and for preparing a site to run the experiment. Field support was provided by Kelly Field and Paul Dinsmore with generous assistance from Cheryl Podemski, Eric Marl, Carol Casey and Mary Ferguson. Laboratory analyses were completed by Trinh Luong, Girdy Hutchinson and Mary Bolin. Drafting was completed by Gordon Hodge.

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1.0 INTRODUCTION

Concepts of relative growth rate in freshwater algae (Goldman, 1980; Bothwell, 1988) and relative peak biomass in periphytic algae (Bothwell 1989) have proven valuable in examining effects of augmentation of a limiting nutrient on the growth and biomass of periphytic algae in rivers. In determining relative indices, specific growth rate (μ) is expressed as a proportion of a maximum μ (μ:μ_{max}) determined experimentally or from an empirical model (Goldman, 1980; Bothwell, 1985), thereby normalizing μ for variation in temperature, light, and other physical factors that can influence measurements of μ. Relative biomass measurements are usually determined in terms of the peak biomass (PB) that is attained during the time course of accrual on artificial substrata (PB:PB_{max}; Bothwell, 1989). In the South Thompson River, British Columbia, these measures were used to show that phosphorus concentration exerted greatest control over diatom growth rates and that temperature had greatest influence on P-replete μ (Bothwell, 1988). Three phases of biomass response to additions of phosphate were found: an initial exponential increase controlled by Monod cellular growth kinetics (Monod, 1942) at 1 $\mu g \cdot L^{-1}$ of P added to ambient soluble reactive P (SRP) concentrations of 1.3 and 2.4 $\mu g \cdot L^{-1}$, a slower linear increase controlled by diffusion kinetics in the algal mat up to P additions of 30 $\mu g \cdot L^{-1}$, and finally a phase of little or no response at P concentrations >30 $\mu g \cdot L^{-1}$ due to effects of factors other than nutrient concentration (Bothwell, 1989). This model and the Monod-type growth models can be very powerful in quantifying responses of periphyton to a given change in phosphorus concentration determined in relation to changes in P loading from allochthonous sources. The approach has been used to identify concentrations of limiting nutrients that cause eutrophication problems (Biggs, 1990), to describe algal growth responses to additions of limiting nutrients as part of fisheries enhancement initiatives (Perrin, 1993), to determine the potential toxicity of chemicals in pulp mill effluent (Perrin and Bothwell, 1992), and to separate effects of light, temperature, and P concentration controlling periphyton growth and biomass, as was done in the South Thompson River (Bothwell, 1989).

For periphyton communities having similar community structure, it can be hypothesized that curves describing μ:μ_{max} or PB:PB_{max} as a function of addition of a growth-limiting nutrient will be similar between rivers. By normalizing growth or biomass data to correct for effects of physical variables, the relative values only change with respect to manipulation of the nutrient that limits growth. This is a physiological response, that should not change between rivers supporting similar algal communities. Hence, it may be possible to extrapolate existing models of μ:μ_{max} or PB:PB_{max} as a function of ambient P concentration to sites remote from where they were developed, thus reducing the need for site specific experimentation.

As part of the Northern River Basins Study (NRBS), an experiment was completed on the Athabasca River at Hinton, Alberta to test two hypotheses. Five pulp mills presently exist in the Athabasca River basin and effluent from these introduces an estimated 537 $kg \cdot d^{-1}$ of total phosphorus into the river (Noton, 1990). With a molar N:P supply ratio well in excess of 100 upstream of the mills, our first hypothesis is that algal productivity is limited by phosphorus. We also hypothesize that algal biomass response to additions of phosphorus can be described by kinetics similar to those determined in other rivers. The South Thompson River, B.C. was selected for specific comparison since extensive experimentation at the South Thompson Experimental Troughs

Apparatus (EXTRA) has produced comprehensive data describing relative response of algal growth to P additions (Bothwell, 1988, 1989).

The terms of reference for this study as described in Schedule A of the NRBS contract are enclosed as Appendix A.

2.0 STUDY SITE

The study was conducted using experimental troughs established on the bank of the Athabasca River at the Weldwood Pulp Mill, Hinton, Alberta (Figure 1). The troughs were well removed from mill and plant operations. The Athabasca River is seasonally turbid due to snowmelt and glacial outwash erosion of sedimentary formations that are characteristic of the east slope of the Rocky Mountains. The river is turbid from May through September after which freezing in the headwaters reduces sediment mobilization and water flow. There is extensive deposition of sediment at Hinton, producing either unstable sand and silt substrata or heavily embedded gravel and cobble. Backwaters are characterized by sand bars with little exposed gravel and cobble. Using methods of MacDonald et al. (1991), we estimated the average cobble embeddedness in riffles and runs accessible from the study site to be $\geq 60\%$. Chlorophyll *a* concentrations on stable substrata at Hinton are < 2 mg $\cdot$ m^{-2} during the spring and summer freshet but with the lower suspended sediment concentrations in September, they can increase by more than an order of magnitude (Anderson, 1989). Hence, algal biomass in the Athabasca can be affected not only by P concentration but also by scour on stable substrata and lack of stable growth substrata in the margin and backwater silt deposits.

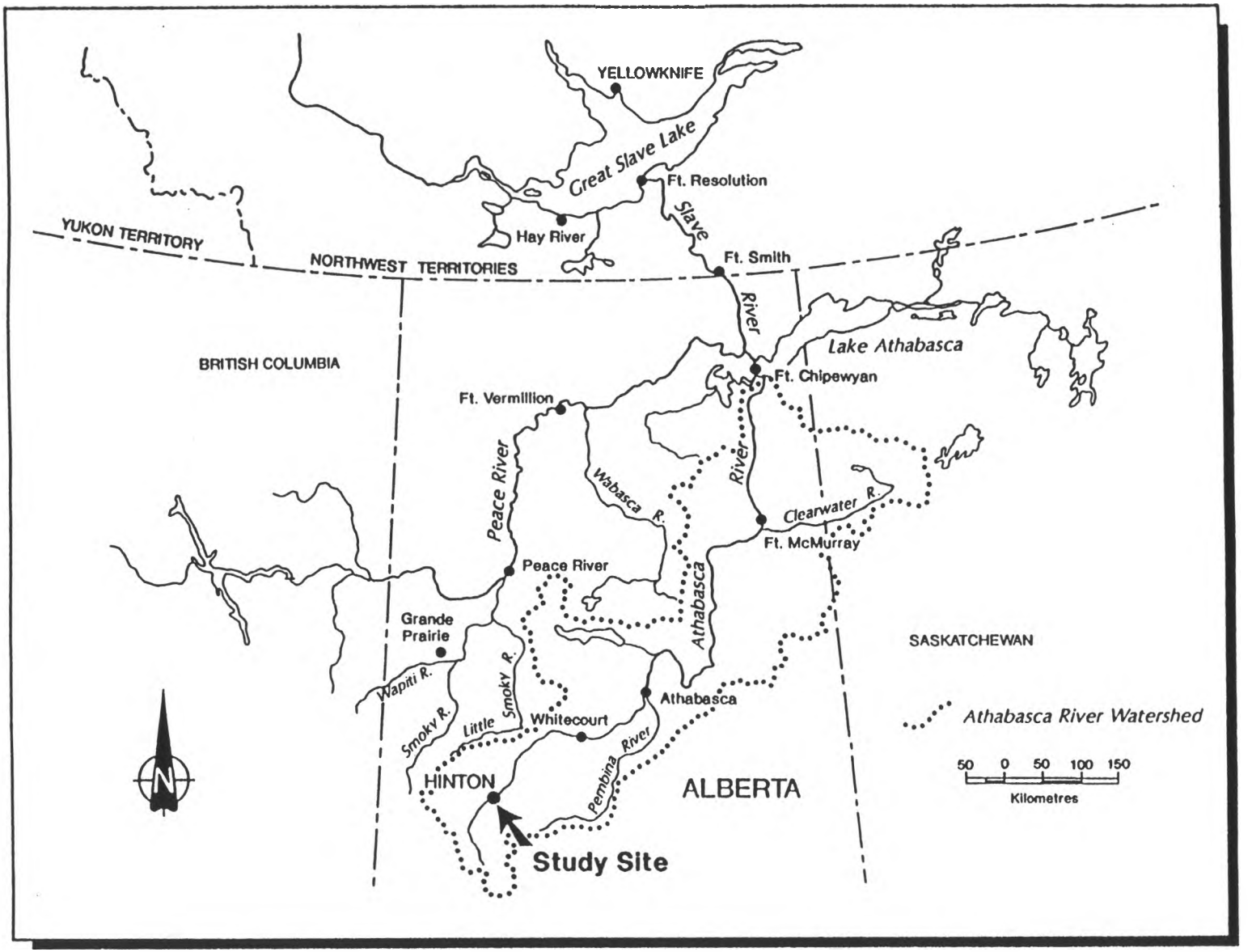

Figure 1. Location of the Hinton study site in relation to other features of the Athabasca watershed.

3.0 MATERIALS AND METHODS

3.1 EQUIPMENT

The experimental apparatus had a similar design to EXTRA, which is described elsewhere (Bothwell, 1988). The apparatus consisted of 12 parallel, flow-through troughs (2m long x 19 cm wide) fabricated from clear acrylic (Figure 2). Fresh river water was supplied from the mill intake via a 5.08 cm diameter pipeline. The water discharged into a double 1200 L head tank that continuously provided constant pressure to a manifold which controlled the flow of water to each trough. Water flow to the troughs was adjusted to 25 $L \cdot min^{-1}$. The bottom of each trough was lined with a sheet of open cell styrofoam-DB (Flora Craft, Pomona, CA) to provide a substratum for periphyton colonization and growth. However, after one week in mid-September of starting water flows to test the equipment, silt and sand settled and covered the styrofoam, preventing the styrofoam from providing a stable substratum for algal colonization. The extent of this accumulation of fines was greater than expected, given that the test was run when turbidity was declining in the river. Hence, to simulate exposed gravel and cobble substrata, styrofoam from the downstream half of each trough was removed and replaced with squares of terra cotta tile (4.5 cm x 4.5 cm x 1.5 cm) that were acquired on short notice from a tile distributor. The tiles were washed prior to use. The tiles were separated 3 cm from each other, creating channels of relatively high velocity water around them, similar to flow conditions around natural gravel. This orientation allowed sediment to accumulate between the tiles but not on the tops and sides, thus mimicking flow conditions on natural gravel. At the time the tiles were installed, new sheets of styrofoam were placed in the upstream half of each trough and the experiment was restarted.

Stock solutions of KH_2PO_4 and $NaNO_3$ were continuously metered into the treated troughs using a Technicon Model III metering pump (Pulse Instrumentation, Saskatoon, Sask.).

3.2 EXPERIMENTAL DESIGN

The original experimental design involved a standard test for N or P deficiency in the periphyton and a test of periphyton biomass response to a gradient of P additions, each to be completed from measurements on styrofoam substrata. With the unexpected accumulation of sand and silt, the experiment was modified to examine biomass accrual on the styrofoam and tile. Treatments for the nutrient deficiency test included a control, N, P, and N+P additions in separate randomly selected troughs. N was added at 100 $\mu g \cdot L^{-1}$ and P was added at 50 $\mu g \cdot L^{-1}$. In six other randomly selected troughs, periphyton biomass response to P additions at 0.1, 0.2, 1, 5, 10, 25, and 50 $\mu g \cdot L^{-1}$ were examined using samples from both the tile and silt substrata.

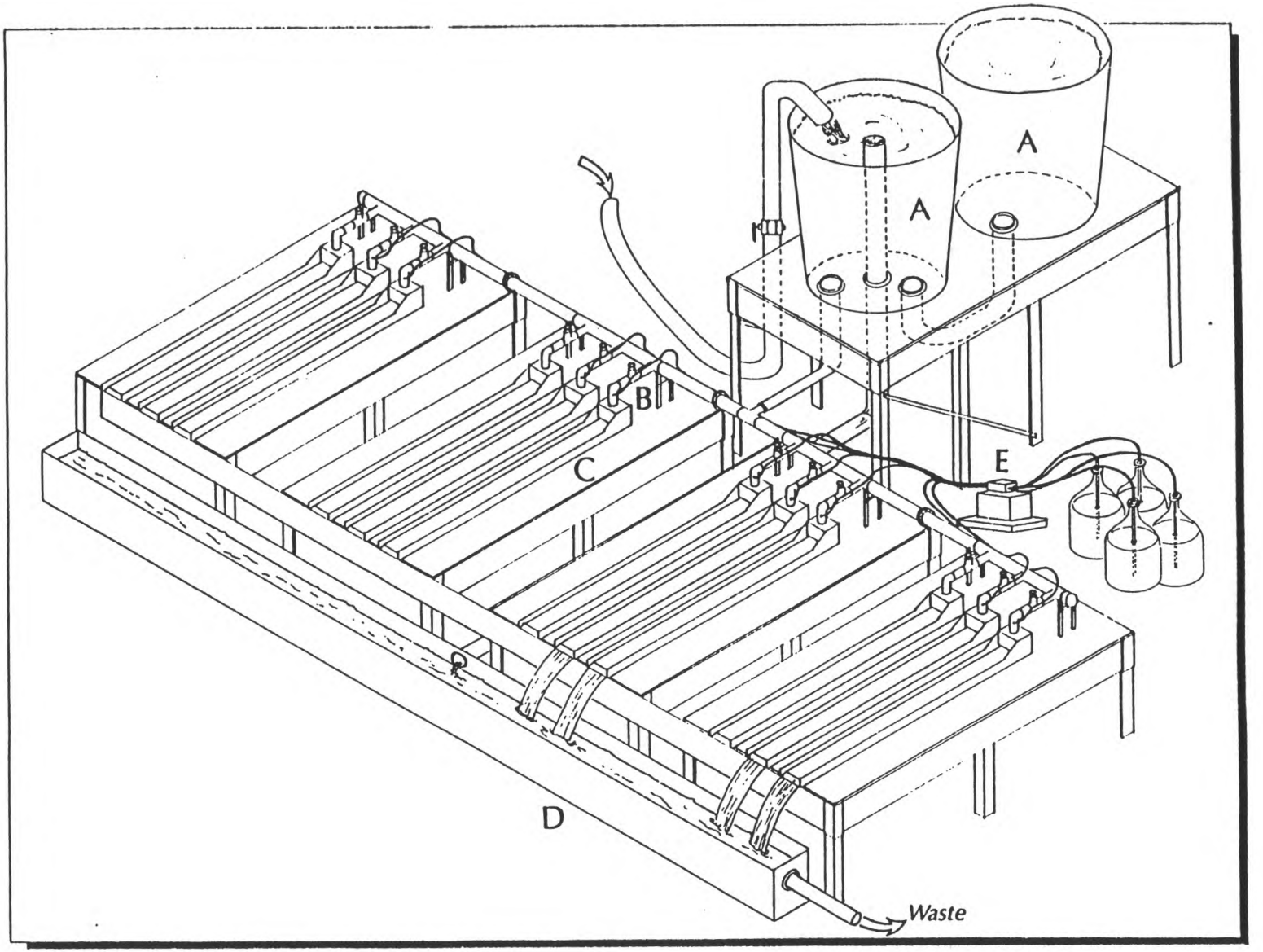

Figure 2. Schematic layout of the trough apparatus used at Hinton: A, double head tank assembly showing the inflow pipe, water distribution inside the tanks, head control, and outflow to the trough manifold; B, flow controlled water supply to each trough with chemical delivery lines; C, trough in which the styrofoam and tile substrata were placed; D, waste collection trough with discharge back to the river; E, chemical reservoirs and metering pump. All plumbing and exposed chemical delivery lines were insulated to prevent freezing.

3.3 SAMPLING AND ANALYTICAL PROCEDURES

Hourly integrated values of photosynthetically active radiation (PAR: 400-700 nm) were recorded with a Li-Cor (Lincoln, NB) Li-1000 data logger equipped with a LII90SA quantum cosine sensor. Daily PAR values ($E \cdot m^{-2} \cdot d^{-1}$) were calculated from the hourly data. A calibrated Ryan TempMentor 1.1 thermograph (Redmond, WA) submerged in the head tank was used to record water temperature in 30 min intervals throughout the experiment. Mean daily temperature was calculated.

The experiment began with the commencement of water flow and chemical additions on September 25, 1993. The tiled reach of each trough was arbitrarily stratified into equidistant upstream, middle, and downstream sections. All sampling was stratified by these sections due to uncertainty about differences in sand accumulation along the trough length. At 2 to 3 day intervals, biomass from 48.15 cm^2 of a randomly selected tile (5 surfaces including top and sides) was removed from each section. The tile was scraped using a razor blade and samples were frozen at -15°C. In the laboratory all samples were thawed, extracted in 90% ethanol in a heated water bath, and analyzed fluorometrically for chlorophyll *a* concentration (Holm-Hansen et al., 1965; Nusch, 1980). The experiment was terminated on October 24, 1993, when biomass sloughing was apparent. On the final day of sampling, triplicate cores of the styrofoam with the attached biomass were randomly removed from each trough and analyzed for chlorophyll *a* concentration using the same procedures used for biomass scraped from the tiles. Also on the final day, an additional core of styrofoam and an additional tile were collected from each trough for taxonomic enumeration. Counts of cells containing cytoplasm were made at 500x magnification in Utermohl chambers. A minimum of 100 individuals of the most abundant species and a total of 300 cells in total were counted.

Water samples for the analysis of soluble reactive phosphorus (SRP), NH_4^+, $NO_3^- + NO_2^-$, and total dissolved phosphorus (TDP) were collected from the outlet of each trough three times during the experiment (start, middle, and end). The samples were filtered in the field and kept cool prior to analysis within 24 h of sample collection. Samples for TDP analysis were digested and analyzed by Menzel and Corwin's (1965) potassium persulfate method. SRP was analyzed using the molybdenum blue method (Murphy and Riley, 1962). NH_4^+ and $NO_3^- + NO_2^-$ were analyzed using a Technicon autoanalyzer (Stainton et al., 1977).

The rate of algal biomass accrual was determined by fitting the time-series chlorophyll *a* data to a significant model (arbitrarily selected $p<0.01$) providing the lowest standard error of the estimate. Where growth clearly dominated the curves (Monod kinetics were obvious), the best model was described by $y=a10^{kt}$ where y is the chlorophyll *a* concentration ($mg \cdot m^{-2}$) at day t, 'a' is the initial chlorophyll *a* concentration, and k is the specific net growth rate. Values of k multiplied by 3.322 gave specific growth rate (μ) in units of divisions per day (Guillard, 1973). In the South Thompson River studies, the natural log model was used, but either approach gives the same division times. In the Athabasca, the $\log_{10}$ model produced half the error of the natural log model. Relative peak biomass ($PB:PB_{max}$) was determined as the ratio of the mean of the three highest chlorophyll *a* values for a given nutrient treatment (PB) divided by the maximum biomass attained under nutrient replete conditions (PB_{max}) (Bothwell, 1989). Differences in growth or biomass curves

over time were distinguished by examining differences between slopes of lines using analysis of covariance (ANCOVA; Wilkinson, 1990) where time, in days, was the covariate, treatment was the category, and chlorophyll *a* concentration was the dependant variable. Comparisons of curves between rivers was done the same way except that river was the category.

4.0 RESULTS

4.1 PHYSICAL AND CHEMICAL

Mean daily water temperatures generally declined over the course of the 29-day experiment (Figure 3). Highest temperatures of 8-10°C were measured within the first 10 days, but thereafter temperatures declined to a minimum of 2.7°C on day 25 (October 20). Mean daily temperature for the duration of the experiment averaged 6.99°C. Irradiance was variable (Figure 3) reflecting changing cloud cover. The highest value, 28.2 $E \cdot m^{-2} \cdot d^{-1}$, was measured on day 2 and the experiment ended with a value of 6.0 $E \cdot m^{-2} \cdot d^{-1}$. The lowest value, 2.95 $E \cdot m^{-2} \cdot d^{-1}$, was measured on Oct. 6 and was due to heavy overcast and light snowfall.

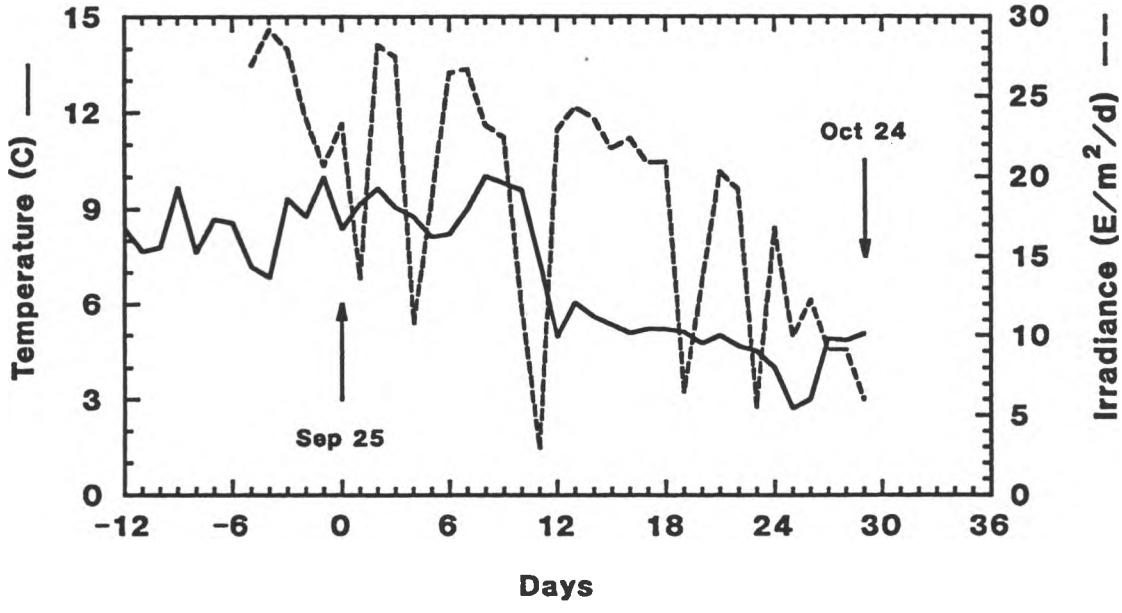

Figure 3. Mean daily water temperature and photosynthetically active radiation (PAR). The experiment started on September 25 (Day 0) and ended on October 24, 1993.

Within 5 days of the start of the experiment, the styrofoam was heavily embedded in silt and within 15 days, the styrofoam was obscured. At the end of the experiment, silt had accumulated to a mean depth of 0.5 cm over the styrofoam in all troughs. Top surfaces of the tile substrata generally remained free of the silt throughout the experiment. Silt deposits were apparent occasionally, but they were temporary, washing off from surfaces within less than a day. Longer lasting silt deposits did accumulate between the tiles, similar to that around gravel in the river.

Unamended river water had large molar N:P supply ratios (Table 1). The mean SRP concentration was 0.9 $\mu g \cdot L^{-1}$ in each trough not receiving P additions. Mean NH_4^+-N and NO_3^--N concentrations in raw river water were 7.9 - 21.2 $\mu g \cdot L^{-1}$ and 47.9 - 54.1 $\mu g \cdot L^{-1}$ respectively. P additions less than 1 $\mu g \cdot L^{-1}$ could not be distinguished from unamended controls using the wet chemistry techniques; the higher additions produced the intended concentration gradient. The NO_3^- additions increased ambient concentrations by the intended amount with no change in NH_4^+-N concentrations relative to the control. These N and P manipulations produced a range of molar N:P ratios from 2 at the highest P addition to 384 at the highest N addition. The mean unamended molar N:P supply ratio was 167.

4.2 TEST OF NUTRIENT DEFICIENCY

The accrual of chlorophyll *a* clearly indicated that periphytic algal growth in the Athabasca River was limited by phosphorus (Figure 4). Using data in Figure 4, a two-way (time and treatment) analysis of variance (ANOVA) was run using orthogonal contrasts to examine significant treatment comparisons (Wilkinson, 1990). Only two contrasts were run; control versus N addition and P added alone versus N plus P. Probability values cited below were those generated from the contrasts. Addition of N produced slight but significantly ($p < 0.001$) greater biomass levels than the control on all sampling dates but the differences were < 2 mg $\cdot m^{-2}$ and these were likely due to the differences in colonization (0.09 mg $\cdot m^{-2}$ chl *a* for N alone and 0.03 mg $\cdot m^{-2}$ chl *a* for the control). The phosphorus addition produced a biomass response up to two orders of magnitude greater than that of the control and there was no significant difference in chl *a* concentrations between the P addition and N plus P addition ($p = 0.62$). The strong response to P addition, little effect of N added alone, and no added effect of N with the P addition indicates that the algal community growth was P-limited.

Table 1. Mean N and P concentrations (± SE) for all treatments. Atomic N:P was determined from the mean DIN and SRP values. DIN is dissolved inorganic N (NH_4^+-N + NO_2^--N + NO_3^--N).

N and P added ($\mu g \cdot L^{-1}$)		Mean Measured N and P concentrations ($\mu g \cdot L^{-1}$) ± SE				molar N:P
KH_2PO_4-P	$NaNO_3$-N	NH_4^+-N	NO_3^--N	DIN	SRP	
0	0	19.6 (4.8)	48.2 (8.5)	67.8 (12.0)	0.9 (0.12)	167
0.1	0	16.4 (7.9)	47.9 (9.5)	64.4 (15.7)	0.7 (0.15)	204
0.2	0	14.3 (4.4)	48.2 (9.5)	62.5 (10.4)	0.97 (0.47)	154
1.0	0	7.9 (2.7)	52.8 (3.4)	60.7 (4.0)	2.0 (0.5)	67
5.0	0	17.5 (4.8)	53.1 (13.3)	70.6 (18.1)	8.9 (1.0)	18
10.0	0	13.5 (4.5)	54.1 (4.2)	67.6 (8.6)	13.4 (2.0)	11
25.0	0	21.2 (7.6)	54.0 (3.3)	75.2 (11.0)	31.6 (2.7)	5
50.0	0	18.6 (5.2)	51.6 (2.3)	70.3 (7.5)	68.9 (4.2)	2
0	100	14.3 (4.2)	142 (10)	156 (7)	0.9 (0.2)	384
50.0	100	9.2 (1.7)	161 (14)	170 (15)	67.9 (5.3)	6

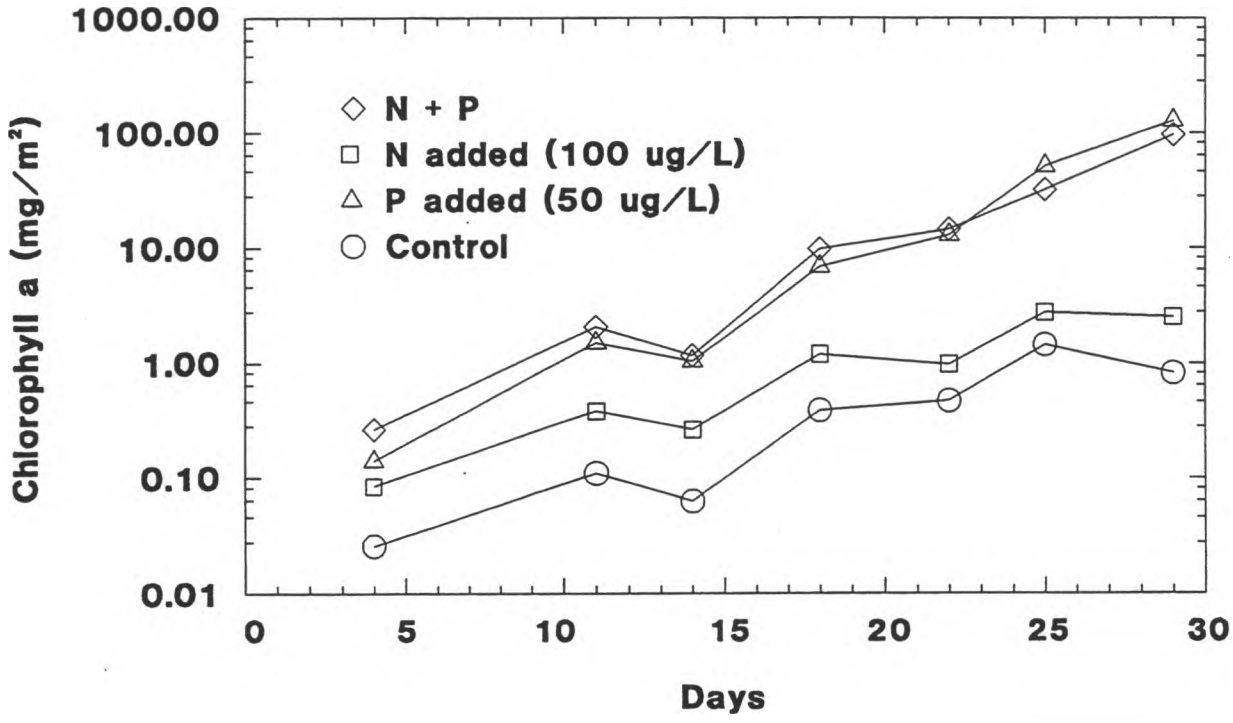

Figure 4. Semi-log plot of time-course accrual of algal periphyton biomass (measured as chlorophyll *a*) on tile substrata to test for N or P deficiency. The strong response to P addition, no effect of N added alone, and no added effect of N with the P additions indicates that the algal community growth was P-limited.

4.3 COMMUNITY COMPOSITION

Algal community composition changed greatly in relation to P addition and type of substrata (Figure 5). On tile substrata receiving P additions < 1 $\mu g \cdot L^{-1}$, the communities were completely comprised of diatoms. Most abundant taxa included *Diatoma tenue*, *Achnanthes minutissima* and *Synedra ulna*. Less abundant diatoms included *Fragilaria sp.*, *Hannaea sp.*, *Cymbella sp.*, *Nitzschia sp. Navicula sp.*, *Gomphonema sp.*, *Diploneis sp.* and *Surirella sp.*. At phosphorus additions ≥ 1 $\mu g \cdot L^{-1}$, the green alga, *Chlorella sp.* became numerically abundant while at P additions ≥ 5 $\mu g \cdot L^{-1}$, it was the single most abundant genus (Figure 5). Diatoms in the troughs receiving > 5 $\mu g \cdot L^{-1}$ P treatments remained the same as those found at low or no P additions but in reduced relative abundance. Nitrogen additions had no effect on community composition on the tile (Figure 5). The increase in abundance of *Chlorella sp.* on tile with P addition was not found in the community growing on the silt matrix (Figure 5). The latter communities remained entirely composed of diatoms of which the most abundant taxa were *Cymbella sp.*, *Hannaea arcus*, *Fragilaria vaucheriae*, and *Synedra ulna*. Relatively rare species on the silt included *Diatoma tenue*, *Achnanthes*

minutissima, Nitzschia sp., Gomphonema sp., and *Surirella sp.*. There was no apparent effect of the P additions on community composition on the silt.

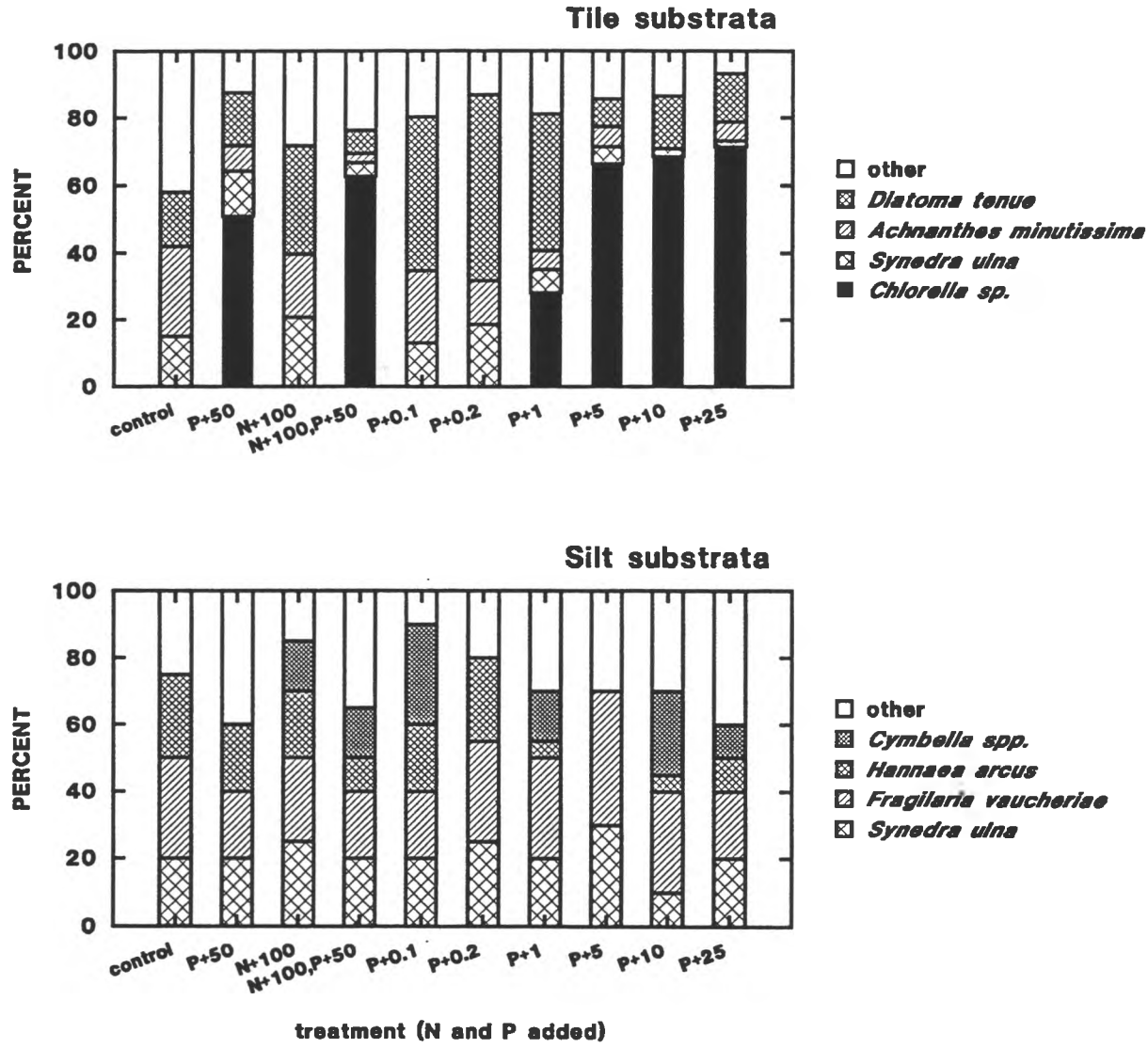

Figure 5. Relative abundance of algal taxa comprising >10% of total cell numbers in each treatment on both types of substrata. Absolute cell counts by species were expressed as a percentage of total cell numbers. The "other" category on tile substrata included the relatively rare diatoms, *Fragilaria sp., Hannaea sp., Cymbella sp., Nitzschia sp. Navicula sp., Gomphonema sp., Diploneis sp.*, and *Surirella sp.* "Other" on silt substrata included *Diatoma tenue, Achnanthes minutissima, Nitzschia sp., Gomphonema sp.*, and *Surirella sp.*.

The diatoms found in the Athabasca troughs were also common to experiments in the South Thompson River (Bothwell, 1989). However, a major difference between communities was the appearance of *Chlorella sp.* which was not found in the South Thompson River with or without P enrichment (Bothwell, 1989). Although an extensive taxonomic survey of periphyton has not been conducted in the Athabasca, it would be very surprising to find it even as a rare genus. *Chlorella sp* is associated with bogs, standing water in soils and shallow ponds, not large rivers. It also was not found in troughs adjacent to the present ones in which another experiment was being run using the same water supply (C. Podemski, Nat. Hydrol. Res. Inst. Saskatoon, Sask. Pers. Comm). The fact that it was found only on the tile, not on the silt substrata, and that it did not appear in the other experiment suggests that *Chlorella sp.* was a contaminant that was not removed when the tile was cleaned prior to starting the experiment.

4.4 GROWTH RATE, BIOMASS AND PHOSPHORUS INTERACTIONS

The change in chlorophyll *a* concentrations over time, by treatment, was examined on the tile substrata. For all P additions, there was a logarithmic increase in biomass (Figure 6). Algal colonization, which is accrual within the first week (Bothwell and Jasper, 1983), ranged between 0.026 $mg \cdot m^{-2}$ in the control to a high of 0.162 $mg \cdot m^{-2}$ in the trough receiving the P addition of 1 $\mu g \cdot L^{-1}$. Approximately two orders of magnitude separated the final biomass levels. Mean peak biomass (PB) in the control was 1.44 $mg \cdot m^{-2}$ and highest PB (PB_{max}) of 127.6 $mg \cdot m^{-2}$ was measured with the P addition of 50 $\mu g \cdot L^{-1}$.

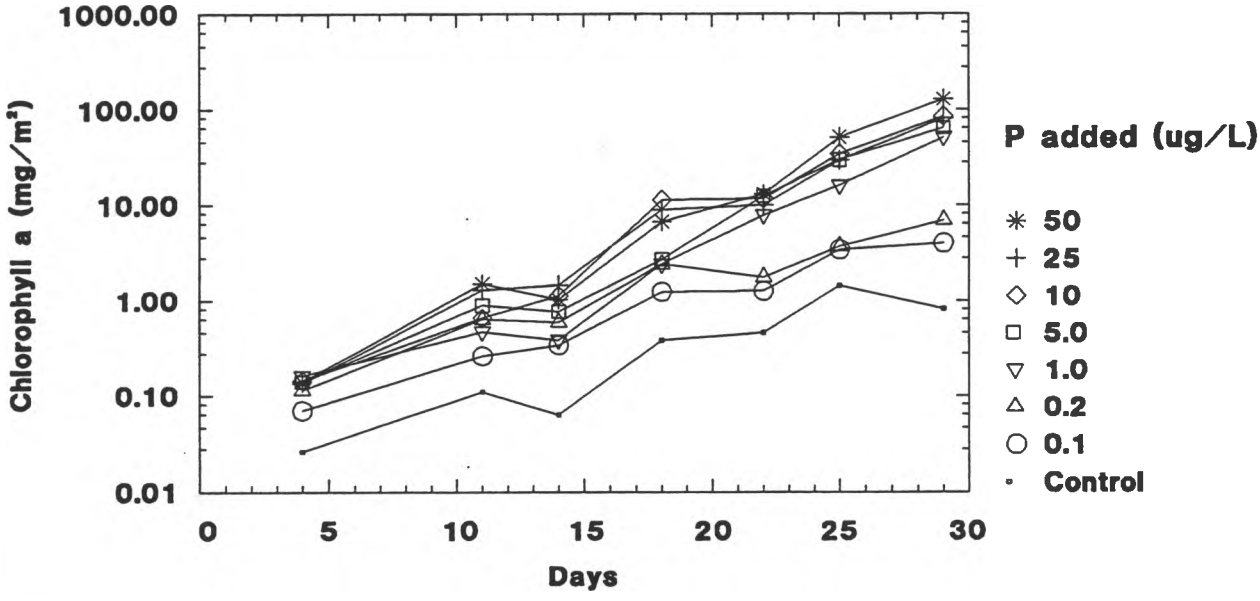

Figure 6. Semi-log plot of accrual of periphyton biomass on tile substrata at several P additions up to 50 $\mu g \cdot L^{-1}$.

Specific growth rates (μ) were determined from these accrual data and plotted with μ determined for diatom communities from South Thompson River artificial stream experiments run at similar temperatures (6.4°C) (Figure 7). The curvilinear relationship in these data indicate Monod growth kinetics with P-saturated μ occurring at 0.5 $\mu g \cdot L^{-1}$ of added P in the Thompson River (discussed by Bothwell, 1988), and between 0.2 and 1 $\mu g \cdot L^{-1}$ in the Athabasca River. Over the range of P additions, μ was generally higher in the Athabasca. Most notable was that μ in the Athabasca at P additions between 1 and 10 $\mu g \cdot L^{-1}$ were 0.36 to 0.39 $div \cdot d^{-1}$. At 25 and 50 $\mu g \cdot L^{-1}$ P added, μ reached a maximum (μ_{max}) of 0.4 $div \cdot d^{-1}$. These values were only achieved in the Thompson River in May or June when water temperatures were up to 9°C. The highest μ for the 6.4°C South Thompson River experiment was 0.31 $div \cdot d^{-1}$ (Figure 7).

We normalized these growth data to maximum μ determined at P-replete conditions to account for the slight differences in temperature between trials (6.4°C in the Thompson and 7.0°C in the Athabasca). The μ_{max} in the Athabasca was 0.4 $div \cdot d^{-1}$ at P additions of 50 $\mu g \cdot L^{-1}$. The equivalent value for the South Thompson was 0.32 $div \cdot d^{-1}$ (Bothwell, 1988). Despite removing physical effects between trials, $\mu:\mu_{max}$ for the Athabasca remained slightly higher than that for the Thompson River at P additions ≥ 1 $\mu g \cdot L^{-1}$. An important distinction, however, is that P additions up to 0.2 $\mu g \cdot L^{-1}$ did not increase growth rates from control levels in the Athabasca. Only with P additions >0.2 $\mu g \cdot L^{-1}$ was a response found and that appeared greater than found in the South Thompson experiment.

A test for differences between slopes of these $\mu:\mu_{max}$ was made with ANCOVA where location was the category, $\mu:\mu_{max}$ was the dependant variable and $\log_{10}$(P added) was the independent variable. The $\log_{10}$ transformation provided the lowest standard error of the estimate. The comparison was made at P additions up to 5 $\mu g \cdot L^{-1}$ to make the ranges of P addition comparable between curves. This analysis indicated no difference between slopes ($p=0.21$), which suggests that despite the apparent separation in magnitude of $\mu:\mu_{max}$ between rivers, the basic kinetics of the response of μ to P addition appeared the same.

Results of the ANCOVA appeared to contradict the differences in response of μ to P additions $\leq$ 0.2 $\mu g \cdot L^{-1}$ between the Athabasca and South Thompson experiments (Figure 7). The closely bunched values of μ at the low end of P additions in the Athabasca experiment suggests that the minimum P concentration that induces a growth response is higher than in the South Thompson experiment and that μ is higher even without P additions. Those bunched values would have introduced more variance in the Athabasca model compared to the South Thompson model at the low range of P additions and reduced the probability of the ANCOVA to detect a difference between models even if it truly existed. The biological importance of higher overall growth rates regardless of P addition in the Athabasca experiment and the fact that the periphyton community required higher additions to elicit a growth response than did periphyton in the South Thompson experiments, should not be overlooked. These functional differences suggest that periphyton responses to P addition were very different between the Athabasca and Thompson experiments and acceptance of the null hypothesis that the two curves are similar, based solely on the ANCOVA, would be misleading.

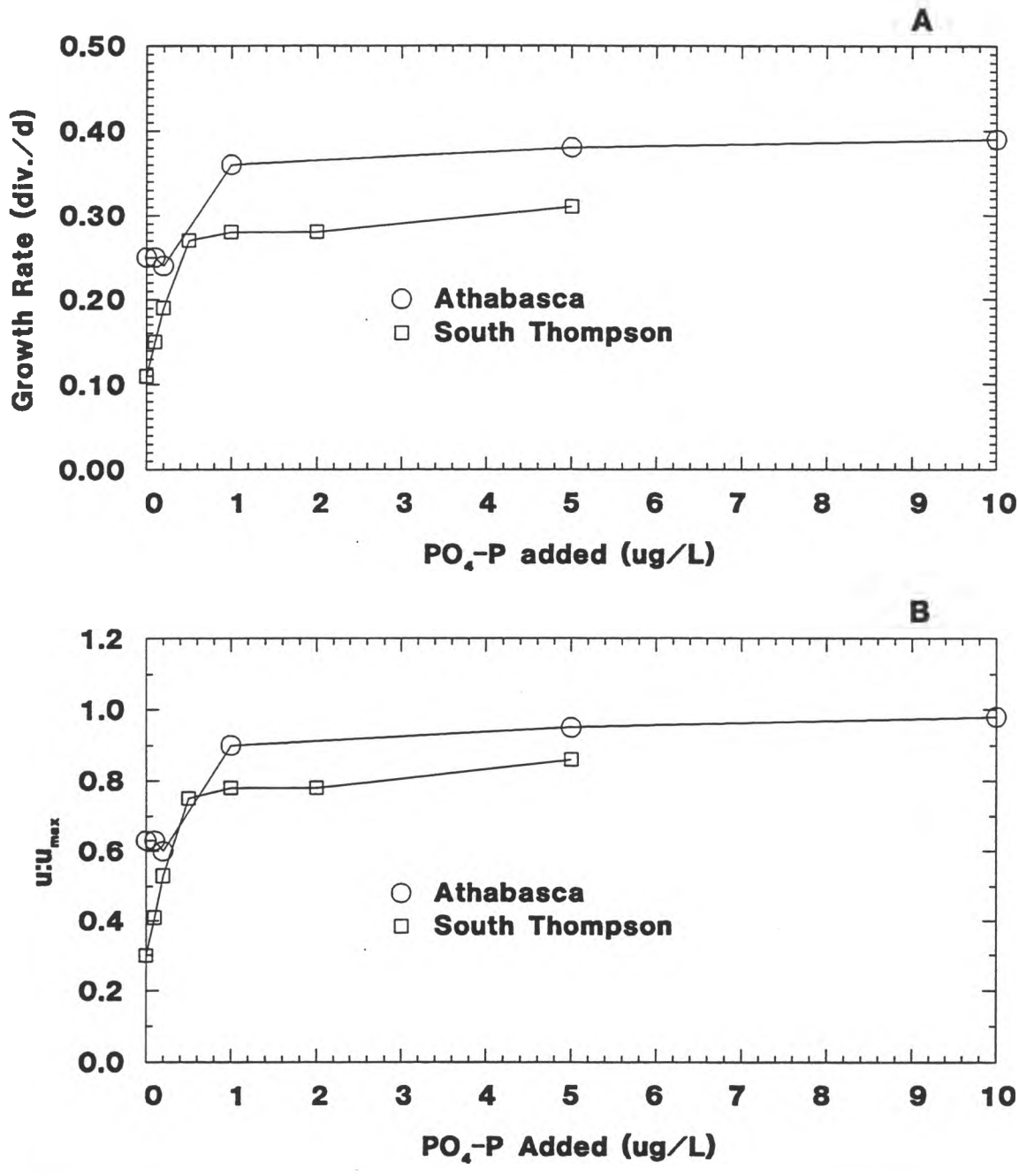

Figure 7. Athabasca and South Thompson River periphyton growth response to P additions up to 10 $\mu g \cdot L^{-1}$. Specific growth rates shown in (A) are normalized to μ_{max} in (B) to remove effects of physical variables. Athabasca μ_{max} was determined at a P addition of 50 $\mu g \cdot L^{-1}$. South Thompson μ_{max} was from an experiment run at 6.4°C reported by Bothwell (1988).

On both the tile and silt substrata, PB followed a curvilinear response to phosphorus addition (Figure 8), thus indicating that it was proportional to community growth rate. A two way analysis of variance was applied to determine if PB differed by substrata type. The two factors were substrata (tile and silt) and P addition (8 levels ranging from the control through 50 $\mu g \cdot L^{-1}$). PB was transformed using $\log_{10}$ to linearize the data. There was a highly significant interaction ($p=0.001$) such that PB on silt was greater than that on tile at concentrations of P added of ≤ 0.2 $\mu g \cdot L^{-1}$ but PB was greater on tile than on silt at P additions ≥ 1 $\mu g \cdot L^{-1}$. In the control trough, PB was almost four times greater on silt than on tile but at such low biomass levels, this difference amounted to only 4 $mg \cdot m^{-2}$ of chlorophyll *a*.

By normalizing PB to PB_{max} we compared the Athabasca and South Thompson River PB data. $PB:PB_{max}$ for both rivers and for silt and tile substrata in the Athabasca are plotted in Figure 9. The curvilinear shape of the relationships again indicate that $PB:PB_{max}$ is proportional to community growth rate in the experiments from both rivers. Slopes of the $PB:PB_{max}$ curves on the different substrata and in the two rivers were compared by ANCOVA where the category term was location or substrata, the dependant variable was $PB:PB_{max}$ and the covariate was the log of ambient SRP concentration. Ambient SRP was the mean background concentration from the control treatment plus the known PO_4-P addition. The P concentration data were $\log_{10}$ transformed to linearize the models and facilitate a slopes comparison. No difference was found between the slopes describing $PB:PB_{max}$ as a function of PO_4-P concentration on the tile and silt substrata ($p=0.923$). Hence, Athabasca $PB:PB_{max}$ data from both substrata were combined for comparison to the South Thompson data reported by Bothwell (1989). The corresponding ANCOVA indicated a barely significant difference between slopes ($p=0.05$). The model for the Athabasca River having the best fit ($r^2=0.93$) and lowest standard error of the estimate ($S_Y=0.096$) was:

$$PB:PB_{max} = 0.075 + 0.536 * \log_{10} P \qquad (1)$$

where P can be approximated by the ambient SRP concentration ($\mu g \cdot L^{-1}$). An important difference between the Athabasca and Thompson curves was that $PB:PB_{max}$ in the Athabasca experiment saturated at a higher PO_4-P concentration than it did in the South Thompson River. Both curves showed most rapid increases in $PB:PB_{max}$ at low PO_4-P concentrations (<10 $\mu g \cdot L^{-1}$). But, the Athabasca curve diverged below the South Thompson curve at 2 $\mu g \cdot L^{-1}$ which corresponded to the added P concentration of about 1 $\mu g \cdot L^{-1}$. At concentrations >1 $\mu g \cdot L^{-1}$, Athabasca $PB:PB_{max}$ was always less than that for the South Thompson River until convergence of the curves at 50 $\mu g \cdot L^{-1}$. This comparison suggests that a given change in relative areal biomass in the Athabasca River requires a higher P concentration than in the South Thompson River. For example, the most rapid increase in biomass accrual in the South Thompson River saturates at a $PB:PB_{max}$ of 0.7 near an ambient PO_4-P concentration of 3 $\mu g \cdot L^{-1}$. This saturation concentration is higher than the 0.5 $\mu g \cdot L^{-1}$ cited by Bothwell (1989) because we added the augmented P concentration to the background SRP concentration. At the same $PB:PB_{max}$ of 0.7 in the Athabasca River, the SRP concentration would be about 14 $\mu g \cdot L^{-1}$.

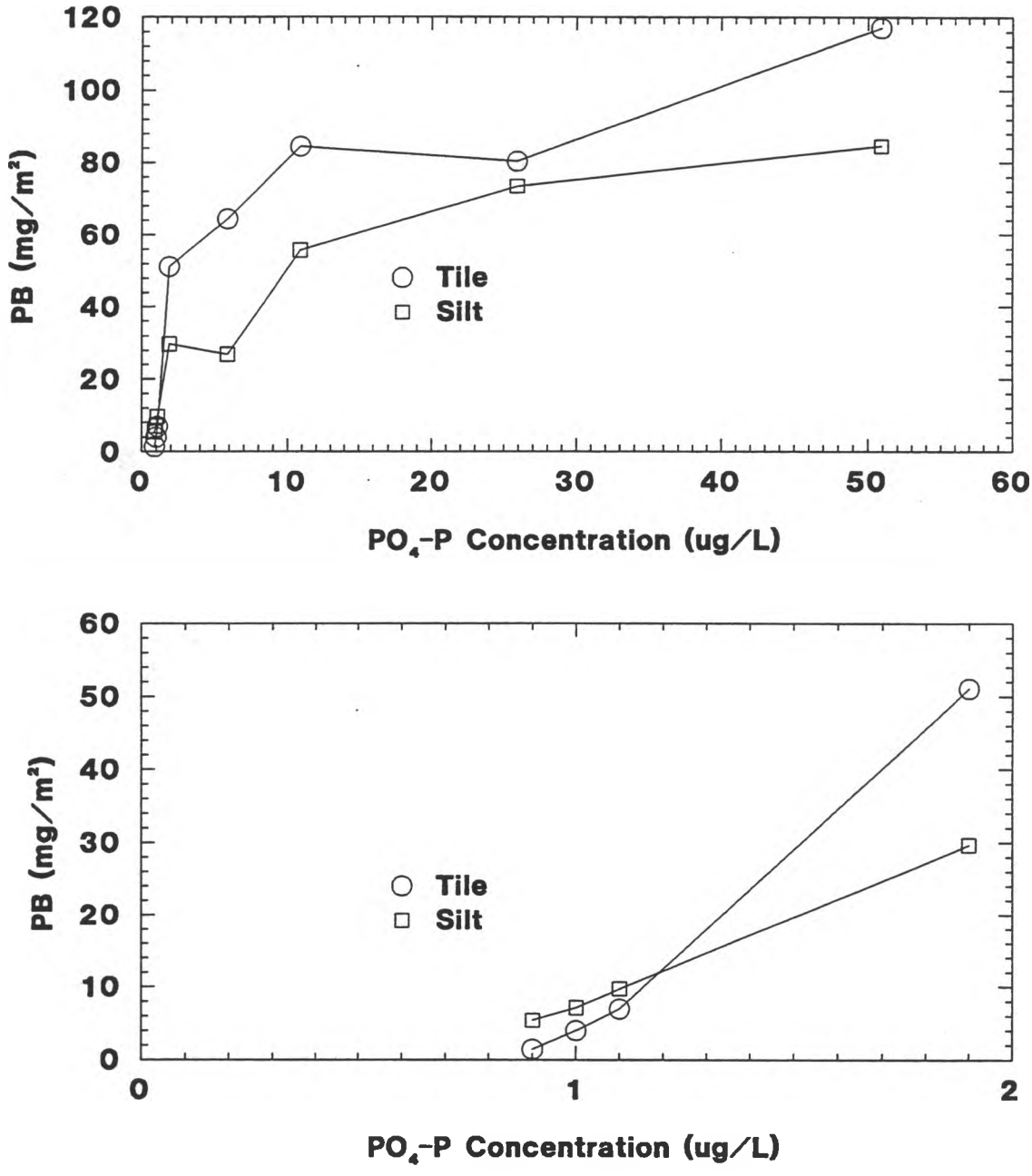

Figure 8. Change in mean PB as a function of ambient PO_4-P concentration on tile and silt substrata in the Athabasca River troughs. Ambient concentration was determined as the unamended SRP concentration (0.9 $\mu g \cdot L^{-1}$; Table 1) plus P added. PB at lowest P additions in (A) are replotted at a higher resolution in (B) to show differences of PB in the control and up to 1 $\mu g \cdot L^{-1}$ of P added (1.9 $\mu g \cdot L^{-1}$ of ambient PO_4-P).

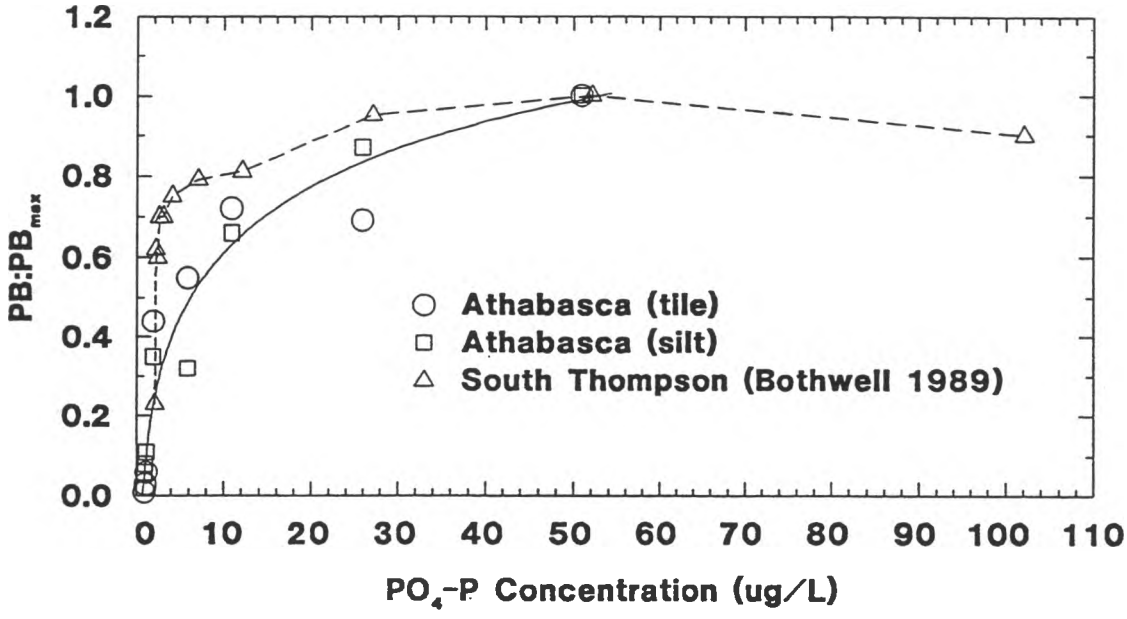

Figure 9. PB:PB$_{max}$ plotted as a function of ambient PO_4-P concentration in the Athabasca and South Thompson Rivers. Because there was no significant difference between responses on tile and silt substrata in the Athabasca (p=0.923), data from both substrata were combined for comparison to the South Thompson River. The difference in response between rivers was barely significant (p=0.05).

5.0 DISCUSSION

Relative specific growth and relative peak biomass have proven to be useful comparative measures for describing periphytic algal responses to phosphorus additions in the Athabasca and Thompson Rivers. Comparison of data from the South Thompson River experiment (Bothwell, 1988, 1989) with our data for the Athabasca experiment, allowed us to validate our findings and test the generality of our predictions of algal growth and biomass response to P enrichment. We hypothesized that relationships between growth or biomass of periphyton and the concentration of added P can be extrapolated between rivers. In this study we have shown that an extrapolation from the Thompson River to the Athabasca can be misleading.

The silt and tile substrata simulated environments for sediment deposition in the Athabasca River at Hinton and selected for different diatom communities (Figure 5). The styrofoam substratum was highly porous and had a high capacity to trap and retain sediment along with entrained algal biomass. Silt accumulated to a depth of 0.5 cm and represented habitat that could be found in river

margin areas and other zones of deposition. The tile substratum was more typical of gravel and cobble found in fast riffle and run flow regimes where the rock surfaces were at least partly exposed above a plane of embeddedness (MacDonald et al., 1991) and had a smaller capacity to retain particles compared to the silt. *Chlorella sp.* never occurred on the silt: it was found to be a contaminant restricted to the tile. *Synedra ulna* was common to both substrata. *Diatoma tenue* and *Achnanthes minutissima* that were common on tile were not able to become established in the relatively unstable silt whereas *Cymbella spp.*, *Hannaea arcus* and *Fragilaria vaucheriae* were able to become established in the silt and become the most abundant taxa. Relatively abundant taxa on one substratum were found as rare taxa on the other. *Nitzschia sp.*, *Navicula sp. Gomphonema sp.*, *Diploneis sp.*, and *Surirella sp.* were rare on both substrata. Thus, there were differences in the affinity of the different diatoms to settle and grow on the two surfaces.

Periphytic algal growth in the Athabasca River is limited by phosphorus (Figure 4) and saturates at P additions between 0.2 and 1 $\mu g \cdot L^{-1}$ (Figure 7). The kinetics of a growth response to P additions were curvilinear in both the Athabasca and South Thompson experiments, but μ in the Athabasca River was consistently greater than that in the South Thompson River for a given concentration of P added (Figure 7). The relative peak biomass response to P additions, which reflects the net effect of growth, further indicated that a given biomass response in the Athabasca required a higher concentration of ambient PO_4-P than in the South Thompson River (Figure 9). Together, these $\mu:\mu_{max}$ and $PB:PB_{max}$ comparisons suggest there are functional differences in the way the respective periphyton communities respond to phosphorus enrichment in the Athabasca and South Thompson Rivers.

Although the diatom communities were similar between this experiment and that in the South Thompson, contamination of the Athabasca tiles by *Chlorella sp.* confounded growth rate comparisons between rivers. For *Chlorella sp.*, the ratio of phosphorus uptake in the light to that in the dark increases greatly with increasing phosphate concentration and can lead to greatly increased growth rates (Healey, 1973). A phosphorus uptake response to P addition can also occur in some diatoms, but not in others (Healey, 1973). The occurrence of *Chlorella sp.* at added SRP concentrations >1 $\mu g \cdot L^{-1}$ suggests that the P concentration required for *Chlorella sp.* to grow at a specific rate is higher than for diatoms in the Athabasca River. Numerical dominance of the diatoms at the lower P concentrations (<1 $\mu g \cdot L^{-1}$ of P added) indicates a competitive advantage at the low P concentrations. This advantage diminishes as the ambient P concentration approached 1 $\mu g \cdot L^{-1}$ and it disappeared above that concentration. At that point, the growth rate of *Chlorella sp.* took off, resulting in an increase in relative abundance. Since we have concluded that *Chlorella sp.* was a contaminant that does not occur in the Athabasca or South Thompson Rivers, its different adaptation to phosphorus augmentation compared to diatoms has confounded an unequivocal comparison of periphyton growth between the two river experiments.

The significant interaction between substrata type and P treatment on PB (Figure 8) can also be explained by the influence of *Chlorella sp.* on chlorophyll measurements. During algal colonization in the first week of the experiment, diatoms would have preferentially seeded with the silt (depositional zone) rather than on the smoother tiles which simulated an erosional zone. Because diatoms are better P-competitors than the *Chlorella sp.* population (Healey, 1973), chlorophyll a

levels associated with diatoms would be expected to be higher on silt than on tile at the low P concentrations ($\leq 0.2\ \mu g \cdot L^{-1}$ added). Silt constantly covering over and mixing with the diatoms on the styrofoam would also cause an increase in the chlorophyll *a*:C ratio in the diatoms due to the shade adaptation response (Jasper and Bothwell, 1986; Perry et al., 1981; Falkowski, 1983). Shade adaptation would be less important on the tile. The competitive advantage of *Chlorella sp* at the P additions $\geq 1\ \mu g \cdot L^{-1}$ on the tile produced chlorophyll *a* concentrations that greatly exceeded those produced even by the shade adapted diatoms on the silt, eventually producing greater chlorophyll *a* concentrations on the tile.

No significant difference was found between tile and sand substrata in the relationship between P concentration and $PB:PB_{max}$ (equation 1) ($p=0.923$). Given the presence of *Chlorella sp* on the tile and its known difference in adaptation to P concentration (Figure 8) compared to the diatoms that were most common on the silt, this result may be fortuitous. Because *Chlorella sp.* does not naturally occur in the Athabasca River, equation 1 cannot be considered representative of a functional process in the river. However, the effect of *Chlorella sp.* can be removed by eliminating the tile $PB:PB_{max}$ data. The modified equation that applies only to diatoms becomes;

$$PB:PB_{max} = 0.069 + 0.54*\log_{10}P \qquad (2)$$

in which the coefficients are only slightly different from those in the original equation (1) and the curve is similar to the combined tile and silt curve (Figure 9). The relationship is highly significant ($p<0.001$), and has a good fit ($r^2=0.95$) to the logarithmic model. The slope of this curve is significantly different from the South Thompson curve (Figure 9) (curve comparison using ANCOVA; $p=0.013$), thus indicating that despite similar community structure (diatoms in the sand also occurred on substrata in the South Thompson experiment), relative peak biomass response to P addition was different between the two rivers. Diatoms on silt substrata in the Athabasca required a higher P concentration than did those in the South Thompson to achieve a given biomass response.

An obvious factor contributing to this difference is the effect of silt and sand scour on PB. In the South Thompson where there was little or no suspended sediment, accrued areal biomass was not affected by scour as it was in the Athabasca experiment. Scouring may tend to reduce the curvilinear nature of a functional response based solely on growth in two ways. The first is to reduce PB_{max} by limiting the extent of mat development and the second is to reduce moderate biomass levels because a larger proportion of cells are exposed to, and could be removed by, moving sediment compared to those forming a mat at PB_{max}. The latter process would tend to flatten out the curve. The high phosphorus concentrations required to saturate a biomass response in the Athabasca suggests that where phosphorus enrichment may produce large and perhaps maximum changes in growth rate of periphytic algae, an actual biomass response can be relatively small. One could speculate that in some cases of extreme naturally occurring suspended sediment concentrations, a biomass response may be indistinguishable from background variation regardless of P concentration.

This comparison between experiments suggests that substrata type can confound change in $PB:PB_{max}$ in relation to P addition. Differences in the adaptation of algal groups to change in P

concentration prevents extrapolation of functional models between systems having different community structure. This study shows that processes that can modify biomass accrual must also be similar between systems to justify extrapolation of algal response models. These criteria prevent models developed in the South Thompson River to be applied to the Athabasca River.

Given the importance of processes that can independently modify biomass accrual, it follows that relationships between P concentration and $PB:PB_{max}$ developed experimentally in the Athabasca River cannot be used to predict *in situ* biomass response to P augmentation. In the troughs, invertebrate grazers were rarely found and they were thought to have little or no effect on PB estimates. *In situ*, however, grazing rates may be much higher and as found elsewhere, may be very effective in cropping available biomass (Jacoby, 1985; Lamberti and Resh, 1983) particularly with nutrient enrichment (Hershey et al., 1988, McCormick and Stevenson, 1991). Current velocity can have a negative effect on biomass by increasing shear stress and reducing colonization (Lohman et al., 1992; Stevenson and Glover, 1993) but it can also have a positive effect by increasing algal metabolism (Whitford and Schumacher, 1964). Although seasonal variation in unshaded irradiance has little influence on algal growth or biomass (Bothwell, 1988), attenuation of light associated with turbidity may reduce PAR sufficiently in the Athabasca to reduce algal growth rates. Increased PAR in heavily shaded streams of the Cascade Mountains has been found to increase diatom community biomass (Lyford and Gregory, 1975). Since the effect of the interaction of these physical and biological factors on areal biomass is not quantitatively predictable from simple mesocosm-scale experiments, actual biomass for a given P concentration cannot be extrapolated from the troughs to the river.

6.0 CONCLUSIONS

This study has unequivocally shown that periphyton growth in the Athabasca River is limited by phosphorus concentration. If we ignore the tile substrata that were contaminated by *Chlorella sp* in the experiment, we have also shown that within an unstable matrix of silt and sand, which is the most common substratum in the Athabasca near Hinton, there is a logarithmic increase in $PB:PB_{max}$ with P addition. This relationship, however, is flatter than that developed elsewhere, which we have attributed to unique effects of silt and sand scouring. There is no obvious saturation of $PB:PB_{max}$ with increasing P concentration up to 50 $\mu g \cdot L^{-1}$ although greatest change occurs at P concentrations ≤ 10 $\mu g \cdot L^{-1}$ (Figure 9 and equation 2). Above 10 $\mu g \cdot L^{-1}$ the change is linear. The differences between this model and that produced for the South Thompson River, for example, show that models of periphyton growth and biomass response to nutrient augmentation developed in one river and based on accrual methodology cannot necessarily be extrapolated to another river. Both Figure 9 and equation 2 only indicate the potential change in $PB:PB_{max}$ as a function of P addition. Factors in the river that were not important in the troughs including insect grazing, variable current velocities, variable turbidity, and others will modify actual biomass responses to P augmentation. In some situations, these factors may prevent any change in biomass from occurring despite phosphorus enrichment. Results in Figure 9 and equation 2 only indicate the potential diatom biomass response to P addition in unstable silt and sand substrata.

The occurrence of *Chlorella sp.* as a contaminant in this experiment has served as a warning that adequate cleaning and sterilization must be part of mesocosm-scale experiments in the field. Authors of this paper have conducted numerous experiments similar to this one and never before encountered confounding by a contaminating organism. Clearly equipment and materials that could be potentially contaminated from other environments must be adequately cleaned and sterilized before use in any subsequent experiment. The importance of this quality control cannot be overemphasized.

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8.0 APPENDICES

8.1 APPENDIX A: TERMS OF REFERENCE

TO EXAMINE PHOSPHORUS-LIMITED GROWTH
OF PERIPHYTON IN THE ATHABASCA RIVER

Submitted to

Northern River Basins Study
c/o National Hydrology Research Institute
Saskatoon, Saskatchewan

by
Limnotek Research and Development Inc.
Vancouver, B.C.

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1.0 INTRODUCTION

As part of the Northern River Basins Study (NRBS), the effect of nutrient addition from pulp mill effluent on periphyton growth and trophic dynamics will be examined in a series of experiments that have been included for funding in 1993-94. Due to the expansion in number of pulp mills on the Athabasca River, most interest centres on whether these mills alter the chemical and biological functioning of the river, both as individual point sources of contaminants and nutrients, and also as a cumulative series of point sources. In 1993-94, recirculating mesocosms will be used to test hypotheses of effects of pulp mill effluent, as a complex chemical mixture, on lotic insect abundance and composition (J. Culp, National Hydrology Research Centre, Saskatoon; pers comm). This proposal outlines a companion study to examine relationships between dissolved nutrient concentrations and periphytic algal growth rates and biomass, with the use of flow-through experimental flumes. Independently and together, these studies will contribute to an understanding of river ecosystem processes affected by pulp mill effluent.

Flow-through flumes have been routinely used to examine functional relationships between algal growth and concentrations of nutrients that limit growth. The apparatus allows for control of water flow across replicated substrata that will support periphyton growth. With several flumes operating concurrently, chemical solutions can be applied in a wide variety of experimental designs to test hypotheses of change in ecological and physiological processes associated with chemical manipulation. The main feature of any periphyton flume is removal of environmental variation to quantify treatment effects. Peterson et al (1983) used the technique to demonstrate phosphorus limitation in the Kuparak River, Alaska. Perrin (1991b) modified the Peterson apparatus to show co-limitation by N and P in the Nechako River, British Columbia and a similar apparatus was used to demonstrate N-deficiency in periphyton of streams in the Queen Charlotte Islands (Perrin 1988). An apparatus established on the bank of the Columbia River showed P-limitation (Perrin 1991a). Perrin and Bothwell (1992) used flumes to show no effect of chlorate discharges from pulp mills on periphyton growth and species composition. Perhaps best known are a series of experiments run by Bothwell (1985, 1988, 1989) on the Thompson River in which P-limitation was demonstrated (Bothwell 1985), temperature was found to secondarily limit periphyton growth and irradiance was found to be of less importance (Bothwell 1988) and models of periphyton growth response to changes in ambient P concentrations were developed (Bothwell 1989). Following from this work, Perrin (1991b) found that periphyton response to nutrient augmentation in flumes could be described by kinetics that are consistent regardless of whether the nutrient that limits growth is N or P. All of these studies have demonstrated unequivocal relationships between a chemical stressor and periphyton response and the data have been accepted for setting environmental policy more than any other technique used to examine effects of chemical manipulation in river communities.

Since flume studies provide for the control of environmental variables, relationships and models produced from them should be applicable to remote sites, given that the composition of the periphyton community and chemical conditions are similar between sites. This extrapolation of data should be possible because flow, substrata texture, light, and temperature, all of which are physical factors that influence periphyton growth are controlled or they are held constant across all flumes. This control should allow any response in some flumes but not others to be attributed only to the treatment that is applied, whether it be a nutrient or contaminant. Hence, a relative change in functional response measured in the flumes should be the same relative change that can be expected in the river due specifically to the nutrient or contaminant that is tested. Absolute levels of biomass that result from a growth response are unlikely to be similar between flumes and natural river substrata, because the ambient environmental factors are not the same in the river as they are in the flumes. The important result from a water management point of view, is that the causal relationship that is established in the flumes should be applied to quantify cause and effect models of ecosystem function both for the site where they were established and at other sites that have similar periphyton communities and similar chemical conditions to the test site.

If this extrapolation is accepted, it will be possible to apply all of the concepts developed by Bothwell and other researchers using similar techniques to questions of periphyton response to nutrient manipulation in the Athabasca River using ambient data describing periphyton community structure and SRP concentrations, given that the taxonomic composition of the Athabasca periphyton is similar to that from the experimental sites. We understand that the periphyton community in the upper Athabasca is mainly comprised of diatoms, as it is the Thompson River where models and relationships between P and algal growth were developed (Bothwell 1989). We also understand that water chemistry data indicate that N:P supply ratios are very high and that soluble reactive phosphorus (SRP) concentrations in the Athabasca are as low as those found in the Thompson River, thus implying P-deficient growth of diatoms in the Athabasca. These similarities between the Athabasca and Thompson Rivers suggest that models developed for the Thompson River could be applied to the Athabasca.

There are three general questions of periphyton response to nutrient augmentation in the Athabasca River that are required for objectives of the NRBS. The first is to determine if P limits periphyton growth, as the water chemistry data imply. The second is to quantify the periphyton response to a gradient of additions of the limiting nutrient, thus producing a model that will predict periphyton growth at a given concentration of a limiting nutrient. The third question is to determine how that model changes with seasonal variation in light and temperature. Since each of these questions have been dealt with by Bothwell in the Thompson River work, this project will test the hypothesis that models from the Thompson River can be extrapolated to the Athabasca. In this first study of nutrient-periphyton interactions in the Athabasca there are four specific objectives:

1. To determine if N or P limits the growth of periphytic algae in the upper Athabasca River.

2. To quantify the model describing change in periphytic algal growth as a function of addition of a limiting nutrient.

3. To determine how the model changes between late summer and winter irradiance levels and temperatures.

4. To determine if kinetics of periphyton growth measured in flumes on the Athabasca are similar to those found from experiments run on the Thompson River.

2.0 WORK PLAN

2.1 Experimental Design

Two experiments will be completed on the Athabasca to meet these objectives. In each experiment, periphyton growth, biomass, and taxonomic response will be tested over a gradient of additions of the limiting nutrient. The first experiment will be run in September, 1993 and a duplicate experiment will be run in January, 1994 to examine responses at relatively low temperature and light conditions. We will assume at this point that phosphorus limits growth, thus a P gradient will be tested. Ambient inorganic N (NH_4^+ and NO_3^-) and P (SRP) concentrations are 80 and <1 $\mu g \cdot L^{-1}$ respectively (P. Chambers, pers comm.) and the molar supply ratio is in excess of 3000 (Table 1). A gradient of P additions will cover the range of concentrations tested in the Thompson River studies to facilitate direct comparison of growth and biomass curves (Table 1). Throughout the phosphorus gradient, N will not be added since it should be in surplus for periphyton growth in September and January. If water samples collected at the time of flume installation indicate lower N concentrations, N augmentation may be included in all treatments to ensure P deficiency. The classic nutrient bioassay (control, N, P, and N+P additions) to determine which nutrient limits growth will also be included in the experiment. N and P will each be added at 100 $\mu g \cdot L^{-1}$ in this test.

Table 1. Layout of treatments across 12 flumes. Molar ratios are determined with the ambient inorganic N (NH_4^+ and NO_3^-) concentration of 80 $\mu g \cdot L^{-1}$ and an SRP concentration of 0.05 $\mu g \cdot L^{-1}$.

Chemical Added ($\mu g \cdot L^{-1}$)	Trough number											
	1	2	3	4	5	6	7	8	9	10	11	12
KH_2PO_4-P	0.1	0.2	0.5	1	5	10	25	50	100	0	0	100
$NaNO_3$-N	0	0	0	0	0	0	0	0	100	0	100	0
Molar ratio (N:P)	1181	709	322	169	35	18	7	4	4	3543	7971	2

2.2 Field Measurements

Periphyton growth rate (cell divisions per day (μ)), relative specific growth rate (μ relative to a maximum at surplus ambient P concentration ($\mu:\mu_{max}$)), biomass (chlorophyll *a*), peak biomass (maximum mean biomass), and relative peak biomass (mean chlorophyll *a* concentration relative to maximum measured at surplus ambient P concentration) as defined by Bothwell (1989) will be used to measure effects of treatment on the general growth response. Curves will be fitted to describe changes in growth due to the N and P additions. If available, power analyses will be used to determine the probability of finding no difference between treatment levels when one might exist.

The abundance of algal taxa will also be determined at the end of the treatment period. If the relative abundance of dominant taxa does not change significantly over the P gradient, the experiment would indicate no taxa-specific response to P additions. If significant changes in relative abundance are found and a growth response is also found, the data would indicate alteration of the periphyton community associated with the nutrient additions.

2.3 Data Analysis

Several response curves (growth versus nutrient concentration) will be plotted and equations derived to produce models of growth at given nutrient concentrations. The independent functional responses to N and P additions will likely have large differences in which case further statistical approaches are not necessary to draw conclusions of relative N and P deficiency. More subtle differences between curves will be distinguished by examining differences between slopes of lines using analysis of

covariance (ANCOVAR) where "day" will be the covariate, treatment will be the category, and chlorophyll *a* concentration will be the dependant variable. ANCOVAR will also be used to compare curves describing the gradient of P additions in a single experiment, between the summer and winter experiments and to compare similar treatments between the Thompson River and Athabasca data. In these latter analyses, "location" will be the category but "day" will remain the covariate. All logarithmic curves will be transformed to linear functions to accommodate the ANCOVAR's. To correct for environmental variation between rivers, relative biomass and growth rate (measured value relative to the maximum possible at a given temperature) will also be considered for comparison of independent data sets. If the ANCOVAR's indicate no significant difference between curves from the Thompson and Athabasca, we can conclude that the kinetics of phosphorus-limited growth is similar between rivers and that findings from the Thompson River work may be extrapolated to the Athabasca. Significant differences between curves will suggest functional differences to be resolved with additional experimentation.

2.4 Study Location

Since we want to produce models of periphyton response which include very low concentrations of the limiting nutrient, the experiment will be run using water not affected by nutrient discharge from upstream sources. Based on this criteria, a survey by Dr. P. Chambers (NHRI) has resulted in the selection of the Weldwood pulpmill at Hinton as the study site. Water will be supplied using a branch line from the existing mill intake. The specific flume location will be away from fly ash and other airborne contaminants that may affect water chemistry in the flumes.

The first experiment will be run in September as this is when spring and summer turbidity is relatively low but temperatures are near the annual maximum. Optimum growth rates and largest differences in growth between treatments will be measured at this time. The second experiment will be run in January or when extremely cold temperatures (near -40°C) can be avoided for experiment start-up.

2.5 Trough Design and Operation

The flume apparatus consists of 12 flow-through troughs (2 m long and 19 cm wide) fabricated from plexiglass and assembled in a parallel layout similar in design to that used on the Thompson River (Bothwell 1983) but modified as a mobile facility for use at remote sites. The equipment was designed and fabricated by LIMNOTEK as part of an earlier contract with NRBS. Water and biota carried in suspension in the river will supplied to the troughs via a head tank system. Chemical solutions will be stored in glass carboys which will be placed near the head tank structure.

The apparatus will be assembled at the Weldwood mill and all systems tested in August, 1993. The assembly will require installation of the head tank and flumes on an aluminum and wood platform, the installation of a water supply pump and pipeline, power to the site from the mill, a chemical delivery pump, flume substrata, and complete systems testing. The experiment will start on September 6 with the installation of a sheet of open-celled styrofoam-DB (Snow Foam Inc. El Monte, California) in the bottom of each trough to provide a substratum for microbial colonization. Water flows will be set at 0.8 $L \cdot s^{-1}$ and stock chemical solutions shown in Table 1 will be metered into assigned treatment troughs using a Technicon® peristaltic pump.

2.6 Sampling and Response Indices

Algal biomass will be sampled by removing triplicate cores of the styrofoam 3 times per week in the first two weeks and 2 times in the following week. The cores will be frozen immediately after sampling and shipped to University of Alberta for analysis of chlorophyll *a* concentrations using fluorometric procedures. Growth rate and biomass indices will be determined according to methods outlined by Bothwell (1989). Visible invertebrates will be routinely removed from the troughs to limit any influence of grazing pressure. The sampling will continue for three weeks, the duration usually required before sloughing begins to interfere with growth rate measurements. Near the end of the experiment, one additional core will be extracted and preserved in Lugol's solution for algal enumeration in Vancouver. Another core will be removed and frozen for later analysis of alkaline phosphatase activity (APA). APA will be determined at NHRI, Saskatoon.

A water sample will be collected from each flume three times during the experiment. The samples will be filtered in the field and immediately shipped to the University of Alberta for analysis of ammonium-N, nitrate plus nitrite-N, soluble reactive phosphorus (SRP), and total dissolved P (TDP) concentrations.

2.7 Reporting

A draft and final report formatted to NRBS specifications will be submitted. We understand that the requirements are for a Times Roman font and that the reports are to include an executive summary, background review, a clear statement of objectives, methods, results, and interpretation of the findings. This format is always followed in LIMNOTEK reports and we do not expect much variation from our usual report production procedure.

The draft report will be produced as if it were a final and it will include a full technical description of the results including data analyses and interpretations from both experiments. It will also

include a review of relevant literature that will assist in interpreting the findings. The draft will be formatted to allow the insertion of reviewers comments.

The final report will be an edited version of the draft and will be the manuscript used for publication as a NRBS document.

All statistics and graphics will be run using SYSTAT and SYGRAPH version 5.1 software and reports and manuscripts will be prepared using WORDPERFECT 5.1.

Schematic drawings and other technical drafting will be provided by Limnotek graphics personnel.

Ongoing progress of project activities will be communicated by telephone and fax to the scientific authority at least monthly. Communications will be weekly when the experiments are in progress and on other dates deemed necessary by the project manager.

3.0 MANAGEMENT PLAN

3.1 Corporate Responsibilities

LIMNOTEK will assume full responsibility for the management and operation of the project. LIMNOTEK will supply the project manager and field crew, provide any equipment not available at the research site, and implement the logistical arrangements necessary to complete the work to our usual high standards. LIMNOTEK will supply the draft and final reports to complete contractual requirements with the NRBS.

3.2 Equipment and Materials

The experimental trough facility will be assembled at the Weldwood mill at Hinton. The equipment includes a head tank, flumes, support tables, and all internal plumbing. A water supply pump and associated plumbing plus waste discharge plumbing is not included with the apparatus and must be purchased separately. We understand that Dr. Patricia Chambers (NHRI) will cover the costs of this water

supply and waste plumbing and those costs will not be included in this contract. Other materials including sampling utensils, vials, chemicals, coolers for shipping samples, styrofoam substratum for the flumes, and other miscellaneous materials will be supplied by LIMNOTEK either at direct cost (for materials that cannot be reused) or for a usage fee (hardware that is part of LIMNOTEK inventory).

3.3 Task Scheduling

Detailed scheduling of project tasks is shown in Table 2. The study site will be prepared in August and plumbing will be installed in the first week of September. The first experiment will begin on September 10 and continue for three weeks. Lab results will be received over the following month after which time data analyses will proceed. Weather permitting, the second experiment is scheduled to start in February and again run for three weeks. After all lab and data analyses are completed, the draft report will be produced for submission on or before March 31, 1994. The final report will be submitted after review by the scientific authority and other members of the NRBS. With submission of the final report, our contract with the NRBS will be completed. Since the winter experiment is scheduled to begin late in the 1994 fiscal year, submission of the final report is not expected until early in the 1995 fiscal year. We understand that this timing is acceptable both in terms of scheduling and invoice processing.

3.4 Team Organization and Responsibilities

LIMNOTEK will assign the company President, one field biologist, and support staff to this project. Company President and Senior Systems Ecologist, Chris Perrin will assume overall responsibility for project management, adminstration, data analysis, and reporting. Chris will direct all activities from Vancouver and will be in field on three trips:

- August 3-6 for initial site review and preparation.
- September 7-10 for trough installation and experiment start-up.
- January 24-26 for start-up of the second experiment.

When not in the field, Chris Perrin will be in constant communication with the field staff and the scientific authority by telephone. Chris will run the data analyses and will be the senior author of the final report. All technical and administrative communications with the scientific authority and the NRBS will be from Chris Perrin.

Kelly Field will responsible for maintaining field operations. Kelly has recently completed his MSc. in limnology and is fully aware of the field requirements. His main tasks will be to ensure that the chemical metering proceeds uninterrupted, to routinely inspect and make adjustments to the water supply and discharge systems, to inspect and remove invertebrates from the flumes, prepare chemical stock solutions, and to maintain all sampling activities under the direction of Chris Perrin. Both Chris Perrin and Kelly Field will assemble the flume apparatus with assistance provided by staff from the NHRI. Kelly Field will report directly to Mr. Perrin.

At the time of equipment installation, another flume apparatus that is required for a study of invertebrate response to pulp mill effluent exposure will be installed. The study will be managed by Dr. J. Culp of NHRI. LIMNOTEK fabricated much of the apparatus that will be used in that project as part of an earlier contract with the NRBS and thus, will be required to provide assistance for its assembly in the field. Since that equipment will also be installed at Hinton, Chris Perrin will supervise both the periphyton and invertebrate flume installations at the same time. We understand that at least two other people from NHRI will be at the site to assist.

To complete the winter experiment, a cold weather shelter complete with heating to prevent water freezing will be required for placement over the head tank, plumbing network, and working area. We understand that this facility will be purchased, installed, and tested by NHRI, ready for the February start-up.

3.5 Manday Allocations

Hourly requirements for all project tasks are shown in Table 2. Administration, client liaison, meetings, etc. has been restricted to 16 hours for Chris Perrin. Approximately four days has been assigned to Chris Perrin and Kelly Field for site preparation in August. An additional five days is provided for flume installation and starting the first experiment in September. A total of 20 hours is assigned for each of Chris Perrin and Kelly Field for start-up of the second experiment. At this time, we will work with NHRI technicians who will be completing the installation of the cold weather shelter. A total of 50 hours have been assigned for Kelly Field to maintain the flumes and complete all sampling over each three week experiment. An additional 14 hours and 16 hours has been allocated at the end of the summer and winter experiment, respectively, for clean up and dewatering (after summer experiment) or dismantling the apparatus (winter experiment). We expect that assistance will be available from NHRI for some of this work since the equipment will likely have to be stored at NHRI facilities. Chris Perrin has been assigned 16 hours for analyses of data from the first experiment. After completion of the second experiment, Chris has been assigned 21 hours for data analyses and 36 hours for completing the draft

report. An additional 36 hours are allocated for completion of the final report. With these time allocations, a total of 445 hours or 59 person days have been allocated to the project.

To assist with installation of the insect experimental flumes, an additional 24 and 48 hours for Chris Perrin and Kelly Field respectively have been assigned for a total of 72 hours.