# PILOT FERTILIZATION OF THE NECHAKO RIVER: A TEST OF NUTRIENT DEFICIENCY AND PERIPHYTON RESPONSE TO NUTRIENT ADDITION

*NECHAKO FISHERIES CONSERVATION PROGRAM*

*Technical Report No. RM88-3*

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## ABSTRACT

Two experiments were conducted in the upper Nechako River to examine relationships between nutrient concentrations and periphyton production and establish chemical criteria for fertilization of the upper Nechako River for conservation of chinook salmon. A side channel was fertilized with a N-P-K blend of 34-0-0 and 12-51-0 resulting in predicted concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) of 40 $\mu g \cdot L^{-1}$ and 10 $\mu g \cdot L^{-1}$ respectively. Actual concentrations were highly variable due to the pulsing nature of nutrient input from the mechanical dispensers and plume dynamics which affected the dispersal of fertilizer across the channel width profile. Elevated N and P concentrations that were detectable up to 3.4 km downstream of the side channel were also related to incomplete mixing of the side channel and mainstem water. The addition of N and P resulted in a shift from a diatom-dominated periphyton community to one having equal proportions of diatoms and chlorophytes. The fertilizer addition increased peak periphyton biomass (PB) (the highest concentration measured) and sustainable biomass (SB) (the highest average concentration) in the side channel to 218 $mg \cdot m^{2}$ and 120 $mg \cdot m^{-2}$ of chlorophyll-a respectively; a 10 times increase over background levels. There was a corresponding increase in the rate of accrual (k) from 0.088 in the control to 0.44 in the fertilized reach. A concurrent bioassay experiment involving manipulation of nutrient concentrations in flow-through chambers established that periphyton production in the upper Nechako River was primarily limited by nitrogen but that phosphorus was co-limiting. By examining responses to various levels of nutrient addition, N and P-limited accrual saturated at not more than 20 $\mu g \cdot L^{-1}$ and 5 $\mu g \cdot L^{-1}$ respectively. These concentrations were also important in limiting taxonomic shifts and were identified as being acceptable as target concentrations for future fertiliza-


## INTRODUCTION

In September, 1987, a settlement agreement between Alcan, the Federal Government represented by the Minister of Fisheries and Oceans and the Provincial Government represented by the Minister of Energy, Mines and Petroleum Resources provided a framework to manage flows in the Nechako River (Settlement Agreement 1987). The agreement allows Alcan to further divert water from the Nechako River with the Kemano Complection Project. The 1987 agreement also provides for a program of protection of the commercially important salmonid stocks (Nechako Fisheries Conservation Program: NFCP) that presently use the Nechako River as a migratory route, as well as for spawning and rearing.

An important component of the NFCP is a requirement for Alcan to construct a cold water release facility at the Kenney Dam. The cold water will be released during the spawning migration of sockeye salmon (*Oncorhynchus nerka*) that begins in mid-July of each year.

Technical questions related to confounding effects of the altered temperature and flow regime on salmonid species were recognized during negotiation of the Settlement Agreement (1987). The more important of these were questions of strategies to reduce the risk of potential effects on spawning and rearing habitat of the Nechako River chinook (*Oncorhynchus tshawytscha*): reduced fry growth rates due to lowering of temperatures in mid-July and loss of rearing and spawning habitat due to additional water diversion. To resolve these questions and "develop a program of measures and plan of implementation which will provide an acceptable level of certainty for the conservation and protection of the chinook fisheries resource of the Nechako River", a working group involving specialists from Alcan, and the Federal and Provincial governments (Strangway 1987) recommended a three-stage process that primarily involved habitat modifications and flow design changes to conserve chinook. This process and ongoing operation of the NFCP is being implemented by the "Technical Committee" which was established from recommendations of the working group (Strangway

1987). The emphasis and priority of the program and activities by the Technical Committee is maintenance of the wild chinook stock of the Nechako River.

As part of stage 1 in the Strangway report, fertilization of the upper Nechako River with inorganic fertilizer was identified as an important strategy to be tested early in the program. The recommendation was based on evidence of successful enhancement of steelhead (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*) by inorganic fertilizer additions to the Keogh River, Vancouver Island (Perrin et al. 1987, Slaney et al. 1986). In addition, McFadden and Cooper (1962) showed that the standing crop of salmonids in streams can be directly related to the concentration of dissolved solids. Huntsman (1948) showed that nutrient loading can affect the mean weights of salmonids. In more recent literature, low nutrient concentrations have been shown to limit trophic production in lotic and lentic ecosystems (Bothwell 1985, Hyatt and Stockner 1985, Peterson et al. 1985, Mills 1985). Many of these studies report increased growth rates of fish with additions of nitrogen and phosphorus. While increased growth rates alone are not evidence of increased production of salmonids (salmonid production expressed as smolt yield and adult returns at maturity are of greatest importance), it is generally recognized that higher juvenile growth rates produce larger smolts that can have greater marine survival and produce less variability in adult returns than smaller smolts (Ricker 1962, Peterman 1982, Bilton et al. 1982).

Although fertilization can increase the production of some salmonids, the effects on production of chinook has not been previously examined. This report addresses the first question associated with examining effects of fertilization on chinook production: what are the quantitative relationships between nutrient concentration and periphyton production in the upper Nechako River? Two experiments were conducted in May through July, 1988. The first involved *in situ* fertilization of a side channel to examine ambient changes in periphyton accrual and species composition that can be expected during fertilization trials. The second experiment was a bioassay designed to identify the nutrient(s) limiting autotrophic production in the upper Nechako River and to examine periphyton responses to a wide range of N and P concentrations. The data were important in selecting target concentrations of N and P that could be applied to future fertilization trials in which changes in growth rates of juvenile chinook in relation to fertilization of the Nechako River would be measured.

# METHODS

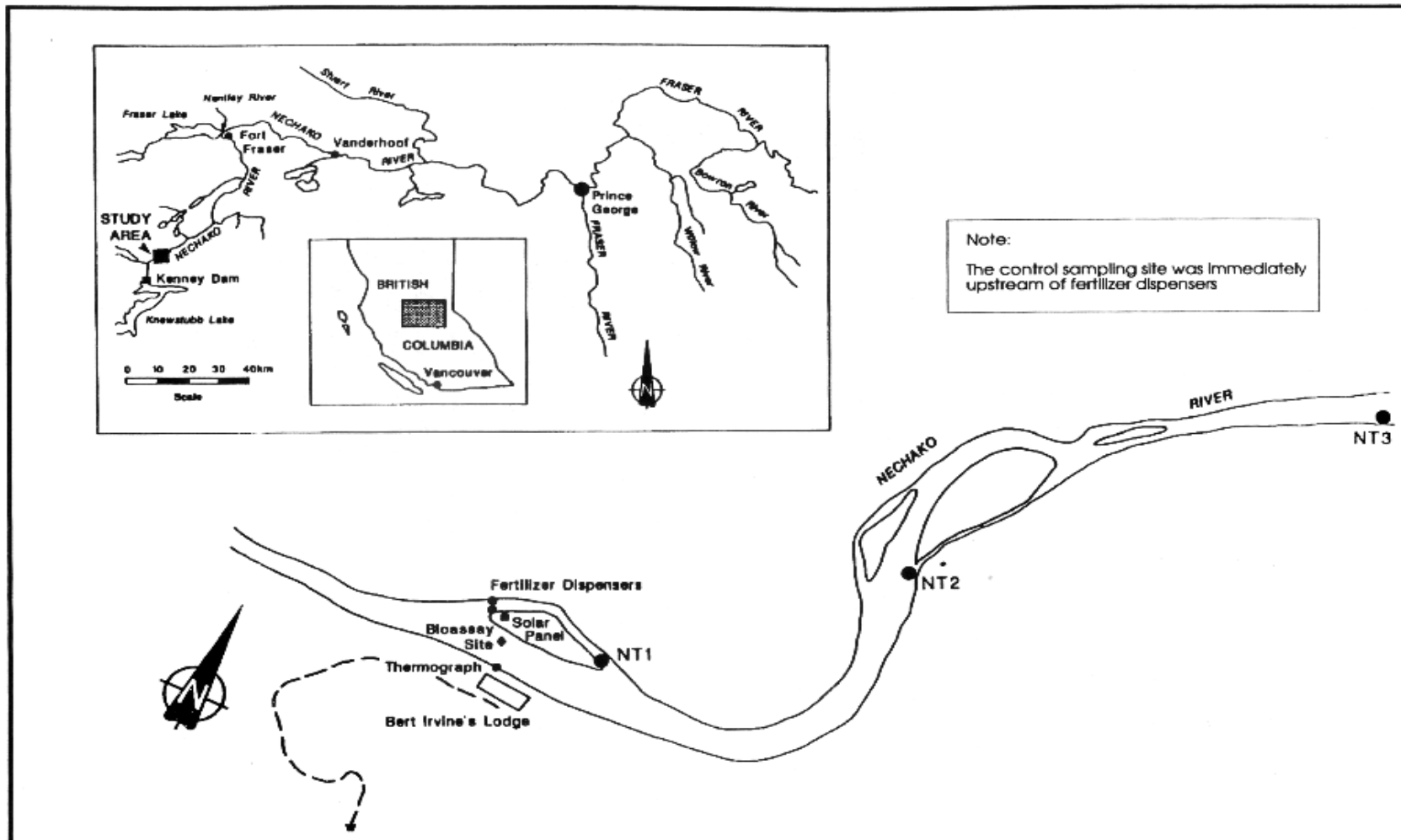
## Study Site

All field work for this project was conducted in the Nechako River near Bert Irvine's Lodge, located approximately 20 km downstream of the Kenney Dam (Figure 1). The side channel immediately across from Bert Irvine's Lodge was the site for *in situ* fertilization. It was 300 m in length, had a average wetted width of 31.5 m and a mean depth of 0.37 m. During the course of the study, mid-channel depths ranged from 0.48 to 0.70 m, current velocities ranged from about 0.4 $cm \cdot s^{-1}$ in the margins to 0.8 $cm \cdot s^{-1}$ in mid-channel, and flows averaged 6.11 $m^{3} \cdot s^{-1}$ (about 10% of the mainstem flow). One tributary stream that was estimated to be flowing at <0.1 $m^{3} \cdot s^{-1}$ entered the channel at the midpoint. The stream was accessible to fry and had extensive cover from grasses and other riparian macrophytes.

The side channel was dominated by a riffle that graded into glide habitat having a mixed gravel (2 - 64 mm) and cobble (64 - 250 mm)[1] substratum. At the downstream end, the channel widened into shallow pool habitat which acted as a deposition area for detritus and other organic matter from upstream. Structures such as log jams, undercut banks, overstream and instream macrophytes, boulders, and other habitat complexes where juvenile salmonids may find refuge (Nechako River Project 1987) were absent from the side channel.

Occasional float surveys during the study revealed marked temporal changes in the presence of chinook fry. Fry were present mostly in margins of the channel from the beginning of the study until mid-June. Although fry counts were not done, the casual observations suggested numbers increased from June 5 through 10, particularly near the inlet stream. Thereafter, numbers dropped markedly and by June 15, fry

[1] Particle size criteria are taken from the Wentworth Particle Size Scale as described by Bovee and Cochnauer (1977).




Nechako Fisheries Conservation Program | Map # RM88-3-1

**FIGURE 1. LOCATIONS OF SAMPLING SITES AND THE BIOASSAY APPARATUS**

were absent.

Access to the site for sampling and equipment maintenance was by jet boat launched from Bert Irvine's Lodge.

## Physical Data

Continuous temperature and side channel flow measurements were collected from April through July 10, 1988.

Temperature data were accessed from files of the Department of Fisheries and Oceans (DFO). The DFO recording facility is located at Bert Irvine's Lodge on the south shore opposite the side channel (Figure 1). The primary instrumentation includes a thermistor probe that is linked to the Water Survey of Canada data collection platform (DCP). Hourly measurements are downloaded to a computerized data base via satellite. Transmission problems were encountered during the course of this study resulting in incomplete temperature records. Backup data were collected from a Bristol mechanical thermograph located at the same site.

Flow measurements of the side channel were determined by rating daily staff gauge readings to a flow calibration curve. The staff gauge was installed at the upstream end of the side channel in slack water near the channel margin. Since surface turbulence was minimal at this location, the installation of a well for water level measurements was considered unnecessary. Flow calibrations were performed on April 21, 1988 and June 26, 1988. A regression equation based on these two points was developed to relate calibrated flows to gauge height. Since only two measurements were made, the rating of gauge height to flow has very low precision and must be viewed with caution. This is particulary relevant when gauge readings were less than or greater than the range that was included in the regression relationship since real flows may not conform to the linearity of model predictions.

## Fertilizer Application

A fertilizer blend of 34-0-0 and 12-51-0 (as N:P:K) was introduced to the side channel at a rate to achieve final concentrations of 40 $\mu g \cdot L^{-1}$ of dissolved inorganic N ($NO_3$ + $NO_2$ + $NH_3$ + $NH_4$-N) and 10 $\mu g \cdot L^{-1}$ of $PO_4$-P at full mixing. Fertilization started on May 6, 1988 and was terminated on July 10, 1988.

The fertilizer was dispensed from two Tessomat high capacity feeders (Aquatess Canada Ltd., Vancouver, B.C.) equipped with spreaders to distribute the fertilizer over a 20 m width of channel. The feeders were suspended from welded aluminum A-frame supports that were anchored to the river bottom with rebar. Power was supplied from a 12 volt, 104 amp hour battery that was continuously recharged from a Genesis G-100 solar panel (Arco Solar, U.S.A.). A Skretting Datess 100 control unit (Modus Elektronic AB, Sweden) was programmed to turn the feeders on for a specified duration (usually a few seconds) every 30 minutes, 24 hours per day. The feeder apparatus required regular adjustments to maintain the required output.

## *In Situ* Sampling

The effects of fertilizer addition to the side channel was measured as changes in dissolved nutrient concentrations, accrual of periphyton biomass, taxonomic composition of the periphyton community, and dissolved oxygen concentrations in spawning gravel. Treatment effects were determined by spatial comparisons of replicate samples collected at a control site located upstream of the fertilizer dispensers and one or more sites located downstream. Since measurements of variables were replicated within the control and treatment areas, not among replicate reaches, conclusions were based on pseudoreplication criteria. Although the use of pseudoreplication in experimental design is controversial, it remains widely used in the primary literature and accepted in field experiments where logistical constraints offer no other alternative.

Water samples were collected weekly from the control site located 50 m upstream of the dispensers and from one treatment site (NT1) located at the downstream end of the side channel, 300 m from the fertilizer dispensers. Biweekly samples were collected at two additional sites on the Nechako River mainstem located 1.5 km (NT2) and 3.4 km (NT3) downstream of the dispensers (Figure 1). All water samples were filtered in the field and shipped to Cantest Laboratories in Vancouver on the day of collection for analysis of $NO_3$ + $NO_2$-N, $NH_3$ + $NH_4$-N, soluble reactive phosphorus (SRP), and dissolved phosphorus (DP) concentrations. The sum of the concentrations of nitrate plus nitrite plus ionized and unionized ammonia represented dissolved inorganic nitrogen (DIN). All analyses were performed according to

techniques described in B.C.M.O.E. (1976) and APHA (1985). In this report, the $NO_3 + NO_2$ combination will be described as $NO_3$ and $NH_3 + NH_4$ will be simplified to $NH_4$.

Dissolved oxygen concentrations were measured at the end of treatment on July 10, 1988 with a YSI model 57 oxygen meter that was calibrated on the day of use. At 5 random sites in each of the control and treatment reaches both surface water and intergravel water at a depth of 15 cm were measured. Surface water was collected as grab-samples in a 1000 mL glass Erlenmeyer flask. Intergravel water was extracted from stainless steel tubing that was placed at the 15 cm depth. Water was drawn into a 1000 mL Erlenmeyer flask for the DO measurements with the use of a hand pump, without creating turbulence.

Changes in periphyton biomass accumulation in control and treatment reaches were measured using the accrual technique reported by Perrin et al. (1987). Three replicate styrofoam-DB (Snowfoam Inc., El Monte, California) substrata were cut into 30cm x 30cm sheets, attached to concrete anchors and installed at each site in random locations. The styrofoam sheet provided a uniform surface for comparison of the net accrual (settlement plus growth less sloughing plus grazing) of periphyton biomass between sites. Samples of periphyton biomass were collected every 3 days over a 21 day time series by removing replicate cores from the styrofoam using the open end of a 10 dram plastic vial. Two time series of periphyton accrual were sampled: series 1; May 13 to June 10 and series 2; June 17 to July 8. For each series, differences in biomass accrual between sites was examined using a two way fixed effect analysis of variance of log transformed chlorophyll-a concentrations where time and location factors were blocked into 7 and 2 levels, respectively.

Curves that describe accrual of periphyton on substrata can usually be separated into three phases. In the first 3 to 5 days, the substrata are colonized and accrual is dominated by passive settlement, not growth (Bothwell and Jasper 1983). The second is a 10 to 17 day period of exponential increase in chlorophyll-a concentrations due mainly to growth of the attached community. In the third and final phase which occurs beyond the 17th day, concentrations decline or become highly variable due to biomass sloughing. In this project, an index of the rate of growth (k) was estimated according to methods reported by Guillard (1973). Rates were expressed in units of cell divisions per day ($div \cdot d^{-1}$) using data from the period of exponential accrual. It is important to recognize that although growth may dominate during this period, the data actually reflected the net accrual of growth plus settlement less sloughing and grazing. Hence k is more properly regarded as an index of growth.

Indices of biomass accumulation were also determined from the accrual curves. Peak biomass (PB) was the highest average concentration of chlorophyll-a that was measured on any one day and sustainable biomass (SB) was the average concentration measured on the last two days of sample collection. Each of these indices were useful in making comparisons of periphyton responses between sites at different times (Bothwell 1988b).

All samples for analysis of chlorophyll-a were stored frozen at -15°C for up to two weeks before shipment on dry ice to Cantest Laboratories in Vancouver for analysis. The fluorometric analysis described in APHA (1985) was used for all samples after chlorophyll extraction in 90% acetone. Instrumentation was calibrated using fresh chlorophyll-a standards purchased from Sigma Chemicals.

At the end of each periphyton accrual series, one additional core was collected from each substrata and preserved in Lugol's solution for later taxonomic examination. The percent composition of each algal taxa was determined from counts taken at 500x magnification along several transects from subsamples that were allowed to settle in Utermohl chambers. Communities between sites were qualitatively compared in terms of presence, absence, and relative proportion of sample volume occupied by dominant species. Dominant species were regarded as those occupying more than 10% of sample volume.

The taxonomic composition of the artificial and natural gravel/cobble substrata were also compared to determine whether the styrofoam substrata were representative of the side channel community. At the time taxonomy samples were collected from the styrofoam, five rocks were randomly selected from each of three locations in the vicinity of the artificial plates. The rocks were brushed clean with a stiff-bristle brush and the removed periphytic material was preserved in Lugol's solution for taxonomic examination using the same methods as for the periphyton on styrofoam. Community comparisons were again made using presence/absence criteria

and by comparing relative proportions of the dominant taxa.

## Bioassay

The bioassay experiment involved measurements of net periphyton accrual as a function of 3 levels of N and 3 levels of P additions plus interactions resulting in 9 separate treatments. An additional control was also included, giving 10 treatments in total. Table 1 provides a layout which matches the treatment allocations in the actual bioassay chambers shown in Figures 2 and 12.

With this design, comparisons of net periphyton accrual among the control (A and J), N alone (B and C), and P alone (D and G) treatments were used to identify the nutrient which primarily limited autotrophic production in the upper Nechako River: addition of the limiting nutrient yielded greatest values of k, PB, and SB. The combinations of N and P showed potential changes in the growth indices with increasing concentrations. To compare biomass accrual among treatments, a fixed effect two-way (time x treatment) analysis of variance was used in which there were 7 time levels (as in the *in situ* time series analysis of periphyton accrual). The number of treatment levels were variable depending on what comparisons were being made. Values of all biomass levels from all sampling days associated with the limiting nutrient had to be significantly greater than those resulting from addition of the other nutrient based on this ANOVA at $p<0.05$ before it was considered to significantly limit autotrophic production.

The bioassay apparatus consisted of an array of 10 flow-through chambers that were floated under the water surface from a styrofoam and wood frame (Figure 2). The chambers were fabricated from plexiglass and had inside dimensions of 10 cm x 10 cm x 123 cm. Sodium fluorescein dye injections to the chambers showed that the current velocity across the substrata was 25 $cm \cdot s^{-1}$ or about 60% of that in the adjacent water. Water flow through the chambers approximated 0.9 $L \cdot s^{-1}$. Flow reductions were caused mainly from a series of 4 baffles that were designed to ensure complete mixing of nutrient solutions with water at the intake to each chamber. A styrofoam substratum that was secured to a removable tray in the base of each chamber provided a surface for periphyton accrual and a sampling surface using the coring method described above.

The nutrient solutions were introduced to the upstream end of each chamber by siphoning concentrated solutions of $NH_4NO_3$ and $KH_2PO_4$ from 2 L polyethylene reservoir bottles through microbore teflon tubing. Nutrient solutions were mixed using filtered river water and stock reagent grade chemicals. Drip rates into the chambers were calibrated daily and adjustments were made by changing the reservoir head height, cleaning the microbore tubing with water pressure from a syringe, or replacing the tubing. Occasionally, nutrient delivery rates declined slightly due to small particulates becoming lodged in the tubing.

Table 1
The Layout of Treatments
in the Bioassay Chambers

| Treatment | Nutrient Added | |
|---|---|---|
| | N | P |
| A | 0 | 0 |
| B | 20 | 0 |
| C | 50 | 0 |
| D | 0 | 5 |
| E | 20 | 5 |
| F | 50 | 5 |
| G | 0 | 10 |
| H | 20 | 10 |
| I | 50 | 10 |
| J | 0 | 0 |

The apparatus was installed in the Nechako River mainstem, adjacent to the fertilized side channel, but well away from any influence of the fertilizer dispensers (Figure 1). The experiment was started on June 3, 1988 with the installation of new styrofoam substrata and the commencement of nutrient flow. Final samples were collected on June 20, 1988.

Sampling procedures were similar to those described for the *in situ* sampling. Water samples were collected from each chamber using the same hand pump and glassware that was used for collections of water for DO measurements. Care was taken to rinse the glassware thoroughly between sample collections. Weekly collections were filtered in the field and shipped to Cantest for analysis of $NO_3$-N, $NH_4$-N, and SRP on the day of sample collection. Triplicate cores were removed once every 3 days from each chamber and processed as described above for analysis of chlorophyll-a concentrations. Sampling continued for 16 days and final samples included cores for taxonomic examination.

Figure 2
View of the Periphyton Bioassay Apparatus Installed *In Situ* in the Nechako River

- Nutients are delivered by gravity through microbore tubing to flow-through chambers that are floated beneath the water surface from a styrofoam and wood frame.
- Chambers are labelled A to J from left to right.

Figure 3
Mean Daily Temperatures of the Upper Nechako River During the Study Period

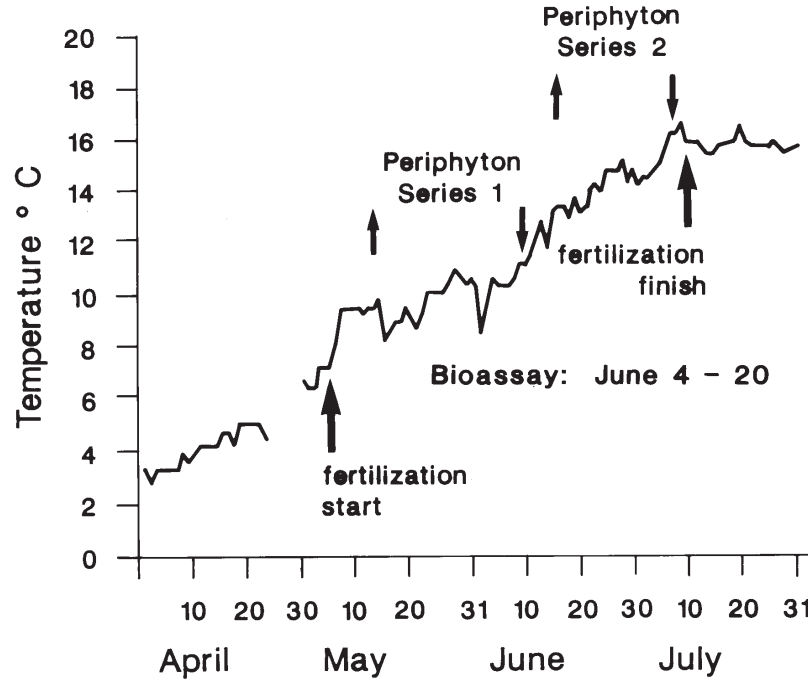
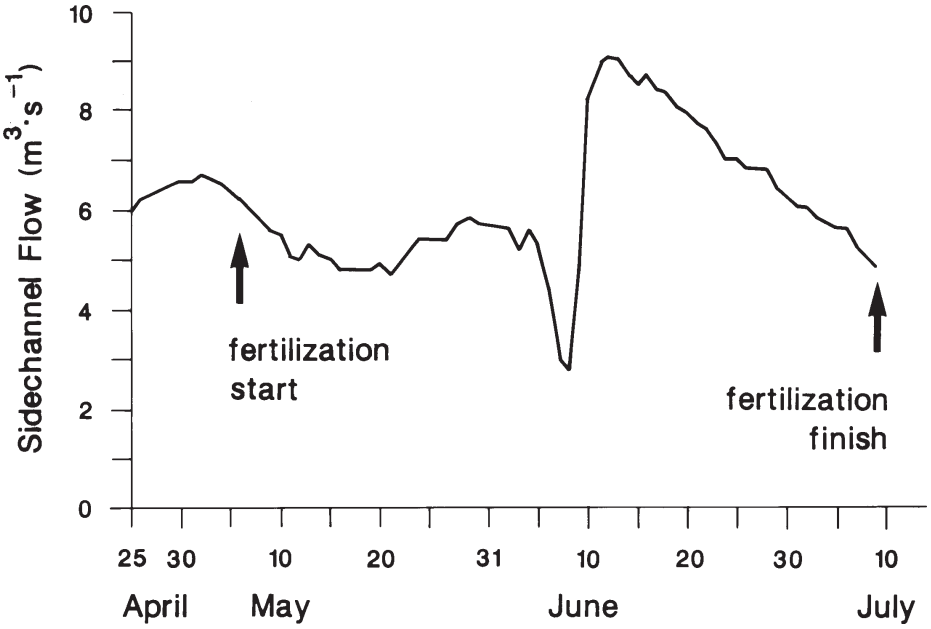



Figure 4
Flow in Fertilized Side Channel During the Study Period



## RESULTS

### Physical Data

Mean daily temperature increased steadily throughout the study period (Figure 3). Temperatures were slightly above 6°C in early May and increased to a maximum of 16.4°C at the time fertilization was finished on July 10. Average temperatures during the periphyton accrual series 1 and 2 were 9.8°C and 14.3°C, respectively. During the bioassay, the average temperature was 11.7°C.

Flows in the side channel ranged from 4.8 to 6.7 $m^3 \cdot s^{-1}$ until June 7 (Figure 4). At that time, the Skins Lake spillway was temporarily closed to assist in the investigation of a drowning accident. Side channel flow declined from 4.5 to 2.8 $m^3 \cdot s^{-1}$ within 24 hours of the closure. Excess water that had accumulated in the reservoir was then released resulting in higher than normal flows from June 10 through about June 25. These changes were reflected in a peak side channel flow of 9.02 $m^3 \cdot s^{-1}$ on June 13 and a steady decline in flows thereafter to reach 4.8 $m^3 \cdot s^{-1}$ by July 10.

### Fertilizer Application

Fertilizer loading to the side channel remained consistent with the intended input rates (Figure 5). The total mix of 34-0-0 and 12-51-0 was initially introduced at about 60 $kg \cdot d^{-1}$, but after the first two weeks of treatment, fertilizer loading was increased to 80-90 $kg \cdot d^{-1}$ because concentrations of N and P determined from water sampling were less than the target levels. The fertilizer blend was maintained at 70% 34-0-0 and 30% 12-51-0.

Predicted concentrations of N and P ($C_i$) at full mixing of the fertilizer was determined as the ratio of element input ($L_i$) to side channel flow ($Q_s$), where a correction factor (r) was used to change units to $\mu g \cdot L^{-1}$: $C_i = (L_i / Q_s)r$.

The time series of $C_i$ for P was very close to the intended concentrations of 10 $\mu g \cdot L^{-1}$ SRP, but for N, $C_i$ was generally less than the target of 40 $\mu g \cdot L^{-1}$ (Figure 6). The abrupt spike in both predicted N and P concentrations on June 7-10 was caused by the unexpected changes in streamflow due to closure and opening of the spillway. As flows declined from June 10 through to the end of the study, the relatively constant input of fertilizer (Figure 5) caused an inverse increase in predicted N and P concentrations (Figure 6).

### Measured Nutrient Concentrations

Actual concentrations of N and P did not match the predicted concentrations (Table 2, Figure 6). Before fertilizer was introduced, nutrient concentrations at the control and NT1 were identical: with one exception, all forms of N and P were less than detectable limits (Table 2). Only $NH_4$-N was detectable but only by a margin of a part per billion. After fertilization started, control nutrient levels remained low and although concentrations of both N and P forms were

Table 2
Nutrient Concentrations at Control and NT1 Sites During Fertilization, 1988

| | $NO_3$ - N | | $NH_4$ - N | | TDP | | SRP | |
|---|---|---|---|---|---|---|---|---|
| | C | T1 | C | T1 | C | T1 | C | T1 |
| **Pretreatment** | | | | | | | | |
| April 26 | <5 | <5 | <10 | 11 | <1 | <1 | <1 | <1 |
| **During Treatment** | | | | | | | | |
| May 9 | <5 | <5 | <10 | <10 | 1 | 2 | <1 | <1 |
| May 16 | <5 | <5 | <10 | 11 | 2 | 3 | <1 | 2 |
| May 25 | 6 | 66 | <10 | 140 | 2 | 52 | <1 | 50 |
| May 30 | <5 | 62 | <10 | 56 | 4 | 37 | <1 | 2 |
| June 6 | <5 | 730 | <10 | 540 | 4 | 79 | <1 | 74 |
| June 20 | <5 | 13 | <10 | <10 | 4 | 41 | 1 | 34 |
| June 28 | <5 | 130 | <10 | <10 | 5 | 34 | 1 | 32 |
| July 4 | <5 | 55 | <10 | 750 | 5 | 50 | 2 | 45 |

Figure 5
Time Series of Fertilizer Discharge to the Treatment Side Channel

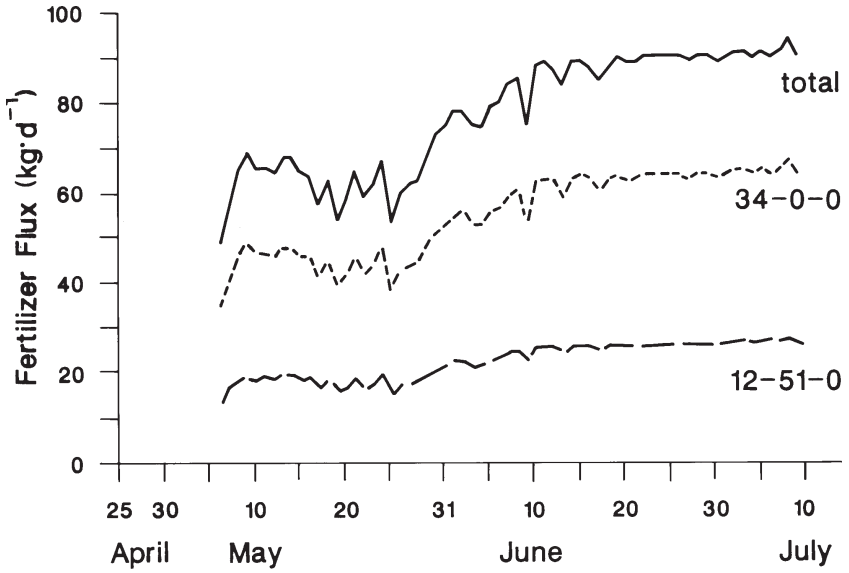



Figure 6
Predicted Concentrations of N and P at Full Mixing in the Fertilizer Side Channel as Determined Using Equation 1

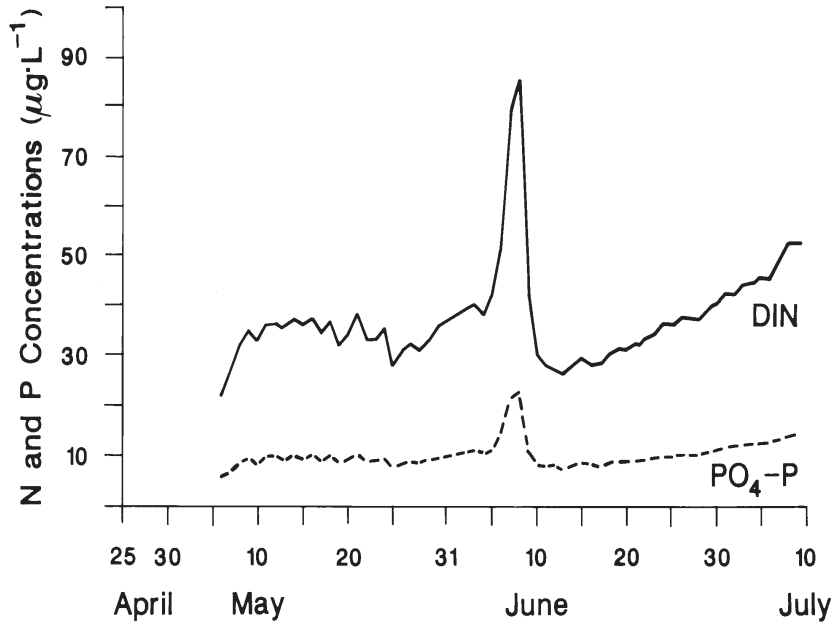
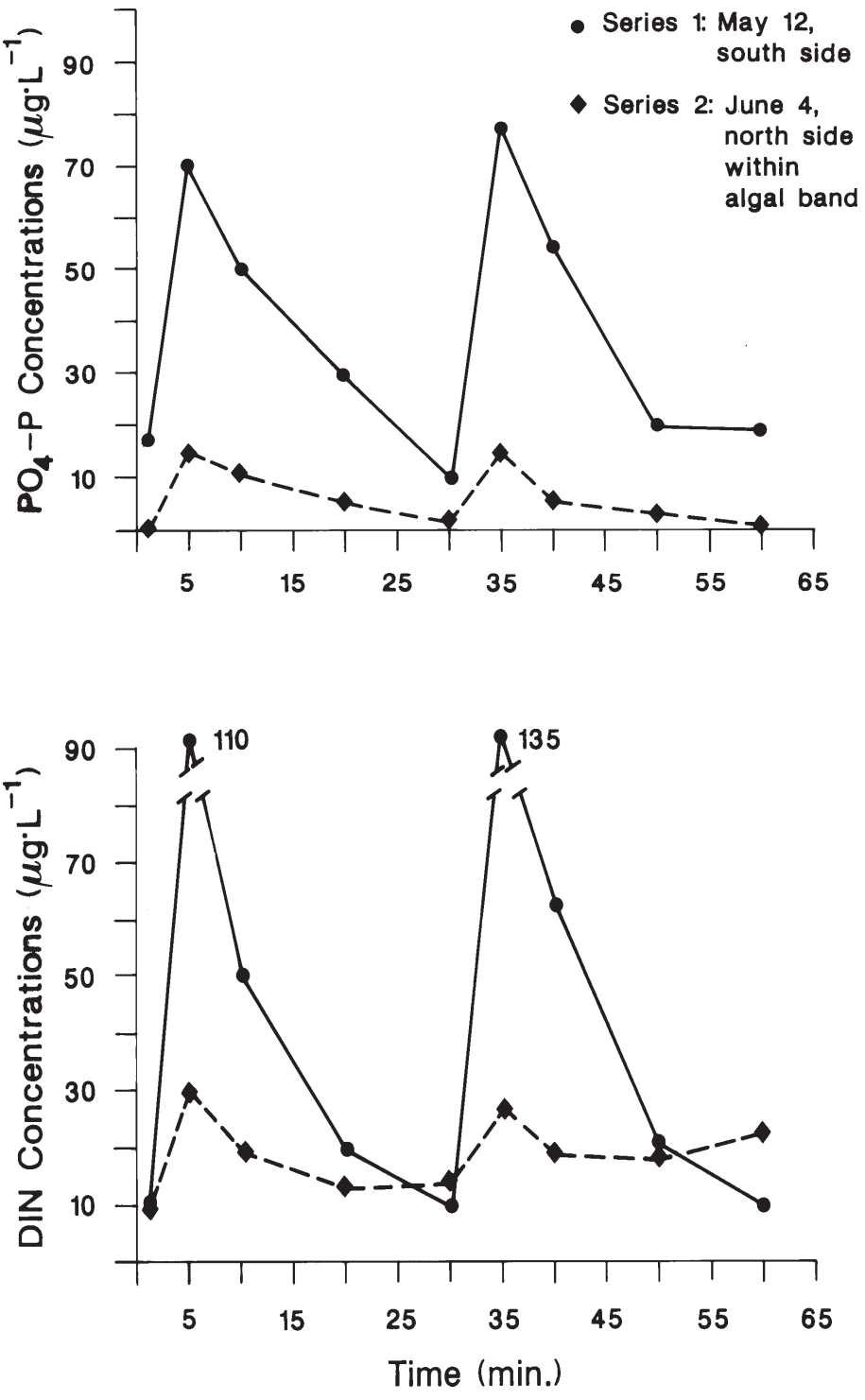


Figure 7
Concentrations of N and P from Short Interval Water Sampling at NT1 to Document Temporal Pulsing and Incomplete Dispersal of Fertilizer in the Side Channel



higher at NT1, they were extremely variable. $NO_3$-N levels ranged from <5 µg·L$^{-1}$ to more than 700 µg·L$^{-1}$. $NH_4$-N concentrations were equally variable with a maximum value of 750 µg·L$^{-1}$. The range of TDP and SRP concentrations was from undetectable levels to about 50 µg·L$^{-1}$.

This extreme variation can be explained by the nature of fertilizer introduction and subsequent downstream transport in the side channel. Since the dispensers were programmed to spread fertilizer in 30 minute intervals, nutrient introduction occurred in pulses which resulted in wide extremes in nutrient concentrations downstream. The effect was greatest in the vicinity of the dispensers and would have lessened downstream due to dispersion effects. The nutrient pulsing was described on two dates (May 12 and June 4) by sampling water at NT1 in 5 to 10 minute intervals over one hour. Results in Figure 7 show that the range of concentrations was equally large to the variation found from analysis of the routine grab samples. Clearly, longitudinal dispersion effects were ineffective in dampening the pulsing action of chemical transport within the side channel, and the timing of sample collection relative to the timing of fertilizer introductions strongly affected the measured concentrations.

Another feature of Figure 7 was the variation in concentrations that resulted from sampling at different locations across the width profile. Highest concentrations were measured on the north side where more than 70% of the channel flow was estimated to occur. Fertilizer must have been carried in this main flow and did not completely mix with water on the south side which had much lower N and P concentrations and was characterized by a quiescent, shallow pool area. This variation in nutrient concentrations across the width profile is fundamental evidence to support the basic rule in water sampling techniques that areas outside of the main flow may not provide representative information on chemical concentrations.

## Downstream Transport of Fertilizer

After fertilizer additions were started, water samples were routinely collected at two downstream locations on the Nechako River mainstem; one, 1500 m (NT2) and the other, 3400 m (NT3) downstream of the fertilizer dispensers (Figure 1). Changes in N and P concentrations at these sites are shown in Figure 8. Note that the data are not statistically summarized so as to avoid misleading interpretations associated with potentially incomplete fertilizer dispersal. Nitrate concentrations were always <5 µg·L$^{-1}$, ammonia levels were <20 µg·L$^{-1}$ and half of these were <10 µg·L$^{-1}$. TDP concentrations ranged between 2 and 15 µg·L$^{-1}$.

If we assume that side channel discharge became fully mixed with mainstem flow, we can predict N and P concentrations in the mainstem, as was done for side channel chemistry. Since the side channel flow was about 10% of that in the mainstem, predicted concentrations of DIN and SRP from fertilizer additions that are added to background concentrations would be 4 µg·L$^{-1}$ and 1 µg·L$^{-1}$ respectively at full mixing. These levels are within background variability of DIN and TDP concentrations at the control site and thus should not be greater than control site concentrations at NT2 or NT3. This was true for $NO_3$-N and although some values were higher, $NH_4$ values were generally similar between control, T2 and T3 samples. However, in 3 out of the 5 samples, TDP concentrations were noticeably higher at T2 and T3 than at the control. Since this result could only occur if side channel water was not fully mixed with mainstem water, the data further suggest that even at a distance of 3.4 km downstream of fertilizer addition, there is incomplete mixing of introduced nutrients.

## *In Situ* Periphyton Accrual

### *Taxonomy*

The periphyton community on artificial substrata was representative of that on the natural gravel and cobble (Table 3). On both sampling dates (June 12 and July 8), diatoms and chlorophytes were equally dominant on both artificial and natural substrata and the species composition within each taxa was similar. The diatoms were dominated by *Gomphonema sp.*, *Synedra sp.*, and *Cymbella sp.* and the chlorophytes were always represented by *Ulothrix sp.* June 12th samples showed that artificial substrata had more dominant diatom species than the natural gravel, but July 8 samples showed that numbers of species were similar on each substrata. The later samples showed that the most dominant species occurred in similar proportions on both substrata, but the relative abundance of less dominant species varied. Generally, there were more diatom species found in

Figure 8
Comparison of N and P Concentrations at the Control and Sites
Up to 3.4 km Downstream of the Fertilizer Dispensers

- Vertical lines show ranges of individual data points.
- Horizontal bars show detection limits for specific ions.
- Numbers below arrows refer to the number of samples in which the concentration was less than the detectable limit.

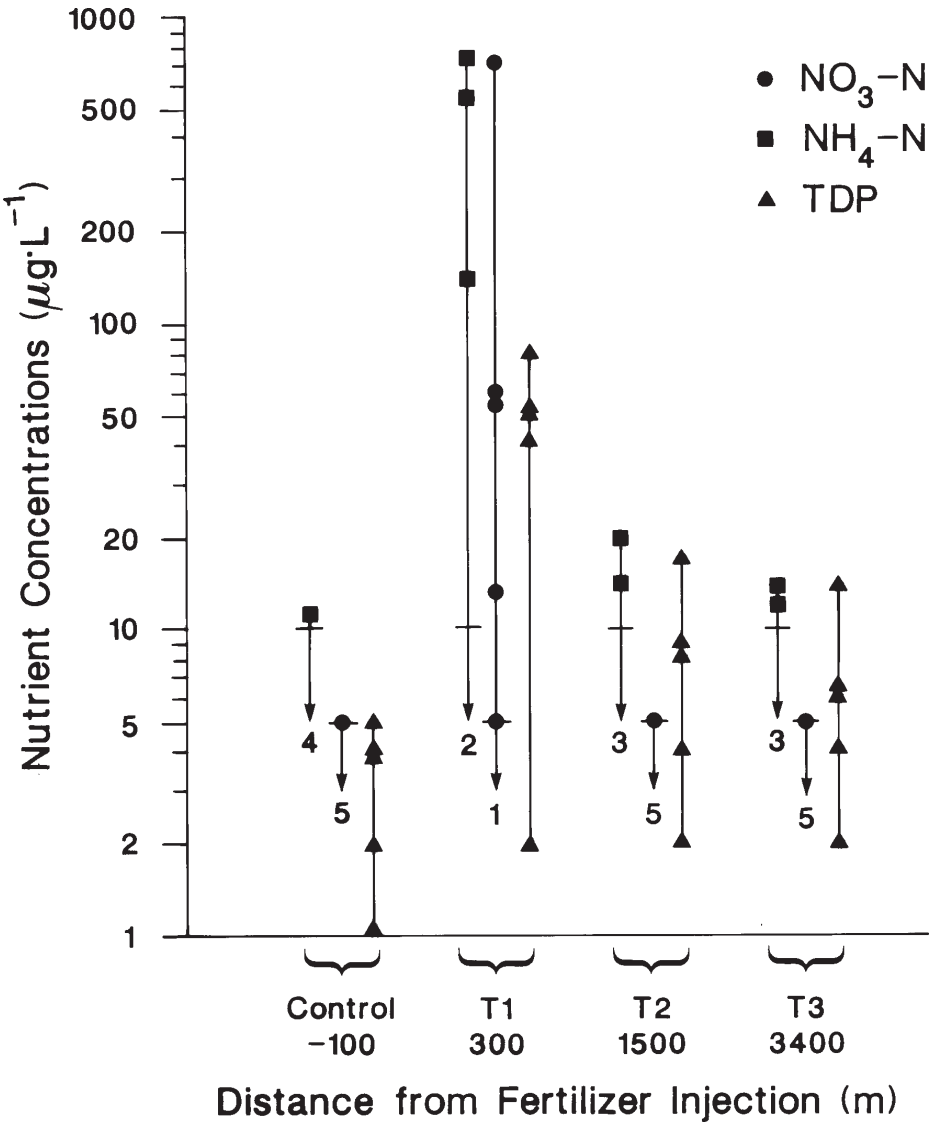


Table 3
Comparison of Taxonomic Composition on Natural and Artificial Substrata
in the Treatment Reach on Two Dates

***1. Phyla or Class***

| Sampling Series | Taxa | Natural (Cobble) | Artificial (Styrofoam) |
|---|---|---|---|
| June 12 | Diatoms | 50% | 48.3% |
| | Greens | 50% | 41.7% |
| July 8 | Diatoms | 55.7% | 57% |
| | Greens | 55.7% | 42% |

***2. Species Composition***

| Date | Taxa | Natural | Artificial |
|---|---|---|---|
| June 12 | Diatoms | Gomphonema sp. (95%) | Gomphonema sp. (15%)<br>Hannaea sp. (27%)<br>Cymbella sp. (15%)<br>Synedra sp. (40%) |
| | Greens | Ulothrix sp. (100%) | Ulothrix sp. (96%) |
| July 8 | Diatoms | Gomphonema sp. (45%)<br>Cymbella sp. (39%)<br>Epithernia turgida (12%) | Gomphonema sp. (33%)<br>Cymbella sp. (17%)<br>Synedra sp. (12%)<br>Fragilaria sp. (15%) |
| | Greens | Ulothrix sp. (52%)<br>Stigeoclonium sp. (27%)<br>Draparmaldia sp. (21%) | Ulothrix sp. (57%)<br>Spirogyra sp. (40%) |

Table 4
Dominant Periphyton Taxa on Styrofoam Substrata at Control
and NT1 Sites During Fertilization, 1988

***1. Phyla or Class***

| Sampling Series | Taxa | Control | Treatment |
|---|---|---|---|
| June 10 | Diatoms | 81.7% | 48.3% |
| | Greens | 17.7% | 51.7% |
| July 8 | Diatoms | 18.5% | 57% |
| | Greens | 17% | 42% |

***2. Species Composition***

| Date | Taxa | Diatoms | Chlorophytes |
|---|---|---|---|
| June 10 | Control | Synedra sp. (77%)<br>Diatoma tenue (10%) | Zygnema sp. (90%) |
| | Treatment | Synedra sp. (40%)<br>Hannaea sp. (27%)<br>Cymbella sp. (15%)<br>Gomphonema sp. (15%) | Ulothrix sp. (96%) |
| July 8 | Control | Synedra sp. (25%)<br>Epithemia tugida (13%)<br>Diatoma tenue (17%)<br>Fragilaria sp. (23%) | Zygnemia sp. (20%)<br>Ulothrix sp. (23%) |
| | Treatment | Synedra sp. (12%)<br>Fragilaria sp. (15%)<br>Cymbella (17%)<br>Comphonema sp. (33%) | Ulothrix sp. (57%)<br>Spirogyra sp. (40%) |

excess of 10% of sample volume on artificial substrata than was found on natural substrata.

Samples collected from the styrofoam substrata at the end of each periphyton accrual series were always dominated by diatoms and chlorophytes at both control and treatment sites. Cyanophytes (blue green algae) were present in less than half the samples, but in those samples they occupied 1% or less of sample volume. The Chrysophyte, *Dinobryon sertularia* was also found in trace amounts. Since the proportions of both the Cyanophytes and Chrysophytes were low, they were considered unimportant with regards to the community response to nutrient addition.

Table 5
Periphyton Growth and Biomass Indices for Accrual Series 1 and 2 Measured on Artificial Substrata in Control and fertilized Sites

| | Series 1 | | Series 2 | |
|---|---|---|---|---|
| | Control | NT1 | Control | NT1 |
| PB[1] | 5.53 | 149.0 | 14.9 | 218.0 |
| SB[1] | 3.92 | 111.0 | 08.12 | 121.0 |
| k[2] | 0.063 | 0.439 | 0.088 | 0.416 |

[1] Units are $mg \cdot m^{-2}$ as chlorophyll-*a*
[2] Units are $div \cdot d^{-1}$

Table 6
Comparison of Dissolved Oxygen Concentrations in Control and Treatment Surface Water and Spawning Gravel at the Completion of Side Channel Fertilization

| **Depth** | **Control** | | **Treatment** | |
|---|---|---|---|---|
| | Measured Value[1] | % Saturation[2] | Measured Value | % Saturation |
| Surface Water | 8.7 ± 0.25 | 95.0 | 8.7 ± 0.16 | 94.0 |
| 15 cm Intergravel | 6.8 ± 0.41 | | 8.3 ± 0.48 | |

[1] Units are $mg \cdot L^{-1}$ . Values are means ±95% confidence intervals of 5 randomly selected samples.
[2] Percent saturation is the DO concentration in surface water relative to the concentration at complete saturation with respect to oxygen solubility at the ambient temperature of 18°C.
Solubility data were taken from Wetzel (1975).

Differences were found in the taxonomic composition between control and treatment sites (Table 4). In the control, diatoms always occupied more than 80% of sample volume and were represented by *Synedra sp.* and *Diatoma tenue* in June 10 samples. *Fragilaria sp.* and *Epithemia tugida* also dominated in the July 8 samples. Chlorophytes occupied about 17% of sample volume with *Zygnema sp.* and *Ulothrix sp.* dominating all samples. However, samples from NT1 showed that chlorophytes and diatoms occurred in similar proportions. While *Synedra sp.* and *Fragilaria sp.* were found to dominate in both the control and at NT1, other diatoms including *Cymbella sp.* and *Gomphonema sp.* were abundant in the fertilized area, but not in the control. Equally striking was that *Diatoma tenue* was frequently more than 10% of diatom volume in control samples, but was never found in NT1.

## Biomass Accrual

Chlorophyll-a concentrations at NT1 reached levels more than an order of magnitude greater than control levels in both sampling series (Figures 9, 10; Table 5). Initial concentrations measured on the third day after incubation of the plates was similar between sites ($p<0.05$) in each series, but large differences were apparent thereafter. In series 1, starting concentrations of 0.5 $mg \cdot m^{-2}$ increased to a SB value of 3.92 $mg \cdot m^{-2}$ in the control representing a k value of 0.063 $div \cdot d^{-1}$. At NT1, SB reached 111 $mg \cdot m^{-2}$ and k was about 7 times that in the control. In series 2, starting concentrations of 3-5 $mg \cdot m^{-2}$ were higher than the earlier measurements, perhaps due to effects of higher temperatures (Bothwell 1988). SB and PB values were also higher with values in the fertilized area being more than an order of magnitude greater than in the control. Values of k differed by almost 5 times between sites.

## Dissolved Oxygen

Dissolved oxygen concentrations did not change due to fertilizer addition (Table 6). Surface water concentrations at both control and treatment sites were 8.7 $mg \cdot L^{-1}$ and represented more than 94% of saturated concentrations with respect to oxygen solu-

Figure 9
Periphyton Biomass Accrual on Styrofoam Substrata at the Control and NT1 Sites: Series 1, May 13 - June 10

- Data are means of triplicate samples with 95% confidence limits.

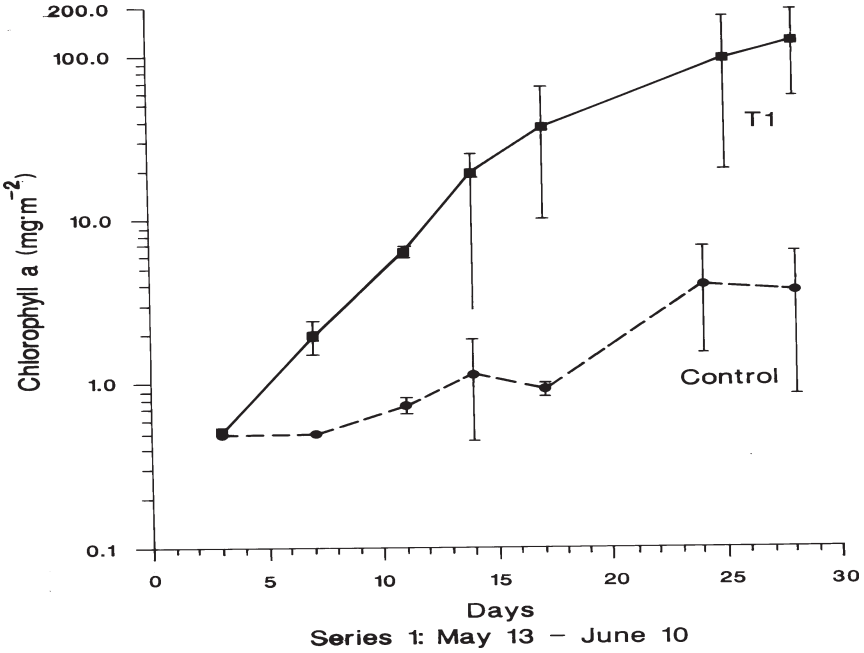



Figure 10
Periphyton Biomass Accrual on Styrofoam Substrata at the Control and NT1 Sites: Series 2, June 17 - July 8

- Data are means of triplicate samples with 95% confidence limits.

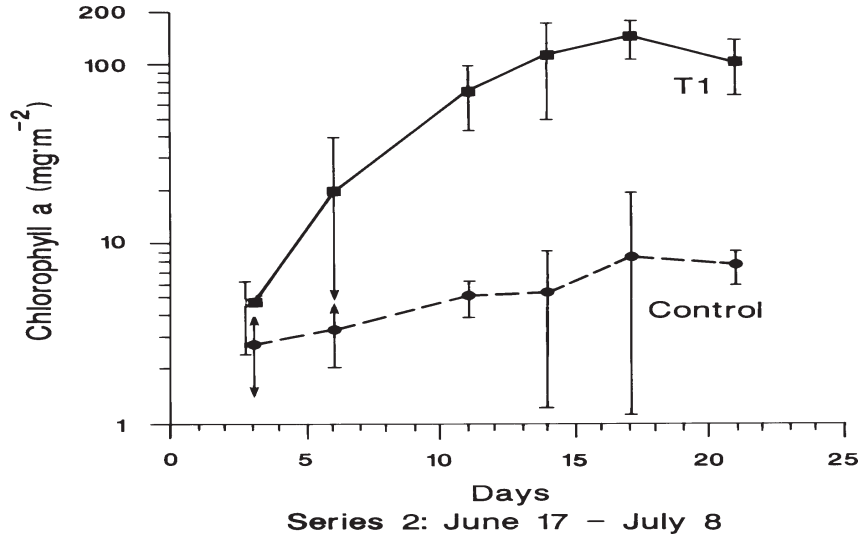


Table 7
Summary of Nutrient Concentrations Measured in Each Chamber During the Bioassay[1]

| | Bioassay Chamber | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Chamber: | A | B | C | D | E | F | G | H | I | J |
| NP added[2] | 0/02 | 0/0 | 50/0 | 0/5 | 20/5 | 50/5 | 0/10 | 20/10 | 50/10 | 0/0 |
| $NO_3$ - N | <5 | 13.0 | 29.3 | <5 | 16.8 | 38.5 | <5 | 16.8 | 38.8 | <5 |
| SE | | 3.5 | 7.9 | | 2.1 | 2.8 | | 2.0 | 5.0 | |
| $NH_4$ - N | <10.0 | 16.5 | 28.0 | <10.0 | 13.7 | 11.0 | <10 | 11.1 | 22.5 | <10 |
| SE | | 1.3 | 6.4 | | 2.7 | 1.1 | | 1.2 | 6.3 | |
| DIN | <10.0 | 29.5 | 50.3 | <10.0 | 27.0 | 48.5 | <10 | 20.5 | 50.0 | <10 |
| SE | | 4.1 | 16.1 | | 4.9 | 11.1 | | 5.1 | 12.0 | |
| SRP | <1.0 | <1.0 | <1.0 | 7.3 | 7.3 | 7.8 | 12.0 | 13.3 | 12.7 | <1 |
| SE | | | | 1.3 | 1.7 | 1.9 | 2.3 | 2.4 | 2.9 | |

[1] Data are means of one sample collected on each of 4 dates (June 4, 8, 16 and 20, 1988) and are expressed in units of $\mu g \cdot L^{-1}$ of N or P. The standard error (SE) is shown below each mean value except where concentrations were undetectable.

[2] The ratios (N/P) are the intended treatment ratios of the final N and P concentrations in the bioassay chambers.

bility at the ambient temperature of 18°C. DO concentrations at a depth of 15 cm from surface gravels were about 22% lower than surface water levels in the control and only about 5% lower in the treatment channel. Greater compaction of gravels that were found in the mainstem gravels, where control samples were collected may have been responsible for the relatively low values at that site. All DO concentrations were greater than levels that are required for successful incubation of salmon eggs (Davis 1975).

## Bioassay

### *Chemistry*

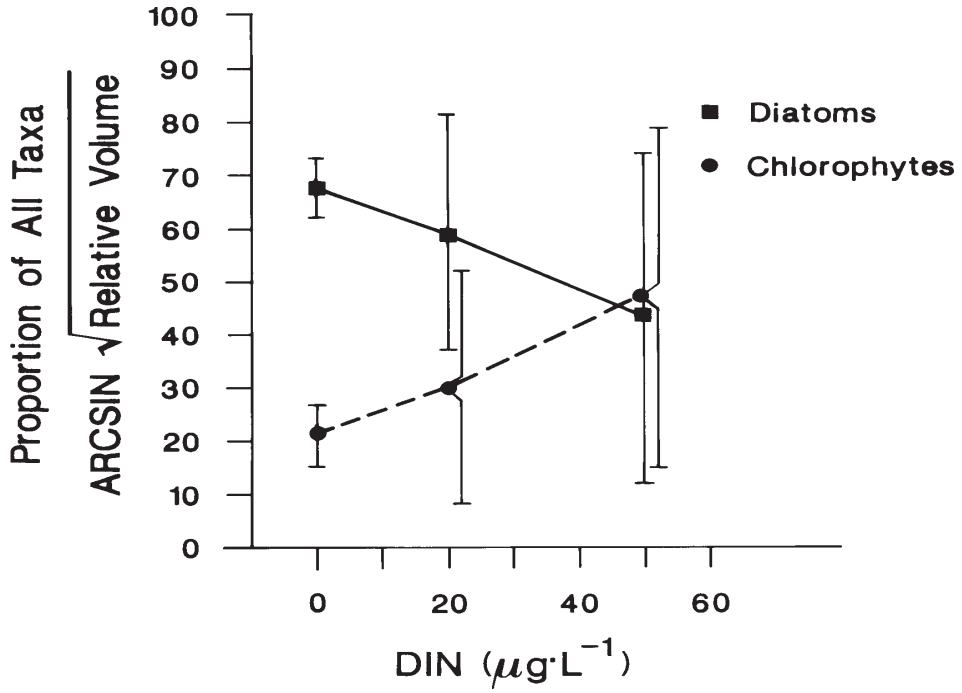
Although variation was found in measured nutrient concentrations, DIN and SRP levels were reasonably close to the intended N / P treatment ratios for each chamber (Table 7). Control samples showed undetectable levels for all nutrient species. In treatment chambers, contributions to DIN were usually split equally between $NH_4$ and $NO_3$. Measured SRP concentrations were generally greater than the target levels by about 2 $\mu g \cdot L^{-1}$. For two of the N addition treatments, measured concentrations were also slightly higher than intended but otherwise, concentrations were at target levels. This variation in nutrient concentrations was likely related to small variations in flows through the chambers, as well as small shifts in nutrient delivery rates that occurred despite daily calibrations.

### *Taxonomy*

The taxonomic composition in the bioassay was similar to that found *in situ*; the community in all treatments was dominated by diatoms and chlorophytes. These two taxa always occupied more than 98% of all samples. The cyanophytes, *Anabaena sp.* and occasionally *Oscillatoria sp.* were found in most samples, but they occupied less than 1% of sample volumes.

The species composition of diatoms and chlorophytes was similar among treatments although there was some evidence that relative dominance among species shifted in relation to nutrient level. Chlorophytes were always dominated by *Zygnema sp.* and *Ulothrix sp.*: the former dominated (> 75%) at low nutrient levels (treatment A, J, B, D), but where N and P were added together, the importance of *Ulothrix sp.* increased, but never more than 55% of sample volumes. *Synedra sp.* was always the most dominant diatom in all samples, occupying 30 - 70% of sample volumes. *Fragilaria sp.* was also found in all samples representing 15 to 35% of the community. Less common species were *Epithemia tugide*, *Diatoma tenue, Hannae*

Figure 11
Shifts in Relative Dominance of Diatoms and Chlorophytes at Varying Concentrations of N and P in the Periphyton Bioassay



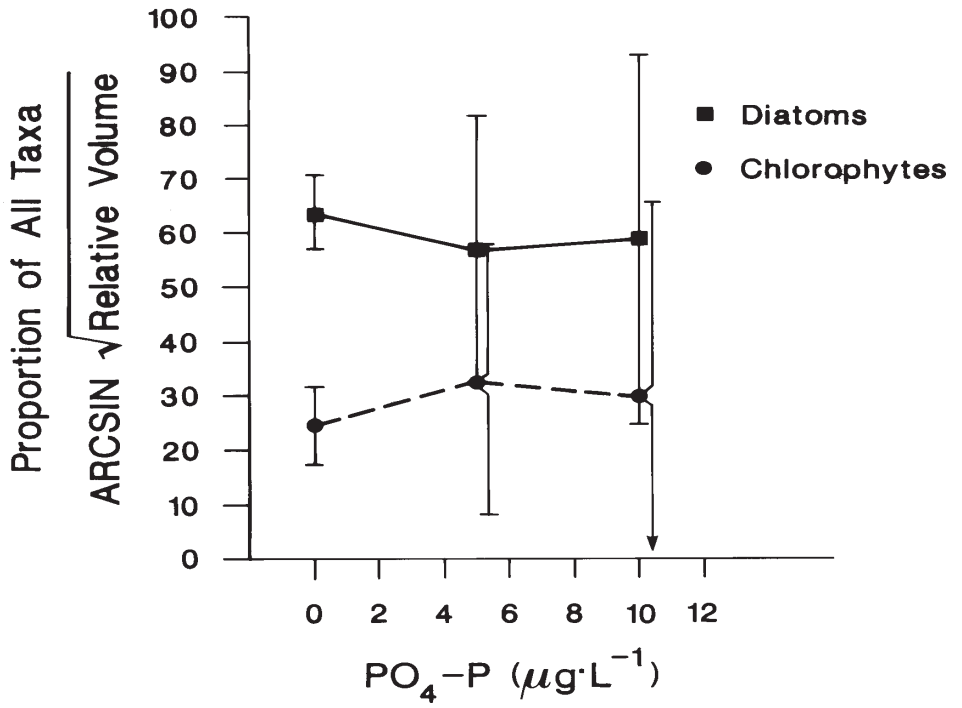



Figure 12
View of Biomass Accumulation in the Periphyton Bioassay Showing the Algal Community at Both Nutrient-Deficient and Enriched Conditions

- The chambers are labelled A to J from left to right.
- Refer to text for other treatment details.

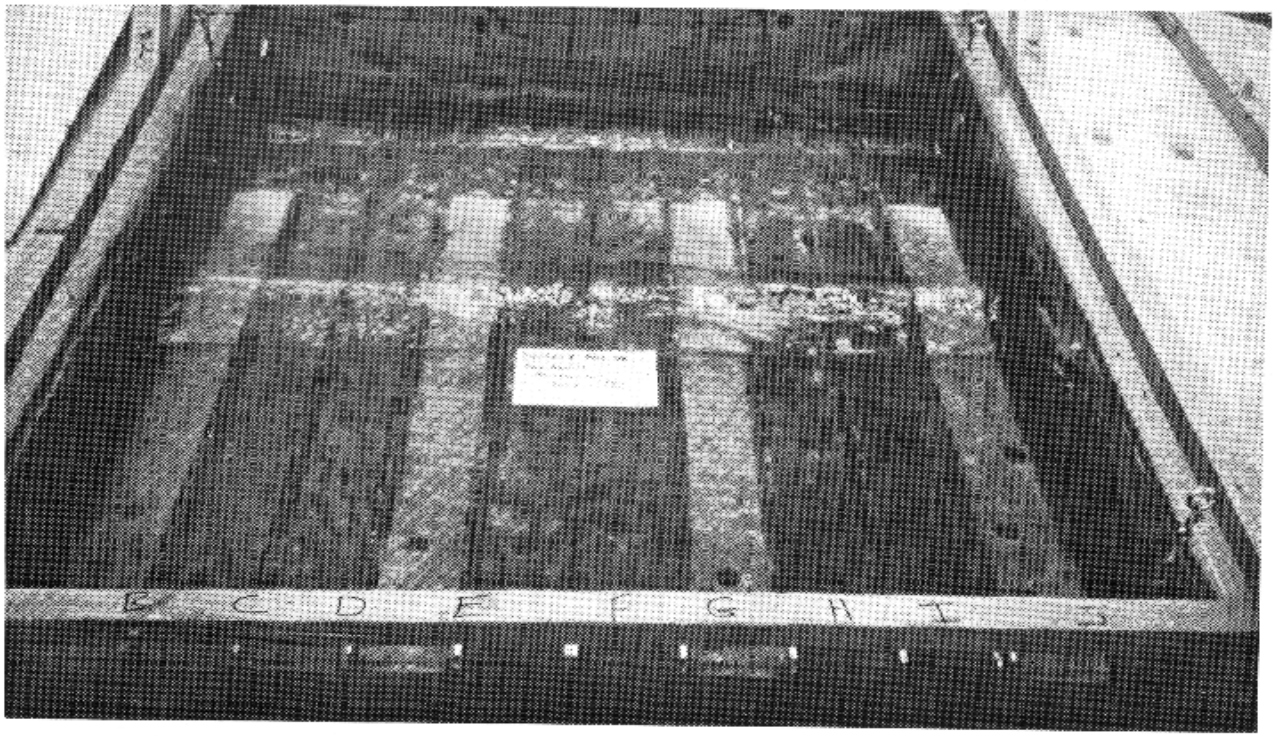


Table 8
Summary of Dominant Algal Taxa in Bioassay Chambers

| Treatment | Diatoms | Chlorophytes |
|---|---|---|
| A & J: N=0, P=0 | Synedra sp. (45%)<br>Fragilaria sp. (25%)<br>Epithemia turgide (15%) | Zygnema sp. (75%)<br>Ulothrix sp. (24%) |
| B: N=20, P=0 | Synedra sp. (45%)<br>Fragilaria sp. (40%) | Zygnema sp. (80%)<br>Ulothrix sp. (19%) |
| C: N=50, P=0 | Synedra sp. (40%)<br>Fragilaria sp. (30%)<br>Diatoma tenue (20%) | Zygnema sp. (59%)<br>Ulothrix sp. (40%) |
| D: N=0, P=5 | Synedra sp. (45%)<br>Fragilaria sp. (25%) | Zygnema sp. (99%) |
| E: N=20, P=5 | Synedra sp. (30%)<br>Fragilaria sp. (15%)<br>Hannaea Arcus (20%)<br>Gomphonema sp. (15%) | Zygnema sp. (64%)<br>Ulothrix sp. (35%) |
| F: N=50, P=5 | Synedra sp. (60%)<br>Fragilaria sp. (15%) | Zygnema sp. (35%)<br>Ulothrix sp. (55%) |
| G: N=0, P=10 | Synedra sp. (70%)<br>Fragilaria sp. (35%) | Zygnema sp. (45%)<br>Ulothrix sp. (45%) |
| H: N=20, P=10 | Synedra sp. (50%)<br>Fragilaria sp. (35%) | Zygnema sp. (59%)<br>Ulothrix sp. (40%) |
| I: N=50, P=10 | Synedra sp. (30%)<br>Fragilaria sp. (30%)<br>Diatoma tenue (15%)<br>Cymbella sp. (15%) | Zygnema sp. (543%)<br>Ulothrix sp. (55%) |

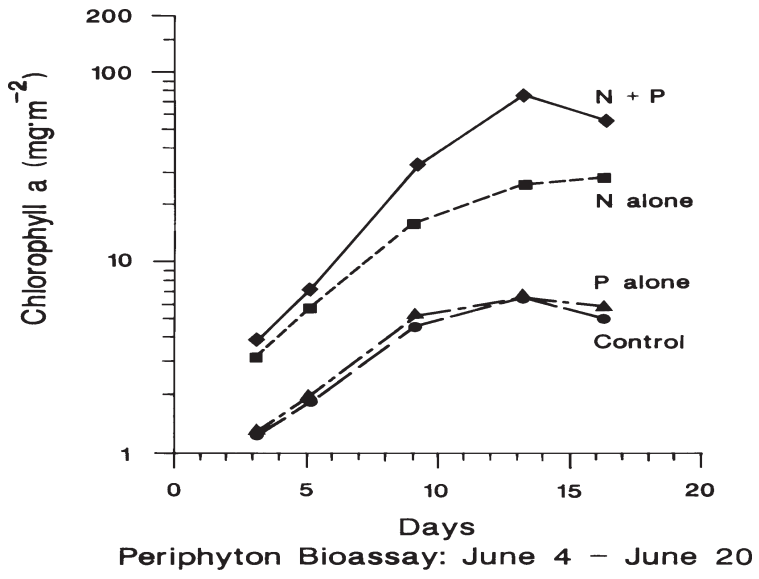
*arcus, Gomphonema sp.* and *Cymbella sp.* Species having relatively large cell volumes (*Gomphonema sp., Hannae arcus, Cymbella sp.*) were only found at higher nutrient levels. This observation is consistent with other work showing that cells with higher ratios of surface area to volume should be better nutrient competitors (Smith and Kalff 1982).

Although dominant species within major taxa were similar among treatments, the relative dominance of diatoms and chlorophytes did not remain consistent. To examine shifts in dominance of the major taxa, means and 95% confidence intervals of the proportionate data were plotted in relation to concentrations of N and P (Figure 11). To improve linearity in the percentage data and allow statistical comparisons of taxonomic composition at various nutrient concentrations, the arcsine square root transformation was applied. Two points emerge from this analysis. First, when no nutrients were added, diatoms were significantly more dominant than chlorophytes ($p<0.05$), comprising more than 60% of the community. This diatom dominated community is typical of nutrient-deficient streams of the Pacific Northwest in which a thin, brown-green coating on stream substrata is commonly observed, not extensive bright-green filamentous algae associated with the chlorophytes. The second point was that as nutrient concentrations increased, variability in relative dominance also increased. In particular, as N concentrations increased, the mean dominance of each taxa converged. At DIN concentrations greater than 20 $\mu g \cdot L^{-1}$, differences among diatoms and chlorophytes were not significant ($p<0.05$). This shift can be seen in Figure 12 as an increase in filamentous biomass particularly in chambers H and I which had N concentrations $\geq 20$ $\mu g \cdot L^{-1}$. A similar increase in variability was associated with additions of P resulting in no significant difference among diatom and chlorophyte dominance at P concentrations $\geq 5$ $\mu g \cdot L^{-1}$.

It is clear from these data that the addition of nutrients increases the potential for dominance by filamentous forms of the chlorophytes. Table 8 suggests that dominant species will be present at all nutrient concentrations that were tested, but that the overwhelming dominance by diatoms at low nutrient levels shifts to a more equitable mix of chlorophytes and diatoms at higher nutrient levels.

Figure 13
Changes in Chlorophyll-a Concentrations Due to Additions of N at 50 µg·L$^{-1}$and P at 10 µg·L$^{-1}$ in the Bioassay Enrichment Experiment

- Data are means of duplicate samples.




## *Nutrient Deficiency*

N and P additions are used in the bioassay to provide insight into limitation of the growth of peri-phyton by N, P or a close coupling of N and P. The accrual curves were plotted to show biomass responses to individual and combined nutrient additions (Figure 13). Except for the control curve which shows the response to no additions, each curve in Figure 13 represents the response at the highest nutrient levels tested: N = 50 µg·L$^{-1}$ and P = 10 µg·L$^{-1}$. The greatest response occurred with the addition of both nutrients from which PB reached 76.7 mg·m$^{-2}$ which was more than 10 times the level measured in both control chambers and the chambers receiving P alone. PB in chambers receiving N additions alone reached 29.1 mg·m$^{-2}$; about a 5 times increase over control levels or half of that found from the N plus P additions. The increase in biomass levels due to N additions alone was evidence that concentrations of N were limiting growth. Since a similar response was not observed from P additions, the community was P-replete relative to N supply.

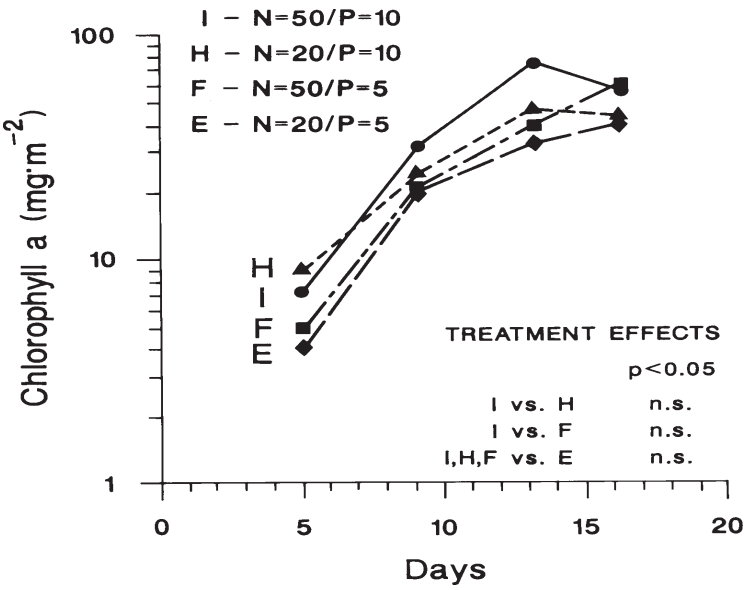
Hence, the bioassay community was primarily N-deficient. If N were in excess, further additions would not produce a response. Although the response from addition of N suggested that internal reserves of P were sufficient to increase growth, greater increases in accrual with the addition of N and P suggested that P reserves were depleted when N was added alone. This response is evidence of a coupling between N and P-deficiency: increased concentrations of N reduced the primary deficiency by N resulting in increased growth rates by the algal community which in turn drove the community into limitation by P once internal P reserves were depleted. If P was in excess, the addition of N alone would result in a several-fold increase in biomass which would be similar to a response from additions of both N and P. This coupling of N and P is also known as N-P co-limitation (Suttle and Harrison 1988).

The effect of varying concentrations of added nutrients can be used to examine the saturation of accrual that is limited by a coupling of N and P. We can explore two scenarios: one where we change N concentrations at surplus P and the other where P is altered under N-replete conditions.

N or P-replete growth infers that cell divisions pro-

Figure 14
Changes in Chlorophyll-a Concentrations at Combinations of N and P Added Together in the Bioassay Enrichment Experiment

- Data are means of duplicate samples.




ceed without limitation caused by depletion of N and P reserves. Growth or the accrual of periphyton is controlled by some other factor, usually temperature in the case of lotic periphyton (Bothwell 1988a). An index that reflects the filling of internal reserves is the substrate affinity or uptake rate ("substrate" refers to a nutrient element) which is relatively high if internal reserves are low and is low when reserves approach maximum capacity (Suttle and Harrison 1988, Rhee 1978). Hence, nutrient uptake rates and particulate nutrient concentrations (concentrations in cell tissue) are important variables in determining if a community is replete or deficient in specific nutrients. By using these and other physiological indices, Bothwell (1985) found that the growth of lotic periphyton cells that were dominated by diatoms in the Thompson River, B.C. was saturated with respect to P at concentrations of <1 $\mu g \cdot L^{-1}$. The critical concentration may increase, however, as the algal mat thickness increases; mainly because diffusion processes can limit the transport of P to all cells at an equal rate (Bothwell 1988b, Lock and John 1979). Similarly, Bothwell (pers. comm.) has found the critical N concentration at the cellular level to be about 20 $\mu g \cdot L^{-1}$, but may be higher in dense algal mats.

Bothwell's experiments are directly relevant to the present work in that the periphyton taxonomy in Bothwell's work was very similar to the community in the Nechako River. Hence, physiological criteria can be compared. To be conservative we suggest that saturation of N and P-limited growth of periphyton in the bioassay likely occurred at concentrations slightly higher than what Bothwell found; for example 5 and 30 $\mu g \cdot L^{-1}$, respectively.

This hypothesis was tested by comparing the biomass accrual between the two levels of N when P was at the maximum concentration (chamber I versus H) and the two levels of P when N was at maximum levels (chamber I versus F). In these comparisons I assume that at the community level, cell reserves of N and P were replete at the highest concentrations tested. Hence, any change in response to manipulation of one nutrient is not confounded by limitation of accrual by the concentration of the other. Results plotted in Figure 14 show that peak biomass at the highest N and P concentrations (chamber I) was 60% greater than was found for any other treatment. However, statistical comparisons showed that differences between peak biomass means for both the I versus H and I versus F comparisons were not signifi-

cant ($p<0.05$). Also, the ANOVA used to examine differences between means at all points through time showed that differences in chlorophyll-a concentrations for the treatments H, I, F, and E shown in Figure 14 were always statistically insignificant at $p<0.05$. Since responses at the higher concentrations were not found to be different from those at the lower concentrations, these data suggest that N and P-limited growth was saturated at concentrations of not more than 20 $\mu g \cdot L^{-1}$ N and 5 $\mu g \cdot L^{-1}$ P. At these concentrations, cell reserves of N and P, at the community level, were replete with respect to requirements for N and P.

An additional line of evidence that can be used to examine the effect of increasing nutrient concentrations is to compare biomass accrual resulting from the lower combined N and P additions (treatment E) to that at the higher combined additions. If the lower concentrations saturate the N and P-limited accrual, then there should be no difference in accrual at the lower and higher combined additions. All comparisons were done by examining differences in peak biomass and average chlorophyll-a concentrations at all sampling dates through time using the 2-way fixed effect ANOVA. Chambers E and I were first compared, and again mean responses from additions of the lower nutrient concentrations (chamber E) appeared less than those resulting from the higher nutrient concentrations (chamber I), but the statistical comparisons showed no difference between the two ($p<0.05$). Statistical similarities were also found when the remaining combinations were tested (H versus E; F versus E; F versus H), thus corroborating conclusions that the lower concentrations were sufficient to saturate N and P-limited accrual in the bioassay chambers.

# DISCUSSION

Several points emerge in this study which are important with regards to the methodology of fertilizer application to the Nechako River, an understanding of biological responses to nutrient addition in the Nechako River, and the selection of treatment rates in future fertilization trials.

## Fertilizer Dispersal

Although methods for dispensing fertilizer are relatively simple in small rivers (Perrin et al. 1987, Peterson et al. 1985), the complexity of plume dynamics becomes increasingly important as the size of the river increases. In this study, considerations of plume dynamics were relatively unimportant as the intent of the side channel fertilization was simply to examine a periphyton response to nutrient addition. Whether the treatment covered the entire channel or not was of little consequence. Water sampling did, however, show the importance of plume dynamics in the dispersal of fertilizer and can be used in identifying an approach for larger scale treatments where there is interest in ensuring that responses do occur across the entire width profile of a designated reach.

Within the side channel, the temporal pulsing of nutrient transport (Figure 7) due to the dispenser mechanics was obviously important in contributing to variation in N and P concentrations. Some of the peak concentrations were relatively high and although they never reached levels that may be considered toxic to aquatic organisms or fish, there is interest to modify the pulses to ensure that no such concern arises in the future. This change can easily be done with alternate dispenser equipment including continuous feed systems using fertilizer in a liquid or slurry form.

The more complicated variation in nutrient transport was related to incomplete lateral dispersal which was evident within the side channel (Figure 7) and at locations more than 1 km downstream (Figure 8). Many of the observations can be explained with calculations of plume transport or more correctly, "pollutant cloud" dynamics (Fisher et al. 1979). The dispersal of a plume can be described by a probability distribution in which increments away from a central axis represents the transverse distance from the centre of the cloud ($\theta$). As is the case for probability distributions, one width interval ($\theta_y$) would include 68% of the cloud and $2\theta_y$ would include 95%. $4\theta_y$ would represent 95% of cloud width on both sides of the central axis. For specific flow regimes, Fisher et al. (1979) and Ward (Peter Ward and Associates Ltd.; pers. comm.) show that $\theta_y$ can be determined as:

$$\theta_y = (2E_y t)^{1/2}$$

Where $E$ = transverse dispersion coefficient and

$t$ = elapsed time the substance is in the water.

Since $t = x/u$

Where $x$ = distance from the injection point and

u = longitudinal velocity and

$E_y = 0.6\ du^*$

Where d = mean depth and

u* = sheer velocity = u / 10 for natural river channels

Then the 95% cloud width

$4\theta_y = 4[2(0.06du / u)x]^{1/2}$ or

$4\theta_y = 4(0.12dx)^{1/2} = 1.39\ d^{1/2}\ x^{1/2}$

At the mean depth of 0.37 m in the side channel used in this study, cloud width of $4\theta_y$ at the half way point (150 m) and the end of the channel (300 m) was 10.3 m and 14.6 m, respectively. Since the channel had an average width of 31.5 m, there is little surprise that fertilizer was not completely dispersed even with the use of two dispensers.

In the Nechako River mainstem the mean depth was near 0.6 m in the vicinity of Bert Irvine's Lodge at the time of this study. At that depth, $4\theta_y$ at 300 m, 1000 m, 2000 m, and 3000 m would be 18.6 m, 24 m, 34 m, and 58.9 m, respectively. At an average width in the mainstem ranging from 75 to 100 m it is clear that complete dispersal of a pollutant cloud from a single point source would require more than 3 km to become dispersed across the width profile. It would in fact require a minimum of 5 km to produce a 95% cloud width of 76 m.

Although these calculations can become more complex to deal with detailed physical effects, they offer an example of what can be used to determine the number of point sources for fertilizer injection ($F_n$) that will be required for complete coverage of the width profile within a desired distance downstream. The distance required for complete dispersal (D) is arbitrarily selected on the basis of required target areas and the mean channel width (W) is divided by the 95% cloud width that is determined for that distance. For example, if we arbitrarily select D = 300 m and W = 75 m and $4\theta_y$ at 300 m = 18.6 m, then $F_n$ = 75 / 18.6 = about 4 point sources across the width profile.

## Nutrient Deficiency

It is clear from both the side channel fertilization and bioassay experiment in this study that periphyton accrual in the Nechako River will increase with additions of N and P. The bioassay in particular, showed that the periphyton community was primarily N-deficient and when N was added, the community was driven into P-deficiency (Figure 13). This co-limitation by P was shown by levels of accrual indices at the N plus P treatment that were greater than when N was added alone.

N:P supply ratios determined during the bioassay provided additional evidence of N-deficiency by indicating the potential limitation of the community for N or P. The relative deficiency for resources among species of a community will vary: critical ratios which determine whether an alga is N or P-deficient are different among species and generally cover the range from 7:1 to 45:1 by atoms (Rhee and Gotham 1980). Supply ratios in the same range indicate potential co-limitation by N and P. At relatively low ratios most species of a community will be potentially limited by N and at high ratios by P, but as long as the supply ratio is within the range known to occur in algae, the potential for co-limitation exists. In the Nechako River, concentrations of N and P that were available for uptake by algae ($NO_3$, $NH_4$, SRP) were largely undetectable. Occasionally, however, DIN concentrations were measured at 5 $\mu g \cdot L^{-1}$ and SRP was measured at not more than 1 $\mu g \cdot L^{-1}$ resulting in an atomic supply ratio of 11.16:1. This value is near the extreme low end of critical ratios in algae and suggests that a greater proportion of the community would be potentially N-limited than P-limited.

The importance of N and P in limiting autotrophic production in lotic and lentic systems is well established and has been investigated in detail since the early descriptive studies by Hasler and Einsele (1948) in lakes and Hunstman (1948) in streams. Usually, phosphorus has been regarded as the nutrient that primarily limits autotrophy; a view that originates largely from classic work by Vallentyne (1970) and the graphic demonstration of phosphorus limitation by Schindler (1974). Much of this early work was based on experiments using water from lakes having excess nitrogen relative to P supply, usually in interior continental areas of North America. More recent studies, particulary those examining primary production in coastal lakes of western Canada, have found that both N and P supplies can be extremely low (Stockner and Hyatt 1984). At these sites, a close coupling between N and P-deficiency may be the rule rather than the exception (Suttle and Harrison 1988) and N must be regarded as equally important to P in the lake trophodynamics.

Table 9
A Comparison of Growth and Biomass Parameters Between Results of the Bioassay (Nutrient Deficiency Component) and the *In Situ* Periphyton Accrual Sampling (Series 2)

| | Bioassay | | | | *In Situ* (Series 2) | |
|---|---|---|---|---|---|---|
| | Control | N Alone | P Alone | N Plus P | Control | Treatment |
| DIN | <15 | 50.3 | <15 | 50.0 | <15 | 30-40 |
| SRP | <1 | <1 | 12 | 12.7 | 1 | 10 |
| PB | 6.81 | 29.1 | 6.56 | 76.7 | 14.9 | 218 |
| SB | 6.29 | 25.3 | 6.37 | 66.8 | 8.12 | 121 |
| k | 0.13 | 0.24 | 0.13 | 0.32 | 0.088 | 0.416 |

Nutrient concentrations are expressed as elemental $\mu g \cdot L^{-1}$. Peak Biomass (PB) and sustainable biomass (SB) are units of mg. chl.-a$\cdot m^{-2}$ and the growth constant (k) is expressed as div$\cdot d^{-1}$.

Similarly in streams, P can be of singular importance in some systems (Bothwell 1985, Perrin et al. 1987), but co-limitation of N and P with primary P-deficiency is characteristic of others (Peterson et al. 1983, Stockner and Shortreed 1978). At a few sites where volcanic parent materials dominate the drainage chemistry, nitrogen is found to be deficient (Bisson 1976) with no co-limitation by P. This relatively unusual condition is related to relatively high dissolved P concentrations in the surface chemistry associated with weathering of the volcanic material.

Considering these various possibilities for relative N and P deficiency, the present bioassay data are not surprising, but they are important in being the first to demonstrate primary N-deficiency and co-limitation of N and P in a lotic system in Canada. Suttle and Harrison (1988) may be correct in their view that co-limitation of primary production by N and P at very low ambient nutrient concentrations may be more typical in freshwater systems than has been perceived up to the present.

## Periphyton Responses to Nutrient Addition

The relative biomass responses by the periphyton to N and P additions in the Nechako River (Figures 9, 10) were similar to periphyton responses measured in other rivers. In the Keogh River, for example, Perrin et al. (1987) reported more than an order of magnitude increase in biomass accrual with peak chlorophyll-a concentrations reaching 150 $mg \cdot m^{-2}$ on styrofoam substrata, virtually identical to that shown in Figures 9 and 10. Peterson et al. (1983) found relative fluorescence on glass slides to increase about 4 times from addition of the primary limiting nutrient (P in this case) and more than 6 times from addition of N and P in a bioassay in the Kuparuk River, Alaska. Since measurements were not continued through the entire exponential phase of accrual in that study, relative differences may actually have been greater, given additional time. In Carnation Creek bioassays (Stockner and Shortreed, 1978), periphyton biomass increased from a peak of 25 $mg \cdot m^{-2}$ after 30 days of no nutrient addition to 110 $mg \cdot m^{-2}$ with P addition alone, and 225 $mg \cdot m^{-2}$ with both N and P added over the same period.

Absolute values in these data may not be directly comparable to the present results because of differences in substrata materials from which measurements were made, as well as differences in temperature, water flow and other environmental factors that can influence periphyton accrual. What is important, however, is that the relative increases in periphyton responses due to addition of the primary limiting nutrient, and further increases due to additions of co-limiting nutrients were consistent among studies. A 4-5 times increase in biomass per unit time during the exponential period of increase was found by addition of the primary limiting nutrient and a 6-11 times increase was typical from combined additions with

co-limiting nutrients.

The variation in ranges of responses was related directly to the length of time in which measurements were continued. Greatest responses were found when measurements continued throughout the exponential phase of accrual and true peak biomass on the substrata was measured.

Although the Nechako River side channel received N and P additions that were less than the highest concentrations tested in the bioassay, *in situ* periphyton responses were greater than the bioassay responses (Table 9). One explanation for the differences involves confounding by passive settlement on measurements of periphyton accrual, an effect that was originally described by Bothwell and Jasper (1983). The rate of settlement of suspended material is directly related to the amount of biomass that is in suspension which in turn is related to the amount of biomass that has accumulated on river substrata upstream of a measurement location. As nutrient addition increases the amount of periphyton as standing crop, it also increases the flux of suspended material because of sloughing effects. Eventually that suspended material settles out at some point downstream from where it was sloughed and will continuously add to the biomass that is produced from actual growth. At NT1, there was 300 m of upstream substrata that had large accumulations of periphyton. The extent of periphyton biomass was relatively small upstream of the bioassay apparatus (Figure 9 and 10). Hence, the flux of suspended biomass and the amount of passive settlement would be expected to contribute relatively high positive bias to results at NT1 compared to those at the bioassay location regardless of the nutrient regime.

The nature of nutrient introduction may offer another explanation to this disparity. The side channel was fertilized using nutrient pulses whereas the bioassay chambers received continuous enrichment. This difference should not be important from a theoretical basis in that cells were expected to be replete with respect to both N and P supply under both conditions. However, relative differences in periphyton responses to different nutrient delivery regimes has not been investigated in streams and remains unresolved in this study.

Current velocity is also important in regulating rates and magnitude of periphyton accrual. Growth rates, respiration rates, nutrient uptake rates and metabolic activity, in general, increases in algae growing in relatively high current velocities mainly because diffusive boundary-layer effects are minimized (Whitford and Schumacher 1964). Diffusion of nutrients that is enhanced at higher current velocities is fundamental to the transport of ions in an algal mat (Schumacher and Whitford 1965, Lock and John 1979). I have mentioned that the bioassay apparatus reduced flows across the substrata to about 60% of ambient flows. This reduction may have been adequate for diffusion processes to be more important in limiting accrual in the bioassay compared to that at NT1, particularly at highest biomass levels attained near the end of each measurement period.

## Periphyton Taxonomy

In both the bioassay and side channel fertilization, higher concentrations of N and P selected for an increase in the relative abundance of filamentous chlorophytes. At the highest concentrations tested, diatoms and chlorophytes equally dominated the community (Figure 11), which was a significant shift from the unfertilized community in which diatoms typically represented 90% of the community. The bioassay showed that the shift was significant at N concentrations greater than 20 $\mu g \cdot L^{-1}$ and P concentrations greater than 5 $\mu g \cdot L^{-1}$. Although the selection of these nutrient concentrations may have been an artifact of the analysis in that only three treatment levels were examined, there is little question that higher nutrient levels did increase the competitive advantage of chlorophytes.

In much of the early eutrophication literature, N-deficiency is often associated with "eutrophy" under the premise that N-deficits could be overcome by increasing dominance by N-fixing cyanobacteria, at least in lakes (Schindler 1977). However, N-fixers and other Cyanophytes were virtually non-existent in the Nechako River. The important point here is that phosphorus is, by definition, always in excess concentrations in a eutrophic condition, but in systems where both N and P are deficient, N-fixers are poor competitors for available P (Smith and Kalff, 1982). The common diatom genus, *Synedra*, for example, can outcompete many other genera including most cyanobacteria for available P. Similar competitive interactions may have been important in the Nechako River. Although production of periphyton

in the Nechako River was limited by N, N-fixers were not found, likely because of their poor ability to sequester limited supplies of P.

A fundamental question associated with the shift in algal community composition is whether the change affected invertebrate community structure which ultimately contributes to the diet of fish. Although work in diet preference by invertebrates is limited, the perception is that stream invertebrates are in a general sense, omnivores (Cummins and Klug 1979). It appears that the ability of mouth parts to efficiently ingest particulates is the main limitation to food utilization (McCullough et al. 1979). In large streams where trophic energetics depend on autotrophy (Minshall 1978, Naiman and Sedell 1979), dominant insects are those that can efficiently ingest unicellular and filamentous algae and detrital material. According to the functional classification of Cummins and Klug (1979), these include collectors, filterers, herbivore shredders and piercers (Hawkins et al. 1982). Surprisingly, scrapers which feed on thin algal films on the surface of rocks may not dominate at high periphyton biomass levels since accumulations of filamentous algae may physically exclude them from clean rock habitat. Also, where a filamentous "slurry" of algae is encountered by the filter feeders, *Simulium spp.* (Diptera) and *Hydropsyche occidentalis* (Tricoptera), the animal's ingestion rate may decline because of time taken to clean and avoid destruction of its net which is used for food collection (McCullough et al. 1979). Otherwise, these same species are opportunistic and will essentially use any food item as long as they can physically handle it with some degree of efficiency. Larvae of the caddisfly, *Helicopsyche borealis* are also opportunistic and where physically possible, will aggregate wherever periphyton biomass is highest (Lamberti and Resh 1983). Herbivore shredders and piercers, a group of insects that exploit macrophytes or large filamentous forms or mats of algae may increase in importance in thick algal mats because they are physically efficient in ingesting such material (Hawkins et al. 1982).

These studies and observations suggest that insect larvae are highly opportunistic, but relative dominance of taxa may be fundamentally limited by the functional ability of mouth part structures to efficiently ingest food particles. Functional limitation of food ingestion has been dealt with in some detail in the studies mentioned above and consequently it is reasonable to expect some shifts in relative dominance among taxa according to their abilities to ingest particles in an environment of greatly increased diatom and green algal biomass. One might speculate that an increase in periphyton production should be limited to a level above which the insects cannot efficiently ingest the food. Unfortunately, the quantitative nature of that response has never been experimentally examined. In general, shifts in insect community structure due to manipulation of algal biomass and community structure remain unresolved.

Despite these uncertainties in real changes to invertebrate community structure, there is evidence that fish do respond to nutrient enrichment where diatoms and filamentous chlorophytes dominate. Undoubtedly, insect species composition will shift but reduced diversity or complete shifts away from the present community structure would appear unlikely. In the Keogh River studies where fertilization also produced a large increase in biomass of diatoms and green algae, a change in the invertebrate community structure was not detectable (Perrin and Johnston 1986) and within two months of treatment, mean weights of coho salmon fry increased by 82% over those measured in a control reach. Steelhead weights increased by 67% over those in the control (Slaney et al. 1986). Clearly, the invertebrate fauna remained suitable as a food supply and invertebrate biomass must have increased to support the increased fish growth. Similar responses were found in the Kuparuk River studies in Alaska where a doubling in mean weights of Arctic Grayling was found (B. Peterson, Woods Hole Oceanographic Institute; pers. comm.). Hence, a "black box" approach from these studies suggests that invertebrate communities remain suitable to support increased fish production when fertilizer additions increase algal biomass that is composed of unicellular and filamentous taxa. We have no evidence to suggest that invertebrate responses in the Nechako River should be any different, despite the shift in algal community structure.

## N and P Concentrations and Fertilization Trials

The taxonomic shifts, biomass and growth responses, and their relationship to nutrient levels in this study form an important relationship that can be used to select treatment rates for future fertilization of the Nechako River. There are two points. First, the bioassay suggested that concentrations of N 20 $\mu g \cdot L^{-}$

[1] were sufficient to saturate N-limited accrual. A corresponding level of P was found to be 5 $\mu g \cdot L^{-1}$. Taxonomic shifts were also evident at concentrations greater than or equal to these levels, but these shifts may have occurred gradually rather than occurring abruptly at a critical concentration. The data are not sufficiently precise to separate these two possibilities. The second point is that an order of magnitude increase in biomass resulted from these same concentrations.

The selection of concentrations of both N and P can be approximated from these data. An N concentration of 20 $\mu g \cdot L^{-1}$ can be selected as a potential target for future treatments with confidence that sustainable biomass will increase about 5 fold (Figure 13) and taxonomic shifts will be minimized (Figure 11). Additions of P alone will not produce a response because the Nechako River is primarily N-deficient (Figure 13). Additions of N at 20 $\mu g \cdot L^{-1}$ and P at 5 $\mu g \cdot L^{-1}$, however, can significantly increase the growth rate index by about 4 times and increase sustainable biomass at least an order of magnitude or a level twice that achieved from N added alone (Figures 13, 14). As discussed above, these relative responses are typical of those which produced a doubling in mean weights of juvenile salmonids in the Keogh River study and grayling in the Kuparuk River study.

It is important to recognize that the selection of these N and P concentrations is not definitive. Analysis of the bioassay data were relatively insensitive to effects of small changes in nutrient concentrations. The critical N and P concentrations may actually be less than 20 and 5 $\mu g \cdot L^{-1}$ respectively but the numbers of treatments required to examine relative responses in small intervals was beyond the capabilities of a single bioassay. Similarly, more precise information on community shifts in smaller ranges of nutrient concentrations would have to be handled in additional bioassays. The scope of the present work was wide ranging and it provided reasonable estimates of critical N and P concentrations. In a large river fertilization, more accurate values may be required, particularly if critical concentrations are found to be lower, thus providing savings in the cost of fertilizer, application equipment and logistics as the spatial scale of the treatment increases. To improve this accuracy in the assignment of critical concentrations, more precise bioassays will be required.

Another factor that can affect assignments of the N and P concentrations was sampling precision in the bioassay and its effect on statistical decisions shown in Figure 14. Although the ANOVA is a powerful test, it is sensitive to outlier data of which there were a few in the bioassay data. All outliers were included in the ANOVA and as such the analysis may have been biased by suggesting that means were similar when they may actually have been different. However, there was no basis for ignoring these outliers as no errors were found in procedures to explain them. Hence, they must be considered representative of variation associated with the algal mat development. Daily observations of the bioassay indicated that much of the variation was related to random sloughing of biomass from the substrata which produced a patchy biomass distribution, particularly near the end of the experiment; the same time that outliers appeared in the data. Since the chambers were sampled randomly, sloughing may have been important in finding no significant differences in responses between combinations of N and P yet there were apparent differences between means in Figure 14.

Sampling precision may be improved despite the effects of sloughing if single daily samples are collected from each chamber in any future bioassay work. Regression analysis can then be used to examine differences in k, SB and PB between treatments rather than using ANOVA. The important difference here is that temporal precision which is clearly important in defining growth and biomass indices in the Nechako River data would increase. Spatial precision which is sensitive to variation due to sloughing effects would decrease and relative accuracy of the analysis would increase. This approach would be particularly useful when examining biomass and taxonomic responses to relatively small changes in nutrient concentrations.

Another factor that must be considered in the bioassay was that the nutrient-accrual relationships were based on responses on substrata that supported relatively small amounts of biomass. The relative responses apply mostly to cellular demands for limiting nutrients. At greater levels of biomass that may occur on natural substrata, particularly with effects of passive settlement, diffusion processes may be important in restricting the flux of ambient N and P to all cells in the periphyton mat (Bothwell 1988b). Hence, order of magnitude increases in biomass may not be maintained if concentrations drop below those which are recommended for further fertilization of the Nechako River.

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